Chapter

Neurofibromatosis Type 2: Current Trends and Future Directions for Targeted Biologic Therapies

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Abstract

Neurofibromatosis type 2 (NF2) is an inherited tumor predisposition syndrome leading to the formation of vestibular schwannomas (VS) and other central nervous system (CNS) tumors. Clinical NF2 follows a genetic alteration to the NF2 gene, which disrupts the function of a cell membrane-related protein, merlin. Though the role of merlin is incompletely understood, it is predominantly thought to achieve tumor suppressive effects by affecting multiple signaling pathways important for contact inhibition, cellular proliferation, and cellular growth. Patients with NF2 have a bimodal age of onset in children and young adults, with the former tending to present with a more severe phenotype involving multiple tumors. Currently available treatments are non-curative. Surgical resection is the mainstay for growing tumors but comes at the cost of significant morbidity, while radiotherapy is generally not advisable due to the risk of secondary malignancy and malignant transformation. Hence, there remains a critical demand for effective anti-neoplastic therapies for NF2-related tumors. There are currently no FDA-approved biologic therapies for the treatment of NF2. Given the complexity and far-reaching effects of Merlin, multiple molecular targets and pathways have been investigated and are currently at various stages of investigation.

Keywords: neurofibromatosis type 2, Merlin, vestibular schwannoma, targeted therapy, Bevacizumab, axitinib, sorafenib, lapatinib, erlotinib, crizotinib, brigatinib, everolimus, vistusertib, AR-42, selumetinib

1. Introduction

NF2 is an autosomal dominant, tumor predisposition syndrome with an incidence of 1:25,000–1:40,000 [1, 2]. The diagnosis is made based on the presence of specific clinical features. Under the NIH criteria, a patient can be diagnosed with NF2 if they have [1] bilateral VS or [2] have a family history significant for NF2 and any one of the following: unilateral VS, other schwannoma, meningioma, glioma, neurofibroma, or juvenile posterior subscapular lens opacity [3]. Notably, the presence of a pathogenically mutated NF2 gene is not necessary to complete the diagnosis.

The NF2 gene is located on the long arm of chromosome 22, and encodes for the cell membrane-related protein, Merlin or Schwannomin. Though the function of Merlin is incompletely understood, it is predominantly thought to achieve tumor suppressive effects by affecting multiple signaling pathways important for contact inhibition, cellular proliferation, and cellular growth [4, 5]. A wide variety of genetic alterations affect the gene predisposing to NF2, including frameshift, nonsense, missense, and less commonly, splice site [6, 7]. Mutations of the NF2 gene are only detected in 70% of affected patients, and the rate of detection is even less for patients without a family history. This discrepancy is explained by mosaicism: about 25–30% of NF2 patients with a de novo mutation of the NF2 gene are mosaics, where only a portion of their cells contain the mutated gene [8].

Patients with NF2 are prone to develop multisystemic clinical features involving the nervous system, eyes, and skin. The most common clinical manifestations include bilateral VS, intracranial and spinal tumors (other schwannomas, meningiomas, and ependymomas), peripheral neuropathy, cataracts, and cutaneous tumors [9, 10]. Less common but prevalent features include epiretinal membranes, retinal hamartomas, skin plaques, and subcutaneous tumors [9, 10].

The age at clinical onset is bimodal: the first peak is in children, with a median age of onset ranging from 8 to 14 years in pediatric patients [11–13], while the second is between the ages of 20 and 22 for adults [9, 10]. Correspondingly, the clinical presentation of patients affected by NF2 is grouped into two main subtypes: the more aggressive Wishart type, more common for childhood-onset, and the less aggressive Gardner type, presenting in adulthood [13]. Adults tend to present with symptoms of tinnitus, hearing loss, and/or imbalance related to VS, while children have a higher likelihood of presenting with symptoms due to spinal cord compression or other CNS-tumors, as well as ophthalmologic involvement [11, 12, 14].

Growth rates for NF2-related tumors vary [15]. Although NF2-related VS are generally slow growing, they are known to cause considerable morbidity and mortality. Left untreated VS leads to decline in auditory function and complete hearing loss over a period of several years [16]. The goal of treatments for NF2 is to reduce the presence of disabling symptoms, and help improve overall survival for patients. Unfortunately, there are no FDA-approved pharmacologic therapies, and currently available treatment options are non-curative. Surgical resection remains the only approved and standard treatment for NF2-related tumors, while radiotherapy is a less desirable option. Targeted biologic therapies may be an emergent treatment option.

Surgical resection is indicated for VS with accelerated growth rate, ≥3 cm in size, and/or symptoms of radiographic evidence due to compression of adjacent structures [17]. However, surgical intervention comes at the risk of hearing loss, facial paralysis, damage of lower cranial nerves, stroke, and CSF leak [17]. In addition, VS can recur following resection: a large retrospective review evaluating 148 NF2 patients with a mean pre-operative tumor size of 3.1 cm and a mean follow-up of 12 years revealed a recurrence rate of 14% [18]. The size of VS also does not correlate with the degree of hearing loss [19, 20], and at times there is profound hearing loss despite a very small size of the VS where surgical resection would unlikely reverse deafness. In contrast to VS, other schwannomas tend to be slower growing and hence surveillance with serial imaging is recommended; surgical resection is reserved for tumors symptomatically affecting the patient [17].

In regards to NF2-related meningiomas, surgical resection is advised when patients become symptomatic or tumors demonstrate increased growth rate [10, 17]. In contrast, the role of surgical resection for spinal ependymomas is not clearly established. Results from a multi-institutional retrospective review suggest that timely resection in the hands of a skilled neurosurgeon may improve a patients' natural clinical history [21].

NF2-related tumors may also be treated with radiotherapy. There is no formal consensus regarding the role of radiotherapy for NF2 patients with growing VS. The use of external beam radiotherapy for progressive VS in NF2 patients is considered controversial due to the increased risk of malignant transformation and secondary malignancy, as compared with patients with sporadic VS [22–24]. Additionally, patients with NF2-related progressive VS treated with radiotherapy have poorer rates of local control and reduced preservation of hearing as compared with non-NF2 patients [17, 25]. In a large retrospective study by Rowe et al., only 40% of 92 NF2 patients with growing VS treated with stereotactic radiosurgery (SRS) retained hearing function at 3 years post treatment; the rest progressed, including 20% of patients who developed complete hearing loss [26]. Overall, radiotherapy for NF2-related VS is not recommended, and is reserved for surgically inaccessible tumors [3]. Moreover, radiotherapy prior to surgical resection has fallen out of favor due to the observation that radiotherapy makes resection of VS more difficult [3]. Data on SRS for NF2-related meningioma is even more scarce. In general, radiation therapy is not advised due to the risk of creating a secondary malignancy, and is reserved for atypical and/or anaplastic meningiomas [17].

2. Biologically targeted therapies

Current treatment options for NF2-related tumors provide only temporary benefits and there are no FDA-approved pharmacologic therapies for NF2. There remains an unmet need for effective anti-neoplastic therapy of NF2-related tumors. Since NF2-related tumors tend to be slow growing, options such as cytotoxic chemotherapies are not appropriate to treat this lifelong condition, thus molecularly targeted therapies represent a better treatment modality and a budding and encouraging prospect.

Merlin is a multifunctional protein that is involved with the regulation of intra- and extracellular molecules and biologic pathways that are important for cellular structure, survival, proliferation, and contact-dependent inhibition. Increased research efforts have enabled the discovery of various molecular pathways and targets relevant to the pathogenesis of NF2-related tumors. These include vascular endothelial growth factor (VEGF), platelet-derived growth factor receptor (PDGFR), and epidermal growth factor receptor (EGFR), phosphoinositide 3-kinase (PI3K)/Akt, histone deacetylase (HDAC), and mammalian inhibitor of rapamycin (mTOR). The mechanisms of these targets and their pathways are briefly discussed here.

NF2-VS have increased expression of VEGF [27, 28], a powerful mediator of tumor angiogenesis [29]. Additionally, VS are highly vascular tumors, making anti-angiogenesis an appealing option that has been evaluated in NF2 patients in clinical studies [30] and is described in further detail in Section 2.1. The precise mechanism of angiogenesis regulation by Merlin is not known, but a downstream pathway implicating the Rac1 GTPase has been described [31]. Merlin is thought to maintain angiogenesis by regulating Rac1 activity. In NF2-deficient Schwann cells, increased Rac1 activity lead to augmented VEGF levels and more tumor burden [31].

Another growth factor relevant to NF2-pathogenesis is PDGFR. PDGFR accumulates on the surface of NF2-deficient Schwann cells [32, 33]. Increased activation of PDGFR leads to downstream phosphorylation of B-catenin, and subsequent destabilization of cell junctions, loss of contact-dependent inhibition, and increased cell cycle activity [32]. Intact Merlin is important for regulating the concentration of cell surface PDGFR through degradation pathways [34]. In preclinical

studies, inhibitors of PDGFR reduced proliferation of NF2-deficient Schwann cells via negative regulation of ERK and Akt [35].

The ErbB family of receptor tyrosine kinases has also been increasingly studied in NF2, primarily ErbB1 (EGFR). NF2-deficient cells have increased levels of EGFR [32, 33], leading to increased cellular proliferation and resistance to apoptosis [36]. Merlin can physically associate with and negatively regulate EGFR by controlling its membrane distribution and subsequent trafficking [37–39], thereby blocking various downstream targets including Raf, extracellular signal-regulated kinase (ERK), and Akt [32]. Moreover, pharmacologic inhibitors of EGFR efficiently revert the phenotypic consequences of NF2-deficiency in several types of cultured cells [37–39].

Merlin is also involved in regulating a number of pathways and intracellular targets relevant to cellular proliferation and survival. In NF2 preclinical studies, loss of merlin activity leads to Schwann cell growth via activation of the PI3K/Akt pathway [40, 41]. Normally, Merlin inhibits PI3K activity, preventing downstream phosphorylation and hence activation of Akt, a protein kinase responsible for proteasome-mediated degradation of Merlin [42]. Akt phosphorylation is also regulated by HDAC; pharmacologic inhibitors of HDAC lead to tumor shrinkage and inhibition of cellular growth in preclinical NF2 studies [43].

In addition, gene-expression profiling of sporadic and NF2-associated VS demonstrated overexpression of the PI3K/Akt/mTOR pathway [44]. MTOR complex 1 (mTORC1) is an evolutionarily conserved protein kinase involved with cell survival, metabolism, and protein translation [45]. In contrast, mTOR complex 2 (MTORC2) regulates the actin cytoskeleton and activation of Akt [45, 46]. Merlin is considered a negative regulator of mTOR. Loss of Merlin function leads to constitutive mTOR signaling in NF2-deficient tumors, including schwannomas, meningiomas, and mesotheliomas. Notably, aberrant signaling of mTOR in NF2-tumorigenesis is independent of PI3K/Akt pathways [45]. In preclinical studies, inhibition with mTORC1 reversed the phenotypic consequences of NF2-deficient schwannoma and meningioma cell lines [45, 47].

In the following sections, specific pharmacologic-targeted therapies and relevant clinical studies are discussed further.

2.1 Angiogenesis inhibitors

2.1.1 Bevacizumab

The use of Bevacizumab, a humanized monoclonal antibody targeting VEGF, has resulted in meaningful imaging and hearing response rates for NF2 patients with progressive VS [28, 48, 49]. Plotkin et al. first showed the intravenous administration of Bevacizumab at 5 mg/kg every 2 weeks resulted in tumor reduction in 9 out of 10 patients, with an imaging response in 6 out of 10 patients, and improved or stabilized hearing (as measured by word-recognition scores) in 6 out of 7 eligible patients [28]. Additional studies with larger patient cohorts have demonstrated imaging and hearing response rates of 39–55% and 45–57%, respectively [48, 49], in addition to improved quality of life [49]. The average time to treatment response was 3 months [48, 49]. Hearing remained stable or improved in 86–90% of patients after 1 year [48, 49] and 61% after 3 years [48]; tumor volume remained stable or reduced in 88–90% of patients after 1 year and 54–63% after 3 years [48, 49]. In a large multi-institution prospective study with 51 NF2 patients, predictors of imaging response included older age: 6 patients <18 years with evaluable VS exhibited significantly reduced responses to Bevacizumab and tended to have faster growing

tumors refractory to treatment, as compared with their adult counterparts [49]. Similarly, a small retrospective study evaluating the efficacy of Bevacizumab for pediatric NF2 patients with progressive VS also yielded poor results: none of the seven patients met criteria for a radiographic response, and of the four evaluable patients for hearing assessments, one improved while three stabilized [50]. These observations are in line with previous studies revealing that younger patients have a higher likelihood of developing more severe phenotypes with accelerated tumor growth rates [15, 51].

This constellation of clinical results for adult and pediatric NF2 patients lead to phase II clinical studies evaluating the effect of bevacizumab for NF2 patients with growing VS. In the phase II study by the National Cancer Institute, a dose of bevacizumab 7.5 mg/kg every 3 weeks for 12 months was administered, the results of which are pending (NCT01207687). In the study by Plotkin et al., patients were treated with bevacizumab 10 mg/kg every 2 weeks for 24 weeks, followed by 5 mg/kg every 3 weeks [30]. Published preliminary results showed that 22 patients with NF2-related progressive VS experienced tumor shrinkage and improved hearing in 43 and 36% of patients, respectively [30]. In line with past studies [49, 50], the seven NF2 patients ≤21 years had much less benefit as compared with patients >21 years [30]. In fact, only one out of seven demonstrated a hearing response, and no patients had a radiographic response [30]. Reported adverse events were similar to prior studies [48, 49], and included hypertension, proteinuria, delayed wound healing, fatigue and irregular menses [30].

Unfortunately, the presence of these adverse events can lead to interruptions and even discontinuation of Bevacizumab therapy. In addition, long-term therapy with Bevacizumab is also limited by cumulative toxicities: primarily hypertension and proteinuria, but also, premature ovarian insufficiency in menstruating females. Responses are not sustained off treatment and discontinuation of Bevacizumab has been associated with accelerated VS regrowth and decline of hearing function [48]. The optimal duration of therapy with Bevacizumab for NF2-related VS remains unknown.

Bevacizumab has also been administered for treatment of other NF2-related tumors, including meningiomas and ependymomas. In a small retrospective study involving 15 patients with NF2-related meningiomas, a volumetric response rate of 29% was observed, however the median duration of response was limited at only 3.7 months [52]. Hence the effectivity for Bevacizumab in treating NF2-related meningioma remains unclear. In regards to NF2-related ependymoma, a retrospective evaluation demonstrated no significant benefit for patients with solid ependymomas, however, 7 out of 12 patients who had ependymomas with a syrinx or cystic component demonstrated radiographic and clinical improvements in response to treatment with Bevacizumab [53].

2.1.2 Axitinib

Axitinib is an orally available small molecule multikinase inhibitor of VEGF, PDGFR, and c-KIT (a receptor tyrosine kinase), leading to reduction of angiogenesis. Preclinical NF2 models have demonstrated the relevance of these molecular targets in the pathogenesis of NF2-related schwannomas [35, 54]. Specifically, *in vitro* studies on human schwannoma cells from NF2 patients revealed inhibition of PDGFR and c-KIT activation lead to inhibition of schwannoma cell growth and induction of schwannoma cell death [35, 54]. Recently, these results have been translated into an ongoing phase II clinical trial, evaluating the efficacy of axitinib in patients with NF2 and progressive VS (NCT02129647).

2.1.3 Sorafenib

Sorafenib is an orally available small molecular multikinase inhibitor against PDGFR, VEGF, and kinase-Raf1 (C-RAF). The relevance of these markers for VS tumorigenesis has been investigated in preclinical studies: increased PDGFR activity lead to increased cellular proliferation of human schwannoma cells *in vitro* by upregulating ERK 1/2 and Akt [35]. Expectedly, treatment with sorafenib reversed the PDGFR-mediated proliferation of schwannoma cells, and decreased the activity of ERK 1/2 and Akt [35]. These results were translated into a clinical study assessing the intratumoral concentration and activity of sorafenib in cutaneous schwannomas in NF2 patients (ISRCTN49989464), the results of which are pending.

2.1.4 Highlights

- Bevacizumab has efficacy in treating NF2-related VS, as evidenced by tumor reduction or stabilization, and improved hearing assessments or clinical stabilization [17, 28, 30, 49], in addition to improved quality of life [49].

- The exact dose remains to be determined, variable doses of 5 mg/kg every 2 weeks, 5 mg/kg every 3 weeks, and 10 mg/kg every 2 weeks have all demonstrated effectivity [17, 30, 49].

- As compared with their adult counterparts, there is less benefit in the treatment of pediatric NF2-related VS [30, 49].

- Although well tolerated for shorter durations, cumulative toxicities limit the long-term use of Bevacizumab, and responses are not sustained off treatment [48]. Optimal duration of treatment remains unknown.

- Bevacizumab is partially effective in treating NF2-related meningiomas, although these responses are not particularly durable [52].

- No benefit has been demonstrated for the treatment of solid ependymomas, however, ependymomas with a cystic component or syrinx had a modest response rate [53].

- Axitinib and sorafenib have demonstrated anti-schwannoma activity in preclinical studies [35, 54] and have been evaluated in clinical studies (NCT02129647 and ISRCTN49989464, respectively), the results of which are pending.

2.2 Tyrosine kinase inhibitors

2.2.1 Lapatinib

Lapatinib is an orally active small molecule, reversible tyrosine kinase inhibitor of EGFR and human epidermal growth factor receptor 2 (HER2/neu). Preclinical studies showed lapatinib had anti-proliferative effects on human schwannoma cells from NF2 patients *in vitro*, via downregulation of ERBB2, survivin, and other downstream receptor kinases [55]. Subsequently, in a phase 2 clinical trial for adults and children with NF2 and progressive VS, treatment with lapatinib resulted in imaging and hearing responses in 4 out of 17 patients and 4 out of 13 evaluable patients, respectively [56]. However, audiological responses were sustained in only one patient, and persisted for 9 months [56]. Based on these results, the 2018

Congress of Neurological Surgeons suggested there is Level 3 evidence to support lapatinib monotherapy in reducing VS volume and/or improving hearing function in NF2 patients with VS [57].

In addition, the effect of lapatinib on meningioma growth was evaluated in NF2 patients who received lapatinib in the clinical study described above [58]. Eight out of 17 patients had a total of 17 volumetrically measurable meningiomas and received at least 5 cycles of lapatinib [58]. Increased meningioma growth was observed in patients off lapatinib therapy (9 out of 17), as compared with those on treatment (2 out of 17). One patient had significant volume shrinkage which sustained for 23 months on lapatinib [58]. Hence, lapatinib may reduce the growth rate of NF2-related meningiomas and delay time to progression [58].

In regards to toxicity, in general treatment was tolerable and the most common adverse events were minor including most commonly skin rash, and less commonly fatigue, headache, diarrhea, nail changes, and elevations of liver transaminases without impact on liver function [56]. One patient developed a grade 3 toxicity of delayed wound healing postoperatively, and no grade 4 or 5 toxicities were observed [56].

2.2.2 Erlotinib

In contrast to lapatinib's pan-Erb inhibitory activity, Erlotinib is an orally active tyrosine kinase inhibitor that is selective for EGFR. Preclinical studies demonstrated persistent EGFR signaling in NF2-deficient cells contributes to cellular proliferation, and that treatment with a selective EGFR inhibitor stopped cellular proliferation at a high cell density via contact-dependent inhibition [37].

In a retrospective study, 11 patients with NF2-related progressive VS ineligible for surgical resection or radiotherapy were treated with erlotinib on the basis of compassionate use [59]. Unfortunately, no significant imaging or hearing responses were observed, though median time to clinical progression was 9.2 months [59]. Notably, the subset of four evaluable patients reported to experience stable disease on erlotinib all had slow VS growth rates (baseline annual volumetric growth rate ranging from 6 to 14%) prior to erlotinib initiation [59]. In summation, erlotinib is not effective in treating fast growing VS, however it may delay time to progression in patients with slow growing VS. The adverse event profile of erlotinib is similar to that of lapatinib, and primarily included minor toxicities of skin rash, diarrhea, and hair thinning [59]. Two patients developed a rare corneal keratopathy that was related to eyelash curling [59].

2.2.3 Crizotinib

Crizotinib is an orally active, small molecule multi-target tyrosine kinase inhibitor of mesenchymal-epithelial transition (MET), anaplastic lymphoma kinase (ALK), and c-ros oncogene 1 (ROS1). In orthotopic mouse models of NF2, treatment with crizotinib resulted in slower growth of VS as compared with those who did not receive the drug [60]. Kissil et al. found that wildtype focal adhesion kinase (FAK1) was necessary for proliferation of NF2-null Schwann cells and treatment with crizotinib lead to inhibition of FAK1 and significantly reduced proliferation of NF2-null Schwann cells [60]. Conversely, treatment with crizotinib-resistant forms of FAK1 reversed the anti-proliferative benefits seen with wildtype FAK1 [60]. Hence, the primary anti-proliferative activity of crizotinib on NF2-null Schwann cells is mediated through inhibition of FAK1 [60]. FAK has previously been recognized as a target involved with NF2-mediated tumorigenesis [61]. In addition, MET has also been implicated in the pathogenesis of NF2-related VS [62, 63]. Recently,

these results have been translated into an upcoming phase II clinical trial evaluating the efficacy of Crizotinib in children and adults with NF2 and progressive VS, set to open for enrollment later this year.

2.2.4 Brigatinib

Brigatinib is an orally available, small molecule tyrosine kinase inhibitor of ALK and EGFR. Based on a cell viability screen, ALK was identified as a promising target for inhibiting growth of NF2-deficient Schwann cells [64]. Subsequently, administration of brigatinib to NF2 mouse models delayed growth of schwannomas and better preserved hearing, as compared with vehicle-treated models [64]. These results have contributed to the development of a phase II clinical study for NF2 patients, which is currently being developed and set to open for enrollment later this year.

2.2.5 Highlights

- Lapatinib has modest effect in reducing VS volume and/or improving hearing function in NF2 patients with VS [56, 57]

- Erlotinib is not effective in shrinking VS or improving hearing function, however, it may delay time to progression in patients with slow growing VS [59].

- Crizotinib and Brigatinib have demonstrated anti-schwannoma activity in preclinical studies and these results have been translated into a phase II clinical studies set to open later this year.

2.3 Mammalian target of Rapamycin (mTOR) inhibitors

2.3.1 Everolimus (RAD001)

Everolimus is an orally available inhibitor of mTOR complex 1 (mTORC1). Merlin is considered a negative regulator of mTORC1, and loss of merlin function leads to increased mTOR signaling and NF2-related tumorigenesis [65]. In preclinical studies, inhibition of mTORC1 with rapamycin lead to VS tumor shrinkage *in vivo* [47] and halted growth of NF2-deficient meningioma cells *in vitro* [45]. These results were subsequently translated into two phase II clinical trials evaluating the efficacy of everolimus in NF2 patients with progressive VS [66, 67]. Both trials included adult and pediatric NF2 patients and subjects were treated with everolimus at 10 mg/day for continuous 28-day cycles. Neither study demonstrated any appreciable tumor shrinkage or hearing improvement after treatment with everolimus [66, 67]. However, in the phase II study by Karajannis et al., three adult patients did experience mild reductions in their target VS (−3.6 to −11.93 cm^3 reduction from baseline); two of these patients discontinued treatment at 3 and 6 months due to personal preference, while the third continued for 12 months with stabilization of their index VS [67]. Goutagny et al. also found that treatment with everolimus resulted in stabilization of tumor volumes, as well as hearing function, in five out of nine evaluable adult patients [66]. Stabilization was parallel to a decrease in the median annual growth rate of VS, from 67% per year before treatment to 0.5% per year during treatment [66]. Overall, time to tumor progression also increased, from 4.2 months before treatments to >12 months with treatment [66].

In the five patients with stabilization of disease on everolimus, discontinuation after 12 months lead to rebound effects on tumor growth rate [66]. All five

patients experienced marked growth of VS volumes 2–6 months after discontinuation; one patient had concomitant decline of hearing and balance and elected to undergo surgical resection [66]. In the other four patients, resumption of everolimus leads to a median VS volume reduction of 6.8% at 24 months and increased time to tumor progression [68]. Thereafter, the study was amended to increase duration of therapy with everolimus for another 2 years [68]. At 4-year follow-up, one patient continued to remain stable on everolimus, and the other three patients progressed radiographically after 36, 39, and 45 months of treatment; hearing function was stable for three out of four patients [68]. Hence, reintroduction of everolimus resulted in delayed time to progression from a median of 2.9 months before treatment, to 13.9 months with treatment [68]. In addition, the previously observed rebound effect on VS growth rate following withdrawal of everolimus, did not occur with discontinuation of therapy after VS progression on treatment [68]. Two of the three patients with progression went on to receive bevacizumab, which resulted in tumor shrinkage for one patient and stabilization for the other [68].

Notably, the patients in the study by Goutagny et al. had received less prior medical therapies as compared with Karajannis et al., potentially reflecting a less refractory group of NF2-related VS. No rebound effects were observed by Karajannis et al., however, as seven out of nine patients were previously treated with lapatinib and/or bevacizumab, these therapies may lead to genetic alterations of VS tumorigenesis prior to everolimus therapy [67].

Everolimus monotherapy also resulted in stabilization of meningiomas in NF2 patients. Two patients with asymptomatic frontal meningiomas had increased time to progression of meningioma while on everolimus, from 5.5 and 8.5 months before therapy, to 17.3 months and not reached, respectively, after 26 months of treatment with everolimus [68].

Overall the side effects due to everolimus were tolerable and mostly minor grade 1 or 2 events including mouth ulcers, rash, headache, fatigue, cholesterol elevation, sinusitis, and delayed wound healing [66, 67]. One patient developed a grade 3 toxicity of basocellular carcinoma which did not require additional treatment beyond excision [66] and another had transient azoospermia which resolved after drug discontinuation [67].

2.3.2 Vistusertib (AZD2014)

AZD2014 is an orally available dual inhibitor of mTOR complex 1 and 2 (mTORC1/2). Plotkin et al. found that combined mTORC1/2 inhibition was more effective than single mTORC1 inhibition in reducing proliferation of NF2-deficient meningioma cells in vitro [69]. These results were translated into a phase II clinical study for NF2 patients with growing or symptomatic meningiomas; the trial is active however it is not currently recruiting patients according to cancer.gov (NCT02831257).

2.3.3 Highlights

- Everolimus is effective in stabilizing VS and delaying time to tumor progression in about 50% of patients [66]; these observations are more notable for patients who have not been previously treated with other biologic agents [66, 67].

- Early discontinuation of everolimus in patients' with stable disease may result in a life-threatening rebound effect on VS growth rate [66] which can be rescued by reintroduction of everolimus [68].

- Notably, this rebound effect was not observed in patients who progressed on everolimus [68] or who had been previously treated with lapatinib or bevacizumab [66]. Hence discontinuation of everolimus in setting of ongoing VS stabilization is not recommended [68].

- While the timing of everolimus administration remains unclear, data suggest it is more likely to achieve tumor stabilization prior to other biologic agents [66, 67], and that bevacizumab can be safely administered after progression on everolimus with some stabilization of VS [68].

- Everolimus can also slow the growth of meningiomas in NF2 patients [68].

- Vistusertib is a dual mTORC1/2 inhibitor and may have more anti-meningioma activity than mTORC1 inhibitors [69]; it is currently being evaluated in a phase II clinical study (NCT02831257).

2.4 Histone deacetylase (HDAC) inhibitors

2.4.1 AR-42

AR-42 is an orally active inhibitor of HDAC with multiple downstream molecular targets, including downregulation of phosphorylated-Akt [70], a protein kinase important for cellular apoptosis. Preclinical studies with AR-42 on schwannoma and meningioma cells *in vitro* demonstrated a dose-dependent inhibitory effect on the cellular proliferation and increased cellular apoptosis, by reducing Akt activation [43]. Subsequently these findings were replicated in schwannoma mice xenograft models, which demonstrated the mice that ate AR-42 had 42% smaller tumor volume and less phosphorylated-Akt as compared with those that did not receive drug [43]. Collectively, these studies demonstrate AR-42 has anti-neoplastic activity against schwannoma and meningioma cells via a dose-dependent suppression of phosphorylated-Akt [43]. These results have been translated into a proof of concept phase 0 study assessing expression of phosphorylated-Akt of VS and meningiomas in adults receiving AR-42 prior to surgical resection (NCT02282917).

2.5 Mitogen-activated protein kinase (MEK) inhibitors

2.5.1 Selumetinib (AZD6244)

Selumetinib is an orally available small molecule inhibitor of MEK 1 and 2. The Ras/MEK/extracellular signal-related kinase (ERK) pathway is implicated in NF2 tumorigenesis and is strongly activated in NF2-deficient schwannoma cell lines [71]. In preclinical studies, inhibition of MEK 1 and 2 with selumetinib lead to cessation of cellular proliferation of human schwannoma cells *in vitro* and interruption of PDFGR-mediated ERK activation [72]. These findings lead to phase II clinical study assessing the efficacy of selumetinib for patients with NF2-related tumors, and are actively recruiting according to clinicaltrials.gov (NCT03095248).

3. Conclusions

NF2 is a tumor predisposition syndrome that leads to the formation of VS, meningiomas, ependymomas, and a variety of ophthalmologic and cutaneous features. The management of NF2-related tumors has primarily been with surgery

and radiotherapy. However, surgical resection is associated with considerable morbidity, and radiotherapy is increasingly less advised due to the risk of malignant transformation and secondary malignancies. Hence, there remains a critical need for the treatment of patients with NF2-related tumors.

Biologically targeted therapies are an emerging and promising role. Dysfunctioning Merlin underlies NF2-associated tumorigenesis and is a multifunctioning protein intimately involved with molecular targets and pathways important for cellular metabolism, structure, proliferation, survival, and contact-dependent inhibition. Targets that have been validated in preclinical models and translated into clinical trials include VEGF, PDGFR, VEGF, PI3K/Akt, HDAC, mTOR, ALK, and Ras/Raf/MEK/ERK. Ongoing studies for patients with NF2-related tumors include AR-42, selumetinib, and vistusertib, and two other clinical trials are set to open later this year: crizotinib and brigatinib. Currently published results from clinical studies suggest bevacizumab, everolimus, and lapatinib have some effectivity in stabilizing NF2-VS and to a lesser degree, NF2-meningiomas. Bevacizumab, especially, has the ability to preserve function and prevent hearing loss in NF2 patients with progressive VS growth or hearing loss. However, these results are not durable and tumor growth eventually occurs. Given the multifunctioning reaches of Merlin, and the eventual drug-resistance of NF2-related tumors seen with monotherapy, developing a multi-drug regimen may be necessary for reducing the emergence of drug-resistant tumor cells, optimizing cell kill, and improving overall survival.

In summary, major advancements in understanding NF2 biology has enabled the translation of biologically targeted therapies for NF2-related tumors. Secondarily, monotherapy with some agents has resulted in stabilization of disease and clinical improvement. However, these results are not sustained off treatment, are limited by cumulative toxicities, and unfortunately, eventual growth occurs on monotherapy. A multi-drug regimen may be the key to overpowering the multifunctioning reaches of Merlin, and developing more efficacious long-term therapies with improved survival outcomes for patients with NF2.

Conflict of interest

The authors declare no conflict of interest.

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