TheNetworkEdge

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

The Network Edge 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 7 of *The Network Edge*:

- Survey of insurance claims shows NF1 may be linked to higher risk of other medical conditions.
- Candidate drugs to improve bone mineralization and treat pseudarthrosis in NF1 are presented; sunshine exposure improves Vitamin D levels in NF1 but still falls short.
- From MPNST biology, a new candidate drug and two new drug targets emerge.
- Impact of NF1 on communication, academic success in early life, and later lifestyle is examined.
- In NF1 clinical trial, drug Sirolimus doesn't shrink plexiform tumors, but it may help with NF1-related learning and behavioral challenges.
- Bevacizumab shows promise in clinical trial for treatment of the rare NF1 high-grade glioma.
- New mouse model of NF2 represents human condition, opens the door to new research.
- Quality of life impact is a key consideration in selecting NF2 treatment regimes.

The Network Edge: Volume 7 – Contents

1. NF1 Clinical Management

a. *Investigating the Likelihood of Diagnosing Other Medical Conditions in People with NF1*

The Bottom Line: People with NF1 may be at greater risk of developing other medical conditions; doctors examine headaches in NF1 and decipher the meaning of brain UBOs; a cautionary note is given on FDG-PET scanning; the benefits of whole body MRI are examined; it may be time to rethink the NIH NF1 diagnostic guidelines.

NF1 can cause a range of well-recognized clinical problems including nerve and brain tumors, bone abnormalities and learning disabilities. But is NF1 associated with an increased risk of other medical conditions? **Madubata *et al.*** [NIH] *(United States)* explored this question by analyzing a large database of private insurance claims made in the United States between 2006 and 2010. The study looked at claims made by 8,579 people with NF1 and 85,790 people without NF1. The findings showed that people with NF1 make around twice as many insurance claims as people without NF1. They also showed that people with NF1 are more likely to have claims related to headaches (this was found to be unrelated to brain tumors); claims related to sleep disturbances, especially in younger patients; and claims related to epilepsy, Parkinson's disease and multiple sclerosis in older patients. Multiple sclerosis and Parkinson's disease are very rare in the general population; the rate was doubled in people with NF1. Interestingly, diabetes was less common in people with NF1.

This seemingly higher risk of developing additional medical conditions could result from underlying biological links with NF1. These findings highlight particularly the increased risk of neurological conditions for people with NF1.

b. *Understanding Headaches in NF1*

Headaches are common in children with NF1, but their cause is not well understood. **Pinho *et al.*** *(Brazil)* surveyed children and young persons aged four to nineteen, fifty with NF1 and fifty without NF1, about experience with headaches and migraine.

In the group without NF1, fourteen percent reported regular headaches ("regular" was defined as at least three times a year and not associated with head trauma), and fourteen percent reported migraine. In the NF1 group, sixty-two percent reported regular headaches and fifty-four reported migraine i.e. at much higher rates than the group without NF1.

This study highlights the frequency of headaches in young persons with NF1. The study team speculated that the cause of migraine may be dysfunction in signaling of dopamine in the brain. Interestingly, alterations in dopamine signaling is also linked to learning disabilities in NF1; therefore, progress in understanding and treating NF1 learning disabilities may also help identify treatments for NF1-associated migraine.

c. *Update on Imaging Technologies*

Unidentified bright objects (UBOs), also known as T2 hyperintensities, are areas seen on scans from brain magnetic resonance imaging (MRI) of children with NF1. Their cause and meaning are not well understood, and they can disappear as the child develops without explanation or effect. There are theories as to what UBOs are, and some of these have been explored in past volumes of *The Network*

Edge; for example, do they represent areas of the brain where the nerve fibers are losing their myelin (insulation)?

Billiet *et al.* FREE *(Belgium, Germany, Italy, Netherlands, United Kingdom)* endeavor to address the meaning of the UBO in NF1. They use a new approach called diffuse magnetic resonance imaging (dMRI) that examines the water content and microstructure of UBOs. Seventeen children with NF1 aged nine to sixteen with a total of 30 UBOs on their scans were examined. The study concluded that the UBOs do not represent areas of nerve demyelination, but they may represent areas where the myelination is being compromised by the occurrence of edema, or fluid build-up, providing a new theory for the origin of UBOs.

Patrick *et al.* FREE *(Belgium)* address one of the great challenges of using imaging in the clinical management of NF1 in an article themed "the more you look, the more you find." Specifically, they look at FDG-PET CT, a type of scanning used to visualize actively growing (therefore potentially malignant) tumors, in one adult with NF1. The "FDG" stands for fluorodeoxyglucose; this is fluorescently tagged sugar that can be injected into someone before scanning. Because tumors are growing faster and have a faster metabolism than the rest of the body, they take up more fluorescent sugar and show up brighter on imaging. However, this study shows that FDG is also taken up by inflammatory granuloma materials, the cause of which turned out to be infectious disease. The authors caution those using FDG-PET CT to carefully assess what has been detected in imaging before determining course of treatment.

The technique of whole body magnetic resonance imaging (MRI) can look at the entire body in one swoop. In NF1, complex formations of tumors can grow in multiple parts of the body, so this technique is tremendously useful for monitoring overall "tumor burden" and recognizing changes in tumor size early on.

Korchi *et al.* *(Switzerland)* report on five years of experience of using whole body MRI in children. The group has used this to examine forty-two children with a range of medical conditions including three boys with NF1. The youngest children imaged were three months of age. Children were sedated if they were between three months and six years of age. The MRI was well tolerated except by one of the boys with NF1 of nine years of age who had cognitive issues and was given sedation before imaging. Overall, the researchers feel whole body MRI is a robust approach to use in the clinic, as it provides invaluable data as a clinical monitoring tool, particularly for tumor growth.

d. Rethinking the Diagnostic Guidelines for NF1

The current National Institutes of Health guidelines for NF1 diagnosis date back over 25 years, to a time before the tremendous progress that has been made in improving the clinical management of NF1, understanding its molecular biology, and developing clinical trial approaches for its treatment. Though independently developed guidelines have since been developed, there have been no updates to the NIH guidelines. With this in mind, **Tadini *et al.*** *(Italy)* raise the critical issue of the need to readdress these guidelines. Of particular interest is the need to develop guidelines that would, given our knowledge today of early indicators of NF1, allow for the diagnosis of NF1 earlier than supported by the current guidelines.

e. NF1 Bony Abnormalities

The Bottom Line: Abnormal bone mineralization occurs in NF1 mouse model, and a new enzyme might restore this; mouse studies use nanoparticles to deliver two drugs in combination to bone and show promise for treatment of NF1 pseudarthrosis; seasonal changes in sunshine lead to fluctuations in Vitamin D levels, but people with NF1 can't fully utilize the bone benefits of sun exposure.

New Biological Findings and Emerging Drug Treatments for NF1

The biology of NF1-related bony abnormalities has been well-studied. It is known that when the NF1 protein, neurofibromin, is not functioning correctly in bone – as is the case in people with NF1 – there are abnormalities in the way new bone is made and in how damaged bone can remodel and repair itself. Since these are normal ongoing processes in the body, this presents a number of problems for people with NF1, including an increased risk of bone breakage.

Ndong, Makowski *et al.* NIH, CDMRP *(Italy, Japan, United States)* examined the underlying biology of these bone differences. They developed mice that are genetically engineered to have NF1 bony abnormalities, including short stature and reduced bone mineralization. In these mice, a substance called pyrophosphate accumulates to unusually high levels in the bone cells. When Ndong *et al.* gave these mice a substance called astophase-alpha enzyme for eight weeks, the mice showed significant improvement in bone health. This enzyme is a synthetic version of a substance called alkaline phosphatase, which is normally present in healthy bone cells. So essentially, the researchers have restored bone metabolism in these NF1 mice, and "normal business" is able to resume in the bone.

This finding has for the first time established the NF1 protein neurofibromin as a key regulator of normal bone mineralization – because when neurofibromin is missing, as in these mice, bone can't mineralize. The processes of bone development are parallel in mice and human, and as a result the researchers suggest these results with astophase-alpha enzyme might open up a new drug pathway to explore for treatment on NF1-related bone abnormalities.

The same group of **Ndong, Stevens *et al.*** NIH *(United States)* used another new NF1 mouse model to further explore bone biology. In this second model, mice were genetically engineered so that they developed normally for a time and did not develop dwarfism (unlike the mouse used in the study described above). Once the bone had a chance to mature, the NF1 gene was "switched off" in osteoblasts - the cells that make new bone - so that these cells could no longer make the NF1 protein neurofibromin. This caused the mice to develop pseudarthrosis, a feature that develops in people with NF1 and renders the bone unable to heal after breakage. This can lead to the need for amputation; therefore, effective drug treatments are badly needed for this NF1 clinical manifestation.

The group explored the use of two test drugs to treat pseudarthrosis in these mice: Trametinib (which inhibits the cell signaling pathway MEK) and inhibitors of the cell signal BMP2. Both signals are known to be overactive in pseudarthrosis. The test drugs "correct" these signals and have previously been explored as drug treatments for NF1 bone abnormalities.

What was different about this study was that it was the first to use Trametinib and BMP2 inhibitor in combination, and it used a novel drug delivery approach: the drugs were contained in slow release nanoparticles and injected directly into the area of bone pseudarthrosis. The mice receiving these injections showed improved bone structure. The two drugs seem to work well in concert. These findings will be pursued further by the group and in the long term could be adapted to human clinical trials.

Vitamin D, Sunshine and Bone Integrity in NF1

The Vitamin D metabolite D3 has a role in maintaining normal bone metabolism, and it is known that exposure to sunlight can increase the body's levels of Vitamin D. **Schnabel *et al.*** *(Germany)* investigated whether there are differences in bone metabolism in people with NF1 versus people without NF1 in winter and summer with seasonal differences in sun exposure. The study looked at children and adults - with or without NF1 - and measured levels of the Vitamin D metabolite 25-Hydroxyvitamin D3 in the blood in winter and summer.

In children there was no significant difference between NF1 and non-NF1 groups in level of 25-Hydroxyvitamin D3, either in winter or in summer. Overall, for both NF1 and non-NF1 groups, levels of D3 in children were much lower than adults. This suggests a difference in metabolism in childhood and adulthood.

NF1 adults had twice as much D3 metabolite in the blood in summer as in winter, while adults without NF1 maintained stable levels of metabolite year round, with just a slight increase in summer. Overall, the levels in the NF1 adults remained lower year round than in the non-NF1 adults.

Based on these findings, the researchers suggest that as people with NF1 age, the metabolism in the bone increases, leading to a higher turnover in Vitamin D metabolites not seen in NF1 during childhood and not seen in adults who don't have NF1. Another confounding factor suggested is that plexiform tumors operate at a higher metabolic rate than normal tissue and they too may be utilizing more Vitamin D. Adults with NF1 benefit from summertime exposure to sunshine, but this is not sufficient to bring their bone metabolism of Vitamin D up to the levels of the NF1 population.

f. NF1 Malignant Peripheral Nerve Sheath Tumors

The Bottom Line: AT101 is introduced as a new candidate drug for MPNST that induces cell death; TGLN, the gene for transgelin, promotes MPNST growth and may represent a new drug target; *Trp53/EGFR* genes interact to promote MPNST growth.

Malignant peripheral nerve sheath tumors (MPNSTs) occur in 10-12% of people with NF1, most commonly when a plexiform tumor becomes malignant. These are tremendously difficult to treat and can be resistant to both chemotherapy and biologically-targeted drugs. Three recent studies explore new ways to treat these challenging tumors, in cells and in genetically engineered mice.

Kaza *et al.* NIH, CDMRP, FREE *(United States)* explore the use of an experimental drug, AT101, for treating MPNST by testing the drug on human MPNST cells grown in a dish. AT101 is a naturally-occurring product derived from cottonseed and is being tested as a cancer drug. It works by inducing cell death: this is done by activating BCL-2, a death-inducing cell signal, and inhibiting a number of functions needed for the cell to stay alive and multiply. MPNSTs are usually very good at eluding drug treatment, but AT101 succeeds in killing the cells. AT101 - and the signaling pathway it targets - merit future exploration as a potential treatment for MPNSTs.

Park *et al.* *(Japan)* examine cells taken from a person with NF1: from their normal tissue, from a plexiform tumor and from an MPNST. The MPNST was found to contain a protein called transgelin at high levels, but this was absent from the plexiform tumor and from the normal tissue. The transgelin gene, TAGLN, was found to be hyperactivated in the MPNST. This happened through a cellular event called hypomethylation, which acts by de-repressing the TAGLN gene and allowing it to be more active. When TAGLN gene activity was blocked in the MPNST cells, their multiplication was slowed; and when

TAGLN activity was promoted, cells multiplied again. TAGLN therefore seems to be involved in MPNST tumor progression in NF1 and may represent a candidate drug target for treatment.

Rahrmann *et al.* [NIH] *(United States)* further examine the molecular cause of MPNSTs in a highly-detailed study that included human gene analysis, cell studies and a new genetically engineered mouse model that has reduced activity of the cancer-related gene *Trp53*. These mice can be induced to develop MPNSTs at a specific point in life that represents adults with NF1 developing these tumors. The researchers found that *Trp53* interacts with another gene associated with tumor growth, *EGFR,* and that together the two promote cancer cell growth. This is the first study to link the *EGFR* and *Trp53* genes together in cancer causation, and it is an important contribution to understanding how MPNSTs develop and therefore may be treated.

2. NF1 Learning & Development: Social Challenges in NF1

The Bottom Line: NF1-related communication and language problems may need special attention; impaired executive function in children with NF1 affects academic success; NF1 impacts reading and mathematical ability; NF1 learning disabilities may have lifelong lifestyle impact and could benefit significantly from intervention early in life.

a. Impact of NF1 on language/communications issues in early life

Some recent publications look at the impact of NF1 on real life – language and communication, academic success, social functioning and lifestyle – and the progression of its impact in children and adults.

In a study funded in part by the NF Network (NF MidAtlantic and NF Midwest), **Brei *et al.*** [NIH] *(United States)* identified difficulties both in communication skills (affecting language) and social functioning problems among thirty children with NF1 aged four to six years. Some of these children also had NF1-related attention difficulties, but these did not necessarily correlate with communication problems.

Are communication problems therefore an independent and direct effect of NF1, separate from attention deficits? This study highlights the importance of directly studying communication/language abilities in NF1, as these may be a root cause of social functioning problems. In particular, communication difficulties may affect a child's interactions from an early age, both with their parents and the world at large, therefore further impeding social development.

b. NF1 effect on academic success in early life, impact on lifestyle in adulthood

It is known that children with NF1 have impaired executive function (the ability to organize information and plan ahead). But does this affect academic success? **Gilboa *et al.*** *(Israel)* say "yes it does" based on their study of twenty-nine children with NF1 and twenty-seven children without NF1, all aged eight to sixteen. The children completed two clinical surveys to measure executive function and a survey to measure academic performance. There were strong correlations between the two i.e. children with NF1 who had reduced executive function also had reduced academic success. The researchers acknowledged that other factors may be affecting the results, such as tumors or other physical manifestations of NF1 that might affect a child's academic performance. The researchers highlighted the importance of providing appropriate supplemental educational support where needed – including in reading, language, and mathematics - to children with NF1.

Orraca-Castillo *et al.* FREE *(Cuba)* tested thirty-two children with NF1 aged seven to fourteen years to assess mathematical and reading ability. In both of these areas, the children with NF1 scored lower than those without NF1. Reduced mathematical ability was less common in NF1 than reduced reading ability; reduced mathematical ability affected less than twenty percent of the children with NF1, and almost eighty percent of those affected were boys. However, reduced reading ability was more common, affecting around fifty percent the children with NF1, and equally affecting girls and boys.

Digging deeper, the researchers found that children with NF1 have specific challenges including difficulties in "decoding words," performing mental arithmetic, and "retrieving" numbers from memory. This interesting study may point to specific areas of focus for children with NF in an educational setting.

Moving to a study of adults, **Granström *et al.*** *(Germany)* had two-hundred-twenty-eight adults with NF1 - some with diagnosed learning disabilities - fill out a questionnaire that asked about their perception of their NF1-related physical disabilities - how severe they felt these were, their living situation/social experiences, and their sensitivity to stress. Participants with learning disabilities had an increased occurrence of depression and attention deficit and hyperactivity disorder (ADHD). Participants with learning disabilities were also less likely to be married/in a partner relationship or to have children, and they were more sensitive to stress. Those with a learning disability also had more difficulty in assessing the severity of their NF1; this is a concern because they may be less likely to recognize changes that would warrant clinical check-up such as a tumor's growth or progression from benign to malignant. These findings highlight some of the real life impacts for adults with NF1 learning disabilities.

Granström *et al.* noted links between their paper and the paper by Orraca-Castillo *et al.* They noted that learning disabilities in childhood can also affect things such as occurrence of bullying, which further impacts academic success, and that these problems in early life can affect later career and lifestyle. They suggest that psychological or psychiatric help in early life could be very important for some people with NF1, to offer some intervention in regard to learning disabilities or other aspects of brain function that may otherwise have long-term detrimental effects.

3. What's New in NF1 Biology?

The Bottom Line: Zebrafish model of NF1 separates the biology of learning and memory deficits, suggesting these issues require separate treatment.

Wolman *et al.* NIH, CDMRP, FREE *(United States)* present an intriguing study in which they examine NF1-related learning disabilities using a genetically engineered zebrafish model. Zebrafish are genetically very similar to humans and mice, and thus they are a popular research model for this reason as well as being easy and inexpensive to breed. They have a simple nervous system and are transparent, which has made them popular for NF1 tumor studies. This study showed that zebrafish genetically engineered to have reduced NF1 gene activity display memory and learning disabilities. When the cell signals MAPK and PI3K are blocked, memory and recall are restored but learning is not. Meanwhile, when the cell signal cAMP is enhanced in these animals, learning is restored but memory is not. This is the first study to suggest that memory and learning in NF1 are regulated by individual signaling pathways and may require different types of treatment.

4. NF1 Clinical Trials Update

The Bottom Line: The drug Sirolimus doesn't shrink plexiform tumors in an NF1 clinical trial, but it may offer benefits for behavioral and learning difficulties; authors reflect on the challenge and importance of getting the Sirolimus dose right; Bevacizumab shows promise for rare but highly malignant NF1 brain tumors; doctors stay focused on finding new drugs for NF2 tumor treatment.

a. Results from the CDMRP Sirolimus Trial Show Unexpected Benefits

Three recent reports provide updates on the CDMRP-funded Neurofibromatosis Clinical Trials Consortium's Phase II clinical trial of the drug Sirolimus (Rapamycin) for the treatment of NF1 plexiform neurofibromas. Trials included children and adults with NF1, from three years of age and older, who all had plexiform tumors that couldn't be surgically removed because of their location in the body.

Weiss *et al.* CDMRP, NIH *(United States)* report on the primary outcome of this clinical trial. At its close, the best result seen from the trials was that tumors had remained stable i.e. they had not grown or shrunk. Since the goal had been to see tumor shrinkage, it was not considered a success in terms of Sirolimus as a plexiform tumor treatment.

However, as it emerged, there were other, unforeseen benefits of Sirolimus for the participants, some who completed a quality of life survey as part of the trial (with young children, parents completed the survey on the child's behalf). The results revealed that the children receiving Sirolimus had improved in "Emotional" and "School" categories – the bottom line being that the children were happier and doing better in the educational setting when taking Sirolimus.

This intriguing result may be due to the fact that the cell signals altered in NF1 tumors are also altered in the brain, where they cause NF1-related behavioral or learning difficulties. Sirolimus may be "correcting" these brain signals. So although the trial results were disappointing in terms of finding a tumor treatment, the drug may have unforeseen benefits for NF1 learning and behavioral differences.

The amount of the drug a person is given is based on various measures including their weight. However, in the above Sirolimus clinical trials, the effectiveness of the drug seemed to vary quite a bit between individuals.

To understand why, **Emoto *et al.*** CDMRP, NIH *(United States)* looked at how Sirolimus is metabolized (broken down and used in the body) by studying blood samples from people aged three to forty-five years, who had participated in the trial and received Sirolimus. It turns out that Sirolimus is processed in the body at different rates in growing children, and this rate may parallel the growth of the liver and gastrointestinal system. Therefore, when determining Sirolimus dose, metabolism should be taken into consideration. This finding should help inform future clinical trials looking at Sirolimus as an NF1 drug candidate.

b. Bevacizumab Shows Promise for Rare NF1 Malignant Gliomas

High grade gliomas are malignant tumors that occur rarely in children and adults with NF1. They are a clinical challenge, because they can be unresponsive to chemotherapy and hard to treat.

Theeler *et al.* CDMRP, NIH *(United States)* explore the possible use of Bevacizumab (Avastin) as a treatment for these tumors. Bevacizumab is already well established as a treatment option for NF2 schwannomas. It acts by targeting the growth of blood vessels, effectively "starving" tumors. Five adults with NF1 and high-grade gliomas were given Bevacizumab. All had longer than expected survival, with two of the five candidates given the drug still alive and free of tumor growth recurrence five years after

treatment began. These are encouraging results and suggest that Bevacizumab and other treatments that aim to reduce blood vessel growth could offer hope as a treatment for these rare but potentially devastating NF1 tumors.

5. NF2 Clinical Management

The Bottom Line: New quality of life survey might help decide on the best treatment option for people with NF2; study examines causes of death in a small NF2 population.

a. Quality of Life in NF2 Could Help Determine Best Treatment Approach

As new NF2 drug therapies emerge, people with NF2 will need to make decisions about which treatment path to follow. For each individual, this may be informed by understanding three measures: a full clinical evaluation to understand the need; an individual's NF2 gene mutations (which might help identify those treatments they will be most responsive to); and a good understanding of the person's quality of life.

Ferner *et al.* FREE *(United Kingdom)* studied the importance of understanding quality of life using a questionnaire survey called "NF2 Impact on Quality of Life" (NFTI-QOL). The survey asked eight questions about the extent and impact of a person's NF2-related symptoms including dizziness, hearing, facial weakness, depression, etc. For each question, participants scored themselves on a scale from zero (feeling best) to three (feeling worst). The survey was completed by two-hundred-eighty-eight men and women with NF2, all sixteen years of age or older (average age forty-two), over a two-year period. Some participants took the survey multiple times. The overall survey "score" was found to be a robust way of measuring the health status of someone with NF2. When people took the survey multiple times, progressive changes in certain measures - such as hearing and balance - could be scored over time.

The outcome of this survey study showed that quality of life score correlated somewhat with the clinical evaluation score, although not with the genetic evaluation score. However, overall the researchers feel that the NFTI-QOL survey offers a broad picture of a person's NF2 status and might be helpful in deciding which treatment approach will be best for a person. Next, the researchers hope to expand the survey for use in people with NF2 as young as twelve years old.

b. Looking at Causes of Death in NF2

Aboukais *et al.* *(France)* surveyed causes of death in seven persons with NF2 who died between 1987 and 2011. These persons had been diagnosed with NF2 at an average age of twenty-six and died at an average age of thirty-nine. Causes of death among the group included one case of a hematoma after removal of a grade IV schwannoma, three cases of aspiration pneumonia after swallowing disturbances, one case of intracranial hypertension due to growing multiple meningiomas, one case of a grade III brachial plexus sarcoma, and one suicide. All of the seven patients who died had spinal tumors, as well as types of NF2 genetic mutation called frameshift or nonsense - features previously associated with poor outcomes in NF2. This small but informative study highlights the importance of understanding the genetic mutation of each person with NF2, and – given the breadth of causes of death - of conducting broad ongoing clinical surveillance for people with NF2.

6. What's New in NF2 Biology?

The Bottom Line: A much-needed new mouse model of NF2 represents the human condition and opens the door for new biological studies; new drug targets for schwannomas and ependymomas are explored; studies suggest genetic changes in NF2 tumors may be age related.

a. A New Mouse Model of NF2

One of the greatest challenges of researching NF2 biology has been the difficulty in creating a genetically engineered mouse model that authentically represented the clinical condition. Now, **Gehlhausen *et al.*** NIH, CDMRP *(United States)* may have successfully done this. The group deleted the NF2 gene in those cells that expressed periostin, a protein quite widely expressed in epithelial cells. Using periostin has previously proved to be effective as a way to alter gene expression in Schwann cells, the cells that make NF2 schwannomas. These mice developed tumors on the eighth cranial nerve and on spinal nerves, as is seen in clinical NF2. Furthermore, the mice have impaired hearing and balance, as do people with advanced NF2. This is a tremendously exciting finding and paves the way for pre-clinical research to better understand how NF2 tumors develop and can be treated.

b. New Candidate Drug Targets for NF2 Tumors

Ammoun *et al.* *(United Kingdom)* report on their study of the role of the tumor suppressor gene *p53* in NF2 tumors. They found *p53* activity to be reduced in human schwannoma cells. In cells lacking functional merlin protein (as is the case in NF2), when merlin is genetically reintroduced to the cell, p53 levels increase again, indicating a restoration of protection from tumor formation. In addition, a new candidate drug called Nutlin-3 was used to treat NF2 schwannoma cells. Nutlin-3 prevents *p53* from interacting with another cell signal MDM2, and together these two appear to be responsible for promoting tumor growth. So Nutlin-3 may have promise as an approach for halting NF2 tumor growth.

Ependymomas are non-malignant spinal tumors that can occur in NF2; when these require treatment they are largely removed by surgery, which might potentially damage the adjacent spinal cord. **Garcia and Gutmann** NIH, FREE *(United States)* examine the biology of ependymomas with a view to understanding these and potentially finding non-surgical approaches to treatment. The researchers find that the cell signal Erb-B2 is important in the growth of these tumors and that this may represent a potential drug target for treatment of ependymomas, for example BY THE Erb-B2 targeted drug Lapatinib, which was previously assessed for the treatment of NF2 related vestibular schwannomas.

Schulz *et al.* FREE *(Germany)* present a thorough and interesting review of the functions of the NF2 gene protein, merlin. Recently this group reported that merlin protein plays a role in the function and communication of healthy neurons (nerve cells) as well as in glial cells (from which NF2 schwannomas arise). This research has opened up new avenues for thinking about NF2 and how to understand and treat it. This detailed FREE review is worth a read.

7. NF2 Clinical Trials Update

a. NF2 Treatments: Moving Beyond Repurposed Cancer Drugs

Significant progress has been made in the area of NF2 clinical trials the past few years. **Lim *et al.*** *(Australia)* present a comprehensive review of the milestones accomplished and the challenges that lie ahead in this area. The majority of drugs that have been tested or considered as NF2 tumor treatments,

including Bevacizumab (Avastin) and Lapatinib (Tykerb), were actually first developed for forms of cancer. These drugs have been repurposed to treat NF2 tumors, which can be a tremendous health burden but are largely non-cancerous. These drugs were intended for short term use in cancer, but NF2 tumors require lifelong treatment to control growth. Because these cancer drugs can have significant side effects, their long term use in NF2 tumor management can outweigh their benefits.

The review by *Lim et al.* emphasizes the importance of continuing to investigate new drug targets for NF2 tumor treatment, rather than just repurposing cancer drugs. It also highlights the value of using cutting-edge clinical trial designs for NF2: for example, in "Phase Zero" trials, a drug is given to someone with NF2 prior to tumor surgery. When the tumor is removed, it can be studied to understand how the drug is acting at the molecular level. Another cutting-edge trial design is the adaptive clinical trial model. These are set up for participants to start out at different drug doses, or with some individuals taking placebo (sugar pills) instead of the drug. Adaptive trials are designed to have very early stage decision points built in so that as soon as a promising result is seen, the effective drug or dose can be made available to all of the participants in the trial. This is very well suited to NF2, where there are currently few options outside of surgical removal of tumors and thus new drug options are badly needed.

8. NF Genetics Update

The Bottom Line: This study examines the NF1 gene mutation landscape; gene expression patterns in NF2 may change with age.

a. Reviewing the Genetic Basis of NF1

Three recent papers review and examine genetic mutation types in NF1. These FREE papers are worth exploring for more details.

Abramowicz and Gos *(Poland)* review the role of genetics as a basis of NF1 manifestations. They discuss the area of genotype-phenotype research, which is the effort to determine whether specific types/genetic locations of NF1 gene mutations might predict the later occurrence of specific NF1 manifestations. About five percent of NF1 cases will be due to a microdeletion, where the whole NF1 gene is missing; these cases have the most severe outcome, whereas people with more localized discrete mutations are likely to have less severe cases.

Xu *et al.* FREE *(United States)* report the identification of fifty-four new mutations of the NF1 gene from a retrospective review of three-hundred-seventy-eight NF1 cases with gene testing results. The researchers comment that the mutation rate seen in the NF1 gene is one of the highest rates for all genes across the genome, which may account for the identification of novel mutations. They identified five cases with gene deletions that were serious NF1 cases. Missense mutations were associated with milder cases. However, both of these papers concluded that much is unknown about linking type of gene mutation to the likely severity of a case of NF1.

Finally, **Yap *et al.*** FREE *(Australia, Singapore)* review the NF1 gene "from bench to bedside." This includes an examination of what is known about tumor types – both common and rare to NF1; biology; and clinical trials in NF1. This extensive FREE review is particularly engaging.

b. Age-Related Genetic Changes in NF2

Toren *et al.* FREE *(United States)* report the identification of age-related patterns in gene expression in NF2 tumors. A panel of 21 genes of interest was assembled and the genes' expression assessed in people of varying ages diagnosed with NF2. Gene expression patterns were found to be different at different ages. Some of the genes expressed early on may represent new indications of the signals that regulate the onset of clinical NF2.

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