In this issue Karanikas and colleagues review various immunization methods directed against a well-defined tumor antigen, MUC1. The clinical trials described highlight a new direction in the study of cancer vaccines. Persons with cancer can be immunized to tumor antigens, but how can vaccination strategies be optimized to result in a favorable clinical outcome? Focuses on patient selection, novel statistical designs of phase I vaccine studies, and a rigorous approach to immune response measurement will help to optimize and prioritize cancer vaccine strategies for clinical study.

The patients enrolled in the trial reported had metastatic or locally advanced adenocarcinomas. An end point of the investigation was an assessment of the antitumor response, with no partial or complete tumor responses observed in any of the patients. In this series of trials, the patients' life expectancy was approximately 12 weeks, most had advanced metastatic disease, and 16 (24%) of them could not complete the study because of progressive disease or death. How much immunity is needed to eradicate established and progressive disease? In infectious disease antigen systems, in which the antigens are highly immunogenic and "foreign," active immunization plays little role in the eradication of established infection. Similarly, the primary role of cancer vaccines may be to prevent cancer relapse or even, eventually, the development of tumors. Studies, such as the one reported here, indicate that a more appropriate clinical end point may be the evaluation of time to progression in patients who have no evidence of disease after definitive treatment and relapse prevention.

Importantly, the primary objective defines the sample size of a study. Phase I trials are the appropriate format for initial testing of a therapy or drug in humans and are designated as the mechanism by which the maximally tolerated dose of an agent is defined. These trials are usually composed of small patient groups to whom escalating doses of an agent are administered. An important goal of cancer vaccine studies, and a stated goal of this study, is to define the immunogenicity of the vaccine. Therefore, more information is needed than can be gleaned from the traditional small sample sizes of a phase I study. In the current trial, although the sample sizes divided between the intraperitoneal or intramuscular routes of administration may be adequate to meet certain end points, there were so many dose levels with so few patients at each that no firm conclusions could be drawn regarding the immunogenicity of a particular approach. The development of cancer vaccines as a therapeutic strategy requires that we answer some unique biologic questions within in the context of the phase I study that do not focus on a definition of the classic maximally tolerated dose but rather on the evaluation of biologic outcome measures. Safety is always an objective of phase I studies and has significant relevance in trials targeting potential self-antigens. However, more patients may be needed in each group to define the immunogenicity of a specific vaccine formulation. Similarly, small incremental dose assessment is appropriate for a toxic agent, but evaluation of the most biologically effective dose may require large differences among doses (e.g., a low-, moderate-, and high-dose approach).

In addition, as more vaccine studies, such as this one, show that patients with cancer can be immunized against tumor antigens, highly quantitative and reproducible methods of measuring those immune responses will be needed. No standard validated methods exist for immunologic monitoring after immunization with a cancer vaccine. The ideal clinical T-cell immunologic monitoring strategy is one that (1) uses a minimal amount of clinical material (i.e., the analysis can be performed with a single tube of blood), 2) allows sensitive and specific analysis of cryopreserved cells, 3) requires no ex vivo manipulation such as in vitro simulation(s), 4) is highly quantitative and sensitive to a broad range of responses, and 5) is amenable to multiple evaluations in a short period of time (i.e., is easily automated).

Several novel methods of T-cell enumeration are becoming available and must be adapted for clinical use, such as ELIspot; intercellular cytokine staining by flow cytometry, as described in this trial; and major histocompatibility complex class I tetramer analysis. The validation of these tests as clinical tools is critical to their evolution from laboratory to clinical tests. Clinicians expect that laboratory tests are backed by rigorous analyses that define baseline responses and validate the reproducibility of the results. Assay validation data are rarely published or discussed in the context of reporting results from cancer vaccine trials. Quality control monitoring and assay validation are composed of several analytical measures. These measures are routine, for the most part, for serologic studies. An example is the definition of positive antibody responses to MUC1 in this clinical trial validated by comparison to a reference population of 99 non-cancer-bearing persons. A rigorous assessment of the robustness of a method is difficult to apply to T-cell-based immunologic monitoring. However, some assessment of the accuracy, reproducibility, limit of detection, specificity, and baseline values in a reference population is needed for adequate evaluation and interpretation of T-cell immunity. Only when assays to assess the magnitude and function of T-cell responses to cancer antigens become standardized can comparisons be made between vaccine formulations and a laboratory surrogate for a clinical effect be defined.