Neuro-Oncology Advances
2(S1), i134–i140, 2020 | doi:10.1093/noajnl/vdaa017

Clinical trial design in neurofibromatosis type 1 as a model for other tumor predisposition syndromes

Andrea M. Gross and Brigitte C. Widemann

Pediatric Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Corresponding Author: Andrea M. Gross, MD, Pediatric Oncology Branch, National Cancer Institute, National Institutes of Health, 9000 Rockville Pike Building 10, Room 1-5742, Bethesda, MD 20892, USA (Andrea.gross@nih.gov).

Abstract

Up to 10% of all pediatric cancer patients may have an underlying germline mutation which predisposed them to develop a malignancy. With more patients being tested for and diagnosed with genetic tumor predisposition syndromes, there has been improved characterization of their many nonmalignant manifestations. However, designing and implementing clinical trials to treat the nonmalignant tumor and non-tumor manifestations of these syndromes poses many unique challenges. Unlike trials for malignancies where tumor response and survival can be used as straightforward trial endpoints, the nonmalignant manifestations are often chronic, evolve more slowly over time, and may not be immediately life-threatening. Therefore, they will likely require a different approach to both testing and treatment with a focus on more functional and patient-reported outcome trial endpoints. The recent success of treatment trials for the benign tumors plexiform neurofibromas in the tumor predisposition syndrome neurofibromatosis type 1 (NF1) can be used as a model for the development of clinical trials in other tumor predisposition syndromes. In this article, we review the unique challenges associated with targeting the nonmalignant aspects of these conditions as well as some of the lessons learned from the NF1 experience which may be applied to other syndromes in the future.

Keywords

clinical trial design | genetic tumor predisposition syndromes | germline predisposition | neurofibromatosis type 1 | rare disease

Background

The use of molecularly targeted and immunotherapy-based therapies is revolutionizing the world of pediatric oncology. Breakthroughs such as the FDA approvals of chimeric antigen receptor-modified T cells for refractory acute lymphoblastic lymphoma[1] and targeted therapy with larotrectinib against TRK fusion-positive cancers[2] would not have been possible without the extensive preclinical and clinical research infrastructure that exists within the oncology space. In particular, the ability to perform international and standardized clinical trials has been crucial for these developments. While all clinical trials have their unique challenges, studying malignant conditions is somewhat simplified by the predictable and usually life-threatening consequences of these diseases, which allow for the use of relatively straightforward clinical trial designs with a focus on tumor shrinkage and overall survival as a key measure for activity and benefit, respectively.

While the importance of these advances cannot be overstated for the patients and families impacted by these conditions, it is important to note that up to 10% of pediatric malignancies arise in the setting of germline mutations,[3–5] which may cause not only the development of cancer but also a wide variety of other tumor and non-tumor related manifestations. The same targeted therapies that have been successfully used to treat the malignant manifestations of these conditions may be able to ameliorate some of these other, often debilitating, manifestations of tumor predisposition syndromes. However, designing efficient and effective clinical trials for these nonmalignant manifestations presents many challenges. The recent success of clinical trials targeting the benign tumors that occur within the genetic tumor predisposition

syndrome neurofibromatosis type 1 (NF1)[6] may serve as a model for the development of targeted therapies for other tumor predispositions syndromes in the future.

A 2017 meeting of the Pediatric Working Group of the American Association for Cancer Research identified 9 major groups of tumor predisposition syndromes: (1) Li-Fraumeni Syndrome, (2) neurofibromatoses, (3) overgrowth syndromes and Wilms tumor, (4) neural tumors, (5) gastrointestinal cancer predisposition, (6) neuroendocrine syndromes, (7) leukemia predisposition, (8) DNA instability syndromes, and (9) miscellaneous syndromes.[7] It is notable that nearly all of these groups include syndromes that have important nonmalignant complications associated with them (Table 1). For example, the overgrowth syndrome WAGR (Wilms tumor–aniridia–genitourinary anomaly–retardation) is characterized by the presence of developmental delay and anatomic abnormalities unrelated to the primary malignant manifestation. Similarly, in the neuroendocrine syndrome multiple endocrine neoplasia type 2B, all patients in a recent retrospective review had at least 1 non-endocrine manifestation, such as marfanoid body habitus, gastrointestinal symptoms, or mucosal neuromas.[8]

The standard drug development and approval pathway in the oncology setting depend on 3 key elements: (1) establishing a maximum tolerated dose (MTD) or optimal dose for further testing, (2) achieving measurable tumor shrinkage, and (3) demonstrating prolonged overall or progression-free survival compared with standard therapies. However, these elements each have limited applicability in the setting of clinical trials for tumor predisposition syndromes. First, establishing a true MTD may not be necessary or appropriate in a population that will likely require treatment over a prolonged period of time and in whom toxicities may be different based on their underlying germline mutation. Second, while achieving tumor shrinkage may be one possible endpoint for these syndromes, the nonmalignant manifestations of tumor predisposition syndromes may include very slow growing or complex benign tumors, such as plexiform neurofibromas (PN), as well as a variety of other features for which standard uni- or bi-directional measurements (RECIST or WHO) may not be appropriate, such as skeletal deformities or cardiovascular defects. Third, while life-altering, potentially life-shortening, and often causing severe morbidity, most tumor predisposition syndromes are not immediately life-threatening in the absence of malignant disease. Survival is therefore not a practical or feasible endpoint for these trials. This highlights the need to focus on other, nontraditional, clinically meaningful endpoints, such as improvements in functional and patient-reported outcome (PRO) measures.

In addition to the difficulty of applying standard oncology-based trial design[22] to tumor predisposition syndromes as described above, there are other more specific challenges associated with these conditions which have likely limited the number of clinical trials conducted to date. As with most pediatric conditions, the relatively small numbers of patients with these syndromes make designing clinical trials with sufficient statistical power to draw meaningful conclusions difficult. However, as the example of pediatric oncology and the collaborative successes of groups such as the Children's Oncology Group, the International Society of Pediatric Oncology, and others have demonstrated, trials for rare diseases are possible with enough motivation and national and international cooperation. In addition, the young age of many of these patients at diagnosis and the fact that they will likely require treatment over a long period of time rather than for a pre-specified treatment course as is often used for malignancies means that the tolerability of these agents must be closely assessed along with their efficacy. Given the underlying tumor predisposition syndromes in these patients, long-term safety outcomes need to be carefully monitored as well to ensure that the treatment itself is not resulting in an increased rate of secondary malignancies or other adverse events. In addition, children with noncancer conditions may not tolerate toxicities the same way children with aggressive cancers do. Determining the most appropriate time to initiate treatment for a nonmalignant condition can also be difficult, as side effects of the treatment could outweigh benefits if one is treating a condition that is not yet symptomatic for the patient with the goal of preventing future morbidity. The final and perhaps largest challenge for designing clinical trials in these diseases lies in defining clinical trial endpoints which are both measurable and clinically meaningful to patients and their families. We are highlighting recent advances in the treatment of NF1-associated PN as an example of how these challenges can be overcome.

Case Example: Neurofibromatosis Type 1

NF1 is a genetic tumor predisposition syndrome characterized by germline mutations in the tumor-suppressor gene *NF1* leading to neurofibromin deficiency and excessive activation of the *RAS* pathway.[23,24] While these patients are at an increased risk for aggressive malignancies such as juvenile myelomonocytic lymphoblastic leukemia and malignant peripheral nerve sheath tumors, these occur only in a minority of patients with NF1.[25] In contrast, manifestations such as PN, histologically benign nerve sheath tumors, occur in up to 50% of patients with NF1.[26,27] Low-grade optic pathway gliomas occur in 15–20% of children with NF1[28] and nearly 100% of patients with NF1 will develop one or more cutaneous neurofibromas in their lifetime.[29] Since the discovery of the role of neurofibromin as a tumor suppressor in the *RAS* pathway,[23,30] a variety of clinical trials targeting these manifestations have been undertaken. Phase I trials of the farnesyltransferase inhibitor tipifarnib,[31] thalidomide,[32] pegylated interferon,[33] pirfenidone,[34] sorafenib,[35] and selumetinib[36] for PN resulted in a number of important lessons being learned. First, the need for long-term tolerability of these agents became apparent, as did the fact that the recommended dose for a tumor predisposition syndrome such as NF1 may be lower than that recommended in the setting of a malignant condition. For example, sorafenib, despite very promising preclinical data showing PN shrinkage in mouse models,[37] was not tolerated by patients with NF1 even at doses well below the pediatric solid tumor MTD.[35]

Table 1 Examples of Wide Variety of Tumor and Non-tumor Manifestations Across a Variety of Tumor Predisposition Syndromes

	Example Tumor Predisposition Syndrome	Affected Gene	Associated Malignant Tumor(s)	Associated Low Grade/Nonmalignant Tumor(s)	Key Non-tumor Manifestations
Neurofibromatoses	Neurofibromatosis type 1[9,10]	*NF1*	Malignant peripheral nerve sheath tumors, juvenile myelomonocytic leukemia (JMML), pheochromocytomas, rhabdomyosarcomas, breast cancer	Plexiform neurofibromas, cutaneous neurofibromas, optic pathway gliomas, gastrointestinal stromal tumors	Skin manifestations (café au lait macules), skeletal deformities (tibial pseudarthrosis, dystrophic scoliosis, sphenoid wing dysplasia), cardiovascular anomalies, neurocognitive defects
Overgrowth syndromes	WAGR syndrome[11]	*WT1*	Wilms tumor	Gonadoblastoma	Aniridia, glaucoma, cataracts, genitourinary defects, pancreatitis, intellectual disability
Neural tumor syndromes	Gorlin syndrome[12]	*PTCH1*, *SUFU*	Basal cell carcinoma, medulloblastoma	Keratocystic odontogenic tumors, cardiac or ovarian fibromas	Macrocephaly, hypertelorism, plantar/palmar pits, lamellar calcification of the falx cerebri
GI cancer syndromes	Peutz–Jeghers syndrome[13,14]	*STK11*	Colorectal cancer, breast cancer, pancreatic cancer, gynecological cancer, small bowel cancer, lung cancer, and gastroesophageal cancers	Gastrointestinal polyps	Mucocutaneous pigmented lesions
Neuroendocrine syndromes	Multiple endocrine neoplasia syndrome, type 2B[8,15]	*RET*	Medullary thyroid carcinoma, pheochromocytoma	Mucosal neuromas	Marfanoid body habitus, skeletal deformities (scoliosis, pectus excavatum), ophthalmologic defects (alacrima, eversion of upper eyelids), gastrointestinal symptoms
Leukemia predisposition syndromes	GATA2 deficiency[16]	*GATA2*	Acute myeloid leukemia	Myelodysplastic syndrome	Pulmonary alveolar proteinosis, lymphedema
DNA instability syndromes	Dyskeratosis congenita[17,18]	*DKC1*, *TERT*, *TERC*, *TINF2*, *N0P10*, *NHP2*, *TCAB1*, *C16orf57*	Acute myeloid leukemia, head and neck squamous cell carcinomas, anorectal cancer	Myelodysplastic syndrome	Pulmonary fibrosis, hepatic cirrhosis, intrauterine growth retardation, developmental delay; microcephaly, nail dystrophy, premature greying; excessive sweating; short stature; hypogonadism; enteropathy; liver disease; esophageal and urethral stenosis; osteoporosis; avascular necrosis of the hips and shoulders
Miscellaneous syndromes	Costello syndrome[19–21]	*HRAS*	Embryonal rhabdomyosarcoma	Cutaneous papilloma	Cardiovascular anomalies, linear growth defects, macrocephaly, generalized hypotonia, pectus deformities, palmoplantar keratoderma

Each of these manifestations could be considered a potential endpoint for a future clinical trial.

Similarly, in the phase 1 trial of selumetinib, the MTD of 25 mg/m^2 b.i.d. was only 60% of the adult recommended dose for malignancies.[36]

The second key lesson revealed by both the phase 1 studies and key phase 2 studies for PN such as the trials of sirolimus[38] and pirfenidone[39,40] was the need to develop a clinical trial endpoint different than those used for most malignant conditions. The phase 1 trial of tipifarnib for refractory solid tumors and PN in NF1 and the phase 1 trial of thalidomide for PN used bi-directional line measurements (WHO criteria) for evaluation of tumor response,[31,32] though the relatively slow growth and complex shape of PN compared with other solid tumors made this difficult and limited the ability to sensitively detect changes. Given these limitations, a more sensitive method for evaluating tumor growth over time was developed at the National Cancer Institute by using volumetric MRI.[41] This methodology allows for more sensitive detection of both response and disease progression on clinical trials, which ensures that patients on trials are not receiving prolonged treatment with an ineffective agent. Volumetric MRI has since been accepted as the standard method for phase 2 trials of PN in the NF1 community.[42]

The phase 1 trial of selumetinib, a MEK 1/2 inhibitor, demonstrated not only drug tolerability but also disease response using volumetric MRI, with tumor shrinkage in 17 of 24 (71%) patients on the trial.[36] While these findings were promising, shrinkage of tumors in a benign condition is not sufficient to warrant the use of a potentially toxic medication in pediatric patients over a prolonged period of time. Therefore, an ongoing phase 2 trial was designed that includes not only the primary endpoint of tumor volumetric response, but also key secondary endpoints evaluating the impact of treatment on functional endpoints, such as pulmonary function tests and overall strength and range of motion, as well as for changes in PN-related pain and other impacts on quality of life using PRO. One challenge with this approach is the fact that PN can be located anywhere in the body, and the tumor location has a large impact on the types of symptoms experienced by the patient. Therefore, specific functional and PRO evaluations must be tailored to each patient's needs. Preliminary results from this study not only confirmed the volumetric responses seen in the phase 1 portion of the trial, with 34 of 50 (68%) patients achieving a confirmed partial response, but also showed improvements in functional outcome measures such as strength and PRO measures of pain and quality of life.[6] The results of this study led to the US FDA to grant selumetinib breakthrough designation for the treatment of NF1 in April 2019. At the same time, other MEK inhibitors have shown promise in PN and NF1 associated low-grade gliomas.[43–45]

Several barriers to the use of these key secondary trial endpoints existed at the time the phase 2 selumetinib trial was designed, most of which apply not only to NF1 but also to most other tumor predisposition syndromes. First, no validated functional measures had been published for NF1 at the time of study initiation. Since that time, there have been several advances in the assessment of functional endpoints for NF1. The Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS)[46] international collaboration has brought together researchers, clinicians, and patient representatives to establish a set of consensus response criteria for NF1 clinical trials, with particular emphasis on airway and visual function.[47,48] This type of multi-disciplinary collaboration could be a good model for other rare diseases to follow. In addition, Mullin et al.[49] recently published on the good inter- and intra-rater reliability of specific functional outcomes (the functional reach test, timed up and go test, and the 10-meter walk test) in adult patients with NF1, which will be useful for the design of future clinical trials. These advances highlight the importance of ongoing collaboration to establish meaningful and validated clinical trial endpoints in this population.

There are similar limitations for PRO and observer-reported outcome measures in the NF1 population. The REiNS group performed a systematic review of existing PRO measures and has published a series of recommendations regarding which ones are most appropriate for this population, including the numeric rating score 11 (NRS-11) for pain intensity, the pain interference index, and the PROMIS physical functioning measure.[50,51] In 2013, Nutakki et al.[52] published on the development, feasibility, reliability, and validity of an NF-specific measure of health-related quality of life, the Adult PedsQL NF1 Module. They followed this with a pediatric version, the PedsQL NF1 Module for pediatric patients age 5–25.[53] In addition, Dr. Pamela Wolters' work on developing validated PRO for pain in NF1 was accepted by the FDA Clinical Outcome Assessment (COA) program (DDT COA #000061). The existence of these types of validated measures will greatly facilitate future clinical trials, and their development can be used as a model for non-NF1 disorders.

A challenge of using functional and PRO endpoints for NF1, which will also likely apply to other tumor predisposition syndromes, has been determining whether improvements in these measures directly correlate with the degree of tumor volumetric response. In NF1-related PN, this correlation is elusive for several reasons. First, the absolute volume of tumors does not necessarily correlate with the degree of symptoms as even small tumors in a sensitive location (eg, the orbit, airway, or spinal canal) can result in significant symptoms. Similarly, even small changes in tumors located in these areas can result in significant symptomatic improvements. Second, the effect of MEK inhibitor treatment on functional and PRO outcomes such as pain is likely multifactorial and may not be exclusively related to the effect on tumor volume. Third, since very few patients experienced disease progression during the course of treatment, establishing a true correlation of symptoms to tumor volume was not statistically feasible. Similar issues are likely to be encountered with other tumor predisposition syndromes in which the absolute size and number of tumors may not be directly correlated with symptoms. While randomized controlled trials would help in establishing true treatment versus placebo effect, this type of design can be challenging in a rare disease population. The use of creative trial designs, such as cross-over or "n of 1" trials may help address this issue.[54] Similarly, rigorous natural history studies are essential for determining the usual progression of symptoms in these populations.

In addition to prospective studies evaluating functional and PRO measures, one of the critical aspects in developing treatment trials for PN in NF1 has been a better

understanding of the natural history of the disease process. The growth rate and pattern of PN had been incompletely described when the first targeted clinical trials for them began in the early 2000s. The use of the volumetric MRI analyses techniques described above allowed for better characterization of the growth rate of PN in children, with particular focus on the fact that PN growth tends to be most rapid in young children and slows down in young adults,[55] and that tumor growth does not accelerate during puberty as had been previously thought.[56] In March 2019, the FDA published draft guidance for industry highlighting the importance of natural history studies to support the development of treatments for rare diseases.[57] To date, there have been no phase 3 trials for the treatment of PN. In part, this is due to the small patient population as well as the lack of a current standard of care against which randomization could occur. Therefore, natural history studies have been essential and are being used as a control group to show the efficacy of targeted interventions such as MEK inhibitors.

Using NF1 as a Model for Other Tumor Predisposition Syndromes

The ability to conduct successful clinical trials for the nonmalignant manifestations of NF1 has hinged on 3 factors: (1) a better understanding of the natural history of the disease, (2) international collaboration in the development of accepted and meaningful clinical trial endpoints, and (3) a network of preclinical and clinical researchers evaluating a pipeline of scientifically justified agents for use in these tumors. These factors can be translated to other tumor predisposition syndromes and used as a model for future clinical trial and drug development. There are several syndromes that stand out as potential opportunities for advancement at this time.

First, while NF1 is caused by mutations in the *NF1* gene leading to activation of the *RAS* pathway, germline mutations in *RAS* or the *RAS* pathway lead to a variety of disorders collectively known as "RASopathies" and include Costello syndrome, Cardiofaciocutaneous syndrome, Legius syndrome, Noonan syndrome, and Noonan syndrome with multiple lentigenes.[58] These disorders share an overlapping phenotype of tumor predisposition, cardiac anomalies, and developmental delay among other features. Given a large number of RAS-targeted agents being developed for malignant indications in adults, these syndromes are prime targets for future clinical trials utilizing these agents to treat the nonmalignant manifestations of these diseases, such as neurocognitive deficits or cardiac dysfunction, and to possibly prevent the development of malignancy.[59,60] Ongoing international efforts spearheaded by researchers and patient advocacy groups are working toward the establishment of a more detailed natural history of these diseases in order to better establish the meaningful clinical trial endpoints needed.[61]

Other conditions, such as Proteus syndrome, a tumor overgrowth syndrome caused by somatic mosaic gain-of-function mutations in AKT1,[62] are also building on the foundation of a strong natural history study in combination with a unique trial design to advance the field. In 2019, Keppler-Noreuil et al.[63] published a study of Miransertib, an AKT1 inhibitor, which used levels of AKT phosphorylation, a pharmacodynamic marker, as the primary endpoint to establish an appropriate dose for this population. A phase 2 trial studying the efficacy of Miransertib for various manifestations of Proteus syndrome, including the disfiguring cerebriform connective tissue nevi, is currently ongoing (NCT03094832).

Conclusions

The future of clinical trials for nonmalignant manifestations of germline tumor predisposition syndromes is bright but will require a significant investment of time and effort from both the research and patient advocacy communities. A large number of targeted therapies now available for the malignant manifestations of these conditions have significant implications for germline mutations in these same pathways and can lead to effective therapies, as demonstrated with the success of MEK inhibitors for NF1-related PN. However, before interventional clinical trials can be successful, robust natural history studies and meaningful clinical trial endpoints must be established. It is important to note that while functional and PRO endpoints may be more essential for determining the benefit of treatment in nonmalignant conditions, the importance of these measures even in trials for malignant conditions is being increasingly recognized as necessary for drug approval by the FDA.[64] Therefore, advancements in the development of these types of meaningful endpoints could provide benefits not just for the tumor predisposition syndrome community but also for more traditional oncologic clinical trials. Ongoing efforts in these areas are promising and should be supported by future research.

Funding

No funding to report.

Conflict of interest statement. No financial conflicts of interest to report.

Authorship Statement Both A.M.G. and B.C.W. directly contributed to the content of this original manuscript.

References

1. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med.* 2013;368(16):1509–1518.

2. Drilon A, Laetsch TW, Kummar S, et al. Efficacy of larotrectinib in TRK fusion-positive cancers in adults and children. *N Engl J Med.* 2018;378(8):731–739.
3. Mody RJ, Wu YM, Lonigro RJ, et al. Integrative clinical sequencing in the management of refractory or relapsed cancer in youth. *JAMA.* 2015;314(9):913–925.
4. Parsons DW, Roy A, Yang Y, et al. Diagnostic yield of clinical tumor and germline whole-exome sequencing for children with solid tumors. *JAMA Oncol.* 2016;2(5):616–624.
5. Zhang J, Walsh MF, Wu G, et al. Germline mutations in predisposition genes in pediatric cancer. *N Engl J Med.* 2015;373(24):2336–2346.
6. Gross A, Wolters P, Baldwin A, et al. SPRINT: phase II study of the MEK 1/2 inhibitor selumetinib (AZD6244, ARRY-142886) in children with neurofibromatosis type 1 (NF1) and inoperable plexiform neurofibromas (PN). *J Clin Oncol.* Abstract presented at ASCO; June 2, 2018; Chicago, IL;36(15 Suppl):10503–10503.
7. Brodeur GM, Nichols KE, Plon SE, Schiffman JD, Malkin D. Pediatric cancer predisposition and surveillance: an overview, and a tribute to Alfred G. Knudson Jr. *Clin Cancer Res.* 2017;23(11):e1–e5.
8. Castinetti F, Waguespack SG, Machens A, et al. Natural history, treatment, and long-term follow up of patients with multiple endocrine neoplasia type 2B: an international, multicentre, retrospective study. *Lancet Diabetes Endocrinol.* 2019;7(3):213–220.
9. Hirbe AC, Gutmann DH. Neurofibromatosis type 1: a multidisciplinary approach to care. *Lancet Neurol.* 2014;13(8):834–843.
10. Ferner RE, Huson SM, Thomas N, et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. *J Med Genet.* 2007;44(2):81–88.
11. Fischbach BV, Trout KL, Lewis J, Luis CA, Sika M. WAGR syndrome: a clinical review of 54 cases. *Pediatrics.* 2005;116(4):984–988.
12. Foulkes WD, Kamihara J, Evans DGR, et al. Cancer surveillance in Gorlin syndrome and Rhabdoid tumor predisposition syndrome. *Clin Cancer Res.* 2017;23(12):e62–e67.
13. Beggs AD, Latchford AR, Vasen HF, et al. Peutz-Jeghers syndrome: a systematic review and recommendations for management. *Gut.* 2010;59(7):975–986.
14. Syngal S, Brand RE, Church JM, Giardiello FM, Hampel HL, Burt RW; American College of Gastroenterology. ACG clinical guideline: genetic testing and management of hereditary gastrointestinal cancer syndromes. *Am J Gastroenterol.* 2015;110(2):223–262; quiz 263.
15. Castinetti F, Moley J, Mulligan L, Waguespack SG. A comprehensive review on MEN2B. *Endocr Relat Cancer.* 2018;25(2):T29–T39.
16. Hsu AP, McReynolds LJ, Holland SM. GATA2 deficiency. *Curr Opin Allergy Clin Immunol.* 2015;15(1):104–109.
17. Fernández García MS, Teruya-Feldstein J. The diagnosis and treatment of dyskeratosis congenita: a review. *J Blood Med.* 2014;5:157–167.
18. Alter BP, Giri N, Savage SA, Rosenberg PS. Cancer in dyskeratosis congenita. *Blood.* 2009;113(26):6549–6557.
19. Abe Y, Aoki Y, Kuriyama S, et al.; Costello and CFC Syndrome Study Group in Japan. Prevalence and clinical features of Costello syndrome and cardio-facio-cutaneous syndrome in Japan: findings from a nationwide epidemiological survey. *Am J Med Genet A.* 2012;158A(5):1083–1094.
20. Villani A, Greer MC, Kalish JM, et al. Recommendations for cancer surveillance in individuals with RASopathies and other rare genetic conditions with increased cancer risk. *Clin Cancer Res.* 2017;23(12):e83–e90.
21. Tidyman WE, Rauen KA. The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. *Curr Opin Genet Dev.* 2009;19(3):230–236.
22 Ivy SP, Siu LL, Garrett-Mayer E, Rubinstein L. Approaches to phase 1 clinical trial design focused on safety, efficiency, and selected patient populations: a report from the clinical trial design task force of the national cancer institute investigational drug steering committee. *Clin Cancer Res.* 2010;16(6):1726–1736.
23. Xu GF, O'Connell P, Viskochil D, et al. The neurofibromatosis type 1 gene encodes a protein related to GAP. *Cell.* 1990;62(3):599–608.
24. Bollag G, Clapp DW, Shih S, et al. Loss of NF1 results in activation of the Ras signaling pathway and leads to aberrant growth in haematopoietic cells. *Nat Genet.* 1996;12(2):144–148.
25. Evans DGR, Salvador H, Chang VY, et al. Cancer and central nervous system tumor surveillance in pediatric neurofibromatosis 1. *Clin Cancer Res.* 2017;23(12):e46–e53.
26. Mautner VF, Asuagbor FA, Dombi E, et al. Assessment of benign tumor burden by whole-body MRI in patients with neurofibromatosis 1. *Neuro Oncol.* 2008;10(4):593–598.
27. Korf BR. Plexiform neurofibromas. *Am J Med Genet.* 1999;89(1):31–37.
28. de Blank PMK, Fisher MJ, Liu GT, et al. Optic pathway gliomas in neurofibromatosis type 1: an update: surveillance, treatment indications, and biomarkers of vision. *J Neuroophthalmol.* 2017;37(Suppl 1):S23–S32.
29. Cannon A, Chen MJ, Li P, et al. Cutaneous neurofibromas in neurofibromatosis type I: a quantitative natural history study. *Orphanet J Rare Dis.* 2018;13(1):31.
30. Viskochil D, Buchberg AM, Xu G, et al. Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. *Cell.* 1990;62(1):187–192.
31. Widemann BC, Salzer WL, Arceci RJ, et al. Phase I trial and pharmacokinetic study of the farnesyltransferase inhibitor tipifarnib in children with refractory solid tumors or neurofibromatosis type I and plexiform neurofibromas. *J Clin Oncol.* 2006;24(3):507–516.
32. Gupta A, Cohen BH, Ruggieri P, Packer RJ, Phillips PC. Phase I study of thalidomide for the treatment of plexiform neurofibroma in neurofibromatosis 1. *Neurology.* 2003;60(1):130–132.
33. Jakacki RI, Dombi E, Potter DM, et al. Phase I trial of pegylated interferon-alpha-2b in young patients with plexiform neurofibromas. *Neurology.* 2011;76(3):265–272.
34. Babovic-Vuksanovic D, Widemann BC, Dombi E, et al. Phase I trial of pirfenidone in children with neurofibromatosis 1 and plexiform neurofibromas. *Pediatr Neurol.* 2007;36(5):293–300.
35. Kim A, Dombi E, Tepas K, et al. Phase I trial and pharmacokinetic study of sorafenib in children with neurofibromatosis type I and plexiform neurofibromas. *Pediatr Blood Cancer.* 2013;60(3):396–401.
36. Dombi E, Baldwin A, Marcus LJ, et al. Activity of selumetinib in neurofibromatosis type 1-related plexiform neurofibromas. *N Engl J Med.* 2016;375(26):2550–2560.
37. Wu J, Dombi E, Jousma E, et al. Preclinical testing of sorafenib and RAD001 in the Nf(flox/flox);DhhCre mouse model of plexiform neurofibroma using magnetic resonance imaging. *Pediatr Blood Cancer.* 2012;58(2):173–180.
38. Weiss B, Widemann BC, Wolters P, et al. Sirolimus for non-progressive NF1-associated plexiform neurofibromas: an NF clinical trials consortium phase II study brain. *Pediatr Blood Cancer.* 2014;61:982–986.
39. Babovic-Vuksanovic D, Ballman K, Michels V, et al. Phase II trial of pirfenidone in adults with neurofibromatosis type 1. *Neurology.* 2006;67(10):1860–1862.
40. Widemann BC, Babovic-Vuksanovic D, Dombi E, et al. Phase II trial of pirfenidone in children and young adults with neurofibromatosis type 1 and progressive plexiform neurofibroma. *Pediatr Blood Cancer.* 2014;61:1598–1602.
41. Solomon J, Warren K, Dombi E, Patronas N, Widemann B. Automated detection and volume measurement of plexiform neurofibromas in neurofibromatosis 1 using magnetic resonance imaging. *Comput Med Imaging Graph.* 2004;28(5):257–265.
42. Dombi E, Ardern-Holmes SL, Babovic-Vuksanovic D, et al.; REiNS International Collaboration. Recommendations for imaging tumor

response in neurofibromatosis clinical trials. *Neurology.* 2013;81(21 Suppl 1):S33–S40.

43. Weiss B, Plotkin S, Widemann B, et al. NF106: phase 2 trial of the MEK inhibitor PD-0325901 in adolescents and adults with NF1-related plexiform neurofibromas: an NF Clinical trials consortium study. *Neuro Oncol.* Abstracts from the 18th International Symposium on Pediatric Neuro-Oncology (ISPNO 2018); June 30–July 3, 2018; Hyatt Regency Hotel, Denver, Colorado, USA; 2018;20(Suppl_2):i27–i213.
44. Fangusaro J, Onar-Thomas A, Young Poussaint T, et al. Selumetinib in paediatric patients with BRAF-aberrant or neurofibromatosis type 1-associated recurrent, refractory, or progressive low-grade glioma: a multicentre, phase 2 trial. *Lancet Oncol.* 2019;20(7):1011–1022.
45. McCowage GB, Mueller S, Pratilas CA, Hargrave DR, Moertel CL, Whitlock J. Trametinib in pediatric patients with neurofibromatosis type 1 (NF-1)-associated plexiform neurofibroma: a phase I/IIa study. *J Clin Oncol.* 2018;36(15).
46. Plotkin SR, Blakeley JO, Dombi E, et al. Achieving consensus for clinical trials: the REiNS International Collaboration. *Neurology.* 2013;81(21 Suppl 1):S1–S5.
47. Plotkin SR, Davis SD, Robertson KA, et al.; REiNS International Collaboration. Sleep and pulmonary outcomes for clinical trials of airway plexiform neurofibromas in NF1. *Neurology.* 2016;87(7 Suppl 1):S13–S20.
48. Fisher MJ, Avery RA, Allen JC, et al.; REiNS International Collaboration. Functional outcome measures for NF1-associated optic pathway glioma clinical trials. *Neurology.* 2013;81(21 Suppl 1):S15–S24.
49. Mullin RL, Golding JF, Smith R, Williams V, Thomas M, Ferner RE. Reliability of functional outcome measures in adults with neurofibromatosis 1. *SAGE Open Med.* 2018;6:2050312118786860.
50. Wolters PL, Martin S, Merker VL, et al.; REiNS International Collaboration. Patient-reported outcomes in neurofibromatosis and schwannomatosis clinical trials. *Neurology.* 2013;81(21 Suppl 1):S6–S14.
51. Wolters PL, Martin S, Merker VL, et al.; REiNS International Collaboration. Patient-reported outcomes of pain and physical functioning in neurofibromatosis clinical trials. *Neurology.* 2016;87(7 Suppl 1):S4–S12.
52. Nutakki K, Hingtgen CM, Monahan P, Varni JW, Swigonski NL. Development of the adult PedsQL™ neurofibromatosis type 1 module: initial feasibility, reliability and validity. *Health Qual Life Outcomes.* 2013;11:21.
53. Nutakki K, Varni JW, Swigonski NL. PedsQL Neurofibromatosis type 1 module for children, adolescents and young adults: feasibility, reliability, and validity. *J Neurooncol.* 2018;137(2):337–347.
54. Lillie EO, Patay B, Diamant J, Issell B, Topol EJ, Schork NJ. The n-of-1 clinical trial: the ultimate strategy for individualizing medicine? *Per Med.* 2011;8(2):161–173.
55. Dombi E, Solomon J, Gillespie AJ, et al. NF1 plexiform neurofibroma growth rate by volumetric MRI: relationship to age and body weight. *Neurology.* 2007;68(9):643–647.
56. Dagalakis U, Lodish M, Dombi E, et al. Puberty and plexiform neurofibroma tumor growth in patients with neurofibromatosis type I. *J Pediatr.* 2014;164(3):620–624.
57. U.S. Department of Health and Human Services FaDACfDEaRC, Center for Biologics Evaluation and Research (CBER), Office of Orphan Products Development (OOPD). *Rare Diseases: Natural History Studies for Drug Development Guidance for Industry—Draft Guidance.* 2019:1–19.
58. Rauen KA. The RASopathies. *Annu Rev Genomics Hum Genet.* 2013;14:355–369.
59. Rauen KA, Huson SM, Burkitt-Wright E, et al. Recent developments in neurofibromatoses and RASopathies: management, diagnosis and current and future therapeutic avenues. *Am J Med Genet A.* 2015;167A(1):1–10.
60. Korf B, Ahmadian R, Allanson J, et al. The third international meeting on genetic disorders in the RAS/MAPK pathway: towards a therapeutic approach. *Am J Med Genet A.* 2015;167A(8):1741–1746.
61. Gross AM, Frone M, Gripp KW, et al. Advancing RAS/RASopathy therapies: an NCI-sponsored intramural and extramural collaboration for the study of RASopathies. *Am J Med Genet A.* 2020.
62. Lindhurst MJ, Sapp JC, Teer JK, et al. A mosaic activating mutation in AKT1 associated with the Proteus syndrome. *N Engl J Med.* 2011;365(7):611–619.
63. Keppler-Noreuil KM, Sapp JC, Lindhurst MJ, et al. Pharmacodynamic study of miransertib in individuals with Proteus syndrome. *Am J Hum Genet.* 2019;104(3):484–491.
64. Health USDo, Human Services FDACfDE, Research, et al. Guidance for industry: patient-reported outcome measures: use in medical product development to support labeling claims: draft guidance. *Health Qual Life Outcomes.* 2006;4:79.