Eliminating barriers to personalized medicine: Learning from neurofibromatosis type 1
David H. Gutmann

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Eliminating barriers to personalized medicine
Learning from neurofibromatosis type 1

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.

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. 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).
Table 1  

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Genetic strain</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optic glioma</td>
<td>Nf1+/−, GFAP-Cre</td>
<td>8,9</td>
</tr>
<tr>
<td>Malignant glioma</td>
<td>Nf1+/−; p53+/−</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Nf1+/−, p53+: Pten+/−, nestin-Cre</td>
<td>15</td>
</tr>
<tr>
<td>Dermal neurofibroma</td>
<td>Nf1flox/flox; CMV-Cre</td>
<td>11</td>
</tr>
<tr>
<td>Plexiform neurofibroma</td>
<td>Nf1flox/flox; PLP-Cre</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Nf1flox/flox; Krox20-Cre</td>
<td>10</td>
</tr>
<tr>
<td>MPNST</td>
<td>Nf1flox/flox; Dhh-Cre</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Nf1+/−; p53+/−</td>
<td>16,17</td>
</tr>
<tr>
<td></td>
<td>Nf1flox/flox; Ptenflox/flox; Dhh-Cre</td>
<td>18</td>
</tr>
</tbody>
</table>

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

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, 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. 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.

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. While neurofibromin controls cell growth in numerous different cell types in a RAS-dependent manner,
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-OPAs 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 neurofibrin loss. As such, GEM strains only lacking Nf1 gene expression in neuroglial progenitors do not form optic glioma40: 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

(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) Postchiasmal optic tract glioma (arrows).
tumor-induced retinal ganglion cell (RGC) axonal dysfunction, leading to RGC death by apoptosis. 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. 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. Similarly, >90% of Nf1−/−/−; GFAP-Cre mice maintained on a C57BL/6 background develop optic gliomas, compared to ~20% maintained on a mixed genetic background. 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. 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. These findings support a role for sex as a critical factor that underlies clinical outcomes in children with NF1-OPG.

**Table 2 Comparison of preclinical and clinical studies**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Human NF1-OPG</th>
<th>Mouse NF1 optic glioma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathologic specimens</td>
<td>Uncommonly acquired</td>
<td>Always acquired</td>
</tr>
<tr>
<td>Age and sex</td>
<td>Variable</td>
<td>Specific age or sex</td>
</tr>
<tr>
<td>Tumor locations</td>
<td>Variable</td>
<td>One location</td>
</tr>
<tr>
<td>Germline NF1 gene mutation</td>
<td>Variety of mutations</td>
<td>One specific targeted mutation</td>
</tr>
<tr>
<td>Genetic background</td>
<td>Heterogeneous</td>
<td>Inbred genetic background</td>
</tr>
<tr>
<td>Timing of somatic NF1 inactivation</td>
<td>Variable</td>
<td>One defined time point</td>
</tr>
<tr>
<td>Clinical behavior</td>
<td>Many &quot;distinct&quot; diseases</td>
<td>One single disease</td>
</tr>
<tr>
<td>Potential therapeutic targets</td>
<td>Many targets</td>
<td>Limited targets</td>
</tr>
</tbody>
</table>

Abbreviations: NF1 – neurofibromatosis type 1; OPG – optic pathway glioma.
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. 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. 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. Moreover, this effect is independent of sex, suggesting that it reflects the primary biology of these tumors. 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. Similarly, individuals with 5’ end NF1 gene frameshift and premature truncation mutations are prone to developing optic gliomas.

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. 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 postchiasmal 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:BRADF 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 biopsies 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 “clinical 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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