Insights into the Pathogenesis of NF1-Associated Neoplasms

Ashley Bui[1], Chunhui Jiang[2], Renee M. McKay[2], Laura J. Klesse[1,3,4] and Lu Q. Le[2,3,4,5]

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.

JID Innovations (2021);**1:**100044 doi:10.1016/j.xjidi.2021.100044

[1]*Department of Pediatrics, The University of Texas Southwestern Medical Center, Dallas, Texas, USA;* [2]*Department of Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, USA;* [3]*Harold C. Simmons Comprehensive Cancer Center, The University of Texas Southwestern Medical Center, Dallas, Texas, USA;* [4]*Comprehensive Neurofibromatosis Clinic, The University of Texas Southwestern Medical Center, Dallas, Texas, USA; and* [5]*Hamon Center for Regenerative Science and Medicine, The University of Texas Southwestern Medical Center, Dallas, Texas, USA*

Correspondence: Lu Q. Le, Department of Dermatology, UT Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390-9069, USA. E-mail: lu.le@utsouthwestern.edu

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; NFI, neurofibromatosis type 1; PDT, photodynamic therapy; pNF, plexiform neurofibroma; SC, stem cell; SCF, stem cell factor; SCP, Schwann cell precursor; SKP, skin-derived precursor; TME, tumor microenvironment; WT, wild type

Received 29 April 2021; revised 2 July 2021; accepted 6 July 2021; accepted manuscript published online 29 July 2021; corrected proof published online 20 August 2021

Cite this article as: JID Innovations 2021;1:100044

Neurofibromatosis type 1

Neurofibromatosis type 1 (NF1) is a tumor-predisposition syndrome affecting 1 in 3,000 individuals (Gutmann et al., 2017; Li et al., 2020). NF1 has a wide phenotypic spectrum and complete clinical penetrance, meaning that all patients will exhibit disease manifestations at some point in their lifetime. However, even within families, the clinical course can vary greatly. Nontumor diagnostic features include café-au-lait macules (CALMs), intertriginous skin freckling, and long bone dysplasia or other skeletal abnormalities (Brosseau at al., 2018b, Legius et al., 2021) (Figure 1). The majority of patients often struggle with neurocognitive deficits, including learning difficulties and behavioral problems (Lehtonen et al., 2013). They may also have cardiovascular defects and secondary hypertension (Hirbe and Gutmann, 2014).

Patients with NF1 are also prone to developing both benign and malignant tumors. Benign tumors predominate, most notably as the characteristic neurofibromas. Other tumors include iris melanocytic hamartomas (termed Lisch nodules), optic pathway gliomas, and other low-grade gliomas of the CNS (Gutmann et al., 2017; Hirbe and Gutmann, 2014;). Malignant tumors seen in this population include malignant peripheral nerve sheath tumors (MPNSTs), juvenile myelomonocytic leukemia, pheochromocytoma, rhabdomyosarcoma, and glioblastoma multiforme (Hirbe and Gutmann, 2014). With advances in next-generation sequencing, it is now appreciated that *NF1* is one of the most commonly mutated genes in other cancers, including melanoma, lung cancer, and colon cancer (Philpott et al., 2017). Strikingly, patients with NF1 do not have an increased incidence of developing these cancers. This could possibly be attributed to the *NF1* heterozygous tumor microenvironment (TME), which may play an antagonistic role in carcinogenesis owing to enhanced immune surveillance (Brosseau and Le, 2019; Brosseau et al., 2018a).

NF1 is driven by mutations in the neurofibromin gene, *NF1*, which encodes a tumor suppressor. The role of *NF1* pathologic variants, in cooperation with other intrinsic and extrinsic factors, is described in more detail later.

Neurofibroma and other cutaneous manifestations of NF1

Pigmentary lesions are the most common clinical feature in NF1. CALMs are flat, hyperpigmented lesions that represent dense collections of $NF1^{-/-}$ melanocytes (Figure 1a) (Gutmann et al., 2017; Hirbe and Gutmann, 2014). Patients with NF1 have ≥6 CALMs, usually appearing in infancy and often increasing in number and/or size during puberty and pregnancy, suggesting a response to hormonal changes (Gutmann et al., 2017). The diagnostic criteria also include skinfold freckling, most often in the axillary or inguinal regions (Figure 1b). These tend to appear later than CALMs,

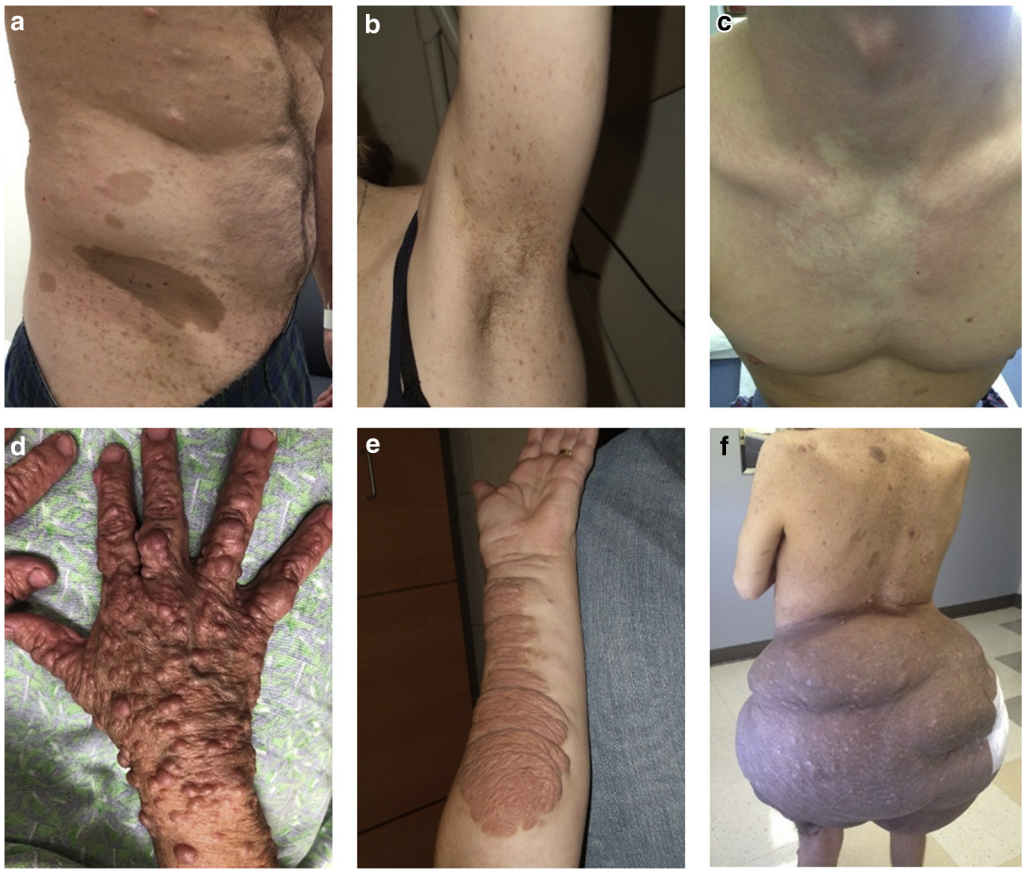

Figure 1. Cutaneous manifestations of NF1. Clinical images of six different cutaneous manifestations of NF1 are shown: (**a**) axillary freckling, (**b**) CALMs, (**c**) nevus anemicus, (**d**) cutaneous neurofibroma, (**e**) localized plexiform neurofibroma, and (**f**) diffuse plexiform neurofibroma. CALM, café-au-lait macule; NF1, neurofibromatosis type 1.

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).

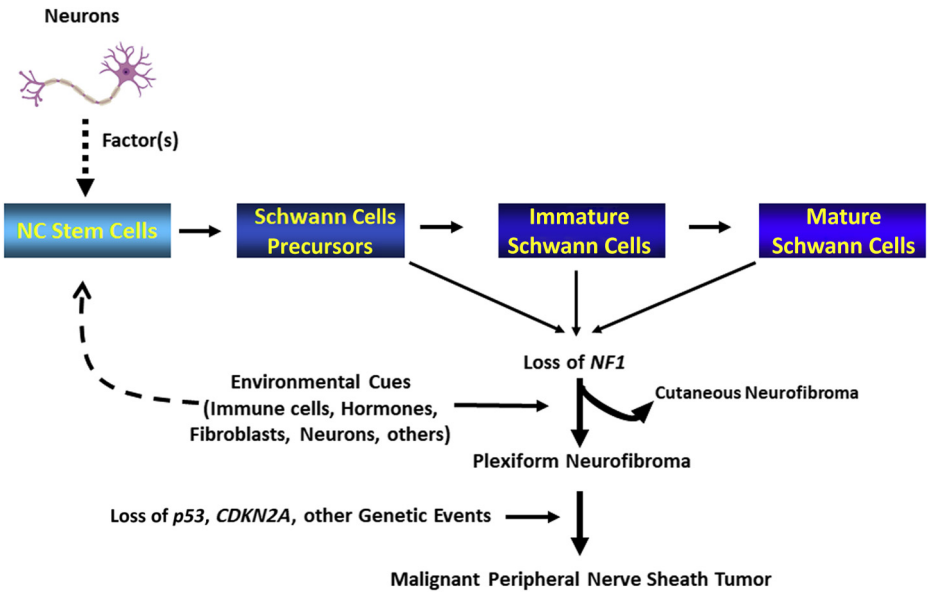

Figure 2. Key factors required for neurofibromagenesis. The development of neurofibroma begins with loss of the neurofibromin gene, *NF1*, in a particular cell type (Schwann cells, NC stem cells) that is in close proximity to a nerve, and then further stoked by interaction with protumorigenic cells of the microenvironment and other environmental cues, such as hormones. Other genetic events, for example, loss of *p53*, are required for the progression of benign plexiform neurofibroma to MPNST. MPNST, malignant peripheral sheath tumor; NC, neural crest.

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^{+} SCPs arising from spinal nerve roots as the pNF cell of origin (Chen et al., 2014). Characterization of these PLP^{+} SCPs 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 cNFs around age 3 months (Saito et al., 2007). The formation of cNFs is consistent with potential neural crest origin, but notably, these mice did not develop pNFs or other typical manifestations of NF1 (Saito et al., 2007). Furthermore, the conditional expression of oncogenic NRAS, which although tumorigenic to a certain degree, might not reflect the gene dosage nor the function of natural NF1 pathogenesis driven by wild-type (WT) RAS alterations, thus making direct comparisons difficult.

In pursuit of a clearer understanding of cNF formation, Le et al. (2009) showed that skin-derived precursors (SKPs)—self-renewing multipotent neural crest–like SCs that have the ability to differentiate along glial lineages—contain the potential cNF cell of origin. When injected into the dermis, *Nf1*$^{-/-}$ SKPs only formed cNFs in pregnant female mice with altered circulating hormones or when topical tamoxifen was administered topically to induce *Nf1* deletion in the surrounding skin (Le et al., 2009). After observing a lack of tumor formation on straightforward intradermal injection of *Nf1*$^{-/-}$ SKPs, they then implanted the *Nf1*$^{-/-}$ SKPs near the sciatic nerve, replicating the close proximity of tumor and nerve in humans. This successfully resulted in pNF formation within 2 months, whereas implantation of *Nf1*$^{+/-}$ SKPs led to no tumor growth (Le et al., 2009). These experiments provided further support that cNFs and pNFs have different tumor cells of origin while also shedding light on the importance of a tumor-permissive microenvironment in addition to *Nf1* LOH in the cell of origin.

More recently, boundary cap cells, another neural crest–derived population with terminal glial potential, have also been identified as a candidate cell of origin. Boundary cap cells are found specifically at entry and exit points for peripheral nerves during early development (Gresset et al., 2015). Radomska et al. (2019) devised a new mouse model with embryonic *Nf1* inactivation in *Prss56*-positive boundary cap cells resulting in cNF formation. They also observed pNF formation in this model. Cell tracing revealed *Prss56*-positive boundary cap cells as a common predecessor for nerve root glia and hypodermal/dermal glia, raising the possibility that boundary cap cells give rise to more specific cNF or pNF cells of origin (Radomska et al., 2019).

Around the same time, Chen et al. (2019) reported another novel mouse model featuring *Nf1* deletion in HOXB7-positive cells: these mice displayed skin hyperpigmentation 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 overactivation 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 cNF and pNF, with distinct methylation profiles that differentially affect downstream RAS signaling. Thus, epigenetic regulation could represent a key intrinsic difference between cNF 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/MEK/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, the 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 Mirsky, 2005). Adameyko et al. (2009) described SCPs as a source of both Schwann cells and melanocytes; SCPs are found in close association with nerves and depend on additional signaling to determine their final cell fates. Using a mouse model, Liao et al. (2016) showed that peripheral nerves are required for neurofibroma formation. When they injected $Nf1^{-/-}$ SKPs into the sciatic nerve versus into a non-nerve (subcutaneous) tissue, they found that pNFs only developed when these tumor cells of origin were injected into nerve tissue (Liao et al., 2016).

Mast cells. Mast cell infiltration is one of the key histopathologic features of neurofibromas, first observed back in 1911 (Brosseau et al., 2018b; Staser et al., 2010; Yang et al., 2003). These immune effector cells, classically known for their role in allergic reactions and secretion of histamine-containing granules, play a protumor, profibrotic role in neurofibromas. The mast cells found in neurofibromas appear to be activated, associated with elevated histamine and circulating IgE levels (Geller et al., 2006; Liao et al., 2018). Schwann cells secrete SC factor (SCF) (also known as Kit ligand), which binds the c-kit tyrosine kinase receptor on the surface of mast cells (Staser et al., 2010; Yang et al., 2003). Activation of the c-kit receptor leads to cell proliferation, migration, and survival (Lennartsson and Rönnstrand, 2012). It was also demonstrated that $NF1^{-/-}$ Schwann cells secrete

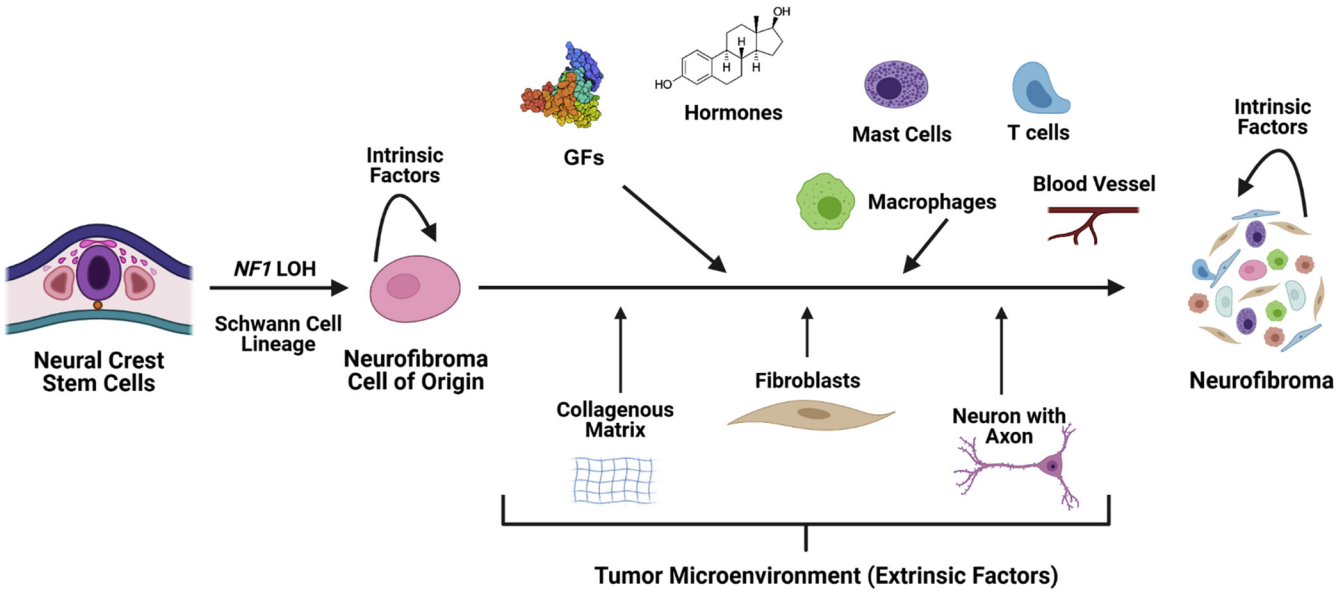

Figure 3. Intrinsic and extrinsic factors in both cNF and pNF act on the cells of origin of neurofibroma to drive tumor formation. Schematic diagram showing the various factors that drive and impact the development of neurofibroma. Illustration created with BioRender.com. cNF, cutaneous neurofibroma; LOH, loss of heterozygosity; pNF, plexiform neurofibroma.

five- to six-fold higher levels of SCF than $NF1^{+/-}$ or 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;*$Nf1^{flox/flox}$ 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 $Nf1^{+/-}$ 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 (Coultas 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 $NF1^{-/-}$ Schwann cells directly stimulated the proliferation of endothelial cells in vitro. They identified differential expression of several GFs—FGF2, PDGF, and midkine—in $NF1^{-/-}$ 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 *Nf1* 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 $Nf1^{-/-}$ 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 (Brosseau 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.

ORCIDs

Ashley Bui: http://orcid.org/0000-0003-2012-9779
Renee M. McKay: http://orcid.org/0000-0001-5976-1996
Lu Q. Le: http://orcid.org/0000-0003-2817-5382

AUTHOR CONTRIBUTIONS

Writing - Original Draft Preparation: AB, CJ, RMM, LJK, LQL; Writing - Review and Editing: AB, CJ, RMM, LJK, LQL

ACKNOWLEDGMENTS

LQL held a Career Award for Medical Scientists from the Burroughs Wellcome Fund and holds the Thomas L. Shield, MD Professorship in Dermatology. He is supported by funding from the National Cancer Institute of the National Institutes of Health (grant number R01 CA166593) and the

Developmental and Hyperactive RAS Tumor SPORE (grant number U54 CA196519), by the Neurofibromatosis Therapeutic Acceleration Program, the NF1 Research Consortium Fund, and the Giorgio Foundation.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

Adameyko I, Lallemend F, Aquino JB, Pereira JA, Topilko P, Müller T, et al. Schwann cell precursors from nerve innervation are a cellular origin of melanocytes in skin. Cell 2009;139:366–79.

Anastasaki C, Wegscheid ML, Hartigan K, Papke JB, Kopp ND, Chen J, et al. Human iPSC-derived neurons and cerebral organoids establish differential effects of germline NF1 gene mutations. Stem Cell Rep 2020;14:541–50.

Beert E, Brems H, Daniëls B, De Wever I, Van Calenbergh F, Schoenaers J, et al. Atypical neurofibromas in neurofibromatosis type 1 are premalignant tumors. Genes Chromosomes Cancer 2011;50:1021–32.

Brems H, Chmara M, Sahbatou M, Denayer E, Taniguchi K, Kato R, et al. Germline loss-of-function mutations in SPRED1 cause a neurofibromatosis 1-like phenotype. Nat Genet 2007;39:1120–6.

Brems H, Legius E. Legius syndrome, an update. Molecular pathology of mutations in SPRED1. Keio J Med 2013;62:107–12.

Brosseau JP, Le LQ. Heterozygous tumor suppressor microenvironment in cancer development. Trends Cancer 2019;5:541–6.

Brosseau JP, Liao CP, Wang Y, Ramani V, Vandergriff T, Lee M, et al. NF1 heterozygosity fosters de novo tumorigenesis but impairs malignant transformation. Nat Commun 2018a;9:5014.

Brosseau JP, Pichard DC, Legius EH, Wolkenstein P, Lavker RM, Blakeley JO, et al. The biology of cutaneous neurofibromas: consensus recommendations for setting research priorities. Neurology 2018b;91(Suppl. 1):S14–20.

Brosseau JP, Sathe AA, Wang Y, Nguyen T, Glass DA 2nd, Xing C, et al. Human cutaneous neurofibroma matrisome revealed by single-cell RNA sequencing. Acta Neuropathol Commun 2021;9:11.

Brossier NM, Carroll SL. Genetically engineered mouse models shed new light on the pathogenesis of neurofibromatosis type I-related neoplasms of the peripheral nervous system. Brain Res Bull 2012;88:58–71.

Cannon A, Chen MJ, Li P, Boyd KP, Theos A, Redden DT, et al. Cutaneous neurofibromas in neurofibromatosis type I: a quantitative natural history study. Orphanet J Rare Dis 2018;13:31.

Carrió M, Gel B, Terribas E, Zucchiatti AC, Moliné T, Rosas I, et al. Analysis of intratumor heterogeneity in neurofibromatosis type 1 plexiform neurofibromas and neurofibromas with atypical features: correlating histological and genomic findings. Hum Mutat 2018;39:1112–25.

Chamseddin BH, Hernandez L, Solorzano D, Vega J, Le LQ. Robust surgical approach for cutaneous neurofibroma in neurofibromatosis type 1. JCI Insight 2019;5:e128881.

Chen Z, Liu C, Patel AJ, Liao CP, Wang Y, Le LQ. Cells of origin in the embryonic nerve roots for NF1-associated plexiform neurofibroma. Cancer Cell 2014;26:695–706.

Chen Z, Mo J, Brosseau JP, Shipman T, Wang Y, Liao CP, et al. Spatiotemporal loss of *NF1* in Schwann cell lineage leads to different types of cutaneous neurofibroma susceptible to modification by the hippo pathway. Cancer Discov 2019;9:114–29.

Choi K, Komurov K, Fletcher JS, Jousma E, Cancelas JA, Wu J, et al. An inflammatory gene signature distinguishes neurofibroma Schwann cells and macrophages from cells in the normal peripheral nervous system. Sci Rep 2017;7:43315.

Cichowski K, Shih TS, Schmitt E, Santiago S, Reilly K, McLaughlin ME, et al. Mouse models of tumor development in neurofibromatosis type 1. Science 1999;286:2172–6.

Cimino PJ, Gutmann DH. Neurofibromatosis type 1. Handb Clin Neurol 2018;148:799–811.

Cohen NA, Zeng S, Seifert AM, Kim TS, Sorenson EC, Greer JB, et al. Pharmacological inhibition of KIT activates MET signaling in gastrointestinal stromal tumors. Cancer Res 2015;75:2061–70.

Coultas L, Chawengsaksophak K, Rossant J. Endothelial cells and VEGF in vascular development. Nature 2005;438:937–45.

Dasgupta B, Gutmann DH. Neurofibromin regulates neural stem cell proliferation, survival, and astroglial differentiation in vitro and in vivo. J Neurosci 2005;25:5584–94.

De Raedt T, Brems H, Wolkenstein P, Vidaud D, Pilotti S, Perrone F, et al. Elevated risk for MPNST in NF1 microdeletion patients. Am J Hum Genet 2003;72:1288–92.

Dombi E, Baldwin A, Marcus LJ, Fisher MJ, Weiss B, Kim A, et al. Activity of selumetinib in neurofibromatosis type 1-related plexiform neurofibromas. N Engl J Med 2016;375:2550–60.

Dombi E, Solomon J, Gillespie AJ, Fox E, Balis FM, Patronas N, et al. NF1 plexiform neurofibroma growth rate by volumetric MRI: relationship to age and body weight. Neurology 2007;68:643–7.

Dumitrescu CE, Collins MT. McCune-Albright syndrome. Orphanet J Rare Dis 2008;3:12.

Fisher MJ, Shih CS, Rhodes SD, Armstrong AE, Wolters PL, Dombi E, et al. Cabozantinib for neurofibromatosis type 1-related plexiform neurofibromas: a phase 2 trial. Nat Med 2021;27:165–73.

Geller M, Ribeiro MG, Araújo AP, de Oliveira LJ, Nunes FP. Serum IgE levels in neurofibromatosis 1. Int J Immunogenet 2006;33:111–5.

Gitler AD, Zhu Y, Ismat FA, Lu MM, Yamauchi Y, Parada LF, et al. Nf1 has an essential role in endothelial cells. Nat Genet 2003;33:75–9.

Gresset A, Coulpier F, Gerschenfeld G, Jourdon A, Matesic G, Richard L, et al. Boundary caps give rise to neurogenic stem cells and terminal glia in the skin. Stem Cell Reports 2015;5:278–90.

Gross AM, Wolters PL, Dombi E, Baldwin A, Whitcomb P, Fisher MJ, et al. Selumetinib in children with inoperable plexiform neurofibromas [published correction appears in N Engl J Med 2020;383:1290]. N Engl J Med 2020;382:1430–42.

Gutmann DH. NF GEMMs already! The power and promise of mouse tumor models. Cancer Cell 2014;26:596–9.

Gutmann DH, Ferner RE, Listernick RH, Korf BR, Wolters PL, Johnson KJ. Neurofibromatosis type 1. Nat Rev Dis Primers 2017;3:17004.

Hirbe AC, Gutmann DH. Neurofibromatosis type 1: a multidisciplinary approach to care. Lancet Neurol 2014;13:834–43.

Ingram DA, Hiatt K, King AJ, Fisher L, Shivakumar R, Derstine C, et al. Hyperactivation of p21(ras) and the hematopoietic-specific Rho GTPase, Rac2, cooperate to alter the proliferation of neurofibromin-deficient mast cells in vivo and in vitro. J Exp Med 2001;194:57–69.

Ingram DA, Yang FC, Travers JB, Wenning MJ, Hiatt K, New S, et al. Genetic and biochemical evidence that haploinsufficiency of the Nf1 tumor suppressor gene modulates melanocyte and mast cell fates in vivo. J Exp Med 2000;191:181–8.

Jessen KR, Mirsky R. The origin and development of glial cells in peripheral nerves. Nat Rev Neurosci 2005;6:671–82.

Jessen WJ, Miller SJ, Jousma E, Wu J, Rizvi TA, Brundage ME, et al. MEK inhibition exhibits efficacy in human and mouse neurofibromatosis tumors. J Clin Invest 2013;123:340–7.

Jett K, Friedman JM. Clinical and genetic aspects of neurofibromatosis 1. Genet Med 2010;12:1–11.

Joseph NM, Mosher JT, Buchstaller J, Snider P, McKeever PE, Lim M, et al. The loss of Nf1 transiently promotes self-renewal but not tumorigenesis by neural crest stem cells. Cancer Cell 2008;13:129–40.

Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer 2006;6:392–401.

Klesse LJ, Jordan JT, Radtke HB, Rosser T, Schorry E, Ullrich N, et al. The use of MEK inhibitors in neurofibromatosis type 1-associated tumors and management of toxicities. Oncologist 2020;25:e1109–16.

Kolberg M, Høland M, Agesen TH, Brekke HR, Liestøl K, Hall KS, et al. Survival meta-analyses for >1800 malignant peripheral nerve sheath tumor patients with and without neurofibromatosis type 1. Neuro Oncol 2013;15:135–47.

Lammert M, Mautner VF, Kluwe L. Do hormonal contraceptives stimulate growth of neurofibromas? A survey on 59 NF1 patients. BMC Cancer 2005;5:16.

Laycock-van Spyk S, Thomas N, Cooper DN, Upadhyaya M. Neurofibromatosis type 1-associated tumours: their somatic mutational spectrum and pathogenesis. Hum Genomics 2011;5:623–90.

Le LQ, Liu C, Shipman T, Chen Z, Suter U, Parada LF. Susceptible stages in Schwann cells for NF1-associated plexiform neurofibroma development. Cancer Res 2011;71:4686–95.

Le LQ, Parada LF. Tumor microenvironment and neurofibromatosis type I: connecting the GAPs. Oncogene 2007;26:4609–16.

Le LQ, Shipman T, Burns DK, Parada LF. Cell of origin and microenvironment contribution for NF1-associated dermal neurofibromas. Cell Stem Cell 2009;4:453–63.

Legius E, Messiaen L, Wolkenstein P, Pancza P, Avery RA, Berman Y, et al. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation [e-pub ahead of print]. Genet Med 2021. https://doi.org/10.1038/s41436-021-01170-5 (accessed July 1, 2021).

Lehtonen A, Howie E, Trump D, Huson SM. Behaviour in children with neurofibromatosis type 1: cognition, executive function, attention, emotion, and social competence. Dev Med Child Neurol 2013;55:111–25.

Lennartsson J, Rönnstrand L. Stem cell factor receptor/c-Kit: from basic science to clinical implications. Physiol Rev 2012;92:1619–49.

Li PH, Liu LH, Chang CC, Gao R, Leung CH, Ma DL, et al. Silencing stem cell factor gene in fibroblasts to regulate paracrine factor productions and enhance c-Kit expression in melanocytes on melanogenesis. Int J Mol Sci 2018;19:1475.

Li S, Chen Z, Le LQ. New insights into the neurofibroma tumor cells of origin. Neurooncol Adv 2020;2:i13–22.

Liao CP, Booker RC, Brosseau JP, Chen Z, Mo J, Tchegnon E, et al. Contributions of inflammation and tumor microenvironment to neurofibroma tumorigenesis. J Clin Invest 2018;128:2848–61.

Liao CP, Pradhan S, Chen Z, Patel AJ, Booker RC, Le LQ. The role of nerve microenvironment for neurofibroma development. Oncotarget 2016;7:61500–8.

Lu T, Chen C, Wang A, Jiang Z, Qi Z, Hu Z, et al. Repurposing cabozantinib to GISTs: overcoming multiple imatinib-resistant cKIT mutations including gatekeeper and activation loop mutants in GISTs preclinical models. Cancer Lett 2019;447:105–14.

Mashour GA, Ratner N, Khan GA, Wang HL, Martuza RL, Kurtz A. The angiogenic factor midkine is aberrantly expressed in NF1-deficient Schwann cells and is a mitogen for neurofibroma-derived cells. Oncogene 2001;20:97–105.

Mautner VF, Asuagbor FA, Dombi E, Fünsterer C, Kluwe L, Wenzel R, et al. Assessment of benign tumor burden by whole-body MRI in patients with neurofibromatosis 1. Neuro Oncol 2008;10:593–8.

Meadows AT, Baum E, Fossati-Bellani F, Green D, Jenkin RD, Marsden B, et al. Second malignant neoplasms in children: an update from the Late Effects Study Group. J Clin Oncol 1985;3:532–8.

Miettinen MM, Antonescu CR, Fletcher CDM, Kim A, Lazar AJ, Quezado MM, 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;67:1–10.

Miller SJ, Jessen WJ, Mehta T, Hardiman A, Sites E, Kaiser S, et al. Integrative genomic analyses of neurofibromatosis tumours identify SOX9 as a biomarker and survival gene. EMBO Mol Med 2009;1:236–48.

Mo J, Anastasaki C, Chen Z, Shipman T, Papke J, Yin K, et al. Humanized neurofibroma model from induced pluripotent stem cells delineates tumor pathogenesis and developmental origins. J Clin Invest 2021;131:e139807.

Munchhof AM, Li F, White HA, Mead LE, Krier TR, Fenoglio A, et al. Neurofibroma-associated growth factors activate a distinct signaling network to alter the function of neurofibromin-deficient endothelial cells. Hum Mol Genet 2006;15:1858–69.

Nguyen R, Jett K, Harris GJ, Cai W, Friedman JM, Mautner VF. Benign whole body tumor volume is a risk factor for malignant peripheral nerve sheath tumors in neurofibromatosis type 1. J Neurooncol 2014;116:307–13.

Oliveira LB, Geller M, Cunha KS, Santos A, Bernacchi A, Rubenstein AE, et al. Clinical assessment of the use of topical liquid diclofenac following laser microporation of cutaneous neurofibromas in individuals with neurofibromatosis type 1. Heliyon 2021;7:e06518.

Ortonne N, Carroll SL, Rodriguez FJ, Miller DC, Nazarian RM, Blakeley JO, et al. Assessing interobserver variability and accuracy in the histological diagnosis and classification of cutaneous neurofibromass. Neurooncol Adv 2019;2(Suppl. 1):i117–23.

Ortonne N, Wolkenstein P, Blakeley JO, Korf B, Plotkin SR, Riccardi VM, et al. Cutaneous neurofibromas: current clinical and pathologic issues. Neurology 2018;91(Suppl. 1):S5–13.

Ozerdem U. Targeting neovascular pericytes in neurofibromatosis type 1. Angiogenesis 2004;7:307–11.

Pemov A, Hansen NF, Sindiri S, Patidar R, Higham CS, Dombi E, 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 Oncol 2019;21:981–92.

Pemov A, Li H, Patidar R, Hansen NF, Sindiri S, Hartley SW, et al. The primacy of NF1 loss as the driver of tumorigenesis in neurofibromatosis type 1-associated plexiform neurofibromas. Oncogene 2017;36:3168–77.

Pemov A, Li H, Presley W, Wallace MR, Miller DT. Genetics of human malignant peripheral nerve sheath tumors. Neurooncol Adv 2020;2(Suppl. 1):i50–61.

Philpott C, Tovell H, Frayling IM, Cooper DN, Upadhyaya M. The NF1 somatic mutational landscape in sporadic human cancers. Hum Genomics 2017;11:13.

Prada CE, Jousma E, Rizvi TA, Wu J, Dunn RS, Mayes DA, et al. Neurofibroma-associated macrophages play roles in tumor growth and response to pharmacological inhibition. Acta Neuropathol 2013;125:159–68.

Quirk B, Olasz E, Kumar S, Basel D, Whelan H. Photodynamic therapy for benign cutaneous neurofibromas using aminolevulinic acid topical application and 633 nm red light illumination. Photobiomodul Photomed Laser Surg 2021;39:411–7.

Radomska KJ, Coulpier F, Gresset A, Schmitt A, Debbiche A, Lemoine S, et al. Cellular origin, tumor progression, and pathogenic mechanisms of cutaneous neurofibromas revealed by mice with *Nf1* knockout in boundary cap cells. Cancer Discov 2019;9:130–47.

Ribeiro S, Napoli I, White IJ, Parrinello S, Flanagan AM, Suter U, et al. Injury signals cooperate with Nf1 loss to relieve the tumor-suppressive environment of adult peripheral nerve. Cell Rep 2013;5:126–36.

Robertson KA, Nalepa G, Yang FC, Bowers DC, Ho CY, Hutchins GD, et al. Imatinib mesylate for plexiform neurofibromas in patients with neurofibromatosis type 1: a phase 2 trial. Lancet Oncol 2012;13:1218–24.

Rodriguez FJ, Folpe AL, Giannini C, Perry A. Pathology of peripheral nerve sheath tumors: diagnostic overview and update on selected diagnostic problems. Acta Neuropathol 2012;123:295–319.

Roth TM, Petty EM, Barald KF. The role of steroid hormones in the NF1 phenotype: focus on pregnancy. Am J Med Genet A 2008;146A:1624–33.

Ryan JJ, Klein KA, Neuberger TJ, Leftwich JA, Westin EH, Kauma S, et al. Role for the stem cell factor/KIT complex in Schwann cell neoplasia and mast cell proliferation associated with neurofibromatosis. J Neurosci Res 1994;37:415–32.

Saito H, Yoshida T, Yamazaki H, Suzuki N. Conditional N-rasG12V expression promotes manifestations of neurofibromatosis in a mouse model. Oncogene 2007;26:4714–9.

Slopis JM, Arevalo O, Bell CS, Hebert AA, Northrup H, Riascos RF, et al. Treatment of disfiguring cutaneous lesions in neurofibromatosis-1 with everolimus: a phase II, open-label, single-arm trial. Drugs R D 2018;18:295–302.

Staser K, Yang FC, Clapp DW. Mast cells and the neurofibroma microenvironment. Blood 2010;116:157–64.

Tartaglia M, Gelb BD, Zenker M. Noonan syndrome and clinically related disorders. Best Pract Res Clin Endocrinol Metab 2011;25:161–79.

Theoharides TC, Conti P. Mast cells: the Jekyll and Hyde of tumor growth. Trends Immunol 2004;25:235–41.

Wegscheid ML, Anastasaki C, Gutmann DH. Human stem cell modeling in neurofibromatosis type 1 (NF1). Exp Neurol 2018;299:270–80.

Well L, Jaeger A, Kehrer-Sawatzki H, Farschtschi S, Avanesov M, Sauer M, et al. The effect of pregnancy on growth-dynamics of neurofibromas in neurofibromatosis type 1. PLoS One 2020;15:e0232031.

Williams KB, Largaespada DA. New model systems and the development of targeted therapies for the treatment of neurofibromatosis type 1-associated malignant peripheral nerve sheath tumors. Genes (Basel) 2020;11:477.

Wise JB, Cryer JE, Belasco JB, Jacobs I, Elden L. Management of head and neck plexiform neurofibromas in pediatric patients with neurofibromatosis type 1. Arch Otolaryngol Head Neck Surg 2005;131:712–8.

Wu J, Williams JP, Rizvi TA, Kordich JJ, Witte D, Meijer D, et al. Plexiform and dermal neurofibromas and pigmentation are caused by Nf1 loss in desert hedgehog-expressing cells. Cancer Cell 2008;13:105–16.

Wu M, Wallace MR, Muir D. Nf1 haploinsufficiency augments angiogenesis. Oncogene 2006;25:2297–303.

Yamamoto T, Hartmann K, Eckes B, Krieg T. Mast cells enhance contraction of three-dimensional collagen lattices by fibroblasts by cell-cell interaction: role of stem cell factor/c-kit. Immunology 2000;99:435–9.

Yang FC, Chen S, Clegg T, Li X, Morgan T, Estwick SA, et al. Nf1+/– mast cells induce neurofibroma like phenotypes through secreted TGF-beta signaling. Hum Mol Genet 2006;15:2421–37.

Yang FC, Ingram DA, Chen S, Hingtgen CM, Ratner N, Monk KR, et al. Neurofibromin-deficient Schwann cells secrete a potent migratory stimulus for Nf1+/– mast cells. J Clin Invest 2003;112:1851–61.

Yang FC, Ingram DA, Chen S, Zhu Y, Yuan J, Li X, et al. Nf1-dependent tumors require a microenvironment containing Nf1+/–- and c-kit-dependent bone marrow. Cell 2008;135:437–48.

Zheng H, Chang L, Patel N, Yang J, Lowe L, Burns DK, et al. Induction of abnormal proliferation by nonmyelinating Schwann cells triggers neurofibroma formation. Cancer Cell 2008;13:117–28.

Zhu Y, Ghosh P, Charnay P, Burns DK, Parada LF. Neurofibromas in NF1: Schwann cell origin and role of tumor environment. Science 2002;296:920–2.

Zhu Y, Harada T, Liu L, Lush ME, Guignard F, Harada C, et al. Inactivation of NF1 in CNS causes increased glial progenitor proliferation and optic glioma formation. Development 2005;132:5577–88.

Muir D, Perrin G, Neubauer D, Whelan H. Photodynamic therapy development using NF1 tumor xenografts. Paper presented at: Children's Tumor Foundation Conference, June 6-10, 2008; Bonita Springs, FL.