Welcome to the 10th International Cancer Immunology Symposium sponsored by the Cancer Research Institute (CRI). These meetings have documented the remarkable progress made over the past ten years in understanding the interactions between the immune system and cancer and in efforts to develop effective cancer immunotherapies. Immunotherapeutic strategies for cancer, mainly antibodies, vaccines, and the adoptive transfer of T cells, are being actively explored in the clinic and are receiving greater and greater attention from the pharmaceutical industry. As we have heard at these meetings, the science of cancer vaccine development has become increasingly strong, and there is now a growing belief that for the first time in the history of the field the idea of therapeutic cancer vaccines can be critically tested. This conviction is based on the revolutionary conceptual and technical progress in our understanding of the immune system and on four major advances in cancer immunology:

(i) The resurgence of cancer immunosurveillance as a unifying principle in the field,
(ii) the development of powerful methodologies to define the human cancer immunome, that is the complete repertoire of cancer products that are immunogenic in humans and therefore potential targets for vaccine development,
(iii) the establishment of a range of sensitive and standardized methods to monitor humoral and cellular immunological responses to tumor antigens, and
(iv) the availability of a wide array of approaches to generate vaccines, from peptides, proteins, DNA, viral and bacterial vectors, to new adjuvants, delivery systems and immunomodulatory cytokines.

It is no surprise that many of these considerations are also central to our thinking about the development of HIV vaccines, and so it seems eminently reasonable that there should be a close coordination between the activities of the two fields. That there has not been more efforts in this direction is understandable, given the daunting organizational, logistic and financial challenges confronting each of our vaccine initiatives, and also because there has been essentially no success stories with either HIV or cancer vaccines, or pressure from national or international funding bodies to coordinate our vaccine efforts. However, this is the precise reason why now would be an appropriate time to form alliances and to use this unique historical opportunity to establish the principles of effective immunization in humans, not just for HIV and cancer, but for the host of other infectious diseases that confront humans and that cannot be controlled by our current vaccines. This was the thinking that prompted this symposium on the development of cancer and HIV vaccines, and I am grateful to Bruce Walker, Andrew McMichael and Alex Knuth for their participation in organizing this meeting.
A stellar group of investigators who are leaders in the HIV and cancer vaccine arena has been gathered here for our discussions. To begin, let me briefly survey the background and current status of cancer vaccines and then Norman Letvin will give a comparable perspective for HIV vaccines.

The idea that cancer is naturally restrained, both in its origins and its growth, by immunological forces is one of the oldest ideas in cancer research. It had its formal introduction by way of the immunosurveillance hypothesis of Burnett and Thomas in the 1950s, and the idea enjoyed an enthusiastic if uncritical acceptance during the 60s and early 70s. However, the finding that immunocompromised nude mice did not develop more tumors, either induced or spontaneous, led to a general, if equally uncritical, rejection of the idea. With the availability of multiple mouse models having selective defects in components of the immune system, cancer immunosurveillance has been reevaluated, and a large body of evidence coming from the work of Bob Schreiber and Mark Smyth and their groups has shown that the immune system recognizes and eliminates cancer cells, as indicated by a higher frequency of cancer in immunocompromised mice. And where immunity does not eliminate cancer, it shapes the immunogenicity of the developing tumor cells.

I know of no concept in cancer research that has been as controversial as cancer immunosurveillance, so it is not surprising that there is still some resistance to its acceptance, although that also seems to be rapidly disappearing. What is clear is that the immune system is involved in far more than determining whether a tumor emerges or not, the endpoint of the original immunosurveillance idea, but that the tumor and the immune system influence one another throughout the life history of the tumor, what Bob Schreiber calls immunoediting, carrying out a continuing Darwinian battle that shapes the immunogenicity of the tumor, and results in either a victory for the host, or more usually, a victory for the tumor after periods of unstable equilibria. A question of immense importance is the magnitude of the immunological forces that the body can marshal against tumor development and growth. A powerful insight into the potential power of immune protection against cancer comes from the work of Zheng Cui at Wake-Forest on what I have called "the remarkable mouse". Cui and his colleagues have identified a mutant BALB/c mouse whose innate resistance to challenge with transplantable cancer cells is unprecedented (1). This mouse can resist millions of tumor cells on initial challenge, and several populations of cells involved in the immune response, including monocytes, NK cells and PMNs, mediate rapid and efficient tumor cell destruction in the absence of prior exposure to the tumor. Identification of the mutation and how protection of this sort can be generated in normal animals are questions of great interest. None of the immunomodulating agents studied over the past 50 years that increased resistance to tumor induction and challenge, from Coley's toxins to bacille Calmette-Guerin (BCG), C. parvum to CpG, IL-12 and alpha-galactosylceramide, comes anywhere close to the effectiveness of Cui's mutation. Nonetheless, the effect of BCG in superficial bladder cancer in humans (Figure 1), the first immunotherapy with a consistent therapeutic response rate in a significant percentage of patients with a solid tumor, provides some insight into the promise of modulating innate immunity in patients with cancer.

Now immunological protection is the expected and desired endpoint of our cancer vaccine strategies. However, we must be aware that our efforts can lead to a state of immunological suppression, in which susceptibility to tumor induction and growth is enhanced, and not inhibited. This phenomenon, originally described by Nat Kaliss in the middle of the last century with allografted tumors, was called tumor enhancement and was induced by immunization with lyophilized tumor tissue and mediated by antibody. A possibly related form of immunological suppression was discovered by Dr. Sakaguchi, a speaker at this symposium, and is mediated by regulatory CD4+ CD25+ T cells, cells crucial in inhibiting the development of autoimmunity. However, what is good for protecting against autoimmunity is bad for protecting against cancer, and this lesson is forcefully coming home from experiments by Dr. Nishikawa and Dr. Shiku and their colleagues in experimental systems. DNA immunization with widely expressed autoantigens leads to a state of enhanced susceptibility to challenge with transplantable tumors (3).
The response is dependent on CD4+ CD25+ cells and these cells have a potent inhibitory action on NKT cells, as well as on CD8+ and CD4+ T cells. As shown in Figure 2, Nishikawa and Shiku have now found a remarkable acceleration in the induction of chemically-induced tumors in mice developing regulatory CD4+ CD25+ cells following immunization with the Dna J-like 2 autoantigen and other autoantigens, and this finding is of the utmost importance with regard to understanding early events in tumor formation. As a footnote, Dick Gershon would not be at all surprised by all of this interest in regulatory T cells in cancer, but might wonder why they are not called suppressor T cells, as he originally named them. From the perspective of efforts to develop cancer vaccines, these findings of Nishikawa and colleagues on CD4+ CD25+ cells alert us to the fact that vaccination and immunization, despite their usual association with protection, can have just the opposite effect and we must be prepared in our clinical trials to spot this undesired effect at the earliest possible time.

Let us now move on to the great success story of cancer immunology over the past decade and a landmark in cancer vaccine development - the identification and molecular characterization of immunogenic human cancer antigens - a discipline I refer to as cancer immunomics. The three major steps in this development were (i) the...
establishment of suitable autologous in vitro systems for identifying specific humoral and T cell immune responses to human tumor antigens by our group, (ii) immunomic cloning methodologies such as T cell epitope cloning, introduced by Thierry Boon and his colleagues, and SEREX (serological identification of antigens by recombinant expression cloning) analysis, by Michael Pfreundschuh and his colleagues, to identify and sequence tumor targets recognized by autologous CD8+ T cells and antibodies respectively, and (iii) remarkable advances in our ability to dissect and analyze immune responses recognized by CD8+ and CD4+ T cells. As a consequence of efforts by groups led by Boon, Rosenberg, Pfreundschuh, and my colleagues Yao Chen and Matt Scanlan, we now have a growing portfolio of cancer antigens that could easily keep us busy for the next decade.

In addition to immunomic analysis, bioinformatics, transcriptomics, and proteomics have now become important disciplines contributing to our knowledge of human tumor antigens. To keep up with this avalanche of new information, Victor Jongeneel of the Ludwig Institute for Cancer Research (LICR) has created a cancer immunome database (4) that incorporates data on structure, homology, expression patterns, and immunogenicity of human tumor antigens, and there are now over 1000 different gene entries in the database.

Six categories of immunogenic human tumor antigens have been identified: (i) differentiation antigens expressed by cancers and a restricted subset of normal cells, such as Melan-A/MART-1 and NY-BR-1, (ii) mutational antigens, (iii) overexpressed or amplified gene products, such as HER-2/neu and NY-C0-58, (iv) splice variant antigens, (v) viral antigens, including the HERV family, and (vi) cancer-testis or CT antigens.

Because of their fascinating characteristics, CT antigens have taken center stage in the cancer antigen identification and vaccine programs carried out by LICR and the CRI. The first CT antigens, MAGE, BAGE and GAGE, were recognized by Boon and his group using T cell epitope cloning. Subsequently, the SEREX cloning technique of Pfreundschuh, mRNA expression assays and bioinformatics analysis identified a large number of gene products with CT characteristics. There are now 44 genes or gene families coding for CT antigens (5), many of which are located on the X chromosome, and Matt Scanlan and his colleagues Yao Chen, Andy Simpson and Victor Jongeneel are putting together a CT database which will be published in Cancer Immunity, the new e-journal in the field.

CT antigens have the following characteristics: their expression in normal tissues is highly restricted, primarily to germ cells in testis and fetal ovary, and to trophoblast cells, and they are aberrantly expressed in a wide range of different tumor types. This restricted expression pattern in normal tissues and broad expression in cancer make these antigens excellent targets for vaccine development, as does the fact that many CT antigens have inherent immunogenicity - that is they elicit an immune response in patients bearing tumors expressing these antigens - indicating that immunological tolerance has not been established against them. Also, it is important that the immunogenicity of these antigens is not associated with any apparent paraneoplastic autoimmune disorder.

There is now intense interest in unraveling some of the unresolved questions surrounding CT antigens. (i) What is their role in normal gametogenesis? Aside from evidence for MAGE products having transcriptional regulatory activity, SCP-1 having a role in meiosis, and OY-TES-1 a role in packaging acrosomal proteins in the sperm head, the function of CT antigens is unknown. (ii) What is the basis for their aberrant expression in cancer? Global hypomethylation associated with cancer is clearly one of the factors leading to their expression, but this finding raises the question of what genetic changes lead to hypomethylation in the first place in cancer cells. (iii) What is the role of CT antigens in cancer etiology and pathogenesis? Nothing has emerged to clarify this issue as yet, but we do know that CT expression appears to increase with tumor progression, as determined by tumor stage and grade. As CT antigen expression appears to be a probabilistic event associated with tumor progression, this raises the fascinating possibility of vaccinating with CT antigens before CT expression in the tumor (anticipatory vaccines), and thus preventing the emergence of presumably more highly malignant CT+ cells. I could go on and on about CT antigens because they are so inherently interesting, and because my colleagues and I have developed a theory that the expression of CT antigens in cancer is evidence that cancer has usurped the genes of normal gametogenesis for its own purposes, thereby acquiring features of the gamete,
such as immortality, migratory characteristics, and invasiveness (6). But more to the point, this discussion raises the issue of what constitutes the perfect tumor antigen. In addition to high potential immunogenicity, broad cancer expression and restricted expression in normal cells, cancer antigens of choice will be ones that are essential for the continued viability or behavior of the cancer cell. Examples of these come from work in mouse systems by Ikeda and Shiku and Hans Schreiber on mutant MAP kinase and ribosomal gene products, but these antigens are uniquely expressed in single tumors. This is why the work of Pramod Srivastava, one of our speakers, is so pertinent to this question of the ideal tumor antigen or antigens.

Heat shock protein (HSP) vaccines from the autologous tumor should theoretically contain the full repertoire of tumor antigens, including the unique mutant antigens that are so immunogenic and that would be absent from vaccines containing only shared tumor antigens, and we shall have the pleasure of hearing Dr. Srivastava speak on this topic.

Surprisingly, none of the common cancer genes such as p53, ras, Rb, or APC have turned out to be promising cancer vaccine targets as yet, but there is continuing interest in HER2/neu, telomerase, survivin and other gene products that are associated with the cancer phenotype.

Given the rich array of immunogenic human tumor antigens that have been found, some would argue that we don't need to find more tumor antigens. I'm reminded of a comment made by Peter Gorer, the discoverer of MHC antigens when someone told him that they had found another MHC antigen and he said, "Oh really, must you! Can't we make do with the ones we've got?"

Of course I disagree with the suggestion that we have enough tumor antigens. I think that we've just begun our analysis of the cancer immunome and the search for vaccine targets. Clearly single-antigen vaccines can lead to tumor escape so polyvalent vaccines containing defined antigens or autologous HSP vaccines are a logical solution to this challenge. And we have not even begun to define the cancer immunome for CD1 and other antigen presenting systems recognized by T cells. So there is much more work to be done, and the international coordinated effort called the Human Cancer Antigen Discovery Collaborative, initiated by the CRI and the LICR in 1999 and made up of 30 investigators from 8 different countries, is a good example of a funding mechanism facilitating the discovery process that has the common objective of finding suitable targets for cancer vaccines.

With the molecular definition of human cancer antigens that have characteristics of promising vaccine targets, the next stage is a detailed analysis of the humoral and cellular immune response elicited by the antigens in nonvaccinated cancer patients. The four antigenic systems we know the most about in this regard are the melanocyte differentiation antigens, Melan-A/MART-1 and gp100, and the CT antigens, MAGE-3 and NY-ESO-1 (Table 1).

Melan-A and gp100 are expressed at high frequency in melanoma, and a number of CD8+ T cell epitopes have been discovered for these antigens. CD8+ T cell reactivity to Melan-A is seen in both normal individuals and melanoma patients, but the frequency is higher in cancer patients and the T cells shift toward a memory phenotype. No humoral immunity to Melan-A/MART-1 or gp100 has been found. Of the CT antigens, MAGE-3 is the most frequently expressed and a large number of CD8+ and CD4+ T cell epitopes have been defined. Humoral and cellular immunity to MAGE-3 is only rarely found in patients with tumors expressing these antigens.

On the other hand, NY-ESO-1 is expressed at a lower frequency than MAGE-1, with the exception of synovial sarcoma, but it appears to be highly immunogenic and induces an integrated humoral and cellular immune response (to a number of CD8 and CD4 epitopes) in a substantial number of patients with advanced NY-ESO-1-expressing tumors. No NY-ESO-1 immunity has been found in normal individuals or individuals with NY-ESO-1 negative tumors or in patients with early stage NY-ESO-1 positive tumors.
Table 1. Immune response to four cancer vaccine targets.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Tumor Expression (% of tumors)</th>
<th>No. of Defined T Cell Epitopes</th>
<th>Spontaneous Immunity (% of patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CD8+</td>
<td>CD4+</td>
</tr>
<tr>
<td>MELAN-A / MART-1</td>
<td>&gt;90</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>frequent</td>
<td>-</td>
</tr>
<tr>
<td>GP100</td>
<td>&gt;90</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>frequent</td>
<td>-</td>
</tr>
<tr>
<td>MAGE-3</td>
<td>30-60</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rare</td>
<td>rare</td>
</tr>
<tr>
<td>NY-ESO-1</td>
<td>25-35</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10-20</td>
<td>10-20</td>
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We have taken advantage of the strong spontaneous immunity to NY-ESO-1 to develop and standardize typing and monitoring methodologies to measure NY-ESO-1 expression in tumors, by RT-PCR and immunohistochemistry, and to quantitate NY-ESO-1 humoral immunity, by ELISA and Western blot, and CD8 and CD4 T cells by tetramer and Elispot. As a consequence, we know that (i) NY-ESO-1 antibody is only found in patients with NY-ESO-1-expressing tumors (usually at an advanced stage of the disease), (ii) the presence of CD8+ and CD4+ T cell immunity is almost invariably associated with the presence of NY-ESO-1 antibody, and (iii) a large number of NY-ESO-1 CD8+ and CD4+ peptide epitopes have now been defined. An important outcome of this strong focus on NY-ESO-1 is the development of a general method for assessing CD8 and CD4 reactivity to NY-ESO-1 in patients, regardless of the MHC haplotype, by Sacha Gnjatic and his colleagues.

A number of new typing and monitoring elements are being put in place to supplement the existing NY-ESO-1 methodologies:

(i) To analyze humoral immunity: antibody class, isotype, epitope reactivity, affinity, and opsonic activity.
(ii) For T cells: detailed phenotypic and T cell receptor characterization using CD4 tetramers, Th0/Th1/Th2 phenotyping, and assays for CD4+ CD25+ regulatory cells.
(iii) For DTH: multipitope skin testing with NY-ESO-1 peptides and proteins.
(iv) For tumor cells: quantitation of MHC, MHC/peptide complexes, and gamma-interferon responsiveness at the single cell level, all important issues with regard to tumor escape mechanisms.

What is becoming increasingly important is characterizing the frequency, reactivity, and specificity of infiltrating lymphocytes in NY-ESO-1 positive tumors, something we call "in situ immunology". Drs. Sato, Jungbluth, and Odunsi have been carrying out such an analysis on ovarian cancer. Figure 3 shows four ovarian cancers with varying degrees of lymphocytic infiltration, from sparse to brisk. Zhang and his colleagues reported this year in the New England Journal of Medicine (7) that T cell infiltrates in ovarian cancer were highly correlated with prognosis, in terms of disease-free interval, response to chemotherapy, and overall survival. As presented in a
poster at this meeting (8), Sato, Jungbluth and Odunsi have confirmed this association between lymphocyte infiltration and prognosis in a series of 111 ovarian cancers, but in their analysis it did not quite reach statistical significance. However, they did find a significant correlation between survival and NY-ESO-1 expression and lymphocyte infiltration, and this would be one of the first indications that spontaneous NY-ESO-1 immunity confers benefit to the patient. These studies are now being extended to examine the specific NY-ESO-1 reactivity of the infiltrating lymphocytes in NY-ESO-1 positive tumors.

Figure 3. CD3+ T cell infiltrates in four ovarian cancer patients.

Now, how does one go about constructing a maximally immunogenic vaccine with a known human tumor antigen such as NY-ESO-1, using this antigen as a model for other human tumor antigens? We might follow Ted Boyse’s and my dictum when we were defining the first lymphocyte differentiation antigens in the mouse in the 60s and 70s. Immunize everything against everything and test everything against everything. Not bad advice but human clinical trials don’t lend themselves to that sort of approach. For a human vaccine and a single antigen, the number of different strategies that can be proposed is daunting. First the form of the antigen - peptides, protein, RNA, naked DNA, or DNA introduced by the way of viral vectors such as vaccinia, fowlpox, canarypox, lentivirus, adenovirus, adeno-associated virus (AAV) or by the way of microbial vectors such as Salmonella, E. coli, Listeria or yeast. Then there are discussions about the adjuvant, the cellular delivery system, and the inclusion of cytokines and other natural or synthetic immunopotentiators. However, these are all theoretical choices - because there is the issue of availability, cost, regulatory requirements, commercial restrictions and funding for the clinical trials and the monitoring.

And that’s only the beginning of the problems confronting the translational vaccinologist, but enough to send most people back to mouse models. For those who proceed, the usual choice is peptide vaccines with an adjuvant like Montanide because these are available and affordable to most academics. Working with companies is the only option for most investigators, but companies are solely interested in the approach covered by their intellectual property, not in direct comparisons of different vaccine constructs to find which is maximally immunogenic. Companies need to go directly to product development and a commercial product, whereas the academic mindset argues for the fine tuning and comparative testing that characterize the discovery modality of the laboratory, and it is this conflict between commercial and academic priorities that makes interactions between the academic enterprise and companies so challenging and progress so slow when we get to the clinical arena.

To confront this challenge, the CRI and the LICR have developed the Cancer Vaccine Collaborative (Figure 4), a global consortium of investigators to carry out direct comparisons of different vaccine constructs in clinical trials that are monitored by standardized methodology. These investigators are at 20 sites in 8 countries. Each site has strong experience in T cell immunology, a large patient population and a strong clinical commitment to cancer vaccine development, and a tight integration of laboratory and clinical activities. The entire effort is
centrally managed by the LICR Office of Clinical Trials Management, under Dr. Eric Hoffman, which is organized, staffed and operated very much like comparable clinical management activities at a pharmaceutical company. Protocol initiation, review and approval are conducted in accordance with established procedures at the LICR and at individual LICR clinical trial centers. The LICR has two sources for its various therapeutic agents. It has established a GMP Biological Production Facility at its Melbourne Branch, and is developing another at Cornell University in Ithaca, to produce and vial clinical grade NY-ESO-1 vaccines containing peptides, proteins and bacterial vectors. Other vaccines are being developed in collaboration with biotech companies having proprietary technology with the understanding that the LICR will sponsor, conduct and monitor the initial phase I/II clinical trials with these NY-ESO-1-based vaccines.

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**Figure 4. Cancer Vaccine Collaborative.**

**Figure 5. NY-ESO-1 vaccine program.** The four major antigen forms (top) - peptides, proteins, vectors, DNA - are shown in red. Single variable vaccine constructs (middle) are shown in blue and the clinical trial centers (bottom) are shown in purple.
In this way, we have organized a NY-ESO-1 vaccine program according to the following model: academic, international, centrally managed and coordinated, multiple sites, parallel NY-ESO-1 vaccine trials with single variable vaccine constructs, standardized immunological monitoring, central data accrual and analysis, and not dominated by commercial interests of companies. Figure 5 summarizes the current and imminent NY-ESO-1 vaccine trials.

Alex Knuth, Sacha Gnjatic, Elke Jäger and Jonathan Cebon will be presenting the current status of the phase I/II NY-ESO-1 peptide, protein, and vaccinia/fowlpox trials in tomorrow’s session and will be emphasizing the dominant and recurring theme of the LICR/CRI program - we will not know how to vaccinate until we know how to immunize, and we will not know how to immunize until we know how to monitor. In addition, this integrated LICR/CRI activity directed toward a common clinical objective and conducted in a coordinated fashion is an important model for other academic and governmental agencies.

The development of HIV and cancer vaccines draws upon the same immunological system, principles, and techniques. They choose among the same array of approaches to vaccine development and immunological monitoring, and they confront the same massive problems of moving science from the laboratory to the clinic, in terms of cost, ethical considerations, regulatory requirements, and challenges of working with industry. Both fields are in a critical phase of development. Frustration is mounting about the slow progress in developing effective AIDS and cancer vaccines, but this is countered by the many new approaches that continue to emerge as we learn more and more about the workings of the immune system and how to manipulate it. In my opinion, the central problem confronting us is the challenge of conducting early phase clinical trials in a regulatory and financial environment dominated by product development rather than academic inquiry. To change this will require that the discovery environment of the laboratory be replicated in the clinic and that academic rather than commercial principles dominate early phase clinical trials. Obviously, this will necessitate a major change in the way we carry out discovery phase clinical investigation, particularly in the area of therapeutics, and will require a coordinated effort by academic institutions, funding bodies and government and regulatory agencies. But surely, we can find more efficient and effective ways to translate the exciting therapeutic opportunities arising from the biological revolution of the 20th century into clinical realities.

Abbreviations
CRI, Cancer Research Institute; CT, cancer-testis; LICR, Ludwig Institute for Cancer Research

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