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## The APL Paradigm and the “Co-Clinical Trial” Project

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### Summary

Tremendous advances in technologies have allowed the attainment of powerful insights into the molecular and genetic determinants that drive human cancers. However, this acquired knowledge has been translated into effective therapeutics very slowly, in part due to difficulty in predicting which drug or drug combination is likely to be effective in the complex mutational background of human cancers. To address this difficulty we have proposed and initiated the “co-clinical trial” project, in which we exploit mouse models that faithfully replicate the variety of mutational events observed in human cancers, to conduct preclinical trials that parallel ongoing human phase I/II clinical trials. Here, we focus on concepts relevant to the application of this novel paradigm and the essential components required for its implementation to ultimately achieve the rational and rapid development of new therapeutic treatments.

### Introduction

The “co-clinical trial” project stems from the realization of the tremendous power of preclinical testing of new drugs and drug combinations in faithful genetically engineered mouse (GEM) models of human cancer.

A “GEM to human” or “preclinical to clinical” approach has already proven to be highly effective toward the eradication of a subset of diseases [e.g., acute promyelocytic leukemia (APL)] in which the genetic defects are defined and “druggable” targets and targeted therapies have been identified. In this article, we discuss how we propose to export this paradigm for the optimization of therapy in other tumor types, and in principle to any disease, using the more refined GEM models and more sophisticated modeling capability currently available.

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We review how the co-clinical trial project is aimed at overcoming four fundamental challenges to making the use of preclinical models transformative in the real-time design of effective and informative clinical trials and ultimately, disease eradication:

1. A preclinical to clinical working model is extremely valuable, but still very slow because data have to be accrued in the mouse model of interest and then translated to inform the clinical trial that will follow (often the translation of the relevant information does not even occur).
2. Pharmaceutical companies are reluctant to delay phase I/II clinical trials pending the outcome of preclinical trials that could require 2 to 3 years to obtain conclusive results.
3. The notion that the preclinical results in GEM models of cancer (as opposed to standard xenograft) are predictive of human response to drugs is still novel and is not broadly accepted by pharmaceutical companies. Lack of success in such efforts in “genetically undetermined” human cell line xenograft models is often considered ahead of efforts in GEM models, although these two approaches are fundamentally different.
4. Because GEM models are often generated in academic laboratories, the Material Transfer Agreements (MTA) granting animal transfers from academia to industry, as well as drug transfers from industry to academia, can require years of lead time.

Taken as a whole, these challenges deny academic scientists and pharmaceutical companies the opportunity to share, in real time, valuable information obtained from preclinical studies, which could predict drug response in specific patients based on their genetic make-up and assist in the design of phase II and III human clinical trials. Finally, these obstacles impede cancer patients from receiving the appropriate and effective treatment that could be developed from these trials.

In this article, we initially discuss the historical milestones that led to eradication of APL through a GEM-to-human strategy as an example of powerful applicability for our proposed approach. We then describe the essential components required to implement the co-clinical trial idea and our vision for the integration of these elements to allow the application of the co-clinical approach as an innovative paradigm for the testing and development of new therapeutic modalities.

## **APL Cure as the Foundation of the Co-Clinical Trial Project**

The preclinical-to-clinical approach implemented to cure APL undoubtedly represents the foundation and inspiration for the co-clinical trial project. The ability to optimize therapeutic treatments in faithful GEM models of APL and to stratify patients on the basis of genetic criteria had a dramatic impact on the evolution of treatment of APL with single agents or drug combinations, and ultimately led to disease eradication.

Here we briefly summarize the stepwise milestones on the journey of discovery that culminated in the eradication of this formerly lethal leukemia. Remarkably, APL is now curable with the sole use of combined targeted therapies (reviewed in ref. 1).

The APL story started at the bedside with the cloning of the APL fusion genes, and came full circle to end at the bedside, with clinical trials that have led to its cure. In the case of APL, the paradigm was to complete the mouse studies first and then to bring this information to the attention of the clinicians, rather than conduct parallel trials. The co-clinical trial project proposes to extend this effort far beyond the APL paradigm and to

further accelerate this process of discovery, enabling a more rapid and successful return to the bedside.

### **APL Is Not One but Many: Gene Identification (1998–2004)**

APL is characterized by a distinctive block in differentiation at the promyelocytic stage of myeloid hematopoietic differentiation and the invariable association with reciprocal and balanced chromosomal translocations involving chromosome 17 (2). In 1991, we and others cloned the breakpoint of the t(15;17) translocation, thereby identifying the two genes involved at the breakpoint: *PML* on chromosome 15q22 and the *RAR $\alpha$*  gene on chromosome 17q21 (3). Although the t(15;17) translocation is the most frequently observed mutation in APL, other translocations, in which chromosome 17 is invariant, have also been described. During the period from 1998 to 2004, each variant translocation was molecularly cloned by our group as well as others, thereby leading to a new molecular classification for APL on the basis of these new genetic criteria (Table 1; reviewed in ref. 4).

To summarize, research showed that APL is not one but many, and that the genetic stratification was of relevance in dictating the biology of the disease and its therapeutic characteristics (Table 1). We all now accept that cancer is not one but many, and that therapy needs to be tailored according to genetics and molecular criteria.

Importantly, the APL paradigm was instructive at yet another level: in light of these findings, we realized that the development of clinical trials in the conventional manner would not be feasible, the reason being that APL is reasonably rare, representing approximately 10% of all myeloid leukemias. Consequently, there are not enough patients to enroll if genetic stratification should be taken into account for guiding the design of such a trial.

### **Faithful Mouse Modeling of APL (1994–1998)**

The mouse modeling efforts proved complex, especially at that time, due to lack of appropriate expression vectors, promoters, and regulatory elements to accurately direct transgenic expression in the appropriate hematopoietic/myeloid cellular compartment *in vivo*.

Ultimately, we succeeded in generating faithful models of APL in transgenic mice harboring the various human fusion genes (Table 1). Indeed, the pathologic features of these leukemia models were strikingly similar to those observed in human APL, even though mice do not spontaneously develop APL (Fig. 1). Furthermore, our systematic analysis of the role of the various fusion genes and proteins in single and double transgenic models allowed us to conclude that although the *X-RAR $\alpha$*  fusion gene is always oncogenic (e.g., *PML-RAR $\alpha$*  or *PLZF-RAR $\alpha$* ), the reciprocal *RAR $\alpha$ -X* product (e.g., *RAR $\alpha$ -PML* or *RAR $\alpha$ -PLZF* generated by the reciprocal and balanced APL chromosomal translocations) is also important in dictating the biological features and the response to therapy of these leukemias (5-10).

Historically, the generation of faithful GEM models of APL represented a fundamental step forward in convincing the scientific and medical community that systematic modeling of human cancer in the mouse was indeed feasible.

### **Model Validation (1997–1998)**

Going forward, we were able to demonstrate that APL models in the mouse were not only faithful to the biological and pathologic features of the human disease, but also that their leukemias behaved exactly as APL in human patients when challenged with drugs.

An initial example was offered by the realization that leukemia in *PML-RAR $\alpha$*  mice was found to transiently respond to retinoic acid (RA) exactly as human t(15;17) APL (5-7). As in human APL, RA was able to induce complete remission in the mouse, but leukemia would eventually relapse if treated with RA alone. In addition, leukemias in APL *PML-RAR $\alpha$*  mouse models would often become RA-resistant, as is the case for human t(15;17) APL treated solely with RA. We also demonstrated that as in human t(11;17) APL, leukemia in *PLZF-RAR $\alpha$*  mice was resistant to RA at presentation (10).

These findings represented a defining moment that radically changed the perception of GEM models of human cancer within the scientific and clinical oncology community. From that point forward, clinicians and physician scientists started to consider mouse models of human cancer not only as useful basic science tools for biological discovery, but also as powerful tools for preclinical testing of novel drugs and drug combination therapies.

### Informing and Predicting Clinical Trials and Outcome (1998–2000)

Subsequently, our mouse models of APL began to inform clinical trials in place. We could advise the clinicians on how to stratify patients for clinical trials on the basis of genetic criteria, using information accrued in preclinical testing of our mouse models of APL. As an example, we demonstrated that leukemia in *PML-RAR $\alpha$*  transgenic mice was responsive to arsenic trioxide (As<sub>2</sub>O<sub>3</sub>), which later proved to be another powerful weapon for the treatment of APL, although once again leukemia in *PLZF-RAR $\alpha$*  mice was found to be resistant (11) (Fig. 2A). Subsequently, t(15;17) APL was treated successfully with As<sub>2</sub>O<sub>3</sub>, and it then became clear that t(11;17) APL patients would require additional therapeutic modalities beyond RA and As<sub>2</sub>O<sub>3</sub>.

### Testing and Optimizing Combined Treatments (1998–2004)

On the basis of the predictive power of APL mouse models of distinct genotypes, we could optimize combined treatments by utilizing these mouse APL models as a preclinical predictive engine. A decisive “home run” in this respect was the realization that although As<sub>2</sub>O<sub>3</sub> and RA in combination were ineffective in *PLZF-RAR $\alpha$*  leukemias, this combination was tremendously effective in *PML-RAR $\alpha$*  leukemia in the mouse (11, 12).

These findings were even more relevant in light of the fact that data obtained from *in vitro* experiments using human leukemia cell lines predicted the opposite (13). This *in vitro* analysis suggested that the two drugs in combination would oppose each other (13). This analysis in turn resulted in several clinical combination trials being halted in many academic centers.

By contrast, this combination in mouse models was not only well tolerated, but also was able to eradicate APL in *PML-RAR $\alpha$*  leukemia models (Fig. 2B) (11, 12). These transgenic animals remained in complete remission and off treatment, even though this drug combination was administered at leukemia presentation for a short period. This finding was a compelling result taking into account that the *PML-RAR $\alpha$*  transgene remains expressed for the entire life of these animals.

On the basis of these convincing results, clinical trials combining both As<sub>2</sub>O<sub>3</sub> and RA were subsequently performed in many academic centers worldwide. Today, the As<sub>2</sub>O<sub>3</sub> and RA combination is considered the standard of care in the treatment of t(15;17) APL (14). These two drugs specifically target the PML-RAR $\alpha$  fusion protein by opposing its aberrant transcriptional activity and targeting it for degradation. As<sub>2</sub>O<sub>3</sub> and RA are offered at presentation to t(15;17) APL patients as frontline therapy with limited toxicity, in the absence of chemotherapy as induction or consolidation treatment. With these two drugs,

complete remission is obtained in 100% of cases, leading to disease eradication. Hence, the “targeted therapy devoid of chemotherapy” of APL has come of age from these efforts.

The preclinical effort in the mouse was decisive at informing and predicting clinical outcome. The APL GEM models were instrumental at multiple stages: 1) defining the oncogene(s) of APL; 2) identifying the druggable target(s); 3) determining the combined treatment that proved curative beyond misleading results obtained *in vitro* in cell lines; and 4) the APL GEM models proved critical for the study and effective treatment of therapy-resistant APL.

### **Developing and Testing Novel Therapeutic Modalities for Therapy-resistant APL (1998–2004)**

In spite of the remarkable success, the subtype of APL associated with PLZF-RAR $\alpha$  fusion proteins remained a resilient subtype of APL, maintaining resistance to conventional chemotherapy as well as to RA and As<sub>2</sub>O<sub>3</sub>.

Once again, GEM models proved critical to test novel therapeutic modalities for therapy-resistant APL. To this end, we recreated the dual complexity of human t(11;17) APL by coexpressing in transgenic animals the PLZF-RAR $\alpha$  and RAR $\alpha$ -PLZF fusion proteins. These transgenic mice developed a classic APL-like leukemia that proved resistant to conventional chemotherapy and to the combination of RA and As<sub>2</sub>O<sub>3</sub> just as observed in human t(11;17) APL (8).

Utilizing this APL model, we could therefore test a novel therapeutic concept and a novel class of drugs: histone deacetylase inhibitors [HDACI (the rationale for “transcription therapy” with HDACIs is reviewed in ref. 15)], which we predicted would revert in combination with RA the transcriptional repressive activity of the PLZF-RAR $\alpha$  fusion protein (16).

Mouse models of t(11;17) APL allowed us to demonstrate not only the efficacy of this combination in therapy-resistant APL, but also the limited toxicity of HDACIs (16). Suberoylanilide hydroxamic acid (SAHA), a novel experimental HDACI, proved well tolerated and capable of inducing complete remission in leukemia in *PLZF-RAR $\alpha$ /RAR $\alpha$ -PLZF* double transgenic animals when administered in combination with RA (16).

Subsequently, we were granted approval from the Food and Drug Administration to test in therapy-resistant human APL a combination of phenyl butyrate (a compound also active as HDACI) and RA, because both drugs were already approved for clinical use. The first patient, a 13-year-old girl, attained complete molecular remission with negligible toxicity after receiving a therapeutic regimen that was entirely derived from the regimen optimized in mouse models of t(11;17) APL with SAHA plus RA (17). By now, SAHA is also approved for clinical use under the name vorinostat.

### **Can the APL Paradigm Be Replicated?**

Although the APL story exemplifies the tremendous utility of a preclinical to clinical or mouse to human approach, there is little doubt that this line of attack could be exported to other forms of cancer and replicated in the immediate future. This statement is based on a number of considerations. All the ingredients for this approach to be possible are at hand: a detailed understanding of the genetic basis of human cancer; faithful mouse models of several cancers; and a wide range of drugs available for testing in the immediate future.

However, a number of things have changed since 1991 when the *PML-RAR $\alpha$*  fusion gene was originally cloned, and several other aspects also need to change if we want to accelerate

this paradigm. It must be noted that in the case of APL, 18 years elapsed from gene identification to cure. Therefore, although success ultimately was achieved, the time period needed to eradicate APL was very lengthy.

What has changed 18 years later is that the volume of information has dramatically increased: researchers are flooded by genetic information, the relevance of which needs to be carefully assessed. Moreover, many more drugs are available to be tested in clinical trials in multiple subtypes of cancer. This complexity and high volume will complicate the development of clinical trials if they are performed in the traditional manner. For instance, patient accrual may become a serious issue because there will not be enough patients to test drugs singly or in combination once the disease is stratified according to molecular and genetic criteria (as is the case for APL, which is rare and has many subtypes).

This complexity could partially be ameliorated by the fact that mouse modeling of human cancer has reached a level of unprecedented sophistication. Researchers can now perform genetic modifications in the mouse that were inconceivable 10 years ago.

Conversely, four fundamental aspects have not changed and should be taken into serious account if the ultimate goal is to accelerate the process of drug testing using preclinical information from GEM models of human cancer:

1. To proceed in a preclinical to clinical fashion would take a very long time, even in the most optimistic scenario of an immediate translation of the preclinical information into intelligent clinical trials, as it was in APL.
2. Pharmaceutical companies are reluctant to allow preclinical testing of their drugs in mouse models by academic laboratories and basic scientists because such efforts frequently do not offer the needed quality control that, in principle, a well-executed clinical trial could offer.
3. Pharmaceutical companies are determined to perform clinical trials in human patients as soon as regulatory bodies allow them to do so, irrespective of the possible accrual of valuable preclinical information in mouse models of human cancer.
4. Perhaps the most serious hurdle is the fact that the “basic science academic vector of discovery,” which emanates from the basic academic laboratories to generate preclinical information, rarely meets the “clinical vector of discovery” that is sustained by the activity of clinicians and pharmaceutical companies. These two worlds seldom occupy the same table, and the information each one provides is infrequently integrated. The data released in scientific papers, even when published in prestigious journals, are rarely utilized in designing clinical trials. On the other hand, basic scientists, who often are not familiar with how to design clinical trials, rarely read clinical papers.

Taking into account these facts and potential hurdles, as well as utilizing the powerful example constituted by the APL paradigm, we have formulated the co-clinical trial project.

### **The Co-Clinical Trial Concept: Synchronicity**

We believe that the most direct way to overcome the aforementioned hurdles is to accelerate the process of data integration and the translation of preclinical efforts into the clinic as well as to facilitate and develop an infrastructure that allows the synchronization and real-time integration of preclinical and clinical efforts.

Many clinical trials will be developed in the immediate future, as the pharmaceutical industry and clinical investigators (the clinical vector of discovery) will test numerous new molecules for cancer treatment in phase I/II trials. This reality can be met and given greater incentive by creating an infrastructure whereby the “basic science academic vector” of preclinical testing operates in parallel with the clinical vector of discovery.

Specifically, we propose that each clinical trial be conducted in parallel with preclinical trials in appropriate GEM models, and that the relevant clinical, biological, and pharmacologic information (i.e., somatic mutational background, germline single nucleotide polymorphism variations, responsiveness to specific regimens, imaging, microarray, and proteomics profiles) are accrued, analyzed in parallel, and integrated to facilitate the identification of biomarkers that predict response to specific treatments (approach 1 in Fig. 3). Furthermore, to complement the GEM models, genetically stratified human cancer explants obtained from patient biopsies during clinical trials can be used to assess responsiveness *in vivo* using xenograft mouse models in *NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/SzJ* (NSG) mice (approach 2 in Fig. 3).

This approach can immediately be developed on a large scale in a prospective manner, because what we propose is straightforward and relatively inexpensive *vis-à-vis* the cost of clinical trials in human patients. The simple working model that we propose, which can be exported beyond cancer research to any disease and biomedical discipline, is exemplified as follows. For example, when a novel experimental drug “X” is tested in the clinic against prostate cancer, it should be systematically tested in each GEM model of prostate cancer that is considered both faithful and informative by the scientific community. Data should be accrued in real time and translated back to the clinic simultaneously, thereby allowing for immediate human patient stratification, among other things (Fig. 4). Assuming that of the mouse models of prostate cancer depicted in Figure 4, 1 (genotype “C”) responds to drug X but the other 2 fail to do so, we could immediately translate all the mouse data for comparison with information obtained from human prostate cancer patients who do or do not respond to drug X. This, in turn, allows for real-time testing of the hypothesis that genotype “C” is also a major determinant of sensitivity in human patients as it is in the mice, whereas the other genotypes are major determinants of upfront resistance. Importantly, even the genotype “C” model will eventually relapse, allowing for the synchronous analysis and identification of determinants of acquired resistance (Fig. 4).

This working model can be applied for the achievement of several fundamental goals (summarized in Table 2), and at once solves each and every one of the above-mentioned hurdles: 1) the general process will be tremendously accelerated by the synchronicity of the analysis; 2) pharmaceutical companies will find it extremely appealing to perform clinical trials with academic centers that offer this infrastructure and perform co-clinical trials with rigor and sophistication; and 3) the data integration will bring basic scientists, clinicians, and pharmaceutical companies to the same table.

In addition, the co-clinical trial project will enable the conduct of accelerated GEM model-based biomarker discovery. It is important to underline that, although cancers that develop in a specific GEM model are initiated by a common genetic alteration, there is substantial heterogeneity in the genomic profiles of the tumors that develop, as shown by differences in mRNA expression signatures, gene copy number alterations, and the mutation status of other complementing genes. There is also substantial heterogeneity when the tumor becomes resistant to a given treatment modality. In turn, these data suggest that tumors in GEM models may reflect the heterogeneity of human tumors—even those initiated by the same genetic event—and therefore vary in response to treatment, enabling the rapid identification of biomarkers of sensitivity and resistance to a treatment modality.

## The Fundamental Components for Co-Clinical Trial Implementation

For the co-clinical trial project to succeed and set a new paradigm for drug testing and development, we need to create the appropriate infrastructure and educate the clinical and preclinical operators. In principle, the number of “preclinical centers” behind the co-clinical trial project could be the same as the number of “clinical centers” that enroll patients in a given clinical trial.

We have identified the components that, in our opinion, are critical for the development of the co-clinical trial project (Fig. 5):

1. A “mouse hospital” with a state-of-the-art infrastructure for *in vivo* mouse imaging and a mouse pharmacy.
2. An infrastructure for comparative pathology.
3. A bioinformatics infrastructure for data mining and analysis.

### The Mouse Hospital

The basic premise of a co-clinical trial strategy is that GEM models, which harbor molecular defects causally associated with human cancers, can be used to focus treatment paradigms at a fraction of the cost and time required for human clinical trials. In concert with the results from phase I/II clinical trials, these mouse models can be used to evaluate therapeutic candidate monotherapy, multiple combination therapy efficacy, and biomarker responses that can then direct the design of early phase human clinical trials. Furthermore, the co-clinical strategy could be expanded to help rescue failed clinical candidates and direct their reevaluation against an appropriate oncogenic mutation.

The global mouse genetics community has generated sophisticated resources that have great potential to support co-clinical cancer trials. These resources include accurate mouse models of multiple human cancer types, imaging technologies ideally suited for determining physiologic and tumor responses to treatment, and comprehensive biomarker analysis centered on high-throughput genomic and gene expression platforms.

A co-clinical trials program requires rigorous attention to standardized protocols; expertise in mouse genetics, biology, and husbandry; pharmaceutical drug development; and efficient communication among basic, clinical, and pharmaceutical researchers. Programs must be designed to involve and educate chemists, pharmacologists, biologists, physicians, geneticists, clinical trial contract research organizations and their study monitors, investors, and pharmaceutical executives. Dosing regimens must be carefully controlled, as must measurements of response. Sophisticated imaging and bioresponse measurement equipment is also required. Data collection and management must be organized to permit community access and integration.

We therefore propose that, to ensure standardized procedures and the requisite attention to experimental details, central cancer mouse hospitals (CCMH) are needed where large-scale mouse trials can be conducted and evaluated by experts in close consultation with clinicians and pharmaceutical companies. The CCMHs will be equipped with a dedicated vivarium, procedure rooms, and tissue culture facilities. A CCMH must also include comprehensive, high-throughput biomarker analysis of and host physiologic response to therapeutic treatment to support co-clinical human-mouse trials. Additionally, a state-of-the-art CCMH will vitally depend on multitechnology comprehensive small-animal imaging centers and a centralized mouse pharmacy.

## Oncology Imaging Center at the Mouse Hospital

We believe that to ensure standardized procedures and necessary attention to technology application and implementation, an oncology imaging center (OIC) is needed to support large-scale mouse trials in close consultation with clinicians and pharmaceutical companies.

The envisioned OIC technologies would include comprehensive noninvasive macroscopic imaging systems, such as magnetic resonance imaging; microcomputed tomography; micropositron emission tomography; ultrasound; and fluorescence-mediated tomography. An OIC would offer several benefits.

First, the availability of an OIC will guarantee that *in vivo* imaging protocols for mice closely match clinical protocols in terms of both data acquisition and data analysis.

Second, the establishment of an OIC will assure creation of state-of-the-art facilities, imaging systems, supporting infrastructure, and capabilities.

Third, standard operating procedures for all imaging modalities and organ-specific imaging protocols will be established and distributed. Standardized procedures will permit multiple laboratories to compare results of drug efficacies, share information, and allow for establishing phenotype databases for all mouse models.

In the context of the co-clinical trial project, we postulate that imaging studies in mouse models will parallel the analysis done in clinical trials (at a fraction of the cost); serve as a platform to rapidly test emerging new research directions; and allow the development of new imaging approaches and agents, which could then be tested clinically. Thus, the fundamental opportunity is to analyze mouse imaging data sets similarly to clinical data sets and integrate the results to better predict treatment response and outcomes.

## A Mouse Pharmacy for the Mouse Hospital

The biggest challenge in obtaining experimental compounds for testing in animal models is the lengthy process needed to obtain MTAs from the pharmaceutical companies who hold patents on these drugs. MTAs can take more than a year to complete and are extremely narrow in regard to models that can be tested. Furthermore, they can invariably exclude the use of drug combinations, and any request for a change in the MTA can require months. Additionally, the compounds obtained in this fashion cannot be shared with collaborators.

We propose to circumvent the complexity of the MTA process and have compounds of interest for the co-clinical trial project synthesized for shared use. This approach has many advantages. First, multiple laboratories can use the same compounds in their mouse models and freely share information. Second, combinations of agents can be tested, which would otherwise be impossible with compounds obtained via MTAs.

Because the scientific community intends to use these compounds only in animal models or in cell lines for noncommercial purposes, and because by the time the compounds enter phase I clinical trials, the structures have almost certainly been published in patents, there is no restriction to access or use them.

## Comparative Pathology Center

The realization of the co-clinical trial project will require extensive human/mouse comparative pathology expertise. As for the OICs, to ensure standardized procedures and technology application and implementation, we propose the development of comparative pathology centers (CPC) to support co-clinical trials in close consultation with clinicians.

We propose the creation of CPCs that constitute a state-of-the-art hub for oncologic and pathology-based research, where pathologists work with molecular biologists, translational researchers, and clinical researchers to develop molecular pathology technologies and resources to support targeted cancer therapy. These include, for instance, the development of tissue-based assays aimed at identifying, validating, and assessing biomarkers of cancers and the development and analysis of experimental models of cancers. Overall, CPCs will be comprised of molecular pathologists interested in mouse models of cancer and their relevance and similarities to human tumors with the ultimate goal of patient stratification using molecular biomarkers.

## Co-Clinical Bioinformatics Centers for Data Storage, Mining, and Analysis

Through co-clinical trial efforts, we will collect extensive data from mouse models concerning specific genetic lesions that drive cancer initiation and progression. Such data will include, for example, large sets of expression transcript profiling, comparative genomic hybridization profiling, whole genome sequencing, immunohistochemical data, and morphologic data that will characterize these tumor models throughout their development at a pathologic and molecular level. These models will be evaluated in a similar manner for the response to various single agent and combined treatments that will be cross-referenced with data collected to characterize the primary models.

This mouse effort will be coordinated with the clinical counterpart to accrue and compile extensive data regarding the genetic profile of tumors before and after treatment. This review will include, for example, analysis of major cancer-associated genes to define alterations to these genes in these tumors (i.e., *p53*, *PTEN*, and *c-Myc* genomic status) and how they relate to prognosis and treatment outcome.

Therefore, all data collected will be compiled, compared, and cross-referenced to provide a comprehensive overview of how the response from preclinical trials in mice can assist in the design of clinical trials and inform the clinic regarding stratification of patients. Data integration is the foundation of the “co-clinical trial” principle. Thus, a bioinformatics platform for mouse and human integrated normalization of data is critical to the successful realization of the project.

## Conclusions

The co-clinical trial project rests on a novel paradigm: parallel testing of new therapeutic modalities in faithful GEM models and patients to streamline the progression from bench to bedside for experimental therapeutics or novel combinations of already approved drugs. Notably, these mouse and human integrated efforts also can be developed postclinically to optimize standard-of-care treatment modalities that are often randomly administered to all comers (see Table 2).

We are aware that tests in animals for co-clinical trials are expensive, involving not only the cost of drugs, but also the operating cost of the “mouse hospital.” However, these expenditures are trivial compared to human clinical trials. Perhaps the biggest challenge is obtaining the quantities of investigational drugs needed for the co-clinical trials. Initially, pharmaceutical companies may be reluctant to provide these drugs for free and will resist blanket MTAs across institutions, hence the need for a mouse pharmacy. However, it is becoming clear that pharmaceutical companies may find it extremely appealing to have access to the prepublication information that originates from the co-clinical trial project. In addition, many of the ongoing trials with drugs that target components of signaling pathways will fail as single agents in phase I/II because the majority of patients in clinical

trials will have combinations of mutations that will circumvent the effect of the drug. The critical information from co-clinical trials is to predict which mutational events will result in resistance to the drugs and to exclude patients with these mutations from phase I/II trials, thus enhancing the likelihood that a drug will benefit a specific genetically determined patient population in early phase clinical trials. This information will in turn accelerate drug approval, albeit for a narrowly defined population of patients, and ultimately allow new clinical trials with combinations of approved drugs where broader efficacy is more likely. Furthermore, this information could diminish clinical trial failure due to insufficient patient enrollment because of patients being misdirected into the wrong trial. Conversely, the co-clinical approach can also enhance the negative predictive value of a drug early in the drug development process: for example, if a drug does not work in any of the faithful GEM models of prostate cancer, it will likely not work in a phase I/II human clinical trial. This information will reduce the enormous costs associated with a failed phase III clinical trial involving many hundreds of patients and the investment of several billion dollars.

Finally, this integrated approach has already started to develop for the benefit of cancer patients at several US institutions, and will soon spread worldwide. Indeed, it is important to underscore that the co-clinical trial project could be easily exported to any medical center and the approach could be applied to other biomedical disciplines and diseases (e.g., degenerative and metabolic disorders), whenever faithful mouse models are available and clinical trials with experimental drugs are ongoing. The co-clinical trial project was originally launched in prostate and lung cancers, with the initial support of stimulus grants from the National Cancer Institute (NCI). Additionally, the co-clinical trial project is at the core of our Stand Up to Cancer–supported “dream team,” which is targeting the phosphoinositide 3-kinase pathway in women’s cancers. For each of the human clinical trials designed by interinstitutional teams of clinicians and scientists, parallel trials are being conducted in GEM models of women’s cancers, using the same therapies. The mouse models are being generated at the participating institutions (Beth Israel Deaconess, Memorial Sloan-Kettering, Massachusetts General Hospital, MD Anderson, Dana-Farber Cancer Institute, Vanderbilt, Columbia, and Vall d’Hebron) and the results are shared with all the team members at quarterly meetings. The early observations from the mouse clinical trials are already influencing hypotheses that will be evaluated in the parallel human clinical trials. We anticipate that these and other ongoing co-clinical trials will provide the proof of concept that establishes the value of this new paradigm.

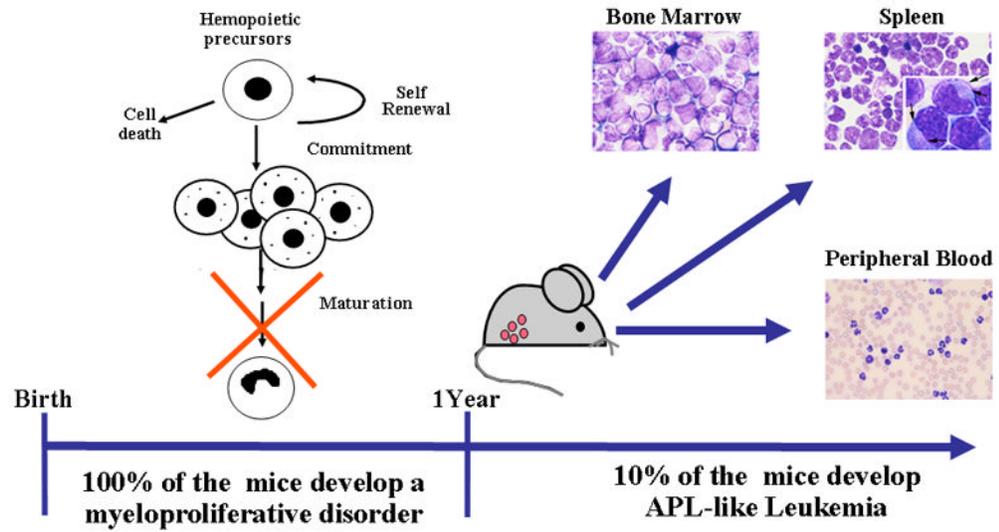
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## References

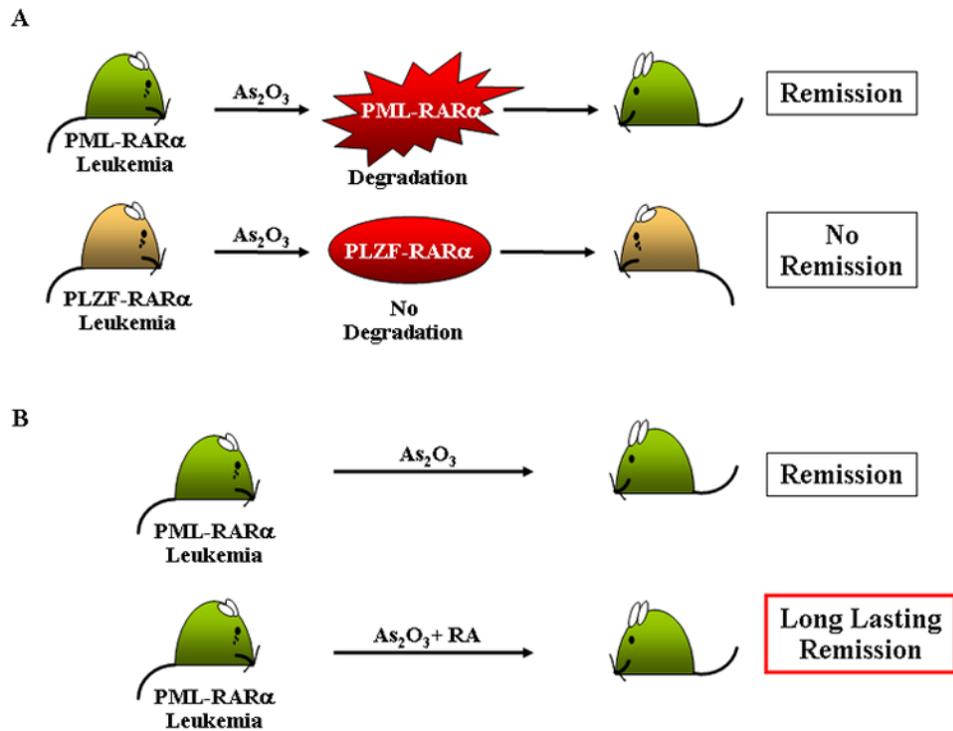
1. Nasr R, Guillemain MC, Ferhi O, Soilhi H, Peres L, Berthier C, et al. Eradication of acute promyelocytic leukemia-initiating cells through PML-RARA degradation. *Nat Med.* 2008; 14:1333–42. [PubMed: 19029980]
2. Rowley JD. Chromosomal patterns in myelocytic leukemia. *N Engl J Med.* 1973; 289:220–1. [PubMed: 4514580]
3. Pandolfi PP, Grignani F, Alcalay M, Mencarelli A, Biondi A, LoCoco F, et al. Structure and origin of the acute promyelocytic leukemia myl/RAR alpha cDNA and characterization of its retinoid-binding and transactivation properties. *Oncogene.* 1991; 6:1285–92. [PubMed: 1650447]
4. Piazza F, Gurrieri C, Pandolfi PP. The theory of APL. *Oncogene.* 2001; 20:7216–22. [PubMed: 11704849]

5. He LZ, Tribioli C, Rivi R, Peruzzi D, Pelicci PG, Soares V, et al. Acute leukemia with promyelocytic features in PML/RARalpha transgenic mice. *Proc Natl Acad Sci U S A.* 1997; 94:5302–7. [PubMed: 9144232]
6. Grisolano JL, Wesselschmidt RL, Pelicci PG, Ley TJ. Altered myeloid development and acute leukemia in transgenic mice expressing PML-RAR alpha under control of cathepsin G regulatory sequences. *Blood.* 1997; 89:376–87. [PubMed: 9002938]
7. Brown D, Kogan S, Lagasse E, Weissman I, Alcalay M, Pelicci PG, et al. A PMLRARalpha transgene initiates murine acute promyelocytic leukemia. *Proc Natl Acad Sci U S A.* 1997; 94:2551–6. [PubMed: 9122233]
8. He LZ, Bhaumik M, Tribioli C, Rego EM, Ivins S, Zelent A, et al. Two critical hits for promyelocytic leukemia. *Mol Cell.* 2000; 6:1131–41. [PubMed: 11106752]
9. Rego EM, Ruggiero D, Tribioli C, Cattoretti G, Kogan S, Redner RL, et al. Leukemia with distinct phenotypes in transgenic mice expressing PML/RAR alpha, PLZF/RAR alpha or NPM/RAR alpha. *Oncogene.* 2006; 25:1974–9. [PubMed: 16331271]
10. He LZ, Guidez F, Tribioli C, Peruzzi D, Ruthardt M, Zelent A, et al. Distinct interactions of PML-RARalpha and PLZF-RARalpha with co-repressors determine differential responses to RA in APL. *Nat Genet.* 1998; 18:126–35. [PubMed: 9462740]
11. Rego EM, He LZ, Warrell RP Jr, Wang ZG, Pandolfi PP. Retinoic acid (RA) and As2O3 treatment in transgenic models of acute promyelocytic leukemia (APL) unravel the distinct nature of the leukemogenic process induced by the PML-RARalpha and PLZF-RARalpha oncoproteins. *Proc Natl Acad Sci U S A.* 2000; 97:10173–8. [PubMed: 10954752]
12. Lallemand-Breitenbach V, Guillemin MC, Janin A, Daniel MT, Degos L, Kogan SC, et al. Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia. *J Exp Med.* 1999; 189:1043–52. [PubMed: 10190895]
13. Shao W, Fanelli M, Ferrara FF, Riccioni R, Rosenauer A, Davison K, et al. Arsenic trioxide as an inducer of apoptosis and loss of PML/RAR alpha protein in acute promyelocytic leukemia cells. *J Natl Cancer Inst.* 1998; 90:124–33. [PubMed: 9450572]
14. Nasr R, de The H. Eradication of acute promyelocytic leukemia-initiating cells by PML/RARA-targeting. *Int J Hematol.* 91:742–7. [PubMed: 20455087]
15. Pandolfi PP. Transcription therapy for cancer. *Oncogene.* 2001; 20:3116–27. [PubMed: 11420728]
16. He LZ, Tolentino T, Grayson P, Zhong S, Warrell RP Jr, Rifkind RA, et al. Histone deacetylase inhibitors induce remission in transgenic models of therapy-resistant acute promyelocytic leukemia. *J Clin Invest.* 2001; 108:1321–30. [PubMed: 11696577]
17. Warrell RP Jr, He LZ, Richon V, Calleja E, Pandolfi PP. Therapeutic targeting of transcription in acute promyelocytic leukemia by use of an inhibitor of histone deacetylase. *J Natl Cancer Inst.* 1998; 90:1621–5. [PubMed: 9811311]



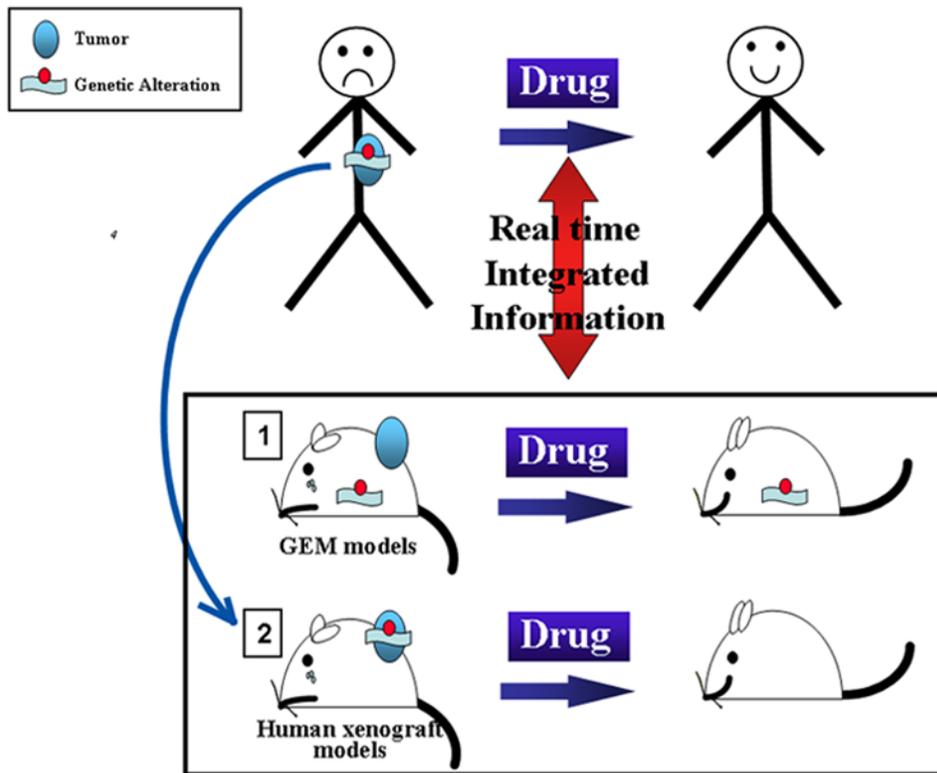
**Figure 1. Modeling APL in the mouse**

Transgenic mice, in which the human *PML-RAR $\alpha$*  fusion gene is under the control of the regulatory elements of the myeloid/promyelocytic specific cathepsin-G gene, develop leukemias with classic APL features.



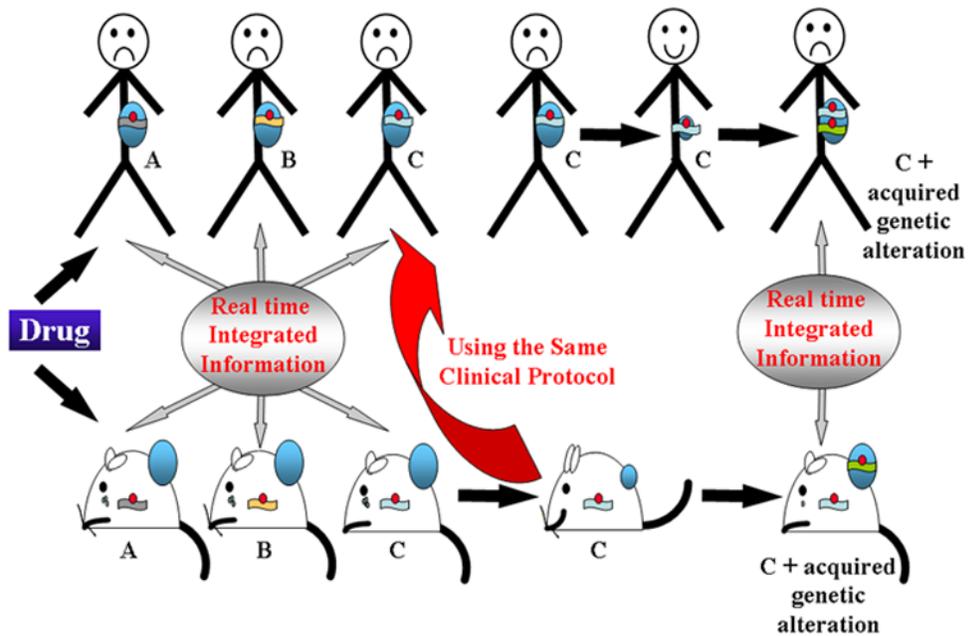
**Figure 2. Preclinical trials in mouse models of APL**

A, mouse models of APL respond differentially to  $As_2O_3$ . *PLZF-RARα* leukemia proved resistant to therapy as it was resistant to RA as single agent. By contrast, *PML-RARα* leukemia proved exquisitely sensitive to  $As_2O_3$ , which triggers the degradation of the fusion protein, suggesting that this drug could be used successfully in large-scale clinical trials. B, treatment of *PML-RARα* mice with RA and  $As_2O_3$  in combination leads to a longer lasting remission compared to the treatment with RA alone.



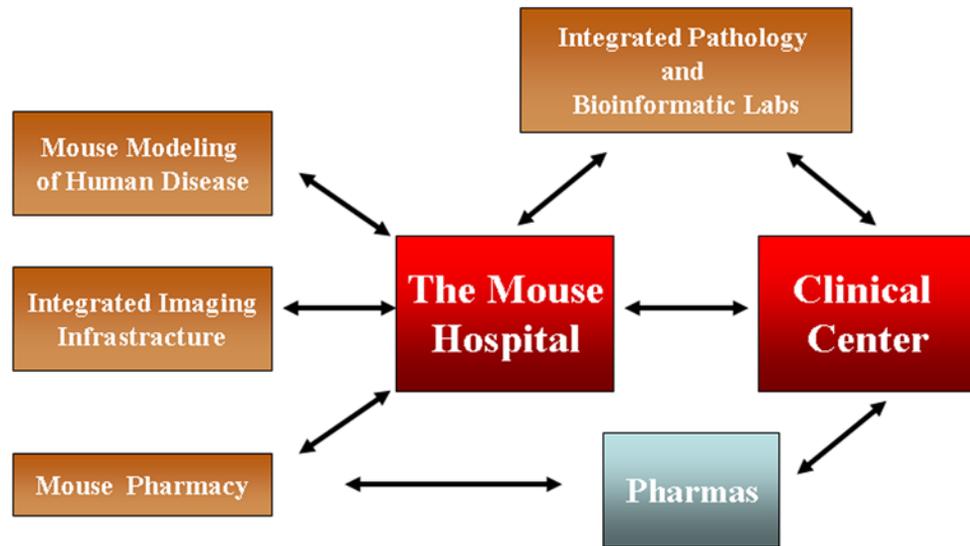
**Figure 3. The co-clinical trial project**

Synchronizing preclinical with clinical trials to be run in parallel in human patients and mouse models, either GEM models (1) or human xenograft models (2), will allow real-time integration of information.



**Figure 4. Co-clinical trials in patients and in mouse models of human cancer**

The same drug and exactly the same protocol will be utilized in mouse models and human patients. Data will be accrued and analyzed in parallel toward disease stratification in primary tumors as well as in acquired treatment resistance.



**Figure 5. Critical components of a co-clinical center**  
Fundamental components of the co-clinical effort (in red) and their relationship with the infrastructures and pharmaceutical companies.

**Table 1**

Molecular classification for APL and its implications for treatment and therapy

Aberrant genes	Translocations	Response to chemotherapy	Response to RA treatment	Risk of treatment failure
<i>PML-RAR<math>\alpha</math></i>	t(15;17)	Good	Good	Low/intermediate
<i>PLZF-RAR<math>\alpha</math></i>	t(11;17)	Poor	Poor	High
<i>NPM-RAR<math>\alpha</math></i>	t(5;17)	Good	Good	Low/intermediate
<i>NuMA-RAR<math>\alpha</math></i>	t(11;17)	Good	Good	Low/intermediate
<i>STAT5b-RAR<math>\alpha</math></i>	t(17;17)		Poor	

**Table 2**

## Fundamental goals of the “co-clinical trial” project

1) Perform co-clinical assessment of novel agents while in phase I/II to permit rapid stratification of responder and resistant populations.
2) Assess single agents or drug combinations in GEM models of genetically distinct and “rarer” cancer types/subsets, in which sizable patient accrual represents a major hurdle.
3) Perform <i>in vivo</i> testing of drug combinations to determine tolerability, efficacy, and pharmacodynamics.
4) Facilitate the prioritization of the most attractive combinations for assessment in humans.
5) Allow the “postclinical” optimization of standard of care treatment modalities.
6) Identify mechanisms of acquired resistance to agents/therapeutic modalities (often before clinical trials start).
7) Assess the system impact of agents and combinations (e.g., tumor immune response or impact on tumor stroma and vasculature).
8) Permit the co-clinical and preclinical identification of novel biomarkers.
9) Determine the importance of the temporal sequence of genetic alterations in dictating response or resistance to treatment modalities.