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Eliminating barriers to personalized medicine

Learning from neurofibromatosis type 1

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ABSTRACT

With the emergence of high-throughput discovery platforms, robust preclinical small-animal models, and efficient clinical trial pipelines, it is becoming possible to envision a time when the treatment of human neurologic diseases will become personalized. The emergence of precision medicine will require the identification of subgroups of patients most likely to respond to specific biologically based therapies. This stratification only becomes possible when the determinants that contribute to disease heterogeneity become more fully elucidated. This review discusses the defining factors that underlie disease heterogeneity relevant to the potential for individualized brain tumor (optic pathway glioma) treatments arising in the common single-gene cancer predisposition syndrome, neurofibromatosis type 1 (NF1). In this regard, NF1 is posited as a model genetic condition to establish a workable paradigm for actualizing precision therapeutics for other neurologic disorders. *Neurology*® 2014;83:1-9

GLOSSARY

cAMP = cyclic adenosine monophosphate; **GEM** = genetically engineered mouse; **GWAS** = genome-wide association studies; **mTOR** = mammalian target of rapamycin; **NF1** = neurofibromatosis type 1; **NSC** = neural stem cell; **OPG** = optic pathway glioma; **PA** = pilocytic astrocytoma; **RGC** = retinal ganglion cell.

Neurofibromatosis type 1 (NF1) is one of the most common monogenic disorders in which affected individuals develop benign and malignant tumors.¹ NF1 impacts 1:2,500 people worldwide, and individuals with NF1 are prone to the development of peripheral (neurofibromas, malignant peripheral nerve sheath tumors) and central (optic pathway glioma, malignant glioma) nervous system tumors.^{2,3} Similar to other autosomal dominant cancer predisposition syndromes,^{4,5} people with NF1 start life with a germline mutation in one copy of the *NF1* tumor suppressor gene; however, tumors require somatic (acquired) inactivation of the remaining functional *NF1* allele, leading to complete loss of *NF1* expression in specific cell types.^{6,7} For example, complete *Nf1* gene inactivation in neuroglial^{8,9} or Schwann cell^{10,11} progenitors is required for murine optic glioma or neurofibroma formation, respectively.

With the development of numerous accurate small-animal (genetically engineered mouse; GEM) models of NF1-associated nervous system tumors (table 1),⁸⁻¹⁸ the creation of the NF Clinical Trials Consortium,¹⁹ and the establishment of response criteria for NF1 clinical trials,²⁰ the stage has been set for the discovery and validation of promising therapeutic strategies and their translation to people affected with NF1. However, despite these advances, there are currently no effective therapies, which likely reflects the striking biological and clinical heterogeneity inherent to this condition. This review uses NF1-associated brain tumors (optic glioma) as an illustrative platform to discuss the barriers and challenges to developing and implementing effective targeted therapies.

NF1-ASSOCIATED OPTIC PATHWAY GLIOMA NF1-associated optic pathway gliomas (NF1-OPGs) are largely pediatric tumors typically arising in children younger than 7 years of age.^{21,22} As such, 15%–20% of children with NF1 will develop World Health Organization grade I pilocytic astrocytomas (PAs) anywhere along the optic pathway, from the retro-orbital optic nerve to the postchiasmatic optic tracts (figure 1). In addition to neoplastic glial cells, 30%–50% of the cells in these tumors are non-neoplastic cells (microglia)

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Table 1 *Nf1* GEM nervous system tumor models

Tumor type	Genetic strain	Reference
Optic glioma	<i>Nf1</i> ^{fllox/mut} ; GFAP-Cre	8,9
Malignant glioma	<i>Nf1</i> ^{+/-} ; <i>p53</i> ^{+/-}	14
	<i>Nf1</i> ^{fllox/fllox} ; <i>p53</i> ^{fllox/fllox} ; <i>Pten</i> ^{fllox/fllox} ; nestin-Cre ^{ER}	15
	<i>Nf1</i> ^{fllox/fllox} ; <i>p53</i> ^{fllox/fllox} ; nestin-Cre ^{ER}	15
Dermal neurofibroma	<i>Nf1</i> ^{fllox/mut} ; CMV-Cre ^{ER}	11
Plexiform neurofibroma	<i>Nf1</i> ^{fllox/fllox} ; PLP-Cre	12
	<i>Nf1</i> ^{fllox/mut} ; Krox20-Cre	10
	<i>Nf1</i> ^{fllox/fllox} ; Dhh-Cre	13
MPNST	<i>Nf1</i> ^{+/-} ; <i>p53</i> ^{+/-}	16,17
	<i>Nf1</i> ^{fllox/fllox} ; <i>Pten</i> ^{fllox/fllox} Dhh-Cre	18

Abbreviations: GEM = genetically engineered mouse; MPNST = malignant peripheral nerve sheath tumor.

harboring one functional copy of the *NF1* gene and one nonfunctional *NF1* allele (germline *NF1* gene mutation).²³

Since baseline MRI is not useful in predicting clinical outcome,²⁴ it is currently not possible to identify children at greatest risk for developing NF1-OPG. In addition, following NF1-OPG identification, there are no reliable methods for determining which child is most likely to experience continued visual decline and require treatment, necessitating frequent MRI (often requiring sedation) and neuro-ophthalmologic evaluations, which can be unreliable in preverbal children.^{25,26} At present, affected children typically receive chemotherapy routinely used for sporadic PA (carboplatin/vincristine), with varying success. Radiation therapy is not used because of the elevated risk of secondary cancer development in this cancer predisposition syndrome.²⁷ Despite radiographic evidence of tumor stabilization or response following chemotherapy, only a minority of children with NF1-OPG experience improvements in vision.^{28,29} In addition, there is increasing concern that chemotherapy in this young age group may result in cognitive decline as a late secondary sequela, negatively impacting long-term quality of life.³⁰ Collectively, these issues highlight the pressing need to develop management strategies that reflect the unique biology of NF1-OPG.

USE OF *NF1* GEM STRAINS TO UNDERSTAND *NF1*-OPG PATHOGENESIS

There are numerous important unanswered questions relevant to the care of children with NF1-OPG that may lead to the improved management of these brain tumors, ranging from explaining the unique spatial and temporal distribution of these tumors to understanding the cellular and molecular constraints that drive glioma formation and progression. Ideally, these answers would emanate from discovery efforts using human

tissue specimens; however, in the case of NF1-OPG, few patients undergo tumor biopsy or removal prior to or following treatment. Moreover, many of the available NF1 brain tumor specimens represent tumors with unusual features, such as those arising in older children or in brain regions other than the optic pathway,³¹ and may not be representative of the more commonly encountered OPGs in children with NF1.

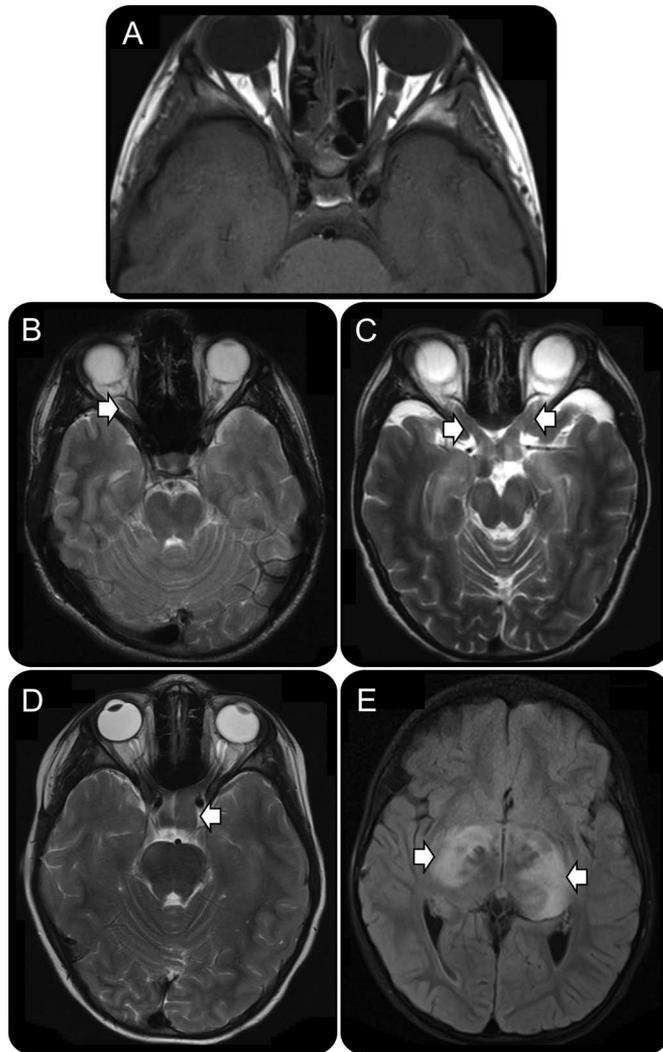
In light of the above challenges with human tissue specimens, *Nf1* GEM strains have been developed that model the optic gliomas arising in children with NF1.^{8,9,32} While not perfect representations of the human condition, *Nf1* optic glioma mice have yielded several unanticipated observations critical to the future design and execution of clinical trials for these tumors. It should be appreciated that these findings were only made possible through the use of *Nf1* GEM strains.

Understanding the unique temporal and spatial pattern of NF1-OPG. To address the question of why NF1 gliomas display a propensity for the optic pathway and brainstem of young children, researchers used a series of *Nf1* mutant mice in which somatic *Nf1* gene inactivation could be experimentally manipulated. Murine optic gliomas require complete *Nf1* gene inactivation in specific neuroglial progenitors (neural stem cells; NSCs) during mid to late embryogenesis, thus creating a narrow developmental window in which tumor initiation must occur.^{32,33} The ability of these NSCs to generate gliomas following *Nf1* loss during embryonic development, but not in more differentiated glial cell types after birth, likely accounts for the predilection for these tumors to arise in young children.

Second, NSCs from some brain regions, but not others, increase their growth following *Nf1* gene inactivation. In these studies, *Nf1* gene inactivation in NSCs residing in the third ventricle or brainstem results in stem cell expansion and increased glial differentiation, whereas no effect is observed following *Nf1* loss in cortical or lateral ventricle NSCs.^{32,34} These findings partly explain the distinct brain region distribution of these tumors (optic pathway/brainstem but rarely in the cortex).

Identifying NF1-OPG targeted therapies. Further analysis of the molecular basis underlying the above brain region- and cell type-specific effects revealed that the impact of *Nf1* gene inactivation is dependent on how the downstream RAS signaling pathway functions in specific cell types. The *NF1* gene codes for the protein neurofibromin, which contains a 300 amino acid domain similar to other proteins that function as negative regulators of the RAS proto-oncogene.³⁵ While neurofibromin controls cell growth in numerous different cell types in a RAS-dependent manner,^{36,37}

Figure 1 MRI reveals different types of optic gliomas arising in children with NF1



(A) Normal optic nerves and chiasm in a child with neurofibromatosis type 1 (NF1) but no optic pathway glioma. (B) Right optic glioma (arrow). (C) Bilateral optic nerve gliomas (arrows). (D) Optic chiasm glioma (arrow). (E) Postchiasm optic tract glioma (arrows).

there are several distinct molecules that transmit the RAS growth signal, including MEK and AKT. Whereas RAS/MEK signaling is critical for murine *Nf1* plexiform neurofibroma growth,³⁸ *Nf1* loss uniquely affects NSCs from the third ventricle and brainstem by activating AKT in a mammalian target of rapamycin (mTOR)-regulated manner,³⁴ thus identifying the mTOR pathway as a key mediator of NF1-OPG growth. This cell type-specific growth dependence led to the evaluation of rapamycin, initially in preclinical mouse optic glioma models³⁹ and then in a clinical trial for children with NF1-associated glioma (ClinicalTrials.gov identifier NCT01158651).

The fact that NF1-PAs are cellularly heterogeneous tumors composed of several distinct cell types raises the intriguing possibility that non-neoplastic stromal cells (e.g., microglia) may actively participate

in NF1-OPG formation and growth. Consistent with this hypothesis, *Nf1* optic glioma formation in mice requires the interplay between noncancerous cells (e.g., microglia) harboring one mutated *Nf1* allele and precancerous cells (astroglial progenitors/astrocytes) with complete neurofibromin loss. As such, GEM strains only lacking *Nf1* gene expression in neuroglial progenitors do not form optic gliomas⁴⁰; optic gliomagenesis necessitates that *Nf1* loss in neuroglial progenitors occur in mice with a germline inactivating *Nf1* gene mutation in all cells in the brain.^{8,9} Additional support for a critical role for microglia in glioma development and growth derives from numerous complementary studies employing *Nf1* GEM strains. In these experiments, silencing microglia function using either pharmacologic or genetic strategies reduces optic glioma tumor growth.^{41,42} Moreover, impairing microglia migration into the region of the developing mouse optic glioma delays tumor formation.⁴³ The obligate role for noncancerous cells in the tumor microenvironment (stroma) offers new potential strategies for stroma-directed therapy. Studies are currently under way in several laboratories to identify these glioma-associated microglial factors.^{44,45}

The key role of the microenvironment in tumor development and growth is further underscored by analogous observations involving mast cells and macrophages in *Nf1* GEM plexiform neurofibromas.⁴⁶ Similar to *Nf1* murine optic gliomas, *Nf1* plexiform neurofibroma formation requires that *Nf1* loss in Schwann cell precursors occur in mice with a germline inactivating *Nf1* gene mutation (*Nf1*+/- mice).¹⁰ Using bone marrow transplantation, *Nf1*+/- bone marrow-derived mast cells, but not those from normal mice, induce plexiform neurofibroma development in normal mice with *Nf1* loss in Schwann cell precursors only.⁴⁷ Moreover, the replacement of *Nf1*+/- bone marrow-derived mast cells with normal ones reduced plexiform neurofibroma growth in *Nf1*+/- mice with *Nf1* loss in Schwann cell precursors. The dependence on mast cells led to the identification of the KIT receptor as a potential target for antitumoral therapy. In addition to promoting mast cell infiltration relevant to plexiform neurofibroma growth, KIT is also critical for tumor macrophage accumulation.⁴⁸ As such, blocking KIT activation in *Nf1* plexiform neurofibroma-bearing mice with imatinib resulted in attenuation of tumor growth, which is currently being explored further in human clinical trials.

Defining the cellular and molecular basis for NF1-OPG-associated visual loss. One of the major disappointments has been the limited visual recovery in children following treatment with chemotherapy for clinically progressive NF1-OPG. Using *Nf1* optic glioma mice, visual impairment was shown to result from

Table 2 Comparison of preclinical and clinical studies

Factor	Human NF1-OPG	Mouse <i>Nf1</i> optic glioma
Pathologic specimens	Uncommonly acquired	Always acquired
	Heterogeneous collection	Homogeneous collection
Age and sex	Variable	Specific age or sex
Tumor locations	Variable	One location
Germline <i>NF1</i> gene mutation	Variety of mutations	One specific targeted mutation
Genomic background	Heterogeneous	Inbred genetic background
Timing of somatic <i>NF1</i> inactivation	Variable	One defined time point
Clinical behavior	Many "distinct" diseases	One single disease
Potential therapeutic targets	Many targets	Limited targets

Abbreviations: NF1 = neurofibromatosis type 1; OPG = optic pathway glioma.

tumor-induced retinal ganglion cell (RGC) axonal dysfunction, leading to RGC death by apoptosis.⁴⁹ The mechanism underlying this neuronal death involves neurofibromin regulation of cyclic adenosine monophosphate (cAMP) production, such that restoring normal cAMP homeostasis in *Nf1* optic glioma mice partially ameliorates the RGC apoptosis.⁵⁰ This finding raises intriguing questions about the role of neuroprotective approaches in reversing visual loss in children with NF1-OPG.

Identifying genomic factors that influence NF1-OPG development and progression. The low incidence of NF1-OPG could reflect the factors discussed above; however, it is also possible that genomic variation contributes to glioma development. As such, defining the genomic determinants that favor optic gliomagenesis has potential value for predictive risk assessment. In this regard, many *Nf1* GEM studies employ mice maintained on a specific inbred genetic background, whereas others use mice on a mixed background. Humans by definition harbor a mixed genomic composition that reflects subtle chromosomal polymorphisms inherited from each of our parents. Studies have begun to unravel the genomic contributions to tumorigenesis in *Nf1* GEM strains: whereas *Nf1*+/-;*p53*+/- mice develop high-grade astrocytomas (gliomas) when maintained on a C57BL/6 background, *Nf1*+/-;*p53*+/- mice maintained on a 129sv background exhibit a significantly reduced frequency of glioma formation.^{51,52} Similarly, >90% of *Nf1*^{flax/mut}; GFAP-Cre mice maintained on a C57BL/6 background develop optic gliomas,⁸ compared to ~20% maintained on a mixed genetic background.⁹ While astrocytoma predisposition genomic modifiers have been identified in mice, their application to the human condition has not been fully explored.

Another genomic factor that might influence NF1-OPG is the sex of the patient. As such, one exciting observation to emerge from *Nf1* GEM studies is the impact of sex on NF1-OPG-induced vision loss. In *Nf1* optic glioma mice, reduced cAMP levels and higher levels of RGC apoptosis are observed in females, leading to reduced visual acuity in female mice only.⁵³ Moreover, while the frequency of OPG development is similar in girls and boys with NF1, 3 times more females with NF1-OPG require treatment for visual decline.⁵³ These findings support a role for sex as a critical factor that underlies clinical outcomes in children with NF1-OPG.

TRANSLATING BASIC SCIENCE DISCOVERIES TO HUMAN THERAPEUTIC CLINICAL TRIALS

Despite these exciting advances in molecular/cellular biology and the ability to efficiently evaluate drugs in accurate preclinical models prior to human clinical trials, there are presently no effective treatments for most NF1-associated tumors. In part, this may reflect the manner in which *Nf1* GEM preclinical drug study results are translated to the clinical workplace. As such, biologically based therapies frequently exhibit dramatic antitumoral responses in mice, but limited tumor shrinkage in human clinical trials. For example, imatinib was shown to be highly effective at attenuating *Nf1* GEM plexiform neurofibroma growth,⁴⁷ but it exhibited far less efficacy for treating human NF1-associated plexiform neurofibromas (<20% tumor response).⁵⁴ Future preclinical studies will need to incorporate outcome expectations (e.g., number of mice exhibiting a response, percent of tumor reduction, and the durability of the effect) that parallel those employed in human clinical trials.

There are significant differences inherent in *Nf1* GEM preclinical and NF1 patient clinical trials (table 2). Taking NF1-OPG as an illustrative example, human NF1 clinical trials do not involve the collection of pathologic specimens and they enroll patients of different ages, sexes, and tumor locations. As such, the genomic background, the timing of the somatic *NF1* gene inactivation event, and the specific germline *NF1* gene mutation vary from patient to patient. For this reason, the clinical outcomes reflect the fact that the patients enrolled in these trials constitute a diverse population of distinct disease subgroups, likely defined by the factors that influence NF1-OPG heterogeneity, with potentially different molecular drivers of tumor growth and druggable therapeutic targets. In addition, the lack of pathologic specimens prevents assessments of CNS drug penetration and target inhibition in the tumor, causing investigators to rely solely on changes in radiographic appearance (tumor volume) and visual improvement. Since tumor volume does not correlate with visual improvement and vision changes may require months

(or even years) to fully manifest,^{28,29} the outcomes of current clinical trials may not be interpreted accurately.

In contrast, each *Nf1* optic glioma GEM strain represents a homogeneous population of mice in which the genetic background, inherited *Nf1* mutation, timing of somatic *Nf1* loss, age, sex, and tumor location are identical. As such, these preclinical *Nf1* GEM strains model only one disease subgroup with a more limited number of druggable targets. Moreover, optic glioma specimens are routinely acquired in these preclinical studies, enabling a demonstration of drug bioavailability (CNS penetration) and target inhibition. In this manner, while the use of a single homogeneous population of *Nf1* optic glioma mice provides more interpretable experimental outcomes, the resulting findings may only be applicable to one subtype of NF1-OPG.

NF1-OPG IS A DISEASE OF HETEROGENEITY As outlined above, it is highly likely that NF1-OPG comprises multiple distinct diseases defined by specific factors, including patient age, sex, tumor location, *NF1* germline mutation, genomic background, and other genetic changes (figure 2). The identification of the responsible risk factors will likely yield clinically relevant subgroups of patients who could be stratified for tailored therapeutic approaches that best match their unique subtype.

Age. In a large multicenter study, children younger than 2 years were more likely to experience visual decline secondary to NF1-OPG.⁵⁵ While the etiology of this observation remains unknown, it may reflect the cell of origin and the developmental period during which *NF1* inactivation occurs.

Sex. As mentioned above, female mice as well as female humans with NF1-OPG are more likely to experience visual decline and require treatment than their male counterparts.⁵³ These observations raise the possibility that females have different epigenetic programming due to X-chromosome influences, which change the intracellular context in which changes in neurofibromin expression affect biological outcomes. Identifying these epigenetic determinants may reveal new genes for risk stratification and therapeutic targeting.

It is also possible that sex influences optic glioma outcomes through differential hormonal production. In this scenario, female hormones (e.g., estrogen) may directly act on RGC neurons to influence intracellular cAMP levels and cell survival. As such, reduced neurofibromin function in RGC neurons may have different consequences in females, in whom heterotrimeric G protein–induced cAMP production is uniquely modulated, relative to their male counterparts. By leveraging this potential hormonal mechanism, it may be possible to envision therapies that

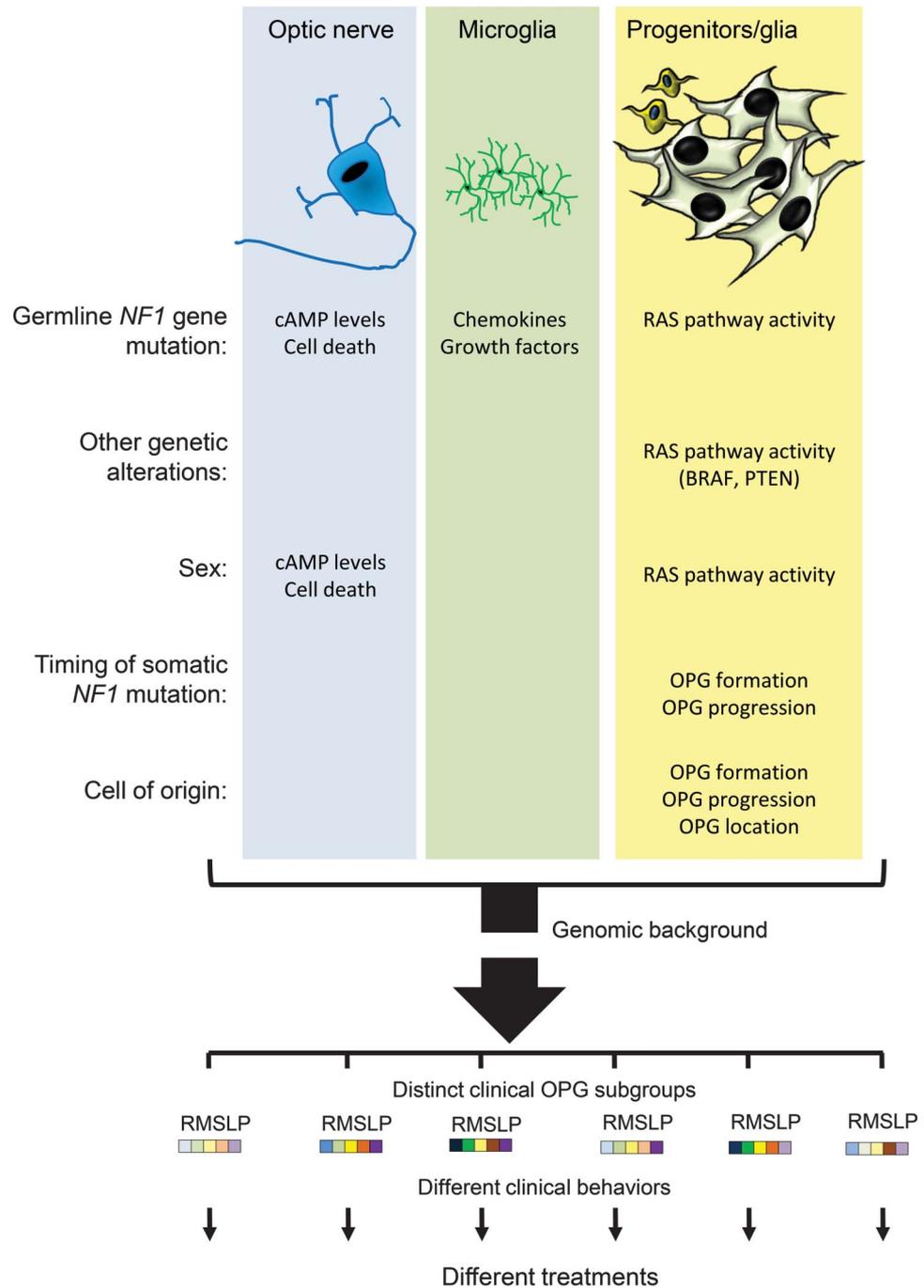
target this receptor by repurposing drugs used to treat hormonally responsive male and female cancers.

Location. Several studies have demonstrated that NF1-OPGs involving the postchiasmatic optic tracts are more likely to cause visual decline and require treatment.⁵⁵ Moreover, this effect is independent of sex, suggesting that it reflects the primary biology of these tumors.⁵³ As more diverse GEM models of NF1-OPG are developed, it might be possible to mechanistically determine why tumors in this location have such different outcomes relevant to the design of targeted therapies.

Germline NF1 gene mutation. Emerging evidence from numerous laboratories has begun to reveal that the *NF1* germline mutation in people with NF1 may have distinct consequences on the spectrum of the clinical features observed. As such, recognized genotype–phenotype correlations include individuals from different families harboring the c1756-1759_delACTA mutation, who, despite having the other features of NF1, do not develop dermal neurofibromas.⁵⁶ Similarly, individuals with 5' end *NF1* gene frameshift and premature truncation mutations are prone to developing optic gliomas.^{57,58} In addition, patients with *NF1* locus microdeletions that delete the entire *NF1* gene are prone to the development of malignancies (malignant peripheral nerve sheath tumor, high-grade glioma), which may reflect the co-deletion of other tumor suppressor genes (e.g., *SUZ12* gene) in the region.^{59,60} Together, these data suggest that not all germline *NF1* gene mutations are equal in their biological effects.

In addition, it is important to recognize that the differential impact of these germline *NF1* gene mutations on non-neoplastic cells (neurons and microglia) may be profound: mutations that mildly impair neurofibromin function in neurons would be predicted to have less deleterious effects on glioma-induced RGC survival and vision than mutations that completely abrogate neurofibromin expression from that allele. Similarly, microglia with *NF1* germline mutations that result in significantly reduced neurofibromin expression may elaborate higher levels or a different spectrum of gliomagens (growth factors and chemokines) that promote glioma growth than those with relatively normal neurofibromin function. In this regard, correlating the germline *NF1* mutation with tissue-specific neurofibromin expression levels may provide meaningful insights into NF1-OPG development and outcome as well as the design of future stroma-directed therapies and neuroprotective strategies. Studies are currently under way to generate *Nf1* GEM strains harboring specific NF1 patient-derived germline *Nf1* gene mutations for such mechanistic studies.

Figure 2 Determinants of NF1-OPG disease heterogeneity



Neurofibromatosis type 1–optic pathway glioma (NF1-OPG) heterogeneity is determined by a confluence of individual factors that individually affect cell biology and glioma risk. For example, the specific germline *NF1* gene mutation creates differential effects on cyclic adenosine monophosphate (cAMP) levels and retinal ganglion cell (RGC) death in neurons (denoted R in the subgroup bar code), chemokine and growth factor production in microglia (denoted M in the subgroup bar code), and RAS pathway activation in neoplastic progenitors/glia (denoted S in the subgroup bar code). Similarly, other genetic alterations (*KIAA1549:BRAF* or *PTEN* mutation) alter the activity of the RAS pathway relevant to *NF1*-deficient tumor cell growth. In addition, patient sex leads to differences in cAMP levels (neurons) or RAS pathway activity (neoplastic progenitors/glia) to affect RGC survival or optic glioma growth. Likewise, the timing of and cell type with somatic *NF1* gene inactivation influences NF1-OPG brain location (optic nerve vs postchiasmatal tracts, denoted L in the subgroup bar code) or clinical features (clinical progression, denoted P in the subgroup bar code). Finally, the genomic background represents another strong determinant of tumor development and progression. Together, these factors (depicted as colored boxes to illustrate their relative effects) could be used to construct risk assessment algorithms that inform clinical practice.

Genomic factors. While genome-wide association studies (GWAS) have not yielded genomic predictors of glioma development in the general population owing to the diversity of initiating and cooperating genetic events required for malignant gliomagenesis, all patients with NF1-OPG share a similar genetic etiology (germline mutation in the *NF1* tumor suppressor gene). Recent studies have begun to reveal single nucleotide polymorphisms that may predict NF1-OPG development (Dr. Joshua Rubin, written communication, 2014). While still early in their validation and application to predictive testing, they may permit early glioma risk assessment in a population with a known propensity for brain tumor formation. Similarly, the use of twin studies may also facilitate the identification of these genomic factors.⁶¹ The preselection of at-risk children for intense monitoring changes the current anticipatory management paradigm to one of more directed medical monitoring.

Additional genetic mutations. While the vast majority of NF1-associated low-grade gliomas analyzed by next-generation sequencing harbor only *NF1* gene inactivation,²³ recent data suggest that some NF1-OPGs may have monoallelic loss of *PTEN* gene expression or concurrent *KIAA1549:BRAF* alterations.⁶² Recent *Nf1* GEM studies in our laboratory in which these genetic changes were introduced revealed differential effects on tumor volume and proliferation. Moreover, the extent and diversity of growth control signaling pathway activation (e.g., MEK or AKT activation) is also different, raising the possibility that tailored treatment regimens will be required to suppress the growth of these NF1-OPGs compared to their counterparts harboring only neoplastic cell *NF1* gene inactivation.

CHALLENGE FOR PRECISION MEDICINE The implementation of individualized therapies will require information not currently available that can only be derived from large collaborative studies that leverage resources not widely available in the NF community. First, large data sets for epidemiologic investigations aimed at identifying clinical associations using population science approaches are required to provide hypotheses for future mechanistic laboratory investigation. For example, with the launch of the NF1 Patient Registry Initiative, a Web-based, patient-driven registry,⁶³ relationships between specific medical conditions and NF1 brain tumor risk have begun to emerge (Dr. Kimberly Johnson, written communication, 2014). Second, the availability of genomic DNA and *NF1* germline mutation data may enable GWAS that reveal new genomic predictors of NF1-OPG development as well as novel genotype-phenotype correlations. The resulting data, when

confirmed, may allow clinicians to more accurately predict which children with NF1 are likely to develop OPG as well as which at-risk children are most likely to require treatment following NF1-OPG formation.

Third, there is a paucity of renewable human biospecimens for drug evaluation. The assembly of large induced pluripotent stem cell repositories for reprogramming into neurons, neuroglial progenitors, and microglia offers unprecedented opportunities for translational research. The use of human cells from patients with NF1 may set the stage for more personalized therapies. Fourth, future preclinical studies might consider the use of multiple GEM models of NF1-OPG that differ with respect to the germline *Nf1* gene mutation, the timing of somatic *Nf1* gene inactivation, the genetic background of the mouse, and the sex of the animal. The availability of a heterogeneous “clinic population” of NF1-OPG GEM strains may allow investigators to identify those distinct disease subgroups most likely to respond to specific targeted therapies.

With the successful deployment of the above strategies, it is possible to envision a future proactive model of NF1 clinical care in which young children with café-au-lait macules (initial presenting feature of NF1) are assessed for their risk of optic glioma development and then stratified into clinical subgroups based on predictive modeling for targeted therapies that reflect their individual underlying disease pathogenesis and biology. Of importance, the lessons learned from and paradigms created for NF1-OPG will likely apply to other neurologic disorders similarly characterized by clinical heterogeneity.

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