Neurofibromatosis type 1-associated optic pathway gliomas: pathogenesis and emerging treatments

A. AMATO¹, B.P. IMBIMBO², B. FALSINI³

¹Ophthalmology Unit, IRCCS Ospedale San Raffaele, Milan, Italy
²Department of Research & Development, Chiesi Farmaceutici, Parma, Italy
³Ophthalmology Unit, Fondazione Policlinico Universitario “A. Gemelli” IRCCS/Università Cattolica del S. Cuore, Rome, Italy

Abstract. – Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder associated with an increased risk of developing a variety of benign and malignant tumors. Fifteen to 20% of children with NF1 are diagnosed with an optic pathway glioma (NF1-OPG) before 7 years of age, and more than half of them experience visual decline. At present, no effective therapy is available for prevention, restoration, or even stabilization of vision loss in subjects affected by NF1-OPG. This paper aims to review the main emerging pharmacological approaches that have been recently assessed in preclinical and clinical settings.

We performed a search of the literature using Embase, PubMed, and Scopus databases to identify articles regarding NF1-OPGs and their treatment up to July 1st, 2022. The reference lists of the analyzed articles were also considered a source of literature information. To search and analyze all relevant English articles, the following keywords were used in various combinations: neurofibromatosis type 1, optic pathway glioma, chemotherapy, precision medicine, MEK inhibitors, VEGF, nerve growth factor.

Over the past decade, basic research and the development of genetically engineered mice models of NF1-associated OPG have shed light on the cellular and molecular mechanisms underlying the disease and inspired animal and human testing of several compounds. A promising line of research is focusing on the inhibition of mTOR, a protein kinase controlling proliferation, protein synthesis rate and cell motility that is highly expressed in neoplastic cells. Several mTOR blockers have been tested in clinical trials, the most recent of which employed everolimus with encouraging results. A different strategy aims at restoring cAMP levels in neoplastic astrocytes and non-neoplastic neurons, since reduced intracellular cAMP levels contribute to OPG growth and, more important-ly, are the major determinant of NF1-OPG-associated visual decline. So far, however, this approach has only been attempted in preclinical studies. Stroma-directed molecular therapies – seeking to target Nf1 heterozygous brain microglia and retinal ganglion cells (RGCs) – are another fascinating field. Microglia-inhibiting strategies have not yet reached clinical trials, but preclinical studies conducted over the last 15 years have provided convincing clues of their potential. The importance of NF1-mutant RGCs in the formation and progression of OPGs also holds promise for clinical translation. The evidence of Vascular Endothelial Growth Factor (VEGF)-Vascular Endothelial Growth Factor (VEGFR) signaling hyperactivity in pediatric low-grade gliomas prompted the use of bevacizumab, an anti-VEGF monoclonal antibody, which was tested in children with low-grade gliomas or OPGs with good clinical results. Neurprotective agents have also been proposed to preserve and restore RGCs and topical eye administration of nerve growth factor (NGF) has demonstrated encouraging electrophysiological and clinical results in a double-blind, placebo-controlled study.

Traditional chemotherapy in patients with NF1-OPGs does not significantly ameliorate visual function, and its effectiveness in halting tumor growth cannot be considered a satisfactory result. Newer lines of research should be pursued with the goal of stabilizing or improving the vision, rather than reducing tumor volume. The growing understanding of the unique cellular and molecular characteristics of NF1-OPG, coupled with the recent publication of promising clinical studies, raise hope for a shift towards precision medicine and targeted therapies as a first-line treatment.

Key Words: Neurofibromatosis type 1, Optic pathway glioma, Vision loss, Targeted-therapies, Precision medicine.
Emerging treatments in NF1-related optic pathways gliomas

Introduction

Neurofibromatosis type 1 (NF1) is an autosomal dominant syndrome with a prevalence of one individual every 3,000, caused by a germline mutation in the neurofibromin 1 (NF1) gene\(^1,2\). NF1 is a tumor suppressor gene located on chromosome 17, which codes for neurofibromin, a cytoplasmic protein predominantly expressed in neurons, Schwann cells, oligodendrocytes, and leukocytes. People with NF1 have a genetic predisposition to developing tumors in both the central nervous system (CNS) and peripheral nervous system (PNS), including benign (such as neurofibromas) and malignant (such as malignant peripheral nerve sheath tumors, or MPNST) neoplasms\(^3-5\).

Neurofibromin is involved in downregulating the activity of proto-oncogene rat sarcoma (RAS)\(^6,7\) and also has other non-RAS-mediated functions relevant to NF1-related tumor initiation and progression\(^8\).

Similar to other tumor predisposition syndromes, patients with NF1 are born with a germline mutation in 1 copy of the NF1 gene, but tumors only arise following a somatic mutation of the other allele, thus leading to the complete loss of neurofibromin in specific and vulnerable cytotypes\(^9-11\).

CNS tumors developed by children with NF1 are mainly low-grade gliomas (LGGs), and most NF1-LGGs are World Health Organization (WHO) grade 1 pilocytic astrocytomas (PAs)\(^2\), whereas high-grade gliomas (HGGs), the most common of which is glioblastoma (GB), represent approximately 2% of brain tumors in children with NF1\(^13\). Fifteen to 20% of children with NF1 develop a LGG anywhere along the optic pathway, and 75 to 85% of these NF1-related optic pathway gliomas (NF1-OPGs) are located in the optic nerves or chiasm, while the remaining 15% of them arise in the post-chiasmatic optic pathway\(^2,20,26,38-42\), which is the main objective in the clinical management of these patients.

At present, indications for the treatment of pediatric NF1-OPGs are clinical progression (intended as a significant visual decline, whose assessment is problematic in preverbal children with frequent comorbid learning and attention deficits\(^33\)) and/or radiological progression detected by magnetic resonance imaging (MRI)\(^19\).

Because these tumors are not amenable to complete surgical resection due to their critical location\(^2\), and since radiotherapy in NF1 patients brings about an elevated risk of secondary malignancies\(^10\), neurocognitive\(^31\) and neuroendocrine disorders\(^32\), and radiation-induced vasculitis\(^33\), chemotherapy is the first line of treatment. Among the most frequently employed cytotoxic agents, carboplatin and vincristine are well tolerated and have been found\(^14\) to yield tumor response rates that are higher than those of children without NF1. In contrast, it is important to avoid alkylator agents in hereditary OPGs, as they can contribute to the development of secondary tumors\(^15\). Although being the best available option among traditional therapies and despite its efficacy in hindering or halting tumor growth\(^16,37\), chemotherapy is fraught with short- and long-term adverse effects and, most importantly, generally fails to improve or even preserve visual function\(^20,26,38-42\), which is the main objective in the clinical management of these patients.

Challenges of Visual Loss Treatment in NF1-OPG

The development of sophisticated technologies and efficient preclinical models [such as genetically engineered mice (GEM) strains], as well as the institution of international cooperation networks (such as the NF Clinical Trial Consortium), has raised the hope of future personalized treatment of NF1-related tumors\(^43\). At present, however, there are no effective therapeutic options for NF1-OPG-associated vision loss.
The development of precision medicine approaches targeting specific molecules involved in NF1-OPG formation, growth, and maintenance and in the associated visual decline requires at least 3 major obstacles to be circumvented.

First, an in-depth understanding of the signaling pathways affected by neurofibromin defect in neoplastic NF1-deficient cells and in non-neoplastic NF1-mutant cells, most notably microglia and retinal ganglion cells (RGCs), should be achieved.

Second, since NF1 is a heterogeneous disease, the therapeutic results obtained in a patient with specific characteristics might not be replicable in a patient bearing different features. Thus, it is crucial to identify patient subgroups with common biological profiles and, thus, with a higher probability of responding to a particular pharmacological treatment.

With regards to these two objectives, the major challenge is the shortage of biospecimens for genomic analysis, since most lesions are not biopsied prior to or following treatment. In addition, there is a lack of human NF1-pilocytic astrocytoma cell lines or patient-derived xenografts (PDXs), due to the low clonogenic nature of these tumors and to the requirement of a permissive microenvironment. GEM models of NF1-associated OPG [i.e., Nfatop1lox/lox glial fibrillary acidic protein-cyclization recombinase (GFAP-Cre) mice] have been developed with the specific purpose of finding ways around these obstacles. Although not perfectly representative of their human counterpart, such models allowed researchers to outline the molecular mechanisms underlying the development and growth of these neoplasms and the associated visual decline, shedding light on a variety of pathogenic aspects and inspiring the design of numerous trials.

Finally, since many NF1-OPGs are asymptomatic, and not all symptomatic forms require treatment, a standardized methodology should be developed to identify patients at risk for vision loss before serious irreversible damage occurs. Consistent with the finding that Nfatop1lox/mut GFAP-Cre mice do not show reduced visual acuity until 6 months of age, clinically evident visual impairment in NF1-OPG patients is generally associated with a 30% or more decrease in RGCs count. Hence, given the difficult assessment of vision in preverbal children with comorbid attention deficits, efforts should be made to identify reliable biomarkers of impending visual loss.

**Molecular Pathogenesis of NF1-OPGs**

Numerous in vitro and in vivo studies have been conducted over the years to reveal the mechanisms by which neurofibromin loss regulates glial cell proliferation and, more generally, the cellular and molecular pathogenesis of NF1-OPG. The results of these studies made it possible to define a multifactorial model of pediatric gliomagenesis, wherein tumor formation requires a number of factors, including activation of cellular growth pathways, signals from the tumor microenvironment, involvement of specific cell types in specific cerebral regions, the patient’s genetic profile, etc., thus explaining why only a minority of children with NF1 develop an OPG.

**Neurofibromin and Downstream Signaling Cascades**

Neurofibromin is a large cytosolic protein (220-250 kDa) containing a 300-amino-acid guanosin triphosphate (GTP)ase activating protein-related domain (GAP-related domain, or GRD) involved in downregulating the activity of proto-oncogene RAS by accelerating the conversion of RAS-GTP to its inactive GDP-bound form. Neurofibromin can also inhibit RAS-dependent growth independently of its GTPase-accelerating function.

Normally, following the interaction of a growth factor with its tyrosin-kinase or G protein-coupled receptor, RAS-GDP (inactive form) is converted to RAS-GTP (active form) by a guanine nucleotide exchange factor. Once RAS is converted to the GTP-bound form, its downstream signaling pathways are activated.

In NF1, due to neurofibromin loss (in neoplastic cells) or heterozygosity (in non-neoplastic cells), three main pathways are dysregulated and involved in the genesis of OPGs and OPG-associated visual loss, thus constituting ideal targets for precision medicine approaches.

First, in neoplastic NF1-deficient astrocytes, RAS-GTP activates phosphoinositide-3-kinase (PI3K), which in turn, phosphorylates and activates protein kinase-B (also known as AKT), a serine/threonine-specific protein kinase playing a key role in multiple cellular processes, including cell proliferation, partly through the mechanistic target of rapamycin (mTOR) complex. Both AKT and mTOR undergo increased phosphorylation and activation in human and murine NF1-associated CNS neoplasms.
Second, activated RAS binds to the rapidly accelerated fibrosarcoma (RAF) kinase molecule, triggering the activation of the whole RAS-RAF-mitogen activated kinase (MEK)-extracellular signal-regulated kinase (ERK) signaling pathway, which is dysregulated in sporadic LGGs, as well.\textsuperscript{59,55,56}

Notably, these two pathways converge toward the activation of mTOR, a protein kinase controlling proliferation, protein synthesis rate, and cell motility in astrocytes,\textsuperscript{57} even though the RAS-RAF-ERK pathway can control cell growth in an mTOR-independent fashion.\textsuperscript{58} These findings indicate mTOR as a potential target for both NF1-associated and sporadic PAs.\textsuperscript{1}

Third, RAS activation reduces cAMP generation in NF1-deficient astrocytes through a downstream effector pathway involving intermediates that converge on adenyl cyclase, the enzyme responsible for the synthesis of cAMP.\textsuperscript{59,60} The decrease in cAMP levels is relevant not only for NF1-deficient neoplastic cells but also for NF1-mutant non-neoplastic RGC neurons. CNS neurons are extremely vulnerable to reduced NF1 gene expression: in vitro, NF1\textsuperscript{-/-} CNS neurons (hippocampal neurons and RGC neurons) show reduced growth cone areas and neurite lengths and increased apoptosis compared to their wild-type counterparts; in vivo, GEM modeling NF1-associated OPGs show axonal damage in the retro-orbital optic nerve proximal to the site of glioma formation and augmented NF1\textsuperscript{-/-} RGC apoptosis.\textsuperscript{61} This abnormal phenotype results from impaired neurofibromin-mediated cAMP generation. Moreover, in CNS neurons, the neurofibromin/cAMP homeostasis operates in a RAS-dependent manner through the activation of atypical protein kinase C zeta (PCKz), rather than through the activation of the MEK/ERK or AKT/mTOR effector pathways, leading to GRK2-mediated G\textsubscript{ai} inactivation.\textsuperscript{62}

Collectively, these findings indicate that reduced cAMP intracellular levels mediate opposite effects in neoplastic (NF1-deficient) astrocytes (increased survival response) and in non-neoplastic NF1-mutant RGCs [decreased survival, with consequent thinning of retinal nerve fiber layer (RNFL) and decline of visual acuity in mice].\textsuperscript{7}

The RAS-regulatory domain, however, only comprises 10% of neurofibromin’s entire coding sequence.\textsuperscript{63} It has been recently shown\textsuperscript{64} that this protein has numerous binding domains and previously undescribed conformational states, arguing that this molecule mediates other RAS-independent functions. Among these newly reported properties, neurofibromin regulation of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels is one of the most relevant for optic gliomagenesis. Indeed, neurons from heterozygous NF1-mutant mice are hyperexcitable at baseline compared to their wild-type counterparts and uniquely sensitive to electrical activity.\textsuperscript{65,66} The mechanisms underlying NF1-mutant neuronal hyperexcitability have yet to be fully understood. However, NF1\textsuperscript{-/-} mice exhibit increased gamma amino butirric acid (GABA)ergic interneuron excitability, resulting from decreased HCN channel activity,\textsuperscript{67} which in turn results in increased midline secretion and downstream activation of the neuron-immune-cancer cell axis,\textsuperscript{68} as described later in this article.

**Role of Non-Neoplastic Cells in the Tumor Microenvironment**

GEM strains provided evidence\textsuperscript{44,46} of a tight relationship between neurofibromin-deficient (NF1\textsuperscript{-/-}) neoplastic glial cells and neurofibromin heterozygous (NF1\textsuperscript{-/+}) non-neoplastic stromal cells in the tumor microenvironment.

As previously described, optic gliomagenesis in mice and humans requires the biallelic inactivation of the NF1 gene in GFAP-positive neural-glial progenitors. Since NF1 knock-out (KO) mice (NF1\textsuperscript{-/-}) die at an embryonic stage,\textsuperscript{68,69} experimental models harboring a conditional inactivation of the NF1 gene in this specific cytotype were created by leveraging LoxP-Cre technologies.\textsuperscript{70} The surprising evidence\textsuperscript{71} that the complete loss of neurofibromin in GFAP-positive neural-glial progenitors, although causing a hyper-proliferative astrocyte response, is insufficient for OPG formation argues that additional factors derived from the surrounding NF1-heterozygous brain are necessary for tumorigenesis.

It has been observed that NF1\textsuperscript{-/-} mice undergoing biallelic inactivation of the NF1 gene in GFAP-positive neural-glial progenitors during embryogenesis [the so-called Nf1flox/mutGFAPCre mice or NF1\textsuperscript{-/-} GFAPcellKO (GFAPCKO) mice or fetal microchimeric cell (FMC) mice] develop OPGs most of the time.\textsuperscript{46,51} However, additional preclinical findings\textsuperscript{46} demonstrate that NF1 optic gliomagenesis only occurs if NF1-deficient astrocytes receive growth-promoting signals from an NF1-heterozygous tumor microenvironment in the surrounding brain.
The Role of Microglia

Microglial cells make up to 30-50% of tumor mass in human NF1-OPGs\textsuperscript{72}. These cells belong to the mononuclear phagocyte system and are involved in the maintenance of cerebral homeostasis through the promotion of neuronal survival, enhancement of synaptic transmission, and synthesis of neurotrophins\textsuperscript{73-76}. In addition, microglia can also produce chemokines, growth factors, and inflammatory mediators\textsuperscript{77,78} with relevance to two aspects of murine optic gliomagenesis.

Elevated microglia numbers represent one of the earliest events in the natural history of murine NF1-OPG and may be observed even before evident tumor formation in the optic nerves of Nf1\textsuperscript{+/-} GFAPCKO mice\textsuperscript{44,79}. It has been hypothesized that Nf1-deficient glial neoplastic cells release soluble molecules (i.e., stromagens) that recruit or activate Nf1-heterozygous microglia, which in turn produces factors promoting tumor proliferation (i.e., gliomagens)\textsuperscript{80-83}.

Recent studies\textsuperscript{84,85} have focused on the role of two gliomagens, namely the Ccl5 chemokine and the CXCL12 growth factor, both emerging as potential targets for stromal-directed molecular therapies. Second, microglia are involved in NF1-OPG-associated RGC death. Specifically, two mechanisms (one intrinsic – or cell autonomous – and one extrinsic – or cell non-autonomous) have been identified to underlie OPG-associated loss of Nf1-heterozygous RGCs, with such loss starting as a tumor-induced axonal dysfunction and culminating in death by apoptosis\textsuperscript{59}.

The intrinsic mechanism, as previously described, consists of a unique vulnerability of CNS Nf1-mutant neurons by virtue of their altered neurofibromin expression and consequent reduction in cAMP intracellular levels\textsuperscript{61}.

The extrinsic mechanism involves microglia and provides insight into the predilection of NF1-OPG-associated visual decline for girls. Gonadal estradiol binds to the estrogen receptor b (ER\textsubscript{b}) expressed by microglial cells and stimulates them to produce neurotoxin cytokines, able to damage RGC axons\textsuperscript{37,44,86}.

The Role of Neurons

Recent studies\textsuperscript{66} have shown that microglial cells are not the only cytotype involved in the tumor-stroma interactions that promote optic gliomagenesis. Preclinical studies\textsuperscript{66} have demonstrated that also central nervous system neurons (i.e., RGCs) may support neoplastic growth by secreting paracrine factors necessary for tumor initiation and progression in an NF1 mutation- and neuronal activity-dependent manner.

Specifically, a neuronal cell surface protein (NLGN3) and a neurite growth-promoting factor (midkine), both released in a RAS-independent fashion, have been called into question (Figure 1). NLGN3 is a synaptic adhesion protein expressed by oligodendrocyte precursor cells and neurons, whose ectodomain is cleaved by a Disintegrin and Metalloproteinase 10 (ADAM10)\textsuperscript{87}. This enzyme, mainly secreted by neurons, is activity-dependent: in the context of the baseline hyperexcitability of Nf1\textsuperscript{+/-} neurons\textsuperscript{8}, light-evoked stimulation of RGCs during a susceptible interval in Nf1\textsuperscript{OPG} mice post-natal life results in an increased secretion of ADAM10 and, therefore,

![Figure 1](flowchart_summarizing_recently_described_molecular_events_occurring_in_Nf1-mutant_RGCs_and_leading_to_NF1-OPG_initiation_and/or_progression.png)
in an increased NLGN3 shedding\textsuperscript{66}. The latter event seems to be crucial for both the initiation and the progression of murine OPGs, consistent with previous studies\textsuperscript{67-69} in xenografts models demonstrating the role of this molecule in the progression of high-grade gliomas (HGGs). For reasons yet to be clarified, Arg1809Cys germline\textsuperscript{90,91} mice, which, just like patients with the cytidine-to-thymidine R1809C germline NF1 gene mutation\textsuperscript{90,91}, never develop OPGs, do not exhibit increased expression of the ADAM10 transcript and consequently do not undergo increased NLGN3 shedding\textsuperscript{66}. These finding underscores both the importance of NLGN3 in optic gliogenesis and the mutation-dependence of NF1-OPGs formation. Similarly, in a recent study, NF1\textsuperscript{+/0} mice that reared in the dark from 6 to 16 weeks did not develop OPGs, whereas this tumor formed in all NF1\textsuperscript{+/0} mice raised in regular light cycles\textsuperscript{66}. Taken together, these results raise the possibility that neuronal activity-triggered NLGN3 shedding into the tumor microenvironment drives the initiation and promotes the maintenance of NF1-OPGs.

The second important OPG trophic molecule is midkine, whose secretion depends on the baseline hyperexcitability exhibited by NF1-mutant RGCs\textsuperscript{66}, caused by HCN channel dysregulation\textsuperscript{67}. Indeed, HCN channel targeting using its agonist lamotrigin decreased firing rates in vitro, while also reducing midkine (Mdk) RNA and protein levels and blocking Nf1-OPG progression, but not initiation, in vivo (i.e., in OPG-bearing NF1\textsuperscript{+/0}; hGFAP-Cre mice)\textsuperscript{90}.

Studies on human induced pluripotent stem cell (iPSC)-derived CNS neurons revealed that, similarly to NLGN3, midkine expression is increased in neurons harboring NF1 mutations that are found in NF1 patients who develop OPGs, but not in NF1\textsuperscript{+/0} neurons with the Arg1809Cys mutation\textsuperscript{92}.

Midkine is part of the so-called neuron-immune-cancer cell axis. In murine models of NF1-OPGs, NF1-mutant neurons secrete midkine to stimulate T-cell C-C Motif Chemokine Ligand 4 (Ccl4) expression, which induces microglial elaboration of Ccl5, an obligate OPG growth factor\textsuperscript{93}. Moreover, NF1\textsuperscript{1809} mice do not develop OPGs, even though their T-cells and microglia are able to secrete Ccl4 in response to midkine and Ccl5 in response to Ccl4, respectively, thus suggesting\textsuperscript{66} that the Arg1809Cys mutation operates at the level of the neuron.

Collectively, the above findings reveal that tumor-causing NF1 mutations in RGCs regulate the production of paracrine factors through both visual experience-evoked neuronal activity and HCN channel dysregulation-mediated baseline neuronal hyperexcitability. In this model of tumorigenesis, NF1-OPG initiation relies on light-induced RGC activation and consequent NLGN3 shedding, whereas NF1-OPG progression requires both NLGN3 shedding and HCN channel-dependent baseline neuronal hyperexcitability with consequent midkine production.

**Determinants of Disease Heterogeneity**

NF1-OPG is a disease of heterogeneity\textsuperscript{43}, meaning that each NF1 patient harbors a unique combination of diversely assorted variables, which influence the probability of developing the tumor and the risk of experiencing tumor-associated visual loss.

The identification of subgroups of patients with similar characteristics is crucial for both risk assessment purposes and for the development of precision medicine approaches aimed at targeting different phenotypes of the same disease.

Many of the determinants of disease heterogeneity were first identified using GEM strains. In addition to age, gender and tumor location (as previously described), other variables have recently been taken into account\textsuperscript{5}.

Several studies\textsuperscript{94-97} leveraging human cell lines or iPSCs derived from NF1-patients and mice strains engineered to harbor specific mutations demonstrated that different NF1 gene germline mutations yield different effects, both in terms of neurofibromin levels and function and in terms of the tumor phenotype. Further studies are required to identify predictive phenotype-genotype correlations, relevant for tumor-development risk assessment and for therapeutic purposes. Importantly, since the NF1 gene germline mutation is likely to impact non-neoplastic cells as well (e.g., RGCs), such phenotype-genotype correlations will hopefully also be useful for stratifying the risk of experiencing vision loss\textsuperscript{43}.

Genomic modifiers have gained interest as potential determinants of disease heterogeneity. The first evidence that genomic modifiers play a role in gliomagenesis came from a study\textsuperscript{98} on NPCis mice (NF1 GEM strains harboring heterozygous mutations in both the NF1 and p53 genes), which revealed that NF1\textsuperscript{+/0} p53\textsuperscript{+/+} mice maintained on a 129 mice substrain 129S4sv/Jae (129) background.
exhibited a low frequency of glioma formation, while \( Nf1^{+/-}; p53^{+/-} \) mice maintained on a substrain C57BL/6J (B6) background even developed HGGs. More recently, the first glioma-modifier locus was identified in humans, as it was shown\(^9\) that single nucleotide polymorphisms (SNPs) in adenylate cyclase 8 (AC8) increases the risk of glioma formation in females while decreasing it in males.

Although most patients with hereditary OPGs only harbor the \( NF1 \) gene inactivation\(^9\), recent data\(^10\) suggest that some of them might present additional genetic alterations, such as the fusion event between proteins KIAA1549:BRAF (typical of sporadic OPGs) or PTEN monoallelic mutations. Studies\(^11\) on GEM harboring these genetic alterations reveal a differential behavior in terms of mass volume and proliferation, as well as a diversity in the activation of the signaling pathways involved in cellular growth, suggesting that additional targeted therapies might be needed in these subgroups of NF1 patients.

### From Animal Studies to Clinical Trials: Difficulties and Limitations

Despite the progress achieved in the field of cellular and molecular biology and the possibility of testing drugs in animal models, at present, no effective treatment is available for the majority of NF1-related tumors.

To a large extent, this is due to the difficulties with which animal studies are transposed to the clinical setting, resulting from the multiple differences between GEM models from humans\(^12\), such as the CNS anatomy, the histological features of human PAs, and the diversity of the studied populations. Indeed, human patients represent a variegated population in terms of age, gender, tumor location, and \( NF1 \) germline mutation, whereas preclinical studies are conducted on a uniform genetic cohort.

Moreover, in murine models, histological samples are always available and homogeneous, as opposed to human neoplastic samples, which are scant and heterogeneous, making it difficult to assess drug bioavailability, including brain penetration, and neoplastic target inhibition\(^13\). Consequently, researchers\(^14\) base their evaluation mainly on tumor volume changes, that are not necessarily predictive of visual outcomes\(^15\), and on visual improvements, that might require months or years to appear\(^16,17,18\). Therefore, results obtained in animal studies are not always interpretable and are even less applicable to human patients.

### On the Road to Precision Medicine

Despite the lack of effective treatment options addressing the unique features of NF1-OPGs, significant progress has been made in the field of molecular and cellular biology, opening the way to promising drug design studies.

Clinical trials adopting tumor growth-halting pharmacologic strategies yielded disappointing results. Moreover, tumor shrinkage cannot be considered the priority endpoint in the management of NF1-OPGs for several reasons. First, tumor expansion in hereditary OPGs tends to naturally decline after 7 years of age\(^19\). Second, patients rarely die as a direct consequence of tumor growth. Third, tumor-related visual loss does not primarily reflect a compressive optic neuropathy but rather results from cell-intrinsic and cell-extrinsic mechanisms triggered by impaired neurofibromin function. Radiological outcomes are, therefore, not closely related to visual outcomes\(^20\).

These considerations suggest that reducing or stabilizing the volume of the mass is therapeutically suboptimal in NF1-OPGs and that future research efforts should focus on the molecular mechanisms underlying tumor-associated visual decline, in order to develop effective and safe neuroprotective strategies.

Herein, we review the main emerging pharmacological approaches recently assessed in pre-clinical and clinical settings (Figure 2, Table I).

#### RAS Effector Inhibition

Since neurofibromin acts as a RAS-GTPase-activating protein, initial targeted therapies for NF1-associated tumors leveraged RAS inhibitors. Specifically, farnesyltransferase inhibitors blocking isoprenylation (necessary for RAS membrane tethering), although showing promising results in animal studies\(^21\), yielded limited success in patients with NF1-related plexiform neurofibromas\(^22\), thus prompting researchers to focus on other molecules in the RAS signaling pathways. Mounting evidence\(^23,24\) suggests that inhibition of the RAS downstream effector pathways reduces tumor growth and, to a lesser extent, improves vision or prevents further visual
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So far, however, these therapies have largely failed to guarantee a durable effect following their interruption.

**Inhibition of mTOR-Converging Pathways**

Recent studies revealed that blocking the PI3K/AKT pathway (by means of PI3K inhibitor, BMK120, and AKT-inhibitor, MK2206) and the MEK/ERK pathway (by means of MEK inhibitor, PD0325901) reduces Nf1-deficient astrocyte proliferation to wild-type levels in vitro and decreases optic nerve volume and glioma proliferation in vivo (Nf1-OPG mice). In addition, both PI3K and MEK inhibition improved retinal dysfunction in Nf1+/− GFAPCKO mice, suggesting a complementary role for these pathways in NF1-OPG-related visual impairment, in addition to RAS-dependent reduced cAMP generation.

Consistently with the convergence of signaling pathways on mTOR and with the high levels of mTOR expressed by neoplastic cells, rapamycin-mediated inhibition of mTOR in cultures of Nf1-deficient astrocytes abrogated proliferation and motility phenotypes. However, decreased tumor proliferation was also observed in rapamycin-treated Nf1+/− GFAPCKO mice, the failure of low-dose regimens to increase tumor cell apoptosis and guarantee a sustained effect in vivo suggests a tumoristatic rather than a tumoricidal effect of rapamycin.

These encouraging results paved the way for clinical studies with mTOR inhibitors. In a recent trial, 19 patients (8 with NF1) received erlotinib and rapamycin for recurrent LGGs failing to respond to conventional treatment. While this 2-drug regimen was well tolerated, no objective responses were documented in patients with sporadic LGGs, while only one NF1 patient had a partial response (PR), defined as a ≥50% reduction in the bi-directional measurement of the tumor. Moreover, only two patients (both with NF1) maintained stable disease for more than 1 year after completion of treatment.

Shortly after the rapamycin-erlotinib trial, other attempts were made to manage pediatric LGGs with non-conventional agents.

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**Figure 2.** Scheme of the main molecular pathways dysregulated in NF1-OPGs and of the associated potential therapeutic targets.
Table I. Summary of the most relevant clinical trials in patients with NF1-OPGs.

<table>
<thead>
<tr>
<th>Tested molecule(s)</th>
<th>Pharmacological class</th>
<th>Trial phase and Identifier</th>
<th>Study population</th>
<th>Primary endpoint</th>
<th>Main results</th>
</tr>
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<tbody>
<tr>
<td>Erlotinib + Rapamycin</td>
<td>mTOR inhibitor</td>
<td>Phase I NCT00901849</td>
<td>19 patients with recurrent LGG, 8 with NF1-LGG</td>
<td>Radiographic and clinical evaluation for one year</td>
<td>No objective response, except for 1 NF1 patient who had PR</td>
</tr>
<tr>
<td>Everolimus</td>
<td>mTOR inhibitor</td>
<td>Phase II NCT01158651</td>
<td>23 patients with radiologic-progressive, NF1-LGG and prior treatment with a carboplatin-containing chemotherapy</td>
<td>Objective response rates (CR, PR, SD assessed by MRI) at 48 weeks</td>
<td>68% of patients exhibited some tumor response (shrinkage of arrest of tumor growth); of these, 66% remained free of progression.</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Multikinase inhibitor</td>
<td>Phase II NCT01338857</td>
<td>11 patients with recurrent LGG, 3 with NF1-LGG</td>
<td>Objective response rates (CR, PR, SD assessed by MRI)</td>
<td>Acceleration in tumor growth within 3 treatment cycles in &gt;80% of patients</td>
</tr>
<tr>
<td>Selumetinib</td>
<td>MEK inhibitor</td>
<td>Phase I NCT01089101</td>
<td>38 patients with progressive LGG, 5 with NF1-LGG</td>
<td>RP2D and DLTs</td>
<td>4/5 NF1 patients had some tumor response, but none exhibited PR; 10/25 patients in stratum 3 (40%) achieved PR with a 2-year PFS of 96±4%. Only 1 patient progressed while on treatment.</td>
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<td></td>
<td></td>
<td>Phase II (ongoing) NCT01089101</td>
<td>Patients assigned to 6 strata. Stratum 3 included 25 patients with NF1-LGG</td>
<td>Objective response rates (CR, PR, SD assessed by MRI)</td>
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<td>Bevacizumab + irinotecan</td>
<td>Angiogenesis inhibitor</td>
<td>Phase II NCT00381797</td>
<td>10 children with multiply recurrent LGG, 3 with NF1-LGG</td>
<td>Objective response rates (CR, PR, SD assessed by MRI) sustained for ≥8 weeks</td>
<td>3/3 NF1 patients exhibited an objective response by MRI. Only 1 patient showed some clinical improvement</td>
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<tr>
<td>Nerve Growth Factor (eye drops)</td>
<td>Neuroprotective agent</td>
<td>Phase I CHF6467-OPG</td>
<td>5 children with advanced optic atrophy due to OPG, 10 patients received NGF eye drops, 8 patients received placebo</td>
<td>Median VEP amplitude and latency, VEP (amplitude and latency), RNFL thickness (assessed by OCT)</td>
<td>Progressive increase in median VEP amplitude</td>
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<td>Phase II CHF6467-OPG</td>
<td>18 OPG patients with stable disease and severe visual loss (10 patients received NGF eye drops, 8 patients received placebo)</td>
<td></td>
<td>Significant improvements in PhNR amplitude at 180 days, PhNR latency at 15 days, and VEP amplitude at 30 days; 3 patients experienced a significant VF enlargement</td>
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</table>

LGGs = low-grade gliomas; NF1 = neurofibromatosis type 1; PR = partial response; CR = complete response; SD = stable disease; MRI = magnetic resonance imaging; RP2D = recommended phase II dose; DLTs = dose-limiting toxicities; PFS = progression-free survival; OPG = optic pathway glioma; VEP = visual evoked potential; BCVA = best-corrected visual acuity; VF = visual field; PhNR = photopic negative response; RNFL = retinal nerve fiber layer thickness; OCT = optical coherence tomography.
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In a multicenter, prospective, open-label, phase II clinical trial, 11 patients with progressive or recurrent LGGs (3 with NF1) who had failed at least 1 regimen of chemotherapy were treated with sorafenib, a multikinase inhibitor targeting vi-raf murine sarcoma viral oncogene homolog B1 (BRAF), vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor alpha (PDGFR), and protooncogene receptor tyrosine kinase (c-kit), which had yielded promising results in preclinical trials. However, since more than 80% of patients (irrespective of their NF1 or BRAF status) experienced an acceleration in tumor growth, the study was terminated early. Subsequent in vitro studies suggested that the observed effect was due to the paradoxical activation of ERK.

To bypass this effect, attention was shifted to MEK inhibitors. A phase I trial with orally-available MEK1/2 inhibitor selumetinib (AZD62DD) enrolled 5 patients with NF1-associated recurrent or refractory LGG, 4 of whom had some tumor response, but none exhibited PR (≥50% reduction in the tumor) to the treatment. The phase II trial assigned patients to 6 strata based on histology, BRAF aberration, and NF1 to allow correlation with tumor response and progression-free survival (PFS). Stratum 3 included 25 patients with NF1-LGG, 10 of whom (40%) achieved PR with a 2-year PFS of 96±4%. Only 1 patient progressed while on treatment.

The most recent mTOR-targeting clinical trials employed everolimus, an oral derivative of rapamycin that acts on neoplastic cells directly, by inhibiting neoplastic growth and proliferation, and indirectly, by down-regulating factors involved in tumor vascularcularity, such as the tumor cell hypoxia-inducible factor 1 and VEGF. The phase II study investigated the safety and efficacy of daily oral everolimus on radiographically progressive NF1-associated pediatric LGGs previously treated with chemotherapy. Everolimus resulted in disease stability or shrinkage at 48 months (primary endpoint) in 68% of the patients enrolled in the trial and was well-tolerated, even though functional end-points were not collected.

cAMP-Elevating Strategies

Since mounting evidence indicates that reduced intracellular cAMP levels contribute to OPG growth and, more importantly, are the major determinant of NF1-OPG-associated visual decline, cAMP-elevating agents have been leveraged in preclinical studies. cAMP-restoring strategies (either with an adenylyl cyclase activator, forskolin, or with a phosphodiesterase-4 inhibitor, rolipram), have been shown to reverse the phenotypical alterations observed in NF1-mutant CNS neurons to wild-type levels in vitro, while attenuating RGC apoptosis and inhibiting OPG growth in vivo.

Moreover, lovastatin, a negative RAS regulator active on both the mTOR-converging signaling pathways and RAS-cAMP-mediated RGC survival, has been used in 12-week-old NF1-OPG mice with the aim of instituting treatment before 30% RGC loss occurred. While the effect on tumor proliferation and volume was limited in time, a long-lasting preservation of RGC numbers and retinal nerve fiber layer (RNFL) thickness was observed, suggesting that there is a therapeutically relevant interval during which the adoption of neuroprotective strategies prevents further damage to the visual pathway.

Toward Stroma-Directed Molecular Therapies

Microglia

As tumor formation and tumor-associated vision loss require a permissive NF1+ cellular microenvironment, a fascinating line of research is looking at microglia to design stromal-directed molecular therapies. Microglia-inhibiting strategies have not yet reached clinical trials, but preclinical studies conducted over the last 15 years have provided exciting clues regarding their great potential. Many complementary approaches have been employed in these proof-of-principle studies. One of the first successful attempts leveraged a Jun N-terminal kinase (JNK) inhibitor, SP600125, to block the corresponding pathway that has been found to be hyperactive in NF1-mutant microglia, but not in NF1-deficient astrocytes: JNK blockade of NF1-mutant microglia ameliorated its increased proliferation and motility phenotypes in vitro and reduced OPG proliferation in vivo. Consistently, ganciclovir-mediated ablation of brain microglia in integrin CD11b-TK transgenic mice and genetic reduction of optic nerve microglia in FMC mice by means of impaired Cx3cr1 (chemokine receptor driving microglia migration) expression reduced tumor proliferation and delayed tumor formation, respectively,
while treatment with Ccl5 neutralizing antibodies even improved NF1-associated retinal dysfunction in vivo. Microglia is thought to be responsible for the sexually dimorphic visual loss observed in murine models, as suggested by the fact that female NF1-OPG mice have 3-fold more microglia than their male counterparts and that minocycline inhibition of these cells decreases RGC apoptosis in vivo. Moreover, pharmacologic inhibition of microglial ERβ function by means of selective estrogen receptor antagonist PHTPP reduced both proliferating Ki-67+ cells and RGC apoptotic TUNEL+ cells in female NF1-OPG mice. While this finding establishes the ERβ-driven activation of microglia as a key determinant in NF1-OPG sexually dimorphic visual loss in mice, further investigation is needed to explain the corresponding gender predilection in pediatric patients.

**Neurons**

The importance of NF1-mutant RGCs in the formation and progression of OPGs has great potential for clinical translation. Over the last years, the ADAM10-NLGN3 axis and the neuron-immune-cancer cell axis have emerged as attractive therapeutic targets. Recently, researchers have demonstrated that treatment of Nf1+/- mice with GI254023X (a specific and brain-penetrant inhibitor of ADAM10) reduces NLGN3 shedding in the optic nerve. In addition, it phenocopies the effects of NLGN3 loss or dark-rearing in Nf1-OPGs. Indeed, optic nerve volumes and proliferation in Nf1+/- mice treated with GI254023X are indistinguishable from those found in wild-type mice. Furthermore, the efficacy of lamotrigine in blocking Nf1-OPG progression in vivo establishes the HCN channel – and the downstream midkine-Ccl4-Ccl5 pathway – as a targetable regulator of neuronal activity-dependent tumor growth for the treatment of childhood NF1-OPGs.

**Angiogenesis Inhibitors**

The evidence of VEGF-VEGFR signaling hyperactivity in pediatric LGGs and the encouraging results yielded in HGG adult patients treated with bevacizumab, an anti-VEGF monoclonal antibody, were the rationale for the administration of bevacizumab and irinotecan in 10 children with multiply-recurrent LGGs (3 with NF1) lacking other treatment options. All three patients with NF1 exhibited an objective response assessed by MRI (2 partial responses and 1 minor response), and 2 of them showed some sort of clinical improvement (including increased vision in 1 patient). In a recent case series, 4 pediatric patients with OPG (2 with NF1) already treated with chemotherapy or radiotherapy received treatment with bevacizumab (alone or combined with irinotecan) for progressive visual acuity or visual field loss. All 4 patients showed a radiologic response (decreased in size and enhancement by MRI) and experienced marked visual improvement, with near-complete visual field restoration in 1 of the 2 NF1 patients. These impressive results on OPG-mediated visual loss may be ascribed to a combined effect on tumor expansion and tumor-associated inflammatory edema. However, caution is required prior to administering bevacizumab, since transient leukoencephalopathy, proteinuria, and hypertension are reported adverse events.

**Nerve Growth Factor (NGF)**

Since the leading cause of morbidity in children with OPGs is progressive and largely irreversible visual loss, future efforts should shift towards neuroprotection and neuroregeneration of the retina and the optic pathway. Nerve growth factor (NGF) is a neurotrophin that acts on peripheral and central neurons, as well as on non-neuronal cells. The therapeutic potential of NGF has been proposed for a number of non-ocular neurological conditions, including Alzheimer’s disease and sensitive neuropathy associated with diabetes and HIV infection. More recently, it has been proposed to use NGF topically. The rationale for the use of topical NGF in OPGs comes from studies showing that, when applied to the conjunctiva, this molecule reaches the retina, the optic pathway, and the cerebral cortex, demonstrating biological activity in these regions.

In 2011, Falsini et al evaluated the effects of topical NGF in 5 children with advanced optic nerve atrophy due to LGGs and showed that, compared to the untreated controls, there was a progressive increase in visual evoked potential (VEP) amplitude (primary end-point) peaking at 90 days post-treatment and declining at 180 days, though still remaining above the baseline level. These promising results led to a randomized, double-blind, phase II clinical trial in 18 OPG patients with stable disease and severe visual loss. Patients were evaluated by testing visual acuity, visual field, VEPs, optic coherence tomography.
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(OCT), electroretinographic photopic negative response (PhNR), negative-going wave following the b-wave of the cone electroretinogram driven by RGCs, and MRI before and after treatment at 15, 30, 90, and 180 days. Treatment with NGF led to statistically significant improvements in PhNR amplitude at 180 days, PhNR latency at 15 days, and VEP amplitude at 30 days, and 3 NGF-treated patients experienced a significant visual field enlargement\cite{129}.

In both these exploratory studies\cite{128,129} no effect was reported on tumor growth. Moreover, treatment was well tolerated in all patients, with no ocular adverse events, other than a short-lasting mild periocular burning in a few of them.

**Conclusions**

Since NF1-OPGs are not amenable to complete resection, their treatment is based on non-surgical strategies. The risk of developing secondary malignancies, radio-induced vasculitis, and neurocognitive and neuroendocrine impairments greatly limits the role of radiotherapy in NF1 pediatric oncologic patients, whereas chemotherapy has a better risk-benefit profile in this setting. Traditional chemotherapy, however, does not significantly ameliorate visual function, and its effectiveness on halting tumor growth cannot be considered a satisfactory result in the management of these patients.

Newer lines of research should therefore be pursued with the goal of stabilizing or improving the vision, rather than the tumor volume, of these patients. Future clinical trials in pediatric patients with low-grade gliomas should plan recruitment stratification and statistical analysis by NF1 status. In addition, these clinical studies should include vision function endpoints as a distinct outcome measure of efficacy.

The growing understanding of the unique cellular and molecular characteristics of NF1-OPG, coupled with the recent publication of promising clinical studies, raise cautious hopes that further progress will be made to break down the barriers that still stand in the way of precision medicine and that targeted therapies will indeed become a first-line treatment option for NF1-OPG in the near future.

**Conflict of Interest**

The Authors declare that they have no conflict of interests.

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**Informed Consent**

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**ORCID ID**

Alessia Amato: 0000-0002-9515-7363
Bruno Pietro Imbimbo: 0000-0002-0327-7262
Benedetto Falsini: 0000-0002-3569-4968.

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