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Review

# Molecular targets for NF1-associated malignant peripheral nerve sheath tumor

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ABSTRACT

Malignant Peripheral Nerve Sheath Tumor (MPNST) is a soft-tissue neurosarcoma. It can occur sporadically, after radiotherapy or in patients with Neurofibromatosis 1 (NF1). The hereditary disorder, NF1, is a common cancer predisposition syndrome. The main genetic feature is the mutation of the NF1 tumor suppressor gene that is inherited in an autosomal dominant, progressive manner. Mutations of the NF1 gene increase the activity of Ras signaling and cause the development of different types of tumors, including subcutaneous and plexiform neurofibromas. These can have further mutations that mediate the transformation into MPNST. Somatic mutations that have been observed are the loss of cell cycle regulators of the CDKN2A gene, and the inactivation of Polycomb Repressive Complex 2 (PRC2), mainly embryonic ectoderm development (EED) or suppressor of zeste 12 homologue (SUZ12). Other molecular pathways that have been targeted for treatment are dual MAPK-mTOR targeting, p53 protein, and MEK-ERK pathway. To advance the therapies focused on delaying or inhibiting malignant tumor formation in NF1, we need to understand the implications of the molecular and genetic pathway that are involved in the transformation into MPNST.



## 1. Introduction

Neurofibromatosis (NF) is known as an autosomal dominant inherited, progressive disorder with signs and symptoms that are different from one person to the other due to the variance in expression.[1,2] It occurs in patients with either a positive family history of the disease or due to spontaneous mutations. Such spontaneous mutations account for 30-50% of de novo with no NF background.[3,4] Originally, NF was divided into two different types in regards to phenotypes and clinical manifestations, Neurofibromatosis 1 (NF1) and Neurofibromatosis 2 (NF2). NF1 was formerly termed von Recklinghausen disease, named after a German pathologist in 1881.[5] In the 1981, NF2 was established as a separate disease by Riccardi.[1] The gene on chromosome 17q11.2 and linked mutations of NF1 were recognized in 1990,[6] while for NF2 mutation in gene *NF2* located on chromosome 22q12.2 was identified in 1993.[4] The third type of neurofibromatosis is called Schwannomatosis and was found to be distinct from neurofibromatosis type 2 in the 1990s. Schwannomatosis is differentiated by the lack of vestibular tumors that are found in NF2. Unlike NF1 and NF2, Schwannomatosis did not have full penetrance.[7] NF1 type is the most common with birth incidence of 1 in 2700 individuals. Comparatively, NF2 has an incidence of 1 in 33,000 individuals.[8] Schwannomatosis has an incidence of 1 in 40,000 individuals and represents the rarest type with regard to familial occurrence.[9] Due to the difference in phenotypes and pathogenicity between NF1 and the other NF types, there are potential clinical targets for designing treatment therapies for each type, with the primary focus on NF1 representing the most common type (Fig. 1).

## 2. Neurofibromatosis 1

In 1881, von Recklinghausen observed benign tumors that were originating from the peripheral nerve sheath, using the term neurofibroma for their depiction.[5] NF1 is classified as a tumor-predisposing syndrome with high penetrance and a change in the severity of NF1 patients.[10] NF1 is caused by the loss of neurofibromin, the protein encoding the *NF1* gene. This protein is abundantly located within the cytoplasm of the nonmyelinating schwann cell, neurons, and oligodendrocytes.[11,12] One of NF1′s functions is inhibiting the Ras pathway, resulting in regulation by inhibition of cell proliferation and survival.[13] NF1 clinical manifestations primarily include: multiple café-au- lait spots (6 or>0.5 cm in children, >1.5 cm in adults), multiple skinfold freckles, two or more Lisch nodules of the iris, bone abnormalities, and first-generation relative with NF1.[1,3] One of the common tumors found

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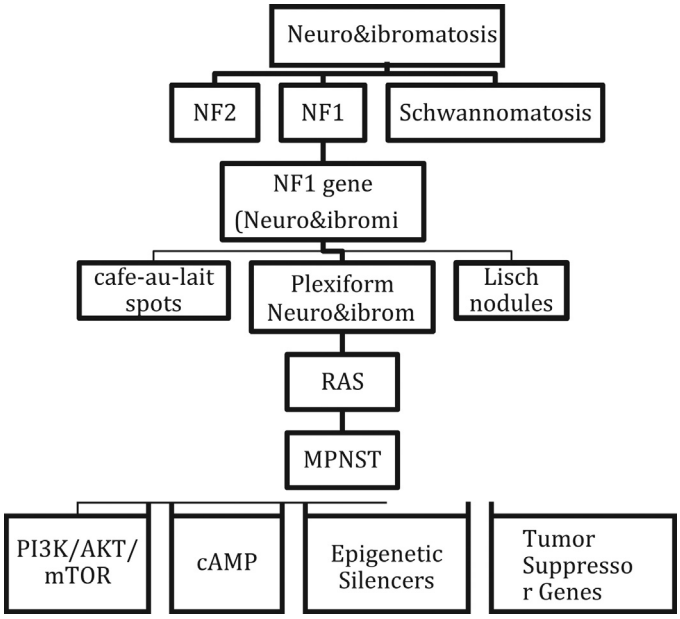


**Fig. 1.** A schematic diagram of the NF1 gene-related symptoms and its signaling cascade.

in NF1 patients are optic pathway glioma in children and can rarely arise in adults.[14] The other common tumors in NF1 are neurofibromas and they were classified as either multiple discrete neurofibromas (NF) or one plexiform neurofibroma (PN).[3] Later in 2017, the National Institute of Health (NIH) proposed three additional classifications of tumors related to NF1. Neurofibroma with atypia, defined as "scattered bizarre nuclei" without the other three alarming histological features (i.e., increased cellularity, damaged neurofibroma architecture, or mitotic activity). With cellular neurofibroma, the only concern is the increased cellularity. Thirdly, atypical neurofibromatous neoplasms of uncertain biologic potential (ANNUBP) are classified by having two of the four alarming features, which marks it as having the highest risk of developing malignancy. Adding the previous classification for MPNST to the list, which are low-grade and high- grade MPNST. The low-grade MPNST has ANNUBP features and mitotic activity without necrosis. Lastly, the high-grade MPNST has the histological feature of necrosis. That comes to a total of seven classifications for NF1-related tumors.[15]

## 3. Malignant peripheral nerve sheath tumor in NF1 patients

MPNST is an aggressive soft-tissue tumor, which originates from peripheral nerves and differentiates into schwann cells, perineurial cells, and fibroblasts.[16] Two population-based studies presented NF1 patients between the ages of 20 and 50 years old have an 8 to 16% lifetime risk of developing MPNST.[17,18] The presence of atypical neurofibroma (ANF) increases the incidence of MPNST progression.[19] In some cases, a malignancy developed in NF1 from deep locations instead of surface plexiform neurofibromas.[17] The embryonic origin would mainly be from the mesoderm, while rarely resulting from neural ectoderm source. Patients diagnosed with a soft-tissue malignant sarcoma have a mortality rate of about 50%.[20] NF1 patients are usually presented with multiple neurofibromas, which increases the risk of NF1 turning into MPNST.[21] As seen in a cohort study by Higham et al., having one ANF will increase the potential of other ANF to emerge, and 15 of 63 patients have more than one ANF (24%).[19] Unfortunately, the 5-year and 10-year disease- specific survival (DSS) are 60% and 45%, respectively for NF1-associated MPNST. In such cases, their outcome is worse than it is for patients with non-NF1-associated MPNST. This is due to the presence of multiple neurofibromas in association with NF1 and difficulty identifying malignant transformation in these tumors.[22]

The World Health Organization (WHO) grading for MPNST is II-IV classified into glandular, spindle, mesenchymal, or rhabdomyoblastic differentiation patterns.[23] Along with the use of magnetic resonance imaging (MRI) for diagnosis, the noninvasive imaging technique 18 F-fluorodeoxyglucose Positron emission tomography (18 F-FDG PET) is used to distinguish between the benign nature of neurofibromas and MPNST. By observing the increase in metabolism of glucose in cancer cells, 18 F-FDG PET shows the proportional correlation in the proliferation and metastatic nature of the MPNST sarcomas.[24] With that in mind, the usage of 18 F-FDG PET is still with constraints and needs further testing for validation of outcomes.[25] The S-100 protein is used as an immunohistochemical marker with a restrained diagnostic efficacy. Another diagnosis marker is Sox10, which is a neural crest transcription factor that is more specific than S-100 protein to neural crest-derived tumors.[26] Although these markers were abundant in neurofibromas, they were found to be deficient or non-existent in MPNST.[15] MPNST has also been termed as neurofibrosarcoma and malignant schwannoma. It develops from a nerve as a de novo, after radiotherapy, or from a preceding neurofibroma, as seen in NF1 patients. It is concurrent with a poor prognosis, with chemotherapy or radiation alone being recognized as an ineffective treatment for MPNST patients. The radiation therapy has a consequential latent MPNST manifestation when used for NF1- associated sarcomas.[27] In addition to the development of secondary sarcomas, radiotherapy has been associated with cardiotoxic effects.[28,29] New approaches in radiotherapy have been emerging as neoadjuvant therapy. The precision of the adaptive radiotherapy technique in soft tissue sarcoma can be used to minimize unwanted side effects.[30] Accordingly, the combination of surgical and radiation therapy, with regard to the age of the patient shows improvement in survival, but having no effect on preventing relapse. Surgery and chemotherapy are the choice of treatment for younger neurofibroma-diagnosed NF1 patients.

Particularly, since recent studies have revealed that inclusion of radiotherapy could promote the development of MPNST further down the line.[22] Therefore, the only approach would be surgical removal of the tumor or amputation. The early detection and operative approach on small tumors increased the survival in comparison to surgically removed large tumors.[19,27] The necessity of surgical removal is presented when the tumor causes painful or problematic lesions.[15,19] The placement of tumors is predominantly in the extremities, more precisely in proximal nerves. Other tumor areas include the head and neck, or within the abdomen. The tumor size in NF1- associated MPNST was commonly larger than 5 cm compared with sporadic MPNSTs.[22,27]

## 4. Molecular pathways as potential targets for pharmaceutical therapies

### 4.1. *NF1 gene*

There are studies proposing novel 'two-hit' hypothesis indicating that for development of MPNST, specific steps in a molecular mechanism have to occur.[31] The first hit occurs when germline heterozygous inactivation of the *NF1* gene causes the formation of nerofibromas.[12] Followed by the second hit, which is the somatic inactivation of the second allele inherited from the other parent.[32] Nonetheless, biallelic *NF1(-/-)* inactivation is inadequate to establish malignancy. Along with *NF1* gene, CDKN2A/B deletion is essential for premalignant ANF formation.[33]

Pemov et al. dictated in their work that the formation of ANF is obtained by the deletion of CDKN2A/B.[34] Then, there is

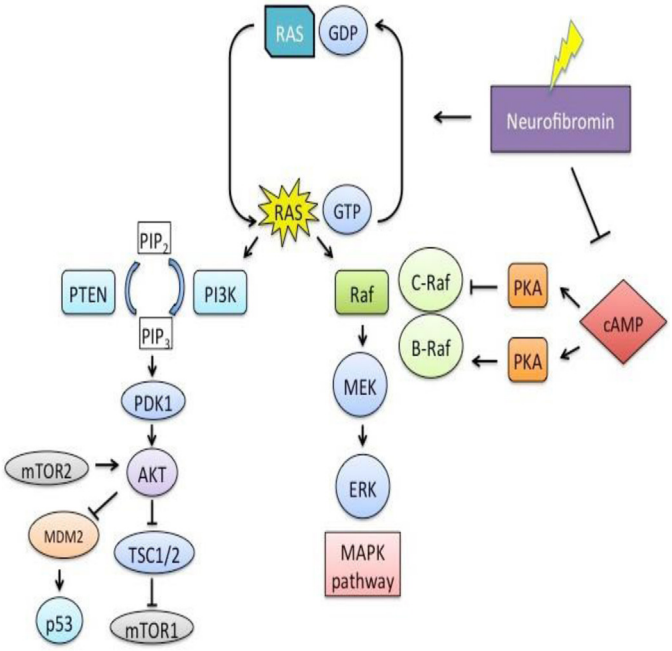


**Fig. 2.** The role of Neurofibromin in RAS pathway and the crosstalk between RAS and PKA-cAMP pathways and their effect on cell proliferation and growth.

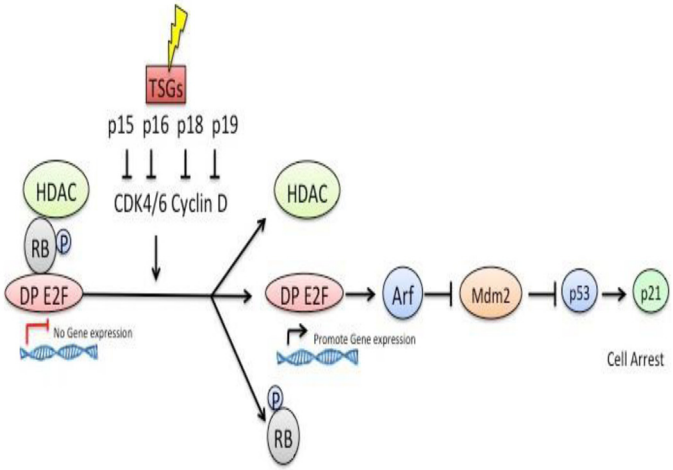


**Fig. 3.** The role of Tumor Suppressor Genes (TSGs) in cell cycle progression.

the third hit that is necessary to trigger MPNST formation; this includes the genetic silencers, for example the suppressor of zeste 12 homologue (SUZ12) mutation in addition to many genetic aberrations.[34,35]

Neurofibromin regulates cell proliferation, differentiation and survival by working as a GTPase-Activating Protein (GAP) through negatively regulating the Ras pathway (Fig. 2). Heterozygous inactivating mutations of the tumor suppressor *NF1* gene suppresses the production of neurofibromin and further increases the activity of the Ras signaling pathway.[13] This observation has led to the finding that the loss of the function of neurofibromin disrupts the MAPK pathway and PI3K/AKT/mTOR molecular pathways further increasing the probability of cellular tumorigenicity.[36] A recent study illustrated the non-growth-factor-related activation of the mTOR pathway due to *NF1* loss-of-function through Ras/phosphatidylinositol 3-kinase (PI3K) pathway. The activation of PI3K pathway enables the inactivation of the tuberin, a protein encoded by *Tuberous Sclerosis Complex 2* (*TSC2*) gene, through the AKT pathway.[37] Studies were aiming to down-regulate mTOR as MPNST- targeted therapy because of its increased activation compared to neurofibromas. Based on these studies rapamycin or its analogue has been considered in NF1 patients.[37,38] Yet the drawback of inhibition of the mTOR pathway is that it does not inhibit the mTORC2 complex, causing the activation of the AKT pathway further demonstrating a reverse effect in the tumor suppression.[39] Both Zou et al. and Johansson et al. in two independent studies presented that combined AKT phosphorylation inhibitor with rapamycin, or its analogue, offset this feedback loop, displaying improved survival in human MPNST cell line.[38,40] In Dombi et al. the use of a MAPK inhibitor selumetinib, an oral selective inhibitor of MEK 1 and 2, resulted in partial responses of chemotherapy with more than or equal to 20% decrease in the baseline of tumor volume in 71% of children with plexiform neurofibromas.[41] Further studies combined the mTOR inhibitor with the MEK inhibitor of MAPK pathway, which resulted in the increase of survival rate for patients with Schwann cell tumors, while there was resistance to the treatment with single drug.[42]

Other functional pathways for neurofibromin would be the inhibition of cAMP due to the loss of the *NF1* gene, resulting in the activation of the cAMP pathway activating MAPK, which results in the transcription of cyclin D1 further activating CDK4 and phosphorylating pRb, resulting in cell cycle progression.[43] However, studies have shown that the loss of neurofibromin lowers the activity of cAMP through the Pituitary Adenylate Cyclase-activating Polypeptide (PACAP) pathway in homozygous loss of *NF1* compared to heterozygous mouse.[44] Opposite to other types of cells, where cAMP inhibits the mitogenic Ras pathway, Schwann cells cAMP has a co-mitogenic effect by interacting with growth factors. For example, this includes insulin-like growth factor (IGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and Reg-1. Otherwise, it can occur with an increase in the expression of growth factor receptors.[43] The explanation for the divergent cAMP behavior is the existence of different Raf isoforms that are affected by cAMP. There are three mammalian Raf isoforms: A-Raf, B-Raf and c-Raf (other name for c-Raf is Raf-1).[45] These isoform exert different effect on the downstream MAPK pathway. This difference in the effect is dependent on cell type, upstream effectors and expression pattern of each Raf isoform.[46] Accordingly, B-Raf is abundant in neuronal cells and is activated by the elevation of cAMP potentiating gene expression and cell differentiation.[47] Furthermore, Ras-dependent activation of c-Raf is inhibited after the increase of cAMP in a PKA-independent pathway in many types of cells.[48] A-Raf is the third isoform, which is less expressed in variant cells and is not activated by cAMP as c-Raf.[49] In a study aimed to inhibit MPNST growth by using sorafenib, a B-Raf kinase inhibitor was used in MPNST human cell lines, which exhibited downstream inhibition of the MAPK pathway and G1 cell arrest.[50]

### 4.2. Tumor suppressor genes (TSGs)

*The p14 ARF, p19 ARF, p15 INK4B p16 INK4A, p21*, and *p27* are some of the genes responsible for the regulation of the cell cycle at the G1 checkpoint and contribute to cancer development if they are disrupted.[51] Cyclin-dependent kinase inhibitor 2A (CDKN2A) gene encodes both p16INK4A and p19ARF. The CDK inhibitor p16 works by binding to Cdk4 while preventing the formation of the Cdk4/Cyclin D1 complex. Therefore, it regulates the retinoblastoma protein (pRb) and causes inhibition of transcription by E2F (Fig. 3). This leads to mediating cell arrest. The function of p19 ARF is binding to Mdm2 to release and regulate p53 by averting its degradation. Many studies linked the development of tumors to CDKN2A and ARF gene aberrations.[52] In a histological-molecular association study done by Carrió et al., by applying the NIH classification indi-

cated that the formation of a premalignant feature (atypia) was due to the deletion of CDKN2A/B locus.[33] The *p53* gene deletion occurs either in sporadic cases, as shown in ref.[53] or only at a later stage, in high-grade MPNSTs.[54] The INK4A gene was found mutated in 60% to 75% of MPNST patients in general.[52,55] While in NF1-associated MPNSTs, the mutations were 50% in the INK4A gene. Immunohistochemical studies were done for p16 protein in NF1 associated neurofibromas and MPNSTs. Staining for p16 was lost in MPNSTs, whereas in neurofibromas, the expression of p16 showed strong reactivity.[56] Methylation of 5′ CpG in *p16* would lead to tumor formation.[57] Methylation-specific PCR results showed that methylation of p14 and p16 occurs in 18% of patients only, while there was no methylation detected in p15.[58] Mutation analysis of microarray-based Comparative Genome Hybridization (aCGH) studies detected the CDKN2A deletion in MPNST samples, the leading hallmark for malignant transformation. Moreover, they observed CDKN2B deletion with CDKN2A in MPNSTs, which is a cell growth inhibitor encoding p15INK4B that also regulate the Rb pathway.[53] Fluorescent In Situ Hybridization (FISH) analysis and duplex-PCR study concluded that homozygous deletion (46%) and loss of expression (80%) in 9p21 gene cluster would result in the inactivation of both *TP53* and *Rb* pathways in MPNSTs.[58] Thus, in both sporadic and NF1-associated MPNSTs the disturbance of *TP53* and *Rb* pathways aid in the MPNST advancement.[55] In the absence of these TSGs, the Cdks and cyclins will be amplified.[59] To further investigate these findings, a study was conducted using CDK4/6 inhibitor, known as palbociclib, in sarcomas. It works by binding to ATP binding pocket in CDK4/6 and then causing inhibition of CDK4/6 Cyclin D complex formation causing cell arrest. The MPNST with low p16INK4A levels tested in this study showed a senescence response in the tumor after the addition of palbociclib.[60] The expression of p21 was influenced by p53, and growth factor signaling pathways. This could explain the overexpression observed in neurofibromas compared to the expression in MPNSTs that was marginally low. On the other hand, an immunoreactivity of p27 genetic phenotype study observed a drastic decrease in MPNSTs (9%) where the expression in neurofibromas was high (94%).[61] A further study demonstrated the p27 loss only in high-grade MPNSTs.[54]

### 4.3. Epigenetic gene silencers

#### 4.3.1. Polycomb (PcG) proteins

PcG proteins are genetic silencers that work as repressors of transcription. Polycomb repressive complex 2 (PRC2) modifies chromatin structure by tri-methylation of H3K27me2/3 and changes gene activity. This protein is crucial during differentiation, proliferation, and tumor development.[62] PRC2 is composed of an enhancer, zeste homologue 1 and 2 (EZH1, EZH2), embryonic ectoderm development (EED), SUZ12 subunits. PRC2 acts as tumor suppressors through the stimulation of H3K27 by catalyzing the trimethylation of lysine 27 of histone H3 and helping the binding of PRC1 to chromatin.[62,63] In recent studies on MPNSTs, with whole-genome sequencing they observed mutations in two PRC2 components, EED and SUZ12.[35,64] In addition, EED facilitates the binding of Ezh2 and PRC2 allosteric regulation by a feedback loop mechanism.[63] Lee W. et al. used whole-exome sequencing and custom IMPACT, and they showed that 80% of MPNSTs have either EED or SUZ12 mutations with no alterations on the other subunits (Fig. 4). PRC2 loss of function activates many downstream regulators like IGF2. They observed that the alterations of PRC2, *NF1*, and *CDKN2A* co-existed in MPNSTs. Use of immunohistochemistry showed the loss of H3K27me3 in MPNST cells compared to the neurofibromas that retained H3K27me3 staining. Thus, it can be used as a detector for the progression into malignant tumors.[65]

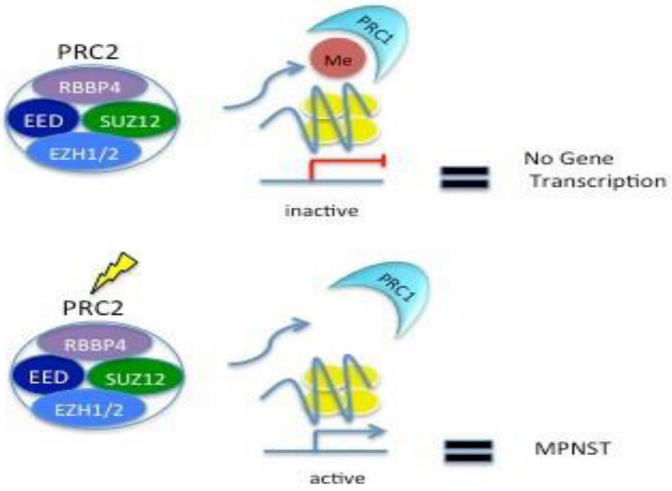


**Fig. 4.** The effect of epigenetic silencers on Gene transcription and the inactivation of PRC2, due to mutations of its subunits SUZ12 or EED, cause loss of methylation leading to MPNST formation.

### 4.4. PcG proteins and TSGs (p14 ARF, and p16 INK4A)

PRC1 is composed of many subunits; however, the most studied are, BMI1 (BMI1 polycomb ring finger oncogene), CBX7 (chromobox homologue 7) and RING1B (ring finger protein 2).[66] PRC2 in association with PRC1 repress CDK inhibitors p16 and p14 tumor suppressor proteins by controlling the INK4A-ARF locus.[67] To illustrate, a study showed the relations of BMI1 with *ink4a* gene through down- regulation of tumor suppressor genes. In turn, the PcG proteins use p16 and p19 as downstream targets to control senescence and cell arrest.[68] In the case of cancer, many studies have related the up-regulation of EZH2 and BMI1 with the development of tumors. Additionally, the EZH2 and BMI1 genes of PRC2 are increased in many cancers, such as aggressive breast, prostate, ovarian, etc.[69] A Western Blot and ChIP analysis study observed a decrease in protein and mRNA levels of the subunit EZH2, only in association with the p53 pathway. This results in the loss of H3K27me3, causing dissociation of BMI1 and CBX8 from the INK4A promoter, activating the expression of p16 and causing cellular senescence. Although EZH2 does not directly cause senescence, other factors may be contribute to the development of cancer other than the up-regulation of EZH2.[67] Conversely, a study indicated that EZH2 related drug resistance is due to a D1 domain mutation.[70] To overcome this issue, a combination therapy was applied. Adding MEK inhibitor to avoid resistance has exhibited an increase in efficacy.[71] Other studies shed light on the toxicity effect after inhibiting EZH2 gene because it prevents the vital role PRC2 has in regulating cellular function.[72]

## 5. Conclusion

Recently, NF1 gene aberrations have been linked to other malignancies like glioblastoma, lung, ovarian, cutaneous melanoma, and other types of cancer.[73] The current therapeutic approach for NF1 is limited to surgical resection. Better understanding of the molecular pathogenesis of NF1 will aid in controlling the malignant transformation of the benign neurofibromas. The development of MPNST is associated with the loss of function of NF1 gene along with many pathways. Several lines of evidence indicate that *NF1*, TSGs and PcG proteins are involved in the progression of MPNSTs. This review covered the correlations and interconnections that are found in these pathways.

The decrease in neurofibromin activity has substantial effects due to the involvement of this protein in many cellular processes, in this case representing involvement in tumor suppression. The cAMP pathway is not clearly understood since it is very complex and depends on many factors like cell types and the expression patterns of the downstream effectors.

The diagnosis of MPNST is challenging and poor survival rate necessitates the development of novel diagnostic techniques to further detect these abnormalities and expand current information of malignant transformation. Due to limited treatment options, future studies will also require investigating new biological pathways with a view to finding potential therapeutic agents for NF1-defecient tumors.

## Conflict of interest

None.

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## References

1. Riccardi VM. Von Recklinghausen neurofibromatosis. *N Engl J Med*. 1981.
2. Gutmann DH, et al. The diagnostic evaluationand multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. *JAMA*. 1997;278(1):51–57.
3. Friedman JM, Riccardi VM. *Neurofibromatosis : Phenotype, natural history, and pathogenesis*. 3rd ed. Baltimore: Johns Hopkins University Press; 1999.
4. Evans DG, et al. A genetic study of type 2 neurofibromatosis in the United Kingdom. I. Prevalence, mutation rate, fitness, and confirmation of maternal transmission effect on severity. *J Med Genet*. 1992;29(12):841–846.
5. Crump T. Translation of case reports in Ueber die multiplen Fibrome der Haut und ihre Beziehung zu den multiplen Neuromen by F. V. Recklinghausen. *Adv Neurol*. 1981;29:259–275.
6. Viskochil, D., et al., Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. Cell. 62(1): p. 187-192.
7. MacCollin M, et al. Schwannomatosis: A clinical and pathologic study. *Neurology*. 1996;46(4):1072–1079.
8. Evans DG, et al. Birth incidence and prevalence of tumor-prone syndromes: Estimates from a UK family genetic register service. *Am J Med Genet A*. 2010;152A(2):327–332.
9. MacCollin M, et al. Diagnostic criteria for schwannomatosis. *Neurology*. 2005;64(11):1838–1845.
10. Easton DF, et al. An analysis of variation in expression of neurofibromatosis (NF) type 1 (NF1): Evidence for modifying genes. *Am J Hum Genet*. 1993;53(2):305–313.
11. Daston, M.M., et al., The protein product of the neurofibromatosis type 1 gene is expressed at highest abundance in neurons,Schwanncells,and oligodendrocytes. Neuron. 8(3): p. 415-428.
12. Pemov A, et al. The primacy of NF1 loss as the driver of tumorigenesis in neurofibromatosis type 1-associated plexiform neurofibromas. *Oncogene*. 2017;36(22):3168–3177.
13. Basu TN, et al. Aberrant regulation of ras proteins in malignant tumour cells from type 1 neurofibromatosis patients. *Nature*. 1992;356(6371):713–715.
14. Campian J, Gutmann DH. CNS tumors in neurofibromatosis. *J Clin Oncol*. 2017;35(21):2378–2385.
15. Miettinen M, Antonescu M, Cristina R, 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*. 2017:1.
16. Thway K, Fisher C. Malignant peripheral nerve sheath tumor: Pathology and genetics. *Ann Diagn Pathol*. 2014;18(2):109–116.
17. Evans DGR, et al. Malignant peripheral nerve sheath tumors in neurofibromatosis 1. *Am J Hum Genet*. 2001;4.
18. Uusitalo E, et al. Distinctive Cancer Associationsin PatientsWith Neurofibromatosis Type 1. *J Clin Oncol*. 2016;34(17):1978–1986.
19. Higham CS, et al. The characteristics of 76 atypical neurofibromas as precursors to neurofibromatosis 1 associated malignant peripheral nerve sheath tumors. *Neuro-oncology*. 2018;20(6):818–825.
20. Clark MA, et al. Soft-tissue sarcomas in adults. *N Engl J Med*. 2005;353(7):701–711.
21. Ferner RE, Gutmann DH. International consensus statement on malignant peripheral nerve sheath tumors in neurofibromatosis 1. *Cancer Res*. 2002;62(5):1573–1577.
22. Stucky C-CH, et al. Malignant peripheral nerve sheath tumors (MPNST): The mayo clinic experience. *Ann Surg Oncol*. 2012;(3):878.
23. Louis DN, et al. The 2016 world health organization classification of tumors of the central nervous system: A summary. *Acta Neuropathol*. 2016;131(6):803–820.
24. Van Der Gucht A, et al. Metabolic tumour burden measured by 18F-FDG PET/CT predicts malignant transformation in patients with neurofibromatosis Type-1. *PLoS One*. 2016;11(3):e0151809.
25. Tovmassian D, Abdul Razak M, London K. *The role of [18F]FDG-PET/CT in predicting malignant transformation of PlexiformNeurofibromasin Neurofibromatosis-1. Vol. 2016*; 2016:7.
26. Karamchandani JR, et al. Sox10 and S100 in the diagnosis of soft-tissue neoplasms. *Appl Immunohistochem Mol Morphol*. 2012;20(5):445.
27. Ducatman BS, et al. Malignant peripheral nerve sheath tumors. A clinicopathologic study of 120 cases. *Cancer (0008543X)*. 1986;57(10):2006.
28. Suchorska WM. Radiobiological models in prediction of radiation cardiotoxicity. *Rep Pract Oncol Radiother*. 2020;25(1):46–49.
29. Šteiner I. Pathology of radiation induced heart disease. *Rep Pract Oncol Radiother*. 2020;25(2):178–181.
30. Abu-Hijlih R, et al. Adaptive radiotherapy in patients receiving neoadjuvant radiation for soft tissue sarcoma. *Rep Pract Oncol Radiother*. 2019;24(3):263–268.
31. Colman SD, Williams CA, Wallace MR. Benign neurofibromas in type 1 neurofibromatosis (NF1) show somatic deletions of the NF1 gene. *Nat Genet*. 1995;11:90.
32. Legius E, et al. Somatic deletion of the neurofibromatosis type 1 gene in a neurofibrosarcoma supports a tumour suppressor gene hypothesis. *Nat Genet*. 1993;3(2):122–126.
33. Carrió M, et al. Analysis of intratumor heterogeneity in Neurofibromatosis type 1 plexiformneurofibromasand neurofibromas with atypical features: Correlating histological and genomic findings. *Hum Mutat*. 2018;39(8):1112–1125.
34. Pemov A, et al. Low mutation burden and frequent loss of CDKN2A/B and SMARCA2, but not PRC2, define premalignant neurofibromatosis type 1–associated atypical neurofibromas. *Neuro-Oncology*. 2019;21(8):981–992.
35. Zhang M, et al. Somatic mutations of SUZ12 in malignant peripheral nerve sheath tumors. *Nat Genet*. 2014;46(11):1170–1172.
36. Peltonen S, Kallionpaa RA, Peltonen J. Neurofibromatosis type 1 (NF1) gene: Beyond cafe au lait spots and dermal neurofibromas. *Exp Dermatol*. 2017;26(7):645–648.
37. Johannessen CM, et al. The NF1 tumor suppressor critically regulates TSC2 and mTOR. *Proc Natl Acad Sci USA*. 2005;102(24):8573–8578.
38. Johansson G, et al. Effective in vivo targeting of the mammalian target of rapamycin pathway in malignant peripheral nerve sheath tumors. *Mol Cancer Ther*. 2008;7(5):1237–1245.
39. O'Reilly KE, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates akt. *Cancer Res*. 2006;66(3):1500–1508.
40. Zou CY, et al. Dual targeting of AKT and mammalian target of rapamycin: A potential therapeutic approach for malignant peripheral nerve sheath tumor. *Mol Cancer Ther*. 2009;8(5):1157–1168.
41. Dombi E, et al. Activity of selumetinib in neurofibromatosis type 1–Related plexiform neurofibromas. *N Engl J Med*. 2016;375(26):2550–2560.
42. Watson AL, et al. Co-targeting the MAPK and PI3K/AKT/mTOR pathways in two genetically engineered mouse models of schwann cell tumors reduces tumor grade and multiplicity. *Oncotarget*. 2014;5(6):1502–1514.
43. Kim HA, et al. Schwann cell proliferative responses to cAMP and Nf1 are mediated by cyclin D1. *J Neurosci*. 2001;21(4):1110–1116.
44. Dasgupta B, Dugan LL, Gutmann DH. The neurofibromatosis 1 gene product neurofibromin regulates pituitary adenylate cyclase-activating polypeptide-mediated signaling in astrocytes. *The Journal Of Neuroscience: The Official Journal Of The Society For Neuroscience*. 2003;23(26):8949–8954.
45. Marais R, Marshall CJ. Control of the ERK MAP kinase cascade by Ras and raf. *Cancer Surv*. 1996;27:101–125.
46. Mercer KE, Pritchard CA. Raf proteins and cancer: B-Raf is identified as a mutational target. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*. 2003;1653(1):25–40.
47. Wiese S, et al. Specific function of B-Raf in mediating survival of embryonic motoneurons and sensory neurons. *Nat Neurosci*. 2001;4(2):137.
48. Sidovar MF, et al. Phosphorylation of serine 43 is not required for inhibition of c-Raf kinase by the cAMP-dependent protein kinase. *J Biol Chem*. 2000;275(37):28688–28694.
49. Bogoyevitch MA, Marshall CJ, Sugden PH. Hypertrophic agonists stimulate the activities of the protein kinases c-Raf and A-Raf in cultured ventricular myocytes. *J Biol Chem*. 1995;270(44):26303–26310.
50. Ambrosini G, et al. Sorafenib inhibits growth and mitogen-activated protein kinase signaling in malignant peripheral nerve sheath cells. *Mol Cancer Ther*. 2008;7(4):890–896.
51. Hanahan D, Weinberg Robert A. Hallmarks of Cancer: The next generation. *Cell*. 2011;144(5):646–674.

52. Kourea HP, et al. Deletions of the INK4A gene occur in malignant peripheral nerve sheath tumors but not in neurofibromas. *Am J Pathol*. 1999;155(6):1855–1860.
53. Beert E, et al. Atypical neurofibromas in neurofibromatosis type 1 are premalignant tumors. *Genes Chromosomes Cancer*. 2011;50(12): 1021–1032.
54. Zhou H, et al. Malignant peripheral nerve sheath tumor: A comparison of grade, immunophenotype, and cell cycle/growth activation marker expression in sporadic and neurofibromatosis 1-related lesions. *Am J Surg Pathol*. 2003;27(10):1337–1345.
55. Berner J-M, et al. Chromosome band 9p21 is frequently altered in malignant peripheral nerve sheath tumors: Studies of CDKN2A and other genes of the pRB pathway. *Genes Chromosomes Cancer*. 1999;26(2):151–160.
56. Nielsen GP, et al. Malignant transformation of neurofibromas in neurofibromatosis 1 is associated with CDKN2A/p16 inactivation. *Am J Pathol*. 1999;155(6):1879–1884.
57. Schutte M, et al. Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. *Cancer Res*. 1997;57(15):3126–3130.
58. Perrone F, et al. p15INK4b p14 ARF, and p16 INK4a Inactivation in Sporadic and NeurofibromatosisType1-related Malignant Peripheral Nerve Sheath Tumors. *Clin Cancer Res*. 2003;9(11):4132–4138.
59. Lim S, Kaldis P. Cdks, cyclins and CKIs: Roles beyond cell cycle regulation. *Development*. 2013;140(15):3079–3093.
60. Perez M, et al. Efficacy of CDK4 inhibition against sarcomas depends on their levels of CDK4 and p16ink4 mRNA. *Oncotarget*. 2015;6(38): 40557–40574.
61. Kourea HP, et al. Expression of p27 and other cell cycle regulators in malignant peripheral nerve sheath tumors and neurofibromas. *Am J Pathol*. 1999;155(6):1885–1891.
62. Cao R, Zhang Y. The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. Current Opinion in histone H3. *Curr Opin Genet Dev*. 2004;14(2):155–164.
63. Jiao L, Liu X. Structural basis of histone H3K27 trimethylation by an active polycomb repressive complex 2. *Science*. 2015;350(6258).
64. De Raedt T, et al. PRC2 loss amplifies Ras- driven transcription and confers sensitivity to BRD4-based therapies. *Nature*. 2014;514:247.
65. Lee W, et al. PRC2 is recurrently inactivated through EED or SUZ12 loss in malignant peripheral nerve sheath tumors. *Nat Genet*. 2014;46(11):1227–1232.
66. Levine SS, King IFG, Kingston RE. Division of labor in Polycomb group repression. *Trends Biochem Sci*. 2004;29(9):478–485.
67. Bracken AP, et al. The Polycomb group proteins bind throughout the INK4A-ARF locus and are disassociated in senescent cells. *Genes Dev*. 2007;21(5):525–530.
68. Jacobs JJL, et al. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature*. 1999;397:164.
69. Raaphorst FM. Deregulated expression of Polycomb-group oncogenes in human malignant lymphomas and epithelial tumors. *Hum Mol Genet*. 2005;14(suppl_1):R93–R100.
70. Baker T, et al. Acquisition of a single EZH2 D1 domain mutation confers acquired resistance to EZH2-targeted inhibitors. *Oncotarget*. 2015;6(32):32646–32655.
71. Zeng D, Liu M, Pan J. Blocking EZH2 methylation transferase activity by GSK126 decreases stem cell-like myeloma cells. *Oncotarget*. 2017;8(2):3396–3411.
72. Xu B, et al. Targeting EZH2 and PRC2 dependence as novel anticancer therapy. *Exp Hematol*. 2015;43(8):698–712.
73. Philpott C, et al. The NF1 somatic mutational landscape in sporadic human cancers. *Hum Genomics*. 2017;11.