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Unmasking targets of antitumor immunity via high-throughput antigen profiling

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More than three decades of evidence has established that antitumor immune responses, initially shown with IL-2 treatment, can result in complete, durable eradication of malignant disease in metastatic patients. Recent studies have demonstrated that immune checkpoint blockade as well as cellular therapies, including dendritic cell activation of T cells and adoptive T cell transfer, can induce long-lasting responses. To elicit cytolysis of tumor cells, effector T cells rely on tumor expression of target antigens. However, the antigens targeted during antitumor responses are largely unknown. Technological advancements and availability of sequencing data have paved the way for more efficient screening and validation of tumor-associated antigens and neoantigens derived from non-synonymous mutations targeted by T cells under baseline conditions and in the context of immunotherapy.

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Elimination of antigen expressing tumors: A common link in immunotherapeutic regimens

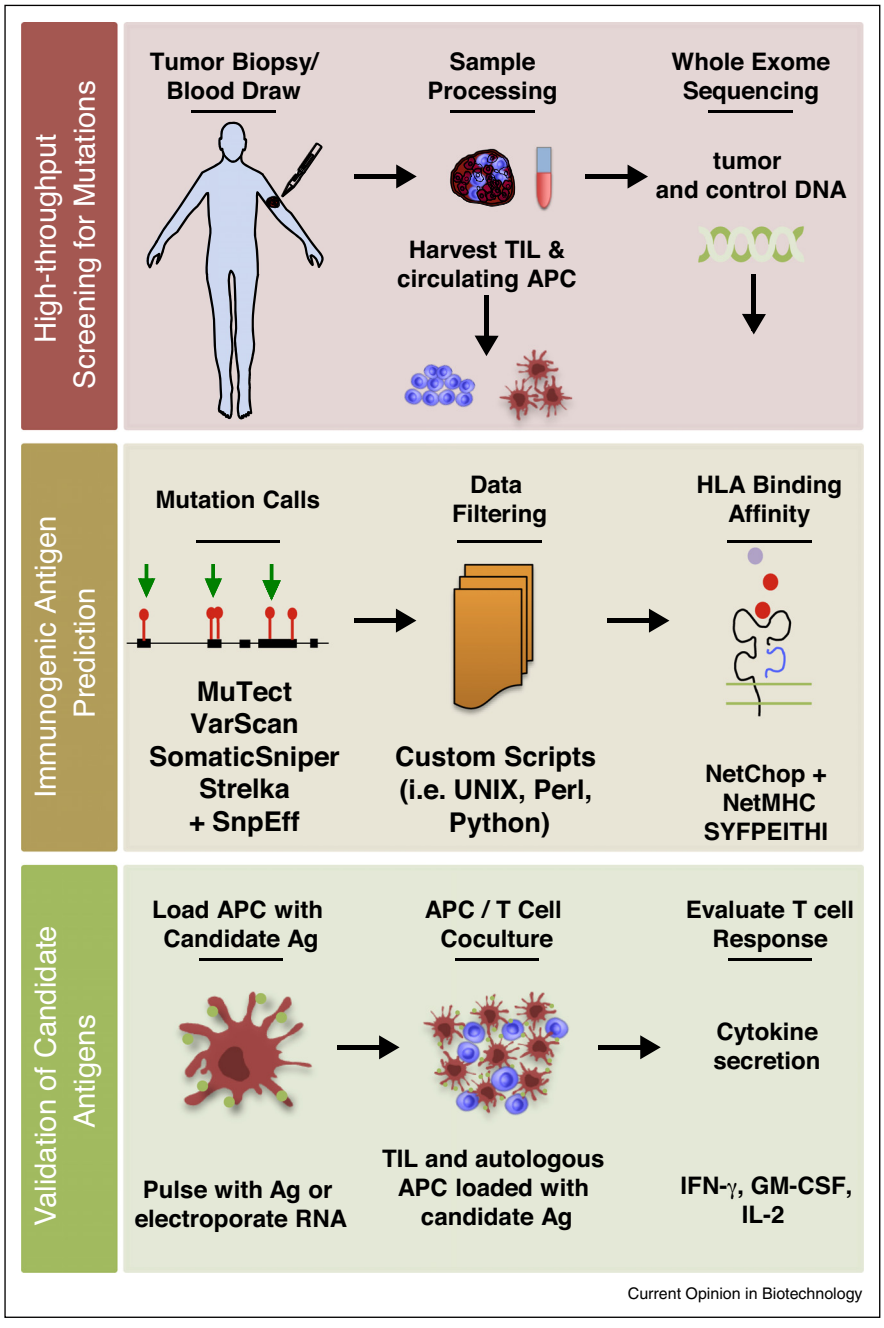
Recent innovations for immune-based treatment of cancer have led to a resurgence of enthusiasm in immunotherapies due to a broadening of responsive tumor types and more well-tolerated treatment programs. In this regard, 8 out of 19 cancer therapies that received FDA breakthrough status in 2015 were designed to improve antitumor immune responses. The identification of novel molecular immune checkpoints [1], along with innovative adoptive T cell transfer [2•,3] and dendritic cell vaccine protocols in clinical trials [4], as well as combinatorial approaches that utilize conventional immunotherapy [5], define immunotherapy as an evolving treatment option with the documented potential to eradicate metastatic malignancies.

The majority of immunotherapy protocols are designed to activate effector T cell responses in order to mediate tumor regression, yet the targets or tumor antigens that are recognized by T cells are often ill-defined. Tumor antigens are divided into two classes: First, tumor-associated antigens that are overexpressed in tumors and have restricted expression in normal tissues, and second, neoantigens originating from non-synonymous mutations in the tumor microenvironment. Pioneering studies performed more than twenty years ago utilized autologous T cell clones and patient-derived melanoma cell lines to identify the first tumor-associated antigen (melanoma antigen-1 or MAGE-1) and the first neoantigen [6–8]. In these studies, autologous T cell clones were painstakingly cultured with melanoma cell lines transfected with potential antigens and the candidate antigen identified on the basis of T cell reactivity. Other methods of antigen identification include mass spectrometry [4], transcript comparison between tumor and normal tissues [9,10], and screening of cDNA library products for reactivity with antibodies present in cancer patient serum [11]. Tumor-associated antigens identified through these conventional methods have been utilized in immunotherapies that in some cases resulted in durable complete responses [3,12]. Importantly, complete and durable responses following therapy designed to target a single tumor-associated antigen can be observed in tumors with heterogeneous expression of that particular antigen [12]. This is speculated to occur due to antigen spreading whereby distinct antigens not targeted by the initial therapy are released during tumor cell cytolysis and processed by antigen presenting cells (APC) which then activate endogenous T cell responses.

Current studies have leveraged unprecedented access to next generation sequencing data and tumor specimens with *in silico* analysis algorithms to profile the neoantigens targeted by intratumoral T cells. Whole exome sequencing opens the possibility of efficiently characterizing neoantigens arising from somatic mutations in coding regions, which are likely to cause changes in the amino acid sequence. Neoantigens represent potentially superior immune targets since neoantigen-specific T cell clones have not been deleted during negative selection of self-reactive cells that occurs during T cell development.

However, in order for an antigen to be targeted by T cells, it must be presented in the context major histocompatibility complex (MHC, known as human leukocyte antigen (HLA) in humans) on tumor cells. Furthermore, clinical tumor samples contain stromal cells, macrophages, tumor infiltrating lymphocytes and other immune cells, which can present technical challenges to efficiently identify truly actionable neoantigens. Therefore, a coordinated workflow, such as the one described below (Figure 1), is required.

Figure 1



Identification and validation of immunogenic neoantigens expressed in tumors. Tumor samples and peripheral blood provide tumor infiltrating lymphocytes (TIL) and antigen presenting cells (APC) respectively that are used for validation. Whole exome sequencing of tumor and comparison with autologous healthy tissue (i.e. circulating leukocytes) is utilized for discovery of somatic mutations. A number of mutation callers can be used in conjunction with data annotation tools. After data filtering, algorithms such as such as NetChop, NetMHC and SYFPEITHI are utilized to predict cleavage length and affinity for MHC since less than 1% of somatic mutations bind a particular class of MHC. Finally, candidate antigens (Ag) are loaded onto APC either by pulsing with peptide or electroporation of RNA. TIL are cultured with these APC and monitored for cytokine secretion (IFN-γ, GM-CSF, IL-2) to determine which neoantigens elicit productive T cell responses.