Trends in Cancer

CellPress

Review

Neoantigen-directed therapeutics in the clinic: where are we?

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In the past decade, immune checkpoint inhibitors (ICIs) and chimeric antigen receptor (CAR) T cell therapy have brought immunotherapy to the forefront of cancer treatment; however, only subsets of patients benefit from current approaches. Neoantigen-driven therapeutics specifically redirect the immune system of the patient to enable or reinduce its ability to recognize and eliminate cancer cells. The tumor specificity of this strategy spares healthy and normal cells from being attacked. Consistent with this concept, initial clinical trials have demonstrated the feasibility, safety, and immunogenicity of neoantigen-directed personalized vaccines. We review neoantigen-driven therapy strategies as well as their promise and clinical successes to date.

Highlights

The generation of personalized neoantigen-directed therapies is complex, time-consuming, and expensive, but has proved to be feasible in the clinic.

Personalized vaccines targeting neoantigens are safe and induce robust, durable anti-vaccine immunity in patients with solid tumors.

Several small, non-randomized clinical trials have demonstrated preliminary signals for antitumor activity mediated by neoantigen-directed vaccines (e.g., radiographic responses including complete responses in individual patients, pathologic responses, and epitope spreading).

Ongoing randomized clinical trials testing personalized vaccines including in patients with melanoma, colorectal cancer, and pancreatic cancer are anticipated to provide more definitive evidence of clinical efficacy.

Adoptive cell therapy using engineered neo-T cell receptor T cells derived from tumor-infiltrating lymphocytes or circulating T cells is feasible and can mediate durable regression of advanced solid tumors.

Main text

The promise of utilizing the body's own immune system to treat cancer is in part linked to the dogma of 'cancer immunoediting' which posits that the immune system not only can play a vital role in the protection of the host against tumorigenesis but can also shape and even promote tumor growth [1]. The understanding that tumors develop upon immune evasion has paved the way for new treatment options to shift the balance from a protumoral environment towards the development of an unfavorable setting for cancer cells by (re)boosting the immune system. ICIs are arguably the most effective class of immune therapeutics for the treatment of solid tumors available to date. ICI therapy in general depends on the presence of tumor-specific T cells that have already infiltrated the tumor site because these mostly exhausted $CD8^+$ T cells, which are in part specific for tumor neoantigens can be reactivated with ICI [2]. Because spontaneously induced T cells are triggered by only a relatively small number of neoantigens, tumors harboring few mutations are less likely to be infiltrated with activated T cells, and are thus less likely to respond to ICI therapy [3]. Indeed, most cancer patients only have spontaneous T cell immunity against a small fraction (<1%) of their mutations [4]. Another limitation of ICIs is the development of acquired resistance upon treatment because tumors relapsing under ICI treatment can exhibit a different mutational landscape with a different spectrum of neoepitope variants [5]. This could be explained by the selective pressure imposed by the ICI-activated immune system on the selected set of spontaneously recognized neoantigens. Broadening and diversifying the tumor-reactive T cell repertoire and increasing the number of infiltrating tumor-specific T cells through other forms of immunotherapy, such as cancer vaccination, is therefore a promising strategy to synergize with ICI therapy, particularly for patients with 'cold' tumors [6]. Neoantigen-directed therapy in general is aimed at strengthening and broadening immune responses to specific neoantigens on the tumor surface so as to enable or enhance the recognition and elimination of cancer cells. Because the majority of neoantigens are unique to the individual tumor and are absent in healthy tissue, targeting neoantigens should not cause off-target toxicity.

Two distinct neoantigen-directed therapy approaches are currently being explored in the clinic: cancer vaccination and adoptive T cell transfer (ACT). Both strategies are illustrated in Figure 1.

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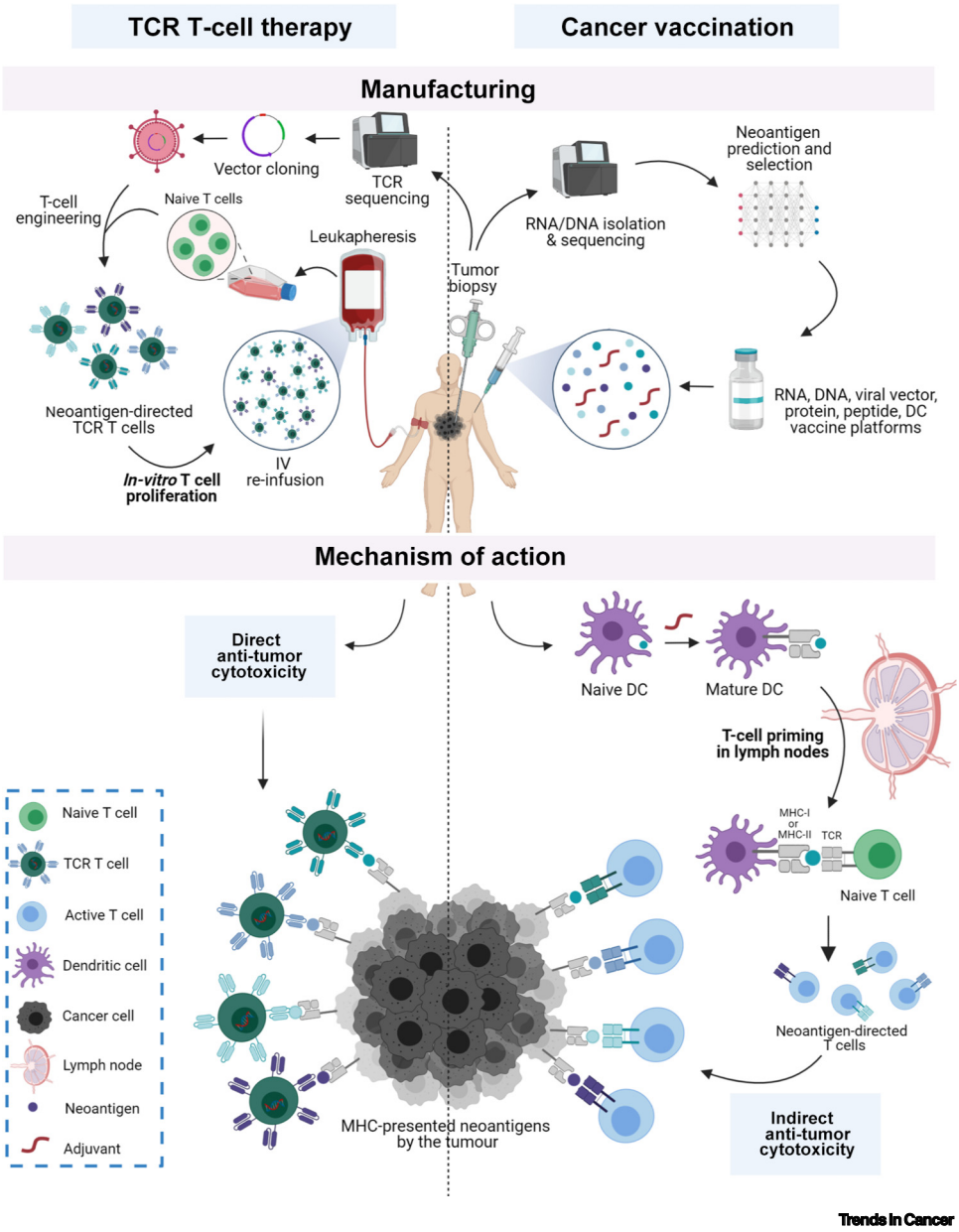

Figure 1. Comparison of cancer vaccination versus transgenic TCR T cell therapy: manufacture and mechanism of action. Abbreviations: DC, dendritic cell; IV, intravenous; MHC, major histocompatibility complex; TCR, T cell receptor.

Cancer vaccination involves immunization of the patient with neoantigens with the aim of priming T cells or boosting existing weak responses *in vivo* in the lymph nodes. ACT involves the administration of *ex vivo* expanded neoantigen-specific T cells to directly attack the tumor. In general, ACT goes beyond administration of a selected set of expanded neoantigen-specific T cell cultures and entails reinfusion of T cells genetically engineered to express a neoantigen-specific T cell receptor (neoTCR) or a CAR. Of the two, only TCR therapy is considered to be a true neoantigen-directed approach because the engineered TCRs are selected to recognize the tumor neoantigens that are processed and presented by the MHC by antigen-presenting cells

(APCs) and tumor cells. Binding of TCRs to these MHC–antigen complexes leads to efficient recognition and strengthening of the antigenic signals. By contrast, CARs recognize tumor-specific molecules independently from the MHC and, unlike TCRs, they are currently unable to efficiently detect and amplify antigenic signals. Hence, because neoantigens usually are present in low(er) densities, CARs are yet not sufficiently efficient to recognize such epitopes [7].

Although ACT therapies can affect the breadth of the immune response through antigen spreading, vaccines can also to some extent substantially increase and broaden the number and diversity of neoantigen-specific T cells more extensively [6]. As such, both approaches could also be considered to be complementary. The development of engineered TCR-ACT therapy directed at neoantigens is still in its early stages and only limited success has been reported in solid malignancies to date. Vaccines targeting neoantigens have shown to be feasible and safe and to induce vaccine-specific immune responses at levels not previously seen with cancer vaccines; early biological signals for vaccine-induced antitumor activity have also been observed [8–12]. In the field of neoantigen-targeting therapies, this review highlights the promise of personalized immunotherapy and the progress that has been made.

How can the immune response be directed most effectively against neoantigens?

Tumor antigens include tumor-associated antigens (TAAs), tumor-specific antigens (TSAs), and aberrantly expressed TSAs (aeTSAs). Both TAAs (antigens overexpressed by tumor cells relative to normal cells) and aeTSAs (antigens aberrantly expressed or presented by tumors) are often shared across tumors, thus allowing the generation of a non-personalized, 'off the shelf', drug. However, TSAs mostly arise from private mutations occurring in the tumor genome or from oncoviral proteins. Because TSAs are exclusively present in tumors and not in normal cells, they do not induce central tolerance or autoimmunity responses, making them preferred targets to induce a tumor-directed immune response [13]. Neoantigens are presented by tumor cells and/or APCs including dendritic cells (DCs), leading to activation of naive T cells and their differentiation into effector T cells. These effector cells can specifically recognize cancer cells as 'foreign'. They eliminate the cells that present the neoantigen on their surface, which the T cells have been primed against, or they push the cells into senescence [14].

Multiple approaches have been used in the past to redirect the immune system against neoantigens. Before next-generation DNA and RNA sequencing enabled the identification of private neoantigens, a more common technique was to use a whole-tumor cell-based approach to serve as the natural source of neoantigens. These cancer vaccines provide the entire spectrum of tumor antigens to the immune system, including neoantigens, but without any selection, in other words without prioritization of the neoepitopes most suitable to induce an immune response. The whole cell-based approach does not require genomic identification of neoantigens, which offers great advantages in terms of complexity, time, and cost. Notably, autologous DCs pulsed with autologous tumor cell lysates were found to induce neoantigen-specific T cell responses including T cell clones with substantially increased avidity compared to baseline [15]. Nevertheless, suboptimal priming of the immune system can occur because the spectrum of tumor antigens contains both immunogenic and presumably many non-immunogenic, irrelevant antigens. This explains at least in part the limited clinical benefit obtained with whole cell-based vaccines [16,17].

Identification of neoantigens enabled by sequencing of the tumor genome has the potential to generate a more 'focused' therapy targeting the most relevant, immunogenic tumor antigens provided that these antigens can be effectively identified – generally using computational and bioinformatic pipelines [18]. Selection of highly immunogenic neoantigens – neoantigens that can elicit a strong, tumor-specific immune response – and targeting multiple neoantigens to mitigate

immune escape are likely crucial for the success of personalized therapy [18–21]. Some important bioinformatic challenges need to be tackled, mainly involving expansion of the neoantigen search space for tumors with a low tumor mutational burden (TMB) and selection of only those neoantigens that are truly immunogenic (able to elicit a strong immune response) given the limited availability of functional neoantigen-specific T cell data.

Vaccination

Although vaccines against cancer have been investigated for decades, compelling clinical activity has been elusive, as evidenced by only a single FDA approval (sipuleucel) to date. Nevertheless, the generation of robust, durable, specific, and broad T cell immunity against tumor cells is likely a cornerstone for the successful induction of long-lasting cancer regression. Vaccination leverages the vital role of DCs, the most potent class of APCs, in connecting the innate and adaptive immune systems thereby inducing durable active immunity. Upon neoantigen vaccination, DCs process and present neoepitopes via the major histocompatibility complex (MHC)-I and MHC-II molecules on their cell surface, leading to $CD8^+$ and $CD4^+$ T cell activation and proliferation. For cancer vaccination, it is essential to induce activated $CD8^+$ T cells or cytotoxic T lymphocytes (CTLs) that can recognize and control the cancer cells against which they are primed. However, $CD4^+$ T helper 1 (T_H1) cells – which are necessary for optimal CTL priming and T memory cell expansion for tumor immune microenvironment optimization, as well as for innate effector cell stimulation, are also important, and they have recently been found to exhibit cytotoxic properties including in the cancer vaccine setting [10,22,23].

The concept of vaccination to elicit neoantigen-directed immunity is an attractive strategy to achieve the goal of long-term tumor control for cancer patients. There are many platforms available for vaccine delivery, as impressively illustrated by the development of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines in the recent past. These platforms include protein, peptides, RNA, DNA, microbial and cellular vectors, and DCs that can be loaded with tumor antigens *ex vivo*. The vaccine platforms as well as the strategy for neoantigen discovery have a direct impact on the manufacturing time as well as the clinical setting in which such a vaccine can be utilized [18,24,25]. Furthermore, there are many choices with regard to the dosing interval and route of vaccine administration, in addition to cotherapies aimed at enhancing DC stimulation and T cell priming, and counteracting immune suppression in the tumor. Studies in mouse models point to the need for intravenous administration of the neoantigen vaccine to optimize antitumor immunity and to induce $TCF1^+PD1^+CD8^+$ T cells and tumor regression that depend upon systemic type I interferon [26,27]. Numerous studies that have characterized SARS-CoV-2 vaccine-induced immune responses have also provided important lessons on the nuances of the multipronged adaptive immune response that is triggered by these vaccines and leads to prevention and/or mitigation of coronavirus disease 2019 (COVID-19) in the majority of vaccinated individuals [28–30].

The successful development of vaccines against SARS-CoV-2 in record time has also demonstrated how a highly focused and collaborative effort spurred by an urgent need and dedicated resources can vastly accelerate progress. Some of these advances should provide direct benefit to ongoing and future personalized cancer vaccine efforts because many companies have scaled up the production of adaptable vaccine platforms – as is most evident from the transition of RNA-based vaccines from a niche approach to being the most widely used and effective SARS-CoV-2 vaccine delivery, further enabled by large-scale manufacturing of billions of vaccine doses that can be rapidly and seamlessly adapted as new SARS-CoV-2 variants emerge.

Although SARS-CoV-2 vaccines have been immensely successful, important differences between cancer and acute infection should be acknowledged. Prophylactic vaccines against

infectious organisms target a foreign, highly immunogenic antigen in the setting of a naive immune state against that antigen (the host has never 'seen' that antigen before the acute infection). By contrast, a therapeutic cancer vaccine needs to take on the challenge of tumor heterogeneity and immune evasion because most cancers have evolved over months to years. Furthermore, coevolution of the cancer and the immune response leads to the emergence of immunosuppressive circuits in the tumor itself and the exhaustion of tumor-specific T cells, which include T cells responding to the epitopes targeted by vaccines. Consequently, although vaccines against infectious agents rely mainly on the generation of neutralizing antibodies by B cells (at least during the initial stage of the immune response), cancer vaccines need to generate or boost a $CD8^+$ and $CD4^+$ effector T cell response directed against a bulk of diverse and continuously changing tumor cells.

There are many platforms and delivery approaches for cancer vaccines. *Ex vivo* vaccination involves the adoptive transfer of autologous or allogeneic DCs that have been loaded with a mix of adjuvants and cancer antigens [31,32]. *In vivo* cancer vaccination, on the other hand, aims to target DCs in the patient through the administration of a vaccine containing both tumor antigens and an adjuvant [33]. A sizable percentage of DCs in cancer patients are dysfunctional, which could potentially be overcome by an *ex vivo* DC vaccine [34]. However, *in vivo* vaccination is less complex, labor-intensive, and costly, and can engender physiological stimuli that lead to a stronger antitumor immune response.

Adoptive T cell therapy

The simplest form of ACT involves the selection of active tumor-targeting T cells, followed by direct administration to the patient. These cells are either obtained by harvesting tumor-infiltrating lymphocytes (TILs) from the tumor or by enriching neoantigen-specific lymphocytes from the peripheral blood mononuclear cells (PBMCs) of the patient. Through reinfusion of large numbers of these effector T cells, the balance shifts from T cell anergy, exhaustion, and tolerance to an antitumoral T cell population.

Retrospective studies have shown that tumors from patients with complete responses (CRs) following TIL therapy had overall higher TMB and that, in general, TILs from responders frequently include neoantigen-specific lymphocytes [35,36]. Indeed, among T cells present in the tumor tissue, those that are cognate to tumor neoantigens appear to be the driving force of any antitumor response in the context of immune checkpoint inhibition [37–40]. Similarly, CRs following adoptive transfer of *ex vivo* expanded TILs have been reported in melanoma, cholangiocarcinoma, and breast cancer patients [41–43]. In melanoma, for example, T cells specific for the identified neoantigens were observed to be persistent at high levels post-treatment in both tumor and peripheral blood [21]. Most recently, a study reported improved outcomes with TIL therapy over blockade of cytotoxic T lymphocyte antigen 4 (CTLA-4) as a second-line treatment, as tested in a randomized, multicenter study [44].

Broader applicability of ACT can potentially be achieved with TCR transgenic ACT therapy. TCR transduction of T cells from peripheral blood has been shown to result in T cells with better proliferation capacity and more functionality [6]. The main challenge is the identification and engineering of potent neoTCR T cells in an acceptable timeframe. It should also be noted that adoptive cell transfer therapy in general faces important clinical challenges. Indeed, substantial toxicity can be observed related to the high-dose chemotherapy and IL-2 therapy that are given to achieve lymphodepletion and to sustain T cell numbers *in vivo*. CAR-ACT on the other hand can lead to cytokine release syndrome and immune effector cell-associated neurotoxicity [45–47].

Personalized adoptive T cell therapy manufacturing

An important challenge for neoantigen-based therapeutics is related to the necessary individualization of the treatment. Personalized analysis of the individual tumor is required, hence negatively impacting on the cost and manufacturing time. Manufacturing should be as time-efficient as possible to ensure rapid treatment availability and to avoid intercurrent detrimental disease progression. Personalized medicine currently involves complex logistics as well as labor-intensive and costly methodologies, particularly for cell-based therapies. To illustrate, the total price of tisagenlecleucel and axicabtagene ciloleucel, for instance, is approximately US$320 000 per treatment regimen. Owing to these high costs, CAR T cell therapy is currently ranked among the most expensive therapeutics [48]. It should be noted that this therapy has the promise to be a curative, one-time therapy, which may help to justify the cost.

One of the main drivers of the time and cost of manufacturing cell-based therapeutics is related to the fact that autologous cells must be amplified. To make neoantigen-driven cell therapies broadly available – in an acceptable time and at an acceptable cost – fully closed, scalable, and cost-efficient manufacturing methods and quality systems optimized for neoantigen-driven therapeutics need to be developed [49,50]. A strategy that is currently being explored involves the use of allogeneic cell lines as off-the-shelf formulation platforms [51,52]. However, this approach needs further optimization in terms of safety and efficacy to reach its full potential.

Compared to *ex vivo* cell therapeutics, *in vivo* therapeutics can also offer a less time- and cost-expensive solution. Different *in vivo* approaches exist involving DNA-, RNA-, synthetic long peptide (SLP)-, and viral vector-based platforms. The recent clinical successes obtained with mRNA-based and SLP-based vaccines in different cancer types show the feasibility and promise of these *in vivo* approaches. As opposed to viral vector or DNA-based vaccines, using mRNA or SLPs as a vaccine platform is particularly interesting because there is no risk of infection or insertional mutagenesis. mRNA faces instability (in contrast to DNA and SLPs) as well as delivery challenges; however, major progress has been made to largely solve these issues [53]. Furthermore, nucleic-based approaches in general are not limited by the solubility issues of peptide vaccines. mRNA, in particular, allows more rapid, low-quantity, and inexpensive manufacturing, both at a small and large scale [54–56]. Given the tight clinical timeframes for personalized therapeutics, the speed of manufacturing is an important advantage and, combined with the recent successes achieved with COVID-19 vaccines, mRNA-based approaches have therefore become a research hotspot in the personalized therapy field [57].

The promise of neoantigen-driven immunotherapy

A key advantage of personalized neoantigen-driven immunotherapy is the potential broad applicability in solid cancer treatment. Several clinical trials have demonstrated that neoantigens can be recognized by $CD4^+$ and $CD8^+$ T cells and can trigger an *in vivo* antitumor response.

An overview of completed neoantigen-directed cancer vaccine and ACT trials is given in Table 1. Between 2015 and 2022, nine neoantigen-directed cancer vaccine clinical trials have been completed as well as one *ex vivo* DC vaccine [8] and eight *in vivo* vaccines (SLP- or RNA-based) [9–11,58–62]. In the same timeframe, four neoantigen-centered ACT trials have been reported involving three TIL therapies [42,43,63] and one TCR-ACT [64]. These results support the preclinically identified potency of this approach in a clinical setting for cancers such as melanoma and glioblastoma.

Neoantigen-directed cancer vaccines

The first reported Phase 1 clinical trial of neoantigen-based vaccines was performed in three patients with advanced melanoma by utilizing a DC vaccine stimulated with a set of seven

Table 1. Overview of completed neoantigen-based immunotherapy clinical trials and their clinical response[a]

Year	NCT number	Therapy type	Number of neoantigens	Cancer type	Number of patients	Clinical response
Neoantigen-directed cancer vaccine trials						
2015 [8]	NCT00683670	DC vaccine	7	Melanoma	3	Induction of neoantigen-specific T cells
2017 [9],[12]	NCT01970358	SLP vaccine	20	Melanoma	6	Four patients NED Two patients recurrence (both CR after anti-PD1)
2017 [11]	NCT02035956	RNA vaccine (IVAC MUTANOME)	10	Melanoma	13	Eight patients NED Five patients recurrence (one patient CR after anti-PD-1)
2019 [10]	NCT02287428	SLP vaccine	20	Glioblastoma	8	Increased neoantigen-specific T cell response
2019 [59]	NCT02149225	SLP vaccine (GAPVAC)	20	Glioblastoma	15	Increased neoantigen-specific T cell response
2022 [60]	NCT04487093	SLP vaccine	5–14	NSCLC	24	Six patients PR, one patient CR Five of seven responders showed neoantigen-specific T cell induction
2022 [58]	NCT03380871	SLP vaccine (NEO-PV-01)	20	NSCLC	21	Induction of neoantigen-specific T cells (in combination with anti-PD-1 and Pt-based chemotherapy)
2022 [62]	NCT03639714	Adenoviral vector (prime) and samRNA vector (boost)	20	MSS-CRC, GEAC, or NSCLC	14	Induction of neoantigen-specific T cells (in combination with anti-PD1) One patient CR for 6 months (GEAC)
Neoantigen-directed adoptive cell transfer trials						
2014 [42]	NCT01174121	TIL-ACT	1	Cholangiocarcinoma	1	Modest regression of metastases Durable response for almost 9 years (after TIL-ACT retreatment in combination with anti-PD-1)
2016 [63]	NCT01174121	TIL-ACT	1	MSS-CRC	1	Regression of all metastases
2018 [43]	NCT01174121	TIL-ACT	4	Breast cancer	6	One patient CR >5 years Two patients PR for 6–10 months (in combination with IL-2 and anti-PD-1)
2022 [64]	NCT04146298	TCR-ACT	1	Pancreatic cancer	1	PR, regression metastases ongoing at 6 months

[a]Abbreviations: ACT, adoptive cell transfer; CR, complete remission; CRC, colorectal cancer; DC, dendritic cell; GEAC, gastroesophageal adenocarcinoma; MSS, microsatellite-stable; NED, no evidence of disease; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; PR, partial response; Pt, platinum; samRNA, self-amplifying mRNA; SLP, synthetic long peptide; TCR, T cell receptor; TIL, tumor-infiltrating lymphocyte; UC, urothelial cancer.

personalized peptide neoantigens [8]. The vaccine enhanced the pre-existing neoantigen-specific T cell responses and triggered *de novo* T cell activity against subdominant neoantigens with no pre-existing immunity before treatment. Neoantigen vaccination thus expands the antigenic breadth and clonal diversity of antitumor immunity.

This relevant clinical impact of neoantigen vaccination was confirmed in two other melanoma trials. In a study conducted at the Dana-Farber Cancer Institute (DFCI), six patients with resected stage 3 or 4 melanoma received a personalized SLP vaccine targeting up to 20 neoantigens formulated with the Toll-like receptor agonist polyinosinic-polycytidylic acid (poly-ICLC) [9]. Four of the six patients were recurrence-free within 25 months after vaccination. Two vaccinated patients

achieved CRs when they received programmed cell death protein 1 (PD-1) antibody treatment upon disease recurrence. In another study, a 'naked' personalized mRNA vaccine termed IVAC MUTANOME containing ten neoantigens was injected into the lymph nodes of 13 melanoma patients. Eight patients without radiographically detectable metastatic disease remained recurrence-free during a follow-up period of up to 23 months, and two of five patients with metastatic disease had objective responses upon vaccination. Trafficking of neoantigen-specific T cells into a lymph node metastasis was demonstrated in one patient [11]. *Ex vivo* T cell responses against vaccine neoantigens were observed in all patients on these two trials, and there was a predominance of $CD4^+$ over $CD8^+$ T cells in both studies. In a follow-up analysis of the SLP vaccine study, long-term persistence of polyfunctional memory T cells was observed [12]. Most T cell responses directed against the immunized peptides were found to be still present after 2–4.5 years, suggesting that neoantigen-directed vaccines have the potential for long-term anti-tumor immunity.

Two studies tested neoantigen-based vaccines in human glioblastoma. This 'cold' tumor generally exhibits limited intratumoral infiltration of immune cells, is characterized by a low number of mutations (averaging 30–50 non-synonymous variants), and is largely unresponsive to immune checkpoint inhibition [59]. In one study using personalized SLP vaccines formulated with poly-ICLC [10], eight patients with glioblastoma treated with neoantigen vaccines presented an increased number of neoantigen-specific $CD4^+$ and $CD8^+$ T cells. Vaccine neoantigen-specific T cells were shown to traffic into intracranial tumor post-treatment, thus favorably altering the immune environment of the glioblastoma. Another Phase 1 trial involved treatment of 15 glioblastoma patients with APVAC-1 (a vaccine derived from unmutated epitopes) followed by APVAC-2 (a personalized neoepitope vaccine). Sustained beneficial immune patterns and predominantly $CD4^+$ T_{H1} T cell responses were elicited in all patients [59]. Although no objective responses were achieved, these findings demonstrate that neoantigen-based vaccines are feasible for 'cold' tumors such as glioblastoma.

The personalized neoantigen SLP vaccines developed at DFCI were tested further in combination with PD-1 inhibition alone and platinum-based chemotherapy plus PD-1 inhibition, respectively. In the first trial, 60 patients with previously untreated melanoma, non-small cell lung cancer, or urothelial cancer received the vaccines in combination with nivolumab [61]. Robust *de novo* $CD4^+$ and/or $CD8^+$ T cell responses against approximately half of the vaccine immunizing neoepitopes were detected in all patients. Response rates and progression-free survival (PFS) compared favorably with historical data for patients treated with PD-1 inhibition alone; furthermore surrogates for vaccine efficacy including epitope spreading, pathologic CRs assessed in core tumor biopsies obtained after vaccination, and cytotoxicity phenotypes of vaccine-specific T cells were observed. In the second study, 21 patients with treatment-naive metastatic non-small cell lung cancer received SLP vaccines in combination with platinum-based chemotherapy and pembrolizumab [58]. More than half of the vaccinating epitopes generated T cell responses. Immune profiling of vaccine-specific $CD4^+$ T cell populations revealed an activated phenotype and cytolytic potential. Similar to the first trial, the observation of epitope spreading post vaccination suggested vaccine-induced tumor cell killing.

A heterologous prime–boost vaccine approach consisting of a prime with an adenovirus vector and a boost using a self-amplifying mRNA (samRNA) vector, both encoding up to 20 personal neoepitopes, was tested in combination with nivolumab in 14 patients with advanced, previously treated microsatellite stable (MSS) colorectal cancer ($N = 7$), gastroesophageal (GE) adenocarcinoma ($N = 8$), or non-small cell lung cancer in a Phase 1/2 trial [62]. The vaccines were found to be feasible and safe, and induced robust, durable $CD8^+$ T cell responses exhibiting memory

phenotypes and cytotoxic potential. One patient with GE cancer had a CR lasting for 6 months; a decrease in circulating tumor DNA post-vaccination correlated with overall survival in a small cohort of patients with colorectal cancer.

Interim data from an investigator initiated trial testing the BioNTech individualized mRNA cancer vaccine in patients with resected pancreatic duct adenocarcinoma, in combination with anti-programmed death-ligand 1 and chemotherapy, demonstrated a strong association of prolonged recurrence-free survival (RFS) with the induction of vaccine-specific T cell responses (NCT04161755). At an early median follow-up of 18 months, patients with a *de novo* immune response ($N = 8$) had a significantly longer RFS as compared to those without vaccine-induced immune responses ($N = 8$) [median not reached vs. 13.4 months, hazard ratio (HR) 0.08, 95% confidence interval 0.01–0.4, $P = 0.003$].

Preliminary Phase 1 results were recently reported in head and neck cancer and in ovarian cancer for TG4050, a virus-based therapeutic vaccine encoding neoantigens. In the head and neck cancer trial (NCT04183166), patients were randomized to immediately receive vaccination with TG4050 (arm A) or at relapse (arm B). All evaluable patients randomized to arm A ($N = 8$) were still in CR whereas two patients in arm B had experienced recurrence. Treatment with the personalized vaccine for ovarian carcinoma patients was started at recurrence defined as elevation of cancer antigen CA) 125 or onset of suspicious radiological findings (NCT03839524). One patient treated after a rise of CA-125 experienced normalization of CA-125 without clinical progression for 9 months until death from an unrelated chronic illness. Another patient was treated upon radiographically detected recurrence and experienced tumor stability for 11.4 months. Furthermore, Moderna recently reported that a personalized mRNA vaccine directed at neoantigens combined with anti-PD-1 reduced the risk of recurrence or death by 44% compared to anti-PD-1 alone in patients with stage 3 or 4 melanoma (NCT03897881).

Collectively, neoantigen vaccines based on DNA, RNA, SLP, and DCs have been tested in Phase 1 clinical trials, and the initial data suggest that neoantigen vaccines are safe, induce neoantigen-specific T cell response including trafficking of vaccine-specific T cells into the tumor, and can mediate anti-tumor activity. However, enhancing the frequency of pre-existing or *de novo* neoantigen responses does not always translate into clinical benefit because, most probably, other components of the TME influence the final efficacy of the immune response [65,66].

Neoantigen-directed adoptive T cell transfer

Neoantigen-targeted adoptive cell transfer is still in early development for solid malignancies [67]; however, several reports of cases and case series have demonstrated proof of principle as evidenced by deep and durable regression of tumors in patients with metastatic gastrointestinal tumors and patients with advanced breast cancer. Different approaches include adoptive therapy of TILs, neoTCR TILs, and neoTCR T cells derived from PBMCs (autologous or off-the-shelf) (Figure 2).

Neoantigen-specific T cells expanded from TILs

In initial reports by the Surgical Branch at the National Cancer Institute (NCI), patients were treated with TILs containing neoantigen-specific T cells that were selectively expanded *in vitro*. The first case reported by the Surgery Branch at the NCI involved a patient with metastatic cholangiocarcinoma who was treated with 40 billion TILs containing ~25% $CD4^+$ T cells specific for a mutation in ERBB2IP, leading to modest regression of liver and lung metastases. Upon disease progression, the patient was retreated with 12.6×10^{10} $CD4^+$ T cells mostly reactive to ERBB2IP followed by additional treatment with PD-1 inhibition, leading to a durable response lasting almost 9 years

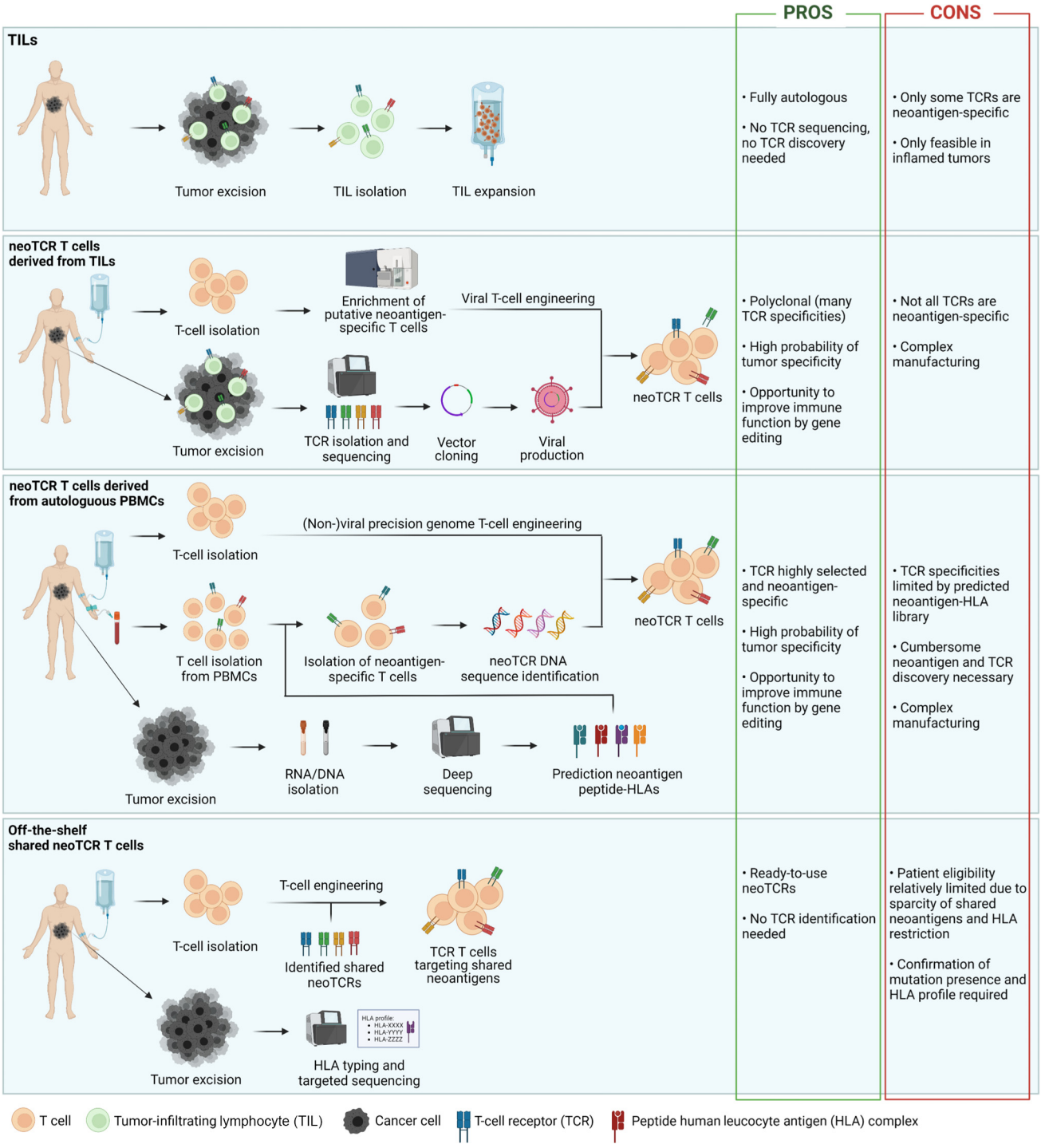

Figure 2. Different approaches for neoantigen-directed T cell therapy. Abbreviations: HLA, human lymphocyte antigen; neoTCR, neoantigen-specific TCR; PBMC, peripheral blood mononuclear leukocyte; TCR, T cell receptor; TIL, tumor-infiltrating lymphocyte.

[41,42]. In another report, a patient with MSS colorectal cancer metastatic to the lungs experienced regression of all tumor lesions after treatment with 14.8×10^{10} $CD8^+$ T cells, of which 75% were specific for the driver mutation KRAS-G12D that had been expanded from KRAS-G12D-specific $CD8^+$ T cell clones contained in TILs harvested from three pulmonary metastases [63]. Although the most frequent of four infused T cell clonotypes was not detectable in the peripheral blood 40 days after adoptive transfer, three of these clones were present at this timepoint and two of these persisted at frequencies of 4.5–10% for up to 9 months. KRAS-G12D-reactive T cell receptors were found to be specific for mutant but not KRAS wild-type peptides.

In a clinical trial also conducted at the NCI, a patient with chemotherapy-refractory hormone receptor-positive metastatic breast cancer who received adoptive transfer with TILs containing $CD4^+$ and $CD8^+$ T cells reactive against four neoantigens (SLC3A2, KIAA0368, CADPS2, and CTSB) in addition to IL-2 and pembrolizumab experienced complete durable tumor regression that is ongoing for >5 years. Notably, eight of 11 TCR clonotypes that recognized the four neoantigens persisted in the peripheral blood for up to 17 months after treatment [43]. An additional two of the six patients with advanced breast cancer treated on the trial to date had partial responses lasting for 6 and 10 months, respectively. Of note, only six patients whose TILs were screened for the presence of neoantigen-reactive clones were eventually treated with adoptive transfer of enriched neoantigen-specific TILs, highlighting a challenge for this approach.

T cells engineered to express neoantigen-specific TCRs

Several ongoing clinical trials are testing neoantigen-directed adoptive cell therapy either based on transfer of autologous TCRs directed at personal neoantigens identified from the tumor of each patient or of allogeneic TCRs reactive against recurrent oncogenic driver mutations [41].

The vast diversity of both human HLA class I alleles and the mutations encoding most neoantigens substantially limits the number of patients eligible for any off-the-shelf neoTCR engineered T cell therapy approach. Personalized adoptive cell therapy is therefore arguably necessary to broaden the applicability of this strategy across patients with cancer, similarly to neoantigen-specific vaccines. The standard method to transfer neoTCRs is viral transduction of peripheral blood T cells. Candidate TCR sequences with known neoantigen reactivity can also be introduced into T cells by transposon or CRISPR/Cas9 systems. A recently developed non-viral precision genome-editing technique can simultaneously knockout the endogenous TCR genes and introduce a neoTCR, allowing faster production of clinical grade T cells [68].

Based on this technology, a first-in-human Phase 1 clinical trial (NCT03970382) of gene-edited autologous neoTCR T cells in patients with solid tumors was recently conducted. The investigators demonstrated for the first time that it is feasible in the clinic to identify neoTCRs from T cells in the peripheral blood of cancer patients, engineer these into autologous T cells, expand those T cells *in vitro*, and adoptively transfer them back into the patients [69]. In the trial, 16 patients with treatment-resistant advanced solid tumors (mostly MSS colorectal cancers) were treated with up to three distinct autologous gene-engineered T cells expressing personalized neoTCRs targeting private cancer mutations. Personal neoantigens were identified using whole-exome sequencing (WES) and RNA-seq of tumor and normal cells for mutation calling and *in silico* HLA binding prediction and RNA expression. Up to 352 neoantigen peptide–HLA candidates across the HLA class I alleles of each patient covered by an HLA library were selected per patient. By fusing neoantigen peptides to β2-microglobulin domains and the HLA, peptide–HLA libraries were generated. Using DNA-barcoding, fluorescent labeling, and multimerization, neoantigen-specific T cells were isolated from peripheral $CD8^+$ T cells. Across the 16 treated patients who were eventually treated, 175 TCRs (median of eight) specific for a median of four mutations were detected.

The neoTCR sequences expressed by the captured neoantigen-specific T cells were then cloned and functionally characterized in healthy donor T cells for adoptive T cell transfer product selection. Confirmation of the reconstituted neoTCR binding to the soluble peptide–HLA complex resulted in 75 out of 127 eventually tested TCRs (57%) being confirmed as specific and functional. Up to three confirmed TCR candidates per patient were selected for the final cell transfer product based on functionality, binding, and diversity across HLAs. Clinical grade neoTCR transgenic T cell products were generated using a non-viral precision genome-engineering approach. NeoTCR T cells were found to be polyfunctional (CD107a, IL-2, TNF-α, IFN-γ) and specific. Engraftment of the transferred neoTCR T cells was in the 10% range (per total T cells in a given patient) and showed persistence for up to 60 days. Infused neoTCR transgenic T cells obtained from the blood of patients after transfusion had similar phenotypes compared to the infused product (mainly T memory stem cells and T effector cells). Clonal mutations encoding neoantigens targeted by the infused neoTCR transgenic T cells were largely consistent between tumor biopsies obtained for neoantigen target selection and the repeat biopsies obtained before treatment. The majority of TCRs were detected in post-treatment tumor biopsy of a subset of patients. The therapy was found to be safe, and no objective tumor responses to the treatment were reported; five patients had stable disease whereas the other 11 patients had disease progression.

This pioneering study demonstrates that personalized neoTCR engineered T cell therapy is feasible in principle; however, it also highlights several challenges that need to be overcome for this therapeutic approach to be broadly feasible in the clinic. Several additional studies using autologous T cells engineered with personalized neoTCRs are being conducted in different types of solid tumors (e.g., NCT05292859, NCT04520711, NCT05194735).

A time-consuming process

Reflecting the aforementioned study, the generation of the personalized therapy took the better part of a year: the median time from screening a potential treatment candidate to the start of treatment for the patient in the study was 269 days (167 days for TCR discovery and validation, and 102 days for manufacture and quality control). Moreover, <10% of patients (16 of 187) who signed consent to participate in the study were eventually treated. This lengthy time-interval is clearly a manifestation of the innovative, but also exquisitely cumbersome personalized production process. The timeline also should be taken in context with other personal adoptive T cell therapies; most notably, clinical trials of adoptive TIL therapy reported in the past have had notoriously high drop-out rates resulting in low numbers of patients receiving the intended therapy. Undoubtedly, it should be possible to streamline the manufacturing process, for example, through economies of scale and further automation of the TCR discovery and validation processes.

Persistence of adoptively transferred neoTCR T cells in the peripheral blood

An important parameter of success of any adoptive TCR-engineered neoantigen-directed T cell therapy is arguably the achievement of relatively large fractions of neoTCR T cells in peripheral blood after adoptive transfer. As expected, neoTCR fractions increased with larger number of cells transferred; in addition, increased doses of the conditioning chemotherapy regimen and addition of IL-2 resulted in a substantial increase of neoTCR persistence, albeit no data beyond the 60 day mark are available from the current study.

TCR affinity and trafficking of neoTCR T cells into metastatic tumor sites

In the current study the affinity of the selected and adoptively transferred neoTCRs ranged widely, and the majority of TCRs' IFN-γ EC_{50} values were above 10 ng ml^{-1}, thus exhibiting lower affinity compared to previously studied TCRs specific for the TAAs MART-1 and NY-ESO-1, the viral antigen human papilloma virus (HPV) E7, and the shared mutations KRAS-G12D and mutated p53.

Comprehensive analysis of post-treatment tumor specimens for the presence of neoTCRs revealed that higher TCR affinity was associated with increased numbers of neoTCRs in the tumors, demonstrating improved trafficking to metastatic sites and suggesting that high TCR affinity is an important criterion for neoTCR selection.

Off-the-shelf engineered neoTCR T cell therapy

Although several trials testing off-the-shelf neoTCR therapy directed at shared mutations are ongoing, no systematic data are so far available. However, there are reports from two patients with advanced pancreatic cancer harboring a KRAS-G12D mutation who were treated with autologous peripheral T cells engineered to express TCRs derived from the above-described patient with MSS colorectal cancer who had an objective response after treatment with enriched KRAS-G12D-specific TILs. One of the two patients experienced a partial response to treatment, and regression of multiple pulmonary metastases was ongoing at 6 months post-treatment [64]. TCR-transgenic adoptively transferred T cells were predominantly $CD8^+$ and persisted over time. These data provide proof of concept of the feasibility and potential antitumor activity of an ACT approach using TCR-engineered neoantigen-specific T cells that could be used off-the-shelf for patients with tumors harboring the respective mutations. The ability to use these preidentified TCRs (noting that large amounts of autologous T cells from the peripheral blood still need to be obtained from each patient) makes this strategy substantially more efficient compared to a personalized ACT-engineered TCR approach given that the time, cost, and labor-intensive process of neoTCR identification can be eliminated. However, as outlined above, patient eligibility for an off-the-shelf approach is limited by both the relative infrequency of shared mutations as well as by HLA restriction of the neoepitopes encoded by these mutations.

Concluding remarks and future perspectives

Targeting tumor- (and patient)-specific neoepitopes with precise therapeutic interventions is a potentially elegant way to strengthen and direct the immune response to the tumor, aiming at elimination of cancer cells without collateral damage. Although the early clinical experience of testing neoantigen-directed vaccines as well as case reports of neoantigen-specific cell therapies have demonstrated that antitumor activity can be achieved in select patients with advanced cancers, it has also become clear that most patients who were treated in the initial trials likely received only modest if any clinical benefit.

The effective selection of only those neoantigens that can induce a strong immune response remains a substantial hurdle (see Outstanding questions). Neoantigen prioritization needs further optimization as only a very small fraction of the predicted neoantigens have found to be immunogenic in cancer vaccine trials [9,59]. The Tumor Neoantigen Selection Alliance (TESLA) initiative has recently revealed that – on the same validation dataset – different discovery pipeline outputs have little overlap and little positive predictive value (≤10%) [70]. Better prediction algorithms could shorten or eliminate the need for screening of neoantigen-reactive T cells. Beyond that, a broader search space of the neoantigen landscape is required to allow neoantigen identification in low TMB tumors by developing different and more thorough neoantigen discovery tools [71]. This involves identifying mutations outside of the current space – single-nucleotide variants (SNVs) and indels – and exploring the domain of gene fusion events, transposable element activity, neoisoforms, and alternative proteasomic splicing, among other events [72].

Faster and more sensitive identification of neoantigen-specific T cells and cloning of their TCRs will also help to expand the field of neoantigen-targeted ACT. Molecular signatures of neoantigen-specific TILs, determined by high-throughput transcriptomic and TCR sequencing, have been discovered that allow enrichment of neoantigen-specific T cells based solely on TIL

Outstanding questions

Is there a platform and delivery approach that is particularly well suited for neoantigen-directed vaccines, or are we 'all in' on RNA vaccines?

What is the most effective trial design to assess cotherapies necessary to activate the innate immune response and modulate the tumor microenvironment?

Are neoantigen-directed vaccines most effective in a preventative or a minimal residual disease intervention setting versus advanced solid tumors?

Conversely, is neoantigen-directed cell therapy the preferred approach for more advanced disease?

How can the neoantigen discovery space be broadened beyond the SNVs and indels that are currently targeted in the clinic?

Which attributes of neoantigens are most informative concerning their ability to elicit an effective antitumor immune response and how can they be identified?

What is the best readout for a cancer vaccine trial to inform decisions regarding further development? (i) Quality, magnitude, phenotype of vaccine-specific immune responses in the peripheral blood versus the tumor; (ii) radiographic signals of vaccine-induced tumor activity (response rate, PFS); (iii) randomized studies with clinical endpoints; (iv) pathologic response and epitope spreading.

Are there opportunities to simplify neoantigen-directed therapy to minimize manufacturing time and cost?

Is it feasible to use non-viral gene editing to improve the persistence and function of neoantigen-specific adoptive T cell therapy?

What is the role of 'off-the-shelf' neoantigen-based T cell therapy?

transcriptomic states [73]. New technologies that enable the expansion and identification of rare tumor neoantigen TILs as well as rapid cloning of the cognate TCRs expressed by these TILs are also being developed [74–76]. These new developments and the simplification of logistics and automation of manufacturing are key to reducing costs and promoting further expansion of this field.

Furthermore, many variables and processes govern a tumor-specific immune response in an individual cancer patient, and likely determine the success or failure of any immune intervention, including neoantigen-directed therapy. The sheer number of different pathways and mechanisms that provide potential therapeutic targets is both promising and daunting. Because realistically only a small proportion of these variables can be tested in the clinic, a 'first principles', hierarchical, and pragmatic approach will be necessary to improve the efficacy of neoantigen-directed cancer vaccines. Beyond selection of high-priority cotherapies for systematic testing in a clinical trial, including anti-CD40 and CD27 antibodies, and the cytokine FLT-3 ligand (or a combination thereof), the choice of an appropriate clinical setting (tumor type, one tumor vs. multiple, early vs. late disease) and design of the trial are crucial (relatively small, non-randomized, multiarm, biomarker-driven vs. larger, randomized studies). We posit that smaller, multiarm studies in a single tumor type, ideally with opportunities for collection of surgical tumor biopsies, might be the most fruitful testing ground. Criteria enabling a 'go/no go' decision for a particular combination should incorporate careful assessment of the immune response post-treatment in both the tumor and peripheral blood (quality/magnitude/phenotype) as well as clinical endpoints for vaccine-induced tumor activity [objective response rate (ORR), PFS, etc.]. Although the induction of a high-magnitude, *ex vivo* neoantigen-specific $CD8^+$ T cell response in peripheral blood would be encouraging, vaccine-induced peripheral immune responses have often not correlated with clinical benefit. The observation of increased frequencies of effector T cells in post-treatment tumors is an arguably more informative marker of success.

To allow rigorous assessment of vaccine and cotherapy effects, combinations should ideally be tested sequentially starting with monotherapy, followed by combination therapy and immune/tumor assessments between the therapy segments (Figure 3). Implementing such a design is challenging: testing a novel agent as monotherapy is only feasible in a clinical setting where there is no compelling standard-of-care therapy. Tumor types that have not shown responsiveness to PD-1 inhibition, ideally with planned tumor debulking as standard of care – as is often done, for example, for ovarian cancer – are worth considering for such studies.

Several reports of durable antitumor activity achieved with neoantigen-specific T cells derived from TILs has provided proof of concept for neoantigen-directed T cell therapy. Recently developed single-cell technologies – specifically the ability to identify and clone tumor-reactive TCRs based on the expression of PD-1 and CD39 on tumor-infiltrating $CD8^+$ T cells – have revealed an association between tumor reactivity, TCR avidity, and T cell functional state. Importantly, most $CD8^+$ TILs recognizing tumor antigens (neoantigens and TAAs) exhibited an exhausted state and a lack of memory properties [2]. Furthermore, recent studies have demonstrated that non-exhausted, stem-like T cells are characterized by expression of the transcription factor TCF7 and are crucial mediators of antitumor activity in the context of cancer vaccines and anti-PD-1-based immunotherapy [27,77,78]. In the adoptive cell transfer setting, neoantigen-specific stem-like TILs lacking expression of CD39 were present in TIL products of patients with antitumor responses and were absent in non-responders [79]. These findings highlight strategies aimed at further improving the efficacy of neoantigen-specific T cell therapies. For TIL therapy, gene editing could be employed to convert tumor-specific, exhausted tumor-infiltrating T cells into cytolytic T cells with stem-like properties capable of mediating robust antitumor activity. Such an

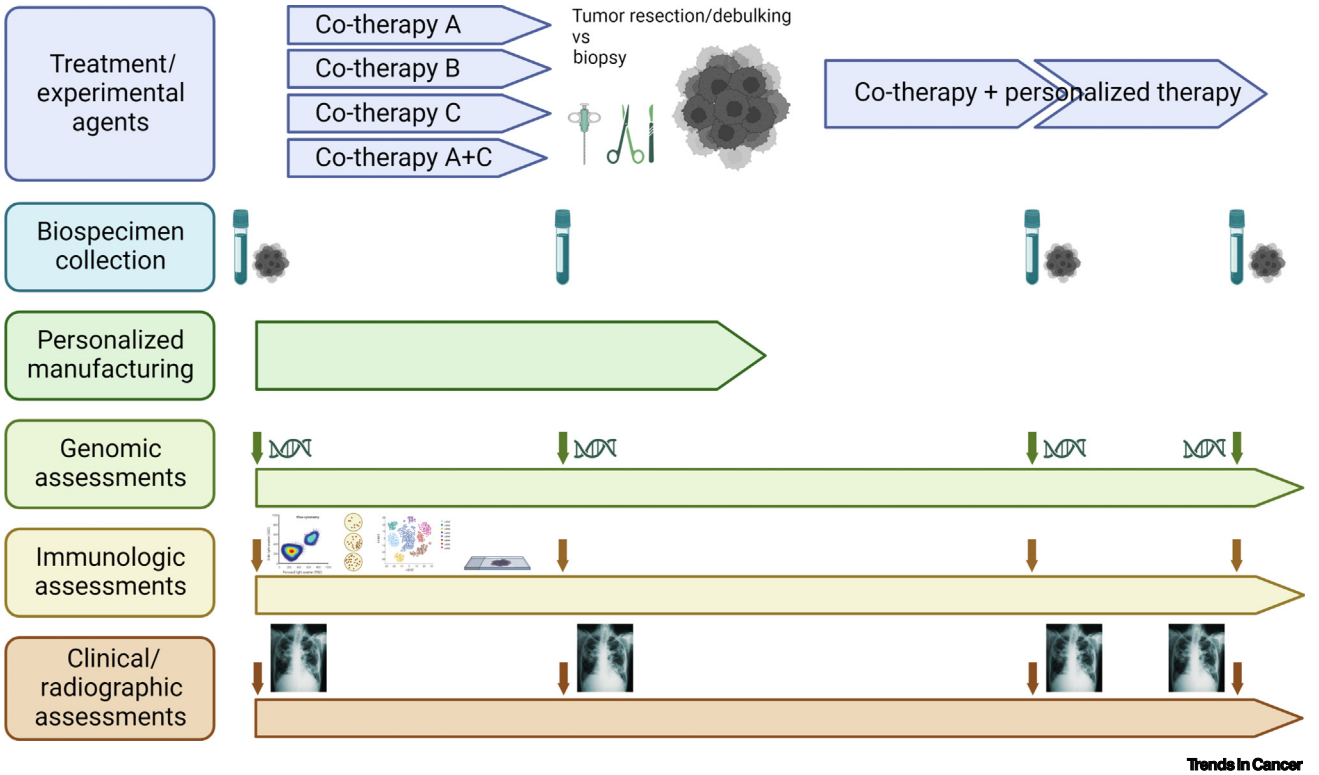

Figure 3. Window of opportunity platform/basket clinical trial design setup suitable for personalized therapeutics to test multiple therapies at the same time, thus enabling informed decision concerning the optimal combination therapy.

'engineered TIL' approach – namely the identification of *in vivo* tumor-reactive T cells identified from the exhausted TIL compartment followed by gene editing to reestablish T cell function and persistence – has potential advantages compared to the adoptive transfer of personalized neoTCR engineered peripheral T cell approach pioneered by the PACT Pharma team: (i) the TILs would contain polyclonal T cells with a spectrum of TCRs specific for multiple tumor antigens, thus accounting for tumor diversity by encompassing a spectrum of tumor antigens, and (ii) the time- and cost-intensive TCR discovery steps would be eliminated. However, the non-viral gene-editing approach employed by PACT Pharma also potentially lends itself not only to replacing wild-type TCRs of peripheral T cells with high-affinity, neoTCRs but also to altering the function of these T cells towards a non-exhausted, stem-like effector state. This approach has the additional advantage that an entirely off-the-shelf product could be generated, encompassing TCRs specific to shared genomic variants and a variety of HLA haplotypes.

Acknowledgments

Funding to P.A.O. from NCI-R01 CA229261; Francis and Adele Kittredge Family Immuno-Oncology and Melanoma Research Fund; Faircloth Family Research Fund; Susan and Bruce Hampton Research Fund; Fisher Family Fund; and Morgan Family Fund for Melanoma Research is acknowledged.

Declaration of interests

P.A.O. has received research funding from and has advised Neon Therapeutics, Bristol-Meyers Squibb, Merck, CytomX, Pfizer, Novartis, Celldex, Amgen, Array, AstraZeneca/MedImmune, Armo BioSciences, Evaxion, Phio, Xencor, Oncorous, Servier, Imnmunetune, and Roche/Genentech.

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