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Algorithm for Codevelopment of New Drug-Predictive Biomarker Combinations: Accounting for Inter- and Inpatient Tumor Heterogeneity

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Background

Personalized cancer therapy, based on molecular profiling of each patient's cancer, is increasingly viewed as likely to increase the overall effectiveness of cancer treatment and to do so in both a clinically meaningful and cost-effective manner by sparing patients who are unlikely to benefit from the costs and adverse effects of ineffective therapies.^{1–3} Thus, in the emerging era of new anticancer agents directed against molecular targets present in only a small subset of patients within a general population, such as non-small-cell lung cancer (NSCLC), it is increasingly important to consider simultaneous and early codevelopment of an associated predictive biomarker. To emphasize this point, one need only recall the poor track record of phase III randomized controlled clinical trials (RCT) of chemotherapy with or without a so-called targeted agent in advanced NSCLC (Table 1), only 2 trials met the criterion gold standard of success, improved survival, regardless of the drug class or molecular target. Importantly, none of these trials incorporated prospective evaluation of a potential predictive biomarker for the new agent being studied, with the sole exception of basic epidermal growth factor receptor (EGFR) protein expression in FLEX.⁴ In retrospect, it now seems overly naive to have thought that these targeted therapies would lead to clinical benefit in the overall population of patients with NSCLC in view of what we now know

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Disclosure

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about the tremendous degree of inter-patient tumor heterogeneity that exists within the umbrella diagnosis of NSCLC, arguably a collection of biologically and molecularly distinct malignancies. This point is emphasized by recent literature of a variety of molecularly defined and drug treatment-specific patient subsets of adenocarcinoma, such as those that harbor activating mutations of EGFR or anaplastic lymphoma kinase (ALK) fusion proteins.⁵⁻⁹ Further, genome-wide profiling of individual patient tumors now offers the possibility of identifying complex mechanisms of tumor proliferation and/or drug resistance, which are actionable by currently available or developing targeted therapies.^{2,10-12} Recent data from the Lung Cancer Mutation Consortium, for example, demonstrated “actionable” abnormalities in 54% of lung adenocarcinomas.¹³ It appears that clinical trial designs enriched for the target population by a predictive biomarker or statistically powered to prospectively evaluate a biomarker as a coprimary or secondary endpoint will most likely demonstrate patient subgroups that benefit. A classic example in support of this concept is provided by the successful biomarker-driven pivotal trial that evaluated chemotherapy with or without trastuzumab in breast cancer, in which mathematical modeling clearly demonstrated that an unselected RCT design would have failed to identify the benefit of this important anti-cancer agent.¹⁴

Transition From Unselected RCTs to Biomarker-Driven Trials

At a minimum, required components for the transition from unselected RCTs to biomarker-driven clinical trials consist of (1) adequate patient tumor tissue for molecular profiling in every patient, (2) early codevelopment of both the new drug and the associated biomarker, and (3) recognition of inter- and inpatient tumor heterogeneity with populations of patients with NSCLC.

In dissecting these 3 components, the first point regarding availability of tumor tissue is becoming more and more obvious: NSCLC, even patients who present in advanced stage, must be thought of in a similar fashion to breast cancer, in which an adequate tumor specimen is a prerequisite for therapeutic decision making. Practically, this means that limited sampling from fine needle aspiration will prove inadequate for molecular testing in a substantial proportion of patients. Next, codevelopment of a new anticancer agent and an associated predictive biomarker requires a change in perspective from that of the classic drug development paradigm, in which an associated biomarker, if any, arises relatively late in the process. A case can be made for viewing the steps associated with biomarker development in a similar fashion to that of the new drug itself (Figure 1), moving from initial target discovery and assay development at the preclinical or early clinical phase through sequential steps to eventual clinical validation of the biomarker during the course of a phase III trial. This strategy requires that improved preclinical models of cancer be integrated into the drug-biomarker development process, because the classic in vitro cell line and accompanying in vivo models have proven to be relatively ineffective in predicting subsequent clinical activity or even refining biomarker-target interactions. Here, use of patient-derived xenografts (PDX), which reflect the complex biology of the human host cancers from which they originated, may provide an advantage and may supplement data available from genetically engineered mouse models (GEMM). Already, efforts to explore this potential in NSCLC are underway, as discussed in greater detail below.¹⁵

Algorithm for Selection of Biomarker-Driven Clinical Trial Strategy

Regardless, the process described here for early “marriage” of new drug development to biomarker development should facilitate subsequent phase III clinical trial designs by using one of a variety of biomarker-driven strategies (Table 2).^{16–18} Alternatives presented here include an all-comers design with a secondary biomarker endpoint, a targeted design in which only patients who are marker positive are enrolled, and a series of hybrid designs, which specify multiple hypotheses as coprimary endpoints, with the type 1 error split between the hypotheses. A current example of the subgroup-focused hybrid design is the ongoing SWOG (Southwest Oncology Group) trial S0819, which is comparing chemotherapy with or without the EGFR-directed monoclonal antibody cetuximab, in which the EGFR gene copy number by fluorescence in situ hybridization represents a coprimary endpoint, whereas protein expression by H score is the secondary endpoint. These designs are differentiated by whether they specify a nested hypothesis in the entire population and the subgroup (such as overall population and subgroup-focused designs) or different hypotheses for the subgroups (discrete hypothesis design). An overarching principle is that higher certainty about a potential biomarker leads to trial designs more strongly driven by that biomarker and vice versa. Irrespective of which strategy is selected, taken together, these trial designs appear likely to improve the chances of successful drug development by comparison with the unselected RCT paradigm used for the past 20 years. Which biomarker trial strategy is most appropriate for a given new drug-biomarker combination will differ, dependent on the particular new drug-biomarker variables and constraints under consideration. For example, whether a biomarker-positive (targeted) design or another design is preferable for a given new drug-biomarker combination depends to a certain extent on the strength of the preliminary bio-marker data as well as the prevalence of the biomarker in the study population. A proposed algorithm for selection of phase III trial design is presented in Figure 2, which addresses both the relative strength of preliminary biomarker knowledge and cut point when applicable, as well as prevalence of the biomarker in the proposed study population, in making the determination regarding choice of trial design.¹⁹

Addressing Inter- and Intrapatient Tumor Heterogeneity

Further, to improve the probability that emerging new drug-biomarker combinations will ultimately be successful in achieving clinically meaningful benefit, future clinical trials must attempt to address tumor heterogeneity of both the interpatient (differences in tumor genomics among patients) and inpatient (differences in tumor genomics within portions of the same patient’s tumor) variety. This concept is especially pertinent to NSCLC, in which diversity in histology and molecular biology is being increasingly recognized. These concepts, in brief, as they apply to new drug development and the intrinsic heterogeneity of human cancers are illustrated in Figure 3. For example, because the classic RCT design assumes that the great majority of patients with NSCLC, as represented in Figure 3, panel 1, by patient A and patient B, are equally likely to benefit from a new targeted agent, it is not surprising that the trials listed in Table 1 were almost uniformly negative. In retrospect, it is less biologically probable that an entire study population of patients with NSCLC would derive a modest benefit from a new targeted anticancer agent but rather that a smaller subset of patients, preferably defined as a marker-positive group, would derive a quantitatively

larger benefit. In contrast, marker-negative populations might be postulated to fall into 1 of 3 categories: derive no benefit but also no harm (a neutral effect), a modest benefit (but less than the marker-positive group), or even a negative effect from the therapy under study. Dependent on the magnitude of benefit anticipated from a new therapy and prevalence of the biomarker-defined patient subset, mathematical modeling can postulate how likely a given trial design would prove successful.

In moving forward, it is important to recognize that, not only must clinical trials account for interpatient tumor heterogeneity (Figure 3, panel 2), but they must also account for inpatient tumor heterogeneity (Figure 3, panel 3). In particular, the concept of inpatient tumor heterogeneity is fundamental to improving clinical trial designs for new anticancer agents directed against acquired resistance mechanisms. Here, recent concepts in evolutionary biology are particularly compelling. For example, mathematical modeling of inpatient tumor heterogeneity, emergent over time, proposes various patterns for development of secondary “driver” and “passenger” genetic alterations, all of which may be demonstrated at the time of acquired resistance to a targeted therapy (Figure 3, panel 3, scenario 3).²⁰ Sorting out which of these emergent molecular abnormalities is a “driver” (such as an oncogene driving cell proliferation) and which is merely a “passenger” (coexistent but not altering clinical course) is daunting to say the least. Nevertheless, early and accurate identification of genotypic evolution, such as emergence of secondary mutations, will be a key to development of successful new agents directed toward these resistance mechanisms.²¹ Current examples in the targeted therapy of NSCLC include T790M mutations in EGFR mutant cancers and secondary mutations in patients with ALK-positive cancers. As described above, use of GEMM or PDX models characterized for EGFR mutation or ALK fusion variants offers potential for advancing knowledge of complex biologic systems inherent to human NSCLC in ways that cannot be duplicated in patients themselves. To illustrate this point, such models can be treated simultaneously with several different combinations of resistance-modulating therapies, all highly characterized for relevant molecular pathways, followed by next-generation sequencing interrogation of posttherapeutic changes. Such an algorithm may prove advantageous in deciphering mechanisms of acquired drug resistance that are emergent over time. An ongoing collaborative research platform, iGXT (integrated GEMM-PDX-Clinical Trials), is exploring this potential. The workflow for this project, demonstrated in Figure 4, is directed toward development of NSCLC PDXs by using the JAX–nod scid gamma mouse model, each having undergone genomic profiling. Examples related to EGFR mutation status are shown here. In turn, each of these models is also highly annotated for clinical characteristics regarding the patients from whom they were derived. Optimally, PDX models are developed both from patient biopsy at the time of diagnosis and again from rebiopsy at the time of progressive disease to study tumor genomic evolution over time and under the influence of therapy.

Unfortunately, no currently available biomarker-driven trial designs, including those described Table 2, adequately address this issue of treatment-emergent inpatient tumor heterogeneity, which is projected to develop during the course of clinical trial therapy but which is commonly unmeasurable at baseline. An appropriate trial design for this purpose

would need to accommodate cross-sectional and longitudinal measures, a goal not easily accomplished within the usual constraints of clinical trial design.

Summary

In summary, we present here a perspective on approaches to improving the success rate of new drug-predictive biomarker combinations, based on early and simultaneous biomarker development, transition to biomarker-driven Phase III trials, and recognition of the therapeutic implications of inter- and intra-patient heterogeneity. While this strategy poses multiple challenges, it also presents unique opportunities for improving treatment options for patients with NSCLC, and other cancers as well.

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Figure 1.
Improved Drug-Biomarker Development Paradigms: “Marriage” of Drug-Biomarker Development

Figure 2.

(A) Algorithm for Biomarker-Driven Trial Designs of Targeted Therapies: Marker Strength.

(B) Algorithm for Biomarker-Driven Trial Designs of Targeted Therapies: Marker Prevalence

Figure 3.
Models for Inter- and Intrapatient Tumor Heterogeneity

Figure 4. Development of Patient-Derived Xenografts (PDX) Platform for Drug Testing in JAX–Nod Scid Gamma Models: Epidermal Growth Factor Receptor–Driven Therapies
Abbreviations: aMT= activating mutation; CNV = copy number variation; MT = mutation.

Table 1

Classic Randomized Controlled Trial Design (Unselected): Recent Phase III Trials of Chemotherapy With and Without a Targeted Agent^a in First-Line Advanced Stage NSCLC

Target	Agent	Survival Benefit
MMP	Prinomastat, others	No
EGFR TKI	Gefitinib or erlotinib	No
Farnesyl Transferase (RAS)	Lonafarnib	No
PKC α	ISIS 3521	No
RXR	Bexarotene	No
VEGFR (TKI)	Sorafenib	No
VEGF (MoAb)	Bevacizumab	Yes
EGFR (MoAb)	Panitumumab	No
TLR9 Agonist	PF-351	No
EGFR (MoAb)	Cetuximab	Yes ^b
IGF-1-R	Figitumumab	No
VDA	ASA-404	No

Abbreviations: EGFR = epidermal growth factor receptor; IGF-1-R = insulin growth factor-1 receptor; MoAb = monoclonal antibody; MMP = matrix metalloproteinase; NSCLC = non-small-cell lung cancer; PKC α = protein kinase C; RXR = retinoid X receptor; TKI = tyrosine kinase inhibitor; TLR9 = toll-like receptor 9; VDA = vascular disrupting agent; VEGFR = vascular endothelial growth factor receptor.

^a In combination with platinum-based chemotherapy vs. chemotherapy.

^b EGFR immunohistochemistry positive.

Table 2

Selected Biomarker-Driven Clinical Trial Designs of Targeted Therapies

Design	Description	Pros	Cons
All-Comers Design (With Biomarker-Driven Secondary Objectives)	Unselected study population	Tests entire study population, fewer barriers to accrual	No prospective validation of treatment in marker-defined subgroups
Overall Population-Focused Design	Enroll all-comers with coprimary hypothesis in overall population and subgroup; type I error split with majority given to overall group	Prospective validation of treatment in subgroup and overall population; subgroup could be identified during the course of the trial	Subgroup analysis is typically underpowered so that a null result could likely be a false negative
Subgroup Population-Focused Design	Enroll all-comers with coprimary hypothesis in overall population and subgroup; type I error split, with the majority given to the subgroup	Prospective validation of treatment in a subgroup and overall population	Needs prospective identification of a subgroup, assessment in the entire population may have some power loss
Targeted Design	Enroll only marker-positive patients	Prospective validation of treatment in marker-positive subgroup only	No evaluation of treatment in marker-negative group
Discrete Hypothesis Design	Enroll all-comers with separate hypotheses for marker-positive and marker-negative groups	All screened patients are enrolled; most proficient for testing interaction	Mandates assessment of both marker-positive and marker-negative groups