

## Consensus Recommendations for Current Treatments and Accelerating Clinical Trials for Patients With Neurofibromatosis Type 2

Jaishri O. Blakeley,<sup>1\*</sup> D. Gareth Evans,<sup>2</sup> John Adler,<sup>3</sup> Derald Brackmann,<sup>4</sup> Ruihong Chen,<sup>5</sup> Rosalie E. Ferner,<sup>6</sup> C. Oliver Hanemann,<sup>7</sup> Gordon Harris,<sup>8</sup> Susan M. Huson,<sup>2</sup> Abraham Jacob,<sup>9</sup> Michel Kalamarides,<sup>10</sup> Matthias A. Karajannis,<sup>11</sup> Bruce R. Korf,<sup>12</sup> Victor-Felix Mautner,<sup>13</sup> Andrea I. McClatchey,<sup>8</sup> Harry Miao,<sup>14</sup> Scott R. Plotkin,<sup>8</sup> William Slattery III,<sup>4</sup> Anat O. Stemmer-Rachamimov,<sup>8</sup> D. Bradley Welling,<sup>9</sup> Patrick Y. Wen,<sup>15</sup> Brigitte Widemann,<sup>16</sup> Kim Hunter-Schaedle,<sup>17</sup> and Marco Giovannini<sup>4</sup>

<sup>1</sup>Johns Hopkins University, Baltimore, Maryland

<sup>2</sup>MAHSC, St. Mary's Hospital, University of Manchester, UK

<sup>3</sup>Stanford University, Stanford, California

<sup>4</sup>House Research Institute, Los Angeles, California

<sup>5</sup>NexGenix Pharmaceuticals, New York, New York

<sup>6</sup>Guy's and St. Thomas' NHS Hospital Trust, London, UK

<sup>7</sup>Peninsula Medical School, University of Plymouth, Plymouth, UK

<sup>8</sup>Massachusetts General Hospital, Boston, Massachusetts

<sup>9</sup>Ohio State University, Columbus, Ohio

<sup>10</sup>Hopital Beaujon, APHP, Clichy, France

<sup>11</sup>New York University Langone Medical Center, New York, New York

<sup>12</sup>University of Alabama, Birmingham, Alabama

<sup>13</sup>Department of Neurology, Hospital Eppendorf, Hamburg, Germany

<sup>14</sup>PTC Therapeutics, South Plainfield, New Jersey

<sup>15</sup>Dana Farber Cancer Institute, Boston, Massachusetts

<sup>16</sup>National Cancer Institute, Bethesda, Maryland

<sup>17</sup>Children's Tumor Foundation, New York, New York

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Neurofibromatosis type 2 (NF2) is a tumor suppressor syndrome characterized by bilateral vestibular schwannomas (VS) which often result in deafness despite aggressive management. Meningiomas, ependymomas, and other cranial nerve and peripheral schwannomas are also commonly found in NF2 and collectively lead to major neurologic morbidity and mortality. Traditionally, the overall survival rate in patients with NF2 is estimated to be 38% at 20 years from diagnosis. Hence, there is a desperate need for new, effective therapies. Recent progress in understanding the molecular basis of NF2 related tumors has aided in the identification of potential therapeutic targets and emerging clinical therapies. In June 2010, representatives of the international NF2 research and clinical community convened under the leadership of Drs. D. Gareth Evans (University of Manchester) and Marco Giovannini (House Research Institute) to review the state of NF2 treatment and clinical trials. This manuscript

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\*Correspondence to:

Jaishri O. Blakeley, M.D., Johns Hopkins University, 1550 Orleans Street, Suite 1M16, Baltimore, MD 21204. E-mail: jblakel3@jhmi.edu

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summarizes the expert opinions about current treatments for NF2 associated tumors and recommendations for advancing therapies emerging from that meeting. The development of effective therapies for NF2 associated tumors has the potential for significant clinical advancement not only for patients with NF2 but for thousands of neuro-oncology patients afflicted with these tumors. © 2011 Wiley Periodicals, Inc.

**Key words:** neurofibromatosis type 2; meningioma; schwannoma; vestibular schwannoma; ependymoma; radiotherapy; molecular therapy; surgery

## INTRODUCTION

Neurofibromatosis type 2 (NF2) is an autosomal dominant tumor suppressor disorder that causes multiple tumor types to form at every level of the nervous system. Although a rare disorder (estimated 1 in 25–33,000 births) [Evans et al., 2005, 2010], the tumor types seen in NF2 are among the most common in neuro-oncology (Fig. 1). The hallmark of NF2 is bilateral vestibular schwannomas (VS) which progressively enlarge leading to sensorineural hearing loss and deafness [Evans, 2009b]. VS may also cause brainstem compression resulting in severe neurologic morbidity and mortality. Half of all individuals with NF2 will also develop intracranial meningiomas and 75% will develop spinal tumors including schwannomas, meningiomas and ependymomas [Evans, 2009b]. The vast majority of individuals with NF2 require surgery, and most will have multiple procedures during their lifetime. The progression of NF2 and requisite surgical intervention

can result in deafness, facial palsy, blindness, seizures, and hemiparesis.

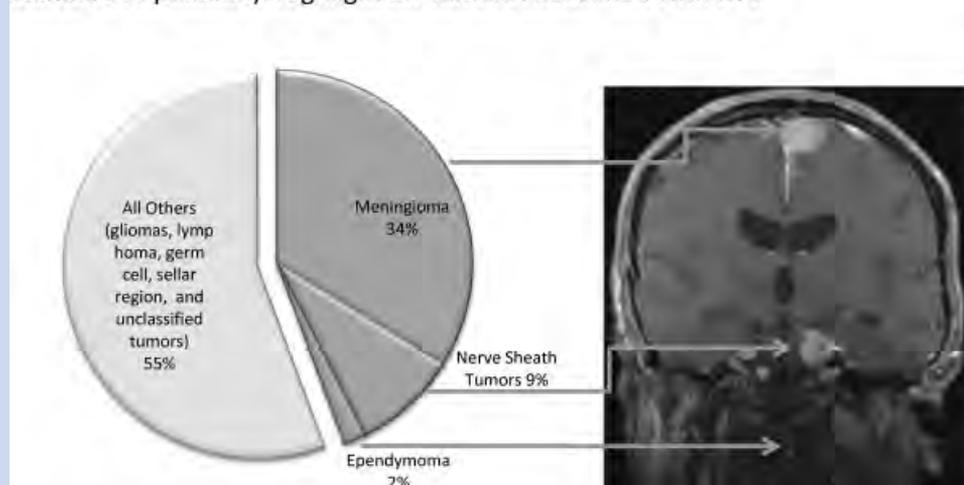
Until recently there were few therapeutic options for individuals with NF2. Thankfully things have progressed with increasing understanding of the genetic basis of NF2, as well as, emerging molecular parallels within various cancers for which drug therapies are in development or already clinically available. Nevertheless challenges lie ahead for the NF2 community, including prioritizing candidate drugs, managing patient recruitment to clinical trials, and integrating new therapies into clinical care across multiple surgical and medical disciplines. This article summarizes the latest recommendations for NF2 clinical management; advances in understanding the molecular underpinnings of this disorder; and progress made to date in implementing NF2 clinical trials.

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Incidence Rates of Central Nervous System Tumors (Age Adjusted to the 2000 U.S. Standard Population): Highlight on Tumors Associated with NF2



**FIG. 1.** The tumors that are found in NF2 [schwannomas, meningiomas, and ependymomas] are some of the most common seen in neuro-oncology overall.

## CURRENT CLINICAL CARE STANDARDS FOR NF2

### Surgical Management of NF2 Vestibular Schwannoma

Idiopathic VS are fairly common, with roughly 3,000 new cases per year in the United States, and growing incidence in recent years [Evans et al., 2005]. These tumors cause unilateral hearing loss, tinnitus, and imbalance. The primary treatment modality for these tumors is surgical resection or increasingly, radiosurgery, especially for tumors <3 cm [Rowe et al., 2003]. NF2 VS do not behave exactly like sporadic VS and require special consideration [Samii et al., 1997]. Care team experience is important in the formulation of a treatment plan for NF2 associated VS which commonly includes observation, surgery, stereotactic radiation or increasingly, drug therapy.

The first important therapeutic consideration for NF2 associated VS is the presence of brainstem compression. Large bilateral VS with obstructive hydrocephalus may cause rapid loss of consciousness, herniation, and death. In such cases urgent surgical removal of at least one VS is the first life-preserving priority along with diverting cerebrospinal fluid via shunt to address the hydrocephalus. For tumors greater than or equal to 3 cm in diameter but no evidence of hydrocephalus or brainstem compression, surgical resection should be considered as first line therapy, giving consideration to brainstem protection and facial nerve preservation [Wiegand et al., 1996; Anderson et al., 2005; Myrseth et al., 2009].

The various available approaches for VS surgery (translabrynthine, middle cranial fossa, and suboccipital) each has advantages and disadvantages; but ultimately surgical outcome is highly dependent on the team's experience overall and with the particular approach [Thomsen et al., 1994; Welling et al., 1999; Bennett and Haynes, 2007]. The primary advantage of the translabrynthine approach is direct access to the tumor where it comes into contact with the facial nerve, allowing development of a clean dissection plane between facial nerve and the VS [Day et al., 2004; Brackmann et al., 2007; Brackmann and Green, 2008]. Necessary cerebellar retraction is also minimized with this approach [Day et al., 2004]. The advantage of the suboccipital approach is a more rapid means to get access to larger VS, but the facial nerve is hidden from early access as it is on the anterior surface of the tumor [Samii and Matthies, 1997; Chen et al., 2010]. This approach may be desirable when the tumor extends out of the internal auditory canal into the cerebellopontine angle. Finally, the middle cranial fossa approach is utilized primarily for smaller (<1.5 cm) tumors limited to the internal auditory canal where hearing preservation is a major goal of surgery [Slattery et al., 2011].

A major concern of surgical intervention for VS greater than 3 cm in diameter is the risk of losing facial nerve function [Anderson et al., 2005; Brackmann et al., 2007]. An intact facial nerve is of critical importance to patients' quality of life (QOL); being required for corneal protection, facial mimetic function, and speech. Facial nerve monitoring during surgery has greatly improved the ability to spare this nerve. However, with tumors greater than 2.5 cm in diameter there is increased risk of facial nerve injury with 17% of patients having facial injury after resection compared to tumors less than 2.5 cm where 100% of patients had satisfactory facial nerve function after resection in a large series [Grey et al., 1996]. Although

size is an important factor, the consensus is that facial nerve outcome is not as good overall in NF2 patients versus patients with idiopathic VS even in the most experienced hands [Evans et al., 2005; Samii et al., 2008].

VS tumors less than 3 cm in diameter may be monitored for growth and hearing loss, or may be surgically excised [Thomsen et al., 1994; Bennett and Haynes, 2007]. There is no consensus about when to pursue surgery for smaller tumors in patients with NF2 (e.g., following hearing loss only; following tumor growth only; or both). A detailed discussion of the risks and benefits is required to help the patient choose the optimal approach for each situation.

Observation of VS without surgical intervention is often the approach taken for a period of time. The natural history of NF2 associated VS was assessed via a consortium of VS expert centers [Slattery et al., 2004; Fisher et al., 2009]. They evaluated 540 patients with NF2 and showed a roughly 7-year delay from the time of symptom onset to diagnosis and that roughly 1/3 of patients had surgery within 2 years of their diagnosis. In this study, the average age at diagnosis was 27 years and there was an average of 1 mm/year rate of tumor growth with a high rate of variability. Importantly, the growth rates of the right and left VS were independent, and rate of growth was not correlated with rate of hearing loss. This variability suggests that a tailored approach must be taken for each patient with multidisciplinary assessment from otolaryngology/neuro-otology, neurosurgery, genetics, and neurology in planning the timing of surgical interventions.

When surgery is planned for NF2 VS less than 1.5 cm in diameter, a common strategy is removal of the VS where hearing is most likely to be preserved post-operatively [Samii and Matthies, 1995]. If useful hearing (greater than 70% speech discrimination) is preserved, the second tumor may also be removed via a hearing preservation approach (either the suboccipital or middle cranial fossa approach) (Fig. 2). If hearing is not successfully preserved in the first ear, but the cochlear division of the eighth nerve is preserved anatomically, the second ear is monitored for tumor growth or hearing loss, allowing preservation of hearing as long as possible before a second surgery [Samii et al., 1997; Friedman et al., 2003; Slattery et al., 2007, 2011]. When hearing is declining in the only hearing ear, internal auditory canal decompression is a surgical approach that may preserve hearing in the affected ear for a period of time [Slattery et al., 2011]. If the remaining hearing becomes non-functional or absent, hearing restoration approaches such as cochlear nerve implant (CNI) or auditory brainstem implant (ABI) can be considered. To assess the utility of CNI, the patient is referred for promontory stimulation [Tran Ba Huy et al., 2009]. Promontory stimulation is a means of electrically stimulating any remaining cochlear nerve fibers. If the patient senses an auditory stimulus with the stimulation, CNI is recommended [Lustig et al., 2006; Neff et al., 2007; Vincenti et al., 2008]. If during the first surgery the cochlear nerve cannot be not anatomically preserved, ABI can be considered on the side where the first tumor is removed [Grayeli et al., 2008]. If the cochlear nerve is anatomically preserved, but has lost hearing and does not detect auditory signals with the promontory electrical stimulation test, then an ABI should be considered at the time of the second VS removal [Lesinski-Schiedat et al., 2000; Schwartz et al., 2008].

Tinnitus is a common complaint of NF2 patients with bilateral VS. It can be severe and in some cases disabling. Hence, it is another

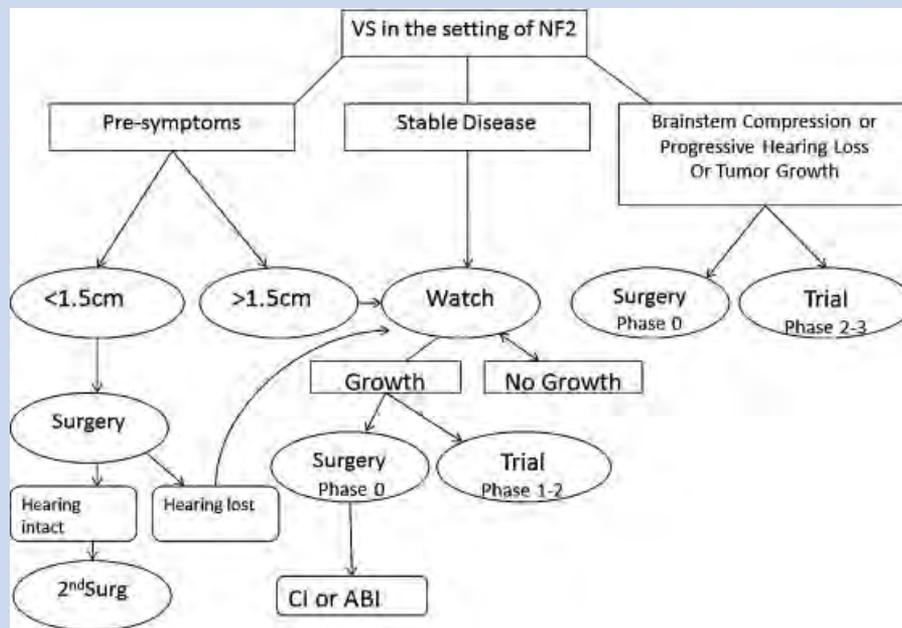


FIG. 2. Decision points in the clinical management of patients with NF2 associated vestibular schwannomas.

important consideration in the discussion of surgical expectations. In some cases tinnitus can be improved after surgery, but in as many as 20% of NF2 patients' tinnitus worsens post-operatively [David Moffat, unpublished data, personal communication to D.G.E.]. Of 121 patients with unilateral VS who had tinnitus at baseline, 19 had resolution post-operatively, 28 had reduction in severity, 45 had unchanged severity and 29 had worsening tinnitus. Importantly, of 21/55 patients (38%) developed new tinnitus post-operatively [Inoue et al., 2001]. For some patients, new or worsening tinnitus combined with loss of functional hearing is an unacceptable complication. This issue deserves detailed pre-operative discussion.

In summary, the range of surgical options for NF2 associated VS is vast in terms of approach, timing, and goals. However, NF2 VS tend to involve more complicated surgeries than idiopathic VS and require expert multidisciplinary management for optimal surgical outcome.

### Radiosurgery for NF2 Vestibular Schwannoma

Stereotactic radiosurgery (SRS), consisting of a single or limited number of highly accurate and conformal sessions of therapeutic radiation, is increasingly popular among patients with idiopathic VS. Although there is no consensus about which VS patients are the best candidates for SRS, it is widely felt that idiopathic VS tumors that are <3 cm, especially when the patient is older, or poor surgical candidates, are optimal SRS candidates [International RadioSurgery Association, 2004]. Tumors over 3 cm are not usually recommended for SRS due to concerns about radiation injury to normal neural structures or post radiation swelling which may further compromise a compressed brainstem.

The role of any form of external beam radiation therapy for NF2 associated VS remains controversial. Radiotherapy (either SRS or intensity modulated radiation therapy, IMRT) has been used in a subset of NF2 tumors that progress despite surgical treatment or in individuals who are at high risk for operative complications. However, many clinicians are hesitant to recommend radiation for NF2 patients with tumors of any size. Radiation should be used with caution in a setting of NF2 since—though the prevalence of nervous system malignancy is very rare in NF2 population studies—secondary malignancies after radiotherapy treatment have been reported [Baser et al., 2000]. In a North American and European study, only 9 of 1,242 patients with NF2 were found to have a spontaneous nervous system malignancy, and all cases were malignant peripheral nerve sheath tumors (MPNST) [Baser et al., 2000]. In contrast, after radiotherapy for benign tumors such as VS the prevalence of nervous system malignancies in patients with NF2 was 4,717 per  $10^5$  (95% CI: 681–8,753 per  $10^5$ ). This represented a substantial increase in the incidence of nervous system malignancies that may be related to the loss of the tumor suppressor gene in patients with NF2 allowing greater susceptibility to the ionizing effects of radiotherapy [Baser et al., 2000]. To date, more than 20 cases of malignancies (i.e., glioblastoma, rhabdomyosarcomas, or malignant meningiomas) have been reported in patients with NF2 after radiation therapy [Balasubramaniam et al., 2007]. Because of the focality and size of the radiation dose, it is possible that SRS has different biologic effects than IMRT or other forms of external beam radiotherapy. However, it is unknown to what extent this alters the risk of secondary malignancy in patients with NF2.

Compared with the treatment of sporadic VS where SRS plays a central role in management, SRS may result in only moderate rates

of tumor control and poorer long-term hearing preservation in the setting of NF2. The current best long-term data are estimated from a center in Sheffield, England. Rowe et al. [2008] reported on 122 VSs in 92 patients with NF2 treated with radiotherapy and estimated tumor control at 8 years to be 50%. At 3 years they estimated 40% of patients to have preserved hearing, 40% of patients to have progressive hearing loss, and 20% to have progressed to deafness. The long-term risk of facial palsy was 5%. In another study of 62 patients with NF2-related VS treated with SRS, hearing preservation was reported to be 73% at 1 year, 59% at 2 years, and 48% at 5 years after SRS. Facial neuropathy occurred in 5% of patients [Mathieu et al., 2007]. Overall, these data suggest that there is often short-term tumor control and hearing preservation after SRS in patients with NF2. However, the longer term efficacy (>5 years) of SRS for NF2-related VS is less robust than with sporadic lesions with <50% of patients retaining hearing.

### Current Clinical Care Standards for Meningioma

Meningioma is the most common primary brain tumor seen worldwide. It arises from the meninges covering the brain and spinal cord, and can involve any portion of the central nervous system. Most commonly, meningiomas are benign and noted incidentally. However, in some cases meningiomas involve critical brain areas causing focal neurologic deficits or brain irritation leading to seizures [Louis et al., 2000]. Standard treatment of idiopathic meningiomas includes observation if asymptomatic and slowly growing; surgery or radiation therapy is required for rapidly growing or symptomatic tumors [Nakamura et al., 2003]. Most commonly symptomatic meningiomas are resected and further intervention is not required. In some cases of atypical or malignant meningioma additional radiation therapy is required due to the invasive nature of the tumor [Durand et al., 2009]. Overall, these tumors account for 25% of all sporadic meningiomas [Willis et al., 2005].

Meningiomas occur in 38–58% of individuals with NF2 and are most often multiple (median = 3/patient) occurring both in cranial and spinal locations [Mautner et al., 1996; Goutagny and Kalamarides, 2010]. Although meningiomas in NF2 may become symptomatic independently, or combine with VS to create “collision tumors” near the brainstem, a recent series in France showed that most meningiomas did not require intervention [Goutagny and Kalamarides, 2010]. Although these tumors may not require surgery in all patients, the presence of meningiomas may portend a worse prognosis. In one series, the risk of mortality was 2.5-fold greater in NF2 patients with meningiomas versus those without meningiomas [Baser et al., 2002]. In children with NF2, meningiomas are more likely to become symptomatic and are more likely than VS to be responsible for the presenting symptom [Evans et al., 1999; Ruggieri et al., 2005]. Hence, although VS are the hallmark tumor of NF2, meningiomas are responsible for a great deal of morbidity in NF2 and therefore require specific attention.

Most meningiomas occur in surgically accessible locations and hence, surgery is generally considered first line therapy if an intervention is needed for a symptomatic meningioma [Asthagiri et al., 2009]. Further considerations on the role of radiosurgery are presented below. An additional consideration is the occurrence of parafalcine meningiomas that encroach upon the

sagittal sinus. In idiopathic meningiomas, some experts advocate for tumor resection prior to sinus invasion [Sindou and Alvernia, 2006; Raza et al., 2010]. In NF2, given the multiple meningiomas that often involve the falx and the diffuse nature of the tumor, it is not recommended to pursue resection in order to prevent sagittal sinus invasion [Goutagny and Kalamarides, 2010]. However, it is important to recognize the chronic venous hypertension that can occur and contribute to chronic headaches, vision changes, or other neurologic deficits in patients with NF2 and parafalcine meningiomas [Acebes et al., 2009; Sztikar, 2010].

### Radiosurgery for NF2 Meningioma

A number of studies have investigated radiosurgery as the primary treatment modality for idiopathic meningiomas [Kondziolka et al., 1998; Bria et al., 2011], but there is very little data about the impact of such treatment in NF2 associated meningiomas. In less surgically favorable locations involving the skull base and cavernous sinuses, SRS may have a role for treating meningiomas [Lee et al., 2002]. In addition, in the uncommon case where the meningioma is atypical or frankly malignant, radiation therapy, or SRS after surgical resection is often considered despite concerns about inducing a second malignancy in the setting of NF2. Small series have reported successful SRS treatment of NF2 meningiomas [Kondziolka et al., 2009; Wentworth et al., 2009]. However, these retrospective, single center series included only small numbers of patients. Hence, there is currently no definitive data for or against the use of SRS as a primary treatment modality for NF2 associated meningiomas. For most NF2 associated meningiomas the optimal approaches are observation or surgery.

### PLANNING NF2 CLINICAL TRIALS: WHICH PATIENTS TO RECRUIT?

Responding to the need for additional treatment options for patients with NF2 associated tumors, new potential therapies are entering the clinical setting via clinical trials. As this option becomes available for a greater number of patients with NF2, investigators will need to clearly define the potential risks and benefits of each proposed intervention in the context of other available therapies so that patients and their care providers may make informed decisions. Initiation of trials will also require close collaboration between the patients' care providers and clinical trialists in order to design trials to allow patients the maximal number of treatment options while preserving the scientific integrity of the study. A key consideration in this area is determining which patients should be recruited to which clinical trials.

### NF2 Vestibular Schwannoma Trials—Which Patients to Recruit?

Clinical trials provide access to new treatments and are required for treatment advances. However, they are intensive for both patients and investigators, expensive, and inherently involve patient risk. Choosing a population for investigation of a new therapy requires consideration of the safety of the intervention, the clinical demands and the question to be answered. For example, for NF2 associated

VS, the meeting consensus is that patients whom are  $>3$  years old and with progressive, symptomatic disease (with or without prior treatment) should be considered for enrollment at this time. These recommendations are based on the fact that some of the most severe forms of NF2 present in childhood. However, compliance with the demands of a clinical trial and the risks of agents to be studied may be unacceptable for children  $<3$  years old or in patients who do not have symptomatic disease. Age considerations are also determined by the pediatric-specific risks of the investigational agent or intervention to be studied.

Strictly defining the clinical question the study seeks to answer helps delineate the required characteristics of patients to be considered for enrollment. For example, if MRI measurements will be the endpoint in a study of VS, patients enrolled on trial should have target VS that are  $\geq 1$  cm in diameter and without implants that would influence interpretation using current MRI techniques. The perceived risks of the intervention to be tested also influence the population for enrollment, as in general higher risk is more acceptable to patients with progressive tumors.

There are essentially two main categories to consider when using progressive disease as the inclusion criteria: radiographic progression and symptom progression. Radiographic progression of a given tumor is a familiar criterion to clinical researchers and regulatory agencies as it is commonly used in oncology trials. Radiographic progression must be specifically defined (i.e., at least 2 mm linear growth in 2 years on MRI or a specified percent change in volume over time). The advantages of using radiographic progression as a criteria is that the measures are generally reproducible across a wide number of centers and provide a baseline measure by which either response or stabilization can be determined. However, this may not always be the optimal choice given that there is no definitive correlation between tumor size and function in the tumors that afflict patients with NF2. To address this limitation, symptomatic progression may be used as the enrollment criteria. For example, progressive hearing loss or facial palsy may be considered as an enrollment criteria. Again, very clear definitions of progression are required (i.e., change in word recognition score over 12 months).

The Workshop consensus was that for NF2 associated VS, patients deemed particularly appropriate for current clinical trials are those with progressive hearing loss in their only hearing ear where the outcomes and risks of the currently available treatment options (deafness from surgery, risks of radiation therapy) are suboptimal. Patients with brainstem compression leading to radiographic evidence of obstructive hydrocephalus due to enlarging VS are also considered high priority candidates for clinical trials with agents that have the potential to halt tumor progression or shrink tumor given the risks associated with surgery for such tumors (when surgical decompression is not needed urgently to reverse hydrocephalus or brainstem compression).

Patients who would not currently be recommended for inclusion in VS drug trials are patients with non-progressive disease and patients with small (less than or equal to 1.5 cm diameter), asymptomatic tumors where functional preservation of hearing is likely without intervention or with specialized surgical tumor removal (Fig. 2). Radiation therapy should also be considered as a therapeutic option prior to clinical trial enrollment in tumors  $<3$  cm, or

in symptomatic tumors on other cranial nerves. Indeed, one avenue for future clinical studies is to incorporate radiosensitizing agents with radiation to improve the long-term tumor control rates in NF2. However, currently it is rare that radiation therapy is an attractive option for tumors  $>3$  cm, in children, or in non-progressive tumors. In these cases, observation, surgery, or a clinical trial specific to the clinical situation is preferred.

Finally, the inclusion of patients with unilateral sporadic VS in clinical drug trials designed primarily for patients with NF2 has several potentially important benefits. NF2-associated VS and unilateral sporadic VS have many similarities both histopathologically and in their underlying genetics. For example, the protein Merlin is absent in all VS studied to date whether related to NF2 or not [Hadfield et al., 2010]. Importantly, idiopathic VS have a lifetime risk of close to 1/1,000 [Evans et al., 2005] and hence are a common brain tumor in the general population. Identification of effective systemic therapies for these tumors through NF2-focused research may ultimately benefit a much larger population of patients.

## Cranial Meningioma Trials—Which Patients to Recruit?

As discussed above, the standard for NF2 associated meningiomas is observation unless these tumors become symptomatic. In symptomatic tumors, the standard therapy is surgery, which is frequently feasible and effective. Currently, the consensus opinion is that patients recommended to be considered for NF2 associated meningioma clinical trials should have recurrent World Health Organization (WHO) grade I or II tumors. Patients with WHO grade III (malignant meningioma) meningiomas have a different natural history and should have dedicated clinical trials. Given that about 70% of sporadic meningiomas carry the NF2 mutation [Goutagny and Kalamarides, 2010], therapeutic trials assessing both NF2 associated and sporadic meningiomas harboring the NF2 mutation may be indicated. These inclusion criteria would require surgical intervention for tissue sampling first, however, in most cases initial surgical management prior to an experimental agent is desirable. Meningioma is the most common primary brain tumor in adults and there are no known effective systemic therapies for this tumor, hence, development of clinical trials for residual or progressive meningiomas after surgery, and radiation therapy is a need for both patients with NF2 and for the tens of thousands of patients with idiopathic meningiomas.

## Clinical Trials for Spinal Meningioma, Spinal Schwannoma, and Spinal Ependymoma

The unclear history and diversity of NF2-associated spinal cord tumors, along with their relative infrequency compared to VS and cranial meningiomas, makes for a difficult tumor population to develop clinical trials for at this time. Whole body imaging (such as MRI) should be incorporated into NF2 clinical trials whenever possible as a secondary endpoint to add to the cumulative knowledge of the natural history of NF2 spinal cord tumors, and to allow monitoring of any serendipitous response to therapies principally targeted to VS or meningioma. When it is determined that

therapeutic intervention is required, it is reasonable to refer NF2 patients to available general population clinical trials designed for the particular spinal cord tumor types.

## The Pediatric NF2 Population—Role in NF2 Clinical Trials

Although NF2 is traditionally thought to present in young adults, registry data have shown that 18% of newly diagnosed NF2 patients are younger than 15 years of age [Evans et al., 1999]. NF2 manifestations in children generally involve skin and ophthalmologic findings as well as meningiomas causing focal neurologic deficits; and there is an association between early development of symptoms and poor prognosis [Baser et al., 2002]. Hence, recognition and treatment of NF2 associated symptoms in children may allow administration of therapies that could delay the development of neurologic morbidity and potentially mortality.

There are rare cases with fulminant disease progression very early in life for which the currently available treatments of surgery and radiation therapy are insufficient [Mautner et al., 1993; Nunes and MacCollin, 2003; Ruggieri et al., 2005; Evans et al., 2009]. In one series of 12 patients diagnosed with NF2 before 18 years old, there was a high tumor burden with >75% of patients having VS, other cranial nerve schwannomas, meningiomas or spinal cord tumors [Nunes and MacCollin, 2003]. At least 75% of the children had hearing loss and in the 58% of patients who underwent surgery for VS, none had preserved hearing post-operatively. Hence, the limited literature regarding NF2 in children suggests that there is a subpopulation that presents with early and severe disease and for whom the currently available treatment options are quickly exhausted.

As diagnostic criteria are applied more uniformly and more families with NF2 are registered, the frequency of identification of children with both asymptomatic and symptomatic tumors is increasing. For example, a United Kingdom series found that 9% of patients (33/343) were diagnosed before age 10 years [Evans et al., 1999]. When considering enrolling children in NF2 clinical trials it is important to recognize that although hearing loss may be one manifestation, presenting symptoms more commonly include unilateral congenital amblyopia that may lead to blindness and focal neurologic deficits due to neuropathy or meningioma. Finally, an important consideration is that children may have unique toxicities to consider including the potential impact on growth and normal development that are not as concerning in adults. The duration of therapy and the long-term impact of experimental therapies have to be considered. Overall, these considerations require a solid understanding of the disposition of the investigational agent in children and a relatively high risk population to offset the unknown potential long-term risks of the experimental agent.

## Incorporating Patients Who Have Received Radiotherapy in NF2 Clinical Trials

Tumors that have undergone radiation therapy may have a different natural history than non-irradiated tumors as radiotherapy may either improve outcome via effective tumor control or worsen the

disease course due to malignant transformation. In the short term following radiation, there can be acute or sub-acute inflammatory changes that can alter MRI interpretation. Careful consideration must be given to these factors as clinical trials for NF2 patients are likely to be small in number and therefore not allow stratification for such variables. However, patients previously treated with radiation therapy should be considered for NF2 trials with these factors in mind. In addition, trials to assess the short- and long-term impact of radiation therapy are needed to better define the risks and benefits of radiation for patients with NF2.

## DESIGN OF CLINICAL TRIALS FOR PATIENTS WITH NF2

The common progression of clinical trials is from dose finding, tolerability studies (Phase I) to efficacy studies designed to explore clinical activity (Phase II) and then to confirmatory efficacy studies to demonstrate that the new therapy is better than either existing therapies or placebo (Phase III). The success or failure of phase II studies is predicated on many factors over and above whether or not the treatment drug has the desired effect, including the endpoints chosen to demonstrate efficacy, the appropriate dose and dosing schedule taken from phase I studies, and the power of the study to detect a statistically significant difference. In order to increase the efficiency of efficacy studies, there has been increasing interest in incorporating translational studies or “Phase zero” studies early in new drug investigations.

### Phase Zero or Translational Studies in NF2

Phase zero is a term popularly applied to translational clinical studies in which a study drug is administered to a small number of patients (often less than 10) over a short period of time to assess pharmacokinetics (PK) and pharmacodynamics (PD). Such studies are designed to accelerate the development of promising drugs by assessing whether the agent of interest behaves in human subjects and tissues as expected from preclinical studies.

Recently, the United States Food and Drug Administration (FDA) published Phase zero guidelines as part of the 2006 Guidance on Exploratory Investigational New Drug (IND) Studies. The FDA-specific definition of Phase zero trials under the exploratory IND includes first-in-human testing of new agents at sub-therapeutic doses and provides for the assessment of drug-target effects and PK–PD relationships in humans [FDA 2006]. In the setting of NF2, Phase zero studies may consist of novel, biomarker-driven early clinical trial concepts that assess drug-target effects in human NF2-specific tumors *in vivo*, using drugs already approved for other tumors or disease indications. A Phase zero study would prescribe the drug of interest at the FDA approved dosing for other indications and assess if the agent reaches NF2-related tumors in active concentrations and shows the expected biological effects such as molecular target and signaling pathway inhibition. This assessment can be done via tissue sampling at the time of a planned surgical procedure, with imaging biomarkers or via other approaches such as microdialysis to assess the intratumoral extracellular drug concentrations [Blakeley et al., 2009]. If the predefined PK or PD endpoints are met, this is an early sign of biologic activity at the site

of disease and provides rationale for advancing to clinical efficacy testing. Since no direct benefit is expected for study participants given the short duration of drug exposure, Phase zero trials are limited to drugs that have a well-known safety profiles, minimal expected side-effects, do not interfere with surgery and wound healing, and do not pose any known long-term risks. Based on encouraging preclinical data [Doherty et al., 2008; Ammoun et al., 2010], a Phase zero trial using lapatinib, a dual EGFR/ErB2 small-molecule inhibitor for patients with NF2-related, and sporadic VSs is currently ongoing. (ClinicalTrials.gov identifier NCT00863122). Additional Phase zero trials with other molecular targeted agents including sorafenib for NF2 are expected to open within the next year. Patients with NF2 who have chosen a surgical therapy as their best treatment are optimal candidates for translational studies. In combined translational/efficacy trial designs, the experimental agent can be restarted post-operatively and assessed for its ability to impact the residual tumors.

### Phase I Studies in NF2

Agents that are new to human studies or that have specific side effects or mechanisms that may affect NF2 patients in unpredictable ways may require a study to specifically address safety and tolerability in patients with NF2. The need for safety assessments prior to efficacy testing is dependent on the treatment to be assessed and the amount of information about the safety of that agent. When it is determined that tolerability and toxicity studies are needed, these can be done simultaneously with phase zero investigations to maximize the information available about the drug prior to efficacy testing.

### Phase 2 Efficacy Studies in NF2

Careful selection of agents for efficacy testing and thoughtful trial design is particularly important in NF2 given the relative rarity of the disease and therefore, limited patient numbers for enrollment. Defining endpoints with precision requires detailed understanding of the natural history of the tumor of interest and the mechanism of the agent under investigation. The trial can then be structured with strict “go/no go” evaluation points based on the pre-determined primary endpoint. For example, VS with progressive growth over months have a less than 1% chance of spontaneous regression [Slattery et al., 2004; Harris et al., 2008]. Rather, 99% will continue to grow in volume. Therefore, a drug that is designed to reduce volume will have to show reduction in only 4 of 10 patients to demonstrate efficacy for that endpoint. Hence, small, single group trials may be sufficient to demonstrate efficacy in the right scenario. For more exploratory phase II studies, small multi-arm trials using novel designs such as adaptive randomization, “pick-the-winner” design, sequential accrual, and randomized discontinuation may be considered. For example, in an adaptive randomization study, patients initially would be randomized equally to one of three agents. As the efficacy of the arms become evident, more patients will be randomized to the arms with promising results, and the less promising arms will be dropped. These novel designs potentially allow more agents to be screened rapidly, and reduce the overall number of patients that will be required. However, these studies

also require strictly defined endpoints and the close collaboration of committed centers with strong, real-time statistical support. The slow growth of VS also allows patients to potentially cross over between study arms after a washout period if the predetermined endpoint is not achieved.

Given the scarcity of resources for NF2 clinical trials, it is important to forge collaborations with others doing clinical trials in similar tumor types. For example, ongoing meningioma efficacy studies may include NF2 associated meningioma patients. Although differences between sporadic and NF2 associated meningiomas must be accounted for to allow accurate interpretation of the results, collaboration with other tumor trials is feasible and may allow greater opportunity for trial development in NF2. Finally, as drugs develop along the clinical pipeline, consideration will have to be given to how to perform confirmatory studies for clinical efficacy. Patients with NF2 requiring treatment for progressive tumors are unlikely to accept a placebo, however, as mentioned above, there is no clear consensus as to the optimal treatment strategies for many NF2 associated tumors. Overall, it is not clear that a traditional phase III (large, double-blind, placebo-controlled) trial will be appropriate for NF2 patients [Neary et al., 2010a].

### NF2 Prevention Trials

Current clinical trials are focused on patients with progressive or symptomatic tumors. However, as the clinical and pre-clinical repertoire grows and as new natural history data become available, it will be desirable to identify therapies that can halt tumor progression before patients are symptomatic. The drugs in development currently are predominantly agents that halt cell growth or communication and hence may not be safe for long-term use, particularly in young patients. Moreover, common endpoints in prevention studies (i.e., time to symptom onset) require better understanding of the natural history of each tumor type and ideally, the development of biomarkers to accurately report tumor change. The consensus opinion is that state of science is not yet mature enough to support prevention studies, but may be in the near future.

### Quality of Life/Disease Severity Considerations in NF2 Clinical Trials

The major threats to QOL in patients with NF2 include: loss of hearing, mobility, and balance; facial disfigurement; pain; and social and emotional problems [Hornigold et al., 2010; Neary et al., 2010a]. Studies assessing QOL in patients with late onset hearing loss without NF2 suggest that there is an association between degree of hearing impairment, social isolation, perceived disability and depression [Nachtegaal et al., 2009]. Moreover, there is some indication that although patients with late-onset hearing loss have decreased reported QOL at baseline, there is the possibility for improvement with effective therapies. Studies in patients receiving CNI for adult onset hearing loss suggest that specific improvement in function such as communication abilities directly influenced perceived QOL [Zhao et al., 2008].

In order to better assess the specific factors effecting QOL in patients with NF2, Neary et al. 2010a,b produced a 32 item closed set

postal questionnaire to identify the extent and severity of QOL issues in 62 NF2 patients and compared responses to the Short Form-36 questionnaire (SF36). Subsequently, Hornigold et al. [2010] developed an independent eight item scoring system validated against the EuroQOL and SF-36 questionnaires expressly for patients with NF2 named The Guy's NF2 Impact Severity Score (NFTI-SS). Both of these measures remain in validation stages currently. As reliable tools to assess QOL are developed, QOL may serve as an important endpoint for future studies in NF2.

## SELECTING APPROPRIATE NF2 CLINICAL TRIAL ENDPOINTS

### Hearing Endpoints for NF2 Clinical Trials

There are two forms of hearing loss in patients with NF2. The first, and most common, is gradual hearing loss. Importantly, the correlation between tumor size and hearing is not strong, hearing loss can occur with tumors of any size and the exact mechanism of hearing loss is unknown. Hearing aids cannot reliably address VS-related hearing loss because amplifying sound levels does not address the issue of poor quality word information that is characteristic of hearing loss related to VS. Eventually, most patients with NF2 suffer complete hearing loss either from tumor growth or from the morbidity of surgical or radiation treatment. Although CNI and ABI devices provide benefit for some patients, there is no widely effective treatment for the gradual hearing loss experienced by NF2 patients.

The second form of hearing loss is sudden hearing loss (defined as hearing loss with onset over a period of less than 72 hr) [Rauch, 2008]. The generally accepted treatment for sudden hearing loss is a tapered course of oral corticosteroids (usually prednisone or methylprednisolone). Although sudden hearing loss is concerning for patients and clinicians, gradual hearing loss is the main cause of deafness in NF2 patients. Most NF2 patients lose hearing after acquisition of language; thus, the effect of hearing loss on their QOL is typically severe.

Common tests of hearing include detection of sound (measured as pure tone thresholds at individual stimulus frequencies or the average of several frequencies [pure tone average, PTA]) and speech discrimination (measured as word recognition score based on standardized word lists and presentation conditions). Other hearing tests such as oto-acoustic emissions (OAEs) or auditory brainstem response (ABR) monitor auditory function that is not clinically significant for patients. The American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) scale, a composite hearing endpoint, is in wide clinical use for reporting hearing preservation results after VS surgery [American Academy of Otolaryngology-Head and Neck Surgery Foundation, 1995]. This scale uses PTA and word recognition scores to classify hearing into four grades (A–D). Because the AAO-HNS scale uses one category to score all hearing ears with word recognition less than 50% (Class D), it is insensitive to changes in hearing within the category that may have clinical relevance for NF2 patients with a single hearing ear.

Speech discrimination (word recognition score) is a good primary hearing endpoint in VS trials because it directly measures a

patient's ability to communicate through speech. An improvement in word recognition would be of special value in NF2 patients, who often have only one hearing ear and whose hearing cannot be significantly improved with amplification. In clinical trials, a hearing response would be defined as the smallest improvement in word recognition score over baseline that meets criteria for statistical significance at the  $P=0.05$  level [Halpin and Rauch, 2006]. Values are defined for detecting a statistically significant change in word recognition at the  $P=0.05$  level for different baseline scores using 50- and 100-word lists [Thornton and Raffin, 1978]. The table of values can be used to compare any two scores from the same patient, such as before and after treatment. Currently, 50-word lists are used in word recognition score testing. However, the confidence interval be reduced (and accuracy increased) by using 100 word lists. A simple "hearing response criteria table" could be widely implemented to standardize clinical trials of chemotherapy, radiation, and surgery for VS.

### Lesion Volume Endpoints for NF2 Clinical Trials

Imaging of tumors is now a well-accepted endpoint for many cancer trials. Imaging is advantageous as it is non-invasive and given careful acquisition and analysis, shrinkage of tumor provides unequivocal evidence of biologic activity. However, imaging can encompass a wide variety of techniques and challenges that have to be carefully considered based on the tumor of interest and the experimental agent. There are two principle issues to consider for managing imaging metrics in NF2 clinical trials: what image acquisition and measurement criteria to use; and how to coordinate the workflow and reporting of metrics for a clinical trial. Options for measurement criteria to track change in lesion size include linear measures, such as RECIST criteria [Therasse et al., 2000] or three-dimensional measures of lesion volume [Harris et al., 2008].

Magnetic resonance imaging (MRI) is the most common diagnostic imaging modality used in patients with NF2 for tracking growth of lesions, including VS or other lesions such as meningioma or spinal schwannoma. In general, linear measures of VS underestimated volumetric growth by an average of 50% on MRI [Harris et al., 2008]. Although not routinely clinically available due to resource constraints, increasingly software is available for making semi-automated lesion volumetric measurements [Sorensen et al., 2001], which can be applied to NF2 lesions [Harris et al., 2008].

Standardization of image acquisition protocol used across sites, patients, and time points for a clinical trial are as important as image analysis. This is true of all body regions to be imaged. Table I summarizes the guidelines of a standardized imaging protocol for VS. Reliable acquisition and measurements are one step in a complex workflow involving organization of measurement requests, image transfers from multiple sites to a central analysis facility, image archiving, reproducible measurement techniques, quality assurance by trained staff, adherence to the clinical trial prescribed metrics, and longitudinal results reporting. To streamline the clinical trials process and to create a reliable and uniform workflow, infrastructure for collecting scans to support clinical trials has been developed. For example, the Tumor Imaging Metrics Core (TIMC; <http://www.tumormetrics.org>), is an integrated

TABLE I. Brain Magnetic Resonance Imaging Sequences for Optimal Assessment of Vestibular Schwannomas in Patients With NF2

Series no.	MRI series name	Type of pulse sequence	Slice thickness (mm)
1	Localizer	GradientEcho	5
2	Calibration_Scan	GradientEcho	6
3	Sag_T1	SpinEcho	5
4	Ax_DIFF	SpinEcho	5
5	Ax_FLAIR_T2	Inversion Recovery	5
6	Ax_T2	Fast Spin Echo	5
7	Ax_T1_IAC_pre-contrast	SpinEcho	3
8	Ax_3D_FIESTA_Hi_Res	Fiesta	0.8
9	Ax_T1_IAC_FS_Post-contrast	SpinEcho	3
10	Cor_T1_IAC_FS_Post-contrast	SpinEcho	3
11	O-Ax_post_FS_T1_SPGR	SPGR	2.5
12	O-Ax_T1_post_Whole_Brain	SpinEcho	5

Sag, sagittal orientation; Ax, axial orientation; Cor, coronal orientation; T1, T1 weighted; T2, T2 weighted; DIFF, diffusion weighted sequence; FLAIR, fluid inversion recovery sequence; IAC, internal auditory canal; FIESTA, fast imaging employing steady-state acquisition; IAC\_FS, internal auditory canal with fat saturation; SPGR, spoiled gradient recalled.

system that currently manages imaging metrics for over 300 imaging clinical trials [Urban et al., 2010]. A parallel site was developed specifically for NF tumor metrics and NF clinical trials (<http://www.nftumormetrics.org>).

## Molecular endpoints and Paradigms for NF2 Clinical Trials

Traditionally, clinical trials in oncology have employed clinical response criteria, such as overall survival (OS) and progression-free survival (PFS) to assess treatment efficacy, ideally compared to a placebo arm. Using such endpoints, however, is typically not feasible for a rare disease with multiple, slow-growing tumors and a variable clinical course, such as NF2. In theory, substituting molecular for clinical endpoints may help identify active drugs for NF2 patients in vivo in a much shorter period of time with fewer patients and at a lower cost, and potentially help eliminate drugs that do not reach the target and/or show insufficient evidence of biological activity in the tumor tissue of interest [Tan et al., 2009]. In addition, this approach provides a unique opportunity to gain valuable insights into the effects of drug on molecular signaling in vivo and help confirm or reject observations gleaned from pre-clinical model systems. For example, positive or negative signaling feedback loops identified in response to treatment in preclinical models may or may not be operational in humans in vivo. In addition, unexpected escape or resistance mechanisms may be uncovered by studying drug-treated human tumor tissue and such data may help drive the future development of molecular targeted therapies. Defining the specific molecular endpoints for a given drug can be challenging, in particular if the drug has several targets or significant off-target effects. Phase zero studies are one approach to investigate these questions and are optimal for drugs with well-described mechanisms of action and PD endpoints [Murgo et al., 2008, Tan et al., 2009].

The optimal investigation of molecular drug effects is to investigate tumor tissue before and after treatment. Repeat sampling of brain and spinal tumors is not feasible requiring the comparison of treated patient samples to non-treated samples from a control group. This limits analysis to biomarkers with a low inter-patient variability and a robust response to treatment. An ongoing study of sorafenib in NF2 patients is assessing drug effect in cutaneous schwannomas since these can be accessed pre- and post-treatment. An inherent limitation of surrogate tissue is that it may or may not truly reflect the primary tissue of interest (i.e., VS), however, it does allow pre- and post-treatment assessments of a in vivo biologic effect.

In summary, carefully designed exploratory clinical trials with molecular endpoints rather than traditional endpoints may help prioritize drugs emerging from the NF2 preclinical pipeline [Evans et al., 2009] for further study in efficacy trials with traditional or NF2-specific clinical endpoints [Plotkin et al., 2009a].

## MOLECULAR MECHANISMS OF NF2 TUMOR SUPPRESSION AND CANDIDATE DRUG TARGETS

Located on chromosome 22q 11.2, the NF2 gene is biallelically inactivated in NF2 tumors. The NF2 gene encodes a tumor suppressor protein called Merlin/Schwannomin (commonly known as Merlin) [Rouleau et al., 1993; Trofatter et al., 1993]. In normal cells, Merlin, a regulator of cell growth and cell-cell interactions, is expressed diffusely across several cell types including Schwann cells, meningeal cells, mesothelial cells, and lens cells [McClatchey and Giovannini, 2005; Curto and McClatchey, 2008].

The roles of Merlin protein are wide-reaching. It impacts several tumorigenic pathways and acts within several intracellular sites. Questions that are now emerging in an effort to fully understand Merlin's function include: how many complexes can Merlin form in

## NF1 and NF2 Gene Signaling Pathways

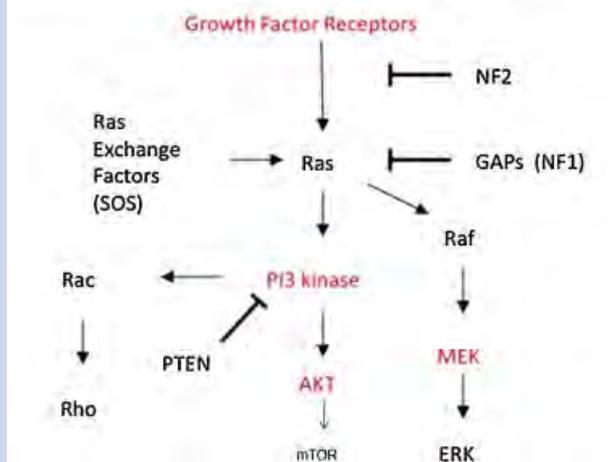


FIG. 3. The primary therapeutic targets in the Ras downstream signaling pathway.

a given cell? Of Merlin's various cellular roles, which are pathogenic when abrogated, and therefore important for drug targeting? Conversely, is Merlin a universal regulator, such that inhibiting a single cellular pathway will have a low likelihood of success in controlling growth of NF2-associated tumors? Ongoing research is addressing these questions to further elucidate the molecular interactions at large in the setting of a *NF2* gene mutation.

Recent evidence suggests that Merlin may also be a negative regulator of growth and progression of several non-NF2 associated cancer types [Stamenkovic and Yu, 2010]. Indeed, many of the pathways that appear important in NF2 tumorigenesis contribute to the growth of a diverse number of cancers such as breast, colon, liver, and renal cell carcinoma as well as many hematologic malignancies. This supports the idea that therapies developed for NF2-associated tumors could well have much broader clinical applications.

As we learn about the pro-tumorigenic pathways in which loss of Merlin function is implicated, cellular targets are identified that may respond to therapeutics (druggable targets). As noted above, some of these drug targets are common to other cancer conditions, and as a result there are several drugs currently in development and clinical use that may inhibit NF2 target pathways. Figure 3 highlights the up- and downstream candidate drug targets currently of most significant interest in NF2.

In the last few years, there has been a concerted effort to accelerate the identification of NF2 therapies by bridging basic discoveries and translational science. The NF Preclinical Consortium (NFPC) sponsored by the Children's Tumor Foundation offers a unique approach to facilitating preclinical trials. Candidate NF drugs are assessed in parallel in a series of validated NF1 and NF2 genetically modified mouse models and xenograft mouse tumor models. NFPC has employed standardization of PK/PD analysis across models and sites with collaborative interpretation of data. Established in 2008, NFPC has been successful in collaborating with

biotechnology and pharmaceutical companies and offered valuable lessons about how to overcome the challenges in NF2 preclinical drug testing. Though promising results in a mouse do not necessarily predict human efficacy, NFPC should inform future selection of drug candidates to be advanced to the clinic, and shed light on relevant drug pathways.

As a general note, it is likely that multiple target inhibition—using combination therapies or by using single agents designed to impact multiple targets—may be a more potent therapeutic approach than single target inhibition. However, in designing combined therapy trials, attention to PK interactions, combined toxicities, and cost is required particularly given that NF2 tumors are largely not malignant. Preclinical investigations are critical for defining the PK relationships and toxicities before committing to clinical investigation. Some of the key drug targets currently under investigation for NF2 are described below.

## Epidermal Growth Factor Family

In NF2, the loss of Merlin protein leads to abnormal activation of the epidermal growth factor family (EGFR) receptor tyrosine kinases (RTKs) namely EGFR, ErbB2, and ErbB3 which span the cell membrane. In the normal cell these EGFR family RTKs activate cell division, contribute to feedback loops, and regulate cell death. In the absence of Merlin, RTKs remain constitutively active allowing increased cell proliferation and resistance to cell death [McClatchey and Fehon, 2009].

Drugs that target the EGFR family of RTK include lapatinib (a dual EGFR/ErbB2 inhibitor), erlotinib, and gefitinib. All are oral agents already approved by the FDA for various cancers. These drugs have been investigated as NF2 therapies, principally for VS and ependymoma. In vitro, merlin deficient cells showed aberrant EGFR activation and inhibition of EGFR signaling decreased cellular proliferation [Curto et al., 2007]. However, in a clinical study, erlotinib (which inhibits EGFR alone) failed to show tumor response in NF2 patients with progressive VS [Plotkin et al., 2010]. Long-acting inhibitors that target multiple EGFR/ErbB family members have recently been developed. These are being tested in clinical trials for various other cancers and are of great interest as potential NF2 therapies.

## The RAS-GTP Pathway

RAS was one of the first oncogenes identified, and is one of the most common, having a role in multiple tumor types. Normal RAS is bound to the internal cell membrane and can assume two formations: active, promoting cell growth and proliferation (RAS-GTP state), or inactive (RAS-GDP state) [Blum and Kluog, 2005]. In normal cells, Merlin forms complexes with Ezrin at the membrane, and silences RAS-GAP [Morrison et al., 2007]. However, in Merlin's absence, RAS can remain active, drive cell proliferation and contribute to tumor growth.

It is difficult to design a drug specifically to target activated RAS. However, there are several agents such as farnesyl transferase inhibitors that indirectly inhibit RAS activation [Blum and Kluog, 2005]. RAS can also be targeted by inhibiting downstream cell signaling pathway pro-survival and pro-proliferation elements

including Raf-MEK1/2-ERK1/2 and PI3-K-Akt (Fig. 3). A number of agents targeting these have been assessed in NF2. For example, AR-12 is a derivative of the anti-inflammatory celecoxib that inhibits PI3K/Akt via phosphoinositide-dependent kinase 1 (PDK-1) inhibition, inhibiting growth of VS and malignant schwannoma cells [Lee et al., 2009]. Honokiol, a natural compound found in the bark and leaves of Magnolia trees that has pro-apoptotic effects in several cancers, has recently been shown to inhibit the growth of VS cells [Lee, 2010], also appears to act via Akt inhibition.

Rac is another Ras downstream element (Fig. 3) and a member of the Rho-like GTPases that contributes to cell migration and invasion in malignancy. In the absence of functional Merlin, Rac becomes activated and allows integrin-RTK signaling resulting in tumor proliferation [Okada et al., 2007]. Rac may represent another target of interest for NF2.

Raf/MEK/ERK is in a parallel signaling pathway downstream of Ras with roles in cell proliferation and cell cycle arrest depending on the signal [Peyssonnaud and Eychène, 2001]. This pathway may also be dysregulated in the absence of Merlin. Sorafenib is a multiple RTK inhibitor that targets Raf/Mek/Erk pathway in addition to PDGF, VEGF, and c-kit, while nilotinib is a new generation RTK inhibitor of BCR-ABL that also targets PDGFR and c-kit. Both sorafenib and nilotinib are entering clinical trials for VS and are discussed in more detail below.

## mTOR

Another downstream element in Merlin's tumor suppressor activity is mammalian target of Rapamycin complex 1 (mTORC1) [James et al., 2009]. mTORC1 is constitutively activated in both schwannomas and meningiomas with Merlin deficiency [James et al., 2009; López-Lago et al., 2009]. Several mTOR inhibitors are in clinical use for other conditions including NF1 associated plexiform neurofibromas, and may also be of potential interest for NF2. These include rapamycin, everolimus, and temsirolimus.

## VEGF and PDGF

Angiogenesis is a requirement for malignant tumor growth, and more recently has been implicated in the benign tumors found in NF2. VS express vascular endothelial growth factor (VEGF) on both tumor cells and associated endothelial cells, suggesting a role for angiogenesis in the growth of these benign tumors [Plotkin et al., 2009b; Wong et al., 2010]. The VEGF-targeted agent bevacizumab has shown promise in some NF2 patients and is now in a clinical trial for progressive VS.

## Heat Shock Proteins

Heat shock protein 90 (Hsp90) is one of the chaperone proteins that are responsible for ensuring the target proteins are correctly folded and transported throughout the cell. It is one of the proteins in the microenvironment that indirectly contributes to cancer cell proliferation. Hsp90 is increasingly investigated as a target for inhibition in CNS malignancies. For example, the agent, NXD30001, induces regression of glioblastoma (GBM) in a mouse model [Zhu et al., 2010].

## DCAF1: Ubiquitin Ligase Inhibitor

It was recently reported that Merlin in its closed formation can accumulate in the nucleus and interact with the E3 ubiquitin ligase CRL4 (DCAF1) [Li, 2010]. Depleting DCAF1 with an inhibitor reduced proliferation of Schwann cells cultured from NF2 patients [Li et al., 2010]. DCAF1 has not previously been investigated widely in cancer research, but is known to be one of the target proteins of a HIV-1 accessory gene that induces cell-cycle arrest [Nonaka et al., 2009]. Modifiers of DCAF1 or related proteins may provide a new NF2 therapeutic avenue for exploration.

## Traditional Cytotoxic Therapies and NF2

Though recent focus has principally been on targeted biologic drugs, there may still be a role in NF2 for traditional chemotherapies. The major concern about using such agents is the potential for unacceptably high toxicities, especially given the likely need in NF2 for prolonged use. Importantly, since VS are largely benign tumors, the tumor cells may be proliferating at such low rates as to render cytotoxic therapies ineffective. Nonetheless, in select cases screening of traditional cytotoxic agents, for example, following VS malignant progression or in meningioma, may be warranted and should be considered.

## ONGOING CLINICAL TRIALS FOR NF2

### Bevacizumab

Bevacizumab is a humanized IgG1 monoclonal antibody (MAb) that binds all biologically active isoforms of human VEGF (or VEGF-A) with high affinity ( $k_d = 1.1$  nM). VEGF is one of the most potent and specific angiogenic factors and is a critical regulator of both normal and pathological angiogenesis. To date, over 7,000 adult patients have been treated in clinical trials with bevacizumab as monotherapy or in combination regimens for a variety of cancers. Bevacizumab has been studied in the pediatric population in three clinical trials and two retrospective reports. Across all of these studies, with evaluation periods ranging from 1 month to 2 years, there have not been any dose limiting or major toxicities seen in children treated with bevacizumab [Benesch et al., 2008; Glade Bender et al., 2008; Liu et al., 2009; Packer et al., 2009; Modak and Cheung, 2010].

Although initially developed for cancers, VEGF is overexpressed compared to normal tissue in sporadic VS by expression analysis [Evans, 2009a], though not when measured by immunohistochemistry [Plotkin et al., 2009a]. It was recently shown that bevacizumab treatment results in clinical improvement in individuals with NF2 and progressive symptoms related to VS [Plotkin et al., 2009b; Mautner et al., 2010a]. In 10 NF2 patients at risk for complete hearing loss, bevacizumab at 5 mg/kg IV every 2 weeks resulted in 4 of 7 evaluable patients having significantly improved word recognition scores and 6 of 10 patients experiencing  $\geq 20\%$  reduction in tumor volume [Plotkin et al., 2009b]. An ongoing prospective study is confirming and expanding these findings in which individuals with NF2 and symptomatic VS (progressive hearing loss over the preceding 24 months) who are not candidates for surgery or

radiotherapy are treated with bevacizumab 7.5 mg/kg IV every 3 weeks (ClinicalTrials.gov identifier NCT01207687). The primary endpoint is hearing improvement. Secondary endpoints are tumor volume, exploratory advanced MRI studies, whole body MRI, plasma biomarkers, and QOL measures. The study is designed to confirm a reliable improvement in hearing with bevacizumab and to explore in detail its mechanism of action and duration of effect in the setting of NF2 associated VS.

Despite promising initial results, many important questions about the role of bevacizumab in NF2 remain. Most importantly, bevacizumab can impair wound healing, and surgery must be scheduled only after the drug has cleared from the system. This can be limiting for NF2 patients whom have multiple tumor types that may need to be addressed concurrently. Also, recent case reports suggest that the bevacizumab effect is temporary and when drug is stopped, radiographic or clinical benefit was reversed [Mautner et al., 2010b].

One possibility is that there is a unique interaction between anti-angiogenic mechanisms and Merlin deficiency that will benefit from combining bevacizumab with another drug therapy. For example, elevated levels of platelet derived growth factor (PDGF) have been observed in NF2 VS and meningiomas [Ammoun and Hanemann, 2011]. PDGF has been suggested to be activated in the setting of VEGF inhibition in some malignancies, allowing angiogenic escape [Pietras et al., 2008]. However, in a Phase II study of recurrent meningiomas treated with the PDGFR inhibitor imatinib mesylate, there was no significant clinical benefit [Wen et al., 2009]. Ultimately, there may be rationale for combining VEGF inhibition with other targeted therapies such as imatinib, nilotinib, or sorafenib.

## Lapatinib

Lapatinib is a small molecule RTK inhibitor that is orally active and reversibly inhibits both EGFR and ErbB2, blocking phosphorylation and activation of Erk1/2 (phospho-Erk1/2) and Akt (phospho-Akt) in EGFR and/or ErbB2-expressing tumor cell lines and animal xenografts [Rusnack et al., 2001]. It is commercially available and FDA approved for metastatic breast cancer in combination with cytotoxic therapies. Lapatinib is well tolerated with manageable side effects in adults and pediatric patients [Fouladi et al., 2010]. For these reasons it is of great interest for NF2. However, a pre-requisite for efficacy of any anti-tumor therapy is that the drug can cross the blood–brain barrier, access the tumor and affect the drug target. To examine this, in an ongoing phase zero trial discussed above, individuals with either idiopathic or NF2 associated VS take lapatinib for 10 days prior to tumor resection. At the time of resection blood and tissue samples are collected to assess the plasma to tumor PK ratio and to assess molecular markers of activity in the tumor. The information gained from this study will inform about the mechanism of action of lapatinib at the level of the tumor and assist in the interpretation and planning of future studies with similar agents.

Concurrently, a phase II clinical trial of lapatinib in children and adults with progressive VS is ongoing. In a two-stage design, NF2 patients older than 3 years of age with progressive VS are eligible (ClinicalTrials.gov identifier NCT00973739).

Lapatinib is administered continuously for 28-day courses. The primary endpoint is defined as a decrease of at least 15% in tumor volume. Enrollment of the first trial stage has been completed with nine eligible patients. Two of the nine patients discontinued protocol therapy after three treatment cycles due to radiological progression. One patient had a 16.6% reduction in the VS tumor volume after three cycles, and two patients remain on treatment for >12 months with stable disease.

## Sorafenib and Nilotinib

Sorafenib is a multiple RTK inhibitor that targets Raf/Mek/Erk pathway in addition to PDGF, VEGF, and c-kit. Sorafenib is currently being investigated in a small pediatric clinical trial for NF1 plexiform neurofibromas, and planning is underway in the United Kingdom to bring this drug into a phase zero NF2 clinical trials based on positive NF2 in vitro data [Ammoun et al., 2011]. This trial will be informed by the ongoing NF1 trial where, especially in younger children, tumor pain and rash made dose reductions necessary. The NF2 trial planned will examine tolerability as well as molecular efficacy via cutaneous schwannoma biopsies pre- and post-treatment in each patient.

Nilotinib, an analog of imatinib, is a new generation RTK inhibitor of BCR-ABL that also targets PDGFR and c-kit. It has also shown promising NF2 in vitro data although less effective than sorafenib [Ammoun et al., 2011]. However, nilotinib is likely to have fewer clinical side effects than sorafenib. A Phase Zero trial with a similar design as described for sorafenib is currently underway. This includes 15 patients receiving 14 days of oral dosing and is a simple trial design (only requiring three clinic visits over 28 days). A Phase II study of nilotinib for VS is planned for 2011 in Canada (ClinicalTrials.gov identifier NCT01201538).

## PTC299

PTC299 is an oral VEGF inhibitor that acts by binding to the 5' untranslated region (UTR) of the VEGF mRNA. In vitro, PTC299 potently inhibits VEGF production in a broad range of tumor types, including breast, colorectal, fibrosarcoma, gastric, and lung cell cancer lines. In vivo, the drug reduced levels of VEGF in plasma and tumors and inhibited the growth of multiple tumor xenografts. A Phase II study of PTC299 in NF2 patients with progressive VS is currently underway (ClinicalTrials.gov identifier NCT00911248).

## ADVANCES IN PATHOLOGICAL ANALYSIS OF SCHWANNOMAS

The detailed pathological review of schwannomas is an important, but often overlooked, resource. The pathological evaluation of resected drug-treated tumors compared to treated tumors, can yield valuable information in both preclinical and clinical settings. Human samples that are collected tend toward tumors that have failed to respond to treatment, since surgery will not be done on responsive tumors; but a close examination of treated tumors can yield valuable information about drug effects—status of signaling

pathway activation, effects on cell proliferation, apoptosis, and vascular density.

Pathologic review of schwannomas can also shed light on new therapeutic targets. Investigation of schwannomas in their earliest phase of development—termed Schwann cell tumorlets—suggests that loss of NF2 gene function is necessary, but not sufficient for schwannoma formation. Something similar to this has been noted in other tumor suppressor syndromes such as retinoblastoma and Von Hippel Lindau Disease [Stemmer-Rachamimov et al., 1998]. Immunohistochemical study of VEGF the pathway in VS has revealed a dramatic decrease of proteins semaphorin 3A (SEMA3A) and 3F (SEMA3F) expression in tumors compared to normal nerves. SEMA3A and SEMA3F compete with VEGF ligand, and their decrease is thought to be associated with VEGF pathway activation [Wong et al., 2010]. An additional necessary change may be loss of SEMA which may contribute to an “angiogenic switch.” The exact timing of this is not clear in humans, but in mouse models it appears that Merlin deficiency contributes to SEMA3 loss of expression and to a pro-angiogenic phenotype [Wong et al., 2010]. Tumorlets may thus provide us with a tool to investigate the tumor progression in schwannomas and aid in the identification of new drug targets that could halt tumor progression.

One major area of need is to standardize how tissue is prepared and stored after resection so that these data that are currently underutilized can inform new treatment options. In 2011, the Children’s Tumor Foundation (CTF) NF Clinic Network will launch an NF BioBank to help address this need.

## INDUSTRY’S INTEREST IN NF2

Given the cost of clinical trials, engaging industry in the development of therapies for NF2 will be vital in order to advance treatments to the clinic. Interest in rare diseases in general is on the increase in industry, with a number of entities establishing rare disease research units. The Children’s Tumor Foundation has succeeded in attracting interest from industry through the NF Preclinical Consortium and through the Drug Discovery Initiative Toolbox, an online listing of drugs that pharmaceutical and biotechnology companies have made available to NF researchers. Individual NF2 investigators have also formed successful collaborations with industry to develop the clinical trials discussed. This growing success is a result of several factors including: (1) a substantial unmet medical need for patients with NF2 as there are no effective drug therapies for the tumors associated with NF2 and no optimal standard of care, (2) significant progress has been made in understanding the molecular underpinnings of NF2, providing potential validated targets for NF2 drug discovery and development efforts, (3) NF2 preclinical models are now available to evaluate potential therapeutics in order to efficiently plan clinical studies and, (4) successful development of therapies for NF2 associated tumors (schwannomas, meningiomas, ependymomas) could have significantly expanded market outreach given the high prevalence of these tumors worldwide. In summary, the time is right for industry to engage closely in identifying and advancing the quest for NF2 drug therapies.

## SUMMARY

A recent surge in translational and clinical research on NF2 tumors—VS, meningioma, ependymoma, and peripheral schwannoma—has culminated in the commencement of the first clinical trials specifically for patients with NF2, with drugs targeting major signaling pathways of interest including ErbB2, VEGF, PDGF, mTOR, PI3K, and AKT. NF2 clinical trials are employing a range of innovative clinical trial designs thus far focused on VS. For efficacy studies in VS, there is consensus that the current optimal endpoints are change in hearing tumor volume, or QOL measures. As NF2 clinical trials expand it will be necessary to determine the most meaningful endpoints for monitoring other tumor types such as meningioma and the various forms of spinal tumors. It is clear that progress in NF2 clinical trials is made with close collaboration between basic and clinical scientists. Translational studies, including the analysis of all tumor samples from patients with NF2 accessed in the course of standard care, are an important source of data to support the efficient discovery of new treatments for patients with NF2.

Looking ahead, as the number of NF2 clinical trials grows, a NF2 clinical trial consortium would provide a venue for reviewing preclinical, translational, and clinical data to prioritize agents to be advanced. This is a particularly vital practical measure in NF2 as without centralized oversight, there are unlikely to be enough patients available to complete all of the proposed studies. One exciting development is that the Phase II NF Clinical Trials Consortium, established in 2005 with funding from the Congressionally Directed Medical Research Program within the Department of Defense, is proposing to expand to offer NF2 specific trials. As the number of treatment options and active clinical trials continues to grow, it will be crucial to maintain communication and organization across all subspecialties that contribute to the care of patients with NF2 to ensure that all caregivers are well informed about standard and experimental treatment options.

In summary, there are an increasing number of opportunities for identifying effective NF2 therapies. The NF2 community needs to remain focused on the importance of careful pre-clinical screening of new agents utilizing meaningful tumor models and patient tumor samples, engaging the biopharmaceutical industry, developing innovative clinical studies that are cost and time efficient and building multidisciplinary collaborations between basic and clinical researchers, clinicians and patients. If these tasks are vigorously pursued, it is very likely that there will be new, effective therapies for patients with NF2 in the very near future.

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