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NEUROFIBROMATOSIS-RELATED TUMORS: EMERGING BIOLOGY AND THERAPIES

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Abstract

Purpose of review—Over the past decade, substantial insight into the biological function of the tumor suppressors neurofibromin (*NF1*) and Merlin (*NF2*) has been gained. The purpose of this review is to highlight some of the major advances in the biology of neurofibromatosis type 1 (NF1) and neurofibromatosis type 2 (NF2) as they relate to the development of novel therapies for these disorders.

Recent findings—The development of increasingly sophisticated preclinical models over the recent years has provided the platform from which to rationally develop molecular targeted therapies for both NF1 and NF2 related tumors, such as within the Department of Defense-sponsored Neurofibromatosis Clinical Trials Consortium (NFCTC).

Summary—Clinical trials with molecular targeted therapies have become a reality for NF patients, and hold substantial promise for improving the morbidity and mortality of individuals affected with these disorders.

Keywords

neurofibromatosis type 1; neurofibromatosis type 2; plexiform neurofibroma; malignant peripheral nerve sheath tumor; optic pathway glioma; schwannoma; meningioma; ependymoma; epidermal growth factor receptor (EGFR); mammalian target of rapamycin (mTOR); mitogen activated protein kinase (MEK)

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CONFLICTS OF INTEREST

There are no conflicts of interest.

INTRODUCTION

In this review, we describe recent key advances in NF biology that provide the basis for emerging molecular targeted therapies. Since both *NF1* and *NF2* have also been implicated in the tumorigenesis of a wide spectrum of sporadic cancers, successful novel therapies may also be of benefit to non-NF patients in the form of “personalized medicine”.

CLINICAL TRIAL DEVELOPMENT AND DISEASE OUTCOME MEASURES

The burgeoning of novel therapy underscores the need to develop a co-ordinated approach to clinical trials and this has been undertaken by the Department of Defense-sponsored Neurofibromatosis Clinical Trials Consortium (NFCTC). The focus is on well-designed, prospective trials with a strong biological rationale in appropriately selected patient populations, using rigorous clinical and molecular endpoints [1]. The impetus of the international REINS group (response evaluation in neurofibromatosis and schwannomatosis) is to develop appropriate endpoints and outcome measures including imaging, neurological, psychological, ophthalmologic and respiratory assessments, as well as validated, patient focused, disease specific quality of life questionnaires [1]. Measurement of visual acuity using quantitative methods is recommended as the primary outcome measure for optic pathway gliomas and the children’s visual functional questionnaire as a secondary endpoint [2]*. Volumetric magnetic resonance imaging is the gold standard for measuring plexiform neurofibromas with a 20% volume change indicative of change in tumor size [3].

NEUROFIBROMATOSIS TYPE 1

Neurofibromatosis 1 (NF1) is an autosomal dominant tumor predisposition disorder with a birth incidence of about 1 in 2,700 and prevalence of 1 in 4,560 [4]. The principal and defining features involve the skin, nervous system, bone and eye and the disease complications are protean [5]. The *NF1* gene was cloned on chromosome 17q11.2 and the cytoplasmic protein neurofibromin is widely expressed with high levels in the nervous system [6] [7] [8].

Neurofibromin

Neurofibromin interacts with the proto-oncogene RAS to suppress tumor formation. Negative regulation of RAS reduces cell proliferation and differentiation by forestalling activation of the downstream signaling pathways phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR)) and rapidly accelerated fibrosarcoma/mitogen activated protein kinase kinase/extracellular signal regulated kinase (RAF/MEK/ERK) [9]. Neurofibromin also regulates adenylyl cyclase and generation of intracellular cyclic adenosine monophosphate (cAMP) via RAS dependent activation of atypical protein kinase C zeta; loss of neurofibromin results in lower levels of cAMP in some cell types including neurons [10]*.

NF1 associated tumors

Individuals with germline inactivation of the *NF1* gene have a propensity to develop both benign and malignant tumors through acquired inactivation of the functioning *NF1* allele.

The emblematic lesion is the benign neurofibroma, but there is an 8–13% lifetime risk of developing malignant peripheral nerve sheath tumor (MPNST) [5] [11]. Gliomas are predominantly low grade pilocytic astrocytomas that occur mainly in the optic pathways and brainstem but may arise elsewhere in the brain and spinal cord [5]. Phaeochromocytoma, gastrointestinal stromal tumor, myeloproliferative disease (i.e., juvenile myelomonocytic leukemia), myelodysplastic syndrome, osteosarcoma and rhabdomyosarcoma have all been described in NF1 individuals [5] [12]. An increased relative risk was reported in NF1 for all cancers outside the nervous system; gastrointestinal neoplasms were highlighted with thyroid, bone, ovary and lung tumors, breast cancer in women under 50, melanoma and non-Hodgkin's lymphoma [12]. Neurofibromas are comprised of Schwann cells, fibroblasts, perineurial cells, mast cells and axons, embedded in a collagenous extracellular matrix [13]. They may form as cutaneous, subcutaneous spinal nerve root or plexiform growths. The latter have a rich vascular supply, frequently involve multiple nerves and may encroach on surrounding structures causing pain, disfigurement, hemorrhage and neurological deficit. Cutaneous neurofibromas are invariably benign but subcutaneous and plexiform neurofibromas may undergo transformation to MPNST and high grade lesions herald a poor prognosis [5]. The clinical presentation includes pain, rapid growth change in texture and neurological deficit; 18^{F} fluorodeoxyglucose positron emission computerized tomography with delayed imaging is a useful diagnostic tool in distinguishing benign neurofibromas from MPNST [5] [14]. The mainstay of treatment for plexiform neurofibromas is judicious surgery, complete excision is recommended for MPNST, radiotherapy and chemotherapy used as palliation for incompletely excised and high grade lesions [5]. Optic pathway gliomas (OPG) are commonest in children under 7 years and are usually asymptomatic but may cause visual loss, hydrocephalus, or precocious puberty secondary to hypothalamic involvement [5]. Females, infants under 2 years, and post chiasmatic OPG have a poorer visual prognosis. Tumors outside the optic pathway are usually indolent but may manifest with neurological deficit or hydrocephalus and gliomas occurring in adulthood are more aggressive [5]. Chemotherapy with vincristine and carboplatin is the treatment of choice for symptomatic OPG, surgery manages proptosis, but radiotherapy is contra-indicated because of the risk of secondary malignancy, neuropsychological, neurovascular and endocrine problems [5].

Molecular targeted therapy and clinical trials

The ultimate aim for NF1 tumors is targeted treatment, tailored to the individual and monitored with reliable clinical, radiological and patient focused outcome measures. Recent research has made great strides in identifying the underlying cellular and molecular mechanisms of NF1 related tumors and facilitated the use of novel therapy (Table 1) [15]* [16] [17] [18] [19] [20] [21] [22]. Genetically engineered mouse models (GEM) have been developed for multiple tumors including plexiform neurofibromas, OPG, malignant glioma and MPNST. Murine models are helpful in representing different types of NF1 malignancy, although they do not exhibit the spectrum of severity encountered in patients with MPNST and OPG [1]. Nonetheless, they are crucial in developing new drugs and exemplify neurofibromin's distinct modes of growth control in different cell types [23] [24]**. Notably, a recent pre-clinical study demonstrated the key role of abnormal signaling in the RAF/MEK/ERK pathway in sustaining the growth of neurofibromas and MPNSTs [24]**.

A highly selective MEK inhibitor produced reduction in neurofibroma size in the majority of mice evaluated and increased survival in mice implanted with human MPNST cells. This work paved the way for clinical trials, and preliminary results from a phase I study of the MEK1/2 inhibitor selumetinib (AZD6244) in children and young adults with NF1 and inoperable plexiform neurofibromas (PNs) was recently published (ClinicalTrials.gov identifier NCT01362803) [19]. Selumetinib was tolerated in children on a continuous dosing schedule at approximately 50% of the adult recommended dose and very encouraging preliminary activity was noted: of 11 patients with ≥ 1 restaging MRI, all had a decrease in PN volume (median maximal decrease 24%, range 8–39). A Neurofibromatosis Clinical Trials Consortium (NCTC) sponsored phase 2 clinical trial with another MEK inhibitor (PD-0325901) for adolescents and adults with NF1-associated PN is currently ongoing (ClinicalTrials.gov identifier NCT02096471).

The importance of the microenvironment has been highlighted in tumor formation, notably microglial cells in OPG and mast cells in plexiform neurofibromas [25] [26]. The tumor cellular environment was spotlighted as a potential target for therapy when glioma formation was delayed in a GEM by impeding microglial function [26].

Furthermore, imatinib (a c-kit inhibitor, involved in mast cell development) was assessed in a clinical trial of symptomatic plexiform neurofibromas, resulting in at least a 20% decrease in volume in tumor size in 6/36 patients [27]. An NCTC sponsored trial with cabozantinib (XL184), a small molecule inhibitor of c-kit, MET, RET and VEGFR2, for adolescent and adult NF1 patients with PN in currently ongoing (ClinicalTrials.gov identifier NCT02101736).

Multiple molecular changes contribute to the progression from plexiform neurofibroma to MPNST. Loss of *NF1* is sufficient for plexiform neurofibroma growth but in vitro and in vivo studies on MPNST demonstrated additional loss or alteration of cell cycle regulators including Tumor protein 53 phosphatase (*TP53)*, retinoblastoma gene 1 (*RB1*), tensin homolog (*PTEN*) and cyclin dependent kinase inhibitor 2A (*CDKN2A*) [28] [29] [30]. Numerous growth factor signaling systems are implicated in MPNST, particularly increased expression and/or amplification of epidermal growth factor receptor (EGFR); Rahrmann and colleagues demonstrated that reduced *p53* gene expression and increased *EGFR* expression co-operate to promote MPNST formation and progression [9] [31].

The potential role of combination therapy in tackling malignancy has been addressed recently. Radiation was used in combination with an mTOR inhibitor and bortezomib (a proteasome inhibitor regulating protein expression and function and removing damaged proteins) [32]. The result was decreased proliferation in vitro and reduced tumor growth and enhanced apoptosis in vivo.

Changes in gene expression may arise from DNA promoter methylation and microRNAs. Reduced *RASSF1A* expression (RAS association domain family member 1, isoform A) was detected in a large series MPNSTs due to promoter methylation and was associated with a poor prognosis independent of tumor size or clinical manifestations [33]. *RASSF1A* acts as a tumor suppressor by controlling microtubules and potentially could be used as a prognostic

marker. In a whole gene sequencing study of gliomas, *NF1* methylation was one of the mechanisms underpinning somatic *NF1* loss in pilocytic astrocytomas [34].

An overview of the most relevant, recently published clinical trials for NF1 are summarized in Table 1.

NEUROFIBROMATOSIS TYPE 2

Neurofibromatosis type 2 (NF2) is an autosomal dominant genetic disorder with a birth incidence of approximately 1/33,000 [4]. It is caused by inactivation of the NF2 gene located on chromosome 22q, which codes for the NF2 gene product, Merlin. In contrast to neurofibromin, Merlin acts both at the cell cortex and the nucleus, directly affecting multiple signaling pathways related to contact inhibition and tumor suppression.

Merlin

Merlin has substantial sequence homology to members of the Ezrin/Radixin/Moesin (ERM) family of proteins, which link a variety of cell-adhesion receptors to the cortical actin cytoskeleton [21], and has emerged as a major effector of cell contact inhibition. In addition, it has been known that Merlin can affect a variety of mitogenic signaling pathways, including Rac–PAK, mTOR, EGFR–Ras–ERK and PI3K–Akt, and contribute to the activation of the Hippo tumor-suppressor pathway. More recently, it has been recognized that Merlin pleiotropically affects cell signaling by migrating into the nucleus and inducing a growth-suppressive program of gene expression through direct inhibition of the $CRL4^{DCAF1}$ E3 ubiquitin ligase [35], and that derepressed $CRL4^{DCAF1}$ promotes activation of the Hippo pathway component YAP by inhibiting Lats1 and 2 in the nucleus [36]**.

The complex biology underlying Merlin's functions, including tumor suppression and contact inhibition, remains incompletely understood and is beyond the scope of this article, but has been summarized in a number of excellent recent reviews [37] [38] [39].

NF2 associated tumors

NF2 patients develop multiple tumors affecting the central and peripheral nervous system tumors, i.e. schwannomas, meningiomas and ependymomas. The majority of NF2 patients develop progressive hearing loss in young adulthood due to bilateral vestibular schwannomas (VS). Schwannomas frequently also involve other cranial nerves, impacting on neurologic function such as swallowing, vision and facial function. Meningiomas and less commonly, ependymomas, involve the brain and spine, leading to mass effect and/or neurological dysfunction, based on size and location. The natural history of VS growth and hearing decline in newly diagnosed, untreated NF2 patients was reported in a recent study [40]*. The rate of hearing decline was 5%, 13% and 16% at 1, 2 and 3 years, respectively; while the rate of tumor progression was 31%, 64% and 79% at 1, 2 and 3 years, respectively. The median time to tumor progression was 14 months and the median time to hearing decline 62 months.

Molecular targeted therapy and clinical trials

The traditional treatment paradigm for NF2 patients consisted of clinical observation and surveillance imaging observation, with judicious use of surgical intervention for symptomatic tumors, and sometimes radiotherapy. Angiogenesis occurs in VS, and VEGF receptors are expressed in these tumors. Bevacizumab, an anti-VEGF monoclonal antibody, has recently emerged as a medical treatment option for NF2 patients with progressive VS. The initial reports, based on case series and off-label use of bevacizumab, suggested that treatment was effective in the majority patients, including not only imaging responses, but also hearing improvement, which in some patients was dramatic [41] [42]. Subsequently it became clear that the responses can only be sustained with continued treatment, which poses a challenge due to dose-limiting long-term toxicities, chiefly hypertension and proteinuria [43]. A further multi-center, prospective phase 2 clinical trial for NF2 patients with symptomatic VS, i.e. hearing loss, was completed recently (NCT01207687) and results are expected to be published in the near future. A similar phase 2 study of bevacizumab in children and young adults with NF2 and progressive VS sponsored by the NFCTC is currently ongoing (NCT01767792).

Based on recent insights into the biology of Merlin-deficient tumors, a number of molecular targeted agents have been repurposed for testing in preclinical models of NF2, including genetically engineered mouse models for schwannomas [44] and meningiomas [45] [46]. Major molecular targets validated in NF2 preclinical models that have been recently translated into clinical trials include EGFR/ErbB2 (lapatinib) [47], mTOR (rapamycin/everolimus) [48] [49] [50] [51]* [52]* and VEGFR/PDGFR/c-kit (sorafenib, axitinib) [53] [54] [55].

In a phase 2 clinical trial for adult and pediatric NF2 patients with progressive VS, lapatinib showed modest activity with objective volumetric and hearing response rates of 24% and 31%, respectively [56]. The hearing responses, however, were predominantly minor and not sustained. In contrast, a similarly designed phase 2 study with everolimus failed to yield any objective volumetric or hearing responses [57]*.

An overview of the most relevant recent and ongoing clinical trials for NF2 are summarized in Table 2.

CONCLUSION

A key challenge is to develop effective personalized targeted therapy for patients with neurofibromatosis. This will be facilitated by the further development of preclinical models that represent the variability in clinical manifestations in NF1 and NF2, and in parallel, the identification of biomarkers to identify individuals most likely to benefit from a given therapy.

In NF1, the MAP-kinase signaling pathway has emerged as a key target; MEK inhibition reduced plexiform neurofibroma and MPNST growth in preclinical studies [22], and small-molecule MEK inhibitors are currently being tested in phase 2 clinical trials for NF1 patients with plexiform neurofibromas

Since NF2 loss affects a plethora of cellular signaling pathways, the most suitable molecular targets for clinical therapy, which may vary between tumor types, remain to be elucidated. While a number of molecular targets have been validated preclinically in NF2 related tumors and some agents have already shown promise in the clinical realm, especially for a subset of patients with VS, effective medical therapies for NF2 that achieve sustained tumor regression remain elusive. In addition, meningiomas remain a tough clinical challenge in NF2 patients, with effective medical therapies urgently needed.

In addition to traditional efficacy trials, several (pharmacokinetic/pharmacodynamic) clinical trials ("Phase 0") are currently exploring the achievable drug concentrations in tumor tissue of NF2 patients and molecular target inhibition (see Table 2). Patients scheduled for tumor surgery are given study drug for a short period of time preoperatively, and tumor tissue is acquired for comprehensive laboratory analysis. The key goals are to estimate the achievable drug concentration in human tumor tissue *in vivo*, as well as molecular target inhibition. In addition, valuable information on potential tissue-specific resistance mechanisms, such as release of negative feedback loops, may be gained.

Opportunities also exist for future drug development aimed at molecular pathways with currently "undruggable" targets, such as the Hippo pathway and $CRL4^{DCAF1}$. Importantly, bi-allelic loss of *NF2* is also found in tumors of non-NF2 patients, including the relatively common sporadic schwannomas and meningiomas, as well as ependymomas and malignant mesotheliomas. The development of effective therapies of NF2 patients is therefore expected to be highly relevant for a much larger patient population.

In summary, our rapidly increasing understanding of NF biology, coupled with increasingly sophisticated preclinical models, is expected to yield novel treatment approaches to be tested in rigorously designed clinical trials with standardized and validated clinical outcome measures relevant to NF patients, including functional outcome measures.

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KEY POINTS

- Major advances in our understanding of NF1 and NF2 biology have opened avenues for novel therapeutic approaches
- NF preclinical models are becoming increasingly sophisticated, yet do not fully capture the diverse clinical manifestations of NF1 and NF2
- NF clinical trials are increasingly developed based on preclinically validated molecular targets and drugs
- The successful development of effective therapies will be aided by validated clinical outcome measures relevant to NF patients, as well as the identification of biomarkers to help with selection of patient subsets most likely to benefit

Table 1

Novel therapies for NF1 associated tumors – recently published studies

Drug	Mode of action	Trial design	Age (years)	n	Results
Inoperable or progressive plexiform neurofibromas (≥20% volume increase on volumetric magnetic resonance imaging)					
Tipifarnib [15]*	Farnesyltransferase inhibitor	Phase 2 Flexible crossover double blind placebo controlled	3–25	62	Time to tumor progression on imaging not significant - Tifarnib versus controls
Pirfenidone [16]	Anti-fibrotic anti-inflammatory ↓ collagen, ↓ fibroblasts	Phase 2 Single arm Open label	3–21	36	Time to tumor progression on imaging not significant Pirfenidone versus control used from Tifarnib study
Sorafenib [17]	Multi-kinase inhibitor (BRAF, VEGFR2, PDGFR, c-kit)	Phase 1 and Pharmacokinetic study	3–18	9	Poorly tolerated at lower maximum tolerated dose than patients with solid tumors
Sirolimus [18]	mTORC1 inhibitor	Phase 2	≥3	13	No shrinkage non-progressive plexiform neurofibromas. Quality of life improved
Optic pathway glioma and low grade glioma					
Bevacizumab [19]	VEGF-A inhibitor	Case series	0.5 – 7	2 NF1 2 sporadic	Improved vision
Rapamycin and erlotinib [20]	mTORC1 inhibitor EGFR inhibitor	Toxicity and efficacy	<21	8 NF1 11 sporadic	Well tolerated Two NF1 patients had prolonged disease control > 1 year
Sorafenib [21]	Multi-kinase inhibitor (BRAF, VEGFR2, PDGFR, c-kit)	Phase 2	≥2	3 NF1 8 sporadic	Median time to progression 2.8 months; with apparent acceleration of tumor growth in both sporadic and NF1

EGFR = endothelial growth factor receptor; mTORC = mammalian target of rapamycin complex; PDGFR = platelet derived growth factor; VEGF = vascular endothelial growth factor

Table 2

Novel therapies for NF2 associated tumors – recently completed or published studies and ongoing clinical trials

Drug (ClinicalTrials. gov identifier)	Mode of action	Trial design	Age [years]	n	Results
Prospective therapeutic studies for NF2 patients with progressive VS					
Lapatinib [55]*	Dual EGFR/ErbB2 inhibitor	Phase 2 (two-stage) Single arm Open label	>3	17	Volumetric response 24%, hearing response 31%
Everolimus [56]*	mTORC1 inhibitor	Phase 2 (two-stage) Single arm Open label	>3	9	No objective hearing or volumetric responses
Bevacizumab (NCT01207687)	VEGF-A inhibitor	Phase 2 Single arm Open label	≥12	14	Enrollment completed, results pending
Bevacizumab (NCT01767792)	VEGF-A inhibitor	Phase 2 (two-stage) Single arm Open label	12–30	14–22	Study ongoing
Axitinib (NCT02129647)	Multi-kinase inhibitor (VEGFR2, PDGFR, c-kit)	Phase 2 (two-stage) Single arm Open label	≥18	9–17	Study ongoing
Phase 0 (pharmacokinetic/pharmacodynamic, non-therapeutic) studies for NF2 VS/meningioma					
Lapatinib (NCT00863122)	Dual EGFR/ErbB2 inhibitor	Phase 0	≥18		Enrollment completed, results pending
Everolimus (NCT01880749)	mTORC1 inhibitor	Phase 0	≥18		Study ongoing

EGFR = endothelial growth factor receptor; mTORC1 = mammalian target of rapamycin complex; PDGFR = latelet derived growth factor; c-kit = cellular homolog of feline sarcoma viral oncogene v-kit; VEGF = vascular endothelial growth factor; HDAC = histone deacetylase