It is my great pleasure to greet you on the occasion of this tenth year since the initiation of the Cancer Research Institute (CRI) International Immunotherapy Symposium series. Each year we celebrate advances in the two main objectives of the field of tumor immunology - the development of effective cancer vaccines and the development of antibody-based cancer therapies. This year we have three reasons to celebrate. The first is the formation of a partnership between the CRI and the Ludwig Institute for Cancer Research (LICR), focusing the joint strengths of these two great institutions on developing effective cancer immunotherapies. The initial manifestation of this partnership is the creation of the Cancer Vaccine Collaborative (CVC), a consortium of basic researchers and clinical investigators at six New York institutions carrying out early phase clinical trials aimed at establishing principles of effective immunization with cancer antigens. The inauguration of the Cancer Vaccine Collaborative represents the second development that we are celebrating at this meeting.

The underlying motivation for forming the CRI/LICR partnership and establishing the Cancer Vaccine Collaborative comes of course from the remarkable developments that have punctuated the recent history of cancer immunology. We have gathered here today the individuals responsible for many of these advances to celebrate the field of cancer immunology and its central role in contemporary cancer research. In the midst of this happy occasion, I have a sad note to add. Dr. Elisabeth Stockert, one of the preeminent cancer serologists of our time and a close colleague and dear friend of many of us here today, died last week of a disease she studied all her life. We will miss her greatly and plan to gather at a future date to celebrate her life and work.

Over the next two days, we will be focusing our discussions on the four areas of central relevance to understanding how to construct effective cancer vaccines:

1. **Cancer immunosurveillance / immunoediting.** The growing evidence that the emergence of cancer is recognized and restrained by immunological forces has led to the renaissance of the cancer immunosurveillance hypothesis.

2. **Cancer antigen discovery: The cancer immunome.** Several speakers will be providing an overview of the current categories of human cancer antigens that have been identified and the prospects for defining the human cancer immunome, the complete repertoire of human cancer gene products that elicit an immune response in humans.
3. **Monitoring immune reactions to cancer.** With the rapidly expanding list of human cancer antigens, the pressing need is to characterize the nature of the immune response to these antigens and to develop ways to monitor the humoral and cellular elements in this response during tumor growth and after therapeutic interventions.

4. **Developing cancer vaccines.** In pursuing the goal of developing cancer vaccines, we first need to know how to induce consistent, strong, and persistent integrated immune responses in humans and, in this regard, we are still woefully ignorant. This is essential before we can sensibly ask whether our cancer vaccines have therapeutic value in humans.

In my opinion, the re-emergence of cancer immunosurveillance as the unifying concept in cancer immunology represents an advance of unparalleled importance (Figure 1). Although the linkage between cancer and immunity remained firmly if vaguely fixed in the general public, the idea that immunity protects against the emergence of cancer as proposed by Ehrlich, Thomas and Burnett was essentially discredited and abandoned by the cancer establishment, primarily because of Osias Stutman's studies in the 1970s in nude mice. In fact, this lack of evidence for cancer immunosurveillance represented a deep embarrassment to the field of cancer immunology for the past thirty years. To me, Stutman's evidence was never convincing, primarily because nude mice were not immunologically null, and as I often jested, if I had to be an immunocompromised animal I wouldn't have minded being a nude mouse. What seemed more important than trying to prove or disprove cancer immunosurveillance with inadequate models at the time was to see if the generality of cancer displayed antigens that could be recognized by the immune system. To me, immune recognition of cancer rather than immune protection against cancer was the central and first issue that had to be addressed. If there was no recognition, there could clearly be no protection, but one could imagine all sorts of reasons why recognition failed to result in protection.

![Figure 1. Landmarks in the development of the cancer immunosurveillance theory.](http://www.cancerimmunity.org/v3suppl1p1/021117.htm (2 of 9))

The development of mouse models with defined defects in essential immune functions has now provided us with convincing evidence for the existence of cancer immunosurveillance, an advance that justifies in my opinion a year-long celebration and dancing in the streets. Thank you Bob Schreiber, thank you Mark Smith and all your colleagues for establishing this critical landmark in cancer immunology.

Cancer antigen discovery, the second topic of our meeting, also has a long and fascinating history. Once again, it was mouse models, in this case inbred mice, that provided the first convincing evidence starting in the 1950s for the existence of tumor-specific antigens in chemically-induced sarcomas and subsequently in virus-induced and even spontaneous tumors. However, demonstrating that such antigens exist in human cancer was a much tougher nut to crack because the transplantation techniques used in the mouse were not possible in humans.
Beginning in 1970, my colleagues and I began a long search for human tumor antigens using an approach that
came to be known as autologous typing (Figure 2) and it was this approach that eventually led to the molecular
definition of the first human tumor antigens recognized by human CD8 T cells by Thierry Boon and his
colleagues and for the dissection of specific humoral immunity to cancer by Michael Pfreundschuh and his
colleagues. At the time that autologous typing was developed, and remember this was before MHC restriction
was recognized, efforts to identify immune reactions to human tumor antigens depended on allogeneic systems,
that is sera or lymphocytes from one patient tested on a limited number of established tumor lines from other
patients, and much of what was reported as tumor-specific was undoubtedly alloreactivity. To develop as
unambiguous a system as possible, we restricted our tests to autologous reactions with cultured tumor cell lines,
lymphocytes and sera from the same patient, and for specificity analysis, normal cells, typically fibroblasts, from
the same patient. In a sense, we were creating in humans a counterpart to an inbred mouse system. In our
original studies, melanoma, kidney cancer, and astrocytoma were the targets because these were the tumor
types that could be established in tissue culture with any degree of regularity. Humoral rather than cellular
immune reactions were analyzed because techniques for testing antibodies to cell surface antigens were far
more advanced at the time than analyzing cellular immune responses. What became clear from our analysis of a
large series of patients was that a very small subset of patients mounted a vigorous immune response with
specificity for autologous tumor cells, and several antigenic systems, including gangliosides and the melanocyte
differentiation antigen gp75, were identified as tumor targets. Most of the antigens, however, remained beyond
our means to identify molecularly. The second phase in the saga of autologous typing came when Alex Knuth
joined our group and began to analyze CD8 T cell reactivity to melanoma. He established the first CD8 T cell-
defined autologous system in human cancer (in this case the SK-MEL-29 melanoma system), and when he
returned to Germany he continued the search for the rare patient with strong specific CD8 T cell reactivity,
eventually identifying patient MZ. Because I was well aware of Thierry Boon's outstanding work on the
development of molecular techniques for the cloning of mouse tumor antigens, I suggested Alex and Thierry
meet and the rest is history. From these two cell lines, SK-MEL-29 and MZ-MEL-2, and the corresponding
reactive autologous CD8 T cells came the identification of MAGE, BAGE, GAGE, Melan-A and tyrosinase.
Michael Pfreundschuh and his colleagues took the third next major step in the development of autologous typing
by developing SEREX (serological analysis of recombinant cDNA expression libraries). Michael worked with me
during the early days of autologous typing and recognized the three limitations of the approach: (i) It was limited
to the detection of cell surface antigens, (ii) it was restricted to tumors that could be grown \textit{in vitro}, and (iii) it left
us ignorant about the molecular structure of most antigens being detected. In combining serological approaches
with expression cloning, Michael Pfreundschuh and his colleagues created the SEREX methodology, and as you
will hear at this meeting, this approach has revolutionized the identification of serologically recognized tumor
antigens, just as the work of Dr. Knuth and Dr. Boon revolutionized the identification of CD8 T cell recognized
targets. So, thank you Dr. Pfreundschuh, thank you Dr. Boon and thank you Dr. Knuth and all your colleagues for
establishing the modern era of human tumor antigen discovery.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Defining human cancer antigens by autologous typing.}
\end{figure}
With the methodologies now well in place for the molecular identification of human tumor antigens recognized by human CD8 T cells, antibodies, and, as Rongfu Wang will tell us, CD4 cells as well, we are entering a phase in tumor immunology where we can expect to have a catalogue of the complete range of immunogenic human tumor antigens, something I have called the "cancer immunome". Victor Jongeneel of the LICR is well along in creating a database that compiles existing information about the cancer immunome that is available to the cancer immunology community.

Now, where do we stand with the existing cancer immunome? Five categories of human tumor antigens have been identified (Table 1), with melanocyte differentiation antigens such as Melan-A, tyrosinase, and gp100, cancer-testis antigens such as MAGE 1 and 3, and NY-ESO-1, and over-expressed antigens such as HER2 and p53 being a few well-known examples of a very large and rapidly expanding list. Our speakers today will be giving us a look at the current status of the cancer immunome.

Table 1. Categories of human cancer antigens.

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differentiation antigens</td>
<td>Tyrosinase, Melan-A, gp100, NY-BR-1</td>
</tr>
<tr>
<td>Cancer/Testis (CT) antigens</td>
<td>MAGE, NY-ESO-1, SSX2, SCP1</td>
</tr>
<tr>
<td>Amplified/mutated antigens</td>
<td>HER-2/neu, p53</td>
</tr>
<tr>
<td>Splice variant antigens</td>
<td>NY-CO-38/PDZ73, ING1</td>
</tr>
<tr>
<td>Viral antigens</td>
<td>HPV, HBV, HERV-K</td>
</tr>
</tbody>
</table>

Figure 3. Tyrosinase expression in melanocytes.
Now, the specificity of some tumor antigens is quite exquisite. Figure 3 shows the expression of the melanocyte differentiation antigen tyrosinase in normal melanocytes in skin - so as a vaccine target for melanoma, antigen expression in normal tissues is remarkably restricted. Figure 4 shows the expression of the CT antigen, MAGE-1, in spermatogonia in testes - a breathtakingly beautiful picture for an old tumor immunologist.

Figure 4. MAGE expression in normal testis.

I must confess enormous affection for the CT category of antigens (Figure 5). The large number of antigens with cancer-testis characteristics (over 15 CT antigens have now been identified), their extraordinary specificity pattern with restricted expression to germ cells and cancer, and the strong spontaneous immunity elicited in a subset of patients by the CT antigen NY-ESO-1, make CT antigens of great interest in tumor biology and tumor immunology.

Figure 5. Cancer/Testis antigens.

It is often forgotten that most of our knowledge about the expression patterns of tumor antigens is based on mRNA, even though we assume the presence of message correlates with protein expression, an assumption that needs to be validated for each antigen in normal tissues and tumors. In developing cancer vaccines, every
effort should be made to know the protein expression pattern of the target antigen in the tumor of patients enrolled in trials, so that this parameter can be correlated with the eventual outcome. Achim Jungbluth has been one of the leaders in this area of immunohistochemical typing of tumor antigens and will be telling us about the current status of his field.

Given the embarrassment of riches with regard to the growing cancer immunome, a very welcome challenge has emerged - which antigens do we focus on to (1) characterize the nature of the immunological responses they elicit and (2) to use as targets for cancer vaccine development. In the Ludwig Institute, Melan-A, MAGE and NY-ESO-1 have been selected as prototypic human tumor antigens for detailed laboratory and clinical study, and you will be hearing a great deal about these antigens at this meeting.

It is a generally held credo in the field that the three basic elements in the development of effective cancer vaccines are:

1. The identification of well-defined antigen targets,
2. The establishment of standardized monitoring methodologies for measuring humoral and cellular immune responses to the vaccines, and
3. The construction of maximally immunogenic vectors to deliver cancer antigens.

Monitoring methodologies have undergone considerable improvement over the past few years, in terms of reliability, specificity and sensitivity, but there is still much work to be done. For measuring CD8 T cell responses, we have Jean-Charles Cerottini to thank for his life-long dedication to the understanding of this cell's role in the immunological philharmonics. The introduction of tetramers has been a wonderful addition to the immunologist's toolbox, and we thank Mark Davis for this gift. The list of typing and monitoring elements is large and getting larger. Because of the strong humoral and cellular immune response induced by the CT antigen NY-ESO-1, this antigen has become a prototype for developing standardized methodologies for monitoring and as a target for vaccine development. Table 2 lists the various elements involved in NY-ESO-1 typing and monitoring: RT-PCR and immunohistochemistry to determine antigen expression in the tumor, ELISA and Western blots to determine antibody responses, tetramer, ELISPOT, and cytotoxicity assays to determine CD8 T cell responses, and skin tests with NY-ESO-1 peptides or proteins to determine delayed type hypersensitivity (DTH). As to assays under development, standardized CD4 monitoring is lacking in our current NY-ESO-1 monitoring, but should be a reality in the near future. Also essential, but still challenging, are assessing the expression of MHC, MHC allotypes, and MHC/peptide complexes at the level of the tumor cell. Another dimension in monitoring that needs to be added has to do with characterizing the nature and specificity of the immunocellular infiltrates in tumors before and after vaccination, what we are calling in situ immunology, and considerable progress is being made in this direction.

I spoke earlier about our embarrassment of riches with regard to cancer antigens. The same challenge confronts us with regard to choosing the most effective way to vaccinate against NY-ESO-1.

To guide us in this effort, we have come up with two maxims that embody our deeply held convictions about how to proceed and the importance of typing and monitoring in developing successful cancer vaccines. The first of these maxims is "If you don't know how to immunize, you won't know how to vaccinate". And the second is "If you don't know how to monitor, you won't know how to immunize".

In other words, you need to know how to monitor if you hope to know how to immunize most effectively, and you must know how to immunize effectively if you hope to develop a successful therapeutic vaccine.
Table 2. NY-ESO-1 typing and monitoring.

<table>
<thead>
<tr>
<th>Standardized Assays</th>
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<tbody>
<tr>
<td><strong>Antigen expression</strong></td>
</tr>
<tr>
<td>RT-PCR, immunohistochemistry (IHC)</td>
</tr>
<tr>
<td><strong>Antibody</strong></td>
</tr>
<tr>
<td>ELISA, Western blot</td>
</tr>
<tr>
<td><strong>CD8+ T cell</strong></td>
</tr>
<tr>
<td>ELISPOT, tetramer, cytoxicity</td>
</tr>
<tr>
<td><strong>DTH</strong></td>
</tr>
<tr>
<td>Skin test: Peptide or protein</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Assays under Development</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD4+ T cell</strong></td>
</tr>
<tr>
<td>ELISPOT, tetramer, cytokine release</td>
</tr>
<tr>
<td><strong>MHC expression</strong></td>
</tr>
<tr>
<td>IHC</td>
</tr>
<tr>
<td><strong>Peptide/MHC complex</strong></td>
</tr>
<tr>
<td>mAb, IHC</td>
</tr>
<tr>
<td><strong>In situ immunology</strong></td>
</tr>
<tr>
<td>IHC</td>
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</tbody>
</table>

Figure 6 lists the NY-ESO-1 vaccines and vaccine strategies that are currently available, and each day seems to add new ones. Daunting, isn't it? No one knows which strategy will be most effective and all of them need to be tested. Of central importance, immunological monitoring of individual trials needs to be carried out by standardized methodology, so that valid comparisons of the results of each vaccine can be made. No one group can do this for even one antigen, and companies usually limit themselves to single approaches based on their proprietary technologies. It was this dilemma that led to the formation of the CRI/LICR partnership and the development of the Cancer Vaccine Collaborative.

The objectives of the Cancer Vaccine Collaborative can be characterized in the following way. The trials will be academic, coordinated, and take place at six institutions in New York. Initial focus will be placed on NY-ESO-1 as the vaccine target. Rather than a series of sequential trials testing one variable after the other, the trials will take place in parallel testing single variables such as peptide, protein, viral vectors, and cDNA, simultaneously at multiple sites. Finally, all trials will be monitored in the same way, with standardized monitoring methodology and standardized reporting.
The current participants in the CVC are:

- Nasser Altorki at the Weill Medical College of Cornell University
- Kyri Papadopoulos and Charles Hesdorffer at the Columbia-Presbyterian Medical Center
- Anna Ferrari at the Mount Sinai School of Medicine
- Pam Sharma, Harry Herr, Dean Bajorin, Bob Maki, and Jakob Dupont at the Memorial Sloan-Kettering Cancer Center
- Nina Bhardwaj and Steve Burakoff at the New York University Cancer Institute
- Kunle Odunsi at the Roswell Park Cancer Institute

These clinical trials in New York will become an integral part of the Ludwig Institute clinical trials network (Figure 7), which is running trials in 10 countries around the world. This large-scale coordinated collaborative network, with an emphasis on early stage clinical trials and with the initial aim of constructing maximally immunogenic cancer vaccines, represents a new era in cancer therapeutics. It also provides a model for how the striking advance in all fields of cancer research can be more effectively and efficiently translated into the clinical arena.
Thank you.

Now I have the pleasure of introducing Bob Schreiber, who will open the first session of our meeting.

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**Abbreviations**

CT, cancer/testis

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