TheNetworkEdge
The NF Network presents A quarterly research review
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The Network Edge: February 2013

The Network Edge brings you quarterly updates on the latest neurofibromatosis (NF) research and clinical trial advances from recent scientific publications. The Network Edge is organized into 'bite sized' pieces by NF topic area, so you can focus in on the information that is of most interest for you.

This edition of The Network Edge opens with a few timely words about Congressionally Directed Medical Research Program for NF research (CDMRP NFRP), the largest single funder of NF research in the world and the backbone of NF research progress. This program has supported many critical NF research advances, a number of which have also shed light on the role of the *NF* gene in cancer and other medical conditions.

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1. CDMRP NFRP – A Critical Funder of NF Research

Each year at this time the NF community gears up to plan visits and write letters to Congressional representatives, to remind them about the importance of the Congressionally Directed Medical Research Program for NF research (CDMRP NFRP). Since 1996, this program has committed over $225 million to NF research. It is the largest single funder of NF research in the world.

Many of the advances made with CDMRP NFRP support have been revolutionary. This much needed support would have been difficult to find from other funding sources. Two such examples are the ***Neurofibromatosis Mouse Models Consortium*** and the ***Neurofibromatosis Clinical Trials Consortium***.

In 2000, CDMRP NFRP recognized that the NF research community needed better genetic mouse models of NF tumors. The program funded the ***Neurofibromatosis Mouse Models Consortium***, a collective of research laboratories that collaborated to develop a new collection of mouse NF tumor models. These models have been invaluable, as they have been used both to understand tumor growth, and to test candidate drug treatments for shrinking tumors. Another gap recognized by CDMRP NFRP was the need for an NF clinical trials network that united multiple research centers to collaborate on

clinical trials. In 2005, CDMRP NFRP funded the ***Neurofibromatosis Clinical Trials Consortium.*** Obtaining this funding has significantly resulted in accelerated NF trials. Now multiple sites are recruiting patients for the same trials and now each trial can be filled and completed more rapidly than by any clinic working solo. CDMRP NFRP continues to fund this important program today.

These two NF research milestones (and many others) would not have been possible without CDMRP NFRP and its unique oversight structure, which allows annual strategic decision making to ensure the most important research priority areas are funded.

Please join the NF Network in asking your Congressional representative for their support of CDMRP NFRP. In doing this, it is critical to share the message that CDMRP NFRP benefits not only those with NF but also those affected by a myriad of other conditions. There is an increasing amount of evidence to show common links between NF, cancer and many other diseases. Neurofibromatosis is a clinical 'model' for advancing research in cancer, pain management, nerve regeneration, bone growth and repair, vascular disease, breast cancer and learning disabilities. CDMRP NFRP is therefore a most valuable investment.

2. A Focus on NF1 Malignant Peripheral Nerve Sheath Tumors

The malignant peripheral nerve sheath tumor (MPNST) is a form of cancer that can occurs in around 10-15% of people with NF1. An MPNST develops when a plexiform neurofibroma (a non-cancerous tumor) undergoes 'transformation' or biological changes, and takes on the features of cancer. MPNSTs grow quickly and will migrate (metastasize) to form tumor masses in other parts of the body. MPNSTs are challenging for doctors to treat because they grow so fast, and because they can undergo further 'transformation' as they grow. As a result, even if a drug is initially effective in slowing MPNST growth, the tumor can later become resistant to the same drug.

This edition of The Network Edge presents a special focus on MPNSTs as there have been a number of recent reports describing advances in this area, from clinical management to emerging treatments.

MPNST Biology, Drug targets and Emerging Treatments

Surgery, radiation therapy and drug treatments are all utilized as MPNST treatments and all of these approaches have potential benefits and drawbacks. In terms of surgery, **Dunn *et al.*** examined the medical records of 23 persons with NF1 related MPNST who underwent surgery for these tumors between 1991 and 2008. The study found that gross total resection (or full surgical removal) of the MPNST offers a more significant long term survival benefit than partial resection. This is not surprising given the ability of MPNSTs to grow rapidly, but does not diminish the fact that these can be difficult surgeries.

The drug Rapamycin has been used as an NF1 MPNST clinical therapy, and it targets the cell signal mTOR. A lot of research to find new drug options is focused around the Pi3k/Akt/mTOR signaling pathway. The cell signal Pten is one important regulator of this pathway. **Bradtmöller *et al***. found that Pten activity was reduced in human MPNSTs compared to benign plexiform neurofibromas. This appears to be not because the *PTEN* gene is mutated, but rather because the *PTEN* gene promotor is 'methylated'. Methylation is a natural biological event and a way in which cells can 'quiet down' a gene's

activity, basically by 'wrapping' it up so the gene cannot be expressed. Methylation is prominent in tumors, especially at the early stage of metastasis (when the tumor cells begin to migrate to other parts of the body to form new tumors). This is an exciting study because it shows a new role for *PTEN* in promoting plexiform neurofibromas to transform to MPNSTs. *PTEN,* and its methylation, could be new drug targets for MPNST treatment.

Aurora kinase A (AURKA) is an emerging candidate drug target in cancer at large, and the aurora kinase inhibitor (AKI) MLN8237, has shown promise against MPNST cell lines. In a Canadian study, **Mohan *et al.*** examined the effect of MLN8237 on primary human MPNSTs which had been implanted into mice. The drug seems to stabilize tumor growth and is more effective when the regulatory gene product HMMR/RHAMM is silenced. Aurora kinase inhibitors may therefore be candidate drug treatments for MPNSTs. Further study of the role of HMMR/RHAMM signaling might further advance this area.

The protein Bcl-xL is anti-apoptotic. This means its normal job in the cell is to prevent cell death. **Park *et al.*** reported that the Bcl-xL protein is increased in NF1 MPNSTs compared to NF1 plexiform neurofibromas. This makes sense, since the malignant tumors cells multiply at a greater rate than normal. Levels of Bcl-xL are also higher in Schwann cells grown from human MPNSTs than in normal human Schwann cells. MPNST Schwann cells are resistant to the drug doxorubicin, but this resistance is reversed when Bcl-xL expression is reduced. The high Bcl-xL levels in MPNST cells do not seem to be linked to reduced NF1 gene activity. These are very interesting findings because they put forward a new perspective on how MPNST growth is propagated, how these cancers can become drug resistant, and new potential avenues for drug therapy.

What Makes a Plexiform Neurofibroma into an MPNST?

What drives a plexiform neurofibroma to undergo 'transformation' and become an MPNST? A number of recent studies have examined this at the molecular level. If we can understand this, it will greatly help with the development of new drug strategies.

Keng *et al.* created a new mouse model in which the *PTEN* and *EGFR* genes are modulated to examine the roles of these genes in MPNST development. In mice where PTEN signaling is missing and EGFR signaling is enhanced, high grade MPNSTs develop. **Wu *et al.*** further examined this same question and found that enhanced EGFR signaling in MPNSTs also enhanced activity of another cell signal, STAT3. They found that a STAT3 inhibitor called FLLL32 delayed the growth of MPNSTs in a mouse with an MPNST tissue implant. Further exploration of this pathway is warranted.

MicroRNAs (miRNAs) are molecular elements with a number of functions in the cell, and as **Sedani *et al.*** highlight, possibly a role in MPNST growth. This report puts forward the idea that miRNAs will promote malignant transformation of plexiform neurofibromas to MPNST and presents miRNAs as a potential MPNST drug target. To 'fingerprint' the difference between plexiform neurofibromas and MPNSTs, **Presneau *et al.*** examined miRNAs from NF1 related neurofibromas and MPNSTs. Sixteen miRNAs were found to be significantly differentially expressed in MPNST compared with neurofibromas, fourteen being downregulated in MPNST and two upregulated. Among those downregulated in MPNST was one, miR-29c that targets extracellular matrix genes and matrix metalloprotease, which are involved in cell migration and invasion seen in MPNSTs. miR-29c may have a key role in MPNST progression and therefore represent a candidate drug target.

Together these findings further unravel the molecular cause of plexiform neurofibromas transforming into MPNSTs, and offer some new ideas for drug therapy to treat malignant tumors.

Clinical Management of MPNSTs

MPNSTs occur in the general population as well as in NF1, so findings from this research will have potential benefit for the broader community. However, an unanswered question has been: is an MPNST more likely to have a worse outcome for someone with NF1 than for someone in the general population? This question was examined by **Kolberg *et al.*** who reviewed published reports of MPNST survival rates from more than 1800 patients from the last 50 years. Overall, this research showed that an MPNST will have a better outcome in someone who does not have NF1 than for someone who does have NF1. However, when the study focused in on data from just the past 20 years, there was less of a difference in outcomes between NF1 and non-NF1 cases of MPNST. A further part of this study analyzed survival in 179 persons with MPNST from 3 European sarcoma centers from recent years. By focusing in further on this smaller group who had received expert care, there was little difference in outcomes between NF1 associated MPNSTs and non-NF1 related MPNSTs.

In a similar vein, **Kamran *et al.*** examined medical records of 84 persons with MPNST from 1999 to 2011. Looking at tumor size and tumor features, the study found that NF1-related MPNSTs tended to be diagnosed at a younger age than non-NF1 MPNSTs (age 35 for NF1 versus 45 for non-NF1), but that there was no difference in rate of tumor growth or outcome between the two groups.

These studies suggest that MPNST in the setting of NF1 may be diagnosed earlier and appears to be benefiting from better clinical management than in the past. Therefore, NF1 MPNSTs seem to have a better outlook today than in the past.

The earliest possible clinical detection of an MPNST is critical to ensure that treatment decisions can be made in a timely manner. But what is the best way to image a tumor? F-fluorodeoxyglucose (FDG) PET/CT is one approach used. Since fluorescently-tagged sugar fluorodeoxyglucose is absorbed by the rapidly growing cancer at a greater rate than by normal body tissues, the tumor 'lights up' and may be visualized under PET/CT imaging. This technique has been widely used to image and monitor MPNSTs.

More recently, the approach of whole body MRI has been adopted into NF1 clinical care. This technique can be used to visualize a person's overall "tumor burden" (or all tumors throughout the body) and is particularly helpful in NF1 where tumors can be numerous and of different shapes, making them difficult to measure individually. **Derlin *et al.*** report compared PET/CT to whole body MRI to see which technique was a better option for MPNSTs. They examined 31 patients with both techniques, measuring various tumor parameters including dimensions and volume. The study showed that PET/CT, the traditionally used technique, has a higher sensitivity for identifying MPNST than does whole body MRI.

Salamon *et al.* also examined MPNSTs using (FDG) PET/CT scanning, but looked within the tumors to see if individual tumors absorbed the FDG (fluorescently tagged sugar) in different ways. They examined MPNSTs in 50 persons with NF1 and found that tumors of higher malignancy took up more FDG than tumors of lower malignancy. However, they also found that the more malignant tumors had more variation of FDG uptake within different regions of each tumor. This highlights the fact that the more malignant MPNSTs have molecular differences throughout the tumor, making them unpredictable in terms of how they will respond to treatment.

MPNST Genetics

Studying MPNST genetic mutations can help inform as to what drugs the cancer might respond to. **Thomas *et al.*** examined a range of molecular mutations in MPNSTs and found that 70% of MPNSTs have different mutations in different regions of a single tumor. This 'intra-tumoral heterogeneity' complements the observation with (FDG) PET/CT described above, and again highlights the challenge of treating MPNSTs with the right targeted drugs.

Alkindy *et al.* examined the genetic mutations of 149 people with NF1 and showed that the occurrence of a type of genetic mutation called a 'splice-site' mutation puts that person at greater risk of developing MPNSTs, as well as brain gliomas. This is important information, as around one-third of persons diagnosed with NF1 have splice-site mutations. This could be used to predict the persons more likely to develop MPNSTs and brain tumors, and their clinical management could be modified accordingly.

3. NF2 Clinical Management Updates

A New Biomarker for Predicting Risk of Hearing Loss in NF2?

For most people with NF2, monitoring the growth rate of their vestibular schwannomas is a top priority since tumor size and growth rate will dictate the need for surgery. However, tumor growth does not always correlate with progression of hearing loss. Because hearing loss is often the greatest concern for those with NF2 a new measure of hearing loss would be very helpful. **Asthagiri *et al.*** have identified such a measure, a new biological marker that may actually help predict hearing loss. This substance, called intralabyrinthine perilymphatic protein, accumulates in the inner ear. This substance was found to be present in greater amounts in the ears of persons with NF2 that were at risk of losing hearing, compared to those with NF2 at lower risk of losing hearing. Interestingly, intralabyrinthine perilymphatic protein is thought to accumulate in the inner ear in part because of changes in blood vessels within the ear. With that in mind, the researchers speculate that one of the reasons why the drug Bevacizumab (Avastin) may show promise in restoring hearing in NF2 clinical trials is because it reduces the level of intralabyrinthine perilymphatic protein in the ear, since this drug targets blood vessels.

Monitoring the levels of intralabyrinthine perilymphatic protein in NF2 may be a useful early 'molecular predictor' of the risk of hearing loss, and could therefore help to inform clinical treatment decisions.

NF2 Surgery and the Facial Nerve

When persons with NF2 are preparing for surgery to have a remove a vestibular schwannoma removed (these are the characteristic tumors of NF2 that grow on the vestibular nerves in the skull), they are often concerned that the surgery will damage their facial nerve and affect their ability to smile and show expression. This is because the facial nerve and vestibular nerve are very close together in the skull and the facial nerve may be at risk of damage during surgery. To overcome this risk, **Schmitt *et al.*** looked at using electrical stimulation and monitoring the facial nerve during and after vestibular schwannoma surgery. They report on 267 surgeries (this included NF2-related schwannomas and non-NF2 cases). The study showed that electrical stimulation and monitoring during surgery is a good predictor of short-term, but unfortunately not long-term, facial function. The investigators do think that monitoring facial nerve function has a use during surgery in guiding the amount of tumor tissue that can be safely removed.

Auditory Technology for NF2

The previous Network Edge (Nov. 2012) reported on some published reviews of the use of auditory brainstem implant (ABI) or cochlear implant (CI) technologies as options for hearing restoration following NF2 vestibular schwannoma surgery. In a new publication, the Portugese group **Monteiro *et al.*** report on 4 NF2 patients, one of whom has had a CI for 12 months; and three who have had an ABI for up to 48 months. All four persons had improvements in their hearing threshold after using the implant. While the CI user did not recognize vowels or sentences, the ABI users recognized over two-thirds of vowels, and two of the three had some sentence recognition. This report adds to the growing body of knowledge about the practical use of the ABI and CI technologies in NF2 following vestibular schwannoma surgery.

NF2 Diagnosis and Management in the Older Population

Most people with NF2 will be diagnosed with bilateral vestibular schwannomas in their teens, twenties, or sometimes at even younger ages. There are cases of NF2 diagnosis much later in life; however there has not been much focus on this population. In a French study, Goutagny *et al.* monitor 7 persons who were diagnosed with NF2 at age 70 or older tracking them for up to 8 years. These cases appear to be due to spontaneous mutations, as none of the persons diagnosed had a germline NF2 mutation (which would be detected in the blood). Overall, there was no or little growth of the vestibular schwannomas over the 8-year period monitored. Where meningiomas or peripheral schwannomas were present, these too seemed to be stable. This study shed light on a population not well studied. It suggests that with these older diagnoses of NF2, 'watching and waiting' may be the best approach to management, since the tumors seem to grow quite slowly.

4. NF1 Learning Disabilities: News from the Clinic and the Bench

One area of NF1 research that has made rapid progress in the past few years is in understanding learning disabilities. This is an area of great potential impact, because two thirds of people with NF1 are estimated to have some form of learning disabilities, ranging from mild to severe. Recently, it has emerged that there is a lot of crossover between the features of NF1 learning disabilities and the features of autism, attention deficit and hyperactivity disorder (ADHD), and other learning and behavioral disorders. As a result, clinicians and scientists focused on different behavioral and cognitive disorders are now sharing knowledge and ideas. What is emerging is a complex picture of NF1-related learning disabilities. A number of recent studies have focused on different aspects of these disabilities. They range from clinical analysis of children and adults with NF1 learning disabilities, to research on the underlying biology, searching for drug targets to treat the various learning disabilities.

Clinical Assessment of NF1 Learning Disabilities

Although it is well established that children with NF1 can have developmental delays, the progression of these delays throughout the different stages of childhood has not been studied. **Wessel *et al.*** assessed 124 children with NF1, grouped by ages 0-2, 3-5 and 6-8. The study found that as children age, developmental delay is seen in more areas, including math, reading, gross motor skills, fine motor skills, and self-help development. Long-term information was available for 43 of the children studied. Interestingly, this showed that over time, many children 'flip-flopped' between being delayed

and being non-delayed in most areas. These findings suggest that early screening, ongoing monitoring, and intervention as needed might benefit children with NF1 developmental delays.

Attention deficit hyperactivity disorder has been well studied in children with NF1, but autism spectrum disorder has not been fully evaluated in NF1. **Walsh *et al.*** address this through a retrospective study of 66 children with NF1, using clinical scales to measure autism features. The study found that 40% of the children had behavioral symptoms of 'clinical significance' and 14% had behavioral symptoms that equaled those seen in kids with a diagnosis of autism spectrum disorder. Interestingly, the presence of autism features was not necessarily associated with more severe cases of NF1. There does, however, seem to be a link between attention/hyperactivity deficits and social awareness/social motivation impairments. **Garg *et al.*** also focus on autism spectrum disorder in NF1. The group developed a questionnaire to assess autism spectrum disorder, ADHD and other psychiatric features. The questionnaire was issued to parents and teachers of over 200 children with NF1 aged 4 to 16. The survey results suggested 30% of the kids with NF1 had severe clinical autism, and about 30% had mild to moderate clinical autism. Overall, 25% of the kids surveyed had both clinical autism and clinical ADHD.

Through another survey-based study**, van Eeghen *et al.*** examine the relationship between autism, epilepsy, and cognitive function in persons with either NF1, tuberous sclerosis complex (TSC) or childhood-onset epilepsy. The results showed that autism severity in TSC and childhood-onset epilepsy is linked with intelligence and epilepsy outcomes, but that this is not the case for NF1.

This is a lot of information from a number of studies, but together, they highlight the emergence of information about autism spectrum disorder in NF1. They also link the NF1 gene as a potential cause of autism. This is also exciting given the high diagnosis rate of autism, because this could be of relevance to the general population.

Garwood *et al.* examined 27 adolescents with NF1 to assess their cognitive functioning, pain, functional disability, quality of life measures and social and emotional function. The study showed that quality of life is substantially driven by emotional functioning, whereas functional disability is more often determined by the physical manifestations of NF1. This finding emphasizes the important role of emotional support for those with NF1.

Many studies examining learning disabilities in NF1 yield contradictory results. This may in part be because these studies often compare individuals with NF1 to a random 'normal' group that is not necessarily parallel in IQ to the NF1 group. **Descheemaeker *et al.*** endeavor to address this with a study that included 20 adults with NF1 and a group of IQ-, age- and gender-matched adults without NF1. The NF1 group showed poorer performance in a battery of tests of visual and hearing memory as well as sustained attention testing and executive function (planning), though it was not conclusive if the IQ of the person played a role in the extent of performance deficits. This is an interesting finding in itself but also highlights the importance of using an appropriate matched control group for these studies.

Imaging the NF1 Brain

Imaging of the brain of persons with NF1- related learning disabilities has shown that these individuals have a larger brain volume. It has been suggested this is due to the increased number of cells in these brains. What is not known is if there are also fine changes in the white matter of the brain (the part of the brain where myelinated, or insulated nerve fibers run). ***Karlsgodt et al.*** examined this by imaging and analyzing the brains of 14 young adults with NF1 and 12 without NF1. They revealed widespread reductions in white matter integrity throughout the NF1 brains. They speculated that this is possibly due either to decreased myelination of nerve fibers or increased space between axons

(individual nerve cell projections). The frontal lobe of the brain was found to be particularly affected. This could be directly linked to certain learning deficits in NF1 that rely on the frontal lobe.

The corpus callosum is a bundle of nerve fibers that connects and facilitates information exchange between the left and right hemispheres of the brain. There has been some evidence that corpus callosum abnormalities contribute to NF1 related learning disabilities in adults. **Filippi *et al.*** demonstrate through imaging studies that children with NF1 learning disabilities also have small (microstructural) abnormalities of the corpus callosum. The study of the corpus callosum could provide an early predictor that a child with NF1 will develop learning disabilities.

Emerging Treatments, Biology and Drug Targets for NF1 Learning Disabilities

Paschou *et al.* examined the contribution of microRNA as a regulating factor in NF1 learning disabilities. They studied neurons (nerve cells) in the hippocampus, a region of the brain whose function is frequently altered in persons with NF1. The study found that a form of microRNA called miR-128 has an important role in hippocampal neuron function, either when working alone or in combination with other microRNAs called miR-103 and miR-137. When these microRNAs are increased in expression, neurofibromin levels are decreased (as they are in NF1 learning disabilities); but conversely, when the microRNAs are inhibited, neurofibromin levels increase (which is more reflective of the normal brain). This study points toward regulation of microRNA activity as a possible approach for the treatment of NF1 learning disabilities. Finally, **Diggs-Andrews *et al.*** present data to implicate the role of reduced dopamine signaling as a key factor in the learning deficits seen in NF1.

5. NF1 Clinical Management Updates

Plexiform Neurofibromas

Around half of the people diagnosed with NF1 will develop a plexiform neurofibroma tumor. These grow on nerves anywhere in the body. They are not malignant, although they carry a risk of transforming into malignant peripheral nerve sheath tumors (MPNSTs) which are cancerous. MPNST research is the subject of section 2 of The Network Edge, above.

Even though they usually remain benign, plexiform neurofibromas can cause major health problems. They often grow around internal organs, making surgery to remove the tumors quite tricky. Surgeons will leave a bit of tumor behind rather than risking damage to the organ, and this in turn increases the risk of tumor recurrence. There are no effective drug treatments for plexiform neurofibromas, and they have been a challenge for clinicians.

The good news is that the biology of plexiform neurofibromas is now reasonably well understood, and a few drugs have been selected based on this biology and taken into clinical trials. Among these drugs, Imatinib Mesylate (Gleevec) has emerged as a promising treatment for plexiform neurofibromas. This work was pioneered at Indiana University, from where **Robertson *et al.*** have now reported the latest findings from a Phase 2 trial of Imatinib Mesylate for plexiform neurofibromas. The study was open-label, which means that all participants received Imatinib Mesylate (no-one received placebo/"sugar pill"). Initially, 36 persons were enrolled in the study. All persons had NF, were between the ages of 3-65 with clinically significant plexiform neurofibromas, and had the 'intent to treat'. However, only 23 of these persons remained on drug for 6 months or more. During that time period (6 months), they were given Imatinib Mesylate twice daily. At the end of the study, the tumors of 6

participants (out of the remaining 23) had shrunk 20% or more. Minor side effects were seen in a few trial participants and included skin rash, edema and weight gain. This outcome confirms Imatinib Mesylate as a promising treatment option for NF1 plexiform neurofibromas, and the trial is now being expanded to additional clinics and more patients.

Plexiform neurofibroma tumor growth involves different cell types in the tumor communicating with each other. **Prada *et al.*** reported new evidence which shows that cells called macrophages can promote plexiform growth. The researchers gave a drug, PLX3397, to mice with plexiform tumors, including young mice with newly growing tumors, and older mice with well established tumors. Interestingly, the drug only worked in the older mice. PLX3397 is a drug that specifically targets the kit/fms kinase signal pathway, a cell communication pathway used by macrophages.

These findings are important for two reasons. Firstly, they show that plexiform neurofibromas undergo changes as they grow and age, one of which is that macrophages increase in the tumor. Secondly, they open new avenues for treating established plexiform tumors in adults with NF1. This is important because this represents a large population of people who today have limited treatment options for these tumors.

Cutaneous Neurofibromas

People with NF1 can develop cutaneous (dermal) neurofibromas that grow in the skin and are visible as bumps on the skin surface. They are benign and not usually a major health concern. However, because of their visibility, these tumors can have a significant impact on quality of life. Treatment is difficult and, because of their large number, surgery is not practical except to remove the largest cutaneous tumors. In any case, they often grow back. New treatment strategies for cutaneous neurofibromas are greatly sought after.

Chiang *et al.* report a follow-up survey of 5 persons with NF1 aged 36-56 whose cutaneous neurofibromas were removed using a carbon dioxide (CO_2) laser. Prior to laser treatment, each person had 20-200 cutaneous neurofibromas of different sizes. Persons were laser-treated in 2-4 sessions. The follow up survey was done no more than 2 years after the last treatment. Of those 5 persons surveyed, 3 reported no recurrence of tumors for up to 2 years after treatment, and 2 persons reported recurrence of a few lesions. All said they would recommend the laser treatment. The only side effect the group reported was some loss of skin pigmentation at the treatment site. This approach may be worth exploring for those with these tumors.

Optic Pathway Gliomas

Clinical trials for optic pathway gliomas are taking shape, but one of the key challenges for clinicians is how to measure resulting improvements in vision (visual acuity). This is particularly challenging because optic pathway gliomas are childhood tumors. Many of the children affected will also have a learning disability (since they have NF1), which could affect their ability to take a visual acuity test. **Avery *et al.*** assessed the most widely used visual acuity tests by using them in children under age 10 years with NF1, and with or without an optic pathway glioma. The study showed that these young children were often unable to complete the tests. This emphasizes the importance of having well developed measures for visual acuity for future optic pathway glioma trials and suggests that these tests need to be better developed.

A Link Between Gastrointestinal Tumors and Pheochromocytoma in NF1

Gastrointestinal problems are rarely a primary focus in NF1, but **Agaimy *et al.*** present findings to suggest that these are actually a significant and under-recognized complication of NF1. The study examined published reports on this topic and show that NF1 carries an increased risk of GI conditions ranging from microscopic changes to gastrointestinal stromal tumors (GIST). The majority of GISTs are benign, but can become malignant. Even when benign, they can compromise health. One of the most interesting aspects of this report is that the authors propose that a clinical diagnosis of gastrointestinal issues could actually uncover and identify previously undiagnosed or missed cases of NF1.

A Dutch study from **Vlenterie *et al.*** provides further study of GIST and, in addition, links these to the occurrence of pheochromocytoma, a tumor on the adrenal gland. Pheochromocytoma causes the release of excess hormones from the adrenal gland, and these can consequently increase blood pressure and heart rate. This study examines a small series of persons with NF1 who have co-occurrence of both pheochromocytoma and GIST and found these persons had a greater than expected occurrence of pulmonary embolism. The study highlighted the importance of screening individuals with NF1 for presence of GIST and pheochromocytoma, and of looking for the presence of pheochromocytoma in anyone with NF1 diagnosed with GIST that requires surgery. This is especially important since a pheochromocytoma carries an enhanced risk of cardiovascular problems during surgery.

Radiation Therapy, Secondary Malignancies and NF Genetic Mutations

The use of radiation therapy for NF tumors remains a debated topic. One of the concerns is whether radiation treatment for a tumor in childhood might increase the risk of cancer in adulthood due to residual impact of the radiation treatment. Children with NF1 seem to be more sensitive to developing these 'secondary malignancies' than do those receiving radiation for treatment of non-NF1 related tumors. This is an area that has been difficult to study because of a lack of good study models. To address this, **Choi *et al.*** exposed Nf1+/- mice (these are mice that lack one copy of the *Nf1* gene), as well as normal mice, to abdominal irradiation. All of the mice went on to develop malignancies in the body areas that had received radiation, but malignancies were more extensive in the Nf1+/- mice, and these mice also had shorter survival times overall than the normal mice that received radiation. The group also examined human clinical samples of secondary malignancies, and they found that even in persons without NF1, mutations appeared in the *NF1* gene in their secondary malignancies. This suggests that NF1 actually plays an important role in promoting the growth of radiation-induced secondary malignancies, both in those with NF1 and in the general population. Understanding how the NF1 gene drives malignancies could therefore benefit not just those with NF1 but also the general population.

A New NF1 Patient Registry

Building a patient registry is an important tool for studying any disease or disorder, especially if it accrues lots of information on a large population of individuals affected by the same condition. In **Johnson *et al.,*** the NF research team at the Washington University School of Medicine reports the creation of the NF1 Patient Registry Initiative (NPRI). Any individual with NF1 may participate by completing a short questionnaire detailing their health status. 1,000 persons from across the United States and around the world have already registered with NPRI. This will be a very valuable resource for NF1 research, as it provides an NF1 population that can be invited at any time to participate in research studies. NPRI can be found online at https://nf1registry.wustl.edu/.

6. What's New in NF2 Biology?

Schwannomas are the hallmark tumors that occur in both NF2 and schwannomatosis. In NF2 these tumors occur in the vestibular nerve, where they impair hearing, or they occur along peripheral nerves. In schwannomatosis, schwannomas also grow along peripheral nerves, but they cause unmanageable pain, which is characteristic of schwannomatosis. Schwannomas have traditionally been managed by surgical removal, although this can yield further clinical problems including advancing hearing loss in NF2, and enhancing pain issues in schwannomatosis. More recently, a few drug therapies have entered clinical trials, including Bevacizumab, which has shown some promise.

Prabhakar *et al.* have used mice carrying schwannoma tumors to explore the use of gene therapy as a candidate treatment for schwannomas. The researchers created an adeno-associated virus (AAV) vector containing the enzyme caspase-1. This is a cell death-inducing signal. The virus was designed so that it would only become activated in Schwann cells (the cells from which schwannomas develop) and would not target other cell types. When the virus was injected into the tumor-bearing mice, the tumors shrank and reduced tumor-associated pain that had been seen in the mice.

In a similar vein, in a Spanish study, **Castellanos *et al.*** test the possibility of using antisense gene therapy to treat NF2. The study selected a person with NF2 based on the specific nature of the mutation, a deep intronic mutation, which caused a truncated form of the NF2 protein, merlin, to be produced by the patient's cells. The researchers took fibroblasts from a biopsy of patient tissue, grew these in a dish and were able to restore normal merlin function by treating the cells with a custom designed antisense oligomer that would rectify gene splicing. The study worked, and the fibroblasts became much more 'normal' in behavior. Though only a cell based, single-patient study, this at least explored the possibility of developing customized gene therapies for NF2.

Gene therapy has not been widely used with success in humans, but these reports are certainly interesting and provide food for thought.

The *NF2* gene region Exon 2 is frequently mutated in persons with NF2. Exon 2 codes for PBD1, which is a section of NF2 protein merlin that will bind paxillin, a protein in the cell skeleton. **Manetti *et al.*** show that mutation of PBD1 could be a contributor to NF2 tumor growth. **Ammoun *et al.*** look for a role in NF2 for a set of molecular signals called the TAM family receptor kinases. The TAM family is overexpressed in schwannoma tumors as well as in some cancers, where they correlate with drug resistance and promote tumor behavior such as invasiveness (which is important in metastasis). One member of the TAM family is the Axl receptor. Axl and its ligand (binding partner) Gas6 appear to be heavily expressed in schwannoma cells. Stimulating Axl/Gas6 also activates cell signals Src and NFκB and this pathway appears to help stimulate tumor cell growth and survival. These studies highlight new potential pathways for drug targeting in NF2.

Mutations in the *NF2* gene are the primary cause of NF2 itself, but the *NF2* gene is also something of a 'master regulator' in the cell. A review by **Beltrami *et al.*** highlights this and reviews the mechanism of action of NF2 in linking receptors at the plasma membrane to cytoplasmic kinases and nuclear proteins to regulate cell division.

Finally, as mentioned above, Bevacizumab has shown promise as a therapy for NF2 vestibular schwannomas; this targets cell signal VEGF. In a German study, **Koutsimpelas *et al.*** conducted a tissue analysis of 182 sporadic vestibular schwannomas to examine the expression of VEGF and its receptors, as well as the extent to which these tumors were growing. Tumor recurrence and previous irradiation were also used as reference points. They found that all of the tumors expressed VEGF and its receptors, and that VEGF levels were significantly higher in recurrent tumors and in preoperatively irradiated tumors. These findings confirm the role of VEGF in tumor growth, but also show that this is enhanced in

recurrent and previously irradiated tumors. Though this data has come from sporadic vestibular schwannomas, this may shed some light on selection of tumor therapies in NF2.

7. Heart and Blood Vessel Abnormalities in NF1: New Findings

There is growing evidence that persons diagnosed with NF1 carry an increased risk of developing a range of heart and blood vessel defects. This can be serious and might include arterial occlusion resulting in tissue ischemia and sudden death. When this does occur in NF1, it often occurs in quite young people. The area of heart and blood vessel defects in NF1 is not well understood though, and NF1 clinical care today does not include routine assessment of cardiovascular function. However, in the past few years, knowledge in this area has expanded quite a bit. Some of the most recent findings are summarized below.

Genetics of NF1 Cardiovascular Abnormalities

Two recent studies have linked specific types of NF1 genetic mutations with increased risk of heart defects. In a German study, **Nguyen *et al.*** evaluated 16 individuals with NF1 and large deletions of the NF1 gene, and compared them to 16 age-matched persons with NF1 who did not have large deletions of the NF1 gene. None of the individuals without large NF1 gene deletions had major cardiac abnormalities, although they did have thickening of the heart walls in parts of the left ventricle and septum. In contrast, 6 of the 16 individuals with large NF1 gene deletions had major cardiac conditions including mitral insufficiency, aortic insufficiency and hypertrophic cardiomyopathy. Two individuals also had tumors lying within the heart.

Pulmonary stenosis, an obstruction of blood flow in the heart from the right ventricle to the pulmonary artery, is more common in NF1 than in the general population and is most likely due to an underlying developmental defect in the heart structure. **Ben-Schachar *et al.*** showed that in persons with NF1, this defect is most often associated with a specific type of NF1 mutation called non-truncating mutations.

Both of these studies highlight the importance of cardiology evaluation and surveillance for all persons with NF1, and that there may be genetic predictors of increased risk of cardiovascular defects in NF1.

Emerging Treatments, Biology and Drug Targets for NF1 Cardiovascular Abnormalities

Stansfield *et al.* examined the molecular basis of cardiovascular issues in NF1 using a genetically engineered mouse model, and showed that myeloid cells from the bone marrow play an important role in the development of blood vessel defects. When myeloid cells lack one copy of the *Nf1* gene, these cells promote the repair of the arterial wall after injury, but the cells' hyperactivity also lead to arterial wall thickening. When the myeloid cells lack both copies of the *Nf1* gene, the myeloid cells' response to arterial injury leads to arterial stenosis. These disease-like features are quieted when the mice are treated with an anti-inflammatory drug rosuvastatin. The results uncover the cellular basis of cardiac disease in NF1 and suggest anti-inflammatory drugs may be helpful for treating this.

Bajaj *et al.* examined the basis of NF1 cardiovascular abnormalities by using the molecular tool short hair RNA (shRNA) to block activity of the *NF1* gene in endothelial cells, the cells that line blood vessels, when these cells are grown in a dish. When NF1 is blocked in endothelial cells, signaling is elevated in the Ras pathway. This also causes endothelial cells to multiply and to fail to develop into three-dimensional networks in the dish, something that normal cells would do. When the shRNA treated

cells are exposed to the drug Rapamycin, normal growth and development patterns are restored. Rapamycin has previously been assessed as an NF1 tumor treatment in NF1, and may also be a candidate drug for the treatment of cardiac abnormalities in NF1.

8. What's New in NF1 Biology?

Pilocytic astrocytomas are a type of brain tumor that can occur in NF1. In humans, NF1 tumors develop due to a congenital (germline) mutation in one copy of the *NF1* gene, followed by a spontaneous (somatic) mutation of the second copy of the *NF1* gene in the same cell at some point in life. However, even when mice are genetically engineered to recapitulate this mutation sequence, the mice never develop pilocytic astrocytomas. This suggests that other "cooperating factors" are needed to promote the growth of these brain tumors. To find out what these cooperating factors might be, **Gutmann, McLellan *et al.*** performed whole genome sequencing of three human NF1-associated pilocytic astrocytoma tumors. What they found was that the somatic mutation in each tumor had occurred due to a different mechanism. Furthermore, only around half of the cells in the tumor had undergone this somatic mutation, and there were a large proportion of stromal cells (connective tissue type cells) in the tumor. The findings suggest that what may be important in driving the growth of the pilocytic astrocytoma in humans are not necessarily additional mutations above and beyond *NF1*, but specific cellular interactions in the tumor.

Keeping to the theme of brain tumors, **Solga *et al.*** aimed to find the cells that underlie the cause of one form of brain tumor, optic pathway gliomas. They focused on a cell type called NG2+, but, through a series of studies, confirmed that NG2+ cells are not the cause of optic pathway gliomas. Though a negative result, this is useful information for future studies to find the causal cell.

In an intriguing study, **Kaufmann *et al.*** examine the behavior of fibroblast cells taken from individuals with NF1 and grown in a dish. The cells were 'challenged' to respond to different mechanical signals in the dish such as grooves and ridges. The NF1 cells were much less responsive to environmental challenges than were cells from individuals without NF1. Interestingly, when the NF1 cells were treated with a farnesyl transferase inhibiting drug to 'normalize' NF1 signaling, this 'normalized' the cells' response to their environment. This is interesting because it suggests that mutations in NF1 gene lead to changes in the way cells respond to the environment, a factor that may contribute to tumor growth.

In the search for new candidate drug targets for NF1, **Vallée *et al.*** looked for new molecules that bind and interact with the NF1 gene. For this, they used a yeast model system. (Yeast is widely used by researchers to study signaling interactions, since yeast cell contain essentially all the components of the human cell. However, yeast cells divide very quickly, so they are much simpler to manipulate and study). Through this study they identified LIMK2, a well-known cell kinase along the Rho/ROCK/LIMK2/ cofilin signaling pathway. Interestingly, this implicates *NF1* signaling in controlling the cell's actin cytoskeleton. This controls how the cell is shaped and how it moves around which is very important in tumor growth. LIMK2 appears to be a selective relationship in binding because the related molecule LIMK1 does not interact with *NF1* in the same way. Thereby this research puts forward a new drug target to be explored for the treatment of NF1.

Drosophila (fruit flies) are another model system widely used to study NF1 biology, again because they reproduce quickly and can readily be used for genetic manipulation; and the findings from these studies may be translated to the human. **Tsai *et al.*** used *Drosophila* to demonstrate a role for neurofibromin protein in maintaining the normal function of synaptic growth and transmission at the neuromuscular junction. They show that neurofibromin's function is downstream of the focal adhesion

kinase called Fak56. These findings demonstrate a new role for neurofibromin that could have relevance to learning disabilities as well as other features in NF1.

Staser *et al.* showed that dual disruption of the cell signals Erk1 and Erk2, but not disruption of either of these signals alone, will prevent the development of myeloproliferative disease in genetically engineered *Nf1* mice who are predisposed to developing this blood disease. This finding lends further support to the use of drugs that inhibit Erk signaling as NF1 therapies.

Fleming *et al.* examined the connection between *NF1* gene function and the regulatory roles of two protein families, muscleblind-like (MBNL) and CUG-BP and ELAV-Like family (CELF) proteins. The MBNL and CELF proteins are called splicing regulatory proteins; their role is to block certain aspects of gene expression. Only a limited number of targets have been identified that are targeted by both of these proteins; Fleming *et al.* show that *NF1* gene exon 23 is such a target, and that this exon of the *NF1* gene is under a tight and complex set of controls. Future analysis of this may be helpful in understanding how *NF1* gene regulation leads to specific physical manifestations in this disorder.

Finally, **Gutmann, Parada *et al.*** provide a summary of a recent symposium held at the Society for Neuroscience, focused on the molecular underpinnings of NF1 and how they relate not only to features such as tumor growth and learning disabilities, but also to the role of *NF1* gene signaling in the normal development of the nervous system.

9. NF1 Bony Abnormalities Update

Clinical Management of NF1 Bony Abnormalities

Bone densitometry in young persons with NF1 is an important measure during early life as it can potentially predict bone problems in later life. **Lodish *et al.*** assessed bone mineral apparent density (BMAD) and whole body bone mineral content (BMC), as a ratio of height, in 69 young persons with NF1 (age approx 5-24) who also have a high plexiform neurofibroma burden. 47% of those assessed had some impaired bone mineral density. 36% had impairment of the lumbar spine; 18% at the femoral neck. BMAD was lower when the plexiform burden was higher and vice versa.

The previous Network Edge reported the work of a Finnish group who were utilizing a long-term NF1 database, accumulating information on persons with NF1 from over 41 years, to better understand fracture risk in NF1. From the same group, **Heervä *et al.*** use the database to examine osteoporosis in NF1 and how this might be predicted. In 2011, the researchers revisited a set of persons with NF1 first examined in 1999, and who actually yielded the first evidence that bone mineral density is reduced and osteoporosis is common in NF1 (a fact now well recognized in NF1 clinical care). This new study found that persons with NF1 diagnosed with osteoporosis in 1999 had an increased risk of fracture in 2011. Also, persons with NF1 diagnosed with osteopenia in 1999 had osteoporosis in 2011. There was no correlation between the findings and general diet/health measures among participants such as smoking, weight, etc. It is therefore important to monitor persons with NF1 with an osteopenia diagnosis in case this advances further.

Finally, given NF1's potential effects on the skeleton, it is worth examining whether it can also affect the integrity of the teeth and dental health. The good news is 'no', following a retrospective study of 34 patients, again in Finland. **Jääsaari *et al.*** found that a clinical diagnosis of NF1 has no effect on the timing of normal dental development in early life. It will be interesting to see whether there are different findings on the aging of teeth and their condition in later life.

Emerging Treatments, Biology and Drug Targets for NF1 Bony Abnormalities

There are good mouse models of NF1 bone abnormalities, and researchers have a reasonable knowledge of how *NF1* gene disruption in bone cells can lead to the poor bone development, osteoporosis and osteopenia and increased risk of bone breakage seen in individuals with NF1. **He *et al.*** focus on the molecular signals behind this, and show that the bony defects are in part due to the growth factor macrophage-colony stimulating factor (M-CSF) binding to its receptor c-Fms. The drug PLX3397 blocks c-Fms, and when given to mice in which *Nf1* gene signaling is reduced and with osteoporosis, bone loss is prevented and the bone cells start behaving more normally. Targeting M-CSF/c-Fms signaling might therefore be a potential treatment for NF1-related bone abnormalities.

10. The Evolving Link Between NF and Cancer

We reported in the last Network Edge about the growing evidence that women with NF1 have an increased risk of developing breast cancer compared to women in the general population. Persons with NF are also at increased risk of developing other non-NF related cancers. **Seminog *et al.*** analyzed medical records from over 8,000 persons with NF (NF1, NF2 and schwannomatosis). Among this group they found 769 cases of cancer of the esophagus, stomach, colon, liver, lung, bone and thyroid, as well as malignant melanoma, non-Hodgkin's lymphoma, chronic myeloid leukemia, and female breast and ovarian cancer. As we learn more about the increased risk of cancer faced by those with NF, it becomes more important for everyone with NF to have thorough and regular medical evaluations and for doctors to be vigilant about the broad health risks that NF carries.

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