The meeting intends to analyze and discuss the state of the art of research in the field of cancer immunotherapy. The development of new strategies for cancer treatment is a major priority of biomedical research with important implications for public health. Recently, the research progress has opened new perspectives for novel and more effective strategies of cancer immunotherapy. However, the success of the immune-based therapies is still limited by the low percentage of patients showing effective and durable clinical responses. A special attention will be given to the analysis of the immunological mechanisms underlying the failure or success of cancer immunotherapy as well as of the strategies that might allow to enhance, evaluate and predict the therapeutic response of cancer patients to immunological interventions. The meeting will also review and discuss the measures adopted at the European and international level to promote clinical research in the field of cancer immunotherapy.

**Key words**: Cancer, Immunotherapy, Vaccines

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Istituto Superiore di Sanità


Edited by Alessandra Mariani, Maria Ferrantini and Filippo Belardelli

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2006, vii, 52 p. ISTISAN Congressi 06/C3 (in inglese)

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Scientific and Organizing Committee:

E. Garaci (Honorary President), F. Belardelli, M. Ferrantini, F. M. Marincola, G. Parmiani, S. Vella

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PROGRAM

Wednesday 24 May

8.00   Registration

9.00   Welcome and Opening Address
       Enrico Garaci, President ISS

Opening Session
Chairpersons: J. Gordon McVie, Alexander M.M. Eggermont

9.10   Keynote lecture
       The global problem of cancer: new solutions needed
       Peter Boyle

9.40   Keynote lecture
       State of the art of anti-cancer vaccines
       Giorgio Parmiani

10.10  Coffee break

Session I
ANIMAL MODELS AND THEIR PREDICTIVE VALUE
FOR THE IMMUNOTHERAPY OF CANCER
Chairpersons: Mario P. Colombo, Guido Forni

10.40  Peptide-based anti-cancer vaccines: from animal model to clinical application
       Rienk Offringa

11.00  Oncoantigens as suitable targets for tumor prevention
       Federica Cavallo

11.20  BORIS - a target antigen for cancer treatment: in vitro and in vivo studies
       Herbert C. Morse III

11.40  A novel dendritic cell subset involved in tumor immunosurveillance
       Laurence Zitvogel

12.00  Mouse myeloid suppressor cells comprise a peculiar population of inflammatory monocytes
       Vincenzo Bronte
12.20  Regulatory T cells depletion or inactivation: is it a problem in cancer immunotherapy?  
Mario P. Colombo

12.40  Discussion  
Leader: Mario P. Colombo

13.00  Lunch

Session II  
IMMUNOLOGICAL BASIS OF THE FAILURE OR SUCCESS OF CANCER IMMUNOTHERAPY  
Chairpersons: Francesco M. Marincola, Peter L. Stern

14.00  The immunological constant of rejection  
Francesco M. Marincola

14.20  Activation of iNKT cells results in dendritic cell maturation and enhanced T cell expansion in vivo  
Vincenzo Cerundolo

14.40  Induction of myeloid cells with suppressive activity by human tumor-released microvesicles  
Licia Rivoltini

15.00  T cell memory, anergy and immunotherapy in breast cancer  
Volker Schirrmacher

15.20  IFN-alpha as an adjuvant in cancer immunotherapy  
Maria Ferrantini

15.40  Discussion  
Leader: Francesco M. Marincola

16.00  Coffee break

Session III  
STRATEGIES FOR ENHANCING THE EFFICACY OF CANCER IMMUNOTHERAPY (I)  
Chairpersons: Gerold Schuler, Giulio C. Spagnoli

16.30  Dendritic cell vaccination of melanoma: looking back and ahead after 100 patients  
Gerold Schuler
16.50 *Dendritic cell-derived exosomes harbour functional NKG2D ligands: restoration of NKG2D levels and functions in metastatic melanoma patients*

Thomas Tursz

17.10 *Immunotherapy with dendritic cells in melanoma patients*

I. Jolanda M. de Vries

17.30 *Combining vaccines with adoptive immunotherapy: restoration of immunity in lymphopenic cancer patients*

Carl H. June

17.50 Discussion

*Leader: Gerold Schuler*

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**Thursday 25 May**

**Session IV**

**STRATEGIES FOR ENHANCING THE EFFICACY OF CANCER IMMUNOTHERAPY (II)**

*Chairpersons: Bernard Fox, Stefano Fais*

9.00 *Factors that influence responses to adoptive immunotherapy*

Paul F. Robbins

9.20 *Combining chemo-induced lymphopenia, adoptive transfer of peripheral T cells and active-specific immunotherapy to augment the anti-cancer immune response: preclinical and clinical studies*

Bernard Fox

9.40 *Dacarbazine to enhance vaccine-mediated antitumor immunity in melanoma patients*

Enrico Proietti, Paola Nisticò

10.00 *DNA and Adenoviral vectors and encoding TAA/fusion proteins elicits superior immune and anti-tumor responses*

Gennaro Ciliberto

10.20 Discussion

*Leader: Bernard Fox*

10.40 Coffee break
Session V
MONITORING AND PREDICTION OF EFFECTIVE RESPONSES TO CANCER IMMUNOTHERAPY
Chairpersons: Pierre G. Coulie, Pier Giorgio Natali

11.10  Breaking tolerance: autoimmunity and peptide vaccination in melanoma
Jeffrey Weber

11.30  Monitoring MAGE vaccination. Insights into mechanisms of tumor regression
Pierre G. Coulie

11.50  Dynamics of specific T cell responses to peptide-based cancer vaccines
Pedro Romero

12.10  Survival and tumor localization of adoptively transferred antigen-specific CD8+ T cells in cancer patients
Andreas Mackensen

12.30  Discussion
Leader: Pierre G. Coulie

12.50  Lunch

Session VI
CRITICAL ISSUES FOR PROMOTING CLINICAL RESEARCH IN CANCER IMMUNOTHERAPY
Chairpersons: Thomas Tursz, Giorgio Parmiani

14.00  Regulatory issues and critical needs of clinical immunotherapy
Alexander M.M. Eggermont

14.20  Cancer Clinical Trials: the European regulatory perspective
Stefano Vella

14.40  US initiatives for promoting clinical research in cancer immunotherapy
Bernard Fox

14.50  The cooperation between academia and industry: comparing priorities and opinions
Roberto Camerini

15.05  SMEs and critical issues for the development of cancer immunotherapy in Europe
Jacques Bartholeyns
15.15  The Italian Network for Cancer Bioimmunotherapy (NIBIT): a model to be exported at European level?
Michele Maio

15.25  Designing clinical immunomonitoring: looking at tumor immune biology where and when it matters
Francesco M. Marincola

15.35  Towards the promotion of clinical research of cancer immunotherapy: the need of initiatives at the European level and opportunities in the context of the EUROCAN+PLUS project
Filippo Belardelli

15.50  General Discussion

16.00  Closing Remarks
Enrico Garaci

16.10  End of the meeting
FOREWORD

The origin of Immunotherapy of Cancer dates back more than 110 years. In 1893, William B. Coley, a New York surgeon, described the effect of the first “biological response modifier”, which represented a mixture of bacterial products, reporting impressive responses in certain cancer patients. Since then, the history of cancer immunotherapy has been characterized by alternate cycles of optimism and discouragement. Several major milestones have been evident. In 1967, D.L. Morton described a marked tumor regression after the injection of the BCG vaccine in melanoma patients. However, the mechanisms underlying the clinical response to biological response modifiers such as the BCG vaccine remained a mystery for years. We have subsequently learned how specific soluble factors released by reactive immune cells are responsible for the antitumor response. Further research progress in the field then led to the cloning and clinical use of certain cytokines, such as interferons, TNF-alpha and IL-2. Some of these cytokines are still used in clinical oncology. Today, two principal types of strategy of cancer immunotherapy can be considered: 1) a strategy aimed at enhancing the antitumor immune response by injecting cytokines, antibodies, or cells of the immune system; 2) a strategy of active immunization (administration of cancer vaccines). For many years, cancer vaccines were believed to be the dream of some scientists. Optimism on the perspectives in the development cancer vaccines stemmed from the cloning, performed by T. Boon and colleagues in 1991, of the first human tumor associated antigen (TAA), the melanoma MAGE-1; this subsequently led to the identification of many other human TAAs. Since vaccines are considered as “ideal medical products” inducing long-term immune protection at a relatively low cost, the interest in the field has recently increased. However, some major difficulties have been encountered. These difficulties include: a) poor immunogenicity, as TAAs are often self antigens; b) tumor-induced immune suppression; c) tumor cell escape from CTL recognition; d) dampening of the vaccine-induced antitumor immune response by T regulatory cell-driven mechanisms. Today, however, the progress in cell biotechnologies and the understanding of the mechanisms regulating the antitumor immune response has opened new and exciting perspectives in overcoming these difficulties. During the last decade, many conferences on cancer immunotherapy have been organized to debate leading edge research in this field. As an example, our institute (The Italian National Institute of Health, Rome) organized a conference on Cancer Vaccines in November 1999. Another international meeting on Cancer Vaccines, co-organized with the US NCI, was also held at this Institute (April 2004). The program of this meeting, “Immunotherapy of Cancer: Challenges and Needs”, was designed with the specific aim of ensuring sufficient time to debate specific scientific issues of particular importance, for the development of more effective strategies of cancer immunotherapy. Today, in view of the new understanding and technologies, there are great perspectives and potentialities for cancer immunotherapy. However, the full exploitation of this new knowledge is somewhat threatened by obstacles, which often preclude the translation of promising preclinical data into clinical trials. One such obstacle is the complexity of the procedures required for the preparation of new cell products to be used in clinical trials; the need in coordinating specific initiatives in promoting and supporting clinical experimentation is an opinion commonly expressed by
researchers involved in this field. Hence, in order to meet fully the expectations of the scientific community on the potential impact of immunotherapy in the fight against cancer, it is timely and important to establish strategic cooperation among public institutions responsible of public health, National Cancer Institutes, industry, and the representatives of regulatory bodies. This international meeting intends also to address these crucial issues in an attempt to identify the needs and possible solutions in promoting clinical research in cancer immunotherapy.

Filippo Belardelli
Opening Session

Chairpersons

J. Gordon McVie, Alexander M.M. Eggermont
STATE OF THE ART OF ANTI-CANCER VACCINES

Giorgio Parmiani
Unit of Immunotherapy of Human Tumors, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy

During the last few years the results of several clinical studies of vaccination have been published. These can be considered as second generation anti-cancer vaccines, considering the cell-based vaccines as those belonging to the first generation.

The clinical studies of the second generation are based on the use of peptide/proteins to construct vaccines aimed at testing in the clinics the principle that tumor-derived CD8 T cell epitopes given with traditional adjuvants (e.g. IFA), with cytokine-based adjuvants (e.g. GM-CSF, IL-2, IFN-α) or loaded on autologous dendritic cells, could generate a T cell immune response targeting tumor cells and translating into a clinical response. More recent studies involved the use of full proteins in an attempt to induce T cell reactions against both class I and II epitopes. Such studies will be summarized and discussed to identify strengths and weaknesses of those approaches whose clinical outcome remains disappointing. However, a phase III study in patients with metastatic prostate cancer showed benefit for the patients arm immunized with autologous dendritic cells loaded with a fusion protein containing PAP and GM-CSF. I will focus also on the many escape mechanisms that allow tumor cells to avoid destruction by immune T cells including myeloid suppressor cells and T regulatory lymphocytes. Such an analysis should allow to design more effective clinical protocols in cancer vaccination.
Session I

Animal models and their predictive value for the immunotherapy of cancer

Chairpersons
Mario P. Colombo, Guido Forni
PEPTIDE-BASED ANTI-CANCER VACCINES: FROM ANIMAL MODEL TO CLINICAL APPLICATION

Rienk Offringa, Sjoerd H. van der Burg, Jan Wouter Drijfhout, Gemma Kenter, Cornelis J.M. Melief

Tumor Immunology Group, Leiden University Medical Center, Leiden, The Netherlands

Cumulative experimentation in our laboratory with peptide-based anti-tumor vaccines in murine tumor models has shown that the immunogenicity and therapeutic efficacy of such vaccines is optimal if the following requirements are met:

– targeting of antigen presentation to DC;
– proper conditioning of DC by potent adjuvants;
– prolonged presentation of vaccine-derived T-cell epitopes by DC;
– activation of a strong - preferentially tumor-specific - CD4⁺ Th1-response.

We found that vaccines consisting of long (20-30-mer) peptides, which comprise epitopes recognized by both CD8⁺ CTL and CD4⁺ T-cell epitopes, and supplemented with Toll-like receptor (TLR) ligands, meet these requirements. Accordingly, we have shown that such a vaccine for HPV16E7, when administered to tumor-bearing mice, elicits strong HPV16-specific CD4⁺ and CD8⁺ T-cell immunity capable of tumor-eradication.

A clinical grade vaccine with similar design, specific for the E6 and E7 oncoproteins of HPV16, is currently being tested in patients with HPV16-positive genital neoplasia. Analysis of the natural HPV16-specific T-cell responses in cohorts of such patients revealed that, whereas a majority of healthy subjects display strong T-cell memory against HPV16, neoplasia is associated with failure of the HPV16-specific T-cell response. Importantly, our HPV16-specific peptide vaccine restores HPV16-specific T-cell immunity in these patients. Analysis of biopsies from vaccinated patients shows that these T-cells are able to home to the HPV16-positive lesions. Clinical efficacy of this vaccine is currently under investigation.

In view of these results, we are currently performing pre-clinical experiments with respect to similar peptide-based vaccines directed against two other classes of tumor antigens:

– a newly discovered class of T-cell epitopes that is selectively expressed at the surface of “immune escaped” tumors with impairments in their antigen processing machinery;
– melanocyte/melanoma-specific antigens.
ONCOANTIGENS AS SUITABLE TARGETS FOR TUMOR PREVENTION

Federica Cavallo, Guido Forni
Department of Clinical and Biological Sciences, University of Turin, Orbassano, Italy

Oncoantigens are tumor associated molecules that play a causal role in the promotion of carcinogenesis and cannot be easily down-modulated or negatively selected by precancerous lesions under the pressure of a specific immune attack. When expressed on the cell membrane they can be the target of both cell-mediated and antibody-mediated immune responses. Oncoantigens have so far been identified on tumor cells. Her2 is a well known oncoantigen that deliver signals affecting the proliferation and survival of normal and tumor cell. In normal and p53-deficient BALB-neuT mice transgenic for the rat Her2 oncogene, mammary and salivary carcinogenesis is driven by Her2 receptor expression. Repeated electroporations of plasmids coding portion of the Her2 activate residual T and B cells that escaped central tolerance. Despite the small repertoire of reactive cells activated in tolerant mice, a complete protection is provided when these vaccinations get started during the early stages of carcinogenesis. Vaccine activated CD4⁺ T cells releasing IFN-γ and IgG2a anti Her2 antibodies are not enough to eradicate neoplastic lesions but prevent the progression of precancerous lesions as shown by histological and gene expression analysis. In mice preimmunized to Her2, a lifelong protection is obtained by repeated expansion of memory cells through CpG administrations. The removal of T CD4⁺, CD25⁺ and Foxp3⁺ regulatory cells further extend in time the protection afforded by the DNA vaccine. In the course of tumor progression, additional genetic hits may make Her2 receptor signaling redundant as other oncogene signaling pathways are activated and anti-Her2 therapeutic vaccinations result in a marginal tumor inhibition. In p53-deficient BALB-neuT mice it leads to an immmunoediting and expansion of Her2 negative tumor clones. The search for new oncoantigens expressed on precancerous lesions through microarray analysis is leading us towards the identification of additional target oncoantigens to be combined with anti-Her2 vaccine. The synergism stemming from these vaccine combinations may permit a vaccine to provide an effective protection even against more advanced neoplastic lesions.
BORIS - A TARGET ANTIGEN FOR CANCER TREATMENT: IN VITRO AND IN VIVO STUDIES

Lucia Gabriele (a), Dmitri Loukinov (b), Giulia Romagnoli (a), Paola Borghi (a), Victor Lohansenkov (b), Michael Agadjanyan (c), Filippo Belardelli (a), Herbert C. Morse III (b)

(a) Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy
(b) National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA
(c) Institute for Molecular Medicine, Huntington Beach, CA, USA

BORIS (Brother Of the Regulator of Imprinted Sites) is an 11 zinc finger (ZF) DNA-binding protein that is normally expressed only in testicular spermatocytes. It is paralogous to CTCF which is expressed in all cells but spermatocytes, but which shares the central ZF domain and thus binds to the same target sites as BORIS. Studies of human cancers showed that BORIS is expressed by the vast majority of tumor cell lines, including the NCI 60, as well as primary cancers, and is thus a cancer-testis gene (CTG). The role of BORIS in cancer includes reactivation of other CTG including MAGE-A1 and NY-ESO-1. The potential for BORIS as a target for immunotherapy in the context of vaccine or cell transfer protocols comes from two recent studies. First, mice were primed with DNA immunogen and boosted with a recombinant adenovirus vector using truncated forms of BORIS and then challenged with the BORIS-expressing 4T1 mouse mammary tumor. Vaccinated mice exhibited prolonged latency and significant inhibition of tumor growth. Second, strong specific CTL responses to BORIS peptides could be elicited in HLA-A*0201 transgenic mice and in the peripheral blood lymphocytes (PBLs) of HLA-matched healthy donors. Therefore, BORIS may be considered as a potent candidate for innovative immunotherapeutic strategies of a broad spectrum of tumors.
A NOVEL DENDRITIC CELL SUBSET INVOLVED IN TUMOR IMMUNOSURVEILLANCE

Nathalie Chaput (a), Julien Taieb (a), Cédric Ménard (a), Lionel Apetoh (a), Evelyn Ullrich (a), Mathieu Bonmort (a), François Ghiringhelli (a), Graça Raposo (b), Thomas Tursz (a), Hideo Yagita (c), Guido Kroemer (d), Laurence Zitvogel (a)

(a) ERM0208 INSERM, Faculté de Médecine Kremlin Bicêtre, Institut Gustave Roussy, Villejuif, France
(b) CNRS-UMR144, Institut Curie, Paris, France
(c) Department of Immunology, Juntendo University School of Medicine, Tokyo, Japan
(d) CNRS-UMR8125, Institut Gustave Roussy, Villejuif, France

Immune surveillance against tumors is mediated by both innate and adaptive components of cellular immunity. IFNγ and lymphocytes are critical effectors against methylcholanthrene-induced and spontaneous tumors in mice, setting the stage for the concept of “tumor immuno-editing”. IFNγ appears to play a fundamental role at the tumoral level enhancing antigen processing and presentation for optimal recognition by T cells. However, the source of IFNγ at the onset of tumor progression is unclear. The IFNγ-induced TRAIL effector mechanism is a vital component of cancer immunosurveillance by NK cells. Here we show that the main source of IFNγ is not the conventional NK cell but a subset of B220+Ly6C- dendritic cells (DC) that are atypical in thus far that they express NK cell surface molecules (Dx5, NK1.1, NKG2D). They represent about 1% of splenic DC but expand by 4-5 fold, traffick and infiltrate tumors when host are treated with c-kit tyrosine kinase inhibitors and IL-2. Upon contact with a variety of tumor cells that are poorly recognized by NK cells, B220+NK1.1+ dendritic cells secrete high levels of IFNγ and mediate TRAIL-dependent tumor cell lysis. Adoptive transfer of these Interferon-producing Killer Dendritic Cells (IKDC) into tumor-bearing Rag^-/-IL-2Rγ^-/- mice prevented tumor outgrowth whereas conventional NK cells failed to do so. In conclusion, we identified IKDC as pivotal sensors and effectors of the innate anti-tumor immune response.
MOUSE MYELOID SUPPRESSOR CELLS COMPRIZE A PECULIAR POPULATION OF INFLAMMATORY MONOCYTES

Vincenzo Bronte
Oncology Section, Istituto Oncologico Veneto, Padova, Italy

Active suppression of tumor-specific T lymphocytes can limit both immune-surveillance and immunotherapy. Several mouse tumors, either transplantable or spontaneously arising, alter the normal myelopoiesis inducing the expansion of a population of CD11b+ cells in the blood, lymphoid organs and at the tumor site. These cells have been named myeloid suppressor cells (MSC) for their proven myeloid origin and the ability to inhibit antigen-activated T cells in an antigen- and MHC-independent fashion. While tumor-recruited CD11b+ MSC cells are known mediators of tumor-associated immune dysfunction, the true nature of these suppressive cells and the fine biochemical pathways governing their immunosuppressive activity remain elusive. By means of combined genome-wide expression profiling, biochemical analyses, and functional studies in knock out mice, we identified a population of CD11b+, inflammatory-type monocytes expressing the alpha chain of the IL-4 receptor (IL-4Rα), that is elicited by growing tumors and activated by IFN-γ released from T lymphocytes. CD11b+/IL-4Rα+ cells produce IL-13 and IFN-γ and integrate the downstream signals of these cytokines to trigger the molecular pathways suppressing antigen-activated CD8+ T lymphocytes. In particular, IL-13 and IFN-γ cooperate in CD11b+/IL-4Rα+ cells to activate the enzymes arginase and nitric oxide synthase that metabolize the amino acid L-arginine and are the final mediators of the suppressive machinery acting on CD8+ T lymphocytes. Although slight differences exist, these pathways are shared by circulating ant tumor-infiltrating CD11b+ MSCs, suggesting a common differentiation route in tumor-bearing hosts. MSCs challenge the current dogma of classic and alternative macrophage activation, possibly representing a novel M2 subtype, and show how the inflammatory response elicited by tumors has detrimental effects on the adaptive immune system.
REGULATORY T CELLS DEPLETION OR INACTIVATION: IS IT A PROBLEM IN CANCER IMMUNOTHERAPY?

Mario P. Colombo, Silvia Piconese, Barbara Valzasina
Immunotherapy and Gene Therapy Unit, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy

Natural arising CD4⁺CD25⁺ regulatory T cells (T reg) originate in the thymus from high avidity interactions of their TCRs with self-peptide/MHC class II expressed on thymic stroma cells. They play an essential role in controlling T cell numbers neutralizing autoreactive T cells. T reg number is tightly regulated, accordingly underneath or excessive number of T reg characterizes pathological conditions like autoimmunity or cancer, respectively. We show that tumors of different origin induce an increase in CD4⁺CD25⁺ T reg number in draining lymph node and spleen but not controlateral node early after tumor injection. Studying CD4⁺CD25⁺ T cells regeneration in thymectomized and CD25 depleted mice either bearing or not a tumor, we established a system in which the majority of the CD4⁺CD25⁺ T cells recovered derive from conversion thus excluding proliferation of pre-existing T reg. In this system, purified CD4⁺CD25⁺ from draining lymph node and spleen of tumor bearing mice suppress the proliferation of CD3-stimulated or allogeneic effector cells and express high levels of Foxp3 at mRNA and protein levels, indistinguishable from natural arising T reg. Transfer of congenic Thy1.1 CD4⁺CD25⁻ T cells, from mice treated or not with vinblastine, into tumor bearing or tumor free mice and analysis of recovered donor lymphocytes indicate that conversion is the main mechanism for acquiring the expression of CD25 and Foxp3 through a process that does not require proliferation.

While conversion of CD4⁺CD25⁻ T cells for generation of T reg has been described as a natural process that maintains peripheral T reg population, this process is used by the tumor for immune escape. The prompt recovery of T reg from mAb-mediated CD25 depletion in TB mice suggests attempts able to inactivate rather than deplete them when treating existing tumors, a process made possible through selected molecules like GITR or OX40.
Session II

Immunological basis of the failure or success of cancer immunotherapy

Chairpersons
Francesco M. Marincola, Peter L. Stern
THE IMMUNOLOGICAL CONSTANT OF REJECTION

Ena Wang, Monica C. Panelli, Eleonora Aricó, Francesco M. Marincola
Immunogenetics Section, Department of Transfusion Medicine, Clinical Center, National Institutes of Health, Bethesda, MD, USA

The complexity determining a pathological process does not necessarily correspond to complexity of the tools required for its resolution. The biology underlying rejection of cancer or allograft, determining autoimmunity or inducing tissues damage during pathogen infections, is complex and multifaceted. However, from this complexity common patterns are starting to emerge that lead to a common final outcome in which tissue destruction occurs that in some cases is associated with resolution of the pathogenic process (cancer, acute pathogen infection) in others unwanted side effect, tissue damage and organ failure. Observations performed through direct human observation in such circumstances indicate that an immunological constant of rejection is required for such occurrences. Clinical observations based on global transcript analysis converge into common signatures characterized by the expression of interferon stimulated genes (ISGs) and others signatures associated to activation of immune effector functions (IEF) both apparently required for tissue destruction. Such signatures are consistently present in tissues biopsied during inflammation. While ISGs appear to be necessary but not sufficient alone to induce immunologically mediated tissue destruction, IEF signatures represent a recurrent theme in distinct pathological conditions and a valuable biomarker of terminal immune differentiation toward the elimination of unwanted tissues. This observation may reconcile the ambiguous role attributed to inflammation in cancer progression. It is clear that chronic inflammation promotes tumor growth while acute inflammatory reactions are required for therapeutic effects against cancer. The presentation will review recent human findings regarding the requirements for tumor destruction in the context of the information available from other immune pathologies in which tissue destruction occur with beneficial or damaging results. Finally we will discuss strategies for future evaluation of the mechanism(s) leading to the switch from a lingering and ineffective inflammatory process to the acute phase necessary for tumor rejection.
ACTIVATION OF iNKT CELLS RESULTS IN DENDRITIC CELL MATURATION AND ENHANCED T CELL EXPANSION IN VIVO

Jonathan D. Silk (a), Mariolina Salio (a), Ian F. Hermans (a), Uzi Gileadi (a), Gurdyal S. Besra (b), Vincenzo Cerundolo (a)

(a) Tumour Immunology Group, Weatherall Institute of Molecular Medicine/Nuffield Department of Medicine, John Radcliffe Hospital, Oxford, United Kingdom
(b) School of Biosciences, University of Birmingham, Edgbaston, Birmingham, United Kingdom

Dendritic cells (DCs) are key regulators of T cell responses. Invariant Natural Killer T cells (iNKT) express a semi-invariant Vα14-Jα18 T cell receptor and are activated in response to glycolipids such as α-galactosylceramide (α-GalCer) presented by non-polymorphic CD1d molecules. Activation of iNKT cells with α-GalCer induces production of a number of different cytokines including IFN-γ, IL-4 and GM-CSF within hours.

Activated iNKT cells also induce rapid DC maturation in vivo, as measured by upregulation of costimulatory molecules such as CD86, in a process that is partially mediated by soluble factors which may include Type I Interferons and IFN-γ. Interestingly, DCs from CD1d-deficient mice (which lack iNKT cells and cannot present α-GalCer) are matured when co-cultured with α-GalCer and CD1d+ splenocytes, again providing further evidence for trans-acting factors that may be involved in DC maturation. Toll-like receptor (TLR) ligands such as monophosphoryl lipid A (MPL), which signals through TLR4, also result in DC maturation. Co-administration of protein, such as ovalbumin, together with such stimuli, result in enhanced antigen-specific CD8+ and CD4+ T cell responses. A synergistic effect on DC maturation is observed, as measured by the upregulation of costimulatory molecules, when iNKT stimulation with α-GalCer occurs together with TLR4 triggering by MPL. When co-injected together with ovalbumin, α-GalCer and MPL results in a significantly larger CD8+ T cell response, which is more effective at killing peptide-pulsed targets than those induced with either α-GalCer or MPL with ovalbumin. We also show synergy on CD86 upregulation on DCs leading to enhanced in vitro stimulatory capacity using α-GalCer together with other TLR ligands that utilise different signalling pathways. Similarly α-GalCer analogues, such as OCH, that stimulate release of different cytokine patterns act together with MPL to enhance DC maturation.

These data suggest that iNKT activation with different α-GalCer analogues, together with different TLR ligands present a useful and potentially flexible method for inducing DC maturation as an adjuvant for effective T cell vaccines.
INDUCTION OF MYELOID CELLS WITH SUPPRESSIVE ACTIVITY BY HUMAN TUMOR-RELEASED MICROVESICLES

Licia Rivoltini, Roberta Valenti, Paola Filipazzi, Veronica Huber, Paola Canese, Lorenzo Pilla, Giorgio Parmiani

Unit of Immunotherapy of Human Tumor, Istituto Nazionale Tumori, Milan, Italy

We recently demonstrated that human tumors, such as melanoma and colo-rectal cancer, release microvesicles (MV) expressing bioactive FasL and TRAIL molecules which induce apoptosis in activated anti-tumor T cells. We additionally showed that MV with comparable phenotypic and functional features can be found in tumor lesions and sera from cancer patients, suggesting a role of this pathway in tumor progression. Here we report that tumor-released MV can profoundly affect myeloid cell function by inducing an abnormal differentiation which results in the generation of suppressive cells. Indeed, when CD14+ monocytes are differentiated into DC (by culture with GM-CSF and IL-4) in the presence of tumor-released MV, they retain CD14 expression and significantly down-modulate co-stimulatory molecules, such as CD80 and CD86. Most importantly, these CD14+ cells become HLA-DR<sup>neg/low</sup> and secrete relevant amounts of TGFβ, which exert a potent suppressive activity on lymphocyte proliferation. Notably, the same CD14<sup>+</sup>HLA-DR<sup>neg/low</sup> TGFβ-secreting cells were generated <i>in vitro</i> when monocytes were differentiated in the presence of MV isolated from the plasma of advanced melanoma patients. Therefore, we searched for the presence of CD14<sup>+</sup>HLA-DR<sup>neg</sup> cells in the peripheral blood of melanoma patients, where the frequency of these cells appeared significantly higher as compared to healthy donors. CD14<sup>+</sup>HLA-DR<sup>neg</sup> cells directly isolated from the peripheral blood of melanoma patients spontaneously secreted higher levels of TGFβ as compared to CD14<sup>+</sup>HLA-DR<sup>+</sup> cells and displayed potent suppressive activity on lymphocyte proliferation. Finally, CD14<sup>+</sup>HLA-DR<sup>neg</sup> cells with an enhanced suppressive activity were significantly expanded in the same melanoma patients after treatment with HSP96 vaccine and GM-CSF, a growth factor that seems to be involved also in myeloid suppressor cell function.

Altogether, our findings show that human tumor cells can induce the generation of myeloid cells with suppressive activity by releasing MV that can exert a long-distance systemic effect, without the need of cell-to-cell contact. These cells, which are expanded in the peripheral blood of melanoma patients, are likely to play a detrimental role in the immunological and clinical efficacy of anti-tumor vaccines.
T CELL MEMORY, ANERGY AND IMMUNOTHERAPY IN BREAST CANCER

Volker Schirrmacher, Philipp Beckhove
German Cancer Research Center, Division of Cellular Immunology, Heidelberg, Germany

T cell immunity in breast cancer is suggested to play a role in tumor dormancy, a period of stability which can correspond to the time interval between primary treatment and tumor recurrence. Bone marrow (BM) in breast cancer patients seems to be particularly important because it is highly enriched with cancer specific memory T cells (MTCs). Similar cells can be found in peripheral blood, but these appear to be functionally anergic. The immune system of primary operated breast cancer patients does not seem to be completely anergized. BM derived MTCs in contrast to naïve T cells can be specifically re-activated ex vivo in a short-term assay with tumor antigen presenting dendritic cells (APCs) and show functional reactivity, including tumor rejection upon adoptive transfer into human breast cancer xenotransplanted NOD/SCID mice. Such adoptive immunotherapy (ADI) leads to tumor infiltration and cluster formation in tumor tissue between MTCs and co-transferred APCs. These findings prompted a first Phase I clinical study of ADI with BM derived MTCs in metastatic breast cancer patients, the results of which will be reported.

Promising results were also obtained from a postoperative Phase II active specific immunotherapy (ASI) study: 32 primary operated patients treated postoperatively with an optimal formulation of a virus-modified autologous tumor vaccine (ATV-NDV) showed augmented anti-tumor memory delayed-type hypersensitivity (DTH) reactivity and appeared to have a significant 5-year survival benefit in comparison to standard therapy. Our results suggest that cancer reactive MTCs which are enriched in the BM of breast cancer patients, can be exploited for immunotherapy. They can be either activated ex vivo via autologous dendritic cells pulsed with breast cancer tumor antigens or they can be activated in situ via a tumor vaccine which combines tumor antigens with danger signals from virus infection. The findings should encourage further studies in breast cancer on ASI with tumor vaccine or ADI with activated memory T cells.
IFN-ALPHA AS AN ADJUVANT IN CANCER IMMUNOTHERAPY

Maria Ferrantini (a), Laura Santodonato (a), Giuseppina D’Agostino (a), Eleonora Aricò (a,c), Tiziana Di Pucchio (a), Imerio Capone (a), Francesco M. Marincola (b), Lorenzo Pilla (c), Licia Rivoltini (c), Giorgio Parmiani (c), Filippo Belardelli (a)

(a) Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy
(b) Immunogenetics Section, Department of Transfusion Medicine, Clinical Center, National Institutes of Health, Bethesda, MD, USA
(c) Unit of Immunotherapy of Human Tumors, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy

IFN-alpha are “old” cytokines with a long record of clinical use. The use of IFN-alpha in clinical oncology has generally been based on the rationale of exploiting its antiproliferative and antiangiogenic activities. Early studies in mouse tumor models performed by our group had demonstrated the crucial importance of host-mediated immune mechanisms in the antitumor response induced by type I IFN, but this information has not been translated into clinical studies for assessing whether this cytokine can enhance an immune response to tumor-associated antigens in cancer patients. Recent studies have shown that IFN-alpha is an enhancer of dendritic cell (DC) activation and a powerful vaccine adjuvant in mice. Likewise, treatment of human monocytes with IFN-alpha in the presence of GM-CSF, results in the rapid generation of highly activated DCs (IFN-DCs) capable of acting as powerful cellular adjuvants for the generation of MHC class I restricted CD8+ T cell responses against EBV, melanoma and CML antigens. In a recent pilot clinical trial aimed at evaluating the immune adjuvant effects of IFN-alpha administered with Melan-A/MART-1:26-35(27L) and gp100:209-217(210M) peptides in stage IV melanoma patients, we observed a remarkable enhancement of blood CD8+ T cells recognizing modified and native MART-1 and gp100 peptides and MART-1+gp100+ melanoma cells, along with a raise in the corresponding tetramer-positive cells and an increased frequency of CD45RA-CCR7- (terminally differentiated effectors) and CD45RA-CCR7+ (effector memory) cells. The IFN/vaccine treatment also resulted in a marked enhancement in the percentage of blood monocyte/DC precursors expressing high levels of costimulatory molecules. Of interest, the gene profile analysis of PBMC after IFN/vaccine administration revealed that a well defined panel of genes (classical IFN-induced signature, including up-regulation of genes of the immune response) was consistently up-regulated at early times after cytokine administration. Of note, a strong direct correlation between enhanced survival in IFN-treated melanoma patients and development of signs of an autoimmune response has been recently shown. This finding is consistent with data from Banchereau’s group and suggests the importance of IFN interaction with DCs in breaking tolerance against self antigens and inducing an antitumor response. In conclusion, now that we have a better understanding of the mechanisms by which IFN-alpha can induce an antitumor immune response in patients, we can design clinical studies for validating new strategies of cancer immunotherapy based on the use of IFN-alpha, either in vivo as a vaccine adjuvant or in vitro for generating patient’s IFN-DCs to be utilized in therapeutic vaccination protocols.
Session III

Strategies for enhancing the efficacy of cancer immunotherapy (I)

Chairpersons
Gerold Schuler, Giulio C. Spagnoli
DENDRITIC CELL VACCINATION OF MELANOMA: LOOKING BACK AND AHEAD AFTER 100 PATIENTS

G. Schuler
Department of Dermatology, University Hospital of Erlangen, Germany

We have performed a series of dendritic cell (DC) vaccination trials accompanied by serial immuno-monitoring in metastasizing melanoma patients after extensive preclinical validation. The DC vaccine consisted of tumour-peptide loaded monocyte-derived DC matured initially by autologous monocyte-conditioned medium and later by its mimic (i.e. IL-1 beta + IL-6 + TNF alpha + PGE2). In initial trials (19 patients) we demonstrated the induction of polyclonal Mage-3 peptide- specific CTL responses. In a “multi-peptide” trial (16 patients) we used DC loaded with MHC class I and II restricted peptides avoiding competition for a particular HLA molecule. A rapid induction of Th1 cells occurred, but the induction of CTL was rather weak in most patients possibly due to a limiting number of 4 million DC per class I peptide. In the next trial (4 cohorts >60 patients) injection of 10 million DC was clearly effective in inducing CTL and Th. Interestingly, we induced T cell reactivity to HSA (human serum albumin) in 10% of the patients some of whom developed rashes. Overall survival continuously increased with the consecutive trials even though objective regressions were rare (11, 24, and currently 31 months median overall survival). In a randomized Phase III Trial of the DC Study Group of the DeCOG peptide-pulsed DC proved equal but not superior to Dacarbazine in the first-line treatment of stage IV metastatic melanoma patients, but this was a first generation vaccine with fewer and less mature DC administered. Following extensive preclinical work we have now started the vaccination with DC transected with defined (Mage-3, Melan-A, survivin) or PCR amplified total tumor RNA. We found ex vivo detectable immune responses in the defined RNA trial, but clinical responses were observed so far only in the total RNA trial. We are also exploring several amplification strategies such as: i) dose escalation trials to test the hypothesis that regulatory T cells can be depleted by either cyclophosphamide or denileukin difitox (Ontak™) for enhancing the immunogenicity of the peptide-loaded DC vaccine; ii) RNA expression of DC which express a chimeric E/L-selectin protein to enable intravenously injected DC to enter lymph nodes directly from the blood.
DENDRITIC CELL-DERIVED EXOSOMES HARBOUR FUNCTIONAL NKG2D LIGANDS: RESTORATION OF NKG2D LEVELS AND FUNCTIONS IN METASTATIC MELANOMA PATIENTS

Thomas Tursz (a), Nathalie Chaput (a), Caroline Flament (a), Sophie Viaud (a), Fabrice André (a), Magali Terme (a), Cedric Ménard (a), Sophie Novault (a), Stéphan Roux (a), Bernard Escudier (a), Caroline Robert (b), Olivier Lantz (c), Sebastian Amigorena (c), Clotilde Théry (c), Philippe Bénaroche (c), Sophie Caillat-Zucman (d), Anne Caignard (e), Laurence Zitvogel (a)
(a) ERM0208 INSERM, Department of Clinical Biology, Institut Gustave Roussy, Villejuif, France
(b) Department of Dermatology, Institut Gustave Roussy, Villejuif, France
(c) U520/U365, INSERM, Institut Curie, Paris, France
(d) INSERM U56, Hôpital Saint-Vincent de Paul, Paris, France
(e) U487, INSERM, Institut Gustave Roussy, Villejuif, France

Dendritic cell (DC) derived-exosomes (Dex) were described as nanovesicles harbouring functional MHC molecules stimultating T cell responses. Our first clinical trial assessing the feasibility and safety of DC derived-exosomes pulsed with MHC class I and II tumor peptides in metastatic melanoma patients revealed that large amounts of exosomal MHC class II molecules can be purified in GMP conditions from autologous immature DC cultures to allow safe and prolonged immunization with exosomes. If occasional tumor regressions were also observed in this study, no DTH responses, peptide dose dependent effects nor T cell responses could be detected. However, elevated circulating numbers of NK cells prompted us to investigate innate effector functions before and after 4 exosome vaccines in these 15 melanoma patients. Here we show that exosomes, but not the immature DC from which they derive, harbour functional NKG2D ligands (ULBP-1 in normal volunteers and MICA/B in patients) mediating NK cell activation. Therapy with DC derived-exosomes could restore NKG2D expression on circulating NK cells of 7/14 metastatic melanoma patients restoring killing of NKG2D ligand expressing K562. Long term administration of NKG2DL bearing-exosomes was not immunosuppressive but instead, maintained NK cell effector functions. Moreover, long term administration of Dex could enhance NKG2D expression levels on CD8+ T cells as well, suggesting non MHC restricted T cell responses independent of the vaccinating epitopes. Such data were corroborated in mouse studies and NK cell dependent antitumor models. It is noteworthy that NKG2D ligands shed by tumors mediate immunosuppressive activity. It is the first demonstration of a particulate form of NKG2DL exhiiting immunostimulatory potentials. Exosomes should be considered as vehicles triggering both the cognate and the innate arms of immunity in vivo.
Dendritic cells (DCs) have the potential to stimulate the immune system and have great promise for the treatment of cancer. Although early clinical trials indicate that DC vaccines can induce immune responses in some cancer patients, careful design and use of standardized clinical and immunological criteria are needed.

In DC therapy, delivery and subsequent migration to regional lymph nodes is essential for effective stimulation of the immune system. We have shown that in vivo magnetic resonance (MR) tracking of magnetically labeled cells is feasible in humans for detecting very low numbers of DCs in conjunction with detailed anatomical information. Autologous DCs were labeled with superparamagnetic iron oxide or $^{111}$In-oxide and were coinjected intranodally in melanoma patients under ultrasound guidance. Before, 24 and 48 hours after intranodal injection of these in vitro-labeled DCs, patients were imaged by body scan and MR. After the last imaging session, the lymph node basin was resected and radioactive lymph nodes were embedded.

In contrast to scintigraphic imaging, MR imaging allowed assessment of the accuracy of DC delivery. Surprisingly, the injections were inaccurate in four of eight patients, despite ultrasound guidance of the injection needle. Subsequent migration could be observed only when DCs were correctly injected into the lymph node, demonstrating the importance for cellular therapy of magnetic resonance verification of accurate delivery.

Migration of labeled DCs was observed with both imaging methods. Mature DCs migrated to the lymph nodes where they could be immunohistochemically detected very efficiently by Prussian Blue staining for iron. From this staining we concluded that injected DCs entered the lymph nodes via the lymphatic vessels, the natural way, subsequently migrating to the T cell areas. A significant proportion of the cells penetrated deep into the T cell areas and were often found to be surrounded by rosetting T cells which were slightly enlarged indicative of T cell activation, a requirement for effective DC vaccines.

In conclusion, high resolution MR imaging allows the detection of small numbers of DC in lymph nodes and appears clinically safe and well suited to monitor cellular therapy in humans.
COMBINING VACCINES WITH ADOPTIVE IMMUNOTHERAPY: RESTORATION OF IMMUNITY IN LYMPHOPENIC CANCER PATIENTS

Aaron P. Rapoport (a), Edward A. Stadtmauer (b), Nicole Aqui (b), Dean Mann (a), Robert Edelman (a), Carl Frasch (c), Bruce L. Levine (b), Alan Cross (a), Carl H. June (b)

(a) University of Maryland Greenebaum Cancer Center and Center for Vaccine Development, Baltimore, MD, USA
(b) Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA, USA
(c) Food and Drug Administration, Rockville, MD, USA

Immunodeficiency is a barrier to successful vaccination in patients with cancer and chronic infection. Combination immunotherapy consisting of adoptive T cell transfer and vaccination may restore immunity and lead to improved outcomes. We performed a randomized phase I/II study in lymphopenic patients following high-dose chemotherapy and autologous hematopoietic stem cell transplantation. Patients were vaccinated with a pneumococcal conjugate vaccine. Combination immunotherapy consisting of a single early post-transplant infusion of in vivo vaccine-primed and ex vivo costimulated autologous T cells followed by post-transplant booster immunizations improved the severe immunodeficiency associated with high dose chemotherapy, and led to the induction of clinically relevant immunity in adults within a month following transplantation. Immune assays demonstrated accelerated restoration of CD4 T cell numbers and function. Early T cell infusions also resulted in significantly improved T cell proliferation to antigens that were not contained in the vaccine, as assessed by responses to staphylococcal enterotoxin B and cytomegalovirus antigens. In the setting of lymphopenia, combined vaccine therapy and adoptive T cell transfer fosters the development of enhanced memory T cell responses.
Session IV

Strategies for enhancing the efficacy of cancer immunotherapy (II)

Chairpersons
Bernard Fox, Stefano Fais
FACTORS THAT INFLUENCE RESPONSES TO ADOPTIVE IMMUNOTHERAPY

Paul F. Robbins
Surgery Branch, National Institutes of Health, National Cancer Institute, Bethesda, MD, USA

Objective clinical responses have been observed in 18 out of 35 melanoma patients treated with the adoptive transfer of autologous tumor reactive TIL following non-myeloablative chemotherapy. Analysis of the T cell receptor beta chain variable region (TR-BV) repertoire of the administered TIL, as well as PBMC obtained following adoptive transfer, revealed that clinical response to therapy was associated with the \textit{in vivo} persistence of dominant TIL clonotypes. Further analysis of multiple persistent clonotypes indicated that they had proliferated extensively \textit{in vivo} following transfer, suggesting that the proliferative potential of the transferred TIL may play a role in the response to therapy. These findings lead to studies in which the lengths of the telomeres present in bulk populations of transferred cells, as well as in dominant TIL clonotypes, were evaluated. The mean telomere length of dominant clonotypes present in the administered TIL that persisted following transfer (6.2±0.4 kb) was significantly longer than that of the non-persistent clonotypes (4.5±0.3 kb) \((p<0.001)\), and the mean overall telomere length of TIL that were administered to responders (6.3±0.4 kb) was significantly longer than that of TIL that were administered to non-responders (4.9±0.3 kb) \((p<0.01)\). Phenotypic analysis was then carried out to determine if the stage of differentiation of the transferred TIL was associated with response, as previous studies demonstrated the loss of expression of markers such as CD27 and CD28 on end stage effector T cells with limited proliferative potential. The result of this analysis indicated that persistent T cell clonotypes expressed higher levels of the co-stimulatory marker CD28 than non-persistent clonotypes \((p<0.05)\). Responding patients also received significantly larger numbers of T cells expressing the co-stimulatory marker CD27 \((1.5±0.3\times10^{10})\) than non-responding patients \((0.54±0.1\times10^{10})\) \((p<0.005)\) when TIL were analyzed following \textit{in vitro} culture in the absence of IL-2 for 2 days to partially mimic the effects of adoptive transfer. These results suggest that the proliferative potential, as well as the stage of differentiation of administered TIL may influence their \textit{in vivo} therapeutic efficacy. Current efforts are focused on the development of methods, based on these observations, which can be used to identify TIL with enhanced therapeutic efficacy.
COMBINING CHEMO-INDUCED LYMPHOPENIA, ADOPTIVE TRANSFER OF PERIPHERAL T CELLS AND ACTIVE-SPECIFIC IMMUNOTHERAPY TO AUGMENT THE ANTI-CANCER IMMUNE RESPONSE: PRECLINICAL AND CLINICAL STUDIES

Bernard Fox
Laboratory of Molecular and Tumor Immunology, Robert W. Franz Cancer Research Center, Earle A. Chiles Research Institute, Portland, OR, USA

Vaccination strategies have failed to improve outcome for cancer patients significantly! Why? For decades clinical trials have administered “cancer vaccines” not knowing whether:

– the vaccine failed to induce a strong tumor-specific immune response or;
– a strong immune response developed, but failed to mediate tumor regression.

Development of new reagents and technology to monitor anti-tumor immune responses in vaccinated patients has provided an opportunity to evaluate tumor-specific responses. These analyses discover low frequencies, generally less than 1%, of circulating tumor-reactive lymphocytes. This is in striking contrast to some preclinical data suggesting that higher frequencies of tumor-specific T cells are required to treat established tumors. Additionally, recent clinical data suggests that 5% of circulating CD8⁺ T cells need to be tumor-reactive and persist for at least two weeks in order to mediate tumor regression. While some controversy exists about this topic, we hypothesize that a primary reason for the failure of tumor vaccine strategies is that the magnitude of the anti-tumor immune response following vaccination has been insufficient to mediate tumor regression. Our own work, as well as that of others, has identified an approach that exploits the increased sensitivity of lymphocytes to respond to antigenic stimuli when they are placed under conditions of homeostasis-driven proliferation. Based upon these observations, our group has initiated four phase I/II clinical trials of this approach for patients with melanoma, NSCLC, ovarian and advanced hormone refractory prostate cancer. The objective of the prostate cancer study is to evaluate the safety and immunologic effects of priming vaccinations, and subsequent boosting vaccinations with an Allogeneic Prostate vaccine immunotherapy in patients made lymphopenic by treatment with chemotherapy and infused with autologous PBMC. We have enrolled and vaccinated 7 men. Of the 6 patients analyzed, three were randomized to receive cyclophosphamide, reconstitution (infusion of peripheral blood lymphocytes) and vaccination. The other three received only vaccination. Analysis of patients who received cyclophosphamide revealed that the Treg cells (CD4⁺/CD25⁺/FoxP3⁺) were at pretreatment levels by four weeks. While preliminary, the apparent failure to deplete Treg cells effectively in the current treatment strategy potentially limits the likelihood that vaccination will trigger the desired effect. This prediction is supported by recent preclinical studies from our group as well as that of others. Based on preclinical studies we are initiating trials that will reconstitute lymphopenic patients with PBMC depleted of Treg cells. While preclinical studies are encouraging, success may
ultimately rely on the adoptive transfer of effector T cells. We continue to evaluate whether our current strategy may provide an in vivo strategy to prime tumor-specific CD4 and CD8 T cells that can be obtained from the peripheral blood, expanded ex vivo and used for adoptive immunotherapy.

These studies were performed as part of a joint Translational Research Program between the Earle A. Chiles Research Institute and Xi’an Jiaotong University, Xi’an, China and Klinikum Grosshadern, Ludwig-Maximilians-University, Munich, Germany.
DACARBAZINE TO ENHANCE VACCINE-MEDIATED ANTITUMOR IMMUNITY IN MELANOMA PATIENTS

Paola Nisticò (a), Pier Giorgio Natali (a), Belinda Palermo (a), Duilia Del Bello (a), Alessandra Ranieri (a), Virginia Ferraresi (a), Mariangela Ciccarese (a), Alessandra Felici (a), Francesco Cognetti (a), Giuseppe Vercillo (a), Gianluca Manni (b), Emanuela Fossile (b), Mario Roselli (b), Mara Valantini (c), Laura Bracci (c), Federica Moschella (c), Filippo Belardelli (c), Immerio Capone (c), Enrico Proietti (c)

(a) Istituto Regina Elena, Rome, Italy
(b) Università degli Studi Tor Vergata, Rome, Italy
(c) Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy

Antitumor immune response is a complex and dynamic process, thus a great effort is ongoing to develop effective immunotherapeutic treatments, but, often, the number of circulating tumor-specific cytotoxic lymphocytes (CTL) does not correlate with in vivo tumor regression suggesting a defect in CTL function or activation. Our previous studies in animal models demonstrated that non-myeloablative treatment by cyclophosphamide (CTX) enhances the activity of antitumor vaccines. Transferring our experimental models to melanoma cancer patients, we carried out a pilot phase I-II clinical trial. Ten HLA-A2 stage II/IV melanoma patients, with no evidence of disease, were enrolled in a phase I-II clinical trial and randomly assigned to one of two treatment groups: 1) Vaccine: Montanide, Melan-A/MART-1:26-35(27L), gp100:209-217(210M), IFN-alfa; 2) Vaccine combined with dacarbazine 800 mg/mq. No major toxicity was observed in either treatment group. IFN-gamma ELISPOT assay and tetramer analysis were used to monitor immune responses before and at different times during vaccination, in either ex-vivo or in vitro expanded CD8\(^+\) T cells. A strong ex-vivo expansion of peptide-specific CD8\(^+\) T cells was observed only in patients treated with dacarbazine plus vaccine (4/5 patients). Moreover, in vitro stimulation of CD8\(^+\) T cells demonstrated that patients receiving the combined treatment developed a peptide-specific immune response and ability of specifically lysing tumor cells consistently higher than the group treated with vaccine alone. Among the five patients treated with vaccine alone, four went into progression. In contrast, 3 out of 5 patients treated with vaccine plus dacarbazine are, presently, disease free. The initial results obtained monitoring the immune response of the patients enrolled in this study suggest that effective immunotherapy of cancer may depend on the creation of a synergy between immune intervention and conventional cancer treatment. The use of dacarbazine in combination with vaccine may represent a novel strategy in the treatment of melanoma patients.
DNA AND ADENOVIRAL VECTORS AND ENCODING TAA/FUSION PROTEINS ELICITS SUPERIOR IMMUNE AND ANTI-TUMOR RESPONSES

Gennaro Ciliberto, Luigi Aurisicchio, Carmela Mennuni, Andrea Facciabene, Sridhar Dharmapuri, Elisa Scarselli, Barbara Cipriani, Daniela Peruzzi, Nicola La Monica
Istituto di Ricerche di Biologia Molecolare (IRBM), Pomezia, Rome, Italy

Development of vaccines for the treatment of cancer is hampered, at least in part, by the lack of effective immunization strategies capable of eliciting antigen specific immune responses and significant anti tumor effect in vaccinated patients. To overcome these limitations we have assessed the immunogenic potency of plasmid DNA electroporation (DNA-EP) and adenoviral (Ad) vectors as gene transfer vehicles for vaccination in a variety of animal models. Additionally, the impact of codon usage optimized cDNA on the immunogenicity of the target antigens has been evaluated. Lastly, the immunogenic potency of vectors encoding tumor antigens fused to immunoenhancing sequences has also been assessed. The results obtained have demonstrated that heterologous prime/boost immunizations based on DNA-EP and Ad vectors elicit significant immune responses to tumor antigens. The immunogenic potency of the genetic vaccine can be further enhanced by utilizing codon usage optimized cDNA and by fusing the target antigen to immunoenhancing proteins such as DOM, LTB, or FcIgG1. Lastly, the amplitude of the antigen specific immune response as well as the extent of tumor protection can be augmented by depletion of T regulatory cells prior to vaccination or by combining the genetic vaccine to immunomodulatory molecules such as TLR agonists or antibodies to suppressive receptors. These observations have great implications on the development of genetic vaccines for clinical applications and we are rapidly moving to apply these new technologies and concepts in a clinical setting.
Session V

Monitoring and prediction of effective responses to cancer immunotherapy

Chairpersons
Pierre G. Coulie, Pier Giorgio Natali
Ipilimumab, a human anti-CTLA-4 antibody has clinical activity in melanoma and renal cancer associated with inflammatory-type adverse events termed immune breakthrough events (IBEs). Twenty-five patients with resected stages IIIC/IV melanoma received MART-1/gp100/tyrosinase peptides with adjuvant Montanide ISA 51 12 times injected subcutaneously with Ipilimumab at 3 mg/kg intravenously every eight weeks for 12 months. The primary endpoints were toxicity and the achievement of a 40% rate of tolerable IBEs, and 20% or less rate of intolerable adverse events. Immune responses measured by ELISPOT and peptide-tetramer assays, and time to relapse were also assessed. Peptides were administered at 1000 mcg/dose each. Median age was 55, with 13 men and 12 women. 15 patients had stage IV, and 10 had stage IIIC resected disease. Thus far, 12/25 (48%) patients had grades 2-3 IBEs; 5 (20%) were dose limiting; 7 had GI toxicity, of which 2 were dose limiting; four had skin toxicity, of which 2 were dose limiting; two patients had hypopituitarism, which was also dose limiting. One patient with dose limiting toxicity required hospitalization and all returned to baseline status with the use of systemic steroids. Serologic assays of IBD-related microbial and auto-antibodies showed that seven of eight patients tested had increases in ANCA IgG or I2 IgA, and 5/8 had increases in ASCA IgA. Five of 25 patients have relapsed with a median of 10 months of follow-up. Two were again rendered NED surgically. Two patients were treated with biochemotherapy, with one CR and one PR. All 25 patients are alive, two with disease. One patient with grade 2-3 IBEs has relapsed. ELISPOT assays showed that 10/11 patients tested responded in fresh PBMC to MART-1 and gp100. IBEs are associated with clinical benefit in patients with resected high-risk melanoma receiving Ipilimumab at 3 mg/kg with a peptide vaccine, and support testing the current regimen in a larger randomized trial. We also tested a human programmed death-1 (PD-1) abrogating antibody in in vitro experiments, and demonstrated that it augmented generation of cytotoxic activity and recognition efficiency of melanoma antigen specific T cells. This antibody merits testing in phase I trials.
MONITORING MAGE VACCINATION. INSIGHTS INTO MECHANISMS OF TUMOR REGRESSION

Pierre G. Coulie, Christophe Lurquin, Bernard Lethé, Nicolas van Baren, Véronique Corbière, Thierry Boon
Christian de Duve Institute of Cellular Pathology, University of Louvain, and Ludwig Institute for Cancer Research, Brussels Branch, Belgium

MAGE antigens have been used for therapeutic vaccination trials of metastatic melanoma patients, either as antigenic peptides, protein, a pox family recombinant virus carrying a $MAGE-3$ sequence, or peptide-pulsed dendritic cells. Clinical responses were observed in 10-20% of the patients. To detect anti-MAGE-3 T cell responses we resorted to an in vitro restimulation with antigenic peptide before labeling with tetramers. In order to evaluate precursor frequencies, these cultures were carried out in limiting dilution condition. Tetramer-positive cells were sorted and cloned. The specificity of the CTL clones was verified, and their TCR sequenced. We found a CTL response in 5 out of 10 regressor patients and in 2 out of 18 progressors, suggesting a correlation between the occurrence of these CTL responses and the tumor regressions. In addition, we examined whether T cells recognizing other tumor antigens might contribute to the tumor regressions. We used autologous melanoma lines from 6 vaccinated patients to estimate blood frequencies of all anti-tumor CTL. After vaccination, frequencies of anti-tumor CTL ranged from $10^{-4}$ to $3 \times 10^{-3}$ of the blood CD8 T cells, i.e. 10 to 10,000-fold higher than those of the anti-vaccine CTL in the same patients. Frequencies of similar magnitude were already present prior to vaccination. Antigens recognized by anti-tumor CTL clones from a patient who showed tumor regression following vaccination were identified. They were encoded by genes $MAGE-C2$ and $gp100$. An anti-MAGE-C2 CTL clone was present in the blood at a frequency of $9 \times 10^{-5}$ and in an invaded lymph node at more than $9 \times 10^{-2}$. Other anti-tumor CTL were also highly enriched in tumors. These results suggest that anti-vaccine CTL may exert their main effect by triggering in the tumor a stimulation of other anti-tumor CTL which destroy the tumor cells.
In contrast to prophylactic vaccines for microbial diseases whose clinical efficacy rests on long term humoral immunity, therapeutic vaccines aim at the induction of tumor antigen specific T cell responses. It is hoped that vaccine formulations able to induce high numbers of cytolytic T lymphocytes (CTL) bearing high avidity receptors for tumor antigens will mediate control of malignant disease.

Numerous vaccine formulations have been tested in phase I clinical trials of cancer immunotherapy, predominantly in patients with advanced melanoma. These include the use of synthetic peptides representing the exact length of antigenic peptides recognized by tumor reactive CTL. We have recently assessed the role of adjuvant in the induction of ex vivo detectable high frequency specific CTL by synthetic modified or native sequence peptides. Subcutaneous injection of emulsions of low dose peptide analogue in Montanide resulted in induction of high frequency CTL in about half of vaccinated patients. In striking contrast, the addition of half milligram of CpG-ODN, a TLR-9 agonist, to such emulsions led to rapid induction of high frequency specific CTL in all vaccinated individuals. The latter adjuvant formulation was equally efficient when using as immunogen a wild type intermediate avidity antigenic peptide. The T cell responses peaked at 7-11 days after the second dose and subsequently after booster injections. All antigen specific CTL displayed an effector memory phenotype and were functionally competent.

To understand the clonal composition of responding specific T cells, we have adapted an approach consisting on the ex vivo isolation of HLA-A2/peptide tetramer 
CD8 
T lymphocytes exhibiting various differentiation phenotypes followed by RNA extraction, cDNA amplification and TCR spectratyping. Oligoclonally or monoclonally expanded TCR are sequenced. Clonotypic PCR is used to track the dominant clonotypes over time. We have documented the boosting of dominant clonotypes that were present in patients with advanced melanoma and preceded the onset of vaccination. We have also observed variations in the composition of dominant clones over time. The decline of certain clonotypes could not be directly attributed to T cell senescence. In my presentation, I will discuss work in progress in the laboratory using this molecular approach to fully characterize the ex vivo properties of vaccine induced T cells and the implications of these results for specific immunotherapy of cancer.
The adoptive transfer of \textit{in vitro} induced and expanded tumor antigen-specific cytotoxic T lymphocytes (CTL) provides a promising approach to the immunotherapy of cancer. We have previously shown that antigen-specific CTL can be generated from HLA-A2.1$^+$ melanoma patients by repetitive \textit{in vitro} stimulation of purified CD8$^+$ T cells with autologous dendritic cells (DC) pulsed with HLA-A2-binding peptides. Based on these results we have performed a phase I clinical study of adoptive T cell therapy in advanced melanoma patients using Melan-A-specific CTL. T cells were generated from HLA-A2.1$^+$ patients by stimulation with Melan-A (ELAGIGILTV)-pulsed DC. Twelve HLA-A2$^+$ melanoma patients received at least three i.v. infusions of Melan-A-specific CTL i.v. at 2-week intervals. Each T cell infusion was accompanied by a 6-day course of s.c. IL-2 (3x10$^6$ IU daily). A total of 51 T-cell infusions were administered, averaging 2.1 x10$^8$ Melan-A specific T cells per infusion. Clinical side effects were mild and consisted of chills and low-grade fever (WHO grade I-II) in 7 out of 12 patients. Clinical responses consisted of antitumor responses in 3 out of 12 patients (1 CR, 1 PR, 1 mixed response). Using the multimer technology which allows for detection and quantification of antigen-specific CTL, we assessed the frequency of circulating Melan-A-specific CTL. Before therapy, the frequencies of Melan-A-specific CTL in patients’ circulating CD8$^+$ T cells ranged from 0.01-0.07\%. Characterization of the multimer frequencies before and at different time points post transfer revealed an increase of circulating Melan-A-specific CTL up to 2\%, correlating significantly with the number of transferred Melan-A-specific CTL. An elevated frequency of Melan-A multimer$^+$ T cells was demonstrated up to 14 days post transfer, suggesting long-term survival and/or proliferation of transferred CTL. Combining multimer analysis with IFN-$\gamma$ secretion and annexin-V staining, unimpaired production of IFN-$\gamma$ and no apoptosis was demonstrated \textit{in vivo} for at least 24h after transfer. $^{111}$ In labeling of Melan-A-specific CTL demonstrated localization of transferred CTL to metastatic sites as early as 48h after injection. Overall, the results suggest that \textit{in vitro} generated Melan-A specific CTL survive intact\textit{ in vivo} for several weeks and localize preferentially to tumor.
Session VI

Critical issues for promoting clinical research in cancer immunotherapy

Chairpersons
Thomas Tursz, Giorgio Parmiani
REGULATORY ISSUES AND CRITICAL NEEDS OF CLINICAL IMMUNOTHERAPY

Alexander M.M. Eggermont
Erasmus University Medical Center, Rotterdam, the Netherlands

Biotherapies comprise a most important field in the development of new treatments in oncology. The field is driven by an explosive increase in knowledge, new technologies and new insights in the biology behind the various phases of cancer. The dynamics in this field demand adapting regulations to facilitate clinical trials that are driven by translational research questions and demand a flexible system to accommodate the field. Only if we can create such circumstances will the promise of the field translate into new treatments of cancer within a short timeframe. Europe faces differences in research and health care systems, differences in culture and differences in economic development and opportunities between the various countries. Yet, Europe presents an array of opportunities to take the lead, as we have a large number of top institutions, a high density population and well developed equal access health care systems, and a culture that is not stifled by litigation. In Europe the opportunities outweigh the drawbacks.

**Vaccines:** European networks of clinical researchers and an improvement in the European regulatory framework for clinical trials are required. Narrow categories of patients must be accrued for each trial. To progress rapidly a Europe-wide recruitment is necessary. Also a coherent series of trials that differ between each other by only one variable, so as to identify factors of improvement. The introduction of preventive vaccines in oncology represents a new challenge.

**Gene Therapy:** Harmonization issues more prominent even than in other fields of biotherapy R&D and Clinical Trial development and confusion over EU-Directives. Lack of harmonization or the interpretation by ethics committees of SOP’s across Europe regarding production as well as application rules.

**Bioassays:** Standardization, Validation and Quality Control are key concerns here, in a field where new bioassays are developed almost daily.

**Tissue Collection:** SOPs and Quality Control are also key here. The EORTC has published and will publish additional SOP’s in these fields. A functional and adaptive regulatory system will need the continuous input of the PROFESSIONALS in the field. This means that we can not only do research, but MUST actively engage in this field to secure progress.
Stefano Vella, Annarita Meneguz
Department of Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy

The European Medicines Evaluation Agency (now the European Medicines Agency, but it still maintains the acronym EMEA) set up in 1995, since the beginning of its activity has considered the opportunity to release specific guidance for anticancer non clinical and clinical investigations, that are in fact operating from 1996. The “Note for guidance on the pre-clinical evaluation of anticancer medicinal products” (CPMP/SWP/997/96)” serves the purpose of avoiding unnecessary non clinical tests, thus enabling the promptest possible introduction of newly developed anticancer medicinal products into clinical trials without compromising safety. The “Note For Guidance on Evaluation of Anticancer Medicinal Products in Man” (CPMP/EWP/205/98 rev 3 ) has been revised three times, the last coming into operation in June 2006, due to continuously evolution of knowledge on this topic, and provides an outline of the requirements in study design for the various Phases (I – III) of clinical development. In section 5 of this guideline “Requirements For Authorisation”, it is stated that Marketing Authorisation Applications (MAA’s) for new pharmaceuticals can be granted in “exceptional circumstances, in cases where full comprehensive data are no available” (Directive 75/318/EEC). Therefore, authorisation can be obtained, in the absence of a Phase III study, if an applicant has compelling Phase II data from which they are able to justify unequivocally that the product has outstanding benefits in the target patient population. Usually in these circumstances, specific obligations and follow-up studies may be required.

As of 2005 it will mandatory for all oncology products MAAs to be filed through the Centralised Procedure. This entails a maximum time frame of 210 days, excluding clock stops for oral or written explanations from the applicant. When a product is deemed suitable for accelerated evaluation (CPMP/495/96/rev1) by the Rapporteur/Co-Rapporteur, a proposal is submitted to the CHMP at the beginning of the assessment procedure to ask if an accelerated timetable for the assessment process would be acceptable.

Moreover EMEA/CHMP established a Scientific Advisory Groups on Oncology (SAG-O) and in the EMEA Management Board two representatives of patients' organisations has been included. This issue is considered particularly relevant for anticancer drugs.
THE COOPERATION BETWEEN ACADEMIA AND INDUSTRIES: COMPARING PRIORITIES AND OPINIONS

Roberto Camerini
Clinical Research Unit III, R&D Department Sigma-Tau, Pomezia, Rome, Italy

Interaction between industries and academia is influenced by different perception of the priorities: market issues, intellectual property and time to market are some of the industries high priorities, on the contrary for the academia the highest priorities are represented by peer-reviewed publications and technology emphasis. A recent survey sponsored by the Italian association of the pharmaceutical industries (Farmindustria) on qualitative analysis of industry-academia collaboration showed a negative evaluation by industries regarding the lack of academia support in terms of technology transfer, development based mentality and bureaucratic-logistic help; on the other hand academia expressed a negative judgement relevant to the poor industry availability to involve public institutes in start-up activities, joint intellectual property as well as in funding R&D activities.

Definitely is necessary to re-design industry-academia interaction focusing the attention on type and aims of collaboration. Partnership should be based on well defined objectives and timelines, scientific program agreement and risk sharing as well as the recognition of academic needs and roles. Translating discoveries in the laboratory into clinical interventions for the diagnosis, treatment, prognosis and prevention of disease with the direct benefit to human health (i.e. translational research) is the ultimate common goal. To reach this goal the removal of translational blocks both in industries and academia is required.
THE ITALIAN NETWORK FOR CANCER BIOIMMUNOTHERAPY (NIBIT): A MODEL TO BE EXPORTED AT EUROPEAN LEVEL?

Michele Maio (a), Paolo Ascierto (b), Filippo Belardelli (c), Paola Queirolo (d), Vincenzo Russo (e), Alessandro Testori (f), Giorgio Parmiani (g)

On behalf of the Italian Network for Cancer Bioimmunotherapy
(a) Medical Oncology and Immunotherapy, University Hospital, Siena, Italy
(b) Clinical Immunology, National Cancer Institute, Naples, Italy
(c) Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy
(d) Medical Oncology A, National Institute for Cancer Research, Genoa, Italy
(e) Cancer Immunotherapy and Gene Therapy Program, Istituto Scientifico San Raffaele, Milan, Italy
(f) Melanoma Unit, European Institute of Oncology, Milan, Italy
(g) Immunotherapy of Human Tumors, Istituto Nazionale Tumori, Milan, Italy

The significant improvement in our knowledge of tumor immunobiology and of the fine immunologic mechanisms regulating tumor-host interactions is still not paralleled by satisfying therapeutic results deriving from the application of immunobiologic therapies in the clinical setting. Different obstacles concur to a better understanding of the clinical potential of immunobiologic therapies. Among these therapies a major role is played by the still very limited interaction among the main actors of the scene: academia, regulatory agencies and pharmaceutical industry. This situation clearly slows-down or even hampers the translation, from the laboratory to the clinic and vice versa, of novel and potentially effective immunobiologic therapies. As a consequence, important therapeutic opportunities might eventually never reach the bed of cancer patients. To contribute to overcome such a negative situation, in September 2004 the Italian Network for Cancer Bioimmunotherapy (NIBIT) was founded in Siena. The NIBIT so far encompasses more than forty national academic, regulatory and industrial groups, and its aims are to: i) promote and foster a stronger scientific and operative interaction among professionals belonging to these three fields; ii) develop innovative, multi-center clinical studies of cancer immuno/biotherapy at national level; iii) develop initiatives to inform cancer patients about potentials and limitations of immunobiologic therapies, as well as about ongoing clinical trials. Concrete early results from the activities of NIBIT include an ongoing open-face dialog with the national regulatory authorities aimed at speeding-up the activation of new clinical trials. Furthermore, academia and pharmaceuticals are becoming increasingly aware of their respective projects, plans and strategies, being reciprocally more conscious of the great advantages of a stronger operative interaction at pre-clinical and clinical level. Additionally, an informative booklet on immuno/biotherapy addressed to lay people is being prepared and will be distributed nationwide in collaboration with a non-profit foundation. Lastly, novel clinical trials of therapeutic vaccination of melanoma patients are being designed and will be conducted in selected institutions that belong to the NIBIT.

Filippo Belardelli, Franca Moretti, Imerio Capone, Giovanni Migliaccio, Maria Ferrantini
Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome Italy

The history of cancer immunotherapy has been characterized by alternate cycles of optimism and discouragement. Recently, as a result of important discoveries in tumor immunology, we have been witnesses of a resurgence of interest in strategies of cancer immunotherapy. Two main types of strategies of cancer immunotherapy can be considered: 1) strategies aimed at enhancing the antitumor immune response by injecting cytokines, antibodies or cells of the immune system; 2) strategies of active immunization (i.e., administration of cancer vaccines). Vaccines have generally been considered as “ideal medical products” capable of inducing long-term immune protection at a relatively low cost. However, some major difficulties have been encountered in the development of therapeutic vaccines against cancer. These difficulties include: a) poor immunogenicity, as TAAs are often self antigens; b) tumor-induced immune suppression; c) tumor cell escape from CTL recognition; d) dampening of the vaccine-induced antitumor immune response by T regulatory cell-driven mechanisms. The recent progress in cell biotechnologies and in the understanding of the mechanisms regulating the antitumor immune response has, however, opened new and exciting perspectives for overcoming these difficulties and, thus, for the development of more effective cancer vaccines. However, the exploitation of this knowledge for designing novel and potentially more effective strategies of cancer immunotherapy is today threatened by regulatory barriers and major obstacles, which often preclude the translation of promising preclinical data into clinical trials. One obstacle is represented by the complexity of the procedures required for the preparation of new cell products to be used in clinical trials; the need of coordination and specific initiatives for promoting and supporting clinical experimentation is commonly felt by investigators involved in this field. Thus, in order to fully meet the great expectations by the scientific community on the potential impact of immunotherapy in the fight against cancer, it is timely and important to establish a strategic cooperation between public institutions responsible for Public Health, National Cancer Institutes, industries and representatives of regulatory agencies. This need is particularly felt by European researchers and clinicians, in view of the regulatory fragmentation and lack of European coordination. These issues can be addressed in the framework of the EUROCAN+PLUS project, the recently started “Feasibility Study for Coordination of National Cancer Research Activities” coordinated by IARC. Some major critical issues for promoting clinical research on immune prevention and treatment of cancer in Europe will be presented and debated.
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