ABSTRACT BOOK

EVENT AGENDA:

SCAN ME

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IN PERSON EVENT

OCTOBER 10-11, 2022

Central Manchester

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Unbalancing the Ras/MAPK pathway and the cAMP/PKA pathway as a therapeutic strategy for cutaneous neurofibromas

Eduard Serra, *Hereditary Cancer Lab; Germans Trias i Pujol Research Institute (IGTP)*

The development of multiple cutaneous neurofibromas (cNFs) constitute one of the major concerns of Neurofibromatosis type 1 (NF1) affected persons. cNFs are complex tumors, composed of different cell types bearing different NF1 genotypes, each component playing its role in cNF growth. Studying the cross-talk between the distinct cNF cell types might be a way to identify potential therapeutic targets. We identified a transcriptional signature due to the interaction of cNF NF1(-/-) Schwann cells (SCs) and NF1(+/-) fibroblasts (FB), being the proton-sensing G protein-coupled receptor GPR68 one of the upregulated genes that caught our attention. Ogerin is an allosteric modulator of GPR68 that potentiates proton activity in GPR68-mediated Gs-cAMP production, thus elevating intracellular cAMP. Since a coordinated activation of cAMP and Ras/MAPK pathways is required for balancing SC proliferation vs differentiation and neurofibromin has been implicated in both pathways, we analyzed the functional impact resulting from unbalancing both pathways by Selumetinib and Ogerin co-treatment in SCs. The coordinated activation of cAMP and inhibition of Ras/MAPK pathways resulted in the SC terminal differentiation and decreased cell viability in cNF-derived SC cultures in 2D. Furthermore, co-treatment also increased cell death in an independent 3D iPSC-based neurofibromasphere model composed of differentiating SCs and cNF-derived FBs (Mazuelas et al., 2022). The use of cAMP analogues replacing Ogerin in co-treatment experiments generated the same response. The simultaneous activation of the cAMP pathway and inhibition of the Ras/MAPK pathway may constitute a new therapeutic approach for treating cNFs that deserves further investigation.

Co-Authors: Helena Mazuelas, Míriam Magallón, Itziar Uriarte, Alejandro Negro, Imma Rosas, Ignacio Blanco, Elisabeth Castellanos, Conxi Lázaro, Bernat Gel, Meritxell Carrió & Eduard Serra Funding: This work has been supported by an Agreement from the Johns Hopkins University School of Medicine and the Neurofibromatosis Therapeutic Acceleration Program (NTAP). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Johns Hopkins University School of Medicine. Mazuelas et al. (2022) DOI: 10.1016/ j.celrep.2022.110385

Aberrant GABAergic neuronal development leads to disrupted contactin expression in a human induced pluripotent stem cell model of Neurofibromatosis 1-Autism

Julieta O'Flaherty, *University of Manchester*

Aberrant GABAergic neuronal development leads to disrupted contactin expression in a human induced pluripotent stem cell model of Neurofibromatosis 1-Autism
Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disease with an unknown cause. Animal studies have suggested that it may result from aberrant neuronal development, leading to an imbalance between excitatory and inhibitory signals (E-I balance), but translation to human models is poor. Neurofibromatosis 1 (NF1) is a useful syndromic model due to its monogenic nature and high prevalence of ASD amongst patients (24%). Loss of function mutations in the NF1 gene result in an overactive RAS/MAPK pathway. We generated four established human induced pluripotent stem cells (hiPSC) lines from two individuals with NF1-ASD (<10yrs, male) and two related parental controls (>30yrs, female), as well as unrelated controls (various ages and sexes), to discern the effects of NF1 mutations during cortical development. The hiPSCs were successfully differentiated into excitatory and inhibitory cortical neurons using established protocols. Their early phenotypic differences were characterised using cellular and molecular techniques. We identified disrupted rosette development and expression patterns of contactins in GABAergic progenitors but not in glutamatergic lineages. This suggests that GABAergic neuron development may be selectively affected in NF1-ASD. As the RAS/MAPK pathway is important for several developmental processes, these findings will also be informative for other developmental systems.

A case-only study in 1,333 neurofibromatosis type 1 patients to identify common genetic modifiers of cutaneous, subcutaneous, and plexiform neurofibromas

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Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder caused by loss-of-function mutations in the tumor suppressor gene NF1. A typical sign of the disease is the highly variable and unpredictable development of benign peripheral nerve tumors that may be cutaneous (cNFs), subcutaneous (scNFs), or plexiform (pNFs) neurofibromas. Many NF1 patients have been genotyped but few NF1 allele-phenotype correlations have been identified. Intrafamilial phenotypic correlations and animal models provided evidence for a strong genetic component in neurofibromas development but no significant contribution of the NF1 locus to the overall phenotypic variation of each neurofibroma type. We present here the first gene-wide association study focused on genomic modifier regions implicated in cNFS, scNFs and pNFs development. Methods: NF1 patients were recruited through the NF-France network between 2003 and 2013 and molecularly characterized. All patients were phenotypically evaluated by a medical doctor using a standardized questionnaire. NF1-mutated patients were enrolled and genotyped with the Illumina OmniExpressExome chip. Imputation and quality controls were applied in 1,333 patients. A total of more than 7 million common genomic variants were obtained for association study. The cohort was divided into discovery (n = 918) and replication (n = 415) cohorts. Results: Association study focused on three major clinical features: cNFs, scNFs, and pNFs. Genome-wide significance threshold (5 × 10-8) was reached in the discovery cohort in 9q21.33 for the pNFs phenotype. Twelve, three and four regions suggestive of association ($p < 1 \times 10\text{-}6$) were identified respectively for pNFs, cNFs, and scNFs in the discovery cohort. Evidence of replication was observed respectively for four, two, and six loci. CRIPSR-Cas9-mediated knock-out experiments were conducted in wild-type and NF1-/- Schwann cells for four candidate modifiers genes: GAS1, SPRED2, TBK1, and MYCL. Proliferation assay suggests that GAS1 may play a more specific role in the NF1-/- Schwann cell growth. Conclusions: We performed the first genome-wide association study exploring the genetic modifiers in the three main types of NFs in NF1, and the largest GWAS in NF1 to date. This study pointed out a number of susceptibility genomic loci and helped to identify 168 candidate modifier protein coding genes in pNFs, cNFs, and scNFs. These results may shed a new light on the pathogenesis of neurofibromas and will hopefully contribute to the development of innovative therapeutics in a deleterious and life-threatening condition.

Co-Authors: Audrey Sabbagh, Pierre Sohier, Djihad Hadjadj, Manuela Yé, Anne Boland-Auge, Delphine Bacq-Daian, Ingrid Laurendeau, Audrey Briand, Laurence Allanore, Jean-François Deleuze, Raphaël Margueron, Michel Vidaud, Salah Ferkal, Nicolas Ortonne, Béatrice Parfait, Dominique Vidaud, members of the NF-France network, Eric Pasmant, Pierre Wolkenstein

Synthetic lethal screening identifies existing autophagy drugs with selective viability effects on Neurofibromatosis type-1 model systems

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Using a Drosophila cell model of NF1, we performed synthetic lethal screens to identify novel drug targets. We identified 54 candidates, five of which could be targeted with existing drugs, including autophagy inhibitors. Inhibition of autophagy using chloroquine (CQ) and bafilomycin A1 resulted in selective reduction in cell viability in a panel of human NF1 mutant cell lines and a Drosophila in vivo model. Finally, we found that combined treatment with CQ and selumetinib resulted in further reduction of NF1 mutant cell viability. The results of this study highlight two key points: 1) the use of Drosophila cells as a model to screen for drugs specifically targeting NF1 mutant cells was highly successful as the candidate interactions were conserved across a panel of human NF1 mutant cells and an in vivo fly NF1 mutant model, and 2) NF1-deficient cells have vulnerability to disruption of the autophagy pathway. Not only does this pathway represent a promising target for the treatment of NF1-associated tumours, but we identified CQ and bafilomycin A1 as candidate drugs for the treatment of NF1 tumours.

Co-Authors: Wang Y, Stevens M, Mandigo TR, Bouley SJ, Sharma A, Sengupta S, Housden A, Perrimon N, Walker JA, Housden BE.

The course of intellectual development in children with Neurofibromatosis Type 1 based on longitudinal history study

A.M. van Abeelen, MSc, *Kempenhaeghe, Center for Neurological Learning Disabilities*

Aim: Neurofibromatosis Type 1 (NF1) is known to have substantial neurocognitive impact, but previous research has not examined which children between preschool and adolescence are most vulnerable with respect to cognitive development. With this prospective cohort study we want to gain insight into the course of cognitive development in children with NF1.

Method:
In the NF1 expertise center, children are regularly referred for neurocognitive evaluation at four timepoints: at preschool (T1), elementary school (T2), secondary school (T3) and secondary/vocational school (T4). This study presents data collected between 2010 to 2020 from 377 Dutch children with NF1 (209 boys, 138 girls, aged 2;6-17 years) who visited one of our outpatient clinics (Erasmus Medical Center and Kempenhaeghe, Center for Neurological Learning Disabilities). This paper reports only on the standard scores of age-appropriate Wechsler Intelligence Scales. In the longitudinal study, 40 children were included at T1 (mean age= 3.9 years) and T2 (mean age= 6.2 years). Furthermore, 29 children were included at T3 (mean age= 11.4 years) and T4 (mean age= 15.0 years). In our presentation, we will focus on possible predictors such as sex and ADHD for the developmental course of intelligence. We will also present cross-sectional data from 308 children.

Results: In cross-sectional data, we did not observe discrepancies in standard scores for intelligence at different timepoints (T1-T2, T3-T4). However, based on longitudinal data, we observed a significant increase in Total (TIQ), Verbal (VIQ) and Performance intelligence (PIQ) between T1 and T2 - see Figure 1, and in PIQ between T3 and T4. Regression-analyses showed that sex or ADHD did not predict these changes in IQ.

Interpretation: In contrast to cross-sectional observations that show no discrepancies in IQ at different timepoints, we find changes in intellectual functioning throughout development in children with NF1, based on longitudinal data. This underscores the importance of longitudinal history studies to understand mechanisms of cognitive development in children with NF1. We aim to present the full longitudinal development of IQ scores over these four age groups. In a future study, we will also focus on the course of development in neurocognitive domains such as attention, visual motor integration, executive function, and memory in these children with NF1.

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Generic Health-Related Quality of Life: Analysis of the Pediatric Quality of Life Inventory and Child Health Questionnaire in children with Neurofibromatosis Type 1

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Background: Neurofibromatosis type 1 (NF1) is a hereditary disorder with a significant impact on health-related quality of life (HRQoL). Multiple questionnaires are available to measure HRQoL. The aim of this study was to investigate HRQoL in Dutch children with NF1 using two questionnaires: the Pediatric Quality of Life Inventory (PedsQL) and the Child Health Questionnaire (CHQ), and to compare the content and psychometric properties of these questionnaires in NF1 patients. Methods: PedsQL and CHQ parent-reports were administered as part of regular care in NF1 patients aged five to twelve years that visited the Sophia Children's Hospital from October 2016 through February 2020. HRQoL scores were compared with a healthy Dutch population using independent t-tests and effect sizes. Psychometric properties were assessed by floor/ceiling-effects and Cronbach's alpha coefficient. Known-groups validity was tested using Mann-Whitney U. A principal component analysis (PCA) with varimax rotation was performed to identify the data's internal structure. Content mapping was conducted to identify unique constructs. Results: PedsQL and CHQ questionnaires were completed for 160 patients. A significantly lower HRQoL was reported on all PedsQL subscales compared to the reference population, except for 'physical functioning' in the 8 – 12 age group. Significantly lower HRQoL was reported on the CHQ on 9 out of 14 subscales in the 5 – 7 age group, and 11 out of 14 subscales in the 8 – 12 group compared to the reference population. Effect sizes ranged from d=0.23 (small) to 1.04 (large) for both questionnaires. The psychometric properties of the PedsQL and CHQ were adequate and comparable. Both questionnaires showed known-groups validity for the manifestations 'moderate to severe cognitive impairment' and ADHD. However, only the CHQ detected significant differences in HRQoL for patients with vs without plexiform neurofibroma and optic pathway glioma. The PCA revealed a two-component model, explaining 61,1% of total variance. The first component represented psychosocial aspects of HRQoL, component two represented the physical aspects. Both questionnaires showed relevant loadings on both components. Content mapping revealed that the subscales on impact on parents and family were unique to the CHQ. Conclusion: Parent-reported HRQoL of children with NF1 was significantly lower compared to the reference population on multiple domains. Both the PedsQL and CHQ showed relevant loadings to the PCA components and adequately measure HRQoL in children with NF1. However, the CHQ has better known-groups validity and covers a unique dimension of HRQoL associated with disease impact on parents and family, which is missing in the PedsQL.

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Platform trials as an opportunity to identify new treatments in NF: identifying potential participating centers

Britt Dhaenens

The European Patient-Centric Clinical Trial Platforms (EU-PEARL) is a collaborative IMI project to establish the use of Integrated Research Platforms (IRPs) for the development of new therapies for patients in four disease areas. One of the disease specific work groups aims to create a platform trial for neurofibromatosis type 1 (NF1) that can be applied to various manifestations and manifestation severities. By reviewing the patient journey through a clinical drug trial, we identified critical decision points that could be incorporated into a generic flowchart. We reviewed previously published clinical trials in NF1, extracting information on eligibility criteria, criteria for removal from treatment, outcome measures, and response definitions. Miniteams were composed to address adaptations for specific manifestations. Meetings with experts on NF1 clinical trial design from the USA have been arranged for each mini-team to align the protocols to trials for NF1 in the USA to ensure future data comparability. Protocols were reviewed and discussed with C4C experts and patients to leverage pediatric expertise. Variability in disease manifestation prevalence, progression, and severity complicates the development of a protocol that fits NF1 manifestations. Generic outcome was tumour size or volume on imaging. Challenges are NF1 patients presenting with combined complaints (tumour growth and/or pain), outcome measures preferably including tumour size and patient reported values, and harmonisation of definitions, measurements and timing of assessments.In this current session we present the generic approach of the platform trial and its manifestation specific eligibility criteria, outcome measures, response definitions and assessment schedule. In this session we aim to discuss feasibility of the suggested protocol, alignment with current clinical practice of NF1 experts and patient perspective, to identify potential clinical centers that can participate in the clinical network for NF platform trials and promoting the platform trial to pharmaceutical companies. This is an important step in the sustainability and implementation of the NF1 platform trial development.

Targeted audience:

-Nf1 expert clinicians
-Patient representatives of NF1
-Representatives from pharmaceutical companies

Methods/content:

The session will have a mixed approach of presentations, survey questions related to acceptability of suggested eligibility criteria, outcomes, assessment schedules, availability of required resources or to be responded by participants and discussion on topics that are identified with most discrepancies from the survey questions.

Presentations/panellists:

Generic flowchart and trial considerations (Rianne Oostenbrink, chair of panel))
Generic approach longitudinal registry (Jonas Leubner)
Manifestation specific presentations:

- OPG (Jonas Leubner)
- Low grade glioma (Eric Legius)
- Plexiform neurofibroma (Britt Dhaenens)
- Cutaneous Neurofibroma (Britt Dhaenens)

Co-Authors: Eric Legius, Charlotte Carton, Jonas Leubner, Gareth Evans, Pierre Wolkenstein, Rosalie Ferner, Uche Iloeje, George Kirk, Guenther Heimann, Claas Rohl, Kassandra Walluks, Annette Bakker, Marco Nievo, Rianne Oostenbrink

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Therapeutics discovery using synthetic lethal pharmacogenomic screens reveals vulnerability in MPNSTs harboring loss of function mutations in the gene encoding products of the polycomb repressive complex 2 (PRC2)

Christopher Moertel, *University of Minnesota, Department of Pediatrics*

Malignant peripheral nerve sheath tumors (MPNST) are a leading cause of mortality in people with neurofibromatosis type 1 (NF1). About 80% of MPNSTs harbor loss of function mutations in gene encoding products the polycomb repressive complex 2 (PRC2), including SUZ12 or EED. The PRC2 regulates chromatin accessibility by writing repressive trimethylation marks on histone H3 at lysine 27 (H3K27TriM). This suggests epigenetic homeostasis perturbation contributes to malignant transformation. However, the altered epigenetic state of the cells might also provide a targetable vulnerability. Therefore, we constructed models of nerve sheath tumors that arise in NF1 patients and conducted therapeutics discovery using synthetic lethal pharmacogenomic screens. Using CRISPR/Cas9, we created immortalized human Schwann cell lines lacking NF1 or NF1 and SUZ12, mimicking known genetic drivers of MPNSTs. Using a targeted epigenetic drug library, we identified compounds showing selective lethality towards NF1/SUZ12 double mutant cells. Hits from our drug screening pipeline were tested for efficacy, as single agents and in combination, in multiple in vivo MPNST models. We identified drugs capable of further perturbing chromatin homeostasis, such as HDAC inhibitors (HDACi). Characterization studies showed NF1/SUZ12 mutant Schwann cells recruit more HDACs to their chromatin, possibly to reduce global transcription and overcome the lack of PRC2 activity. When an HDACi was tested in combination with MEK inhibition (MEKi) strong synergy was observed against NF1/SUZ12 deficient human Schwann and MPNST cell lines. Given both selumetinib, a MEKi, and vorinostat, an HDACi, are FDA approved for other indications, these clinically interesting candidates were tested as single agents and in combination using multiple in vivo MPNST models. The vorinostat/selumetinib combination showed striking synergy across all models tested, including MPNST cell line and patient derived xenografts. We observed durable responses in survival studies and the ability to dramatically shrink large established tumors. Our results implicate targeting of epigenetic homeostasis, in combination with MEKi, as a major vulnerability of MPNSTs (and possibly other cancers) deficient for PRC2 activity. This combination was then used off label to treat a patient with NF1 and H3.3 gene H3K27M mutant glioblastoma of the spinal cord. Treated with surgery and this chemotherapy regimen only (no radiation), the patient is alive with no evidence of disease at 1 year of therapy. This experience and preclinical results from the studies described above are forming the basis for a Phase 0 “window of opportunity” clinical trial we are proposing for the treatment of PRC2 deficient MPNSTs.

Clinical characteristics and management of patients with neurofibromatosis 1 and plexiform neurofibromas in Denmark: A nationwide study

Cecilie Ejerskov[1] *Centre for Rare Diseases, Aarhus University Hospital*

Background: Plexiform neurofibromas (PN), benign nerve sheath tumours, are a common complication in patients with neurofibromatosis 1 (NF1), which may cause moderate-to-severe and even life-threatening morbidities. Complete surgical removal is often difficult due to their infiltrative nature, location and size. Our objective was to describe clinical characteristics and treatment of NF1 patients with PN from a nationwide perspective in Denmark.

Methods: Patients with NF1 were identified at the two Danish national specialist centres for NF1 between 2000 and 2020. Data were retrieved from the individual medical records. Patients with PN were grouped according to tumour size (small PN, ≤3 cm; large PN, >3 cm) and age (<18; ≥18 years).

Results: Of the 1099 patients with NF1 12% (35/296) of the paediatric and 21% (172/803) of adult patients had ≥1 large PN (≥3 cm).

Detailed information about the PN was available for 31/35 paediatric patients with large PN and 132/172 adult patients with large PN; a total of 40 PN in paediatric patients and 191 PN in adult patients. Approximately half of the paediatric (16/31) and 40% of adult patients (69/172), with large PN had at least one symptomatic large PN. Pain was the most frequently documented symptom among both paediatric (9/31) and adult patients (42/132) followed by neurological deficits, cosmetic issues and disfigurement, Figure 1. Analgesia was used by 3 of 9 paediatric patients and 13 of 42 adult patients; analgesia type varied from paracetamol to other non-steroidal anti-inflammatory drugs, opioids, antiepileptic, and tricyclic antidepressants as well as combinations of these.

Complete resection or debulking to relieve symptoms was performed in 38% (15/40) of the large PN in paediatric patients and 45% (86/191) of large PN in adult patients. A total of 35% (14/40) of large PN in paediatric patients and 32% (62/191) in adult patients were inoperable and no surgery was performed.

Conclusion: More than one third of children and adolescents and two thirds of adults with NF1 in Denmark developed at least one PN. Half of the large PN were symptomatic with pain as the dominant symptom. When used, the type of analgesia varied. and analgesia type varied. One third of the large PN in both paediatric and adults were inoperable. This highlights the severe sequelae and unmet need in both the paediatric and adult NF1 patients with PN.

Study sponsor: AstraZeneca

Co-Authors: Stense Farholt[2], Flemming Secher Kromann Nielsen[3], Ingunn Berg[1], Aparna Udupi[4], Trude Ågesen[5], Sofie de Fine Licht[5], Mette Møller Handrup[1]

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A Phase 1 study to assess the effect of food on the pharmacokinetics (PK) and gastrointestinal (GI) tolerability of selumetinib in adolescents with neurofibromatosis Type 1 (NF1)-related plexiform neurofibromas (PN)

David Viskochil, *Department of Pediatrics, University of Utah, Salt, Lake City, Utah, USA* [1],

Selumetinib (ARRY-142886, AZD6244) is a MEK1/2 inhibitor approved for pediatric patients with NF1 and symptomatic, inoperable PN in regions including Europe and the USA (EMA, aged ≥3 years; FDA, aged ≥2 years). Based on single-dose food-effect (high-fat and low-fat meal) studies in adults,[1–3] selumetinib is dosed in a fasted state (2 hours pre- and 1 hour post-dosing twice daily). This Phase 1 study (NCT05101148) evaluated the effect of a low-fat meal on steady-state exposure and GI tolerability in adolescents with NF1 and inoperable PN to confirm a recommendation for dosing selumetinib with food.

Eligible patients aged ≥12 to <18 years received selumetinib 25 mg/m^2 twice daily for 1 cycle (28 days) with a low-fat meal according to FDA guidance[4] (T1; fed C1), then in a fasted state for 1 cycle (T2; fasted C1) following 1-week washout. T2 continued beyond 1 cycle until study end or dose-adjusted selumetinib in a fed state (T3), if required.

Primary endpoints were $AUC_{0\text{-}12,ss}$, GI adverse events (AEs; CTCAE v5.0), GI patient-reported outcomes (PROs), and GI concomitant medications in fed vs fasted state. Secondary endpoints included other PK parameters and safety.

Overall, 24 participants received selumetinib (safety population); 19 were evaluable for fed vs fasted paired PK comparison. Meanduration of actual exposure for fed and fasted states was 28.3 (standard deviation [SD] 1.1) and 28.3 (SD 3.0) days, respectively. Steady-state extent of absorption was similar for fed vs fasted state ($AUC_{0\text{-}12,ss}$ geometric mean ratio [GMR] 0.919; lower 1-sided 90% confidence interval [CI] bound 0.841). Fed-state maximum serum concentration (C_{max}) was reduced (GMR 0.763; lower 1-sided 90% CI bound 0.667) and time to C_{max} was delayed (34 minutes; 90% CI 28, 60; **Figure**) vs fasted state. Similar findings were observed for N-desmethyl selumetinib metabolite.

Similar proportions of participants experienced GI AEs (fed C1, 29.2%; fasted C1, 33.3%). GI PROs were consistent between fed C1 vs fasted C1. Overall use of concomitant GI medications was low. No new safety signals were identified. No patients in either state experienced grade ≥3 or serious AEs.

At steady state, dosing selumetinib with a low-fat meal delayed absorption rate and lowered C_{max} but had no clinically significant effect (<30% $AUC_{0\text{–}12,ss}$ reduction) on the extent of absorption. There was no clinically significant impact on GI tolerability. Therefore, a dose adjustment is unlikely required. This study shows that dosing selumetinib with a low-fat meal has no significant impact on selumetinib AUC nor GI tolerability.

Co-Authors; Mariusz Wysocki[2], Maria Learoyd[3], Peng Sun[4], Karen So[4], Azura Evans[4], Francis Lai[5], Héctor Salvador Hernàndez[6]

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Phase 2a randomized, double-blind, vehicle-controlled trial of NFX-179 Gel in Persons with Neurofibromatosis Type 1 patients with cutaneous neurofibromas.

Gerd Kochendoerfer, *PhD Affiliation: NFlection Therapeutics*. Purpose: Cutaneous neurofibromas (cNFs) occur in virtually all persons with neurofibromatosis type 1 (NF1) and are a major cause of morbidity due to local irritation, pain, disfigurement, and social anxiety. Loss of NF1 gene function in Schwann cell leads to upregulation of the RAS/MAP kinase pathway. Selumetinib, an oral MEK inhibitor, was approved for treatment of inoperable plexiform neurofibromas in children with NF1. However, systemic toxicity including rash and diarrhea, limits the utility of oral MEK inhibitors for treatment of cNF. We developed a novel MEK inhibitor, NFX-179, specifically designed for topical treatment. NFX-179 efficiently penetrates the stratum corneum, potently suppresses MEK, and is rapidly degraded in blood, thereby minimizing systemic exposure. Methods: We completed a double-blind, vehicle-controlled Phase 2a trial of NFX179 in 48 individuals with NF1. NFX-179 gel (0.05%, 0.15%, and 0.5%) or vehicle was applied topically once daily to 5 target cNF tumors for 28 days. cNF were measured by calipers and photographed during treatment. Tumors were excised for p-ERK biomarker analysis at the end of study. The primary endpoint of the study was difference in p-ERK levels among treatment groups. Secondary endpoints included safety and tolerability and change in tumor volume. Results: Forty-seven subjects (mean age 47.2 years, 67% female) completed treatment (1 did not complete treatment due to COVID-19 infection). Treatment with NFX-179 gel led to a doserelated reduction in p-ERK in cNFs at Day 28 with a 47% reduction in the 0.5% NFX-179 gel group compared with vehicle ($p < 0.0002$). No treatment-emergent local or systemic toxicities were observed, including acneiform rash which has been reported with systemic inhibitors. A dose-related reduction in tumor volume at Day 28 was observed, with a 17% mean reduction in the 0.5% NFX-179 Gel group compared to 8% in the vehicle group ($p = 0.073$) Conclusions: This first-in-human study of topical NFX-179 in cNF demonstrated an excellent safety and tolerability profile and potential therapeutic benefit based on biomarker and clinical endpoints. A multi-center, randomized, placebo-controlled phase 2b study of NFX-179 Gel in NF1 patients with cNFs is currently enrolling. In this study, subjects will be treated with NFX179 gel or placebo for six month with a primary endpoint of change in tumor size.

Disclosure of relevant financial relationships: This study was funded by NFlection Therapeutics

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Prevention of neurological signs and symptoms in children with NF1 and Brain stem gliomas-When is too little too late?

Grace Vassallo, *HSS NF1 Genomic Centre for Medicine Manchester University Hospitals NHS Foundation*

Background: NF1 is a common syndrome with a predisposition to central nervous system tumours.

In children these tumours are commonly WHO grade 1 (low grade) gliomas. The commonest sites are the optic pathway (OPGs) and the brain stem. The clinical course of brain stem gliomas in children with NF1 is less well characterised than that of OPGs.[i]

Aim:

To determine the outcome of children with brainstem gliomas in NF1.

Methods:

The NHS funded HSS for Complex NF1 in Manchester UK is one of two HSS in England and serves the North of England. Records of all the children from 0-18 years known to the service during 2022 were reviewed (251) and all children with brain stem gliomas identified.

Results

20 children with brain stem gliomas were identified (7 females and 13 males) Median age 12.5 years Mean 10. (3) children had involvement of most of the brainstem, (5) had a lesion located predominantly in the pons, (9) in the medulla and (3) in the midbrain. Two children required CSF flow diversion with an Endoscopic third ventriculostomy. One child with whole brain stem involvement presented with headache and episodic head tilt with nystagmus-she required chemotherapy, CSF diversion (VP and ETV), posterior fossa decompression and chemotherapy. She is now 16 years of age and well. Three children are on MEK inhibitors for symptomatic progression plexiform neurofibromas in other areas and their lesions are stable. Four children presented with or developed neurological signs. Two had cranial nerve palsies at presentation and one developed them while he was being monitored . All three received chemotherapy with stabilization of tumour size but no improvement in neurology. One child presented with a new onset hemiparesis and is now on chemotherapy. 14 (70%) children had no signs or symptoms.

Conclusion:

In our series most children with NF1 brain stem gliomas are asymptomatic 13 (65%) 2 required only CSF flow diversion. (10%). In the children who presented with neurological deficit or developed it during monitoring chemotherapy stabilized the BSG but their neurological deficit persisted. Better identifiers of this group of children are required as for them chemotherapy is often too little and too late.

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Comparison of a STIR- and T1-weighted-based radiomics model to differentiate between plexiform neurofibromas and malignant peripheral nerve sheath tumors in neurofibromatosis type 1

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Purpose: Plexiform neurofibromas (PNF) and malignant peripheral nerve sheath tumors (MPNST) are typically visualized on short TI inversion recovery (STIR) sequences on MRI due to their uniform ability to suppress fat. However, depending on the imaged body region, STIR sequences are not routinely acquired in the clinical setting. T1-weighted pre-contrast (T1W) sequences are acquired in a more standardized fashion but provide insufficient contrast to identify tumors. Using radiomics – a high-throughput computational method to extract quantitative MRI features – we developed a model based on STIR and T1W sequences to differentiate between NF1-associated PNF and MPNST. Methods: Using a 3D quantitative imaging analysis software (3DQI), 68 MPNST and 79 PNF from nine centers were segmented on STIR sequences (if available) or T2 fat-saturated or T1-weighted fat-saturated post-contrast sequences. Tumor regions of interest (ROIs) were co-registered to T1W sequences. Standard pre-processing included N4 bias field correction to reduce the effect of magnetic field non-uniformity, intensity normalization based on a mean of 120 SI and standard deviation of 80 SI, and resampling to 1 mm3 voxel resolution. 107 radiomic features were extracted from the ROIs on STIR and T1W sequences using PyRadiomics. To classify tumors as PNF or MPNST, we applied the Boruta algorithm and correlation removal for selection of important features on 3DQI. Feature importance was estimated by the decrease in accuracy of a Random Forest model if the feature was randomly permuted. A Random Forest model was built using the top five selected features. The data were divided into a training/validation and independent test set in a 7:3 ratio. Within the training/validation set, five-fold cross-validation was performed and repeated 100 times. Model performance was evaluated using the area under the ROC curve (AUC), sensitivity, specificity, accuracy, and 95% confidence intervals (CI). Results: The top five features selected in the STIR-based model were two shape features (sphericity, flatness) and three texture features (all representing voxel interrelationships within the tumor ROI). The STIR-based model demonstrated an AUC of 0.875 (95% CI 0.868-0.883) in the training/validation set and 0.856 (95% CI 0.727-0.984) in the test set (Table 1, Fig. 1). The top five features selected in the T1W-based model were three shape features (sphericity, flatness, surface-to-volume ratio) and two texture features. The T1W-based model had an AUC of 0.903 (95% CI 0.897-0.910) in the training/validation set and 0.867 (95% CI 0.743-0.990) in the test set (Table 1, Fig. 2). Conclusions: Using MRI sequences that are most sensitive for peripheral nerve sheath tumors and routinely acquired during clinical care, our radiomics models demonstrate high and comparable performance to distinguish between PNF and MPNST on STIR and T1W sequences. Our inclusion of multicenter MRIs acquired on different scanners with heterogeneous imaging protocols enhances model generalizability. In the future, our models can potentially be integrated into the radiologic workflow to help clinicians in the early identification of MPNST or pre-malignant atypical neurofibromas on clinical MRIs.

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RISK FACTORS IN PEDIATRIC MALIGNANT PERIPHERAL NERVE SHEATH TUMORS (MPNST): RESULTS FROM THE FRENCH PEDIATRIC ONCOLOGY SOCIETY (SFCE) COHORT.

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Introduction. MPNST are very rare and aggressive tumors, which affect children, adolescents, and young adults. These tumors appear frequently in the context of type 1 neurofibromatosis (NF1). This study aims to determine risk factors in unselected pediatric MPNST in order to define an adapted therapeutic strategy.

Methods. Multicentric national retrospective study of all pediatric patients (0-18 yo) treated for MPNST, confirmed by a systematic pathology review, in France, from 1995 to 2017 and included in the SIOP MMT-95, EpSSG NRSTS-05 protocols and the French National Pediatric Tumor Registry (RNCE).

Results. Overall, 66 patients (median age 13.0 yo [range, 0.1-18.0]) developed a MPNST located in the limbs (36%), trunk (27%), head and neck (21%) and para-vertebral area (15%). Among them, 48% of patients had NF1, 44% had grade I-II FNCLCC grade tumors while 50% had grade III (not gradable, 4 cases). A majority of patients had localized (94%) and N0 (96%) tumors. In patients with NF1, MPNST developed in preexisting plexiform neurofibroma in 59% of cases. The therapies consisted of surgery (94%), chemotherapy (neoadjuvant (36%) and/or adjuvant (27%)) and radiotherapy (52%). After a median follow-up of 3.8 y [range, 0.0-18.7], 5-year overall (OS) and event-free survivals (EFS) were respectively 46.7% [95%CI, 35.8-60.8] and 40.8% [95%CI, 30.3-54.9]. In multivariate analysis, unfavorable risk factors for overallsurvival were: presence of metastases (OR 6.4 [1.8; 22.0], p=0.001), FNCLCC grade 3 (OR 4.77 [1.4; 15.8], p=0.01), large tumor size (≥10 cm; OR 2.3 [1.1; 4.8], p=0.02) and NF1 status (OR 2.2 [1.1; 4.4], p=0.02).

Conclusion. MPNST had overall poor outcome especially in NF1 patients and in high grade, large and metastatic tumors. These risk factors should be considered to develop a more adapted risk stratification and develop new strategies to improve the survival of the patients. Regular monitoring for early MPNST detection in patients with NF1 also needs to be developed.

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Increased risk of endocrine morbidity in individuals with NF1: A Danish population-based cohort study

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Background: Previous studies have reported an increased risk for endocrine disease in individuals with neurofibromatosis 1 (NF1). However, larger population-based studies giving a comprehensive overview of different endocrine morbidities, including osteoporosis, fractures and chromosomal disorders, and surgeries are lacking. Thus, we conducted a population-based cohort study of 2,467 individuals registered with NF1 in Denmark to assess their risk of endocrine morbidity. Methods: We identified 2,467 individuals with NF1 in the RAREDIS Database and using NF discharge diagnoses of the International Classification of Diseases (ICD) version 8 (743.49) and 10 (Q85.0) in the Danish National Patient Registry. The 2,467 individuals with NF1 were matched on sex and birth year and month to 20,132 population comparisons randomly selected from the Danish Civil Registration System. Endocrine morbidity was assessed using discharge hospitalization diagnoses and information on surgeries from the Danish National Patient Registry and prescribed medication. Cox proportional hazard models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for endocrine morbidity. Results: Individuals with NF1 had an incrased HR for any discharge diagnosis of an endocrine disorder of 1.33 (95% CI 1.26–1.42) compared to the population comparisons. The HR was increased for 9 out of 12 main groups of endocrine disorders, including diseases of the pituitary gland (HR 8.64, 95% CI 6.18–12.08), chromosomal disorders (HR 6.00, 95% CI 3.42–10.48), diseases of the adrenal gland (4.86, 95% CI 3.19–7.42) and parathyroid glands (HR 3.16, 95% CI 2.00–5.00), gonadal (HR 2.68, 95% CI) and bone disorders (HR 2.65, 95% CI 2.19–3.21), osteoporosis (HR 2.19, 95% CI 1.88–2.57), various insuffiencies, including malnutrition (HR 2.12, 95 CI 1.54–2.91), and thyroid diseases (HR 1.62, 95% CI 1.35–1.95). No increased HR was observed for diabetes, obesity or non-osteoporotic fractures. We also found that individuals with NF1 more often had endocrine surgeries than the population comparison (HR 2.07, 95% CI 1.42–3.02), with an increased HR for women with NF1 relative to men with NF1 (HR 2.21 95% CI 1.06–4.61). The HR for any first prescription for diabetic medication was decreased in individuals with NF1 (0.73, 95% CI 0.59–0.89) as well as for estrogens and progesteron in women with NF1 (0.83, 95% CI 0.77–0.91). We found increased HRs for a first prescription of vitamins (1.55, 95% CI 1.18–2.05), minerals (1.77, 95% CI 1.58–1.99) and diuretics (1.32, 95% CI 1.06–1.66) as well as for different types of systemic hormones, including systemic glucocorticoids (1.38, 95% CI 1.27–1.51), thyroid (1.52, 95% CI 1.27–1.81), thyroid hormones (1.60, 95% CI 1.32–1.94) and parathyroid hormones (2.57, 95% CI 1.12–5.87). Conclusion: Individuals with NF1 are at increased risk for a wide range of endocrine hospitalizations, surgeries and medication. Thus, awareness of endocrine morbidity is important in follow-up of individuals with NF1.

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Pregnancy and the risk for cancer among women with neurofibromatosis type 1

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Background: Neurofibromatosis type 1 (NF1) is known for the associated high risk for cancer, amounting for up to 60% over lifetime. Pregnancy may modify the risk for cancer in the general population, yet it is not known whether pregnancy affects the risk for cancer in NF1. Cutaneous neurofibromas may increase in size and number during pregnancy, suggesting a pregnancy-related effect on the growth of NF1-associated tumors. Moreover, Schwann cells from individuals with NF1 exhibit increased cell proliferation in response to sex hormones. The present study aims at characterizing the risk for cancer during and after pregnancy in women with NF1. Methods: The Finnish NF1 cohort encompasses all individuals with verified NF1 who have visited a secondary or tertiary referral center in mainland Finland during 1987–2011. A control cohort has been collected by matching ten controls for each individual with NF1 based on age and area of residence. Information on the pregnancies of women with NF1 and controls was retrieved from the Finnish Medical Birth Register and information on cancer diagnoses was obtained from the Finnish Cancer Registry. Cancers occurring during pregnancy or in the following ten years were examined by computing a standardized incidence ratio (SIR) with a 95% confidence interval (CI) using the general population cancer incidence rates provided by the Finnish Cancer Registry. Results: Totals of 263 pregnancies among 136 women with NF1 and 3,176 pregnancies among 1,720 controls were observed. Two cancers were diagnosed in the NF1 group during pregnancy and the year following the delivery, yielding a SIR of 6.44 (95% CI 1.07 to 19.89). One cancer was observed among controls with a SIR of 0.25 (95% CI 0.01 to 1.08). Within 1-10 years after pregnancy, 13 individuals with NF1 and 21 controls were diagnosed with cancer. The respective SIRs were 7.54 (95% CI 4.15 to 12.41) and 1.12 (95% CI 0.71 to 1.67). The SIR for cancer among women with NF1 aged 20-49 years and without a known history of deliveries was 8.63 (95% CI 6.08 to 11.81). Conclusions: Even though individuals with NF1 show an increased SIR during and after pregnancy, the cancer incidence related to pregnancy is similar to women with NF1 aged 20-49 years in overall. Pregnancy may therefore not increase the cancer incidence among women with NF1, yet further studies are needed.

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Overall survival amongst patients with neurofibromatosis type 1 and malignant peripheral nerve sheath tumor

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Background: Patients with neurofibromatosis type 1 (NF1) have a lifetime risk of 8-13% of developing a malignant peripheral nerve sheath tumor (MPNST). MPNSTs are highly aggressive sarcomas that are difficult to detect and have often metastasized at the time of diagnosis. The prognosis is generally poor, with high mortality and poor treatment outcomes. Several studies have identified NF1 as a poor prognostic factor, but the prognostic role of NF1 is still unclear. This descriptive study will provide new knowledge on the survival of NF1-associated MPNST and compare it to sporadic MPNST within Denmark. Methods: The study is based on two cohorts: 1) Patients with NF1 from the two Danish National Centers of Expertise for NF1, which follows all Danish patients with NF1 of any age and socioeconomic background, and 2) All patients diagnosed with MPNST from the national Danish sarcoma database. All dead and alive patients with NF1 and MPNST will be included in the NF1-subgroup with an observation period from year 2000-2020. All dead and alive patients with MPNST will be included in the MPNST subgroup with an observation period from year 1979-2020. Demographics, NF1 characteristics and MPNST characteristics, including treatment and survival, will be collected. The primary endpoint is overall survival. Results: For the NF1 subgroup, we identified 21 patients with MPNST out of 1066 in the cohort. Two patients were ≤ 17 years, and the median age at MPNST diagnosis was 29.7 years [12.8;67.5]. As of today, eight patients (38 %) are still alive. For the MPNST group, we identified 153 patients with MPNST. Out of those, ten patients also appeared in the NF1 subgroup. Further data and results will be presented at the meeting. Conclusion: Data on NF1-associated MPNST compared to sporadic MPNST will be presented. Results from the study will highlight differences in overall survival between the two subgroups.

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High dimensional imaging highlights the T-cell compartment as a viable therapeutic target in the vestibular schwannoma tumour microenvironment

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Neurofibromatosis type 2 (NF2) is a rare autosomal dominant condition that causes growth of multiple central nervous system (CNS) tumours. Individuals with NF2 almost always develop bilateral vestibular schwannoma (VS), which leads primarily to sensorineural hearing loss, but left unchecked can lead to various other co-morbidities. NF2 is currently incurable and treatment options are very limited beyond surgery. T-cell-based therapies, like checkpoint inhibitors, have shown much success in various cancers, and may serve as promising therapeutic targets for individuals with NF2. The immune tumour microenvironment of VS is poorly understood, and understanding the subcellular architecture of these tumours, and the immunological signature that underpins VS pathogenesis, would aide in the identification of new drug targets for NF2 patients. To this end, we have used Hyperion imaging mass cytometry (IMC) on 7 sporadic VS to uncover the tumour microenvironment and decipher the T-cell compartment of these tumours. Firstly, we characterise the T-cell compartment of these tumours. We illustrate that the majority of tumour infiltrating leukocytes (TILs) present with sporadic VS have an effector-memory CD8+ phenotype, characterised by co-expression of CD8 and CD45RO (data currently not shown). Secondly, we use bioinformatic algorithms to spatially map single cell data from these tumours to examine the localisation of T-cells within VS (Figure 1). Our data demonstrates that T-cell populations within VS co-localise with tumour-associated macrophages (TAMs). Regions of VS dominated by TAMs elicit vast infiltration with TILs, however, where TAMs are sparce, almost no TILs are present. Taken together, our data indicates that TILs present with VS exhibit a functional effector memory phenotype, yet their co-localisation with TAMs suggests that TILs in VS may be sequestered from interacting with schwannoma cells. TAMs are known to form niches of immunosuppression within many different tumour types that limit T-cell responses, and so further investigation into receptor-ligand interactions between TILs and TAMs within VS will highlight the reasons T-cells co-localise with TAMs, which serve as promising targets for therapeutic intervention in NF2.

Improving sensitivity for detection of pathogenic variants in familial NF2-related schwannomatosis.

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NF2-related schwannomatosis is a tumour predisposition disorder resulting from pathogenic variants (PVs) in the NF2 gene. Current clinical genetic screening techniques include next generation sequencing (NGS) analysis of the coding region, and multiplex ligation-dependent probe amplification (MLPA) analysis to identify copy number changes. Less commonly, karyotype analysis is used to detect structural abnormalities that affect the NF2 gene. This screening approach detects over 90% of germline PVs in non-mosaic NF2-related schwannomatosis, but still leaves a proportion of cases meeting clinical criteria with no identified molecular cause. Here we present extended genetic analysis to identify additional variants that were initially missed through standard screening techniques. We analysed DNA extracted from lymphocytes of a cohort of 168 second generation familial NF2-related schwannomatosis cases. Routine genetic testing by NGS and MLPA only, detected PVs in 151 cases (89.99%). No PVs were identified for 6 individuals in our cohort. Splice variants were the most common, accounting for 30% cases, followed by copy number variants (CNVs) and frame-shift deletions (FSDs), which accounted for 17% of total cases each. Nonsense variants, in turn, accounted for approximately 16% of total variants. Extended genetic screening and analytical interrogation enabled identification of PVs in 11 additional cases, increasing sensitivity to 96.43%. The additional variants included chromosomal translocations (5/11), deep intronic splice variants (4/11), and two variants located in the NF2 5' UTR which are thought to disrupt an upstream open reading frame. We characterised the complex structural rearrangement further using whole genome long-read sequencing analysis using the Sequel II platform from Pacific Biosciences (PacBio). This analysis identified additional breakpoints to those observed in short-read WGS analysis, as well as a number of smaller rearrangements within the region structural variants. As the number of cases associated with non-coding PVs and large structural rearrangements continues to increase, it becomes clearer that these variants are important contributors to the genetic landscape of NF2-related schwannomatosis. Extended screening of second generation NF2-related schwannomatosis cases is a valuable tool to address missing heritability and may aid implementation of these techniques for mosaic cases, for which detection rate is currently much lower. This in turn may help improve accuracy of genetic diagnosis within the schwannomatoses and enable better targeted clinical management.

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The inflammatory and microvascular microenvironment in sporadic and neurofibromatosis type II related vestibular schwannoma (VS): a comparative imaging and pathology study

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Objective: Sporadic and neurofibromatosis type II (NF2) related VS are known to differ in their macroscopic structure and clinical behaviour. The extent to which these tumours are similar in their inflammatory and microvascular tumour microenvironment has not, however, been established. In this combined MR imaging and pathology study we sought to compare differences in the inflammatory and microvascular characteristics across both VS groups.

Design: Non-randomized, unblinded prospective study

Subjects: Twelve patients with NF2-related VS and 24 patients with sporadic VS

Methods: Diffusion tensor imaging (DTI) and dynamic contrast enhanced (DCE) MRI datasets from 20 NF2 related and 24 size matched sporadic VS were prospectively acquired. Diffusion metrics (mean diffusivity, fractional anisotropy) and DCE-MRI derived microvascular biomarkers (Ktrans, vp, ve, R1N, tumour blood flow) were compared across the two VS groups. Tissue from 17 imaged sporadic and a separate cohort of 12 NF2-related VS were examined with immunohistochemistry markers for vascular permeability (fibrinogen), microvessels (CD31) and tumour associated macrophage density (Iba1). Expression of VEGF and VEGF receptor 1 (VEGFR-1) was evaluated through immunohistochemistry, western blotting and double immunofluorescence.

Results: Imaging data demonstrated that both sporadic and NF2 related VS displayed increases in Ktrans (Spearman's Rho, $p<0.001$), ve ($p\leq0.004$) and mean diffusivity ($p<0.001$) with increasing tumour size and pre-treatment tumour growth rate, and decreases in both R1N ($p\leq0.003$) and fractional anisotropy ($p\leq0.08$). Regression analysis demonstrated that with the exception of mean diffusivity ($p < 0.001$), NF2 status had no statistically significant effect on any of the imaging parameters or the observed relationship between the imaging parameters and tumour size ($p > 0.05$). Immunohistochemistry confirmed the imaging metrics in sporadic VS and demonstrated that across both sporadic and NF2-related VS there was a close association between tissue microvessel area and Iba1+ macrophage density ($r=0.55$, $p=0.002$). In both tumour groups, there was expression and co-localization of VEGF and VEGFR1 within intratumoural macrophages.

Conclusions: We present the first in vivo comparative study of inflammatory and microvascular characteristics across sporadic and NF2 related VS. We demonstrate that despite the considerable variation in clinical behaviour between these tumours, in terms of their underlying inflammatory and microvascular characteristics, they are strikingly similar. Our results indicate that in both these tumour groups inflammation is a key contributor to the microenvironment and should be viewed as potential therapeutic target.

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Sporadic and NF2 Schwannomatosis Vestibular Schwannomas Share Homogeneous Tumour Microenvironments.

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In the tumour predisposition syndrome NF2-related schwannomatosis – 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 have also been noted in sporadic unilateral VS, which also share histopathological features such as Antoni A and B regions, scarring, and whorls that appear in both NF2 schwannomatosis and sporadic VS tumours. However, previous studies comparing NF2 schwannomatosis and sporadic VS have yet to combine bulk differential gene expression analysis alongside high dimensional tissue imaging at the single cell spatial level to explore the similarities or differences in their tumour microenvironments. The current study drew together bulk transcriptomic data from three individual Affymetrix microarray datasets (GSE54934, GSE108524 and GSE141801) to compare the gene expression profiles of sporadic and NF2 schwannomatosis VS. Additionally, single cell spatial mapping of the VS tumour microenvironment was completed with Hyperion imaging mass cytometry in 12 sporadic VS and 18 NF2 schwannomatosis VS in order to characterise similarities and differences between NF2 schwannomatosis VS and their sporadic counterparts. After differential gene expression and functional enrichment analysis with Ingenuity Pathway Analysis (IPA), NF2 schwannomatosis and sporadic VS samples in all three datasets GSE54934, GSE108524 and GSE141801 showed no distinct separation (Figure 1 using GSE108524 as a representative example), few differentially expressed genes (Figure 2 using GSE108524 as a representative example), and strong similarities in the most significantly dysregulated pathways on IPA (Figure 3 using GSE141801 as a representative example). IPA highlighted the 'Neuroinflammation Signalling Pathway' as the top significantly upregulated pathway in both NF2 schwannomatosis and sporadic VS, indicating similar changes in the immune component of the tumour microenvironments compared to healthy nerve. Furthermore, imaging mass cytometry revealed high Iba1+ macrophage staining in both NF2 schwannomatosis and sporadic VS samples (Figure 4 in sporadic VS as a representative example), with spatial mapping of distinct populations of cells within the tumour microenvironment (Figure 5 sporadic VS as a representative example). Taken together, these data suggest a strong homogeneity in the tumour microenvironments of NF2 schwannomatosis and sporadic VS, which both contain a macrophage rich immune compartment that differs from healthy nerve samples.

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Reprogram the tumor microenvironment to prevent tumor-induced hearing loss and augments treatment efficacy in NF2 schwannoma rodent models

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Hearing loss is one of the most common symptoms of neurofibromatosis type 2 (NF2) caused by vestibular schwannomas (VSs). Fibrosis in the VS tumor microenvironment is associated with hearing loss in patients with NF2. Fibrosis is due to the excessive deposition of extracellular matrix (ECM) components, such as collagen and hyaluronan (HA). The abnormal ECM deposition leads to a buildup of compressive forces that induce vessel collapse, hypoperfusion, poor delivery of drugs, and compromised trafficking of cytotoxic T-cells to these tumors. The resulting tumor microenvironment (TME) is also hypoxic, acidic, and immunosuppressive. Our over-arching hypothesis is that losartan, an FDA-approved antihypertensive drug that blocks angiotensin signaling, can normalize the TME to prevent tumor-induced hearing loss and enhance treatment efficacy. Using NF2 mouse models, we found that losartan treatment normalized the TME by i) reducing neuroinflammatory IL-6/STAT3 signaling and preventing hearing loss, ii) normalizing tumor vasculature and alleviating neuro-edema, iii) increasing oxygen delivery and enhancing the efficacy of radiation therapy, and iv) increasing the intratumoral infiltration of immune effector cells and drug delivery. In preparation to translate these exciting findings into the clinic, we used patient samples and data and demonstrated that IL-6/STAT3 signaling was inversely associated with hearing function, elevated production of tumor-derived IL-6 was associated with reduced viability of cochlear sensory cells and neurons in ex vivo organotypic cochlear cultures, and that patients receiving angiotensin receptor blockers have no progression in VS-induced hearing loss compared with patients on other or no antihypertensives based on a retrospective analysis of patient with VS and hypertension. Our study provides the rationale and critical data for a prospective clinical trial of losartan in patients with VS.

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Susceptibility locus for sporadic vestibular schwannoma identified by genome-wide association analysis

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Vestibular schwannomas (VS) are nerve sheath tumours that arise on the vestibulocochlear nerve and can cause hearing loss and balance dysfunction. VS are known to occur in the context of the predisposition syndromes NF2-related and LZTR1-related schwannomatosis but the majority of VS present sporadically without an identified predisposing germline variants. To identify novel genetic associations with risk of VS, we conducted a genome-wide association study on a cohort of 911 sporadic VS. Genotypes from this cohort were compared to 5,500 control samples identified from the UK Biobank resource. One risk locus, at 9p21.3, reached genome-wide significance in our association analysis. 9p21.3 is a genome-wide association study association hotspot, and a number of genes are localised to this region, CDKN2B-AS1 and CDKN2A/B, also known as the INK4 locus. Dysregulation of the INK4 locus has been associated with multiple pathologies, including neoplasms, and the genes in this region have been observed to regulate the expression of one another directly. Identified associations of the INK4 locus with components of well-described oncogenic pathways provide compelling evidence that the 9p21.3 region is truly associated with risk of VS tumourigenesis.

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Antisense oligonucleotides targeting exon 11 are able to partially rescue the Neurofibromatosis Type 2 phenotype in vitro.

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Neurofibromatosis type 2 (NF2) is an autosomal dominant condition caused by loss of function variants in the NF2 gene, which codes for the protein Merlin, and characterized by the development of multiple tumours of the nervous system (1). The clinical presentation of the disease is variable and related to the type of the inherited germline variant. In this work, we evaluated the use of phosphorodiamidate morpholino oligomers (PMOs) (2) in vitro, to reduce the severity of the effects of NF2 truncating variants with the aim of generating milder hypomorphic forms in vitro through the induction of the in-frame deletion of the exon-carrying variant. With this aim, primary fibroblasts cultures from NF2 patients carrying nonsense variants were treated with the corresponding pair of PMOs to induce exon skipping of exons 4, 8, and 11 of the NF2 gene, according to the exon in which the mutation was located. We were able to specifically induce the skipping of exons 4, 8 and 11 maintaining the NF2 gene reading frame at cDNA level, that was assessed through RT-PCR and confirmed by Sanger sequencing. In order to evaluate the effect of the treatment, we analysed merlin levels, fibroblasts actin cytoskeleton organization and the proliferation capacity. Only the skipping of exon 11 produced a hypomorphic Merlin (Merlin-e11), able to partially rescue the phenotype observed in primary fibroblast cultures from NF2 patients, being encouraging for the treatment of patients harbouring truncating variants located in exon 11. Furthermore, we tested if PMOs could be used to correct the splice signalling caused by variants at +/- 13 from the intron-exon boundary region. Here we show that the PMOs designed for these variants do not constitute a therapeutic approach.

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Multiple meningiomas as a criterion for the diagnosis of Neurofibromatosis Type 2 and other tumor predisposition syndromes

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Background Bilateral vestibular schwannomas (VS) are pathognomonic of Neurofibromatosis type 2 (NF2), but the diagnostic criteria also include unilateral vestibular schwannomas (UVS) in combination with multiple meningiomas (MM) and other schwannomas, as well as MM without VS. Objective To investigate the diagnostic value of these criteria and establish the presence of other genetic conditions in patients presenting in this manner. Methods The (BLINDED FOR REVIEW) NF2 database was accessed to obtain information on patients presenting with a UVS and MM or ≥2 non-intradermal schwannomas (NIDS). We gathered data on patients diagnosed with NF2 due to MM without VS, and on patients presenting with MM without meeting NF2 criteria. Analysis was performed for pathogenic variants (PV) in NF2, SMARCE1, SMARCB1 and LZTR1. Results 131/131 patients presenting with a UVS and MM were diagnosed with NF2 after molecular studies, in comparison with 85/96 patients presenting with UVS and ≥2 NIDS (p=<0.00001). 50% presenting with a UVS and ≥2 NIDS with NF2 developed bilateral VS, compared to only 26% of those who presented with a UVS and MM (p=0.0046). 11/152 patients presenting with MM without fulfilling NF2 criteria were found to have a PV in SMARCE1, and 7/152 were confirmed to have mosaic NF2. Conclusion Patients presenting with UVS and MM are significantly more likely to have NF2 than patients presenting with UVS and ≥2 NIDS, but significantly less likely to develop bilateral VS. 7% of those presenting with MM without meeting NF2 criteria had PV in SMARCE1, and 5% had mosaic NF2.

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POPLAR-NF2: A Parallel-group, Two-staged, Phase 2/3, Randomized, Multicenter Study to Evaluate the Efficacy and Safety of REC-2282 in Participants with Progressive NF2 Mutated Meningiomas

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a) Disclosure of Financial Relationships: All authors are employees of Recursion Pharmaceuticals, Inc.

b) Purpose of the Study: POPLAR-NF2 (NCT05130866) is a Phase 2/3, randomized, multi-center study designed to investigate the efficacy and safety of REC-2282, a pan-HDAC inhibitor, in patients with progressive NF2 mutated meningiomas who have either NF2 disease-related meningioma or sporadic meningiomas that have NF2 mutations.

c) Methods: REC-2282 is an orally bioavailable, CNS-penetrant, pan-HDAC inhibitor. HDAC inhibitors induce cell cycle arrest, apoptosis, autophagy, antiangiogenesis, immune response modulation, and facilitate Akt dephosphorylation resulting in decreases in meningioma and schwannoma cell proliferation. Pharmacologic activity of REC-2282 has been demonstrated in various cancer cell lines and mouse xenograft models. REC-2282 has been dosed in 77 participants in investigator-initiated trials with treatment duration up to 4.4 years. In a limited number of participants with solid tumors median progression-free survival (PFS) was 9.1 months in participants with CNS tumors and 1.7 months in participants with non-CNS tumors. POPLAR-NF2 will be conducted in 2-parts. This study will enroll approximately 90 participants with progressive NF2 mutated meningiomas in North America and Europe. Approximately 20 adult participants will be randomized to one of two dose levels of REC-2282 in Cohort A (Part 1) which will provide early data on efficacy, safety, and pharmacokinetics of REC-2282 and provide guidance for the dose in the Part 2 of the study (Cohort B). The first 8 participants enrolled in Cohort A will complete a food effect run-in sub study. In Cohort B, participants will be randomized to a single dose of REC-2282 (dose to be determined from Cohort A) or placebo in 2:1 ratio. Cohort B will assess the efficacy and safety of REC-2282 compared with placebo in participants with progressive NF2 mutated meningiomas. In both cohorts, there will be a screening period (up to 8 weeks), a treatment period, a 4-week safety follow-up period, and a 6-month follow-up. The primary endpoint in Cohort B is PFS.

d) Results: All participants will have progressive NF2 mutated meningioma with either a confirmed diagnosis of NF2 disease or sporadic meningioma with an NF2 mutation. Efficacy of REC-2282 will be assessed by PFS, objective response rate, time to response, duration of response, and quality of life. Safety will be assessed through monitoring of AEs, changes in laboratory and vital signs, physical examination, and ECG. Plasma and urine PK of REC-2282 will be assessed.

e) Conclusions: POPLAR-NF2 is designed to investigate the efficacy and safety of REC-2282 representing a potential new pharmacologic treatment in patients with progressive NF2 mutated meningioma. Enrollment is ongoing.

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"Immunogenic subtype" characterised by macrophages infiltration predominantly comprise meningiomas in neurofibromatosis type 2 patients

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Whereas recent molecular analysis has revealed that sporadic meningioma has various genetic, epigenetic, and transcriptomic profiles, those of meningioma in NF2 patients are not fully elucidated. This study probed meningiomas' clinical, histological, and molecular characteristics in NF2 patients. Methods: A long-term retrospective follow-up (13.5 ± 5.5 years) study involving 159 meningiomas in NF2 patients was performed. We assessed their characteristics by performing immunohistochemistry (IHC), bulk-RNA sequencing, and copy number analysis. All variables of meningiomas in NF2 patients were compared with those of 189 sporadic NF2-altered meningiomas. Results: Most meningiomas in NF2 patients were stable, and the mean annual growth rate was 1.0 ± 1.8 cm3/yr. Twenty-eight meningiomas (17.6%) in 25 patients (43.1%) were resected during the follow-up period. WHO grade I meningiomas in NF2 patients` were more frequent than in sporadic NF2-altered meningiomas (92.9% vs 80.9%). Transcriptomic analysis for NF2 patients`/sporadic NF2-altered WHO grade I meningiomas (n = 14 versus 15, respectively) showed that tumours in NF2 patients had still higher immune response and immune cell infiltration than sporadic NF2-altered meningiomas. Furthermore, RNA-seq/IHC-derived immunophenotyping corroborated this higher immune response by identifying myeloid cell infiltration, especially macrophages. Conclusions: Clinical, histological, and transcriptomic analyses for meningiomas in NF2 patients demonstrated that meningiomas in NF2 patients showed less aggressive behaviour and elicited a marked immune response than sporadic NF2-altered meningiomas. We suggest that meningiomas in NF2 patients predominantly comprise the immunogenic group with macrophage infiltration and that this predilection contributes to less aggressive tumour behaviour.

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Creation of the international NF Variant Curation Expert Panel to improve genetic testing of NF-SCHW genes

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Purpose of the study: Neurofibromatosis type 1 (NF1), NF2-related schwannomatosis (NF2), SMARCB1 and LZTR1-related schwannomatosis (SWN), and Legius syndrome (LGSS) require a genetic diagnosis (1) to confirm clinical suspicion in patients with indeterminate phenotype, (2) to better understand a patient's prognosis and (3) for family planning1–4. Variant interpretation and classification of the five genes causing these disorders (NF1, NF2, SMARCB1, LZTR1 and SPRED1) is challenging due to the broad mutational spectrum, the paucity of clear mutational "hotspots", and the high proportion of non-coding and splicing variants5–7. In addition, 50% or more of NF and SWN-affected patients have de novo variants; many of these patients do not meet diagnostic criteria in the early stages of these disorders, and for whom an accurate genetic test is critical for clinical use and follow-up. Methods: Thirty-nine individuals from North and South America, Australia and Europe with expertise in NF1, NF2, LGSS, and SWN, (or other related hereditary tumor predisposition pathologies) or from high volume diagnostic labs (public and private) volunteered to develop specific ACMG/AMP rules8 for NF-SCHW genes as members of a Variant Curation Expert Panel (VCEP) in the framework of the NIH-funded ClinGen Hereditary Cancer Clinical Domain Working Group (9, 10). This panel of experts includes clinical and molecular geneticists, variant scientists, genetic counselors, epidemiologists, neurosurgeons, etc.) who regularly participate in the diagnosis and/or clinical management of this group of pathologies. Results: The Neurofibromatoses and Schwannomatoses VCEP is comprised of 5 sub-VCEPs that will address causative genes associated with Neurofibromatosis type 1 (NF1), Legius Syndrome (SPRED1) and NF2, SMARCB1 and LZTR1-related Schwannomatoses (NF2, SMARCB1 and LZTR1). The overall project will begin developing rules for NF1, and subsequent efforts will focus on the remaining four genes. The NF1 sub-VCEP has been organized into 3 working groups (functional, phenotypic, and computational) that will modify the 26 general ACMG/AMP rules to establish specific criteria for the NF1 gene. Conclusions: The NF-SWN Variant Curation Expert Panel has been created to modify and refine specific ACMG /AMP rules for NF-SWN genes to develop a compendium of NF/SCHW gene-specific ACMG/AMP evidence rules that can ultimately ensure more accurate interpretations for clinical use for NF patients.

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Clinical and molecular characterization of patients with neurofibromatosis type 1 and breast cancer

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Studies suggest that women with NF1 have a moderately elevated risk for breast cancer, especially under age 50, and that specific NF1 mutations predispose to BC in NF1. To better elucidate the relationship between NF1 and BC, we reviewed the medical records and analyzed germline NF1 variants in NF1 women affected with BC. Retrospective analysis of 719 NF1 consecutive subjects (311 males, 408 females) identified 41 (5.7%) individuals (all females) with breast cancer (BC). Age at diagnosis for BC was between 34-75 y (median age 47.5 y). 19 patients (44%) had BC diagnosis <50 y. 23 had invasive ductal carcinoma, 3 in situ ductal carcinoma, 4 invasive lobular carcinoma, and 11 unknown. 8 tumors were luminal A, 12 luminal B, 5 HER2 positive, 4 basal like/triple negative, and 12 unknown. Of 24 patients with tumor stage, 3 were stage 0, 8 stage I, 8 stage II, 5 stage III, and 17 unknown. 4/41 (10%) patients had tumor relapse between 4 and 14 years from diagnosis. 27% of NF1-BC patients had concomitant neoplasia: 4 MPNST, 1 GIST, 1 ependymoma, 1 pituitary adenoma, 1 thyroid cancer, 1 pancreatic adenocarcinoma, 1 MPNST and adrenal adenoma, 1 MPNST and GIST. NF1 mutation analysis of 28 NF1-BC cases identified 10 nonsense, 8 frameshift, 5 splicing, 4 missense, and 1 intragenic CNV variants, and absence of WGD. 24 variants localized in the first half and 4 in the second half of the gene. 3/4 missense variants mapped at the N-term of the protein. 10/28 variants mapped within the CSRD domain and 8/28 in the TBD-GRD domain. Unequal distribution and clustering of NF1 variants within specific protein domains suggest the existence of genotype-phenotype correlations predisposing to BC in NF1.

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Whole-body vibration training in addition to muscle-strengthening exercises alone in improving muscle function in children with Neurofibromatosis Type 1 – a randomised interventional trial

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Introduction: Children with Neurofibromatosis Type 1 (NF1) have muscle weakness. Currently no evidence-based intervention exists for improving this. Whole-body vibration (WBV) therapy has been shown to improve muscle function in children with other neuromuscular disorders. Objectives: This randomised trial investigated whether WBV therapy in combination with muscle-strengthening exercises would improve muscle function compared to muscle-strengthening exercises alone, in children with NF1 who have muscle weakness. Methods: Children with NF1 aged 6-16 years with evidence of muscle weakness [grip force standard deviation score (SDS) <-1.0] were randomised to daily muscle-strengthening exercises for 6 months (EXER group), or these daily exercises plus a WBV therapy programme for 6 months (EXER+WBV group). The primary outcome was jumping power SDS measured using mechanography, with secondary and pre-determined exploratory outcomes including jumping efficiency, hopping force, grip force, 6-minute walk test, balance, physical activity intensity, perceived fatigue and quality of life. Qualitative data regarding safety, feasibility and compliance were also collected. Results: Forty-four children were recruited (20 males; age range 6.1-16.5 years, mean age 10.6 years), with equal allocation to EXER group and EXER+WBV group. There was no effect noted on the primary outcome of jumping power SDS between the two groups at the end of the trial (absolute effect size=-0.1, 95% confidence interval -0.5 to +0.3, P=0.53). Similarly, no difference was detected with regards to secondary and exploratory outcomes. No significant changes in muscle function SDS were noted from baseline in either intervention group. Mean reported compliance to muscle-strengthening exercises was 57%, mean measured compliance to WBV therapy was 23%. Compliance was affected by the Covid-19 pandemic and waning of enthusiasm over time. No adverse events were reported. Conclusion: Six months of daily muscle-strengthening exercises alone or in combination with a WBV therapy programme is safe, although compliance was an issue. No difference in muscle function, balance, physical activity, fatigue or quality of life was detected when WBV therapy was added to daily muscle-strengthening exercises. However, this was a pilot study, not powered for moderate effect sizes, and therefore larger trials would be needed to determine the true effect.

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Optic Pathway Gliomas in children with Neurofibromatosis Type 1 – impact on growth and puberty

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Background Neurofibromatosis 1 (NF1) is known to affect children's growth and puberty. One of the characteristic pathologies of NF1 are optic pathway gliomas (OPGs), which are often in close proximity to the hypothalamo-pituitary axis, and could therefore contribute to the growth and pubertal phenotype recognised in NF1. This study explored whether location of OPG and associated treatments could be associated with changes in height and pubertal onset in NF1 children. Methods A retrospective review of case notes was undertaken in 75 children with NF1 known to the Complex NF1 service at Manchester. Information on children's heights, parental heights, sex, age, OPG location, interventions, NF1 inheritance and pubertal timing were collected, with comparison of growth data compared to reference population data to compare standard deviation scores (SDS). Results Mean height SDS at diagnosis of OPG was -0.3 (standard deviation (SD) 1.6). Mean latest height SDS was -0.4 (SD 1.4). No statistically significant differences were notes in height SDS between sex, NF1 modes of inheritance, age at diagnosis of OPG, or when accounting for parental heights. No difference was noted in height SDS between those that had received chemotherapy or not, although a difference was noted between children that had surgical resection (mean height SDS -1.9, SD 1.5) and those that did not (mean height SDS -0.2, SD 1.4) (p=0.02). Location of OPG did not affect height SDS. 28% of children with OPG developed precocious puberty, with hypothalamic (p=0.018) and bilateral (p=0.008) involvement of the OPG significantly associated with increased risk of precocious puberty. Conclusion Generally, NF1 children are shorter than the heights found in this study, suggesting OPGs are not a variable directly influencing height. Furthermore, no specific factors associated with the OPG or its treatment (apart from the largely historical treatment of surgery) appears to impact height. However, precocious puberty was observed at much higher rates in this study than in the NF1 or general population, with hypothalamic or bilateral OPG involvement being even more predictive. This has important clinical implications for the endocrine monitoring of children with NF1 who develop OPGs.

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The Benefits of a Neuro Multidisciplinary Team within an NF1 service

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Neurofibromatosis 1(NF1) is an inherited neurocutaneous disease that predisposes affected individuals to the development of benign and malignant nervous system tumours including brain and spine gliomas. There is also a pre-disposition to other abnormalities of the brain and spine which may require management such as aqueduct stenosis, scoliosis, intradural neurofibromas causing cord compression, and dural ectasia.

In 2009 NHS England commissioned a highly specialised service for individuals with Complex NF1. In the Manchester service we have set up a dedicated monthly Neuro Clinic and MDT to help provide an efficient and high-quality service for adults with brain and spine complications related to their NF1 diagnosis. This consists of a Neurologist, Neurosurgeons, Neuro Radiologist, Geneticist and NF1 clinical nurse specialists. Some of the benefits identified from joint neuro clinics are better treatment planning and compliance, increased service provision due to reduced need for individual specialist appointments, increased monitoring of patients at increased risk of NF1 related malignancy, efficient long term follow up/monitoring due to combined surveillance for those with multiple co-morbidities or requiring surgical intervention. This model of consolidated care has allowed the development of a team specialists with extensive experience in the recognition and management of neuro complications within NF1, a rare disease. Furthermore, it facilitates holistic care and timely treatment decisions with direct communication between specialists and with patients on the same day. The single point of contact may also improve patient satisfaction and qualitative outcomes.

Since the service began, we have seen 421 patients and facilitated 70 interventions via the MDT joint neuro clinic consultation. The clinic has provided a unique opportunity to understand and manage neuro issues in NF1 and enabled the team to work collaboratively as a multi-disciplinary team to take decisions concerning complex situations. The degree of organisation and the type of communication in these MDTs has a direct impact on the quality of patient care provided. The MDT joint neuro clinic is also an opportunity for the team to learn more about the disease and its known and unknown complications and educate the team about the disease and various complexities.

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The Role of a Specialist Pain Clinic within the Complex NF1 Service

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Neurofibromatosis (NF1) is a genetic condition with an incidence of 2500 to 3000. The severity of disease can vary from to person. Patients may face different co-morbidities in their lifetime such as MPNST'S (malignant peripheral nerve sheath tumour) brain and spine gliomas, neurological disabilities, plexiform, subcutaneous and skin neurofibromas, gastrointestinal disorders, and orthopaedic problems such as scoliosis and pseudoarthrosis. All these may pre-dispose to chronic pain in NF1 patients. In 2009 NHS England commissioned a two centre highly specialised service for individuals with Complex NF1. Managing chronic pain effectively in NF1 is challenging due to the potential for multiple aetiology. To try to better understand, manage and personalise the care we provide to our patients with chronic pain, a dedicated pain clinic was started in March 2021. The pain clinic consists of a multi-disciplinary team (MDT) which includes consultant in anaesthesia and pain medicine, consultant clinical psychologist, neurologist, geneticist, and clinical nurse specialists (CNS). Patients are referred to the clinic from within the service and this is coordinated by the CNS. Many of the patients reviewed have suffered chronic pain despite taking significant amounts of analgesia long term. It is therefore vital to review efficacy of medications and re-evaluate symptoms reported and clinical signs to exclude alternative cause of pain particularly in NF1 particularly where patients are at risk of getting malignancies such as MPNST. To date we have completed 6 dedicated pain clinics and 17 patients have been reviewed in total. Patients were asked to complete a validated pain questionnaire and quality of life questionnaire prior to their clinic appointment. Patients reported pain, depression, and anxiety as severe (50%) and moderate (50%). Funding has been approved to continue the clinic based on pilot clinic reviews and outcomes. Although the current post clinic data evaluated does not show significant reduction in pain score, our patients felt it was good to be able to discuss with professionals who can fully understand pain and related issues due to NF1. Overall patient experience has showed this has a positive impact on patients' quality of life. Ongoing outcomes will be evaluated and inform future service development and pathways.

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Beliefs, Screening Attitudes, and Breast Cancer Awareness of Young Women with Neurofibromatosis Type 1: A Reflexive Thematic Analysis

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PhD is sponsored by the Childhood Tumour Trust. No affiliation for MSc. Health Psychology.

Recognising the increased risk of developing breast cancer at a younger age among women with Neurofibromatosis Type 1 (NF1), compared to the general population, qualitative research was conducted using reflexive thematic analysis to examine the beliefs, screening attitudes, and breast cancer awareness of young women with NF1. This was done to explore whether a tailor-made breast cancer awareness intervention would be of benefit to this population. Semi-structured interviews were conducted with women diagnosed with NF1 that were aged 18-40 years, and were recruited via the Childhood Tumour Trust charity. Analysis conducted using reflexive thematic analysis placed the theme of 'the metastasis of malignant information barriers' hierarchically, to communicate the participants' perceptions and personal experiences of information deficiencies surrounding NF1 and breast cancer risk, but these deficiencies also included their direct dealings with health professionals, predominantly General Practitioners. The women interviewed found these professionals as not only lacking awareness about their condition, but also perceived them as unsupportive. These information deficiencies within the main theme are further explored through sub-themes throughout the research. Clinical recommendations include the establishment of accessible and accurate NF1 breast awareness information, and the development and implementation of a breast awareness intervention for young women with NF1. These materials are also envisaged to act as educational resources for health professionals.

Co-Author: Melissa Pilkington

Preliminary results of the TRAIN study: Trametinib in Neurofibromatosis type 1 related symptomatic plexiform neurofibromas.

Christine Noordhoek PhD candidate at the neurology department

Preliminary results of the first two years after the start of the TRAIN study: trametinib in NF1 related symptomatic plexiform neurofibromas. Describing effect on pain, tumor volume, safety and toxicity.

Co-Author: D.C. Noordhoek

Selective lethality screening yields active agents in the treatment of polycomb repressive complex 2 MPNST in Neurofibromatosis, type 1

Christopher Moertel, *University of Minnesota, Department of Pediatrics*

Noting that 80% of NF1-associated malignant peripheral nerve sheath tumors harbor mutations on H3K27M, we pursued synthetic lethality screens using CRISPR/Cas9 generated cell lines, then confirmed our results in patient derived xenografts. The selumetinib/vorinostat regimen derived from these experiments was used successfully in a single NF1 patient with H3K27M mutant GBM and a future phase zero "window of opportunity" trial is planned.

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SELUMETINIB THERAPEUTIC EFFECTS AND SAFETY AT DIFFERENT TIME POINTS IN NF1 PATIENTS WITH PLEXIFORM NEUROFIBROMAS

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Introduction Neurofibromatosis type 1 (NF1) is a multisystemic neurocutaneous syndrome characterized by an altered function of the MAP-kinase pathway. In NF1, this dysfunction results in tumor predisposition, with about 50% of the affected individuals developing one or multiple plexiform neurofibromas (pNFs). pNFs mainly grow during infancy and cause pain, disfigurement, functional loss, and possible threat to life by gaining malignant potential (MPNST). In 2019 selumetinib, a MEK 1/2 inhibitor, was approved by US FDA for the treatment of pNFs in children with NF1. From January 2020, it is also available on a compassionate basis at Gaslini Institute in Genoa. Materials and methods Patients affected by inoperable pNFs and treated with selumetinib for at least six months at Gaslini Hospital were enrolled. Clinical data, laboratory results, the volume of pNFs, and adverse effects were collected in a digital database. Each pNF MRI was segmented in slices and volume calculated using a free open-source image analysis software (Horos). Statistical analysis was performed (Wilcoxon test, and Spearman's correlation coefficient) searching for correlations among drug efficacy, age at NF1 diagnosis or at first pNF occurrence, age at the start of selumetinib, and adverse effects. Moreover, considering pNFs volumes at baseline and after 6 months (T6), the intraobserver agreement was evaluated using the graphical method by Bland and Altman. Results 7 patients with a mean age of 12.3 years at the start of the treatment were included, of whom five presenting a disfiguring pNF. 3/7 children had a one-year-long follow-up, one an 18-month-long. No correlation was found between the age of each patient at the start of treatment and the volumetric variation in the main pNF. Comparing volumes at T6 vs baseline, in 5/6 cases (83%) a reduction was detected, in three over 20%. Wilcoxon test demonstrated a significant reduction in volume. Intraobserver agreement was sufficient, according to Bland and Altman graphical methods. The most reported adverse effects were perionyxis/paronychia in 4/7 patients (57%) of whom 2 requiring minor surgery, facial and/or body acne in 3/7 (43%), cutaneous xerosis in 3/7 (43%), maculopapular rash, thinning of the hair and increased effluvium in 2/7 (29%). No cardiac, ophthalmological, pulmonary, or gastrointestinal adverse events were observed. Conclusions We confirm the therapeutic efficacy of selumetinib on pNFs, with adverse effects being mainly mild and limited to the skin. Volumetric assay of the lesions through open-source software showed sufficient reliability. Prospective data collection will let further statistical analysis.

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Pigment epithelium derived factor regulates melanocyte proliferation and migration in café au lait macules

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Background: RASopathies represent a group of clinically related disorders defined by Ras/mitogen-activated protein kinase (Ras/MAPK) pathway activation. Multiple Café au Lait Macules (CALMs) are a common feature of RASopathies including: neurofibromatosis type 1, 3bp neurofibromatosis type 1 and Legius syndrome. Objectives: Determine (1) the basis for CALM formation by transcriptomic analysis of three genetic disorders and (2) why only neurofibromatosis type 1 is associated with an increased risk for tumour formation that are less prevalent in 3bp neurofibromatosis type 1 and Legius syndrome. Methods: Patients with neurofibromatosis type 1 (n=2), 3bp neurofibromatosis (n=3) and Legius syndrome (n=1) underwent skin biopsies from CALM and unaffected skin. Melanocytes were isolated and propagated so that there were five independent replicated from each tissue sample for sequencing. DNA and RNA were extracted for mutational analysis and transcriptomic profiling. Melanocyte and melanoma cell lines were then used for mechanistic determination. Results: In all cases CALMs were associated with biallelic loss, resulting in amplification of Ras/MAPK and Wnt pathway signalling. In classical neurofibromatosis type 1 there was also concordant activation of the PI3K/AKT pathway. Herein we determined that all CALMs were associated with reduced PEDF, a downstream target of MITF, led to increased melanocyte proliferation, migration and invasion. Importantly re-introduction of PEDF reduced cell proliferation and invasion melanoma with NF1 loss. Conclusions: The melanocyte proliferation and migration leading to CALMs is due to biallelic loss that in turn reduces expression of the tumour suppressor PEDF. Furthermore, the concordant activation of the PI3K/AKT pathway in classical neurofibromatosis type 1 may account for increased risk of tumour formation that is absent in 3bp neurofibromatosis and Legius syndrome.

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Satellite hubs for the Complex NF1 service in the North of England-Fact or Fiction?

Grace Vassallo

Background:

In 2009 NHS England commissioned two Highly Specialized Services (HSS) for the care of patients with complex NF1 [1]based in London and Manchester. The ultimate aim of these services was to provide high quality care for individuals with complex NF1 led by an experienced multidisciplinary specialist team able provide input across geographical boundaries in England thus reducing morbidity and mortality from the disease (with special emphasis on malignancy most prominently MPNST) and inexpert interventions. Geographical distance from the HSS centres could lead to inequality of access for some patients. One way to reduce this would be through Satellite Hubs in key Foundation Tertiary University Hospitals. The challenges for the setting up of these satellite hubs are significant and may be considered hopeless and unnecessary.

We present the data from one of the Manchester Satellite hubs based in Leeds Teaching Hospitals NHS Trust that illustrates that this model is not only possible but essential in a multitude of ways

Aim:

To analyse the demographics of complex NF1 individuals served by the Satellite Hub in Leeds Teaching Hospitals NHS Trust and holistic benefits generated from the Satellite Hub.

Methods:

The satellite hub based at Leeds Teaching Hospitals NHS Trust is Led by a Consultant Clinical Geneticist with a special interest in Cancer Genetics and NF1 with the support of a specialist complex NF1 nurse located in Leeds.

In clinics there is a joint representation of a lead consultant from the Leeds Hub and Manchester Complex NF1 HSS service both providing clinic letters with clear lines of responsibility.

The complex NF1 service supports four Adult Complex NF1 clinics and Six Complex Paediatric NF1 clinics (Two with paediatric neuro-oncologists) per year-both Complex NF1 Consultants are Adult and Paediatric Neurologists respectively.

Results:

The complex NF1 Leeds satellite hub serves 65 patients annually. (Total complex NF1 population served by Manchester=398 (16%)) 26 individuals are adults. (M=10 F=16 age range 18-64 years, Median=33 years) and 39 are paediatric patients (M= 21 F=18 age range 3-17 years Median 11 years). Many have more than one complex indicator. Clinics and investigations are organised locally. Patient knowledge and clinical learning experience is retained locally when patients need access to emergency treatment.

Conclusion:

Satellite hubs are an effective and efficient method of providing expert care for individuals with complex NF1 close to home with far reaching benefits on patients and NHS centres alike.

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NEUROFIBROMATOSIS TYPE 1 IN AUSTRIA: ANALYSING UNMET NEEDS USING DEEP SEMANTIC ANALYSIS

Irina Efimenko PhD, *Semantic Hub, Switzerland*

Neurofibromatosis is a tumour-predisposing genetic disorder. Early diagnostics and general awareness play an important role in successfully managing the disease manifestations and reducing the psychosocial burden both on patients and their caregivers. Deep semantic analysis has been used to analyse unmet needs of patients with Neurofibromatosis and their caregivers in Austria based on their stories and messages shared online in public groups. More than 100,000 online texts extracted from various German- and English-speaking social media have been analysed. Among 30,000 users from Austria (anonymized), several hundred people represent Austrian patients with NF and their caregivers. Knowledge on the following aspects of patient experience was extracted: - patient language (i.e. the way patients / caregivers describe symptoms) with multiple linguistic expressions extracted; - psychosocial burden and Quality of Life: 20+ categories of problems. According to the findings, patients use multiple synonyms and descriptive verbal constructions to explain their symptoms. For example, the description of café-au-lait spots (CALs) is made in different ways by Austrian patients: Café-au-lait-Flecken, CAL-Flecken, CalF, Cafe-Ole-Flecken, Kaffee Ole Flecken, milchkaffeefarbene Hautflecken, bräunliche Flecken, braune Flecken etc. There seems to be a high dependency on the choice of the word depending on a person's general awareness about the disease. This finding could become an important tool in early detection and awareness campaigns. As regards the psychosocial burden, patients share multiple stories mentioning social isolation, challenges in personal and sexual life, difficulties with learning and career, inability to engage in sports, as well as bullying which concerns not only kids and teenagers but also adults. These problems are partly related to symptoms (e.g. inability to do diving or fly in case of tumours) but also to own perceptions (e.g. "hating summer" or speculating that their partner is ashamed of their appearance). Another insight deduced from the results of the analysis points to the problem of public perception with respect to the 2022 monkeypox outbreak. At least one story referred to a photo of a person with facial manifestations of Neurofibromatosis posted online as a monkeypox case. This further stigmatises people with NF. The obtained data represent the "reality check" on NF patients experience in Austria. They reflect the actual burden on patients and caregivers, and allow systematising examples of patient language used to depict manifestations of the disease. These findings could help to improve communication strategies to identify and support NF patients and caregivers looking for information online.

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Malignant Peripheral Nerve Sheath Tumours (MPNST) in Neurofibromatosis Type 1 (NF1) – A 13-year experience from The Manchester Complex NF1 service, UK

Judith Eelloo, RGN, RSCN, MPhil, *HSS Complex NF1 Service, Manchester University NHS Foundation Trust*

NF1 carries a lifetime risk of developing a MPNST in 8-13% of individuals, which may be higher in those with high internal burden or certain type of pathogenic variant. MPNST's in NF1 are associated with a poor prognosis, reflecting the diagnostic challenges, typically high-grade behaviour and limited treatment options. In particular, anatomical location and position within a larger surrounding plexiform neurofibroma often makes complete surgical resection challenging and morbid. In 2009, a two centre Highly Specialised Service was commissioned by NHS England to provide holistic multi-disciplinary care for Complex NF1. A primary focus being the improvement of mortality and morbidity in NF1- associated MPNST. We will present data and disease characteristics, including 5 and 10 year survival, for 99 patients with at least 1 confirmed MPNST, per local and/or central histology. Patients were identified from a prospectively populated database encompassing all patients seen in the Manchester Complex NF1 service since inception in 2009. This will provide us with a unique set of data with which to inform future management pathways for patients with NF1 and MPNST. This is with the aim of increasing survival time by improving referral pathways to ensure rapid diagnosis, central histology review and in collaboration with regional Sarcoma teams develop and deliver clinical trials for treatment of early stage and advanced NF1-associated MPNST.

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OPTIC-PATHWAY MRI IMAGING FINDINGS IN CHILDREN WITH NEUROFIBROMATOSIS TYPE-1 (NF-1)

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BACKGROUND/OBJECTIVES: Optic-pathway-glioma (OPG) represents the most common central-nervous tumor in children with neurofibromatosis type-1(NF1), occurring at an incidence of 15-20%. It is estimated that 1/3 of NF1 patients (pts) with OPG will need treatment. DESIGN-METHODS: We performed a retrospective-review of all NF1-pts examined in the First Hellenic Multidisciplinary-Clinic – Center of Expertise for Neurocutaneous-disorders. Gender, age, MRI-radiological and ophthalmological findings, presence of OPG, management and outcome were analyzed. RESULTS: Since the establishment of the Clinic in 2016, 198 pts with clinical diagnosis of NF1 based on NIH1988-criteria were evaluated and of them, 165 (73 females, median age: 5.5y, range:0.3-17.1y), who had imaging studies were included in this analysis. Eighty three pts(50.3%) had NF1-positive genetic-testing and 45 NF1-family-history(27.3%). Imaging findings from optic pathway were found in 55/165pts(28females). Percentage of pts with findings were 51.7% for <3y, 45.4% for 3-5y, 34.7% for 5-10y and 8.5% for >10y, respectively. The median age of their first brain-MRI imaging was 2.82y. Upon 1st MRI imaging, 70.9% presented thickness of the optic nerves (ON)(25,4%bilateral, 20% optic chiasm,18.1% right ON, 10.0% left ON), 14.5% ON-tortuosity, 38.1% OPG (43,5% in the optic-chiasm) and 34.5% contrast enhancement. Of notice, 14pts presented an OPG after a median follow-up time of 1.79y. According to LGG2004-protocol indications for treatment, only 15/55pts had to be treated(27,2%, 5pts with family history, 33.3% between 5-10y). Severe vision loss with need for immediate start of treatment upon 1st MRI imaging was found in 4 patients, of whom 75% had family history and first evaluation after the 5th year of age. Of notice, only 2pts under the age of 3y had to receive treatment, one with family-history and one with symptoms (diecenephalic syndrome). CONCLUSIONS: Pts with NF1 should be followed by a multidisciplinary team. Management should be individualized and imaging studies can be limited to patients at high risk. Positive family history may be a negative prognostic factor for OP lesions.

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Development of a core outcome set for cutaneous neurofibromas trials: results of a systematic review, focus groups and e-Delphi

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Cutaneous neurofibromas (cNF) affect more than 95% of individuals with neurofibromatosis 1 (NF1). They are considered by patients as one of the greatest medical burden because of physical disfigurement. To date, procedural-based methods are the only treatments available for cNF; however, we are currently witnessing the early stages of the development of medical treatments. Because no consensus on outcomes for NF1 exists, researchers use various instruments, which may or may not be valid. In order to tackle these issues, the REiNS Skin Working Group, part of the REiNS international consortium was formed. Its main goal is to develop a core outcome set (COS) of domains for cNF. This COS represents a minimum set of agreed-upon outcomes to be measured in the upcoming clinical trials targeting cNF, allowing the comparison of treatment effectiveness. Supported by C3 collaboration, the methodology was based on the following steps. First, we performed a systematic review of the literature (from inception to 08/20/21, on PubMed, Embase et Clinicaltrials.gov) to identify the outcomes reported in studies investigating cNF treatments (Figure 1). Second, to identify items of importance to patients focus groups were held with French (n=6) and international NF1 individuals or representatives (n=2), alongside to a survey sent to a French national e-cohort (ComPaRe, n=234). To identify items of importance to health-care professionals (HCP), we performed focus groups with French (n=10) and international NF1 experts (n=12), semi-structured interview with a psychologist and a nurse coordinator involved in the care of NF1 individuals. Third, an international e-Delphi was held (3 rounds) alongside regular meeting of the Skin Working Group. Thirty studies met the inclusion criteria. A total of 24 outcomes were identified in those studies. Seven additional outcomes were identified by individuals with NF1 and HCP. We thus generated a list of 31 candidate outcomes (Table 1). The first two rounds of the Delphi revealed differences between patients' and HCP's responses demonstrating the need for new focus groups. The next steps of the project are: i) to submit this list of candidate outcomes and their definitions to 3 focus groups including 5 persons living with NF1 who had already participated in the two delphi rounds (1 in France, 1 in the US and 1 in the rest of Europe), ii) to launch the third and last round of the delphi, iii) the final international consensus meeting. This project will hopefully allow the comparison of treatment effectiveness across trials. Figure 1 – PRISMA diagram illustrating the systematic review of the literature (from inception to 08/20/21, on PubMed, Embase et Clinicaltrials.gov). Table 1- List of candidate outcomes generated after the systematic review of the literature, and the focus groups and the survey sent to a French ComPaRe NF1 e-cohort.

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Application of droplet digital PCR to the molecular characterization of recurrent deletions of the NF1 locus

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Application of droplet digital PCR to the molecular characterization of recurrent deletions of the NF1 locus
Synopsis: Background: Neurofibromatosis type 1 (NF1) is caused by loss-of-function variants in the NF1 gene, among which ~5-10% are deletions of the whole NF1 locus. Such deletions are associated with a more severe form of the disease (Pacot et al. Cancers 2021). Three recurrent types of deletions have been described this far and are caused by non-allelic homologous recombination mediated by low copy repeats at the NF1 locus. Here, we describe a new approach using droplet digital PCR (ddPCR) to quickly and easily specify the three different types of recurrent deletions of the NF1 locus and distinguish them from atypical non- recurrent deletions. Methods: A total of 121 index cases with an NF1 deletion were included. All patients were phenotypically described and 109had previously been molecularly characterized using an MLPA typing (Pacot et al. Cancers 2021). Seven ddPCR probe sets distributed all along the NF1 locus were selected to closely delimitate the three recurrent deletion types, and an additional probe set was selected in the fourth exon of NF1 as an internal control. Results: Among the 121 deletions analyzed: 76 were type-1 (63%), 22 were type-2 (18%), 6 were type-3 (5%), and 17 showed an atypical profile (14%). Five patients had a mosaic deletion, four of which were type-2. Comparison with MLPA results showed perfect match results with ddPCR, with the exception of one patient (99% concordance). The only discordant result showed a type-2 profile in MLPA, but the results in ddPCR clearly indicate the non-deletion of the SUZ12 gene, a profile that was previously named "group #2A". Mosaicism levels were comparable between the two techniques. Conclusions: We developed a quantitative, sensitive, and efficient ddPCR approach to characterize recurrent deletions of the NF1 locus. This technique is comparable to MLPA for type-1 and type-3 deletions and is more resolutive to distinguish between type-2 and "group #2A" deletions. Given the importance of the SUZ12 co-deletion in the tumor phenotype more often associated with deletions, it is important to finely characterize these rearrangements at the molecular level to allow more precise and specific genetic counseling in patients with large NF1 deletions in the future.

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NF1 point mutations associated with a specific phenotype: the French experience

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Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder caused by loss-of-function variations in the NF1 gene. NF1 has a high mutation rate, responsible for the high frequency of the disease (about half of cases are sporadic) and for a great variability of pathogenic variants (more than 3,000 reported to date). Only a few NF1 variants have been associated with a specific clinical presentation, either causing a milder form of the disease without any neurofibromas (Forde et al. Eur J Hum Genet 2022; Koczkowska et al. Hum Mutat 2020; Rojnueangnit et al. Hum Mutat 2015), or on the contrary, leading to a more severe phenotype (Koczkowska et al. Hum Mutat 2020; Koczkowska et al. AJHG 2018). Deletions of the NF1 locus, in particular, are responsible for a higher risk of developing several symptoms associated with NF1 (Pacot et al. Cancers 2019). We present here the clinical description of a French cohort of patients harboring a variant of the amino acids previously correlated with a specific form of NF1: Met1149, Arg1809, Arg1276, Lys1423, and codons 844-848 of neurofibromin. Methods: Patients were enrolled and phenotypically characterized with a standardized questionnaire between 2005 and 2020 from a large French NF1 cohort. Phenotypic descriptions of the different groups were compared to that of a "classic NF1" reference population (Koczkowska et al. AJHG 2018). Results: In total, clinical data were collected for 2 patients with a missense mutation at position Met1149 of neurofibromin, 21 at Arg1809, 23 at Arg1276, 31 at Lys1423, and 25 at codons 844-848. Median ages were 16.5, 18, 17, 19, and 17 years old respectively. As previously described, none of the patients mutated at the Met1149 or Arg1809 positions had any neurofibroma. Also, cutaneous neurofibromas were less frequent in Arg1276 patients than in the "classic NF1" cohorts (46% vs 90.7%), while spinal neurofibromas were more frequent (50% vs 1.7%). Arg1276, Lys1423, and 844-848 patients developed more frequently plexiform neurofibromas (60%, 63%, and 43% vs 18.5%, respectively). Bone abnormalities were more frequent in Lys1423 and in 844-848 patients (80 and 61% vs 15.2%). Contrary to what was described in other cohorts, cutaneous neurofibromas were not less frequent and optic pathway gliomas were not more frequent in the 844-848 patients. Conclusions: Altogether, our cohort description is in line with previous reports in international cohort studies. Some discrepancies were observed with 844-848 patients, suggesting individual-codon correlations might be more relevant in this region of neurofibromin.

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Contribution of whole genome sequencing in the molecular diagnosis of mosaic partial deletion of the NF1 gene in neurofibromatosis type 1

Laurence Pacot, d' organeFédération de génétique et médecine génomique, Hôpital Cochin, *AP-HP.Centre - Université Paris Cité*

Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant disease with complete penetrance but highly variable expressivity. In most patients, Next Generation Sequencing (NGS) technologies allow the identification of a loss-of-function pathogenic variant in the NF1 gene, a negative regulator of the RAS-MAPK pathway. Methods: We describe the 5-year diagnosis wandering of a patient with a clear NF1 clinical diagnosis, but no molecular diagnosis using standard molecular technologies. Results: The patient presented with a typical NF1 phenotype but NF1 targeted NGS, NF1 transcript analysis, MLPA, and array comparative genomic hybridization failed to reveal a genetic aberration. After 5 years of unsuccessful investigations, trio WGS finally identified a de novo mosaic (allele frequency ~14%) 24.6kb germline deletion encompassing the promoter and first exon of NF1. Conclusions: This case report illustrates the relevance of WGS to detect CNVs and other structural variants that would be missed by targeted NGS, genotyping, or aCGH. WGS outperforms microarrays for the detection of clinical relevant CNVs, arguing for its use as a single assay for genetic variation detection. In the present case, the mosaic deletion of a GC-rich region was not previously detected by standard approaches. The identification of the causal pathogenic variant allowed a tailored genetic counseling with a targeted non-invasive prenatal diagnosis by detecting the deletion in plasmatic cell free DNA from proband's pregnant partner. This report clearly highlights the need to make WGS a clinically accessible test, offering a tremendous opportunity to identify a molecular diagnosis for otherwise unsolved cases (Pacot et al. Hum Genet 2022).

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Pathogenic neurofibromatosis type 1 (NF1) RNA splicing resolved by targeted RNAseq

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Pathogenic neurofibromatosis type 1 (NF1) RNA splicing resolved by targeted RNAseq M.J. Blok, PhD Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, The Netherlands GROW Institute for Developmental Biology and Cancer, Maastricht University Medical Center, The Netherlands Neurofibromatosis type 1 (NF1) is caused by loss-of-function variants in the NF1 gene. Approximately 10% of these variants affect RNA splicing and are either missed by conventional DNA diagnostics or are misinterpreted by in silico splicing predictions. Therefore, a targeted RNAseq-based approach was designed to detect pathogenic RNA splicing and associated pathogenic DNA variants. For this method RNA was extracted from lymphocytes, followed by targeted RNAseq. Next, an in-house developed tool (QURNAs) was used to calculate the enrichment score (ERS) for each splicing event. This method was thoroughly tested using two different patient cohorts with known pathogenic splice-variants in NF1. In both cohorts all 56 normal reference transcript exon splice junctions, 24 previously described and 45 novel non-reference splicing events were detected. Additionally, all expected pathogenic splice-variants were detected. Eleven patients with NF1 symptoms were subsequently tested, three of which have a known NF1 DNA variant with a putative effect on RNA splicing. This effect could be confirmed for all 3. The other eight patients were previously without any molecular confirmation of their NF1-diagnosis. A deep-intronic pathogenic splice variant could now be identified for two of them (25%). These results suggest that targeted RNAseq can be successfully used to detect pathogenic RNA splicing variants in NF1.

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Employment, occupation and income in adults with neurofibromatosis 1 in Denmark: A population- and register-based cohort study

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Neurofibromatosis 1 (NF1) is a genetic disorder associated with an increased risk for a high somatic disease burden, psychiatric disorders and neurocognitive deficits as well as a lower educational level, which might impact both employment and income. However, little is known of the employment status and disposable income in adults with NF1. Thus, the aim of the present study was to assess employment status, occupational position and disposable income in adults with NF1 using a population-based study design. Methods: From the Danish National Patient Registry and the two national Center for Rare Diseases, we identified 1,469 adults with NF1, who were matched to 11,991 randomly selected population comparisons on sex and birth year and month. Annual information on employment, unemployment and health-related unemployment as well as occupation and disposable income was ascertained from Statistics Denmark between 1980 and 2019. Logistic and linear regression with 95% confidence intervals (CIs) was conducted to assess the association between NF1 and employment status, income and occupation. Results: Adults with NF1 had a lower OR for employment (OR 0.54, 95% CI 0.47–0.63) and higher OR for health-related unemployment (OR 4.72, 95% CI 3.55–6.26) at age 30 years than population comparisons, which persisted at age 40 and 50 years. Hospitalizations for somatic and psychiatric diseases decreased the OR for employment, but increased the risk of health-related unemployment. Adults with NF1 had around 90% of the disposable income of the comparison cohort, but were not more often in the lowest income group (ORage30 0.92, 95% CI 0.70–1.20). Finally, adults with NF1 were less likely to be in a high skilled occupation at age 30, 40 and 50 year (ORage30 0.48, 95% CI 0.52–0.80). Conclusion: Adults with NF1 have a lower employment rate, which was mainly due to health-related reasons and a slightly lower disposable income then adults without NF1.

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Selumetinib-induced cutaneous reactions in children: a single-center interventional study

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Neurofibromatosis type 1 (NF1) affects nearly 1 in 3000 individuals worldwide. Plexiform neurofibromas (pNF), congenital benign peripheral-nerve tumors, occur in around 50% of NF1 individuals and carry a 15% lifetime risk to progress to malignancy. Surgical approaches often are unsuccessful in eradicating the tumor. Selumetinib is an oral selective inhibitor of mitogen-activated protein kinase 1/2 (MEK), recently approved for the treatment of symptomatic inoperable pNF in children. Cutaneous reactions (CR) are the most common side effects of this therapy in children. Most of the literature regarding the management of CR describes the adult experience and very few recommendations regard pediatric patients. The aim of this study is to determine the frequency and spectrum of CR in a pediatric cohort receiving selumetinib and to propose an algorithm for a prompt and effective management of selumetinib-induced CR in children. On a cohort of 18 patients receiving selumetinib at Giannina Gaslini Children's Hospital, 18 (100%) presented at least one CR; the most frequent side effect was paronychia, reported by 13 patients (72%), of whom 4 (31%) classified as grade 3 according to the National Cancer Institute’s common terminology criteria for adverse events, version 5.0. Therefore, according to the current literature on this topic, we proposed a management algorithm to improve the children's quality of life under treatment and avoid drug discontinuation. Future aims should include randomized clinical trials to validate these recommendations and optimize care for NF1 children with selumetinib-induced CR.

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Morbidity underlying the reduced labor market participation in neurofibromatosis 1

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Introduction: Neurofibromatosis 1 (NF1) is a multisystem disorder associated with various morbidities such as cancer, cardiovascular disease, osteoporosis and a variety of behavioral and cognitive deficits. NF1 is associated with a lower level of educational attainment. NF1 also negatively affects the income earned by the affected individuals, partly due to a reduction in labor market participation. Methods: The Social Insurance Institution of Finland records the causes of sickness allowances and disability-related pensions. We analyzed these data from the years 1996-2014 using the Finnish cohort of individuals with verified NF1 and a ten-fold control cohort matched with age, sex and area of residence. The analysis was focused on individuals aged 18-59 years. Rate ratios (RRs) and their 95% confidence intervals (CIs) were estimated using generalized linear mixed effects models with Poisson distribution. Results: Among the 924 individuals with NF1 and 10,126 controls aged 18–59 years in 1996-2014, 482 individuals with NF1 and 4,458 controls had at least one period of sickness allowance within an average follow-up time of 9.8 years (SD 6.3) per individual with NF1 and 11.0 years (SD 6.2) per control. The causes of sickness were highly concordant with the previously reported morbidity profile of NF1 including neoplasms (RR 7.67, 95% CI 7.51-7.84), diseases of the circulatory system (RR 2.18, 95% CI 2.10-2.27), mental and behavioral disorders (RR 1.28, 95% CI 1.25-1.30), diseases of the nervous system (RR 2.07, 95% CI 2.00-2.15), and diseases of the musculoskeletal system (RR 1.05, 95% CI 1.03-1.07). The same diseases also contributed to the significantly higher risk for disability-related pension in NF1 compared to the controls. Conclusions: Several NF1-related co-morbidities contribute to the high rates of sickness absence and disability-related pension in NF1 and thereby reduce the labor market participation of working-age individuals with NF1.

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Current perspectives on the transition from pediatric to adult care for adolescents with Neurofibromatosis type 1: a 3 years' experience in the Netherlands

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BACKGROUND The transition from pediatric to adult Neurofibromatosis type 1 (NF1) care is known to be challenging. Given the nature of NF1, a chronic neuro-cutaneous disease with increased rates of cognitive and social problems and with a life-long risk of developing various malignancies, the importance of continuity of care is emphasized. In particular the adolescent period may be at risk for adverse outcomes and loss to follow up, which is associated with a negative impact on life [1,2]. Previous studies in the Netherlands have shown that the transition period for patients with NF1 did not meet the needs [3]. Therefore, we started in 2019 the NF1-Transition Outpatients Clinic (NF1-TOC) at the Erasmus MC in Rotterdam. The aim of the NF1-TOC is foremost to prevent loss to follow up. It also provides structured care for patients and their families and it stimulates local and national collaboration between health professionals in clinical care for NF1. METHODS The NF1-TOC starts with a multidisciplinary meeting with a dedicated pediatric and adult team for NF1. In the meeting we discuss medical complexity, neurocognitive-, psychosocial functioning and whether patients are to be referred to the Erasmus MC NF1 Expertise Centre or to one of the eleven Treatment Centers within the national NF1 network. Efforts are made to provide care close to home if possible, but centralized complex care is provided when necessary. A joint consultation with the patient, their family, and health professionals from pediatric and adult care takes place on the same day. The NF1-TOC is being coordinated by nurse practitioners. In a cohort study, we evaluated the number of patients seen at the NF1-TOC and the number of loss to follow up three years after transfer. RESULTS Between May 2019 and June 2022 a total of 67 patients in the age of 16-18 years were discussed at the multidisciplinary meeting for transition at the NF1-TOC. Only 58 were ready for transfer to adult care. Therefore, 42 patients were referred to the adult care in the Erasmus MC NF1 Expertise Center. Evaluating medical records, 33 patients showed up at the first consultation in adult care, 7 patients have future appointments and only 2 did not show up at the first consultation in adult care. CONCLUSION A structured NF1-TOC with a joint consultation of dedicated pediatric and adult care health professionals for patients with NF1 seems effective, although based on relative short period and small sample. Our experiences may contribute to other NF1-TOC's to be developed. Critical factors for successful transition will be further discussed.

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Familial transmission of a ring chromosome 22 associated with features of neurofibromatosis type 2 (NF2) without intellectual disability

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Familial transmission of a ring chromosome 22 associated with features of neurofibromatosis type 2 (NF2) without intellectual disability Dr. Aliza Imam (BSc (Hons) Kings College London, MBBS Hull York Medical School) Yorkshire Regional Genetics Service, Chapel Allerton Hospital, Leeds Teaching Hospitals NHS Trust, Leeds, UK The presence of a constitutional ring chromosome 22 (r(22)) can lead to the development of neurofibromatosis type 2 (NF2) related tumours due to loss of the r(22) during mitosis with subsequent somatic abrogation of the remaining NF2 allele. Due to the unstable nature of ring chromosomes, most r(22) are de novo and often associated with intellectual disability due to a chromosome deletion. They are also a cause of Phelan-McDermid syndrome, when associated with a specific deletion of 22q13.3 involving at least part of SHANK3. 16% of patients with Phelan-Mcdermid syndrome due to a r(22) have NF2-related tumours. MRI surveillance is recommended in this group of patients, starting at the age of 10 and continuing into late adulthood (Ziats et al. 2020). There are rare reports of families with inherited r(22), although NF2-related features have not been described in these pedigrees. We present a three-generation family with a heritable r(22) not associated with intellectual disability or a chromosome deletion, but with radiological evidence of NF2-related tumours. A r(22) was identified incidentally in the proband at the age of 22 on karyotype analysis following an intrauterine death. At the age of 42, the patient developed neurological symptoms and an MRI revealed multiple cranial and spinal meningiomata. Her two sons have inherited the r(22), one of whom had a small left-sided vestibular schwannoma and two small meningiomas identified on his first screening scan at the age of 29, thus meeting clinical diagnostic criteria for NF2. Her other son is awaiting screening. The proband's mother also has multiple meningiomas identified on scan in her forties, but has not engaged with having genetic testing. This family shows that NF2 tumours due to loss of a r(22) during mitosis can occur in families with a r(22) that appears stable during meiosis, highlighting the need for prenatal genetic counselling and appropriate surveillance regimens to identify NF2-related tumours. The tumours in this family were identified in adult life, however no childhood screening was performed, so further research is needed to define the NF2-related phenotype of families with heritable r(22) without a deletion. Before this information is available, we would propose a similar surveillance regimen for those with an intact r(22) to those with NF2 due to a pathogenic germline NF2 variant.

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Impact of essential interventions in Paediatric NF2- A significant added burden on children and their families

Eusra Hassan, *Paediatric Neurology Department, Manchester University NHS Foundation Trust, UK. HSS NF2 Service, Manchester University NHS Foundation Trust, UK*

Impact of essential interventions in Paediatric NF2- A significant added burden on children and their families Author: Dr Eusra Hassan (1,2) MBChB, MPhil, MRCPCH (1)HSS NF2 Service, Manchester University NHS Foundation Trust, UK (2)Paediatric Neurology Department, Manchester University NHS Foundation Trust, UK Background: The care of individuals with Neurofibromatosis Type 2 (NF2) is complex and highly specialised. In 2009 NHS England set up 4 highly specialised services in the UK to centralise this expertise and improve long-term survival. Aim: To explore the impact of invasive interventions on children with NF2 who are cared for by the Manchester NF2 service. Methods: Records of all the patients seen by the NF2 service were analysed. 33 children from 0-18 years were identified. 22 children had a family history (8 F/ 14M). Median age 13 years and mean age 9 years. Severity spectrum and grades were as follows: 1 child had mild clinical phenotype/grade 1, 9 children had moderate severity /grade 2a, 7 children had moderate severity /grade 2b and 5 had severe phenotype/grade 3 (Halliday D, 2017). There were 11 children with no family history of NF2 (4 F/7 M), with a mean age of 12 years and median age of 14 years. 8 children had severe phenotype grade 3, 2 children had moderate phenotype grade 2b and one had mild grade 2a. Results: Children with grade 3 severe phenotype were most likely to have had interventions. 10 children had severe NF2 phenotype grade 3 and 2 children had moderate 2b. The negative impacts of these interventions are listed below: (1) Severe needle and hospital phobia (3 children) (2) Loss of vision due to resection of optic nerve meningioma (1 child) (3) Severe Hemiparesis secondary to brain stem ischaemic arterial stroke (1 child) (4) Young age at major surgery (2 children) (5) Rapid regrowth of resected meningiomas (1 child) (6) Kidney impairment secondary to Avastin needing Enalapril (1 child) (7) Raised haematocrit secondary to Avastin (1 child). (8) Cervical vertebral fusion after two major surgeries. (1 child). Conclusion: Improved therapeutic options for children with NF2 come with their own cost. Children with the more severe phenotypes (3 and 2b) are more likely to be at risk of complications. Targeted support for these children is essential.

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Proton Radiotherapy for Vestibular Schwannomas in Patients with NF2-related schwannomatosis: a Case Series

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Objective: To evaluate the efficacy and treatment-related toxicity of proton radiotherapy (PRT) for vestibular schwannoma (VS) in patients with neurofibromatosis type 2-related schwannomatosis (NF2). Materials & Methods: Charts of NF2 patients treated with PRT for VS between 2004 and 2016 at the Massachusetts General Hospital (Boston, USA) were retrospectively reviewed. Diagnosis of NF2 was made according to the 1997 Manchester criteria. Prescribed dosage was 12 Gy(relative biological effectiveness: RBE) in a single fraction (radiosurgery) or fractionated therapy with 1.8 Gy(RBE) daily in 28 to 31 fractions, resulting in a total dose of 50.4 to 54 Gy(RBE). Outcomes of interest were tumor volumetrics, tumor control (defined as not requiring salvage treatment during follow-up), facial and trigeminal nerve function, hearing, tinnitus and vestibular symptoms. Results: Eight NF2 patients treated with PRT for a vestibular schwannoma were included (median age 36 years, 50% female). Five (63%) received fractionated PRT and three (38%) PRT radiosurgery. Median follow-up duration was 70 months. Six patients (75%) received prior VS surgery, of which three also received bevacizumab. Local tumor control was achieved in 6/8 patients (75%). Two patients (25%) with residual hearing lost it after PRT, six had already lost ipsilateral hearing prior to PRT. Tumor and/or treatment-related morbidity was evaluated in six patients. After PRT, facial paresis occurred or worsened in five (83%), two developed trigeminal hypoesthesia (33%), two developed tinnitus and vestibular symptoms occurred or worsened in four (67%). Conclusions: PRT for NF2-related VS provides good local tumor control. Tumor and/or treatment-related cranial nerve deficits seem common. This is at least partly explained by the use of PRT as salvage treatment after surgery or bevacizumab in the majority of this cohort. The efficacy and toxicity of PRT as primary treatment for VS in NF2 patients remains therefore to be determined.

SPECTRUM OF MALIGNANCIES IN CHILDREN WITH NEUROFIBROMATOSIS (NF): 6-YEARS-EXPERIENCE FROM THE FIRST GREEK NF-CENTER

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Background/Objectives: Neurofibromatosis (NF) type-1(NF1) and type-2(NF2) are distinct genetic clinical syndromes in which affected individuals develop both benign and malignant tumors that predominantly affect the nervous system. In order to standardize and improve the clinical care of NF patients, the first national center of reference was established in 1/1/2016. Design/Methods: To describe the spectrum of malignancies in patients (pts) with NF examined from January 2016 until December 2022 in our NF reference center. Clinical diagnosis of NF1 was based on National Institutes of Health diagnostic-criteria (NIH,1988), while for NF2 on National Neurofibromatoses Foundation Criteria (NNFF,1997). Pts were evaluated by a multidisciplinary team including neurologist, dermatologist, oncologist, ophthalmologist as well as psychologist. Genetic counseling was provided in all the families and regular follow up for the pts was arranged. Results: During the study-period 203pts (age 4mo-17y, median 6.1y) were examined and included in the analysis. Clinical diagnosis of NF1 and NF2 had 198pts and 5pts respectively. NF1 confirmation with molecular testing was available in 83pts (45 NF-1 familial history). Among the 83pts, there were 4 and 2 families with 2 and 3 affected children respectively, while 7 patients were referred due to positive family history. Optic-Pathway-Gliomas (OPGs) were found in 43.5% of NF1pts, of whom 15 had visual deterioration during follow up and received chemotherapy. Two siblings with NF1 and OPGs presented with non-reversible blindness. Non OPGs were diagnosed in 8pts, 4 located in brainstem. Neurofibromas were diagnosed in 20pts (10.1%), Plexiform neurofibromas in 18pts(9.1%), and malignant peripheral sheath nerve tumors in 3pts(1.5%). Of notice, symptomatic large plexiform neurofibromas with need for treatment were observed in 5 pts(2.5%). Bilateral vestibular schwannomas causing mild deafness with previous history of two resected meningiomas were observed in two out of three patients with NF2. Meningioma was histologically diagnosed in 1 NF2pt. Indication for oncologic intervention was needed in 13.1% of the patients. Overall survival was 98.4% for NF1 pts and 100% for NF2 pts. Conclusion: Establishment of a Multidisciplinary Center for Neurofibromatosis can improve clinical care by providing necessary multidimensional approach and contributing to early diagnosis and timely therapeutic intervention.

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