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Personalized T cell-mediated cancer immunotherapy: progress and challenges

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Immunotherapies are yielding effective treatments for several previously untreatable cancers. Until recently, vaccines and adoptive cell therapies have been designed to target public tumor antigens common to multiple patients rather than private antigens specific to a single patient. Due to the difficulty of identifying public antigens that are expressed exclusively on tumor cells, these studies have yielded both clinical successes and serious immune-related adverse events. Multiple avenues of research now underscore the centrality of tumor-specific mutated private antigens to endogenous anti-tumor immunity. Immunotherapies that target these neoantigens may enable safer and more durable tumor regression, but personalized targeting presents a number of challenges. Foremost among these is to develop processes that accelerate advancement from neoantigen discovery to use of these neoantigens as vaccines or as targets for adoptive cell therapies. Exome sequencing has facilitated discovery of neoantigens for melanoma and other highly mutated cancers. New technologies – possibly proceeding from T cell receptor repertoire sequencing – are needed to identify antigens for cancers with low mutational burden and few neoantigens. In this review, we discuss progress toward personalizing T cell-mediated immunotherapy for cancer as well as challenges going forward.

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Immunotherapy: a new paradigm for cancer treatment

The field of cancer immunology grew from tumor transplantation studies in syngeneic mice [1]. These studies (reviewed in Ref. [2]) provided evidence for the immunosurveillance hypothesis, which posited that tumors express 'new antigenic potentialities' arising from somatic mutation and that these antigens are specifically targeted by the immune system [3]. Concurrently, clinicians observed that lymphocytic infiltration into resected carcinomas correlated with longer post-operative survival for human patients [4]. Subsequent attempts to mobilize anti-tumor immunity through vaccination generally failed, however, and surgery, radiotherapy, and chemotherapy remained the mainstays of cancer treatment for the majority of the twentieth century [5].

Three avenues of research revitalized interest in cancer immunotherapy, particularly as mediated by T lymphocytes. First, the role of T cells in tumor immunity was cemented by the observation that mice deficient for lymphocytes ($RAG2^{-/-}$) or for T cell effector molecules (perforin and interferon-γ) exhibited a higher incidence of spontaneous carcinoma [6••,7]. Second, clinical interventions that boosted T cell responses – either through infusion of interleukin-2 (IL-2) or through adoptive transfer of autologous tumor-infiltrating lymphocytes (TILs) – resulted in tumor regression in a subset of patients with metastatic melanoma [8–11,12•]. Third, interventions that relieved suppression of T cell responses – most notably antibody-mediated blockade of the checkpoint receptors cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) – resulted in durable tumor regression in mice and humans [13,14,15•]. It is now established that endogenous T cells can recognize and kill cancer cells. Cancer immunotherapies exploiting this capacity constitute a paradigm shift in cancer treatment, yielding successes where conventional cancer therapies fail [16]. Recent advances in this rapidly moving field are focused on characterizing the antigens targeted by anti-tumor immunity and learning from these how to design immunotherapies of greatest clinical benefit.

Focus shifts from public to private antigen targeting

A unique T cell receptor (TCR) expressed on each T cell enables the cell to scan for antigens presented on major histocompatibility complex (MHC) molecules on the tumor cell surface. Broadly, these tumor antigens are of two types: (1) non-mutated public antigens, including tissue-specific or cancer-testis (CT) antigens that are aberrantly expressed in cancerous cells; and (2) private neoantigens resulting from non-synonymous somatic mutations within the cancerous cells [17]. Public antigens

can be shared by multiple cancers but can also be expressed on normal tissues, whereas neoantigens are tumor-specific but generally also patient-specific [18,19].

Due to the clearer path to clinical application, targeted immunotherapies have predominantly focused on public antigens. Therapeutic vaccines designed to stimulate T cell responses to public antigens have yielded modest success (*e.g.*, sipuleucel-T was approved for treatment of metastatic castration-resistant prostate cancer in 2010 [20]). However, vaccination with public antigens has not generally worked well, likely because T cells specific for such antigens are either absent or functionally suppressed by the tumor microenvironment [5]. TCR gene therapy is a more effective means of targeting specific public antigens. Because the TCR is the sole determinant of T cell specificity [21], viral transfer of genes encoding a public antigen-specific TCR can impart tumor reactivity to autologous peripheral T cells [22]. Following expansion and re-infusion, these engineered T cells specifically kill those cells presenting their cognate (targeted) antigen. TCR gene therapy has achieved objective response rates of 13–67% for multiple cancer types – most notably melanoma and synovial cell carcinoma – by targeting the melanocyte differentiation antigen, MART-1/Melan-A, or the CT antigen, NY-ESO-1, respectively [23,24•,25,26].

Notwithstanding these successes, targeting public antigens presents safety and efficacy concerns. Public antigens are often tumor-associated rather than truly tumor-specific in their expression. As such, targeting them can result in on-target but off-tumor reactivity [27]. Public antigen-specific TCRs have low affinity for their non-mutated targets due to central tolerance and attempts to evolve higher affinity TCR variants can introduce cross-reactivity that is difficult to predict [28,29]. Consequences of off-tumor reactivity range from manageable morbidity to serious adverse events and even death (Table 1) [24•,28,30–33].

Due in part to these challenges and in part to the advent of deep-sequencing technologies, there has been a recent surge in interest in targeting patient-specific neoantigens. Because these antigens occur only within tumor tissue, they are not presented by thymic epithelial cells and do not induce central tolerance. Therefore, neoantigen-specific TCRs may be both more specific and higher affinity than TCRs targeted to public antigens. It is also becoming increasingly clear that – at least for tumors with high mutational burden – neoantigens are important targets of endogenous immunity. Mutated neoantigens contribute to tumor recognition by TILs [34••]. Adoptive TIL therapy is generally well-tolerated and achieves up to 72% objective responses for metastatic melanoma [35], suggesting this targeting is clinically relevant. Moreover, adoptive transfer of TILs specific for a single neoantigen is sufficient to mediate tumor regression [36••]. Checkpoint blockade trials similarly indicate that neoantigens are central to anti-tumor immunity. In melanoma, the tumor mutational burden and number of predicted neoantigens correlate with clinical response to ipilimumab (anti-CTLA-4) [37,38]. High tumor mutational burden is a prognostic biomarker for PD-1 blockade as well, predictive of higher response rates for non-small cell lung cancer (NSCLC) [39•] and mismatch repair-deficient colorectal carcinoma [40]. Tumors with many shared (truncal) mutations express high levels of the ligand for PD-1 and respond particularly well to checkpoint blockade [41]. Collectively, these results suggest that neoantigens are targets of endogenous immunity and may be ideal targets for cancer immunotherapy.

Table 1

Select examples of adverse events resulting from clinical application of immunotherapies targeting public antigens

Antigen	Immunotherapy	Adverse event	Cause	Ref.
MART-1/MelanA	TCR	Fatal neural and cardiac toxicity	High levels of inflammatory cytokines alone or in combination with semi-acute heart failure and epileptic seizure	[30]
		Uveitis, Hearing loss, Loss of pigmentation	On-target activity of TCR-engineered T cells targeting normal cells expressing the cognate epitope	[24•]
	TCR + DC vaccination	Acute respiratory distress	High levels of inflammatory cytokines	[31]
NY-ESO-1	TCR (Affinity enhanced)	Skin rash with lymphocytosis, diarrheal syndrome	Autologous GVHD-like syndrome possibly due to loss of self-tolerance	[32]
MAGE-A3	TCR (Affinity enhanced)	Fatal cardiogenic shock	Cross-reactivity with an unrelated epitope from the Titin protein presented on cardiac tissue	[28]
	TCR (Affinity enhanced)	Mental status changes, comas, necrotizing leukoencephalopathy with extensive white matter defects	Reactivity to similar MAGE-A12-derived epitope presented on neural cells	[33]

Targeting neoantigens with T cell-mediated immunotherapies

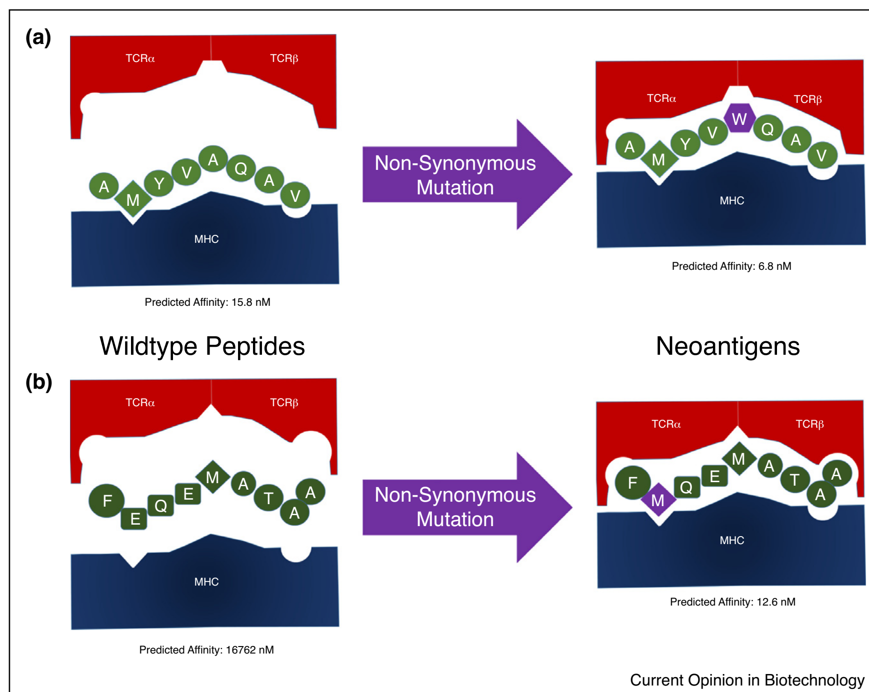
Checkpoint blockade mobilizes endogenous T cells by breaking tolerance to self-antigens, including but not limited to neoantigens. While this approach achieves remarkably durable tumor regression in a subset of patients, there are limitations. For example, tumor-resident effector cells mobilized by checkpoint blockade can possess stable epigenetic markers of exhaustion, hampering their ability to form memory cells [42]. Additionally, broadly breaking self-tolerance results in adverse events in over 50% of patients when multiple checkpoints are inhibited [43]. By contrast, effector cells used in adoptive therapies can be selected or engineered for optimal phenotype and targeted specifically to neoantigens. Thus, personalized T cell-mediated immunotherapies may be more effective and engender less morbidity than checkpoint blockade, provided we can overcome the challenges of personalization.

There is a degree of personalization intrinsic to T cell-mediated cancer immunotherapies. Public antigens are expressed heterogeneously across cancers, among patients with the same cancer, and even among an individual patient's cancer cells. Additionally, MHC restriction limits the pool of patients who are candidates for treatment with any given TCR. Finally, adoptive TIL therapy, TCR gene therapy, and dendritic cell-based vaccines already require laborious manipulation of autologous cells. Nonetheless, targeting neoantigens on a patient-by-patient basis presents unique challenges of both scientific and logistic nature. These include accelerating antigen and receptor discovery, devising alternate discovery strategies for cancers with few neoantigens, and improving response rate and durability, all while negotiating new regulations necessary for safe implementation.

Neoantigen discovery

A neoantigen is formed only when somatic mutation creates a peptide epitope that is expressed, processed, presented by one of the patient's MHC molecules, and recognized by a subset within the patient's T cell repertoire (Figure 1). This stochastic outcome has been compared to a lottery in which each mutation increases the odds of neoantigen formation [44]. Most mutations do not create neoantigens and those that do are generally bystander mutations that are incidental to cancer progression and unique to each responding patient [38,45•,46]. Thus, high-throughput methods are required to identify

Figure 1



Self-peptides are tolerated by the immune system because nascent T cells that bind self-peptides presented on self-MHC are deleted during thymic selection. Neoantigens are somatic, non-synonymous mutations in expressed genes. They are immunogenic because they are not expressed in the thymus and do not induce central tolerance. **(a)** Mutation of a TCR-facing residue results in a previously tolerated self-peptide acquiring immunogenicity. **(b)** Mutation of a MHC-facing residue results in a previously unpresented and untolerated self-peptide binding to a patient's MHC molecule and eliciting immunogenicity. Wild-type peptides depicted are derived from human β-actin (ACTB) protein. Predicted affinities for wild-type and mutated peptides binding to HLA-A2.1 were calculated using NetMHC 4.0 [48,49].

the subset of patient-specific mutations that result in neoantigen formation.

The first molecular identification of a neoantigen was achieved by Boon *et al.*, who used laborious expression cloning to identify a single mutation responsible for tumor rejection by cytotoxic T cells from syngeneic mice [47•]. Modern neoantigen discovery studies use a 'reverse immunology' approach, identifying hundreds of patient-specific mutations by deep-sequencing the exome from resected tumor tissue and comparing these data to reference data from normal tissue. MHC class I-restricted neoantigens arising from these mutated peptide sequences are predicted using algorithms that calculate the affinity of derived mutant peptides for MHC-I alleles relevant to the patient [48–50]. These putative neoantigens are then validated by testing for recognition by $CD8^{+}$ T cells (*e.g.*, using MHC-I multimers or peptide-pulsed presenting cells). Following demonstration in mouse models [51,52•], this approach was first applied to human clinical samples to identify neoantigens targeted by adoptive TIL therapy and checkpoint blockade in patients treated for melanoma [34••,53]. Exome-guided discovery has revealed that melanoma-infiltrating T cells frequently respond to MHC class II-restricted neoantigens as well [45•]. Because epitope prediction and tetramer production are challenging for MHC class II epitopes, this was demonstrated by pulsing autologous cells with peptides comprising all mutations identified through exome sequencing and then determining which of these elicit $CD4^{+}$ T cell reactivity [45•]. Finally, neoantigens can be identified directly from tumor-derived MHC eluates using mass spectrometry [54•,55,56,57••], provided its sensitivity can be made comparable to immunological methods. This approach may be particularly well-suited to detecting neoantigens arising from aberrant splicing, cryptic start sites, or post-translational modifications, none of which would be evident from exome sequencing [57••,58,59].

Targeting neoantigens directly through vaccination or tailored adoptive TIL therapy

Once identified, neoantigens can be used diagnostically (*e.g.*, as biomarkers to characterize and monitor T cell responses to checkpoint blockade) or therapeutically (*e.g.*, as targets for therapeutic vaccines or adoptive cell therapies). The most straightforward means of targeting neoantigens is through therapeutic vaccination. Mice injected with dendritic cell vaccines incorporating long peptide neoantigens induced tumor rejection comparable to checkpoint blockade [54•]. Importantly, clinical application of neoantigen vaccination induced expansion of neoantigen-specific T cells without impacting disease progression [56], suggesting vaccination may be best employed as an adjunct to adoptive cell therapy or checkpoint blockade.

Personalized adoptive T cell therapies require the selective expansion or capture of neoantigen-specific T cells. This has been achieved with various technologies. Schumacher *et al.* pioneered the use of UV-induced peptide exchange to enable rapid assembly of peptide-MHC multimer libraries [60]. Combined with multi-color encoding and fluorescence-activated cell sorting [61], these libraries enable the isolation of neoantigen-specific T cells from TILs and peripheral blood [53,62]. A variation of this approach employs UV-exchanged peptide-MHC multimers labeled with DNA barcodes rather than fluorochromes to capture neoantigen-specific T cells in a spatially-encoded manner on a DNA-spotted microfluidic device [63] (and unpublished results). Alternately, Rosenberg and colleagues have demonstrated that neoantigen-specific T cells can be expanded from TILs and blood using autologous presenting cells expressing tandem neoantigen minigenes [64,65••].

A critical question facing the field is how to employ neoantigen-specific T cells on a clinically useful timescale while navigating a novel regulatory landscape. Adoptive therapy with TILs of undefined composition achieves durable tumor regression in 20% of melanoma patients [66]. It may be possible to improve this response rate by enriching for neoantigen-reactive T cells prior to infusion (*e.g.*, based on antigenic specificity or surface expression of PD-1) [36••,67,68••,69••,70]. Such personally tailored adoptive TIL therapies – which provide direct evidence for the clinical relevance of neoantigen-specific TILs – have several attractive features. First, there are indications that the proportion of TILs that are neoantigen-specific may affect clinical efficacy [68••,69••], though this has not been rigorously demonstrated. Second, production of tailored cell products requires only marginally more labor and turn-around time than is already required for on-demand preparation of unsorted TILs. Indeed, tumor-reactive TILs can be selected based on expression of activation markers such as PD-1 or CD137 even without identifying their cognate epitopes [69••,70]. Third, regulatory approval may be more readily granted for enrichment for pre-existing specificities than for introduction of new specificities through TCR gene transfer [71], particularly when targeting multiple neoantigens. Indeed, both monoclonal and polyclonal tailored TIL products have been administered to patients already [36••,68••].

Personalized TCR gene therapy

The alternative to tailored adoptive TIL therapy is to clone TCR genes from neoantigen-reactive T cells for heterologous expression in a distinct population of effector cells. While more laborious and likely more challenging from a regulatory standpoint, personalized TCR gene therapy provides greater customizability and potentially better efficacy than adoptive TIL therapy (Figure 2).

Figure 2

Current Personalization

Target Public Antigen (Off-the-shelf)
- Well-characterized
  - No antigen discovery phase
- Fewer regulatory hurdles
  - Risk relatively well-known

TCR Antigen-specific TCR (Off-the-shelf)
- Well characterized
  - No discovery phase
  - Toxicity assessment done
- Easier implementation
  - Regulatory approvals such as IND ready
  - Vector manufacturing established

Effectors T cells/HSCs (On demand)
- Autologous cell product
  - Limited potential for engineering
- Product processing methods well-established
  - Testing release criteria on the fly

Prospective Personalization

Target Private Antigen (On demand)
- Newly identified
  - Additional time required for neoantigen discovery
- Regulatory hurdles
  - Need to obtain regulatory approvals
  - Potentially unknown risks

TCR Neoantigen-specific TCR (On demand)
- Newly discovered
  - Additional time required for discovery
  - Toxicity assessment required
- Tougher implementation
  - Need to obtain regulatory approvals
  - Need to manufacture new vector on a short timescale

Effectors T cells (Off-the-shelf)
- Heterologous cell product
  - Can be engineered to have better efficacy
- Pre-manufactured and cryopreserved product
  - Minimal processing before release

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Comparison of the current state-of-the-art practice of TCR gene therapy with prospective modifications to enhance efficacy and facilitate implementation.

Adoptive TIL therapy requires surgical access to the tumor to acquire TILs, and these fail to expand from 30% of resected melanoma samples [72]. By contrast, neoantigen-reactive TCR genes can be captured from patient peripheral blood [62,65••] or even from T cells from unrelated blood samples [73•]. Unrelated donor blood may in fact be a superior source of reactive TCRs, because immunoediting promotes the loss of neoantigens recognized by the autologous TCR repertoire during tumor development [51,73•,74]. In combination with sequencing of circulating tumor cells or cell-free tumor DNA, it is conceivable that identification of both neoantigens and reactive receptors will be achievable from blood in the future.

Personalized TCR gene therapy provides considerable flexibility in terms of effector cell identity as well. Whereas expansion of TILs for adoptive therapy can lead to exhaustion, TCR gene transfer to naïve peripheral T cells or hematopoietic stem cells provides a large – even continually renewable – supply of tumor-reactive T cells of superior phenotype [75,76]. Recent advances in gene editing and synthetic biology can further enhance the potency and safety of engineered adoptive therapies by modulating expression of endogenous TCRs or checkpoint receptors [77–80]. Notably, genome-edited allogeneic T cells expressing chimeric antigen receptors targeted to CD19 were employed recently as an off-the-shelf immunotherapy [81]. Targeting neoantigens precludes this degree of universality, but off-the-shelf allogeneic effector cells transduced with patient-specific TCR genes offer other advantages. For example, this approach would simplify lot testing for the infused effector cell product. Moreover, this approach allows the use of logical programming to limit off-tumor reactivity, prevent escape, or impart novel effector functions on these engineered cells [82–84]. Graft-vs-host disease is a prominent risk of using allogeneic donor effector cells. Careful lot testing to ensure extirpation of donor TCR and/or MHC expression – possibly in combination with a fail-safe mechanism to eradicate graft cells – will be necessary to enhance safety of allogeneic effector cell grafts [81].

Expanding personalized T cell immunotherapies to cancers with low mutational burden

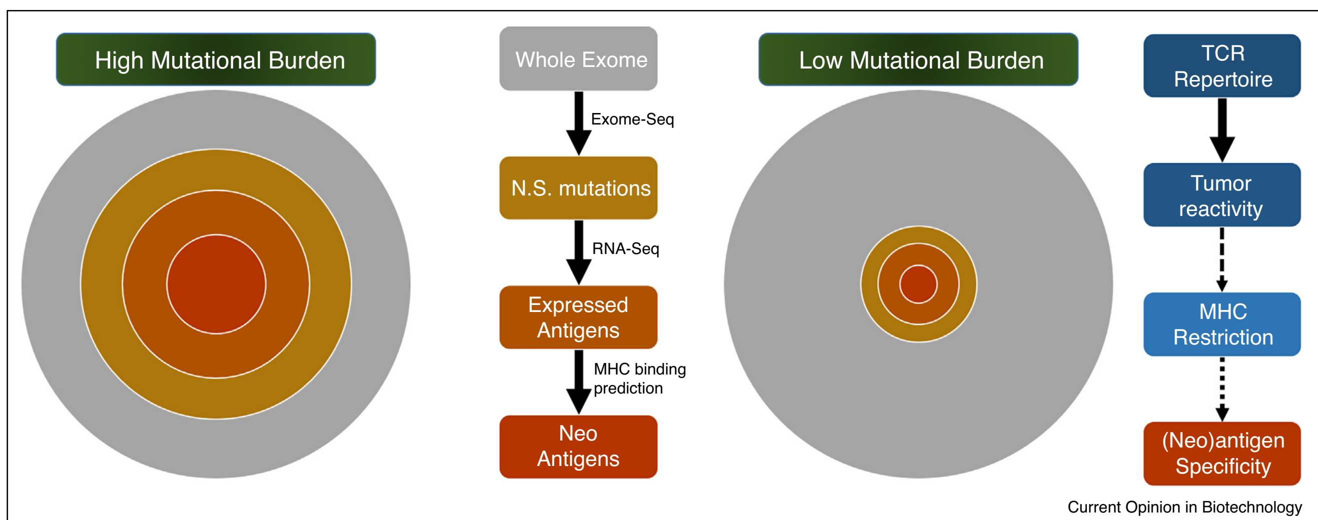
Mutational burden varies widely across tumor types [85••]. Many of the successful immunotherapy trials

implicating neoantigens have been conducted for ultraviolet- or carcinogen-induced tumors with high mutational burden (>10 mutations/Mb). For tumors with intermediate to low mutational burden (<1 mutation/Mb), neoantigens are relatively rare and less likely to be operative in eliciting anti-tumor immunity (Figure 3). Nonetheless, TILs are present in such tumors [36••,86,87]. These include neoantigen-specific T cells [36••], but there are likely to be non-mutated antigens recognized as well. Exome-guided approaches cannot identify such targets, so antigen-agnostic alternatives are needed to elaborate anti-tumor immunity for tumors with low mutational burden.

One such strategy is to use a personalized TIL-guided approach: isolate T cells mediating endogenous immunity to a particular tumor and then use these cells or their TCRs to identify those antigens that are driving the immune response (Figure 3). Tumor-reactive T cells infiltrate the tumor stroma, become enriched in this context, and express PD-1 [69••,88,89]. In multiple cancer types, single tumor-reactive clones constitute as much as 25–50% of the TIL TCR repertoire and mediate tumor regression following tailored TIL therapy [36••,68••]. High clonality and PD-1 expression on infiltrating cells are also associated with clinical response to PD-1 blockade [90]. Thus, tumor-reactive T cells can be identified from TILs based on their location, frequency, and/or phenotype without a priori knowledge of their antigenic specificity.

Just as deep-sequencing technologies have enabled high-throughput discovery of patient-specific neoantigens, so too are they enabling comprehensive description of patient-specific TCR repertoires. Specifically, it is now possible to identify the frequencies of unique TCR clonotypes among TILs [91]. Multiple technologies have emerged to obtain paired TCRα and TCRβ sequence reads [63,92,93•], facilitating functional cloning of the most frequent TCR genes. The bottleneck for this approach is that there are no high-throughput technologies for identifying the cognate antigens for these orphan TCRs. A low-throughput approach has been described that involves expressing the orphan TCR ectodomains as a soluble, fluorescently-labeled reagent, and then using this TCR reagent to selectively identify its cognate antigen from a yeast cell surface-displayed peptide-MHC library [94]. However, because soluble expression of TCRs is laborious and non-robust, and MHC restriction for a given TCR is difficult to predict, it will be challenging to scale this approach to identify antigens targeted by multiple TCRs involved in a polyclonal immune response. Ideally, TIL TCR specificity can be evaluated *in situ*, for example by using TILs directly to capture or mark those target cells expressing their cognate ligand from among a cellular library expressing tumor-derived cDNA.

Figure 3



Exome-guided discovery of neoantigens employs whole exome sequencing to identify non-synonymous mutations, followed by a number of filtering steps to identify those mutations likely to be immunogenic. Cancers with high mutational burden (>10 mutations/Mb) may have 100–200 non-synonymous mutations, a subset of which will be immunogenic neoantigens. For cancers with low mutational burden, the number of non-synonymous mutations may be 10–100-fold lower and neoantigens will be rare or absent. An alternate approach for target discovery in these cancers may be to identify T cells mediating immunity (*e.g.*, based on TIL TCR frequency or expression of PD-1) and clone the TCRs from these cells. TCRs for which tumor reactivity is confirmed and MHC restriction can be determined would then be used to capture their cognate ligands. Dashed arrows indicate process steps for which no high-throughput methods are yet available.

Conclusions

Mutation drives the unchecked growth, heterogeneity, and evolvability of cancer cells, rendering many cancers refractory to conventional treatments. It is now clear that mutation is also cancer's Achilles' heel, distinguishing it from self and opening it to immunological attack. Personalized immunotherapies have scored stunning victories against cancer by exploiting this vulnerability. Even so, we have only just sampled the potential for personally tailored immunotherapies to yield more effective and safer cancer treatments. More victories – and many challenges – are ahead.

We have highlighted here several key challenges related to target discovery and therapeutic implementation. First, while deep-sequencing technologies have enabled rapid neoantigen identification, targeting these antigens with gene therapy will require an equally efficient pipeline for cloning TCR genes from reactive T cells. Notably, neoantigen-reactive T cells present in blood must be expanded *ex vivo* for weeks before they are detected by current methods. This timescale may be incongruent with therapeutic needs for rapidly progressing cancers. Second, alternatives to exome-guided approaches are needed to extend immunotherapy to cancers with few mutations. Patient-specific analysis of the TIL repertoire may be an effective starting point to interrogate the antigenic targets of anti-tumor immune responses that are not focused chiefly on neoantigens. To do this will require high-throughput methods to capture ligands for orphan T cells identified as tumor-reactive based on frequency and/or phenotype. Importantly, developing such technologies for low mutational burden cancers will benefit efforts to 'de-orphanize' T cell responses in pathologies unrelated to mutation (*e.g.*, autoimmune inflammatory diseases). Third, tumors can escape initially effective T cell responses through myriad mechanisms [68••,95], suggesting combination of T cell therapies with orthogonal treatments may be beneficial. Finally, cancer immunotherapies are complex 'drugs' that employ the immune system as the active ingredient against a complex disease. The immune repertoire and cancer exome in every patient are unique and mutable. Personalizing the immune effectors used and the cancer antigens targeted will require reconciling timescales of clinical need, on-demand manufacturing, and regulatory compliance. These challenges, by no means insurmountable, will prompt advances leading to safer and more effective cancer immunotherapies.

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In addition to demonstrating that lymphocytes protect against carcinogen-induced and spontaneous cancers, this study showed that tumors from immunocompetent mice have reduced immunogenicity relative to those from immunocompromised ($RAG2^{-/-}$) mice. Thus, the immune system not only protects the host from cancer (immunosurveillance) but also exerts selective pressure that sculpts the antigenic character of the developing tumor (immunoediting).

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This study and Dudley *et al.* (Ref. [11]) established that clonal adoptive T cell therapy (using clones reactive with MART1 and/or gp100) can cause tumor regression in patients with metastatic melanoma, laying the groundwork for tailored adoptive TIL therapy with neoantigen-specific TILs.

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This study illustrated that checkpoint (PD-1) blockade can mobilize antitumor immunity for several cancers with high mutational burden. Response was achieved in patients with melanoma (28%), renal cell cancer (27%), and non-small-cell lung cancer (18%). These responses were durable (>1 year in 20/31 responders) and were associated with PD-L1 expression on the tumor cells. Notably, no responses were seen among patients with prostate cancer, which has a generally low mutational burden.

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