Advances in the treatment of neurofibromatosis-associated tumours

Andrew L. Lin and David H. Gutmann

Abstract | Neurofibromatosis (NF) comprises two distinct genetic disorders—neurofibromatosis type 1 and 2 (NF1 and NF2)—in which affected individuals develop both benign and malignant tumours. NF1 results from germline mutations in the *NF1* gene that encodes neurofibromin, while NF2 results from germline mutations in the *NF2* gene that encodes merlin (or schwannomin). The major tumour types arising in individuals with NF1 include neurofibromas, malignant peripheral nerve sheath tumours, and gliomas, whereas NF2 is characterized by the formation of schwannomas, meningiomas, and ependymomas. With the identification of the *NF1* and *NF2* genes and the generation of robust preclinical mouse models of NF-associated neoplasms, novel treatments that specifically target the growth control pathways deregulated in these tumours have been discovered, some of which are now being tested in clinical trials in individuals with NF1 and NF2. In this Review, we will highlight the key clinical features of NF1 and NF2 and the advances in future clinical management based on an improved understanding of the function of the *NF1* and *NF2* genes and the development of small-animal models.

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Introduction

Neurofibromatosis type 1 (NF1) and type 2 (NF2) represent distinct tumour predisposition syndromes that largely affect the nervous system. The major tumour types arising in individuals with NF1 are neurofibromas, malignant peripheral nerve sheath tumours, and gliomas, whereas individuals with NF2 are prone to the formation of schwannomas, meningiomas, and ependymomas.[1] While these disorders share some similarities, they are distinct conditions with unique genetic aetiologies and pathogenetic mechanisms.[2] The diagnosis of NF1 and NF2 is established using diagnostic criteria that was originally formulated by the National Institutes of Health Consensus Development panel in 1987,[3] and was critically re-evaluated for NF2 in 2002.[4]

NF1 is the more common of the two disorders, with a prevalence of approximately 1 in 2,500 to 3,000 worldwide.[5,6] By contrast, NF2 has a much lower birth incidence, with estimates ranging from 1 in 25,000 to 1 in 40,000.[7,8] Over half of individuals diagnosed with NF1 and NF2 carry *de novo* mutations and represent the first person in their family with the condition.[8,9] As NF1 and NF2 are autosomal dominant syndromes with complete penetrance, once an individual has received a diagnosis, that given individual typically harbours a 50:50 chance of transmitting the disorder to their offspring.[3] However, individuals with NF2 can also be mosaic for a mutation in the *NF2* gene, resulting in less than a 50% risk of transmission to their offspring.[10]

In a systematic review of death certificates, the mean age of death for individuals with NF1 was 54.4 years (versus 70.1 years in the general population) with death from malignancy disproportionately high among individuals that died before the age of 30.[11] Similarly, individuals with NF2 have a reduced life expectancy from disease-related causes. In a recent observational study, the estimated life expectancy for individuals with NF2 was 69.0 years compared to a life expectancy of 71.5 years for individuals with NF1 and 80.0 years for the general population.[12]

Prior to the 1990s, the treatment of NF-associated tumours was largely managed by surgeons and oncologists in an identical fashion to histologically-similar tumours arising in the general population. With the cloning of the *NF1* and *NF2* genes, the generation of preclinical mouse models for many of the NF-associated tumour types, and the establishment of a clinical trials consortium, there have been enormous advances in the therapeutic options available for affected individuals. In this Review, we discuss the clinical features, genetic aetiologies, and pathogenesis of neoplasia in NF1 and NF2. We also describe the current treatment options for patients with NF1 and NF2, and advances in clinical and preclinical science that will impact on the future treatment of these two hereditary tumour predisposition syndromes.

Clinical features of NF1

Individuals with NF1 have a predisposition to the development of Schwann cell neoplasms (neurofibromas, malignant peripheral nerve sheath tumours), gliomas (optic pathway gliomas, malignant gliomas), leukaemia, pheochromocytoma, and several other tumours (such as gastrointestinal stromal tumours, rhabdomyosarcoma, and breast cancer; Box 1 and Figure 1).[13–26]

Neurofibromas, the most common type of tumour associated with NF1, are benign peripheral nerve sheath

Competing interests
The authors declare no competing interests.

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Key points

- Neurofibromatosis type 1 and type 2 are distinct genetic disorders characterized by an increased incidence of tumour development
- Identification of the *NF1* and *NF2* genes has resulted in the discovery of new targets for therapeutic drug design
- Numerous small-animal models of NF1-associated and NF2-associated tumours have been developed
- Current investigational therapies for tumours arising in individuals with NF1 and NF2 target both deregulated growth control pathways as well as the tumour microenvironment

Box 1 | Tumours arising in individuals with NF1 and their frequency

Cutaneous neurofibroma: frequency 40–60%[13,14]
Internal nerve sheath tumour (intraneural and plexiform neurofibroma): frequency 60%[15]
Malignant peripheral nerve sheath tumour: frequency 8–13%[16]
Optic pathway glioma: frequency 15–20%[17,18]
Malignant glioma: frequency 0.8%[19,20]
Leukaemia: frequency <1%, although a 200 to 500-fold increased risk of JMML[20–22]
Pheochromocytoma: frequency 0.1–13%[23,24]
Rhabdomyosarcoma: frequency 1–6%[20,25]
GIST: frequency 5–30%[24]
Breast cancer: frequency 8.4% by age 50[26]

Abbreviations: GIST, gastrointestinal stromal tumour; JMML, juvenile myelomonocytic leukaemia; NF1, neurofibromatosis type 1.

tumours (PNSTs) that arise from Schwann cell progenitors, embedded in a microenvironment composed of perineural cells, fibroblasts, mast cells and a rich collagenous extracellular matrix (Figure 1a).[27] These benign PNSTs can occur on nerves anywhere in the peripheral nervous system. Cutaneous neurofibromas are the most common type of neurofibroma and can appear as nodular masses, peduncular lesions, or diffuse plaques. These tumours frequently begin to develop during early adolescence and continue to increase in number throughout adulthood.[27] Importantly, cutaneous neurofibromas do not transform into malignant PNSTs (MPNSTs).[27,28] Internal PNSTs are also very common and are present in as many as 60% of individuals with NF1,[15] where they can present as intraneural or plexiform neurofibromas. Intraneural neurofibromas are fusiform expansions of a peripheral nerve, typically involving the spinal roots, which frequently develop a dumbbell appearance as they enlarge.[29] By contrast, plexiform neurofibromas involve multiple fascicles of a nerve or a plexus and can extend down its branches (Figure 1b). Hence, plexiform neurofibromas may feel like a 'bag of worms' on palpation. These tumours are usually detected in young children and grow most rapidly during the first decade of life.[30] These more diffuse PNSTs can exert a mass effect, compressing nearby structures (such as the trachea or blood vessels), and either stimulate bone growth or lead to bone erosion. PNSTs can also cause spinal cord compression, weakness, cranial neuropathy, disfigurement, and pain.[30] Moreover, these tumours have a rich vascular network and can bleed profusely, especially during surgery.

Unlike cutaneous neurofibromas, plexiform neurofibromas can undergo malignant transformation into MPNSTs—an aggressive spindle-cell sarcoma. The lifetime risk for individuals with NF1 of developing a MPNST is between 8% and 13%, with a peak incidence in their mid-30s.[16] MPNSTs are difficult to treat and can be widely metastatic to the lungs, soft tissue, and bone.[31] Unfortunately, current therapies have shown little long-term benefit, and most individuals succumb to these cancers within 5 years following diagnosis.

Individuals with NF1 are also prone to the development of gliomas, most commonly pilocytic astrocytomas (WHO grade I) involving the optic pathway (optic pathway glioma [OPG]; Figure 1c).[32] These tumours are predominantly observed in children younger than 7 years of age, with a mean age at presentation of 4.2 years.[17] They can affect one or both optic nerves, the chiasm, or the postchiasmal optic tracks.[17,33,34] Bilateral optic nerve involvement is common in children with NF1-OPG. Although the incidence of OPG in patients with NF1 is 15–20%, fewer than half of these tumours cause symptoms.[17,18,35] When symptomatic, children typically present with decreased vision or precocious puberty secondary to tumour infiltration of the hypothalamus.[36]

In addition to OPGs, gliomas can develop in the brainstem. Brainstem gliomas occur with an estimated lifetime incidence of 4% or higher, and typically become symptomatic in children younger than 10 years of age.[37,38] Many of these tumours occur in the medulla with occasional extension into the pons. They can result in brainstem dysfunction, including dysarthria, cranial neuropathies and incoordination, and can compromise the ventricular system, resulting in hydrocephalus and headache.[37–39] Most often, these tumours are pilocytic astrocytomas or, less commonly, grade II astrocytomas. In rare circumstances, cerebellar gliomas are observed, but when detected, these are typically pilocytic astrocytomas, which are usually asymptomatic at the time of diagnosis.[40]

Although the majority of brain tumours in this patient population are low-grade gliomas (astrocytomas) that occur during the first decade of life, adults with NF1 are at higher risk of developing high-grade tumours (WHO grade III and IV). Compared to the general population, adults with NF1 are 50 to 100 times more likely to develop a symptomatic non-optic pathway brain tumour.[19] The majority of these tumours arise in the cerebral hemispheres (more frequently within cortical than subcortical areas), and only a minority are pilocytic astrocytomas. In this regard, 40% of non-optic pathway tumours reported in one cohort were grade II, 27% were grade III, and 13% were grade IV.[19] The high-grade tumours (WHO grade III and IV) occurred in patients older than 20 years of age.[19]

Other less-common cancers that occur in individuals with NF1 include leukaemia (juvenile chronic myelogenous leukaemia or myelodysplastic syndrome), rhabdomyosarcoma, gastrointestinal stromal tumours, and pheochromocytoma.[21–24] Pheochromocytomas deserve particular mention owing to their association with unexplained hypertension in this at-risk population.[23] These endocrine tumours generate excess catecholamines, leading to dramatic changes in blood pressure. It has also been shown that women younger than 50 years of age with NF1 have a fivefold increased risk of breast cancer.[26] Secondary malignancies, such as MPNST or malignant

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glioma, are also reported in individuals with NF1, typically as a result of ionizing radiation administered to treat another cancer earlier in life.[41,42]

Clinical features of NF2

Tumours arising in individuals with NF2 are distinct from those seen in NF1 (Box 2, Figure 2). Vestibular schwannomas (VS) are a hallmark of NF2 and are present in >95% of individuals with this condition (Box 2; Figure 2a).[43,44] Previously called acoustic neuromas, the name of these neoplasms was changed to reflect their Schwann cell lineage origins and the primary involvement of the vestibular, rather than the cochlear, branch of the eighth cranial nerve.[45] Although the vast majority of unilateral VS occur sporadically, it is rare for an individual without NF2 to develop bilateral VS.[7] Histologically, NF2-associated VS tend to be more lobular and less vascular relative to their sporadic counterparts, and become symptomatic earlier in life.[43,46] In individuals with NF2, 44% of affected persons who become symptomatic as adults present with symptoms attributable to VS, including hearing loss, tinnitus, and vestibulopathy.[43] Over time, progressive growth of these cranial nerve tumours can cause brainstem compression. When individuals with NF2 present as children, they are less likely to become symptomatic from an underlying VS; rather, they are more likely to become symptomatic as a result of mass effect from an intracranial or spinal cord lesion, a peripheral nerve tumour, or a cutaneous lesion.[47]

Over their lifetime, 24 to 51% of individuals with NF2 will develop a schwannoma of another cranial nerve in addition to VS, most commonly along the fifth, seventh, ninth, or twelfth cranial nerves.[48,49] Schwannomas also develop near the spinal cord and along peripheral nerves, resulting in peripheral neuropathies, appearing as cutaneous tumours in 59–68% of individuals with NF2.[43,48] Schwannomatosis[50] is a syndrome that shares some clinical features with NF2, but is not caused by germline mutations in the *NF2* gene. Instead, these tumors arise in individuals with mutations in the *SMARCB1* tumour suppressor gene.[51] Individuals with this condition can also develop multiple schwannomas, but in contrast to individuals with NF2, they rarely develop VS.[52]

Meningiomas occur in 45–58% of persons with NF2 (Box 2, Figure 2b).[43,48] NF2-associated meningiomas tend to occur at an earlier age than sporadic meningiomas (during the third and fourth decade of life versus the middle of the sixth decade), and individuals with NF2 are prone to the development of multiple meningiomas.[43,53] Meningiomas can affect the spinal cord and are difficult to distinguish from other spinal tumours arising in individuals with NF2. They may develop along the optic nerve, which can appear on neuroimaging studies as optic gliomas arising in the context of NF1.[54]

Finally, ependymomas are also observed in persons with NF2. These are intramedullary glial cell neoplasms most often involving the cervical cord (Box 2, Figure 2c).[55,56] The frequency of these tumours in the NF2 population is unclear; however, estimates range from 33% to 53% of affected individuals.[56] Fortunately, only a minority of these tumours cause symptoms and require treatment.

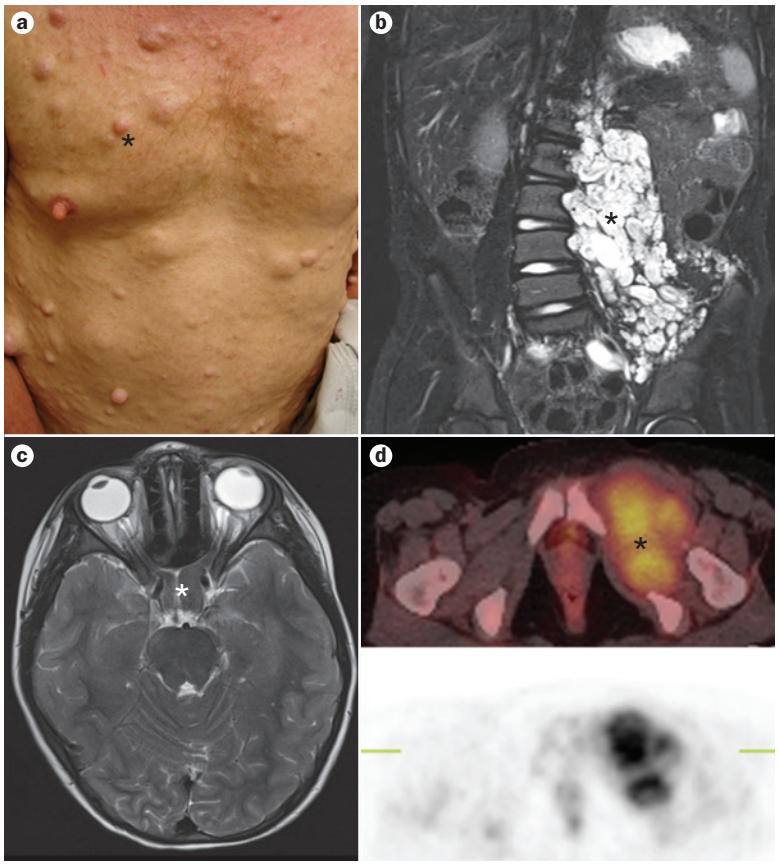

Figure 1 | Tumours arising in individuals with NF1. **a** | Cutaneous neurofibromas on the chest in an adult male with NF1. The asterisk denotes a representative cutaneous neurofibroma. **b** | MRI shows a large left intra-abdominal plexiform neurofibroma in a young adult with NF1. The asterisk denotes the tumour mass. **c** | MRI shows a chiasmal optic glioma in a child with NF1. The asterisk denotes the tumour. **d** | A mass with increased radioligand tracer (fluorodeoxyglucose) uptake visualized by PET, denoted by the asterisk. This pelvic mass was biopsied and found to be a MPNST. Abbreviations: MPNST, malignant peripheral nerve sheath tumour; NF1, neurofibromatosis type 1.

Box 2 | Tumour frequency arising in individuals with NF2

Vestibular schwannoma: frequency >95%[43,44]
Other cranial nerve schwannoma: frequency 24–51%[48]
Cutaneous schwannoma: frequency 59–68%[43,48]
Intracranial meningioma: frequency 50%[43]
Peripheral nerve schwannoma: frequency 42%[15]
Ependymoma: frequency 33–53%[56]
Mesothelioma: frequency rare, associated with asbestos exposure[124,125]
Malignant schwannoma: frequency rare[126]

Abbreviation: NF2, neurofibromatosis type 2.

Genetics of NF1 and NF2

The genes mutated in *NF1* and *NF2* are tumour suppressor genes, which encode for proteins involved in divergent signalling pathways (Figure 3). While individuals with NF1 and NF2 start life with one functional copy of *NF1* and *NF2* genes in every cell of their bodies (with the exception of those with mosaic forms of NF1 or NF2), NF1-associated and NF2-associated tumours exhibit total loss of the protein products encoded by the respective genes.[57–59]

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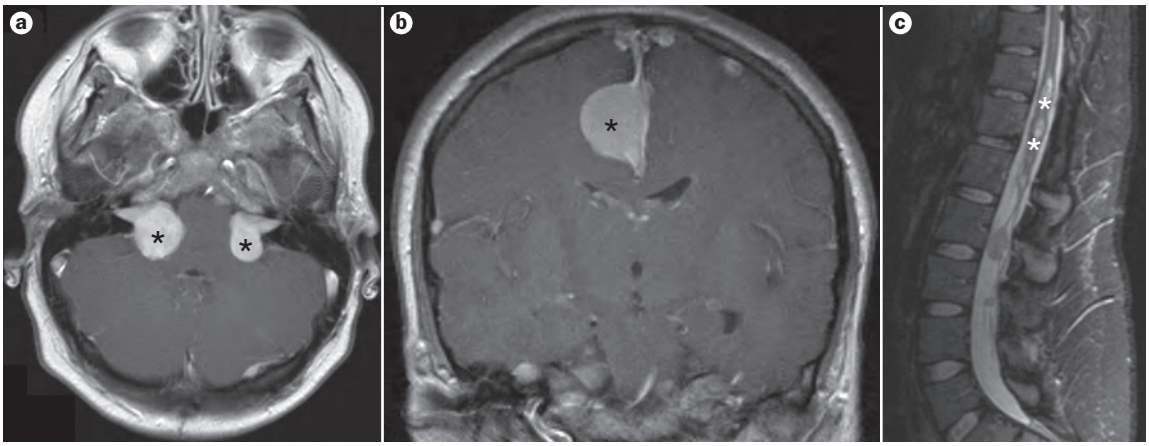

Figure 2 | Tumours arising in individuals with NF2. **a** | MRI shows bilateral vestibular schwannomas (asterisks) in a teenage girl with NF2. **b** | MRI shows a meningioma (asterisk) in a young girl with NF2. **c** | MRI shows multiple intraparenchymal spinal tumours, most likely ependymomas, in a young woman with NF2. Abbreviation: NF2, neurofibromatosis type 2.

The *NF1* gene resides on chromosome 17 and codes for neurofibromin. Neurofibromin is a 220 kDa cytoplasmic protein containing a region of 300 amino acids with significant homology to domains found in GTPase-activating proteins (GAPs).[60–62] GAPs comprise a family of proteins that function as negative regulators of the *RAS* proto-oncogene, an important regulator of cell proliferation, differentiation, apoptosis and migration.[63] Ras cycles between one of two states—an active GTP-bound conformation and an inactive GDP-bound conformation. Interaction with neurofibromin accelerates the intrinsic GTPase activity of Ras, leading to Ras inactivation and reduced cell growth (Figure 3).[62,64,65] In NF1-associated tumours, loss of neurofibromin results in high levels of activated Ras, leading to increased cell growth.[62,66,67] *NF1* loss leads to hyperactivation of the downstream effector proteins that transduce Ras signalling, including the mammalian target of rapamycin (mTOR) and mitogen-activated kinase kinase (MEK) signalling intermediates.[68,69] In addition to its role in the negative regulation of Ras, neurofibromin is a positive regulator of adenylyl cyclase, the enzyme responsible for the generation of intracellular cyclic AMP (cAMP).[70,71] In some cell types, including neurons, reduced neurofibromin levels leads to decreased intracellular cAMP levels and attenuated cell survival.[72]

The *NF2* tumour suppressor gene is located on human chromosome 22q and encodes another cytoplasmic protein called merlin (or schwannomin). Merlin is a 595-amino-acid protein with structural similarity to proteins of the Band 4.1 family.[73,74] The shared band 4.1 domain spans 300 amino acids at the N-terminus of merlin, where it likely facilitates interactions with membrane-associated molecular partners, including SCHIP1 and fodrin (β2-spectrin).[75] Merlin regulates the activation of several growth factor receptors (the EGFR and HER2 receptors) and suppresses the Rac1, mTOR, and Hippo/YAP intracellular mitogenic signalling pathways.[76–79] Loss of merlin results in the increased activation of a number of these growth control signalling pathways (Figure 3), although there is currently no consensus as to which pathway is most important for tumorigenesis in any given tissue.

Treatment of NF-associated neoplasms

Until recently, treatments for tumours arising in individuals with NF1 and NF2 were similar to those employed for histologically-similar tumours encountered in the general population. In the case of NF1, current therapies for cutaneous neurofibromas that cause disfigurement include surgery and, in some instances, CO_2 laser treatment or electrodessication.[80] Plexiform neurofibromas are largely debulked when clinically indicated, although their infiltrative nature presents significant challenges during surgery, and some patients will experience nerve damage or significant haemorrhage.[30] As a result of advances in the understanding of NF1 at the molecular and cellular level, new investigational agents are now being evaluated in clinical trials (Table 1). One such promising therapeutic agent is imatinib, which was recently shown in a small phase II study to reduce plexiform neurofibroma growth by ≥20% in 17% of treated individuals.[81] These individuals exhibiting therapeutic responses harboured plexiform neurofibromas in the neck and pelvic regions, and these results prompted a planned clinical trial focused specifically on imatinib treatment in this subgroup of NF1-affected individuals.

Malignant transformation of plexiform neurofibromas into MPNSTs is a significant problem in individuals with NF1 and is a leading cause of death. Individuals with NF1-associated plexiform neurofibromas must be monitored for a change in tumour growth and for signs and symptoms of transformation, including the development of pain, neurological deficit (weakness), or constitutional symptoms (weight loss, night sweats). While MRI can define the anatomic location and extent of a PNST, it does not provide accurate information regarding malignant transformation.[82] FDG-PET is a helpful imaging modality for distinguishing benign PNSTs from malignant PNSTs (Figure 1d).[83] In several studies, metabolically-active tumours with FDG standard uptake values greater than 4 were most often MPNSTs.[84,85] Likewise, chest CT scans are useful for detection of metastatic disease, particularly when tumours spread to the lungs.[86] Treatment of a MPNST involves surgical resection with subsequent

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radiotherapy and chemotherapy. The best outcomes are achieved following radical excision of the tumour with wide surgical margins.[31] When chemotherapy is employed, doxorubicin and ifosfamide have been shown to be effective; however, there is currently no standard care for these deadly cancers.[87] Unfortunately, long-term survival is rare when MPNST occurs in patients with NF1 because of lung and bone metastases as well as local tumour recurrence.[88]

Another important consideration when managing patients with NF1 is the occurrence of OPG. Since the majority of NF1-associated OPGs are asymptomatic, surveillance in children younger than 12 years of age entails annual examinations by an experienced ophthalmologist. Age-appropriate visual screening tools should be employed, since vision loss typically occurs in young preverbal children.[89] Screening neuroimaging is not recommended, as early identification of an OPG does not improve clinical outcome.[17,35] When a patient with NF1-associated OPG exhibits clinical progression, as evidenced by declining vision or precocious puberty, treatment with chemotherapy is usually initiated. The typical first-line treatment is combination therapy with carboplatin and vincristine, although other alkylating agents (such as vinblastine) are sometimes used.[89] Surgery is usually reserved for individuals with unilateral optic gliomas causing proptosis and a blind eye. Radiation therapy is not employed in individuals with NF1 because of the increased risk for radiation-induced secondary malignancies, the majority of which are high-grade gliomas. A retrospective study found a threefold increase in the relative risk of developing a second nervous system malignancy among 18 patients with optic gliomas treated with radiotherapy compared to 40 patients who did not receive radiation.[42]

The treatment of NF2-associated neoplasms predominantly entails conservative monitoring and, when appropriate, surgical intervention.[90,91] In the case of VS, the surgeon aims to achieve a complete resection; unfortunately, this is often not possible owing to adherence of the tumour to the facial nerve or, rarely, to the brainstem, and instead, deliberate incomplete resections may be performed to preserve nerve function. The decision to operate requires the coordinated efforts of multiple medical services (otolaryngology, neurosurgery, medical oncology) to best determine when surgery is most likely to preserve hearing and result in minimal potential secondary consequences. In some centres, VS may also be treated with stereotactic radiosurgery. Hearing preservation has been reported in 40% of patients with NF2 3 years after radiosurgery to treat VS.[92] However, similar to conventional surgery, radiosurgery can also result in facial and trigeminal cranial nerve dysfunction.[93] Bevacizumab, an anti-VEGF monoclonal antibody, has emerged as a first-line treatment for VS in individuals with NF2. In the largest case series to date, 57% of individuals had a hearing response (improvement in word recognition), and 55% exhibited a radiographic response (20% decrease in tumour volume) following bevacizumab treatment. At 3 years, 61% had stable or improved hearing and 54% had stable or decreased tumour size.[94] A phase II clinical trial (NCT01767792) is currently underway to further evaluate the use of bevacizumab for this indication.[95] In another study, adults and children with NF2 and progressive VS were treated with lapatinib, a dual inhibitor of EGFR and HER2, with some success. In this study of 17 evaluable

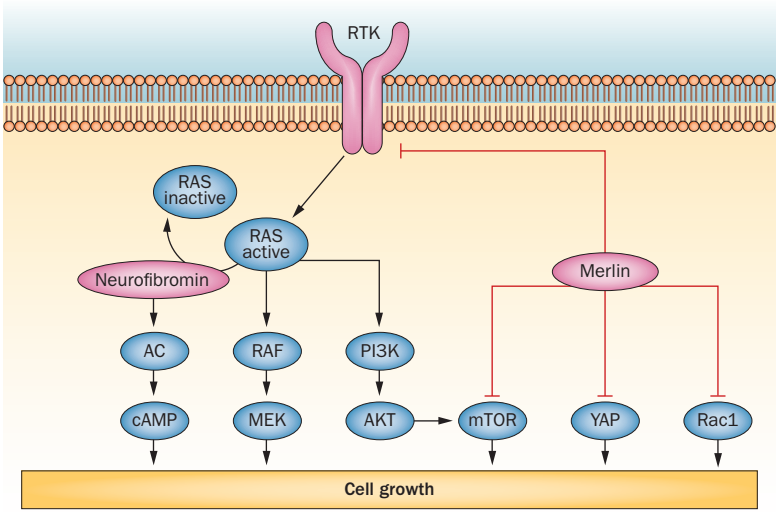

Figure 3 | Neurofibromin and merlin growth control pathways. Neurofibromin accelerates the conversion of active Ras to inactive Ras. Ras in its active conformation increases cell growth by activating PI3K/AKT/mTOR signalling as well as increased RAF kinase and MEK signalling. In addition, neurofibromin functions as a positive regulator of AC to increase cAMP levels and inhibit cell growth. Merlin has been implicated as a negative regulator of Rac1, mTOR, and Hippo/YAP signalling, the activation of which leads to increased cell growth. Abbreviations: AC, adenylyl cyclase; AKT, protein kinase B; cAMP, cyclic adenosine monophosphate; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; RTK, receptor tyrosine kinase; YAP, yes-associated protein.

Table 1 | Clinical trials for NF-associated tumours

Drug	Cellular or molecular target	Reference
Neurofibromatosis type 1 (plexiform neurofibroma)		
Thalidomide	Angiogenesis	Gupta *et al*. (2003)[127]
13-cis-retinoic acid (CRA) or interferon α-2a	Differentiation, angiogenesis	Packer *et al*. (2002)[128]
Tipifarnib (I, II)	Farnesyltransferase inhibitor	Widemann *et al*. (2006)[129]
Pirfenidone	Tumour-associated fibroblasts	Babovic-Vuksanovic *et al*. (2006),[130] Babovic-Vuksanovic *et al*. (2007)[131]
Photodynamic therapy	Tumour tissue disruption and pro-inflammatory	Kissil *et al*. (2010)[132]
Sirolimus	mTOR	Widemann *et al*. (2010)[133]
Pegylated-interferon α-2b	Immune modulator and angiogenesis	Jakacki *et al*. (2011)[134]
Sorafenib	Raf, PDGFRβ, c-kit,VEGFR2	Kim *et al*. (2013)[135]
Imatinib	c-kit, PDGFRβ	Robertsen *et al*. (2012)[81]
Erlotinib	EGFR	Albritton *et al*. (2006)[136]
Dasatinib	Dual inhibitor of Src/Abl and multiple other kinases	Schuetze *et al*. (2009)[137]
Topical rapamycin	mTOR	Koenig *et al*. (2012)[138]
Neurofibromatosis type 2 (vestibular schwannoma)		
Lapatinib	EGFR/ErbB2	Karajannis *et al*. (2012)[96]

Abbreviation: mTOR, mammalian target of rapamycin.

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Table 2 | Genetically-engineered mouse models of NF-associated tumours

Tumour type	Genetic strain	Reference
NF1 optic glioma	*Nf1*$^{flox/mut}$; GFAP-Cre *Nf1*+/-; LSL-KRas; GFAP-Cre mice	Bajenaru *et al*. (2003)[100] Zhu *et al*. (2005)[101] Dasgupta *et al*. (2005)[102]
NF1 malignant glioma	*Nf1*$^{flox/flox}$; *p53*$^{flox/flox}$; *Pten*$^{flox/flox}$ mice *Nf1*$^{flox/flox}$; *p53*$^{flox/flox}$ mice	Kwon *et al*. (2008)[139] Zhu *et al*. (2005)[140]
NF1 cutaneous neurofibroma	*Nf1*$^{flox/mut}$; PLP-Cre^{ER} mice	Mayes *et al*. (2011)[97]
NF1 plexiform neurofibroma	*Nf1*$^{flox/flox}$; Plp-Cre mice *Nf1*$^{flox/flox}$; Dhh-Cre mice *Nf1*$^{flox/mut}$; Krox20-Cre mice	Mayes *et al*. (2011)[97] Wu *et al*. (2008)[98] Zhu *et al*. (2002)[99]
NF1 MPNST	*Nf1*+/-; *p53*+/- mice	Cichowski *et al*. (1999)[103] Vogel *et al*. (1999)[104]
NF1 leukaemia	*Nf1*$^{flox/flox}$; Msx1-Cre mice	Le *et al*. (2004)[105]
NF1 pheochromocytoma	*Nf1*+/- mice	Tischler *et al*. (1995)[141]
NF2 schwannoma	*Nf2*$^{flox/flox}$ mice +Ad-Cre *Nf2*Δ^{f2-Cre}; P0-Cre mice	Giovannini *et al*. (2000)[106] Giovannini *et al*. (1999)[107]
NF2 meningioma	*Nf2*$^{flox/flox}$; PGDS-Cre mice *Nf2*$^{flox/flox}$ mice +Ad-Cre	Kalamarides *et al*. (2011)[108] Kalamarides *et al*. (2002)[109]
NF2 mesothelioma	*Nf2*$^{flox/flox}$; *p16*$^{flox/flox}$ mice +Ad-Cre *Nf2*$^{flox/flox}$; *p53*$^{flox/flox}$ mice +Ad-Cre	Jongsma *et al*. (2008)[142]

Abbreviations: MPNST, malignant peripheral nerve sheath tumour; NF1, neurofibromatosis type 1; NF2, neurofibromatosis type 2.

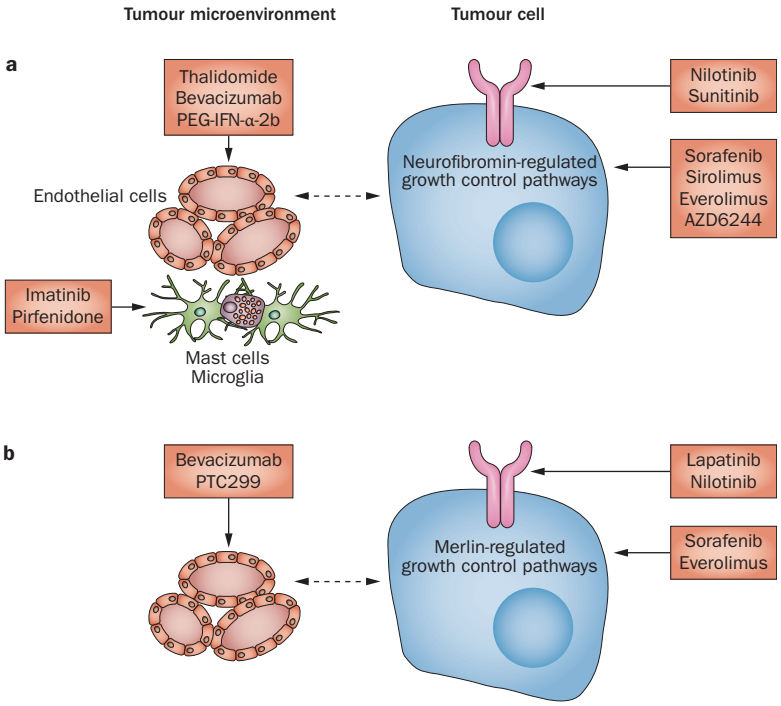

Figure 4 | Targets for NF therapeutic drug design. **a** | Therapies that target either the tumour microenvironment (such as endothelial cells, mast cells, microglia) or the abnormal neurofibromin-controlled growth regulatory pathways in *NF1*-deficient cancer cells have been developed for evaluation in both preclinical mouse models and in human clinical trials. **b** | Similarly, therapies that target either the tumour microenvironment (endothelial cells) or the abnormal merlin-controlled growth regulator pathways in *NF2*-deficient cancer cells have been developed for evaluation in both preclinical mouse strains and in human clinical trials. Abbreviation: PEG-IFN-α-2b, pegylated-interferon alpha-2b.

patients, fewer than 25% of treated patients experienced objective volumetric decreases in tumour volume, and 30% of those patients with radiographic responses met hearing criteria for response.[96]

Surgery is the primary treatment modality for symptomatic and progressively enlarging NF2-associated ependymomas, non-vestibular schwannomas, and meningiomas. Mortality following surgery for meningiomas has been estimated to be two-to-three times higher in individuals with NF2 compared to the general population, since NF2-associated meningiomas tend to be larger at the time of surgery.[90] Effective medical treatments for meningiomas are not yet available, although treatment with stereotactic radiosurgery and biologically-based therapies are currently being explored for these tumours in early phase studies.

Advances in NF therapeutics

One of the most exciting advances in the field of NF clinical therapeutics was the development and intelligent use of mouse models of NF-associated malignancy. Over the past decade, numerous genetically-engineered mouse (GEM) strains have been developed and used in preclinical therapeutic studies of NF (Table 2). The availability of robust preclinical mouse models not only provides an experimental platform to discover new therapeutic targets for drug design, but also enables the rapid evaluation of new classes of compounds prior to testing in human clinical trials.

GEM strains for NF1-associated cutaneous neurofibroma, plexiform neurofibroma, optic glioma, MPNST, and leukaemia have been developed.[97–105] Similarly, mouse models of NF2-associated schwannoma and meningioma have also been generated.[106–109] While each of these models has their limitations, they have provided instructive information to guide the design and execution of human clinical trials.[110] For example, *Nf1* GEM strains of optic glioma and plexiform neurofibroma have revealed the critical role of non-neoplastic stromal cells (tumour microenvironment) in cancer maintenance. In these studies, mice lacking *Nf1* expression in Schwann cell or astroglial cell precursors alone do not develop tumours; however, *Nf1*+/- mice (genetically similar to individuals with NF1) with loss of *Nf1* expression in Schwann cell or astroglial cell precursors form neurofibromas and optic gliomas, respectively.[99,100,111] Other studies have established that the mast cell is an important stromal cell type in mouse plexiform neurofibromas as they provide chemokines and growth factors critical for maintaining tumour growth.[112] The identification of mast cells as microenvironmental drivers of plexiform neurofibroma growth led to the evaluation of imatinib, which inhibits c-kit, in preclinical *Nf1* mouse studies. These studies have now led to human clinical trials of imatinib for plexiform neurofibromas in adults with NF1.[81]

Results from other GEM studies support future clinical trials using sorafenib, rapamycin analogues (for example, everolimus), and MEK inhibitors for NF1-associated plexiform neurofibroma,[69,113] rapamycin analogues and chemokine receptor inhibitors for NF1-associated glioma

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and MPNST,[114–117] and the tyrosine kinase inhibitors nilotinib and lapatinib for NF2-associated VS.[118,119]

While GEM models have great potential for clinical translation, it is important to establish standards for the interpretation of results from mouse preclinical studies. Attention should be paid to the number of mice exhibiting radiographic responses, the durability of these responses, and the magnitude of tumour shrinkage. Pharmacokinetic and pharmacodynamic effects in these mouse models should also be taken into consideration when designing clinical trials.

A second, but equally critical, advance in NF clinical therapeutics was the establishment of the Department of Defense-sponsored Neurofibromatosis Clinical Trials Consortium (NFCTC).[120] This consortium was developed to address the issues associated with conducting efficient therapeutic trials in NF where there is inherent clinical variability between patients and insufficient numbers of affected individuals at any one single institution. The mission of the NFCTC is to create an infrastructure to accelerate the development and completion of biologically-informed, statistically-sound translational clinical trials for adults and children with NF1 and NF2. To date, this consortium has initiated several clinical trials, including sorafenib for NF1-associated plexiform neurofibromas (NCT00727233),[121] bevacizumab and everolimus for MPNST (NCT01661283),[122] and everolimus for progressive NF1-associated glioma (NCT01158651).[123] Future early phase studies will entail the critical evaluation of bevacizumab for NF2-associated VS and additional Ras effector targeted therapies for NF1-associated plexiform neurofibroma.[120]

Conclusions

The tumours that develop in individuals with NF1 and NF2 predisposition syndromes are molecularly and cellularly heterogeneous neoplasms, representing complex cancers in which distinct cell types and control pathways contribute to continued tumour growth (Figure 4). With the development of robust preclinical mouse models for many of the common tumour types in NF1 and NF2, coupled with the availability of a clinical trials infrastructure for rapid drug evaluation in people, unique opportunities for translational medicine have emerged, which will increasingly inform the future management of patients with NF.

Review criteria

Information for this Review was compiled by searching the PubMed database for articles published before March 2013. Search terms included "neurofibromatosis", "NF1", and "NF2". Full articles were reviewed for additional material when appropriate, and articles that cited key references were also reviewed. Relevant clinical trials were identified by searching http://clinicaltrials.gov in the same manner.

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Author contributions

Both authors researched the data for the article, made substantial contributions to the discussion of the content, wrote the article and reviewed and edited it prior to submission.

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