The diverse and fundamental biology involved in the disease we collectively call “cancer” has presented a formidable problem to scientists and medical laboratories. The complexity of the disease warrants an integrated methodology for research, diagnostics, and therapies. At the center of the biology of the disease is the human leukocyte antigen (HLA) system (also referred to as the major histocompatibility antigen [MHC]), the body’s immune-surveillance system that screens cell-surface proteins and targets diseased cells for elimination. Understanding the complexity of this natural protein-screening chip is possibly the clinician’s greatest ally in conquering cancer but also presents an enormous technical challenge for laboratories striving to understand how this information can be used to diagnose and treat disease.

Innovative tools are being developed that hold promise for speeding the discovery of new HLA-related cancer biomarkers that both enable a reliable diagnostic test and also present validated targets for therapy. Further, disease-related HLA complexes can be targeted by specific antibodies that facilitate the development of vaccine and therapeutic solutions. This review summarizes the new approaches to cancer and presents how knowledge of HLA proteomics, or “HLA-omics,” is yielding powerful, specific diagnostics for tomorrow’s medical laboratory and effective cancer treatments for the clinician.

Cancer is often characterized as a single disease. In fact, cancer is a general term describing over 100 individual diseases characterized by uncontrolled cellular proliferation. Worldwide, cancer affects >10 million people each year. In the United States alone, more than 1.4 million new cases of cancer are diagnosed each year. In contrast
with the reduced occurrence of many communicable, infectious diseases (generally through prophylactic-vaccination, public-health, and sanitation strategies), cancer incidence continues to rise — with a 2.4% growth rate projected for the next decade. \(^5\) More than 75% of new cancer cases occur in patients above the age of 60. As the world population ages, the frequency of cancerous events, as well as cancer-associated mortality and mortality statistics, will increase.

The most prevalent form of cancer is non-melanoma, basal-cell carcinoma — with ~900,000 new cases diagnosed in the United States each year. \(^2\) Although locally invasive, basal-cell carcinoma almost never metastasizes and, if identified and treated early, long-term prognosis is excellent. Of invasive cancers, prostate, lung, and breast cancer have the highest incidence of new cases (15.1%, 14.7%, and 12.5%, respectively), but lung cancer far exceeds other cancer in terms of mortality, accounting for 28.5% of all cancer deaths \(^7\) (see Figure 1).

### Cancer diagnosis and detection

Cancer diagnosis relies primarily on histological examination, which has its limitations that may lead to the method being augmented by several innovative approaches. Once diagnosed, cancer staging is primarily based on the TNM (tumor, node, metastasis) system, \(^3\) which aggregates data regarding tumor size/depth, lymph-node spread, and presence or absence of metastases to establish reliable predictors of survival, choices of treatment, and “fit” into clinical trials. \(^5\) Although widely used, the TNM system exhibits several problems, including the fact that it does not distinguish anatomically similar tumors that may not exhibit the same physiology or response to treatments. \(^5\)

New treatments have shown ability to successfully address subsets of tumors with similar TNM staging. Additional criteria, including the characterization of various molecular markers, can differentiate visually similar tumors into categories that can receive more focused or appropriate treatments. \(^5\) Certain therapeutics show effectiveness only if the respective molecular marker they target is expressed on the cancer cell — these therapeutics include trastuzumab (Herceptin), cetuximab (Erbitux), imatinib (Glivec/Gleevec), and others. These molecular markers that allow predictions as to responsive tumors to targeted treatments have led to the recognition that at least some biomarkers can successfully be used not only to stage but also to predict best treatments for at least some cancers.

Cancer biomarkers fall into two primary categories, shared-antigen and unique antigens. \(^5\), \(^6\), \(^7\), \(^8\) Shared antigens are proteins or peptides that are expressed in normal tissues but are over expressed in cancerous cells. In contrast, unique antigens appear to be only expressed in cancer cells. Treatments targeting unique antigens benefit from the linkage of a diagnostic test that identifies patients for treatment with the therapy targeting the cancer, such as detection of the c-erbB-2 antigen. \(^9\), \(^10\), \(^11\) Approaches targeting unique antigens focus the therapeutic effects on cancer cells and show little effect on non-target tissues. In contrast, therapies focused on shared antigens show a preferential effect on cancer cells due to the over expression of the antigen. Since the target is shared with other non-diseased cells, however, off-target effects can occur. The challenge has been to identify additional unique antigens to diagnose cancers with high degree of specificity and sensitivity, and integrate these into therapeutic approaches.

Various methods have been used to identify effective diagnostic biomarkers, including DNA-based, RNA-based, and protein-based or protein post-translational modification status. \(^5\), \(^6\) It has been challenging to validate the presence of a particular biomarker with a particular cancer, to demonstrate lack of off-target expression, and, finally, to develop a specific and sensitive assay to detect the antigens. Curiously, most Food and Drug Administration- (FDA-) approved biomarkers are not used in standard clinical practice and relatively few have been successfully used for therapeutic targeting. \(^5\) Discovery of new cell-surface biomarkers that are both easily detectable and effectively treatable is an important strategy employed by cancer researchers.

### Cancer therapies

Despite the fact that more than 138 new cancer therapeutics have been approved for use in the United States over the last 60 years, the primary treatment regimen for most cancers still includes surgery and radiation therapy. \(^1\) The approved drugs include various cytotoxic chemotherapeutic agents, hormone therapies, radiation therapies, adjunctive therapeutics, and immunotherapeutic approaches. \(^7\) The effectiveness of cytotoxic therapies has been called into question by recent studies, and the contribution and cost effectiveness of these therapies must be seriously considered. \(^12\) The advent of immunotherapeutic approaches has provided a treatment avenue with less morbidity and a greater level of patient improvement. \(^7\)

Immunotherapeutic approaches vary from the application of cytokine therapy, \(^13\) treatments with disease-associated peptides bound to HLA class I molecules, cellular vaccines, gene-based expression, oncolytic viruses, and monoclonal-antibody therapies. \(^7\), \(^9\), \(^10\), \(^14\) Although encouraging results have been documented for several vaccination approaches, no approved therapy has emerged. The use of monoclonal antibodies for therapeutic intervention in cancer has witnessed the most success. For women expressing the Her2/neu antigen in breast tumors, the use of trastuzumab, a recombinant monoclonal antibody recognizing the Her2/neu antigen, is accompanied with improved prognosis. \(^11\), \(^15\) The use of rituximab, a monoclonal antibody binding the CD20 antigen predominantly expressed on mature B cells, has shown durable clinical effect and is now a first-line treatment for non-Hodgkin’s lymphoma. \(^6\), \(^17\)

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**Cancer biomarkers**

**Figure 1.** T-cell receptor mimics (TCRms, lower panel) exhibit similar binding affinity to cytotoxic T lymphocytes (CTLs, <150 pM), and as a soluble reagent serve as an alternative to cell-based binding assays.

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*Cancer therapy continues on page 14*
In spite of these new and promising treatment options, cancer morbidity and mortality continues to be on the rise. Over the past decade, mortality from cancer shows, at best, single digit reductions — indicating that present treatment approaches are not adequately meeting the growing medical need. New methods to diagnose cancer at earlier, more treatable stages and new therapeutic modalities are urgently needed.

Characterization of HLA-peptide complexes

Characterization of peptides presented on the surface by HLA has been the subject of intensive investigation, both predictive and empirical. Many algorithms have been developed to predict which peptides will likely emerge from cellular processing and HLA presentation of a disease-related protein. The limited number of actual peptides presented on diseased cells by class I molecules, coupled with the relatively few molecular structures characterizing HLA-peptide interactions, limit the predictive power of these algorithms. Although attractive, the difficulties with existing predictive methods necessitate a more empirical approach in order to understand the nature of the actual HLA-peptide complexes.

A range of reverse immunological approaches has been used to identify peptide interactions with specific HLA molecules. These approaches generally rely on the ability of active CTLs to guide researchers to relevant peptides presented on HLA class I molecules. Using this reverse immunological approach, a few peptides have been identified that are specifically related to diseased cell expression and CTL activity. 

Alternatively, researchers directly characterize the peptides loaded on HLA class I molecules present on the surface of diseased cells, but traditional approaches relying on immunoprecipitation suffer from poor yields of protein-peptide complexes and an incomplete picture of the HLA proteome of a diseased tissue.

To facilitate the identification of bound peptides, Hildebrand and colleagues have developed an innovative approach for characterizing the HLA-expressed peptides in an efficient and non-biased manner through the expression of soluble HLA, or sHLA, molecules in diseased cells. In short, the sHLA complexes are engineered to be secreted from the cell, enabling an efficient isolation and characterization of the HLA-peptide complex through peptide separation and mass spectrometry. Using approaches for direct identification of an isolated peptide, disease-related peptides have been identified from cancer cells and virally infected cells. The peptides represent specific markers that may identify diseased cells from a population of healthy cells.

Initial validation studies of a newly discovered disease-related peptide include confirming that the RNA transcript and/or protein associated with the identified peptide are, indeed, differentially expressed in diseased cells versus healthy controls. Preferably, expression of the antigen will be strongly associated with the disease condition. HLA presentation of the peptide, however, may be uniquely associated with a diseased condition, in spite of ubiquitous expression of the parental intracellular protein, as found for several peptides associated with HIV infection. Peptides that qualify based on the expression criteria are then tested for immunological relevance. Tests may differ; but, often, peripheral blood mononuclear cells, or PBMCs, are isolated from an infected or otherwise diseased individual; and the ability of the peptide to stimulate interferon-gamma, or IFNγ, or another surrogate for CTL activity is measured, usually by enzyme linked immunosorbent spot, or ELISpot, assay and intercellular cytokine production. A peptide may emerge from these studies as a vaccine candidate (stimulating appropriate T-cell responses) or as a target for other therapeutic agents.

The final validation is to measure the expression on the surface of diseased cells. Such analysis is essential since vaccine and therapeutic antibody approaches to treat disease, either cancer or pathogen infection, require a threshold amount of peptide displayed on diseased cells to effectively distinguish for treatment success.
standard reagents, quantitation of peptide expression is a very challenging prospect. Recent discoveries, however, have shown the value of T-cell receptor mimics, or TCRm — monoclonal antibodies recognizing a specific peptide-HLA complex — to measure the expression of particular HLA-peptide complexes on the surface of cells (see Figure 2). TCRm antibodies can be generated by either screening phage libraries for reactive antibodies or by immunization of animals and isolation of monoclonal antibodies through standard hybridoma techniques. The phage-display methodology benefits from the speed at which a candidate can be identified but may be limited by the lack of affinity maturation in phage libraries, providing insufficient complexity to generate a highly discriminating antibody. Although requiring more time, standard immunization and hybridoma techniques promise high-affinity TCRm antibodies that readily discriminate HLA-peptide complexes and that can be immediately used for quantitative purposes. These TCRm antibodies have specific detection abilities at concentrations <150 pM, similar to the high-avidity CTL lines classically used in binding assays. The ability of TCRm antibodies to discriminate specific HLA-peptide complexes on diseased cells enables empirical discovery of cancer biomarkers that may soon be screened routinely in the medical laboratory.

**Use of HLA-bound peptide discoveries in therapeutic vaccine development**

Peptide-based vaccines have been reported to be effective in treating both viral diseases and cancer in experimental models and in humans. The use of validated peptides offers a facile manner to directly stimulate the immune system to respond to relevant pathogen antigens using a readily defined product. In contrast to the use of defined antigens, many have used tumor-cell lysates, proteins associated with heat-shock proteins, or tumor-derived RNAs as the vaccine modality. In these approaches, proteins or RNA are used to stimulate autologous professional antigen-presenting cells (pAPCs), usually dendritic cells due to their efficiency in presenting MHC-peptide complexes in concert with appropriate co-stimulatory molecules. The stimulated pAPCs process the protein antigens provided as direct protein or translated from transfected RNA. Once reinfused into patients, the pAPCs present relevant tumor antigens via HLA class I to the immune system and stimulate a potent, systemic immune response against diseased cells. These approaches are complicated by the lack of precision defining the nature of the antigens in most cancer vaccines and that each vaccine must be prepared for each patient — so no batch-based release criteria can be applied to the manufacturing process. This, coupled with the fact that less than 50% of the predicted peptides identified as potential immunogens actually generate CTL-mediated killing of tumors, strongly suggests that assays that demonstrate the potency of the prepared vaccines need to be performed before clinical application.
Application of TCRm antibodies to quantitatively measure vaccine potency

Potency of cancer vaccines needs to be measured at two points: 1) at the end of the manufacturing process when the drug substance is prepared for clinical use and 2) during clinical use measuring the patient’s response to a particular vaccine composition, administration regimen, vaccine dose, and schedule. Present means of measuring vaccine potency are generally qualitative or semiquantitative in nature. Labeling of cells using flow cytometry or traditional enzyme-linked immunosorbent assay, or ELISA, methods do not adequately address the function of the vaccine — including antigen uptake, processing and presentation, and subsequent immune response. These assays rely on inherently empirical biological materials, including T-cell clones or human peripheral blood lymphocyte populations, to produce quantitative, precise, and reproducible results when the condition of the cell culture, other biological samples, instruments, and users can differ between applications. In addition, the reliance on cell-based reagents, with their inherent drift in properties, and experience of assay bias complicates quality-assurance efforts in assay standardization. These current shortcomings encourage the development of new methods providing a quantitative measure of potency for both defined-antigen and mixed-antigen vaccines.

The laboratory of Dr. Jon Weidanz has recently used TCRm antibodies to characterize the potency of cancer vaccines by directly detecting and quantitating the MHC-peptide complexes on dendritic cells as a surrogate marker for assessing CTL responses (see Figure 3). The TCRm antibodies confirmed antigen-specific CTL activities by inhibiting the stimulation of T-cells in a specific manner and confirming epitope presentation on vaccine-treated dendritic cells. The density of peptide display directly correlates to the degree of CTL response to vaccines. TCRm antibodies allow the kinetics of peptide-HLA class I presentation to be characterized in dendritic cells exposed to vaccine antigens as well as quantitation of the density of specific peptides displayed in class I complexes. In a related application, TCRm antibodies can be used to establish a real baseline to standard immunological assays, such as ELISpot assays.

Use of TCRm antibodies as diagnostic reagents and therapeutics

TCRm antibodies have been shown to possess exquisite binding specificity — identifying specific HLA-peptide complexes on cell lines induced to present the target peptide, identifying dendritic cells primed with vaccine antigen or, conversely, blocking CTL stimulation by occupying relevant HLA-peptide complexes. Further, TCRm antibodies can identify specific HLA-peptide complexes on the surface of diseased cells and trace intercellular trafficking of these complexes with high precision. The impressive specificity of TCRm antibodies coupled with their ability to recognize validated disease biomarkers in the form of particular HLA-peptide complexes suggests they represent new tools for disease diagnosis (see Figure 4). TCRm antibodies could be used in several existing and innovative diagnostic platforms. Through in-situ tissue-staining methodologies, the penetrance of a cancer phenotype can be directly visualized by TCRm binding to disease-related HLA-peptide complexes, thereby delineating potential tumor boundaries. The data emerging from these in-situ analyses is complementary to standard TNM anatomical analysis and provides confirmatory evidence regarding the disease...
state of biopsied tissue. Based on this diagnosis, treatment strategies can be more confidently pursued or additional tests can be ordered to address areas of concern revealed through the analysis of cancer markers. As a more innovative approach for disease diagnosis, TCRm antibodies conjugated with a contrast agent can be infused into patients, passively circulated through the body, and allowed to bind disease-related HLA-peptide complexes wherever present in the body so that they can be imaged. These binding events provide a non-biased survey of most body tissues without surgical intervention. Suspect sites identified by TCRm binding may require evaluation by other, more traditional tomographic imaging methodologies. Finally, TCRm antibodies could be deployed using a microfluidics platform to capture and identify diseased cells obtained from tissue biopsy or body fluid samples.

Therapeutic antibodies successfully treat a range of diseases, including infectious disease (palivizumab: Synagis), chronic inflammatory disease (e.g. infliximab: Remicade), or cancer (e.g. trastuzumab: Herceptin, rituximab: Rituxan, bevacizumab: Avastin). The success observed with treatment of cancer has lead to remarkable growth in this particular sector with greater than 50% of all new biologics being therapeutic monoclonal antibodies. The mode of action of antibodies used to combat cancer varies — but their exquisite binding specificity to disease-associated surface antigens supports their therapeutic action with predictable patient safety. Further, the ability of therapeutic antibodies to recruit components of the adaptive immune system appears central to their success as cancer or infectious-disease therapies. Trastuzumab and rituximab have been shown to promote antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and researchers have attributed at least a portion of their therapeutic success with these activities. Indeed, TCRm antibodies have shown remarkable promise as therapeutic modalities. TCRm antibodies showed the ability to induce both efficient CDC and ADCC when exposed to cells expressing the appropriate HLA-

Figure 4. TCRms may provide a useful tool for cancer research, diagnostics, and therapeutics in a single, soluble reagent.

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peptide complex.\textsuperscript{36} Further, specific TCRm antibodies were able to inhibit breast-cancer carcinoma-tumor growth in murine models when provided prophylactically and also resolve existing tumors when provided as a therapeutic agent.\textsuperscript{36,54} TCRm antibodies show similar specificity to cell-surface-expressed disease markers, leading to the intriguing possibility that these molecules may represent a new modality for the treatment of cancer.

**Emerging technologies**

Cancer continues as a major force negatively impacting human health and is predicted to grow in frequency due to our aging population. Biomedical researchers face numerous challenges to develop rapid and accurate methods to diagnose and treat the various cancer conditions.

An emerging suite of technologies offers new options for
1. discovery of new disease associated antigens;
2. validation of the new antigens as targets for the human adaptive immune response;
3. integration of newly discovered antigens into diagnostic assays;
4. establishment of potency measurements for cancer vaccines and human immune responses; and
5. development of new therapeutic modalities to specifically and effectively treat cancer and potentially other diseases.

These technologies provide a continuous process extending from disease-biomarker discovery to TCRm-antibody integration into relevant diagnostic and therapeutic options. Further, the development of TCRm antibodies, in particular, offers the potential to use a single agent in several different applications, including vaccine-potency measurements, diagnostic assays, and therapeutic interventions. Continued research is necessary to confirm the potential of these systems. Published data, however, provide strong support for this new integrated approach.

In summary, laboratories will be continuously challenged to deliver timely answers for both early detection of cancer and effective management of the disease. Newly emerging technologies will lead to new diagnostic tools that provide hope for laboratories to keep pace with the ever-growing health need for the rapid and accurate diagnosis of the multitude of cancers that adversely affect the global population.

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**References**

31. Byrne JA, Oldstone MB. Biology of cloned cytotoxic T lymphocytes specific for lympho-

30. Expert Rev Proteomics

28. Sandberg JK, Fast NM, Nixon DF. Functional heterogeneity of cytokines and cytolytic

26. Witzig TE, Gordon LI, Cabanillas F, et al. Randomized controlled trial of yttrium-90-


24. Rosenberg SA, Yang JC, Schwartzentruber DJ, et al. Immunologic and therapeutic evalua-


20. Clark CE, Vonderheide RH. Getting to the surface: a link between tumor antigen discovery


14. Whiteside TL, Gooding W. Immune monitoring of human gene therapy trials: potential appli-


2. Kalorama Information. Monoclonal Antibodies, Vaccines and Other Immunological Cancer