GCRI INTERVIEW

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How much progress have we made in the war on cancer?

In the past four or five years, tremendous progress has been made in the field of cancer immunotherapy. For some of the cancers with a high mutation load, in particular melanoma and lung cancer, new drugs, the so-called checkpoint antibodies activating cancer-specific T cells, are about to become the standard of care.

The strategy of T cell-mediated cancer immunotherapy was initiated about 30 years ago with the adoptive transfer of donor lymphocytes into recipients of hematopoietic stem cell transplantations, resulting in so-called graft-versus-leukemia reactions. This was pioneered by Hans-Jochem Kolb, and has proven to be very successful in certain types of leukemia. The associated toxicities, graft-versus-host reactions, can be severe.

The application of checkpoint antibodies, which unleash the otherwise suppressed T cell responses in patients against tumor antigens, was pioneered by Jim Allison and the first FDA approval of such an antibody, Ipilimumab, marked a breakthrough in cancer immunotherapy. The associated toxicities, again, can be severe, and are probably caused by the activation of autoimmune T cells combined with that of tumor-specific T cells reactive to so-called neoantigens on the tumor. A number of other checkpoint antibodies have either already been approved or are in development and have lower side effects. Limitations of the checkpoint antibodies are that they only work in a fraction of patients, and this fraction increases with higher numbers of tumor mutations. Thus, patients with tumors of relatively lower mutation numbers, such as bowel, breast, or prostate carcinoma, respond to checkpoint inhibition more infrequently.

Another successful way of using T cells to kill cancers is to employ CAR-T cells. These are patient T cells genetically engineered in vitro to express antibody-like receptors that recognize cell surface molecules on cancer cells. Pioneered by Zelig Eshhar, Carl June, Michael Jensen and others, this approach has been shown to achieve long-term remission of leukemia. Some of the limitations are the complex technology involved in producing T cells for every single patient, toxicity, and in particular, the scarcity of target antigens; CD19, a B cell surface antigen, is the

most widely used so far. CD19 is not cancer-specific, but cell-type specific and is only expressed on a cell type that is not required for survival. Adoptive transfer of T cells bearing the natural or engineered T cell receptors is also being developed, with occasional spectacular benefit. If the T cell receptor has not matured in the patient, as is the case with tumor infiltrating cells, but comes from an individual differing in the genes coding for HLA (that is, the molecules presenting antigen on cells) or is engineered to have higher T cell receptor affinity than in a natural setting, there is a high risk of "unexpected" toxicities due to the intrinsic and unpredictable tendency for cross-reactivity of T cell receptors to peptides not seen during negative selection in the thymus.

A related approach is to target T cells to cancer cells by bispecific antibodies, whereby one part recognizes a tumor antigen, the other the T cell receptor. Pioneered by Gundram Jung, this strategy is now being widely exploited, most commonly with the same CD19 popular with CAR-T cells, or with the similar CD20. However, both also suffer similar limitations. One, Blinatumomab, developed by Gert Riethmüller, is FDA approved.

Please elaborate on some of your current research projects concerning translational immunology.

The cancer-specific T cells, which either spontaneously develop in patients or are induced by checkpoint inhibition, or are transferred by donor lymphocyte transfusions, recognize peptides presented by specialized molecules on the surface of all our cells. These molecules have the task of collecting samples of cellular protein fragments/peptides and presenting them to T cells. They are the so-called HLA molecules, which are also found on tumor cells. These peptides are either neoantigens corresponding to tumor mutations, as first shown by Thierry Boon and Thomas Wölfel, or they are of viral origin, or are non-mutated cancer specific or overexpressed antigens, as first described by Lloyd Old and colleagues. Common to all these cancer peptides is that they are different in every patient. Thus, to induce tumor-specific immunity, the best line of action is to personalize the approach.

Identification of neoepitopes can be done by exome sequencing, prediction of the peptides fitting to the patient's HLA, verification by mass spectrometry of peptides isolated from tumor cells or by T cell assays. In recent years, we and others have found that there are far fewer neoepitopes than previously expected; we assume that only between one and ten in a thousand mutations at the DNA exome level will result in an HLA presented peptide on tumor cells. However, we find dozens or more non-mutated tumor specific- or at least overexpressed peptides on any HLA expressing tumor of any entity we analyze. We do this by first isolating HLA molecules from tumor and normal tissues and then eluting the HLA-bound peptides for identification by mass spectrometry. Many of these peptides are immunogenic,

and immune responses against these correlate with overall patient survival, both if one looks at spontaneous immune responses or those observed after vaccination. Based on these considerations, our strategy for antigen-specific cancer immunotherapy is to perform HLA ligandome analysis for a patient's tumor, select the peptides specific to the tumor, and synthesize these for vaccination of this very patient. Alternatively, the antigens can be delivered by the mRNAs encoding them; mRNAs are more immunogenic than peptides, because single stranded RNA works as TLR7 ligand and thus as an in-built adjuvant; it is, however, more demanding to produce mRNAs in clinical grade as compared to peptides. Clinical trials with both approaches are already underway. One problem with peptide vaccination is the weak immune responses observed so far, mainly due to inefficient adjuvants. We are presently developing a new adjuvant, a TLR2/TLR1 ligand that is extremely efficient in inducing human T cell responses upon injection together with free peptides.

Which aspects of your research would you like to focus on next?

We have already started a personalized peptide vaccination trial in glioma patients (EU consortium GAPVAC, gapvac.eu) and will soon begin a similar trial in hepatocellular cancer patients (hepavac.eu). Presently we are developing personalized clinical vaccination trials in patients with minimal residual disease, that is, immediately after surgical resection of the tumor or after a first round of therapy. We are close to starting trials with chronic lymphatic leukemia (CLL), ovarian cancer, and prostate cancer. We are planning to carry out the first clinical trials with our new adjuvant, a synthetic TLR2/TLR1 ligand based on an immunostimulatory substance from the outer cell membrane of E. coli, and to optimize its delivery. At the moment, subcutaneous injection of the vaccine and the adjuvant in a water-in-oil depot works extremely well.

What are the advantages of personalized immunotherapy?

Personalized cancer immunotherapy, as we understand it, calls for molecular identification of the relevant antigens on tumor cells not expressed on normal body cells. Subsequently, the immune system of the patient is induced to elicit T cell responses against such antigens, in our approach, by vaccinating the patient with synthetic peptides together with an efficient adjuvant.

Such a personalized approach should be as efficient as checkpoint inhibition, but should not be associated with severe toxicities. Further, it should be applicable for nearly all tumors, not only those with a high mutation load.