Molecular Therapy
Perspective

Safety Starts with Selecting the Targets

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https://doi.org/10.1016/j.ymthe.2021.01.011

Targeting tumor antigens with immunotherapy is rapidly emerging as a promising approach for cancer treatment. The accumulation of genetic and epigenetic aberrations, a hallmark of malignancy, generates transcriptional and proteomic diversity. This diversity also affects the cancer surfaceome, thus providing targets promoting disease initiation and progression and for use of immunotherapy. However, off-target effects resulting from inadvertent targeting of non-malignant cells expressing the same or similar antigen represent a serious safety concern.

Identifying biologically and therapeutically relevant surface targets is critical to ensuring effective and safe cancer immunotherapies. However, our knowledge of the contribution of an altered surface proteome to cancer pathogenesis is still in development. Recent technological advances are now providing the necessary means to comprehensively map and analyze the cancer-specific surfaceome. These include high-quality mass spectrometry methodologies and integrative bioinformatics tools. These are helping to overcome the challenge of studying surface proteins that present with low abundance, high hydrophobicity, and heavy post-translational modifications compared to intracellular proteins. Nevertheless, our ability to predict the therapeutic value of candidate molecules remains imperfect and requires careful assessment of their abundance in cancer stem cells and dominant cancer clones in large patient cohorts. Just as importantly, the safety of candidate molecules requires evaluation in normal tissues beyond normal counterparts. Finally, their immunogenicity needs validation in functional assays.

It is now well established that the tumor mutational burden, measuring the total number of non-synonymous somatic mutations per megabase of the genome coding area of DNA, influences the ability of the immune system to recognize potential neo-antigens.[1] As such, solid tumors are generally more responsive to immune checkpoint blockade compared to hematologic malignancies, including acute myeloid leukemia (AML). However, little is known about the effects of mutational quality rather than quantity and immune responses. This might be relevant for malignancies presenting a relatively low number of somatic mutations. For example, the average AML genome may have only 13–16 coding mutations, with a striking frequency of epigenetic derangements that contribute to its pathogenesis. The anti-leukemic response in allogeneic hematopoietic stem cell transplantation mediated by a graft-versus-leukemia effect, in which donor immune cells eliminate cancer cells, has been known for a long time and supports the concept of leukemia's immunogenicity. However, while there is evidence of T cell suppression due to leukemic manipulation of the immune microenvironment, the most relevant therapy targets and patient subsets remain unclear. Epigenome rewiring contributes to profound changes in a plethora of transcriptional signatures, which may include genes regulating antigen expression and antitumor immunity.[2] In addition, unconventional target antigens may arise by means of aberrant RNA splicing, translation, or post-translational modifications[3,4] as a result of those frequently recurring mutations, also including splicing factor mutations, with some of these processes being entirely tumor-specific and behaving as neoantigens. Thus, identifying such target antigens brings to light the possibility for the development of more precise and safer immune intervention approaches.

More relevant clinical experience in hematologic malignancies so far comes from targeting tumor-associated antigens, which are overexpressed on normal surface proteins by chimeric antigen receptor (CAR) T cell therapies. Unlike the physiological T cell receptor, which engages HLA-peptide complexes, CARs bind to native cell surface molecules, which do not require antigen processing or HLA expression for tumor recognition. CAR T cells can therefore recognize any surface molecule on any HLA background or in tumor cells that have downregulated HLA expression or proteasomal antigen processing, two mechanisms promoting tumor immune escape. Targeting CD19 has had a transformative effect in B cell hematologic malignancies, including acute lymphoblastic leukemia and non-Hodgkin's lymphoma. CD19 presents a "fortunate" expression profile as it is highly and homogenously expressed in malignant B cell lymphoid cells, including cancer stem cells and, significantly expressed in normal B cells only, causing a treatable B cell aplasia. Unfortunately, this is not the case for most tumor-associated antigens.

A lack of suitable target antigens in addition to genetic heterogeneity and the increased recognition of immunosuppressive factors within the tumor microenvironment have hindered therapeutic success of engineered T cells for adoptive cell therapy (ACT) of CD19-negative malignancies. For instance, in a study involving three patients with colorectal cancer, ACT with T cells bearing a high-affinity T cell receptor (TCR) against the carcinoembryonic antigen (CEA) resulted in objective cancer regression in one patient, but also induced severe irreversible destruction of normal colonic mucosa in all three cases. In one report, targeting carbonic anhydrase IX (CAIX) in patients with metastatic kidney cancer resulted in unintended toxicities due to CAIX expression in the biliary duct epithelium. In an attempt to treat

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breast cancer patients with CAR T cells based on the widely used monoclonal antibody Trastuzumab, within 15 minutes after cell infusion, the patient experienced respiratory distress and displayed a dramatic pulmonary infiltrate on chest X-ray. The patient died 5 days after treatment, likely due to the recognition of low levels of ERBB2 on normal lung epithelial cells.

These results exemplify the safety concerns regarding on-target off-tumor toxicity representing a significant risk in clinical trials and highlight the need for precise protein annotation of newly discovered antigens in several normal tissues to inform rational pre-clinical developments.[5] While there is increasing interest and activity in the development of cell therapies because of their potential to address unmet medical needs, the design of early-phase clinical trials often differs from the design of clinical trials for other types of pharmaceutical products because of considerable uncertainty about the nature and frequency of safety problems.

As such, in search of suitable targets, we also need to take into consideration that the surfaceome is more dynamic than the total proteome and that detailed knowledge about candidate surface proteins, their mechanisms of action, and their functional requirements during distinct stages of normal tissue development may help prevent severe toxicity when targeting with living drugs that persist in humans upon the time of administration.

Efforts are underway to implement target discovery strategies, standardize validation assays, and engineer effective multi-specific CAR T cells that have the potential to reduce the risk of on target off/cancer toxicity, tackle tumor heterogeneity, a major roadblock for all targeted therapies, including immunotherapies, and prevent antigen escape. In fact, targeting one single molecule often leads to disease relapse due to the outgrowth of antigen-low or -negative relapsed tumor cells. This is not surprising when achieving durable antitumor efficacy with CAR T cells depends on the density and stability of the target antigen. Antigen loss variants have emerged as one mechanism of therapeutic failure in patients with leukemia and lymphoma receiving CD19 CAR T cells. In addition, there is an intense focus on engineering T cells to increase their specificity for antigens expressed on tumor cells. These include modulating the affinity of the single-chain variable fragment (scFv), upregulating the antigen density on targeted cells, restricting activity to tumor sites, and developing inducible safety switches that can be activated in the event of adverse events, including on target/off cancer toxicity. After all, the choice of target antigen remains the most important determinant of an effective and safe immunotherapy.

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