Engineering Immunotherapy

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Introduction – the new age of immunotherapy

Immunotherapies are treatments aimed at promoting an immune response against cancer or other diseases. Immunotherapy has been pursued for more than 30 years as a potential treatment for cancer, due to the potential for the immune system to safely distinguish healthy cells from tumor cells, to be resistant to mutational escape by tumors, and for the possibility of immune memory to be established that could prevent recurrence. However, treatments targeting the immune system for many years showed only anecdotal efficacy in clinical trials, leading many to become disillusioned with the field by the late 1990's. Contemporaneously, the 1990's were a period when many critical elements of fundamental biology regulating the immune response became defined: the identification of the first tumor antigens, the discovery of Toll like receptors and many other related receptors governing inflammation and the immune system's ability to identify "danger", the discovery of regulatory receptors that either promoted or blocked T cell activation, and the characterization of specific signaling pathways and mechanisms used by tumor cells to avoid immune destruction.

These discoveries led to a transformation in the field of immuno-oncology, which was most prominently impacted by clinical studies in the early 2000's of an antibody that blocks a key negative regulatory receptor on T cells known as Cytotoxic T Lymphocyte Antigen-4 (CTLA-4). Treatment of melanoma patients with this antibody enabled endogenous anti-tumor immune responses to be mounted that led to tumor regressions in a small proportion of heavily pretreated patients with metastatic disease- leading to ~20% of patients to survive >5 years,

well beyond the expected lifespan for advanced disease.[1, 2] This "tail of the curve" effect in overall survival reflects a dramatic change in outcome from the best modern "targeted" therapies, where early tumor regression is uniformly followed by drug resistance, relapse, and death. Following these early findings, a second class of antibodies blocking another negative regulator axis in T cells, antibodies against PD-1 on T cells (or against its ligand, PD-L1 expressed on tumor cells), showed even more dramatic effects in large clinical trials, with 30-50% of melanoma, renal cell carcinoma, and lung cancer patients showing objective responses.[3] These drugs, although acting by distinct mechanisms, are collectively referred to as "checkpoint blockade" therapies, as they disrupt regulatory checkpoints restraining the immune response against cancer.

In parallel to these developments, a second type of immunotherapy approach known as adoptive cell therapy (ACT), based on the transfer of autologous tumor-specific T-cells into patients, has also been developed with growing success. In ACT, T-cells are isolated from the peripheral blood or from tumor biopsies, cultured with the patient's own tumor cells to identify tumor-reactive clones, and then expanded to large numbers for reinfusion into the patient.[4] The creation *ex vivo* of an army of tumor-specific T-cells has been shown to elicit objective tumor regressions when combined with appropriate adjuvant treatments that promote the functionality of the transferred T-cells (e.g., administration of adjuvant drugs like interleukin-2). In addition, strategies to genetically modify T-cells for patients, introducing a synthetic T-cell receptor (chimeric antigen receptor, or CAR) that allows any T-cell to become a tumor-specific T-cell, has shown particular promise in treating certain leukemias- where >75% of patients have experienced complete responses.[5]

Thus, in the space of a few short years the field of cancer immunotherapy has been revolutionized in the clinic, from a peripheral approach notorious for high toxicity and low efficacy, to a frontline treatment with the prospect of eliciting durable responses, and perhaps cures, in a fraction of patients.

Roles of engineering in the future of cancer immunotherapy

Immunology has traditionally embraced new technologies as a means of driving the field forward, from the early days of monoclonal antibody technology to the recent inventions of powerful mass spectrometry-based cellular analysis tools. However, the field has also recently become home to a growing number of interdisciplinary scientists bringing to bear a unique mindset and new approaches to problems in immunology and immunotherapy rooted in engineering, leading to exciting advances in basic science and new approaches to vaccines and immunotherapies. Engineers excel at creating model systems that break complex problems down into manageable hurdles, and drawing on applied chemistry, physics, and mathematics to create *de novo* technologies that solve practical problems. The contributions of engineers to the evolution in cancer immunotherapy can be illustrated by a few recent examples of progress in the field- in the areas of cancer vaccines and ACT. Importantly, these two areas by no means represent all of the topics where engineers are interfacing with cancer immunotherapy, but rather are two representative examples.

***Enhancing cancer vaccines*.** Checkpoint blockade with anti-CTLA-4 or anti-PD-1 has elicited objective tumor regressions in a small proportion of patients, and this incomplete response rate has motivated a strong interest in finding additional treatments that can be combined with these drugs to further expand the responding population. Because these drugs act to enhance T-cell responses against tumors, one obvious strategy is to combine checkpoint blockade with therapeutic cancer vaccines, since some patients may have spontaneous T-cell responses against tumors that are too weak to be rescued by checkpoint blockade alone. To this end, a renewed interest in cancer vaccines has been kindled in both preclinical and clinical studies. However, cancer vaccines to date have generally been perceived as a failure, both due to their lack of objective responses in patients and their inability to elicit the kind of robust T-cell priming

that is believed to be necessary for tumor regression- T-cell responses more like what are seen to live infectious agents.

How do we do better? Engineering approaches offer some new ideas of how to create more potent and effective cancer vaccines. Vaccines are generally based on the delivery of antigens (the protein, peptide, or polysaccharide target of the immune response) together with inflammatory cues that stimulate the immune system to respond to the associated antigen. One of the simplest approaches that has been most extensively explored in the clinic is the use of peptide antigens combined with adjuvants as T-cell-focused vaccines. However, short peptides injected in vivo have several significant limitations: they are quickly degraded, they largely flush into the bloodstream rather than trafficking to lymphatics and lymph nodes, and short peptides can be presented by any nucleated cell to T-cells. The latter phenomenon, where T-cells are stimulated by random tissue cells rather than professional antigen presenting cells (APCs) in lymph nodes, leads to tolerance or deletion of tumor-specific cells. One example approach to deal with all of these challenges at once is to conjugate so-called “long” peptide antigens (that can only be presented by professional APCs) to an albumin-binding lipid tail through a water-soluble polymer spacer. Albumin constitutively traffics from blood to lymph, and thus linking antigens to an albumin-binding lipid “tail” redirects these molecules efficiently to lymph nodes instead of the bloodstream following parenteral injection. In addition, the polymer/lipid linkage protects the peptide from degradation. A similar strategy can be used to create “albumin hitchhiking” adjuvants. Thus, these simple chemical modifications lead to 15-30-fold increases in vaccine accumulation in lymph nodes, both increasing the safety of the vaccine and dramatically increasing vaccine potency.[6]

Engineers have also used methods developed in the regenerative medicine field to create implantable vaccine “centers”, that coordinate multiple steps in an anti-cancer vaccine response. A common strategy in regenerative medicine is to create biodegradable polymeric scaffolds as artificial environments that can protect and nurture therapeutic cells on implantation

in vivo. Mooney, Dranoff and colleagues demonstrated that a similar approach can be used to regulate the response to a vaccine: By loading polymeric sponges with tumor antigens, chemoattractants for APCs, and adjuvants, they showed they could coordinate a 3-step process of (1) APC attraction to the implanted scaffold, (2) uptake of antigen and adjuvant by the APCs, and (3) migration of the now activated APCs to draining lymph nodes, where they could initiate a potent anti-tumor immune response.[7] Thus, chemistry and biomaterials approaches offer a number of ways to create enhanced cancer vaccines.

***Engineering adoptive cell therapy*.** As noted above, adoptive transfer of tumor antigen-specific T-cells is one of the two classes of immunotherapies to demonstrate significant durable responses in the clinic so far, but strategies to improve this treatment for elimination of solid tumors are still sought. One approach by which engineers are impacting the evolution of ACT treatments is through the application of synthetic biology principles for the creation of novel genetically-engineered T-cells. Recently for example, bioengineers have generate completely artificial ligand-receptor-transcription factor systems, which allow a synthetic receptor and transcription factor pair to be introduced into T-cells, in order to allow T-cell recognition of a tumor-associated ligand to be transduced into transcription of an arbitrary biological response.[8, 9] Another strategy has been to introduce synthetic fragmented antigen receptors that are only activated when a small molecule drug is present, to allow precise control over the activity of therapeutic T-cells in vivo.[10] These are only a few representative examples of a rapidly-moving and exciting area of research.

A second strategy is to chemically engineer T-cells, using approach from the nanotechnology and drug delivery communities to “adjuvant” T-cells with supporting drugs. One promising approach is to attach drug-releasing nanoparticles directly the plasma membrane of ACT T-cells, such that the modified cells carrying supporting drugs on their surface wherever they home in vivo. This approach has been shown to greatly augment the expansion and anti-

tumor activity of T-cells when used to deliver supporting cytokines to the donor cells.[11] This basic demonstration also opens the potential for targeting supporting drugs directly to T-cells in vivo, through targeted nanoparticle formulations.[12] Such studies show promise in preclinical models and are entering the early stages of translation into clinical testing.

Conclusions

In summary, the revolution in cancer therapy being brought about by the first truly successful immunotherapy treatments has revitalized the field of cancer immunotherapy. This ongoing revolution has also created exciting new opportunities for engineers to impact the field of cancer, by solving challenging problems to safely and potently enhancing the immune response against tumors. Marriage of cutting edge tools from engineering with the latest understanding of the immune response to tumors offers the promise of further advances toward the goal of curing cancer or rendering many cancers a manageable, chronic condition.

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