NF2/Schwannomatosis State of the Art 2023

ABSTRACTS

GENETICS

Abstract 1: Unexpected findings in *LZTR1*: Results of a single center's approach to referral and assessment of patients with incidental variants

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Objectives:
Starting in 2020, *LZTR1*, a gene previously limited to phenotype specific panels for neurofibromatosis, schwannomatosis, and Noonan syndrome, was added to large hereditary cancer panels at multiple commercial laboratories. There have now been several cases of incidental *LZTR1* variants identified in individuals without a prior history of schwannomatosis or Noonan syndrome. Here we describe the approach of a single institution to these incidental variants.

Methods:
A chart review was conducted of all patients with an incidental likely pathogenic or pathogenic variant in *LZTR1* on a germline hereditary cancer panel, including referral pattern and outcome, medical history, clinical evaluation, and imaging results.

Results:
11 cases of incidental *LZTR1* variants were identified, one referred by an external genetic counselor, and the remainder internal. All 10 with variants consistent with loss-of-function and/or splicing effects were referred to the NF center, with 6 completing an appointment. 5/6 patients had history of chronic pain, including 1 noted to have a tender subcutaneous forearm mass on exam. All patients were offered and accepted screening MRI of the brain and total spine, and the individual with the forearm mass also completed a targeted MRI. At the time of submission, 5/6 have completed imaging. No intracranial or spinal lesions were identified. The MRI of the forearm confirmed a small T2 hyperintense enhancing mass consistent with schwannoma and penetrant *LZTR1*-related schwannomatosis for one individual.

Conclusions: Much remains to be defined about the penetrance of *LZTR1*-related schwannomatosis, and there are not yet consensus guidelines on the surveillance of incidentally identified individuals. However, our center's experience to date demonstrates the value of expert assessment, as 1/6 individuals seen at our center was found to have a likely schwannoma consistent with penetrant disease. The utility of MRI screening for asymptomatic masses remains to be determined.

Abstract 2: Differential rates of germline heterozygote and mosaic variants in *NF2* may show varying propensity for meiotic or mitotic mutation

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Background:
NF2-related schwannomatosis-(NF2), an autosomal dominant tumour predisposition condition resulting from *NF2* gene perturbation, is very frequently caused by *de novo NF2* nonsense mutations. Over 50% of these occur at just six CpG dinucleotides.

Methods:
We have analysed germline DNA from over 1200 de *novo* cases with NF2 and assess differences in *de novo* heterozygote and mosaic cases.

Results:
We show that the six CpG dinucleotides make up 54%-(136/252) *de novo* nonsense variants despite constituting <10% of nonsense positions in the germline-(total=62). The *NF2* c.586C>T;p.(Arg196Ter) stands out as having higher *de novo* heterozygote to mosaicism ratio than the five other CpG variants-(73.1%vs53.7%;p=0.03) and its neighbouring CpG variant NF2 c.592C>T;p.(Arq198Ter)-38.5%-p=0.02. There appeared to be three hotspots for mosaic variants with different mutational mechanisms. One hotspot was found for a frameshift deletion c.41_42deI;p.(Leu14GlnfsTer34) in exon-1 with 5/6-(83.3%) *de novo* cases being mosaic compared to 53/131-(40.4%) for all frameshift deletions (p=0.08). There was also a hotspot for in-frame-deletions (IFD) across two codons in exon-3 (His116,Phe119*). This was the only site for likely pathogenic IFD variants in NF2, yet 8/9 variants (89%) were mosaic. The final hotspot is in exon 7 with five mosaic cases at a valine residue NF2 c.655G>A;p.(Val219Met)(REVEL-score=0.718;SpliceAI indicates potential donor loss/donor gain). All other 28 likely pathogenic *NF2* germline variants were heterozygous-(p<0.0001).

Conclusion:
The present study has confirmed the high rate of mutations both at zygotic level in meiosis and in early embryogenesis at six CpG dinucleotides in the NF2 gene. However, this disguises a differential rate of zygotic versus post-zygotic early mutation with NF2 c.586C>Tp.(Arg196Ter) having the highest rate of zygotic mutation. Mechanisms to explain this difference with CpG dinucleotides so close together require further investigation. We also describe three apparent hotspots for mosaic mutation in exons 1, 3 and 7 for frameshift deletion, IFDs, and missense variants that requires further explanation.

PAIN AND OTHER SYMPTOMS

Speaker Abstract 1: SMARCB1 is a transcriptional repressor of factors in Schwann cells that directly mediate pain

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Schwannomatosis patients typically present with intractable pain. A significant proportion of patients with schwannomatosis have mutations in the SMARCB1 gene (also called INI1, BAF47 and SNF5). We found that inducible conditional disruption of the Smarcb1 gene in mouse Schwann cells causes increased sensitivity to capsaicin. Dorsal root ganglion (DRG) neurons from mice with Schwann cell-targeted disruption of Smarcb1 express elevated levels of the TRPV1, a non-selective cation channel that can be activated by a number of noxious stimuli including capsaicin. We also find that TRPA1, an ion channel that acts as a sensor for environmental irritants, and the calcitonin gene related peptide (CGRP), which has been implicated in pain signaling, are elevated in the DRG and trigeminal neurons of these mice. Wild type DRG cells grown in Smarcb1-null Schwann cell conditioned media or conditioned media from schwannoma cells derived from schwannomatosis patients with SMARCB1 mutations expressed elevated levels of TRPV1, TRPA1 and CGRP as indicated by immunocytochemistry. Proteomic analysis demonstrated that the secretome of Smarcb1-mutant Schwann cells is distinct from wild type Schwann cells and includes elevated levels of cytokines and chemokines that have been implicated in pain. Smarcb1 interacts with the promoters of these genes and directly represses their transcription. Furthermore, agents that block at least some of these proteins can reverse the induction of TRPV1 in DRG cells treated with SmarcB1-mutant Schwann cell conditioned media and reduce pain responses to conditioned media in mice. Collectively, these data indicate that loss of Smarcb1 in Schwann cells leads to the increased transcription of factors that induce the expression of pain mediators in sensory neurons, and suggest a mechanism for schwannomatosis pain in patients with SMARCB1 mutations.

Abstract 2: Patient reported measures of tinnitus for individuals with neurofibromatosis 2

Heather Thompson for the REiNS Patient Reported Outcomes Working Group
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Introduction:
Neurofibromatosis Type 2 is a genetic disease characterized by the development of benign bilateral vestibular nerve schwannomas, as well as ependymomas, meningiomas, and cataracts. Mild to profound hearing loss with associated tinnitus are common symptoms reported by individuals with NF2. While tinnitus has a significant and negative impact on the quality of life (QoL) of individuals with hearing loss from the general population, the impact on individuals with NF2 is unknown. Consensus regarding the selection of ideal patient reported outcome measures (PROMs) for assessment could advance further research into tinnitus for NF2 patients.

Objective:
To achieve a consensus recommendation by the Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) International Collaboration for PROMs used to evaluate quality of life in the domain of tinnitus for NF2 clinical trials.

Methods:
The REiNS Patient-Reported Outcomes Working Group systematically evaluated PROMs of QoL in the domain of tinnitus for individuals with NF2 using previously published REiNS rating procedures. Of the 24 identified PROMs, eight measures were excluded because they were not published, not validated as an outcome measure, had a naming error or name change, or would not have been useful as a single outcome for a clinical trial. Sixteen published PROMs for the domain of tinnitus were scored and compared on their participant characteristics, item content, psychometric properties, and feasibility for use in clinical trials.

Results:
The Tinnitus Functional Index was identified as the most highly rated measure for the assessment of tinnitus in populations with NF2, due to strengths in the areas of item content, psychometric properties, feasibility and scores available.

Conclusions:
REiNS recommends the Tinnitus Functional Index for the assessment of tinnitus for NF2 clinical trials. Additional work is required to evaluate this measure with individuals with NF2 across a variety of ages through a variety of clinical trial types.

SURGERY & RADIOSURGERY

Abstract 1: Radiation treatment of benign tumours in NF2-schwannomatosis: a national study of 266 irradiated patients showing a significant increase in malignancy

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Background:

Radiation treatment of benign tumours in tumour-predisposition syndromes is controversial, but short-term studies from treatment centres suggests safety despite apparent radiation associated malignancy being reported. We determined whether radiation treatment in NF2-related schwannomatosis patients is associated with increased rates of subsequent malignancy-(M)/malignant progression-(MP).

Methods:

All UK patients with NF2 were eligible if they had a clinical/molecular diagnosis. Cases were NF2 patients treated with radiation for benign tumours. Controls were matched for treatment location with surgical/medical treatments based on age and year of treatment. Prospective data collection began in 1990 with addition of retrospective cases to 1969. Kaplan-Meier analysis was performed for malignancy incidence and survival. Outcomes were CNS M/MP (2cm annualised diameter growth) and survival from index tumour treatment.

Results:

1345 NF2 patients, 266 (133-Male) underwent radiation treatments between 1969-2021 with median first radiotherapy aged 32.9-(IQR=22.4-46.0). Nine subsequent CNS malignancies/malignant progressions were identified in cases with only four in 1079 untreated-($p<0.001$). Lifetime and 20-year CNS M/MP was ~6% in all irradiated patients-(4.9% for VS radiotherapy) versus <1% in the non-irradiated population-($p<0.001/0.01$). Controls were well-matched for age at NF2 diagnosis and treatment-(Males=133-50%) and had no M/MP in the CNS post index-tumour treatment-($p=0.0016$). Thirty-year survival from index tumour treatment was 45.62%-(95%CI=34.0–56.5) for cases and 66.4%-(57.3–74.0) for controls-($p=0.02$), but was non-significantly worse for VS radiotherapy.

Conclusion:

NF2 patients should not be offered radiotherapy as first line treatment of benign tumours and should be given a frank discussion of the potential 5% excess absolute risk of M/MP**.** The study has implications for other tumour-predisposition syndromes.

Abstract 2: Long-term follow-up of schwannoma growth behavior in adult neurofibromatosis type 2 and schwannomatosis patients using whole-body MRI

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Objective:
Characterize the long-term growth behavior of schwannomas in adults with neurofibromatosis type 2 (NF2) and schwannomatosis (SWN).

Background:
NF2 and SWN are related genetic tumor predisposition syndromes caused by distinct germline pathogenic variants on chromosome 22, and are characterized by the presence of cranial, peripheral, and/or spinal nerve schwannomas. The long-term growth behavior of schwannomas is unknown but knowledge thereof would help guide patient surveillance and selection for treatment. Whole-body MRI (WBMRI) can detect whole-body schwannoma burden.

Design/Methods:
12 NF2 and 10 SWN patients who underwent a WBMRI between 2007-2010 underwent a repeat WBMRI between 2018-2019. Schwannomas were segmented on short tau inversion recovery (STIR) sequences. Tumor volume was calculated using a three-dimensional tumor quantification software (3DQI). Tumor growth and shrinkage were defined as a volume change ≥20% over the entire study period.

Results:
Median time between scans was 10 years. A total of 103 schwannomas (46 NF2-associated, 57 SWN-associated) were analyzed. In both NF2 and SWN, 50% of tumors grew. Median growth was 88.3% in NF2 and 100.4% in SWN. All growing NF2-associated schwannomas grew in the setting of exposure to systemic therapy whereas only one growing SWN-associated tumor had been treated systemically. Excluding resected tumors, 19.4% of schwannomas shrank. Median shrinkage was 48.5% in NF2 and 37.4% in SWN. All shrinking NF2-associated tumors had been treated with systemic therapy whereas none of the shrinking SWN-associated tumors had been. 19 new tumors (7 NF2-associated, 12 SWN-associated) developed in 8 patients.

Conclusions:
Half of NF2- and SWN-associated schwannomas grow significantly over a decade. In NF2 patients, growth occurs despite systemic treatment whereas, in SWN patients, schwannomas may shrink spontaneously without treatment. These findings suggest a more aggressive tumor phenotype in NF2 patients. Continued patient enrollment and correlation of MRI findings with functional outcomes and hormone exposure history are ongoing.

BASIC AND TRANSLATIONAL RESEARCH

Speaker Abstract 1: Targeting the Hippo pathway in NF2-null schwannoma and meningioma

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Objectives:
We aim to demonstrate the efficacy of TEAD inhibition in preclinical NF2-null *in* vivo models and identify novel targets of aberrant Hippo pathway activity.

Methods:
In *NF2*-null tumours, the Hippo pathway is aberrantly activated and has been shown to drive multiple tumour phenotypes. Here we use small-molecule TEAD autopalmitoylation inhibitors in the two most clinically relevant models of schwannoma and meningioma, the Periostin;Cre-$NF2^{fl/fl}$ schwannoma model, and an orthotopic xenograft meningioma model.

Results:
Genetic deletion of either YAP or TAZ on an *NF2*-null background significantly reduced proliferation and macrophage infiltration in Periostin;Cre-$NF2^{fl/fl}$ mice schwannomas. Deletion of TAZ but not YAP also ameliorated the severe neuronal loss seen in DRGs, highlighting the overlapping but distinct functions of YAP and TAZ in schwannoma development. TEAD inhibitor drug treatment in Periostin;Cre-$NF2^{fl/fl}$ mice resulted in significant reductions in proliferation and increased apoptosis in both VGs and DRGs compared to controls. TEAD inhibition also significantly reduced DRG and VG schwannoma tumour volume following 21d treatment in 9-month old animals compared to vehicle controls. qPCR and western blot analysis showed target engagement by downregulation of multiple Hippo target genes such as CTGF and Cyr61.
TEAD inhibitors were also seen to significantly reduce proliferation in primary human meningioma cells. Orthotopic injection of meningioma cell lines led to tumour establishment in NSG mice and treatment with TEAD inhibitors significantly reduced proliferation.

Conclusions:
For *NF2*-related schwannomatosis patients, drugs that effectively target schwannomas, meningiomas and ependymomas are the ultimate goal. Aberrant Hippo pathway activity is recognised to drive tumour phenotypes in *NF2*-null schwannoma and meningioma. Here we show that TEAD autopalmitoylation inhibitors can potently block and even reduce tumour size in schwannoma *in vivo,* whilst in the meningioma model, we show preliminary data that TEAD inhibition is also efficacious.

Speaker Abstract 2: A VEGF receptor vaccine for the treatment of neurofibromatosis type 2

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The anti-VEGF antibody bevacizumab has shown efficacy for the treatment of neurofibromatosis type 2 (NF2). Theoretically, vascular endothelial growth factor receptors (VEGFRs)-specific cytotoxic T lymphocytes (CTLs) can kill both tumor vessel cells and tumor cells expressing VEGFRs. Recently, we have performed an exploratory clinical study of VEGFRs peptide vaccine in patients with progressive NF2-derived schwannomas. Tumor volume reduction and hearing improvement in some patients have been observed. There were no severe adverse events related to the vaccine. Both VEGFR1-specific and VEGFR2-specific CTLs were induced in most patients. Surgery was performed after vaccination in two patients, and significant reductions in the expression of VEGFRs in schwannomas were observed. Therefore, this clinical immunotherapy study demonstrated the safety and preliminary efficacy of VEGFRs peptide vaccination in patients with NF2.

Abstract 3: Sporadic and *NF2*-related Schwannomatosis Vestibular Schwannomas Share Similar Tumour Microenvironments.

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BACKGROUND:
In the tumour predisposition syndrome *NF2*-related schwannomatosis (NF2) – previously known as Neurofibromatosis Type 2 – pathogenic variants in the tumour suppressor *NF2* gene elicit the growth of bilateral vestibular schwannoma (VS) tumours. Mutations in *NF2* also occur in the more frequent non-syndromic sporadic unilateral VS, which share histopathological features such as cellular and acellular tissue, microvessel architecture and chronic inflammatory regions. However, previous studies comparing NF2 and sporadic VS have yet to compare the immune microenvironments, which will aid in assessing the viability of targetted immune therapies in these tumours.

METHODS:
This current study drew together bulk transcriptomic data from three individual published Affymetrix microarray datasets (GSE54934, GSE108524 and GSE141801) to compare the gene expression profiles of sporadic and NF2 VS subsequently validated with quantitative PCR. Additionally, single cell spatial mapping of the VS tumour microenvironment was completed with Hyperion imaging mass cytometry (IMC) in 12 sporadic VS and 18 NF2 VS in order to characterise similarities and differences between NF2 VS and their sporadic counterparts.

RESULTS:
Comparative bioinformatics analyses revealed close similarities in NF2 and sporadic VS tumours across the three datasets. The types of inflammatory markers and pathways were closely matched in NF2 and sporadic VS, with upregulation of the ‘Neuroinflammation’ and ‘Tumour Microenvironment’ pathways. From the three datasets, macrophages were predicted to be the most abundant immune cell population

within these tumours. This was further confirmed with IMC, showing equivalent niches of macrophages within the tumour microenvironments of sporadic and NF2 VS.

CONCLUSIONS:

Taken together, these data suggest the tumour microenvironments and immune landscapes of NF2 and sporadic VS are comparable and macrophage-rich. This suggests that targetted immune therapies will have equivalent efficacy in patients with sporadic VS and *NF2*-related schwannomatosis.

Abstract 4: Alteration of pluripotency and differentiation capacities in Merlin-deficient iPSCs

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The appearance of bilateral vestibular schwannomas (VS) is one of the most characteristic features of Neurofibromatosis Type 2 (NF2), an autosomal dominant syndrome that predispose to the development of tumors of the nervous system. VS are caused by the bi-allelic inactivation of the NF2 gene in a cell of the Schwann cell lineage. Our current understanding of VS initiation and progression as well as the development of new effective therapies is hampered by the absence of human non-perishable cell-based models. With this aim, we generated and characterized induced pluripotent stem cell (iPSC) lines with single or bi-allelic loss of function of NF2 by combining the direct reprogramming of VS cells with the use of CRISPR/Cas9 editing. In our hands, NF2 function seems to be essential for reprograming and critical for maintaining a pluripotent state. Despite the difficulty of maintaining pure pluripotent merlin-deficient cell clones, we were able to differentiate them into neural crests (NC) cells. However, at this stage, the spontaneous expression of S100B marker and the impossibility to generate Schwann cells in 2D cultures due to a lack of cell-plate attachment, denoted also an altered differentiation capacity of NF2-deficient cells towards the NC-SC axis, in these *in vitro* conditions. To overcome this deficiency and applying a 3D Schwann cell differentiation protocol, we successfully generated NF2(+/-) and NF2(-/-) spheroids expressing classical markers of the NC-SC axis, showing potential as a genuine *in vitro* model of NF2 schwannoma.

The authors declare no conflict of interest.

Funding: Associación Chromo 22, Fundación Proyecto Neurofibromatosis, the Catalan NF Association (AcNeFi), the Ministry Science and Innovation (PI20/00215), Fundació La Marató de TV3 (126/C/2020) and the Generalitat of Catalonia (2017 SGR 496).

Abstract 5: Development of a novel in vitro model for neurofibromatosis type 2 (NF2) to study disease mechanisms and test potential therapeutics

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Current treatment options for NF2 are very limited and include repeated surgeries and radiotherapy which pose a major burden on the patients and families. A major hurdle to the development of novel treatments for NF2 is the lack of reliable animal and cellular disease models representing the diversity of the patients. To overcome this hurdle, we have developed a new in vitro model system patient skin fibroblasts which are much more readily available compared to fresh tumor samples. The fibroblasts are reprogrammed to induced Schwann cells (iSCs) using a direct conversion method.

We successfully derived iSCs from 3 NF2 patients and 4 healthy controls. We verified reduction in fibroblast marker expression alongside expression of Schwann cell markers prior to testing. Interestingly, when comparing patient and control iSCs, we observed alterations that could predispose NF2 patient iSCs to become tumorous even though the iSCs still contained one healthy copy of the NF2 gene at that point. Changes observed include increased expression of stem cell markers such as c-Myc and Sox2 and increased proliferation patterns compared to healthy control iSCs. We also observed elevated expression levels of retroviral elements such as HervK in the NF2 patient-derived iSCs. Importantly, we tested two different antiretroviral drugs and demonstrate their ability to slow down the proliferation in NF2 patient iSCs. As a next step, we are simulating the second hit to further reduce NF2 expression with two different methods. Together, these new tools allow to study the impact of losing either one or both copies of the gene using the same source cells. Our new model system helps studying disease mechanisms and is well suited for evaluating novel therapeutic options.

Abstract 6: A mouse model of neurofibromatosis type 2 with Lin28 associated Schwann cell proliferation

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Background:
Neurofibromatosis type 2 (NF2) is associated with loss of *NF2*/Merlin, which leads to schwannomas of the vestibular nerve and varying degrees of sensorineural hearing loss (SNHL). The etiology of the hearing loss remains to be elucidated, although leading current theories implicate the secretion of pro-inflammatory and potentially neurotoxic factors. Interestingly, loss of Merlin has also been associated with an increase of RNA binding protein Lin28 in cell lines, and dysregulation of Lin28 can negatively affect myelination. In this study, we examined the auditory and vestibular nerves in a mouse model for NF2 to further investigate the underlying cochlear NF2 phenotype.

Materials and Methods:
NF2 mice or controls were aged up to 11 months. Animals underwent serial measurements of auditory

brainstem responses (ABR) and 5-Ethynyl-2′-deoxyuridine (EdU) injections. Inner ear histology was performed at 11 months. Proliferation was assessed after EdU labeling. Schwann cells and neurons were quantified on serial sections. Cochlear whole mounts were stained and quantified for synaptic markers. Schwann cells at early and late time points were isolated using fluorescence-activated cell sorting (FACS), and microRNA and mRNA were isolated for quantitative PCR.

Results:
At 10-11 months, ABR demonstrated significant hearing loss in all NF2 animals. EdU labeling revealed increased proliferation of Schwann cells with concurrent Lin28 upregulation which was associated with increased loss of spiral ganglion ribbon synapses in the cochlea, followed by neuronal loss.

Conclusions:
NF2 mice display a cochlear phenotype that may be associated with dysregulation of Lin28 after loss of Merlin. This could explain the increased proliferation of Schwann cells in the cochlea, initiating a loss of auditory synapses and neurons. These findings may in part account for the sensorineural hearing loss in patients with vestibular schwannomas.

Abstract 7: High dimensional imaging highlights the T-cell compartment as a potential therapeutic for both NF2-related Schwannomatosis and sporadic vestibular schwannoma

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Vestibular schwannomas (VS) are intracranial tumours that grow along the vestibulocochlear nerves. 95% of VS cases are sporadic, unilateral tumours, with the other 5% of VS cases being bilateral tumours caused primarily by the tumour-predisposition syndrome *NF2*-related Schwannomatosis (*NF2* SWN), previously known as Neurofibromatosis Type 2. VS primarily causes sensorineural hearing loss but left unchecked can lead to various other co-morbidities. Whilst surgery is often used to treat sporadic tumours, *NF2* SWN is currently incurable with limited treatment options.

The tumour microenvironment of VS is poorly understood, and resolving the subcellular architecture of these tumours, and the various cell interactions that underpin VS pathogenesis, would aide in the identification of new drug targets for *NF2* SWN patients. To this end, we have used Hyperion imaging mass cytometry (IMC) on 12 sporadic VS and 18 *NF2* SWN VS to decipher the spatial single cell landscape of these tumours. We illustrate that the majority of tumour infiltrating leukocytes (TILs) present in both sporadic and *NF2* SWN VS have an effector-memory $CD8^+$ phenotype, characterised by co-expression of CD8 and CD45RO. T-cells were observed to co-localise with tumour-associated

macrophages (TAMs) in both *NF2* SWN and sporadic vestibular schwannomas. The distribution of TILs appears highly dependent on density of TAM populations, where they are abundant at high TAM densities, and absent at low TAM densities. Furthermore, many of these TILs can be seen at TAM-intratumoral or TAM-vascular niches.

Taken together, our data indicates that TILs present within both NF2 SWN and sporadic VS exhibit an effector memory phenotype, with their co-localisation tightly associated with TAMs. The influence of these TIL-TAM localisations on T-cell-mediated antitumoral immunity within VS remains elusive, thus further investigation into receptor-ligand interactions between TILs and TAMs within VS will provide greater clarity on our observations and prioritise these interactions as therapeutic targets.

CURRENT MEDICAL TREATMENTS

Abstract 1: Phase II Study of Axitinib in Patients with Neurofibromatosis Type 2 and Progressive Vestibular Schwannomas

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Introduction:
Vascular endothelial growth factor receptor (VEGFR), platelet derived growth factor receptor (PDGFR), and c-KIT represent clinically and/or preclinically validated molecular targets in vestibular schwannomas. We conducted a single institution, prospective, open-label, two-stage phase II study (ClinicalTrials.gov identifier NCT02129647) to estimate the response rate to axitinib, an oral multi-receptor tyrosine kinase inhibitor targeting VEGFR, PDGFR and c-KIT, in neurofibromatosis type 2 (NF2) patients with progressive vestibular schwannomas (VS).

Methods:
NF2 patients older than 5 years with at least one volumetrically measurable, progressive VS were eligible. The primary endpoint was to estimate the objective volumetric response rates to axitinib. Axitinib was given continuously in 28-day cycles for up to of 12 cycles. Response was assessed every 3 months with MRI using 3-D volumetric tumor analysis and audiograms. Volumetric response and progression were defined as ≥20% decrease or increase in VS volume, respectively.

Results:
Twelve eligible patients (ages: 14–56 years) were enrolled on this study. Seven of twelve patients completed 12 cycles (range: 2 to 12 cycles). We observed 2 imaging and 3 hearing responses. Best volumetric response was -53.9% after nine months on axitinib. All patients experienced drug-related toxicities, the most common adverse events were diarrhea, hematuria and skin toxicity, not exceeding grade 2 and hypertension, not exceeding grade 3.

Conclusion:

While axitinib has modest anti-tumor activity in NF2 patients, it is more toxic and appears to be less effective compared to bevacizumab. Based on these findings, further clinical development of axitinib for this indication does not appear warranted.

Abstract 2: Guideline Concordant Care in *NF2*-Related Schwannomatosis

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Objectives:
We previously reported that *NF2*-related schwannomatosis (*NF2*-SWN) clinicians agree or strongly agree with published recommendations that patients have an annual *NF2*-SWN clinic visit (98%), annual audiogram (96%), annual brain MRI (84%), and a surveillance spine MRI at least every 3 years when spinal tumors are present (80%). Here, we measured guideline-concordant care for children and adults with *NF2*-SWN, as reported by patients or caregivers.

Methods:
An electronic survey was sent to United States-based *NF2*-SWN patients and caregivers enrolled in the Children's Tumor Foundation NF Registry in May 2021 and again in May 2022, both of which asked about care patterns over the prior year. For simplicity, only the most recent year's survey responses were included for subjects that responded both years.

Results:
One hundred survey responses were received (49 in 2021 and 51 in 2022). Four responses were omitted from 2021 data due to duplicate entries by the same subject, totaling 96 responses for analysis, which was a 13.9% response rate. Subjects were 85% adult, 65% female, and median age was 40 years (range 6-79). Fifty-nine percent of respondents attended an NF Clinic Network (NFCN) site, and 83% of respondents had seen their NF care team within the last year. Eighty-five percent had a brain MRI performed within the last year, and among patients with known spinal tumors, 92% underwent a spine MRI within the last three years. Among non-deafened patients, 84% had an audiogram within the last year.

Conclusions:
We present patient and caregiver-reported data that suggest opportunities for improvement in guideline-concordant care for NF specialty care, as well as annual imaging and audiology. Specifically, this highlights the need for greater access to NFCN clinic sites and for further study and guideline consensus-building among NF2-SWN clinicians, which may translate to improved care for patients.

FUTURE THERAPY

Abstract 1: A Highly Promising Adeno-Associated Viral (AAV) Gene Replacement Therapy Vector Effectively Reduces Tumor Growth in In Vitro and In Vivo Schwannoma Models

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Neurofibromatosis 2 (NF2) is a rare autosomal dominant disorder caused by loss-of-function mutations within the NF2 gene. This leads to tumor formation in the cranial nerves responsible for hearing and balance (bilateral vestibular schwannomas) as well as meningiomas and ependymomas in the brain and spine, which ultimately increases morbidity and reduces life expectancy. Current standard treatments including surgery and radiotherapy do not address tumor recurrence and risk further complications, thus highlighting the need for more efficacious therapies. In recent years, gene therapy has emerged as a promising treatment option for rare monogenic disorders with limited treatment options. Particularly, adeno-associated viral vectors (AAVs) have been demonstrated to effectively target the nervous system with a remarkable safety profile. Here we describe an AAV gene replacement strategy that we developed for NF2 loss-of-function pathology. We generated AAV vectors comparing several truncated *NF2* promoters expressing either reporter green fluorescent protein (AAV.*NF2*.GFP) or wild-type NF2 (AAV.*NF2.NF2*). *In vivo* testing of the AAV.*NF2*.GFP constructs in wild-type mice showed the GFP transgene delivered to disease-relevant cell types. Injection of the corresponding AAV.*NF2.NF2* construct in wild-type mice confirmed upregulation of NF2 expression in the brain and allowed identification of the best candidate vector to move forward in follow up efficacy studies. No apparent safety issues were observed 1yr post-injection. In addition, we tested our top AAV.*NF2.NF2* vector in a human NF2 *in vitro* model we generated by direct chemical conversion of NF2 patient skin fibroblasts into induced Schwann cells (iSCs). Molecular characterization suggests that NF2 patient iSCs tend to remain in a more stem cell-like state, which was rescued by transduction of our lead vector. Last, our lead construct also reduced tumor growth when injected directly into growing human tumors in a xenograft mouse model. Together, our gene therapy approach is highly promising and warrants further development towards IND-enabling studies.

Abstract 2: WP1066 induces cell death in a schwannomatosis patient–derived schwannoma cell line.

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Schwannomatosis is a rare genetic disorder that predisposes individuals to development of multiple schwannomas mainly in spinal and peripheral nerves and to debilitating chronic pain often unrelated to any schwannoma. Pathogenic variants of two genes, SMARCB1 and LZTR1, are causal in familial

cases. However, many schwannomatosis patients lack mutations in these genes. Surgery is the standard treatment for schwannomas but leaves patients with increasing neurological deficits. Pain management is a daily struggle controlled by the use of multiple analgesic and anti-inflammatory drugs. There is a need
for both nonsurgical treatment to manage tumor growth and nonaddictive, nonsedative pain control. Because standard clinical trials are exceedingly difficult for patients with rare disorders, precision medicine approaches offer the possibility of bespoke therapeutic regimens to control tumor growth. As a proof of principle, we obtained a bio-specimen of paraspinal schwannoma from a schwannomatosis patient with a germline point mutation in the SMARCB1/INI gene. We created an hTERT immortalized cell line and tested the ability of targeted small molecules with efficacy in neurofibromatosis type 2–related schwannomas to reduce cell viability and induce cell death. We identified WP1066, a STAT3 inhibitor, currently in phase 2 clinical trials for pediatric and adult brain tumors as a lead compound. It reduced cell viability and STAT-3 phosphorylation and induced expression of markers for both necroptosis and caspase-dependent cell death. The results demonstrate feasibility in creating patient-derived cell lines for use in precision medicine studies.

Abstract 3: Combining brigatinib with mTOR inhibition to effectively treat NF2-deficient meningiomas

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Presently, an FDA-approved medical therapy for meningiomas, the most common intracranial tumors and frequently found in patients with NF2, is not available. To identify a novel targeted therapy, we, as members of the Synodos for NF2 Consortium together with the NIH-NCATS, previously identified brigatinib, an inhibitor of multiple receptor tyrosine kinases (RTKs) including anaplastic lymphoma kinase, to cause tumor shrinkage in the *NF2*-deficient sporadic meningioma Ben-Men-1-LucB model. Additionally, we found the dual mTORC1/2 inhibitor INK128 to potently suppress meningioma growth. To examine whether combining INK128 with brigatinib exhibits anti-tumor synergy, we generated an NF2-associated meningioma cell line AG-NF2-Men-1 from a grade-I tumor from an NF2 patient. Like Ben-Men-1 cells, AG-NF2-Men-1 cells are *NF2*/merlin-null but expressed several RTKs, such as EGFR, ErbB3, and IGF-1R, and responded to their cognate ligands. Brigatinib and INK128 alone inhibited AG-NF2-Men-1 cell proliferation at IC50 values similar to those in Ben-Men-1 cells. Combining brigatinib with INK128 showed growth-inhibitory synergy. Mechanistically, the combined treatment not only decreased p-AKT(S473) more than either drug alone, but also prevented INK128-mediated re-phosphorylation of AKT on T308, as well as suppressing the downstream signaling of mTOR. RNA sequencing analysis indicated that the brigatinib+INK128 combination elicited major changes in the expression of genes including the upstream regulators of several signaling networks important for meningioma growth. By generating luciferase-expressing AG-NF2-Men-Luc2 cells and stereotactically implanting them at the skull base, we established an orthotopic, quantifiable NF2-assoicated meningioma model. As in the Ben-Men-1-LucB model, brigatinib effectively blocked AG-NF2-Men-Luc2 tumor growth. The combined brigatinib+INK128 treatment enhanced tumor regression in both the AG-NF2-Men-Luc2 and Ben-Men-1-LucB models. Also, the brigatinib+INK128 combination can be repeatedly used to shrink NF2-associated meningiomas. These results suggest that a clinical trial to evaluate the combination of brigatinib with an mTOR inhibitor in *NF2*-deficient meningiomas is warranted. (Supported by CancerFree KIDS, DOD, NINDS, Rally Foundation)

YOUNG INVESTIGATOR AWARDS

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