

Consensus Recommendations to Accelerate Clinical Trials for Neurofibromatosis Type 2

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Abstract Purpose: Neurofibromatosis type 2 (NF2) is a rare autosomal dominant disorder associated primarily with bilateral schwannomas seen on the superior vestibular branches of the eighth cranial nerves. Significant morbidity can result from surgical treatment of these tumors. Meningiomas, ependymomas, and other benign central nervous system tumors are also common in NF2. The lack of effective treatments for NF2 marks an unmet medical need.

Experimental Design: Here, we provide recommendations from a workshop, cochaired by Drs. D. Gareth Evans and Marco Giovannini, of 36 international researchers, physicians, representatives of the biotechnology industry, and patient advocates on how to accelerate progress toward NF2 clinical trials.

Results: Workshop participants reached a consensus that, based on current knowledge, the time is right to plan and implement NF2 clinical trials. Obstacles impeding NF2 clinical trials and how to address them were discussed, as well as the candidate therapeutic pipeline for NF2.

Conclusions: Both phase 0 and phase II NF2 trials are near-term options for NF2 clinical trials. The number of NF2 patients in the population remains limited, and successful recruitment will require ongoing collaboration efforts between NF2 clinics. (Clin Cancer Res 2009;15(16):5032-9)

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The neurofibromatoses encompass three distinct inherited rare disorders: neurofibromatosis type 1 (NF1), neurofibromatosis type 2 (NF2), and schwannomatosis. For many years, these disorders were viewed as part of "generalized neurofibromatosis" (originally termed von Recklinghausen disease). Three forms of NF were formally recognized as separate clinical entities at a NIH consensus meeting in 1987 (1). NF1 and NF2 are autosomal dominant disorders with well-characterized genes: the *NF1* gene on chromosome 17 and the *NF2* gene on chromosome 22. More recently, a candidate gene for schwannomatosis was identified also on chromosome 22 (2, 3).

NF2 mutations are found in >92% of NF2 individuals in the second or greater generation of families (4). Interestingly, although NF2 affects only 1:25,000 persons (5), population studies suggest that up to 1:300 people will develop in their lifetime a tumor with an underlying sporadic NF2 mutation (5-8). This includes persons with unilateral vestibular schwannomas (UWS) as well as meningiomas. It is not clear if the underlying biology of VS is the same in NF2 patients and those with sporadic tumors; however, VS in NF2 patients are known to grow faster than their sporadic counterparts. Therefore, what we learn

Translational Relevance

Despite the fact that significant progress has been made in our understanding of the biology of neurofibromatosis type 2 (NF2), patients with this disorder still face a poor clinical outcome, significant morbidity, and a decreased life span. Here, we provide recommendations for accelerating clinical trials for NF2 with a goal of stimulating rationally designed trials using molecular-targeted agents that will improve both survival and quality of life for NF2 patients.

in developing therapies for NF2 VS could have much broader clinical applications.

Natural History and Clinical Management of VS in NF2

The hallmark of NF2 is the growth of bilateral VS on the vestibular nerves. Meningiomas are also predominant. Recent improvements in microsurgery and the advent of radiotherapy offer initial treatment options. Nevertheless, NF2 has a significant level of morbidity and early mortality (9, 10).

NF2 is diagnosed by the presence of bilateral VS (1, 11), although NF2 diagnosis can also result from a UVS and at least two other NF2-related tumors (11, 12). Although benign in their primary state, VS are the main concern for most NF2 patients because they are responsible for the larger part of morbidity and, to a lesser extent, mortality.

Today, surgery is the primary clinical tool for NF2 management (9). VS tumors can require frequent surgeries: these are intricate because the vestibular nerve is close to the acoustic (cochlear) and facial nerves. VS surgery frequently leads to deafness and loss of facial nerve function, infections, and headaches (9, 13). A confounding aspect of NF2 is that there is no correlation between tumor size and hearing loss (9). Although there are challenges on the path to doing NF2 clinical trials, there is a pressing need to identify an alternative or adjunct to surgery.

Some physicians have observed NF2 VS growing more rapidly in children, in whom a benefit of early interventional surgery may be preservation of long-term hearing (13). Some studies suggest that VS grow intermittently up to 4 to 10 mm a year in diameter and can be dormant up to 10 years before resuming growth (9, 14).

Much remains to be understood about VS natural history. A recent study enrolled 86 newly diagnosed NF2 patients over 3 years, but being newly diagnosed, very few had tumors that progressed during this time, probably underrepresenting the NF2 phenotype. Further data will be mined from this study to see what we can learn about tumor growth. Looking ahead, it may be difficult to get advanced NF2 patients with progressing tumors to enroll in a study, but it is vital to pursue this to fully understand the natural course of NF2 and establish appropriate outcome measures for clinical trials.

Cochlear Implant and Auditory Brainstem Implant for NF2 Patients

Interventional surgery is now being done at earlier ages to preserve hearing, although, unfortunately, many patients identify expert care too late to save the cochlear nerve. NF2 patients with VS of 1.5-cm maximal diameter, and speech discrimination scores of $\geq 70\%$ in both ears, are good candidates for cochlear preservation surgery (suboccipital or middle cranial fossa). Generally, when bilateral VS are present, the more "favorable" tumor for hearing preservation is removed first. Keys to successful surgical hearing preservation are early VS detection, small tumor size, incomplete filling of the lateral end of the internal auditory canal by the tumor, surgical team experience, superior vestibular nerve origin (means reduced caloric response), and good preoperative hearing preservation. If hearing is preserved following surgery, the second VS is carefully removed, attempting to preserve the cochlear nerve. If, however, hearing is not preserved following surgery on the first tumor, and the unoperated ear retains serviceable hearing, careful observation is required before further surgery. If hearing is lost with $<60\%$ word discrimination on the unoperated side, promontory stimulation is done on the first ear. If there is positive auditory stimulation, a cochlear implant may be considered, with hope for long-term sustained viability and excellent speech discrimination (9, 15).

If neither cochlear nerve is stimulated, a multichannel auditory brainstem implant (ABI) is considered (9, 13, 16). The ABI is effective and safe, providing useful auditory sensations in most deaf NF2 patients and improves ability to communicate compared with "lip reading only." ABI allows detection and recognition of many environmental sounds and can even offer significant ability to understand speech using only sound from the ABI (with no lip reading cues; ref. 16).

Natural History and Clinical Management of NF2 Meningiomas

Meningiomas are the second most frequent tumor of NF2 (1, 9, 11), generally slow growing, attached to the dura mater, and composed of neoplastic arachnoidal cells (17, 18). Fifty percent to 60% of NF2 patients will present with an average of three meningiomas. Intriguingly, 20% of all primitive central nervous system tumors are sporadic meningiomas with NF2-associated mutations (18). Both surveillance and surgery are used in meningioma clinical management, depending on tumor growth history. For surveillance, clinical and radiological [magnetic resonance imaging (MRI)] follow-up are required at 6 months and then yearly with very few patients developing new meningiomas (9). Surgery is done where there is significant growth, parenchymal edema, or clinical symptoms such as neurologic deficit, intractable seizures, or intracranial hypertension. Preoperative transarterial embolization allows resection of hypervascularized convexity meningiomas. The goal in NF2 meningioma surgery is to get a maximal safe resection; if meningioma remnants remain, further treatment will be postponed until new growth or symptoms occur.

Reoperation is an important part of meningioma management. The relative risk of mortality is 2- to 3-fold greater in NF2 patients with meningiomas (9, 10) and determined by tumor size and location. The most straightforward surgeries are in

the convexity far from eloquent brain areas (important areas for function speech and motor function). The most risky are in front of eloquent brain areas, midline tumors (parasagittal and falx), and skull base.

Meningioma recurrence depends on completeness of resection and tumor pathologic grade. Thirty percent of NF2 meningiomas are aggressive (grade 2 and 3) versus 10% to 15% in sporadic meningiomas, although this may be biased by the fact that only growing or symptomatic tumors are removed in NF2 patients (8, 17).

Natural History and Clinical Management of NF2 Spinal Tumors

Spinal tumors are seen in 70% of NF2 patients. These are heterogeneous in type (meningioma, schwannoma, and ependymoma), distribution, and behavior. Larger tumors are more likely to progress, and if symptomatic, surgery is currently the only option. Overall, NF2 ependymomas are usually indolent rarely requiring surgery. Spinal tumors are unlikely to be a primary measure in NF2 clinical trials because volumetric assessment is complex.

Radiosurgery and Radiotherapy for NF2 Tumors

Use of radiosurgery and radiotherapy for NF2 management is highly controversial, with some physicians using this and others not. There are differences of opinion as to whether observation, microsurgery, or radiosurgery is more effective in hearing preservation. Although there is no NF2 meningioma–dedicated literature report on radiosurgery or radiotherapy, for cavernous sinus meningiomas surgery is not recommended, and radiosurgery may be helpful for rare symptomatic growing tumors. A major concern of many is that radiosurgery and radiotherapy may induce malignancy, including secondary meningiomas and multiple small tumors. However, other than individual case reports, the NF2 community lacks thorough data on the risks, benefits, and outcome of radiotherapy and radiosurgery.

What Do We Know—and Do Not Know—about NF2 Biology

The NF2 gene encodes Merlin, a novel tumor suppressor protein that lacks enzymatic function (19). Strategies to identify targeted NF2 therapies begin with the delineation of the molecular function of Merlin. Merlin resembles the ERM proteins (ezrin, radixin, and moesin) sharing considerable homology across their NH₂-terminal FERM domain, a trilobed structure that seems to bring together multiple proteins at the cell membrane.

A growing body of information suggests that Merlin coordinates and transmits multiple extracellular signals. Merlin/ERM proteins undergo regulated conformational changes governing localization and interaction with target proteins. Phosphorylation downstream of the small GTPases Rho (for the ERM proteins) or Rac (for Merlin) represents the best-studied mechanisms of induced conformational change and altered localization/activity. Merlin can, in turn, negatively regulate Rac signaling at the level of the serine/threonine kinase Pak; Pak inhibitors are being developed as potential NF2 therapies. Merlin can be phosphorylated on multiple residues by multiple kinases. The consequences of these potentially regulatory events are only beginning

to be understood but should reveal multiple potential steps for NF2 therapeutic intervention. In addition, different molecular strategies may be necessary for different tumor types.

Study of *Nf2*-deficient cells from genetically engineered mice (GEM) suggests that loss of *Nf2* gene function leads to loss of contact-dependent inhibition of proliferation. This fits with the benign nature of tumors seen in NF2 patients. What is the molecular basis? Merlin associates with the hyaluronic acid receptor CD44, which can be important for mediating contact-dependent inhibition of proliferation. Merlin is also necessary for the stabilization of cadherin-containing adherens junctions between other types of *Nf2*-deficient cells. In both cases, Merlin seems to sense the physical extracellular milieu through adhesion receptors.

Recent studies in mammalian and *Drosophila* models have suggested that Merlin controls surface availability of epidermal growth factor receptor (EGFR) and other membrane receptors (20, 21). Merlin coordinates cell contact stabilization with EGFR silencing. Pharmacologic EGFR inhibitors can restore contact-dependent inhibition of proliferation to several types of *Nf2*^{-/-} mouse cells, including Schwann cells. EGFR inhibitors may therefore be of value in NF2, and preclinical *in vitro* studies suggest that EGFR inhibitors affect a cytostatic response: *Nf2*^{-/-} cells proliferate again after drug withdrawal. Merlin may also control surface availability of and signaling from other ErbB family members, and microarray analyses suggest that the phosphatidylinositol 3-kinase (PI3K) pathway is strongly elevated in *Nf2*^{-/-} Schwann cells and schwannomas, which could be due in part to deregulated ErbBs (22). Thus, drugs that target multiple ErbB family members or the PI3K pathway should be considered for NF2. Studies in *Drosophila* suggest that Merlin can also control the surface availability of other membrane receptors, including Notch, and link Merlin to signaling via the Hpo/Wts/Yki pathway (23, 24). Much work still needs to be done to fully define the molecular function of Merlin and to fully unravel candidate drug targets.

What Is the Status of NF2 Preclinical Screening Tools?

As candidate NF2 drug targets emerge, good predictive preclinical models are vital for assessment of new drug therapies. Preclinical drug screening is a particularly critical step in NF2 where there is a limited patient population. Human schwannomas are challenging to grow *in vitro*, although Schwann cell tumor lines are now available (25). Cell line xenografts into mice allow drug testing on the engrafted tumors (26).

Overall, there are three tiers of NF2 preclinical drug screening: (a) cell lines derived from human NF2 tumors or tumors of NF2 GEM, (b) human or mouse NF2 tumor xenografts in immunodeficient mice, and (c) GEM NF2 tumor models. GEM mouse models ideal for drug screening are characterized by (a) high penetrance of tumor development, (b) early initiation and time to progression, and (c) ability to be bred in large numbers. Developing NF2 GEM mouse models has been challenging, but advances in conditional somatic mutagenesis have led to the development of mice with tumors representative of NF2 (27, 28). However, these mice are resource intensive with long latency and low penetrance of tumor lesions. Consequently, tiered screening is vital with GEMs reserved for screening the most promising drugs. Recently, implementation of MRI and other technologies for small-animal imaging has eased the

use of GEMs (29, 30). Further, the observation that strain background modifies the latency and penetrance of tumor development in a mouse model of NF2 schwannoma (31), resulting in a high rate and more predictable time frame of tumor development, provides a more tractable GEM for drug testing.

Which Tumor Should Be Our First Focus in NF2 Clinical Trials?

VS and meningiomas could both provide initial targets in NF2 clinical trials. VS seem to be the more consistent tumors; they are also the most pressing concern for most NF2 patients and therefore may be the preferred initial target. In general, meningiomas are less of a concern if growing without edema or seizures. However, if delivered systemically for a VS trial, drugs would also hit meningioma as a secondary target. An interesting population for NF2 clinical trials could be postsurgery meningioma patients with tumor tissue left behind; drug treatment could be tested as an alternate to radiosurgery. Drug might be delivered to meningioma via transarterial embolization delivery of small drug-loaded microspheres.

What Is the Role of Placebo in NF2 Clinical Trials?

Controls will be critical for NF2 VS and meningioma trials, especially if tumor growth is sporadic. Although a placebo control is the ideal, NF2 patients are unenthusiastic about placebo-controlled trials unless they can cross over to drug part way through the trial. The inclusion of placebo will also significantly increase the number of patients required. One option is that NF2 patients can serve as their own control using pretreatment MRI and audiology data if available.

These are specific recommendations about which tumor type to target in NF2 clinical trials:

1. Define primary tumor of consideration as either VS or meningioma (can be measured as separate arms of the same study or in different studies). Meningiomas more likely to be found in advanced- or end-stage patients. Volumetric analysis is a critical part of the trial and will define tumors to be studied. If possible, MRI information should be submitted to Harvard/Massachusetts General Hospital volumetric imaging facility (14). Imaging should be done at least every 6 months (every 3 months is preferred).
2. Spinal tumors should not be used as an entry criteria tumor, but changes can be measured before and after the trial.
3. All cranial tumors should be assessed before and after the trial.
4. Overall, consider all tumors assessable in addition to primary tumor.
5. If available, consider whole-body MRI at entry and exit for assessment of overall tumor burden.
6. Trials should include VS or meningiomas of at least 1 cm in diameter and/or that show a documented growth of ≥ 2 mm in a period of ≤ 2 years on linear assessment.
7. If outcome of trial is anticipated to include shrinkage, it could potentially include VS of any measurable size.
8. UVS patients may play an important role in phase 0 trials to quickly accrue enough patients to gather preliminary data of drug reaching target. However, UVS patients should only be included in an NF2 trial if (a) the trial has stratification (UVS have different biology so you may see differ-

ent results) and (b) the drug used is very safe, as UVS present less of a threat.

9. If sporadic meningiomas are included, they must be NF2 related and be WHO grade <3 .
10. Ideally, both the tumor and the patient should be tested for NF2 mutation verification.
11. We may need to include patients with decreasing (>10 dB) hearing but who have no documented tumor growth.
12. How many patients are already on unproven therapies? Need to steer patients to proper trials.

What Are Critical End Points in Evaluating NF2 Therapeutic Efficacy and Outcome?

Although multiple questions may be investigated within a clinical trial, to maximize statistical power and show efficacy, a single question should serve as the primary end point (32). This is used to determine design and duration of the study and number of participants. Determining the primary end point demands consideration of what can logistically be assessed and what is clinically meaningful to those living with NF2. Clinically meaningful end points may include hearing loss, facial weakness, vision loss, limb weakness, surgical intervention and outcome, and survival. Objective measures are most feasible and reliable to measure. To avoid prolonged trials (and as a result excessive financial and patient resources), the end point should be attainable in a reasonable period of time. Natural history and current clinical practices must be taken into account so that the end point has meaning within the existing clinical landscape.

Overall, the most clinically meaningful and feasible end point is lesion changes on MRI of the brain because (a) NF2 patients routinely undergo extensive neuroimaging and tumor progression is a common criteria for interventions such as surgery, (b) basic MRI technology required is widely available, and (c) MRI assessment of tumor change is objective when volumetric tumor measures and central review are applied. However, it is critical that image acquisition (magnet strength and slice thickness) and imaging protocols (dose and timing of exogenous contrast and imaging sequences) be standardized across participating sites to minimize technical differences that confound image interpretation.

Volumetric tumor measures provide a more accurate assessment of tumor growth than linear measures in both NF1 and NF2 (14, 32–34). Indeed, assessment of VS changes on linear measures may underestimate growth by $>50\%$ based on comparison with volumetric measures (14, 33, 34). Volumetric measures provide an important tool to detect small changes, critical given the slow-growing nature of VS. In summary, there is consensus among NF2 experts that tumor volume MRI changes are a desirable primary end point for NF2 clinical trials.

Is reduction in tumor size required to show efficacy? Or is delay of growth progression sufficient as a result? If tumor reduction is required, trials may be shorter and will require smaller patient numbers. Achieving tumor reduction does present a high bar and could result in discarding drugs that control but do not shrink tumors. In addition, the percentage reduction required to declare a drug efficacious is essentially arbitrary and determined in part by the sensitivity of volumetric measure. In contrast, an end point of tumor stabilization is more sensitive for drug activity but will require more patients and prolonged

observation. A compromise of these two is to assess the percentage change in tumor growth over time based on patients' natural history of tumor growth before starting the trial. This does not require a reduction in tumor size but in tumor growth rate.

Of course, there is no reason that shrinkage could not be observed in NF2, especially with a biologically targeted drug, as these have been seen to result in marked regression or even eradication of other tumors. Therefore, although stabilization of disease would be a move forward, we should not aim too low. Our ultimate goal should be to shrink and eliminate these tumors.

Hearing preservation is a major quality of life (QoL) measure for NF2 patients and indeed may motivate trial recruitment. However, it may be difficult to identify sufficient patients if recruitment is hearing based due to the fact that although some centers do early "hearing preservation" tumor surgery, many centers let hearing drop significantly (e.g., to 30%) before intervention, in case surgery leads to complete hearing loss.

Secondary end points may include functional measures, including audiology (e.g., ≥ 5 dB difference on 4-frequency pure-tone average), $\geq 15\%$ difference in speech discrimination, measures of facial nerve function, and QoL measures.

Finally, because drugs are likely to be given for extended periods of time in NF2 trials, long-term assessment of toxicity and tolerability will be important.

The specific recommendations as to which tumor outcome measures should be used in NF2 clinical trials are as follows:

1. Effect on tumor growth rate. Determine whether the end point is (a) slowing or stabilizing growth of rapidly growing tumors or (b) tumor shrinkage. Reduction in absolute tumor size is preferable.
2. Assess volumetric reduction or absence of growth. Minimum reliable change is on the order of 10% to 20% volume decrease for lesions over 1 cm in diameter (this corresponds to 3-7% linear diameter decrease). Similar imaging technology and sequences should be applied across centers.
3. For phase II studies, tumor shrinkage should be the end point used due to study power concerns.
4. Loss of T2 signal may also serve as an outcome measure.
5. Hearing change may serve as an outcome measure (significant changes would be 4-frequency pure-tone average > 5 dB change or 15% difference in speech discrimination). There is good natural history available on hearing, which is an important measure to the patients' QoL.
6. QoL measures. SF36, self-report measures.
7. Structured validated questionnaire to capture deficits (eye, facial nerve, mobility, etc.).
8. Video assessment of facial nerve function (especially if a surgical/stereotactic radiosurgery (STRS) arm).
9. Duration of drug effect: is efficacy dependent on chronic drug delivery?
10. Record all toxicities.
11. In phase 0 trials: drug concentration, local drug effect on molecular targets, and histologic features.

Which Patient Population Should We Target First in NF2 Clinical Trials?

As NF2 is a rare disorder, clinical trials should aim to include as broad a patient range as possible. Even major NF2 clinics report having insufficient patients to conduct clinical trials, where-

as many NF2 patients are not under the care of an NF2 clinic and may never learn about clinical trial enrollment opportunities. This issue will grow as we move toward a time of multiple NF2 trials ongoing, and toward phase II trials, which require hundreds of patients. Moving forward, a collaborative consortium between clinics is necessary to implement future clinical trials. Patients are typically stratified in clinical trials by age, tumor type, or severity. There are three broad NF2 populations: patients with tumors being conservatively followed for progression; patients who have had tumor treatment but are at risk for recurrence; and patients with recurrent, progressive, or multiple tumors who have no further surgical or radiotherapy options. Different trial options may apply to different patient groups. Each trial will require one tumor type for primary monitoring, and the tumor should have measurable ongoing growth so that drug efficacy—stopping tumor growth or tumor shrinkage—can be monitored. Including only patients with progressive disease (measurable tumor growth over some period of time) may limit several possible recruits and therefore should be avoided.

There is growing interest in early NF2 VS microsurgery when the tumors are small, and there is maximal opportunity for preserving hearing and cranial nerve function. These individuals may represent another population for future NF2 trials (e.g., a trial to assess need for repeat surgery later in life). Patients receiving early surgical intervention could be followed and, when recurrence occurs, be recruited to a drug clinical trial. For example, in minimally affected patients, with preserved hearing and small tumors, hearing-sparing microsurgery could serve as the first-line approach; then, if hearing or speech deteriorates, patient is considered for clinical trial intervention. For patients with larger tumors and preserved hearing, drug treatment may be the first therapeutic option due to higher risk of hearing loss faced with surgical therapy.

NF2 patients with multiple tumors who cannot undergo further surgery offer a compelling study group and one in desperate need of options. Further, these trials are likely to be efficient if response is used as an end point. Here, drug treatment could be offered in adjunct to surgery or if surgery has been exhausted. Alternatively, patients could be enrolled into ongoing trials for other cancers (generally phase I trials) that are biologically applicable to NF2, accessing treatment options otherwise available. This may be a good approach for end-stage patients with few options.

However, one patient population that may prove more challenging is those who have failed radiosurgery and as a result may have become therapy resistant.

Finally, it is wise to keep in mind that NF2 patients may suffer from "burnout": after multiple surgeries and treatments, how can we engage them in a clinical trial? A trial with a somewhat predictable and optimal outcome will be most attractive.

The specific recommendations as to which patients should be enrolled in NF2 clinical trials are as follows:

1. Three broad patient populations:

- a. Treatment naive: zero to one neurosurgical central nervous system procedure. No major complication barring hearing loss or minor mononeuropathy.

- b. Few treatments and/or some NF2 morbidity. Two to three central nervous system surgeries. Ambulant with no more than one major complication.

- c. NF2 severe. Multiple surgeries or severe morbidity

2. Two broad trial types:
 - a. Therapeutic: symptomatic patients for whom the goal would be stabilization or response.
 - b. Preemptive: in patients with measurable presymptomatic disease (perhaps children) for whom the goal would be stabilization.
3. Children ages 3 years and above may be included if the tumor can reliably be imaged and monitored. However, some studies may need to be restricted to adults only, dependent on properties of an experimental agent and age-related drug effects and toxicities.
4. Patients who have been through multiple surgeries or radiation may have to be excluded (some risk for tumors to be malignant; subsequent imaging of and tumor monitoring may be impaired by surgical artifacts). However, if there are sufficient numbers, these patients may be treated as a separate study arm.
5. Include individuals awaiting surgery in phase 0 studies. This may include sporadic UVS with caveat that data should be handled separately (different biology).
6. Before enrollment, patients must be given usual treatment options of surgery/radiosurgery.
7. Ask patients to consent to data collection if they do not want to participate.
8. Patients must have normal organ function (renal, liver function test, and complete blood count).
9. Patients cannot be or become pregnant during trials.
10. Patients must be able to give consent (including parental for children).
11. Use of certain medications such as antiepileptics may present a contraindication for inclusion (dependent on pharmacologic properties of used agent). However, many modern antiepileptics do not influence the hepatic P450 system.
12. Patients with cochlear implants or ABIs will need to have the device magnet removed for trial participation (imaging).

What Are the Models for NF2 Clinical Trials?

Clinical trials cover phases I to IV from safety to postmarket approval studies. Phase I trial end points assess safety and toxicity. Phases II and III assess efficacy and, for brain tumors, may use end points such as tumor response, improvement in symptoms, delay in tumor growth, and improved survival (35). The path to an approved drug is often inefficient, requiring up to 10 years of investigation, and an estimated 90% of new agents fail to secure approval for clinical use (36).

The NF Phase II Clinical Trials Consortium was established in 2005 with funding from the United States Army Congressionally Directed Medical Research Program for Neurofibromatosis. The Consortium consists of nine U.S. centers with international collaborators focused on planning and executing phase II NF clinical trials. Participating centers were selected based on ability to recruit patients and conduct phase II trials and on institutional commitment to the program. The Consortium has a strong infrastructure, including a data coordination center. The initial focus of the Consortium is NF1. The first phase II trial testing rapamycin in plexiform neurofibromas began in 2008,

and a second trial testing lovastatin in NF1-associated learning disabilities follows in 2009. Further trials are in planning.

This program encountered some hurdles during its inception from which we can learn as we plan NF2 trials. Although participating centers collectively can recruit a significant number of patients, the phenotypes of these individuals will dictate what trials can be done. In the future, alternative approaches may be used. Another approach is to piggy back NF patients onto other trials (e.g., patients with malignant peripheral nerve sheath tumor, a rare malignancy in NF1, could enter sarcoma trials). The Consortium is also adding expert collaborators as required.

There is an opportunity for NF2 trials with secured funding to access the NF Consortium infrastructure. This will require the addition of NF2 expert centers to the Consortium (existing Consortium Centers are largely based in pediatric facilities, which see few NF2 patients). The opportunity that phase II NF2 clinical trials may be able to access Consortium resources will be kept in mind as planning proceeds.

Nevertheless, full-scale phase II trials are challenging. These require (a) drug with a strong level of confidence, (b) funding support for costs, and (c) successful patient recruitment. These demands, as illustrated above, are magnified for the NF2 population.

In planning for NF2 phase II clinical trials, physicians must share information on patient numbers and demographics. A nearer-term opportunity for NF2 may be phase 0 clinical trials to test drugs with known safety profiles from trials in other tumors. In a phase 0 trial, a test drug is administered for a brief window of time (hours, days, or, less commonly, weeks) before surgery. Once the tumor is surgically excised, it can be analyzed to see if the drug has reached its target, and for molecular changes. Phase 0 trials offer, in a fairly short time, valuable information about whether a drug is reaching its target, and molecular changes it is inducing, using end points of tissue concentration or *in vivo* biological effect. This information can be used to improve the design of subsequent trials. This approach is particularly attractive when several promising agents must be triaged for trials.

Phase 0 trials can be rapidly conducted and meaningful data can be accrued from fairly small patient populations. This provides initial clinical data for a drug shown as promising in preclinical testing to determine if it should advance to full clinical testing.

Phase 0 trials are used increasingly in oncology and may be well suited to jump starting clinical trials for NF2. The limitation is that these trials do not offer therapeutic benefit to participants. In addition, they may inadvertently indicate that a potentially active drug is inactive if the expected biological effect is not seen.

In summary, the best way forward for NF2 clinical trials seems to be to start with multiple small pilot trials using the most relevant agents available. Patients who do not respond to a drug could move on to a different trial.

What Are the Candidate Drugs and Potential Therapeutic Approaches for NF2?

Multiple agents could be considered for NF2 clinical trials. It is possible that drugs targeted to NF2-induced signal abnormalities could result in significant responses because, in cancer at large, single gene disorders can respond dramatically to the right therapy. Today, we know enough about NF2 biology to plan clinical trials that consider a roster of potential agents.

Perspective

Some of these drugs are currently in clinical testing for other disorders and therefore present near-term opportunities as NF2 therapies. The roster of potential agents for NF2 clinical trials is summarized in Table 1.

Many signaling pathways are potential targets for NF2 therapy. Because EGF family growth factors are critical for the survival of Schwann cells, the ErbB family of receptors represents a compelling target class. Among the therapeutics that merit consideration for NF2 in this group are the pan-ErbB inhibitor lapatinib and the EGFR-selective inhibitors erlotinib and gefitinib, as well as additional compounds in development. Blood-brain barrier penetration may not be a requirement for therapeutic efficacy, so ErbB-targeted antibodies such as trastuzumab, panitumumab, cetuximab, and pertuzumab may be considered.

Downstream of growth factor receptors in the pathway that affects cytoskeletal and proliferative responses is the small GTPase Rac that binds and activates PAK1 kinase. PAK seems to phosphorylate and inactivate Merlin. Therefore, a PAK1 inhibitor could act through two different mechanisms: direct block of the growth factor-dependent tumor cell survival and reactivation of Merlin if functional protein remains expressed in the NF2 tumor. No PAK1 inhibitors are currently available for clinical testing, but this approach merits consideration. Of interest, Bio30, a natural therapy containing propolis isolated from bee venom, has been proposed as a PAK1 inhibitor and is available.

Additional candidate drug targets include PI3K/Akt, Raf/mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) kinase (MEK)/ERK, and mammalian target of rapamycin (mTOR). NF2-derived tumors have been shown

to elevate phospho-Akt, and Akt-dependent phosphorylation of Merlin has been shown to target this protein for ubiquitin-dependent degradation. Although no PI3K or Akt inhibitors are yet Food and Drug Administration approved, multiple compounds are in development, primarily for oncology indications. The Raf/MEK/ERK pathway has been implicated in NF2 in part due to elevated levels of phospho-ERK and phospho-MEK in tumors. One potential role for this pathway derives from a study showing that Merlin can disrupt a pro-proliferative complex involving assembly of Raf-1/B-Raf heterodimers on the MLK3 kinase; this complex forms independently of MLK3 kinase activity. This offers rationale for testing Raf inhibitors such as sorafenib, or MEK inhibitors, although the literature suggests that Raf inhibition is complicated by a robust feedback loop normalizing flux through the pathway in response to inhibitors.

A further therapeutic approach targets multiple pathways simultaneously. Hsp90 stabilizes a group of client proteins, including ErbB, Raf, and Akt, so a single inhibitor could affect many pathways dysregulated in NF2. Multiple Hsp90 inhibitors are in clinical development. Hsp90 inhibitor 17-allylaminomethyl-17-demethoxygeldanamycin (17AAG) is entering phase III trial for cancer, the first of this class to go to the clinic. However, 17AAG has some liver toxicity. A further compound that inhibits multiple NF2 targets is the curry-derived natural product curcumin, which seems to block many of the same targets as Hsp90 inhibitors as well as additional targets such as focal adhesion kinase.

An innovative new therapeutic overcomes translational blocks due to nonsense mutations. This compound, PTC-124, allows

Table 1. Candidate drugs for NF2

Target	Candidate drugs	Comments
EGFR/Erb2/Erb3	Iressa (gefitinib) Tarceva (erlotinib) Tykerb (lapatinib) Antibodies (trastuzumab, panitumumab, cetuximab, pertuzumab)	Currently in meningioma trials Reported compassionate use Pan-EGFR inhibitor
PI3K/Akt/mTOR	Triciribine Perifosine Rapamycin Torisel (temsirolimus, CCI-779) Certican (everolimus, RAD-001) BEZ235 XL147 XL765 XL418	Akt inhibitor Active in preclinical model, combination with EGFR inhibitor mTOR inhibitor mTOR inhibitor Targets PI3K Targets PI3K Targets PI3K and mTOR Targets Akt and S6K
PAK	Propolis NexGenix candidate	Clinical formulation available
Hsp90	17AAG NVP-AUY-922 (VER-52296) Curcumin	Inhibits multiple other targets
Angiogenesis	Sutent (sunitinib) Nexavar (sorafenib) Avastin (bevacizumab) Zactima (vandetanib, ZD6474) XL647	Multiple kinase targets Multiple kinase targets VEGF antibody Multiple kinase targets Multiple kinase targets
Others	PF-562,271 Valproic acid PD-325901, ARRY-142886 PTC-124	Targets focal adhesion kinase Targets histone deacetylase Target MEK Targets nonsense mutations

selective read through of nonsense mutations, frequently found in NF2 patients. This drug is currently in phase II non-oncology clinical trials but may offer a candidate NF2 treatment.

Finally, NF2 tumor development could also be affected by blocking tumor blood supply using angiogenesis inhibitors. Food and Drug Administration–approved angiogenesis inhibitors include the vascular endothelial growth factor (VEGF)–targeted antibody Avastin and the VEGF receptor–targeted small molecules sunitinib and sorafenib.

In summary, the most promising candidates for NF2 trials suggested included Tykerb, Tarceva, Iressa, and Zactima, followed by Hsp90 inhibitors (17AAG). These are likely to be on the front-runner candidate list for near-term phase 0 or even phase II clinical trials. Looking ahead, it will be critical to integrate data emerging from preclinical drug screening into a pipeline of new candidate NF therapeutics for clinical trials. The Children's Tumor Foundation²⁰ endeavors to facilitate this via a bench-to-bedside roster of programs, including Drug Discov-

ery Initiative Awards (pilot funding for NF preclinical drug screening) and a \$5M NF Preclinical Consortium and pilot Clinical Trial Award program, as well as by serving as a conduit for communication within the community of NF physicians and researchers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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²⁰ <http://www.ctf.org>

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Correction: Consensus Recommendations to Accelerate Clinical Trials for Neurofibromatosis Type 2

In this article (Clin Can Res 2009;15:5032–9), which published in the August 15, 2009 issue of *Clinical Cancer Research* (1), the name of an author, Dusica Babovic-Vuksanovic, was spelled incorrectly.

Reference

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