

Trends in the Use and Role of Innovative Biomarkers in Phase I

Oncology Trials

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ABSTRACT

Purpose: There has been interest in using biomarkers that aid the evaluation of new anti-cancer agents. We evaluated trends in the use of biomarkers and their contribution to the main goals of phase I trials.

Experimental Design: A systematic review of abstracts submitted to the American Society of Clinical Oncology annual meeting from 1991 to 2002. We described the use of biomarkers and provided an analysis of their contribution to published phase I trials.

Results: 22% of ASCO Phase I abstracts (541/2458) from 1991 to 2002 included biomarkers. This proportion increased over time (14% in 1991 compared to 26% in 2002, $P < .02$). Independent predictors of the use of biomarkers included NIH sponsorship, submission in the time period of 1999-2002, adult population, and drug family (biological agents). Biomarkers made substantial contributions to dose and schedule selection for phase II studies for 13 % and 8% of drugs, respectively, decisions to proceed or not with further drug development for 41% of drugs and confirmation of the mechanism of action in 54% of the trials, respectively.

Conclusions: The use of biomarkers in phase I trials has increased over the period from 1991-2002. Trials most likely to include biomarkers are those funded by the NIH, those testing biological agents, and those designed more recently. Biomarkers contributed modestly to determining a dose and schedule for phase II studies, but added moderately to the “go or no go” decision, and helped confirming that the drug modulates a target in 54% of the trials.

Introduction

Cancer biomarkers are emerging as appealing end-points for new anti-cancer agents undergoing phase I trials (1). Several factors explain the growing interest for cancer biomarkers. First, the advent of molecularly targeted drugs entering clinical trials has stimulated the use of biomarkers to correlate clinical empirical data with target modulation. Second, the advances in biotechnology have allowed the creation of accessible test modalities that measure specific tumor targets, such as special imaging studies (e.g., FDG-PET; dynamic MRI) and immunohistochemistry studies such as HER-2 protein expression; protein phosphorylation, etc (2-5). Third, the failure of cytostatic, molecularly targeted drugs to show objective response rates has motivated drug sponsors to look for molecular and biochemical evidence of target engagement during early stages of drug development (6).

The actual importance of novel biomarkers as end-points in phase I trials has not been carefully studied. Biomarkers may help identify several end-points in phase I trials. Those include the definition of a biologically active dose and schedule for phase II studies, and confirmation of mechanism of action. The use of biomarkers may also identify surrogates for patient selection and end points for drug resistance, clinical toxicity, and clinical efficacy (1). Biomarkers have served as critical determinants in trials of trastuzumab and hormonal therapies of breast cancer and in trials of imatinib mesylate (Gleevec, Novartis Pharmaceuticals East Manruer, NJ) in chronic myelogenous leukemia (7-9). Despite the successful use of biomarkers in the development of these drugs, a comprehensive analysis of the role of biomarkers as end-points in phase I trials could be informative in assessing their general value in Phase I trials.

Therefore, we conducted a systematic analysis of trends in the use of biomarkers in phase I trials. We provide a classification of different types of innovative biomarkers, an evaluation of predictors of inclusion of biomarkers in phase I trials, and an analysis of the contribution of biomarkers to the primary goals of phase I trials.

Materials and Methods

Data Acquisition. We developed a database of abstracts of phase I clinical trials submitted to the American Society of Clinical Oncology (ASCO) annual meeting from 1991 to 2002, as previously described (10). Briefly, we reviewed all the abstracts of phase I therapeutic trials and analyzed their baseline characteristics and time trends for inclusion of biomarkers. We collected data from abstracts regarding year of submission, inclusion of biomarkers, type of biomarkers, source of biomarkers, drug family, inclusion of pharmacokinetics (PK), National Institute of Health (NIH) sponsorship, industry sponsorship, trial location, and patient population (adult vs. pediatric). In order to analyze the contribution of biomarkers to phase I trials, we searched MEDLINE for published articles that met the eligibility criteria using the first and last author's names and the generic name of the subject compound as mentioned in the abstracts.

Eligibility Criteria. We considered eligible for the analysis of abstracts all the reports of phase I therapeutic trials. We excluded abstracts that reported PK or biomarker results separately, trials without therapeutic intent (e.g., chemopreventive), supportive treatment trials (e.g., Colony Stimulation factors), and trials with healthy volunteers. For the analysis of the published articles, we included only single-agent phase I trials for compounds that were not yet approved for marketing by the Food and Drug Administration (FDA) at the time of abstract submission. All trials had to describe a

systematic use of at least one biomarker and could not include radiation therapy or a phase II component in their design.

Definition of Innovative Biomarker. We defined an innovative biomarker as any biological variable, either genotypic or phenotypic, that was systematically measured by molecular, biochemical, or imaging techniques during the study and was described in details in the *Materials and Methods* topic of each article. We did not consider common markers of tumor response to treatment, such as reduction of tumor size on standard imaging tests (CT scans or MRI) or serum tumor markers (e.g., PSA, CA 19-9, CEA), as biomarkers for the purpose of our study. Common clinical end-points of toxicity, such as blood counts, were not considered biomarkers for this study, as well as pharmacokinetics. For trials that included multiple biomarkers, we analyzed the one we considered to be the most relevant in order to simplify data acquisition. **Table 1** shows a classification of the types of biomarkers involved in our study.

Source of Biomarkers. We used abstracts to determine the sources of biomarkers as serum, tumor tissue (including malignant effusions), Peripheral Blood Mononuclear Cells (PBMC), special imaging methods, normal tissue (defined by biopsies of solid organs not involved by tumors such as skin or bone marrow), cellular material not otherwise specified, cerebral spinal fluid (CSF), and non-identified sources. We classified the biomarkers according to their sources (**Table 1**).

Baseline Characteristics of Phase I trials. We determined the number and proportion of trials that included biomarker studies according to each baseline characteristic of the trials. Baseline Characteristics include *period* (1991 to 1994 vs. 1995 to 1998 vs. 1999 to 2002), *drug family* of the studied compound (cytotoxic vs. biologic

vs. target vs. other), *PK* studies (yes vs. no), *NIH sponsorship* (yes vs. no), *industry sponsorship* (yes vs. no), *trial location* (USA vs. other country), and *patient population* (adult vs. pediatric).

Predictors of Inclusion of Biomarkers in Phase I trials. We used the above baseline characteristics as explanatory variables to develop a multivariate model for prediction of the inclusion of biomarker studies in phase I trials.

Time Trends of Use of Biomarkers. For each year, we determined the proportion of trials that included biomarkers. Proportions were obtained by dividing the number of abstracts with biomarkers by the total number of phase I abstracts for each year from 1991 to 2002.

Contribution of Biomarkers to Phase I trials. After we identified eligible articles through the MEDLINE search, we analyzed the contribution of biomarkers to the following goals of phase I trials: dose selection for phase II studies, schedule selection for phase II studies, decision to proceed or not proceed with further drug development, and confirmation of the proposed mechanism of action of the drug as demonstrated, for example, by target engagement or inhibition of a pathway. The contribution of the biomarker to these goals was classified either as substantial or indeterminate/irrelevant. A substantial contribution was defined as a biomarker measurement that significantly influenced the achievement of one of the mentioned goals. An indeterminate/irrelevant contribution means that the biomarker had an unclear or irrelevant influence on the achievement of one of the mentioned goals. We performed the analysis of contribution independently for each goal (i.e., a biomarker could make a substantial contribution to more than one goal in the same article). Subsequently, we determined the proportion of

biomarkers derived from various sources (serum, tumor tissue, etc.) that made a substantial contribution to each of the goals (dose selection, etc.). For each article, we selected the one biomarker we considered to be the most relevant.

We considered that a biomarker made a substantial contribution to decisions regarding further drug development when the authors clearly indicated that the decision to proceed to phase II or III trials (“go” decisions) or not to proceed was based on the results provided by the biomarker. We also classified the contribution of biomarkers to “go” decisions either as primary (the biomarker was the primary end-point that supported the decision) or complementary (the biomarker reinforced a decision that was primarily based on other end-points).

Because our classification system is subject to investigator bias, two participants with expertise in cancer biomarkers and phase I trials (H.P., BHG) classified the contribution of biomarkers independently. Discrepant results were resolved by discussion and then tabulated.

Statistical Analysis. We used the Cochran-Armitage test for linear trend to determine the time trends for the inclusion rate of biomarkers over time. We used stepwise logistic regression for the multivariate model of predictors of inclusion of biomarkers. We considered $P < .05$ as statistically significant for both analyses. We report the Odds-Ratio of inclusion of biomarkers only for the baseline characteristics that we found to be independent predictors after the logistic regression analysis. We did not perform a formal statistical analysis of the contribution of biomarkers to phase I trials.

Results

Search results. We identified 2458 abstracts of phase I clinical trials eligible for analysis. Of these, 22% (541/2458) included a biomarker. Our MEDLINE search identified 87 published articles based on these abstracts (**Figure 1**).

Types and sources of biomarkers. **Table 1** shows a description of the biomarkers and their relative frequency within each source in 541 abstracts. Serum markers were the most common source of biomarkers [185/541 (34.2%)], followed by tumor tissue [129/541 (23.8%)], PBMC [114/541 (21%)], and imaging studies [55/541 (10.2%)]. Biomarkers measured in other normal tissues were described in only 20 abstracts (3.7%), with histologic analysis, immunologic studies (skin biopsies for local immunization reactions), and enzyme activity measurements representing the most common biomarkers of this category. Cellular material not otherwise specified (i.e., either malignant or benign cells, or PBMC) comprised 17 (3.1%) abstracts. Twenty-eight abstracts reported biomarkers from non-identified sources.

Overall, innovative biomarkers composed a highly heterogeneous group. Of the 541 biomarkers (one for each abstract), the most common were immunologic studies [88/541 (16.2%)], and included tests of the responses of different components of the immune system to a therapeutic intervention (**Table 1** – footnote). Measurement of an enzyme activity was the second most prevalent type of biomarker [47/541 (8.7%)]. The remaining biomarkers were classified among several different and less frequent types. Fifty-five different types of biomarkers were adopted in less than 1% of the abstracts each (**table 1** – footnote).

Baseline Characteristics of the Trials. The largest number of the trials [1048/2458 (43%)] belong to the last period of the study (1999-2002). Thirty-one percent (760/2458) were submitted in the second period (1995-1998) and 26% (650/2458) reported in the first period (1991-1994). Most of the abstracts [1472/2458 (60%)] report use of cytotoxic agents (i.e., drugs that act by affecting the DNA or its synthesis); 16% (398/2458) were molecularly targeted agents (small molecules), and 15% (383/2458) were biologic agents (obtained by recombinant technology). Forty-eight percent (1169/2458) of the trials had PK studies. NIH sponsored 13% (326/2458) of the trials, while industry sponsorship accounted for 44% (1080/2458). Information about sponsorship for the remaining 1052 abstracts was not available. The United States represented the trial location for 64% (1575/2458) of the studies. Only 3% (71/2458) of the trials included pediatric patients. The number and proportion of trials that used biomarkers within each of the baseline categories of trials is shown in **table 2**.

Predictors of inclusion of biomarkers. A multivariate model identified 4 baseline characteristics that independently predict for the use of biomarkers: *drug family* (biologic vs. cytotoxic), *study population* (adult vs. pediatric), *NIH sponsorship* (yes vs. no), and *period* (1999-2002 vs. 1991-1994). Of these, the strongest determinant was drug family (Odds-Ratio [OR] = 17.0; Confidence Interval [CI] = 13.0 – 25.0). The overall concordance of the multivariate model was 87.4%. The respective Odds-Ratio for each predictor is shown in **table 2**.

Time trends for inclusion of biomarkers. The proportion of trials that included biomarkers has significantly increased over the 11 years of this study, as shown in **figure 2**. In 1991, 14% of the abstracts reported the inclusion of biomarkers, as compared to

26% of the abstracts reported in 2002 ($P < .02$ for the trend). The time trend remains significantly positive even if we switch the time unit from years to periods (**table 2**).

Contribution of biomarkers to the goals of phase I trials. We reviewed 87 published articles of phase I trials that used biomarkers. Fifty-one (59%) trials evaluated biologic (recombinant) agents, 26 (30%) studied targeted (small molecules) drugs, and 10 (11%) tested cytotoxic drugs. The contribution of biomarkers to the goals of these trials is summarized in **table 3**. Overall, biomarkers made a substantial contribution for dose selection for phase II studies in 13% of the trials, i.e., in 13% of the trials the information provided by the biomarker influenced the choice of the dose for a phase II study. For schedule selection for phase II studies, biomarkers made a substantial contribution in 9% of the trials. However, biomarkers made a substantial contribution to the decision whether to proceed or not proceed with drug development in 41% of the trials and, in 54% of the trials, biomarkers confirmed the proposed mechanism of action of the drug. In 54 trials (62%), biomarkers made a substantial contribution to at least one of the goals.

The contribution of biomarkers to the goals of phase I trials varied according to the source of biomarkers (**Table 3**). The proportion of substantial contribution of biomarkers to dose selection for subsequent phase II studies ranged from 0% (studies of PBMC, urine, normal solid tissue, and imaging biomarkers) to 28% (tumor tissue biomarkers). For schedule selection, PBMC was the source of biomarkers with the highest proportion of substantial contribution (12%), followed by serum biomarkers (11%), and tumor tissue tests (5%). Imaging and tumor tissue biomarkers had the highest proportions of substantial contribution to a decision to proceed or not with further drug development (67% and 53%, respectively). Studies of tumor tissue made the most

important proportion of substantial contribution to confirmation of mechanisms of action (95%), followed by studies of PBMC (53%), normal tissues (50%), imaging studies (43%), and serum biomarkers (39%). The one biomarker studied in urine did not contribute to any of the goals (urinary levels of angiogenic factors). The four trials represented in the normal tissue category assessed biomarkers in normal bone marrows (2 trials of solid tumors) and skin (2 trials).

Tumor tissue represented the source of biomarkers with the highest utility, as reflected by the 100% (19/19) rate of contribution to at least one of the goals of the phase I trial. The remaining sources of biomarkers showed substantial contributions to at least one goal in 46% to 75% of the trials (**table 3**).

Imaging biomarkers and tumor tissue biomarkers supported a decision to proceed to phase II in 6 and 10 trials, respectively (“go” decisions). A tumor tissue biomarker (recombinant gene transfection from plasmid in liver metastasis) was the primary end-point for the “go” decision in only one trial of gene therapy (11). The remaining 9 tumor tissue biomarkers that contributed to decision did so by complementing a decision already based on another end-point (tolerability or clinical responses). None of the 87 biomarkers substantiated a decision to interrupt drug development.

Biomarkers had a higher rate of substantial contribution to decisions among trials of agents that received approval by the FDA after the publication of the phase I study compared to never-approved agents (**table 4**). Of 12 trials of agents that became approved in subsequent years, biomarkers contributed to dose selection in 3 trials (25%), to schedule selection in 1 trial (8%), to “go-no go” decisions in 9 trials (75%), and to confirmation of the mechanism of action in 5 trials (42%). Again, all the 9 contributions

to decisions supported a “go” decision. In one trial of Bortezomib (Velcade, Millennium Pharmaceuticals, Cambridge, MA), the biomarker (inhibition of subunit 20S of proteasome in cell lysates) was the primary end-point that substantiated the decision (12). In the remaining 8 trials, the biomarkers complemented a decision supported by response rates and/or tolerability of the drug.

Studies of 18 (95%) tumor tissue biomarkers proved engagement of target or inhibition of a targeted pathway. These end points were identified by selective antibody binding to tumor targets (2 studies), enzyme inhibition (6 studies), recombinant gene transfection from plasmids (5 studies), wild type P53 protein expression after gene transfection (1 study), in vitro tumor cell lyses after exposure to the drug (tumor cells were obtained from patients enrolled in 2 studies), intracellular detection of oncolytic adenovirus (by electronic microscopy; 1 study), and inhibition of DNA methylation (1 study).

Discussion

In phase clinical I trials, biomarkers are appealing for their potential to serve as a surrogate for drug-efficacy, toxicity, and to disclose effects on molecular targets. Thus, the use of biomarkers in early trials may help predict the likelihood of success or failure of drug development. Although appealing, the value of biomarkers in phase I trials is poorly understood. Equally unknown is whether biomarkers actually help investigators to achieve the main goals of phase I trials, which include the selection of dose and schedule for phase II studies, decision about future studies, and estimates of safety and efficacy. Our systematic review included a representative sample of phase I trials of cytotoxic, biologic, and targeted agents that adopted novel biomarkers.

Our study has limitations. First, our definition of innovative biomarkers was broad and included a highly heterogeneous group of biologic variables. However, we evaluated the contribution of biomarkers to phase I trials in a selected and more homogeneous sample of published articles. Second, our analysis of contribution of biomarkers to phase I trials depended on subjective interpretation of articles. We attempted to reduce the subjectivity of this analysis by having two independent investigators review the data and accepted their interpretation only when they had concordant opinions. Third, we did not attempt to include biomarkers used to select a patient population more prone to respond to therapy, as the benefit of these biomarkers can only be assessed in prospective studies that compare the outcomes of populations with and without the expression of a given tumor target. Fourth, the results of this study should be interpreted with caution because of the retrospective design. Nevertheless, these findings make several important points.

The finding that phase I trials of biologic agents (e.g., interferon [IFN]- α , interleukin [IL] 2) were more likely to include a new biomarker compared to trials of cytotoxic agents is somewhat expected. The optimal dose for biological and target agents may be lower than the Maximum Tolerated Dose (MTD), making toxicity a questionable end-point for dose escalation (13). Thus, investigators may adopt biomarkers as alternative end-points to establish a dose for phase II studies of biological agents. Alternatively, new biomarkers can potentially elucidate the complex mechanisms of action of biologic drugs by showing the modulation of a specific molecular target. For example, IL-2 trials have adopted T-cell CD markers in order to evaluate cellular immune responses triggered by treatment (14). In a phase I trial of ISIS 5132, an antisense

oligonucleotide inhibitor, investigators evaluated the suppression of its target, the *c-raf-1* mRNA, as the possible mechanism of action of the compound (15).

NIH-sponsored trials frequently employed biomarkers. The National Cancer Institute (NCI) has been supporting the development of novel biomarkers that are useful as surrogates of clinical efficacy in clinical trials (16). In addition, both governmental and industry sponsors concern themselves about the fact that 50% of the experimental drugs that fail do so in phase III trials (reference available at the Food and Drug Administration web site⁷). Therefore, it is not surprising that the NCI favors the sponsorship of phase I trials that incorporate biomarkers that could help predict early success or failure of novel agents. The fact that industry sponsorship was not associated with inclusion of biomarkers with the same frequency is intriguing. Possibly, industry has started to sponsor biomarker research more recently, and our study may not have detected this trend. Alternatively, 51% (551/1080) of the industry-sponsored trials tested cytotoxic agents, which could partially explain the lack of association between industry sponsorship and use of biomarkers. However, 45% (149/326) of the NCI-sponsored trials were of cytotoxic agents, a difference that is unlikely to explain the findings. Despite the growing interest in biomarker research, there is no evidence showing that the use of biomarkers in clinical trials has increased the number of approved drugs per year.

The number of phase I trials that included biomarkers has steadily increased over the course of the last decade. Trials published in a later period (1999-2002) included biomarkers more frequently than trials published in the first period (1991-1994). These findings probably reflect the advent of biologic and target therapies, better understanding of tumor biology, and development of new technology to access tumor targets. They also

⁷ Internet address: <http://www.fda.gov/oc/speeches/2004/bascrty0707.html>

suggest that the design of clinical trials has evolved from solely based on empirical clinical observations to designs based on both clinical and biological endpoints.

The finding that serum constituted the most common source of biomarkers suggests that easy accessibility is an important factor in determining the value of a biomarker. Tumor tissue was the second leading source of biomarkers, suggesting that reliability in the assessment of tumor markers is also an important factor for the conception of biomarkers. Normal tissue biopsies (e.g., skin, bone marrow, etc.) also represent a convenient source of biomarkers, because they may include sites that are more assessable than tumors and reflect effects of drugs on molecular targets and pathways (17). The fact that normal tissue biopsies accounted for only 3.7% of the sources of biomarkers suggests that normal tissues still need to be validated as surrogates for tumor biopsies in future trials. Targets known to be expressed in both normal and tumor tissue might hold greater chances of guiding drug development. The contributions made to “go” decisions and mechanisms of action by the evaluation of EGFR activity in skin in phase I trials of Erlotinib and Gefitinib constitute examples of successful normal tissue biomarkers (18, 19).

Our dataset of published articles used to analyze the contribution of biomarkers (87 trials) contains mainly trials of biologic (59%) and targeted (30%) agents. Analysis of these trials displays the same association between trials of biologics and inclusion of biomarkers as seen in the larger set of phase I abstracts. Therefore, our sample of published trials confirms that the major motivations to develop markers arise in trials of molecularly targeted drugs and biologic agents.

Toxicity and pharmacokinetics remain the main end-points for dose and schedule selection for phase II studies. Our analysis of published articles revealed that biomarkers contributed to dose and schedule selection in only 13% and 8% of the phase I trials, respectively. This finding is in agreement with a retrospective analysis of 65 phase I trials, where toxicity and pharmacokinetics were the primary basis for dose recommendation in 67% and 21% of the trials, respectively (20). In our study, the reported reasons why biomarkers infrequently affected dose selection include the unclear relationship of dose to biomarker, the difficulty of making a quantitative assessment of the biomarker, and significant inter-patient and intra-patient variability in the results of biomarkers. Another possible reason is the older paradigm that the highest dose tolerated should be used, regardless of the availability of adequate new biomarkers (21).

Biomarkers made substantial contributions to a “go or no go” decision in a moderate fraction of phase I trials (41%). Moreover, biomarkers more often corroborated a decision to proceed with a phase II study rather than interrupt drug development. In all trials to which imaging studies or tumor tissue biomarkers contributed to the decision (6 and 10 trials, respectively), a phase II or III was recommended or initiated. In all but one of these trials, biomarkers reinforced a decision that was primarily based on another end-point (usually tolerability or objective responses). These findings suggest that biomarkers will more often complement the conclusions driven by more traditional end-points (such as tolerability, pharmacokinetics, and tumor responses) rather than serve as the primary basis for decisions. So far, we have no evidence that innovative biomarkers contribute to decisions to interrupt drug development after a phase I trial.

Biomarkers apparently supported “go” decisions in trials of biologic and targeted agents that received further approval by the FDA more frequently than in trials of unapproved agents (75% vs. 37% of the trials, respectively). However, in only one of the 12 trials of recently approved agents did the biomarker constitute the primary end-point for decision (Bortezomib trial). The contribution of biomarkers to dose and schedule selection, and to confirmation of mechanisms of action in these trials do not differ dramatically from the contribution of biomarkers to trials of unapproved agents. These findings suggest, therefore, that the use of biomarkers in the early trials of effective targeted and biologic agents provides only modest support to advance the drugs in their clinical development. It seems unlikely that, in the absence of the studied biomarkers, these drugs would not go to phase III trials and ultimately receive approval by the FDA.

The most prominent contribution of biomarkers to phase I trials was their ability to confirm the proposed mechanisms of action (binding to a target or modulation of a pathway) of the studied compound (54% of the trials). Yet, the contribution of biomarkers in this setting ranged substantially from 38% (serum biomarkers) to 95% (tumor tissue biomarkers) if we exclude urine as a source (1 trial only). This variability may be due to the heterogeneity of biomarkers included in this analysis, different purposes for the adopted biomarkers and differences in the adequacy of biomarkers. For example, one could expect that tumor tissue biomarkers represent more adequate tumor targets than serum markers, which usually measure serum metabolites originated from intracellular enzyme reactions. The latter hypothesis may partially explain the higher rate of confirmation of mechanisms of action of tumor tissue biomarkers as compared to the other sources of biomarkers. However, the importance of confirming mechanisms of

action for clinical research is not entirely clear. Most if not all of the biomarkers in our study were not prospectively validated as surrogate end-points of clinical outcomes such as overall survival or time to tumor progression. In order to help investigators predict early success or failures of new agents, biomarkers need prospective validation in large clinical trials (22, 23).

In summary, innovative biomarkers compose a heterogeneous group of new tests that have been incorporated into clinical trials with increasing frequency over the 1991-2002 period. In this period, NIH sponsored trials, trials testing biologic agents, and trials in adult populations were most likely to use biomarkers. Biomarkers contributed modestly to dose and schedule selection for phase II studies, but had greater impact on complementing the decisions to proceed with further trials and in confirming the mechanisms of action of novel agents. In most trials to which biomarkers substantiated a “go” decision, they did so by supporting a decision already based on another end-point such as tolerability or clinical responses. In no case did biomarkers become the basis for the interruption of drug’s development. The importance of confirming mechanisms of action is still unclear and will depend on prospective validation of biomarkers. The concept of phase I trials is changing from purely based on clinical observations to a new model of biologically correlated clinical research. We hope that refinements in the use of biomarkers will enable investigators to better predict the chances of success or failure in the early stages of drug development.

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Table 1. Description and proportions of biomarkers included in 541 ASCO phase I abstracts from 1991 to 2002.

Types of biomarkers according to source	Number of trials (%)
Serum	185 (34.2)
Immunologic studies †	74 (13.7)
Cytokine levels	22 (4.0)
Other protein levels	22 (4.0)
Hormone levels	10 (1.8)
Angiogenic factors §	7 (1.3)
Nucleoside levels	6 (1.1)
Not Specified	12 (2.2)
Tumor tissue	129 (23.8)
Histopathologic analysis	21 (3.9)
Protein expression #	21 (3.9)
Gene expression #	18 (3.3)
Protein expression – IHC	16 (3.0)
PCR – DNA or RNA	11 (2.0)
Receptor expression – IHC	9 (1.7)
Enzyme activity &	8 (1.4)

Table 1. Continued

PBMC	114 (21.0)
Enzyme activity &	27 (5.0)
DNA synthesis	19 (3.5)
Protein expression #	9 (1.7)
Immunologic studies †	8 (1.5)
Gene expression #	6 (1.1)
Not Specified	9 (1.7)
Cellular Material NOS	17 (3.1)
Enzyme activity &	7 (1.3)
Special Imaging	55 (10.2)
Dynamic MRI	16 (3.0)
Immunoscintigraphy	15 (2.8)
PET scans	10 (1.8)
Radio-labeled drugs	9 (1.7)
Non-Identified Source	28 (5.2)

Abbreviations: ASCO: American Society of Clinical Oncology; PBMC: Peripheral Blood Mononuclear Cells; NOS: Not Otherwise Specified; PCR: Polymerase Chain Reaction; RNA: Ribonucleic acid; DNA: Deoxyribonucleic acid; MRI: Magnetic Resonance Imaging; IHC: Immunohistochemistry; PET scans: Positron Emission Tomography scans; SPECT: Single Photon Emission Computerized Tomography.

† Immunologic studies included skin biopsies for local immunization reactions, lymphoproliferative responses in blood and PBMC's, T-cell subpopulation counts (CD4; CD8), Natural Killer cell counts, specific antibody levels, immunoglobulin fractions (Fab, Fc), complement levels, Delayed Hypersensitivity Test (DHT), markers of lymphocyte differentiation, Human Anti-Monoclonal Antibody (HAMA) levels, and Human Anti-Toxin Antibody (HATA) levels.

§ Vascular Endothelial Growth Factors (VEGF) and Fibroblastic Growth Factors (b-FGF).

Techniques of measurement were not reported.

& Examples of enzyme activity include receptor-tyrosine kinase activity, thymidylate synthase (TS) activity, dihydrofolate reductase (DHFR) activity, Protein Kinase C (PKC) activity, etc.

£ Signal Transduction Protein studies include markers of expression and/or activity of proteins involved in cell-cycle regulation pathways, such as Cycline-Dependent kinases (CDK), mitogen-activated protein kinase (MAP kinase), etc.

The following biomarkers were adopted in 1% or less of the abstracts: *Serum*: Lipid levels (2 abstracts), MRI – spectrophotometry (2 abstracts), amino-acid levels (1 abstract), neurotransmitter metabolites (1 abstract). *Tumor tissue*: Immunohistochemistry – NOS (4 abstracts), DNA synthesis (3 abstracts), epigenetic studies (DNA methylation and histone acetylation tests; 3 abstracts), Flow cytometry – tumor cells (2 abstracts), immunologic studies (2 abstracts), nucleoside levels (2 abstracts), RNA expression (2 abstracts), P-glycoprotein activity (1 abstract), radio-labeled drug (1 abstract), receptor expression (1 abstract), Signal transduction proteins (1 abstract), immunofluorescence

NOS (1 abstract), tumor cell proliferation studies(1 abstract), Not Specified (2 abstracts). *PBMC*: RNA expression (4 abstracts), tubulin polymerization (3 abstracts), protein expression – western blot (3 abstracts), protein expression – IHC (3 abstracts), receptor expression (3 abstracts), cytokine levels (3 abstracts), flow cytometry – leucocytes (3 abstracts), signal transduction proteins (2 abstracts), epigenetic studies (2 abstracts), radio-labeled drug (1 abstract), PCR – RNA (1 abstract), receptor expression – IHC (1 abstract), gene microarray (1 abstract), intracellular glutathione levels (1 abstract), P-glycoprotein activity (1 abstract), intracellular pharmacokinetics (1 abstract), drug efflux studies (do not include MDR-1 or P-glycoprotein expression studies – 1 abstract), karyotype studies (1 abstract). *Cells NOS*: Flow cytometry (5 abstracts), intracellular glutathione levels (3 abstracts), PCR – RNA or DNA (3 abstracts), DNA synthesis (2 abstracts), gene expression (1 abstract), IHC – NOS (1 abstract), Receptor expression – IHC (1 abstract), circulating tumor cells (1 abstract). *Normal Solid Tissues*: histologic analysis (4 abstracts), immunologic studies (3 abstracts), enzyme activity (3 abstracts), Receptor expression – IHC (2 abstracts), IHC – NOS (2 abstracts), protein expression - IHC (2 abstracts), PCR – NOS (1 abstract), flow cytometry (1 abstract), other (1 abstract). *Special Imaging*: SPECT (4 abstracts), other (1 abstract). *Cerebral Spinal Fluid*: PCR-NOS (1 abstract).

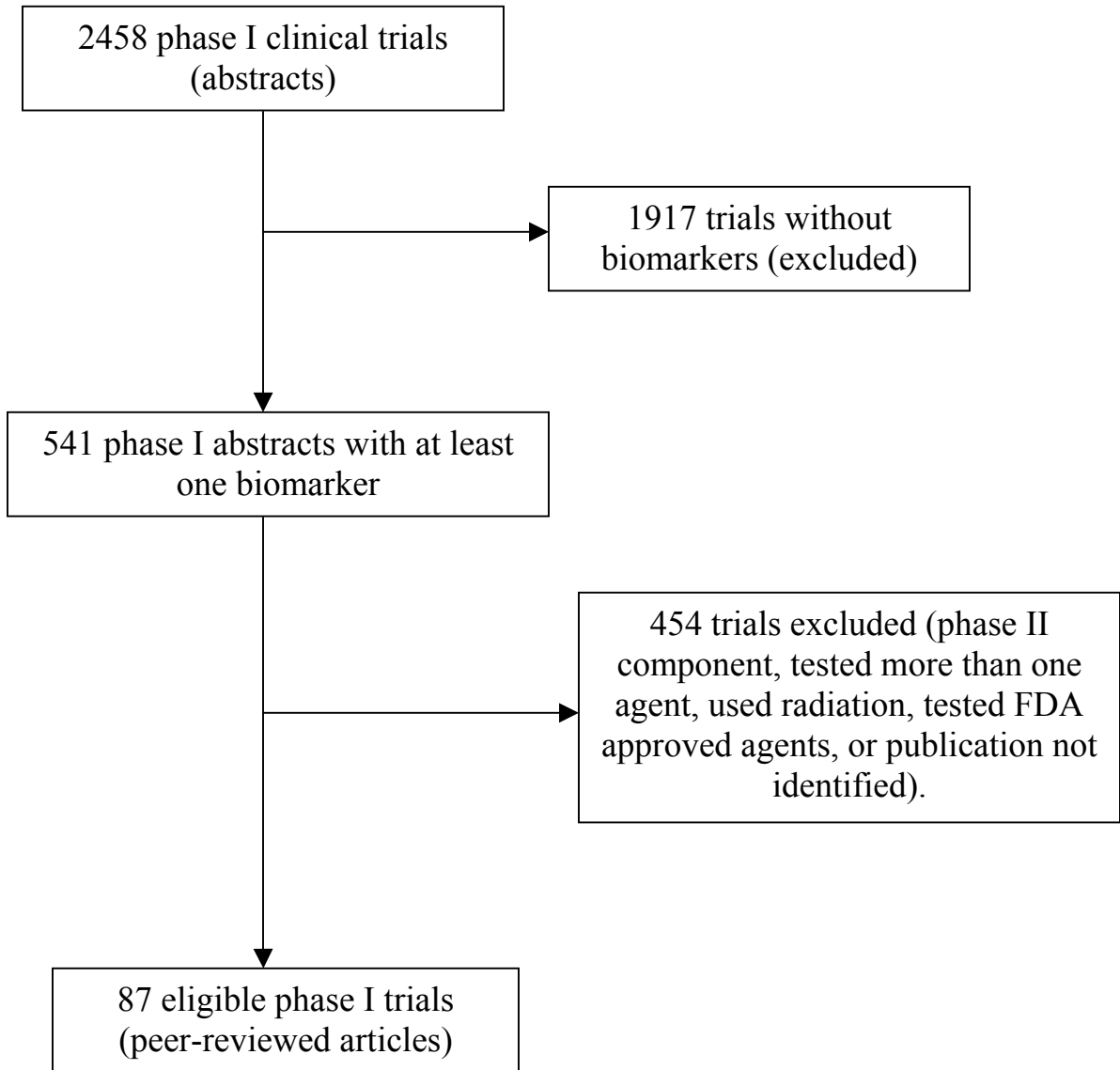


Fig. 1. Flow chart showing the selection of the 79 eligible phase I trials published in the literature.

Table 2. Multivariate analysis of predictors of inclusion of biomarkers in 2458 phase I abstracts from 1991 to 2002

Baseline Characteristics	Trials with biomarkers/total number of trials (%) *	Odds-Ratio (95% CI) ¶
Drug Family (biologic vs. cytotoxic)		17.0 (13.0 – 25.0)
cytotoxic †	122/1472 (8)	
biologic ††	226/383 (59)	
target ‡	149/398 (37)	
other	44/205 (21)	
Population (adult vs. pediatric)		8.0 (2.4-27.0)
adult	538/2387 (23)	
pediatric	3/71 (4)	
NIH sponsorship (yes vs. no)		2.8 (2.0 – 3.7)
yes	128/326 (39)	
no	413/2132 (19)	
Period (1999-2002 vs. 1991-1994)		2.2 (1.7 – 2.9)

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1991-1994	120/650 (18)	
1995-1998	155/760 (20)	
1999-2002	266/1048 (25)	
Pk		NA
yes	285/1169 (24)	
no	256/1289 (20)	
Industry sponsorship		NA
yes	264/1080 (24)	
no	277/1378 (20)	
Trial Location		NA
USA	397/1575 (25)	
other	142/875 (16)	
not given	2/8 (25)	

Abbreviations: Pk, pharmacokinetics; NIH, National Institute of Health; USA, United States of America; CI: confidence interval; NA: not applicable.

* Total number of trials was determined for each characteristic (row).

† cytotoxic: compounds that kill tumor cells by targeting the DNA or its machinery.

†† biologic: macromolecules similar to endogenous molecules produced by recombinant technology (e.g., antibodies, cytokines, antisense oligonucleotides, etc.).

‡ target: small molecules that kill tumor cells or produce cell-cycle arrest by affecting specific biochemical pathways.

¶ We report the Odds-Ratio of significant independent predictors only ($p < 0.05$).

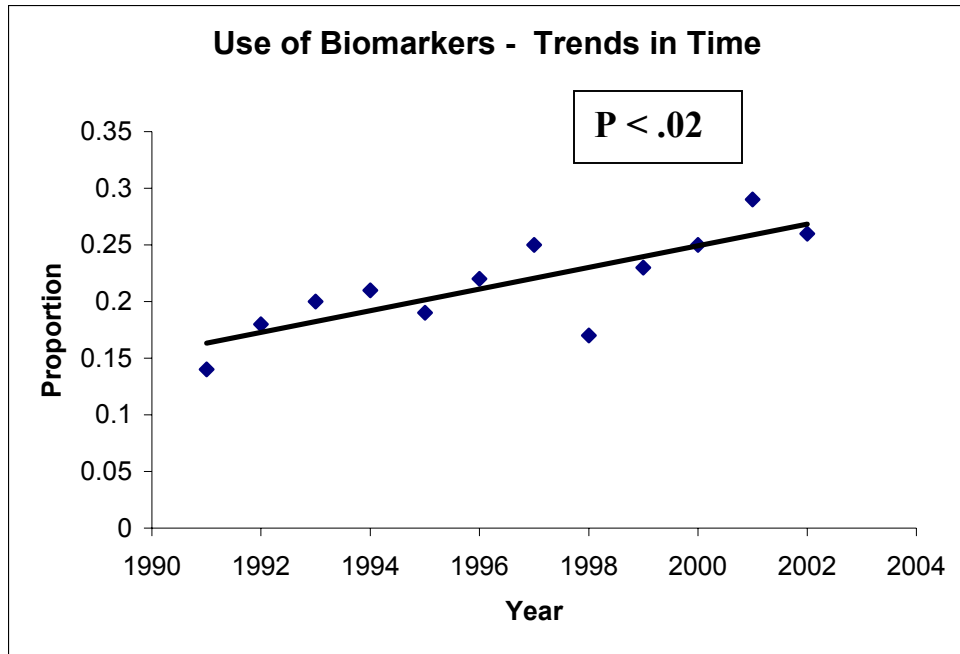


Fig. 2. Graph of the proportion of inclusion of biomarkers in phase I clinical trials from 1991 to 2002. The proportions correspond to the number of trials that adopted at least one biomarker in each year divided by the total number of phase I trials in that year. The P value was obtained by the Cochran-Armitage test for linear trend.

Table 3. Proportions of substantial contributions of biomarkers to dose and schedule selection for phase II studies, decision about further drug development and confirmation of mechanism of action of the studied compound in 87 published phase I clinical trials.

Source of biomarkers	Dose (%)	Schedule (%)	Decision (%)	Mechanism of action (%)	Any contribution*
Serum	4/37 (11)	4/37 (11)	12/37 (32)	14/37 (38)	17/37 (46)
PBMC	1/17 (6)	2/17 (12)	6/17 (35)	8/17 (47)	10/17 (59)
Urine	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Tumor tissue†	6/19 (32)	1/19 (5)	10/19 (53)	18/19 (95)	19/19 (100)
Normal tissue‡	0/4 (0)	0/4 (0)	2/4 (50)	3/4 (75)	3/4 (75)
Imaging ¶	0/9 (0)	0/9 (0)	6/9 (67)	3/9 (34)	6/9 (67)
Total	11/87 (13)	7/87 (8)	36/87 (41)	47/87 (54)	54/87 (62)

Abbreviations: PBMC: Peripheral Blood Mononuclear Cells.

† Included specimens from both solid and hematologic malignancies.

‡ Included normal bone marrow specimens from patients with solid malignancies (2 studies) and normal skin biopsies (2 studies).

¶ Included 5 studies of body distribution of the compound: 5 radioimmunosciintigraphy and 1 Single Photon Emission Computerized Tomography (SPECT) studies, 2 tumor vascular perfusion studies (dynamic MRI), and 1 FDG¹⁸-PET scan.

* Biomarkers that made a substantial contribution to at least one goal.

Table 4. Contribution of biomarkers to dose and schedule selection for phase II studies, “go or no go” decisions, and confirmation of mechanism of action in 12 phase I trials of biologic and target agents that became approved by the FDA.

Drug	Author	Biomarker	<u>Contribution to</u>				Primary end-point for go-no go decision
			Dose	Schedule	Decision	Mec. of action	
Bevacizumab (24)	Gordon <i>et al.</i>	Serum VEGF levels	Irrelevant	Irrelevant	Relevant – “go”	Indeterminate	Minor tumor response
Bortezomib (12)	Aghajanian <i>et al.</i>	proteasome inhibition in cell lysates	Relevant	Relevant	Relevant – “go”	Relevant	Biomarker
Cetuximab * (25)	Baselga <i>et al.</i>	Serum HACA	Irrelevant	Irrelevant	Relevant – “go”	Irrelevant	Tolerability
Erlotinib (18)	Hidalgo <i>et al.</i>	EGFR activity in skin	Irrelevant	Irrelevant	Relevant – “go”	Relevant	Tolerability and minor tumor response
Exemestane (26)	Paridaens <i>et al.</i>	Serum estrogens levels	Irrelevant	Irrelevant	Relevant – “go”	Relevant	Tumor response
Gefitinib (19)	Herbst <i>et al.</i>	EGFR activity in skin	Irrelevant	Irrelevant	Relevant – “go”	Relevant	Tumor response and stable disease
Gemtuzumab Ozogamicin (27)	Sievers <i>et al.</i>	Antigen-antibody bound in PBMC	Relevant	Irrelevant	Relevant – “go”	Irrelevant	Hematologic response

Table. Continued

Imatinib (28)	Oosterom <i>et al</i>	FDG-PET scan	Irrelevant	Irrelevant	Relevant- “go”	Irrelevant	Tumor response
Imatinib (29)	Druker <i>et al</i>	TK activity in CML blasts	Relevant	Irrelevant	Relevant- “go”	Relevant	Hematologic response
Rituximab (30)	Maloney <i>et al</i>	CD20 B lymphocyte depletion	Irrelevant	Irrelevant	Irrelevant	Irrelevant	Tumor response
Tositumomab (31)	Wahl <i>et al</i>	Drug to tumor binding by immunoscintigraphy	Irrelevant	Irrelevant	Relevant- “go”	Irrelevant	Tumor response
Trastuzumab* (32)	Tokuda <i>et al</i>	Serum HER-2 extracellular domain	Irrelevant	Irrelevant	Irrelevant	Irrelevant	Tumor response and tolerability

Abbreviations: Mec. Of action: Mechanism of action; VEGF: vascular endothelial growth factor; HACA: human antichimeric antibody; EGFR: epidermal growth factor receptor; FDG-PET: flurodeoxyglucose- positron emission tomography; TK: tyrosine kinase; CML: chronic myeloid leukemia;

* Biomarkers designed to select the study population (e.g., HER-2 and EGFR overexpression) were not considered for this study.