# TheNetworkEdge

The NF Network presents A quarterly research review
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## The Network Edge: Volume 6 – September, 2014

***The Network Edge*** **brings you quarterly updates on the latest neurofibromatosis (NF) research and clinical trial advances from recent scientific publications. The newsletter is organized into "bite-sized" pieces by specific subtopic, so you can focus in on the information that interests you most.**

### *The Network Edge* Also Features…

- **The Bottom Line**: Each section starts with a **summary sentence** highlighting the "take home" points from that section.

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### Highlights from Volume 6 of *The Network Edge*:

- **Understanding aneurysms and stenosis in NF1**
- **Exploring Methylphenidate (Ritalin) for treatment of attention deficits in NF1**
- **Real-life impact of NF1 learning disabilities on individuals and families**
- **Interpreting MRI hyperintensities in the NF1 and the NF2 brain**
- **New candidate drug targets and treatment approaches for NF1 and NF2**
- **Surgical decision making in NF2 clinical management**
- **Updates on clinical trial and drug treatment reports for NF1 and NF2**
- **A new schwannomatosis gene is identified**
- **Legius Syndrome updates; possible genetic links to leukemia**

## The Network Edge: Volume 6 - Contents

# 1. NF1 Clinical Management

## *a. NF1 and the Eye: Optic Pathway Gliomas and Other Features*

**The Bottom Line:** A new approach for monitoring vision changes in very young children with NF1 is discussed.

Children with NF1 have around a 20% risk of developing an optic pathway glioma, the most common type of central nervous system tumor in NF1. These tumors can reduce vision and may cause blindness. To avoid unnecessary drug treatments, optic pathway gliomas are monitored and treated only when and if they interfere with vision. Monitoring this can be difficult, however, because the techniques used to assess vision changes require cooperation, which is challenging when dealing with very young children.

The last *Network Edge* (Volume 5) included an update on some of the techniques used to monitor optic pathway glioma changes including ophthalmic exam/visual acuity measurement: magnetic resonance imaging (MRI) and visual evoked potential (VEP). As discussed in the last *Network Edge,* these techniques all have benefits and disadvantages, and they may be most helpful when used together to complement each other.

**Avery, Hwang, Ishikawa *et al.*** [NIH] *(United States)* now report on another approach for monitoring vision changes in children – called handheld optical coherence tomography (HH-OCT), which measures changes in the thickness of the nerve fiber layer in the retina at the back of the eye. This same measurement is used in adults to identify visual field loss in glaucoma. This study investigated HH-OCT use for vision assessment in thirty-three children of average age just under five with optic pathway gliomas, either from the general population or with NF1. In children who had vision loss, the nerve fiber layer of the retina was found to be thinner than in children with unaffected vision.

The authors note that HH-OCT is a useful measure for assessing very young children with NF1-related optic pathway gliomas, although they point out that children do have to be sedated for this. They suggest this technique may be used when appropriate in conjunction with other methods, including a standard ophthalmologic exam, to inform clinical decisions.

## *b. NF1 Dermal and Plexiform Neurofibromas*

**The Bottom Line:** Promising results in removal of dermal neurofibromas using YAG-laser; plexiform tumor growth not influenced by puberty.

***Assessing a New Laser Treatment for Dermal Neurofibromas***

Cutaneous (dermal) neurofibromas are benign skin growths that commonly affect persons with NF1. These tumors can number in the hundreds on the face and body, often affecting an individual's self-confidence. Dermal neurofibromas can be removed by traditional surgery, though this can leave scars, and only a small number of tumors can be removed at any one time.

Alternative techniques for dermal tumor removal have emerged, including carbon dioxide ($CO_2$) laser ablation and electrosurgery; these approaches can treat many growths in a single session. **Kriechbaumer *et al.*** *(Austria)* have developed a third technique, called erbium:yttrium–aluminium–garnet(Er:YAG) laser treatment. The wavelength of the Er:YAG laser is absorbed by water in the tumor,

drying it out, and the laser also clots blood at the tumor site to limit bleeding. The authors directly compared the results of Er:YAG laser treatment to $CO_2$ laser ablation and electrosurgery for the removal of dermal neurofibromas.

Twenty-one persons with NF1 and hundreds of dermal neurofibromas were treated for tumor removal between 2004 and 2011. Only tumors of 1cm diameter or smaller were treated. To allow a direct comparison between the Er:YAG laser and the other two techniques, in a few cases, one full side of a person's body was treated with the Er:YAG laser, and the other side was treated with one of the other two techniques.

Overall, the Er:YAG laser yielded a better visible result with reduced scarring, faster healing and a lower level of pain reported. In the follow-up period after treatment, skin areas where tumors had been removed by $CO_2$ laser ablation or electrosurgery were red, and over time these areas lost skin pigment color. In contrast, Er:YAG treated areas regained skin pigment color after treatment and looked more like normal skin. Individuals were followed for up to five years after treatment, and most showed long-lasting effects.

The authors emphasize that this is a small study and a technique still in experimental stages. In personal communication with author Dr. Lukas Kriechbaumer, he reported that he is aware of only two other small reported studies using this laser technique; not aware of a clinic in the United States using this approach. However he feels this method may offer a better option for the treatment of NF1 dermal neurofibromas and is keen to advance its use and increase awareness of this technique among fellow physicians.

***Does Puberty Affect Plexiform Neurofibroma Growth?***

It is known that plexiform and dermal neurofibromas can grow in pregnant women with NF1 and that this is due to hormone fluctuations during this time. Similarly, during puberty, dermal neurofibromas have been reported to increase in number. But can puberty also promote plexiform neurofibroma growth?

**Dagalakis *et al.*** NIH *(United States)* explored this question in a study of fifteen females and twenty-six males with NF1, all experiencing puberty or the period immediately prior to puberty. Both tumor size and hormone levels were monitored for an average of twenty months. Overall, the study found no relation between puberty and plexiform tumor growth. The authors did note that the young people included in this study were all attending a specialist NF clinic at the National Institutes of Health, which sees individuals with difficult-to-manage plexiform neurofibromas. These tumors may therefore be growing more rapidly than plexiforms in the general NF1 population, and this may have affected the results. A follow-up study on a larger population, over a longer period, will resolve this question.

## *c. Heart and Blood Vessel Abnormalities in NF1*

**The Bottom Line:** Updates are relayed regarding identifying the molecular basis of NF1-related aneurysms and arterial stenosis in NF1 - clinical imaging, biology and drug targeting.

A variety of vascular abnormalities can occur in NF1, including aneurysms (where the blood vessel wall weakens, balloons out and can rupture) and stenosis (narrowing of the arteries). Most persons with NF1 are not routinely screened for vascular abnormalities; however, as a result, these often progress silently without detection, and unfortunately they can be fatal. As well as occurring more commonly in NF1, aneurysms can also occur at a much younger age than in the general population. There is a growing body of information about the clinical and biological nature of aneurysms and

stenosis in NF1 and how to detect and manage these conditions. Some recent reports are highlighted below.

***Biology of Aneurysms in NF1***

Persons with NF1 can have an increased number of circulating white blood cells called monocytes. When monocyte level is elevated in the general population, it indicates cardiovascular disease. **Li *et al.*** [NIH] *(United States)* explored what high levels of circulating monocytes means in NF1. The group used mice lacking one copy of the *Nf1* gene in all cells of the body and injected them with the drug angiotensin type II (which can promote aneurysms). These *Nf1* mice were more likely to develop aneurysms than were normal mice.

Next, the group developed a mouse where one copy of the *Nf1* gene was disrupted only in myeloid cells in the blood; myeloid cells give rise to monocytes. These mice developed aneurysms when injected with angiotensin type II, just as the original *Nf1* mice had. This narrowed things down, suggesting that the myeloid cells are the cause of the aneurysm. When the second *Nf1* mouse was treated in advance of the angiotensin injection with one week of Simvastatin (a cholesterol-lowering drug, similar to Lovastatin) or one week of the antioxidant apocynin, the extent of aneurysm was significantly reduced, which suggests that these drugs had "protected" the mouse from aneurysm.

These very interesting findings focus on a possible role for monocytes in causing aneurysm in NF1 and highlight potential drug strategies for protecting persons with NF1 from aneurysm.

***Stenosis in NF1 - Biology, Drug Targeting and Clinical Imaging***

The same team as above also looked at the biology of stenosis in NF1, again using genetically engineered mouse models to do so. **Stansfield *et al.*** [NIH] *(United States)* again used the mice lacking one copy of the *Nf1* gene specifically in the myeloid cells (the second mouse from the study reported above). Myeloid cells include monocytes and macrophages, both of which are found in inflamed or injured blood vessels. These mice were treated surgically to mimic the events of stenosis. Some of the mice were given the drug PD0325901, which inhibits the cell signal Mek, before and/or after surgery. Drug treatment ahead of surgery reduced the extent of some of stenosis-related events, such as blood vessel wall thickening and accumulation of macrophages and monocytes in the affected blood vessel. The drug had some protective effects even when it was given not before surgery but as late as one week after surgery. Interestingly, the authors examined *Nf1* mice that had not received the stenosis surgery and found that their blood vessels were naturally more "inflamed" than blood vessels in normal mice. This inflammation was also reduced when the mice were given PD0325901. These findings suggest that there are stenosis-associated changes present when some of the genetic changes of NF1 are present, but that there is a possibility that affected individuals may be "protected" from later progression of stenosis. It is anticipated the authors will explore these exciting biological mouse-based findings further in the clinic.

Shifting focus to the examination of stenosis in the clinic, **D'Arco *et al.*** *(Italy)* assessed the use of clinical imaging of brain arterial stenosis in eighty-one persons with NF1. Brains were examined using MRI as well as magnetic resonance angiography (MRA). MRA visualizes blood vessels using a modified form of MRI; unlike other angiography techniques, MRA is non-invasive and does not require catheter placement in a blood vessel. The study found fourteen areas of stenosis in six of the persons examined – around eight percent of the group. Only seven areas of stenosis were visualized by MRI; the others were only seen when MRA was used. One region of the brain's blood supply, called the Circle of Willis, was difficult to image using only MRI, but by using MRA, was, at least in some cases, found to be the site of stenosis. From these findings the authors suggest that MRA be used more commonly to examine NF1 brain vasculature to be sure of identifying problem areas.

## d. NF1 Malignant Peripheral Nerve Sheath Tumors

**The Bottom Line:** Updates on clinical imaging of MPNST are relayed.

Though they are a rare tumor type in NF1, malignant peripheral nerve sheath tumors (MPNSTs) are a concern because they can develop from any benign plexiform tumor. They are fast-growing cancers, and can be difficult to treat, because as they grow they can adapt biologically and become resistant to treatment. As a result, developing methods for early detection of these tumors, as well as finding effective drugs to treat them, are NF1 research priorities.

***Assessing Potential MPNSTs via PET Imaging***

A widely used imaging technique for studying fast growing cancers is to inject the patient with radioactively-tagged sugar, then visualize the sugar usage via positron emission tomography (PET) scanning. Because fast-growing tumors use up more sugar than healthy tissue, MPNSTs "light up" more on PET scans. Most clinical centers assess tumor growth by taking a PET reading called "standardized uptake value." However, this is not a growth scale as such, which makes it difficult to compare these PET readings between centers.

With this in mind, **Combemale *et al.*** FREE *(France)* set out to identify a new method for measuring results from PET scans. They looked back at 113 persons with NF1 who had undergone PET imaging at their respective centers over a period of twelve years. Forty MPNSTs had been detected in this group. On the PET scan, the reading level of sugar uptake in the tumor was divided by the reading level of sugar uptake into the liver. When this figure was 1.5 or greater, the tumor was deemed to be a "suspected malignancy." The authors suggest this "ratio" approach could be used by other clinical centers so that data can be more readily compared between clinical centers. This technique can easily be used to analyze archival stored records as well as newly captured images.

## e. Other Clinical Features of NF1

**The Bottom Line:** NF1 gastrointestinal stromal tumors reports highlight unique monitoring and treatment needs for these tumors.

***Update on NF1-Related Gastrointestinal Stromal Tumors (GISTs)***

Gastrointestinal stromal tumors (GIST) affect around one-third of persons with NF1, and they also occur in the general population. The last *Network Edge* (Volume 5) highlighted the fact that NF1 GISTs have unique molecular features and therefore require different treatment strategies from GISTs in the general population. **Salvi *et al.*** FREE *(Italy)* review some past publications in this area that include reports of 126 persons with NF1 and GISTs. What they find concurs with what was reported in the last *Network Edge* (Volume 5): NF1-related GISTs do have different characteristics from GISTs in the general population, occurring as they do earlier in life, and often appearing as multiple small benign tumors. This last feature is an important difference, because in the general population multiple small GIST tumors are a sign of malignancy, whereas in NF1 they may exist as multiple benign tumors. However, twenty percent of NF1-related GISTs may become cancerous, so it is very important that these benign tumors be carefully monitored and appropriately managed.

Monitoring for GISTs in NF1 is, however, challenging. In over half of the cases included in this review, NF1-related GISTs had no symptoms but had been discovered during a clinical examination for other purposes. NF1 GISTs also lack mutations in the genes *KIT* and *PDGFRα*, which are normally GIST

markers in the non-NF1 population. Since NF1-related GISTs lack these gene mutations, they are not responsive to Imatinib (Gleevec), a drug that is quite effective for GISTs in the general population.

This review emphasizes the importance of monitoring for GISTs in NF1 and the need for the development of appropriate treatment strategies.

## 2. NF1 Learning & Development

### *a. NF1 Learning Disabilities*

**The Bottom Line:** Doctors seek early signs of learning disabilities in young children with NF1; a link between physical and learning challenges in NF1 is identified; visual processing in the NF1 brain and the impact of distractions is reviewed; a link between T2 hyperintensities in the NF1 brain and learning disabilities is discovered; Methylphenidate is explored as a treatment for NF1-related attention deficits; 'spiny' nerve cell changes may contribute to NF1 learning disabilities.

It is estimated that at least two-thirds of children with NF1 will develop some form of learning disability, and these can have a lifelong impact. In recent years, this research area has expanded significantly, branching out from the global term of "learning disabilities." The field is now looking at a number of different aspects such as the contributions of altered visual responses and motor function to learning disabilities in children with NF1, links between NF1 learning disabilities and other conditions such as autism and attention deficit disorder, and the impact of these learning differences on real life experiences. Updates on some of these areas are provided below.

***Early Life Indicators of NF1 Learning Disabilities***

The last *Network Edge* (Volume 5) reported a study outlining how signs of learning difficulties can be identified in very young children with NF1. This is an important area of research, because early identification of NF1 learning disabilities could allow for earlier – and potentially more effective - treatment.

Focusing on this issue, **Klein-Tasman, Janke *et al.*** NIH *(United States)* examined forty children with NF1, and thirty-seven children without NF1, aged three to six years. The children were tested with established scales for measuring learning disabilities. Half of the children with NF1 showed some type of learning disability, described as a "mild downward shift" and a "general vulnerability." At least one broad area was affected in these children, which collectively included verbal skills, non-verbal skills and spatial skills. This study also found that communication, memory and expressive language were impaired in some children with NF1. These differences were not seen in the children without NF1. In the children with NF1, these differences could be setting the stage for additional learning disabilities in later life.

The authors note that existing (non-drug) therapy approaches that are used to treat young children in the general population with learning disabilities may also be helpful treatments for at least some of the differences here in young children with NF1. Future studies may look at even younger children with NF1 to see if any differences may be detected even earlier.

***"Real Life" Challenges for Children with NF1***

In a second report from the above group, **Klein-Tasman, Colon *et al.*** NIH, FREE *(United States)* looked at adaptive behavior to see if this is affected in children with NF1. Adaptive behavior means how we apply our learned experiences to modify our behavior in "real life" situations - in other words, "real-

world living." Learned experiences include things as diverse as social skills (making friends) and practical skills (learning how to bathe). This is an area of NF1 research that has not previously been looked at.

Sixty-one children with NF1 aged three to eight were included in this study and compared to fifty-five children without NF1 of a similar age group. A number of tasks and challenges were set for the children, and their parents were interviewed and asked to complete questionnaires about the children. The children with NF1 had overall weaker adaptive skills in areas including community living and independence, language expression and motor function skills. The adaptive skill weaknesses seen in children with NF1 correlated with the children having learning disabilities, though deficits in motor skills did not always correlate with children having learning disabilities. The authors did note that parent reporting may have biased the results of this study, and they plan to expand the research to verify and better understand these initial findings.

***Connecting NF1-Related Motor Skill Challenges and Learning Disabilities***

As discussed in the last *Network Edge* (Volume 5), people with NF1 have been reported to have muscle weakness, and **Champion *et al.*** *(Australia)* explored this area further to see if there is a connection between muscle weakness and learning disabilities in NF1. Their study included thirty-nine young persons with NF1 aged seven to seventeen, all without existing physical challenges (such as having had recent orthopedic surgery). All participants underwent a range of physical and learning assessments including balance, running speed, agility and gait. Participants with NF1 were found to have reduced stride and to walk more slowly and carefully compared to participants without NF1. The participants with NF1 also had a reduced running speed and showed less agility than participants without NF1; this seemed to correlate with the occurrence of learning disabilities. Links were also identified in two other areas: firstly, between stride width and spatial working memory; and secondly, between running speed/agility and the ability to generate strategy.

From these findings, the authors speculated that there are connections between NF1 learning disabilities and the way that the brain develops in its ability to control physical behavior. They also proposed that the brain signal dopamine might be involved in this, since recent findings in a mouse model of NF1 learning disabilities suggested dopamine signaling may be involved. Fluctuations in dopamine could also be involved in the physical changes seen. If this is the case, then these functional challenges might be targeted with drug therapies that regulate dopamine function. This intriguing research study has opened a very interesting avenue that the authors plan to pursue further.

***Visual Responses and Distractions in NF1***

To what extent do limitations in attention contribute to NF1 learning disabilities? **Michael *et al.*** *(France)* examined this question, using a visual test. Twenty persons with NF1 aged seven to thirteen and twenty persons of similar age group without NF1 were included in the study. The participants were first shown a computerized background cartoon picture; then, to this background, two visual objects were added: a "target" (a picture of a white chicken) and a "distractor" (a picture of a black cat). In some tests only the target/chicken was present; in others the distractor/cat appeared at the same time as, or three hundred milliseconds either before or after, the target/chicken. Participants sat through 10 repeats of each of these tests, which were mixed up in random order, and had to press a button to indicate when they saw the target/chicken and whether it had appeared on the left or right hand side of the background picture.

Overall, the children with NF1 showed significantly slower response rates to the test than the children without NF1. This was true under any of the test conditions. All of the children – with or without NF1 – had a faster response time when the distractor appeared first. The children with NF1 had much slower responses when the distractor appeared *after* the target, compared to when *no* distractor appeared; however, this scenario did not slow the responses of children without NF1.

Taken together, these results are very interesting, suggesting that distractions that appear while visual processing is going on (i.e. when the distractor appears after the target has appeared), can alter the brain function of children with NF1. These findings may contribute to refining how we understand NF1 learning disabilities.

***Interpreting MRI Hyperintensities in the NF1 Brain***

T2 hyperintensities are areas of high image intensity that are seen in brain magnetic resonance imaging (MRIs) scans of up to 80% of children with NF1. They appear in multiple brain regions and typically decline with age. T2 hyperintensities have been proposed as being potential indicators of future learning disabilities. To examine this further, **Payne *et al.*** *(Australia)* looked at MRIs from eighteen persons with NF1 and five of their siblings without NF1, taken periodically over 18 years from childhood into adulthood. Over time, the T2 hyperintensities disappeared, and as this happened, the persons showed correlating improvement in learning disabilities. This is the first study to directly link changes in NF1-related T2 hyperintensities and learning disabilities.

To further interpret this finding, consider the heavily debated question of what T2 hyperintensities actually are, as there is no uniform agreement on this. One school of thought is that they represent areas of the brain where nerve fibers have abnormal myelination (nerve insulation) and that this might affect brain function in early life. Presumably as the T2 hyperintensities disappear, myelination becomes normalized, and brain function also improves. This theory of T2 hyperintensities would fit with the results seen in this study. The authors plan a follow-up study with a larger population, and will use broader range of learning ability tests to strengthen these findings.

***Exploring Methylphenidate Intervention to Treat NF1 Learning Disabilities and ADHD***

Up to a half of children with NF1-related learning disabilities will also develop attention deficit disorder (ADHD), with or without hyperactivity. The rate of ADHD in children with NF1 is much higher than in their siblings or parents who don't have NF1. One of the drugs prescribed for children with NF1 and ADHD is Methylphenidate (trade names Ritalin, Concerta and others). **Lidzba *et al.*** *(Germany, United States)* explored the effectiveness of this drug treatment in NF1, by specifically looking at alterations in intelligence test scores in children taking this drug.

The group reviewed data collected for a number of children treated in a single clinic in Hamburg, Germany over a twenty-one year period. This included sixteen children with NF1/without ADHD, fourteen children with NF1/ADHD who received no medication, and thirteen children with NF1/with ADHD who received Methylphenidate. Overall the study found that Methylphenidate, when given on an ongoing basis, significantly improved intelligence test scores in children with NF1 and ADHD, in addition to causing some improvement in attention.

The authors of this research strongly emphasized that this is a very early-stage report and that they are not broadly recommending Methylphenidate for children with NF1. The data reported here was collected over a long period in the clinic and is not a carefully designed or controlled clinical trial; therefore, there are many variables between persons receiving the drug and how their data was recorded at the time. However, it is a promising finding, and the group is planning a larger study to further explore this area.

***Do Changes in Brain Cell 'Spines' Contribute to NF1 Learning Disabilities?***

**Oliveira and Yasuda** [NIH] *(Portugal, United States)* highlight a new role for the *NF1* gene protein neurofibromin that may also add to our understanding of the basis of NF1 learning disabilities. The group looked at the nerve cells from a region of the brain called the hippocampus, which is involved in learning and memory formation and storage. They focused on cells in this region called pyramidal neurons. These cells have lots of "dendritic spines" on their surface, which are protrusions that help the

cells communicate with each other. These spines change shape during learning and memory formation. The group found that in the normal course of brain function, neurofibromin protein controls the cell signal Ras in these cells, and the dendritic spines actively undergo physical changes. This is called "plasticity" and is an important part of information processing in these brain cells. However, when neurofibromin protein is reduced or absent (as it is in NF1), Ras signaling is continually overactive, and the pyramidal neurons lose their plasticity and the spines are less able to change shape. From this finding, the authors propose that the ability of these pyramidal neurons to physically change and adapt may be impaired in brains of persons with NF1 and may contribute to learning disabilities.

## *b. Social Challenges in Neurofibromatosis*

**The Bottom Line:** Mothers of children with NF1 experience increased levels of stress; the article explores the daily life experiences of young persons living with NF1.

Two recent publications explore different aspects of the impact of living with NF1 on the dynamics of both family life and personal relationships.

***"NF1 Moms" and Stress***

A diagnosis that a child has NF1 can bring a lot of stress to family dynamics, but this issue has not been extensively explored. **Esposito *et al.*** FREE *(Italy)* examined a group of thirty-seven children with NF1 - eighteen girls and nineteen boys aged five to eleven - and a comparison group of over 400 children without NF1. The mothers of these children all completed a "parenting stress" survey that asked thirty-six questions relating to three areas: 1) Parental Distress –i.e. the mother's distress about parenting in light of other personal stresses; 2) Parent-Child Dysfunctional Interaction – i.e. the mother's perception that the child is not being responsive to parental expectations; and 3) Difficult Child – i.e. a child's behaviors that make parenting easier or more difficult.

The survey showed that mothers of children with NF1 experienced significantly greater levels of stress than mothers of children without NF1. This increased stress was seen in the areas Parental Distress and Parent-Child Dysfunctional Interaction, but not in the area Difficult Child. This increase in stress was attributed to a variety of factors, including the variability and unpredictability of NF1 and how it will manifest itself, the learning disabilities that may make a child more challenging to care for, and the overall impact of the child's clinical and special needs on family dynamics.

The authors note that "NF1 moms" would benefit from counseling and support and that this should always be considered as a component the NF1 clinical care giving network. The authors propose further studies to look at the experiences of the father as well as the broader family dynamics.

***Experiences of Young Persons Living With NF1***

**Barke *et al.*** *(United Kingdom)* explored the impact of living with NF1 through interviews with nine young people aged fourteen to twenty-four. Discussion topics included the impact of NF1 on their life, the role of friends and family, and experiences of NF1 treatment and support received, with a focus on the impact of NF1 on their appearance.

Three major themes were identified through these interviews: the challenge of living without knowing exactly how your case of NF1 will progress clinically, differences in public perception and even friends' responses when you tell them you have NF1, and stigma from how NF1 is portrayed in the media, such as being misnamed "Elephant Man disease."

The interviewees were overall very positive about the support and information they had received from the Neuro Foundation (United Kingdom neurofibromatosis foundation), and particularly

from attending camps organized by this foundation for people of their own age with NF1. Online NF forums were reported as being very useful. However, some interviewees reported that they had very bad experiences with some doctors they had seen who were not NF specialists and really had little knowledge of NF1 and thus were somewhat insensitive in their responses to these individuals.

This small study offers insight into the experiences and challenges encountered by young persons living with NF1. Overall, the authors were impressed by the resilience of these young people who were living with NF1, succeeding in the world, and not letting NF1 define who they are.

## 3. What's New in NF1 Biology?

**The Bottom Line:** Newly identified naturally occurring compounds could be future NF1 drug therapies; Wnt signaling is highlighted as a drug target in tumor development; challenges and opportunities of targeting the Ras signaling with drugs are discussed; a deeper understanding of the structural and molecular changes that occur in NF1-related bone weakness is relayed; experimental drug kills MPNST cells, but not healthy cells; there is a new mouse model for studying MPNST formation and growth; scientists have a new candidate drug target for MPNST treatment.

***Naturally-Occurring Compounds as Candidate NF1 Drugs***

Around the world, many plants, trees, etc. contain naturally-occurring compounds of medicinal value and have long standing use in local tribal health practices. In recent times, scientists have purified a number of these compounds from their original source then "copied" them in the lab to make synthetic versions. These can then be further optimized in chemical structure to be more potent. This area of research prominently includes searching for anti-tumor agents in the natural world. Now a single team reports progress in two publications detailing their research testing natural products for anti-tumor effects on glioma (brain tumor) cells from mice lacking functional *Nf1* genes as well as lacking function of another gene associated with tumor signaling, called *p53.*

**Devotka *et al.*** *(Paper A)* NIH, FREE *(United States)* examined extract of Simarouba berteroana, which belongs to a family of trees and shrubs that grow in Central and South America. The bark of Simarouba family trees has long been used locally in medicinal tea. Study of the plant extract previously showed the active ingredient to be antiviral, antifungal, antimalarial and cell-killing. In this new study, the group identified eleven compounds of interest, one entirely novel. The majority of these compounds inhibited glioma tumor cell growth. **Devkota *et al.*** *(Paper B)* NIH *(United States)* reported the identification of a series of compounds extracted from Zanthoxylum armatum (a small shrub tree), another important medicine and spice plant, collected in the Himalayas of Nepal. Locally, the tree is known as the "toothache tree" because the bark and seeds of the tree are used to treat pain and other sickness. Extracts made from this tree have previously been shown to halt breast cancer cell growth and human MPNST cell growth. This new study identified four new and six known compounds that were found to inhibit glioma tumor cell growth.

These reports highlight the potential value of natural product derivatives as candidate drug therapies. These compounds can provide a backbone for further study and optimization of these compound structures to see if they can be harnessed as candidate drug therapies. In many cases these compounds are also effective at very low doses, which will be helpful if and when they advance to clinical trials.

***Wnt Signaling Pathway Involvement in NF1 Tumors***

The cell signal Wnt plays a central role in embryonic development, but it is also activated in a number of cancers. However, no role has previously been identified for Wnt in NF1 tumors. **Luscan *et al.*** *(France, United States)* explore this area by examining fifty-seven human NF1 tumors including dermal neurofibromas, plexiform neurofibromas and MPNSTs. They found eighty-nine genes associated with the Wnt signaling pathway to be increased in these tumors, with specific gene alterations associated with the three different tumor types. Furthermore, when the *NF1* gene activity is blocked in normal Schwann cells (the origin cell for NF1 tumors), Wnt signaling is increased, whereas when NF1 gene activity is blocked in epithelial cells, this change is not seen.

These findings highlight a link for Wnt signaling in NF1 tumors and, based on the different gene expression patterns seen, a possible role for Wnt in the progression of tumors from benign to malignant. Indeed, Wnt signaling may be involved in different ways at various stages in the development and progression of NF1 tumors.

Overall, Wnt signaling may represent a new drug target for treating NF1 tumors. One of the roles for Wnt in the developing embryo is in controlling bone development, so it may also emerge in the future that alterations in Wnt signaling is involved in NF1-related bone abnormalities.

***Challenges of Targeting Ras Signaling***

When the NF1 gene does not work, the Ras cell signaling pathway goes into overdrive, and this is a central cause of tumor growth and other clinical features of NF1. In theory, this makes Ras protein a prime candidate NF1 drug target, but researchers have found it challenging to target Ras signaling directly.

**Mattingly** CDMRP, NIH, FREE *(United States)* review current knowledge in this area. One of the challenges is that Ras protein exists in different isoforms (i.e. versions that have slightly different structures) and these can "fill in" for each other when one version is knocked out by a drug. This feature of Ras stymied early NF1 clinical trials for the Ras-targeting farnesyl transferase inhibitors (FTIs, such as the drug Tipifarnib). As a result, more recent NF1 drug therapies have focused on blocking cell signals "downstream" of Ras – i.e. those cellular elements that become overactive as a consequence of Ras behaving abnormally. These include the cell signals MEK, ERK, MAPK, and PI3K/AKT. However, targeting these signals individually has proved insufficient for blocking tumor growth. Ras, therefore, though still elusive, remains a target of interest.

One Ras success story is the drug Lovastatin, currently in clinical trials for the treatment of NF1-related learning disabilities. Lovastatin appears to modulate Ras function, although its exact mechanism of action is still being unraveled.

A more recent approach for targeting Ras has come from drugs that inhibit Ras from integrating properly into the cell membrane (this being a normal part of Ras signaling). The drug Salirasib is such a drug, and it has shown some promise in pancreatic cancer.

***NF1 Bony Abnormalities***

Around a third of persons with NF1 will develop some type of bone abnormality. This may include scoliosis, long-bone dysplasia, bone structural weaknesses, and osteopenia or osteoporosis, which can lead to fractures.

**Kühnisch *et al.*** FREE *(Germany, United States)* used genetically engineered mouse models to study the impact of NF1 on bone development and integrity. One mouse had *Nf1* gene function deleted only in the limbs (Among its features, this mouse has muscle weakness, as discussed in Volume 5 of *The Network Edge.*). The second mouse had *Nf1* gene function deleted only in osteoblasts, which are the cells that create new bone during development and repair.

Studies on these two mice showed that whether *Nf1* gene function is absent from limbs or only from osteoblasts, there is reduced ability to mineralize bone and maintain its structural integrity when faced with mechanical strain. Interestingly, the bone defects seen were more significant at areas of high mechanical load. The group looked at molecular changes in the bone when under mechanical strain and found the cell signal MAPK to be hyperactive. This may contribute to these mice being unable to create and maintain stable bone structure.

Another important finding from these studies was that the mouse model lacking *Nf1* gene function in the limbs had an expanded number of blood vessels in the bone. This seemed to contribute to disorganization of collagen matrix, an important structural element of bone.

As a follow-up to these mouse studies, the group looked at a small number of human bone biopsies taken from people with NF1-related bone dysplasia. They found similar features to those seen in the mice. These mouse models are therefore quite representative of the human condition and will be useful for studying the molecular basis of NF1-related bone abnormalities and how they might be treated.

***New Candidate Drugs for MPNSTs***

**Chau *et al.*** NIH *(United States)* tested a panel of 20,000 synthesized drugs for their ability to halt or reduce growth of either cancer cells or healthy cells grown in a dish. The group identified Compound 21 (Cpd21) as a drug of interest. Cpd21 was then tested on MPNST cells and healthy cells (mouse fibroblasts and Schwann cells) growing in a dish as well as MPNST cells implanted into a mouse where they form a tumor. Both in the dish and in the animal, Cpd21 selectively killed the MPNST cells - by halting the normal cell cycle - while leaving healthy cells intact. Preliminary results also suggested that Cpd21 was even more effective when used together with a second drug, LY294002, which targets the PI3K cell signaling pathway.

These are very early stage studies, but Cpd21 certainly looks promising as a candidate drug treatment to explore further, especially since it kills tumor cells but not healthy cells.

***New Mouse Model for Studying MPNSTs Highlights New Candidate Drug Target***

An important part of studying MPNST growth and effects of drugs are utilizing mouse models of this cancer; these can be used to study tumor biology and test new drugs to stop growth.

**Patel *et al.*** NIH *(United States)* report the development of a new MPNST mouse model. To create this, they took cells from the skin of mice, called skin precursor cells (SKPs), and inactivated the *Nf1* gene as well as another gene, *p53*, in these cells. When these cells are then implanted into the sciatic nerve of a mouse, they form an MPNST. The group looked at genes that became switched on or switched off in the growing tumor cells. The gene *Brd4* was switched on. In the normal cell, *Brd4* controls chromatin (the compacted form of DNA that resides inside the cell's nucleus). When *Brd4* is hyperactivated, as in MPNST cells, they continue to grow. Another cell signal, *Bim*, seems to be involved in this, making *Brd4* and *Bim* of interest as potential candidate drug targets for MPNST treatment.

## 4. NF2 Clinical Management

**The Bottom Line:** Decision making in clinical management is explored, including evaluating the cochlear implant and surgery approaches; an English survey reveals disparities in NF2 radiation therapy services provided by different centers.

***Decision Making in NF2 Clinical Management***

Many factors contribute to decision making for persons considering surgery to remove NF2 vestibular schwannomas. **Lloyd *et al.*** *(United Kingdom)* explore the benefits of one approach: removing a vestibular schwannoma when it is still small (in order to preserve cochlear nerve function) and placing a cochlear implant on the same side either during the same surgery or later (to facilitate hearing). The authors report on six persons with NF2 aged fifteen to thirty-six who, at the same clinical center, underwent this type of procedure.

Four persons had the cochlear implant put in at the same time as tumor removal, while two persons had the cochlear implant put in at a subsequent surgery up to 18 months later. All participants found the cochlear implant useful, even when they still had functional hearing in the un-operated ear, though there were some problems with integrating hearing in the untreated ear with the cochlear implant-facilitated hearing. One of the participants was followed for over seven years and continued to do well with hearing scores.

The authors proposed that the strategy of removing a small vestibular schwannoma and placing a cochlear implant presents an alternative to "watch and wait," where the vestibular tumor is monitored until it reaches a critical stage when it must be removed, but by which time the cochlear nerve may have to be sacrificed due to the complexity of surgery. They also noted that surgery for smaller tumors carries a smaller risk, and using a cochlear implant may give the person with NF2 better hearing/quality of life over a longer period. This new report should add information to the "decision tree" for those diagnosed with NF2.

In this study, the tumors were removed using either the translabyrinthine or retrosigmoid surgical approach, which are two different techniques for vestibular schwannoma removal. Overall outcomes were better for persons undergoing translabyrinthine surgery compared to retrosigmoid surgery. This may be attributed to the fact that the translabyrinthine approach allows the surgeon a full view along the length of the cochlear nerve, which may yield a better outcome for the cochlear nerve.

To see translabrynthine surgery in progress, **Schwartz *et al.*** FREE (VIDEO) *(United States)* have created a teaching video illustrating this technique.

***Radiation Therapy Updates***

Radiation therapy - which includes sterotactic radiosurgery, radiotherapy, gamma knife treatment, etc. - remains controversial in its use in NF2 tumor management. Two recent reports address this.

**Gilkes and Evans** *(United Kingdom)* gathered data from 2000 to the present time from care centers across England that had seen persons with NF2 and treated them with some form of radiation therapy. This was quite a challenge, as in England, there are four NF2 "hubs" -specialty NF2 care centers - as well as twenty care centers with varying degrees of NF2 capabilities. Many persons with NF2 have visited multiple clinics, which further complicated the picture. The study revealed a broad diversity of opinion among the clinical care providers about using radiation services in NF2. None of the four NF2 hubs routinely uses radiation services, but during the study period the other centers treated a total of 90 persons with NF2 with radiation therapy. Two-thirds of treatments were given at a single center. The

number of persons with NF2 receiving radiation therapy in England annually remained level over time, suggesting that those clinics using radiation therapy continue to do so at a consistent rate.

From these findings, the authors highlighted a need for ongoing audits of NF2 services in England and a national database to pool this data and make it accessible to all doctors seeing persons with NF2. Though this would be a challenge, it could be possible under the framework of the National Health Service. Doing this would better track the pattern of decision making and outcomes of giving radiation therapy for NF2 treatment, which is very important, especially as alternatives such as drug treatments become available. This study from England highlights an important issue in NF2 clinical care.

In another report, **Mallory *et al.*** *(United States)* reviewed data from 1990 to 2010 from twenty-six persons with thirty-two NF2-related vestibular schwannomas between them, all of whom underwent stereotactic radiosurgery in one clinic. From the results of this review, the authors suggested that NF2 tumors require higher marginal doses of radiosurgery, as compared to non-NF2 related vestibular schwannomas. However, they noted that radiosurgery treatment yielded poor hearing outcomes and risk of facial neuropathy, which needs to be monitored. The authors propose that to offer hearing augmentation, high dose radiation should be done in conjunction with a cochlear implant.

## 5. What's New in NF2 Biology?

**The Bottom Line:** Imaging findings in the NF2 brain are explained; further insight is given into Merlin's role in nerve fiber myelination and NF2 neuropathy; the effects of human NF2 gene mutations are pinpointed by using a fly model; SIRT2 is a new candidate drug targeted for NF2 tumor therapies; TERT mutations shed light on meningiomas; colom cancer drugs may benefit NF2 patients.

***What Do Hyperintensities in NF2 Brain Imaging Indicate?***

T2 hyperintensities are areas of high image intensity that are visible in magnetic resonance imaging (MRI) scans of the brain. These are found in up to 80% of children with NF1, where they have been proposed to have a link with NF1 learning disabilities. They tend to disappear from the NF1 brain with age. *(For recent updates on T2 hyperintensities in the NF1 brain, see the "NF1 Learning Disabilities" section later in The Network Edge).*

Though T2 hyperintensities have not been a focus of study in NF2, **Vargas *et al.*** FREE *(United States)* investigated this area through a retrospective analysis of thirty-four persons with NF2 who have undergone brain MRIs between 2000 and 2012. T2 hyperintensities were found in twenty-three of the thirty-four persons, and these were greater in number when the person was diagnosed with NF2 at a young age. Looking at the brain structure underlying these T2 hyperintensities, clusters of abnormal cells were frequently identified (these were not tumors, just abnormally located cells).

Other reports have suggested that the NF2 gene protein Merlin helps to organize cells in the developing brain; when Merlin is reduced or absent, as it is in the brains of persons with NF2, the cells may be disorganized, and this could contribute to what is seen here. The authors noted that because they are a specialist NF2 center they see quite aggressive cases of NF2, which may explain the significant occurrence of hyperintensities seen in their population. However, they have certainly opened up an area of study that could shed light on new clinical and biological aspects of NF2.

***More Updates on How Merlin Protein Works***

Nerve biopsies from persons with NF2 contain disorganized nerve fibers; this appears to be due to lack of the NF2 protein Merlin, and this nerve disorganization may be at the root of the neuropathic pain experienced in NF2. These findings were included in *The Network Edge (Vol. 3)* last spring, and now

the same research group has unveiled further insight into this role for Merlin. **Schulz *et al.*** FREE *(Germany)* show that Merlin promotes nerve fiber myelination ("insulation") by signaling from within the neuron (nerve cell), causing the nerve fibers to express a molecule called "neuregulin I type III." This molecule is reduced or absent in human NF2 nerve biopsies. Meanwhile, neuregulin's receptor (called ERBB2) is normally expressed on the Schwann cells that insulate the nerve fibers, but it is at increased levels in the human NF2 nerve biopsies in order to compensate for the lack of neuregulin available. These neuregulin and ERBB2 changes are specific to NF2, and they are not seen in nerves from people with non-NF2-related nerve conditions such as inflammatory neuropathy.

The authors then created genetically engineered mouse models to look at this issue further. When Merlin/neuregulin/ERBB2 signaling is disrupted in mice, the nerve fibers contain *extra* myelination rather than too little. Though initially puzzling, it was suggested this might be due to another signaling pathway "compensating" for the missing neuregulin I type II.

These are extremely important findings continuing on from this group's report last spring, and they help clarify what happens inside nerves when Merlin does not "work," as is the case in NF2. These types of studies can lay the groundwork for the future development of new drug treatments.

*Drosophila* (fruit flies) are very useful models for studying NF genetics because of their genetic parallels with humans. The NF2 gene protein Merlin is 55% identical to the equivalent protein in fruit flies. **Gavilan *et al.*** FREE ***(United States)*** used fruit flies to examine the effects of various NF2 gene mutations on the functioning of Merlin protein. The group identified a number of genetic mutations of interest identified in persons with NF2. The group deleted the flies' own NF2 genes/Merlin protein, and then by genetic engineering introduced various mutated forms of the human NF2 gene back into these flies. Certain forms of the NF2 gene were able to successfully make Merlin protein in the flies, and within the cell, forms of Merlin localized in certain patterns that might be informative as to how this protein functions normally and how it becomes disabled in the cells of persons with NF2. This research highlighted key aspects of NF2 gene function for future follow-up study.

***New Candidate NF2 Drug Targets***

In a search for new candidate NF2 drug therapies, **Petrilli *et al.*** NIH, FREE *(United States)* screened a large library of drugs on mouse Schwann cells with inactivated NF2 gene (therefore presenting a model of NF2 tumor cells). One drug, which targeted the cell signal sirtuin2 (SIRT2), caused the cells to die; however, the drug had no effect on healthy cells. SIRT2 levels were higher in the NF2 tumor model cells than in normal cells, so this likely plays a role in tumor growth. This work provides very early stage evidence of a potentially interesting target pathway, SIRT2, for NF2 tumor treatment.

***'TERT', a New Marker for Meningiomas***

Meningiomas account for a third of all brain tumors and are seen in over half of persons with NF2. Though largely benign, meningiomas can become problematic as they can continue to grow in the limited space of the skull, and they can eventually become malignant.

Looking inside a cancer cell can show physical indications that something is wrong. In the healthy cell nucleus, genes are present like beads along strands of DNA; the DNA is organized into larger structures called chromosomes. The ends of chromosomes have "caps" called telomeres that prevent the chromosome from "fraying." However, these ends do seem to "fray" as people age, and they also "fray" prematurely in cancer. One telomere regulator is called the TERT promoter. **Goutagny *et al.*** *(France)* looked at TERT promoters in meningioma to see if they are altered in any way. The group examined seventy-three persons with meningioma, including some persons with NF2. The TERT promoter was found to have mutations in about a third of cases where the meningiomas were becoming malignant, but there were no mutations in benign tumors. This new finding suggests that

TERT mutations in meningiomas could be a predictor of malignant progression, potentially providing a method for earlier detection.

***NF2 Gene Mutations Common in Colon Cancer***

**Čačev *et al.*** *(Croatia)* explore the potential involvement of *NF2* gene mutations, and therefore lack of functional Merlin protein, in the development of colorectal cancer. The authors were interested in exploring this issue after recent reports suggesting Merlin is a regulator of the Wnt cell signaling pathway. Wnt gene activation already has an established link to colorectal cancer (and as discussed earlier in *The Network Edge,* Wnt may also have a role in promoting growth in NF1 tumors).

The group analyzed tumor samples from one-hundred-and-eighty-five persons who had surgery to remove colon adenocarcinomas. One-hundred-and-thirteen of these samples lacked one functional copy of the *NF2* gene, and twenty-three samples lacked both functional copies of the *NF2* gene. In cases where both copies of the *NF2* gene were non-functional, the tumors tended to be more advanced in cancer stage and to be larger (bigger than five centimeters) than in cases lacking only one functional copy. The more advanced of the tumors contained the lowest amounts of Merlin protein and *NF2* mRNA (an indicator of gene activity and protein production).

These findings support the idea of a role for *NF2* gene mutations in colorectal cancer. These findings should ultimately benefit NF2, since any drugs effective in the colorectal tumors with *NF2* mutations may also be effective in treating NF2 tumors.

## 6. NF Clinical Trials Update

**The Bottom Line:** Anti-angiogenic therapy may be effective in NF1 optic pathway gliomas; there is no clear benefit of bisphosphonates for increasing bone mineral density in adults with NF1; Everolimus doesn't slow NF2 tumor growth, but the related drug Sirolimus shows promise in one person; more positive results of anti-angiogenic therapy for NF2 tumors are revealed; NF2 clinical trials and drug targets are reviewed.

This section includes a number of reports of candidate drugs tested in persons with NF1 and NF2. Although some of these reports are clinical trials, others are either small-scale or retrospective studies. However, since they all report the outcomes of giving drugs to individuals for the treatment of a particular manifestation of NF1, they are included and should add to the growing body of knowledge about NF treatments.

***Is Bevacizumab Effective for the Treatment of NF1 Optic Pathway Glioma?***

Optic pathway gliomas occur in about 20% of children with NF1, and though they are benign tumors, they can ultimately impact vision. Unfortunately, they are often unresponsive to chemotherapy, so there is an urgent need for effective drug treatments.

In a small retrospective study, **Avery, Hwang, Jakacki *et al.*** *(United States)* describe findings from two children with NF1 and optic pathway gliomas. These children had not responded to chemotherapy, and both had progressive vision loss. One of the children with NF1 received Bevacizumab (Avastin) in combination with Irinotecan (a chemotherapy agent), while the other child with NF1 received only Bevacizumab. Both children showed vision improvements, suggesting that Bevacizumab might be a useful treatment for these tumors. The authors plan to follow up on this promising result with a larger clinical study.

***Assessing Bisphosphonate Treatment to Reduce Bone Fracture in NF1***

Bisphosphonates are drugs that are used widely in the general population to treat osteoporosis. Bisphosphonates promote the death of osteoclasts, the cells that "chew up" bone during normal turnover and repair. These drugs are also prescribed for children with NF1 to treat pseudarthrosis where overactive osteoclasts are believed to contribute. Adults with NF1 frequently have reduced bone mineral density, but the benefit of bisphosphonates here is less clear, since experiments have shown that osteoclasts taken from adults with NF1 and grown in a dish appear to be resistant to bisphosphonates.

To look more closely at this, **Heervä *et al.*** *(Finland)* conducted a small study, giving the bisphosphonate drug Alendronate to six persons with NF1, five men and one woman, aged from twenty-eight to seventy-six. Alendronate was given weekly, along with a daily vitamin D supplement, for twenty-three months. After this time, five of the six participants had some increased bone mineral density, but this was not significantly greater than the density seen in a group that received no drug. In addition, one participant developed a new bone stress fracture during the study. Molecular analysis of participants' bone biopsies showed that markers of bone turnover were reduced, suggesting the drugs had been somewhat effective; however, blood levels of TRAP5b, a marker of osteoclast activity, were not reduced.

These are somewhat conflicting results, but, at least so far, there is no clear benefit to utilizing bisphosphonates in adults with NF1 to increase bone mineral density. The authors plan to conduct a larger study to look more closely at this.

***Targeting NF2 Tumors with Sirolimus or Everolimus***

The drug Rapamycin has been evaluated as a tumor treatment in NF1 clinical trials, and its use in NF2 is now being explored. Rapamycin acts by blocking the cell signal mTORC1, which is overactive in many tumor types. Two recent papers report small studies targeting mTORC1 to treat NF2 tumors.

**Giovannini *et al.*** *(France, United States)* assessed Rapamycin on mouse cells, which had no functional *Nf2* gene and therefore are a model of tumor cells. The cells were either grown in a dish or implanted into a mouse, where they form a tumor. Rapamycin was found to halt the growth of these cells both in the dish and as tumors in the animals.

The group also gave Sirolimus (a version of Rapamycin used in the clinic) to one man, aged twenty-nine, with NF2 and fast-growing peripheral schwannomas. The drug was given for "compassionate use" when a tumor in the brachial plexus (shoulder) area was growing but couldn't be removed by surgery due to its location. Over the next four years or so, Sirolimus stabilized the growth of the tumor as well as the man's two vestibular schwannomas. There were no major side effects of the drug. The man was taken off Sirolimus at one point for a few months prior to surgery to remove a meningioma. During this time off of the drug, the schwannomas began to grow again. This was only a one-man study, but it presents positive findings. The authors propose an expanded study to verify the findings.

**Karajannis *et al.*** NIH, FREE *(United States).* They conducted a small Phase II clinical trial of another clinical version of Rapamycin, called Everolimus, for treatment of NF2 related vestibular schwannomas. The trial included eight adults and two children, all with actively growing vestibular schwannomas. Participants were given the drug for up to twelve courses at twenty eight days each. Every three months, their vestibular schwannomas were visualized by magnetic resonance imaging (MRI), tumor volume was measured, and they underwent hearing tests. One participant left the study early by choice, but the remaining nine participants completed the full period of drug treatment. During this time, none showed significant tumor size change, neither growth nor shrinkage.

Karajannis *et al.* do plan a follow up study, called a "Phase Zero" trial, in which they will give Everolimus to people with NF2 immediately prior to vestibular schwannoma surgery. Once the tumors

are removed, they can be studied at the molecular level, to understand the tumor response to Everolimus.

Both of these studies show forms of Rapamycin potentially holding tumor growth at bay. Future NF2 clinical trials may include perhaps using Sirolimus or Everolimus together with another drug to endeavor to also shrink tumors.

***A Small Review of Anti-Angiogenic Drug Treatment of NF2 Tumors***

Anti-tumor drugs that act by inhibiting the formation of blood vessels in tumors – essentially "starving" the tumor of nutrients - are called anti-angiogenic drugs. The most prominent of these drugs is Bevacizumab (Avastin), which has shown great promise for the treatment of NF2 vestibular schwannomas, reducing tumor size and even restoring some hearing ability.

**Hawasli *et al.*** *(United States)* present a retrospective review of a small number of persons with vestibular schwannomas or meningiomas, including six persons with NF2 who all took anti-angiogenic drugs for periods of five to twenty-one months. Five participants with NF2 were given Bevacizumab (Avastin) and one was given a related drug, called Pazopanib (Votrient). The six persons with NF2 all showed tumor stabilization or shrinkage over their treatment period. Hearing remained stable in three of these persons and improved in three persons. Side effects of the drugs included nausea and weight loss. This study, though not a clinical trial, adds to the growing body of knowledge about anti-angiogenic drugs and NF2 tumor management.

***Review of NF2 Drug Targets and Clinical Trials***

Mutations in the *NF2* gene are found in the general population who don't have clinical NF2. **Schroeder *et al.*** FREE *(United States)* review this topic. For example, these mutations are found in liver cancer, mesothelioma and breast cancer tumors. When seen in the general population, *NF2* gene mutations are often called "missense mutations" due to the type of disruption in the gene. In most cases, the *NF2* gene mutation in these tumors will be accompanied by mutations in additional tumor suppressor genes. Information gleaned from studying and treating these general cancers should help advance the development of treatments for NF2 tumors.

This review is worth a read, as it also provides a nice overview of the signaling pathways involved in NF2 tumors, important NF2 studies that have been conducted using cells and mouse models are helping to inform NF2 clinical trials, and the rationale behind a number of past and current NF2 clinical trials with the drugs Bevacizumab, Lapatinib, PTC299, Sunitinib, Nilotinib, Everolimus (RAD-001) and PD-0332991 (CDK inhibitor).

## 7. Schwannomatosis Update

**The Bottom Line:** The *LTZR1* tumor suppressor gene is associated with some cases of the inherited form of schwannomatosis.

Schwannomatosis is the rarest and least-well-understood form of NF, affecting an estimated 1:40,000 persons. The first clinical diagnostic criteria for schwannomatosis were published in 2005, and in 2007, the tumor suppressor gene *SMARCB1* was the first identified candidate gene for schwannomatosis. Subsequent work has suggested an involvemwnt of other genes; however, *SMARCB1* is found in only 50% of inherited cases of schwannomatosis and in 10% of sporadic (first-in-family) cases. More recently it was shown that non-inherited, spontaneous mutations in the *NF2* gene occurring locally in the tumor also seem to be involved in driving schwannomatosis tumor growth.

A search is ongoing for additional genes that are involved in schwannomatosis, and **Piotrowski *et al.*** *(Canada, Poland, United States)* have now reported that another gene, *LTZR1,* is implicated in some inherited cases of schwannomatosis. Like *SMARCB1* and *NF2, LTZR1* is a tumor suppressor gene. It was first identified in the brain tumor glioblastoma multiforme; and, like the *SMARCB1* and *NF2* genes, *LTZR1* is located on Chromosome 22. In a study of human samples, *LTZR1* mutations were found in 80% of inherited cases of schwannomatosis that had disruptions in Chromosome 22 but also lacked a *SMARCB1* mutation.

The identification of *LTZR1* is both significant and exciting, further unraveling the schwannomatosis story. It will be interesting to see what else emerges about how *LTZR1* contributes to the development of schwannomatosis.

## 8. Legius Syndrome Update

**The Bottom Line:** As the number of documented cases of Legius Syndrome grows, and genetic and biology studies expand, a more detailed picture of Legius Syndrome emerges; a link between the SPRED1 gene and leukemia is identified.

Legius Syndrome, or NF1-like syndrome, occurs due to mutations in the SPRED1 gene. It is of great interest to both families living with NF1 and physicians managing persons with NF1, because it shares some common features with NF1. Legius Syndrome is characterized by the presence of café-au-lait spots and learning disabilities, but it does not lead to the development of tumors. Approximately one to four percent of persons who have café-au-lait spots, a hallmark of NF1, will ultimately be diagnosed with Legius Syndrome rather than NF1. Clinicians and families alike are therefore keen to know more about Legius Syndrome, since a diagnosis of Legius Syndrome will require far less aggressive clinical surveillance than does NF1. Definitive diagnosis of NF1 versus Legius Syndrome can be accomplished by a genetic test. Like NF1, Legius Syndrome can be inherited, or can be identified as first-in-family cases.

***Legius Syndrome Update***

**Brems and Legius** FREE *(Belgium)* review the most recent updates on Legius Syndrome research. To date there have been 159 reported clinical cases of Legius Syndrome. In these persons, a number of SPRED1 missense gene mutations have been identified. There have been rare reports of malignancies in individual cases of Legius Syndrome, but as yet there is not enough evidence to suggest that Legius Syndrome is associated with malignancies (rather than these cases just being a general population statistic).

An understanding of the biology of the protein made by the SPRED1 gene is emerging. Like the NF1 protein neurofibromin, Spred-1 protein functions as a tumor suppressor signal, and recent evidence suggests that part of its role in the healthy cell may actually be to interact with neurofibromin and aid in its function. To better understand the underlying biology and cause of Legius Syndrome, genetically engineered mice have been developed that lack either one or both functioning copies of the SPRED1 gene. These mice have learning deficits that are somewhat similar to the learning disabilities seen in mice lacking the NF1 gene. These learning disabilities are worse when both copies of the SPRED1 gene are not functioning (versus one copy remaining functional).

This is a helpful review of the field from the group that first characterized Legius Syndrome. Much remains to be learned, especially as additional clinical cases are documented.

***SPRED1 Shows Links to Leukemia***

**Pasmant *et al.*** *(France)* highlight another role for SPRED1 gene mutations: as a potential cause of childhood leukemias. In the general population, one of the jobs of Spred-1 protein is to regulate blood development by inhibiting growth factors that cause blood cells to multiply too fast. Blood samples from two-hundred-and-thirty children with lymphoblastic or acute myeloblastic leukemia were analyzed for signs of SPRED1 mutations. The majority of samples studied had significantly reduced levels of Spred-1 protein and Spred-1 RNA (low RNA indicates a low level of gene activity/protein production). From this, the authors suggest that SPRED1 may function as a tumor suppressor in some forms of leukemia. Furthermore, they implicate the RAS-MAPK cell signaling pathway in this.

One child from the group of two-hundred-and-thirty examined also had multiple café au lait spots and had received a diagnosis of Legius Syndrome. This child had only one functional copy of the SPRED1 gene in his blood cell lineage and, like the other children in this study, developed a form of leukemia.

From these findings, the authors suggest that leukemia may occur as a rare tumor event in Legius Syndrome. However, as noted above, only a small number of Legius Syndrome cases have as yet been documented; therefore, the question of whether or not leukemia may be a rare complication associated with Legius Syndrome will only be confirmed as the number of documented cases grows.

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