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Testing of New Agents in Childhood Cancer Preclinical Models: Meeting Summary

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Abstract

A workshop on pediatric preclinical testing, sponsored by the National Cancer Institute and the Children's Oncology Group Phase 1 Consortium, was held on June 26–27, 2001 in Bethesda, Maryland. Drs. Peter Adamson, Peter Houghton, and Malcolm Smith organized and hosted the meeting. There were 20 participants from 12 institutions. The primary objectives of the workshop included: (*a*) development of a working inventory of available preclinical models (including human tumor xenografts in immunodeficient mice, transgenic and syngeneic tumors, and selected *in vitro* models), with a basic understanding of the strengths and weaknesses of each as possible components of a preclinical testing program; (*b*) identification of the key scientific issues related to establishment of a program for preclinical testing of new agents for their applicability to childhood cancers; and (*c*) identification of the key infrastructure requirements for a program for preclinical testing of new agents for their applicability to childhood cancers. This report is a synthesis of the workshop's presentations and discussions.

Introduction

Approximately 400 new agents are currently under evaluation for cancer indications in adults (1). Only a small fraction of these can be evaluated in children with cancer as a result of the thankfully small number of children eligible for clinical trials evaluating new agents and the inherent limitations this places on the number of pediatric clinical trials that can be conducted. Because of this increasing imbalance between the number of new agents potentially available for pediatric evaluation and the number that can actually be evaluated, it is essential to develop predictive preclinical models of pediatric cancers to help clinical investigators prioritize new anticancer agents for testing in children.

Preclinical testing of anticancer agents using pediatric cell lines and *in vivo* model systems has occurred for more than two decades. However, pediatric preclinical testing has never been consistently supported, and neither pharmaceutical companies nor the NCI[2] has included pediatric models as standard components of preclinical testing programs. Consequently, a substantial proportion of pediatric Phase I trials have been conducted with limited or no prior testing of the agents in pediatric preclinical models.

There are, however, a select number of pediatric preclinical tumor models that appear to be predictive for future clinical activity. The best studied tumor has been rhabdomyosarcoma. Retrospective studies have demonstrated a strong correlation between the activity of agents against rhabdomyosarcoma xenografts and clinical activity of the same agents (2). Melphalan was first identified as an active agent against rhabdomyosarcoma in xenograft models, and subsequent clinical experience with melphalan in children with newly diagnosed rhabdomyosarcoma mirrored the activity observed in the xenograft models (3). The prospective identification of topoisomerase I inhibitors as active agents against rhabdomyosarcoma xenograft models (4) and the subsequent demonstration of antitumor activity for these agents in children with rhabdomyosarcoma further support the predictive ability of these xenograft models (5).

Xenograft models of neuroblastoma, similar to rhabdomyosarcoma, also have predicted for the future clinical activity of

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[2] The abbreviations used are: NCI, National Cancer Institute; JMML, juvenile myelomonocytic leukemia; ALL, acute lymphoblastic leukemia; PNET, primitive neuroectodermal tumor; MTD, maximum tolerated dose.

topoisomerase I inhibitors in this disease (4, 6). The observation that neuroblastoma cell lines established at different points of therapy acquire a sustained drug-resistant phenotype that mirrors the clinical resistance pattern of patients supports the clinical relevance of the drug sensitivity pattern of these neuroblastoma cell lines (7).

Despite these and other examples of correlations between drug activity in pediatric preclinical models and clinical activity against tumors in children, the absence of a coordinated and consistent approach to the preclinical evaluation of new agents has prevented a definitive determination of the role of preclinical testing in prioritizing new agents for evaluation in children with cancer. Because of this lack of a systematic approach to preclinical testing, questions remain about the validity of preclinical models as predictors of clinical benefit in children with cancer.

As an initial step in developing a systematic approach to the prioritization of new agents for pediatric evaluation, the Children's Oncology Group Phase 1 Consortium and the Cancer Therapy Evaluation Program (NCI) sponsored a meeting on June 26–27, 2001 in Rockville, Maryland of investigators who have studied preclinical models potentially applicable to childhood cancer. Meeting participants were asked to address the following objectives: (*a*) development of a working inventory of available preclinical models, including a summary of the potential strengths and weaknesses of currently available models; (*b*) identification of the key scientific issues related to establishment of a program for preclinical testing of new agents for their applicability to childhood cancers; and (*c*) identification of the key infrastructure requirements for a program for preclinical testing of new agents for their applicability to childhood cancers.

Data presented at the meeting documented the existence of potential models (either xenograft, transgenic, or *in vitro*) for a number of childhood cancers, including rhabdomyosarcoma, osteosarcoma, Ewing's sarcoma/peripheral PNET, neuroblastoma, various types of brain cancers, JMML, and ALL. The meeting summary that follows focuses primarily on the scientific issues related to the establishment and design of a system for analytically testing new anticancer agents in pediatric preclinical models and on issues related to the infrastructure requirements of such a system. Also included is a brief summary of existing preclinical models.

Existing Pediatric Tumor Models

***In Vitro* Model Systems.** *In vitro* model systems have been widely used to test the activity of anticancer agents against childhood cancer primary cells and cell lines, and such models continue to be used as a component of the NCI cell line screen for adult tumors (8, 9). Most *in vitro* model systems use either a clonogenic survival end point, measured by colony formation, or a cell proliferation end point, measured by cell metabolism [*e.g.*, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2*H*-tetrazolium-5-carboxanilide inner salt] or biomass (*e.g.*, SRB) assays (10). One proliferative end point assay presented at the meeting utilizes a fluorescence-based system (DIMSCAN), which has a wide dynamic range (3–4 logs of cell kill) with moderately high throughput. DIMSCAN has been used to study neuroblastoma cell lines established from tumors at various stages of treatment [diagnosis, postinduction, and post bone marrow transplantation (7, 11, 12)]. The DIMSCAN assay and other *in vitro* assays are particularly well suited for defining synergistic interactions between drugs (13, 14). Whereas *in vitro* testing has generally shown limited success in predicting tumor-specific clinical activity in studies of adult tumors, it remains an open question whether testing against a broad panel of carefully selected and well-characterized childhood cancer cell lines can provide clinically useful information.

Primary tumor cells have also been used for *in vitro* drug testing. For example, primary blast cells from children with ALL have been used to test the antileukemia activity of agents and to correlate *in vitro* sensitivity to molecularly targeted agents with molecular alterations in the leukemia cells (15, 16).

***In Vivo* Model Systems.** Most pediatric preclinical *in vivo* testing has involved tumor xenografts, for which several host animal options exist, including *SCID* mice, NOD/*SCID* mice, athymic nude mice, and athymic rats. Most recent work in pediatric solid tumor models has used *SCID* mice, whereas for leukemia models, use of NOD/*SCID* mice has been more common. Tables 1 and 2 list some of the pediatric xenograft models and the anticancer drugs that have been studied in the models. Perhaps the most influential xenograft experiments have been those evaluating the activity of the camptothecin analogues against pediatric tumors. These experiments demonstrated the schedule-dependent activity of the topoisomerase I inhibitors and their high levels of activity against neuroblastoma and rhabdomyosarcoma xenografts at drug exposures achievable in humans (4, 17, 18). The predicted activity of topotecan and irinotecan against neuroblastoma and rhabdomyosarcoma has been confirmed in clinical trials in children with these diagnoses (5, 6, 18). Testing in pediatric xenograft models has not been limited to conventional cytotoxic agents. For example, antiangiogenic agents [*e.g.*, TNP-470 (19), the α_v integrin antagonist EMD-121974 (20), and an antibody to vascular endothelial growth factor (21, 22)] and signal transduction inhibitors [*e.g.*, the Trk family tyrosine kinase inhibitor CEP-751 (23) and the epidermal growth factor receptor inhibitor ZD1839 (24)] have all been evaluated in xenograft models. Although most work with pediatric xenograft models has been for solid tumors, xenograft models of childhood ALL have been developed that have the requisite characteristics of rapid, high-level, uniform leukemia engraftment (25, 26). These ALL xenograft models appear to have potential utility for preclinical testing (27, 28).

Most xenograft testing of anticancer agents has been done with tumors implanted s.c., which has a number of practical advantages (*e.g.*, the ease of evaluating tumor response). The biology of pediatric tumors growing s.c. may differ from that of tumors growing in environments that more closely mimic their site of origin. Alternatives to s.c. models include orthotopic xenograft models (20) and models in which i.v. injection of tumor cells into SCID mice achieves various organ distributions (29). Assessing response is a challenge with any of these intracavitary mouse tumor models, but new capabilities for small animal imaging may help address this challenge (30–32).

Transgenic models may make important contributions to a pediatric preclinical testing program. At the pretesting stage,

Table 1 Pediatric murine and xenograft tumor models presented at the meeting

Tumor type	Investigator	Classification	No. of lines	Comments
ALL	R. Lock (25, 28)	c-ALL	11	Xenografts produce systemic disease with infiltration of bone marrow, spleen, and liver. Engraftment and drug responses are measured by tail vein bleed. 14 lines were obtained at diagnosis, 6 at relapse (1 matched pair).
		T-ALL	3	
		Pre-B	2	
		Early pre-B	1	
		Ph+ ALL	1	
		Biphenotypic	1	
	J. Yu (27)			Preconditioning NOD/*SCID* mice with cord blood-derived cells allows a high level of engraftment for primary ALL and AML[a] cells.
CNS: glial tumors	P. Houghton	Ependymoma	5	
		Low-grade astrocytoma	2	
		High-grade glioma line	2	
CNS: medulloblastoma and other embryonal tumors	P. Houghton	PNET	2	
		Medulloblastoma	4	
		Atypical teratoid rhabdoid	6	
Ewing's sarcoma/peripheral PNET	P. Reynolds	Ewing's sarcoma/PNET	12	Most of these cell lines grow as s.c. xenografts in athymic mice. Selected cell lines have been shown to uniformly form pulmonary metastases (detectable by high-resolution radiographs) when given by i.v. injection into SCID mice.
Neuroblastoma	P. Houghton		6	4/6 tumors obtained at relapse; and 5/6 were *MNYC* amplified. Tumors from patients at relapse were generally resistant to standard drugs. 97 new agent and combination studies were completed (1998–2001).
	J. Maris	MYCN amplified	1	Also presented was a metastatic model using cell lines transfected with GFP and an orthotopic model for adrenal tumors.
		11q23 deleted	2	
	P. Reynolds		18	Cell lines established after various phases of therapy show drug resistance patterns expected for the phase of therapy. All lines have been characterized for response *in vitro* to most drugs used for neuroblastoma therapy.
Osteosarcoma	R. Gorlick	Primary site	4	
		Pulmonary recurrence	2	
	P. Houghton	Primary site	10	
		Pulmonary recurrence	3	
	L. Helman (51, 52)	Rodent model		Rodent model of metastatic osteosarcoma was used to identify functional and genetic differences between highly metastatic and poorly metastatic tumor cells.
Rhabdomyosarcoma	P. Houghton	Embryonal diagnosis	4	All lines express MyoD and/or myogenin, and the alveolar lines have t(2;13) and express Pax3/FKHR. 95 new agent and combination studies were completed (1998–2001).
		Alveolar diagnosis	2	
		Embryonal relapse	3	
		Alveolar relapse	4	
Wilms' tumor	P. Houghton	Favorable histology	9	The tumors are very sensitive to cyclophosphamide, vincristine, topotecan, and irinotecan. The anaplastic tumor is less responsive to chemotherapy than the favorable histology tumors.
		Anaplastic	1	

[a] AML, acute myeloid leukemia; CNS, central nervous system; GFP, green fluorescent protein.

transgenic models offer the opportunity to identify and validate targets for specific tumor types. Transgenic models may be particularly useful in evaluating targeted therapies for which the model has been engineered to focus on the target pathway of the agent of interest. As an example, JMML has been modeled using *Nf1* mutant mice. Approximately 10% of mice with heterozygous inactivation of *Nf1* develop a myeloproliferative disorder during the second year of life (33), although the limited penetrance precludes use of *Nf1* heterozygous mice for drug testing. However, adoptive transfer of homozygous *Nf1* mutant fetal liver hematopoietic cells to irradiated recipients consistently produces a JMML-like myeloproliferative disorder (34, 35). This model of pediatric JMML has been used to evaluate the antileukemia activity of a farnesyl transferase inhibitor, although the process for drug testing is time- and labor-intensive (36).

A mouse model of neuroblastoma involving overexpression of the *MYCN* gene has also been used for drug testing. In this model, N-myc expression is targeted to neural crest cells by linkage to the tyrosine hydroxylase promoter (37). Homozygous *MYCN* transgenic mice develop neuroblastoma within a tight window at 6–7 weeks of age, with amplification of the trans-

Table 2 Preclinical testing of new agents in pediatric tumor models

Tumor type	Examples of published reports of anticancer agents studied in pediatric xenograft models
ALL	B43 (anti-CD19)-pokeweed antiviral protein immunotoxin (53, 54)
	TXU (anti-CD7)-pokeweed antiviral protein immunotoxin (55)
	Vincristine (25, 28)
CNS:[a] glial tumors	EMD-121974 (20)
	Irinotecan (56)
	Temozolomide (57, 58)
	ZD1839 (24)
CNS: medulloblastoma and other embryonal tumors	Busulfan (59, 60)
	CEP 751 (23)
	Ecteinascidin-743 (61)
	EMD-121974 (20)
	Irinotecan (62)
	Temozolomide (57, 58)
Neuroblastoma	9-Aminocamptothecin (45)
	Busulfan (60)
	CEP-751 (23)
	Ecteinascidin-743 (61)
	Human IgM (63)
	Irinotecan (64, 65)
	MGI 114 (66)
	Temozolomide (58)
	TNP-470 (19)
	Topotecan (29, 67)
	Vincristine (29, 67)
	ZD1839 (24)
Osteosarcoma	Antiosteosarcoma immunotoxin (68, 69)
	Topotecan (70)
Rhabdomyosarcoma	9-Aminocamptothecin (45)
	Irinotecan (71)
	Melphalan (3)
	MGI 114 (66)
	Rapamycin (47)
	Temozolomide (58)
	Topotecan (67)
	Vincristine (67)
	ZD1839 (24)
Wilms' tumor	Antibody to vascular endothelial growth factor as either a single agent (22) or in combination with low-dose chemotherapy (21)

[a] CNS, central nervous system.

gene observed in the majority of tumors (38). The high rate of tumor development occurring with a predictable time course makes this model quite tractable for drug testing. *MYCN* transgenic mice respond to the conventional chemotherapy agent cyclophosphamide, and *MYCN* transgenic mice treated with *MYCN* antisense oligonucleotides have reduced tumor incidence and develop smaller tumor mass than animals treated with control oligonucleotides (39). Table 3 provides a listing of some of the genetically engineered murine models for pediatric tumors that have been developed.

Core Model Requirements

The primary goal of a preclinical testing program is to improve outcome for children with cancer by the early identification of potential pediatric applications for new agents in clinical development. The premise for establishing a testing program is that its activities will allow agents (or combinations of agents) to be selected for clinical evaluation that have increased likelihood for clinical benefit. In the absence of an effective preclinical testing program, a preponderance of ineffective agents will be selected for evaluation, thus slowing progress in improving outcome. This hypothesis, although not testable in a randomized manner, requires validation. A pediatric preclinical testing program, although based on scientific principles and designed to pursue scientific opportunities as they arise, would have the very pragmatic objective of providing reliable information to clinical investigators to allow an enlightened prioritization among the multiple agents available for clinical evaluation in children.

A leading issue in the design of a preclinical testing program is the selection of tumor models to study. The first determination will be the tumor types to be studied, because it will likely not be possible to study every type of childhood cancer initially. Factors influencing the selection of tumor types for study include tumor incidence, the adequacy of current therapy for the tumor (*i.e.*, the need for new treatment strategies), overlap with adult tumor types (*i.e.*, can data from adult preclinical testing be applied to the pediatric setting), the availabil-

Table 3 Mouse genetic models of childhood cancers

Tumor type	Comments concerning mouse model
B-cell lymphomas	Mice transgenic for the c-*myc* gene driven by either the IgH enhancer (*Emu-myc*) (72, 73) or regulatory elements of the *Ig*λ locus develop B-cell lymphomas. The *Emu-myc* transgenic lymphoma model has been used to genetically analyze chemotherapy resistance, with the identification of *INK4a/ARF* and *p53* mutations and Bcl-2 expression as factors associated with resistance (74–76).
CNS[a], gliomas	Mice engineered to express oncogenic V(12)Ha-ras using the glial fibrillary acidic protein (*GFAP*) promoter uniformly developed malignant astrocytomas (77). Specific strains of mice harboring null *Nf1* in and *p53* alleles in cis frequently develop high-grade gliomas (78). Astrocytomas develop at low frequency in mice heterozygous for *ARF* (79) and in transgenic mice expressing the v-src kinase under control of the glial fibrillary acidic protein (*GFAP*) gene regulatory elements (80).
CNS, medulloblastoma	Mice heterozygous for *Ptch* have increased propensity for developing medulloblastoma (~10% develop medulloblastoma by 9–10 months) (81–83). Development of tumors requires Igf-2 (83) and is accelerated by loss of *p53* (84). Medulloblastoma also develops in transgenic mice containing the JC virus early promoter and T-antigen gene (85, 86).
Leukemia, *MLL*	Mice heterozygous for the *MLL-AF9* fusion protein develop acute leukemia (primarily AML), with a median time to leukemia of approximately 5 months (87).
Leukemia, Bcr-Abl p190	Mice engineered to express Bcr-Abl p190 consistently develop pre-B-cell leukemia (88, 89). This model has been used to test the activity of a farnesyl transferase inhibitor (90) and to demonstrate the role of the adapter protein Crkl in leukemia development (91).
Leukemia, *AML1-ETO*	Mice engineered to inducibly express AML1-ETO (92) or to express AML1-ETO in myeloid precursor cells (93) do not develop leukemia. However, mutagen treatment of *AML1-ETO* transgenic mice produces AML (93), which is not observed in control mice treated with mutagen.
Leukemia, *PML-RAR*α	Mice engineered to express the PML-RARα fusion protein in myeloid-promyelocytic precursor cells show myeloproliferation in the first year of life with subsequent development of leukemia in some animals (94). Leukemia cells isolated from transgenic mice and transplanted into nude mice were used to evaluate the activity of retinoic acid and arsenic trioxide (95). Transgenic mice expressing PML-RARα have been used to demonstrate that Bcl-2 can cooperate with the fusion protein in APL development (96) and that that the PML protein acts as a tumor suppressor (97).
Leukemia, JMML	See description in text (33–36).
Leukemia, T-cell ALL/ lymphoma	Mice with ectopic expression of Tal-1/Scl in lymphoid tissues can develop T-cell ALL late in life with incomplete penetrance (98, 99). Leukemogenesis is accelerated by transgenic coexpression of casein kinase Iiα (99, 100) and by expression of LMO1 (101) or LMO2 (102). T-cell lymphoid malignancies also develop at high frequency in E2A-deficient mice (103, 104).
Neuroblastoma	See description in text (37–39).
Rhabdomyosarcoma	Mice heterozygous for *Ptch* have increased propensity for developing rhabdomyosarcoma (~10% of animals at weeks 6–13) (82). Development of tumors requires Igf-2 (82) and is accelerated by loss of *p53* (84).
Rhabdoid tumor	A small percentage (10–30%) of mice heterozygous for mutant *Ini1/Snf5* develop malignant rhabdoid tumors (105–107). Tumor location is strain-dependent, with tumors primarily arising in the soft tissue of the face in some strains (106) and with CNS tumors being more common in other strains (107).
Soft tissue sarcoma (non-rhabdomyosarcoma)	Mice that carry linked germ-line mutations in *Nf1* and *p53* develop soft tissue sarcomas at high frequency, including malignant peripheral nerve sheath tumors, malignant Triton tumors, and rhabdomyosarcoma (108, 109).

[a] CNS, central nervous system; AML, acute myelogenous leukemia; RAR, retinoic acid receptor.

ity of tractable preclinical models, and the scientific opportunities related to evaluation of targeted therapies that require study in tumor models for which the target is relevant. If selection of tumor types was based on incidence and mortality rates, then ALL would be at the top of the list, and JMML would be near the bottom. However, if adequacy of current therapy and availability of engineered models of scientific interest is considered, then tumor types such as JMML, for which current therapy is relatively ineffective and for which there are transgenic models suitable for testing targeted agents, would be near the top of the list. In making the initial selection of tumor types to study in a preclinical testing program, it may be preferable to focus on a limited number of tumor types. Once the preclinical testing program's ability to efficiently and expeditiously test new agents is established with the initial panel of tumors, then expansion to other tumor types could be considered.

***In Vitro* Model Systems.** Preclinical testing programs have historically included an *in vitro* testing component. In comparison with *in vivo* testing, *in vitro* testing is less expensive and less time-consuming. Thus, an *in vitro* testing panel could potentially allow more tumor cell lines to be tested per tumor type and could allow additional agents to be tested. Furthermore, the initial testing of combinations of agents can be conducted on a cost-effective basis *in vitro*, whereas testing multiple drug combinations in animal models is difficult. It is important to perform *in vitro* testing using clinically relevant drug concentrations and schedules.

***In Vivo* Model Systems.** For *in vivo* tumor model systems, once the tumor types for initial inclusion in a preclinical testing program are identified, decisions need to be made about the number of models and the specific models that should be studied for each tumor type. The selection of tumor models will depend to some extent on the type of agent to be tested. For example, an immune modulating agent would require testing in immunocompetent animals and would not be testable in SCID mice. Ideally, however, the tumor models selected should be robust enough to be suitable for studying multiple classes of agents. Another consideration is whether models should be

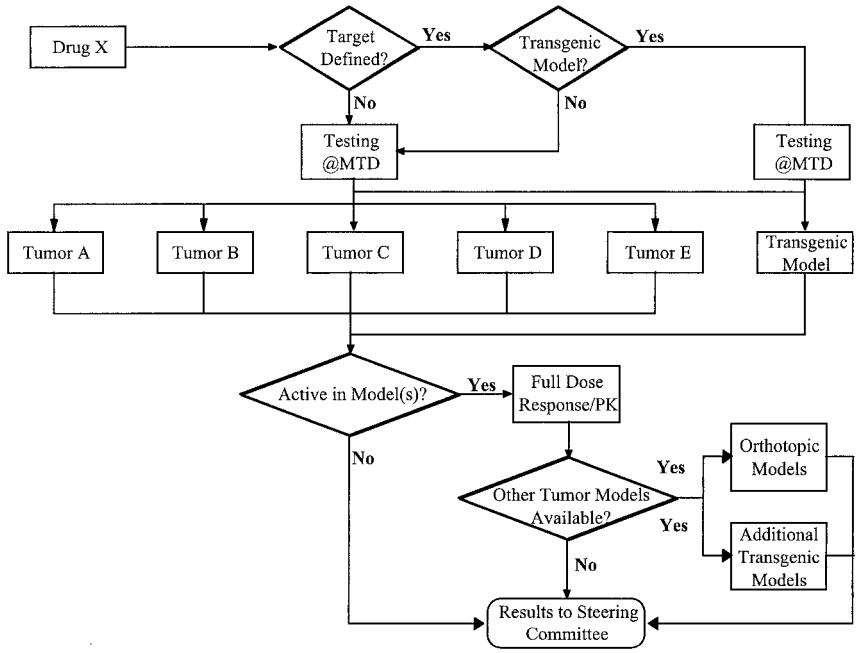

Fig. 1 Diagram of proposed pediatric testing program (see the text for description).

based on tumors from diagnosis or on recurrent tumors. A potential benefit for studying tumors from relapse is that these tumors have clinically relevant mechanisms of drug resistance. Testing using drug-resistant tumors may select for agents that are active against tumors incurable with current therapy. Agents identified as active in relapsed/recurrent tumor models may better predict activity in conventional Phase II studies, whereas models based on tumors at diagnosis may be more applicable to predicting activity in Phase II window studies. Selecting the number of models for each tumor type represents a compromise between the ideal of studying a multitude of models for each tumor type and the resource constraints that limit the number of models that can be studied. This practical limit is probably between 5–10 tumor models for each tumor diagnosis. The number of models studied for each tumor type should reflect the diversity of biological subtypes for the tumor type (*e.g.*, embryonal and alveolar rhabdomyosarcoma and the various molecular subtypes of ALL).

Characteristics of transgenic models suitable for inclusion in a preclinical testing program include: (*a*) uniform tumor development among independent transgenic lines; (*b*) high penetrance; (*c*) early initiation and time to progression; and (*d*) ability to be bred in large numbers. Transgenic animal models may not be optimal for testing against all agents because many of these models are resource-intensive when used for testing. For transgenic models that are resource-intensive, testing might be restricted to agents shown to be active in xenografts of the same tumor type and to agents that target biochemical pathways that are deregulated as a result of the engineered genetic lesion(s) of the model. For example, the JMML model described above may be useful for testing agents that target constitutively active Ras, but not other molecularly targeted therapeutics. One concern about the applicability of transgenic models is that to have high penetrance and early initiation, some transgenic models are engineered to have two or more specific genetic hits in relatively large numbers of cells. It is reasonable to question how accurately models of this type recapitulate the pathogenesis of human cancer. Also, transgenic models likely reflect more the status of the human cancer at diagnosis (*i.e.*, before treatment) than the status at the time of disease recurrence (*i.e.*, after exposure to cytotoxic therapy). Also, the issues related to the importance of pharmacokinetics in interpreting testing results for xenograft models (see below) apply to transgenic models.

A potential approach that has not been exploited extensively involves combining traditional xenograft and transgenic strategies. That is, cancers from genetically engineered mice or cell lines derived from these tumors might be injected into syngeneic immunocompetent recipients to increase the efficiency of drug testing *in vivo*.

Once a set of candidate tumor models is selected, there may be value in testing these models against conventional agents with a known clinical response rate to characterize the operating characteristics of each tumor model. This would serve to demonstrate that benchmark agents are active in appropriate tumor models and would enhance confidence in the preclinical testing program. It will also be important to have a periodic assessment of the screen in successfully identifying effective new drugs. Benchmarks for success should be identified prospectively, and analytic methods for determining whether these benchmarks have been met should be developed.

Proposed Approach

A possible design for a preclinical testing program is presented in Fig. 1. The design anticipates that many of the

agents available for testing will have known molecular targets and that relevant transgenic models will exist for some of these targeted agents. The design additionally recognizes that even when an agent has a putative cellular target, there may be other cell signaling pathways affected by the agent that may have relevance to the therapy of childhood cancers [*e.g.,* CEP-701 inhibits both Trk receptor tyrosine kinase activity and Flt3 (40, 41), and STI571 inhibits Bcr-Abl, c-Kit, and platelet-derived growth factor receptor (42–44)].

For agents with general cytotoxic activity for which a molecular target is not defined, the agent would initially be tested at its MTD against the entire xenograft panel. For tumor types in which responses were observed, a full dose-response curve would be developed, and the agent would also be studied in orthotopic or transgenic models of these tumor types when they are available. This sequential design would minimize the resources required to study drugs by avoiding complete dose-response studies for agents with little or no activity in tumor models.

For those agents with defined molecular targets, testing could be performed at doses below the MTD if there were convincing data documenting significant target modulation at lower doses. Even for agents with known molecular targets, there may be an advantage to performing the initial testing at the MTD because antitumor activity associated with modulation of the agent's known target would likely be detected by testing at the highest dose level tolerated, and activity associated with previously unrecognized targets may be recognized. Regardless of the dose used for the initial testing, if activity was observed, a full dose-response study would be performed to relate antitumor response to target inhibition. If the target of an agent was one for which there was a murine genetic model with suitable characteristics for preclinical testing, evaluation against this model would occur in the first tier of testing. If activity was observed in the first tier of testing for specific tumor types, then the agent would also be studied in available orthotopic models for these tumors.

The role of *in vitro* testing is not specified in Fig. 1. For those tumor types with well-characterized cell lines, *in vitro* testing could occur in parallel with *in vivo* testing. For those agents showing activity in one or more xenograft or transgenic models, *in vitro* testing could be especially useful in identifying combinations including the active agent that warrant further preclinical testing.

The infrastructure of a preclinical testing program would include a steering committee responsible for the overall direction and activities of the consortium, the institutions performing the testing for specific tumor types, and core resources for biostatistical, pharmacokinetic, and correlative study support. The institutions participating in the testing program would need to have the animal care infrastructure to support the requisite tumor model systems. They would need to maintain a cell line bank and/or animal tumor models ready for testing when required. Each institution performing testing would need to meet quality control guidelines, report data in a timely manner, share models and data as necessary, and abide by terms of negotiated material transfer agreements. Institutions would standardize treatment protocols to the extent possible across the tumor types being tested.

Essential Core Functions for a Preclinical Testing Program

Three of the essential core functions for a preclinical testing program are presented below.

Pharmacology Core. Pharmacokinetic data are essential for interpreting the activity observed in preclinical models. Mice may tolerate much higher systemic exposures of some drugs than humans (*e.g.,* DMP0840, 15–20× higher; carzelesin, 80× higher; and sulofenur, 8× higher) and may tolerate lower systemic exposures of other drugs (*e.g.,* etoposide).[3] An example of the importance of pharmacokinetic data in interpreting the likely clinical antitumor activity of "active" agents identified in animal models is that of 9-aminocamptothecin and irinotecan (the former is an agent with disappointing clinical activity, whereas the latter has substantial activity against a range of tumor types). Comparison of the pharmacokinetic behavior of 9-aminocamptothecin and irinotecan in mice and humans indicated that the systemic exposures associated with antitumor activity in mice were achievable in man for irinotecan (18), but not for 9-aminocamptothecin (45). Thalidomide is another example of the importance of identifying systemic exposure to active metabolites because the active metabolite of thalidomide produced in man is not produced in mice (46).

A preclinical testing program would need a central resource to generate pharmacokinetic data. Such a central resource could be at a single institution or, alternatively, could be a "pharmacology committee" representing researchers at a several institutions who would support the performance of pharmacokinetic analyses as needed by the consortium. To minimize the pharmacokinetic studies that need to be performed for each agent tested, it would be advantageous for the preclinical testing program to standardize its testing schema to a limited number of animal strains.

Correlative Science Core. The ability to perform correlative tumor biology studies in the preclinical setting will be increasingly important as agents targeting specific signal transduction pathways are evaluated. Correlative studies may establish whether molecularly targeted agents have achieved target inhibition under test conditions (*e.g.,* Ref. 47). Results from these studies will be important in understanding a targeted agent's activity or lack of activity (*i.e.,* illustrating an association between pathway modulation and antitumor activity) and may serve as a basis for establishing doses of targeted agents for use in combination studies (*i.e.,* selecting doses that modulate target, even though these doses may not have antitumor activity as a single agent). Proteomic and gene expression profiling of tumor models could be used to characterize the presence and activation status of specific cell signaling pathways, to compare gene expression in preclinical model specimens with specimens from patients, and to study changes in pathway activation status and gene expression after drug treatment (*e.g.,* Ref. 48). Assays developed for use in the preclinical setting may also prove applicable in evaluating target modulation in the clinical setting, using either tumor tissue or a surrogate normal tissue.

[3] P. J. Houghton, unpublished observations.

Biostatistical Core. A preclinical testing program would need biostatistical support to design and analyze its experiments. Design and analytic methods for testing new agents using xenograft models are established and are generally based on comparisons of either response rate or tumor volume in control animals and animals receiving the tested agent. Five to 10 animals are used for each dose of the tested agent. Methods for using transgenic animals for testing experimental agents are less well developed. Specifically, the challenge of using time from symptom development to death as an end point needs to be addressed. For example, to detect a 2-fold difference in this time between control and experimental animals with standard statistical parameters (90% power and $\alpha = 0.05$) would require 45 events/group.

Obstacles to Implementation of a Pediatric Preclinical Testing Program

Multiple challenges require resolution before a pediatric preclinical testing program becomes a reality. There are scientific issues, such as the most appropriate tumor models (be they *in vitro*, xenograft, or transgenic) for inclusion in a testing program and the most efficient strategies for testing different classes of new agents. Resource issues must be addressed because establishment of a testing program will require a substantial initial and ongoing investment. Overcoming the perception that preclinical models have inherently poor predictive value in identifying disease-specific activity for new agents (49) must be addressed by validating models used in the program. Perhaps most importantly, a preclinical testing program will need consistent and ongoing support from pharmaceutical companies, as demonstrated by the timely provision of agents for systematic preclinical testing.

Addressing intellectual property right issues and other issues related to collaborations with pharmaceutical sponsors (*e.g.,* data rights and confidentiality, publications, timely access to data, and control of experimental design) will be critical to the ultimate success of a pediatric preclinical testing program. The NCI has established collaborations with a number of sponsors for clinical development of investigational agents, and the solutions developed in the clinical context may be applicable in some instances to preclinical collaborations. With regard to data rights, the clinical collaborative agreements between the NCI and pharmaceutical collaborators stipulate that clinical trial primary data regarding proprietary compounds generated in NCI-sponsored clinical trials are made available exclusively to NCI, the Food and Drug Administration, and the pharmaceutical collaborator. With regard to publications, pharmaceutical collaborators are given 30 days to review manuscripts before submission to assure that no collaborator confidential/proprietary information is released. Collaborators may request an additional 30-day delay in publication to allow time for patent filing. With regard to patent rights, an issue for pharmaceutical collaborators is their access to inventions of federally funded researchers. Federal legislation (Bayh-Dole) prevents academic institutions from assigning intellectual property rights to another party except under narrowly specified conditions. The intellectual property option offers the rights of first negotiation to the pharmaceutical collaborator that supplied the investigational agent when there is an invention related to the agent as a result of federally funded research. If federal funds support some part of the preclinical testing consortium, then a similar option may be appropriate for the consortium to use. The Cancer Therapy Evaluation Program is introducing language into agreements with pharmaceutical sponsors for companies to provide agents for pediatric preclinical testing in accordance with the overall development objectives of the drug. For agents being developed with this stipulation, the agent is distributed under a material transfer agreement with intellectual property and data rights provisions similar to those that govern clinical trials.

Concluding Remarks

Meeting participants considered that the scientific opportunities and clinical need discussed at the meeting provide a strong rationale for establishing a pediatric preclinical testing program to prioritize new agents for evaluation in children with cancer. There is reason to be optimistic that current tumor model systems may have tumor-specific predictive value. Among the recent advances that support this optimism are the increased understanding of the importance of comparisons of systemic drug exposures in mouse models and man (18, 45, 50), the increased availability of pediatric cell lines and xenograft tumor models that have undergone molecular characterization, and the increasing availability of mouse genetic models for many cancer types. Meeting participants recognized, however, that validation of the predictive value of preclinical models is essential and that appropriate analytic methods will be needed to define the ability of specific preclinical testing methods to predict clinical activity for new agents in children.

The initial framework for the successful establishment of a pediatric preclinical testing program has now been outlined. Moving forward with such a program will require ongoing commitment from academia, the pharmaceutical industry, the Children's Oncology Group, the NCI, and the Food and Drug Administration. Acceptance by pharmaceutical companies of the concept of early access for pediatric preclinical testing to agents under clinical development will be a major factor determining the success or failure of a pediatric testing program. Because one of the purposes of publishing the meeting summary is to obtain feedback from academic investigators and pharmaceutical companies concerning the feasibility and utility of a preclinical testing program, persons with interest in such a program or with suggestions regarding how it should be developed are invited to contact the authors.[1]

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