21 INVITED

Legal and ethical requirements of human tissue research

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The increasing possibilities for using tissues for research and the developments in genetics and biotechnology have made stored human biological materials more important than ever. Using stored human biological materials raises a lot of legal and ethical questions (commercialization, protection of privacy, implementation of informed consent, new findings, role of research ethics committees etc.). Research with human subjects and research with personal data is covered by detailed European regulation. The research use of human biological materials however has not been regulated in a detailed manner so far. After discussing the relevant regulation on the level of the European Union and the Council of Europe (EU Directive for the legal protection of biotechnological inventions; Council of Europe Convention on Human Rights and Biomedicine), we will briefly indicate the major issues in retrieving tissues for future research use and in using existing tissue banks for research purposes. A special focus will be on the implementation of the informed consent requirement in daily practice.

Wednesday 29 September 10:15–12:00

WORKSHOP 5

Tumour vaccines

22 INVITED

Human dendritic cell subsets and their implications for clinical studies

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Dendritic cells (DC) are the professional antigen presenting cells of the immune system. They possess the unique capacity to take up and process antigen, migrate to the draining lymphnode, and present antigen to resting lymphocytes. This is the reason why several groups have embarked on the use of these cells for vaccination purposes. We have investigated the capacity of immature and mature monocyte-derived dendritic cells (DC) pulsed with melanoma-associated peptides (gp100 and tyrosinase) to induce a primary cytotoxic T lymphocyte response in vivo. Advanced melanoma patients were vaccinated with peptide- and keyhole limpet hemocyanin (KLH)-pulsed DC, either immature or matured DC Blood, Delayed type hypersensitivity (DTH) reactions against antigen-pulsed DC and tumor sites have been investigated for immunological responses and will be discussed. Results indicate that mature DC are superior to immature DC in the induction of immunological responses in melanoma patients. In other studies we have compared the capacity of immature and mature monocyte-derived DC to migrate to draining lymph nodes. To this end DC were radiolabeled with indium. Melanoma patients who were vaccinated with DC-vaccines received in addition to their first vaccine, radiolabeled DC to study their migratory capacity in vivo. In some cases lymph-nodes were resected and autoradiography was used to determine the number of DC that migrated into the T cell area of the lymph node. Results show that mature DC migrate much better when compared to immature DC. In this presentation I will not only talk about the current state of the art but also discuss issues that still have to be investigated and new opportunities that will broaden the use of this form of immunotherapy in the clinic.

23 INVITED

Development of heat shock cancer vaccine strategies

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Heat Shock Proteins (HSPs) play an essential role in protein metabolism and exert stimulatory activities on innate and adaptive immunity. Vaccination with tumor-derived HSPs induces $CD8^+$ T cell-mediated tumor regressions in animal models. I will show that HLA-A*0201-restricted $CD8^+$ T cells recognizing antigens (Ags) expressed by human melanoma (e.g.Melan-A/MART-1) or colon carcinoma (CEA and EpCAM) were triggered to release IFNγ and to mediate cytotoxicity by HLA-A*0201 matched antigen presenting cells pulsed with HSP96 purified from tumor cells expressing the relevant Ag. Such activation occurred in class I HLA-restricted fashion and appeared to be significantly higher than that achieved by direct peptide loading. Immunization with autologous tumor-derived HSP-peptide complex 96 (HSPPC-96) in either stage IV metastatic melanoma or liver metastasis-resected colon-rectal carcinoma (CRC) subjects, induced a significant increase in the recognition autologous and/or HLA-compatible tumor cells in approximately 50% of patients in both trials. Moreover, anti-Melan-A/MART-$1_{27-35,}$ anti-$CEA_{571-579}$ and anti-$EpCAM_{263-271}$ T cell reaction was elicited in 3 out of 5 and in 2 out of 5 HLA-A*0201 melanoma and CRC patients tested, respectively. Analysis of NK activity also demonstrated an increase in vaccinated patients in both studies. The increments in Ag-specific T cell responses were associated with a favourable disease course after HSPPC-96 vaccination.
A more recent phase II trial was conducted in metastatic melanoma patients with HSPPC-96 and GM-CSF as adjuvant. However, the addition of GM-CSF did not increase the frequency of patients that mounted a tumor-specific T cell response nor the clinical outcome as compared to the previous study of melanoma patients given the HSPPC-96 only.
Altogether, our data provide evidence that HSPPC-96 derived from human tumors can activate the innate immunity and present antigenic peptides to patients' $CD8^+$ T cells and activate them both *in vitro* and *in vivo*. Thus HSPPC-96 appears to represent an important tool for vaccination in cancer patients. Phase III prospective studies are ongoing in melanoma and renal cell carcinoma and will determine the efficacy of HSPPC-96-based vaccination in these diseases.

24 INVITED

Experimental strategies to enhance the potency of dendritic cell-based cancer vaccines for clinical use

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The clinical application of immunotherapy for cancer is rapidly moving forward in multiple areas, which incorporates the adoptive transfer of antitumor-reactive T cells and the use of "therapeutic" vaccines. Both clinical and immunologic endpoints have shown new promise to the field. Novel dendritic cell-based vaccine strategies designed in the laboratory and proven in preclinical animal tumor models are now entering the clinic, with the intent to provide therapeutic efficacy. Improvements on this approach involve breaking tolerance to tumor "self" antigens by inhibiting regulatory cells, boosting T cell co-stimulation, and combinations of recombinant cytokines and other defined molecules with "immuno-enhancing" activities. This review presentation revisits our earlier reported dendritic cell-based vaccine trials in cancer patients and focuses on current and future approaches in the clinic.
Based on encouraging data from our murine studies (1), we initiated and completed two phase I clinical trials of autologous tumor lysate/KLH-pulsed dendritic cells (TP-DCs) in pediatric and adult patients with advanced solid tumors under an IND approved by the FDA in 1996 (2–4). The DCs were produced from adherent PBMC of leukapheresis collects by culture in GM-CSF and IL-4; they were characterized as immature by phenotypic marker profiling. Both immunologic and very modest patient clinical responses in melanoma were noted in these early studies. Autoimmune manifestations were not detected. Although immunologic assays (e.g., IFN-gamma ELISPOTs) revealed evidence of the induction of peripheral blood T cell reactivity to both KLH and tumor lysates post-immunization, particularly in our pediatric patients, durable and complete regressions of established tumors were not achieved through the administration of TP-DCs alone. Based on our recent animal studies, we have now embarked on a new series of clinical trails to evaluate potential improvements in our DC-based vaccine strategy that incorporate the intratumoral delivery of KLH-pulsed DCs (5); the systemic administration of IL-2 (6,7); the use of locally-produced secondary lymphoid tissue chemokine (SLC; 8–10); and the setting of lymphopenia-induced, homeostatic-driven T cell proliferation (11). In this regard, we are planning to conduct the following clinical trials in advanced cancer patients: 1) a phase I trial of escalating doses of Fludarabine/Cy followed by intranodal delivery of MHC class I/II peptide-pulsed DCs in patients with chemotherapy-naïve metastatic melanoma; 2) a randomized phase II trial of Fludarabine/Cy followed by intranodal delivery of peptide-pulsed DCs with or without autologous lymphocyte infusion; and 3) a phase I clinical study assessing autologous DCs gene-modified to secrete SLC as a vaccine to enhance T cell priming in patients with advanced melanoma or colorectal cancer.
Although vaccinations involving TP-DCs have been performed, little, if any, information is available on the effects of phagocytic uptake oftumor lysates on DC biology and function, and how this activity can be influenced to enhance the therapeutic potency of tumor vaccines. We have investigated gene expression pattern differences between unpulsed DCs (UP-DCs) and melanoma TP-DCs, using Affymetrix MG-U74Av2 oligonucleotide arrays, which contain ~12,000 genes and ESTs (expressed sequence tag) (12). Upon 24 hr tumor lysate pulsing, the levels of 87 transcripts increased at

least threefold while the levels of 121 transcripts were reduced by one-third or more, with accompanying p-values less than 0.01. Most of these genes encoded proteins important for DC effector functions including cytokines, chemokines and receptors; antigen presentation; cell adhesion; and T cell activation. We observed a high level of expression of a novel member of the class A scavenger receptor family, MARCO. MARCO is thought to play an important role in the immune response by mediating binding and phagocytosis, but also in the formation of lamellipodia-like structures, of dendritic processes, and in cell trafficking. With respect to the latter, we have now shown in mice that approaches that block MARCO can have profound effects on DC migration from tumor vaccination sites to peripheral lymphoid tissues. Our future clinical vaccine trials in cancer patients may incorporate this strategy as well to enhance potency.

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Wednesday 29 September 10:15–12:00

WORKSHOP 6

Pharmaceutical industry, investigators and institutions: partners or tools? Practical issues in clinical cancer drug development

25 INVITED

Pharmaceutical industry, investigators and institutions: partners or tools? Practical issues in clinical cancer drug development. Erosion of the principal investigator in a climate of industry dominance

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Although institutional review boards, government agencies, and the scientific community consider the principal investigator as the individual who is ultimately responsible and accountable for the design, execution, and analysis of clinical trials of novel therapeutics, the role of the academic investigator is becoming dangerously ambiguous. There is no doubt that the phenomenon is largely due to the progressively greater control that the pharmaceutical and biotechnology industries are insidiously assuming behind the scenes. The heightened interests of the pharmaceutical and biotechnology industries in cancer therapeutic development, along with the somewhat seasonal interests of the investment community, have undoubtedly catalyzed cancer therapeutic development efforts over the last decade, but the notion of the principal investigator as the "captain of the ship" is drifting by the wayside. The pressures and inherent objectives of the "business of oncology" have interfered with the role of the principal investigator as the individual in charge, which is impacting on the optimal development of cancer therapeutics on both microscopic (individual studies) and macroscopic (overall drug development paradigm) scales. The early years of anticancer drug development in the United States were largely dominated by efforts sponsored by the National Cancer Institute (NCI). During this period, principal investigators became "attached" to therapeutics and the proponent for their optimal development, shepherding them through an unencumbered process. The NCI development process encouraged "foundation building", in which clinical scientists orchestrated clinical and translational studies autonomously, and the development of each subsequent study and clinical developmental stage was based on bedside and laboratory investigations performed during the previous stage. Compared to the present, the investigator was truly the "captain of the ship" and intimately understood the therapeutic-maximal efficiency. The investigator held clinical data close to hand; the investigator was aware of all pharmacokinetic and translational data generated at the site; and results were discussed and debated in an unimpeded, uncensored fashion at major meetings.

However, a brave new world is upon us. At present, the principal investigator performs even the most uncomplicated phase 1 trials as a cog in a clinical trial machine, often dominated by large Clinical Research Organization (CRO) and multiple autonomous factions of pharmaceutical companies (e.g. experimental medicine, product oriented medicine, marketing, regulatory, business unit, pharmacology, quality assurance, and imaging groups), each of which enacts its own standard operating procedures and insists that the investigator work according to their directives. This fractionation has result in a true loss of control and the overall principal investigator (principal responsible investigator) is often unnamed and assigned by default on the basis of maximal patients accrued. For the individual investigator, even trying to grasp the organizational aspects of the pharmaceutical sponsor and contend with the paperwork have become inordinate tasks. Although the organizational structure of less complex, smaller biotechnology companies might be much easier to grasp, the overall "investment side" ramifications of the project are always close at hand to the academic scientists. Concerns about investor perception of the new therapeutic are progressively dominating early clinical trials and these pressures are pressures are palpable to the investigator, even at the earliest developmental stage. Although the participation of patient populations with refractory cancers known to have excellent performance status and once considered ideal for the evaluation of toxicity in phase 1 studies, are now considered suboptimal since subliminal corporate pressures have shifted towards the maximizing the potential to demonstrate clinical activity to prop up the drug's perception for investment community. Such efforts, which are often based on teleological reasoning and misperceptions about the mechanism of action of new drugs, impede the achievement of the toxicological objectives.

Sponsors have become obsessed with meeting timelines and milestones and this obsession is indeed coming at a cost. The single institution, single principal investigator trial is becoming archaic because of the notion that time-lines are much more likely to be met by using more investigators and institutions, even when it is clear that the bottleneck is study design, not patient accrual. Championed by the pharmaceutical and biotechnology industries, the growing trend in the conduction of phase 1 studies is in the direction of large, multi-functional evaluations in lieu of smaller, more intimate trials that have been traditionally conducted at a single institutions and strictly focused on dose finding and characterizing the toxicological and pharmacological profiles of new anti-cancer therapeutics. This trend undoubtedly stems from mounting competitive pressures in these industries, resulting in a quest for maximal efficiency in patient resource utilization and a strict adherence to often-unrealistic management-driven timelines. These competitive corporate pressures have been progressively shifted over the years to the clinic and are now being observed downstream at the earliest phase of therapeutic evaluations, which were once considered immune from any study design imperfection that would even slightly increase the risk for patients, since the overriding theme in the design and conduction of the phase 1 studies has always been related to minimizing risk and the principal dictum has been to cut no corners and leave no stones unturned. Phase I studies of anti-cancer agents, which have much narrower therapeutic margins and higher risk-benefit ratios have traditionally been performed by a small number of experienced investigators at a maximum of one or two highly specialized sites. These practices encouraged investigators to become intimate with their clinical and pharmacologic data, whereas current multi-institutional practices encourage investigators from different sites to compete with one another for treatment slots. Similar to good laboratory practices, which mandate the use of the fewest well calibrated instruments as possible to minimize experimental variability due to instrumentation, the seemingly minor, albeit powerful, characteristic of the intimate study has traditionally facilitated the acquisition of expertise by both investigators and research staff since it enables them to make detailed observations over the entire