Management of neurofibromatosis type 1 associated tumors of central and peripheral nervous system

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Purpose of review
In recent years emerging evidence suggests that some tumor types, extremely rare in general population and understudied, can be observed in NF1 and neoplasms related with this condition harbor peculiar genetic and epigenetic features. The aim of this review is to summarize recent advances that, delving into the tumor complexity, have identified new diagnostic tools and potential tumor subtype that may have been associated with clinical implications.

Recent findings
The available data confirmed the presence of peculiar molecular signatures in those tumors, different from those observed in sporadic neoplasms and suggest that a specific reference to NF1 associated neoplasms would deserve to be mentioned in tumor WHO classification. Comprehensive multiomic analysis shows that the histologic assessment does not always match the methylation group assignment and facilitates tumor subclassification into categories predictive of clinical behavior. The non-invasive assessment of tumor genetic profiles by the analysis of plasma ctDNA is representative of tumor features, may help differential diagnosis and may identify malignant transformation, sparing the patient from repeated biopsies.

Summary
A better knowledge of NF1 associated tumors at the molecular level may suggest changes in the clinical management of the disease and open new frontiers of personalized treatment.

Keywords
high-grade glioma, liquid biopsy, low grade astrocytoma with piloid features, MEK inhibitors, MPNST, neurofibromatosis type 1

INTRODUCTION

Neurofibromatosis type 1 (NF1) is known as a tumor predisposition syndrome caused by heterozygous loss-of-function mutations in the *NF1* gene that encodes neurofibromin and inhibits the RAS-MAPK (mitogen-activated protein Kinase) pathway [1]. The main clinical features are café-au-lait spots, iris Lisch nodules, axillary and inguinal freckles, and multiple neurofibromas, but the hallmark of this condition is the development of neoplasms in the peripheral and central nervous system. NF1 related neoplasms include wide spectrum of histology ranging from benign tumor or premalignant lesion to aggressive malignant tumors. The biallelic inactivation of the *NF1* gene is essential for the development of NF1 tumors, however, the understanding of additional genetic and epigenetic factors driving tumor genesis and neoplasm malignant transformation should be expanded.

In recent years emerging evidence suggests that those tumors show different genetic and epigenetic features from those observed in the corresponding sporadic histologic type [2–5]. However, due to the sample sizes and limited NGS performed, those findings need to be confirmed.

Not only can some neoplasms, very rare in the general population, such as subependymal giant cell astrocytoma be observed in NF1 but, through the

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KEY POINTS

- Gliomas and MPNSTs in NF1 have a distinct genetic signature, different from those observed in sporadic neoplasms.
- A specific reference to NF1 associated neoplasms might be mentioned in WHO Classification of tumors of the Central Nervous system.
- Comprehensive multiomic analysis of NF1 related neoplasms allow a more accurate tumor classification into categories predictive of clinical course.
- Analysis of plasma ctDNA is a promising non-invasive tool to assess the tumor molecular genetic profile.

use of a multiomic analysis, new potential epigenetic tumor subtypes, with associated clinical implications, have also recently been identified.

This review does not consider all NF1 related tumors. It is restricted to those with a major impact on morbidity and mortality. In particular, we will discuss comprehensive genomic studies that suggested changes in the clinical management of the disease and opened new frontiers of personalized treatment, identifying new tumor subtypes or innovative diagnostic tools.

NON-OPTIC PATHWAY GLIOMAS

Gliomas of the central nervous system occur in about 20% of patients affected by NF1 [1]. Optic pathway gliomas (OPG) is a child cancer [6], at diagnosis it is symptomless in about 50–75% patients [7], its natural history is more benign than that of sporadic gliomas, only a minority of patients require treatment [8]. Instead non-optic gliomas (non-OPG) are more frequently reported later in life with a wide range of histology and behavior [1]. The new ''WHO Classification of tumors of the Central Nervous system'', published in 2021 includes molecular evaluation into the diagnostic criteria across the histologic subtype of gliomas. In particular the presence of IDH1/2 mutations to determine glioma grade and to subdivide glioblastoma from astrocytoma [9]. However, all the samples of NF1 associated gliomas, tested for IDH1/2 until now, were wild type regardless of grade or histologic subtype [2] and the recently updated WHO classification does not specifically reference NF1 associated tumors [9]. Therefore, data on genetic alterations and tumor behavior are needed to validate previously described molecular features and update tumor classification as well as identifying potential tumor subtypes that may have associated clinical implications.

The clinical, radiological histologic and molecular features of forty-five adults NF1 affected with a median of age 37 (18–68 years) and performance status of 80% (30–100) were retrospectively evaluated [10▪]. Tissue was available for 35 patients. Diagnoses included infiltrating (low-grade) astrocytoma (9), glioblastoma (7), high-grade astrocytoma with piloid features (HGAP) (4), pilocytic astrocytoma (PA) (4), high-grade astrocytoma (3), WHO diagnosis not reached (4) and one each of gliosarcoma, ganglioglioma, embryonal tumor, and diffuse midline glioma. Thirty-two people (71%) had neoplasms involving midline structures, and underwent only biopsy. All the gliomas analyzed (27/45) for IDH1/2 status were wild type. In the 10 cases with molecular testing, the most common genetic variants were in *NF1, EGFR, ATRX, CDKN2A/B, TP53, TERT*, and *MSH2/3* genes as previously mentioned [2]. The median overall survival (OS) was 24 months (2–267 months) in all cases and 38.5 (18–109) months in individuals with low grade gliomas: a poor result considering the young age in the majority of cases (median age of 32 years), good performance status (median KPS 80), and the various treatments provided. Due to the short survival in low grade gliomas (LGG), no statistically significant difference in OS was observed among the different histologic subtypes.

Conversely, an excellent OS of subjects was observed (98% cases still alive after a median follow-up of 3.9 year*)* in a pediatric cohort of 70 non-OPG low grade gliomas NF1 cases under the age of 19 years (median age 9.5 yrs., range 1.9–18.9) with clinical data only in 48 individuals [11▪▪]. The majority of tumors were PA. Notably, the histologic assessment did not always match the methylation group assignment: 88% of the tumors were classified as PA by methylation, while 64% based on histology. Non-PA tumors (18.4% of samples based on DNA methylation analysis) arose often outside the optic pathway or hypothalamus, such as cortex, cerebellum, brainstem and resulted significantly more likely to harbor an additional non-NF1 mutation considered to be ''glioma relevant and thus a possible co-driven event. The most common secondary alteration was *FGFR1* mutation which conferred an additional growth advantage in multiple complementary experimental murine Nf1 models. Other pathogenic variants were also detected in P*IK3CA*, and *SETD2* genes and the *MYB: QKI* fusion was also observed. In light of those results, the current indication of biopsy and pathological confirmation, not foreseen in NF1 LGG [12], could be to reconsider, in particular for tumors refractory to conventional treatment.

A distinct molecular subgroup of NF1-associated gliomas with additional oncogenic variants including *CDKN2A* homozygous deletion and *ATRX* mutation, was described in a cohort of 47 LGG and high grade glioma developed in children and adults NF1 patients, using next-generation sequencing, copy number analysis, and DNA methylation profiling [13▪]. When additional mutations were present, the gliomas more likely occurred during adulthood, had a more aggressive clinical course, and included HGAP or various subclasses of IDH-wild type glioblastoma. The other subgroup harbored bi-allelic *NF1* inactivation only, occurred primarily during childhood, followed a more indolent clinical course, included pilocytic astrocytoma diffuse astrocytoma or ganglioglioma located at different anatomic sites, but at DNA methylation profiling in the majority aligned with a novel methylation subclass of NF1-associated pilocytic astrocytomas.

HGAP is a recently reported new tumor entity that can be diagnosed only by DNA methylation profiling, that can develop throughout the neuroaxis, but often occurs within the posterior fossa [14]. From a histopathologic point of view it shows lower-grade or higher-grade features, with characteristics overlapping those of pilocytic astrocytoma and glioblastoma. Its epigenetic profile is unique, while alterations in the MAPK pathway, in combination with homozygous deletion of *CDKN2A/B* and/or *ATRX* mutations are frequent but not specific [15]. Recently a high proportion of HGAP has been reported in cohort of NF1 associated gliomas [10▪,13▪]. Cimino evaluated an expanded series of sporadic and NF1 associated HGAP: DNA methylation profiling and clustering analysis showed the presence of three distinct HGAP subtypes (or groups = g) which were called as gNF1, g1, and g2 [16▪]. Subtype gNF1 is notable for enrichment with NF1 patients (33.3%, 6 out of 18 cases) increased *ATRX* alteration, increased methylation in the *NF1* enhancer region, evidence of RNA processing dysregulation, increased non-neoplastic glia and neuron cell content, and is confined to the posterior fossa with trends towards worse patient outcome [16▪].

PLEXIFORM AND ATYPICAL NEUROFIBROMAS

Plexiform neurofibromas (PNFs) together with cutaneous neurofibromas are the most common tumor types in NF1 patients. Histologically, all subtypes of neurofibromas are classified as benign (WHO grade 1) tumors, except atypical neuro fibromatous neoplasm of uncertain biological potential (ANNUBP) which has not yet been assigned a grade. PNFs, present in about half of NF1 patients, are considered congenital, even if they cannot be apparent at birth. Their macroscopic appearance is characterized by multinodular/multifascicular nerve expansions described as ''a bag of worms''. Unfortunately, at present, there is not a unique PNF definition among experts, but a new classification, MRI based, was recently proposed [17▪].

PNFs were classified according to morphology or internal structure, depth, and relationship to adjacent tissues and subdivided in fascicular/multi-nodular (consisting of a collection of smaller components that are tubular or spherical or both), superficial (tumor arising superficial to the muscle fascia and/or tumor with cutaneous/subcutaneous involvement), circumscribed (lesions with smoothly defined borders which may compress and/or displace adjacent structures

This classification system is not fully applicable to some tumors, such as paraspinal PNF, (which can extend from the spinal nerve root causing diffuse nerve thickening) and distinct nodular lesion (DNL) a term recently introduced in literature to describe well demarcated lesions, appearing encapsulated, large $\geq$3 cm, without the central target sign characteristic of classic PNF peripheral nerve, arising within or outside of a PNF [17▪]. The same article provides a comprehensive review on diagnostic evaluation, surveillance strategy, and treatment indications in those tumors [17▪].

A biallelic genetic inactivation of *NF1* gene in the schwann cell population is the only somatic recurrent event detectable. At present, there is no clear molecular understanding of PNF malignant transformation. PNF may become atypical neurofibromas (ANF), atypical neurofibromatous neoplasm of uncertain biological potential (ANNUBP), or malignant peripheral nerve sheath tumor (MPNST). ANFs, distinct from both PNF and MPNST, exhibit one or more histological atypical features, but do not fulfill the criteria to be classified as an MPNST, lacking brisk mitotic activity or necrosis (18). ANNUBPs are characterized by at least two of the following features: cytological atypia, hypercellularity, loss of neurofibroma architecture, and an increased mitotic index [17▪,18]. Along with biallelic NF1 inactivation, a recurrent loss of the CDKN2A/B locus at 9p21.3 has been identified both in ANFs and ANNUBPs [19].

ANFs and ANNUBPs are hypothesized to be premalignant lesions with an higher risk of progression to MPNST than to classic PNFs [20] although the true incidence of malignant transformation is still undetermined. To date, complete surgical resection is the only treatment suggested to avoid malignant transformation. A phase 1/2 clinical trial on Abemaciclib, a cyclin-dependent kinase 4 and 6 inhibitor is ongoing to investigate tolerability and

tumor response rate in patients with NF1 associate ANFs (NCT04750928).

The approval of the MEK inhibitor selumetinib by FDA and EMA for the treatment of symptomatic, inoperable PNF in children ≥ 3years has changed the clinical management of those tumors in childhood, even if some questions such as the most appropriate time to start the treatment, a univocal definition of PNF progression, the duration and durability of the treatment should be assessed. A randomized, double-blind, placebo controlled, phase III study is ongoing to assess the efficacy and safety of selumetinib compared with placebo in adults with symptomatic, inoperable PNFs. Recent studies showed an objective partial response and a reduction of pain both in adults and children with PNFs using other MEK inhibitor such as mirdametinib [21] and binimetinib (NCT03231306) [17▪]. Furthermore, also cabozantinib, a small tyrosine kinase inhibitor of c-Kit, VEGFR2, MET, RET, FLT3, and the TAM family receptors, proved efficacy in a recent phase 2 trial for adolescents and adults (NCT02101736).

MALIGNANT PERIPHERAL NERVE SHEATH TUMOR (MPNST)

NF1 patients have an 8–16% lifetime risk of developing a MPNST, a highly aggressive soft-tissue sarcoma [22]. They often arise from preexisting PNFs and atypical neurofibromas ANFs [19]. Therefore, the monitoring of plexiform is crucial in the clinical management of NF1 patients. Pain or rapid growth should raise suspicion of malignant transformation. However, from a clinical point of view it is not easy to differentiate PNF, ANF and MPNST because the majority of clinical symptoms is similar. MRI and FDG-PET in particular when using a SUV cutoff on 3.5 can be useful in the diagnosis, but specificity is not so high [23,24] and the use of radionuclides is needed. Liquid biopsy, a non-invasive assessment of tumor genetic profiles by an analysis of tumor-derived nucleic acids, constitutes a promising tool to identify malignant transformation of PNFs. On plasma circulating tumor DNA (ctDNA) aneuploidy and sub-chromosomal copy number alterations analysis (CNAs), as well as mutation analysis were performed in 28 NF1 patients with neurofibromas (7 benign NF, 9 PNF,12 MPNST) and in 883 healthy controls to distinguish benign from malignant tumors [25▪▪]. Overall sensitivity for detecting MPNST using genome wide aneuploidy scoring was 33%, and the analysis of CNAs improved sensitivity to 50%, while retaining a high specificity of 97%. Furthermore, in a subset of patients, mutations in *NF1, NF2, RB1, TP53BP2*, and *GOLGA2* genes on plasma cell free (cfDNA) were detected.

A similar result was obtained by Szymanski [26] using fragment size analysis and ultra-low-pass whole genome sequencing. The Authors observed that plasma cfDNA from NF1 patients with MPNST (14) harbors a shorter fragmentation profile compared to NF1 patients with PN (23) or healthy donors (16) [26]. Using sequencing reads from this fragmentation profile, they quantified genome-wide copy number alterations (CNAs) in cfDNA and used CNAs to estimate the fraction of plasma cfDNA originating from tumor.

Tumor fraction in plasma cfDNA distinguished not yet treated MPSNT from PNF with 86% accuracy. Plasma cfDNA from MPNST and PNF subjects retained focal copy number loss of *NF1* not found in healthy controls. Furthermore, MPNST patient cfDNA also showed significantly greater tumor genomic instability compared to PNF, with CNAs in key genomic loci previously observed in MPNST tissue (i.e. focal copy number losses in *SUZ12, SMARCA2, CDKN2A/B*), which allowed sensitive and specific liquid biopsy discrimination of MPNST from PNF.

Specific somatic copy-number aberrations (SCNA) in cfDNA was also used to predict prognosis in two novel subtypes of MPNST with different clinical outcome [27▪▪].

Multiomic analysis, including whole-genome sequencing (WGS), whole-transcriptome sequencing RNA sequencing (RNA-seq), and whole-genome DNA methylation arrays, coupled with multiregional deep exome sequencing of 95 samples of 90 MPNST (61 NF1 associated and 29 sporadic) with various degree of malignancy (72 high-grade MPNSTs, 6 low-grade MPNSTs, 3 ANNUBPs, 2 additional tumors probably NF and 7 cases in which the diagnosis could not be reviewed) showed that after biallelic inactivation of *NF1*, loss of *CDKN2A* or *TP53* with or without inactivation of polycomb repressive complex 2 (PRC2) conducts to extensive SCNA. At this stage two different subtypes of MPNST can be identified. MPNSTs with H3K27me3 loss evolve through extensive chromosomal losses followed by whole genome doubling and chromosome 8 amplification and show lower levels of immune cell infiltration. MPNSTs without H3K27me3 are characterized by extensive genomic instability and by abundant immune cell infiltration [27▪▪]. cfDNA, obtained from a separate cohort of patients (n = 14) with NF1 associated MPNST at different disease staging and analyzed by ultra-low-pass WGS, contained the copy number patterns associated with H3K27 loss [27▪▪]. Based on those results, it was possible to predict patient survival.

Multiomic studies suggest that epigenetic dysregulation is a defining feature of MPNSTs [27▪▪,28▪▪].

Based on transcriptional networks two distinct subgroups of MPNST have been proposed also by Suppiah [28▪▪] using multiomic analysis including WGS, whole-transcriptome sequencing RNA-seq, single-cell RNA sequencing, and whole-genome DNA methylation. The comprehensive analysis of 108 samples of sporadic or associated tumors including 19 MPNSTs, 22 premalignant neurofibromas, 34 PNFs and 33 cutaneous neurofibromas revealed the presence of two subgroup based on transcriptional networks: sonic hedgehog SHH pathway activation in MPNST-G1 and WNT/ß-catenin/CCND1 pathway activation in MPNST-G2. In both subgroups about half of the patients were affected by NF1. MPNST-G1 and MPNST-G2, have a distinct outcome. The PFS of patients with MPNST-G2 was more than double compared to that observed inMPNSTs-G1 [28▪▪].

CONCLUSION

More recently published studies confirmed the observation that the genetic and epigenetic signature of glioma and MPNST NF1 related of all ages is different from that observed in the sporadic matching tumors.

Since the tumor histologic assessment did not always match the methylation group assignment and some rare tumors can be diagnosed only by methylation analysis i.e. HGAP, studies using comprehensive multiomic analysis are needed in this field. This kind of investigation not only allows us to identify additional relevant non-*NF1* mutations i.e. *FGFR1* conferring growth advantage in animal models, but also opens new frontiers of personalized treatment. Analysis of plasma ctDNA is a promising non- invasive tool to assess the tumor molecular genetic profile. It might help the differential diagnosis and the identification of the malignant transformation, sparing the patient from repeated biopsies.

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Conflicts of interest

There are no conflicts of interest.

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