Bridging Tumor Genomics to Patient Outcomes Through an Integrated Patient-Derived Xenograft Platform

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Abstract

New approaches to optimization of cancer drug development in the laboratory and the clinic will be required to fully achieve the goal of individualized, precision cancer therapy. Improved preclinical models that more closely reflect the now recognized genomic complexity of human cancers are needed. Here we describe a collaborative research project that integrates core resources of The Jackson Laboratory Basic Science Cancer Center with genomics and clinical research facilities at the UC Davis Comprehensive Cancer Center to establish a clinically and genomically annotated patient-derived xenograft (PDX) platform designed to enhance new drug development and strategies for targeted therapies. Advanced stage non–small-cell lung cancer (NSCLC) was selected for initial studies because of emergence of a number of “druggable” molecular targets, and recent recognition of substantial inter- and intrapatient tumor heterogeneity. Additionally, clonal evolution after targeted therapy interventions make this tumor type ideal for investigation of this platform. Using the immunodeficient NOD scid gamma mouse, > 200 NSCLC tumor biopsies have been xenotransplanted. During the annotation process, patient tumors and subsequent PDXs are compared at multiple levels, including histomorphology, clinically applicable molecular biomarkers, global gene expression patterns, gene copy number variations, and DNA/chromosomal alterations. NSCLC PDXs are grouped into panels of interest according to oncogene subtype and/or histologic subtype. Multiregimen drug testing, paired with next-generation sequencing before and after therapy and timed tumor pharmacodynamics enables determination of efficacy, signaling pathway alterations, and mechanisms of sensitivity-resistance in individual models. This approach should facilitate derivation of new therapeutic strategies and the transition to individualized therapy.

Clinical Lung Cancer, Vol. 16, No. 3, 165-72 © 2015 Elsevier Inc. All rights reserved.

Keywords: Clinical trials, Lung cancer, Mouse model, Patient derived xenograft

Introduction

Substantial advances have been made in understanding the molecular biology that drives carcinogenesis and cancer-associated proliferative and antiapoptotic signaling pathways. A wide variety of potentially “druggable” molecular targets for cancer therapy have emerged from these studies. Although a large number of molecular targeted agents have subsequently been tested, most of which showed substantial activity in available preclinical models, relatively few have been successful
Figure 1 Algorithm of Patient-Derived Xenograft (PDX) Creation. Candidate Patients (PTs) Were Identified and Provided Consent for Tumor Collection. After Biopsy, Pleural Effusion Fluid Collection, or Surgical Resection, Viable Portions of the Fresh Specimens Were Rapidly Transported to the Jackson Laboratories-West Facilities for Implantation Into the NSG Mouse Model. Concurrently, Remaining Portions of the PT Specimen Were Fixed and Subsequently Characterized and Molecularly Profiled. A PDX Model Was Considered “Established” After Demonstrating Growth in Passage 1. After Successful Implantation, Development and Transplantation From Passage 0. Histomorphologic Evaluation and Molecular Profiling of the PDX Model Was Conducted and Results Compared With the Contributing Human Specimen. When PDX Models Reach Passage 2, Cohorts Can Be Prepared for Growth Inhibition and Tumor Pharmacodynamic Studies.

Characterization of NSCLC Patient Tumors and Patient-Derived Xenografts

1. Histomorphologic Evaluation:
   - FFPE
   - IHC and Morphoproteomics
2. Clinically Applicable Molecular Biomarkers:
   - Mutations: EGFR and KRAS
   - Fusion oncogenes: EML4-ALK, ROS1 fusion
   - mRNA levels of EGFR, ERCC1, RRM1, and TS
   - Additional: PIK3CA, MET, RET, HER2
3. NGS Molecular Profiling:
   - CNV/SNP and gene expression arrays
   - NGS (Illumina)
4. Targeted in vivo drug testing:
   - Growth Inhibition Studies and Tumor Pharmacodynamics

Abbreviations: CNV = copy number variation; FFPE = Formalin-fixed, paraffin-embedded; IHC = immunohistochemistry; NGS = next-generation sequencing; NSCLC = non—small-cell lung cancer; SNP = single-nucleotide polymorphism.


Abbreviations: AMP = amplification; mut = mutation.
in the clinic.\textsuperscript{1-5} Thus, there remains an unmet need in drug development for new approaches toward testing patient-relevant treatment models in the laboratory, before or concurrent with initiation of clinical trials. The present preclinical evaluation process for new anticancer agents, based predominantly on human cancer cell lines or cell line-based xenograft models, has proven largely ineffective at predicting therapeutic potential in patients.\textsuperscript{6,7} Despite promising preclinical results, only 5\% of cancer drugs under development are eventually approved for use. Most fail because of lack of efficacy in phase III clinical trials, as exemplified by recent trials for non–small-cell lung cancer (NSCLC).\textsuperscript{8} Chief among the many obstacles to development of effective anticancer regimens is the complexity of tumor signal transduction networks, with parallel pathways, crosstalk, compensatory feedback mechanisms, and extensive interactions between tumor cells and their microenvironment, which cannot be replicated in conventional preclinical models taken in isolation. Adding to these complexities are the inter- and intrapatient tumor heterogeneity characteristic of human cancers and the innate adaptability of tumor mutator phenotypes, resulting in rapid development of resistance mechanisms. Taken together, these challenges necessitate new ways of thinking regarding the role of preclinical models in cancer drug development.\textsuperscript{9}

Addressing an Unmet Need for Improving Drug Development Strategies in NSCLC

This need for improved preclinical platforms is particularly relevant to NSCLC, a worldwide health care epidemic for which most systemic therapies offer only modest benefit. For most patients with advanced NSCLC, the therapeutic decision-making process has remained largely empiric, based on factors such as historical treatment precedent, individual patient characteristics, and physician or patient preferences. Although the feasibility of selecting treatment for individual cancer patients based on tumor molecular profiles (personalized therapy or precision medicine) is already being explored in NSCLC, these efforts have been hampered considerably by tumor heterogeneity and the complexity of the underlying biologic pathways, and suboptimal preclinical models in which individualized therapeutic strategies can be tested.\textsuperscript{1,3,7} Further, improved strategies to identify and overcome mechanisms of de novo and acquired resistance to treatment are essential to increasing survival and cure rates.\textsuperscript{10-13}

Already, transgenic or knock-in preclinical systems such as genetically engineered mouse models (GEMMs) have proven to be invaluable tools for understanding carcinogenesis, tumor biology, and target validation for therapeutics in ways not addressable with other modeling approaches.\textsuperscript{14-18} Nevertheless,
Abbreviations: PDX = patient-derived xenograft; PT = patient tumor.

GEMMs represent nonhuman cancers lacking the range of tumor heterogeneity and complexity of biologic pathways inherent to human cancers and present within patient-derived xenografts (PDXs). Although preliminary studies have already demonstrated that chemotherapeutic agents and biologic therapies can be administered and assessed in a systematic fashion using in vivo xenografts, PDXs can also be used to evaluate the activity of new chemotherapeutic agents and biologic therapies that chemotherapeutic agents and biologic therapies can be administered and assessed in a systematic fashion using in vivo xenografts. PDXs can also be used to evaluate the activity of new chemotherapeutic agents and biologic therapies that may have the potential to target specific subtypes of cancer cells. This NSCLC PDX research platform integrates core components of The Jackson Laboratory (JAX) National Cancer Institute (NCI)-designated Basic Science Cancer Center and the JAX In Vivo Pharmacology and Clinical Lab Services together with laboratory resources and the clinical research program of the NCI-designated University of California Davis Comprehensive Cancer Center (UCD). The platform is designed to dynamically engage external institutions, investigators, additional organizations, and pharmaceutical partners as appropriate on project-specific bases. Altogether, > 25 medical centers and other partners have participated in the Primary Human Tumor Consortium. Since the launching of the program in 2009, > 1700 tumor specimens from individual cancer patients, including > 200 lung tumors, have been xenotransplanted into immunodeficient NOD scid gamma (NSG; NOD.Cg-Pkdcscid Il2rgtm1Wjl/SzJ) mice. Information and data about the PDX models are publicly accessible from the PDX portal at the Mouse Tumor Biology database (MTB; http://tumorinformatics.jax.org/mthbi/PDXsearch.do).24

The Jackson Laboratory NSG Model for PDX

The JAX NSG model is of particular interest for PDX development. Lacking functional B and T cells and natural killer cell activity, the NSG mouse is the most immunodeficient yet physiologically durable murine model available for consistent xenografting of human primary tumors.25 These attributes of the NSG mouse model facilitate engraftment of a wide variety of human cancers, with excellent correlation of histomorphological and molecular features between PDX tumors and the original human cancers as described herein. Ongoing studies at JAX are focusing on optimization of the engraftment algorithm, such as use of small biopsy samples acquired using core needle biopsy (minimum of 1 mm3 of tissue) and “transportability” of viable patient tumor specimens from other institutions. For example, engraftment rates appear similar between specimens acquired locally in Sacramento versus those express-shipped overnight from sites across the country, overall exceeding 40% for NSCLC specimens from advanced-stage disease.

Integrative Clinical and Genomic Annotation of Patient Tumor and Corresponding PDX

The algorithm for PDX creation and analysis after patient tumor biopsy is shown in Figure 1. In a synchronized evaluation process, patient tumors (PTs) and subsequent PDXs undergo histomorphologic assessment by a single reference pathologist, biomarker testing at the Clinical Laboratory Improvement Amendments (CLIA) laboratory at Response Genetics, Inc, and genomic analysis using multiple platforms including gene expression arrays and next-generation sequencing. PDXs of interest, based on molecular subtyping (eg, EGFR-mutated, Kirsten rat sarcoma or KRAS-mutated, squamous, etc) are then grouped into panels for subsequent drug testing. Figure 2 demonstrates how a panel (eg, EGFR-mutated) is subgrouped according to mutation type, resistance mechanism (if known), and clinical annotation (sensitive to EGFR tyrosine kinase inhibitor, de novo resistance, or acquired resistance). Patient clinical annotation is tracked (case example as shown in Figure 3) to account for all pertinent patient demographic characteristics, including smoking status, treatment records, and timing of PDX.

Development of the Integrated University of California Davis Comprehensive Cancer Center-The Jackson Laboratory PDX Resource in NSCLC

Herein we describe development of a clinically and genomically annotated PDX resource in NSCLC and initial pilot projects that focus on epidermal growth factor receptor (EGFR) pathways and EGFR-directed therapies, to directly address and overcome these limitations. This NSCLC PDX research platform integrates core components of The Jackson Laboratory (JAX) National Cancer Institute (NCI)-designated Basic Science Cancer Center and the JAX In Vivo Pharmacology and Clinical Lab Services together with laboratory resources and the clinical research program of the NCI-designated University of California Davis Comprehensive Cancer Center (UCD). The platform is designed to dynamically engage external institutions, investigators, additional organizations, and pharmaceutical partners as appropriate on project-specific bases. Altogether, > 25 medical centers and other partners have participated in the Primary Human Tumor Consortium. Since the launching of the program in 2009, > 1700 tumor specimens from individual cancer patients, including > 200 lung tumors, have been xenotransplanted into immunodeficient NOD scid gamma (NSG; NOD.Cg-Pkdcscid Il2rgtm1Wjl/SzJ) mice. Information and data about the PDX models are publicly accessible from the PDX portal at the Mouse Tumor Biology database (MTB; http://tumorinformatics.jax.org/mthbi/PDXsearch.do).24

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creation. Mutational fidelity is demonstrated in Table 1 for a representative group of PTs and PDXs characterized for EGFR and KRAS mutations and anaplastic lymphoma kinase gene rearrangements in a CLIA laboratory environment (Response Genetics, Inc).25,26

Drug Testing Paradigm

A variety of projects are ongoing to test PDX panels of interest for new drug treatment strategies, and to determine mechanisms of resistance and how to overcome them. A combination of tumor growth inhibition studies and real-time tumor pharmacodynamics are incorporated into each drug testing project (Figure 4). An advantage of this testing paradigm in these is that degrees of responsiveness can be quantitatively measured to provide a more nuanced assessment of the pharmacologic effect of any drug or drug combination. Moreover, the availability of an annotated panel of xenografts for a particular genetic mutation or histology should increase the likelihood of discovery of new genomic signatures of drug sensitivity and resistance. The use of these models in coclinical trials is of particular interest.

An ongoing pilot study to investigate mechanisms of acquired drug resistance in EGFR-mutated lung cancer, for example, is using a panel of EGFR-mutated PDXs to investigate a drug regimen of afatinib with or without cetuximab, using erlotinib and vehicle as controls. Results in 2 PDX models, one in which the host patient proved to subsequently be responsive to the afatinib-cetuximab combination and the other patient was unresponsive, mimicked the clinical outcomes, as shown in Figure 5. Associated tumor pharmacodynamics (Figure 6) illustrates abrogation of multiple signal transduction pathways globally mediated by maximal EGFR inhibition in the sensitive model (LG0703), in contrast to incomplete EGFR inhibition coupled with compensatory upregulation of survival pathways in the resistant model (LG1049). These data suggest that PDX models can recapitulate drug treatment outcomes in patients from whom the PDXs were derived, and that investigations can be designed to increase understanding of underlying biologic pathways and to devise strategies to improve effectiveness of therapy. This hypothesis will be tested in a future Southwestern Oncology Group phase II/III clinical trial (S1403) that will compare afatinib with or without cetuximab in the first-line therapy of patients with EGFR-mutated lung cancer, in which selected patients will undergo repeat tumor biopsy at the time of progressive disease, for genomic assessment and for PDX creation and drug testing.

Summary

The integrated preclinical-clinical modeling strategy described herein, that uses a large annotated NSG mouse resource of PDX
models, provides a novel research tool for drug development and for understanding the genomic complexity of lung cancer. Prospective development of PDXs from mutation-specific cohorts of patients treated in a homogeneous fashion, such as the planned PDX project associated with the S1403 clinical trial, should provide a unique resource for future study.

It is anticipated that availability of such an annotated resource will assist in optimization of therapeutic strategies and lead to improved patient outcomes.

**Acknowledgments**

Research reported in this publication was partially supported by the NCI award to UCD (P30CA034196) by JAX Director’s Innovation Fund, the Addario Lung Cancer Foundation, and the HOPE Foundation. The MTB database is supported by the National Institutes of Health (CA089713).

**Disclosure**

The authors have stated that they have no conflicts of interest.

Abbreviations: ANOVA = Analysis of Variance; p.o. = orally; q.d. = once per day.
Figure 6 Comparison of Tumor Pharmacodynamic Effects After Afatinib/Cetuximab in Two Erlotinib-Resistant Patient-Derived Xenograft (PDX) Models LG0703 (T790M-Negative) and LG1049 (T790M-Positive). (A) LG0703 Untreated, Revealing Intense Signaling Through ERK and p38. (B) LG0703 After Treatment With Afatinib/Cetuximab for 24 Hours, Showing Substantially Diminished MAPK and PI3K-AKT Signaling Concurrent With Loss of Epidermal Growth Factor Receptor (EGFR) Phosphorylation. (C) LG1049 Untreated, Showing Enhanced AKT Activity With Moderate ERK Activity at Baseline. (D) LG1049 After Treatment With Afatinib/Cetuximab Showing Minimal Diminishment of EGFR Phosphorylation and Signal Transduction, With Compensatory Upregulation of p38. Phosphorylation States of Receptors and Signaling Intermediaries Were Measured Using A Combination of immunoblot and Kinase Arrays (Data Not Shown, Publication In Preparation)

Abbreviations: ERK = extracellular signal-regulated kinase; MAPK = Mitogen-activated protein kinase; mTor = mammalian target of rapamycin; Mut = mutant; PI3K = phosphatidylinositol-3-kinase.

References

Tumor Genomics to Patient Outcomes Through PDX Platform


