Drugs of the Future 2007, 32(4): 375-384

CCC: 0377-8282/2007
DOI: 10.1358/dof.2007.032.04.1087933

Neurofibromatosis type 1

Elisa Ferrer, Mª Àngels Moral, Jordi Bozzo
Prous Science, P.O. Box 540, 08080 Barcelona, Spain

CONTENTS

Abstract

Neurofibromatosis type 1 (NF1) is a common genetic disorder characterized by the development of cutaneous manifestations and tumors that grow along nerves in the skin and other parts of the body. It is caused by mutations in a single gene encoding for neurofibromin, which acts as a tumor suppressor. NF1 currently has no cure and treatment is aimed at alleviating the symptoms. However, genetic studies and increasingly sophisticated animal models have allowed a better understanding of the molecular mechanisms underlying this disease. Targeted therapies have shown promise in preclinical studies and are gradually being assessed in human clinical trials. This article will review recent progress in preclinical research, with particular emphasis on targets for therapeutic intervention, and summarize the current status of clinical studies assessing potential therapies for NF1.

Introduction

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder, first described in the late 1800s by Friedrich von Recklinghausen, which affects the skin and nervous system. It was long confused with neurofibromatosis type 2 (NF2), which in fact is a much rarer disorder featuring a different clinical phenotype, consisting mainly of bilateral tumors on the eighth cranial nerve. The prevalence of NF1 is 25/100,000 cases (1) and its clinical presentation is highly variable, ranging from mild cutaneous signs to life-threatening nerve sheath tumors. The symptoms of NF1, especially skin manifestations, are usually present at birth or appear during infancy, and its severity increases with age. There is currently no cure for NF1 and treatment is only palliative and aimed at managing symptoms. However, increasing knowledge of the molecular pathophysiology of NF1 gathered from animal models is providing clues for the development of potential targeted therapies. Here, we review recent discoveries regarding the pathogenesis of NF1, with an emphasis on potential drug targets and experimental strategies under preclinical or clinical investigation.

Clinical manifestations

The clinical phenotype of NF1 consists essentially of nontumor and tumor manifestations. The most common type of tumor is the neurofibroma, composed predominantly of Schwann cells, as well as fibroblasts, pericytes and mast cells. Neurofibromas originate in the nerve sheath and can be focal or may spread along peripheral nerves. They appear as cutaneous or subcutaneous neurofibromas and tend to manifest during late childhood or adolescence. Cutaneous neurofibromas can also occur in non-NF1 patients. Nodular plexiform or diffuse plexiform neurofibromas are exclusive of NF1 and can be highly invasive and disfiguring (2). The presence of plexiform neurofibromas can prompt the development of malignant peripheral nerve sheath tumors (MPNSTs), which are usually painful and associated with a poor prognosis and survival rate (3).

NF1 patients are prone to develop tumors of different origin, such as optic pathway gliomas, a form of astrocytoma found in 15% of patients. The growth of optic gliomas may lead to visual impairment, but otherwise patients may be asymptomatic (2, 4). A growing number of gastrointestinal tumor (GIST) cases in adult patients with NF1 has been identified in recent years. Unlike sporadic GISTs, NF1-associated GISTs may not be related to mutations in the receptor tyrosine kinase *KIT* or platelet-derived growth factor receptor α (*PDGFRA*) genes and their development may be caused by abnormal mitogen-activated protein kinase (MAPK) activation due to an *NF1* gene mutation (5).

Among nontumor manifestations, cutaneous café-au-lait macules are the most common and can be found in 95% of NF1 patients. These flat pigmented skin lesions are oval or rounded spots of at least 0.5 cm in diameter that appear in early childhood (3, 4). Freckling is also very

characteristic of NF1 (present in 90% of patients) and appears in inguinal and axillary areas (2). In general, NF1 is diagnosed when two or more of the following clinical criteria are present: café-au-lait spots, neurofibromas, skin fold freckling, iris Lisch nodules (benign iris proliferation of melanocytes and fibroblasts) and bone lesions (dysplasia). Mutation tests are used to corroborate the diagnosis.

Frequently, neurological complications appear to be associated with the NF1 phenotype, with learning disabilities, attention deficit and visual-spatial skill deficits being fairly common in children with NF1 (6). Bone complications may arise from the mechanical pressure exerted by neurofibromas, which can demineralize and debilitate adjacent bone structures. Scoliosis is relatively frequent among skeletal complications, occurring in around 10% of NF1 patients. Dysplastic lesions (sphenoid dysplasia) have also been reported. Both can be severely disfiguring (2, 4). Dysplasia can also affect the vascular system, leading to cardiovascular complications such as essential hypertension, which may lead to premature death (3).

Pathophysiology

NF1 is caused by a mutation in a single gene located on chromosome 17 (locus 17q11.2). Although NF1 is inherited in autosomal dominant fashion, up to 50% of NF1 cases may originate from spontaneous mutations. The disease is associated with germline mosaicism (*i.e.,* the co-existence in a person of normal and mutated cell populations), whereby mutations in parent germ cells may spread the disease to the offspring while parents are phenotypically normal. If mutations affect cells other than gametes, somatic mosaicism may also occur, usually featuring a milder phenotype (7).

Neurofibromin is the protein product of the *NF1* gene and negatively regulates cellular proliferation and differentiation. Neurofibromin contains a central small amino acid domain, namely the GTPase-activating protein (GAP)-related domain, which targets the intracellular small G-protein Ras, involved in different cell processes such as cell proliferation, survival and differentiation. Neurofibromin GAP acts as a negative regulator by mediating the conversion from active GTP-bound Ras protein (Ras-GTP) to the inactive form (Ras-GDP), as it accelerates the intrinsic rate of Ras-GTP hydrolysis by more than 10^5-fold (8). Thus, a lack of normal neurofibromin GAP activity leads to excessive Ras-dependent signaling, which in turn activates downstream pathways such as the MAPK and/or the phosphatidylinositol 3-kinase (PI3K) signaling cascade, hence contributing to uncontrolled proliferation and tumor formation (8) (Fig. 1).

In mouse models, the lack of *Nf1* has been linked to increased cell proliferation, enhanced Ras activation and tumorigenesis (9). It has been proposed that *NF1* functions as a tumor suppressor gene with both copies mutated in tumor cells (7). In fact, in both neurofibromas and MPNSTs, mutation of both *NF1* alleles (one inherited and one acquired) results in the loss of neurofibromin function. However, it appears that only Schwann cells in neurofibromas display mutations in both *NF1* alleles, while the rest (mast cells and pericytes) are heterozygous for an *NF1* mutation ($NF1^{+/-}$). This genotype appears to be required for the development of tumorigenesis. Mice with a conditional knockout of *Nf1* ($Nf1^{-/-}$) in Schwann cells but heterozygous *Nf1* inactivation ($Nf1^{+/-}$) in all other somatic cells develop classic plexiform neurofibromas with massive mast cell infiltration, resembling the human pathology (10). Other NF1 manifestations, such as café-au-lait macules (7) or cognitive dysfunction in mice (11), present another domain, namely Sec14, homologous to the yeast Sec14p, which mediates intracellular protein and lipid trafficking. However, the function of Sec14 in neurofibromin has not yet been identified (12).

Molecular targets

Ras

As discussed earlier, deregulation of the Ras signaling pathway due to neurofibromin gene loss of function accounts, at least in part, for abnormal cell proliferation and tumorigenesis in NF1. Therefore, directly targeting Ras, or rather the enzymes involved in Ras processing, may be an adequate strategy to interfere with aberrant Ras signaling. At its four *C*-terminal residues (the "CaaX" box), Ras undergoes a series of post-translational modifications that are responsible for membrane translocation and Ras-transforming capacity. CaaX box processing consists roughly of three types of modifications, all susceptible to pharmacological targeting: farnesylation, cleavage and methylation. The key step for Ras membrane translocation is farnesylation, which is mediated by the enzyme farnesyltransferase, which catalyzes the attachment of the farnesyl group from farnesyl pyrophosphate to Ras. Farnesylation can be suppressed by statins, as hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibition blocks the synthesis of mevalonate, which is required for the production of farnesyl pyrophosphate (8).

In addition to statins, farnesyltransferase inhibitors are a promising approach to block the farnesylation phase. The farnesyltransferase inhibitor tipifarnib is currently being investigated in children with NF1 presenting with progressive plexiform neurofibromas (discussed below). However, N- and K-Ras isoforms (but not H-Ras) can undergo geranylgeranylation, a post-translational modification that also allows them to translocate to the membrane and overcome the effects of farnesyltransferase inhibitors (8). As activation of N- and K-Ras isoforms appears predominant in NF1, more specific anti-Ras strategies should therefore be pursued.

Downstream and upstream Ras effectors

Targeting downstream effectors of Ras, such as the MAPK/extracellular signal-regulated kinase (ERK) or the PI3K/protein kinase B (PI3K/PKB/Akt) pathway, which control promitotic and prosurvival cascades, respectively,

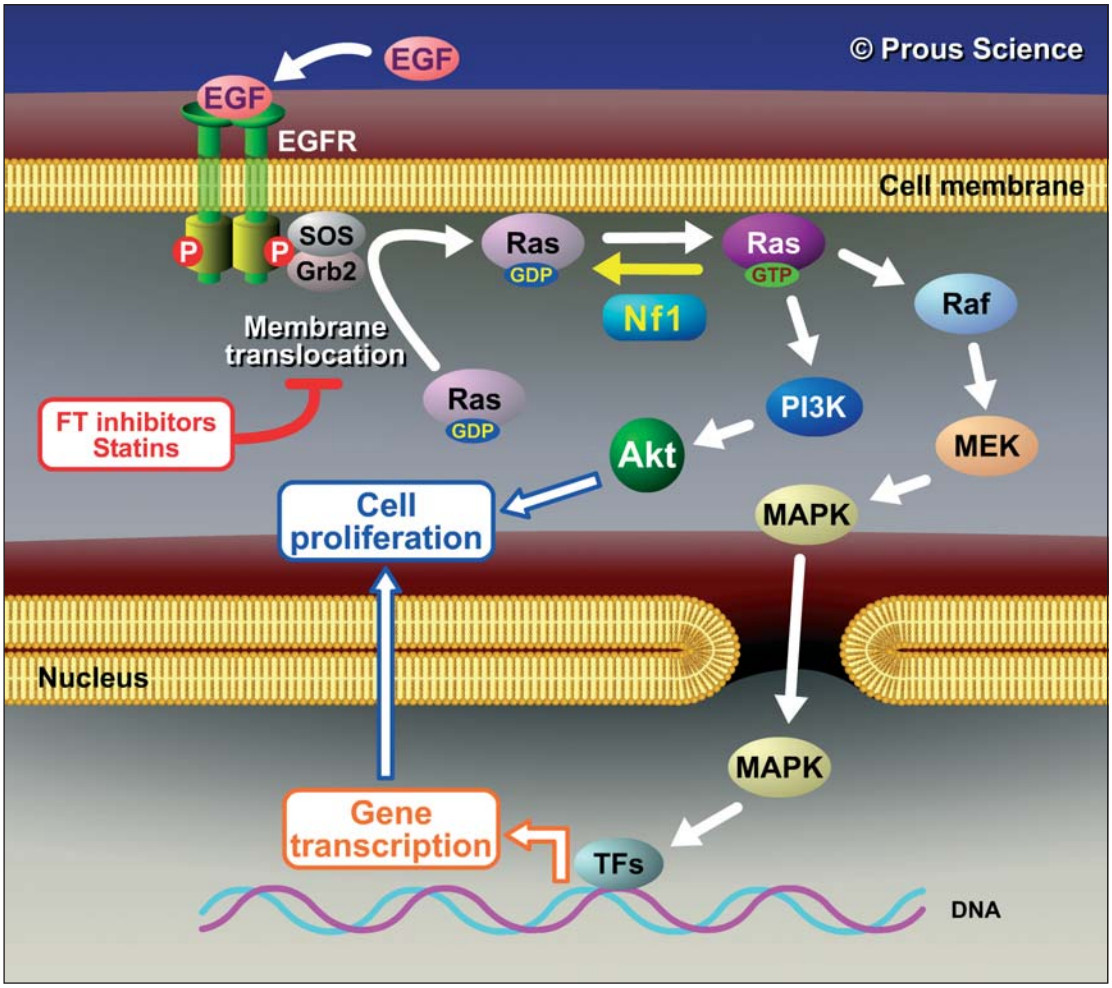

Fig. 1. Neurofibromin, via its GTPase-activating protein (GAP)-related domain, mediates the conversion from active Ras-GTP to inactive Ras-GDP by accelerating Ras-GTP hydrolysis. Functional defects in neurofibromin result in excessive activation of Ras-dependent signaling pathways, ultimately leading to uncontrolled cell proliferation and favoring tumorigenesis. Directly targeting Ras with farnesyltransferase inhibitors or statins, which block key steps in Ras translocation to the membrane and posterior activation, suppresses the cell proliferation cascade. Activation of signaling pathways downstream of Ras, such as phosphatidylinositol 3-kinase (PI3K)/Akt or mTOR (mammalian target of rapamycin), also contributes to the hyperproliferative state in type 1 neurofibromatosis.

has demonstrated *in vitro* antiproliferative effects (8). Another potential therapeutic target is the mixed-lineage kinase 3 (MLK3), a member of the MAPK kinase kinase (MAP3K) family required for mitogen activation of B-Raf and cell proliferation. In *NF1*-mutant cells, as well as in cells with oncogenic Ras mutations, *MLK3* gene silencing has been shown to inhibit tumor cell proliferation. In contrast, targeting MLK3 in cells with activated B-Raf signaling had no effect, which confirms that MLK3 acts upstream of B-Raf and mediates Ras-induced proliferation (13).

Alternatively, upstream activators of Ras could also be potential therapeutic targets in NF1. MPNST cells have been shown to overexpress the epidermal growth factor receptor (EGFR), a known Ras activator, and EGFR overexpression correlates with loss of neurofibromin function. In this context, EGFR inhibitors have blocked EGF-stimulated proliferation in MPNST cells, hence opening a new avenue for the treatment of NF1 malignancies (8).

cAMP/PKA

In addition to modulating Ras signaling, neurofibromin has also been implicated in the regulation of intracellular cyclic adenosine monophosphate (cAMP). Normal astrocyte growth is negatively regulated by elevations in intracellular cAMP, which triggers the activation of the small G-protein Rap1, which in turn inhibits Raf-1 activation and subsequent Ras signaling. Inactivation of the *Nf1* gene in murine astrocytes resulted in a reduction in cAMP production in response to pituitary adenylate cyclase-activating peptide (PACAP), decreased cAMP-dependent calcium influx and impaired Rap1 activation (14). Thus, tumor

proliferation in NF1 could also be related to defective cAMP signaling due to neurofibromin loss.

Besides Ras, neurofibromin has been found to regulate adenylyl cyclase activity and cAMP levels in mice (15) and in *Drosophila*, where this cAMP-dependent pathway was found to be essential for odor learning (16). A recent study reported that both Ras and neurofibromin are required for adenylyl cyclase activation and that binding of endogenous ligands to the EGFR is able to stimulate this neurofibromin/Ras-dependent adenylyl cyclase pathway (17). In addition, neurofibromin mutations in the GAP-related domain interfere with growth factor-stimulated adenylyl cyclase signaling. Another study has shown that *NF1* gene deletion in Schwann cells is associated with increased outward potassium currents via activation of cAMP-dependent protein kinase, or PKA (18). Together, these findings suggest a potential role for cAMP/PKA pathways in cell proliferation in NF1.

mTOR

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase that controls cell growth and proliferation via the synthesis of new proteins. Proteomic analysis performed in astrocytes from *Nf1*-deficient mice revealed increased expression of ribosomal constituents of the protein synthetic machinery, together with an overall elevation of the protein synthesis rate, and enhanced cell proliferation compared to wild-type astrocytes. This neurofibromin-dependent hyperproliferative activity could be suppressed by rapamycin (sirolimus) treatment, while sparing the proliferation of normal astrocytes. This preferential inhibition suggested a hyperactivation of mTOR signaling in *Nf1*-null astrocytes responsible for continued proliferation (19). Further studies demonstrated that mTOR is constitutively activated in mouse *Nf1*-deficient primary cells and in *NF1*-null Schwann cells from human neurofibromas. In addition, proliferation of MPNSTs from NF1 patients was suppressed at very low rapamycin concentrations (IC_{50} = 1-10 nM), indicating its therapeutic potential and confirming mTOR deregulation in NF1 (20).

TGF-β

As mentioned earlier, neurofibromas are a mixture of Schwann cells, mast cells, pericytes and fibroblasts embedded in a collagen matrix. Fibroblasts contribute to carcinogenesis by secreting interstitial collagen and promoting extracellular matrix remodeling, which provides a favorable microenvironment for tumor growth, spread and survival. In turn, activated mast cells have been shown to release proinflammatory growth factors that promote fibrosis, extracellular matrix remodeling and angiogenesis. One of these molecules is transforming growth factor (TGF)-β, the secretion of which is increased in haploinsufficient *NF1* (*NF1*$^{+/-}$) mast cells, enhancing fibroblast proliferation, migration and collagen synthesis (21). TGF-β was found to hyperactivate the nonreceptor tyrosine kinase c-ABL via a Ras-dependent mechanism. Interestingly, pharmacological treatment with the BCR-ABL tyrosine kinase inhibitor imatinib mesilate (Gleevec®) in haploinsufficient NF1 mice was able to suppress excessive collagen synthesis and fibroblast invasion in response to TGF-β. Clinical trials assessing imatinib for the management of neurofibromas are under way and will be discussed below.

Tumor microenvironment

As mentioned earlier, NF1 tumors contain *NF1*$^{-/-}$ Schwann cells and heterozygous *NF1* mutations in surrounding mast cells. In fact, it appears that heterozygosity of mast cells favors tumor formation, as large mast cell infiltrates have been found in neurofibromas harboring *Nf1*$^{-/-}$ Schwann cells and *Nf1*$^{+/-}$ mast cells, while those containing *Nf1*$^{+/+}$ mast cells showed fewer infiltrates (10). Moreover, inflammatory cells are thought to contribute to cancer initiation and progression by secreting factors that facilitate angiogenesis to maintain tumor growth and promote metastasis. Yang *et al*. have demonstrated that *Nf1*$^{-/-}$ Schwann cells secrete Kit ligand, a growth factor that makes *Nf1*$^{+/-}$ mast cells hyperproliferative and hypermotile and promotes their migration, thus generating autocrine/paracrine signaling that ensures neurofibroma progression (22).

Further evidence has suggested targeting angiogenesis as a potential treatment for NF1. In fact, augmented angiogenesis, together with hyperproliferation of pericytes, which are highly abundant in neurofibromas, and endothelial cells has been demonstrated in mouse models of NF1 (23). In this study, targeting the NG2 proteoglycan present in pericytes of MPNST xenografts decreased angiogenesis by more than 50%. Moreover, endothelial cells from NF1 patients have shown increased proliferation, migration and ERK activation in response to vascular endothelial growth factor (VEGF) (24), indicating another potential target for therapeutic intervention. Anti-VEGF experimental therapies for NF1 will be discussed below.

Treatment options

Currently no available therapy can cure or halt the progression of NF1. Treatment of this disorder is mainly symptomatic, depending on the patient's clinical status, and often requires surgery or radiation therapy to remove or reduce individual neurofibromas. Here we will discuss experimental therapies in preclinical or clinical investigation for NF1 and provide a summary of relevant clinical studies in Table I.

Plexiform neurofibromas and MPNSTs

Neurofibromas are peripheral nerve sheath tumors characteristic of NF1. While dermal neurofibromas are usually localized, nodular plexiform or diffuse plexiform neurofibromas, which affect multiple nerve branches, can be very invasive and aesthetically disfiguring and under-

Table I: Clinical studies of experimental therapies for neurofibromatosis type 1 (NF1) (from Prous Science Integrity®).

Drug/ Intervention	Design	Treatments	n	Conclusions/Objectives	Ref.
Plexiform neurofibromas and malignant peripheral nerve sheath tumors (MPNSTs)					
Tipifarnib	Open Dose-finding	Tipifarnib, 150 mg/m^2 p.o. b.i.d. x 21 d 1x/28 d Tipifarnib, 200 mg/m^2 p.o. b.i.d. x 21 d 1x/28 d Tipifarnib, 275 mg/m^2 p.o. b.i.d. x 21 d 1x/28 d Tipifarnib, 375 mg/m^2 p.o. b.i.d. x 21 d 1x/28 d	42	Oral tipifarnib was investigated in children with refractory solid tumors (n=25) or NF1-related plexiform neurofibromas (n=17). The drug was well tolerated at the maximum tolerated dose of 200 mg/m^2	27
	Multicenter Randomized Double-blind Crossover	Tipifarnib, p.o. b.i.d. x 21 d 1x/28 d Placebo	63	A phase II study is evaluating the efficacy, toxicity and effect of tipifarnib on disease progression and quality of life of patients with NF1 and progressive plexiform neurofibromas	28
Pirfenidone	Open	Pirfenidone, 800 mg p.o. t.i.d. [titrated from 400 mg b.i.d. over 3 wks] x 2 y	24	Pirfenidone induced a reduction of more than 15% in tumor volume in 16.7% of patients with NF1 and produced stable disease in 70.8% of patients	32
	Open Multicenter	Pirfenidone, p.o. t.i.d.	36	A phase II study will evaluate the efficacy of pirfenidone for the treatment of NF1 and recurrent or progressive plexiform neurofibroma	33
Imatinib mesilate	Open Multicenter	Imatinib mesilate	32	A phase II/III study will assess the efficacy of imatinib mesilate in patients with MPNSTs	35
Combination chemotherapy	Open Multicenter	Methotrexate, i.v. + Vinblastine, i.v. 1x/wk x 26 wks → 1x/2 wks x 26 wks	35	A phase II study will evaluate the efficacy, tolerability and effect on quality of life of combination chemotherapy with methotrexate and vinblastine in patients with NF1 and progressive plexiform neurofibromas	36
	Open Multicenter	Doxorubicin, i.v. + Etoposide, i.v. + Ifosfamide, i.v.	75	A phase II study will evaluate the efficacy of neoadjuvant chemotherapy with doxorubicin, etoposide and ifosfamide in patients with stage III/IV MPNSTs	37
Thalidomide	Open Multicenter Dose-finding	Thalidomide, 1 mg/kg/d x 12 mo Thalidomide, 1 mg/kg/d x 1 mo → id, 2 mg/kg/d x 11 mo Thalidomide, 3 mg/kg/d [escalated by 1 mg/kg/d 1x/4 wks] x 12 mo Thalidomide, 4 mg/kg/d [escalated by 1 mg/kg/d 1x/4 wks] x 12 mo	20	Thalidomide was only moderately effective in reducing tumor growth and improving pain symptoms in NF1 patients with plexiform neurofibroma	38
Cediranib	Open Multicenter	Cediranib, p.o. o.d. x 2 y	65	A phase II study will evaluate the efficacy, toxicity and effect on quality of life of cediranib in patients with NF1 and extensive plexiform and/or paraspinal neurofibroma	39
Peginterferon alfa-2b	Open	Peginterferon alfa-2b, s.c. 1x/wk x 2 y	87	A phase II study will determine the efficacy and tolerability of peginterferon alfa-2b for the treatment of children and adolescents with plexiform neurofibroma	40
Dermal neurofibromas/café-au-lait macules					
Tacalcitol	Case report	Tacalcitol, top. x 6 mo	1	Topical administration of the vitamin D_3 analogue tacalcitol decreased pigmentation of café-au-lait spots on the back of a patient with NF1	43

Continuation

Table I (Cont.): Clinical studies of experimental therapies for neurofibromatosis type 1 (NF1) (from Prous Science Integrity®).

Drug/ Intervention	Design	Treatments	n	Conclusions/Objectives	Ref.
Gastrointestinal stromal tumors					
Imatinib	Case report	Imatinib, 400 mg/d p.o.	1	The case of a patient with metastatic gastrointestinal stromal tumor associated with NF1 favorably responding to imatinib was reported	46
Cognitive dysfunction					
Lovastatin	Randomized Double-blind	Lovastatin x 8 wks Placebo	NA	This phase I study will evaluate the safety of lovastatin in adult patients with NF1	52
Methylphenidate	Open	Methylphenidate, 5-10 mg p.o. o.d. [titrated from 2.5 mg over 2 d] x 1 y	20	Methylphenidate improved cognitive, academic and social problems in children with NF1 and attention deficit hyperactivity disorder	53
	Multicenter Randomized Double-blind Crossover	Methylphenidate Placebo	80	A study will investigate the effect of methylphenidate on attention, depression, anxiety and specific neuropsychological issues in children with neurofibromatosis compared with children with attention deficit hyperactivity disorder	54
Endocrine disorders					
Growth hormone	Retrospective	Growth hormone x 6 [max.] y	102	Growth hormone was well tolerated and improved growth rate in children with neurofibromatosis and growth hormone deficiency	57
Pegvisomant	Case report	Pegvisomant, 10 mg/d p.o. x 6 wks → 10 mg p.o. 1x/2 d → [@ age 5.6] 10 mg p.o. 1x/3 d → [@ age 5.8] 10 mg p.o. 1x/4 d	1	The case of a girl with NF1 in whom pegvisomant normalized insulin growth factor-1 and -3 levels. Growth velocity was normalized after initial catch-down growth	58

NA: Not available

go malignant transformation. MPNSTs affect about 2-5% of NF1 patients compared with an incidence of 0.001% in the general population, and are associated with low survival (25).

The growth pattern of plexiform neurofibromas is usually slow but sustained, which may cause significant morbidity, as they can reach large proportions and affect adjacent structures. For instance, spinal cord compression may cause pain and neurological dysfunction, or orbital neurofibromas may compress the optic nerve, leading to vision loss, or affect orbital osseus structures, causing sphenoid dysplasia (4, 26). Malignant transformation of plexiform neurofibromas into MPNSTs has been related to the loss of cell cycle regulators, particularly mutations in *p53*, allowing uncontrolled cell proliferation (26).

Targeted therapy

Protein farnesyltransferase inhibitors (FTIs) are promising molecules in the management of NF1, as they target essential steps in Ras activation. Tipifarnib (Zarnestra™) is an oral small molecule with potent farnesyltransferase-inhibitory activity *in vitro*, which is currently being investigated at the National Cancer Institute (NCI) for a variety of human cancers and is preregistered for acute myeloid leukemia (AML). A phase I trial concluded that tipifarnib was well tolerated in NF1 children with refractory solid tumors or inoperable plexiform neurofibromas at a maximum tolerated dose of 200 mg/m^2, inhibiting farnesyltransferase activity by 35% (27). The utility of tipifarnib in preventing cancer in pediatric NF1 patients presenting with progressive plexiform neurofibromas is being evaluated at the NCI in a randomized double-blind, placebo-controlled phase II clinical study that is expected to enroll 63 patients (28).

Farnesylthiosalicylic acid is a potent Ras inhibitor that competes with Ras-GTP for binding to specific plasma membrane sites, thus causing mislocalization of active Ras and promoting its degradation. In neurofibromin-defective MNPST cell lines, farnesylthiosalicylic acid decreased Ras-GTP levels, suppressed downstream Ras signaling and inhibited cell growth. When orally administered to mice bearing MNPST xenografts, farnesylthiosalicylic acid dose-dependently inhibited tumor growth (29).

The nonsteroidal antiinflammatory drug (NSAID) sulindac and its sulfone derivative exisulind also reduced Ras-GTP levels and downstream effectors of the Ras pathway, such as phosphorylated ERK1/2, and sup-

pressed the growth of primary MPNST cell cultures from NF1 patients (30).

Targeting excessive TGF-β production in neurofibromas is another potential pharmacological strategy. Pirfenidone is an orally active small-molecule tumor necrosis factor (TNF)-α production and TGF-β inhibitor, thought to downregulate the production of multiple cytokines and to block fibroblast proliferation and collagen synthesis in response to these cytokines. Evidence gathered in immunocompromised mice bearing human neurofibroma xenografts showed a reduction in xenograft survival upon pirfenidone treatment, with no associated toxicity (31). An open-label phase II clinical study in adults with NF1 showed neurofibroma tumor regression in 29.2% of patients treated with pirfenidone, which correlated with improved neurological function (32). The efficacy of pirfenidone will be further examined in an open phase II clinical trial that will enroll young NF1 patients with recurrent or progressive plexiform neurofibromas (33).

Interestingly, recent investigations have proposed the receptor tyrosine kinases Kit and PDGFRα as drug targets in the treatment of MPNSTs. *PDGFRA* and *KIT* gene amplication was found in selected MPNST samples from NF1 patients, together with *PDGFRA* (75% of patients) and *KIT* (7%) gene expression (34). However, *KIT* mutations were not observed and somatic *PDGFRA* point mutations were only detected in 2 of 31 patients. Imatinib, a known inhibitor of Kit and PDGFRα signaling, blocked MPNST cell culture proliferation and ligand-induced PDGFRα phosphorylation, suggesting potential therapeutic utility. Currently, an open study is examining the safety and efficacy of imatinib in NF1 patients with MPNSTs (35).

Nontargeted therapy

Several strategies used in other types of malignancies have also been tested in NF1 patients to treat peripheral nerve sheath tumors. Hence, combination chemotherapy with methotrexate and vinblastine is currently being investigated in a phase II clinical study in patients with NF1 associated with progressive plexiform neurofibromas (36). Another clinical trial will examine the effects of combination chemotherapy with doxorubicin, etoposide, filgrastim, ifosfamide and pegfilgrastim in patients with stage III or stage IV MPNSTs (37).

Inhibition of angiogenesis is another common approach in cancer therapy. Peripheral nerve sheath tumor development also involves the recruitment of new blood vessels that facilitate tumor invasion. However, angiogenesis inhibitors such as thalidomide have shown only moderate improvements in tumor size and related symptoms (pain) (38). Angiogenesis inhibition may be used in combination with targeted therapies to achieve greater efficacy. AZD-2171 (cediranib, Recentin®), a highly potent inhibitor of VEGFR tyrosine kinases in advanced clinical development at AstraZeneca for the treatment of lung and colorectal cancer, will be tested in adult patients with NF1 and extensive plexiform and paraspinal neurofibromas (39).

Peginterferon alfa-2b (PEG-Intron®), commercialized for the treatment of hepatitis C and undergoing clinical investigation for different cancers, has shown positive preliminary results in treating NF1 patients with plexiform neurofibromas. Further phase II evaluation will determine the efficacy and tolerability of weekly treatment with peginterferon alfa-2b for the treatment of children and adolescents with plexiform neurofibroma (40).

Finally, NX-105 is currently undergoing advanced preclinical screening at NexGenix Pharmaceuticals as a potential treatment for MPNSTs (41).

Dermal neurofibromas and café-au-lait macules

Cutaneous manifestations are the hallmark and usually the first clinical sign of NF1. Café-au-lait macules are hyperpigmented, well-limited spots that may be present at birth and may increase in size and number during childhood. Around 95% of NF1 patients develop café-au-lait spots (2, 4). There is no specific treatment for café-au-lait macules. Dermal neurofibromas are localized and may be surgically removed. Antiprogesterone therapy may be useful to treat neurofibromas, as progesterone receptors may be implicated in the regulation of neurofibroma growth given their abundant expression in human neurofibroma samples (42). Progesterone receptor expression appeared to be predominant in cutaneous neurofibromas compared to plexiform neurofibromas and MPNSTs, whereas normal peripheral nerves are negative for progesterone receptors. However, it remains to be determined whether progesterone receptor expression does actually regulate neurofibroma growth.

Topical administration of the vitamin D_3 analogue tacalcitol, used for the treatment of psoriasis, was found to decrease pigmentation of café-au-lait spots on the back of a patient with NF1 after 6 months of treatment (43). Furthermore, topically applied 22-oxacalcitriol to café-au-lait spots grafted onto nude mice suppressed DNA synthesis by cells from the basal skin layer, likely melanocytes and keratinocytes (43). Moreover, a recent study highlighted that low vitamin D_3 serum levels in NF1 patients are inversely correlated with the number of dermal neurofibromas (44). Together, these results indicate a potential for vitamin D_3-related compounds in the treatment of dermal manifestations of NF1.

NexGenix Pharmaceuticals has finished proof-of-principle trials with NX-101, a topical compound for the treatment of dermal neurofibromas (41).

Gastrointestinal stromal tumors

NF1 is a condition that predisposes to further tumor development. Among tumor manifestations, gastrointestinal stromal tumors, or GISTs, have gained attention in NF1 patients, although the exact incidence has not been established. Maertens *et al*. reported that, compared to sporadic GISTs, NF1-related GISTs are not associated

with receptor tyrosine kinase Kit- or PDGFRα-activating mutations, show marked expression and activation of MAPK and reduced neurofibromin levels, and are primarily found in the small intestine (5). However, other reports have found inherited mutations in the *PDFGRA* gene of NF1 patients presenting with intestinal *KIT*-negative GISTs (45). Imatinib is the treatment of choice for GISTs, but response to treatment usually depends on tumors harboring *KIT* or *PDGFRA* mutations. Surprisingly, a reduction in tumor size after 4 weeks of imatinib treatment has been reported in an NF1 patient presenting with an intestinal GIST (46). A recent study reported loss of heterozygosity of a germline NF1 mutation in GISTs of NF1 patients, likely due to mitotic recombination, as a potential new pathogenetic mechanism in *KIT/PDGFRA*-negative NF1-associated GISTs (47). As discussed earlier, imatinib has shown *in vitro* activity against MPNST cell proliferation.

Cognitive dysfunction

Learning disabilities are very common in NF1 patients and can have different manifestations, such as poor performance on tasks requiring visual-spatial memory, attention deficits or motor disorders (6). In fact, *Nf1*-null heterozygous ($Nf1^{+/-}$) mice also showed impaired spatial learning, which turned out to be Ras-dependent since mutations that decreased Ras function rescued $Nf1^{+/-}$ mouse deficits (11). Similar reversal of cognitive impairment was obtained after treatment with an FTI, BMS-191563, which also interfered with Ras signaling. In addition, $Nf1^{+/-}$ mice exhibited decreased hippocampal long-term potentiation (LTP), a known cellular correlate for learning and memory, compared to wild-type animals. LTP deficit in $Nf1^{+/-}$ mice appeared to be Ras-dependent and caused by increased γ-aminobutyric acid (GABA)-mediated inhibition, thus impairing synaptic plasticity in $Nf1^{+/-}$ mice and potentially explaining learning and memory disabilities.

Activation of the MAPK/ERK pathway plays an important role in synaptic plasticity and long-term memory formation. Upon MAPK/ERK activation and nuclear translocation, phosphorylation of downstream targets, such as cAMP response element-binding protein (CREB), triggers the transcription of immediate early genes encoding regulatory transcription factors and effector proteins required for synaptic plasticity and memory storage (48). A recent study found that hyperactivation of the ERK signaling pathway, evidenced by elevated levels of phosphorylated ERK and CREB in the hippocampus of $Nf1^{+/-}$ mice, correlated with LTP deficits. Treatment with U-0126, which inhibits MEK (an upstream MAPK activator) was able to restore ERK and CREB phosphorylation to control levels and rescued impaired LTP (49).

Furthermore, researchers at the University of California discovered that the HMG-CoA reductase inhibitor lovastatin may be useful for the treatment of cognitive impairment in NF1 (50). Inhibition of mevalonate synthesis by statins results in a reduction of intermediates required for Ras protein farnesylation (*i.e.*, farnesyl pyrophosphate), thus impeding Ras membrane anchorage, essential to perform its biological function (51). Lovastatin treatment caused a downregulation of increased Ras-GTP and MAPK activity in $Nf1^{+/-}$ mouse brain, as well as a reversal of spatial learning and attention deficits. In addition, lovastatin was also able to bring decreased LTP in $Nf1^{+/-}$ mice back to wild-type levels (50). The potential of lovastatin treatment in humans with NF1 will be investigated in a randomized, placebo-controlled clinical trial (52).

Several studies have indicated an association between NF1 and attention deficit hyperactivity disorder (ADHD). It has been estimated that about one-third of children with NF1 present ADHD (6). Methylphenidate, one of the few drugs approved for the treatment of ADHD, has been found to improve cognitive, academic and social problems in pediatric patients presenting with neurofibromatosis and ADHD in a small open clinical study (53). A larger controlled clinical trial will further evaluate the efficacy of methylphenidate in such patients (54).

Endocrine disorders

Growth and hormonal abnormalities such as large head size, short adult stature, precocious puberty or hypothalamic dysfunction, have been reported in NF1 patients, although the exact incidence is unknown (4, 55). Interestingly, both growth hormone (GH) deficiency (56, 57) and hypersecretion (58) have been encountered in children with NF1 and both have been correlated with the presence of intracranial tumors, especially optic nerve gliomas. A retrospective study assessing the safety and efficacy of GH replacement therapy in a cohort of 102 pediatric patients with NF1 and GH deficiency found a good response during the first year of treatment, as the median height velocity increased from 4.2 to 7.1 cm/year. GH replacement therapy was well tolerated and did not influence the progression of other NF1 features, including intracranial tumors (57). In contrast, treatment with the GH antagonist pegvisomant, used to treat acromegaly, normalized insulin-like growth factor-1 (IGF-1) and IGF-3 and reduced growth velocity to target levels in a girl with NF1 presenting with GH hypersecretion (58). In addition, neurofibromas have been found to express the GH receptor, thereby suggesting a potential role in their development (59), although this has not been proven.

Conclusions

Since the identification of the genetic causes of NF1 more than a decade ago, substantial progress has been made in the understanding of the molecular pathophysiology of NF1. Animal models not only mimic disease complications, but have also provided new insights into the biological role of neurofibromin. Results from these studies have translated into the clinical assessment of potential therapies targeting different disease manifestations, in particular NF1-related malignancies. Therapies

addressed to reduce increased Ras-GTP levels are not the only strategy to be pursued to relieve NF1 clinical symptoms, as other signaling pathways have been shown to be important for tumor proliferation. Targeting cell interactions within the tumor microenvironment may also help to prevent the formation of neurofibromas and their further malignant transformation.

References

1. *Prevalence of rare diseases: A bibliographic survey*. Orphanet Reports Series 2007.

2. Yohay, K. *Neurofibromatosis types 1 and 2*. Neurologist 2006, 12(2): 86-93.

3. Ferner, R.E. *Neurofibromatosis 1*. Eur J Hum Genet 2007, 15(2): 131-8.

4. Tonsgard, J.H. *Clinical manifestations and management of neurofibromatosis type 1*. Semin Pediatr Neurol 2006, 13(1): 2-7.

5. Maertens, O., Prenen, H., Debiec-Rychter, M. et al. *Molecular pathogenesis of multiple gastrointestinal stromal tumors in NF1 patients*. Hum Mol Genet 2006, 15(6): 1015-23.

6. Rosser, T.L., Packer, R.J. *Neurocognitive dysfunction in children with neurofibromatosis type 1*. Curr Neurol Neurosci Rep 2003, 3(2): 129-36.

7. Theos, A., Korf, B.R. *Pathophysiology of neurofibromatosis type 1*. Ann Intern Med 2006, 144(11): 842-9.

8. Dilworth, J.T., Kraniak, J.M., Wojtkowiak, J.W. et al. *Molecular targets for emerging anti-tumor therapies for neurofibromatosis type 1*. Biochem Pharmacol 2006, 72(11): 1485-92.

9. Gutmann, D.H., Giovannini, M. *Mouse models of neurofibromatosis 1 and 2*. Neoplasia 2002, 4(4): 279-90.

10. Zhu, Y., Ghosh, P., Charnay, P., Burns, D.K., Parada, L.F. *Neurofibromas in NF1: Schwann cell origin and role of tumor environment*. Science 2002, 296(5569): 920-2.

11. Costa, R.M., Federov, N.B., Kogan, J.H. *Mechanism for the learning deficits in a mouse model of neurofibromatosis type 1*. Nature 2002, 415(6871): 526-30.

12. Le, L.Q., Parada, L.F. *Tumor microenvironment and neurofibromatosis type I: Connecting the GAPs*. Oncogene 2007, Epub ahead of print.

13. Chadee, D.N., Kyriakis, J.M. *MLK3 is required for mitogen activation of B-Raf, ERK and cell proliferation*. Nat Cell Biol 2004, 6(8): 770-6.

14. Dasgupta, B., Dugan, L.L., Gutmann, D.H. *The neurofibromatosis 1 gene product neurofibromin regulates pituitary adenylate cyclase-activating polypeptide-mediated signaling in astrocytes*. J Neurosci 2003, 23(26): 8949-54.

15. Tong, J., Hannan, F., Zhu, Y., Bernards, A., Zhong, Y. *Neurofibromin regulates G protein-stimulated adenylyl cyclase activity*. Nat Neurosci 2002, 5(2): 95-6.

16. Guo, H.F., Tong, J., Hannan, F., Luo, L., Zhong, Y. *A neurofibromatosis-1-regulated pathway is required for learning in Drosophila*. Nature 2000, 403(6772): 895-8.

17. Hannan, F., Ho, I., Tong, J.J., Zhu, Y., Nurnberg, P., Zhong, Y. *Effect of neurofibromatosis type I mutations on a novel pathway for adenylyl cyclase activation requiring neurofibromin and Ras*. Hum Mol Genet 2006, 15(7): 1087-98.

18. Xu, Y., Chiamvimonvat, N., Vazquez, A.E., Akunuru, S., Ratner, N., Yamoah, E.N. *Gene-targeted deletion of neurofibromin enhances the expression of a transient outward K^+ current in Schwann cells: A protein kinase A-mediated mechanism*. J Neurosci 2002, 22(21): 9194-202.

19. Dasgupta, B., Yi, Y., Chen, D.Y., Weber, J.D., Gutmann, D.H. *Proteomic analysis reveals hyperactivation of the mammalian target of rapamycin pathway in neurofibromatosis 1-associated human and mouse brain tumors*. Cancer Res 2005, 65(7): 2755-60.

20. Johannessen, C.M., Reczek, E.E., James, M.F., Brems, H., Legius, E., Cichowski, K. *The NF1 tumor suppressor critically regulates TSC2 and mTOR*. Proc Natl Acad Sci USA 2005, 102(24): 8573-8.

21. Yang, F.C., Chen, S., Clegg, T. et al. *Nf1+/- mast cells induce neurofibroma like phenotypes through secreted TGF-β signaling*. Hum Mol Genet 2006, 15(16): 2421-37.

22. Yang, F.C., Ingram, D.A., Chen, S. et al. *Neurofibromin-deficient Schwann cells secrete a potent migratory stimulus for $Nf1^{+/-}$ mast cells*. J Clin Invest 2003, 112(12): 1851-61.

23. Ozerdem, U. *Targeting neovascular pericytes in neurofibromatosis type 1*. Angiogenesis 2004, 7(4): 307-11.

24. Munchhof, A.M., Li, F., White, H.A. *Neurofibroma-associated growth factors activate a distinct signaling network to alter the function of neurofibromin-deficient endothelial cells*. Hum Mol Genet 2006, 15(11): 1858-69.

25. Ferner, R.E., Gutmann, D.H. *International consensus statement on malignant peripheral nerve sheath tumors in neurofibromatosis*. Cancer Res 2002, 62(5): 1573-7.

26. Packer, R.J., Gutmann, D.H., Rubenstein, A. et al. *Plexiform neurofibromas in NF1: Toward biologic-based therapy*. Neurology 2002, 58(10): 1461-70.

27. Widemann, B.C., Salzer, W.L., Arceci, R.J. et al. *Phase I trial and pharmacokinetic study of the farnesyltransferase inhibitor tipifarnib in children with refractory solid tumors or neurofibromatosis type I and plexiform neurofibromas*. J Clin Oncol 2006, 24(3): 507-16.

28. *Tipifarnib in preventing cancer in children with neurofibromatosis type 1 and progressive plexiform neurofibromas (NCT00029354)*. ClinicalTrials.gov Web site, March 29, 2007.

29. Barkan, B., Starinsky, S., Friedman, E., Stein, R., Kloog, Y. *The Ras inhibitor farnesylthiosalicylic acid as a potential therapy for neurofibromatosis type 1*. Clin Cancer Res 2006, 12(18): 5533-42.

30. Frahm, S., Kurtz, A., Kluwe, L., Farassati, F., Friedrich, R.E., Mautner, V.F. *Sulindac derivatives inhibit cell growth and induce apoptosis in primary cells from malignant peripheral nerve sheath tumors of NF1-patients*. Cancer Cell Int 2004, 4(1): 4.

31. Babovic-Vuksanovic, D., Petrovic, L., Knudsen, B.E., Plummer, T.B., Parisi, J.E., Babovic, S., Platt, J.L. *Survival of human neurofibroma in immunodeficient mice and initial results of therapy with pirfenidone*. J Biomed Biotechnol 2004, 2004(2): 79-85.

32. Babovic-Vuksanovic, D., Ballman, K., Michels, V. et al. *Phase II trial of pirfenidone in adults with neurofibromatosis type 1*. Neurology 2006, 67(10): 1860-2.

33. *Pirfenidone in treating young patients with neurofibromatosis type I and recurrent or progressive plexiform neurofibromas (NCT00078936)*. ClinicalTrials.gov Web site, February 13, 2007.

34. Holtkamp, N., Okuducu, A.F., Mucha, J. et al. *Mutation and expression of PDGFRA and KIT in malignant peripheral nerve sheath tumors, and its implications for imatinib sensitivity*. Carcinogenesis 2006, 27(3): 664-71.

35. *Imatinib mesylate treatment of patients with malignant peripheral nerve sheath tumors (NCT00427583)*. ClinicalTrials.gov Web site, February 13, 2007.

36. *Combination chemotherapy in treating patients with neurofibromatosis and progressive plexiform neurofibromas (NCT00030264)*. ClinicalTrials.gov Web site, February 13, 2007.

37. *Combination chemotherapy in treating patients with stage III or stage IV malignant peripheral nerve sheath tumors (NCT00304083)*. ClinicalTrials.gov Web site, February 13, 2007.

38. Gupta, A., Cohen, B.H., Ruggieri, P., Packer, R.J., Phillips, P.C. *Phase I study of thalidomide for the treatment of plexiform neurofibroma in neurofibromatosis 1*. Neurology 2003, 60(1): 130-2.

39. *AZD2171 in treating patients with neurofibromatosis type 1 and plexiform neurofibroma and/or neurofibroma near the spine (NCT00326872)*. ClinicalTrials.gov Web site, February 13, 2007.

40. *Study of PEG-Intron for plexiform neurofibromas (NCT00396019)*. ClinicalTrials.gov Web site, February 13, 2007.

41. NexGenix Pharmaceuticals Website. http://www.nexgenix-pharm.com/.

42. McLaughlin, M.E., Jacks, T. *Progesterone receptor expression in neurofibromas*. Cancer Res 2003, 63(4): 752-5.

43. Nakayama, J., Kiryu, H., Urabe, K., Matsuo, S., Shibata, S., Koga, T., Furue, M. *Vitamin D3 analogues improve cafe au lait spots in patients with von Recklinghausen's disease: Experimental and clinical studies*. Eur J Dermatol 1999, 9(3): 202-6.

44. Lammert, M., Friedman, J.M., Roth, H.J. et al. *Vitamin D deficiency associated with number of neurofibromas in neurofibromatosis 1*. J Med Genet 2006, 43(10): 810-3.

45. de Raedt, T., Cools, J., Debiec-Rychter, M. et al. *Intestinal neurofibromatosis is a subtype of familial GIST and results from a dominant activating mutation in PDGFRA*. Gastroenterology 2006, 131(6): 1907-12.

46. Lee, J.L., Kim, J.Y., Ryu, M.H. et al. *Response to imatinib in KIT- and PDGFRA-wild type gastrointestinal stromal associated with neurofibromatosis type 1*. Dig Dis Sci 2006, 51(6): 1043-6.

47. Stewart, D.R., Corless, C.L., Rubin, B.P. et al. *Mitotic recombination as evidence of alternative pathogenesis of gastrointestinal stromal tumours in neurofibromatosis type 1*. J Med Genet 2007, 44(1): e61.

48. Sweatt, J.D. *Mitogen-activated protein kinases in synaptic plasticity and memory*. Curr Opin Neurobiol 2004, 14(3): 311-7.

49. Guilding, C., McNair, K., Stone, T.W., Morris, B.J. *Restored plasticity in a mouse model of neurofibromatosis type 1 via inhibition of hyperactive ERK and CREB*. Eur J Neurosci 2007, 25(1): 99-105.

50. Li, W., Cui, Y., Kushner, S.A. et al. *The HMG-CoA reductase inhibitor lovastatin reverses the learning and attention deficits in a mouse model of neurofibromatosis type 1*. Curr Biol 2005, 15(21): 1961-7.

51. Alegret, M., Silvestre, J.S. *Pleiotropic effects of statins and related pharmacological experimental approaches*. Methods Find Exp Clin Pharmacol 2006, 28(9): 627-56.

52. *Trial to evaluate the safety of lovastatin in adults with neurofibromatosis type I (NF1) (NCT00352599)*. ClinicalTrials.gov Web site, February 15, 2007.

53. Mautner, V.F., Kluwe, L., Thakker, S.D., Leark, R.A. *Treatment of ADHD in neurofibromatosis type 1*. Dev Med Child Neurol 2002, 44(3): 164-70.

54. *NF1-Attention: Study of children with neurofibromatosis type 1 treated by methylphenidate (NCT00169611)*. ClinicalTrials.gov Web site, February 15, 2007.

55. Carmi, D., Shohat, M., Metzker, A., Dickerman, Z. *Growth, puberty, and endocrine functions in patients with sporadic or familial neurofibromatosis type 1: A longitudinal study*. Pediatrics 1999, 103(6, Pt. 1): 1257-62.

56. Vassilopoulou-Sellin, R., Klein, M.J., Slopis, J.K. *Growth hormone deficiency in children with neurofibromatosis type 1 without suprasellar lesions*. Pediatr Neurol 2000, 22(5): 355-8.

57. Howell, S.J., Wilton, P., Lindberg, A., Shalet, S.M. *Growth hormone and neurofibromatosis*. Horm Res 2000, 53(Suppl. 1): 70-6.

58. Main, K.M., Sehested, A., Feldt-Rasmussen, U. *Pegvisomant treatment in a 4-year-old girl with neurofibromatosis type 1*. Horm Res 2006, 65(1): 1-5.

59. Cunha, K.S., Barboza, E.P., Da Fonseca, E.C. *Identification of growth hormone receptor in localised neurofibromas of patients with neurofibromatosis type 1*. J Clin Pathol 2003, 56(10): 758-63.