Clinical Proteomics and Biomarker Discovery in Cancer Research

National Cancer Institute Symposium

Quadrus Conference Center
Menlo Park, California

November 5, 2004

Background

An effective platform for clinical biomarker discovery would revolutionize clinical oncology. Such a platform would enable detection of disease early, when it is curable. It would also provide a means of distinguishing quickly those patients who are responding to a given therapy from those who are not, as well as yielding perhaps the ultimate preventive tool capable of identifying those at risk for cancer and even predicting their response to prevention interventions.

To catalyze the development of an effective platform for clinical biomarker discovery, the National Cancer Institute (NCI) is proposing to develop a strategic scientific initiative that would bring the power of proteomic technologies to bear on the problem of discovering biomarkers for cancer. The premises behind this technology-based initiative are the following: Such biomarkers exist in readily accessible body fluids, panels of such markers will be required to achieve high specificity and sensitivity, current technology is capable of discovering these panels, and current application of this technology can be improved. Since no current technology interrogates more than 1 percent of the proteome at a time, a systematic approach to biomarker discovery requires teams of investigators sharing and aggregating data. Achieving this will involve setting standards, ensuring quality control, and developing an informatics platform capable of aggregating and comparing data across laboratories.

Because of his extensive experience in proteomics, the NCI has enlisted Dr. Lee Hartwell, President and Director of the Fred Hutchinson Cancer Research Center, to assist the Institute in its planning activities in clinical proteomics. To catalyze discussion among researchers, Dr. Hartwell prepared a white paper for a focused clinical biomarker discovery initiative. This white paper includes six components organized as shown in Figure 1 and discussed below:

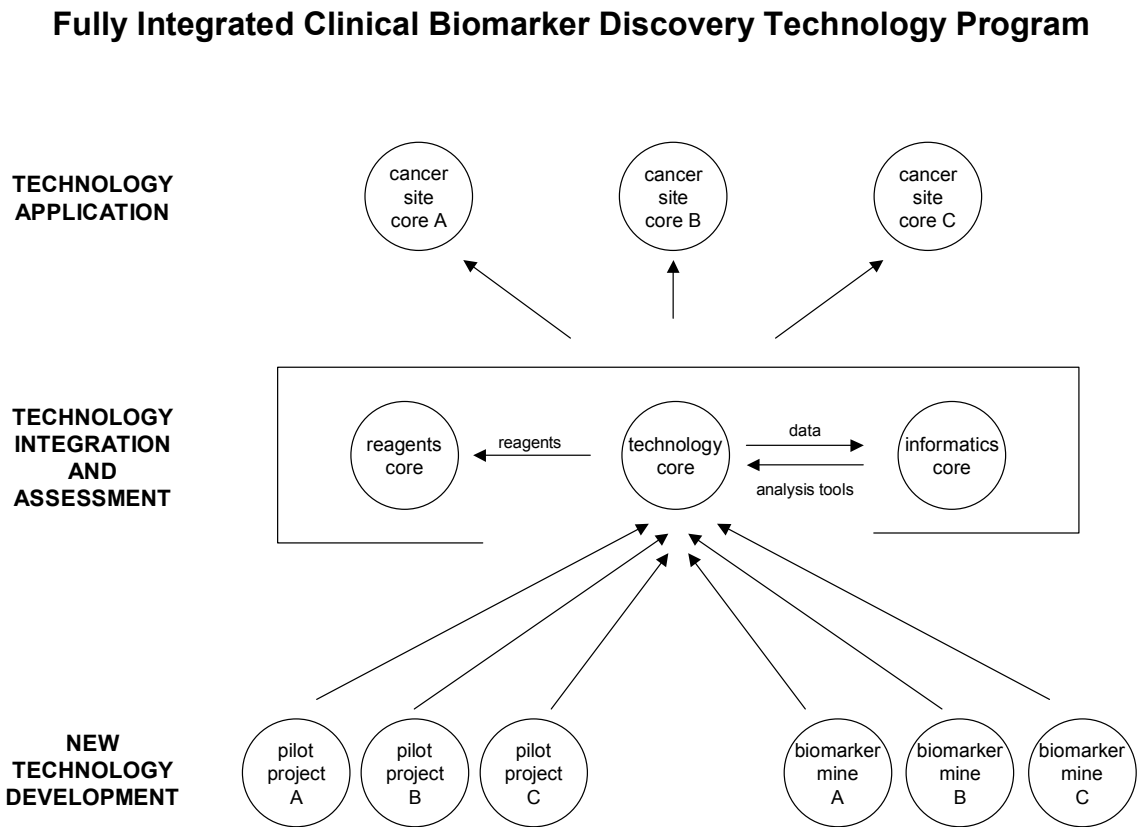

Figure 1. Organization of an effective clinical biomarker discovery program

1. The **Informatics Core** will develop tools to allow laboratories to communicate efficiently and to compare data. This Core will develop a standardized data format to facilitate cross-platform comparisons, and it will provide an open-source suite of analytical tools compatible with this standard data format to facilitate standardization of data review and analysis across laboratories and allow meaningful comparisons of results. Additionally, a central database for storing the curated data of the programs will be housed in the Informatics Core and made accessible to the public.

2. The **Reagents Core** will organize tools for biomarker discovery. This Core will provide a central virtual source for reagents, including human and mouse tissue samples, mouse models, antibodies, and other reagents as needed. Reagents, along with data on reagent performance and quality, will be acquired and dispersed quickly to other core facilities and satellites.

3. The **Technology Assessment Core** will assess technologies central to biomarker discovery in order to provide laboratories with the best possible techniques and protocols. Initially, this Core will systematically compare existing technologies in

each component of the biomarker discovery platform using standard reference plasma. The best-performing technologies from each component will then be integrated into an optimized platform against which new technologies (discovered via pilot projects and biomarker mines; see Figure 1 and below) can be tested. The ability of the integrated and optimized platform to identify biomarkers will be assessed using mouse models. This Core will also provide data to the Informatics Core for algorithm development and will also deposit useful reagents (including reference plasma and mouse tissues) into the Reagents Core for dissemination. Finally, this Core will collaborate with Cancer Site Components to implement optimized technologies to find biomarkers in human samples.

4. **Cancer site components** are a team of investigators dedicated to biomarker discovery at a particular cancer site, such as breast, lung, prostate, colon, and others.

5. **Biomarker mine components** are investigators or small groups dedicated to optimizing the methods for discovery in a particular class of biomarkers, such as cell surface or secreted proteins.

6. **Pilot projects** are single investigator projects designed to test a new technology for biomarker discovery, e.g., protein chips, antibody production, etc. Where appropriate, promising new technologies will be reproduced and tested against current standards by the Technology Assessment Core.

One of the major points of the white paper is that a large, concerted effort is required to advance the field of biomarker discovery. The white paper also argues that the field of proteome-based biomarker discovery must answer the following questions:

- What combination of ionization source and mass analyzers will give the best achievable reproducibility, dynamic range, mass accuracy, and throughput for biomarker discovery?

- What techniques for fractionating plasma simplify the proteome sufficiently to allow significant depth of coverage, given the dynamic range of conventional mass spectrometers?

- What techniques for fractionating plasma are capable of the level of reproducibility (tested on multiple repeats of the same sample) that will be required to allow biomarkers to be detected while analyzing samples from a large number of cases and controls in high-dimensional data?

- Which techniques for quantitative mass spectrometry are robust enough for biomarker discovery?

- Can a standardized data analysis pipeline be established (and available electronically) so that results obtained from different laboratories can be analyzed using the same tools, allowing direct comparison of results?

- Can the best conventional fractionation schemes and mass spectrometry instrumentation interrogate a large enough "space" of the plasma proteome to discover diagnostic biomarkers?

Answering these questions will require a large, concerted effort in which the best existing instrumentation and data analysis tools, being developed in multiple laboratories throughout the scientific community, are integrated into one platform for data collection and analysis. Only then will researchers be able to compare rigorously multiple schemes for processing plasma, head to head on a controlled platform using identical samples, using multiple plasma processing schemes in a controlled manner.

Based on experience with planning other technology development programs, the NCI has committed to holding a series of symposia designed to introduce the proposed program to the research community and to solicit comments from a broad range of experts in proteomics, clinical oncology, biomarker discovery and technology, bioinformatics, and drug and diagnostic technology development. The second of the series of meetings was held on November 5, 2004.

Presentation of the Proposed Plan

Dr. Hartwell began the meeting with a presentation of the proposed plan. He noted that he believes that science can make a significant impact on medicine but wonders how science will have an impact on patients. It is this concern that led him to conclude that scientists can have the biggest impact by developing technology capable of dramatically improving early detection of cancer and, in his mind, proteomics is the technology that can best accelerate the discovery of the cancer biomarkers that will drive early detection. From this conclusion, he went on to develop a white paper titled *Clinical Biomarkers Discovery Initiative*, which he put forth as **one** possible development model that he hopes will serve as a vehicle for discussion and input from the research and development community.

To begin this discussion, Dr. Hartwell laid out his arguments for NCI to fund an initiative in clinical proteomics, stating that, at present, discovery of biomarkers is the limiting step for improved early detection of cancer. While this will be a challenging endeavor, given the potential of various proteomics technologies, it should be possible to identify a sufficient number of biomarkers, at a relatively low price, to revolutionize early detection. Dr. Hartwell chose to emphasize early detection because it has been proven to be the best way of dramatically improving clinical outcomes for patients. As examples of early detection strategy, he cited the progress made in treating colon cancer and cervical cancer.

As far as cost is concerned, it should be possible to develop a panel of 60 biomarkers for early detection for as little as $6 million (or $100,000 each) if the validation trials are

conducted along with existing clinical trials. This is compared to development costs of $800 million for a new drug and $100 million to conduct a prevention trial. Dr. Hartwell noted that not many biomarkers are in the testing and validation pipeline, so reagents are a primary expense at this point.

Indeed, he added, this has not moved forward rapidly despite technological advances because the individual investigator does not have access to reagents; these need to be supplied to the research community globally. He added that validation of biomarkers should not be done in individual trials, but rather in multiplexed diagnostic validation trials run in tandem with therapeutic effectiveness trials. In contrast to the drug development pipeline, which begins by screening huge numbers of molecules and then testing only one at a time, the biomarker pipeline will identify candidate biomarkers and then test each one in tissue and blood of 100 cancer patients and healthy patients to ensure that the marker is capable of distinguishing cancer from non-cancer (Figure 2).

Blood protein biomarkers for cancer are likely to exist, Dr. Hartwell noted, since tumors and the vessels around them are leaky. It is easy to estimate that 1 million different types of proteins are produced by the body, but our current tools can measure only about 100 types. However, even with current technologies, a more systematic approach should allow us to go deeper into the proteome. In fact, he added, it should be relatively straightforward to screen 1,000 biomarker candidates at a time and 10,000 random proteins.

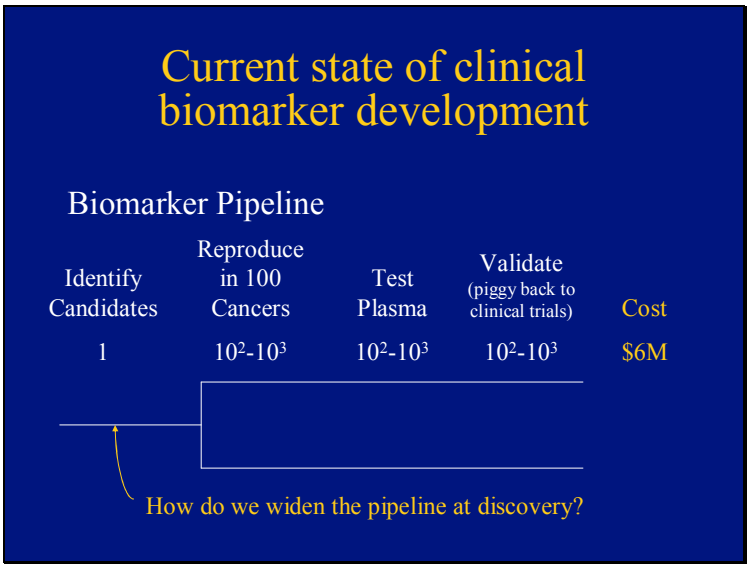

Figure 2. A model for a biomarker development pipeline

Next, Dr. Hartwell discussed two possible methods for identifying biomarker candidates from tissue and fluid samples. One method involves a search for candidates based on likely biological properties; the other is based on the function of likely candidates (Figure 3). He also noted a third method—looking randomly at all proteins—but did not discuss this approach in detail.

Searching for biomarkers by properties would entail looking for proteins that are differentially expressed, are found on the cell surface or secreted, are found either with or without albumin, are of low molecular weight, or are glycosylated, phosphorylated, ubiquinated, or methylated. Searching by function would involve looking for biomarkers associated with angiogenesis, lymphogenesis, metastasis, DNA repair, proteolysis, mitosis, stress response, growth signaling, wound healing, and other processes that may be associated with cancer. Dr. Hartwell noted that the reagents needed for this approach have yet to be developed to any great extent.

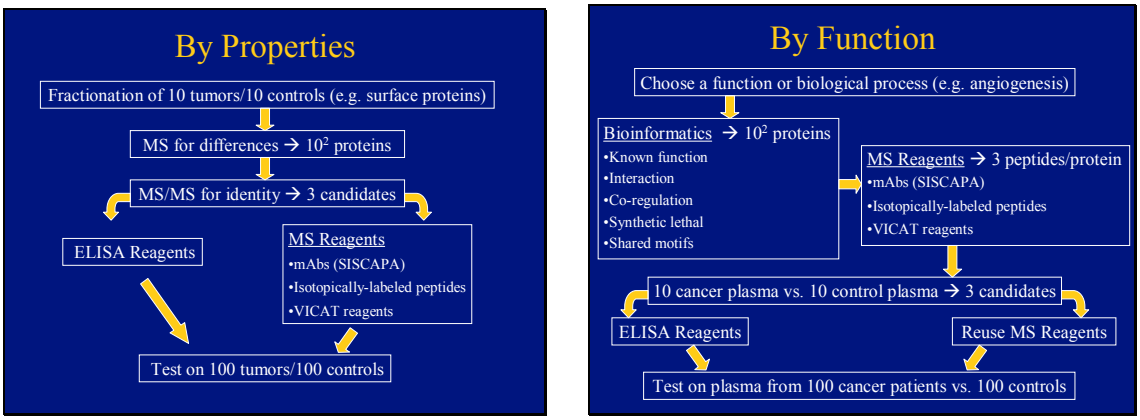

Figure 3. Schemes for biomarker discovery based on properties and function

Outlining how development efforts would proceed for the two approaches, Dr. Hartwell laid out two different development pathways, both of which would lead to testing a panel of biomarkers. Both approaches would also entail development of a host of new reagents and refinement of mass spectroscopic methods. The goal of both pathways would be to reach what Dr. Hartwell called validation stage 1: showing that a biomarker is capable of distinguishing those individuals with cancer from those without it.

Once a biomarker-based diagnostic test completed this stage of validation, the private sector would assume responsibilities for the final stages of development and validation. Dr. Hartwell estimated that this approach would yield 3 validated biomarkers from every 100 identified as possible candidates. For the "properties" approach, reaching validation stage 1 for a single cancer site would cost approximately $2 million, while the "function" approach would be more costly for the first biomarker identified, perhaps as much as $18 million to $20 million (Figure 4). Since many of the reagents developed and used in the functional approach can be reused for other cancer sites, the cost for subsequent sites would drop to approximately $4 million. Dr. Hartwell also noted that having a supply of reagents available to the research community is critical for either of these two approaches to succeed at the lowest cost and in the shortest time possible.

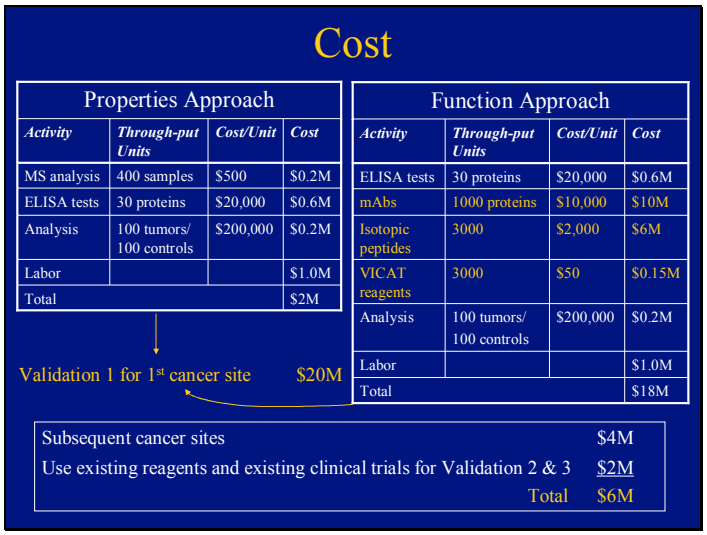

Figure 4. Projected costs of biomarker discovery and validation through stage 1

Dr. Hartwell then closed his presentation with a list of goals for a coordinated clinical proteomics and biomarker discovery initiative:

- Establish criteria and centers for testing biomarker discovery technologies in order to define an effective pipeline for discovery:
  - Develop a technology assessment site to compare competing technologies head to head on identical samples from mouse models of cancer.
  - Identify technology innovations:
    - Pilot grant funding
    - NCI's UIP and Innovative Molecular Analysis Technologies (IMAT) programs
  - Encourage academic/industry collaborations.
  - Promote engineering to improve reproducibility, throughput, and automation.
- Develop a publicly available informatics platform that permits data storage, analysis, searching, and comparison.
- Establish a consortium of collaborating laboratories to discover biomarkers in particular cancer sites and for particular classes of biological molecules.
- Establish repositories of reagents for clinical biomarker discovery, available to the community.
- Promote the translation of new imaging agents to clinical trials.

General Discussion Session

Dr. Hartwell opened the discussion by asking whether anyone in the assembled group took exception to the idea that the systematic approach and program defined in the white paper would be very important in advancing the field of proteomics. The general agreement was that such an effort was needed at this time. Several participants asked whether NCI is fully committed to the initiative. Dr. Greg Downing, Director of NCI's Office of Technology and Industrial Relations (OTIR), assured participants that it is. Attendees also expressed concern that other proteomic efforts, including individual research laboratory programs, might receive less support as a result of the larger initiative. The general consensus was, however, that the initiative's emphasis on shared resource development and technology validation is an appropriate—and necessary—approach. Participants noted that this kind of "bricks and mortar" science—developing thousands of probes, setting standards, and comparing technologies—is best done in a consortium-directed environment rather than by individual investigators. Questions were asked about the future of the IMAT program, which has played a critical role in cancer technology development. Dr. Downing said IMAT, which is directed from the OTIR office, would be continuing.

The remainder of the meeting was devoted to a discussion of what is needed to drive a successful effort using proteomics for biomarker discovery. Participants repeatedly noted that one of their key concerns is the current lack of shared resources, such as common reagents, among proteomics researchers. Currently, each research laboratory must make its own antibodies or other kinds of probes and must collect its own tissue specimens. It was noted that registries tend to be reluctant to part with their samples. Several attendees emphasized that any new initiative should work with existing programs, such as the Early Detection Research Network (EDRN), Specialized Programs of Research Excellence (SPOREs), and mouse consortia, that are developing models of human cancer.

On the topic of shared resources, attendees also said that although it would be beneficial for laboratories to gain access to other laboratories' data to compare and confirm their own findings, safeguards must be built into the process so that data do not become released to the larger community before the original investigators are able to publish their findings. Without such safeguards, participants were not sure that all laboratories would participate in setting up a common set of data platforms. Another concern voiced was that any data sharing must occur within the context of intellectual property concerns and the ability for laboratories, both private and public, to translate discoveries into products. Attendees agreed that one solution would be for the NCI to set up a central laboratory and data repository and act as a disinterested party for data sharing and dissemination.

The topic of searching for early versus late markers was raised, with one participant saying it was important to focus on early biomarkers as well as late ones because early markers would offer greater therapeutic success for a greater number of patients. Another participant stressed that a systematic proteomics initiative must look for biomarkers for general poor health as well as specific cancers. Biomarkers for lung cancer, for example, must be compared with those for other lung diseases in order to determine which of the

biomarkers are specific for cancer versus markers that are indicative of overall poor health or ongoing inflammatory processes, for example.

Part of the remainder of the discussion focused on the need for high-quality human tissue samples. One researcher said that NCI must spend the time and money now on this issue to avoid a situation 5 years from now in which, as a result of new advances in proteomics technology, we are unable to analyze clinical tissues in a meaningful way because of poor quality of the samples—a situation that could result in 99 percent false positives. Many participants called for the establishment of reference centers that could determine how to uniformly process and store samples, developing standard operating procedures for the field to use. One researcher noted that this was successfully done in the development of cholesterol measurements. Some participants felt that if the protocols for collecting and storing human tissue samples were precise and robust enough, the same level of homogeneity could be reached with human samples as with mouse models. It was acknowledged, however, that the technology needed to ensure homogeneity of human samples does not yet exist, which is why it is important to work on mouse models in parallel with human studies. The importance of collecting longitudinal human tissue samples was also stressed, although it was noted that such studies are very challenging.

Dr. Hartwell asked participants to discuss what technologies, other than mass spectrometry and protein chips, are available for protein detection. Both antibody-based detection methods and aptamer technology were mentioned. Later in the discussion, concern was expressed that a large, centralized proteomics initiative might cause some technologies to be ruled out too soon. Attendees cautioned that premature judgments about proteomics technologies could stifle innovation. One researcher suggested that NCI support parallel development of technologies. Those working on the various technologies could then be brought together periodically to discuss and compare their findings. This approach, the researcher said, would help scientists find synergies among the different technologies.

The question was also raised as to how proteomics technologies will translate into useful, clinical diagnostic instruments. Several participants urged NCI to be a stronger advocate for biomarker detection, noting that the private sector has expressed little interest in it thus far. It was acknowledged that if biomarkers could be shown to be good indicators of therapeutic response, the private sector might become more interested in supporting proteomics research. One participant suggested that NCI, through its sponsorship of clinical trials, could leverage researchers to include validation of biomarkers as part of the overall design of a clinical trial. Several participants commented that such dual-purpose trials would enable biomarker validation in a very cost-effective manner. Attendees also felt that NCI could help the proteomics field by encouraging the U.S. Food and Drug Administration (FDA) to allow biomarkers to be used as surrogate endpoints, particularly when validated as part of the clinical trials process. Dr. Downing said that NCI is currently working with the FDA to define biomarkers and their various potential uses, including their use in diagnostics.

Another broad area of discussion involved intellectual property issues. Attendees generally agreed that such issues were of paramount importance to the progress of proteomics research, but how these issues were going to be successively resolved was unclear. Dr. Hartwell said that, ideally, markers would be licensed nonexclusively to any company that wants to develop them to market, but he acknowledged that persuading laboratories and academic institutions to license nonexclusively is going to be difficult. Attendees concurred, with some noting that nonexclusive licensing is not a problem at their respective institutions and others noting that it is. The general consensus was that NCI should take a role in coordinating proteomics-related intellectual property issues.

Dr. Downing closed the meeting by thanking the participants for their comments and suggestions on how to best design a proteomics initiative that would accelerate clinically useful biomarker discovery. He reiterated that Dr. Andrew von Eschenbach, Director of NCI, and Dr. Anna Barker, NCI Deputy Director for Strategic Scientific Initiatives, are fully committed to the initiative. He then assured the participants that NCI will continue to solicit their input and help in developing this plan.

Synopsis of Key Points

Following the meeting, Dr. Hartwell offered the following synopsis of the key recommendations that came from the day's discussions:

- There was general agreement that a more systematic effort in biomarker discovery is warranted at this time.
- High-quality reagents, such as antibodies and isotopically labeled peptides, are needed.
- A high-quality database with algorithms for processing and analyzing mass spectrometry data is needed.
- Any new proteomics program should integrate with existing programs, such as EDRN, mouse consortia, and SPORE grants.
- A new program should encourage innovation, especially improvements in technology.
- Access to high-quality tissues is crucial.
- It is important to use mouse models as well as human tissues.
- A new program should recruit innovations already existing within the community.
- A database of candidate biomarkers already discovered in the community should be constructed.
- There is a need to define a molecular diagnostic pipeline. Standards for validation should be established at each step.
- Academic investigation of diagnostic markers needs to progress to higher levels of validation in order to attract industry.
- Other diseases and institutes at NIH should be considered for inclusion.
- It is necessary to consider how a marker will be used clinically.
- The program should start with one tumor site to prove that a systematic approach is effective.
- The emphasis should be on functional information and therapeutic response for end goals as much as on early detection.

- The program should recruit the most effective scientists and maximize resources through a GLU grant mechanism.
- A systematic examination of collection and storage issues should be done.