Insights into the Pathogenesis of NF1-Associated Neoplasms

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Neurofibromatosis type 1 (NF1) is one of the most common neurocutaneous genetic disorders, presenting with different cutaneous features such as café-au-lait macules, intertriginous skin freckling, and neurofibromas. Although most of the disease manifestations are benign, patients are at risk for a variety of malignancies, including malignant transformation of plexiform neurofibromas. Numerous studies have investigated the mechanisms by which these characteristic neurofibromas develop, with progress made toward unraveling the various players involved in their complex pathogenesis. In this review, we summarize the current understanding of the cells that give rise to NF1 neoplasms as well as the molecular mechanisms and cellular changes that confer tumorigenic potential. We also discuss the role of the tumor microenvironment and the key aspects of its various cell types that contribute to NF1-associated tumorigenesis. An increased understanding of these intrinsic and extrinsic components is critical for developing novel therapeutic approaches for affected patients.

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Abbreviations: ALA, 5-aminolevulinic acid; CALM, café-au-lait macule; cNF, cutaneous neurofibroma; ECM, extracellular matrix; FDA, Food and Drug Administration; GEM, genetically engineered mouse; hiPSC, human induced pluripotent stem cell; iSC, immature Schwann cell; LOH, loss of heterozygosity; MEK, MAPK/extracellular signal-regulated kinase kinase; MPNST, malignant peripheral nerve sheath tumor; MSC, mature Schwann cell; NCSC, neural crest stem cell; NF1, neurofibromatosis type 1; PDT, photodynamic therapy; pNF, plexiform neurofibroma; SC, stem cell; SCF, stem cell factor; SCP, Schwann cell precursors; SKP, skin-derived precursor; TME, tumor microenvironment; WT, wild type

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usually around ages 5–8 years (Gutmann et al., 2017; Hirbe and Gutmann, 2014). Revised diagnostic criteria for NF1 were published in 2021 (Legius et al., 2021). Patients who meet the clinical features usually do not require genetic testing. Legius syndrome, an overlapping disease characterized by a pathogenic variant of SPRED1, should be considered in patients who have CALMs and skin freckling but no other stigmata of NF1. With the many cutaneous manifestations, patients with NF1 may seek medical care from a dermatologist first. Thus, dermatologists play a vital role in diagnosing individuals with NF1, identifying potential complications, and referring patients to the right specialists. This is particularly important because there are other dermatologic conditions that have clinical overlap with similar pathophysiology to NF1, such as Legius syndrome, McCune-Albright syndrome, and Noonan syndrome (Brems and Legius, 2013; Brems et al., 2007; Dumitrescu and Collins, 2008; Tartaglia et al., 2011).

Neurofibromas are the most common tumors in NF1 and are the primary focus of this review. Cutaneous neurofibromas (cNFs) and plexiform neurofibromas (pNFs) are the two broad categories of neurofibromas (Figure 1d–f). Both feature benign nerve sheath tumors composed of Schwann cells, fibroblasts, immune cells, endothelial cells, and surrounding structural components (Brosseau et al., 2018b; Le et al., 2009; Li et al., 2020). However, there are key characteristics that distinguish cNFs and pNFs as separate entities following different clinical trajectories.

cNFs, also called dermal neurofibromas, are benign, localized, slow-growing tumors located solely in the dermis along cutaneous nerve twigs (Liao et al., 2018). Nearly all patients with NF1 will develop cNFs. These lesions can appear at any age and have been reported to increase in number and size with puberty and pregnancy, again suggesting a potential role for hormones in their development (Roth et al., 2008), although there is conflicting data in the field (Lammert et al., 2005; Well et al., 2020), and this is still debated. They vary in size and may appear as large plaques (diffuse cNF) or small nodules (discrete cNF) or may not even be visible in earlier stages; clinically, cNF appearance is described as flat, sessile, globular, or pedunculated (Ortonne et al., 2018). They can occur anywhere on the body and can be numerous, up to thousands in some cases (Figure 1d). Some cNFs may have hyperpigmentation suggestive of melanocytic or pigmentary involvement or a reddish–purplish coloration suggestive of vascular involvement. Although cNFs do not carry a risk of malignant transformation, they can be burdensome and disfiguring, painful, and pruritic. Lesions can have skin breakdown, bleeding, or superimposed infections. They can also result in significant psychosocial impact. When indicated, cNFs can be surgically removed with minimal risk of recurrence (Brosseau et al., 2018b; Chamseddin et al., 2019; Li et al., 2020). An important effort in the field is to arrive at a robust and consistent classification system and a gold standard of diagnostic criteria that will lead to improved accuracy in diagnosis (Ortonne et al., 2019).
pNFs are composed of similar cells as cNFs but develop more internally and grow along multiple nerves or a nerve plexus (Rodriguez et al., 2012). Because some infants are born with visible disfigurement due to pNF, these neurofibromas are thought to be congenital lesions that typically progress throughout childhood and early adolescence (Dombi et al., 2007). However, an adequate natural history study to assess the potential development of pNF over time from an embryonic stage has not yet been done. pNFs are highly infiltrative and often disfiguring tumors (Figure 1f). Depending on their location, they can exert pressure on surrounding nerves and other important structures, potentially with life-threatening complications. More than 50% of patients with NF1 will develop pNFs, although more than half of these tumors are not detectable on physical examination (Cimino and Gutmann, 2018; Gutmann et al., 2017; Hirbe and Gutmann, 2014; Li et al., 2020). Thus, some centers advocate for the use of whole-body magnetic resonance imaging for monitoring of pNFs and detection of potential malignant changes (Mautner et al., 2008). In addition, it is important to differentiate between a pNF that has extended into the skin and a true cNF because atypical changes in a cNF are not concerning but could represent a malignant transformation in a pNF (Ortonne et al., 2018); in contrast to cNFs, pNFs do carry a risk of transformation into MPNST, an aggressive cancer that is difficult to treat and represents the leading cause of death in patients with NF1. Individuals with NF1 develop MPNSTs at earlier ages and have inferior survival rates to patients with sporadic MPNST (Kolberg et al., 2013; Pemov et al., 2020).

**Neurofibroma developmental origin: The tumor cell of origin**

Patients with NF1 exhibit germline monoallelic loss of NF1, a tumor suppressor gene. Tumor formation requires a somatic mutation in the second NF1 locus, a process termed loss of heterozygosity (LOH). The tumor cell of origin is thus the cell that first undergoes biallelic loss of NF1 (Le et al., 2009; Li et al., 2020). NF1 murine models that completely replicate human NF1 have been difficult to generate given the complexity of tumor development and the spatiotemporal variations (Gutmann et al., 2017).

Whereas various cell types have been identified in neurofibromas, there is strong evidence that the tumor cell of origin arises from the Schwann cell lineage (Li et al., 2020). However, within this lineage, there is significant plasticity, which begs the question of when exactly during Schwann cell development does the tumor cell of origin appear (Zheng et al., 2008). Development of Schwann cells begins with multipotent migratory neural crest stem cells (NCSCs) that differentiate into Schwann cell precursors (SCPs) (Figure 2). SCPs then give rise to immature Schwann cells (ISCs), which differentiate into mature Schwann cells (MSCs) postnatally. These mature cells include (i) myelinating cells that generate myelin sheaths to wrap around axons and (ii) nonmyelinating cells with cytoplasmic processes that intermingle with small-diameter axons to form Remak bundles. Alternatively, SCPs have some multilineage potential and have also been shown to differentiate into melanocytes and fibroblasts (Li et al., 2020; Zheng et al., 2008). The distinct clinical features, location, and timing of cNFs and pNFs support different cellular origins, which have been investigated further in preclinical models (Li et al., 2020).

Zhu et al. (2002) found that Cre/LoxP deletion of the Nf1 gene from Schwann cell lineage cells in mice led to pNF development. In addition to Nf1 LOH, Nf1 heterozygosity in surrounding support cells was also found to be required for tumorigenesis (Li et al., 2020; Zhu et al., 2002). In 2008, several groups provided further insight into pNF development. Using Cre recombinase genetically engineered mouse (GEM) models, Joseph et al. (2008) showed that NCSCs with complete Nf1 loss did not persist in areas of tumor development past gestation and are not needed for pNF or MPNST formation. Loss of Nf1 in SCPs, through a P0a-Cre knockout, resulted in pNF formation in adult mice (Joseph et al., 2008). Zheng et al. (2008) also observed pNF formation when using
the same mouse model. Furthermore, they found abnormal Remak bundles with hyperplasia of fully differentiated non-myelinating Schwann cells, implicating them as potential cells of origin (Zheng et al., 2008). Wu et al. (2008) showed that Dhh-Cre inactivation of Nf1 specifically during embryonic development of dorsal root ganglion cells resulted in SCP colony formation and subsequent neurofibroma development, pointing toward an earlier Schwannian lineage origin.

Considering that most of these Cre drivers are expressed early and thus affect Nf1 function during MSC development, Le et al. (2011) examined the spatiotemporal aspects of pNF formation using an inducible transgenic mouse line, Plp-CreERT2, and induced Cre activity with tamoxifen to delete Nf1 at specific time points, from the embryonic stage to adulthood. Loss of Nf1 in Schwann cells lineage during the SCP and ISC stages resulted in pNF formation almost universally, adding additional support for an earlier origin of the tumor-initiating cells (Le et al., 2011). Chen et al. (2014) also honed in on paraspinal pNFs, which comprise a significant subset of internal tumors, employing lineage tracing to identify embryonic GAP43+ PLP+ SCs arising from spinal nerve roots as the pNF cell of origin (Chen et al., 2014). Characterization of these PLP+ SCs notably did not demonstrate expression of S100β, a marker for ISC and MSC, thereby highlighting the crucial role of stem cells (SCs) and their immediate progenitors in tumor initiation (Chen et al., 2014).

Although there has been more success in generating preclinical models of pNFs, cNFs are by far the more common tumor, present in virtually all patients with NF1. In 2007, Saito et al. (2007) reported the first mouse model to develop cNF. Using a Camk2-Cre transgenic mouse model with overexpression of oncogenic NRAS (G12V) specifically in nerve and neural crest–derived cells, they found that these mice had hyperpigmentation of the epidermis and developed both cNF and pNF. The HOXB7 protein is a marker of cells that arise from migrating neural crest cells. The identification of another population of cells early in embryonic development that can give rise to neurofibroma supports the hypothesis that there is a common SC-like SCP expressing HOXB7 and Prss56 that diverges into the tumor-initiating cells of either cNFs or pNFs. In addition, through whole-exome sequencing and mutational pathway analysis, this group identified the Hippo pathway as a modifier that further promotes MAPK signaling and likely contributes to the significant variations observed in neurofibroma characteristics (Chen et al., 2019). These exciting advances provide not only models for the elucidation of cNF pathogenesis but also new platforms to test potential therapies for patients with NF1.

Intrinsic factors driving neurofibroma development

NF1 is the result of a pathologic variant in the NF1 gene on chromosome 17. In North America and Europe, NF1 is inherited in an autosomal dominant pattern in 50% of cases and occurs de novo in the remaining cases (Gutmann et al., 2017; Jett and Friedman, 2010; Le et al., 2009). Isolated somatic driver mutations in NF1 have also been identified and appear to drive a more restricted phenotype with a segmental or mosaic pattern (Gutmann et al., 2017). The NF1 gene encodes a GAP-related domain–containing protein that negatively regulates the RAS/MAPK signaling pathway, a pathway that is critical to cell proliferation and survival. The neurofibromin protein converts RAS from the active guanosine triphosphate–bound state to the inactive guanosine diphosphate–bound state through hydrolysis (Gutmann et al., 2017; Laycock-van Spyk et al., 2011; Le et al., 2009; Staser et al., 2010).

Loss-of-function mutations in NF1 result in the over-activation of RAS and its downstream signaling pathway MAPK/extracellular signal–regulated kinase kinase (MEK)/MAPK, leading to cell overgrowth and survival. NF1 is expressed in all cells but at higher levels within the nervous system (Gutmann et al., 2017). In NF1, clinical manifestations depend on the cell type affected by the functional loss of NF1 (Brosseau et al., 2018). In line with Knudson’s
two-hit hypothesis, a second somatic mutation in NF1 and the resultant LOH are required for complete loss of NF1 function and formation of neurofibromas and other tumors (Cichowski et al., 1999; Laycock-van Spyk et al., 2011; Zhu et al., 2002).

Although cNFs and pNFs have similar histological features, cNFs typically undergo quiescence after an initial period of growth and do not progress to malignancy. Notably, genetic analyses of cNFs and pNFs revealed the absence of recurring gross genetic alterations or point mutations besides those affecting the NF1 gene (Beert et al., 2011; Carrió et al., 2018; Miller et al., 2009; Pemov et al., 2017), indicating the primacy of NF1 in driving tumorigenesis. Loss of neurofibromin results in dysregulation of not only cell survival and proliferation but also cell differentiation. For example, loss of NF1 in the CNS results in aberrant differentiation, with excessive astrocytic differentiation resulting in astrogliosis and optic glioma formation (Dasgupta and Gutmann, 2005; Zhu et al., 2005). Genetic studies further support the dysregulation of differentiation programs (Miller et al., 2009). Recently, a report by Miller et al. (2009) showed that there are epigenetic differences between cNFs and pNFs, with distinct methylation profiles that differentially affect downstream RAS signaling. Thus, epigenetic regulation could represent a key intrinsic difference between cNFs and pNF development.

Malignant tumors, including MPNST, require additional genetic hits to drive their cancerous behavior. Patients with NF1 most at risk for development of MPNST include those with increased whole-body pNF burden, the presence of nodular, and atypical lesions as well as patients with genetic whole-gene deletions, those with microdeletions, and patients who underwent previous radiation therapy (De Raedt et al., 2003; Meadows et al., 1985; Nguyen et al., 2014). In addition to NF1 inactivation, known genetic alterations in MPNST include inactivation of other tumor suppressor genes, including TP53, PTEN, CDKN2A, and PRC2, or amplification of growth-promoting genes such as EGFR or PDGFR (Laycock-van Spyk et al., 2011; Le and Parada, 2007; Pemov et al., 2020). In addition, there appears to be a progression from pNF to atypical neurofibromatous of unknown biological potential to MPNST that involves a step-wise accumulation of relevant mutations and chromosomal rearrangements specific to each step (Beert et al., 2011; Miettinen et al., 2017; Pemov et al., 2019). To date, however, limited effective therapy options exist beyond complete surgical resection, which remains the only curative therapy. Advances in genomic profiling will likely continue to provide more information on the potential drivers of tumorigenesis and, hopefully, novel therapeutic targets.

Recently, Mo et al. (2021) provided additional evidence supporting the role of NF1 pathologic variants and RAS/MAPK MAPK pathway deregulation in affecting not only cellular proliferation but also Schwann cell lineage differentiation. They utilized human induced pluripotent SCs (hiPSCs) harboring patient-based NF1 gene mutations and observed that NF1 loss increased the number of SCPs and delayed further differentiation in the Schwannian lineage (Mo et al., 2021). In addition, NF1-/- hiPSCs displayed higher baseline RAS activity levels than NF1+/- or NF1+/- hiPSCs, peaking in the SCP phase. Cells in this persistent stem-like state carried tumor-forming potential: NF1-/- hiPSC SCPs expressing the neurofibroma markers SOX10 and HOXB7 were implanted into murine sciatic nerves where they successfully engrafted and formed pNFs (Mo et al., 2021). Thus, this study supports previous reports that in addition to its role in regulating cell proliferation, NF1 loss also results in impairment of differentiation, in this case, Schwannian lineage cell differentiation. This is a critical finding because this impairment results in an expansion of the pool of progenitor cells that contain the tumor cell of origin, thus promoting tumor development.

Extrinsic factors driving neurofibroma development

The surrounding support cells and structural components of the TME are also critical in the development and maintenance of both cNFs and pNFs (Figure 3). Beyond Schwann cells, other key components found in neurofibromas include nerves, mast cells, macrophages, fibroblasts, collagen, and endothelial cells. Numerous studies have shown the importance of an NF1+/ background in addition to NF1 LOH in the tumor cells of origin for tumor formation and progression, emphasizing the intricate interactions between the various components of the tumor (Staser et al., 2010; Zhu et al., 2002). The NF1+/ background is not an absolute requirement but does significantly enhance neurofibroma progression and, as such, is considered a crucial aspect of tumorigenesis (Brosseau et al., 2018b; Liao et al., 2018; Zhu et al., 2002). Although these various cell types are found in the microenvironment of both cNFs and pNFs, it is likely that there are differences in their relative contributions to tumorigenesis.

Nerves. Nerves are central to neurofibroma formation in NF1. Normal development of Schwann cells, as early as the SCP phase, occurs in direct contact with nerves (Jessen and Ro¨ nnstrand, 2012). Activation of the c-kit receptor leads to cell proliferation, migration, and survival (Lennartsson and Ro¨ nnstrand, 2012). It was also demonstrated that NF1+/ Schwann cells secrete...
five- to six-fold higher levels of SCF than NF1/WT cells, in turn promoting the migration of NF1/mast cells to the Schwann cells and surrounding tumor region (Yang et al., 2003). These NF1/mast cells demonstrate increased proliferation and survival in response to SCF in both in vitro and in vivo models and appear to be hypersensitive to SCF (Ingram et al., 2001, 2000; Yang et al., 2003).

In murine pNF models, the infiltration of NF1/mast cells into peripheral nerves begins before tumor appearance. Yang et al. (2008) reported on the necessity of NF1/haploinsufficient bone marrow–derived cells, such as mast cells, for neurofibroma development in Krox20-Cre;Nf1flox/flox mice with a small population of NF1 Schwann cells and otherwise functionally WT non-Schwann cells. Compared with mice transplanted with WT bone marrow that survived without evidence of pNF formation, mice transplanted with NF1/mast cell bone marrow developed pNF and significant morbidity and mortality (Yang et al., 2008). Consistent with findings in other mouse and human samples, these tumors displayed mast cell infiltration. Notably, these mast cells were NF1/ and thus were derived from the donor bone marrow. In addition, in mice bearing point mutations in the c-kit receptor leading to disrupted signaling in mast cells, NF1/ donor mast cells did not migrate to or infiltrate nerves, and tumor formation was not observed (Yang et al., 2008).

Liao et al. (2018) employed a different GEM model with Scf mice and validated that mast cell infiltration relies on neoplastic Schwann cell secretion of SCF. However, removal of SCF did not prevent neurofibroma development, supporting the notion that there are other components that contribute to tumorigenesis (Liao et al., 2018). Mast cells themselves also secrete stimulatory factors that are thought to contribute to tumor growth. These include VEGF, which could sustain tumor growth through neovascularization, and PDGF, which promotes mitogenesis (Le and Parada, 2007; Theoharides and Conti, 2004).

**Macrophages.** Macrophages are another group of immune cells that infiltrate neurofibromas (Choi et al., 2017; Liao et al., 2018; Prada et al., 2013). Macrophages can serve either proinflammatory (antitumor) or protumor roles and are separated into M1 and M2 subsets, respectively. In addition to SCF, NF1 Schwann cells secrete VEGF and CCL5, which may help recruit macrophages to tumor regions (Prada et al., 2013). Prada et al. (2013) discovered that macrophage infiltration correlates with disease severity, from neurofibroma to MPNST, in mouse and human tumors. In their GEM NF1 model with Dhh-Cre inactivation of Nf1 in Schwann cells, the macrophages and other non-Schwann cells were WT. However, at different stages, macrophages appeared to both prevent and enhance tumor growth, with macrophages being tumor permissive once the tumor is established (Prada et al., 2013). Additional studies are needed to uncover the mechanisms underlying this phenomenon.

Liao et al. (2018) found that independent of mast cell activity, macrophages were present in high concentrations in neurofibromas and were primarily of the M1 proinflammatory subset. Notably, they also observed preferential development of paraspinal pNFs in cervical and T5–T8 thoracic nerves, anatomic regions associated with more frequent flexion and extension and thus more injury (Liao et al., 2018). Interestingly, these hot spots for pNF growth observed in this mouse model are similar to that seen in human patients (Nguyen et al., 2014; Wise et al., 2005). Other studies have also reported nerve injury as a trigger for neurofibroma formation (Ribeiro et al., 2013). These observations require a deeper investigation into the relationship between the macrophage response and underlying nerve injury.

**Fibroblasts and collagen.** Fibroblasts are connective tissue cells that are involved in structural support, regulation of the extracellular matrix (ECM), and wound healing (Kalluri and
Zeisberg, 2006). They represent another prominent cell type in neurofibromas and play a crucial role in secreting collagen; abnormal, excessive collagen deposition is another hallmark of neurofibromas, with collagen constituting approximately 50% of the tumor weight (Yang et al., 2006). The nerve and other associated cells in neurofibromas are interspersed with a disorganized ECM that is rich in collagen and other proteins (Kalluri and Zeisberg, 2006). Remodeling of this infrastructure allows for tumor invasion and neoangiogenesis. Recently, transcriptome analysis of cNF using single-cell RNA sequencing revealed that collagen VI, a proangiogenic and protumorigenic collagen, rather than the classic collagen I is the predominant collagen secreted by the fibroblasts in these tumors (Brosseau et al., 2021). The mechanisms by which this collagen signals to Schwann cells and other cell types within the tumor remains to be elucidated.

Fibroblasts also represent another source of SCF (Li et al., 2018; Ryan et al., 1994). SCF serves as a paracrine GF between fibroblasts and mast cells (Yamamoto et al., 2000). Using an Nf1 model, Yang et al. (2006) revealed the relationship between murine Nf1-/- fibroblasts and Nf1-/- mast cells in multiple in vitro experiments. Nf1-/- fibroblasts produced a three-fold increase in collagen in response to Nf1-/- mast cell–conditioned media. The synergistic interactions between Nf1-/- mast cells and fibroblasts were confirmed in a three-dimensional collagen lattice model. Using a proteomics approach, they found that Nf1-/- mast cells secrete increased amounts of TGFβ, which activates Nf1-/- fibroblasts, driving their proliferation and production of collagen. They confirmed this enhanced fibroblast activity to be dependent on TGFβ by adding TGFβ-neutralizing antibodies, which effectively reduced collagen levels. Mechanistically, they also showed that the Nf1-/- fibroblast response to TGFβ is driven by hyperactivation of RAS and its downstream c-abl (Yang et al., 2006).

**Endothelial cells.** Neurofibromas are highly vascular tumors. Endothelial cells are the main structural and regulatory cells of the circulatory system; however, they also influence surrounding cells through paracrine signaling (Coutlas et al., 2005). In neurofibromas, in particular, Schwann cells and mast cells produce angiogenic factors such as VEGF, which can stimulate endothelial cells to promote vascular development and contribute to both benign and malignant tumorigenesis, thus reinforcing the complex interactions between various tumor compartments (Le and Parada, 2007; Pemov et al., 2020; Theoharides and Conti, 2004). Mashour et al. (2001) showed that Nft1-/- Schwann cells directly stimulated the proliferation of endothelial cells in vitro. They identified differential expression of several GFs—FGF2, PDGF, and midkine—in Nft1-/- Schwann cells that are potentially angiogenic and tumorigenic (Mashour et al., 2001).

Gitler et al. (2003) used conditional gene inactivation in mice to show that Nft1 loss in endothelial cells resulted in endothelial dysfunction and amplified RAS signaling, leading to cardiovascular anomalies consistent with the human NF1 phenotype. The effects of Nft1-/- endothelial cells on the development of the cardiovascular system are beyond the scope of this review, but this study provided new insights into the role of Nf1 in endothelial cells. Further studies revealed increased angiogenesis in Nf1-/- mice (Munchhof et al., 2006; Ozerdem, 2004; Wu et al., 2006). Munchhof et al. (2006) used endothelial cells from human patients with NF1 to determine that their enhanced proliferation and migration in response to angiogenic factors were related to hyperactivation of the RAS/MEK/MAPK pathway.

**Tumor cell of origin and tumor microenvironment interactions initiate (and maintain) neurofibroma formation**

The development of neurofibromas involves a complex interplay between intracellular and intercellular components. Although tumorigenesis begins at the molecular level with NF1 LOH activating the RAS pathway, the process involves interdependent growth signaling loops between the tumor cells of origin and nearby nerves, mast cells, fibroblasts, and blood vessels. GEM models have revealed the importance of NF1 loss not only in the cells of origin but also within their environment. As evidenced by the studies summarized in this review, the continued effort to expand our knowledge of the complexity of neurofibromas will help in the development of more accurate preclinical models that will pave the way for therapeutic advances.

**Decoding tumor pathogenesis to inform future therapies for neurofibroma**

A curative molecular treatment does not exist for NF1, and surgical resection of neurofibromas remains the mainstay of therapy where feasible. Identifying new therapeutic targets is critical for developing new, effective treatments, and mouse models are a powerful tool toward this end, allowing for a detailed study of the intricacies of NF1 molecular pathogenesis. For example, mouse models were invaluable in identifying the role of MEK inhibitors in restraining tumorigenicity (Jessen et al., 2013), leading to the approval of a MEK inhibitor as the first and, to date, only Food and Drug Administration (FDA)-approved drug for an NF1-associated tumor, pNF (Gross et al., 2020). In addition, key findings in mice can predict what will happen in humans: the finding that continuous treatment with MEK inhibitor is required for sustained suppression of tumor growth was first shown in a mouse model of pNF (Chen et al., 2014) and was later borne out in a phase I clinical trial in human patients (Dombi et al., 2016). The numerous mouse models of neurofibromas available today are therefore vital tools for identifying novel therapeutics and for preclinical testing (Brossier and Carroll, 2012; Chen et al., 2019; Gutmann, 2014; Mo et al., 2021; Williams and Largaespada, 2020) as well as for predicting human clinical trial outcomes. Finally, the use of hiPSCs harboring patient-based NF1 mutations to generate patient-specific mouse avatars offers the exciting opportunity for personalized medicine in the future (Anastasaki et al., 2020; Mo et al., 2021; Wegscheid et al., 2018).

As knowledge of the interactions within and between the cells that compose neurofibromas has evolved, so have the approaches for patient management in the clinical setting. Armed with better preclinical models of NF1 and a better understanding of neurofibroma development, efforts have been made to target individual components of the neurofibroma and/or its microenvironment.
Considering the role of mast cells in neurofibromas, the c-kit tyrosine kinase receptor has been targeted with imatinib, a common tyrosine kinase inhibitor that has demonstrated activity against c-kit, among other tyrosine kinases, and has inhibited the formation of pNFs in preclinical studies (Yang et al., 2008). After observing imatinib activity in mouse models, Yang et al. (2008) reported the use of imatinib in a child with NF1 and an unresectable pNF that progressed to compress her airway. After 3 months of treatment with imatinib, repeat imaging revealed a 70% reduction in tumor volume (Yang et al., 2008). These experiences were followed by a phase 2 clinical trial aimed at determining whether imatinib could decrease pNF tumor volume (Robertson et al., 2012). Although significantly varied clinical responses were observed, 6 of the 36 patients had more than a 20% decrease in tumor volume (Robertson et al., 2012).

Another tyrosine kinase inhibitor, cabozantinib, has also shown promise in treating pNFs in both preclinical and clinical studies, with a phase 2 trial showing >42% of evaluable patients having a partial response (Fisher et al., 2021; Lu et al., 2019). In addition to inhibiting c-kit, cabozantinib also inhibits other receptor Y kinases such as AXL, MET, and VEGFR2 and has been shown to be effective in gastrointestinal stromal tumor models where resistance to imatinib has arisen (Cohen et al., 2015; Lu et al., 2019).

MEK inhibitors have moved to the forefront of NF1 treatment. MEK is a kinase that activates MAPK in the RAS/MAPK signaling cascade. Loss of NF1 results in prolonged activation of RAS and results in the dysregulation and activity of its downstream signaling pathway components, particularly MEK. Selumetinib, an oral MEK inhibitor, was approved in 2020 by the FDA for the treatment of symptomatic, inoperable pNFs and is currently the only FDA-approved medication for pNF (Gross et al., 2020; Klesse et al., 2020). This approval was greeted with much-warranted excitement by the neurofibroma community, but there is still much work to be done: although selumetinib treatment results in tumor shrinkage for the majority of patients, it does not completely eradicate the tumor, can have intolerable side effects, and may require prolonged therapy because tumor regrowth after halting therapy is reported (Dombi et al., 2016; Gross et al., 2020; Klesse et al., 2020). In addition, because pNFs are a very heterogeneous tumor, the cell type(s) within the pNF that are responsive to MEK inhibition and responsible for tumor shrinkage remain to be elucidated. Whereas one target of MEK inhibition is certainly the NF1-null neoplastic Schwann cells, other cells of the TME that are NF1 heterozygous have also been shown to be hyperproliferative (Broseau et al., 2018a; Ingram et al., 2001, 2000) and thus could also be responding to MEK inhibition and contributing to tumor shrinkage. Identifying the target(s) of MEK inhibition is an area of keen interest and continued study.

Clinical trials are also ongoing to test the treatments for cNFs, including testing the efficacy of selumetinib on cNF with both oral (NCT02839720) and topical (NCT04435665) administration. Past trials include a phase 2 clinical trial that tested the effects of systemic (oral) administration of everolimus, an inhibitor of mTOR, and showed a statistically significant reduction in dermal lesions for 19% of patients (Slopis et al., 2018). However adverse events were common, and larger study populations followed over a longer period of time are required. A small study testing the efficacy of cNF microporation with a laser device followed by topical treatment with diclofenac, a nonsteroidal anti-inflammatory drug, showed no reduction in cNF size and no histological changes (Oliveira et al., 2021). However, this was a small study (six patients), and the lack of response may have been due to drug penetration issues. Another phase 1 trial, based on preclinical data obtained in a mouse model of cNF (Muir et al., 2008), tested the efficacy and safety of photodynamic therapy (PDT) for treatment of cNF using 5-aminolevulinic acid (ALA) and 630 nm light (Quirk et al., 2021). Although they found that there was an inflammatory response after ALA-PDT treatment, there was no observed effect on tumor size. Finally, an important step in designing effective drug treatment is understanding the natural history of the target tumor. A quantitative natural history study conducted for over 8 years by Cannon et al. (2018) looked at this question. They found that the number and growth of cNFs increased at variable rates, with differences observed between individuals as well as different body regions on the same person. These findings have implications for the design of clinical trials and highlight the importance of establishing a baseline dataset.

The hope remains that new insights into the intricate pathogenesis of NF1 and neurofibromas will lead to novel therapeutic approaches and significant improvements in patients’ lives. Further refinement of preclinical models will continue to inform new clinical trials and the development of targeted therapies, including combination therapy aimed at the multiple components of the tumor, TME, and/or associated signaling cascades.

**Conclusion and perspectives**

The pathogenesis of NF1 and neurofibromas is complex, interweaving various intrinsic and extrinsic factors with the tumor cells of origin. There is heterogeneity at every level within the tumor system, complicating efforts to replicate the disease in the laboratory. From preclinical models to clinical trials, incredible progress has been made in the understanding of NF1 and its related tumors. The identification of the Schwann cell lineage as the origin for neurofibromas, specifically with a common early progenitor that appears to branch off into cNF or pNF development, provides valuable insight into the tumor initiation stage. Characterization of surrounding cells and support structures in the TME reveals mechanisms of tumor growth and maintenance. By merging our understanding of these components, we can find significant opportunities for novel therapeutic approaches in a unique patient population.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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