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A Collaborative Model for Accelerating the Discovery and Translation of Cancer Therapies

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Abstract

Preclinical studies using genetically engineered mouse models (GEMMs) have the potential to expedite the development of effective new therapies; however, they are not routinely integrated into drug development pipelines. GEMMs may be particularly valuable for investigating treatments for less common cancers, which frequently lack alternative faithful models. Here we describe a multi-center cooperative group that has successfully leveraged the expertise and resources from philanthropic foundations, academia, and industry to advance therapeutic discovery and translation using GEMMs as a preclinical platform. This effort, known as the Neurofibromatosis Preclinical Consortium (NFPC), was established to accelerate new treatments for tumors associated with neurofibromatosis type 1 (NF1). At its inception, there were no effective treatments for NF1 and few promising approaches on the horizon. Since 2008

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participating labs have conducted 95 preclinical trials of 38 drugs or combinations through collaborations with 18 pharmaceutical companies. Importantly, these studies have identified 13 therapeutic targets, which have inspired 16 clinical trials. This review outlines the opportunities and challenges of building this type of consortium and highlights how it can accelerate clinical translation. We believe that this strategy of foundation-academic-industry partnering is generally applicable to many diseases and has the potential to markedly improve the success of therapeutic development.

Keywords

NF1; Ras; Therapeutic development; Pediatric cancers; Sarcoma/soft-tissue malignancies; Animal models of cancer

Introduction

Approximately 90% of potential anti-cancer therapies fail in early clinical trials (1). This dismal statistic underscores the need to better predict efficacy before drugs are tested in humans. While GEMMs are beginning to inform clinical trials, they are not routinely integrated into most drug development pipelines. From an industry perspective the reasons for this include the complex network of proprietary and commercial rights surrounding GEMMs, the expense associated with maintaining large animal colonies, and the lack of comprehensive data illustrating that GEMMs are superior to xenografts for predicting efficacy. These issues become even more complex when combining drugs from different companies. Academic laboratories, on the other hand, have pioneered the development of GEMMs but are often unable to perform rigorous *in vivo* preclinical studies due to barriers including prohibitive costs, inaccessibility to proprietary compounds, and the need to focus on “innovative (individual) science” to ensure academic success. While these problems are applicable to testing drugs for common cancers, developing treatments for rare tumor types is even more challenging.

One population affected by these challenges are individuals with the familial cancer syndrome neurofibromatosis type 1 (NF1). Accordingly, in 2008 the Neurofibromatosis Preclinical Consortium (NFPC) was established. This effort, originally conceptualized and funded by the Children’s Tumor Foundation (CTF) and later co-funded by the CTF and the Neurofibromatosis Therapeutic Acceleration Program (NTAP), was designed to support preclinical translational research and facilitate collaborative studies between academic labs and pharmaceutical companies, using GEMMs of NF1-associated tumors. Since its inception the NFPC has established a robust structure for conducting preclinical testing, identified multiple new therapeutic targets, and directly advanced clinical translation. Here we review our experience with the expectation that elements of this approach can be used as a template to accelerate the development of therapies for other neoplasms and human disorders.

NF1: Background and Mouse Models

Neurofibromatosis type 1 (NF1) is a cancer predisposition syndrome with an incidence of 1 in 3500 (2). Affected individuals can develop benign and malignant tumors that arise in diverse tissues. The *NF1* gene also more broadly functions as a tumor suppressor in sporadic cancers including glioblastoma, melanoma, leukemia and lung cancer (3). *NF1* encodes a GTPase activating protein (GAP) that negatively regulates Ras by accelerating GTP hydrolysis (4). As such, Ras and its downstream kinases become aberrantly activated when NF1 function is lost. However, despite an extensive understanding of Ras signaling networks and the availability of compounds that target various Ras effectors, there are currently no effective treatments for cancers with *RAS* or *NF1* mutations.

GEMMs that recapitulate distinct NF1-associated tumor types have been developed. The most common tumors, neurofibromas, affect the peripheral nervous system and occur in 50% of individuals with NF1 (2). While these tumors are benign they can be painful, debilitating, disfiguring, and lethal. Internal tumors, termed plexiform neurofibromas (PN), can progress to malignancy. Neurofibromas are histologically complex. While *NF1*-deficient Schwann cell precursors drive tumor development (5), these lesions are also comprised of nerves, macrophages, fibroblasts, mast cells, and Schwann cells (4). Accordingly, cell line models do not accurately recapitulate the cellular complexity of neurofibromas and patient-derived xenografts do not exist. Fortunately, two Cre-lox based GEMMs are used by consortium members (5, 6). This tumor type illustrates how GEMMs may be essential for preclinical studies. Importantly, these models have now been shown to predict both clinical successes and failures.

The most common malignancies associated with NF1 are Malignant Peripheral Nerve Sheath Tumors (MPNSTs) (2). These cancers emanate from plexiform neurofibromas, but also develop spontaneously in individuals with and without NF1. MPNSTs are lethal in approximately 70% of cases. A GEMM that develops tumors indistinguishable from human MPNSTs has been developed (7, 8). This model harbors compound mutations in *Nf1* and *p53* but also relies on the spontaneous loss of wild-type alleles. It is used by consortium members because it carries the hallmark genetic alterations of human MPNSTs, and because it recapitulates the high stochastic mutational load of human tumors, thus permitting the evaluation of immunotherapies.

Finally, consortium members also study GEMMs that recapitulate juvenile myelomonocytic and acute myeloid leukemia (JMML and AML). Because mutations in *NF1* or *KRAS* also occur in sporadic myeloid diseases, Cre-lox based models that incorporate these alterations have been developed (9, 10). Studying these models in parallel allows for the evaluation of targeted agents in benign versus malignant disease and also permits a comparison of responses in *NF1* versus *KRAS* mutant tumors. Altogether, the NFPC is currently comprised of investigators studying two models of PN (5, 6), MPNST (7), JMML and AML (9, 10). In all instances these strains faithfully recapitulate the genetic, biochemical, cell biologic, and pathologic features of the corresponding human tumors. Additional GEMMs were considered, but legal issues at some institutions precluded integration into the consortium.

Benefits of the NFPC Structure

In addition to having robust GEMMs in hand, the initial design of the NFPC structure also contributed to its success. The NFPC was developed with three essential components: (1) academic scientists with expertise in NF1 and mouse models; (2) an External Advisory Board (EAB) comprised of scientists from pharmaceutical and biotechnology companies, NF1 clinicians, and individuals with expertise in legal affairs; and (3) a Project Manager (PM). Because NFPC laboratories study different tumors, an initial goal was to evaluate the same drugs across models. Taking this approach early on yielded several benefits. First, laboratories rapidly shared information regarding drug formulation and administration, maximally tolerated doses (MTD), and toxicities. Second, standard operating procedures (SOPs) were developed for collecting, processing, and analyzing pharmacodynamic endpoints. Finally, this parallel analysis permitted the assessment of the same target in models of early (e.g. PN and JMML) versus late (e.g. MPNST and AML) stage tumors. This concerted effort not only enhanced the speed at which the group learned how to execute rigorous preclinical trials, but also demonstrated that agents exert different effects in distinct tumor types.

The EAB initially advised academic sites in conducting trials according to industry standards with respect to sample size, endpoints, and pharmacokinetic profiling. Academic labs have now developed additional productive interactions with scientists in industry, which offers insight into compound handling, formulation, and permits the comparison of GEMM data with prior experience in xenograft models. Thus, partnering with industry adds value that extends well beyond simply obtaining drugs for testing. Finally, the PM coordinates NFPC activities, oversees the implementation of SOPs, maintains relationships with industry partners, and serves as a scientific liaison to supporting philanthropic organizations, thereby enabling a “center without walls”.

Addressing Legal Issues in Industry-Academic Partnerships

To facilitate material transfer agreements (MTAs) a Contracting Collaborative Group was established that consisted of contracting offices from each academic institution, the PM, and CTF legal counsel. While this structure facilitated complex negotiation processes, MTA agreements frequently remain a rate-limiting step. Pharmaceutical companies have complex approval processes and are justifiably wary of risk that could arise with molecules in active clinical development. On the other side, academic institutions are protective of potential intellectual property rights. The Supreme Court ruling in *Merck v. Integra* broadened the permissibility to make or use patented material for research that would “contribute relatively directly to the FDA approval of a drug”, and opened the door to synthesizing and testing compounds without industry agreements. Despite this, the NFPC seeks to develop collaborative relationships whenever possible. In our experience, the benefits of directionally sharing information and expertise generally outweigh the drawbacks of cumbersome MTA processes. Nevertheless, increased flexibility and willingness to compromise on the part of companies and academic institutions would facilitate future efforts of this nature.

Conducting Preclinical trials Using Industry Standards

NFPC studies are conducted using industry practices. This includes implementing group-wide SOPs, determining the MTD in tumor-bearing mice, and standardizing pharmacodynamic (PD) and pharmacokinetic (PK) analyses. SOPs contain detailed drug formulation protocols, drug delivery methods, schedules and doses, and details for PK and PD sample collection and processing. Our experience supports establishing the MTD in each model as we have observed substantial variability across strain backgrounds and in healthy versus tumor-bearing mice.

Kinetic PK/PD studies have led to important practical and mechanistic insights. Tissue samples are routinely collected from three mice per time-point at baseline and multiple time-points after treatment. PK/PD are first assessed in wild-type strain-matched mice and a surrogate tissue (e.g. lung) is used to compare PD across different models, which has allowed NFPC investigators to predict efficacious drug doses based on the degree and duration of target inhibition across models and/or with related drugs. When possible, collaborating pharmaceutical companies perform PK analysis, which permits comparisons of drug exposure to prior studies in xenografts and correlations with tolerated doses in humans. As such, efficacy is either evaluated at the MTD or, preferably, at a dose that most closely reflects the tolerated human dose when known. This practice has greatly facilitated clinical translation.

Each GEMM differs with respect to phenotypic characteristics, rates of progression, and causes of death. For example, MRI-based comparisons of tumor burden is the primary endpoint in one PN model while survival is used in mice with AML. Therefore, it is essential to perform prospective power analyses and use relevant model-specific endpoints and cohort sizes for statistical analysis. After an endpoint for assessing efficacy is established in an individual model, it is applied consistently in sequential trials. This strategy allows NFPC investigators to compare the relative efficacy of different compounds within and across different tumor models.

Successful Target Identification and Translation

Tables 1 and 2 summarize the therapeutic targets that have been identified and the ongoing or planned clinical trials based on work performed by NFPC investigators. The following paragraphs describe a subset of the relevant preclinical studies that informed subsequent clinical trials.

Targeting Ras Effectors With Single Agents

Because aberrant Ras activation drives *NF1*-mutant tumors, NFPC members have extensively examined single agents that target various Ras effector kinases. One of the earliest agents evaluated was the “first generation” MEK inhibitor CI-1040 (Pfizer). While it was ineffective in *Nf1*-mutant JMML, it unexpectedly induced transient remissions in *Nf1*-deficient AMLs (11). Based on these initial data, NFPC investigators systematically tested PD0325901 (PD901, Pfizer), which has superior PK properties and longer target inhibition. In JMML PD901 induced dramatic disease regression in the same model that was

unresponsive to CI-1040 (12), demonstrating that sustained pathway inhibition *in vivo* is essential for efficacy. Interestingly, genetic analysis of bone marrow cells revealed the persistence of *Nf1* mutant cells, suggesting that MEK inhibition normalized the proliferation and differentiation of *Nf1* mutant hematopoietic cells, but did not eradicate them. Similar effects were observed in the $Kras^{G12D}$ JMML model (13). Together, these preclinical studies directly informed the design of a clinical trial of the FDA-approved MEK inhibitor trametinib (GlaxoSmithKline) in JMML. Moreover these studies demonstrated the importance of measuring PK/PD and mutant allele burden in children enrolled on this trial.

MEK inhibition also exhibited efficacy in two mouse models of plexiform neurofibroma (PN) ((14) and unpublished data). Specifically, PD901 and selumetinib (Astra Zeneca) each induced tumor regression at clinically achievable doses. These studies inspired 4 ongoing clinical trials evaluating different MEK inhibitors (PD901, selumetinib, and trametinib) in NF1 patients with plexiform neurofibromas. In the first completed Phase 1 study, partial responses were observed in more than 70% of patients and regressions were maintained (15). This finding is unprecedented for these tumors, which are largely unresectable and currently untreatable, and prospective phase 2 studies are now underway.

Despite the promising activity of MEK inhibitors in early stage neoplasms, these agents only slightly attenuated MPNST growth in mice and *Nf1*-deficient AMLs became resistant (11, 16). Moreover, in MPNSTs the mTORC1 inhibitor rapamycin exerted more potent effects than MEK inhibitors, albeit responses were still cytostatic (17, 18). These findings suggest that different *NF1*-mutant tumor types may be more or less dependent on different Ras effector pathways. In addition, it should be noted that while MEK inhibitors promoted neurofibroma regression in both mouse models and human clinical trials (14, 15), sirolimus did not induce regression in either mice or humans (19, 20). Thus, the comparable positive and negative effects of MEK and mTOR inhibitors on neurofibroma regression, respectively, illustrate the predictive value of these GEMMs.

Combination Trials

Given the large number of available therapeutic agents, mouse models represent a powerful tool for identifying the most promising combinations among numerous possibilities. This approach is further enhanced when guided by complementary basic scientific studies. For example, while MEK and various PI3K pathway inhibitors are ineffective as single agents in MPNSTs, MEK and mTORC1 inhibitors together cause potent tumor regression in GEMMs (16). Transcriptional profiling and imaging studies further identified the glucose transporter, GLUT1, and consequently ^{18}F-FDG uptake as unique biomarkers of sustained (dual) inhibition of MEK and mTOR (16). These observations have directly informed the design of a clinical trial evaluating the efficacy of this combination and early FDG-PET imaging as a biomarker. A similar basic/preclinical approach, aimed at identifying other cancer-related vulnerabilities in MPNSTs, also led to the development of an ongoing clinical trial evaluating the effects of combined HSP90 and mTOR inhibitors ((21) and NCT01427946). As drug combinations will likely be required to confer maximal therapeutic responses in both benign and malignant tumors characterized by *NF1* mutations, GEMMs will be essential for identifying the most promising combinations.

Leveraging basic research to identify new therapeutic targets

A distinct advantage of this consortium model is the extensive (combined) expertise of academic investigators in studying this complex disease, which is fortified by ongoing basic studies. Thus, the academic nature of the NFPC represents both an intellectual and financial asset in this translational endeavor. For example, the neurofibroma model was used in an insertional mutagenesis screen to identify new effector pathways in *NF1*-mutant tumors. Using this approach a Stat3-Arid1b/β-catenin pathway was found to be essential for neurofibroma formation (22), supporting the investigation of JAK/STAT and Wnt/β-catenin pathway inhibitors as potential therapeutic agents. Human genetic and mouse modeling studies also identified the epigenetic regulator, *SUZ12,* as an important tumor suppressor in MPNSTs (23). Mechanistic studies further revealed that bromodomain inhibitors could effectively counteract the effects of *SUZ12* loss, prompting a preclinical study to evaluate the effects of MEK and BRD4 inhibitors. When combined these agents triggered potent tumor regression of MPNSTs *in vivo*, thus revealing another promising therapeutic combination (23). This observation is currently being extended to investigate BRD4 and MEK inhibition in murine AMLs and neurofibromas.

Targeting the Tumor Microenvironment

Another advantage of GEMMs is that tumors develop with an intact microenvironment. This permits the evaluation of agents that target cancer cell-host interactions. Notably, genetic and cell biologic studies using GEMMs showed that infiltrating haploinsufficient bone marrow-derived mast cells contribute to PN development (24). Because mast cells are critically regulated by c-KIT, the therapeutic effects of imatinib mesylate (Gleevec, Novartis) were evaluated. Treatment decreased tumor size and number by 50% and 25%, respectively. Based on these findings, a child with a life-threatening airway obstruction from an inoperable PN was treated on a compassionate use basis and responded dramatically (24). A subsequent phase 2 study using imatinib demonstrated responses in 25% of individuals and further suggested that neurofibromas located in the head and neck have the highest probability of responding (25). Additional trials are ongoing in this population with imatinib (NCT01140360, NCT02177825 and NCT01673009), and a broader trial is evaluating the effects of cabozantinib (Exelixis). Interestingly, while cabozantinib inhibits c-KIT, it also inhibits additional kinases, including MNK, a newly identified in MPNSTs that, when suppressed, synergizes with MEK inhibitors (26). Finally, macrophages are also important for the maintenance of plexiform neurofibromas (27). Specifically, the CSF1R/KIT inhibitor PLX3397 (Plexxikon) was shown to trigger macrophage depletion and neurofibroma regression in GEMMs and inspired the development of another ongoing clinical trial (NCT02390752). It is important to note that a critical feature of the NFPC is its collaborative culture, which fosters data sharing in real time, ultimately increasing the rate at which we discover, understand, and translate new therapies.

Identifying Additional Therapeutic Targets

Other studies by NFPC members have identified additional promising therapeutic targets in NF1 tumor models including EGFR (28), Aurora kinase A (29), HDAC, and PAK1 (30), with new candidates undergoing active investigation. Importantly, the NFPC offers a

powerful platform to rapidly evaluate these targets, new clinical drugs, and drug combinations. Because therapeutic development is an iterative and collaborative process, members of the NFPC and the Department of Defense-funded NF Clinical Consortium interact on a regular basis, both formally and informally. This bidirectional preclinical/clinical pipeline facilitates the development of the most promising preclinical findings into mechanism-driven clinical trials and will enable clinical and laboratory investigators to work together to improve standard-of-care therapies as they emerge.

Summary

The NFPC experience demonstrates the feasibility of exploiting GEMMs in a multi-center setting that is reminiscent of human cooperative clinical trials networks. While throughput is lower than conventional xenografts, GEMMs accurately reflect the underlying disease in the appropriate environment and harbor an intact immune system. In principle, these attributes should improve predictive value, particularly when similar drug exposures can be achieved in mouse and humans. Moreover, the ability to rapidly assess agents can be exploited to identify the best clinical candidate against a specific therapeutic target, as well as the most promising drug combinations. The comparable therapeutic effects of MEK and c-KIT inhibitors observed in human and mouse neurofibromas, both illustrates and validates this approach.

Several principles have emerged from NFPC trials. First, the degree and duration of target inhibition are often critical factors dictating response. This observation underscores the value of performing PK/PD analysis in both the preclinical setting and in clinical trials. While the success of any trial may ultimately be dependent on whether sustained target inhibition in tumors can be achieved without toxicity, the duration of target inhibition is difficult to measure in humans. Therefore, GEMMs may be invaluable for developing tractable biomarkers of target inhibition and/or early efficacy. Second, parallel basic studies that elucidate mechanisms of tumor development and maintenance *in vivo* can fuel new therapeutic approaches. While *in vitro* drug screening strategies are important, unique insight can be gained by studying tumors in immunocompetent animals with an intact tumor microenvironment. Finally, the collaborative nature of this foundation-academic-industry partnership has unquestionably increased the speed of target identification and translation. This approach has obvious appeal for developing therapies for less common diseases, but can also be extended to therapeutic development in any tumor type for which accurate mouse models exist.

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Abbreviations

GEMMs	genetically engineered mouse models
NFPC	neurofibromatosis preclinical consortium
NF1	neurofibromatosis type 1
CTF	children's tumor foundation
NTAP	neurofibromatosis therapeutic acceleration program
PN	plexiform neurofibromas
MPNST	malignant peripheral nerve sheath tumor
JMML	juvenile myelomonocytic myeloid leukemia
AML	acute myeloid leukemia

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Table 1

Clinical trials resulting from NFTC investigators

Drug	Tumor Type	Phase	Clinical Trial
Imatinib	Plexiform NF	2 2 2	NCT01140360 NCT02177825 NCT01673009
Selumetinib	Plexiform NF	1 2 2	NCT01362803 NCT02407405 NCT02644512
Trametinib	Plexiform NF	½	NCT00920140
Bevacizumab + Everolimus	MPNST	2	NCT01661283
Ganetespib + Sirolimus	MPNST	½	NCT01427946
PD0325901	Plexiform NF	2	NCT02096471
Trametinib	JMML	2	In development
Cabozantinib	Plexiform NF	2	NCT02101736
Nilotinib	Plexiform NF	2	NCT01275586
PLX3397	Plexiform NF	½	NCT02390752
Selumetinib + Vistusertib	MPNST	½	In development
BRD4i + MEKi	MPNST, Plexiform NF	½	In development

Table 2

Potential therapeutic targets identified as single agent[a], in combination[b] or in genetic study[c]

	Target	Tumor Type	Reference
1	mTORC1	MPNST[b]	(16, 21)
2	MEK	Plexiform NF[a], JMML[a], MPNST[b]	(12–14, 16)
3	STAT3	Plexiform NF[c]	(22)
4	c-KIT	Plexiform NF[a,c]	(24)
5	BRD4	MPNST[b,c], Plexiform NF[b], JMML[b]	(23)
6	eIF4E	MPNST[c]	(16)
7	MNK	MPNST[b,c]	(26)
8	VEGFR	MPNST[b]	unpublished
9	HDAC	MPNST[b]	unpublished
10	PI3K/p110α	MPNST[a]	(16)
11	AKT	Plexiform NF[a], JMML[a]	unpublished
12	Hsp90	MPNST[b]	(21)
13	CSF1R	Plexiform NF[a]	(27)