AN APPROACH TO THE VALIDATION OF MARKERS FOR USE IN AIDS CLINICAL TRIALS

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Dr. Mildvan and co-authors have thoroughly reviewed and documented what is known about the validation of surrogate markers for use in clinical trials. They have proposed a classification system based on the usefulness of available immunologic and virological assays as measures of prognosis, drug activity, and therapeutic efficacy. The latter, a type II marker in the proposed classification, should estimate the proportion of treatment effect explained by change in the marker induced by therapy and, if complete, can substitute for clinical endpoints. HIV clinical trialists have had a long-standing interest in using surrogates for clinical endpoints to facilitate conduct of experimental protocols and to decrease the time and effort required to develop new treatment strategies. The approach outlined in this review by experienced clinicians, biostatisticians, and immunologists provides a framework to evaluate currently available and potential surrogate markers.

— *John P. Phair*

One of the major scientific goals in AIDS clinical research is the development of surrogate markers that reflect the effects of treatment on clinical outcomes to the extent that the markers themselves may substitute for clinical endpoints in therapeutic trials. The identification and validation of effective surrogates, particularly in early HIV infection, have the potential not only to expedite the evaluation of new AIDS therapies through the use of smaller, shorter trials, but also to provide insights into underlying pathogenetic elements that should be targeted for intervention. Premature acceptance of surrogates, however, could result in misleading interpretation of data on investigational therapies.

Since the earliest days of the HIV/AIDS epidemic, numerous studies have addressed the apparent relationships in HIV infected individuals between immunologic and virological parameters and clinical disease. Relatively few measures, however, have been translated into practical application as "markers" for use in prognostics, therapeutics, vaccine development, or individualized case management.

Several explanations could account for this; salient among these are: (1) the failure to validate preliminary observations made in small cross-sectional ("snapshot") studies in larger, better-characterized cohort studies with longitudinal follow-up; (2) obscure (or unknown) relationship of the marker to HIV disease pathology and lack of specificity for HIV disease; (3) technical barriers (e.g., difficulties in performing/reproducing the measurements) that have limited broader applicability; and (4) inherent complexity of the marker validation process, with the potential for serious underestimates and overestimates of clinical benefit when surrogate marker responses alone are relied upon [1, 2].

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Moreover, despite an extensive literature search that has enabled evolution of both the framework and criteria necessary to define a useful surrogate marker [3-5], there has remained a lack of consensus among clinical and basic research investigators as to the best approach to marker validation and application.

The T-helper (CD4) lymphocyte count represented the first and most widely used laboratory measure for assessing the degree of immunodeficiency and risk of clinical disease progression in HIV-positive individuals [6-28]. However, results of clinical trials of antiretroviral drugs (e.g., zidovudine and didanosine) have shown that, despite its value both as a prognostic marker and as an indicator of drug activity, the CD4 lymphocyte count is a weak surrogate marker: that is, treatment-induced increases in CD4 lymphocyte counts only partially account for the clinical benefit observed in patients receiving antiretroviral therapies [25, 29-32].

Other biological markers that correlate with disease severity and respond to antiretroviral treatment include 82-microglobulin, neopterin, cytokines, and HIV p24 core antigen [12 15, 33-72]. More recently, attention has concentrated on the phenotypic and functional immunologic correlates of protection against HIV infection and progression to AIDS [73-139]. In addition, studies employing sensitive measures for the quantitation of viral burden have identified virion-associated RNA, detectable in plasma in the majority of HIV-infected persons, as a responsive indicator of antiretroviral drug activity and a strong predictor of clinical outcome [140-148].

However, in validation studies conducted to date, no single marker has been shown to explain fully the varying clinical effects observed with therapeutic interventions, a fact that demonstrates the need for new indicators or combinations of existing indicators. Indeed, because of the complexity posed by HIV disease, definition of a composite marker bears consideration: one comprising several individual markers, each reflecting different key elements of pathogenesis and serving, collectively, as a candidate surrogate for clinical endpoints [e.g., 37, 45, 149].

Recognizing the need for a coordinated strategy by laboratory investigators, clinicians, and statisticians, we have proposed a paradigm for the definition and validation of immunologic and virological markers for HIV disease [150]. In this manuscript, the paradigm is presented as a common platform to expedite the development of laboratory markers for application in a variety of clinical interventions.

Implicit in the approach is the requirement to conduct marker validation studies in the context of interventions known to confer clinical benefit. In order to define the essential host elements that contribute to clinical benefit in patients with HIV infection, candidate immunologic markers must be validated in the context of trials of antiretrovirals, i.e., the only drugs whose clinical efficacy in the treatment of HIV infection has been substantiated to date. Once validated, the markers can, in turn, be used to evaluate new treatments. In addition, if novel interventions such as immune-based therapies prove efficacious in future clinical-outcome trials, new opportunities will emerge for marker validation studies.

Classification System

The following classification system standardizes the approach to validation of biological markers. This hierarchical schema can be applied to the analysis of any candidate marker (virological, clinical, or immunologic), across stages of HIV disease.

Type 0: Natural History Marker

A natural history marker (type 0) is defined as a marker of disease severity that reflects underlying pathogenetic mechanisms and predicts clinical outcome independent of treatment.

Type 0 markers are identified as prognostic in longitudinal cohort studies examining the natural history of HIV disease, e.g., the Multicenter AIDS Cohort Study (MACS), Women's Interagency HIV Study (WIHS), and Women and Infants Transmission Study (WITS). Type 0 markers provide the bio logical plausibility for further developing a candidate marker.

The frequency and magnitude of abnormality of a type 0 marker should correlate with stage of disease. The most important applications of type 0 markers are: (1) for use as baseline stratification variables in clinical trials, differentiating individuals at varying degrees of risk for clinical progression, and (2) as milestones of disease progression for monitoring patients. The best examples, to date, of type 0 markers as independent predictors of risk are the CD4' T-cell count and the HIV 1 plasma RNA level; the former represents a measure of disease severity on the target organ, the immune system [151], and the latter, a measure of viral burden as an indicator of the activity and extent of infection.

Type I: Biological Activity Marker

The next stage in marker development is to assess the influence of treatment on a biological indicator that has already shown potential as a prognostic (type 0) marker. A type I marker is defined as one that responds to therapy; the frequency and magnitude of the response should correlate with the degree of therapeutic potency. Type I marker activity is determined in the context of early phase clinical trials.

The most important application of a type I marker is in phase I/II trials in infected patients, where proof of concept focuses on whether or not a new treatment has promising activity, such as whether or not it displays antiretroviral, immunomodulatory, or antiproliferative effects on the appropriate marker to indicate such activity. The degree of response of a type I marker can be used to estimate an optimal dosing regimen and to indicate whether a combination of treatments is more active than a single treatment.

Triple-drug antiretroviral combinations, for example, appeared superior to single drugs and double-drug combinations in vitro and have been shown in clinical trials to have superior activity with respect to rises in CD4 T-cell counts and declines in HIV 1 plasma RNA levels [152-1551. In fact, such favorable responses have led to accelerated licensing for many of the currently available antiretroviral agents, including the new class of highly "active" compounds, HIV 1 protease inhibitors. Conversely, absence of type I marker responses, which may be due to an inadequate dose or the lack of promising activity altogether, can accelerate the decision to abort development of a new therapeutic agent. Examples include compounds of historic interest such as AL721, the tat protein antagonist Ro 24-7429, and, more recently, a highly protein-bound protease inhibitor, SC-52151, all of which were discarded when the examined doses failed to induce viral load declines or CD4+ T-cell count rises in treated patients [156-158].

Type II: Surrogate Marker of Therapeutic Efficacy

The ultimate stage in marker development is to establish, in the context of an efficacious therapeutic intervention, the relationship between an early change in the marker and clinical outcome. A type II marker, either a single marker or composite of several markers, is defined as one that accounts fully for the efficacy of an agent. That is, a beneficial effect on the marker signifies a subsequently favorable clinical outcome or, as defined by Prentice, knowledge of the marker value implies knowledge of the prognosis with or without treatment [5].

Ideally, type II markers represent "complete" surrogates of clinical outcome; their most important application is as substitutes for clinical endpoints in phase II/III efficacy studies. The extent

to which a candidate type II marker explains an observed clinical benefit of therapy in a controlled trial can be modeled, and an estimation of the proportion of treatment effect explained (PTE), together with its standard error, can be used to evaluate type II surrogacy [159, 160]. A PTE value of 1 represents the ideal.

Although there is general agreement on the merit of the PTE approach, its interpretation requires an understanding of underlying mechanisms of disease and drug action: a value of PTE near 1 implies that the marker is a good surrogate only if it is known that the drug operates primarily through its action on the marker and that the marker measures quantities that play a central role in the causal pathway of AIDS. Values of PTE spuriously close to 1 can arise, for example, when the agent has harmful or toxic effects that increase rates of clinical progression but that are not mediated through the marker [160].

Another important issue in the interpretation of the PTE is the extent to which the actual treatment status of patients at the time the marker is measured corresponds to the treatment status later, when the clinical endpoints are measured. While the standard intent-to-treat analysis provides a valid estimate of the clinical effects of randomization in a comparative treatment trial, it becomes problematic in the analysis of surrogacy, particularly so in long-term trials such as in patients with HIV infection.

If one is investigating the efficacy of a short-term change in a marker while estimating a clinical effect that occurs much later and includes subsequent treatment changes (e.g., crossovers to regimens other than the original treatment assignment on which the marker was measured, including no treatment at all, because of discontinuation or the loss of drug action through the development of viral resistance i.e., an intent-to-treat regimen), then the PTE can be spuriously increased to a value near 1 or even greater (in fact, to any value) because the net treatment effect on clinical outcomes that the marker must explain will be reduced by such changes.

Although accumulating evidence suggests that HIV 1 RNA may explain a substantial proportion of the treatment benefit seen with antiretroviral therapy, confirmation will require that the above factors be taken into account in future analyses.

Marker Validation

The marker validation process begins with the development of a hypothesis about pathogenesis and is completed with the establishment of the marker's role and applicability in clinical trials. The initial scientific rationale for developing a marker is often based on the documentation of an apparent relationship to HIV infection, disease stage, or a concept of underlying disease mechanisms. Initial studies are frequently descriptive in nature and cross-sectional in design. The validation process should then follow a step wise approach, carefully matching the type of marker with the appropriate retrospective or prospective study design.

Type 0

Natural history (prognostic) markers are validated by demonstration of a strong relationship between the frequency and magnitude of the marker abnormality at baseline and eventual clinical outcome in a longitudinal study. Epidemiological cohort studies such as the MACS, WIHS, and WITS offer the potential for long-term follow-up and confirmation of clinical events. The placebo arms in the original controlled trials of antiretroviral agents offered yet another opportunity for longitudinal follow-up, albeit usually of shorter duration.

Markers requiring real-time determinations or fresh samples (e.g., delayed-type hypersensitivity, cell function assays, and immunophenotypes) must be incorporated into the study

design at the outset. An important factor is that cohort studies usually establish and maintain extensive specimen repositories, which permit delayed or retrospective marker analyses for those marker assays not requiring fresh specimens (e.g., soluble immune activation markers such as 02-microglobulin, neopterin, and cytokines; plasma or serum HIV 1 RNA quantitation). Such cohort studies represent enormously valuable resources, since the combined access to both longitudinal clinical data and patients' specimens offers the potential to validate new candidate natural-history markers years after a study has been conducted, as was accomplished by Mellors and colleagues for plasma RNA levels [143].

Type I

New biological activity (type I) markers are validated in controlled phase I/II clinical trials, where it can be demonstrated that an active intervention favorably affects the marker, a less active agent or a lower dose has reduced effect, and a placebo results in no significant effect on the marker at all (i.e., a dose-response relationship can be demonstrated). Previously validated type I markers (e.g., HIV p24 antigen and CD4 lymphocytes) provide useful comparisons against which new candidates may be evaluated.

Because of its sensitivity, precision, and applicability to stored samples, HIV 1 plasma RNA, as detected by increasingly sophisticated molecular techniques, represents the current standard against which new type I activity markers will be measured. Optimally, placebo-controlled trials afford the greatest opportunity for type I marker development. However, in settings where placebo controls are not possible, early- versus delayed-initiation-of-therapy designs can also enable successful validation of type I markers.

Type II

A marker validated as type 0 and type I is next validated as a type II or surrogate marker of therapeutic efficacy in the context of studies that demonstrate positive clinical treatment effects, i.e., positive outcome trials. The ideal study is a phase II/III trial that has identified a large difference in clinical outcome between treatment arms and in which, as outlined above, little or no change has taken place in the treatment regimens over time and little dropout has occurred, such that an early change in marker values can be reasonably interpreted to predict or, in fact, mediate a subsequent treatment effect.

In addition, validation of candidate type II markers requires cross-study analyses for the reasons that (1) a marker may be useful at one stage of disease but not another; (2) a marker may be applicable to one type of treatment but not another (e.g., antiretrovirals vs. immunomodulators); and, significantly, (3) conditions that affect the PTE analysis will differ from one trial to another. Results that are consistent across studies are more believable and allow for greater confidence when utilizing the marker as a surrogate for clinical endpoints in efficacy trials.

In particular, PTE values consistently near 1 across studies of treatments with different toxicities and mechanisms of action provide much stronger evidence of surrogacy than could results from any single study. Operationally, the PTE value can be estimated by several different techniques [25, 29-32, 145, 159, 160], and type II markers can be validated in either retrospective or prospective studies, each having its advantages and limitations.

Retrospective Validation Studies in Which Clinical Data Preexist

If the marker(s) of interest were measured but not analyzed during the course of a trial or can be assayed on samples collected and stored during the study, completed positive outcome trials represent important resources for the logical next step in the type II marker validation process. It is important, however, to recognize the limitations of retrospective marker validation studies, especially the constraint imposed by whether or not the requisite data and/or laboratory specimens were collected and appropriately stored during the study.

In many instances, the study may not have been optimally designed to permit marker validation; for example: (1) an inadequate number of patients' samples may be available; (2) specimens may have been stored only from a subset of patients that proves not to be representative of the larger study population with respect to baseline characteristics, treatment assignment, or clinical outcomes; or (3) the sub sample may be too small to permit a meaningful analysis. In selecting retrospective studies for investigation of candidate markers, distinction should be made between specimen banks derived from positive phase II/III trials and those derived from phase I/II trials.

The former represent the limited and invaluable resource required for validation of type II surrogates of clinical efficacy and should be reserved for that purpose and not to define type I markers. Table 1 lists selected positive-outcome trials to which the authors have access that are currently supporting type II marker validation studies. Note that these are limited to soluble markers measured in either frozen plasma or serum, which were the only specimens stored during the trials and available for subsequent study.

Prospective Phase II/III Studies of Clinical Efficacy

Designing prospective phase II/III trials for the investigation of type II markers offers the opportunity to (1) evaluate candidate markers that require real-time testing, (2) target a specific patient population or category of intervention, and (3) optimize specimen quality assurance. The incorporation of optimized specimen collection and storage methods into the protocol should permit the greatest flexibility and yield for future marker development should a significant difference in treatment outcome occur in the trial.

In practical terms, for example, immunophenotypic and functional assays usually need to be evaluated in both real-time and on frozen stored cells in order to assess the applicability of these assays to large multicenter trials. Optimally, the comparisons should be made in trials that have a high likelihood of showing important differences in clinical outcome; for example, the inclusion of a placebo control, where appropriate, could increase the chances for a positive outcome.

On the other hand, it should not be overlooked that negative outcome studies are essentially natural-history studies in carefully selected and homogeneously treated populations and can therefore be used to contribute excellent type 0 marker information. Real-time marker determinations can be costly and labor intensive and therefore should be reserved for those markers that can be measured only during the course of the study, such as with in vivo tests (e.g., delayed hypersensitivity skin test reactivity) or laboratory tests for labile markers (e.g., CD38. relative fluorescence intensity), and for which compelling type 0 and type I data have been accumulated.

It is also important to emphasize that although a marker may be validated according to the schema presented above, limitations are imposed

by the context (study) in which the validation takes place. A marker validated with an antiretroviral intervention in HIV-infected adults may not be applicable in HIV-infected children. Likewise, a marker validated for antiretroviral interventions may not prove suitable for immunorestorative interventions. A marker may be validated as type 0 or type I (e.g., CD4 and p24), but it may not be as useful as a type II marker.

In addition to age and type of intervention, other factors that may influence marker applicability include risk group, stage of disease, and geography [23, 24, 28, 44 47, 51, 66, 70, 74-78, 85, 128, 168]. Virological factors, such as HIV strain, phenotype, and localization of measurement (peripheral blood, lymphatic tissue, CNS, etc.), are also likely to be important determinants [83, 86, 91, 148, 163, 169-181]. Thus, there is a need for multidimensional validation of markers, across populations and across studies, before they may be incorporated with confidence into AIDS clinical trials as substitutes for clinical endpoints.

Current Validation Status of Frequently Measured Markers

In Table 2, we have categorized commonly assayed markers in antiretroviral trials according to the hierarchical schema presented herein (types 0, I, and II), based on critical analysis of published literature, including several excellent review articles [13, 144, 182-184]. The table serves to illustrate the lack of consistent reporting of results, lack of head-to-head comparisons of markers, the paucity of validated type II markers, and the limited availability of type I markers.

Factors taken into account in rating the markers include degree of substantiation (number of reports, size of the data base, and reproducibility of findings), responsiveness of the marker to treatment, and applicability across disease stages. (Note that the p24 rating should be considered relatively reduced in comparison with that of RNA, on the basis of the sensitivity of available assays and prevalence in the target population; p24 antigen, unlike RNA, is detectable in only a minority of patients.)

While many of these studies have led to significant insights into pathogenesis and pointed to new directions for therapy, it is hoped that the proposed systematic and streamlined approach to marker validation will further serve to sharpen the focus of new investigations and hasten the appropriate use of surrogate endpoints in HIV/AIDS clinical trials.

Although the impetus for developing the present paradigm was originally based on the need to expedite clinical trials involving patients with HIV infection, it should be noted, in conclusion, that the schema may also find potential application in other clinical settings in which the identification of biological markers is required to explore fundamental disease mechanisms and to accelerate the development of novel therapies.

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Table 1. Validation of Type II Markers: Key Positive-Outcome Studies

HIV disease stage	Trial	Treatment effective (relative hazard)	Markers under retrospective study	Reference(s)
Advanced	BW02	<0.1, ZDV vs. placebo	BMI, IFN, TG, $ß_2$/neopterin, RNA	[6, 7, 55]
Advanced	ACTG 114/N3300	0.84,* ZDV vs. ddC	BMI, cytokines, $ß_2$/neopterin, RNA	[161]
Advanced	ACTG 116b/117	0.72, ZDV vs. ddI (500mg/d); 0.91, ZDV vs. ddI (750mg/d)	$ß_2$/neopterin, IFN/TG, sTNFR, IL6, RNA, viral resistance, viral phenotype	[146, 162, 163]
Intermediate	ACTG 016	0.48, ZDV vs. placebo	$ß_2$/neopterin, sCD4/8, sIL2R, ICDp24	[8, 164]
Intermediate	ACTG 019	0.53, ZDV vs. placebo	$ß_2$/neopterin	[9, 50]
Intermediate	ACTG 175	0.70, ZDV vs. ddI, ZDV/ddI, ZDV/ddC	$ß_2$/neopterin, sTNFR, RNA, viral phenotype	[148, 165]
Early	ACTG 076	0.33, ZDV vs. placebo†	RNA	[166, 167]

NOTE: ACTG = AIDS Clinical Trials Group; $ß_2$ = $ß_2$-microglobulin; BMI = body mass index; BW = Burroughs Wellcome; ddC = zalcitabine; ddI = didanosine; ICDp24 = immune complex-dissociated p24; IFN = endogenous interferon; IL6 = interleukin 6; sCD4/8 = soluble CD4/8; sIL2R = soluble interleukin 2 receptor; sTNFR = soluble TNF-_ receptors; TG = serum triglycerides; ZDV = zidovudine

* = Ratio of proportion of progressors (relative hazard unavailable)

† = Ratio of proportion of infected children

Table 2. Current Validation Status of Frequently Measured Markers

Markers	Surrogate marker category			Reference
	Type 0	Type I	Type II	
Immunophenotypic				
CD4 (no.), CD4 (%)	+ +	+ +	+	[6-32, 35, 46, 47, 50, 70, 76, 98, 149, 153]
CD8 (no.), CD8 (%)	+	+	?	[11-14, 16, 20, 22, 23, 40, 50, 73-80, 122, 123]
Activation (CD8/CD38)	+	+	?	[38, 78, 81-88, 93, 122, 123]
Memory/naive (CD45RA/CD45RO)	+	+	?	[38, 49, 82-85, 87-90, 93]
Function (CD28)	+	?	?	[75, 83, 88, 91-93]
Immune functional				
LPA	+	?	?	[89, 94-97, 122]
Inducible cytokines	+	?	?	[36, 75, 98-100, 122]
Natural killer cell activity	?	?	?	[101-104]
CTL	+	?	?	[105-114]
CD8 antiviral activity	+	?	?	[115-121]
Apoptosis	?	?	?	[124-126]
Vß repertoire	+	?	?	[86, 127, 128]
Soluble immune				
$ß_2$/neopterin	++	++	+	[12, 14-16, 33-50, 53, 54, 60, 61, 75, 76, 135, 145, 149, 153, 168]
Cytokines/receptors	+	+	?	[37-39, 43, 50-66]
Chemokines/receptors	+	?	?	[129-134]
In vivo				
DTH	+	+	?	[6, 49, 98, 135-139]
Viral				
p24 antigen	++	++	--	[12, 33, 37, 39, 50, 56, 60, 61, 67-72, 135, 156, 157, 164]
RNA	++	++	++*	[65, 88, 128, 140-148, 153-155, 158, 167]
SI phenotype	++	?	?	[83, 148, 163, 169-175]

NOTE: CTL=cytotoxic T-lymphocyte activity; DTH=delayed type hypersensitivity; LPA=lymphoproliferative activity; SI=syncytium-inducing; ++=useful; +=possibly useful; --=not useful; ?=limited data available.
*Pending confirmation of preliminary promising evidence

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