Plexiform Neurofibromas in Neurofibromatosis Type 1

Daniëlle Christine Noordhoek

Propositions as part of the thesis

Plexiform Neurofibromas in Neurofibromatosis Type 1

1. Early detection of malignant peripheral nerve sheath tumors in patients with neurofibromatosis type 1 requires regular follow-up, education on warning signs and identification of patients at risk.
   (this thesis, chapter 2)

2. High-resolution nerve ultrasound can be used to screen patients with neurofibromatosis type 1 to identify patients with high plexiform neurofibroma tumor load.
   (this thesis, chapter 3 and 4)

3. Clinical implementation of MEK inhibitors for the treatment of neurofibromatosis type 1-related plexiform neurofibromas is limited by gaps in knowledge regarding toxicity management and longterm effectiveness.
   (this thesis, chapter 5)

4. Routine cardiotoxicity monitoring during MEK inhibitor monotherapy in patients with neurofibromatosis type 1 is necessary in the first year of treatment and thereafter only if clinically indicated.
   (this thesis, chapter 6)

5. Patient-reported outcome measures should be prioritized when evaluating treatment response for benign manifestations such as plexiform neurofibromas. (this thesis)

6. Adolescent and young adult (AYA) cancer interferes with key developmental phases of life and requires psychosocial care to be actively integrated in standard medical practice.
   (Based on insights from: Hughes et al, Cancers 2024)

7. Intercultural communication training should be a mandatory component of healthcare education, as culturally sensitive communication is a prerequisite for high-quality care.
   (Based on insights from: Schouten et al, Patient Education and Counseling, 2023)

8. Given its beneficial effects on pain, anxiety, stress, sleep disturbance and delirium, listening to music (preferably according to the patient's own preference) should be standard on the neurology wards, where it may even positively impact doctors' workday as well.
   (Based on insights from: Becker et al,Acta Psychologica 2026)

9. The normalization of women feeling unsafe in public space, frequently leading to adapting their routes or behavior, reflects a societal failure that has become routine.
   (Based on insights from: CBS, Emancipatiemonitor 2024)

10. The most effective way to solve a problem may be to take a break from trying to solve it.
    (Based on insights from: Ritter, Front Hum Neurosci 2014)

11. Goed is goed genoeg.

Plexiform Neurofibromas in Neurofibromatosis Type 1

Daniëlle Christine Noordhoek

Layout and printing by ProefschriftMaken | proefschriftmaken.nl

Plexiform Neurofibromas in Neurofibromatosis Type 1

Plexiforme neurofibromen bij neurofibromatose type 1

Thesis

to obtain the degree of Doctor from
Erasmus University Rotterdam
by command of the
rector magnificus

Prof.dr.ir. A.J. Schuit

and in accordance with the decision of the Doctorate Board.
The public defence shall be held on

Tuesday 8 September 2026 at 15.30 hrs

by

Daniëlle Christine Noordhoek
born in Voorschoten, the Netherlands.

Erasmus University Rotterdam

DOCTORAL COMMITTEE

Promotors:	Prof. dr. M.J. van den Bent Dr. R. Oostenbrink
Co-promotor:	Dr. W. Taal
Assessment committee:	Prof. dr. H.M.W. Verheul Prof. dr. R. Ferner Dr. T.J. Snijders

TABLE OF CONTENTS

Chapter 1	General introduction	7
PART I	**Clinical characteristics of plexiform neurofibromas**	23
Chapter 2	Plexiform Neurofibromas and Malignant Peripheral Nerve Sheath Tumors in Adults with Neurofibromatosis Type 1: Clinical Insights from a Large Cohort	25
PART II	**Diagnosing plexiform neurofibromas**	45
Chapter 3	High resolution nerve ultrasound in neurofibromatosis type 1: a prospective and descriptive study	47
Chapter 4	High resolution nerve ultrasound in adults with NF1: an accessible and reproducible imaging tool for plexiform neurofibromas	63
PART III	**Treatment of plexiform neurofibromas**	83
Chapter 5	Trametinib in adults with neurofibromatosis type 1-related symptomatic plexiform neurofibromas	85
Chapter 6	Cardiotoxicity of trametinib monotherapy in adults with neurofibromatosis type 1: results from the TRAIN study	109
Chapter 7	General discussion	127
Chapter 8	Summary / samenvatting	147
Appendices	Dankwoord	159
	About the author	165
	List of publications	166
	PhD portfolio	167

1

General introduction

NEUROFIBROMATOSIS TYPE 1

Neurofibromatosis type 1 (NF1) is a relatively common rare disease, yet it remains largely unknown to the general public and even among healthcare professionals. NF1 can affect almost every organ system and is often further complicated by neuropsychological and cognitive challenges. Over the course of my 4-year PhD project, I have encountered a wide range of adults with NF1. What struck me most was the variability in phenotype, even within the same family. One individual may go through life with few or no problems, while another is in-and-out of the hospital with numerous complications and/or malignancies. The burden of living with a tumor predisposition syndrome with such an unpredictable disease course should not be underestimated. However, I was impressed by the optimism and motivation of our patients to participate in research: often not for their own benefit, but to help others. This patient population deserves continued efforts to improve monitoring and treatment.

Epidemiology and pathophysiology

NF1 has a prevalence of approximately 1 in 2,000 individuals[1, 2]. It is caused by pathogenic variants in the *NF1* gene located on chromosome 17q11.2. NF1 is inherited in an autosomal dominant pattern, but about half of the cases result from de novo pathogenic variants[3, 4]. The gene product of the *NF1* gene is the GTPase-activating protein neurofibromin that negatively regulates the RAS/MAPK pathway. The lack of functional neurofibromin in patients with NF1 leads to overactivation of the RAS/MAPK pathway, resulting in the clinical features such as tumor development (Figure 1). [5] Targeting this pathway is therefore an important strategy in the development of new therapies for NF1. Most manifestations of the disease develop after a "second hit" on the functional *NF1* allele, which explains the differences in phenotype even in family members with the same gene variant[6-12]. NF1 can also occur in mosaic form, caused by postzygotic mutations in the *NF1* gene that affect only a subpopulation of cells. The type and/or location of affected cell populations depicts the clinical phenotype in patients with mosaic NF1, sometimes involving only a segment of the body[13, 14].

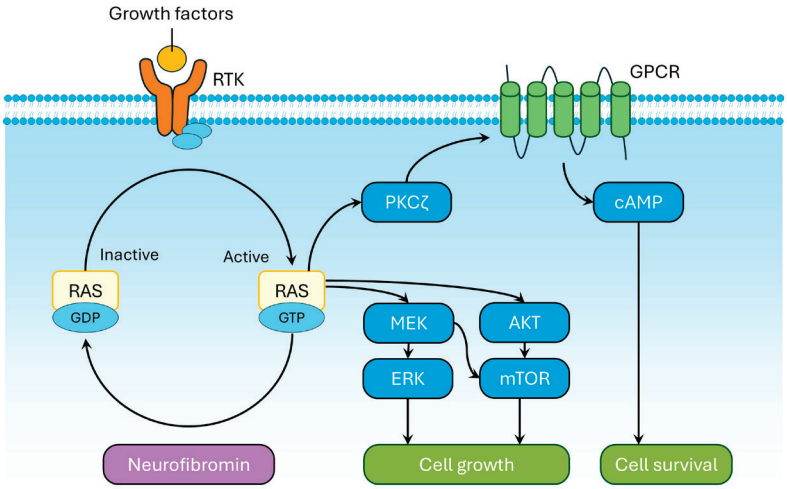


Figure 1. Neurofibromin signaling pathway. AKT=protein kinase B, cAMP=cyclic adenosine monophosphate, ERK=extracellular signal-regulated kinase, GDP=guanosine diphosphate, GPCR=G protein-coupled receptor, GTP=guanosine triphosphate, MEK=mitogen-activated protein kinase kinase, mTOR=mechanistic target of rapamycin, PKCζ=protein kinase C zeta, RAS=rat sarcoma, RTK=receptor tyrosine kinase.

Clinical characteristics

The most common feature of NF1 is café-au-lait macules which are present in >99% of patients with NF1 and are often one of the earliest signs of the disease[15]. Other common pigmentary lesions in NF1 include skin-fold freckling, usually seen in the axillary or inguinal regions and present in about 85% of the patients[15] (Figure 2A). Further diagnostic features are neurofibromas, iris Lisch nodules (Figure 2B) and choroidal abnormalities, distinctive osseous lesions and the presence of a heterozygous pathogenic *NF1* gene variant[13]. Approximately 15-20% of children with NF1 develop an optic pathway glioma (OPG) [16-18]. OPGs are typically detected before 7 years of age, and are symptomatic in 20-30% of the children with OPG[18]. Table 1 shows the current diagnostic criteria for NF1.

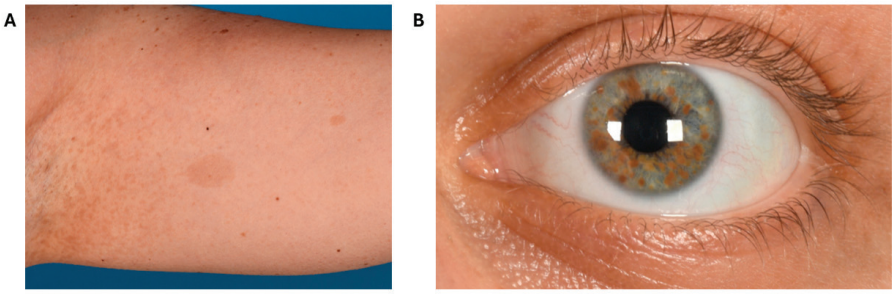


Figure 2. Panel A shows café-au-lait macules (CALM) and axillary freckling, panel B shows Lisch noduli.

Table 1: Revised diagnostic criteria for neurofibromatosis type 1 (NF1) [13]

A: the diagnostic criteria for NF1 are met in an individual who does not have a parent diagnosed with NF1 if to or more of the following are present:
• Six or more café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in post pubertal individuals
• Freckling in the axillary or inguinal region
• Two or more neurofibromas of any type or one plexiform neurofibroma
• Optic pathway glioma
• Two or more iris Lisch nodules identified by slit lamp examination or two or more choroidal abnormalities (CAs)—defined as bright, patchy nodules imaged by optical coherence tomography (OCT)/near-infrared reflectance (NIR) imaging
• A distinctive osseous lesion such as sphenoid dysplasia, anterolateral bowing of the tibia, or pseudarthrosis of a long bone
• A heterozygous pathogenic *NF1* variant with a variant allele fraction of 50% in apparently normal tissue such as white blood cells
B: a child of a parent who meets the diagnostic criteria specified in A merits a diagnosis of NF1 if one or more of the criteria in A are present

Neurofibromas are benign tumors that rise from the endoneurium of the peripheral nerve sheath, and were given their name by the German pathologist von Recklinghausen in 1882[19]. Three categories are distinguished: cutaneous, subcutaneous and plexiform neurofibromas (Figure 3).

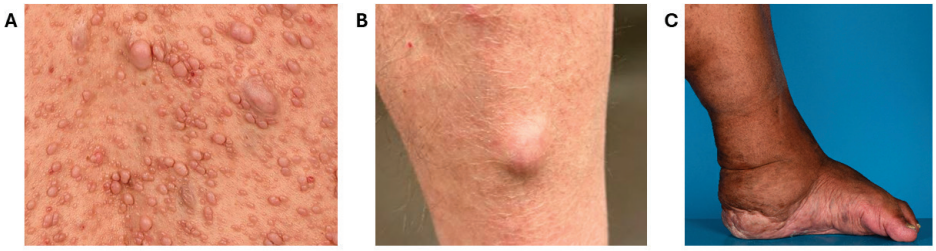


Figure 3. Panel A shows cutaneous neurofibromas, panel B subcutaneous neurofibroma and panel C plexiform neurofibroma.

Cutaneous neurofibromas are tumors in the skin of patients with NF1. Cutaneous neurofibromas typically begin to appear in childhood and increase in number and size with age[20, 21]. Patients with cutaneous neurofibromas often report pruritis and cosmetic concerns[22]. The visibility of the disease plays an important role in the psychological well-being of patients with NF1[23]. Cutaneous neurofibromas never undergo malignant transformation, and therefore histology is not needed before treatment[24]. Treatment consists of local surgery, CO2 laser removal and recently upcoming topical treatment with MEK inhibitors[22, 24, 25].

Subcutaneous neurofibromas develop as nodules on peripheral nerves. They may cause pain and neurological deficits, and they can occasionally transform into malignant tumors. [15] Treatment generally consists of surgical removal in case of symptoms.

Plexiform neurofibromas (PNs) involve multiple nerve fascicles and most often arise from large nerves or plexus. We differentiate between nodular PNs and diffuse growing PNs that impinge on surrounding soft tissue (Figure 4). [26] Approximately 50% of the patients with NF1 have PNs on whole-body MRI[27, 28]. PNs can cause pain, neurological deficits, disfigurement, vision loss, and compression of vital structures[29]. PNs can transform into malignant peripheral nerve sheath tumors (MPNST). Patients with NF1 have an estimated lifetime risk of 15.8% for developing MPNST[30]. Patients with NF1 and internal PNs are 20 times more likely to develop MPNSTs than patients without internal PN[31]. PNs will be discussed more thoroughly later in this introduction, as this is the main topic of this thesis.

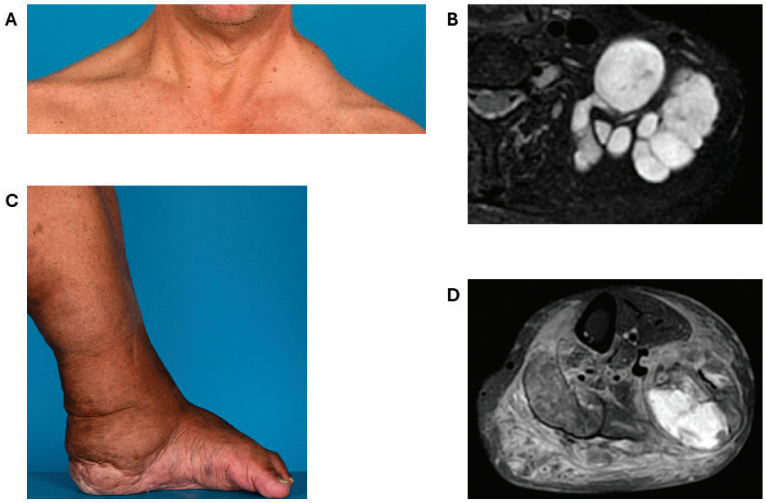


Figure 4. Panel A and B show a nodular plexiform neurofibroma, panel C and D show a diffuse plexiform neurofibroma.

Besides the physical manifestations of the disease, patients with NF1 also face neuropsychological difficulties. Symptoms of attention deficit hyperactivity disorder and autism spectrum disorder, as well as learning disabilities are more common in patients with NF1[15, 32, 33]. Patients with NF1 have on average a significantly lower IQ than the general population[34]. The high prevalence of fatigue among patients with NF1 is most likely associated with these neuropsychological problems[35]. The cognitive and neuropsychological difficulties of patients with NF1 can pose challenges in clinical practice, particularly in symptom interpretation, tumor surveillance, shared decision-making and compliance to treatment. In my experience, these aspects often call for extra attention and a tailored approach.

Malignancies and neurofibromatosis type 1

The lack of neurofibromin in patients with NF1 predisposes them to the development of various types of malignancies. The estimated lifetime cancer risk for patients with NF1 is approximately 60%[30]. MPNSTs are among the most life threatening complications of NF1. A meta-analysis showed a 5-year overall survival rate after diagnosis of MPNST of 45%[36]. MPNSTs often exhibit rapid growth and metastasis, which further reduces survival chances[37, 38]. Given the high incidence of MPNSTs in NF1 and their poor survival outcomes, early diagnosis is essential.

The most common brain tumors in NF1 patients are pilocytic astrocytomas, which typically develop in children and follow an indolent course. In contrast, new brain tumors in adults with NF1 are rare but tend to be more aggressive.[39-41]

In addition to MPNSTs and brain tumors, patients with NF1 are also at increased risk of other malignancies, such as breast cancer, which is approximately 5 times more common in patients with NF1 than in the general population[30].

The high risk of malignancies and the unpredictable nature of the disease, ask for regular monitoring by physicians with NF1 expertise and experience. In the Netherlands, the care of NF1 patients is standardized within a network of collaborating hospitals (*Zorgnetwerk NF1*), with Erasmus Medical Center in Rotterdam as the national expertise center. Every patient with NF1 is eligible for follow-up with regular intervals at a pediatrician or neurologist in one of the hospitals affiliated with the network, as it is also recommended in the recent ERN GENTURIS (European Reference Network Genetic Tumour Risk Syndromes) tumor surveillance guidelines[42].

Plexiform neurofibromas

As of my own experience in the outpatient NF1 clinic, I can confirm that despite their benign nature, plexiform neurofibromas are a major contributor to the disease burden of patients with NF1. In a survey amongst patient representatives, plexiform neurofibromas were assigned the highest need for new treatment[43].

Characteristics and clinical presentation

Plexiform neurofibromas develop mostly before birth, as immature/precursor schwann cells are most susceptible for tumorigenesis[44]. They typically grow at a median rate of 3.7% per year, although growth generally slows down with age[45]. Besides the schwann cells, PNs consist of perineurial cells, fibroblasts and mast cells[26].

In addition to the symptomatic PNs, asymptomatic PNs are also detected with whole-body MRI[28]. PNs significantly impact quality of life, and symptoms of anxiety and depression are frequently reported[46, 47]. Alarming symptoms associated with transition to MPNST

include: new and progressive pain, progressive neurological deficits, rapid tumor growth and change in tumor texture upon palpation [48, 49].

Benign PNs can progress to an atypical neurofibromatous tumor of uncertain biological potential (ANNUBP) by the homozygous loss of cyclin-dependent kinase inhibitor 2A (*CDKN2A*) [50]. ANNUBPs are histologically defined by at least two of the following features: atypical nuclei, loss of neurofibroma architecture, high cellularity and/or increased mitotic index[51]. ANNUBPs are seen as a premalignant stage before transformation into an MPNST, they have a low risk of recurrence or metastasis[52]. There is no consensus on the standard of treatment of ANNUBPs, but resection, if possible, would be preferred to avoid progression to an MPNST[53]. Loss of expression of trimethylation at lysine 27 of histone 3 (*H3K27me3*) marks the transformation to an MPNST[54].

Imaging

Regional MR imaging is performed in case of a symptomatic PN, in preparation for surgical treatment and/or for detection of malignant characteristics. Presence of symptoms and signs suspicious for MPNST development require further diagnostic measures. MRI with gadolinium contrast is the first step. Certain MRI characteristics are associated with malignant transformation, e.g. perilesional edema, contrast enhancement, irregular shape, absence of the target sign[55]. However, none of these features are sufficiently sensitive or specific to determine whether the tumor is benign or malignant. An FDG-PET scan and biopsy are required for the final diagnosis.

Besides acquiring regional MRI scans in case of symptoms, the ERN GENTURIS guideline for tumor surveillance recommends screening for PN with a whole-body MRI in all NF1 patients during transition from adolescence to adulthood[42]. The rationale is to determine the PN tumor load, as this is associated with the risk of developing MPNSTs, and decide the required follow-up frequency[56]. Important to note is that whole-body MRIs often lead to incidental findings, possibly causing mental burden for the patient and costs for extra often invasive diagnostic procedures[57]. High resolution nerve ultrasound (HRUS) has been proposed as a useful tool to screen patients with NF1 for the presence of (asymptomatic) PNs[58]. More studies with larger sample size are needed to determine the added value of HRUS in patients with NF1.

Treatment

Given their benign nature, PNs generally only require treatment in case of significant morbidity or suspicion of malignant transformation. Surgery is the first choice of treatment; however, depending on the size and location of the tumor, PNs are often considered inoperable. Due to their increased vascularity, PN surgery caries a high risk of peri-operative blood loss[59]. Debulking or partial resection often only temporary relief, as tumors tend to

regrow after surgery[60, 61]. Many patients with large size PNs and significant morbidity, are managed conservatively with only symptomatic treatment such as analgesics.

The need for improved treatment options for patients with PN has long been recognized. Since the late 90s, numerous clinical trials have been conducted with various systemic treatment options[26]. The majority of these trials were rather unsuccessful and did not result in any promising treatment options.

The first phase I trial with mitogen-activated protein kinase (MAPK) kinase (MEK) inhibitors marked a breakthrough in PN treatment[62]. MEK inhibitors directly target the RAS pathway that is overactive in NF1. The first phase II clinical trial with selumetinib in pediatric patients resulted in 71% partial response rate (defined as >20% decrease of tumor volume), as well as a positive effect on pain and quality of life[63]. This resulted in FDA (2020) and EMA (2021) approval of selumetinib for PN treatment in children. In the last five years, many trials with MEK inhibitors (selumetinib and mirdametinib) in pediatric and adult patients were published showing similar results[64-66]. These findings have led to the inclusion of adult patients and the MEK inhibitor mirdametinib in the recent FDA approval (2025). In the Netherlands, treatment of pediatric patients with selumetinib is now covered by health insurance. The well-known MEK inhibitor trametinib, often used in combination with dabrafenib for melanoma treatment, had not yet been investigated for its efficacy and safety in the treatment of NF1-related PNs.

SCOPE

The aim of this thesis is to improve patient care and management of plexiform neurofibromas, by focusing on the clinical characteristics, diagnostic methods and treatment.

Part I describes the clinical characteristics and management of plexiform neurofibromas. In **Chapter 2**, we investigate the natural history of NF1 with a focus on plexiform neurofibromas and malignancies by describing the data we collected from our cohort of more than 1000 adult NF1 patients. We aim to further understand these challenges to improve clinical care and patient monitoring.

Part II focuses on a promising additional imaging modality for plexiform neurofibromas: high resolution nerve ultrasound. In **Chapter 3**, we explore the value of high resolution nerve ultrasound and nerve conduction studies in screening for plexiform neurofibromas. In **Chapter 4,** we examine the use of high resolution nerve ultrasound for longitudinal follow-up of plexiform neurofibromas and assess interobserver variability in NF1 patients.

Part III presents the findings of our clinical trial with trametinib (TRAIN) for treating plexiform neurofibromas. In **Chapter 5,** we investigate the efficacy and safety of trametinib in adults with NF1 and inoperable plexiform neurofibromas. We report the effect on tumor volume, pain, and quality of life. In **Chapter 6,** we evaluate the cardiotoxicity of trametinib in adults with NF1 and provide recommendations for cardiac monitoring during treatment.

Chapter 7 discusses the key findings of this thesis in the context of existing literature and offers future perspectives for clinical care and research in NF1 patients.

REFERENCES

1. Lee TJ, Chopra M, Kim RH, Parkin PC, Barnett-Tapia C. Incidence and prevalence of neurofibromatosis type 1 and 2: a systematic review and meta-analysis. Orphanet J Rare Dis. 2023 Sep 14;18(1):292.
2. Kallionpaa RA, Uusitalo E, Leppavirta J, Poyhonen M, Peltonen S, Peltonen J. Prevalence of neurofibromatosis type 1 in the Finnish population. Genet Med. 2018 Sep;20(9):1082-6.
3. Evans DG, Howard E, Giblin C, et al. Birth incidence and prevalence of tumor-prone syndromes: estimates from a UK family genetic register service. Am J Med Genet A. 2010 Feb;152A(2):327-32.
4. McKeever K, Shepherd CW, Crawford H, Morrison PJ. An epidemiological, clinical and genetic survey of neurofibromatosis type 1 in children under sixteen years of age. Ulster Med J. 2008 Sep;77(3):160-3.
5. Gutmann DH, Ferner RE, Listernick RH, Korf BR, Wolters PL, Johnson KJ. Neurofibromatosis type 1. Nat Rev Dis Primers. 2017 Feb 23;3:17004.
6. Stevenson DA, Zhou H, Ashrafi S, et al. Double inactivation of NF1 in tibial pseudarthrosis. Am J Hum Genet. 2006 Jul;79(1):143-8.
7. De Schepper S, Maertens O, Callens T, Naeyaert JM, Lambert J, Messiaen L. Somatic mutation analysis in NF1 cafe au lait spots reveals two NF1 hits in the melanocytes. J Invest Dermatol. 2008 Apr;128(4):1050-3.
8. Serra E, Puig S, Otero D, et al. Confirmation of a double-hit model for the NF1 gene in benign neurofibromas. Am J Hum Genet. 1997 Sep;61(3):512-9.
9. Rasmussen SA, Overman J, Thomson SA, et al. Chromosome 17 loss-of-heterozygosity studies in benign and malignant tumors in neurofibromatosis type 1. Genes Chromosomes Cancer. 2000 Aug;28(4):425-31.
10. Steinmann K, Kluwe L, Friedrich RE, Mautner VF, Cooper DN, Kehrer-Sawatzki H. Mechanisms of loss of heterozygosity in neurofibromatosis type 1-associated plexiform neurofibromas. J Invest Dermatol. 2009 Mar;129(3):615-21.
11. Garcia-Linares C, Fernandez-Rodriguez J, Terribas E, et al. Dissecting loss of heterozygosity (LOH) in neurofibromatosis type 1-associated neurofibromas: Importance of copy neutral LOH. Hum Mutat. 2011 Jan;32(1):78-90.
12. Colman SD, Williams CA, Wallace MR. Benign neurofibromas in type 1 neurofibromatosis (NF1) show somatic deletions of the NF1 gene. Nat Genet. 1995 Sep;11(1):90-2.
13. Legius E, Messiaen L, Wolkenstein P, et al. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. Genet Med. 2021 Aug;23(8):1506-13.
14. Legius E, Brems H. Genetic basis of neurofibromatosis type 1 and related conditions, including mosaicism. Childs Nerv Syst. 2020 Oct;36(10):2285-95.
15. Ferner RE. Neurofibromatosis 1 and neurofibromatosis 2: a twenty first century perspective. Lancet Neurol. 2007 Apr;6(4):340-51.
16. Listernick R, Charrow J, Greenwald MJ, Esterly NB. Optic gliomas in children with neurofibromatosis type 1. J Pediatr. 1989 May;114(5):788-92.
17. Lund AM, Skovby F. Optic gliomas in children with neurofibromatosis type 1. Eur J Pediatr. 1991 Oct;150(12):835-8.
18. Listernick R, Charrow J, Greenwald M, Mets M. Natural history of optic pathway tumors in children with neurofibromatosis type 1: a longitudinal study. J Pediatr. 1994 Jul;125(1):63-6.
19. Recklinghausen Fv. Ueber die multiplen Fibrome der Haut und ihre Beziehung zu den multiplen Neuromen. Berlin1882.

20. Duong TA, Bastuji-Garin S, Valeyrie-Allanore L, Sbidian E, Ferkal S, Wolkenstein P. Evolving pattern with age of cutaneous signs in neurofibromatosis type 1: a cross-sectional study of 728 patients. Dermatology. 2011;222(3):269-73.
21. DeBella K, Szudek J, Friedman JM. Use of the national institutes of health criteria for diagnosis of neurofibromatosis 1 in children. Pediatrics. 2000 Mar;105(3 Pt 1):608-14.
22. Ortonne N, Wolkenstein P, Blakeley JO, et al. Cutaneous neurofibromas: Current clinical and pathologic issues. Neurology. 2018 Jul 10;91(2 Suppl 1):S5-S13.
23. Wolkenstein P, Zeller J, Revuz J, Ecosse E, Leplege A. Quality-of-life impairment in neurofibromatosis type 1: a cross-sectional study of 128 cases. Arch Dermatol. 2001 Nov;137(11):1421-5.
24. Peltonen S, Jannic A, Wolkenstein P. Treatment of cutaneous neurofibromas with carbon dioxide laser: Technique and patient experience. Eur J Med Genet. 2022 Jan;65(1):104386.
25. Sarin KY, Bradshaw M, O'Mara C, et al. Effect of NFX-179 MEK inhibitor on cutaneous neurofibromas in persons with neurofibromatosis type 1. Sci Adv. 2024 May 3;10(18):eadk4946.
26. Fisher MJ, Blakeley JO, Weiss BD, et al. Management of Neurofibromatosis Type 1-Associated Plexiform Neurofibromas. Neuro Oncol. 2022 Jun 3.
27. Mautner VF, Asuagbor FA, Dombi E, et al. Assessment of benign tumor burden by whole-body MRI in patients with neurofibromatosis 1. Neuro Oncol. 2008 Aug;10(4):593-8.
28. Nguyen R, Kluwe L, Fuensterer C, Kentsch M, Friedrich RE, Mautner VF. Plexiform neurofibromas in children with neurofibromatosis type 1: frequency and associated clinical deficits. J Pediatr. 2011 Oct;159(4):652-5 e2.
29. Ejerskov C, Farholt S, Nielsen FSK, et al. Clinical Characteristics and Management of Children and Adults with Neurofibromatosis Type 1 and Plexiform Neurofibromas in Denmark: A Nationwide Study. Oncol Ther. 2022 Dec 1.
30. Uusitalo E, Rantanen M, Kallionpaa RA, et al. Distinctive Cancer Associations in Patients With Neurofibromatosis Type 1. J Clin Oncol. 2016 Jun 10;34(17):1978-86.
31. Tucker T, Wolkenstein P, Revuz J, Zeller J, Friedman JM. Association between benign and malignant peripheral nerve sheath tumors in NF1. Neurology. 2005 Jul 26;65(2):205-11.
32. Garg S, Lehtonen A, Huson SM, et al. Autism and other psychiatric comorbidity in neurofibromatosis type 1: evidence from a population-based study. Dev Med Child Neurol. 2013 Feb;55(2):139-45.
33. Hyman SL, Shores A, North KN. The nature and frequency of cognitive deficits in children with neurofibromatosis type 1. Neurology. 2005 Oct 11;65(7):1037-44.
34. Ferner RE, Hughes RA, Weinman J. Intellectual impairment in neurofibromatosis 1. J Neurol Sci. 1996 Jun;138(1-2):125-33.
35. Rosenberg AGW, Mochel K, Hahner LM, et al. Endocrine and non-endocrine causes of fatigue in adults with Neurofibromatosis type 1. Front Endocrinol (Lausanne). 2023;14:1119159.
36. Kolberg M, Holand M, Agesen TH, et al. Survival meta-analyses for >1800 malignant peripheral nerve sheath tumor patients with and without neurofibromatosis type 1. Neuro Oncol. 2013 Feb;15(2):135-47.
37. Cai Z, Tang X, Liang H, Yang R, Yan T, Guo W. Prognosis and risk factors for malignant peripheral nerve sheath tumor: a systematic review and meta-analysis. World J Surg Oncol. 2020 Sep 30;18(1):257.
38. Sharma MR, Puj KS, Salunke AA, Pandya SJ, Gandhi JS, Parikh AR. Malignant peripheral nerve sheath tumor with analysis of various prognostic factors: A single-institutional experience. J Cancer Res Ther. 2021 Jan-Mar;17(1):106-13.

39. Rodriguez FJ, Perry A, Gutmann DH, et al. Gliomas in neurofibromatosis type 1: a clinicopathologic study of 100 patients. J Neuropathol Exp Neurol. 2008 Mar;67(3):240-9.
40. Guillamo JS, Creange A, Kalifa C, et al. Prognostic factors of CNS tumours in Neurofibromatosis 1 (NF1): a retrospective study of 104 patients. Brain. 2003 Jan;126(Pt 1):152-60.
41. Byrne S, Connor S, Lascelles K, Siddiqui A, Hargrave D, Ferner RE. Clinical presentation and prognostic indicators in 100 adults and children with neurofibromatosis 1 associated non-optic pathway brain gliomas. J Neurooncol. 2017 Jul;133(3):609-14.
42. Carton C, Evans DG, Blanco I, et al. ERN GENTURIS tumour surveillance guidelines for individuals with neurofibromatosis type 1. EClinicalMedicine. 2023 Feb;56:101818.
43. Dhaenens BAE, Ferner RE, Bakker A, et al. Identifying challenges in neurofibromatosis: a modified Delphi procedure. Eur J Hum Genet. 2021 Nov;29(11):1625-33.
44. Le LQ, Liu C, Shipman T, Chen Z, Suter U, Parada LF. Susceptible stages in Schwann cells for NF1-associated plexiform neurofibroma development. Cancer Res. 2011 Jul 1;71(13):4686-95.
45. Nguyen R, Dombi E, Widemann BC, et al. Growth dynamics of plexiform neurofibromas: a retrospective cohort study of 201 patients with neurofibromatosis 1. Orphanet J Rare Dis. 2012 Oct 4;7:75.
46. Lai JS, Jensen SE, Charrow J, Listernick R. Patient Reported Outcomes Measurement Information System and Quality of Life in Neurological Disorders Measurement System to Evaluate Quality of Life for Children and Adolescents with Neurofibromatosis Type 1 Associated Plexiform Neurofibroma. J Pediatr. 2019 Mar;206:190-6.
47. Yoo HK, Porteous A, Ng A, et al. Impact of neurofibromatosis type 1 with plexiform neurofibromas on the health-related quality of life and work productivity of adult patients and caregivers in the UK: a cross-sectional survey. BMC Neurol. 2023 Nov 23;23(1):419.
48. Evans DG, Baser ME, McGaughran J, Sharif S, Howard E, Moran A. Malignant peripheral nerve sheath tumours in neurofibromatosis 1. J Med Genet. 2002 May;39(5):311-4.
49. Ferner RE, Huson SM, Thomas N, et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. J Med Genet. 2007 Feb;44(2):81-8.
50. Rhodes SD, He Y, Smith A, et al. Cdkn2a (Arf) loss drives NF1-associated atypical neurofibroma and malignant transformation. Hum Mol Genet. 2019 Aug 15;28(16):2752-62.
51. Miettinen MM, Antonescu CR, Fletcher CDM, et al. Histopathologic evaluation of atypical neurofibromatous tumors and their transformation into malignant peripheral nerve sheath tumor in patients with neurofibromatosis 1-a consensus overview. Hum Pathol. 2017 Sep;67:1-10.
52. Beert E, Brems H, Daniels B, et al. Atypical neurofibromas in neurofibromatosis type 1 are premalignant tumors. Genes Chromosomes Cancer. 2011 Dec;50(12):1021-32.
53. Vaassen P, Feldkamp A, Scholz M, Blau T, Durr NR, Rosenbaum T. A chance to cut is a chance to cure: complete resection of an atypical neurofibroma prevents further progression to malignancy. Childs Nerv Syst. 2023 Jun 21.
54. Lu VM, Marek T, Gilder HE, et al. H3K27 trimethylation loss in malignant peripheral nerve sheath tumor: a systematic review and meta-analysis with diagnostic implications. J Neurooncol. 2019 Sep;144(3):433-43.
55. Martin E, Geitenbeek RTJ, Coert JH, et al. A Bayesian approach for diagnostic accuracy of malignant peripheral nerve sheath tumors: a systematic review and meta-analysis. Neuro Oncol. 2021 Apr 12;23(4):557-71.
56. Nguyen R, Jett K, Harris GJ, Cai W, Friedman JM, Mautner VF. Benign whole body tumor volume is a risk factor for malignant peripheral nerve sheath tumors in neurofibromatosis type 1. J Neurooncol. 2014 Jan;116(2):307-13.

57. Preez HD, Lasker I, Rajakulasingam R, Saifuddin A. Whole-body magnetic resonance imaging: Incidental findings in paediatric and adult populations. Eur J Radiol. 2020 Sep;130:109156.
58. Telleman JA, Stellingwerff MD, Brekelmans GJ, Visser LH. Nerve ultrasound: A useful screening tool for peripheral nerve sheath tumors in NF1? Neurology. 2017 Apr 25;88(17):1615-22.
59. Canavese F, Krajbich JI. Resection of plexiform neurofibromas in children with neurofibromatosis type 1. J Pediatr Orthop. 2011 Apr-May;31(3):303-11.
60. Nguyen R, Ibrahim C, Friedrich RE, Westphal M, Schuhmann M, Mautner VF. Growth behavior of plexiform neurofibromas after surgery. Genet Med. 2013 Sep;15(9):691-7.
61. Needle MN, Cnaan A, Dattilo J, et al. Prognostic signs in the surgical management of plexiform neurofibroma: the Children's Hospital of Philadelphia experience, 1974-1994. J Pediatr. 1997 Nov;131(5):678-82.
62. Dombi E, Baldwin A, Marcus LJ, et al. Activity of Selumetinib in Neurofibromatosis Type 1-Related Plexiform Neurofibromas. N Engl J Med. 2016 Dec 29;375(26):2550-60.
63. Gross AM, Wolters PL, Dombi E, et al. Selumetinib in Children with Inoperable Plexiform Neurofibromas. N Engl J Med. 2020 Apr 9;382(15):1430-42.
64. Moertel CL, Hirbe AC, Shuhaiber HH, et al. ReNeu: A Pivotal, Phase IIb Trial of Mirdametinib in Adults and Children With Symptomatic Neurofibromatosis Type 1-Associated Plexiform Neurofibroma. J Clin Oncol. 2024 Nov 8:JCO2401034.
65. Gross AM, O'Sullivan Coyne G, Dombi E, et al. Selumetinib in adults with NF1 and inoperable plexiform neurofibroma: a phase 2 trial. Nat Med. 2025 Jan;31(1):105-15.
66. Weiss BD, Wolters PL, Plotkin SR, et al. NF106: A Neurofibromatosis Clinical Trials Consortium Phase II Trial of the MEK Inhibitor Mirdametinib (PD-0325901) in Adolescents and Adults With NF1-Related Plexiform Neurofibromas. J Clin Oncol. 2021 Mar 1;39(7):797-806.

PART I

Clinical characteristics of plexiform neurofibromas

2

Plexiform Neurofibromas and Malignant Peripheral Nerve Sheath Tumors in Adults with Neurofibromatosis Type 1: Clinical Insights from a Large Cohort

D.C. Noordhoek, L. Orlandini, R. Oostenbrink, R. van Minkelen, S.A. van Dijk, A. Taal, C. Taal, W. Taal

Accepted for publication in Neuro-Oncology Practice

ABSTRACT

Background

Plexiform neurofibromas (PNs) and malignant peripheral nerve sheath tumors (MPNSTs) are major complications in adults with neurofibromatosis type 1; we analyzed their clinical features and management in a large cohort of 1,067 adults.

Methods

Clinical data from all adults seen at the Dutch NF1 expertise center (2004-2025) were retrospectively collected, including NF1 diagnostic features, genetic data and details on PNs and MPNSTs. Detailed information was recorded on up to three largest or most symptomatic PNs per patient. Five-year survival after MPNST and risk factors for MPNST were analyzed. Known genotype-phenotype associations were evaluated.

Results

Among 1,067 adult patients with NF1 (1,019 generalized, 48 mosaic), PNs were present in 47%. Surgical management was frequently limited by regrowth and complications. Overall, 5% of patients were deceased; the median age of death was 48 years, most commonly due to MPNST. MPNST occurred in 4% of the patients, predominantly in proximal body regions. The presence of nodular PNs was an independent risk factor for MPNST. Five-year survival after MPNST was 54.5%. 17q11.2 microdeletion was associated with a higher risk for MPNST.

Conclusions

This large cohort study highlights that PNs and the associated risk of malignant transformation represent a significant burden for individuals with NF1. The limitations of surgical management of PNs underscore the need for effective systemic therapies. The prognosis for MPNST remains poor. Regular follow-up is essential for all patients with NF1, particularly those in high-risk subgroups.

Keywords

Neurofibromatosis type 1
Plexiform neurofibroma
Malignant peripheral nerve sheath tumor

Keypoints

- Plexiform neurofibromas are a major burden; surgery is often ineffective due to regrowth and complications.
- Nodular plexiform neurofibromas and 17q11.2 microdeletion are associated with higher risk of malignant peripheral nerve sheath tumor; prognosis remains poor.

Importance of the study

This is the first large-scale cohort study presenting real-world data of adults with neurofibromatosis type 1 (NF1) of the Dutch national expertise center on NF1. Data is manually collected and validated, ensuring detail and accuracy. Our findings confirm the high burden of plexiform neurofibromas. The limitations of surgical treatment (regrowth and complications) highlight the need for effective systemic therapies. We have reported the frequency of non-OPG brain tumors. Malignancies were common, especially under 50 years old. Malignant peripheral nerve sheath tumors remain a major cause of mortality. We identified two high-risk subgroups requiring close surveillance: patients with nodular plexiform neurofibromas and patients with 17q11.2 microdeletion. Given the size and detailed phenotyping of this cohort, these findings contribute to the international understanding of NF1.

INTRODUCTION

Neurofibromatosis type 1 (NF1) is a genetic tumor predisposition syndrome affecting approximately 1 in 2000 individuals[1, 2]. NF1 is a RASopathy that results from a variant in the *NF1* gene that is autosomal dominantly inherited in one half of cases and results from a *de novo* variant in the other half. Clinical manifestations may affect nearly every organ system and vary greatly in severity between individuals. The most common and visible characteristics, that have inspired the NF1 diagnostic criteria, include café-au-lait macules, axillary- and inguinal freckling, Lisch nodules, (sub)cutaneous neurofibromas, and plexiform neurofibromas (PNs)[3]. The most severe complications of NF1 include various neoplasms such as optic pathway gliomas (OPGs), other gliomas, malignant peripheral nerve sheath tumors (MPNSTs) and other types of malignancies[4]. The malignant tumors mainly contribute to the shortened life expectancy in patients with NF1, accounting for an average loss of approximately 15 years[5]. Because of the unpredictable course of NF1 and its consequences for affected individuals and health care professionals, there is a need for studies on the natural history of the disease. Although previous publications on the natural disease course of NF1 are available [6, 7], detailed studies remain important, particularly in adult patients and in relation to the clinical course and treatment of plexiform neurofibromas and associated complications.

A consensus meeting with NF1 experts and patient representatives in 2020 established that PN and MPNST are considered to be among the most urgent challenges in NF1 care[8]. PNs are benign peripheral nerve sheath tumors that arise from multiple nerve fascicles. About 50% of adults with NF1 are affected [9]. Despite being frequently asymptomatic and not visible, certain PNs can induce pain, neurological deficits, disfigurement, and/or compression of vital structures, markedly increasing morbidity in NF1 [6]. Until recently, treatment options for symptomatic PNs were limited to surgery, but many PNs are considered inoperable due to size, location and risk of major peri-operative blood loss [10]. Recent studies with MEK inhibitors demonstrated promising results, achieving partial response (>20% decrease of tumor volume on MRI) in 41-70% of patients, along with pain reduction and improvement in quality of life [11-17]. This novel therapy represents an advance in PN management.

PNs carry a risk of malignant transformation into MPNST. The lifetime risk of developing an MPNST is 8-15.8% for people with NF1, a thousandfold higher than in the general population[18, 19]. This risk is the highest between the ages of twenty and forty[18]. Survival outcomes of MPNST remain poor, with a 5-year overall survival of 44% [20]. For non-metastasized MPNSTs, the gold standard of treatment is surgery with or without radiotherapy [21]. Metastatic MPNSTs require systemic therapy, most commonly chemotherapy, which achieves a low response rate of approximately 20% [22].

In conclusion, PNs and MPNSTs have a major impact on morbidity and mortality of patients with NF1. Current consensus guidelines provide recommendations for tumor surveillance[23], but the heterogeneity of NF1 means that real-world clinical data continues to be valuable. The aim of this study is to describe the clinical characteristics and management of PNs and MPNSTs in a large adult NF1 cohort in the Netherlands. Additionally, descriptive data on other NF1-associated malignancies are included.

METHODS

Study design

This retrospective, single-center cohort study was conducted in Erasmus Medical Center Rotterdam (Erasmus MC). Erasmus MC is recognized as the national expertise center for NF1 and collaborates in the national NF1 care network with 12 regional treatment centers and 2 intervention centers. The outpatient clinic at Erasmus MC provides care for patients with NF1 from the surrounding region as well as complex cases referred from across the country. All patients receive standardized, regular follow-up providing us with up-to-date patient data for research. This study was approved by the local Institutional Review Board (MEC-2023-0730).

Patients and clinical care

All patients with a clinical diagnosis of NF1, according to the revised international diagnostic criteria[3] were included if at least 18 years old at the time of data collection and with a minimum of one visit to the NF1 outpatient clinic in Erasmus MC between 2004 and 2025. Patients were excluded if they objected against the use of their data for research purposes, which would be clearly stated in the patient file.

Clinical follow-up intervals in the NF1 outpatient clinic are tailored to disease severity. In general, patients are seen at least once every three years. Appointments can be scheduled several years in advance, with patients automatically receiving a notification letter three months prior to their scheduled visit. Individuals with more complex manifestations, such as large plexiform neurofibromas or NF1 microdeletions, are typically evaluated annually. The presence of complications, such as gliomas or MPNSTs, required more frequent clinical assessment. Screening procedures, including whole body MRI, were not routinely performed; however, in the event of new pain or other symptoms, imaging is performed with a very low threshold to promptly assess potential tumor progression or malignant transformation. Patients are followed longitudinally within the NF1 expertise center, however, some individuals may be referred back to a regional NF1 affiliated hospital within the national NF1 network.

Data

Study data was collected manually from electronic medical patient records. All available data of any timepoint was used, this included inpatient as well as outpatient records. Data was pseudonymized and stored in *Castor Electronic Data Capture*. Collected data included demographic characteristics, NF1 disease characteristics and diagnostic criteria, genetic diagnosis, presence of cutaneous neurofibromas, PNs and treatment, MPNSTs and treatment, presence of OPGs and non-OPG brain tumors and occurrence of other malignancies. Education was classified according to the international standard classification of education (ISCED)[24]. For the data on PNs, we selected the up to three largest or most symptomatic PNs per patient in order to collect data on symptoms, complications and treatment

Generalized NF1 and mosaic NF1 were defined as per the patients' clinical diagnosis based on the diagnostic criteria[3]. PN locations were categorized as head/neck, spinal, thorax, abdomen/pelvis, proximal extremities and distal extremities. PNs were reported as absent if not found on physical examination or available imaging. PN-related symptoms and complications were defined as: pain needing intervention, motor neurological deficit, sensory neurological deficit, disfigurement, hemorrhage, airway complication, visual complication, bowel complication, bladder complication or bone deformity.

Genetics

Genetic diagnoses were collected from our clinical genetics database, where available. Most genetic testing in the Netherlands has been performed in our center and genetic testing is available for all patients with NF1. Since 2004, various techniques for detecting the *NF1* variant have been used, including: Sanger sequencing, multiplex ligation-dependent probe amplification, targeted next generation sequencing, whole exome sequencing and genome sequencing. Genetic diagnoses were categorized as: not tested, no pathogenic variant found, variant of unknown significance (VUS) or pathogenic *NF1* variant (including variants classified as "likely pathogenic"). Classification of variants was based on the guidelines of the American College of Medical Genetics[25]. In case of multiple variants in one patient, the variant with the highest classification was reported in table 1. *NF1* pathogenic variants with known genotype/phenotype associations were identified and grouped in our population. These variants include: 17q11.2 microdeletion, p.Met992del, p.Arg1809, p.Leu844, p.Cys845, p.Ala846, p.Leu847, p.Gly848, p.Met1149, p.Arg1276 and p.Lys1423. The clinical data of these patients regarding the presence of cutaneous neurofibromas, PNs, MPNSTs, OPGs and other malignancies were compared to the related phenotype that is previously described[26].

Statistical analysis

Descriptive statistics were computed, including median and range for continuous variables and frequencies for categorical variables. For some analysis, the patients were

divided into two groups: generalized NF1 and mosaic NF1. Highest completed education was dichotomized in two categories: ISCED 0-3 (none, primary and secondary education) and ISCED 4-7 (any type of post-secondary education). A multivariable logistic regression was performed to test the association between presence of nodular PN, high PN tumor load (>10 PNs) and age as risk factors for developing MPNST. For survival analysis after MPNST diagnosis, patients were censored at their last recorded follow-up visit. Due to the limited number of cases, subgroup analyses by sex were exploratory and not powered for statistical comparison. Data was analyzed using *R studio version 4.4.1*.

RESULTS

Patient characteristics

For this study, data were collected from all 1,067 adult patients who attended the NF1 outpatient clinic between 2004 and 2025. Of these, 1,019 patients (96%) had generalized NF1 and 48 patients (4%) had mosaic NF1. Median time between the first and last available visit to the adult NF1 clinic was 3.1 years (range: 0-19.8 years), this includes patients with only one visit. The patient characteristics and diagnostic features are presented in Table 1. The *NF1* pathogenic variant was found in 64% of the patients with generalized NF1 and 17% of the patients with mosaic NF1. Fifty-three patients (5%) were deceased at the time of data collection, in 40% of cases because of an MPNST.

Table 1 Patient characteristics

	Generalized NF1 n=1,019	**Mosaic NF1 n=48**	**Total n=1,067**
Sex, n (%)			
Female	575 (56)	34 (71)	609 (57)
Years since first visit, median (range)	3.2 (0-19.8)	2.4 (0-14.7)	3.1 (0-19.8)[a]
Age at last visit, median (range)	35 (17-83)	38 (18-71)	36 (17-83)[a]
Highest completed education, n (%)	N=790	N=39	N=829
ISCED 0-3[25]	279 (35)	11 (28)	290 (35)
ISCED 4-7	511 (65)	28 (72)	539 (65)
Family history NF1, n (%)	N=770	N=36	N=806
De novo	428 (56)	36 (100)	464 (58)
Inherited	342 (44)	0 (0)	342 (42)
Diagnostic criteria, n (%)			
>6 CALM	809/931 (87)	15/47 (32)	824/978 (84)
Freckling	845/949 (89)	22/47 (47)	867/996 (87)
1 PN or 2 or more neurofibromas	875/975 (90)	37/45 (82)	912/1020 (89)
OPG	93/679 (14)	2/26 (8)	95/705 (14)
Lisch nodules	219/347 (63)	9/32 (28)	228/379 (60)
Typical osseous lesion[b]	66/389 (17)	1/20 (5)	67/409 (16)

Table 1 Patient characteristics (*continued*)

	Generalized NF1 n=1,019	Mosaic NF1 n=48	Total n=1,067
Genetic diagnosis, n (%)	N=1019	N=48	N=1067
Not tested	251 (25)	6 (12)	257 (24)
No pathogenic variant	100 (10)	34 (71)	134 (13)
VUS	15 (1)	0 (0)	15 (1)
NF1 pathogenic variant	653 (64)	8 (17)	661 (62)
Deceased, n (%)	52 (5)	1 (2)	53 (5)
Age of death, median (range)	48 (19-76)	unknown	48 (19-76)
NF1 related cause, n (% of deceased)			
MPNST	20 (39)	1 (100)	21 (40)
Brain tumor	13 (25)	0 (0)	13 (25)
Other malignancy	7 (14)	0 (0)	7 (13)
Other cause/unknown	12 (22)	0 (0)	12 (22)

[a]: 9 missing values. [b]: includes distinctive osseous lesions such as sphenoid dysplasia, anterolateral bowing of the tibia, or pseudarthrosis of a long bone. NF1=neurofibromatosis type 1. ISCED=international standard classification of education. ISCED 0-3 includes patients without education, with primary education and with secondary education completed. ISCED 4-7 includes patients which completed any form of post-secondary education. PN=plexiform neurofibroma. OPG=optic pathway glioma. VUS=variant of unknown significance.

The presence of neurofibromas and malignancies is summarized in table 2. Cutaneous neurofibromas are more common in patients with generalized NF1 (80%) compared to mosaic NF1 (44%). Plexiform neurofibromas are present in 47% of all patients. Among the patients with plexiform neurofibromas, 8% have a high benign tumor load of more than 10 PNs. Non-OPG brain tumors were present in 5% of the patients, 3% low-grade glioma and 2% high-grade glioma. Ten percent of the patients had other malignancies. Most common malignancies were breast cancer and gastrointestinal stromal tumor. Six percent of the patients were diagnosed with a malignancy before 50 years old. Median age at diagnosis of breast cancer was 44.7 years old (range: 15.5-69.0 years). Among the patients with other malignancies, 15 patients (13%) were deceased at the time of data collection, of which 7 patients (6%) died because of the malignancy. The median age of death due to other malignancies was 68.1 years (range 50.3-76.2 years).

Table 2 Neurofibromas and malignancies

	Generalized NF1 n=1019	Mosaic NF1 n=48	Total n=1067	
Cutaneous neurofibromas, n (%)	819 (80)	21 (44)	840 (79)	
Plexiform neurofibromas, n (%)	481 (47)	20 (42)	501 (47)	
Yes, <10	345 (34)	14 (29)	359 (34)	
Yes, >10	80 (8)	3 (6)	83 (8)	
Yes, quantity unknown	56 (6)	3 (6)	59 (6)	
MPNST, n (%)	43 (4)	1 (2)	44 (4)	
Non-OPG brain tumors, n (%)	51 (5)	1 (2)	52 (5)	
Low-grade glioma	34 (3)	1 (2)	35 (3)	
High-grade glioma	17 (2)	0 (0)	17 (2)	
			Total	**<50 y**
Other malignancies[a], n (%)	112 (10)	0 (0)	112 (10)	61 (6)
Breast cancer	23 (2)	0 (0)	23 (2)	15 (1)
Lung cancer	5 (0.5)	0 (0)	5 (0.5)	3 (0.3)
GIST	23 (2)	0 (0)	23 (2)	8 (1)
Pheochromocytoma (benign or malignant)	19 (2)	0 (0)	19 (2)	12 (1)
Prostate cancer	2 (0.2)	0 (0)	2 (0.2)	0 (0)
Melanoma	8 (0.8)	0 (0)	8 (0.7)	1 (0.1)
Other	46 (5)	0 (0)	46 (4)	27 (3)

[a]: total number of unique patients with a malignancy are given, followed by the distribution amongst different type of malignancies. Some patients had multiple malignancies. Incidence of malignancy diagnosis before age of 50 years are given separately. NF1=neurofibromatosis type 1. OPG=optic pathway glioma. GIST=gastrointestinal stromal tumor. MPNST=malignant peripheral nerve sheath tumor.

Plexiform neurofibromas and treatment

Among the 501 patients with plexiform neurofibromas, 243 patients (49%) had diffuse PNs, 233 patients (47%) had nodular PNs and 43 patients (9%) had mixed PNs with diffuse and nodular components. Details of the 635 symptomatic PNs are reported in table 3. Most common PN-related symptoms and complications were disfigurement (43%), pain (22%), motor deficits (12%) and sensory deficits (8%). PN-related symptoms, especially pain and sensory deficits were relatively more common among the mosaic NF1 patients (figure 1). Median age at onset of PN symptoms was 25 years (range 0-74 years), in some cases the PN was symptomatic at birth (e.g. externally visible).

Table 3 Plexiform neurofibromas

	Symptomatic PNs N=635
Type of PN, n (%)	N=601
Diffuse	281 (47)
Nodular	274 (46)
Solitary nodule[a]	97 (43)
Multilobular[a]	128 (57)
Mix of diffuse and nodular	46 (8)
Location of PN, n (%)	
Spinal	116 (18)
Head/neck	153 (24)
Thorax	66 (10)
Abdomen/pelvis	101 (16)
Proximal extremities	121 (19)
Distal extremities	78 (12)
Surgery[b], n (%)	N=405
Partial resection/debulking[c]	226 (69)
Complete resection[c]	101 (31)
Peri/postoperative complications, n (%)	53 (13)
Hemorrhage	15 (4)
Neurological deficit	21 (5)
Other	23 (6)
Regrowth after resection, n (%)	145 (36)
Radiotherapy, n (%)	3 (0.5)
Systemic treatment[d], n (%)	29 (6)
MEK inhibitor	28 (6)
Imatinib	1 (0)

[a] 49 missing values. [b]: contains multiple resections per PN. [c]:78 missing values. [d]:systemic treatment was calculated per patient. PN=plexiform neurofibroma. MEK=mitogen-activated protein kinase kinase.

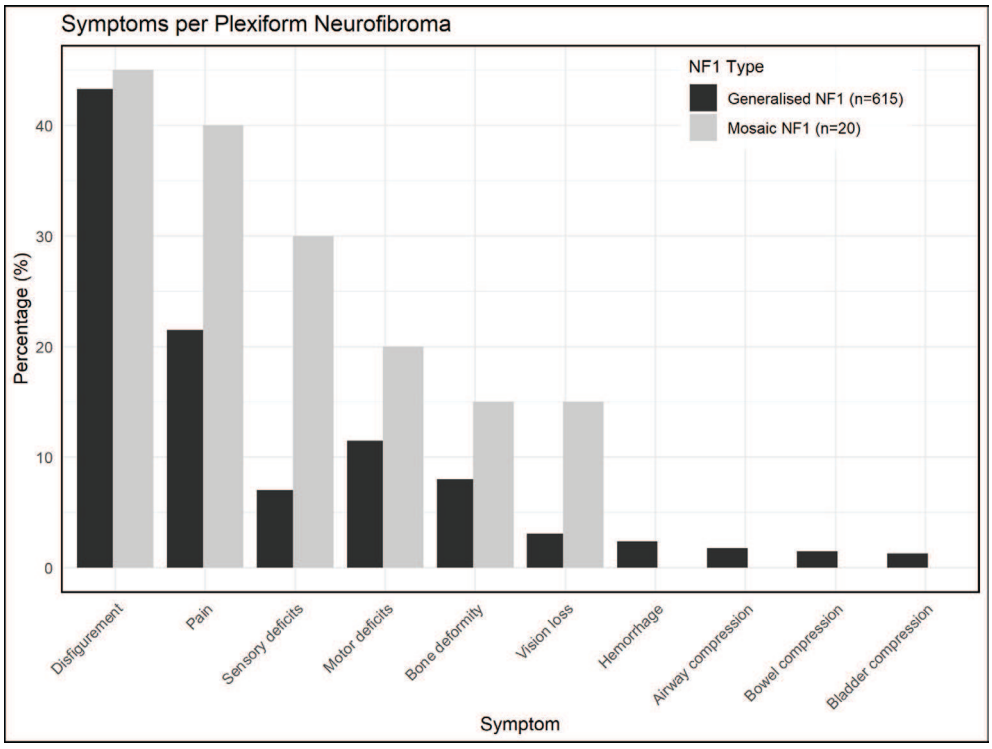


Figure 1: prevalence of symptoms or complications in plexiform neurofibromas with individual bars for plexiform neurofibromas of patients with generalized NF1 vs mosaic NF1.

The most frequently reported indications for treatment were pain (37%), disfigurement (35%) and neurological deficits (14%). Of the total number of patients with plexiform neurofibromas, 229 patients (46%) had one or multiple surgeries for symptomatic PNs. Partial resection or debulking surgery was more common than complete resections. Seventy-six PNs (12%) had multiple resections for the same tumor. The highest number of resections per PN was 14. Three patients received radiotherapy for PNs between 1998 and 2005. In one patient, an MPNST developed 18 years later within the irradiated field. Twenty-six patients were treated with trametinib in the TRAIN study[16]. Two patients were treated with selumetinib. One adult patient was treated with imatinib from 2009 until 2015 in another hospital.

MPNST

Forty-four patients (4%) were diagnosed with MPNST, of whom 43 patients (98%) had generalized NF1 and 1 patient (2%) had mosaic NF1. Nineteen patients with MPNSTs (43%) were male and 25 (57%) were female. In total, 54 MPNSTs were identified in our cohort, with 7 patients presenting with multiple MPNSTs (table 4). A history of plexiform neurofibromas was present in 34 patients (77%) with MPNSTs. MPNSTs were predominantly (91%) located in the proximal body regions, including the proximal extremities.

Multivariable logistic regression showed a strong association between the presence of nodular PNs and MPNST risk (OR 7.42, 95% CI 3.45-16.69, p <0.001). Age (OR 1.01 per year, 95% CI 0.99–1.03, p = 0.27) and high PN tumor load (OR 1.37, 95% CI 0.58–3.11, p = 0.46) were not significantly associated with MPNST in our population.

Table 4 MPNST

	MPNSTs n=54
Age at diagnosis, median (range)	38 (2-74)
Location of MPNST, n (%)[a]	
Spinal	5 (9)
Head/neck	9 (17)
Thorax	9 (17)
Abdomen/pelvis	14 (26)
Proximal extremities	17 (32)
Distal extremities	5 (9)
Surgery, n (%)[b]	n=57
Partial resection/debulking[c]	12 (24)
Complete resection[c]	38 (76)
Peri/postoperative complications, n (%)	11 (19)
Hemorrhage	1 (2)
Neurological deficit	4 (7)
Other	7 (12)
Regrowth after resection, n (%)	30 (53)
Radiotherapy, n (%)	31 (57)
Metastasis, n (%)[d]	21 (48)
Systemic treatment, n (%)[d]	14 (32)
Doxorubicine	13 (30)
Pazopanib	3 (7)
Ifosfamide	7 (16)
Other	4 (9)

[a]: multiple or overlapping locations possible. [b]: contains multiple resections per MPNST. [c]:7 missing. [d]:data of metastasis and systemic treatment are reported per patient. MPNST=malignant peripheral nerve sheath tumor.

Seventy-seven percent of the patients with MPNST underwent at least one surgery. Metastatic disease occurred in 21 patients (48%) and 14 (32%) were treated with systemic therapy. During follow-up, 50% of patients died, with 21 deaths (48%) attributable to MPNST and one (2%) to suicide. The median time between first diagnosis of MPNST and death was 12 months (range 2-109). The 5-year survival after first diagnosis was 54.5%. The 5-year survival stratified by sex was 52.6% for males and 56% for females.

Genotype-phenotype correlations

The known genotype-phenotype correlations as described by Peluto et al. were compared to the findings in our cohort in table 5[26]. No patients were identified with variants p.Leu844, p.Cys845, p.Ala846, p.Gly848 and p.Met1149. The mild phenotypes associated with p.Met992del and p.Arg1809 could be confirmed in our population. Two out of 10 patients with p.Arg1276 had spinal PN, none of the patients had OPGs and most patients had none or <10 cutaneous neurofibromas. The three patients in our cohort with p.Leu847 did not have malignancies. Thirty-four patients were identified with 17q11.2 microdeletion with a median age of 36 years (range 19-78 years). A history of MPNST was reported in 2 patients (6%) and other malignancies were reported in 3 patients (9%) (breast, endometrium and ovarian cancer). We could not confirm the known genotype-phenotype correlation for the variant p.Lys1423, as we identified only one patient with this variant.

Table 5 Genotype-phenotype correlation

Genotype	Known phenotype correlation[a]	Phenotype in our population
p.Met922del (n=8)	Absence of PN	No PNs
	Absence of OPG	No OPGs
p.Arg1809 (n=7)	Absence of CN	Only one patient with a single CN
	Absence of PN	No PNs
	Absence of OPG	No OPGs
p.Arg1276 (n=10)	Spinal PN	Spinal PN in 2 patients[b]
	Less OPG	No OPGs
	Less CN	8 patients (80%) had no or <10 CN
p.Leu847 (n=3)	Risk of other malignancy	No malignancies
p.Lys1423 (n=1)	Visible PN	No PNs
17q11.2 microdeletion (n=34)	Many CN	7 patients (21%) had >100 CN
	Spinal PN	19 patients (56%) had PNs
		6 patients (30%) had spinal PNs
	Higher risk of MPNST	2 patients (6%) had MPNST
		3 patients (9%) had other malignancies

No patients were identified with variants: p.Leu844, p.Cys845, p.Ala846, p.Gly848, p.Met1149

[a]: phenotype correlation as described by Peduto et al[27]. [b]:MR imaging of the spine only available in 5 out of 10 patients. PN=plexiform neurofibroma. CN=cutaneous neurofibroma. OPG=optic pathway glioma. MPNST=malignant peripheral nerve sheath tumor.

DISCUSSION

The primary objective of this study was to describe the clinical characteristics of PNs and MPNSTs in a large real-world cohort of adult NF1 patients in The Netherlands. Given the size and detailed phenotyping of this cohort, these findings contribute to the international understanding of NF1 and complement existing data from other European and global registries.

This analysis included 1,067 adults with NF1: 1,019 with generalized NF1 and 48 with mosaic NF1. The proportion of mosaic NF1 patients in our population (4%) is consistent with the expected ratio, when comparing to the estimated prevalence of mosaic NF1 in the general population (18-20 times less frequent than generalized NF1) [27]. The presence of the diagnostic features, including CALM and freckling, was comparable to previous reports[28-31]. Lisch nodules were likely underreported, as they are often overlooked and not all patients visited an ophthalmologist[32]. Our hospital population seems to be relatively more highly educated, compared to education levels in a registry study [33]. This could imply that patients with higher education are more likely to be under follow-up in a hospital than patients with lower education. The incidence of non-OPG brain tumors was consistent with previous studies, with low-grade gliomas being more common than high-grade gliomas[34]. Our study showed a high prevalence of malignancies, especially under 50 years of age. MPNST accounted for 40% of deaths, with a median age at death of 48 years, underscoring the aggressive nature of NF1-associated sarcomas and their impact on life expectancy. This aligns with previous mortality studies in NF1 populations from North West England and Finland, although our cohort showed a higher percentage of deaths due to MPNST[35, 36]. The relatively young age distribution of our cohort and the referral of more severely affected patients to our expertise center may have led to an overrepresentation of premature NF1-related mortality.

PNs were present in 47% of the adult patients with NF1. This aligns with earlier studies [6, 37, 38], although this likely underestimates true prevalence in our cohort given the absence of routine whole-body MRI. Pain, disfigurement and neurological deficit were the most common symptoms of PNs and often the indication for treatment. Patients with mosaic NF1 exhibited a higher frequency of PN-related pain and neurological deficits, although this likely reflects selection bias because diagnosis in mosaic NF1 typically occurs when symptoms prompt clinical evaluation. Surgical management typically involves partial resection or debulking rather than complete excision, which is associated with a high risk of regrowth and often necessitates repeated resections for the same PN. Furthermore, post-operative complications, such as hemorrhage and neurological deficit, are common. Although radiotherapy has historically been used, it is now avoided because of the risk of secondary malignancies, particularly MPNSTs. Of note, one of the three patients who received radiotherapy, developed an MPNST in the irradiated field. These limitations

highlight the need for effective new therapies for PN. The introduction of targeted therapies, especially MEK inhibitors, is a major step forward in the treatment of patients with PNs and severe symptoms[11-17].

In this cohort, 4% of the patients developed an MPNST, which is lower than the reported life-time risk of 8-15.8% [18, 19]. This discrepancy likely reflects the relatively young age distribution of our cohort. Previous studies have shown that MPNSTs are mostly located in the trunk and extremities[18, 39, 40]. Most MPNSTs (91%) in our study were located in the proximal body and likely not externally visible, underscoring the importance of regular surveillance and prompt imaging when warning signs, such as pain, neurological deficits or rapid growth occur[18, 41]. Most of the patients with MPNSTs were known to have PNs. Presence of nodular PNs was an independent risk factor for MPNST development. The 5-year survival following MPNST diagnosis was 54.5% in our population. Contrary to Ingham et al, who observed improved survival in women, our analysis revealed no sex-related differences [42], consistent with findings from a recent meta-analysis[43].

When comparing with established genotype-phenotype correlations [26], our findings confirmed the milder phenotype associated with p.Met992del and p.Arg1908 variants, which may warrant less intensive surveillance, e.g. once per 3 to 5 years. Consistent with prior evidence, patients with 17q11.2 microdeletion demonstrated an increased risk of MPNSTs, highlighting the need for close monitoring, e.g. once per year, in this subgroup[44]. We could not find an association between 17q11.2 microdeletion and other malignancies in our population. Other variants were observed too infrequently or available imaging was not sufficient to allow meaningful interpretation.

This study has limitations inherent to its retrospective design, including missing data and variable follow-up duration, partly due to treatment in other hospitals. Since this cohort was not established as a prospective longitudinal natural history study, the available follow-up reflects clinical encounters rather than systematic, protocol-driven assessments. Interpretation of survival outcomes is further limited, as the majority of patients were still alive at the time of data extraction and the availability and duration of long-term follow-up varied substantially across individuals. Detailed PN data were restricted to the three largest and/or most symptomatic PNs per patient, which may have led to an underestimation of the total tumor burden. Conversely, as a national tertiary NF1 expertise center, our cohort may overrepresent patients with more severe disease, introducing referral bias. Finally, the number of patients per genotype variant was relatively small, limiting the strength of the genotype-phenotype correlation analyses. These limitations should be taken into consideration when interpreting the results. This is, however, one of the largest reported cohorts of adult patients with NF1, monitored at the national NF1 expertise center and all data were manually collected and verified, ensuring accurate and detailed clinical information.

In conclusion, we confirmed that NF1 is a complex tumor predisposition syndrome characterized by a highly variable clinical expression, an unpredictable disease course, elevated risk of malignancy, and reduced life expectancy. Early identification of malignant transformation remains critical for improving outcomes. Regular surveillance is therefore recommended for all patients with NF1, including adults, accompanied by systematic patient education on warning signs such as pain, neurological deficits, and/or rapid tumor growth. High-risk subgroups, including patients with a 17q11.2 microdeletion or nodular PNs, require intensified monitoring. The emergence of targeted therapy, such as MEK inhibitors, represents a significant advance in addressing the long-standing therapeutic gap for patients with severe PN-related morbidity and will likely shape future clinical guidelines and research priorities. This retrospectively collected dataset will serve as the foundation for a Dutch national longitudinal registry with both adult and pediatric patients with NF1.

REFERENCES

1. Lee TJ, Chopra M, Kim RH, Parkin PC, Barnett-Tapia C. Incidence and prevalence of neurofibromatosis type 1 and 2: a systematic review and meta-analysis. Orphanet J Rare Dis. 2023;18(1):292.
2. Kallionpaa RA, Uusitalo E, Leppavirta J, Poyhonen M, Peltonen S, Peltonen J. Prevalence of neurofibromatosis type 1 in the Finnish population. Genet Med. 2018;20(9):1082-6.
3. Legius E, Messiaen L, Wolkenstein P, Pancza P, Avery RA, Berman Y, et al. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. Genet Med. 2021;23(8):1506-13.
4. Landry JP, Schertz KL, Chiang YJ, Bhalla AD, Yi M, Keung EZ, et al. Comparison of Cancer Prevalence in Patients With Neurofibromatosis Type 1 at an Academic Cancer Center vs in the General Population From 1985 to 2020. JAMA Netw Open. 2021;4(3):e210945.
5. Rasmussen SA, Yang Q, Friedman JM. Mortality in neurofibromatosis 1: an analysis using U.S. death certificates. Am J Hum Genet. 2001;68(5):1110-8.
6. Ejerskov C, Farholt S, Nielsen FSK, Berg I, Thomasen SB, Udupi A, et al. Clinical Characteristics and Management of Children and Adults with Neurofibromatosis Type 1 and Plexiform Neurofibromas in Denmark: A Nationwide Study. Oncol Ther. 2022.
7. Prada CE, Rangwala FA, Martin LJ, Lovell AM, Saal HM, Schorry EK, Hopkin RJ. Pediatric plexiform neurofibromas: impact on morbidity and mortality in neurofibromatosis type 1. J Pediatr. 2012;160(3):461-7.
8. Dhaenens BAE, Ferner RE, Bakker A, Nievo M, Evans DG, Wolkenstein P, et al. Identifying challenges in neurofibromatosis: a modified Delphi procedure. Eur J Hum Genet. 2021;29(11):1625-33.
9. Mautner VF, Asuagbor FA, Dombi E, Funsterer C, Kluwe L, Wenzel R, et al. Assessment of benign tumor burden by whole-body MRI in patients with neurofibromatosis 1. Neuro Oncol. 2008;10(4):593-8.
10. Canavese F, Krajbich JI. Resection of plexiform neurofibromas in children with neurofibromatosis type 1. J Pediatr Orthop. 2011;31(3):303-11.
11. Gross AM, Wolters PL, Dombi E, Baldwin A, Whitcomb P, Fisher MJ, et al. Selumetinib in Children with Inoperable Plexiform Neurofibromas. N Engl J Med. 2020;382(15):1430-42.
12. Dombi E, Baldwin A, Marcus LJ, Fisher MJ, Weiss B, Kim A, et al. Activity of Selumetinib in Neurofibromatosis Type 1-Related Plexiform Neurofibromas. N Engl J Med. 2016;375(26):2550-60.
13. Weiss BD, Wolters PL, Plotkin SR, Widemann BC, Tonsgard JH, Blakeley J, et al. NF106: A Neurofibromatosis Clinical Trials Consortium Phase II Trial of the MEK Inhibitor Mirdametinib (PD-0325901) in Adolescents and Adults With NF1-Related Plexiform Neurofibromas. J Clin Oncol. 2021;39(7):797-806.
14. Gross AM, O'Sullivan Coyne G, Dombi E, Tibery C, Herrick WG, Martin S, et al. Selumetinib in adults with NF1 and inoperable plexiform neurofibroma: a phase 2 trial. Nat Med. 2025;31(1):105-15.
15. Chen AP, Coyne GO, Wolters PL, Martin S, Farschtschi S, Blanco I, et al. Efficacy and safety of selumetinib in adults with neurofibromatosis type 1 and symptomatic, inoperable plexiform neurofibromas (KOMET): a multicentre, international, randomised, placebo-controlled, parallel, double-blind, phase 3 study. Lancet. 2025;405(10496):2217-30.
16. Noordhoek DC, Hanff DF, French PJ, van Dijk SA, Wiemer EAC, Manintveld OC, et al. Trametinib in Adults with Neurofibromatosis Type 1-Related Symptomatic Plexiform Neurofibromas. Ann Neurol. 2025.

17. Moertel CL, Hirbe AC, Shuhaiber HH, Bielamowicz K, Sidhu A, Viskochil D, et al. ReNeu: A Pivotal, Phase IIb Trial of Mirdametinib in Adults and Children With Symptomatic Neurofibromatosis Type 1-Associated Plexiform Neurofibroma. J Clin Oncol. 2024:JCO2401034.
18. Evans DG, Baser ME, McGaughran J, Sharif S, Howard E, Moran A. Malignant peripheral nerve sheath tumours in neurofibromatosis 1. J Med Genet. 2002;39(5):311-4.
19. Uusitalo E, Rantanen M, Kallionpaa RA, Poyhonen M, Leppavirta J, Yla-Outinen H, et al. Distinctive Cancer Associations in Patients With Neurofibromatosis Type 1. J Clin Oncol. 2016;34(17):1978-86.
20. Kolberg M, Holand M, Agesen TH, Brekke HR, Liestol K, Hall KS, et al. Survival meta-analyses for >1800 malignant peripheral nerve sheath tumor patients with and without neurofibromatosis type 1. Neuro Oncol. 2013;15(2):135-47.
21. Dunn GP, Spiliopoulos K, Plotkin SR, Hornicek FJ, Harmon DC, Delaney TF, Williams Z. Role of resection of malignant peripheral nerve sheath tumors in patients with neurofibromatosis type 1. J Neurosurg. 2013;118(1):142-8.
22. Kroep JR, Ouali M, Gelderblom H, Le Cesne A, Dekker TJA, Van Glabbeke M, et al. First-line chemotherapy for malignant peripheral nerve sheath tumor (MPNST) versus other histological soft tissue sarcoma subtypes and as a prognostic factor for MPNST: an EORTC soft tissue and bone sarcoma group study. Ann Oncol. 2011;22(1):207-14.
23. Carton C, Evans DG, Blanco I, Friedrich RE, Ferner RE, Farschtschi S, et al. ERN GENTURIS tumour surveillance guidelines for individuals with neurofibromatosis type 1. EClinicalMedicine. 2023;56:101818.
24. International standard classification of education ISCED 2011. Montreal, Quebec: UNESCO Institute for Statistics; 2012.
25. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24.
26. Peduto C, Zanobio M, Nigro V, Perrotta S, Piluso G, Santoro C. Neurofibromatosis Type 1: Pediatric Aspects and Review of Genotype-Phenotype Correlations. Cancers (Basel). 2023;15(4).
27. Garcia-Romero MT, Parkin P, Lara-Corrales I. Mosaic Neurofibromatosis Type 1: A Systematic Review. Pediatr Dermatol. 2016;33(1):9-17.
28. Miraglia E, Moliterni E, Iacovino C, Roberti V, Laghi A, Moramarco A, Giustini S. Cutaneous manifestations in neurofibromatosis type 1. Clin Ter. 2020;171(5):e371-e7.
29. Trevisson E, Cassina M, Opocher E, Vicenzi V, Lucchetta M, Parrozzani R, et al. Natural history of optic pathway gliomas in a cohort of unselected patients affected by Neurofibromatosis 1. J Neurooncol. 2017;134(2):279-87.
30. Listernick R, Charrow J, Greenwald M, Mets M. Natural history of optic pathway tumors in children with neurofibromatosis type 1: a longitudinal study. J Pediatr. 1994;125(1):63-6.
31. Delucia TA, Yohay K, Widmann RF. Orthopaedic aspects of neurofibromatosis: update. Curr Opin Pediatr. 2011;23(1):46-52.
32. Lewis RA, Riccardi VM. Von Recklinghausen neurofibromatosis. Incidence of iris hamartomata. Ophthalmology. 1981;88(4):348-54.
33. Johansson E, Kallionpaa RA, Bockerman P, Peltonen J, Peltonen S. A rare disease and education: Neurofibromatosis type 1 decreases educational attainment. Clin Genet. 2021;99(4):529-39.
34. Sellmer L, Farschtschi S, Marangoni M, Heran MK, Birch P, Wenzel R, et al. Non-optic glioma in adults and children with neurofibromatosis 1. Orphanet J Rare Dis. 2017;12(1):34.

35. Evans DG, O'Hara C, Wilding A, Ingham SL, Howard E, Dawson J, et al. Mortality in neurofibromatosis 1: in North West England: an assessment of actuarial survival in a region of the UK since 1989. Eur J Hum Genet. 2011;19(11):1187-91.
36. Uusitalo E, Leppavirta J, Koffert A, Suominen S, Vahtera J, Vahlberg T, et al. Incidence and mortality of neurofibromatosis: a total population study in Finland. J Invest Dermatol. 2015;135(3):904-6.
37. Plotkin SR, Bredella MA, Cai W, Kassarjian A, Harris GJ, Esparza S, et al. Quantitative assessment of whole-body tumor burden in adult patients with neurofibromatosis. PLoS One. 2012;7(4):e35711.
38. Nguyen R, Kluwe L, Fuensterer C, Kentsch M, Friedrich RE, Mautner VF. Plexiform neurofibromas in children with neurofibromatosis type 1: frequency and associated clinical deficits. J Pediatr. 2011;159(4):652-5 e2.
39. Ducatman BS, Scheithauer BW, Piepgras DG, Reiman HM, Ilstrup DM. Malignant peripheral nerve sheath tumors. A clinicopathologic study of 120 cases. Cancer. 1986;57(10):2006-21.
40. Dubbink HJ, Bakels H, Post E, Zwarthoff EC, Verdijk RM. TERT promoter mutations and BRAF mutations are rare in sporadic, and TERT promoter mutations are absent in NF1-related malignant peripheral nerve sheath tumors. J Neurooncol. 2014;120(2):267-72.
41. Ferner RE, Huson SM, Thomas N, Moss C, Willshaw H, Evans DG, et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. J Med Genet. 2007;44(2):81-8.
42. Ingham S, Huson SM, Moran A, Wylie J, Leahy M, Evans DG. Malignant peripheral nerve sheath tumours in NF1: improved survival in women and in recent years. Eur J Cancer. 2011;47(18):2723-8.
43. Cai Z, Tang X, Liang H, Yang R, Yan T, Guo W. Prognosis and risk factors for malignant peripheral nerve sheath tumor: a systematic review and meta-analysis. World J Surg Oncol. 2020;18(1):257.
44. Kehrer-Sawatzki H, Mautner VF, Cooper DN. Emerging genotype-phenotype relationships in patients with large NF1 deletions. Hum Genet. 2017;136(4):349-76.

PART II

Diagnosing plexiform neurofibromas

3

High resolution nerve ultrasound in neurofibromatosis type 1: a prospective and descriptive study

D.C. Noordhoek, T.A. Ennik, S.A. van Dijk, E. Mann, S. van der Vlist, J. Drenthen*, W. Taal*

*authors contributed equally
Clinical Neurophysiology. 2025 Nov:179:2110992.

ABSTRACT

Objective

To explore the value of high resolution nerve ultrasound (HRUS) and nerve conduction studies (NCS) in screening for plexiform neurofibromas (PNs) in neurofibromatosis type 1 (NF1).

Methods

Adult patients with NF1 were eligible. Patients were divided into two groups: with peripheral nervous system related symptoms (PNS group) and without PNS-related symptoms (non-PNS group). Study visit included neurological examination, NCS and HRUS.

Results

Sixty patients were enrolled, 37 in the PNS group and 23 in the non-PNS group. HRUS was abnormal in 52 patients (87%), 34 in the PNS group (92%) and 18 in the non-PNS group (78%, p=0.24). Patients with continuous nerve enlargements (high PN tumor load) were easily distinguished with HRUS. 85% of patients with normal NCS have nerve enlargements on HRUS.

Conclusions

HRUS helps to assess PN tumor burden in NF1. NCS did not have additional value.

Significance

HRUS might be useful as a screening tool for PN in NF1, especially in settings with less MRI availability.

INTRODUCTION

Neurofibromatosis type 1 (NF1) is a relatively common tumor predisposition syndrome with a birth incidence of approximately 1 in 2000-2500 individuals (Uusitalo et al, 2015). NF1 is caused by an autosomal dominantly inherited or *de novo* mutation in the NF1 tumor suppressor gene. Clinical manifestations include café-au-lait macules, axillary and inguinal freckling, Lisch nodules and cutaneous, subcutaneous and plexiform neurofibromas.

Plexiform neurofibromas (PNs) are benign peripheral nerve sheath tumors that arise from multiple nerve fascicles. Whole body-MRI (WB-MRI) detects PNs in about 50% of adults with NF1 (Mautner et al, 2008). PNs are present at birth but continue to grow during childhood and early adulthood (Nguyen et al, 2012). They can cause pain, neurological deficit, cosmetic burden and/or compression of vital structures (Ejerskov et al, 2022). We define two types of PNs based on morphology: diffuse and nodular. In general, no imaging is needed to detect diffuse PNs as they are often externally visible in the skin. Contrarily, nodular PNs are usually internal and can be asymptomatic, which requires imaging for detection. However, patients with nodular PNs are 20 times more likely to develop malignant peripheral nerve sheath tumors (MPNSTs) compared to patients without nodular PNs (Tucker et al, 2005). MPNSTs are a life-threatening condition with a 5-year overall survival of 44% (Kolver et al, 2013). Therefore, it is important to identify patients with nodular PNs and monitor carefully for malignant transformation. If a patient presents with symptoms related to a PN, it can be difficult to identify the symptomatic PN as patients often have multiple PNs along the same nerve.

Currently, the imaging modalities used to screen patients with NF1 for nodular PNs are regional or WB-(PET-)MRI, which are expensive, time-consuming and have a large carbon footprint (McAlister et al, 2022). As a quick and cost-effective alternative, high-resolution nerve ultrasound (HRUS) has been suggested. Previously published studies describing HRUS in patients with NF1 have shown frequent subclinical involvement of the peripheral nerves, varying from 85% to 94% of patients displaying HRUS abnormalities, such as focal neurofibromas (hypoechoic enlarged fascicles) and/or nerve enlargement with normal fascicular pattern. (Telleman et al, 2017; Winter et al, 2017) However, these studies had small sample sizes and the majority of the patients were asymptomatic or clinical presentation was not taken into account. HRUS nerve abnormalities and their impact on nerve function, measured by clinical examination and nerve conduction studies (NCS), have not been studied in a larger cohort of patients with NF1.

Our aim is to describe the variety of HRUS and NCS abnormalities in a larger and more diverse group of patients with NF1, with or without symptoms related to the peripheral nervous system (PNS). In addition, we explore the potential value of HRUS in clinical practice as a screening tool for asymptomatic nodular PNs or to identify symptomatic PNs.

METHODS

Study design

We performed a prospective cross-sectional explorative study in the Erasmus Medical Centre (Erasmus MC) in Rotterdam, the Netherlands. Erasmus MC is the Dutch national expertise center on NF1. The study protocol was approved by the local medical ethics committee of Erasmus MC. Patients were eligible for participation if at least 18 years of age and having a clinical diagnosis of NF1 according to the NIH diagnostic criteria (Legius et al, 2021). Patients were recruited by their treating physician during a regular visit to the outpatient clinic. Patients with, as well as without symptoms related to the PNS were eligible. Exclusion criteria were: comorbidity associated with (poly)neuropathy (e.g. alcohol abuse or diabetes mellitus) and inability to give written informed consent or to undergo HRUS and/or NCS. Written informed consent was obtained from all participants prior to inclusion. Patients were invited for a study visit including clinical examination, NCS and HRUS.

Clinical examination

Standardized clinical examination was performed by the patients' treating physician during a regular visit to the outpatient clinic or by a trained neurology resident during the study visit. A short history was taken of the patients' symptoms regarding pain, sensory and motor function. Patients were asked to rate their pain on a Numerical Rating Scale from 0 to 10 (NRS-11) (Downie et al, 1978). The motor function of 9 muscle groups (deltoid, biceps, triceps, wrist flexors and extensors, interossei, abductor pollicis brevis, ankle flexors and extensors) was graded bilaterally using the Medical Research Council scale (MRC) (Medical Research Council, 1976). Sensory deficits and reflexes (biceps, triceps, patellar, achilles and plantar) were documented. Patients were divided into two groups: with PNS related symptoms (PNS group) and without PNS related symptoms (non-PNS group). Assigning the participants to the groups was done in a consensus meeting with two experienced neurologists (WT, JD) and a neurology resident (DN). Patients were assigned to the PNS group if either self-reported symptoms (i.e. pain, motor and/or sensory complaints) and/or findings at neurological examination indicated involvement of one or more peripheral nerves.

Nerve conduction studies

Standardized NCS were performed and analyzed by a trained clinical neurophysiologist or a neurology resident supervised by a trained clinical neurophysiologist. A one-sided NCS protocol was performed on the side on which the patient experienced the most symptoms or, in asymptomatic patients, the non-dominant side. Sensory nerve action potentials (SNAP) and sensory nerve conduction velocity of the median (3rd digit), ulnar (5th digit), radial (1st digit) and sural (ankle) nerves were recorded. Compound muscle action potentials (CMAP), motor nerve conduction velocity, distal motor latency and F-waves of the median, ulnar, radial, fibular and tibial nerves were also recorded. Recording sites were the abductor pollicis brevis, abductor digiti minimi, extensor indicis proprius, extensor digitorum brevis and the abductor hallucis muscles, respectively. Reference values of Buschbacher were used (Buschbacher et al, 2006). Occasionally the NCS protocol was extended with specific tests for carpal tunnel syndrome if clinical symptoms were present.

High-resolution ultrasound

Standardized HRUS was performed and analyzed by a trained clinical neurophysiologist or a neurology resident supervised by a trained clinical neurophysiologist, using the Philips EPIQ Elite ultrasound or the Philips IU22 ultrasound machine, with a 4- to 18-MHz linear array transducer. The median, ulnar and radial nerves were visualized from wrist to axilla; the brachial plexus at the truncal level (superior, medial and inferior trunk); the peroneal nerve from fibular head to popliteal fossa; the tibial nerve at the medial malleolus and the sural nerve approximately 10-14 cm proximal to the lateral malleolus. All nerves were examined bilaterally. Nerve cross-sectional area (CSA) was measured at standard predetermined anatomical sites (supplementary files 1) and at additional sites in case of isolated nerve enlargements. The probe was held perpendicular to the nerve and nerve CSA was measured in mm^2 by drawing a line along the inner border of the epineurium. Doppler technique was used to asses vascularization of the nerve at sites of nerve enlargement. Previously published reference values were used for CSA (Goedee et al, 2017). Regarding additional measurements of the radial nerve in the forearm, the reference value published by Bae et al was used (Bae et al, 2021). For comparison of the CSA across the patient groups, CSA was calculated as percentage of the upper limit of normal (ULN). Besides quantitative measurement of CSA, the overall pattern of HRUS findings was categorized per patient as: A) normal, B) nerve enlargement(s) with normal nerve morphology, C) focal nerve enlargement(s) with abnormal nerve morphology, such as enlarged nerve fascicles as seen in PN (focal PNs), D) continuous nerve enlargements with PN morphology (continuous PNs). (Fig 1)

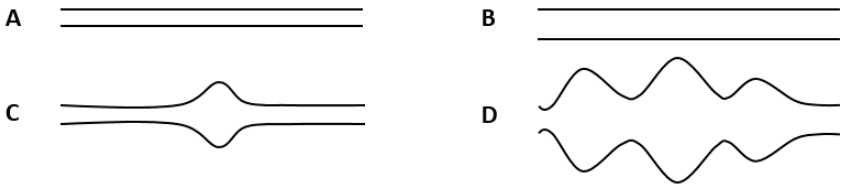


Fig. 1 High-resolution ultrasound patterns. A: normal. B: nerve enlargement(s) with normal nerve morphology. C: focal nerve enlargement(s) with abnormal nerve morphology, such as enlarged nerve fascicles as seen in plexiform neurofibromas (focal PNs). D: continuous nerve enlargements with PN morphology (continuous PNs).

Additionally, patients with nerve enlargement at compression sites (but with intact fascicular pattern) were identified. The following criteria were used: median nerve: enlarged at carpal tunnel with wrist/forearm ratio > 1.5 and normal nerve CSA in the forearm, ulnar nerve: isolated enlargement at the cubital tunnel, peroneal nerve: enlarged at fibular head but normal CSA at popliteal fossa.

Analysis

IBM SPSS statistics version 28 was used to analyze the data. Chi-squared test or Fishers' exact test was used to assess categorical data. Independent samples T-test or Mann-Whitney U test was used to compare continuous variables. *Graphpad prism version 9* was used to create graphs.

RESULTS

Patient characteristics

We enrolled 60 patients between July 2019 and February 2023 (Table 1). Thirty-seven patients had PNS-related symptoms (PNS group), while the remaining 23 were allocated in the non-PNS group. The majority of patients in both groups were female (32 (86%) in the PNS group, 15 (65%) in the non-PNS group). The median age was 45 years in the PNS group and 44 years in the non-PNS group. Pain was the most reported symptom in both groups, but was significantly more common in the PNS group. Patients in the PNS group reported loss of strength and sensory disturbances more often, but self-reported symptoms were not always confirmed by neurological examination. All patients reported at least one symptom. One patient in the non-PNS group had motor deficits during neurological examination, caused by cervical spinal cord compression by neurofibromas.

Table 1: patient characteristics.

	Total (n=60)	PNS group (n=37)	non-PNS group (n=23)	P-values
Female sex – no. (%)	47 (78)	32 (86)	15 (65)	0.05[a]
Median age – years (range)	45 (18-72)	45 (18-62)	44 (18-72)	0.64[b]
Self-reported symptoms				
Pain – no. (%)	46 (77)	34 (92)	12 (52)	**0.001[c]**
Loss of strength – no. (%)	26 (43)	19 (51)	7 (30)	0.11[a]
Sensory disturbances – no. (%)	33 (55)	29 (78)	4 (17)	**<0.001[c]**
Neurological examination				
Motor deficit – no. (%)	6 (10)	5 (14)	1 (4)	0.39[c]
Sensory deficit – no. (%)	19 (32)	19 (51)	0 (0)	**<0.001[c]**

PNS=peripheral nervous system. [a]chi2, [b]t-test, [c]fishers' exact

Ultrasound

HRUS was abnormal in 52 of 60 patients (87%), 34 in the PNS group (92%) and 18 in the non-PNS group (78%). The percentage of patients with abnormal HRUS was not significantly different between the groups (p=0.24). Figure 2 shows two images with measurements of the median nerve CSA at one third of the left forearm in two patients, one with normal CSA and one with severe enlargement of the median nerve including enlarged hypoechoic fascicles.

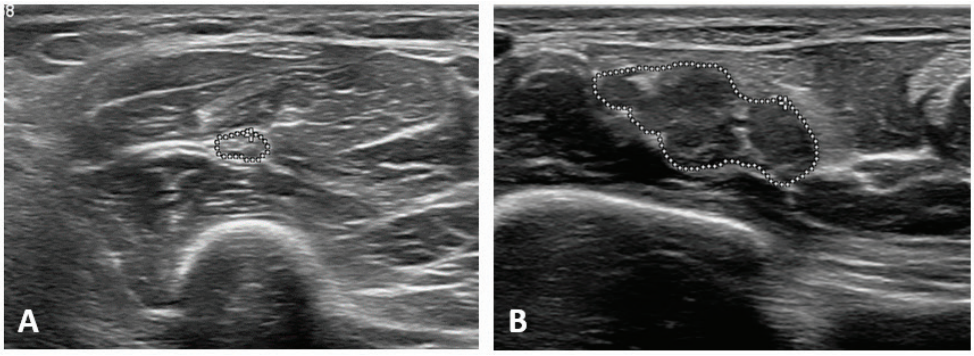


Fig. 2 Median nerve cross-sectional area (CSA) at one third of the left forearm in two patients.
A: CSA 5 mm2 (normal), B: CSA 71 mm2 (789% of upper limit of normal).

Average CSA as percentage of ULN was significantly higher in the PNS group compared to the non-PNS group (p<0.001) (Fig 3). A graph with the CSA as percentage of ULN per nerve is enclosed in supplementary file 2. Vascularization was seen at 2.2% of all measuring sites (including extra measuring sites), 2.5% in the PNS group and 1.6% in the non-PNS group.

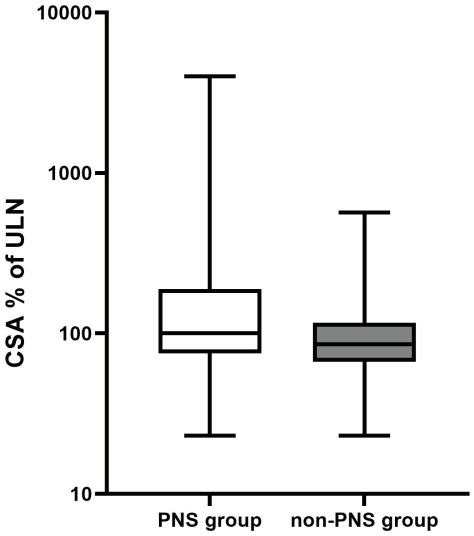


Fig. 3 Box-and-whisker plot of the cross-sectional area (CSA) of all measuring sites calculated as percentage of the upper limit of normal (ULN) per patient group. Boxes representing interquartile range, with the line indicating the median and whiskers indicate min-max range. PNS=peripheral nervous system.

HRUS pattern was not significantly different between the groups (p=0.49) (Fig 4). Almost half of the patients (43%) had continuous PNs on HRUS, this phenotype was easily distinguished from the smaller group of patients (18%) with focal PNs pattern. Nerve enlargements with normal nerve morphology were found in 25% of the patients.

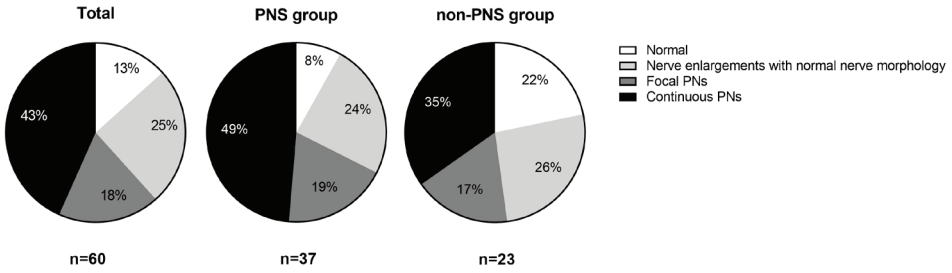


Fig. 4 Pie charts with distribution of high-resolution nerve ultrasound (HRUS) patterns in the two patient groups and total. PNS=peripheral nervous system. PN=plexiform neurofibroma.

Nerve conduction studies

NCS abnormalities were found in 20 patients (33%). The percentage of patients with abnormal NCS was significantly higher in the PNS group (p=0.01) (Table 2).

Table 2: cross table of high-resolution nerve ultrasound (HRUS) findings versus nerve conduction studies (NCS) findings per patient group.

		NCS abnormal	NCS normal	total
PNS group	**HRUS abnormal**	15	19	34
	HRUS normal	2	1	3
	Total	17	20	37
non-PNS group	**HRUS abnormal**	3	15	18
	HRUS normal	0	5	5
	Total	3	20	23

PNS=peripheral nervous system.

HRUS was abnormal in 90% of the patients with abnormal NCS, however HRUS was also abnormal in 85% of the patients with normal NCS. Table 3 shows the NCS findings in relation to HRUS pattern and clinical symptoms. NCS showed various types of abnormalities, of which some had no relation with the HRUS findings or clinical symptoms. A consistent relation between HRUS, clinical symptoms and NCS findings could only be found in case of median nerve entrapment at the carpal tunnel.

Table 3: Patients with abnormal NCS and the relation with HRUS pattern and clinical symptoms.

Patient number	HRUS pattern	Relation NCS-HRUS	Relation NCS-clinical symptoms
NCS: Axonal polyneuropathy			
1	Continuous PNs	Yes	Yes
23, 29	Continuous PNs	Yes	No
33	Normal	No	No
NCS: Mixed axonal and demyelinating polyneuropathy			
27	Continuous PNs	Yes	Yes
NCS: Focal slowing, conduction block or temporal dispersion outside compression points (demyelinating neuropathy)			
3[a], 24, 26, 49	Continuous PNs	Likely	No
39	Focal PNs	Yes: Conduction block at site of PN	No
NCS: Ulnar nerve neuropathy at cubital tunnel			
6[a]	Focal PNs	No: normal CSA at cubital tunnel	No
26	Continuous PNs	likely	No
53	Continuous PNs	No: normal CSA at cubital tunnel	No
NCS: Median nerve neuropathy at carpal tunnel			
36	Nerve enlargement, normal morphology	Yes: significantly enlarged at carpal tunnel	Yes
46, 60	Continuous PNs	Yes: significantly enlarged at carpal tunnel	Yes

Table 3: Patients with abnormal NCS and the relation with HRUS pattern and clinical symptoms. (*continued*)

Patient number	HRUS pattern	Relation NCS-HRUS	Relation NCS-clinical symptoms
Focal decreased CMAP or SNAP amplitudes (axonal neuropathy)			
38	Nerve enlargement, normal morphology	No: no enlargements at nerve(s) with NCS abnormalities	No
41	Normal	No	Yes
45	Focal PNs	Yes: enlargements at nerve site(s) with NCS abnormalities	Yes
50[a]	Focal PNs	Yes: enlargements at nerve site(s) with NCS abnormalities	No
53, 59	Continuous PNs	Likely	No

[a]patients in the non-PNS group. NCS=nerve conduction studies, HRUS=high-resolution nerve ultrasound, PN=plexiform neurofibroma, CSA=cross-sectional area, CMAP=compound muscle action potential, SNAP=sensory nerve action potential.

Symptomatic PN on ultrasound

Amongst the 37 patients in the PNS group, 26 patients (70%) had nerve enlargements in the nerve(s) where the symptoms could be located. In only one patient, a single symptomatic nerve enlargement could be identified. In all other cases, the nerve enlargement responsible for the symptoms could not be located, because of multiple nerve enlargements in the same nerve.

Increased CSA at compression sites

Focal increased CSA at compression sites was found in 23 patients (38%), 14 patients (38%) in the PNS group, and 9 patients (39%) in the non-PNS group (p=0.92). Most common were increased CSA of the median nerve at the carpal tunnel (23 nerve segments) and increased CSA of the ulnar nerve at the cubital tunnel (16 nerve segments). We found one case of increased CSA of the fibular nerve at the fibular head. In 12 nerve segments (30%) we found clinical symptoms consistent with entrapment neuropathy, 11 in the median nerve and one at the ulnar nerve.

In 5 nerve segments (13%), NCS showed corresponding signs of entrapment neuropathy, all in the median nerve and with corresponding clinical symptoms of carpal tunnel syndrome. In 20 nerve segments the NCS was normal at the compression site and in 15 nerve segments it was not specifically measured. Twenty-eight (70%) of nerve segments with focal increased CSA at compression sites were asymptomatic and had no NCS abnormalities related to an entrapment neuropathy.

DISCUSSION

In this prospective study, we described the variety of HRUS and NCS abnormalities in patients with NF1 with or without PNS-related symptoms and explored the potential value of HRUS in clinical practice.

HRUS abnormalities were present in the majority of the patients. We confirmed that nerve enlargements and PN can often be asymptomatic in NF1, as shown by the similar prevalence of HRUS abnormalities in patients with PNS-related symptoms and those without. NCS abnormalities were found more often in the PNS group than in the non-PNS group, but we found that only a minority of the HRUS abnormalities lead to NCS abnormalities. However, HRUS was performed two-sided and NCS only one-sided, which could mean that NCS abnormalities were missed on the side that was not examined. Patients with PN cannot be identified solely on the basis of clinical presentation or NCS. A large part of the patients without PNS symptoms (non-PNS group) were found to have HRUS abnormalities: 52% of them had PNs and an additional 26% had nerve enlargements with normal nerve morphology. Twenty-five percent of all the patients had nerve enlargements with normal nerve morphology. The origin of these nerve enlargements with normal morphology remains unclear due to the absence of histological analysis. Considering the nature of the disease, it is plausible that these enlargements could be attributed to PNs. Our findings are in line with the study of Telleman et al (Telleman et al, 2017). Vascularization was rarely seen in our population.

HRUS patterns were similar in the PNS group and non-PNS group. We did find that HRUS enabled us to make a clear distinction between patients with continuous PNs and patients with other HRUS patterns. The risk of developing MPNST is associated with PN tumor load (Nguyen et al, 2014), so identifying the patients with continuous PNs is important. The newly published NF1 guideline by the European Reference Network (ERN) of Genetic Tumor Risk Syndromes (GENTURIS) recommends screening for PNs with at least one WB-MRI at transition from adolescence to adult care, in order to assess internal PN tumor load and determine the risk of developing an MPNST (based on expert majority decision without consistent evidence). In the absence of PNs, further monitoring with WB-MRI is not needed. (Carton et al, 2023) Using HRUS for screening might have several advantages over WB-MRI. HRUS is quick and cost-effective and, depending on the hospital, more easily available. HRUS has much less environmental impact compared to WB-MRI (McAlister et al, 2022). In addition, WB-MRI has a risk of finding incidentalomas (Preez et al, 2020). It should be noted that screening for PNs with HRUS is limited to the extremities, as HRUS cannot visualize internal PNs along the spine or in the chest and abdominal area. However, HRUS could be used for a first screening to determine the patient's phenotype with regard to PN tumor load. Implementation of HRUS could be particularly useful in

situations where accessibility of MRI is less, such as in low income countries or in pediatric patients that would need sedation for MRI.

Despite CSA being significantly higher in patients with PNS-related symptoms, our findings suggest that pinpointing the PN responsible for the patient's symptoms with HRUS is often not possible. HRUS might not be a suitable technique for this clinical application. An unexpected finding in our data was that 23 patients (38%) had isolated nerve enlargement at compression sites. The majority were asymptomatic and without NCS abnormalities related to entrapment neuropathy. Therefore, in patients with NF1, entrapment neuropathy cannot be diagnosed solely with HRUS. We did not find any literature on entrapment neuropathies in patients with NF1.

Compared to earlier studies on this topic, our study included a larger number of patients and a variety of symptomatic and asymptomatic patients. However, our data might be subject to selection bias since our centre is the national referral centre for NF1, in which patients are on average more severely affected than in the general NF1 population. Consequently, a large proportion of symptomatic patients may have taken part, with a corresponding overestimation of the prevalence of HRUS and NCS abnormalities in patients with NF1.

CONCLUSIONS

Our study has shown that it is possible to get an impression of the patients' PN tumor load on the basis of HRUS findings. HRUS might be useful as a first screening tool for patients with NF1, while NCS has no added value in screening for PNs.

Nerve enlargements at compression sites are common in patients with NF1. We recommend to diagnose entrapment neuropathy in these patients not solely on the basis of HRUS findings, but also taking NCS findings and symptoms into account. Follow-up of our study participants is currently ongoing to study PN growth and interobserver variability with HRUS in patients with NF1. Extended follow-up is needed to determine whether HRUS can effectively differentiate between low risk patients and high risk patients, who are prone to developing MPNST in time. A prospective study comparing HRUS with MRI would help to validate the diagnostic value of HRUS. Future research could also explore the possibility to distinguish between PN and MPNST with HRUS and the role of HRUS in longitudinal monitoring, such as during MEK inhibitor treatment.

REFERENCES

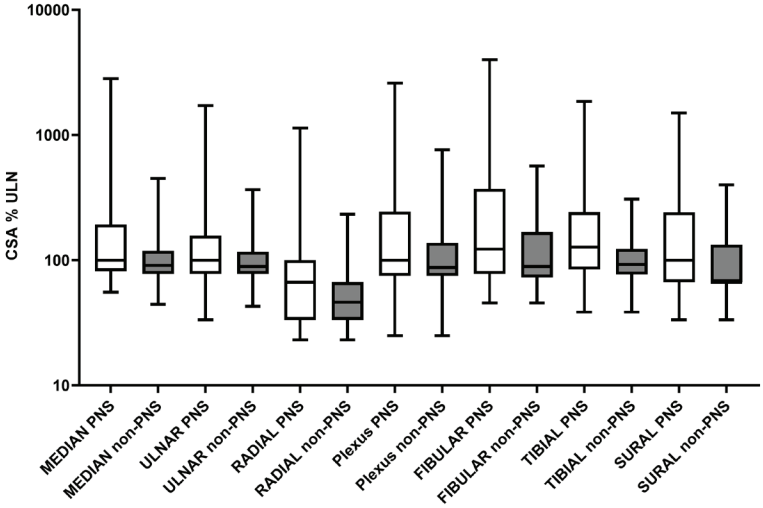
- Bae DW, An JY. Cross-sectional area reference values for high-resolution ultrasonography of the upper extremity nerves in healthy Asian adults. Medicine (Baltimore). 2021;100(18).
- Buschbacher RM, Prahlow, N.D. Manual of Nerve Conduction Studies. Second ed: Demos Medical Publishing; 2006. 299 p.
- Carton C, Evans DG, Blanco I, Friedrich RE, Ferner RE, Farschtschi S, et al. ERN GENTURIS tumour surveillance guidelines for individuals with neurofibromatosis type 1. EClinicalMedicine. 2023;56:101818.
- Downie WW, Leatham PA, Rhind VM, Wright V, Branco JA, Anderson JA. Studies with pain rating scales. Ann Rheum Dis. 1978;37(4):378-81.
- Ejerskov C, Farholt S, Nielsen FSK, Berg I, Thomasen SB, Udupi A, et al. Clinical Characteristics and Management of Children and Adults with Neurofibromatosis Type 1 and Plexiform Neurofibromas in Denmark: A Nationwide Study. Oncol Ther. 2022.
- Goedee HS, van der Pol WL, van Asseldonk JH, Franssen H, Notermans NC, Vrancken AJ, et al. Diagnostic value of sonography in treatment-naive chronic inflammatory neuropathies. Neurology. 2017;88(2):143-51.
- Kolberg M, Holand M, Agesen TH, Brekke HR, Liestol K, Hall KS, et al. Survival meta-analyses for >1800 malignant peripheral nerve sheath tumor patients with and without neurofibromatosis type 1. Neuro Oncol. 2013;15(2):135-47.
- Legius E, Messiaen L, Wolkenstein P, Pancza P, Avery RA, Berman Y, et al. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. Genet Med. 2021;23(8):1506-13.
- Mautner VF, Asuagbor FA, Dombi E, Funsterer C, Kluwe L, Wenzel R, et al. Assessment of benign tumor burden by whole-body MRI in patients with neurofibromatosis 1. Neuro Oncol. 2008;10(4):593-8.
- McAlister S, McGain F, Petersen M, Story D, Charlesworth K, Ison G, et al. The carbon footprint of hospital diagnostic imaging in Australia. Lancet Reg Health West Pac. 2022;24:100459.
- Medical Research Council. Aids to Examination of the Peripheral Nervous System. London: Her Majesty's Stationary Office; 1976.
- Nguyen R, Dombi E, Widemann BC, Solomon J, Fuensterer C, Kluwe L, et al. Growth dynamics of plexiform neurofibromas: a retrospective cohort study of 201 patients with neurofibromatosis 1. Orphanet J Rare Dis. 2012;7:75.
- Nguyen R, Jett K, Harris GJ, Cai W, Friedman JM, Mautner VF. Benign whole body tumor volume is a risk factor for malignant peripheral nerve sheath tumors in neurofibromatosis type 1. J Neurooncol. 2014;116(2):307-13.
- Preez HD, Lasker I, Rajakulasingam R, Saifuddin A. Whole-body magnetic resonance imaging: Incidental findings in paediatric and adult populations. Eur J Radiol. 2020;130:109156.
- Telleman JA, Stellingwerff MD, Brekelmans GJ, Visser LH. Nerve ultrasound: A useful screening tool for peripheral nerve sheath tumors in NF1? Neurology. 2017;88(17):1615-22.
- Tucker T, Wolkenstein P, Revuz J, Zeller J, Friedman JM. Association between benign and malignant peripheral nerve sheath tumors in NF1. Neurology. 2005;65(2):205-11.
- Uusitalo E, Leppavirta J, Koffert A, Suominen S, Vahtera J, Vahlberg T, et al. Incidence and mortality of neurofibromatosis: a total population study in Finland. J Invest Dermatol. 2015;135(3):904-6.
- Winter N, Rattay TW, Axer H, Schaffer E, Decard BF, Gugel I, et al. Ultrasound assessment of peripheral nerve pathology in neurofibromatosis type 1 and 2. Clin Neurophysiol. 2017;128(5):702-6.

Supplementary file 1: Standard anatomical sites for measuring nerve cross-sectional area with high-resolution nerve ultrasound.

Nerve	Anatomical site	CSA reference values (mm^2)
Median	Wrist	11
	⅓ Forearm	9
	½ Upper arm	10
Ulnar	Wrist	7
	Forearm	6
	Distal sulcus	9
	Sulcus	9
	Proximal Sulcus	9
	½ Upper arm	9
Radial	Upper arm	9
	Wrist (superficial nerve)	3
Brachial plexus	Superior trunk	8
	Middle trunk	8
	Inferior trunk	8
Peroneal	Popliteal fossa	9
	Fibular head	11
Tibial	Ankle	13
Sural	Lower leg[a]	3

[a]10-14 cm proximal to the lateral malleolus. CSA=cross-sectional area.

Supplementary file 2: Box-and-whisker plot of nerve cross-sectional area of all measuring sites as percentage of the upper limit of normal, per nerve and patient group.



Boxes representing interquartile range, with the line indicating the median and whiskers indicate min-max range. CSA=cross-sectional area. ULN=upper limit of normal. PNS=peripheral nervous system.

4

High-Resolution Nerve Ultrasound in Adults with NF1: An Accessible and Reproducible Imaging Tool for Plexiform Neurofibromas

D.C. Noordhoek, K.C. van Tulder, T.A. Ennik, W. Taal*, J. Drenthen*

*authors contributed equally

Diagnostics. 2025 Dec 10;15(24):3146.

ABSTRACT

Background/Objectives

High-resolution nerve ultrasound (HRUS) is a promising imaging modality in patients with neurofibromatosis type 1 (NF1). The aim of this study was to evaluate the use of HRUS in adults with NF1 by assessing changes in HRUS findings over a two-year follow-up time and reporting interobserver variability.

Methods

Sixty adult patients with NF1 were invited for a study visit including a clinical examination, nerve conduction studies (NCSs) and HRUS, at baseline and after two-years follow-up. The nerve cross-sectional area (CSA) was measured at standard anatomical sites and at additional sites in cases of nerve enlargements. In 16 patients, the CSA measurements of the median nerve on one side were performed by two observers to assess interobserver variability.

Results

Fifty-two patients participated in the follow-up visit. During follow-up, 40% of nerve enlargements increased, 46% decreased and 14% remained stable. Especially larger CSA measurements at baseline showed substantial increases and decreases at follow-up. The presence or absence of plexiform neurofibromas remained the same. Interobserver agreement of median nerve CSA measurements with HRUS was 0.982 (95% CI: 0.969–0.99).

Conclusions

HRUS can be an important additional imaging tool in patients with NF1. It is helpful to distinguish between patients with and without plexiform neurofibromas, which is relevant for estimating the risk of developing malignant peripheral nerve sheath tumors (MPNSTs). The good interobserver agreement supports the use of HRUS in clinical practice. The majority of nerve enlargements decreased spontaneously in size within two years, which limits the reliability of tumor volume as sole marker for treatment response.

INTRODUCTION

Regular imaging of plexiform neurofibromas (PNs) is often needed in patients with NF1, for instance, for preoperative evaluation or distinguishing between benign and malignant lesions. PNs can cause significant morbidity such as pain and neurological deficits [1]. Furthermore, PNs can transform into malignant peripheral nerve sheath tumors (MPNST), which are a life-threatening condition [2,3]. Early detection of MPNSTs is essential, as tumor size and the presence of metastasis are associated with poor survival [4,5]. Patients with a suspicion of (symptomatic) PN regularly undergo regional MR imaging, and screening for PN tumor load with whole body-MRI (WB-MRI) is recommended in the NF1 guideline by the European Reference Network (ERN) of Genetic Tumor Risk Syndromes (GENTURIS) [6]. High-resolution nerve ultrasound (HRUS) has been suggested as a more accessible additional imaging tool with a lower carbon footprint compared to MRI [7,8,9].

Our recent study of HRUS in patients with NF1 with and without symptoms of the peripheral nervous system (PNS) reported that nerve enlargements were present in the majority of patients and can often be asymptomatic and without abnormalities in nerve conduction studies (NCSs). It was shown that a clear distinction could be made between patients with no or very few neurofibromas and patients with continuous PNs [9]. This distinction between patients with low and high tumor load could be helpful in estimating the risk for MPNST and, therefore, deciding the frequency with which patients should be monitored [10].

Longitudinal follow-up of nerve enlargements with HRUS has been reported in a group of 16 patients with NF1 [7]. Telleman et al. showed that patients with only minor HRUS abnormalities at baseline did not show a large increase in nerve size or number of nerve enlargements at 1-year follow-up. Yet in patients with severe HRUS abnormalities, both a substantial increase and decrease in nerve size were observed, which poses the question of whether these are actual anatomic changes or are related to the interobserver variability of HRUS. A study with WB-MRI showed that the majority (63%) of internal PNs reduced in size within a ten-year follow-up of adults with NF1 [11].

The aim of this study was to further evaluate the use of HRUS in a broad spectrum of adults with NF1 by assessing changes in HRUS findings over a two-year follow-up time and reporting the interobserver variability of CSA measurements. Changes in PNS symptoms and NCS findings during follow-up were also reported.

METHODS

Study Design

Sixty adult patients with NF1 participated in the previously published cross-sectional explorative study on HRUS [9]. The baseline study visit included clinical examination, NCSs and HRUS. For this prospective longitudinal study, we invited the same participants for a follow-up visit. The follow-up visit was planned approximately 2 years after the baseline visit and consisted of the same study procedures, including clinical examination, NCSs and HRUS. The study was conducted in accordance with the Declaration of Helsinki and approved by the local medical ethics committee of Erasmus MC. All patients provided written informed consent at enrollment, which included the baseline and follow-up study visit. Adult patients with NF1, with or without symptoms related to the peripheral nervous system (PNS), were included in the study at baseline. Exclusion criteria at enrollment were having a comorbidity associated with (poly)neuropathy (e.g., alcohol abuse or diabetes mellitus) and inability to give written informed consent or to undergo HRUS and/or NCS.

Clinical Examination

Standardized clinical examination was performed by the patient's treating physician during a regular visit to the outpatient clinic or by a trained neurology resident during the study visit. A short history was taken of the patients' symptoms regarding pain and sensory and motor function. The motor function of 9 muscle groups (deltoid, biceps, triceps, wrist flexors and extensors, interossei, abductor pollicis brevis, ankle flexors, and extensors) was graded bilaterally using the Medical Research Council scale (MRC) [12]. Sensory deficits and reflexes (biceps, triceps, patellar, achilles and plantar) were documented. PNS-related symptoms were defined as either self-reported symptoms or findings at neurological examination indicating involvement of one or more peripheral nerves. Clinical findings were compared to baseline to identify new PNS-related symptoms.

Nerve Conduction Studies

A one-sided NCS protocol was performed on the same side as the baseline visit and by repeating the same protocol. NCSs were performed and analyzed by a trained clinical neurophysiologist or a neurology resident supervised by a trained clinical neurophysiologist. Sensory nerve action potentials (SNAPs) and sensory nerve conduction velocity of the median (3rd digit), ulnar (5th digit), radial (1st digit) and sural (ankle) nerves were recorded. Compound muscle action potentials (CMAPs), motor nerve conduction velocity, distal motor latency and F-waves of the median, ulnar, radial, fibular and tibial nerves were also recorded. Recording sites were the abductor pollicis brevis, abductor digiti minimi, extensor indicis proprius, extensor digitorum brevis and the abductor hallucis muscles, respectively. Reference values of Buschbacher were used [13]. If the NCS protocol was extended with extra tests at baseline, these were repeated at the follow-up visit. NCS findings at follow-up were compared to baseline to identify new NCS abnormalities.

High-Resolution Nerve Ultrasound

A two-sided standardized HRUS was performed and analyzed by a trained clinical neurophysiologist or a neurology resident supervised by a trained clinical neurophysiologist, using the Philips EPIQ Elite or Philips IU22 ultrasound machine (Philips, Eindhoven, The Netherlands), with a 4 to 18 MHz linear array transducer. The median, ulnar, radial, fibular, tibial and sural nerves were examined, as well as the brachial plexus at the truncal level. Nerve cross-sectional area (CSA) was measured manually at standard anatomical sites (Supplementary Table S1) and at additional sites in case of isolated nerve enlargements, similar to the baseline visit. Measurements at additional sites were registered with a distance in centimeters to the nearest external anatomical landmark, to ensure measuring the same site at the follow-up visit. The trunks of the brachial plexus were measured separately when possible; however, in cases where the individual trunks could not be identified, the brachial plexus was measured as a whole at both baseline and follow-up. Reference values for CSA by Goedee et al. were used [14]. Additional measurements of the radial nerve in the forearm were compared to the reference value published by Bae et al. [15]. Extreme outliers of CSA difference between baseline and follow-up were retrospectively reviewed by observers CN and JD. HRUS images were examined to verify the correct site of CSA measurement by comparing the surrounding anatomical structures. Additionally, the completeness of the measurement was compared, as was whether it was indeed performed at the inner border of the hyperechoic epineural rim. If both positioning and measurement integrity were correct, the outlier was classified as a true outlier. The Doppler technique was used to assess vascularization of the nerve at sites of nerve enlargement.

The overall pattern of HRUS findings was categorized per patient: (A) normal; (B) nerve enlargement(s) with normal nerve morphology; (C) focal nerve enlargement(s) with abnormal nerve morphology, such as enlarged nerve fascicles as seen in PN (focal PNs); (D) continuous nerve enlargements with PN morphology (continuous PNs); Figure 1 [9].

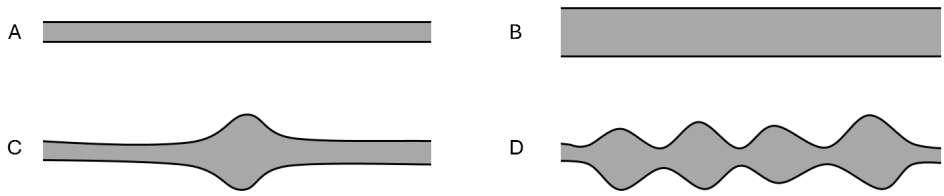


Figure 1. High resolution ultrasound patterns. A: normal. B: nerve enlargement(s) with normal nerve morphology. C: focal nerve enlargement(s) with abnormal nerve morphology, such as enlarged nerve fascicles as seen in plexiform neurofibromas (focal PNs). D: continuous nerve enlargements with PN morphology (continuous PNs). Figure modified, based on Noordhoek et al [9].

In 16 patients the CSA measurements of the median nerve on one side were performed by two observers (observer 1, CN, and observer 2, JD). The repeated measurements were taken in the same session, while the second observer was blinded for the CSA measurements of the first.

Statistical Analysis

Descriptive statistics were used to describe the clinical characteristics, HRUS and NCS findings. The difference in CSA between the baseline and follow-up was compared using a paired t-test. When comparing the difference in CSA between the baseline and follow-up across HRUS patterns, the HRUS pattern at follow-up was used. Correlation between age and CSA increase was evaluated with a linear regression model. The difference in the presence of vascularization between baseline and follow-up was compared using a chi-squared test. The intraclass correlation coefficient (ICC) for interobserver variability was calculated using a two-way random effects model for absolute agreement based on average measures. p-values of 0.05 were considered statistically significant, and 95% confidence intervals were reported where appropriate. Data was analyzed using R studio version 4.4.1.

RESULTS

Between July 2021 and February 2025, 52 out of 60 patients visited our outpatient clinic for the follow-up visit. Table 1 shows the patient characteristics and results of HRUS and NCSs for the patients who participated in the follow-up visit. The median time between the baseline and follow-up visit was 26 months (range 22–40 months). Eight patients were not able to attend the second visit: six because of personal reasons, one because of a newly diagnosed brain tumor, and one patient passed away from an MPNST during follow-up.

Table 1. Patient characteristics, n=52.

	Baseline	Follow up
Female sex – no. (%)	40 (77)	
Median age – years (range)	44 (18-72)	
Median follow-up time – months (range)		26 (22-40)
PNS-related symptoms – no. (%)	30 (58)	23 (44)
HRUS pattern		
Normal – no. (%)	7 (13)	7 (13)
Nerve enlargement(s) with normal nerve morphology – no. (%)	13 (25)	13 (25)
Focal PNs – no. (%)	9 (17)	9 (17)
Continuous PNs – no. (%)	23 (44)	23 (44)
Abnormal NCS – no. (%)	17 (33)	13 (25)

PNS: peripheral nervous system. HRUS: high resolution nerve ultrasound. PN: plexiform neurofibromas. NCS: nerve conduction studies.

Clinical Symptoms and Interventions

In total, 23 of 52 patients (44%) had PNS-related symptoms at the follow-up visit, of which 9 patients (39%) had neurological deficits at examination. Three asymptomatic patients at baseline had new PNS-related symptoms at follow-up, without corresponding HRUS changes.

Two patients had an intervention within the study period affecting one of the peripheral nerves that were examined in this study. One patient had a carpal tunnel release, and another patient had multiple corticosteroid injections in both wrists for carpal tunnel syndrome. In both cases the symptoms persisted.

One participant (age 27 years) developed an MPNST in the pelvis during the study period and passed away. At baseline, this patient had a continuous PNs pattern (high tumor load) on HRUS.

NCS

Thirteen patients (25%) had abnormal NCS results at the follow-up visit. Two patients with normal NCS results at baseline had new NCS abnormalities at follow-up. In both cases this could be attributed to neuropathy at compression sites. CSA measurements at those sites did not change compared to baseline. Two patients with abnormal NCS results at baseline had different NCS abnormalities at follow-up, in both cases with a corresponding increase in nerve CSA on HRUS.

HRUS Pattern

HRUS at follow-up was abnormal in 45 patients (87%), showing a similar distribution of patterns as at baseline: nerve enlargement(s) with normal nerve morphology in 13 patients (25%), a focal PN pattern in 9 patients (17%) and a continuous PN pattern in 23 patients (44%). All patients with a focal PN pattern and continuous PN pattern at baseline had the same HRUS pattern at follow-up. Four patients (8%) had a change in HRUS pattern between baseline and follow-up. The changes in pattern were only between normal HRUS and nerve enlargement(s) with normal nerve morphology, and the overall distribution of HRUS patterns remained the same. The change in pattern was due to fluctuations in CSA measurements that were not clinically relevant.

HRUS CSA Measurements

The mean CSA difference between the baseline and follow-up of all measurements was 0.50 mm2 due to both increases and decreases in CSA with SD 9.33 mm2 (95% CI: 0.11–0.88 mm2, $p = 0.012$). Although this mean difference was statistically significant, a difference of 0.50 mm2 is not considered clinically relevant. Figure 2 shows a scatterplot comparing the difference in CSA between baseline and follow-up to the CSA at baseline. Especially larger CSA measurements at baseline show substantial increases and decreases

at follow-up. A panel of individual scatterplots per nerve is included in Supplementary Figure S1.

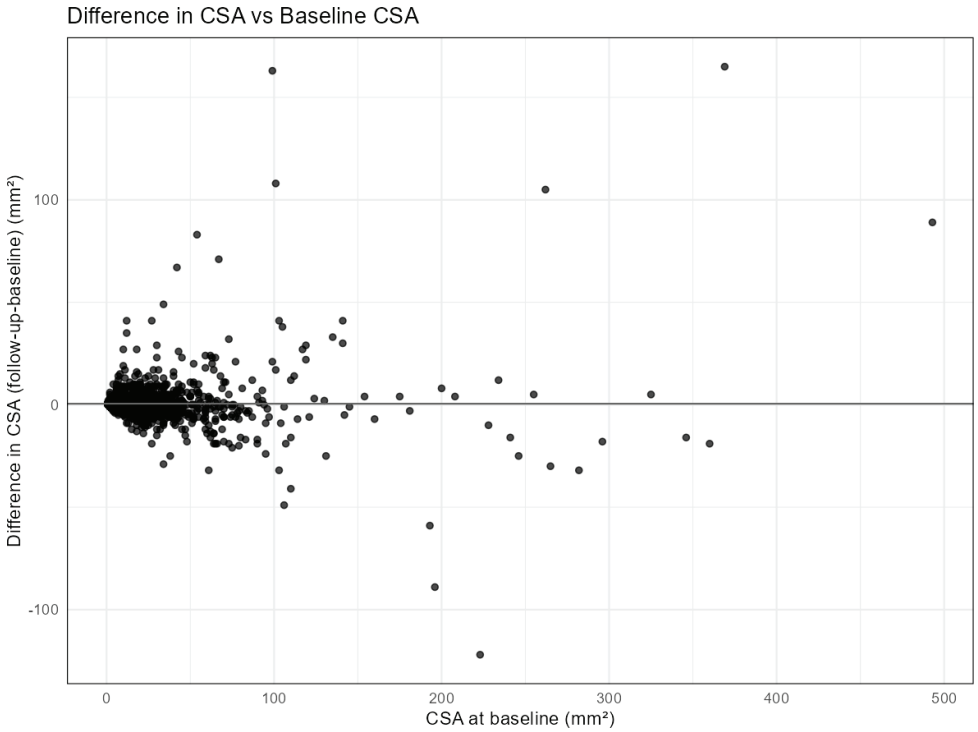


Figure 2. Scatterplot comparing difference in CSA between baseline and follow-up to the CSA at baseline in mm2. CSA = cross-sectional area.

Eight CSA measurements were identified as extreme outliers when compared to the baseline measurement and were subsequently reviewed. Four of these outliers reflect actual changes in nerve CSA. The remaining four outliers were attributable to a variation in measurement approach (e.g., different site of measurement or incomplete capture of the nerve CSA). Of these, three measurements involved the brachial plexus and one the median nerve in the axilla. Figure 3 shows baseline and follow-up images of two of these cases. The measurement of the fibular nerve (A and B) was classified as a true outlier, the measurement of the brachial plexus (C and D) was classified as a variation in measurement.

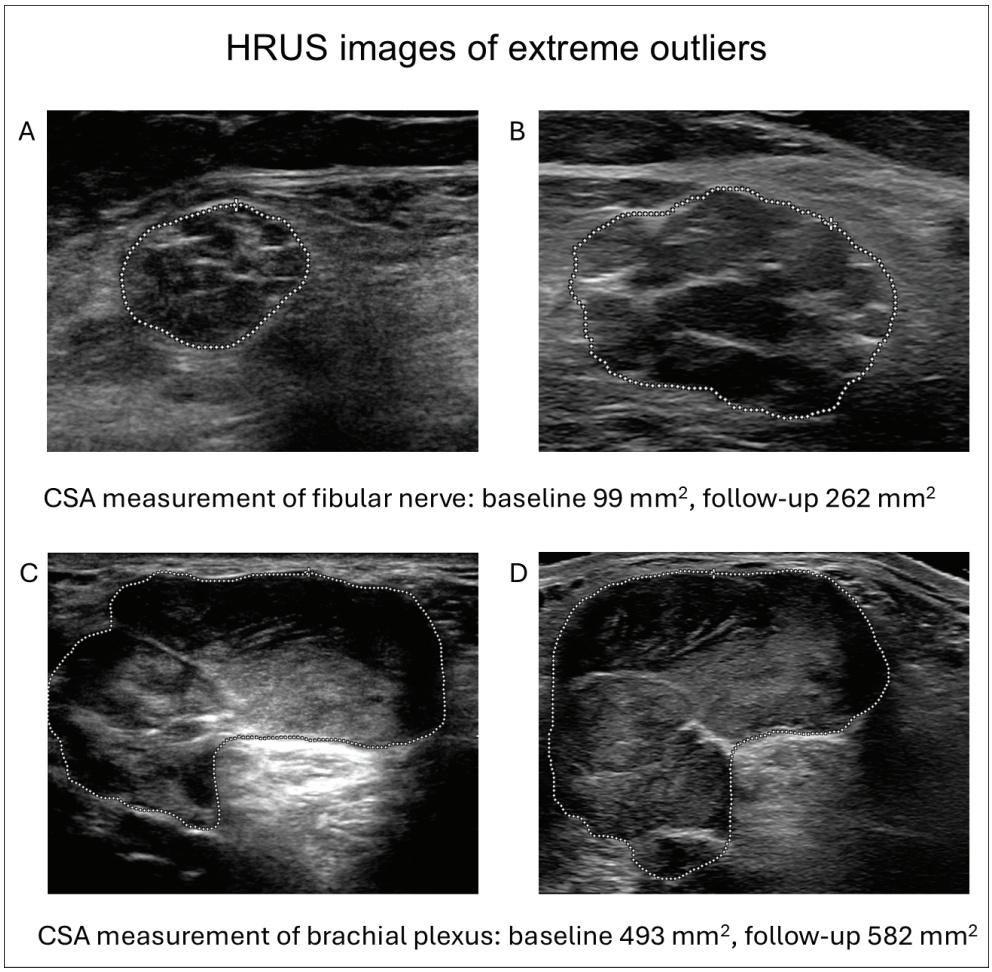


Figure 3. HRUS images of extreme outliers. Panels (A,B) show cross-sectional (CSA) measurements of the fibular nerve at the baseline and follow-up. The extreme difference between the baseline and follow-up was classified as a true outlier. Panels (C,D) show CSA measurement of the right brachial plexus, which was classified as a result of variation in measurement approach upon retrospective review.

Figure 4 compares CSA differences between baseline and follow-up across HRUS patterns. Patients with a normal HRUS pattern, nerve enlargement(s) with normal nerve morphology and a focal PN pattern show only minimal differences in CSA between the baseline and follow-up. In the continuous PN pattern group, however, the mean increase is 0.76 mm2, with substantial variance of both increases and decreases (SD 12.63 mm2).

The mean increase was 8 mm2 (range: 1–165 mm2). Of the 202 nerve enlargements (46%) that decreased, the mean decrease was −6 mm2 (range: −0.5−−122 mm2). Sixty-two nerve enlargements (14%) remained stable. Most patients had both an increase and a decrease in nerve enlargements (83%). Four patients (14%) had only an increase in the

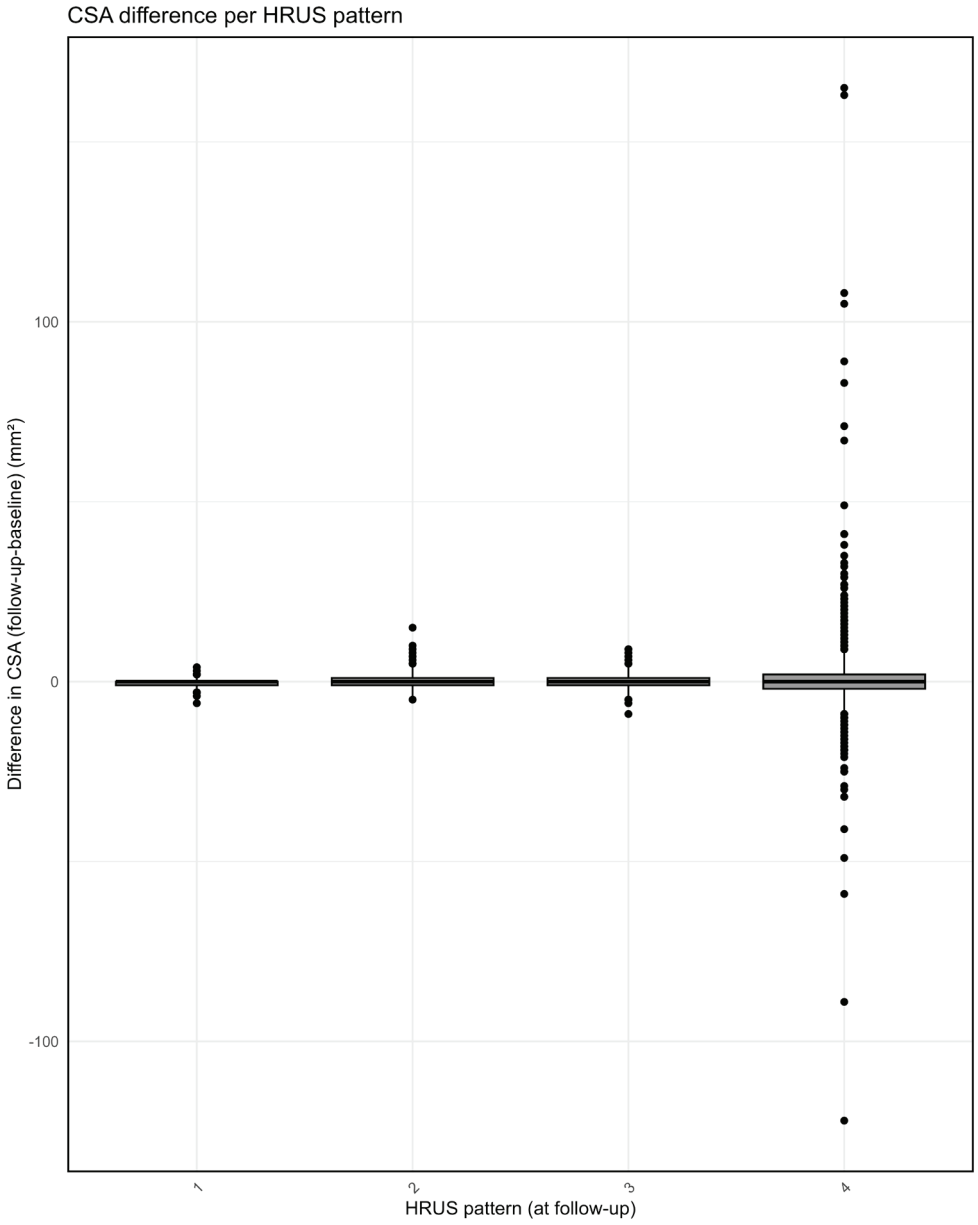


Figure 4. Box-and-whisker plot of difference in CSA (cross-sectional area) between baseline and follow-up per HRUS (high-resolution nerve ultrasound) pattern at follow-up. HRUS patterns: 1 normal, 2 nerve enlargement(s) with normal nerve morphology, 3 focal plexiform neurofibromas (PNs), 4 continuous PNs.

nerve enlargements, and one patient (3%) had only a decrease in the nerve enlargements. None of the four patients with only an increase in nerve enlargements had new PNS symptoms at follow-up and the age range of these patients was 32 to 58 years old. The correlation coefficient of age and CSA difference between the baseline and follow-up was 0.06 mm2, with a p-value of 0.24. Figure 5 shows three examples of CSA measurements with a decrease, stable CSA and an increase.

Vascularization was present in 2.3% of all measuring sites at baseline, compared to 3.0% at follow-up (p = 0.22).

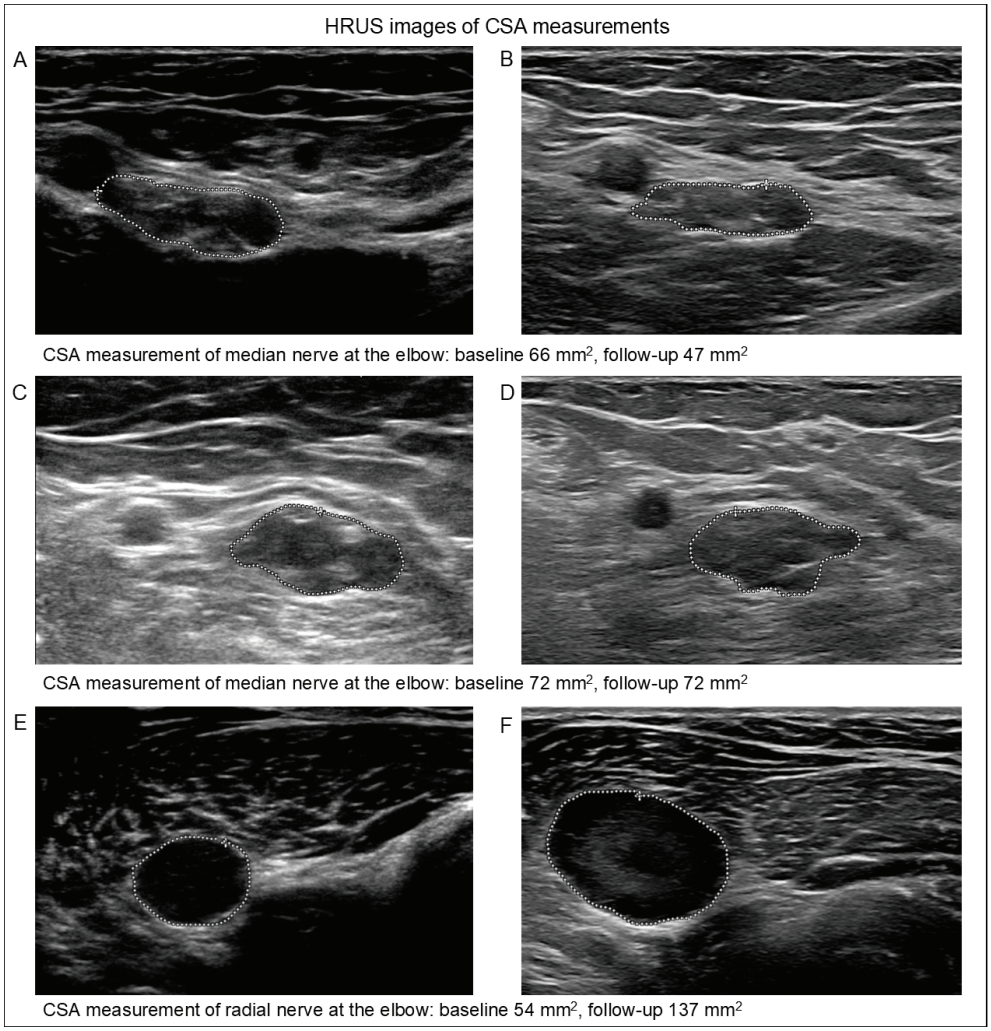


Figure 5. Cross-sectional area (CSA) measurements of different patients at baseline and follow-up. Panels (A,B) show a decrease in CSA, panels (C,D) a stable CSA, and panels (E,F) an increased CSA. HRUS = high-resolution nerve ultrasound.

Interobserver Variability of HRUS in NF1

The mean difference in CSA of the median nerve between observer 1 (CN) and observer 2 (JD) was −1.28 mm2 (95% CI −2.40–−0.17, SD 4.32 mm2), as seen in Figure 6. The ICC for interobserver variability for all CSA measurements was 0.982 (95% CI 0.969–0.99). The mean difference in CSA for only the measurements at standard anatomical sites of the median nerve was −1.38 mm2 (95% CI −2.76–−0.00, SD 4.62 mm2). The ICC for interobserver variability for CSA measurement at standard anatomical sites was 0.95 (95% CI 0.907–0.973). The mean difference in CSA for only additional measurements in case of nerve enlargements in the median nerve was −1.00 mm2 (95% CI −2.00–0.00, SD 3.36 mm2). The ICC for interobserver variability for only additional measurements was 0.995 (95% CI 0.985–0.998).

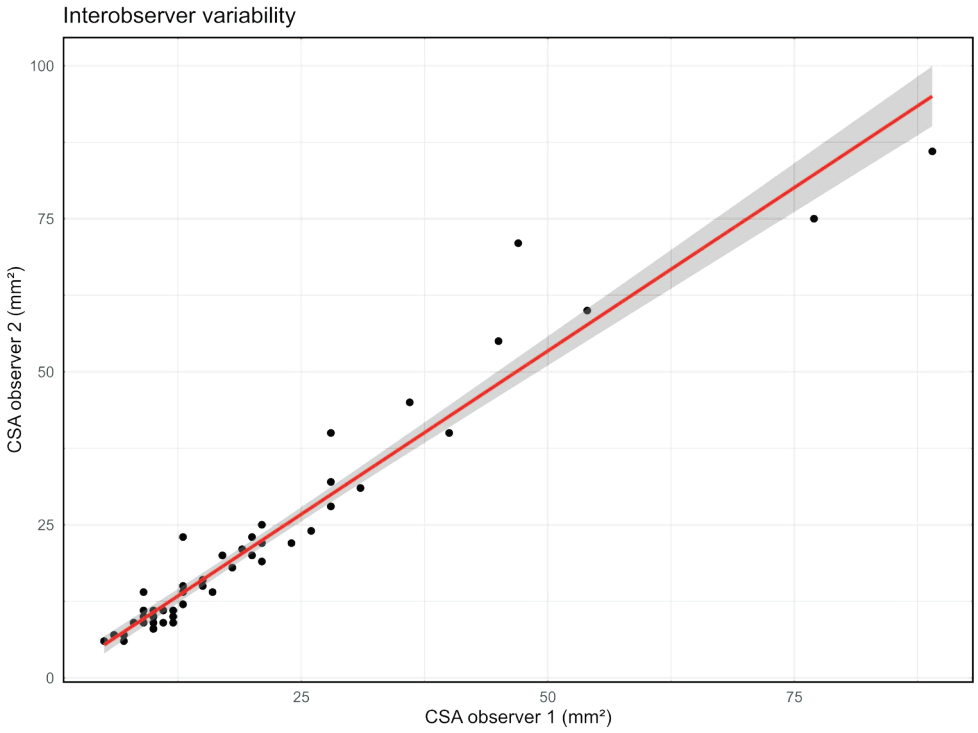


Figure 6. Agreement between median nerve cross-sectional area (CSA) as measured by observer 1 versus observer 2 with regression line (red) and 95% confidence interval (shading), in mm2.

DISCUSSION

HRUS is a promising additional imaging modality for the evaluation of peripheral nerve involvement in patients with NF1. However, the literature on HRUS in NF1 remains limited. This study provides a follow-up analysis of HRUS findings in adult patients with NF1 and reports changes in clinical PNS symptoms and NCS. To our knowledge, this is the first study to report interobserver variability for HRUS in patients with NF1.

Although the total mean CSA of all measurements increased significantly compared to baseline, the average increase of approximately 0.5 mm2 is not clinically relevant. More importantly, we observed that PNs can both increase and decrease in size over a two-year follow-up period, with substantial variance, especially in the continuous PN group. This pattern of bidirectional change aligns with findings from MRI studies [11,16,17] and with a small follow-up study on the use of HRUS in NF1 [7]. Interestingly, in the majority of patients with PNs, both increases and decreases in PN size were observed within the same patient. Among the four patients with only an increase in PN size, no new PNS symptoms were reported. This indicates that changes in PN size are not reliably reflected in clinical symptoms and supports the conclusion that symptom monitoring alone is insufficient to detect PN progression. Given that HRUS is a fast and relatively low-cost imaging modality, regular screening could be considered to assess whether PN growth is progressive and consistent over time, or whether apparent changes reflect natural fluctuations or measurement variability.

Although rapid PN growth is known to be associated with younger age and is typically observed in children [16], we did not find a clear correlation between age and either an increase or decrease in PN size within our adult cohort.

The percentage of patients with abnormal HRUS findings and the distribution of HRUS patterns remained unchanged over time. This suggests that, at least in the adult population, the HRUS-based phenotype is stable, and that patients with no PNs or only minor peripheral nerve involvement do not progress to develop PNs. These findings are consistent with earlier studies showing that adult patients without PNs typically do not develop them over time [7,16]. The patients with this phenotype may require less frequent follow-up to monitor for MPNST development.

Vascularization in nerve enlargements was rarely detected in our study. This did not change during follow-up time. Larger studies with longer follow-up time are needed to determine whether vascularization as detected with HRUS could be a sign of malignant transformation of PNs.

While interobserver agreement in HRUS assessments has generally been reported as good in healthy individuals and in patients with other peripheral neuropathies [18,19,20,21], these findings cannot be directly extrapolated to NF1 due to the often complex and atypical morphology of affected nerves. Accurately measuring complex, irregularly shaped structures such as PNs in a two-dimensional plane is inherently challenging and prone to considerable variability. A key strength of our study is the evaluation of interobserver agreement for CSA measurements in the median nerve of patients with NF1, which was excellent—even in the presence of PNs. Although a small but consistent difference was found between the two observers, this difference is not clinically relevant. It is, however, important to note that observer 1 was trained by observer 2, so the interobserver agreement between unrelated observers may vary.

During follow-up, eight measurements showed marked differences in CSA compared to baseline. Four of these were considered true anatomical changes, while the remaining four were attributed to variation in measurement approach. These discrepancies occurred mainly at the level of the brachial plexus, a region known for high variability in other disorders such as chronic inflammatory demyelinating polyneuropathy (CIDP) [22]. Another discrepancy was found in the axillary region. In both the axillary region and brachial plexus region, the presence of multiple nerve and vascular structures complicates HRUS measurements. The correct measurement position and angle for the brachial plexus requires skill and experience. Caution is therefore warranted when interpreting serial HRUS measurements in these areas.

Our study has several limitations. Interobserver variability was assessed only in the median nerve, while it is known that interobserver agreement with HRUS is generally poorer in the lower extremities [18]. This may have led to an overestimation of the overall interobserver agreement in our study. Several limitations of the HRUS technique itself have to be acknowledged, particularly in comparison to MRI. The visualization of deeper-located lesions, such as in the trunk, is limited with HRUS due to the limited penetration depth and field of view. Additionally, with the current two-dimensional technique of measuring CSA, volumetric assessment cannot be reliably performed. Differentiating between benign and malignant lesions is also not possible with the current HRUS technique. These limitations should be considered when implementing HRUS as an additional imaging tool in clinical practice.

In conclusion, HRUS can be a valuable addition to the clinical tools we have for the evaluation of peripheral nerve involvement in patients with NF1. It is particularly useful for distinguishing between individuals with and without PN tumor load, which may aid in assessing the risk for MPNST development. The observed spontaneous decrease in PN size might limit the use of tumor volume as a sole marker of treatment response and stresses the importance of placebo control groups in therapy trials for PNs. HRUS has shown good reproducibility and interobserver agreement for measuring nerve enlargements in the median nerve. However, CSA measurements at the level of the brachial plexus and axillary region remain challenging.

HRUS, due to its limitations, cannot replace MRI, but could be suitable for use in clinical settings requiring frequent monitoring, in healthcare systems with limited MRI availability or in patients who are unable to undergo MRI without sedation. The findings of this study should be validated in prospective comparative studies involving MRI. The interobserver variability of HRUS in patients with NF1 should be further assessed in the other peripheral nerves and by independently trained observers.

REFERENCES

1. Ejerskov, C.; Farholt, S.; Nielsen, F.S.K.; Berg, I.; Thomasen, S.B.; Udupi, A.; Agesen, T.; de Fine Licht, S.; Handrup, M.M. Clinical Characteristics and Management of Children and Adults with Neurofibromatosis Type 1 and Plexiform Neurofibromas in Denmark: A Nationwide Study. Oncol. Ther. 2022, 11, 97–110.
2. Tucker, T.; Wolkenstein, P.; Revuz, J.; Zeller, J.; Friedman, J.M. Association between benign and malignant peripheral nerve sheath tumors in NF1. Neurology 2005, 65, 205–211.
3. Kolberg, M.; Holand, M.; Agesen, T.H.; Brekke, H.R.; Liestol, K.; Hall, K.S.; Mertens, F.; Picci, P.; Smeland, S.; Lothe, R.A. Survival meta-analyses for >1800 malignant peripheral nerve sheath tumor patients with and without neurofibromatosis type 1. Neuro Oncol. 2013, 15, 135–147.
4. Cai, Z.; Tang, X.; Liang, H.; Yang, R.; Yan, T.; Guo, W. Prognosis and risk factors for malignant peripheral nerve sheath tumor: A systematic review and meta-analysis. World J. Surg. Oncol. 2020, 18, 257.
5. Sharma, M.R.; Puj, K.S.; Salunke, A.A.; Pandya, S.J.; Gandhi, J.S.; Parikh, A.R. Malignant peripheral nerve sheath tumor with analysis of various prognostic factors: A single-institutional experience. J. Cancer Res. Ther. 2021, 17, 106–113.
6. Carton, C.; Evans, D.G.; Blanco, I.; Friedrich, R.E.; Ferner, R.E.; Farschtschi, S.; Salvador, H.; Azizi, A.A.; Mautner, V.; Rohl, C.; et al. ERN GENTURIS tumour surveillance guidelines for individuals with neurofibromatosis type 1. EClinicalMedicine 2023, 56, 101818.
7. Telleman, J.A.; Stellingwerff, M.D.; Brekelmans, G.J.; Visser, L.H. Nerve ultrasound in neurofibromatosis type 1: A follow-up study. Clin. Neurophysiol. 2018, 129, 354–359.
8. McAlister, S.; McGain, F.; Petersen, M.; Story, D.; Charlesworth, K.; Ison, G.; Barratt, A. The carbon footprint of hospital diagnostic imaging in Australia. Lancet Reg. Health West. Pac. 2022, 24, 100459.
9. Noordhoek, D.C.; Ennik, T.A.; van Dijk, S.A.; Mann, E.; van der Vlist, S.; Drenthen, J.; Taal, W. High resolution nerve ultrasound in neurofibromatosis type 1: A prospective and descriptive study. Clin. Neurophysiol. 2025, 179, 2110992.
10. Nguyen, R.; Jett, K.; Harris, G.J.; Cai, W.; Friedman, J.M.; Mautner, V.F. Benign whole body tumor volume is a risk factor for malignant peripheral nerve sheath tumors in neurofibromatosis type 1. J. Neurooncol. 2014, 116, 307–313.
11. Ly, K.I.; Merker, V.L.; Cai, W.; Bredella, M.A.; Muzikansky, A.; Thalheimer, R.D.; Da, J.L.; Orr, C.C.; Herr, H.P.; Morris, M.E.; et al. Ten-Year Follow-up of Internal Neurofibroma Growth Behavior in Adult Patients with Neurofibromatosis Type 1 Using Whole-Body MRI. Neurology 2023, 100, e661–e670.
12. Medical Research Council. Aids to Examination of the Peripheral Nervous System; Volume Memorandum, No. 45; Her Majesty's Stationary Office: London, UK, 1976.
13. Buschbacher, R.M.; Prahlow, P.N. Manual of Nerve Conduction Studies, 2nd ed.; Demos Medical Publishing: New York City, NY, USA, 2006.
14. Goedee, H.S.; van der Pol, W.L.; van Asseldonk, J.H.; Franssen, H.; Notermans, N.C.; Vrancken, A.J.; van Es, M.A.; Nikolakopoulos, S.; Visser, L.H.; van den Berg, L.H. Diagnostic value of sonography in treatment-naive chronic inflammatory neuropathies. Neurology 2017, 88, 143–151.
15. Bae, D.W.; An, J.Y. Cross-sectional area reference values for high-resolution ultrasonography of the upper extremity nerves in healthy Asian adults. Medicine 2021, 100, e25812.
16. Nguyen, R.; Dombi, E.; Widemann, B.C.; Solomon, J.; Fuensterer, C.; Kluwe, L.; Friedman, J.M.; Mautner, V.F. Growth dynamics of plexiform neurofibromas: A retrospective cohort study of 201 patients with neurofibromatosis 1. Orphanet J. Rare Dis. 2012, 7, 75.

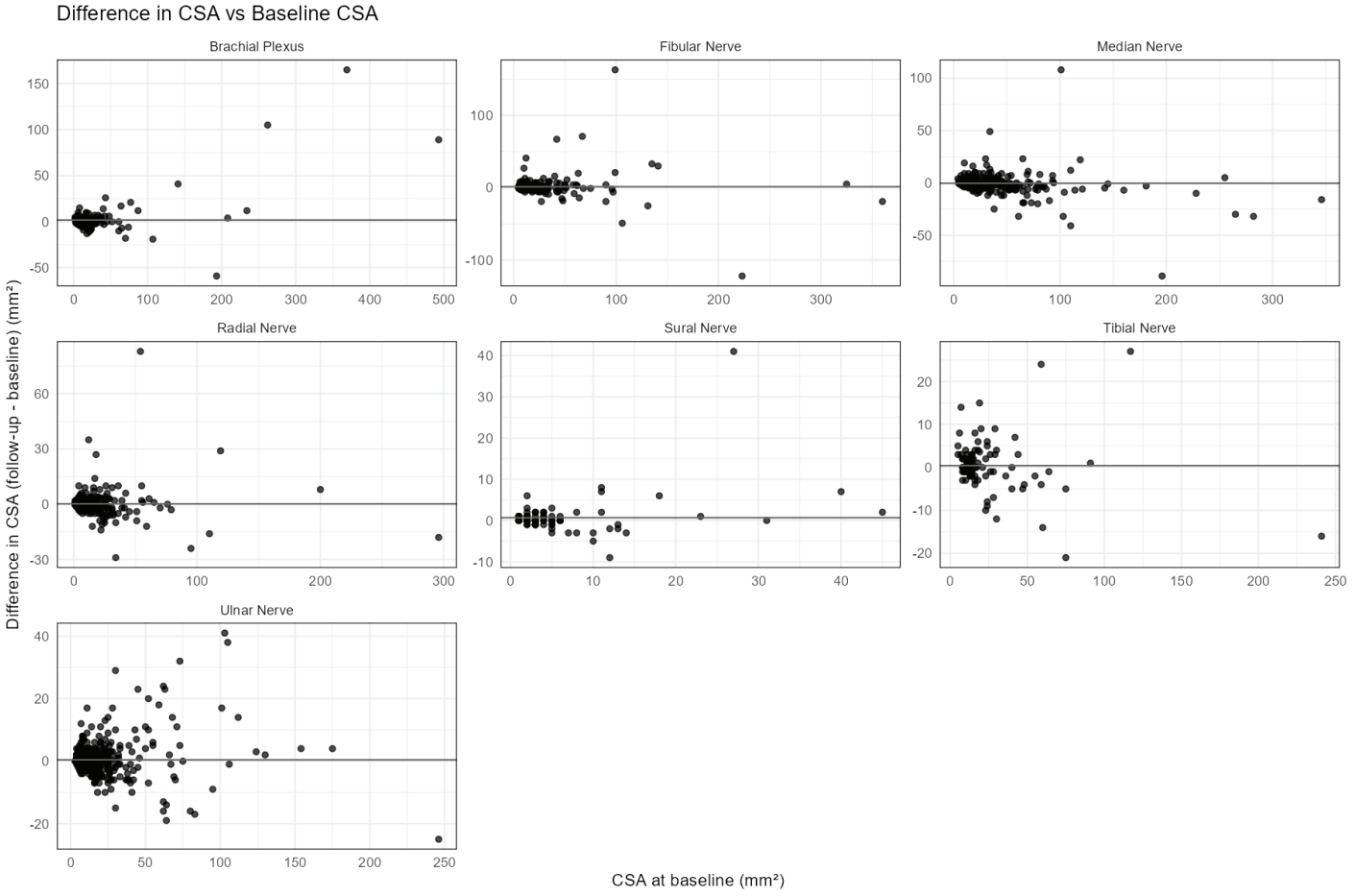
17. Akshintala, S.; Baldwin, A.; Liewehr, D.J.; Goodwin, A.; Blakeley, J.O.; Gross, A.M.; Steinberg, S.M.; Dombi, E.; Widemann, B.C. Longitudinal evaluation of peripheral nerve sheath tumors in neurofibromatosis type 1: Growth analysis of plexiform neurofibromas and distinct nodular lesions. Neuro-Oncology 2020, 22, 1368–1378.
18. Telleman, J.A. Nerve ultrasound: A reproducible diagnostic tool in peripheral neuropathy. Neurology 2019, 92, e443–e450.
19. Boehm, J. High-resolution ultrasonography of peripheral nerves: Measurements on 14 nerve segments in 56 healthy subjects and reliability assessments. Ultraschall. Med. 2014, 35, 459–467.
20. Tagliafico, A. Reliability of side-to-side ultrasound cross-sectional area measurements of lower extremity nerves in healthy subjects. Muscle Nerve 2012, 46, 717–722.
21. Tagliafico, A.; Martinoli, C. Reliability of side-to-side sonographic cross-sectional area measurements of upper extremity nerves in healthy volunteers. J. Ultrasound Med. 2013, 32, 457–462.
22. Herraets, I.J.T.; Goedee, H.S.; Telleman, J.A.; van Eijk, R.P.A.; Verhamme, C.; Saris, C.G.J.; Eftimov, F.; van Alfen, N.; van Asseldonk, J.T.; Visser, L.H.; et al. Nerve ultrasound for diagnosing chronic inflammatory neuropathy: A multicenter validation study. Neurology 2020, 95, e1745–e1753.

Supplementary table S1: Standard anatomical sites for measuring nerve cross-sectional area with high resolution nerve ultrasound.

Nerve	Anatomical site	CSA reference values (mm^2)
Median	Wrist	11
	⅓ Forearm	9
	½ Upper arm	10
Ulnar	Wrist	7
	Forearm	6
	Distal sulcus	9
	Sulcus	9
	Proximal Sulcus	9
	½ Upper arm	9
Radial	Upper arm	9
	Wrist (superficial nerve)	3
Brachial plexus	Superior trunk	8
	Middle trunk	8
	Inferior trunk	8
Peroneal	Popliteal fossa	9
	Fibular head	11
Tibial	Ankle	13
Sural	Lower leg[a]	3

[a]10-14 cm proximal to the lateral malleolus. CSA=cross-sectional area.

Supplementary figure S1:



PART III

Treatment of plexiform neurofibromas

5

Trametinib in Adults with Neurofibromatosis Type 1-Related Symptomatic Plexiform Neurofibromas

D.C. Noordhoek, D.F. Hanff, P.J. French, S.A. van Dijk, E.A.C. Wiemer, O.C. Manintveld, M.J. van den Bent, W. Taal

Annals of Neurology. (2026) 99: 73-83. doi: 10.1002/ana.78010.

ABSTRACT

Objective

Mitogen activated protein kinase kinase inhibitors have shown promising results in treatment of plexiform neurofibromas in neurofibromatosis type 1 patients, but data in adults are limited. The aim of this phase 2 study was to investigate the efficacy and safety of trametinib in adults with neurofibromatosis type 1.

Methods

Thirty patients with a diagnosis of generalized or mosaic neurofibromatosis type 1 and a symptomatic inoperable plexiform neurofibroma were treated with 2 mg trametinib per day. Primary outcome measures were partial response rate on tumor volume at cycle 12 and overall partial response rate. Partial response was defined as ≥20% decrease in tumor volume compared to baseline as measured with 3D volumetric analysis on MR imaging every 6th cycle. Secondary outcome measures were pain, pain interference, quality of life and toxicity.

Results

Partial response rate after cycle 12 was 47% (n=14/30). Overall partial response rate was 50% (n=15/30). Median best response was -22% decrease of tumor volume (range -63% to +7%), and median time until best response was 12 cycles (range 6 to 42 cycles). Pain score and pain interference were significantly decreased after 12 cycles of treatment. No change in quality of life was reported. Acneiform rash and fatigue were the most reported adverse events. Overall rate of treatment discontinuation because of adverse events was 40%.

Interpretation

We observed a positive effect of trametinib on tumor volume and significant pain reduction in adults with plexiform neurofibromas. However, adverse events are common and frequently led to early termination of treatment.

INTRODUCTION

Plexiform neurofibromas (PNs) are benign peripheral nerve sheath tumors and represent a common manifestation of neurofibromatosis type 1 (NF1). These tumors occur in up to 50% of patients with NF1, and can lead to serious complications[1]. Common symptoms of PNs are pain, neurological deficits, and cosmetic burden. Additionally, PNs can compress or grow invasively in adjacent structures resulting in complications such as airway compression or bowel dysfunction. [2] The most life threatening complication of PNs is transformation into a malignant peripheral nerve sheath tumor (MPNST), which is the major cause of reduced life expectancy in NF1 patients[3, 4].

PNs should be considered for treatment in case of substantial morbidity or progressive growth[5]. Until recently, the standard of care for symptomatic PNs was debulking surgery or complete resection. However, surgery is not always feasible because of the location and size of the PN, and the high risk of postoperative complications such as significant blood loss and persisting neurological deficits[2, 5-7]. Moreover, tumor recurrence rates are high, even after complete resection[7, 8]. Hence, PNs are often considered inoperable, raising the need for other treatments.

Pathogenic variants of the *NF1* gene result in the loss of functional neurofibromin, a protein that inhibits the RAS-pathway[9, 10]. Activation of the RAS-pathway in turn drives tumor growth and progression. Mitogen activated protein kinase (MAPK) kinase (MEK) is part of the RAS-pathway and its inhibition provides an opportunity for targeted therapies. Indeed, several phase 2 trials of MEK inhibition for PNs have been reported. The first trial of selumetinib in pediatric NF1 patients with inoperable PNs showed a partial response in 70% of the children[11]. A recent phase 2 trial with selumetinib reported a partial response rate of 64% in adults[12]. Studies on mirdametinib mentioned partial response rates of 41-42% in adolescents and adults and partial response rates of 52% in pediatric patients[13, 14].

We investigated the efficacy and safety of the MEK inhibitor trametinib in adults with generalized and mosaic NF1 and symptomatic inoperable PNs.

METHODS

Trial design

The TRAIN trial is a prospective single arm, open-label, phase 2 trial, which assessed the efficacy and safety of trametinib for treatment of symptomatic inoperable PNs in patients with NF1.

Patients

Patients of at least 18 years old were selected by their treating physician if they had a clinical diagnosis of generalized NF1 or mosaic NF1, a symptomatic inoperable PN with a need for treatment and a Karnofsky Performance Scale (KPS) of at least 70%. Inoperable PN is defined as a PN that cannot be surgically completely removed without risk of substantial morbidity due to its invasiveness, high vascularity or encasement of, or close proximity to vital structures. Female subjects of childbearing potential were required to have a negative pregnancy test within 7 days prior to the start of study treatment and agreed to use highly effective contraception. Male subjects also had to agree to not conceive a child while on study treatment. Patients were not eligible if they received treatment with MEK inhibitors before, if they had a malignancy within 5 years prior to inclusion or major surgery within 4 weeks prior to inclusion. The complete list of eligibility criteria is provided in supplementary file 1. All patients provided written informed consent according to institutional and national guidelines. The study was approved of by the Institutional Review Board (MEC-2019-0463) and registered in the Dutch Trial Registry (NL-OMON28237, registration date: 27-09-2019).

Treatment

Patients received 2 mg trametinib once per day orally in cycles of 4 weeks continuously. All patients received prophylactic antibiotics (tetracyclines) at the start of treatment to reduce the risk of MEK inhibitor-associated skin infections. Adverse events were reported in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0 (CTCAEv5.0) [15]. Dosage was reduced by 0.5 mg or treatment was temporarily halted in case of toxicity (intolerable CTCAE grade 2 or higher). Treatment was permanently discontinued in case of unacceptable toxicity (recurrent intolerable CTCAE grade 2 or higher), progressive disease (≥20% increase of tumor volume compared to baseline) or on patient wish. Patients who did not meet any of these discontinuation criteria, were allowed to continue treatment as long as clinical benefit was observed, based on shared decision-making between patient and the study team. Patients visited the outpatient clinic after every cycle to monitor safety and efficacy. Laboratory tests (including blood cell count, kidney and liver function, N-terminal pro B-type natriuretic peptide (NT-proBNP) and creatinine phosphokinase (CPK), and urine analysis) and physical examination were performed at every visit. Cardiotoxicity of trametinib was monitored by electrocardiogram and transthoracic echocardiography at baseline, after the first cycle and after every third cycle. Screening for ophthalmological toxicity was performed at baseline, after the first cycle of treatment and in case of new ophthalmological symptoms. Regional MRI scans of the target PNs, neurological examination, medical photography (in case of externally visible PNs) and questionnaires with patient reported outcome measures (PROMs) were taken at baseline and after every 6th cycle. PN tissue samples were obtained from all patients at baseline and after 3 cycles of treatment, unless treatment

was discontinued before the 3rd cycle or patients refrained from undergoing the second biopsy.

Primary endpoint

The primary endpoints of this study were partial response rate on tumor volume at cycle 12 and overall partial response rate at any timepoint. The treating physician (WT) selected a maximum of three most clinically relevant (symptomatic) PNs per patient as target lesions for volumetric analysis. PN type was categorized as nodular (including distinct nodular lesions and multinodular PNs) or diffuse. Regional MR imaging of the target PNs was performed after every 6th cycle following a standardized protocol, maintaining the same field-of-view in each follow-up MRI. Volume measurements were executed by a trained investigator (CN) by using *ITKsnap version 3.8.0* (www.itksnap.org) for manual 3D tumor segmentation on T2-weighted fat suppression sequence[16]. Treatment response after cycle 12 was reviewed by an experienced musculoskeletal radiologist (DH) who performed identical volume segmentations of the baseline and cycle 12 MRI scan. The radiologist was blinded for treatment status and clinical response. Response was defined according to the recommendations of the Tumor Measurement Working Group of the REiNS (Response Evaluation in Neurofibromatosis and Schwannomatosis) [17]. The sum of the tumor volume of all target PNs per patient was used for analysis. Partial response (PR) was defined as at least 20% decrease in tumor volume of the target PNs compared to baseline. Stable disease (SD) was defined as an increase or decrease in tumor volume of less than 20%. Progressive disease (PD) was defined as an increase of more than 20% in tumor volume as compared to baseline.

Secondary endpoints

Clinical effects were evaluated by the numeric (pain) rating scale for average (NRS-11) and maximum (max NRS-11) pain score[18], the patient-reported outcome measurement information system on pain interference short form 8a (PROMIS-PI) [19] and the 36-item short form health survey version 2 (SF-36) [20] to assess quality of life. Other secondary endpoints were time-to-progression, incidence of surgical interventions, effect on neurological deficits and disfigurement as seen on medical photographs. Effect on neurological deficits was assessed at every 6th cycle by measuring motor function with the Medical Research Council (MRC) [21] scale and reporting sensory deficits. Effect on disfigurement was scored on medical photographs at every 6th cycle for patients with externally visible PN. Photographs at cycle 12 were compared to baseline by the investigator (CN) in order to determine the number of patients with a reduction in the externally visible tumor.

Tissue sample analysis

All PN tissue samples were processed according to the guidelines of the *MD Anderson functional proteomics RPPA core facility*, where all reverse phase phospho-protein array (RPPA) experiments were run, similar to de Wit et al[22]. Tissue-Tek was removed before

protein extraction. Samples with a final concentration of ≥ 0.5 µg/µl (after adding lysing buffer) were used for RPPA with 497 antibodies (RPPA pipeline version 6.0.0.). Quality control was performed after antibody staining, samples with sufficient total protein content according to the RPPA quality control guidelines were included in the analysis. Data with clustered heatmap (CHM) normalization were used for the analyses.

Statistical analysis

Sample size calculation was based on the null hypothesis that 10% of patients would achieve a spontaneous partial response at 2 years. The alternative hypothesis was that trametinib treatment leads to response in 30% of patients. Thirty patients were needed to be able to reject the null hypothesis with alpha 0.05 and power of 80%[23]. All patients who received at least 1 dose of trametinib were included in the analysis. For all outcome measures, change over time was described using descriptive statistics. For the PROMs we compared baseline with cycle 12, using a paired t-test. 95% confidence intervals were reported where appropriate. Data was analyzed using *R studio version 4.4.1*.

RESULTS

Patient characteristics

Between 17 July 2020 and 10 May 2023, 30 patients with inoperable symptomatic PNs were enrolled in this trial (Table 1). Data up to 10 May 2024 were included in this report. Median follow-up time, including off-treatment follow-up, at the data cut-off was 24 months (range: 2-46 months). All patients had a clinical diagnosis of NF1. Seven had mosaic NF1. However, one patient with a clinical diagnosis of mosaic NF1 received a genetic diagnosis on neurofibroma tissue during the course of the trial, which showed a *KRAS G12D* variant responsible for the phenotype with histologically proven neurofibromas. The patient still met the eligibility criteria and was therefore not taken off study treatment. Median age at enrollment was 45 years (range: 19-67 years). Median target PN volume was 77 mL (range: 2-3703 mL). Most common target PN related signs and symptoms were pain (n=28, 93%), disfigurement (n=14, 46%) and neurological deficit (n=13, 43%).

Table 1 Characteristics of patients and target plexiform neurofibroma at baseline

		N=30
NF1 type (%)	Generalized	23 (77)
	Mosaic	7 (23)*
Median age at registration, years (range)		45 (19-67)
Sex, female (%)		16 (53)
BMI		26 (18-39)
ECOG (%)	0	3 (10)
	1	22 (73)
	2	4 (13)
	3	1 (3)
Previous surgery for target PN (%)		11 (37)
Median volume of target PN in mL (range)		77 (2-3703)
Location of target PN (%)	Head/neck	3 (10)
	Thorax/abdomen	10 (33)
	Upper extremities	2 (7)
	Lower extremities	15 (50)
Type of target PN (%)	Diffuse	8 (27)
	Nodular	22 (73)
Target PN-related morbidity (%)	Neurological deficit	13 (43)
	Pain	28 (93)
	Disfigurement	14 (46)
	Compression of vital structures	1 (3)

NF1: neurofibromatosis type 1, BMI: body-mass index, ECOG: Eastern Cooperative Oncology Group Performance status, PN: plexiform neurofibroma. *One patient with clinical diagnosis of mosaic NF1 had histologically proven neurofibromas, however genetic analysis showed a KRAS G12D variant responsible for the phenotype.

Treatment

Median treatment duration was 15 months (range: 2-45 months). Eighteen patients permanently discontinued study treatment, of which 12 patients stopped treatment within the first 12 months. The most common reason for discontinuing study treatment was toxicity (n=12/18, 67%). Other reasons were lack of treatment effect (n=4/18, 22%) and new contra-indications for trametinib (the start of anticoagulants and mamma carcinoma) (n=2/18, 11%). Twenty-three patients had one or more dose reductions because of toxicity. Nineteen patients required one or more dose interruptions, mostly because of toxicity (n=17) and in some cases because of surgery not related to the target PN (n=5) or personal reasons (n=3). Three patients were not evaluated for response with MRI and PROMs because they stopped trametinib within the first 3 cycles. Two patients had surgical intervention for the target PN after study treatment was discontinued because of toxicity in one patient and because of progressive disease in the other patient.

Tumor volume response

Partial response rate after 12 cycles of treatment was 47% (n=14/30) as assessed by the investigator (CN) and 53% (n=16/30) as assessed by the radiologist (DH). Response classification differed in 5 patients between the two observers. Tumor volume change and disease status at cycle 12, as assessed by the two observers, are reported in Supplementary table 2. All other data on tumor volume response are based on the volume measurements of the investigator (CN). Overall partial response rate (at any timepoint) was 50% (n=15/30), figure 1 shows the waterfall plot with the maximum response in each patient. The median time to initial response was 6 cycles (range: 6-18 cycles). Median time to best response was 12 cycles (range: 6-42 cycles) and median best response was -22% of tumor volume (range: -63 to 7%). None of the patients reached PD on study treatment. One patient developed PD after discontinuation of study treatment. Figure 2 shows a spider plot with the tumor volume change over time in % compared to baseline for each patient. The most substantial relative decrease in tumor volume happens before cycle 6. After cycle 18 the volume seems to stabilize in the thirteen patients still on treatment. In all but one patient, tumor volume remained stable or increased after discontinuation of study treatment, indicated by the dotted lines.

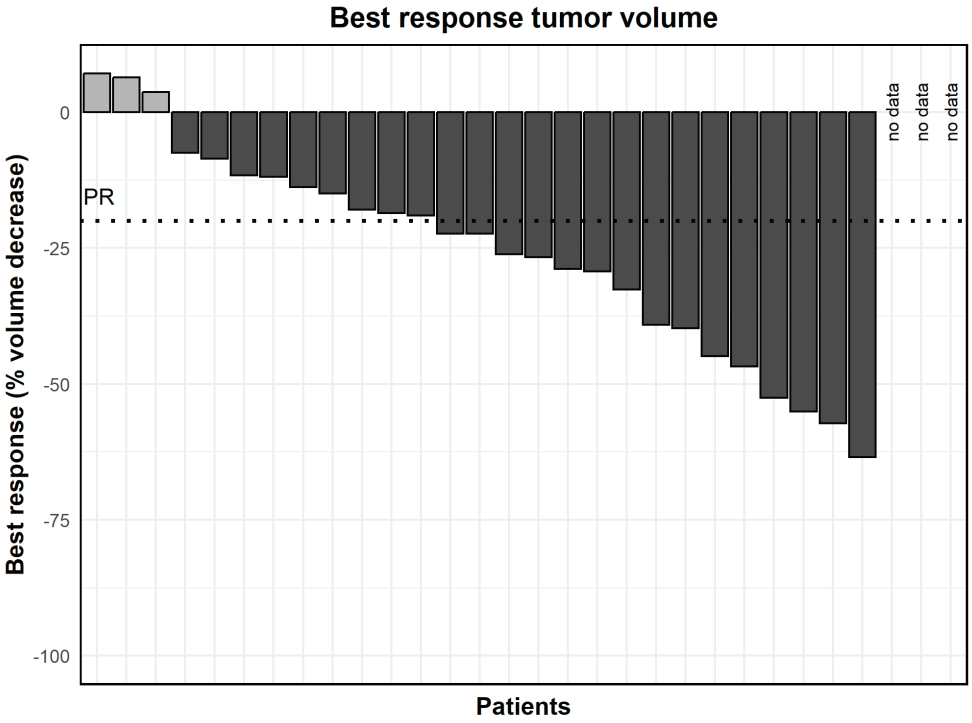


Figure 1. Best response in % of the target plexiform neurofibroma tumor volume compared to baseline. A decrease of 20% of tumor volume is considered partial response. Overall response rate was 15/30 (50%). Three patients did not have any follow-up MRI.

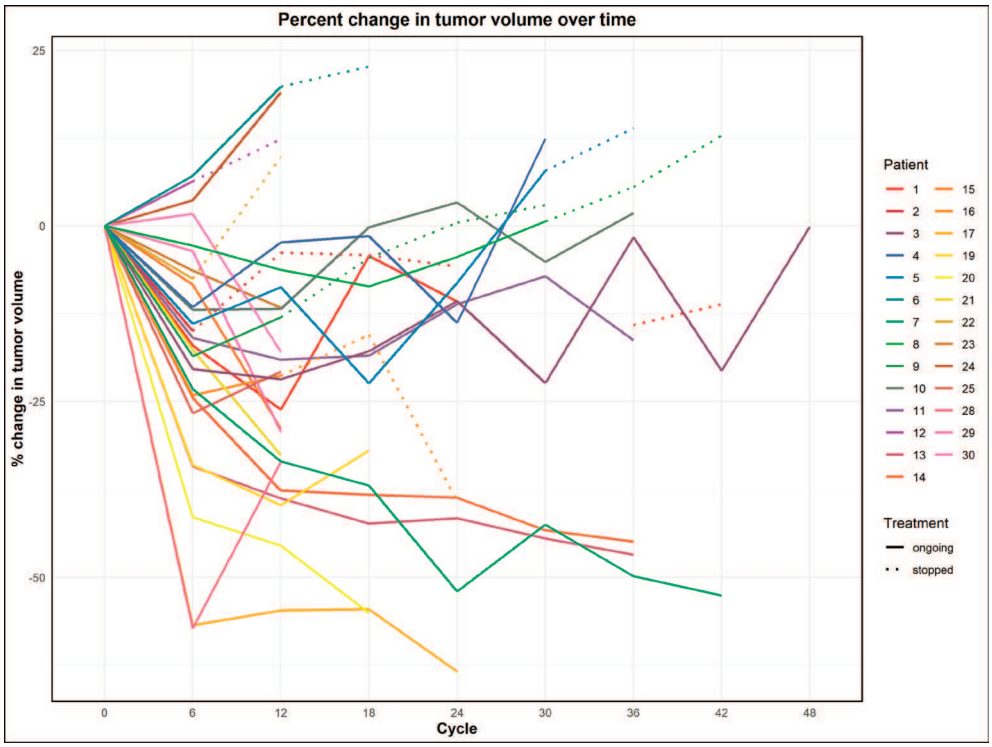


Figure 2. Target plexiform neurofibroma tumor volume change in % compared to baseline for each individual patient. Treatment status is indicated by solid lines for ongoing treatment and dotted lines for treatment stopped.

Partial response was seen in 5 out of 8 patients (63%) with diffuse PNs and 10 out of 22 patients (45%) with nodular PNs. Patients with mosaic NF1 had partial response in 2 out of 7 cases (29%) versus and 13 out of 23 (57%) with generalized NF1. A tumor volume decrease of 18% was observed in the patient with a mosaic *KRAS G12D* variant, but did not reach partial response.

Secondary outcome measures

Neurological deficits

Thirteen patients (43%) had neurological deficits at baseline, of which 12 patients had motor deficits and 9 patients had sensory deficits. Four patients (31%) had improvement of motor deficits during study treatment. One patient had improvement of sensory deficit and one patient had improvement of both motor and sensory deficits. In total, four patients had worsening of motor deficit or developed new motor deficits during the course of the study. In two cases this could be attributed to weakness after a femur fracture and in one case this was caused by growth of a vascular fistula in the target PN

causing compression on the cervical nerve roots. One patient had deterioration of motor function during study treatment which improved after discontinuing treatment.

Disfigurement

Twelve patients had medical photographs taken every 6th cycle to evaluate disfigurement over time. Three of these patients had a clear reduction of externally visible tumor volume after the 12th cycle. Figure 3 shows an example of the decrease in tumor volume in a patient with target PN in the shoulder region.

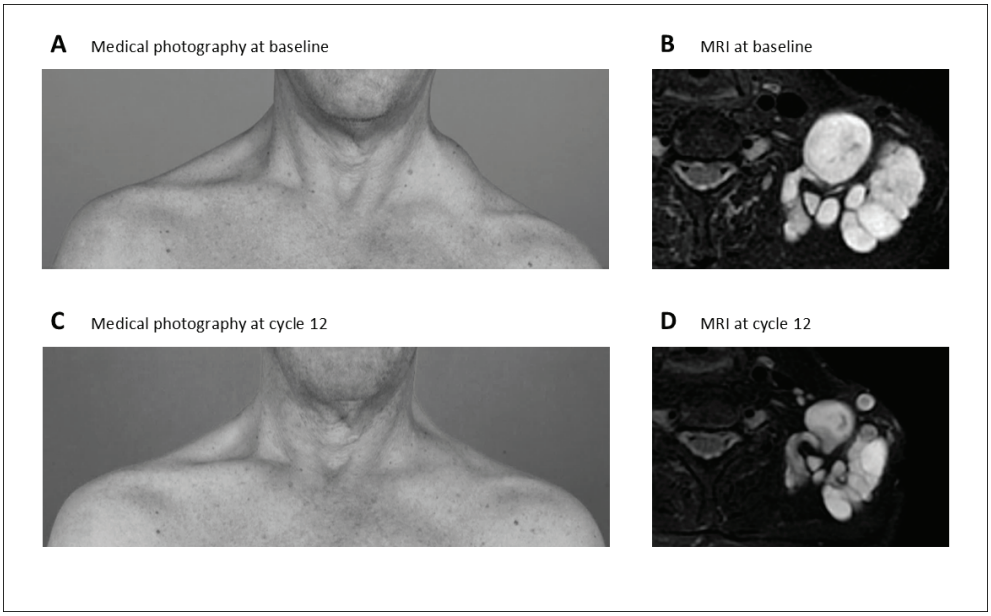


Figure 3. Medical photography and MRI of target plexiform neurofibroma in the left shoulder region with externally visible decrease of tumor volume between baseline (A, B) and at cycle 12 (C, D). Tumor volume decrease at cycle 12 was 34% as measured by both the investigator (CN) and the radiologist (DH).

Patient-reported outcome measures

Pain

Mean NRS-11 decreased from 5.2 (n=29, range 1-9) at baseline to 3.5 after the 12th cycle (n=21, range 0-8). Amongst the 21 patients with paired baseline and 12th cycle data available, mean difference was 2 (p=0.015, 95% confidence interval 0.44-3.56). Eleven out of 21 patients (52%) had a clinically meaningful decrease of at least 2 points on NRS-11. Mean max NRS-11 decreased from 6.4 at baseline (n=29, range 1-10) to 4.6 after the 12th cycle (n=21, range 0-9). Amongst the 21 patients with paired baseline and 12th cycle data available on max NRS-11, mean difference was 2 (p=0.027, 95% confidence interval 0.26-3.84). Eleven out of 21 patients (52%) had a clinically meaningful decrease of at least 2

points on max NRS-11. Out of the 16 patient using analgesics at baseline, 4 patients (25%) were able to reduce or stop. In the patient with a *KRAS G12D* variant NRS-11 decreased from 7 to 2 and max NRS-11 decreased from 9 to 2.

Pain interference (PROMIS-PI)

PROMIS-PI decreased from 61.6 (n=29, range 47.9-68.4) at baseline to 57.4 (n=21, range 40.7-66.9) after the 12th cycle. Mean difference in PROMIS-PI was 4.71 (p=0.011, 95% confidence interval 1.21-8.23) amongst the 21 patients with paired data available at baseline and after the 12th cycle. Ten out of 21 patients (48%) had a clinically meaningful decrease of at least 2 points on the PROMIS-PI.

SF-36 Quality of life

None of the subscales of the SF-36 showed a significant change at cycle 12 compared to baseline. Improvement on the pain subscale with an increase of 10.7 points from baseline to 12th cycle, approached significance (p=0.051, n=21). Similarly, the health change scale improved with an increase of 11.9 points (p=0.076, n=21). General health subscale decreased with 7.6 points (p=0.082, n=21). All data on all subscales of the SF-36 can be found in supplementary table 3.

Tissue sample analysis

Forty-two PN tissue samples were obtained successfully for RPPA. After protein extraction, 24 samples had sufficient final protein concentration to proceed for RPPA processing of which only 13 samples passed the assay's QC; 9 baseline samples and 4 on treatment samples. A heatmap of all RPPA data is shown in figure 4A; figure 4B shows phospho-MEK1 expression at baseline and on treatment. Three out of four samples show relatively low expression of phospho-MEK1 on treatment; figure 4C shows MAPK expression at baseline and after 3 cycles of treatment, which remains the same.

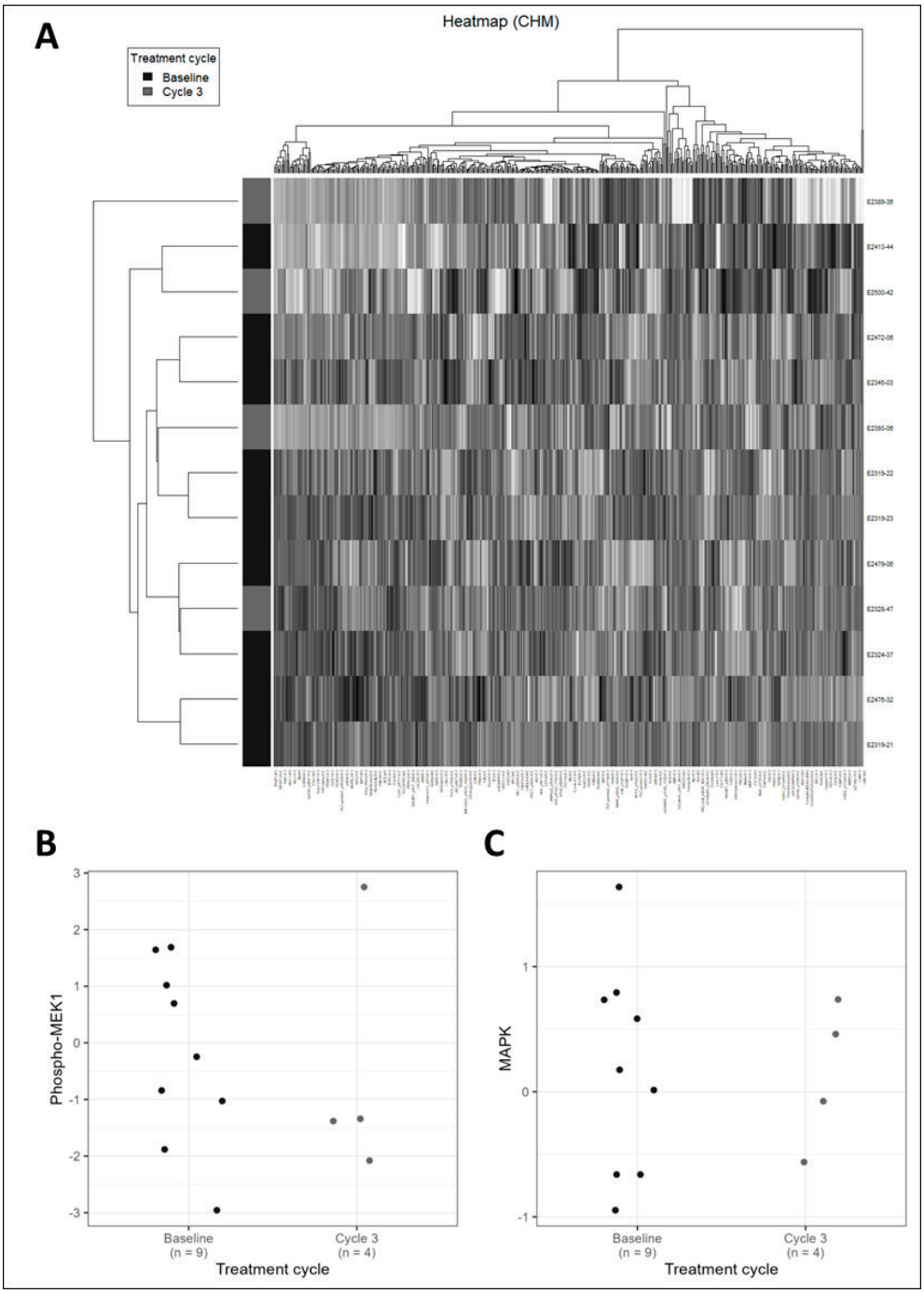


Figure 4. Panel A shows a heatmap of the reverse phase phospho-protein array data, with clustered heatmap normalization CHM, n=13. Panel B shows expression of phospho-MEK1 before treatment and after 3 cycles of treatment. Panel C shows expression of MAPK before treatment and after cycle 3.

Toxicity

All patients reported at least one treatment related adverse event (TRAE). All TRAE are listed in supplementary table 4. Most common TRAEs were acneiform rash, hypoalbuminemia, fatigue and increase of liver enzymes. Acneiform rash, fatigue and gastrointestinal problems such as nausea, diarrhea or constipation were most common TREAs that led to dose modification. Four TRAEs led to hospitalization: pneumonitis (grade 3), rhabdomyolysis (grade 4) and erysipelas twice in the same patient (grade 3). In all cases, patients fully recovered after study treatment was stopped and appropriate treatment was given. One patient developed asymptomatic small serous detachments of the retina which diminished over time without discontinuing study treatment.

DISCUSSION

In this phase 2 trial with trametinib, we found a partial response rate of 47% at cycle 12 in adults with NF1 related symptomatic PNs and the overall partial response rate was 50%. This response rate is similar to those found in studies with other MEK inhibitors in adults [13, 14, 24, 25]. provides an overview of response rates and median time to response in other phase 2 clinical trials with MEK inhibitors in NF1-related PN. We observed responses both in patients with diffuse plexiform neurofibroma and in patients with mosaic NF1. The patient with *KRAS G12D* variant had a tumor volume decrease of 18% and the patient reported a dramatic decrease on the pain score. There are case reports describing an effect of MEK inhibitors in patients with somatic mosaic *KRAS* variants[26-28].

Pain and pain interference improved significantly after 12 cycles of treatment emphasizing clinical patient benefit, and some patients had improvement of neurological deficits and disfigurement. However, quality of life as measured by the SF-36 did not change, except for the pain subscale which showed improvement with an increase of 10.7 points which approached statistical significance. Other studies showed similar significant decrease on pain and pain interference, but conflicting results on health related quality of life[13, 14, 24]. The lack of improvement on quality of life in our study could be due to the use of a generic quality of life measuring tool and the absence of a control arm. Using a disease specific quality of life tool such as the PlexiQol (patient-reported outcome measure of quality of life for NF1-associated plexiform neurofibromas), might be more sensitive to detect improvement during treatment with MEK inhibitor[29, 30]. Other reasons for lack of improvement of the overall quality of life may be the treatment related toxicities and the burden of hospital visits and examinations during the study.

Table 2. Summary of other studies with MEK inhibitors

Trial*	Study population, age (years)	Partial response rate**	Median time to initial response	Toxicity drop-out rate
Gross et al. 2020[11] Selumetinib	**Children**: n=50, median age 10.2 (range 3.5-17.4)	74%	8 cycles (range 4-20)	10%
Kim et al. 2024[25] Selumetinib	**Children:** n=60, median age 8 (range 3-18) **Adults:** n=30, median age 26.5 (range 19-47)	91% ***	NR	0%
Gross et al. 2025[12] Selumetinib	**Adults:** n=33, median age 35.6 (range 18.3-60.2)	63.6%	12 cycles (range 4-54)	6%
Weiss et al. 2021[14] Mirda-metinib	**Adults:** n=19, median age 24 (range 16-39)	42%	12 cycles (range 8-12)	0%
Moertel et al. 2024[13] Mirda-metinib	**Children**: n=56, median age 10 (range 2-17)	52%	7.9 months (range 4.1-18.8)	9%
	Adults: n=58, median age 34 (range 18-69)	41%	7.8 months (range 4-19)	22%

**Only fully published reports of phase 2 clinical trials with MEK inhibitors for neurofibromatosis type 1 related plexiform neurofibromas were included. **definition of partial response was similar in all studies: ≥20% decrease of tumor volume. ***partial response is given for the total group of children and adults combined*

Toxicity was the most common reason for patients to stop study treatment, with a rate of treatment discontinuation because of toxicity of 40%. It has been suggested that stopping treatment because of toxicity may be more common in adults than in pediatric patients treated with MEK inhibition, occurring in respectively 22% versus 9%[13]. However, drop-out rates because of toxicity were lower in other studies with MEK inhibition as seen in table 2. As no clinical trials have directly compared MEK inhibitors in NF1 patients, it remains unclear whether differences in toxicity-related discontinuation rates are due to drug-specific properties or reflect variations in study design, patient characteristics or handling and reporting adverse events.

The most frequently reported adverse events were acneiform rash and fatigue. As treatment with MEK inhibition in NF1 patients is increasingly being used and toxicity is common, it is crucial to develop guidelines for the management of MEK inhibition associated adverse events. In our study, patients received prophylactic tetracyclines to reduce the risk of MEK inhibitor-related skin infections. Given the potential burden of acneiform rash, prompt and proactive topical treatment and, if necessary, dose reduction is crucial. Fa-

tigue requires a more supportive approach, often involving lifestyle adaptations. Several publications provide consensus-based recommendations for managing MEK inhibitor–associated adverse events in patients with NF1[31, 32].

Using RPPA on target PN tissue samples before and after 3 cycles of treatment, we found that three out of four samples showed relatively low expression of phospho-MEK1 on treatment and MAPK expression remained the same. However, unfortunately only two paired samples were included in the analysis and the relationship between target inhibition and treatment response could therefore not be evaluated. PN tissue sample analysis by Gross et al. showed that selumetinib significantly decreased phosphorylation ratios of ERK1 and ERK2, but not of MEK1 and MEK2[24]. Selumetinib and trametinib, although both targeting MEK1 and MEK2, have different mechanisms of action[33, 34].

Our study has some limitations. It is a single center phase 2 trial, in which placebo-effect may have interfered with the assessment of pain and quality of life. Also, the evaluator (CN) of tumor volume response was not blinded for treatment status or clinical response. Therefore, a second assessment was incorporated for the response rate at cycle 12 by an experienced radiologist (DH) who was blinded for treatment status and clinical response. The data as presented in supplementary table 2 show that in 5 cases variability of the tumor measurement around the cut-off point of response assessment can have an impact on study outcome.

In conclusion, the TRAIN study is the first to investigate trametinib in adults with NF1 and symptomatic PN. We observed a tumor volume response in half of the patients; however, treatment toxicity frequently led to treatment discontinuation. Therefore, future research should prioritize optimal patient selection for MEK inhibition therapy and compare different MEK inhibitors to balance efficacy and toxicity.

REFERENCES

1. Nguyen R, Dombi E, Widemann BC, et al. Growth dynamics of plexiform neurofibromas: a retrospective cohort study of 201 patients with neurofibromatosis 1. Orphanet J Rare Dis. 2012 Oct 4;7:75.
2. Ejerskov C, Farholt S, Nielsen FSK, et al. Clinical Characteristics and Management of Children and Adults with Neurofibromatosis Type 1 and Plexiform Neurofibromas in Denmark: A Nationwide Study. Oncol Ther. 2022 Dec 1.
3. Evans DG, O'Hara C, Wilding A, et al. Mortality in neurofibromatosis 1: in North West England: an assessment of actuarial survival in a region of the UK since 1989. Eur J Hum Genet. 2011 Nov;19(11):1187-91.
4. Evans DG, Baser ME, McGaughran J, Sharif S, Howard E, Moran A. Malignant peripheral nerve sheath tumours in neurofibromatosis 1. J Med Genet. 2002 May;39(5):311-4.
5. Fisher MJ, Blakeley JO, Weiss BD, et al. Management of Neurofibromatosis Type 1-Associated Plexiform Neurofibromas. Neuro Oncol. 2022 Jun 3.
6. Canavese F, Krajbich JI. Resection of plexiform neurofibromas in children with neurofibromatosis type 1. J Pediatr Orthop. 2011 Apr-May;31(3):303-11.
7. Prada CE, Rangwala FA, Martin LJ, et al. Pediatric plexiform neurofibromas: impact on morbidity and mortality in neurofibromatosis type 1. J Pediatr. 2012 Mar;160(3):461-7.
8. Needle MN, Cnaan A, Dattilo J, et al. Prognostic signs in the surgical management of plexiform neurofibroma: the Children's Hospital of Philadelphia experience, 1974-1994. J Pediatr. 1997 Nov;131(5):678-82.
9. Gutmann DH, Ferner RE, Listernick RH, Korf BR, Wolters PL, Johnson KJ. Neurofibromatosis type 1. Nat Rev Dis Primers. 2017 Feb 23;3:17004.
10. Viskochil D, White R, Cawthon R. The neurofibromatosis type 1 gene. Annu Rev Neurosci. 1993;16:183-205.
11. Gross AM, Wolters PL, Dombi E, et al. Selumetinib in Children with Inoperable Plexiform Neurofibromas. N Engl J Med. 2020 Apr 9;382(15):1430-42.
12. Gross AM, O'Sullivan Coyne G, Dombi E, et al. Selumetinib in adults with NF1 and inoperable plexiform neurofibroma: a phase 2 trial. Nat Med. 2025 Jan;31(1):105-15.
13. Moertel CL, Hirbe AC, Shuhaiber HH, et al. ReNeu: A Pivotal, Phase IIb Trial of Mirdametinib in Adults and Children With Symptomatic Neurofibromatosis Type 1-Associated Plexiform Neurofibroma. J Clin Oncol. 2024 Nov 8:JCO2401034.
14. Weiss BD, Wolters PL, Plotkin SR, et al. NF106: A Neurofibromatosis Clinical Trials Consortium Phase II Trial of the MEK Inhibitor Mirdametinib (PD-0325901) in Adolescents and Adults With NF1-Related Plexiform Neurofibromas. J Clin Oncol. 2021 Mar 1;39(7):797-806.
15. Common Terminology Criteria for Adverse Events (CTCAE) v5.0. U.S. Department of Health and Human Services; 2017; Available from: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_5x7.pdf.
16. Yushkevich PA, Piven J, Hazlett HC, et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. Neuroimage. 2006 Jul 1;31(3):1116-28.
17. Dombi E, Ardern-Holmes SL, Babovic-Vuksanovic D, et al. Recommendations for imaging tumor response in neurofibromatosis clinical trials. Neurology. 2013 Nov 19;81(21 Suppl 1):S33-40.
18. Downie WW, Leatham PA, Rhind VM, Wright V, Branco JA, Anderson JA. Studies with pain rating scales. Ann Rheum Dis. 1978 Aug;37(4):378-81.

19. Amtmann D, Cook KF, Jensen MP, et al. Development of a PROMIS item bank to measure pain interference. Pain. 2010 Jul;150(1):173-82.
20. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992 Jun;30(6):473-83.
21. Medical Research Council. Aids to Examination of the Peripheral Nervous System. London: Her Majesty's Stationary Office; 1976.
22. de Wit M, Gao Y, Mercieca D, et al. Mutation and drug-specific intracellular accumulation of EGFR predict clinical responses to tyrosine kinase inhibitors. EBioMedicine. 2020 Jun;56:102796.
23. Fleming TR. One-sample multiple testing procedure for phase II clinical trials. Biometrics. 1982 Mar;38(1):143-51.
24. Gross AM, O'Sullivan Coyne G, Dombi E, et al. Selumetinib in adults with NF1 and inoperable plexiform neurofibroma: a phase 2 trial. Nat Med. 2025 Jan 6.
25. Kim H, Yoon HM, Kim EK, et al. Safety and efficacy of selumetinib in pediatric and adult patients with neurofibromatosis type 1 and plexiform neurofibroma. Neuro Oncol. 2024 Dec 5;26(12):2352-63.
26. Claire Hou YC, Evenson MJ, Corliss MM, et al. A comparative analysis of RAS variants in patients with disorders of somatic mosaicism. Genet Med. 2023 Mar;25(3):100348.
27. Chang CA, Perrier R, Kurek KC, et al. Novel findings and expansion of phenotype in a mosaic RASopathy caused by somatic KRAS variants. Am J Med Genet A. 2021 Sep;185(9):2829-45.
28. Dionysiou M, Makri SC, Ahlawat S, et al. Case report: MEK inhibitor as treatment for multilineage mosaic KRAS G12D-associated epidermal nevus syndrome in a pediatric patient. Front Neurol. 2024;15:1466946.
29. Dhaenens BAE, van Dijk SA, Taal W, et al. The PlexiQoL, a patient-reported outcome measure on quality of life in neurofibromatosis type 1-associated plexiform neurofibroma: translation, cultural adaptation and validation into the Dutch language for the Netherlands. J Patient Rep Outcomes. 2024 Mar 18;8(1):33.
30. Heaney A, Wilburn J, Rouse M, et al. The development of the PlexiQoL: A patient-reported outcome measure for adults with neurofibromatosis type 1-associated plexiform neurofibromas. Mol Genet Genomic Med. 2020 Dec;8(12):e1530.
31. Azizi AA, Hargrave D, Passos J, et al. Consensus recommendations on management of selumetinib-associated adverse events in pediatric patients with neurofibromatosis type 1 and plexiform neurofibromas. Neurooncol Pract. 2024 Oct;11(5):515-31.
32. Klesse LJ, Jordan JT, Radtke HB, et al. The Use of MEK Inhibitors in Neurofibromatosis Type 1-Associated Tumors and Management of Toxicities. Oncologist. 2020 Jul;25(7):e1109-e16.
33. Caunt CJ, Sale MJ, Smith PD, Cook SJ. MEK1 and MEK2 inhibitors and cancer therapy: the long and winding road. Nat Rev Cancer. 2015 Oct;15(10):577-92.
34. Cook SJ. Second MEK Inhibitor Shows Promise in Neurofibromatosis Type 1 With Symptomatic Plexiform Neurofibromas Signaling Hope for Other RASopathies. J Clin Oncol. 2024 Dec 11:JCO2402375.

Supplementary file 1 complete list of eligibility criteria

Inclusion criteria

1. Patients with (mosaic) NF1
2. Patients with a clinically significant symptomatic plexiform neurofibroma (PN), such as (but not limited to) head and neck lesions that could compromise the airway or great vessels, brachial or lumbar plexus lesions that could cause nerve compression and loss of function, lesions that could result in major deformity (e.g., orbital lesions) or are significantly disfiguring, lesions of the extremity that cause limb hypertrophy or loss of function, and painful lesions. This will be determined by the treating physician.
3. Signed, written informed consent
4. Age: 18 or higher
5. Karnofsky performance level of ≥70%
6. No standard treatment options = inoperable PN. PN that cannot be surgically completely removed without risk for substantial morbidity due to invasiveness, high vascularity or encasement of, or close proximity to, vital structures of the PN.
7. At least one measurable PN, defined as a well-demarcated lesion of at least 3 cm measured in one dimension
8. Able to swallow and retain orally administered medication
9. Female subjects of childbearing potential must have negative pregnancy test within 7 days prior study treatment and agrees to use highly effective contraception.
10. Normal hematological function: Hemoglobin (Hb)≥6 mmol/l, absolute neutrophil count (ANC)≥1.5x109/l, and platelets≥100x109/l
11. Normal hepatic function: bilirubin <1.5x the upper limit of normal (ULN), unless gilbert then: bilirubin <3xULN and AST/ALT <5xULN
12. Normal renal function: creatinine <1.5xULN

Exclusion criteria

1. Prior treatment with MEK inhibitor(s)
2. Inability to undergo MRI and/or contraindication for MRI examinations
3. History of a malignancy within 5 years of inclusion, except squamous cell carcinoma of the skin, cervical premalignant lesions and other curatively treated malignancy
4. Prior radiotherapy less than 6 weeks prior to enrollment
5. Prior major surgery less than 4 weeks prior to enrollment
6. An investigational agent within the past 30 days
7. Enzyme-inducing anticonvulsants, anti-coagulants (including platelet aggregation inhibitors) or other prohibited medication(s) or requirement for prohibited medications
8. Left ventricular dysfunction, New York Heart Association Class II, III, or IV heart failure, acute coronary syndrome within the past 6 months, clinically significant uncontrolled arrhythmias, and uncontrolled hypertension
9. A history of retinal vein occlusion (RVO) or predisposing factors for RVO, including uncontrolled glaucoma or ocular hypertension, uncontrolled hypertension, uncontrolled diabetes mellitus, or a history of hyperviscosity or hypercoagulability syndromes
10. Risk factors for gastrointestinal perforation, including history of diverticulitis, metastases to the gastrointestinal tract and concomitant use of medications with a recognized risk of gastrointestinal perforation
11. Any evidence of severe or uncontrolled systemic disease, active infection, active bleeding diatheses, or renal transplant, including any patient known to have hepatitis B, hepatitis C, or human immunodeficiency virus (HIV) will be excluded.

Supplementary file 1 complete list of eligibility criteria (*continued*)

Exclusion criteria
12. Refractory nausea and vomiting, chronic gastrointestinal diseases (e.g., inflammatory bowel disease), or significant bowel resection that would preclude adequate absorption
13. Any serious and/or unstable pre-existing medical disorder, psychiatric disorder, or other conditions that could interfere with subject's safety
14. Known severe hypersensitivity to trametinib or any excipient of trametinib or history of allergic reactions attributed to compounds of similar chemical or biologic composition to trametinib
15. Pregnant, lactating or actively breastfeeding female subjects

Supplementary table 2: differences in tumor volume response between the two observers

		Tumor volume response at cycle 12				
Patient	**Type of PN**	**Observer 1: Investigator (CN)**		**Observer 2: Radiologist (DH)**		
		Change (%)	**Disease status**	**Change (%)**	**Disease status**	**Agreement**
1	Diffuse	-26.1	PR	-26.8	PR	Yes
2	Nodular	-3.8	SD	-3.5	SD	Yes
3	Diffuse	**-21.8**	**PR**	**-10.7**	**SD**	**No**
4	Nodular	-2.3	SD	-6.9	SD	Yes
5	Diffuse	**-8.7**	**SD**	**-29.7**	**PR**	**No**
6	Diffuse	19.8	SD	17.7	SD	Yes
7	Nodular	-33.5	PR	-34.0	PR	Yes
8	Nodular	-6.3	SD	-5.7	SD	Yes
9	Nodular	**-13.0**	**SD**	**-23.5**	**PR**	**No**
10	Nodular	-11.8	SD	-14.3	SD	Yes
11	Nodular	-19.0	SD	-18.1	SD	Yes
12	Nodular	12.4	SD	6.0%	SD	Yes
13	Nodular	-38.8	PR	-30.9	PR	Yes
14	Nodular	-37.7	PR	-38.8	PR	Yes
15	Diffuse	-28.9	PR	-48.9	PR	Yes
16	Nodular	-21.3	PR	-61.2	PR	Yes
17	Nodular	-54.7	PR	-55.8	PR	Yes
18	Diffuse	*NA*	*NA*	*NA*	*NA*	*NA*
19	Nodular	-39.8	PR	-40.3	PR	Yes
20	Nodular	-45.5	PR	-46.8	PR	Yes
21	Nodular	-32.6	PR	-34.3	PR	Yes
22	Nodular	9.8	SD	-6.9	SD	Yes
23	Nodular	-11.6	SD	-14.0	SD	Yes
24	Nodular	**19.0**	**SD**	**23.9**	**PD**	**No**
25	Nodular	-20.7	PR	-21.4	PR	Yes
26	Nodular	*NA*	*NA*	*NA*	*NA*	*NA*
27	Diffuse	*NA*	*NA*	*NA*	*NA*	*NA*
28	Nodular	-33.6	PR	-55.4	PR	Yes

Supplementary table 2: differences in tumor volume response between the two observers (*continued*)

		Tumor volume response at cycle 12				
29	Diffuse	-29.3	PR	-22.1	PR	Yes
30	Nodular	**-17.9**	**SD**	**-22.1**	**PR**	**No**

PN: plexiform neurofibroma, PR: partial response, SD: stable disease, PD: progressive disease.

Supplementary file 3 full data on SF-36 Quality of life subscales

Subscale	Baseline mean (range), n=28	Cycle 12 mean (range), n=21	Paired t-test ,n=21
Physical functioning	51.8 (0-95)	53.6 (10-100)	Diff: 1.0, 95% CI: -7.2 – 9.0 P=0.808
Social functioning	58.9 (12.5-100)	67.9 (25-100)	Diff: -7.1 95% CI: -18.0 – 3.7 P=0.186
Role functioning/physical	43.8 (0-100)	44.1 (0-75)	Diff: 2.1 95% CI: -7.9 – 12 P=0.667
Role functioning/emotional	52.7 (0-100)	51.2 (25-83)	Diff: 1.6 95% CI: -8.1 - 11.2 P=0.735
Mental well-being	60.5 (20-85)	61.9 (30-90)	Diff: -1.9 95% CI: -6.5 – 2.7 P=0.396
Energy	42.9 (6-69)	45.8 (19-75)	Diff: 0.6 95% CI: -5.8 – 7.0 P=0.849
Pain	46.5 (12-90)	60.5 (22-90)	Diff: -10.7 95% CI: -21.4 – 0 P=0.051
General health	47.3 (20-75)	42.1 (20-75)	Diff: 7.6 95% CI: -1.1 – 16.3 P=0.082
Health change	42.0 (25-100)	56.0 (25-100)	Diff: -11.9 95% CI: -25.2 – 1.4 P=0.076

Full data on SF-36 version 2 quality of life questionnaire, higher scores indicates better quality of life.
Diff=difference of mean. CI=confidence interval.

Supplementary table 4. Treatment related adverse events

TRAEs No. of patients (n=30)	Grade 1	Grade 2	Grade 3	Grade 4
Rash acneiform	22	8	2	
Hypoalbuminemia	20	9		
Aspartate aminotransferase increased	24	2	1	
Fatigue	20	4		
Alanine aminotransferase increased	21	1		
Blood lactate dehydrogenase increased	22			
Diarrhea	18	1		
Peripheral edema	14	3		
Anemia	14	2		
Alopecia	11	2		
Constipation	7	6		
Palmar-plantar erythrodysesthesia syndrome	11	2		
Nausea	8	3		
Dry skin	7	3		
Hypertension	4	4	1	
Proteinuria	6	1		
Vomiting	6	1		
Pruritus	6	1		
Skin infection		6		
Paronychia	4	1		
Hirsutism	4			
White blood cell decreased	4			
Mucositis oral	1	3		
Dry mouth	3			
Abdominal pain	3	1		
Anorexia	1	1		
Blurred vision	1	1		
Dizziness	2			
Kidney stones	1	1		
Ejection fraction decreased	3		1	
CPK increased			1	1
Wound infection		1	1	
Dry eyes		1		
Dyspnea		1		
Epistaxis	1			
Hair texture abnormal	1			
Headache		1		
Insomnia	1			

Supplementary table 4. Treatment related adverse events (*continued*)

TRAEs No. of patients (n=30)	Grade 1	Grade 2	Grade 3	Grade 4
Nail changes	1			
Skin hyperpigmentation	1			
Urticaria	1			
Vision decreased	1			
Oral dysesthesia	1			
Anal hemorrhage	1			
Vulval infection	1			
Gastroesophageal reflux disease	1			
Pneumonitis			1	
Multiple small serous retinal detachments	1			

TRAE=treatment related adverse events. CPK=creatinine phosphokinase. Graded according to CTCAE v5.0.

6

Cardiotoxicity of trametinib monotherapy in adults with neurofibromatosis type 1: results from the TRAIN study

D.C. Noordhoek*, S.A. Ditta*, F. Jongbloed, E. Oomen-de Hoop, W.C. Meijers, M.J. van den Bent, M.E.M.M. Bos, R.H.J. Mathijssen, W. Taal*, O.C. Manintveld*.

*authors contributed equally

Biomedicine and Pharmacotherapy. 2026 May:198:119251.

ABSTRACT

Background and aims

MEK1/2 inhibitors are increasingly used in neurofibromatosis type 1 (NF1) and can cause cancer therapy–related cardiac dysfunction (CTRCD). Cardiotoxicity rates of up to 12% have been reported in combination therapy, and up to 7% in monotherapy trials. Cardiac monitoring is recommended, but data on trametinib in NF1 remain limited. Therefore, we evaluated the incidence, timing, and clinical characteristics of CTRCD in adults with NF1 receiving trametinib monotherapy.

Methods

The phase II single-arm TRAIN trial included NF1 patients receiving trametinib 2 mg daily. Serial cardiovascular assessments and N-terminal pro–B-type Natriuretic Peptide (NT-proBNP) measurements were collected over 2 years. CTRCD was defined as mild, moderate or severe per 2022 European Society for Cardiology cardio-oncology guidelines.

Results

Of 30 patients, 7 (23%) developed CTRCD: 1 severe, 4 moderate and 2 mild. Median time to onset was 5 months (range 1 - 12 months). All cases were asymptomatic and reversible. One severe case required permanent discontinuation, after which LVEF recovered. NT-proBNP remained within normal limits in 6 cases; only the severe case showed a marked rise. No baseline clinical or cardiovascular parameters could predict CTRCD.

Conclusions

In this NF1 cohort, cardiotoxicity occurred in 23% of patients but only one case required trametinib discontinuation. Events occurred within the first year, were usually moderate, and reversible. NT-proBNP remained normal in most cases but may help identify patients needing echocardiography. We recommend continuing echocardiographic monitoring during the first year, with consideration for less frequent monitoring thereafter based on clinical findings. However, further data are needed for long-term surveillance.

INTRODUCTION

Trametinib is a selective MEK1/2 inhibitor used in oncology to target the mitogen-activated protein kinase (MAPK) pathway; a key regulator of cell proliferation and survival [1]. Trametinib is frequently administered in combination with dabrafenib (a BRAF inhibitor) and is FDA-approved for the treatment of BRAF V600E–mutant melanoma, non–small cell lung cancer, and anaplastic thyroid carcinoma [2, 3]. While trametinib has shown clinical efficacy, it is associated with cardiotoxic effects, including reduced left ventricular ejection fraction (LVEF), hypertension, and arrhythmias [4, 5]. These adverse events are thought to result from inhibition of the MAPK pathway in cardiomyocytes, which plays a critical role in maintaining mitochondrial function, oxidative stress balance, and myocardial remodelling [6, 7].

Early clinical trials, such as the COMBI-d and COMBI-v studies, reported cardiotoxicity rates of 2% to 12% with combined dabrafenib plus trametinib therapy [8, 9]. However, more recent studies have observed higher cardiotoxicity rates. Namely, a retrospective study by Glen et al. reported a 27% incidence of Cancer Therapy-Related Cardiac Dysfunction (CTRCD) in melanoma patients receiving BRAF/MEK inhibitors, while Oddershede et al. found 21% CTRCD in a similar cohort. Both studies noted early onset, typically within 2 to 4 months of treatment [4, 5, 10]. Glen et al. suggested that CTRCD may improve with cardio protective therapy, though recovery rates were not systematically reported [5]. While most cardiotoxicity data derive from combination therapies, monotherapy with trametinib has also shown an incidence of reduced LVEF in up to 7% of patients, with grade ≥3 events in 1% of patients [11].

Given these concerns, patients receiving MEK inhibitors should be monitored for CTRCD. Among these are patients with neurofibromatosis type 1 (NF1); a genetic disorder caused by mutations in the *NF1* gene leading to RAS-MAPK pathway hyper activation [12]. Approximately 50% of these patients develop plexiform neurofibromas (PNs), which can be painful, disfiguring, and difficult to remove surgically [13, 14]. MEK inhibitors have shown substantial benefit in reducing PN volume and pain [15-20].

Limited data exist on the cardiovascular safety of MEK inhibition in the NF1 population. One cross-sectional echocardiographic study by Caiffa et al. reported that NF1 patients treated with the MEK inhibitor selumetinib had significantly lower global longitudinal strain (GLS) and fractional shortening compared to untreated NF1 controls, although LVEF remained preserved [21]. Studying cardiotoxicity in this population is of particular importance, as patients with NF1 not only have a higher prevalence of hypertension, structural heart defects, and early signs of systolic and vascular impairment, but are also typically young and require prolonged treatment, increasing the relevance of potential long-term cardiac effects [22-24]. These findings raise concerns that the risk of CTRCD

may be pronounced in this population and that there is a higher need for cardiovascular screening before initiating treatment.

Therefore, we prospectively evaluated the incidence, timing, and severity of trametinib-associated cardiotoxicity in patients with NF1. The TRAIN trial, registered at the Dutch Trial Registry under number NL8050, is a phase two single arm clinical trial evaluating the effect of trametinib on symptomatic inoperable PN in adults with NF1. In this trial cardiovascular assessments have been performed for a period of 2 years. Understanding the risk for cardiotoxicity in this specific group is critical for optimizing monitoring strategies and improving the long-term safety of MEK inhibition in NF1 patients.

METHODS

Study design and patients

This is a sub-analysis on prospective data collected during the TRAIN trial, which included adult patients (≥18 years) with a confirmed diagnosis of NF1, a symptomatic inoperable PN and a Karnofsky Performance Status of ≥70%. Patients were not eligible for the TRAIN study if they had known LVEF dysfunction (LVEF <50%), New York Heart Association (NYHA) class II–IV heart failure, recent acute coronary syndrome (within 6 months), clinically significant arrhythmias, or uncontrolled hypertension (i.e. blood pressure >140/90mmHg despite maximally tolerated antihypertensive therapy). An electrocardiogram (ECG) and transthoracic heart ultrasound (TTE) were performed at baseline to assess the patient's eligibility. A complete list of in- and exclusion criteria of the TRAIN trial can be found in the supplementary files of the original trial article [20]. Patients received oral trametinib 2 mg daily, administered in 4-week cycles. This is the standard dose given in both monotherapy and combination trials, as well as in routine clinical practice. In case of toxicity, the dose was reduced by 0.5 mg or treatment was paused until toxicity was resolved. Treatment was permanently discontinued in case of unacceptable CTRCD, progressive underlying disease or on patient wish due to side effects. This report presents data collected during the first two years of treatment. The study was approved by the Medical Ethics Committee of the Erasmus University Medical Centre (MEC 19-0463). All patients provided written informed consent prior to participation and the study was conducted according to the declaration of Helsinki.

Assessments

Patients underwent cardiac evaluations consisting of TTE, ECG, office blood pressure and heart rate measurements and NT-proBNP analysis to monitor for cardiotoxicity during outpatient visits. These were performed at baseline, after the first treatment cycle (week 4), and every third cycle thereafter (every 12 weeks) as long as they were still using trametinib. Echocardiography assessments were performed on the Philips EPIC CVX using

standardized protocols and included measurement of left ventricular ejection fraction using 3D measurements and LV global longitudinal strain (GLS) measurements, where possible. A cardiac MRI was performed in cases with severe cardiotoxicity.

In cases of asymptomatic LVEF decrease (>10% compared to baseline and below the institutional lower limit of normal), trametinib treatment was interrupted and echocardiography was repeated within two weeks. Treatment was resumed with a reduced dose once recovery was observed, with further monitoring every 12 weeks. In cases of symptomatic LVEF decrease (Grade 3 or 4), permanent discontinuation of trametinib treatment was required, with additional monitoring.

Endpoints

The primary endpoint of this study was the incidence of CTRCD. CTRCD was defined and graded according to the 2022 European Society of Cardiology (ESC) cardio-oncology guidelines, which incorporate both the degree of LVEF and GLS decline and the presence of symptoms [25]. Mild CTRCD was defined as an LVEF of ≥50%, accompanied by abnormal GLS (>15% relative worsening from baseline) or elevated NT-proBNP. Moderate CTRCD was defined as a ≥10% absolute reduction in LVEF to 40-49%, and severe CTRCD as a drop in LVEF to <40% or the presence of symptomatic heart failure [25].

Secondary endpoints were changes in LVEF and GLS over time, trends in NT-proBNP, blood pressure and heart rate, and the clinical associations between these parameters and the development of CTRCD.

Statistical analysis

Continuous variables were reported as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on data distribution, and were compared using unpaired t-tests or Mann-Whitney U tests, as appropriate. Categorical variables were expressed as counts and percentages and analysed using Fisher's exact test. Patients were stratified into two groups based on the development of CTRCD during follow-up (the CTRCD group and the non-CTRCD group). Baseline demographic and cardiovascular characteristics were compared between patients with and without CTRCD to assess potential predisposing factors. Due to the limited sample size, descriptive statistics and individual trajectory plots were used to assess changes over time and explore visual patterns between groups. All statistical analyses were conducted using SPSS 28.0.1.0 software (IBM SPSS, Chicago, IL) and GraphPad Prism Software Version 9.0 (GraphPad Software Inc., San Diego, CA). Two-sided P-values <0.05 were considered statistically significant.

RESULTS

Patient characteristics

A total of 30 patients were included in the TRAIN-trial between July 2020 and May 2023. Of these, seven patients (23%) developed CTRCD based on the predefined criteria. Two of these patients (7%) classified as mild, four (13%) as moderate and one (3%) as severe CTRCD. The median time to onset was five months (range 1 - 12 months) after initiating trametinib. In the total cohort, 19 patients had a follow-up longer than 12 months and the median follow-up duration was 22 months. Baseline characteristics (**Table 1**) and cardiovascular assessments at baseline (Supplementary **Table 1**) were similar between the CTRCD group and the non-CTRCD group. No clinically relevant ECG abnormalities or structural abnormalities were found at any timepoint.

Table 1: patient characteristics

	Cardiotoxicity (n = 7)	No cardiotoxicity (n = 23)	*p*-value
Patient demographics			
Age	36 [26 – 64]	48 [42 – 59]	0.490
Male	4 (57%)	10 (44%)	0.675
BMI (kg/m^2)	23.1 [21.4 – 27.3]	25.7 [22.3 – 29.8]	0.359
Cardiovascular risk factors			
History of smoking	2 (29%)	9 (39%)	1.000
History of DM2	0	0	-
Hypertension	1 (14%)	2 (9%)	0.515
Cardiovascular medications			
Antihypertensive therapy	1 (14%)	2 (9%)	0.446
Antihyperlipidemic therapy	0 (0%)	3 (13%)	1.000

Values are median (Q1- Q3) or n (%). P values represent the difference between groups.

Abbreviations: BMI = body mass index; CTRCD = cancer therapy–related cardiac dysfunction; DM2 = Diabetes Mellitus type 2.

LVEF and GLS over time

Median LVEF for the total cohort remained stable over the 2-year study period and did not drop below the lower limit of normal (LLN) of 50% **(Figure 1a)**. Median GLS also remained stable over time, with minimal variation across patients (**Figure 1b**). For the group who developed CTRCD, **Figure 2** shows the individual LVEF and GLS trajectories. Five patients in this group experienced an LVEF decline of >10% that dropped below the lower limit of normal of 50%. The lowest recorded LVEF was observed at visit 3 for patient 23, with a corresponding relative decline in GLS of 38% from baseline **(Figure 2**). In total, five patients had a relative decline in GLS of >15% from baseline.

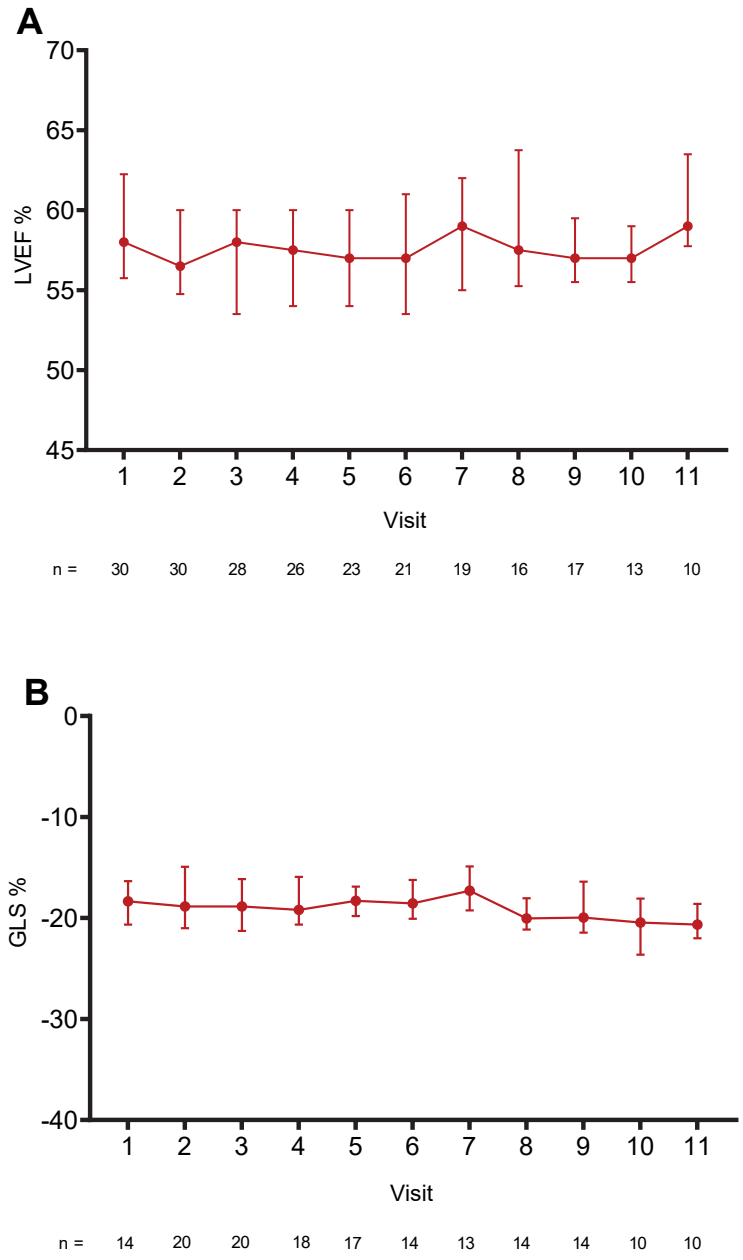


Figure 1. Median LVEF and GLS over 2-year follow-up. Panel A shows median LVEF; panel B shows median GLS. Lines indicate cohort medians, whiskers indicate interquartile ranges. Measurements were obtained at baseline and every study visit during the 2-year follow-up period. Abbreviations: GLS = global longitudinal strain; LVEF = left ventricular ejection fraction.

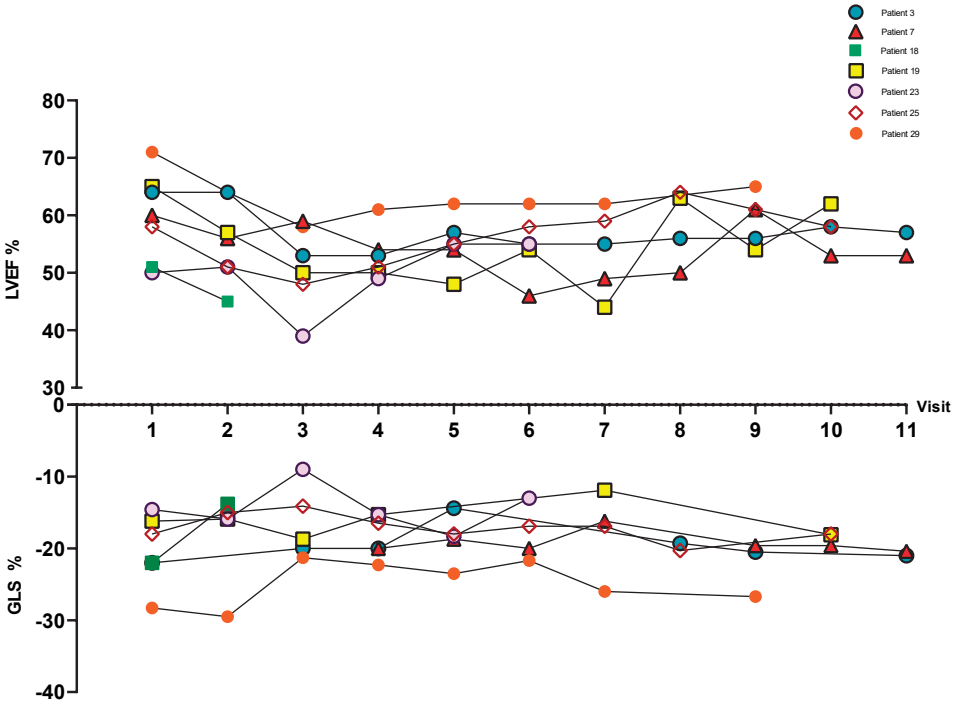


Figure 2. LVEF and GLS over time in patients with CTRCD. LVEF and GLS plotted in time for all patients with CTRCD. Each symbol represents a patient with CTRCD. The blue circle and orange circle represent mild CTRCD. The red triangle, green square, yellow square and red diamond represent moderate CTRCD. The purple circle represents severe CTRCD. Abbreviations: CTRCD = cancer therapy–related cardiac dysfunction; GLS = global longitudinal strain; LVEF = left ventricular ejection fraction.

Other secondary parameters over time

In six out of seven patients with CTRCD, the NT-proBNP levels remained below the upper limit of normal (ULN: 125 pg/ml) throughout the study period (**Figure 3**). One patient, patient 23, had an increase in NT-proBNP at visit 3 (237 pg/ml) and at visit 6 (330 pg/ml). No other laboratory values were correlated with CTRCD in our patients. Only one patient developed increased CPK levels, which was due to rhabdomyolysis.

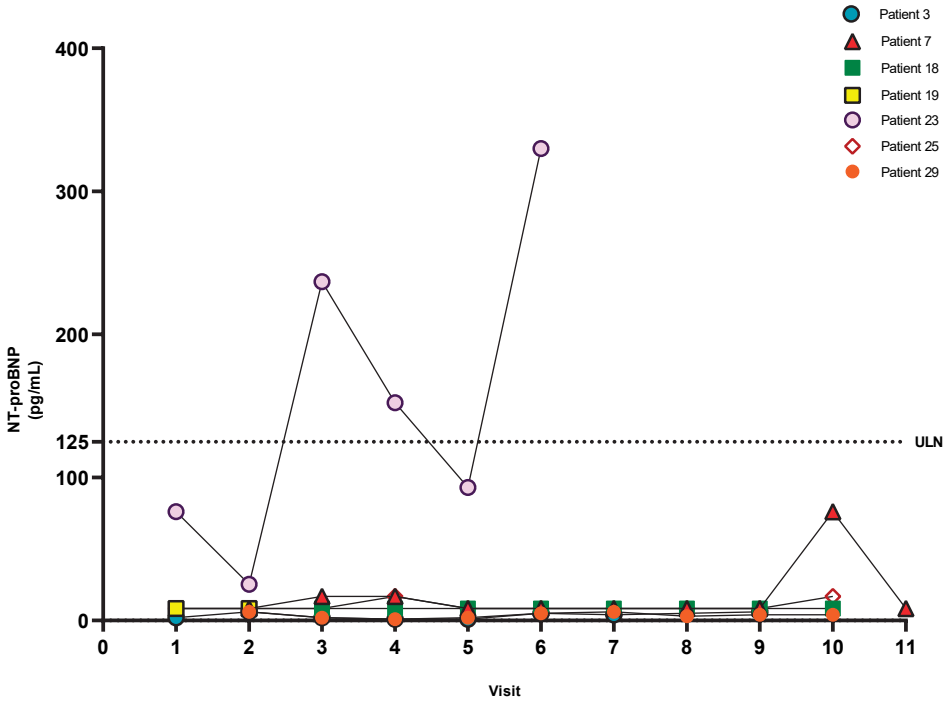


Figure 3. NT-proBNP levels of patients with CTRCD. Each symbol represents a patient with CTRCD and their NT-proBNP levels at the given timepoint. The blue circle and orange circle represent mild CTRCD. The red triangle, green square, yellow square and red diamond represent moderate CTRCD. The purple circle represents severe CTRCD. Abbreviations: CTRCD = cancer therapy–related cardiac dysfunction; NT-proBNP = N-terminal pro–B-type natriuretic peptide.

Median blood pressure (BP) remained stable overall. The CTRCD group showed a higher median baseline systolic BP. In three out of seven patients with CTRCD systolic blood pressure reached or exceeded 140 mmHg around the time of or prior to the LVEF drop. These patients were treated with an angiotensin-converting enzyme (ACE) inhibitor (perindopril). Four patients of the non-CTRCD group developed high blood pressure without LVEF decrease and were treated with perindopril as well. Median heart rate (HR) across the total cohort also remained stable over the study period.

Clinical case descriptions

Severe CTRCD

One patient, patient 23, developed cardiotoxicity meeting the criteria for severe CTRCD and had to permanently stop treatment. After the 3rd treatment cycle, LVEF decreased to 39% and NT-proBNP was 338 pg/ml. Study treatment was paused and the patient's existing dose of lisinopril was increased, and treatment with bisoprolol, spironolactone and empagliflozin was started. Rechallenge with trametinib treatment at 1 mg per day

(50% of the original dose) led to an NT-proBNP increase to 1133 pg/ml between study visits, after which treatment was permanently discontinued before a new LVEF evaluation was done. At evaluation one month after trametinib was stopped, NT-proBNP decreased to 331 pg/ml and LVEF recovered to 55%. A cardiac MRI for further analysis did not show any abnormalities 6 months after treatment was stopped.

Moderate CTRCD

Four patients developed cardiotoxicity meeting criteria for moderate CTRCD (patient 7, 18, 19 and 25). Two of these patients (without cardiac complaints; patient 19 and 25) with LVEF decrease but normal blood pressure and NT-proBNP did not receive additional medication and LVEF improved while study treatment was continued on the same dose. One patient (without cardiac complaints; patient 7) started with an ACE-inhibitor after the first cycle of treatment because of hypertension. After 12 cycles this patient developed an absolute LVEF decrease from 54% to 46% without an increase of NT-proBNP or change in GLS (-20%). Trametinib was continued on the same dose and LVEF improved over time. One patient with moderate CTRCD (patient 18) discontinued trametinib due to dermatological toxicity unrelated to cardiotoxicity.

Mild CTRCD

Two patients developed cardiotoxicity meeting criteria for mild CTRCD (patient 3 and 29). Patient 3 had a 10% LVEF decrease after cycle 3 but remained > 50%. GLS and NT-proBNP did not change and the patient did not have cardiac symptoms. Trametinib was therefore continued on the same dose, but an ACE-inhibitor was started. An additional cardiac MRI that was performed 2,5 months later revealed an LVEF of 48% while echocardiography at the same treatment cycle showed an improved LVEF of 53%. Patient 29 had an increased GLS from -28.3% at baseline to -21.3% at cycle 3, and a decrease of LVEF from 71% at baseline to 58% at cycle 3. NT-proBNP remained stable and patient did not have cardiac symptoms. CTRCD resolved spontaneously without intervention.

DISCUSSION

In our cohort, 23% of NF1 patients treated with trametinib developed CTRCD, with all cases occurring within the first 12 months and being reversible. This incidence is notably higher than reported in other clinical trials based on which trametinib was registered. Flaherty et al. observed a decreased LVEF in 7% out of 214 patients receiving trametinib monotherapy, with grade ≥3 events in only 1% of those [11]. In the COMBI-d and COMBI-v studies, 8% of 561 patients had LVEF reductions and 2% had grade ≥3 LVEF reduction [8, 9]. However, despite the higher overall incidence in our cohort, the severity profile was similar to these previous trials, as only one patient (3%) in our cohort experienced severe cardiotoxicity requiring permanent discontinuation. The other patients with CTRCD did

not require dose reductions or interruptions. The increased incidence of CTRCD observed in our study may be attributed to a potential underlying susceptibility in patients with NF1. Several studies have shown that individuals with NF1 may have pre-existent subclinical cardiac abnormalities and early signs of systolic or diastolic dysfunction [23, 26, 27]. However, the participants of the TRAIN trial underwent cardiovascular screening before inclusion in the trial and no signs of underlying cardiac dysfunction were present when using echocardiography. Additionally, this higher incidence may in part be attributed to the smaller sample size compared to the previously mentioned studies and the more frequent cardiac monitoring schedule used in our study.

The Heart Failure Association - International Cardiovascular Oncology Society tool (HFA-ICOS) is a widely used scoring system designed to assess the risk of CTRCD in patients undergoing cancer treatment [28]. Although the HFA-ICOS tool would classify the risk of CTRCD in our cohort as low, based on factors such as normal baseline LVEF, relatively young age, and the absence of prior cardiovascular disease, it is important to note that the tool may not fully capture the risk in patients receiving trametinib monotherapy. Additionally, the tool has been shown to have limited sensitivity in predicting mild CTRCD, which often presents with subclinical changes in LVEF or GLS that are not accompanied by significant biomarker elevation, as highlighted in a recent systematic review on predictive models for CTRCD [29]. This limitation in predicting mild CTRCD in our cohort underscores the need for regular and thorough cardiac monitoring, particularly in the early phases of treatment, when cardiotoxicity is most likely to develop. While our study demonstrates the utility of routine cardiac monitoring, it is important to highlight that LVEF alone may not fully capture subtle cardiac changes in patients receiving trametinib. Moreover, we observed discrepancies between LVEF and GLS in detecting cardiac dysfunction, with GLS often identifying changes before LVEF did. Significant reductions in GLS were observed in some patients, despite stable LVEF measurements. This suggests that GLS may be a more sensitive marker for early cardiac dysfunction, particularly in the context of trametinib treatment, where changes can be subtle and asymptomatic in the early stages. The ESC guidelines for cardio-oncology also define CTRCD based on both reductions in LVEF and relative changes in GLS [25].

We collected cardiac data per protocol over a two-year follow-up period. With a median time to onset of CTRCD at five months and all within 12 months from the start of treatment, our findings suggest that trametinib associated cardiotoxicity in NF1 patients tends to occur within the first year during treatment. No new cases of CTRCD were observed beyond the first year of treatment. These findings are consistent with previous reports indicating that MEK inhibitor related cardiotoxicity typically manifests within the first few months of therapy [5, 10]. These studies, and our own data, support the rationale for intensified cardiac monitoring during the initial months of trametinib treatment.

Importantly, the majority of cardiotoxic events in our cohort were mild to moderate and reversible. Only one patient experienced severe CTRCD requiring permanent discontinuation of trametinib. Most mild to moderate cases resolved spontaneously without the need for intervention. In the remaining cases, cardiac function improved with ongoing treatment and blood pressure treatment. This is consistent with earlier studies where MEK inhibitor related LVEF decline was often reversible after drug interruption or dose adjustment [4, 9]. It is important to note that the ESC criteria for classifying CTRCD as mild or moderate are strict, and the evidence supporting these classifications in the context of MEK inhibitor therapy is limited [29]. Notably, despite meeting the criteria for CTRCD, none of the patients in our cohort developed symptomatic heart failure. These findings suggest that in NF1 patients, trametinib associated CTRCD is often mild and improves over time with adequate measures.

Interestingly, NT-proBNP levels remained within the normal range in six out of seven patients who developed CTRCD, despite clear evidence of LVEF decline in four of these patients. Only one patient showed a marked rise in NT-proBNP, in line with both LVEF reduction and GLS deterioration. This suggests that while NT-proBNP may lack sensitivity for detecting early and asymptomatic CTRCD, it could still serve as a useful marker for identifying clinically meaningful or progressive cardiotoxicity.

Study strengths

One strength of this study is its prospective design with predefined cardiac surveillance over a two-year period. This allowed consistent follow-up and timely identification of cardiac changes including asymptomatic LVEF decline. By evaluating patients at regular intervals and using both functional assessments (LVEF, GLS) and biomarkers (NT-proBNP), we were able to closely track changes over time. Using each patient as their own control allowed us to better distinguish treatment effects from baseline variability. To our knowledge, this is the first prospective study evaluating trametinib-associated cardiotoxicity in NF1 patients using guideline-based definitions and cardiac surveillance over a two-year period.

Study limitations

Some limitations should be considered when interpreting our findings. The small sample size limited our ability to perform adjusted analyses or identify risk factors for CTRCD beyond descriptive trends. Larger cohorts are needed to explore predictors of cardiotoxicity and confirm our observations. Additionally, although echocardiographic assessments were performed at consistent intervals, some data points were missing due to logistical (or technical) issues and patient population reduced over time as patients discontinued study treatment for other reasons such as non-cardiac toxicity. Finally, while our follow-up period was relatively long, we cannot exclude the possibility of late-onset cardiotoxicity beyond two years of treatment.

Conclusion

In this prospective study of NF1 patients treated with trametinib, cardiotoxicity occurred in 23% of cases, all within the first 12 months of treatment. Most events were asymptomatic, mild/moderate in severity, and reversible without intervention. Only one (3%) patient required permanent discontinuation due to severe CTRCD. NT-proBNP remained within normal limits in most affected patients, though a rise was observed in one case with severe CTRCD with concurrent LVEF and GLS deterioration. These findings suggest that trametinib-associated CTRCD in patients with NF1 is typically mild with an early onset but may be more common than previously reported in oncology trials.

Although LVEF remains the most widely used measure of cardiac dysfunction in clinical settings, our findings support the integration of GLS as an additional tool for detecting subclinical cardiac changes in patients treated with MEK inhibitors. Combining LVEF and GLS could offer a more comprehensive assessment of cardiac function, thereby improving monitoring strategies for patients undergoing these therapies.

When no cardiotoxicity is observed within the first year of trametinib treatment, we have provided data that echocardiography surveillance after that may be redundant and less frequent monitoring might be considered, based on clinical findings. This leads to more durable and patient-friendly medical care. Further studies are needed to optimize risk stratification and monitoring strategies in this population.

REFERENCES

1. Akinleye, A., et al., *MEK and the inhibitors: from bench to bedside.* J Hematol Oncol, 2013. **6**: p. 27.
2. Gouda, M.A. and V. Subbiah, *Expanding the Benefit: Dabrafenib/Trametinib as Tissue-Agnostic Therapy for BRAF V600E-Positive Adult and Pediatric Solid Tumors.* Am Soc Clin Oncol Educ Book, 2023. **43**: p. e404770.
3. Shimoi, T., et al., *Dabrafenib and trametinib administration in patients with BRAF V600E/R or non-V600 BRAF mutated advanced solid tumours (BELIEVE, NCCH1901): a multicentre, open-label, and single-arm phase II trial.* eClinicalMedicine, 2024. **69**.
4. Glen, C., et al., *Mechanistic and Clinical Overview Cardiovascular Toxicity of BRAF and MEK Inhibitors: JACC: CardioOncology State-of-the-Art Review.* JACC CardioOncol, 2022. **4**(1): p. 1-18.
5. Glen, C., et al., *Cardiotoxicity of BRAF/MEK Inhibitors: A Longitudinal Study Incorporating Contemporary Definitions and Risk Scores.* JACC CardioOncol, 2023. **5**(5): p. 628-637.
6. Johnson, E., et al., *Mitochondrial dysfunction and calcium homeostasis in heart failure: Exploring the interplay between oxidative stress and cardiac remodeling for future therapeutic innovations.* Curr Probl Cardiol, 2025. **50**(3): p. 102968.
7. Muslin, A.J., *MAPK signalling in cardiovascular health and disease: molecular mechanisms and therapeutic targets.* Clin Sci (Lond), 2008. **115**(7): p. 203-18.
8. Robert, C., et al., *Improved overall survival in melanoma with combined dabrafenib and trametinib.* N Engl J Med, 2015. **372**(1): p. 30-9.
9. Long, G.V., et al., *Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial.* Lancet, 2015. **386**(9992): p. 444-51.
10. Oddershede, J.K., et al., *Cardiotoxicity in patients with metastatic melanoma treated with BRAF/MEK inhibitors: a real-world analysis of incidence, risk factors, and reversibility.* Acta Oncol, 2025. **64**: p. 507-515.
11. Flaherty, K.T., et al., *Improved survival with MEK inhibition in BRAF-mutated melanoma.* N Engl J Med, 2012. **367**(2): p. 107-14.
12. Gutmann, D.H., et al., *Neurofibromatosis type 1.* Nat Rev Dis Primers, 2017. **3**: p. 17004.
13. Ejerskov, C., et al., *Clinical Characteristics and Management of Children and Adults with Neurofibromatosis Type 1 and Plexiform Neurofibromas in Denmark: A Nationwide Study.* Oncol Ther, 2023. **11**(1): p. 97-110.
14. Mautner, V.F., et al., *Assessment of benign tumor burden by whole-body MRI in patients with neurofibromatosis 1.* Neuro Oncol, 2008. **10**(4): p. 593-8.
15. Gross, A.M., et al., *Selumetinib in adults with NF1 and inoperable plexiform neurofibroma: a phase 2 trial.* Nat Med, 2025. **31**(1): p. 105-115.
16. Gross, A.M., et al., *Selumetinib in Children with Inoperable Plexiform Neurofibromas.* N Engl J Med, 2020. **382**(15): p. 1430-1442.
17. Moertel, C.L., et al., *ReNeu: A Pivotal, Phase IIb Trial of Mirdametinib in Adults and Children With Symptomatic Neurofibromatosis Type 1-Associated Plexiform Neurofibroma.* J Clin Oncol, 2025. **43**(6): p. 716-729.
18. Weiss, B.D., et al., *NF106: A Neurofibromatosis Clinical Trials Consortium Phase II Trial of the MEK Inhibitor Mirdametinib (PD-0325901) in Adolescents and Adults With NF1-Related Plexiform Neurofibromas.* J Clin Oncol, 2021. **39**(7): p. 797-806.
19. Kim, H., et al., *Safety and efficacy of selumetinib in pediatric and adult patients with neurofibromatosis type 1 and plexiform neurofibroma.* Neuro Oncol, 2024. **26**(12): p. 2352-2363.

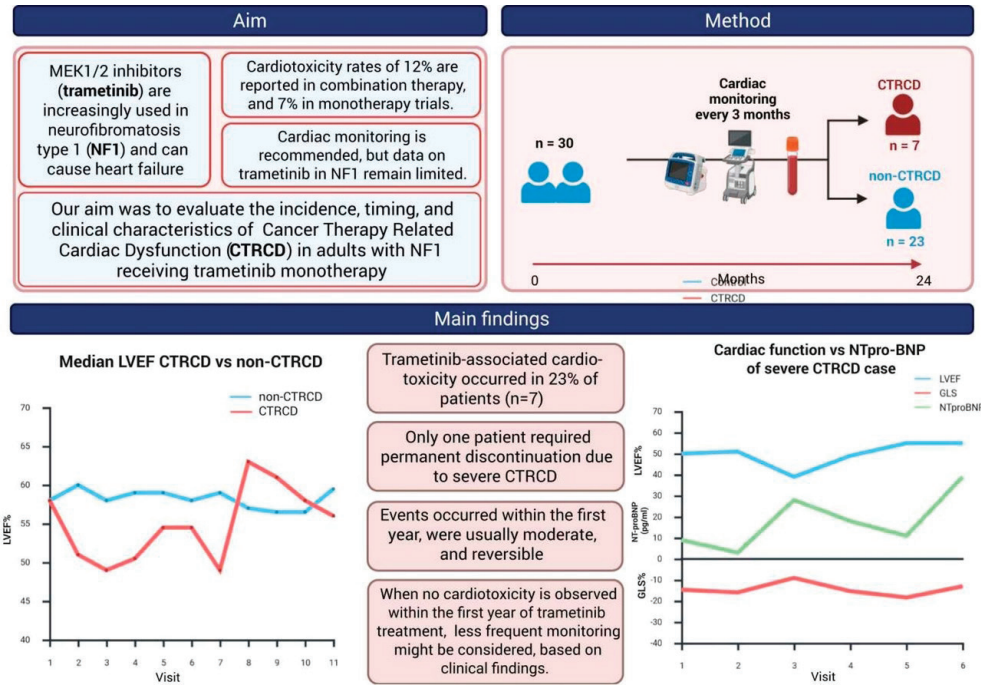
20. Noordhoek, D., *Trametinib in adults with neurofibromatosis type 1 related symptomatic plexiform neurofibromas.* Annals of Neurology, 2025.
21. Caiffa, T., et al., *Characterization of Cardiac Function by Echocardiographic Global Longitudinal Strain in a Cohort of Children with Neurofibromatosis Type 1 Treated with Selumetinib.* Paediatr Drugs, 2023. **25**(2): p. 217-224.
22. Cutruzzolà, A., et al., *Functional and morphological cardiovascular alterations associated with neurofibromatosis 1*. Sci Rep, 2020. **10**(1): p. 12070.
23. Tedesco, M.A., et al., *The heart in neurofibromatosis type 1: an echocardiographic study.* Am Heart J, 2002. **143**(5): p. 883-8.
24. Loponen, N., et al., *Hypertension in NF1: A closer look at the primacy of essential hypertension versus secondary causes.* Mol Genet Genomic Med, 2024. **12**(1): p. e2346.
25. Lyon, A.R., et al., *2022 ESC Guidelines on cardio-oncology developed in collaboration with the European Hematology Association (EHA), the European Society for Therapeutic Radiology and Oncology (ESTRO) and the International Cardio-Oncology Society (IC-OS).* Eur Heart J, 2022. **43**(41): p. 4229-4361.
26. Lama, G., et al., *Blood pressure and cardiovascular involvement in children with neurofibromatosis type1*. Pediatr Nephrol, 2004. **19**(4): p. 413-8.
27. Pinna, V., et al., *Prevalence, Type, and Molecular Spectrum of NF1 Mutations in Patients with Neurofibromatosis Type 1 and Congenital Heart Disease.* Genes (Basel), 2019. **10**(9).
28. Lyon, A.R., et al., *Baseline cardiovascular risk assessment in cancer patients scheduled to receive cardiotoxic cancer therapies: a position statement and new risk assessment tools from the Cardio-Oncology Study Group of the Heart Failure Association of the European Society of Cardiology in collaboration with the International Cardio-Oncology Society.* Eur J Heart Fail, 2020. **22**(11): p. 1945-1960.
29. Gomes, C., et al., *Risk prediction models for cancer therapy related cardiac dysfunction in patients with cancer and cancer survivors: systematic review and meta-analysis.* Bmj, 2025. **390**: p. e084062.

Supplementary Table 1. Baseline cardiovascular assessments

Values	CTRCD (n = 7)		No CTRCD (n = 23)		p-value
	N valid	Value	N valid	Value	
BP systolic (mmHg)	7	128 ± 14	23	130 ± 12	0.866
BP diastolic (mmHg)	7	73 ± 14	23	79 ± 10	0.289
HR (bpm)	7	78 ± 10	20	87 ± 14	0.146
NT-proBNP (pg/ml)	5	34 ± 24	18	51 ± 42	0.120
E-top (cm/s)	7	71 ± 23	23	67 ± 16	0.600
A-top (cm/s)	7	60 ± 19	23	68 ± 16	0.268
E/A ratio	7	1.4 ± 0.79	23	1.01 ± 0.27	0.062
E/E'	7	8.1 ± 1.9	22	8.0 ± 2.5	0.771
DET (ms)	7	208 ± 26	22	194 ± 38	0.368
LVDD (mm)	7	49 ± 4	20	47 ± 6	0.296
LVDS (mm)	3	31 ± 0	10	28 ± 4	0.306
LVVS (mL)	4	33 ± 7.9	15	43 ± 10	0.103
LVVD (mL)	4	95 ± 15	15	106 ± 18	0.283
FS (%)	3	37 ± 3	10	38 ± 6	0.668
SV (mL)	4	62 ± 9	15	63 ± 10	0.535
TAPSE (mm)	7	24 ± 3	20	23 ± 3	0.807
GLS (%)	6	-19.8 ± 5.5	8	-18.2 ± 1.8	0.300
LVEF (%)	7	61 ± 7	23	58 ± 3.8	0.533

Values are given as mean ± standard deviation. N valid shows how many patients had a valid measurement of that specific parameter at baseline. Abbreviations: A-top = peak late diastolic transmitral flow velocity (A wave); BP = blood pressure; CTRCD = cancer therapy–related cardiac dysfunction; DET = deceleration time of early mitral inflow; E-top = peak early diastolic transmitral flow velocity (E wave); FS = fractional shortening; GLS = global longitudinal strain; HR = heart rate; LVEF = left ventricular ejection fraction; LVDD = left ventricular diastolic diameter; LVDS = left ventricular systolic diameter; LVVD = left ventricular volume (diastole); LVVS = left ventricular volume (systole); NT-proBNP = N-terminal pro–B-type natriuretic peptide; SV = stroke volume; TAPSE = tricuspid annular plane systolic excursion.

Graphical abstract



Overview of cardiotoxicity monitoring and findings in NF1 patients treated with trametinib. Thirty patients received 2 mg of trametinib daily and underwent serial NT-proBNP and echocardiographic monitoring. CTRCD occurred in 23% (n=7), typically within the first year. Two cases were mild and four cases were moderate and reversible, one patient had severe CTRCD. Left graph shows median LVEF trends in patients with and without CTRCD. Right graph illustrates NT-proBNP elevation and concurrent GLS/LVEF decline in the severe case. Findings support echocardiography for only the first year of treatment. Created with BioRender.com

7

General discussion

Neurofibromatosis type 1 (NF1) is an unpredictable, complex disease with a high risk of malignancies and with limited treatment options. The aim of this thesis was to improve clinical care of patients with NF1 and plexiform neurofibromas (PNs), by describing the clinical characteristics, by evaluating the role of an imaging tool for tumor load screening and by investigating a new therapeutic option.

In this chapter, the main findings of this thesis are discussed in the context of existing literature, and future perspectives are provided. The discussion is structured in the same parts as the thesis:

- Part I: Clinical characteristics of plexiform neurofibromas
- Part II: Diagnosing plexiform neurofibromas
- Part III: Treatment of plexiform neurofibromas

PART I: CLINICAL CHARACTERISTICS OF PLEXIFORM NEUROFIBROMAS: CLARIFYING RISK AND GUIDING SURVEILLANCE

Plexiform neurofibromas (PNs) and the risk of malignant peripheral nerve sheath tumors (MPNSTs) are a major burden for patients with NF1 (1-4). In order to improve clinical care, it is important to better understand the variability in natural history of these tumors. **Chapter 2** describes the clinical characteristics and management of PNs and MPNSTs in a cohort of more than 1000 adult patients with NF1. In contrast to previous registry-based studies on this topic, our data were obtained through detailed review of the electronic patient files, allowing collection and verification of clinically relevant information.

1.1 Clinical characteristics: plexiform neurofibromas

Chapter 2 demonstrated that PNs are present in half of our patients, despite the fact that they were not routinely assessed with whole body-MRI. The most common symptoms were pain, neurological deficits and cosmetic concerns. Until recently, surgical treatment was the only therapeutic option for PNs. We reported that partial resection or debulking surgery of PNs was more common than complete resection and at least 36% of the patients had regrowth after resection. Additionally, postoperative complications, observed in more than 10% of the PN surgeries, remain a challenge in the surgical treatment of PNs. Previous studies have demonstrated that complete resection is often not feasible because of tumor size, anatomical location, and the risk of substantial intraoperative blood loss (1, 5). Furthermore, a significant correlation was found between less extensive resection and shorter time until progression (6). In our cohort, three patients were treated with radiotherapy for PNs more than 20 years ago. One of these three patients developed an MPNST in the PN that received radiotherapy. This aligns with previous evidence

of radiation-induced MPNSTs in patients with NF1 (7), which is why radiotherapy is no longer used in the treatment of PNs.

These findings highlight the need for new (systemic) therapies for PNs to improve outcome for these patients. The introduction of mitogen-activated protein kinase (MAPK) kinase (MEK)-inhibitors is an important step forward in addressing this treatment gap (8). Some patients in our cohort received MEK inhibitors, mostly trametinib in the context of the TRAIN study (**Chapter 5**), a novel treatment option which is discussed in **Part III** of this discussion.

1.2 Clinical characteristics: malignant peripheral nerve sheath tumors and other malignancies

PNs may undergo malignant transformation into MPNSTs (example in Figure 1) and this was the main cause of death in our population. Prognosis of MPNST remains poor, with a 5-year survival after MPNST diagnosis of 54.5%. Most MPNSTs were located deep in the proximal body regions and therefore not externally visible, consistent with previous studies (4, 9, 10). Additionally, PNs and MPNSTs can both present with pain and neurological deficit, making it difficult to distinguish between them based solely on clinical symptoms. Consequently, early detection of MPNSTs is challenging, however, timely diagnosis is crucial as tumor size is associated with poor prognosis (11). Recognizing high risk subgroups might help to guide tumor surveillance. We found that presence of nodular PNs was strongly associated with an increased risk for MPNSTs (OR 7.4). Other studies have shown similar correlations between tumor load and MPNST risk (12, 13). We also confirmed previously described genotype-phenotype correlations: patients with 17q11.2 microdeletion had a higher risk of developing MPNSTs, whereas patients with p.Met992del and p.Arg1908 variants had a milder phenotype (14). These risk factors for MPNSTs can be taken into account when deciding on the frequency of patient monitoring.

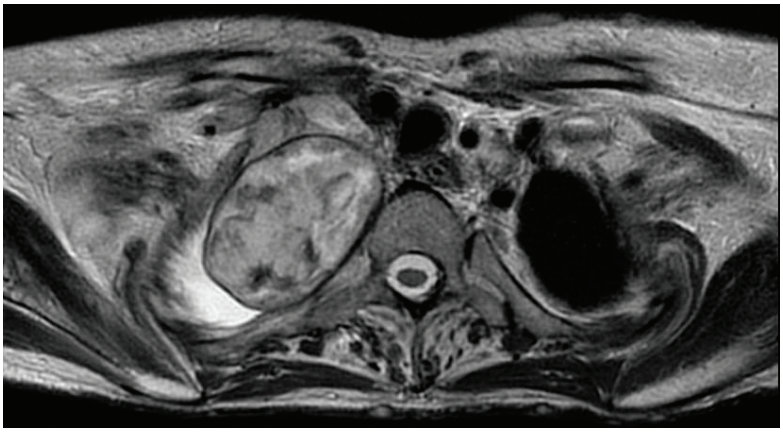


Figure 1. MPNST in the right lung apex.

Besides the peripheral nerves sheath tumors, we also reported the increased occurrence of brain tumors (5%) and other malignancies (10%) in our cohort. The increased risk of malignancies was especially evident in patients under 50 years of age, similar to the age-related risks reported by Uusitalo et al (15).

1.3 Clinical characteristics: key points

- PNs have a major disease burden in patients with NF1 and the role of surgical treatment is limited by postoperative complications and regrowth due to the limited possibility of complete resections.
- MPNSTs are located in deeper regions in the body and prognosis remains poor.
- Certain subgroups such as patients with 17q11.2 microdeletion or nodular PNs have a higher risk of developing MPNSTs and therefore need more frequent surveillance.

1.4 Implications for clinical practice and future research

The high risk of MPNSTs, brain tumors and other malignancies and the associated mortality underscore the importance of early detection in order to improve outcome. Standardized follow-up with regular intervals by a physician with expertise on NF1 is essential (16). Focus should be on tumor surveillance and educating patients on the warning signs of MPNSTs, e.g. rapid growth, change of consistency of the tumor, new persistent pain or new neurological deficits (17). It is important that patients receive this information repeatedly, to increase awareness and enable them to contact their physician in case one of these signs arises. In the Dutch health care system, symptoms are often initially presented at the General Practitioner (GP), so it is helpful to also inform the patient's GP about these warning signs.

The frequency of monitoring should be decided based on the patient's symptoms and risk profile. Since the presence of nodular PNs is independently associated with an increased risk of MPNSTs, the internal tumor load should be assessed to identify patients with high risk. The ERN GENTURIS (European Reference Network Genetic Tumour Risk Syndromes) guideline for tumor surveillance in patients with NF1 recommends performing one whole body-MRI at the moment of transition from pediatric to adult care, in order to assess tumor burden and decide on frequency of monitoring (16). In **Part II**, we discuss an alternative imaging method for tumor load screening that could be used for this purpose.

The recommended frequency of monitoring of adult patients with NF1 is at least once in 3 years (16). However, based on the increased risk for MPNSTs, patients with multiple nodular PNs and/or 17q11.2 microdeletion syndrome should be monitored at least once per year, including MR imaging of symptomatic PNs (e.g. with severe pain, neurological deficit or signs of growth).

The retrospective data that were collected from our cohort of adults with NF1 in Erasmus Medical Center will be the starting point for a Dutch national longitudinal registry for NF1 with the same focus on PNs and malignancies. This will be a great source of data for future research and improvement of NF1 care, by facilitating data to evaluate our patient monitoring strategy. A larger cohort will also provide an opportunity to identify new genotype-phenotype correlations that can help selecting high risk subgroups of patients. Additionally, detailed data on plexiform neurofibromas from the longitudinal registry could be used in future intervention studies as a control arm.

PART II: DIAGNOSING PLEXIFORM NEUROFIBROMAS: THE ROLE OF HIGH RESOLUTION NERVE ULTRASOUND

As discussed in **Part I**, patients with NF1 often need imaging, e.g. in case of the above mentioned warning signs for malignancy, in preparation for surgical treatment, or for PN screening. However, MRI is an expensive imaging technique that comes with a risk of incidental findings which can lead to mental burden for the patient and potentially unnecessary extra (invasive) diagnostic procedures (18, 19). Besides that, some countries have limited availability of MRI and certain patient groups, such as pediatric patients, need to be sedated before undergoing MRI. Given these challenges, a complementary imaging technique that is efficient and more patient-friendly could significantly improve care. In **Chapter 3 and 4**, we investigated the potential value of high resolution nerve ultrasound (HRUS) in clinical care for patients with NF1.

In **Chapter 3**, sixty patients, with or without symptoms of the peripheral nervous system, were invited for a study visit including neurological examination, one-sided nerve conduction studies (NCS) and two-sided HRUS protocol. Using HRUS, the cross-sectional area (CSA) of the nerve was measured at standard anatomical sites and at sites of nerve enlargement. The overall pattern of HRUS findings was categorized as depicted in Figure 2.

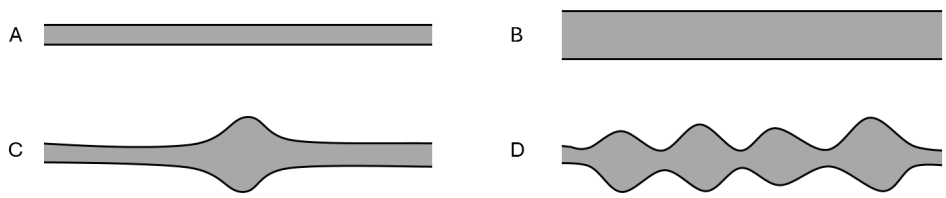


Figure 2: High-resolution nerve ultrasound patterns: A) normal, B) nerve enlargements with normal nerve morphology, C) focal nerve enlargements with abnormal nerve morphology, such as enlarged nerve fascicles as seen in PN (focal PNs) or D) continuous nerve enlargements with PN morphology (continuous PNs). Image from Chapter 4 of this thesis.

The same study procedures were repeated approximately two years after the first visit, these results were presented in **Chapter 4**.

2.1 Diagnosing plexiform neurofibromas: screening with HRUS

In **Chapter 3**, we observed that nerve enlargements were often asymptomatic, which highlights the need for screening for the presence of PNs. As discussed in **Part I** of this discussion section, PN tumor load and especially nodular PNs are an independent risk factor for MPNST development. Using HRUS, we were able to clearly distinguish patients with low tumor load (no or few PNs, pattern A-C) from those with high tumor load (continuous PNs, pattern D). NCS did not have additional value in screening for PNs. At the follow-up visit, we concluded that the tumor load (pattern A-C versus D) remained unchanged over a two-year period.

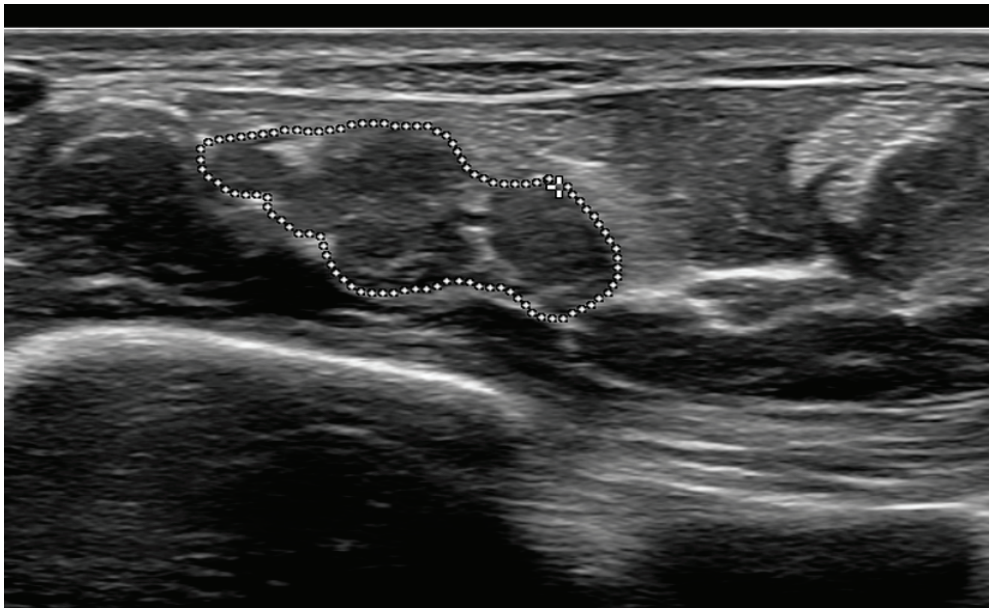


Figure 3. High resolution nerve ultrasound of a neurofibroma in the left median nerve of a patient with continuous plexiform neurofibromas pattern.

2.2 Diagnosing plexiform neurofibromas: follow-up measurements with HRUS

In **Chapter 4**, we reported that PNs can both increase and decrease in size within two years, and even within one patient. This pattern is also seen in prior MRI and HRUS studies (20-23). Such spontaneous size decreases underscore the need for response markers beyond tumor volume and placebo or active control groups in trials. Additionally, this finding may affect clinical decision-making.

2.3 Diagnosing plexiform neurofibromas: interobserver agreement of HRUS

Previous studies in healthy subjects and patients with other peripheral neuropathies have demonstrated good interobserver agreement with HRUS (24-27). However, HRUS in patients with NF1 comes with unique challenges, as irregularly shaped nerve enlargements and altered morphology make accurate CSA measurement more difficult. In **Chapter 4**, we reported that the interobserver agreement was excellent. This indicates that HRUS is a reliable and reproducible method for assessing peripheral nerve enlargements in patients with NF1. However, our assessment of the reproducibility of HRUS had two important limitations. Firstly, we only performed the double measurements in the median nerve, whereas it is known that measurements in the lower extremities are more challenging (24). Secondly, our observers were not independent, as one observer was trained by the other and this may have led to an overestimation of the interobserver agreement.

2.4 Diagnosing plexiform neurofibromas: recognizing signs of malignant transformation with HRUS

In our HRUS studies, we only visualized benign PNs and we did not investigate whether HRUS can distinguish between benign or malignant lesions. A previous study described that signs of malignancy such as inhomogeneity, rich vascularization, intratumoral lobulation, size > 5 cm and calcifications could be detected by HRUS and later confirmed with MR imaging (28). Subsequent histological assessment in this study, however, did not show malignant transformation. There is currently no other literature on diagnosing MPNST with HRUS in patients with NF1. In our cohort, vascularization in nerve enlargements was almost never detected and did not change over the two-year follow-up time.

2.5 Diagnosing plexiform neurofibromas: limitations of HRUS

HRUS has several limitations inherent to the imaging technique itself. Visualization of neurofibromas is restricted to the peripheral nerves in the extremities and the brachial plexus, as penetration depth of HRUS is limited. MPNSTs, however, usually develop in deeper locations (**Chapter 2**). Also, there is currently no evidence that HRUS can distinguish between PNs and MPNSTs. Additionally, HRUS cannot facilitate 3D tumor volume assessment as with MRI.

2.6 Diagnosing plexiform neurofibromas with HRUS: key points

- HRUS can clearly distinguish patients with low or high PN tumor load (figure 2, patterns A-C versus pattern D), which remained unchanged over the two-year follow-up.
- PNs show spontaneous decrease in size, even within two-year follow-up, complicating assessment of treatment effects.
- Interobserver agreement for CSA measurements in the median nerve was excellent.

2.7 Implications for clinical practice and future research

The use of HRUS in clinical practice with NF1 patients has several advantages; besides the above mentioned reasons on costs and patient burden, ultrasound also has approximately 35 times less carbon emission than MRI (29). Considering the results presented above, HRUS would be most suitable for screening for PN tumor load for risk assessment as an alternative to whole body-MRI. Before HRUS can be implemented in clinical practice for this purpose, a comparative study with whole body-MRI is needed to determine whether PN tumor load as assessed by HRUS can be confirmed with MR imaging, which is still the gold standard. Moreover, because HRUS cannot visualize deeper body regions, comparison with whole body-MRI is required to determine whether patients with only deeper located PNs (such as spinal PNs) might be missed with HRUS.

However, during our study, we observed that when many nerve enlargements were present in one nerve, they were typically found throughout the whole body. This raises an interesting question: could HRUS of just one body region (e.g. one arm) be sufficient to identify patients with high PN tumor load? If this hypothesis is confirmed with whole body-MRI comparison, this approach could dramatically reduce the cost, time and effort needed from both the patient and the health care professional for the tumor load screening for deciding monitoring frequency.

To assess whether HRUS could be used to diagnose MPNSTs, further research with histological confirmation is needed. However, it seems that HRUS is not the best technique for this purpose, as most MPNSTs develop in areas that cannot be visualized with HRUS.

In order to use HRUS for PN growth measurements, further studies on interobserver variability are needed including also assessment of the lower extremities and involving independent observers.

PART III: TREATMENT OF PLEXIFORM NEUROFIBROMAS: INSIGHTS INTO MEK INHIBITION IN ADULTS

As described in **Chapter 2** of this thesis, PNs can cause a substantial disease burden and treatment options were traditionally limited. This long-standing treatment gap has left many patients with PN-related pain and neurological deficits relying on symptomatic treatment options only. The introduction of MEK inhibitors that directly target the overactive RAS pathway was a breakthrough in the management of PNs (8). Selumetinib and mirdametinib have demonstrated efficacy in pediatric and adult patients with NF1 and were subsequently approved by the FDA and EMA (30, 31). In the Netherlands, treatment of pediatric patients with selumetinib is now covered by health insurance, whereas MEK inhibitor treatment for adults is still not accessible outside of trial setting.

3.1 Treatment of plexiform neurofibromas: trametinib (TRAIN trial)

The TRAIN trial (**Chapter 5)** was the first clinical phase 2 trial assessing the efficacy and safety of the MEK inhibitor trametinib in adults with symptomatic inoperable NF1-related PNs. Tumor volume on 6-monthly MRI was the primary outcome. Half of the patients achieved ≥20% PN volume reduction(partial response); none showed progression on treatment. Most substantial volume decrease occurs in the first 6 months, with stabilization after 18 months. Patient reported outcome measures (PROMs) on pain and pain interference improved significantly on treatment. Some patients had improvement of neurological deficits and disfigurement, but overall quality of life did not improve. Adverse events were common, most reported were acneiform rash and fatigue. The rate of treatment discontinuation because of adverse events was 40%.

3.2 Treatment of plexiform neurofibromas: cardiotoxicity of trametinib

Chapter 6 evaluated the cardiotoxicity of trametinib monotherapy in adults with NF1, by reporting the results from the TRAIN study. Patients were monitored for cardiotoxicity with 3-monthly transthoracic echocardiography (TTE), electrocardiography (ECG), blood pressure measurement and N-terminal pro-B type Natriuretic Peptide (NT-proBNP) analysis. Seven of 30 patients developed cancer therapy-related cardiac dysfunction (CTRCD). Only one case was severe and required permanent treatment discontinuation after which the left ventricular ejection fraction (LVEF) recovered. In the mild and moderate CTRCD cases, cardiotoxicity spontaneously resolved or improved after initiation of blood pressure treatment (ACE-inhibitor), allowing patients to continue treatment. All CTRCD events occurred in the first 12 months of treatment with trametinib, consistent with previous studies (32, 33). Routine TTE monitoring for cardiotoxicity is therefore only required in the first year of treatment and afterwards only in case of symptoms.

3.3 Treatment of plexiform neurofibromas: key points

- Trametinib reduces PN tumor volume and pain, but quality of life remains the same.
- Adverse events are common and led to treatment discontinuation in 40% of the patients.
- Trametinib-associated cardiotoxicity is mild, reversible, and only occurs within the first year of treatment.

3.4 MEK inhibitor treatment for plexiform neurofibromas: an overview

In the last 5 years, several clinical trials investigating MEK inhibitors for the treatment of PNs have been published. **Chapter 5** included a summary of these trials in table 2. Below, an updated version of this table is presented, including recent trials and additional details on follow-up time and adverse events. In addition to the TRAIN trial (**Chapter 5**), this update includes the first phase 3, placebo controlled trial with selumetinib in adults (34).

The purpose of this table is to provide an overview of the phase 2 and 3 MEK inhibitor trials; details on study design are not included. Although response definitions are similar, comparisons between studies are limited by differences in study design, tumor volume measurements and patient populations. As of today, there is no data available on studies directly comparing efficacy and safety of different MEK inhibitors for the treatment of PNs.

Table 1. Summary of phase 2 and 3 trials with MEK inhibitors for the treatment of plexiform neurofibromas

Trial	Study population, age (years)	Partial response rate	Median time to initial response	Median follow-up time	Toxicity drop-out rate	Most reported adverse events
Gross et al. 2020 (30) Selumetinib Phase 2	**Children**: n=50, median age 10.2 (range 3.5-17.4)	74%	8 cycles (range 4-20)	Treatment duration 36 cycles (range 0-47)	10%	Gastro-intestinal, CPK increase, acneiform rash, paronychia
Kim et al. 2024 (35) Selumetinib Phase 2	**Children:** n=60, median age 8 (range 3-18) **Adults:** n=30, median age 26.5 (range 19-47)	91%	NR	Treatment duration 22 cycles (range 4-26)	0%	Paronychia, acneiform rash, skin infection
Gross et al. 2025 (36) Selumetinib Phase 2	**Adults:** n=33, median age 35.6 (range 18.3-60.2)	63.6%	12 cycles (range 4-54)	NR, but up to 80 cycles completed	6%	Acneiform rash, CPK increase, dry skin, pruritis, ALAT increase, limb edema
Chen et al. 2025 (34) Selumetinib Phase 3	**Adults (selumetinib arm)**: n=71, median age 31 (range 18-60)	20%	3.7 months (CI 3.6-11.1 months)	Treatment duration 20 cycles (range 16-22)	14%	Acneiform rash, CPK increase, gastro-intestinal, fatigue
Weiss et al. 2021 (37) Mirdametinib Phase 2	**Adults:** n=19, median age 24 (range 16-39)	42%	12 cycles (range 8-12)	NR, but up to 24 cycles completed	0%	Acneiform rash, fatigue, nausea
Moertel et al. 2024 (31) Mirdametinib Phase 2	**Children**: n=56, median age 10 (range 2-17)	52%	7.9 months (range 4.1-18.8)	Treatment duration 22.0 months (range 1.6-40.0)	9%	Acneiform rash, gastro-intestinal, paronychia, LVEF decrease, increased CPK
	Adults: n=58, median age 34 (range 18-69)	41%	7.8 months (range 4-19)	Treatment duration: 21.8 months (range 0.4-45.6)	22%	Acneiform rash, gastro-intestinal, fatigue

Table 1. Summary of phase 2 and 3 trials with MEK inhibitors for the treatment of plexiform neurofibromas (*continued*)

Trial	Study population, age (years)	Partial response rate	Median time to initial response	Median follow-up time	Toxicity drop-out rate	Most reported adverse events
Noordhoek et al. 2025* Trametinib Phase 2	**Adults:** n=30, median age 45 (range 19-67)	50%	6 cycles (range 6-18)	Treatment duration: 15 months (range 2-45) Follow-up (including off treatment): 24 months (range: 2-46)	40%	Acneiform rash, hypoalbuminemia, fatigue, increase liver enzymes

Table copied from chapter 5 of this thesis, updated with new trials and additional data. *: Chapter 5 of this thesis. MEK: mitogen-activated protein kinase kinase. NR: not reported. CPK: creatine phosphokinase. ALAT: alanine aminotransferase. LVEF: left ventricular ejection fraction.

Selumetinib is the most investigated MEK inhibitor in NF1 patients; this has also been studied in a placebo controlled phase 3 trial (34). Remarkable is the exceptionally high partial response rate in the uncontrolled study by Kim et al. (35) in contrast to the low response rate in the phase 3 trial. This reflects how differences in study design and study populations can substantially impact trial results, and highlights the need for real-world data to assess the effectiveness of MEK inhibitor treatment in clinical practice without the selection bias inherent to clinical trials (38). In addition, differences in tumor volume measurement technique, observers and the use of central review in clinical trials can impact response rate. As discussed in **Chapter 5**, variability of tumor volume measurements between observers can affect study outcomes, particularly when measurements are near the cut-off for response.

Interestingly, in the placebo controlled selumetinib trial, two patients achieved more than 20% decrease in tumor volume after 12 cycles of placebo treatment (34). Although this represents only a small proportion of the total placebo group, this once again indicates that spontaneous decrease of PNs do occur as also discussed in **Part II**.

3.5 MEK inhibitor treatment for plexiform neurofibromas: challenges

The most important challenge for the implementation of MEK inhibitor treatment in clinical practice is the management of toxicity. Adverse events are common, most frequently reported amongst all studies were acneiform rash, gastro-intestinal symptoms, paronychia and fatigue. Our clinical trial (**Chapter 5**) with trametinib stands out with a high toxicity-related discontinuation rate (40%) compared to other studies. As there are currently no studies directly comparing MEK inhibitors, it remains unclear whether this is related to drug-specific properties or variations in, for instance trial design, follow-up duration, patient population or reporting of adverse events. Nevertheless, this finding is

important, as such high rate of discontinuation because of toxicity may limit the feasibility of long-term treatment with MEK inhibitors. Since PNs are benign tumors and long term treatment is needed, it is essential that side effects do not overshadow the treatment benefits, as patient tolerance for toxicity is lower than when treating patients with life-threatening malignancies.

Another limitation is the current lack of long-term data. As shown in table 1, clinical trials have a relatively short follow-up duration, and because MEK inhibitors were only recently introduced into NF1 care, little is known about their long-term effectiveness and safety. NF1-related PNs are benign tumors, and the effect of MEK inhibition is only maintained while on treatment, meaning patients may require lifelong treatment. Findings from the TRAIN trial (**Chapter 5**) suggest a plateau phase in tumor volume reduction from cycle 18 onward and patients treated with MEK inhibitors are known to develop therapy resistance over time (39). Understanding long-term outcomes, including long-term toxicity, is particularly important as treatment is now also initiated in young children, for instance in the ongoing SPRINKLE study (NCT05309668). The first phase 2 trial with selumetinib by Gross et al. did report long-term follow-up of up to 100 cycles (approximately 7.7 years), showing durable responses without new safety concerns, but also cases of continued PN growth despite treatment, suggesting resistance (40).

Lastly, clinical trials evaluating PN treatment use tumor volume reduction as the primary outcome measure. However, these measurements are challenging due to variability in MRI field of view, imaging angles, and the complex morphology of the tumors. Besides that, we discussed in **Part II and III** that spontaneous tumor volume reduction can occur within the timeframe of a clinical trial. Although volumetric response is a quantitative endpoint, it remains observer-dependent, and measuring techniques vary between centers. Given that PNs are benign tumors, the goal of therapy is not to cure or prolong survival but to improve quality of life. A reduction in tumor volume of >20% is clinically meaningless if the quality of life does not improve.

3.6 Implications for clinical practice and future research

MEK inhibitors are the first effective systemic therapy for patients with NF1-related PNs, however, many challenges remain. As MEK inhibitors are increasingly being used, real-world data should be collected on effectiveness and safety. In the Netherlands, selumetinib is currently approved for the treatment of children with NF1 through the Orphan Drug Access Protocol (ODAP). This provides an opportunity to gain experience and systematically collect real-world data in order to develop clinical guidelines for MEK inhibitor treatment. The TRAIN trial contributes to existing evidence of MEK inhibitor treatment in adults with NF1 and will hopefully support future health insurance coverage for adult patients.

The most important challenge is the adverse events related to MEK inhibitors. Potential strategies to reduce toxicity include improved management of adverse events and identification of patients most likely to benefit with less side effects. Expert consensus recommendations and guidelines have been published to support managing toxicity (41, 42). Dermatologic adverse events, primarily acneiform rash and paronychia, are the most common and generally treatable. Prescribers should be familiar with the initial treatment steps, as timely intervention can significantly improve quality of life. Patients should be referred to a dermatologist if the initial measures are not effective. Fatigue remains challenging, as no specific treatment exists. Management of fatigue can focus on patient education regarding energy balance, activity pacing and optimization of sleep. If fatigue outweighs the treatment benefits and patients have to limit their daily activities, quality of life is clearly not improving. In our experience with the TRAIN trial (**Chapter 5**), finding the most suitable approach to managing toxicity varies between patients requiring an individualized approach. Some patients benefit from short treatment interruptions to allow recovery, while others tolerate continuous dosing better. Overall, patients should be closely monitored by clinicians experienced with the prescription of MEK inhibitors, and consensus recommendations are a valuable guide.

Another step forward in clinical implementation of MEK inhibitor treatment is identifying subgroups that benefit the most. In the TRAIN trial (**Chapter 5**), we reported significant improvement of pain and pain interference with trametinib. In our clinical experience, we saw that patients with severe baseline pain could benefit the most from treatment. These patients may also be more willing to tolerate side effects when treatment benefit is substantial. Both efficacy and toxicity varied greatly between individuals and it often remains a matter of trial and error. Future research with cohorts of larger sample size should compare responders with non-responders, and patients with and without toxicity, to identify predictive features of successful treatment, such as patient characteristics, tumor properties or genetic variants.

Data on long-term follow-up will be important to evaluate durability of treatment response and safety. Patients from the TRAIN trial have been on treatment and follow-up after the manuscript (**Chapter 5**) was published and long-term results on efficacy and safety will be reported in the coming years. We are also awaiting the long-term follow-up results from the other clinical trials in table 1.

Finally, in order to accurately reflect the clinical benefit of MEK inhibitor treatment, patient-reported outcome measures (PROMs) should receive more emphasis in future trials as well as in routine clinical practice.

Although these are all steps to be taken for implementing MEK inhibitor treatment in clinical practice, MEK inhibitors are unfortunately not the "silver bullet" we had hoped for. Adverse events are common and often a reason to discontinue treatment, and the recently published phase 3 trial only found a response rate of 20% (34). Therefore, it is important to continue exploring new therapeutic options arising from basic research and new drug combinations are being investigated. A clinical trial combining cabozantinib with selumetinib is currently being initiated (NCT06502171), and a preclinical study repurposing montelukast in combination with selumetinib has received a Drug Discovery Initiative grant by the Children's Tumor Foundation (https://www.ctf.org/news/2024-drug-discovery-initiative-awardees/).

4.0 Conclusion

In conclusion, this thesis contributes to the understanding of clinical characteristics and challenges in the management of PNs in NF1. It highlights the substantial burden of PNs and the existing treatment gap, as surgical options are limited. Prognosis of MPNST remains poor, and tumor surveillance and continuous education on warning signs of malignant transformation are essential. HRUS is a promising and reproducible complementary imaging technique, particularly for identifying patients with nodular PNs, that are at risk for developing MPNSTs. The introduction of MEK inhibitors for PN treatment is an important step forward with clinical trials demonstrating positive effects on tumor volume and pain. However, challenges for implementing MEK inhibition remain, including management of toxicity and long-term treatment tolerance.

REFERENCES

1. Ejerskov C, Farholt S, Nielsen FSK, Berg I, Thomasen SB, Udupi A, et al. Clinical Characteristics and Management of Children and Adults with Neurofibromatosis Type 1 and Plexiform Neurofibromas in Denmark: A Nationwide Study. Oncol Ther. 2022.
2. Dhaenens BAE, Ferner RE, Bakker A, Nievo M, Evans DG, Wolkenstein P, et al. Identifying challenges in neurofibromatosis: a modified Delphi procedure. Eur J Hum Genet. 2021;29(11):1625-33.
3. Kolberg M, Holand M, Agesen TH, Brekke HR, Liestol K, Hall KS, et al. Survival meta-analyses for >1800 malignant peripheral nerve sheath tumor patients with and without neurofibromatosis type 1. Neuro Oncol. 2013;15(2):135-47.
4. Evans DG, Baser ME, McGaughran J, Sharif S, Howard E, Moran A. Malignant peripheral nerve sheath tumours in neurofibromatosis 1. J Med Genet. 2002;39(5):311-4.
5. Canavese F, Krajbich JI. Resection of plexiform neurofibromas in children with neurofibromatosis type 1. J Pediatr Orthop. 2011;31(3):303-11.
6. Needle MN, Cnaan A, Dattilo J, Chatten J, Phillips PC, Shochat S, et al. Prognostic signs in the surgical management of plexiform neurofibroma: the Children's Hospital of Philadelphia experience, 1974-1994. J Pediatr. 1997;131(5):678-82.
7. Yamanaka R, Hayano A. Radiation-Induced Malignant Peripheral Nerve Sheath Tumors: A Systematic Review. World Neurosurg. 2017;105:961-70 e8.
8. Fisher MJ, Blakeley JO, Weiss BD, Dombi E, Ahlawat S, Akshintala S, et al. Management of Neurofibromatosis Type 1-Associated Plexiform Neurofibromas. Neuro Oncol. 2022.
9. Ducatman BS, Scheithauer BW, Piepgras DG, Reiman HM, Ilstrup DM. Malignant peripheral nerve sheath tumors. A clinicopathologic study of 120 cases. Cancer. 1986;57(10):2006-21.
10. Dubbink HJ, Bakels H, Post E, Zwarthoff EC, Verdijk RM. TERT promoter mutations and BRAF mutations are rare in sporadic, and TERT promoter mutations are absent in NF1-related malignant peripheral nerve sheath tumors. J Neurooncol. 2014;120(2):267-72.
11. Cai Z, Tang X, Liang H, Yang R, Yan T, Guo W. Prognosis and risk factors for malignant peripheral nerve sheath tumor: a systematic review and meta-analysis. World J Surg Oncol. 2020;18(1):257.
12. Tucker T, Wolkenstein P, Revuz J, Zeller J, Friedman JM. Association between benign and malignant peripheral nerve sheath tumors in NF1. Neurology. 2005;65(2):205-11.
13. Nguyen R, Jett K, Harris GJ, Cai W, Friedman JM, Mautner VF. Benign whole body tumor volume is a risk factor for malignant peripheral nerve sheath tumors in neurofibromatosis type 1. J Neurooncol. 2014;116(2):307-13.
14. Peduto C, Zanobio M, Nigro V, Perrotta S, Piluso G, Santoro C. Neurofibromatosis Type 1: Pediatric Aspects and Review of Genotype-Phenotype Correlations. Cancers (Basel). 2023;15(4).
15. Uusitalo E, Rantanen M, Kallionpaa RA, Poyhonen M, Leppavirta J, Yla-Outinen H, et al. Distinctive Cancer Associations in Patients With Neurofibromatosis Type 1. J Clin Oncol. 2016;34(17):1978-86.
16. Carton C, Evans DG, Blanco I, Friedrich RE, Ferner RE, Farschtschi S, et al. ERN GENTURIS tumour surveillance guidelines for individuals with neurofibromatosis type 1. EClinicalMedicine. 2023;56:101818.
17. Ferner RE, Huson SM, Thomas N, Moss C, Willshaw H, Evans DG, et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. J Med Genet. 2007;44(2):81-8.

18. O'Sullivan JW, Muntinga T, Grigg S, Ioannidis JPA. Prevalence and outcomes of incidental imaging findings: umbrella review. BMJ. 2018;361:k2387.
19. Preez HD, Lasker I, Rajakulasingam R, Saifuddin A. Whole-body magnetic resonance imaging: Incidental findings in paediatric and adult populations. Eur J Radiol. 2020;130:109156.
20. Telleman JA, Stellingwerff MD, Brekelmans GJ, Visser LH. Nerve ultrasound in neurofibromatosis type 1: A follow-up study. Clin Neurophysiol. 2018;129(2):354-9.
21. Ly KI, Merker V, Cai W, Bredella M, Muzikansky A, Thalheimer RD, et al. Ten-Year Follow-up of Internal Neurofibroma Growth Behavior in Adult Patients With Neurofibromatosis Type 1 Using Whole-Body MRI. Neurology. 2022.
22. Nguyen R, Dombi E, Widemann BC, Solomon J, Fuensterer C, Kluwe L, et al. Growth dynamics of plexiform neurofibromas: a retrospective cohort study of 201 patients with neurofibromatosis 1. Orphanet J Rare Dis. 2012;7:75.
23. Akshintala S, Baldwin A, Liewehr DJ, Goodwin A, Blakeley JO, Gross AM, et al. Longitudinal evaluation of peripheral nerve sheath tumors in neurofibromatosis type 1: growth analysis of plexiform neurofibromas and distinct nodular lesions. Neuro Oncol. 2020;22(9):1368-78.
24. Telleman JA, Herraets IJT, Goedee HS, Verhamme C, Nikolakopoulos S, van Asseldonk JH, et al. Nerve ultrasound: A reproducible diagnostic tool in peripheral neuropathy. Neurology. 2019;92(5):e443-e50.
25. Boehm J, Scheidl E, Bereczki D, Schelle T, Aranyi Z. High-resolution ultrasonography of peripheral nerves: measurements on 14 nerve segments in 56 healthy subjects and reliability assessments. Ultraschall Med. 2014;35(5):459-67.
26. Tagliafico A, Cadoni A, Fisci E, Bignotti B, Padua L, Martinoli C. Reliability of side-to-side ultrasound cross-sectional area measurements of lower extremity nerves in healthy subjects. Muscle Nerve. 2012;46(5):717-22.
27. Tagliafico A, Martinoli C. Reliability of side-to-side sonographic cross-sectional area measurements of upper extremity nerves in healthy volunteers. J Ultrasound Med. 2013;32(3):457-62.
28. Winter N, Rattay TW, Axer H, Schaffer E, Decard BF, Gugel I, et al. Ultrasound assessment of peripheral nerve pathology in neurofibromatosis type 1 and 2. Clin Neurophysiol. 2017;128(5):702-6.
29. McAlister S, McGain F, Petersen M, Story D, Charlesworth K, Ison G, Barratt A. The carbon footprint of hospital diagnostic imaging in Australia. Lancet Reg Health West Pac. 2022;24:100459.
30. Gross AM, Wolters PL, Dombi E, Baldwin A, Whitcomb P, Fisher MJ, et al. Selumetinib in Children with Inoperable Plexiform Neurofibromas. N Engl J Med. 2020;382(15):1430-42.
31. Moertel CL, Hirbe AC, Shuhaiber HH, Bielamowicz K, Sidhu A, Viskochil D, et al. ReNeu: A Pivotal, Phase IIb Trial of Mirdametinib in Adults and Children With Symptomatic Neurofibromatosis Type 1-Associated Plexiform Neurofibroma. J Clin Oncol. 2024:JCO2401034.
32. Glen C, Adam S, McDowell K, Waterston A, Tan YY, Petrie MC, et al. Cardiotoxicity of BRAF/MEK Inhibitors: A Longitudinal Study Incorporating Contemporary Definitions and Risk Scores. JACC CardioOncol. 2023;5(5):628-37.
33. Oddershede JK, Meklenborg IK, Bastholt L, Guldbrandt LM, Schmidt H, Friis RB. Cardiotoxicity in patients with metastatic melanoma treated with BRAF/MEK inhibitors: a real-world analysis of incidence, risk factors, and reversibility. Acta Oncol. 2025;64:507-15.
34. Chen AP, Coyne GO, Wolters PL, Martin S, Farschtschi S, Blanco I, et al. Efficacy and safety of selumetinib in adults with neurofibromatosis type 1 and symptomatic, inoperable plexiform neurofibromas (KOMET): a multicentre, international, randomised, placebo-controlled, parallel, double-blind, phase 3 study. Lancet. 2025;405(10496):2217-30.

35. Kim H, Yoon HM, Kim EK, Ra YS, Kim HW, Yum MS, et al. Safety and efficacy of selumetinib in pediatric and adult patients with neurofibromatosis type 1 and plexiform neurofibroma. Neuro Oncol. 2024;26(12):2352-63.
36. Gross AM, O'Sullivan Coyne G, Dombi E, Tibery C, Herrick WG, Martin S, et al. Selumetinib in adults with NF1 and inoperable plexiform neurofibroma: a phase 2 trial. Nat Med. 2025;31(1):105-15.
37. Weiss BD, Wolters PL, Plotkin SR, Widemann BC, Tonsgard JH, Blakeley J, et al. NF106: A Neurofibromatosis Clinical Trials Consortium Phase II Trial of the MEK Inhibitor Mirdametinib (PD-0325901) in Adolescents and Adults With NF1-Related Plexiform Neurofibromas. J Clin Oncol. 2021;39(7):797-806.
38. Wilson BE, Booth CM. Real-world data: bridging the gap between clinical trials and practice. EClinicalMedicine. 2024;78:102915.
39. Smith MP, Wellbrock C. Molecular Pathways: Maintaining MAPK Inhibitor Sensitivity by Targeting Nonmutational Tolerance. Clin Cancer Res. 2016;22(24):5966-70.
40. Gross AM, Dombi E, Wolters PL, Baldwin A, Dufek A, Herrera K, et al. Long-Term Safety and Efficacy of Selumetinib in Children with Neurofibromatosis Type 1 on a Phase 1/2 Trial for Inoperable Plexiform Neurofibromas. Neuro Oncol. 2023.
41. Azizi AA, Hargrave D, Passos J, Wolkenstein P, Rosenbaum T, Santoro C, et al. Consensus recommendations on management of selumetinib-associated adverse events in pediatric patients with neurofibromatosis type 1 and plexiform neurofibromas. Neurooncol Pract. 2024;11(5):515-31.
42. Klesse LJ, Jordan JT, Radtke HB, Rosser T, Schorry E, Ullrich N, et al. The Use of MEK Inhibitors in Neurofibromatosis Type 1-Associated Tumors and Management of Toxicities. Oncologist. 2020;25(7):e1109-e16.

8

Summary/samenvatting

In this thesis, we investigated plexiform neurofibromas (PNs) in adults with (mosaic) neurofibromatosis type 1 (NF1), focusing on clinical characteristics, diagnostic modalities and treatment.

In **Part I**, we described the clinical characteristics and management of PNs.

In order to improve clinical care, it is important to better understand the natural history of PNs. **Chapter 2** reports on the clinical characteristics and management of PNs and malignant peripheral nerve sheath tumors (MPNSTs) in our adult NF1 population. We collected data from more than 1000 adult patients at the Dutch national expertise center for NF1. PNs were present in half of the patients of this cohort, even without routine use of whole body-MRI. The most common symptoms were pain, neurological deficits and cosmetic concerns. In the majority of the patients, only partial resection of the PN was possible, consequently, regrowth after resection was common. Postoperative complications occurred in 10% of the PN surgeries. These limitations of surgical treatment underscore the need to develop new systemic therapies.

PNs can develop into MPNSTs, mainly in the proximal body regions such as the trunk. Four percent of the patients in our cohort had an MPNST. Furthermore, MPNSTs were the leading cause of death and prognosis was poor, with a five-year survival of 54.5%. Presence of nodular PNs and 17q11.2 microdeletion were associated with a higher MPNST risk. Regular follow-up is essential for all patients with NF1, and high risk subgroups should be monitored more frequently.

Part II explores a promising additional imaging modality for PNs: high resolution nerve ultrasound (HRUS).

Patients with NF1 and PNs often require imaging, either regional or whole body-MRIs. In **Chapter 3**, we evaluated the potential value of HRUS and nerve conduction studies (NCS) in screening for PNs. Sixty adult patients with NF1, with and without peripheral nervous system symptoms, were invited for a study visit that included neurological examination, one-sided NCS and two-sided HRUS. Nerve enlargements were found in 87% of the patients, and they were often asymptomatic. We reported that patients with no or few PNs could be easily distinguished from patients with continuous PNs (high tumor load) by using HRUS. Identifying patients with high PN tumor load can help deciding on frequency of surveillance, as tumor load is associated with a higher risk of developing MPNST. NCS did not have added value for PN screening.

Chapter 4 presents data from the follow-up visits in the same cohort, two years after the baseline visit. HRUS and NCS were repeated and additionally we evaluated the interobserver variability of the cross-sectional area (CSA) measurements in the median nerve.

Patients identified with high tumor load at baseline remained the same at follow-up. Comparing CSA measurements over time, we observed that PNs can both increase and decrease in size within two years. There was no correlation between age and PN growth. The spontaneous decrease of PN size within two years limits the use of tumor volume as a sole marker of treatment response in uncontrolled studies. Interobserver agreement of CSA measurements in the median nerve was excellent between our observers.

In **Part III**, we investigated the efficacy and safety of trametinib as a treatment for PNs.

In the past years, MEK inhibitors have emerged as the first effective systemic therapy for inoperable PNs. **Chapter 5** contains the main findings of the TRAIN trial: trametinib in adults with NF1-related symptomatic PNs. The TRAIN trial was a single-center phase 2 trial in which 30 adult patients with either generalized or mosaic NF1 and a symptomatic, inoperable PN were treated with 2 mg trametinib per day in cycles of 4 weeks. The primary outcome measure was partial response rate on tumor volume defined as at least 20% volume decrease. Tumor volume response was assessed with 3-dimensional volumetric measurements on MRI after every 6th cycle. We reported an overall partial response rate of 50%, with a median time until best response of 12 cycles. None of the patients reached progressive disease (>20% tumor volume increase) on treatment. Patient-reported outcome measures on pain and pain interference reduced significantly after one year of treatment, but quality of life remained unchanged. Adverse events were common, especially acneiform rash and fatigue. The rate of treatment discontinuation because of adverse events was 40%. In conclusion, trametinib is effective in the treatment of PNs but management of adverse events remains a challenge.

Chapter 6 reported the results of the TRAIN trial on cardiotoxicity of trametinib monotherapy in adults with NF1, and provided recommendations for monitoring. In the TRAIN trial, patients were monitored for cardiotoxicity every 3rd cycle with transthoracic echocardiography, electrocardiography, blood pressure measurement and N-terminal pro-B type Natriuretic Peptide (NT-proBNP) analysis. Cancer therapy-related cardiac dysfunction (CTRCD) occurred in 7 out of 30 patients. Only one case was classified as severe CTRCD, this was also the only patient with NT-proBNP increase. Ultimately, this patient required permanent treatment discontinuation, after which the left ventricular ejection fraction recovered. A cardiac MRI did not reveal any abnormalities. In the remaining CTRCD cases, the left ventricular ejection fraction recovered spontaneously or after initiation of an ACE-inhibitor and trametinib could be continued. All cardiotoxicity occurred within the first year of treatment. We therefore recommend to perform routine monitoring for cardiotoxicity only during the first year of MEK inhibitor treatment and afterwards only when clinically warranted.

In **Chapter 7**, the findings of this thesis were discussed in the light of other international research and recommendations were given for future studies and clinical implementation.

NEDERLANDSE SAMENVATTING

In dit proefschrift onderzochten we plexiforme neurofibromen (PN) in volwassenen met (mozaïek) neurofibromatose type 1 (NF1), met de focus op de klinische karakteristieken, diagnostiek en behandeling.

In **Deel I** beschreven we de klinische karakteristieken en behandeling van PN.

Om de zorg te verbeteren is het van belang om een goed begrip te hebben van het natuurlijk beloop van PN. **Hoofdstuk 2** beschrijft de klinische karakteristieken en behandeling van PN en maligne perifere zenuwschede tumoren (MPNST) in onze volwassen NF1 populatie. We hebben data verzameld van meer dan 1000 volwassen patiënten in het nationale expertise centrum voor NF1. Bij de helft van de patiënten bleken PN aanwezig, zelfs zonder dat er gebruikt werd gemaakt van routinematig uitgevoerde whole body-MRI. De meest voorkomende symptomen waren pijn, neurologische uitval en cosmetische klachten. In de meeste gevallen was slechts een partiële resectie van het PN mogelijk en als gevolg daarvan kwam recidief groei veel voor. Daarnaast kwamen postoperatieve complicaties voor bij 10% van de PN operaties. Deze beperkingen van de chirurgische behandeling van PN benadrukken het belang voor de ontwikkeling van nieuwe systemische therapieën.

PN kunnen transformeren tot een MPNST, vooral in de proximale lichaamsregio's zoals de romp. Vier procent van de patiënten in ons cohort ontwikkelde een MPNST. MPNST was tevens de meest voorkomende doodsoorzaak en de prognose is slecht met een 5-jaars overleving van 54.5%. Nodulaire PN en de 17q11.2 microdeletie waren geassocieerd met een verhoogd risico op het ontwikkelen van een MPNST. Het regelmatig opvolgen van patiënten met NF1 is essentieel en subgroepen met een hoog risico moeten met hoge frequentie gemonitord worden.

In **Deel II** onderzochten we een veelbelovende, aanvullende beeldvormingstechniek voor PN: hoge resolutie zenuwechografie.

Bij patiënten met NF1 en PN is er vaak beeldvorming nodig, meestal een regionale MRI of van het gehele lichaam. In **Hoofdstuk 3** evalueren we de potentiële waarde van zenuwechografie en zenuwgeleidingsonderzoek in de screening voor PN. We nodigden zestig volwassen patiënten uit met NF1, met of zonder symptomen van het perifere zenuwstelsel, voor een studiebezoek met neurologisch onderzoek, enkelzijdig zenuwgeleidingsonderzoek en tweezijdig zenuwechografie. Zenuwverdikkingen werden gevonden in 87% van de patiënten en vaak zonder dat er symptomen waren. We beschreven dat er met zenuwechografie goed onderscheid kon worden gemaakt tussen patiënten met geen of weinig PN en patiënten met heel veel PN (en dus hoge tumorlast). Het

identificeren van patiënten met een hoge tumorlast kan helpen bij het beslissen over de frequentie van monitoring, omdat hoge tumorlast geassocieerd is met een hoger risico op het ontwikkelen van een MPNST. Zenuwgeleidingsonderzoek had geen toegevoegde waarde in het screenen voor PN.

In **Hoofdstuk 4** laten we de data zien van de vervolg studiebezoeken van dezelfde patiënten, twee jaar na het eerste bezoek. Opnieuw werd het zenuwgeleidingsonderzoek en de zenuwechografie uitgevoerd en aanvullend onderzochten we de interobserver variabiliteit van de zenuwechografie metingen van de dwarsdoorsnede van de nervus medianus. Patiënten die bij het eerste bezoek een hoge tumorlast hadden, hadden dit bij het vervolg bezoek ook. Als we de metingen van de dwarsdoorsnedes van de zenuwen vergeleken over de tijd zagen we dat PN zowel toe- als afnemen binnen twee jaar. Er was geen verband tussen leeftijd en groei van PN. De spontane afname van PN binnen twee jaar is een beperking voor het gebruik van tumorvolume als enige marker voor behandelrespons in studies zonder controle groep. De interobserver overeenkomst van de zenuwechografie metingen in de nervus medianus tussen de beoordeelaars was uitstekend.

In **Deel III** onderzochten we de effectiviteit en veiligheid van trametinib als behandeling voor PN.

In het afgelopen decennium zijn de MEK remmers geïntroduceerd als eerste effectieve systemische therapie voor inoperabele PN. **Hoofdstuk 5** omvat de bevindingen van de TRAIN studie: trametinib in volwassen patiënten met NF1 en symptomatische PN. De TRAIN studie was een fase 2 studie waarin 30 volwassenen met gegeneraliseerd of mozaïek NF1 en een symptomatisch, inoperabel PN werden behandeld met 2 mg trametinib per dag in cycli van 4 weken. De primaire uitkomstmaat was het percentage partiële respons van het tumor volume, gedefinieerd als minimaal 20% afname van het PN volume. Tumor volume response werd gemeten middels 3-dimensionale analyse van MRI's na elke 6^{e} behandelcyclus. De helft van de patiënten had een partiële respons. De mediane tijd tot de beste respons was 12 cycli. Geen van de patiënten ontwikkelde progressie van het PN (gedefinieerd als meer dan 20% toename van tumor volume) tijdens de behandeling. Uit vragenlijsten bleek dat de pijn en pijn interferentie significant afnamen na een jaar behandeling, maar de kwaliteit van leven bleef gelijk. Bijwerkingen kwamen veel voor, vooral acne en vermoeidheid. Veel patiënten (40%) moesten stoppen met de behandeling vanwege bijwerkingen. Concluderend is trametinib een effectieve behandeling voor PN, maar de bijwerkingen vormen een uitdaging.

In **Hoofdstuk 6** beschreven we de cardiotoxiciteit van monotherapie trametinib bij volwassenen met NF1 in de TRAIN studie en gaven we aanbevelingen over de monitoring. In de TRAIN studie werden patiënten na elke 3^{e} cyclus onderzocht voor cardiotoxiciteit,

dit werd gedaan middels een transthoracale echocardiografie, elektrocardiografie, bloeddruk meting en meting van het N-terminaal pro-B-type natriuretisch peptide (NT-proBNP). Kankertherapie-gerelateerde hartdysfunctie (CTRCD) kwam voor bij 7 van de 30 patiënten. Slechts in één geval betrof het ernstige CTRCD en dit was ook de enige patiënt waarbij een stijging van het NT-proBNP werd vastgesteld. Uiteindelijk moest de trametinib bij deze patiënt definitief gestaakt worden, waarna de linkerventrikel ejectiefractie volledig herstelde. Er werd een MRI hart verricht welke geen afwijkingen toonde. In de overige gevallen van CTRCD was de linker ventrikel ejectiefractie spontaan hersteld of na het instellen van een ACE-remmer, en kon de trametinib ononderbroken worden voortgezet. Alle cardiotoxiciteit ontstond binnen het eerste jaar van de behandeling met trametinib. Daarom bevelen we aan om alleen in het eerste jaar van behandeling met een MEK remmer routinematig te monitoren voor cardiotoxiciteit en daarna op indicatie.

In **Hoofdstuk 7** werden de bevindingen bediscussieerd ten opzichte van de huidige stand van internationaal onderzoek en werden aanbevelingen gedaan voor vervolg onderzoek en implementatie in de klinische praktijk.

A

Dankwoord
About the author
List of publications
PhD portfolio

DANKWOORD

Met het schrijven van deze woorden komt er een einde aan mijn promotietijd. Een tijd van hele leuke momenten en overwinningen, maar ook omgaan met tegenslagen en teleurstellingen. Een tijd van elke dag jezelf motiveren om weer verder te gaan, ook al voelt het soms alsof je stil staat. Ik wil graag mijn woorden van dank uit spreken aan de volgende mensen zonder wie het niet was gelukt:

Allereerst wil ik alle ontzettend lieve, gemotiveerde **patiënten en hun naasten** bedanken voor de tijd en energie die ze hebben gegeven voor het onderzoek. Dat is bij een aandoening als NF1, waarbij vermoeidheid en verminderde belastbaarheid een grote rol speelt, zeker niet vanzelfsprekend. De motivatie om, niet jezelf te helpen maar andere mensen met NF1 in de toekomst, vind ik bewonderenswaardig!

Beste **Martin**, ik prijs me gelukkig dat ik net voor(/tijdens) jouw pensioen nog onder jouw hoede mocht promoveren. Het was mij direct duidelijk dat jij met jouw jarenlange ervaring heel goed wist wat je tegen mij moest zeggen. Af en toe maakte je me een beetje zenuwachtig met opmerkingen als "je moet nu echt hier aan beginnen, anders krijg je het niet af", maar achteraf was ik je heel dankbaar voor dat duwtje in de rug. Daarnaast zei je ook op de juiste momenten als ik iets vooral niet moest doen. Bedankt voor de wijze lessen, enorm nuttige feedback en je bemoedigende woorden. Je gaf mij elke keer het gevoel dat je volledig achter mij stond.

Beste **Rianne**, bedankt dat je ook als promotor betrokken wilde zijn bij mijn onderzoek. Vanuit de kindergeneeskunde ben jij de kartrekker van de NF1 zorg en het onderzoek, niet alleen in Nederland, maar ook in de internationale samenwerking. Met jouw enthousiasme en drive krijg je iedereen mee en kan er veel bereikt worden! Ik wil je bedanken voor je kritische blik op mijn werk en vooral voor de gezelligheid bij de congressen en de bbq bij jou in de tuin.

Beste **Walter**, het zit erop! Ik kan me nog goed herinneren dat jij mij eind 2021 belde toen ik als ANIOS op de spoedeisende hulp zat. Tijdens ons eerste gesprek hebben we het denk ik 5 minuten over het onderzoek gehad en daarna vooral over de belangrijke vraag of ik wel mee ging op de Babinski?? Met dank aan een klein duwtje van **Thijs van der Vaart** begonnen we samen aan dit traject. Over de jaren hebben we een hoop meegemaakt en af en toe waren we het ook eens grondig met elkaar oneens! Ik wil je bedanken dat je mij altijd de ruimte gaf om een eigen plan te maken en een eigen mening te vormen. Ik snap wel dat alle patiënten fan van jou zijn. Je bent goudeerlijk en zegt waar het op staat, ik denk dat die directe benadering precies is wat mensen met NF1 prettig vinden. Bedankt voor alles wat je me geleerd hebt over deze bijzondere patiënten populatie, over het onderzoek doen en over het leven.

Beste **Judith**, wat hebben we veel zenuwecho's gedaan samen! Het soms eindeloos zoeken naar het juiste meetpunt leidde tot grappige uitspraken, zoals "beetje terugger!". Bedankt dat je mij de kunst van de zenuwecho geleerd hebt en voor je begeleiding bij het schrijven van de ZEN artikelen. Weet je nog toen we geconcludeerd hadden dat ik het eerste ZEN artikel zo ongeveer opnieuw moest schrijven? Je medeleven verzachtte de situatie nog een beetje toen de tranen achter m'n ogen prikten. Bij elke meeting vroeg je eerst oprecht hoe het met me ging en daar kon ik dan ook een eerlijk antwoord op geven. Bedankt voor al je wijze adviezen als echte PhD ervaringsdeskundige. Ik ben heel dankbaar dat je nu ook mijn mentor wil zijn tijdens mijn opleiding tot neuroloog.

A special thanks to **Professor Rosalie Ferner** for taking the time and effort to assess the manuscript of my PhD thesis and coming all the way from London to take place in the PhD committee at my defence, it is a huge honour! Ook veel dank aan **Prof. Dr. Henk Verheul en Dr Tom Snijders** voor het beoordelen van mijn proefschrift en aan de overige leden van de promotiecommissie: **Prof. Dr. Marion Smits, Prof. Dr. Leo Visser, Dr. Martijn Broen en Dr. Yvette van Ierland** voor hun tijd en belangstelling om op deze feestelijke dag met mij van gedachten te wisselen

Beste **Olivier**, bedankt voor je bijdrage aan de TRAIN studie, je beoordelingen van de echo's en ECG's, je ultrasnelle reacties op e-mails en bereikbaarheid voor overleg over de patiënten. Tijdens het schrijven van het cardiotoxiciteit stuk hebben we nog wat meer samen gewerkt en was ik super blij met jouw gestructureerde aanpak en duidelijkheid. Dit brengt me gelijk bij **Sheraz**. Als eenzame PhD'er binnen de NF1 had ik in het begin nog niet gedacht dat ik toch nog samen met iemand een artikel zou schrijven! Ik vond het ontzettend leuk en gezellig dat we dit samen gedaan hebben en ik denk dat we vanuit onze eigen invalshoeken veel van elkaar konden leren. Je hebt ontzettend veel tijd gestoken in het artikel en daar wil ik je heel erg voor bedanken. Beste **Henny**, we hebben helaas nooit het geluk gehad elkaar te ontmoeten, maar ik wil je hartelijk bedanken voor je eindeloze geduld en moeite voor het inplannen van de echo's voor de TRAIN studie.

Beste **Pim**, in het begin vond ik je een beetje intimiderend en voelde ik me vooral erg dom als we in jouw kamer zaten. Dat eerste is gelukkig gaandeweg helemaal omgeslagen, dat laatste nooit.. Ik wil je heel erg bedanken voor je inspanningen en hulp bij de TRAIN studie. Wat me het meest zal bij blijven is ons fijne gesprek bij het Rotterdamse etentje voor de EANO 2023. We hadden een persoonlijk gesprek en jouw oprechte blijdschap toen ik vertelde dat ik de liefde had gevonden zal ik niet vergeten. Ik zal zeker even bij je langskomen met foto's van de bruiloft.

Heel veel dank ook aan **Rania** en **Iris** voor alle hulp rondom de samples van de TRAIN studie, en aan **Erik Wiemer** voor de betrokkenheid bij de TRAIN studie en de feedback op het artikel.

Beste **David**, bedankt dat je in jouw overvolle agenda tijd hebt kunnen vinden voor het mee beoordelen van de tumor volumes in de TRAIN studie, ik weet als geen ander hoeveel werk dat was... Ik vond het een hele prettige samenwerking, zowel tijdens het onderzoek als bij overleg over een MRI uitslag. Wellicht kunnen we die nog voortzetten in de toekomst!

Lieve **Sarah, Saar,** jij was als een rode draad door mijn hele onderzoekstijd betrokken. Ik bewonder je voor je onuitputtelijke inzet voor de NF1 zorg en je ontzettend belangrijke onderzoek waarmee je taboes durft te doorbreken. Je bent een onmisbare kracht binnen de NF1 wereld. Bedankt voor al je harde werk o.a. bij het opzetten van de TRAIN studie, maar ook vooral voor je steun en luisterend oor. Snel weer koffie drinken!

Lieve **Hafida, Lisette en Marjoleine,** het is altijd gezellig om bij het secretariaat langs te lopen voor een kletspraatje. Bedankt voor jullie secretariële én mentale ondersteuning! Ook bedankt dat jullie me steeds hielpen als ik Walter niet kon vinden ;-)

Lieve **Lara en Britt**, mijn NF1 PhD collega's! Bedankt dat jullie mijn buddies waren op de NF1 bijeenkomsten en congressen. **Lara**, ik ben ontzettend blij dat jij nog verder gaat met een aantal staartjes van mijn projecten. Het opzetten van de longitudinale registry is een enorme organisatorische kluif waar je je dapper doorheen slaat. Ik blijf de mooie stappen die je zet graag volgen en als je eens een mental support koffie nodig hebt weet je mij te vinden.

Voor de ZEN studie waren een aantal collega's werkelijk onmisbaar: **Willemijn, Irene en Myrthe**, bedankt voor alle EMG's en zenuwecho's die jullie voor de studie met ons gedaan hebben. Bedankt voor jullie eindeloze geduld en vooral voor de gezelligheid en hilariteit (**Willemijn** op donderdagmiddag = de echo probe niet meer stil kunnen houden van het lachen). Daarnaast ook dank aan alle **KNF AIOS** die ook het geduld hebben gehad om de studie onderzoeken te doen en veel dank aan het **secretariaat** voor de ondersteuning bij de planning en het oproepen van de deelnemers. **Koen en Eline,** jullie waren betrokken als masterstudenten op de ZEN studie en hebben een grote bijdrage geleverd aan het verzamelen van de data en de eerste hand gelegd aan de analyse, super gedaan! **Koen**, wat ontzettend gezellig dat we daarna ook nog collega's werden op de 22e!

Axel en Cas, jullie hebben een gigantische bijdrage geleverd aan het verzamelen van data van de meer dan 1000 (!!!) NF1 patiënten. Hierdoor hebben we een grote start gemaakt voor de longitudinale registry en een mooi beschrijvend artikel kunnen schrijven met gedetailleerde data, omdat dit allemaal handmatig door jullie verzameld is. Super bedankt voor jullie harde werk!

Ik wil de **Neurofibromatose vereniging Nederland (NFVN) en Let's beat NF** enorm bedanken voor het steunen van de onderzoeken en de hulp bij het werven van studiedeelnemers. Het werk wat jullie doen voor de NF community is van onschatbare waarde.

Ute, je bent een ontzettend lief en grappig mens en we hebben heel erg geluk gehad met jou te kunnen werken voor de TRAIN studie. Je hebt je ook met moederlijke zorg over mij ontfermd, misschien omdat ik dezelfde voornaam heb als jouw dochter... Ook al doe je je werk met veel passie, ik gun je van harte om binnenkort van je welverdiende pensioen te gaan genieten.

Bedankt **Thijs** (ben je weer..), dat jij hebt geregeld dat ik op de 22e als stiekeme indringer in de neuro-immuno kamer mijn werkplek mocht hebben. Ook al heb ik altijd aan het feutenbureau gezeten (direct voor de deur), mijn promotietijd was niet hetzelfde geweest zonder de gezelligheid van het beruchte "kippenhok". Ik hoop dat ik geen namen ben vergeten: **Katelijn, Arlette, Cato, Romy, Michiel, Alfred, Koen, Sandy, Marlies, Linda, Sophia, Gamida, Lianne, Yelda, Farah, Maudy, Tessa br, Tessa bi, Jeroen, Yvette, Juliette en Robin**, bedankt! Bedankt voor de steun, bij overwinningen en bij mental breakdowns, voor de hulp, voor de koekjes en taart, de koffietjes en lunch, de borrels en sinterklaas avonden. Het voelde als een familie op de 22e. Sorry dat ik tegen het einde anti-sociaal werd, het kon niet anders... Ik heb onvergetelijke herinneringen aan onze tijd samen (denk aan een Disney themafeest..) en ben jullie voor altijd dankbaar.

Lieve **Isabelle**, we zijn al zo lang vriendinnen en hebben elkaar door allerlei hoofdstukken van ons leven gekend. Van brugpiepers die Wii tennis speelden tot dertigers die elkaar steunen met de struggles van het volwassen leven. Als we elkaar zien is het altijd als vanouds en helemaal vertrouwd. Je bent als familie voor mij en ik hoop dat we voor altijd in elkaars leven blijven. Je maakt mij altijd blij!

Lieve **Sietske, Matthijs en alle andere muziekvrienden** die ik over de jaren heb leren kennen, bedankt voor de gezelligheid en de momenten van ontspanning. Samen muziek maken is het allermooiste wat er is. Dear **friends from EMSOC,** it has been a pleasure to meet you every summer over the past 10 years to make music together. We have made unforgettable memories and every one of you has a special place in my heart. I hope we keep seeing each other in the next chapters of our life.

Lieve **Rosa en Sophie**, ontzettend bedankt dat jullie naast mij willen staan als paranimfen op deze bijzondere dag en mij gesteund hebben in de voorbereidingen. Lieve **Rosa**, onze (bijna wekelijkse) koffiemomenten waren ontzettend waardevol. Je bent zo'n enorme steun geweest tijdens dit hele traject. Bedankt dat je er altijd voor mij bent, voor je luisterend oor en je opbeurende woorden. Bedankt voor de foto die je van mij maakte toen ik net mijn discussie van het proefschrift had afgemaakt. Door ups en downs waren

we samen, maar ook: samen op de EANO in Glasgow, samen fietsen langs de Rotte, samen cello spelen, samen boeken lezen en luisteren naar elkaars concerten. Ik had het niet zonder jouw support gekund en ik kan je niet genoeg bedanken.

Lieve **Sophie**, bedankt voor alles. Van gezellige (verjaardags)etentjes tot elkaar steunen in moeilijke tijden. Je geeft mij het gevoel dat ik altijd bij je terecht kan, ik hoop dat je dat andersom ook zo ervaart. We raken nooit uitgekletst en kunnen open over moeilijke onderwerpen praten. Ik kan bij jou altijd eerlijk mezelf zijn en dat is zo enorm waardevol. Je support gedurende deze periode is onmisbaar geweest. Ik vind het een grote eer dat je mijn paranimf wil zijn en een nog grotere eer je mijn vriendin bent.

Als laatste wil ik mijn familie bedanken. Lieve **Oma Friesland**, als nieuwkomer in Rotterdam is het heel leuk om jouw verhalen over het Rotterdam van vroeger te horen. Ik herken in de mensen hier jouw Rotterdamse directheid en nuchterheid, ik voel me er helemaal in thuis. Bedankt dat je in je rode Mazda de reis vanuit het hoge noorden maakt om bij deze bijzondere dag aanwezig te zijn.

Lieve **Pappa en Mama**, ik kan jullie niet genoeg bedanken voor alle kansen die jullie mij gegeven hebben en voor de onvoorwaardelijke liefde en support. Als ik iets van jullie heb geleerd is het doorzettingsvermogen. Jullie hebben allebei altijd heel hard gewerkt voor wat jullie bereikt hebben, niets is komen aanwaaien. Lieve **Pappa**, van jou heb ik geleerd om altijd voorbereid te zijn. Jij maakt elke week met militaire precisie een boodschappenlijstje op volgorde van je looproute door de supermarkt. Ik heb het niet van een vreemde… Bedankt dat je er altijd bent, bijvoorbeeld om me naar Schiphol te brengen toen ik naar een congres moest en de planning te strak was om nog de trein te nemen. Bedankt voor alle momenten dat je mijn verhaal aan hebt gehoord en zegt "komt wel goed meissie..". Je zorgt met zoveel liefde voor de mensen om je heen. Ik hoop dat je snel van het pensionado leven kan gaan genieten, je hebt het verdiend. Lieve **Mam**, je bent een inspiratiebron. Je hebt als vrouw met een gezin van vier kinderen te maken gehad met discriminatie in een tijd waarin vooral mannen het voor het zeggen hadden. Ik ben daarom extra trots dat je in tweede instantie alsnog de carrière gemaakt hebt waar je met zoveel passie voor werkt en waar je overduidelijk ALLE capaciteiten voor hebt. Mijn plezier in het werken met mensen en communicatieve kwaliteiten daarin heb ik 100% van jou. Bedankt voor al je moederlijke adviezen. Ook de adviezen waar ik in eerste instantie te eigenwijs voor ben om naar te luisteren. Moeders hebben altijd gelijk…

Lieve **Marike**, bedankt voor alle zorg die je aan mij gegeven hebt, ook al was ik er destijds niet altijd dankbaar voor. Bedankt voor de gezellige kletsmomenten en dat ik altijd welkom ben bij jullie. Lieve **Wout**, ik hoop dat ook jij trots op me bent, ook al zijn die medische artikelen wel heel kort ;-).

Lieve **Yassin**, ik bewonder je dapperheid en kracht. Je verwacht het misschien niet, maar ik heb veel van je geleerd. Je maakt mij een betere dokter. Ik beloof je dat ik altijd mijn werk zal doen met oog voor de mens achter de patiënt, zoals ik weet dat zo ontzettend belangrijk is geweest voor jou.

Lieve **Elise, Marije en Rik**, ik vind het zo'n enorme rijkdom om uit een groot gezin te komen. Het lijkt wel gisteren dat we in Voorschoten boven "avondgym" gingen doen na het eten. Of proberen Elise om te duwen als we spinazie hadden gegeten om te kijken of we al sterker waren geworden… Kijk ons nu: allemaal een mooie carrière in een ander vakgebied en allemaal een fijne thuisbasis. Ook al zien we elkaar soms wat minder, ik ben zo blij dat we elkaar hebben. Bedankt voor jullie gezelligheid, liefde en steun. Ook veel dank aan mijn bonusbroers en zus **Quinten**, **Thomas** en **Kim**.

Mijn lieve nichtjes **Merel, Jasmijn en Luna**, jullie hebben geen idee hoe jullie bijgedragen hebben aan dit proefschrift. Als ik het even niet meer zag zitten, konden die schattige filmpjes en foto's van jullie mij altijd weer vrolijk maken. Jullie zijn zulke leuke meiden!

Dear **Kusum, Harish, Abhi, Julia, Noah and the Weißberger family,** thank you so much for welcoming me into your hearts and homes from day one and for supporting me always, even from a distance. I not only found the most amazing partner, but also the best family in law + bonus family in law. I am not taking it for granted. Thank you so much for accepting me.

Mijn allerliefste **Dhruv**, mijn rots in de branding. Ik bedank jou als laatste, maar vanzelfsprekend komt jou de grootste dank toe. Je hebt mijn leven kleur gegeven, en smaak ;-). Je bent mijn alles. Jij bent degene die mij elke ochtend weer een dikke knuffel gaf, waardoor ik de dag aan kon. Je bent degene die in oplossingen denkt, ook op momenten dat ik alleen maar problemen zie. Je bent degene die mij een zelfgebakken koekje komt brengen omdat ik weer de hele zaterdag aan mijn proefschrift moest. Je hebt zo ontzettend veel geduld gehad in de afgelopen periode en jezelf regelmatig op de tweede plaats gezet. De waardering die ik voor jou voel is niet in woorden uit te drukken. Samen met jou ben ik niet bang, want we kunnen alles aan zolang we elkaar maar hebben. Ik prijs me zo ontzettend gelukkig dat je het leven met mij wil delen en ik kijk vol verwachting uit naar de mooie toekomst die we samen voor ogen hebben. Mai tumse bohot zyada pyaar *kart-HI* hoon!

ABOUT THE AUTHOR



Christine Noordhoek (1995) was born and raised in Voorschoten, the Netherlands. She obtained her high school diploma from Alfrink College in Zoetermeer in 2013 and pursued her medical education at Leiden University. During her bachelor studies, she completed an international internship at the (neuro)radiology department of the University Hospital in Jena, Germany. Her first experience with medical research was at the rheumatology department of the LUMC, where she investigated the improvement of microangiopathy after hematopoietic stem cell transplantation in patients with systemic sclerosis.

Her interest in neurology developed during her clinical internship at the neurology department of Alrijne Hospital in Leiderdorp. She graduated cum laude from her masters in medicine in 2019 and worked for one year as a resident not in training at the neurology department of Alrijne hospital. In 2021, Christine moved to Rotterdam to further her career in neurology at Erasmus Medical Center. After one year as a resident at Erasmus MC, she began her PhD research on neurofibromatosis type 1 under the supervision of Dr. Walter Taal, Prof. Dr. Martin van den Bent, and Dr. Rianne Oostenbrink. In 2026, she started her neurology residency program at Erasmus MC.

Beyond medicine, Christine has played the cello from a young age, taking lessons at, among others, the conservatory in Rotterdam. She has performed with numerous orchestras in the Netherlands and abroad. Combining her two passions, she participated annually in the European Medical Students' Orchestra and Choir (EMSOC) from 2016 to 2024 and served as treasurer of the organizing committee for EMSOC 2018 in the Netherlands. In 2025, she performed with the World Doctors' Orchestra in the Teatro Massimo in Palermo, Sicily.

Christine lives with her fiancé, Dhruv Gulhar, in the city center of Rotterdam.

LIST OF PUBLICATIONS

In this thesis

D. Christine Noordhoek, Lara Orlandini, Rianne Oostenbrink, Rick van Minkelen, Sarah A. van Dijk, Axel Taal, Castor Taal, Walter Taal. Plexiform Neurofibromas and Malignant Peripheral Nerve Sheath Tumors in Adults with Neurofibromatosis Type 1: Clinical Insights from a Large Cohort. *Accepted for publication in Neuro-Oncology Practice*.

D. Christine Noordhoek, Tessa A. Ennik, Sarah A. van Dijk, Eline Mann, Sophie van der Vlist, Judith Drenthen, Walter Taal. High resolution nerve ultrasound in neurofibromatosis type 1: a prospective and descriptive study. Clin Neurophysiol. 2025 Nov:179:2110992.

D. Christine Noordhoek, Koen C. van Tulder, Tessa A. Ennik, Walter Taal, Judith Drenthen. High-Resolution Nerve Ultrasound in Adults with NF1: An Accessible and Reproducible Imaging Tool for Plexiform Neurofibromas. Diagnostics. 2025 Dec 10;15(24):3146.

D. Christine Noordhoek, David F. Hanff, Pim J. French, Sarah A. van Dijk, Erik A.C. Wiemer, Olivier C. Manintveld, Martin J. van den Bent, Walter Taal. Trametinib in Adults with Neurofibromatosis Type 1-Related Symptomatic Plexiform Neurofibromas. Annals of Neurology. (2026) 99: 73-83.

D. Christine Noordhoek, Sheraz A. Ditta, Franny Jongbloed, Esther Oomen-de Hoop, Wouter C. Meijers, Martin J. van den Bent, Monique E.M.M. Bos, Ron H.J. Mathijssen, Walter Taal, Olivier C. Manintveld. Cardiotoxicity of trametinib monotherapy in adults with neurofibromatosis type 1: results from the TRAIN study. Biomedicine and Pharmacotherapy. 2026 May:198:119251.

Other publications

M.Boonstra, J.Ciaffi, **D. Christine Noordhoek**, S.I Liem, L.J. Beaart-Van de Voorde, S.T. van Bijnen, T.W. Huizinga, M.C. Vonk, J.K. de Vries-Bouwstra. Improvement of microangiopathy after haematopoietic stem cell transplantation in systemic sclerosis. Clin Exp Rheumatol. 2022 Oct;40(10):1993-1998.

Walter Taal, Sarah A. van Dijk, **D. Christine Noordhoek**, Martijn Broen, Anja Gijtenbeek, Rianne Oostenbrink. Symptomatische tumoren bij neurofibromatose type 1. Ned Tijdschr Geneeskd. 2023;167:D7864

Britt A.E. Dhaenens, Sarah A. van Dijk, Walter Taal, **D. Christine Noordhoek**, Anna Coffey, Stephen P. McKenna, Rianne Oostenbrink. The PlexiQoL, a patient-reported outcome measure on quality of life in neurofibromatosis type 1-associated plexiform neurofibroma: translation, cultural adaptation and validation into the Dutch language for the Netherlands. J Patient Rep Outcomes. 2024 Mar 18;8(1):33.

PHD PORTFOLIO

1. PhD training	Year	ECTS
Courses		
PhD introduction session	2022	0.2
Biostatistics I	2022	4.5
Biostatistics II	2022	4.5
Intervision for PhD candidates	2023	1.0
Presenting skills for PhD candidates	2023	0.8
Scientific integrity	2023	0.3
Personal leadership & communication	2023	1.0
Biomedical English Writing	2024	2.0
Oral presentations		
Annual meeting of the national NF1 care network (zorgnetwerkdag)	2022	1.0
Neurofibromatosis association (NFVN) patient day	2022, 2024, 2026	1.0
Brain tumor center retreat	2022, 2024	2.0
Pizza Drinks & Science meeting neurology department	2023	0.5
KNF (Clinical neurophysiology) days	2023	1.0
Lecture at clinical meeting of neurology department + revision of local guideline	2023	1.5
CTF conference Brussels + Young investigators day	2024	1.0
Lecture at NF1 young ambassador's academy	2024	0.5
EANO meeting Glasgow	2024	1.0
Poster presentations		
Poster at European NF conference Manchester	2022	0.5
Winner of poster prize at national neuro-oncology (LWNO) meeting	2023	0.5
Two posters at EANO Rotterdam	2023	1.0
Poster at CTF conference Brussels	2024	0.5
Attended conferences/meetings (without presentation)		
Online CTF conference Philadelphia	2022	0.2
Annual meeting of the national NF1 care network (zorgnetwerkdag)	2023, 2024	0.4
Neurofibromatosis association (NFVN) patient day	2023	0.2

2. Teaching	Year	ECTS
Teaching neurological exam to medicine students	2022-2025	0.2
Supervising master student thesis	2022	2.0
Supervising master student thesis	2022	2.0
Supervising master student thesis	2023	2.0
Emergency department introduction for new neurology residents	2022-2024	0.2
		ECTS
Total		33.5