Personalized Cancer Vaccines: Clinical Landscape, Challenges, and Opportunities

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Tremendous innovation is underway among a rapidly expanding repertoire of promising personalized immune-based treatments. Therapeutic cancer vaccines (TCVs) are attractive systemic immunotherapies that activate and expand antigen-specific CD8^{+} and CD4^{+} T cells to enhance anti-tumor immunity. Our review highlights key issues impacting TCVs in clinical practice and reports on progress in development. We review the mechanism of action, immune-monitoring, dosing strategies, combinations, obstacles, and regulation of cancer vaccines. Most trials of personalized TCVs are ongoing and represent diverse platforms with predominantly early investigations of mRNA, DNA, or peptide-based targeting strategies against neoantigens in solid tumors, with many in combination immunotherapies. Multiple delivery systems, routes of administration, and dosing strategies are used. Intravenous or intramuscular administration is common, including delivery by lipid nanoparticles. Absorption and biodistribution impact antigen uptake, expression, and presentation, affecting the strength, speed, and duration of immune response. The emerging trials illustrate the complexity of developing this class of innovative immunotherapies. Methodical testing of the multiple potential factors influencing immune responses, as well as refined quantitative methodologies to facilitate optimal dosing strategies, could help resolve uncertainty of therapeutic approaches. To increase the likelihood of success in bringing these medicines to patients, several unique development challenges must be overcome.

Therapeutic cancer vaccines (TCVs) have been heavily investigated in clinical trials for the past 50 years as investigational immunotherapies that aim to elicit new, or strengthen existing, CD8^{+} cytotoxic T cell lymphocyte (CTL) tumor antigen-specific responses.[1,2] As TCVs target antigens predominantly associated with tumor cells, this approach can be safer than other therapies by avoiding off-target effects. TCVs have evolved as a promising class of drugs in the immuno-oncology space, and they comprise a diverse set of antigens, adjuvants, delivery vectors, and administration methods.[3] Historically, hundreds of TCV clinical trials including dozens of pivotal investigations were largely unsuccessful in demonstrating a clear clinical benefit.[4,5] This is likely due to a combination of factors not limited to (1) suboptimal antigens, (2) lack of effective adjuvants, (3) poorly immunogenic platforms, and (4) an insufficient number of CTLs entering the tumor due to immunosuppression related to high disease burden, poor immune fitness, or an immunosuppressive tumor microenvironment.[6,7]

Renewed investment and innovation are now underway, among a rapidly expanding repertoire of advanced TCV platforms. Among recent advances are personalized neoantigen-based TCVs with selective individualized antigens and new combination approaches to enhance immune activities compared to conventional TCVs against shared antigens.[8–10] Given that neoantigen load has been correlated with response to existing immunotherapies,[11,12] these represent compelling targets for personalizing TCVs to enhance activity. More than 799 TCVs are in the global drug development pipeline as of 2019, with more than 400 active clinical trials.[13] Of these, at least 23 are personalized vaccination approaches, which are well suited to investigate therapeutically as custom-tailored medicines in patients. Our review aims to provide a detailed account of the key components and common mechanisms of action of TCVs, while focusing on personalized TCVs, including (1) assessing the current clinical trial landscapes, (2) summarizing vaccination strategies, combination immunotherapies, common obstacles in development, and the regulatory framework of personalized TCVs, and, lastly, (3) by providing insight into additional development aspects important for the clinical development of personalized TCVs.

Mechanism of Action and Key Elements

Many of the mechanisms of action and biology relevant for TCVs also apply to personalized TCVs. To successfully induce anti-tumor T cell responses in the human body, TCVs act on and exploit multiple aspects of cancer immunity, including cancer antigen presentation, T cell priming and activation, recognition of cancer cells by T cells,
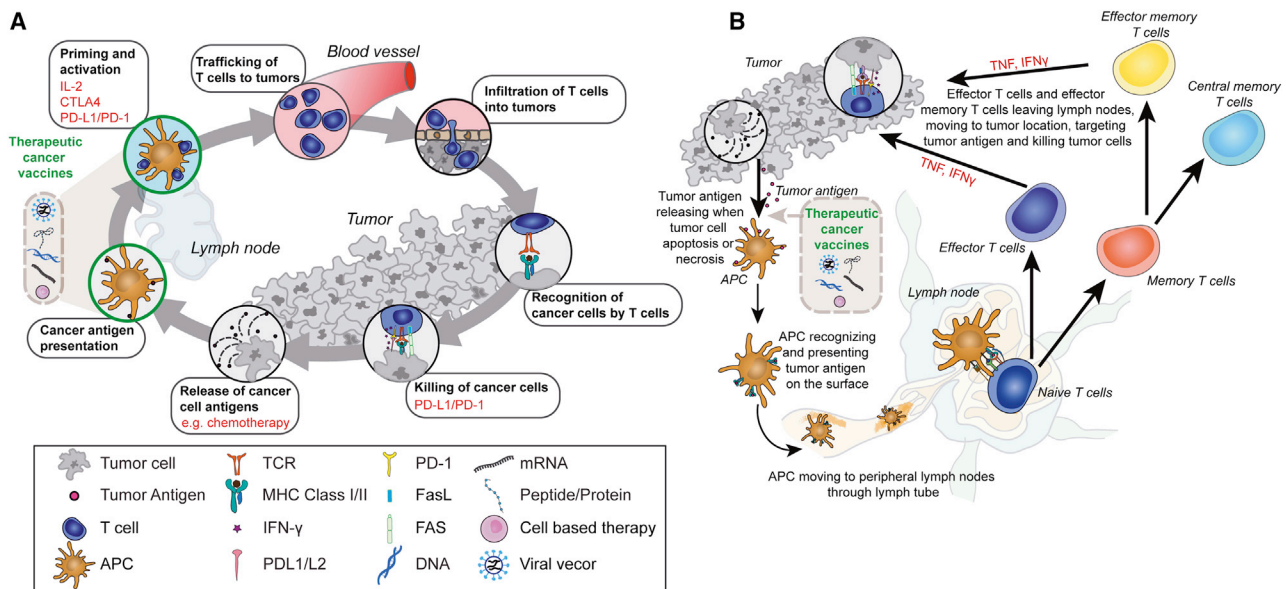
Figure 1. Immune Mechanisms That Underlie Tumor Immunity to Successfully Induce Anti-tumor T Cell Responses in the Human Body

Therapeutic cancer vaccines (TCVs) aim to generate potent immune responses by presentation of antigens to dendritic cells that traffic through the lymphatics and present cancer antigens to naive T cells. Activated cytotoxic T lymphocytes proliferate, multiply, and traffic throughout the body, and they can provide long-lasting immunologic memory. (A) TCV action and combination immunotherapy impacts on specific components of the cancer immunity cycle. (B) T cell activation, effector function, and immunological memory specific to TCV therapy. Adapted with permission from presentation by Chen and Mellman[14] and Song et al.[16]

and several effector mechanisms to eliminate tumor cells.[14] TCVs engage both innate and adaptive immunity with the use of an adjuvant and antigen to trigger an innate and adaptive response, respectively. Nonspecific innate immune responses are activated via pattern recognition receptors, such as Toll-like receptors, that recognize and respond to pathogen- or damage-associated molecular patterns. Engagement of these receptors activates transcription factor nuclear factor κB (NF-κB), stimulates cytokine and chemokine production, and recruits and activates lymphocytes.[15] To induce adaptive CTL-mediated anti-tumor responses, TCVs must assist in (1) presentation and recognition of immunogenic tumor antigens by antigen-presenting cells (APCs); (2) recruitment, antigen processing, and maturation of APCs; (3) induced expression of T cell costimulatory signals and cytokines by APCs; (4) interaction of APCs with the adaptive immune system to prime and activate CD8$^+$ T cells; and, lastly, (5) localization of these elements to the tumor.[16–18] A schematic of common TCV immune mechanisms to induce human anti-tumor T cell responses is provided in Figure 1. Through these processes TCVs can generate long-lasting immunological memory capable of controlling tumor growth and inhibiting relapse and metastasis. In preclinical studies, it has been shown that long-lived memory T cell responses regenerate effector T cells to eliminate tumor cells.[19] Unfortunately, TCVs have rarely met the criteria among the number of biological processes that must be engaged for a TCV to be efficacious; however, new approaches hold promise for improved performance.[20]

To strengthen anti-tumor immunity, TCVs must activate tumor-associated antigen-specific CTLs, and thus targeting neoantigens expressed on a tumor cell surface via the use of a personalized TCV may be an effective strategy. The choice of target antigen is a major determinant of immunogenicity and takes advantage of distinctions between tumor and normal cells. Numerous approaches are used to identify one or multiple antigens for a TCV. These include selection of overexpressed or dysregulated tumor-associated proteins, such as the melanoma antigen gene (MAGE), New York esophageal squamous cell carcinoma 1 (NY-ESO01), or human epidermal growth factor receptor 2 (HER2) proteins, or by personalized approaches targeting cancer-associated neoantigens that arise by mutations found in a specific tumor.[16,21] Most TCVs to date have been directed against the first type, i.e., antigens overexpressed in malignant cells and expressed at lower levels in healthy tissues.[1]

CD4$^+$ T cells play a complex and pivotal helper role orchestrating cancer immunity by regulating and priming antigen-specific CD8$^+$ T cells (boosting function, magnitude, quality, persistence, and memory); additionally, these cells provide protective immunity through effector function, cytokine secretion, and activation of tumoricidal macrophages.[22–24] Interferon (IFN)-γ release by CD4$^+$ T cells is required for elimination of tumor cells[25,26], and immune attack against tumors depend on both CD4$^+$ and CD8$^+$ T cells, with depletion of either subset limiting tumor inhibition.[27] Mechanistic insights into the crosstalk between T lymphocyte subsets, as well as future work to optimize and modulate CD4$^+$ T cells against specific tumor antigens, are likely crucial determinants for improved clinical response of personalized TCVs.[27–31]

TCVs currently in development rely on various methods of antigen delivery, including cell-based, protein/peptide-based, RNA- or DNA-based, and viral/bacterial-based approaches. Each of these has unique considerations related to manufacturing, delivery, antigen selection, immunogenicity, and tolerability.[1] A wide spectrum of adjuvants may also be used to enhance immune responses.[32] The optimal adjuvant must contain attributes to produce more immunity than the antigen alone and succeed at increasing cell-mediated immunity to an optimal amplitude, specificity, and effector profile, some of which can be optimized preclinically.[32,33] Most adjuvants activate damage- or pathogen-associated pattern recognition receptors, initiating a cascade of innate immune response aiding presentation of antigens on APCs. The multitude of diverse approaches for selection, engineering, packaging, and delivery of antigens, in conjunction with the complex biology, give rise to great complexity in comparing TCV platforms systematically.

Personalized TCVs

Neoantigen-based vaccines are individualized tumor-specific therapies,[34] typically targeting multiple tumor antigens unique to each patient. To identify and confirm expression of non-synonymous somatic mutations expressed in the tumor for inclusion in a personalized TCV, a biopsy of tumor tissue is taken for whole-exome and RNA sequencing. Mutations are analyzed using major histocompatibility complex (MHC) class I epitope prediction algorithms and prioritized. Ranked lists of candidate antigens are further refined based on *in vitro* binding assay results in which synthetic peptides are tested for binding to the appropriate class I human leukocyte antigen allele of interest.[9] Selected neoantigens are tumor-specific and, hence, unlike tumor-associated antigens, neoantigen-specific T cells are less likely to have been eliminated during development of immune self-tolerance. This enhances their immunogenicity and ability to stimulate robust T cell responses and increases the breadth and diversity of the response.[8] Various types of variant mutations can be targeted by neoantigen-based vaccines. These include the common use of single nucleotide variants resulting from a single nucleotide change from one base to another, or indels as an insertion or deletion of a sequence of nucleotides from the genome resulting in a frameshift mutation that may alter protein function. Neoantigens selected may be of clonal origin present in all tumor cells or subclonal, which are present only in a subset, both of which influence immunoreactivity.[35] Mutations can also be classified by their role in tumor growth, and either passenger mutations lacking intrinsic growth advantages, or driver mutations that provide growth advantages selected during tumor evolution, can be incorporated into personalized TCVs.[36]

Patients harbor extensive variability in tumor neoantigen expression and clonality that gives rise to evasion of immune effectors and formation of resistance mechanisms, which are key challenges to reducing variability and increasing efficacy for immunotherapies such as TCVs.[37,38] Tumors with high neoantigen intratumoral heterogeneity have a higher degree of branched mutations that give rise to an increased amount of subclones expressed with specific neoantigens, resulting in weaker neoantigen-specific T cell responses.[39]

Provided that T cell infiltration and anti-tumor effect are related to selected antigens and the percentage of tumor cells expressing selected antigens, high fractions of subclonal neoantigens have had a negative impact on the response to immunotherapy.[35] Innovative multi-epitope approaches targeting more neoantigens by multiplexed personalized TCVs in addition to multi-regional tumor sampling that account for temporal changes following longitudinal liquid biopsy at follow-up may be key to combating tumor antigen heterogeneity,[39,40] allowing for diverse targeting of both dominant subclones and low-abundance neoantigens to increase T cell reactivity.

Following vaccination, augmentation of initial CTL responses through epitope spread leads to distinct immune responses to additional untargeted cryptic tumor antigens not present in TCVs, which have been reported in mouse and human cancers.[31,41–45] These become additional targets of ongoing immune response important in control and protection against heterogeneous tumors in a robust, durable, and adaptive process that broadens and expands over time.[46] Correlations between epitope spread and tumor regression show significant value in immune response diversification by TCV therapies.[47]

Foundational pre-clinical work of tumor neoantigens led to the first proof-of-concept study in mice, which revealed that mice treated with a peptide-based neoantigen vaccine against mLama4 and mAlg8 using poly(IC) as an adjuvant conferred strong anti-tumor immunity and high response rates in treated animals.[48] Further supportive preclinical efforts in other tumor models using other neoantigen-based vaccines have since confirmed anti-tumor immunity through *de novo* CD8^{+} T cell responses that are capable of inducing tumor rejection of aggressive tumors in mice, conferring a survival benefit.[49] The anti-tumor efficacy of neoantigen-based cancer vaccines has been established with the first neoantigen vaccine clinical trials in patients with melanoma, and later in patients with glioblastoma, validating the potential benefit of neoantigen-based cancer vaccines as effective and even curative cancer therapies.[50–53]

Now more than a dozen companies and academic institutions have partnered to explore personalized TCVs and have many studies ongoing or poised to begin enrolling patients. Advances in DNA/RNA sequencing, epitope prediction algorithms, and artificial intelligence are helping to identify more potent neoantigens. Additionally, optimization and expansion of manufacturing capacity is underway with the goal of supporting more widespread use of personalized TCVs.[54,55] Many personalized TCVs have entered this space with accelerated development plans, despite considerable investment risk and uncertainty regarding the best platform given the many unproven methodologies among diverse algorithms for neoantigen prediction. Additional questions remain around optimal delivery, dosing, and identifying the best therapeutic setting.[56–58] More human data are needed to substantiate the evolving field of diverse immunizing platforms.

Clinical Trial Landscape of Personalized TCVs

An analysis of personalized TCVs in clinical trials conducted by 13 major companies and partnerships was performed to investigate the

various platforms, antigens, algorithms, delivery systems, clinical settings, endpoints, and preliminary clinical findings. Parameters for trial selection were limited to a search of personalized TCVs acting through neoantigens, which included both completed and ongoing trials in 2017 through March 2020. Sources included ClinicalTrials. gov, company websites and press releases, conference presentations, and abstracts within the last 5 years at the American Society of Clinical Oncology (ASCO), the European Society for Medical Oncology (ESMO), the Society for Immunotherapy of Cancer (SITC), and the American Association for Cancer Research (AACR), in addition to PubMed search results for relevant publications. Keywords included personalized cancer vaccines, personalized neoantigens/neoepitopes, personalized immunotherapy, personalized vaccines, and relevant national clinical trial (NCT) numbers. Our survey of innovative approaches to personalized TCVs revealed at least 23 distinct phase 1 and 2 trials, most which are ongoing (Table 1). Among these trials, 20 (87%) are phase 1 investigations, with up to three trials per sponsor, with most (17; ~75%) using mRNA-, DNA-, or peptide-based antigen delivery platforms (Figure 2). The bulk of trials are enrolling patients with multiple solid tumor types; trials in non-small-cell lung cancer (NSCLC) and melanoma are the next most common personalized TCV trial indications (Figure 2E). We identified 15 unique antigen-selection algorithms based on proprietary artificial intelligence, machine learning, advanced data analytics, and deep analysis bioinformatics that are being used to predict, rank, and validate high-quality and high-frequency neoepitopes for optimal MHC binding and T cell recognition for personalized TCV therapy (Table 1). The studies show a broad range in the number of target antigens selected, with more than 75% of personalized TCVs currently in phase 1–2, targeting a total of ≥ 10 neoantigens (Figure 2D). As it stands, the best delivery method and route of administration are not yet defined. Lipid nanoparticles are the most commonly used delivery vehicle, and the route of administration is primarily intravenous (30%), intramuscular (30%), or subcutaneous (25%; Figure 2G). A dosing frequency of every 3 weeks (q3w) is used in 40% of trials (Figure 2I). The annual number of vaccinations varies widely from 6 to 26 (Figure 2J). At least 40% of these trials will evaluate multiple dose levels (Figure 2K). Many TCVs are being tested in combination with other treatments, including chemotherapy, interleukin (IL)-2, and anti-programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1), given as single agents or in combination with anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4) (Figure 2F). Taken together, these diverse efforts recall earlier TCV trials, where a similar lack of consensus on the best approach to the systematic testing of antigen and vaccine efficacy was evident.[59] As shown in Figure 2, immunogenic platforms that (1) benefit broader patient populations, (2) are comprised of fewer antigens, (3) offer a less frequent immunization schedule, (4) require a less invasive delivery route, and (5) are more amendable to combination approaches hold practical advantages for late-stage development.

Personalized TCV Platform Influence on ADME Processes

Limited absorption, distribution, metabolism, and excretion (ADME) characterization due to poorly conducted dose-finding trials jeopar-

dize the chance of approval of some personalized TCVs that might have otherwise provided clinical benefit to patients.[84–86] Figure 3 illustrates the complexity of the components impacting dosing strategy and dose response for a personalized TCV. Absorption and biodistribution of personalized TCVs are influenced by a myriad of factors, including delivery method, lymphatic uptake by mononuclear phagocytes, formulation, critical quality attributes, payload/carrier ratio, and in vivo "leakage" (premature release) of antigen-encoding material from carriers.[87] Absorption is influenced by the route of administration, and biodistribution is dependent on the carrier, which impacts antigen uptake, expression, and presentation. After processing of neoantigens, immune responses can be detected and evaluated. However, due to heterogeneity in dose-response relationships across multiple antigens or epitopes in a personalized TCV, it can be difficult to determine specific associations between various personalized TCV components and any resulting clinical response. Hence, favorable absorption and distribution of antigen-encoder/antigen to lymphoid organs are precursors to successful immune activation, anti-tumor CTL activity, and effective tumor killing.[88,89]

The presence of antigen, or amount of antigen surrogate (such as a lipid carrier), in systemic circulation after personalized TCV administration may or may not reflect distributed amounts of antigen to lymphoid organs. Understanding how novel adjuvants, excipients, and carrier systems, such as nanoparticles, bacterial ghosts, heat shock proteins, or other vesicles (see Table 1), influence the ADME of personalized TCVs may provide insights to improve efficacy and/or safety.[87,90,91] Additional aspects related to interactions and use in special populations should be considered in development of these novel modalities.[92–94]

Immune Cell Responses as Surrogate Biomarkers for Efficacy

Certain human immune cell functions allow for quantitative measurement of responses to personalized TCV therapy. This includes tumor antigen-specific T cell responses that potentially lead to tumor rejection, and hence these techniques are an essential component of optimal dose selection in cancer vaccine trials. Immune-monitoring techniques include (1) analysis of cell populations by flow cytometry, including phenotypes, functionalities, and activation status; (2) enzyme-linked immunospot (ELISPOT) assays to measure cytokine release after antigen-specific immune responses, and peripheral cytokine profiling using and enzyme linked immunosorbent assay (ELISA) to evaluate innate immune responses; (3) tetramer analysis using MHC multimers loaded with antigen peptides to measure antigen-specific CD8^{+} T cells; and (4) T cell receptor analysis by sequencing and polymerase chain reaction to elucidate the immune repertoire, including genetic arrangement, and specificity.[95] Together, these assays are applied to immune cells in peripheral blood mononuclear cells (PBMCs) acquired from patients to detect and describe T cell responses (Figure 4). ELISPOT is one of the most common techniques to identify CD8^{+} T cell responses to a given antigen.[96,97] Quantitative ELISPOT output is correlated with the strength of the antigen-specific T cell response, although it cannot be used to determine the absolute number of antigen-specific T cells. An alternative

Table 1. Clinical Trials of Personalized Therapeutic Cancer Vaccines

NCT No., Patients Expected (Trial Status): ClinicalTrials.gov	Sponsors	Antigen Platform	Antigen Selection Algorithm	Delivery	Dose and Schedule	Indication	Combination Therapy	Biomarkers	Endpoints	References
NCT03289962, n = 770 (phase 1 recruiting, 2020 completion)	BioNTech and Genentech	neoantigen mRNA	MHC class I & II prediction, mutations ranked by HLA allele-specific antigen binding affinity	i.v. infusion RNA-lipoplex, size and charge optimized	25–100 μg qw prime + boosters	multiple solid tumors	atezolizumab	IFN-γ ELISPOT, flow cytometry, TCR analysis, T cell killing	AEs, imAEs, DLTs, ORR, DOR, PFS, OS	[60]
NCT03815058, n = 132 (phase 2 recruiting, 2022 completion)	BioNTech and Genentech	neoantigen mRNA	^	^	not specified; qw prime + boosters	melanoma	pembrolizumab	^	AEs, ORR DOR, PFS, OS	[61]
NCT03313778, n = 90 (phase 1 recruiting, 2021 completion)	Moderna and Merck	neoantigen mRNA	based on HLA type; analyzes IEDB data for immunogenicity prediction	intramuscular via lipid nanoparticles	40–1, 000 μg nine q3w cycles	multiple solid tumors	pembrolizumab	neoantigen-specific T cell response	AEs, DLTs, RFS, DOR, PFS, OS	[62]
NCT03897881, n = 150 (phase 2 recruiting, 2023 completion)	Moderna and Merck	neoantigen mRNA	^	^	1,000 μg nine q3w cycles	melanoma	pembrolizumab	^	AEs, RFS, DMFS, OS	[63]
NCT03948763, n = 100 (phase 1 recruiting, 2024 completion)	Moderna and Merck	TAAs mRNA	targets four most prevalent KRAS mutations	^	not specified	CRC, NSCLC, pancreatic	pembrolizumab	T cell receptor clonality and diversity	AEs, DLTs, discontinuation, ORR	[64,65]
NCT03380871, n = 15 (phase 1 active, not recruiting, 2021 completion)	Neon Therapeutics and Merck	neoantigen peptides	Recon bioinformatics engine quantifies epitope quality from mass spectrometry binding to predict peptide presentation	subcutaneous (up to four sites) with poly(ICLC)	5 priming doses then qw + boosters	NSCLC	pembrolizumab, pemetrexed, carboplatin	IFN-γ ELISPOT, T cell phenotyping	AEs, SAEs, ORR, DOR, RCR, CBR, PFS, OS	[66]
NCT02897765, n = 55 (phase 1 active not recruiting, 2020 completion)	Neon Therapeutics and BMS	neoantigen peptides	^	^	not specified	multiple solid tumors	nivolumab	IFN-γ ELISPOT, cytokines, flow cytometry	AEs, SAEs, ORR, DOR, RCR, CBR, PFS, OS	[67] and P.A. Ott et al., 2019, Soc. Immunother. Cancer, conference
NCT03639714, n = 214 (phase 1/2 recruiting, 2022 completion)	Gritstone Oncology and BMS	neoantigen adenovirus vector + self-amplifying mRNA	Edge, novel AI model for antigen prediction for optimal immunogenicity/T cell recognition	intramuscular via viral vector	ChAdV prime 1 × 10^12 vp SAM boosts 30–300 μg	NSCLC, MSS-CRC, EC, BC	nivolumab ipilimumab	*ex vivo* ELISPOT	AEs, immune response, ORR, DOR, CBR, PFS, OS	[68]

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Table 1. *Continued*

NCT No., Patients Expected (Trial Status): ClinicalTrials.gov	Sponsors	Antigen Platform	Antigen Selection Algorithm	Delivery	Dose and Schedule	Indication	Combination Therapy	Biomarkers	Endpoints	References
NCT03953235, n = 144 (phase 1/2 recruiting, 2023 completion)	Gritstone Oncology and BMS	neoantigen tumor-specific shared neoantigen peptides	^	^	not specified	NSCLC, CRC, pancreatic, other solid tumors	^	*ex vivo* ELISPOT	^	69,70
NCT02992977, n = 5 (phase 1 terminated in 2019)	Agenus	neoantigen peptides linked to heat shock protein	AIM algorithm for ID and immunogenicity prediction *in vitro* mass spectrometry validation	subcutaneous peptides are linked to recombinant HSP70 protein carrier + QS-21 Stimulon adjuvant	30–240 μg of AutoSynVax + 50 μg of QS adjuvant q2w up to 1 year	melanoma, NSCLC, bladder, TNBC, RCC, HNC, CRC, solid tumors	none	ELISPOT, cytokines	AEs, T cell response, ORR, PFS, OS	71
NCT03673020, n = 3 (phase 1 recruiting, 2020 completion)	Agenus	neoantigen peptides linked to heat shock protein	^	^	50 μg + 240 μg of Hsc70 + 50 μg of QS q2w	multiple solid tumors	none	^	AEs. time of recurrence	71
NCT03633110, n = 99 (phase 1/2 recruiting, 2022 completion)	Genocea	neoantigen peptides	Atlas proprietary system, *ex vivo* assay for ID, epitope prediction based on predicted binding to HLA	subcutaneous with Hiltonol poly(ICLC) adjuvant	not specified; days 1, 22, and 43 with boosters at weeks 12 and 24	melanoma, NSCLC, RCC, HNC, UC	nivolumab or pembrolizumab	IFN-γ ELISPOT, cytokines, immuno-phenotyping	anti-tumor activity	72
NCT03265080, n = 5 (phase 1 active not recruiting, 2020 completion)	Advaxis Immunotherapies and Amgen	neoantigen *Listeria monocytogenes*-secreting TAAs	MINE system, machine learning to predict and weigh rankings, allele frequency, tumor drivers, MHC binding	i.v. infusion −15 min attenuated bacterial vector + adjuvant fusion protein tLLO-NEO	1 × 10^8–1 x 10^9 CFU, q3w for 2 years	NSCLC, MSS-CRC, HNC, UC	pembrolizumab	ELISPOT, cytokines	AEs, ORR, DOR, DCR, PFS, OS	73
NCT03847519, n = 74 (phase 1/2 recruiting, 2023 completion)	Advaxis Immunotherapies and Personalis	TAAs (public or shared hotspots) *Listeria monocytogenes* secreting TAA	^ + ImmunoID NeXT deep analysis platform	^	^	NSCLC	pembrolizumab	ELISPOT, cytokines, gene expression, immuno-sequencing of T cell repertoire	AEs, anti-tumor activity, PFS, OS	74
NCT02325557, n = 51 (phase 1/2 completed 2018)	Advaxis Immunotherapies and Merck	TAAs *Listeria monocytogenes*-secreting TAAs	MINE system with machine learning to predict and weigh rankings, allele frequency, tumor drivers, MHC binding	^	1 x 10^9–1 × 10^{10} CFU q3w for 2 years	prostate	pembrolizumab	ELISPOT, gene expression, flow cytometry, TCR sequencing	AEs, immune responses, PSA, anti-tumor activity, PFS, PROs	75

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Table 1. *Continued*

NCT No., Patients Expected (Trial Status): ClinicalTrials.gov	Sponsors	Antigen Platform	Antigen Selection Algorithm	Delivery	Dose and Schedule	Indication	Combination Therapy	Biomarkers	Endpoints	References
NCT03189030, n = 28 (phase 1 active, not recruiting, 2020 completion)	Aduro Biotech	TAAs *Listeria monocytogenes*-secreting TAAs	ZoomX workflow for neoantigen ID and selection	i.v. infusion −1 h with live attenuated *Listeria monocytogenes*	1×10^8–1×10^9 CFU q3w 17 injections per year	MSS-CRC	none	IFN-γ ELISPOT	AEs	[76]
NCT01675765, n = 60 (phase 1completed 2019)	Aduro Biotech	TAAs *Listeria monocytogenes*-secreting TAAs	^	^	1×10^9 CFU two infusions q2w apart followed by two boosts q3w	malignant pleural mesothelioma	pemetrexed, cisplatin	IFN-γ ELISPOT, flow cytometry	AEs, immune responses, serum mesothelin, ORR, TTP, OS	[77]
NCT03548467, n = 65 (phase 1/2 recruiting, 2023 completion)	Vaccibody and Nektar-Therapeutic	neoantigen DNA plasmid	NeoSELECT neoepitope selection for high-frequency, high-quality neoepitopes	intramuscular needle-free jet injection DNA plasmid pUMVC4a vector	3 mg of multiple dose induction q4w until week 50, 14 injections	melanoma NSCLC, RCC, UC, SCCHN	bempegaldesleukin	IFN-γ ELISPOT, flow cytometry	AEs, immune responses, ORR, DOR, PFS	J. Krauss et al., 2019, Soc. Immunother. Cancer, conference
NCT03552718, n = 16 (phase 1 recruiting, 2020 completion)	NantBioscience	neoantigen yeast based	not specified	injectable suspension recombinant yeast-based vector	not specified	CRC, BC, HNC, NSCLC, pancreatic, liver	none	not specified	AEs, RR, DFS, PFS, OS	[78,79]
NCT03164772, n = 56 (phase 1/2 recruiting, 2024 completion)	CureVac, Boehringer Ingelheim, and MedImmune	neoantigen mRNA	MutSig algorithm and sparse partial correlation estimation algorithm	intradermal injection needle-free lipid nanoparticles	2×200 μL as six components, total of 14 doses in 12 cycles	NSCLC	durvalumab, tremelumumab	ELISPOT, flow cytometry, cytokines, CD8$^+$ T cell response	AEs, ORR, DOR, PFS, OS	[80]
NCT03199040, n = 24 (phase 1 recruiting, 2022 completion)	MedImmune and Washington University St. Louis	neoantigen DNA	public tools, including NetMHC, NetMHCpan, and NetChop algorithms to predict binding and epitope processing	intramuscular electroporation administration delivery system with two injections at separate sites	not specified; D1, D29, D57, D85, D113, D141 q3w between injections	TNBC	durvalumab	ELISPOT, flow cytometry	AEs, immune responses	[81]
NCT03598816, n = 48 (phase 2 not yet recruiting 2022 completion)	MedImmune and Washington University St. Louis	neoantigen DNA	^	intramuscular electroporation administration delivery system one injection into each deltoid or lateralis, two injections at each vaccination	not specified; C1D1, C1D15, C2D1, C3D1, C4D1, and C5D1 total of six doses with two injections per dose	RCC	durvalumab, tremelumumab	ELISPOT, tetramer staining	AEs, ORR, PFS, OS	[81]

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Table 1. Continued

NCT No., Patients Expected (Trial Status): ClinicalTrials.gov	Sponsors	Antigen Platform	Antigen Selection Algorithm	Delivery	Dose and Schedule	Indication	Combination Therapy	Biomarkers	Endpoints	References
NCT02933073, n = 15 (phase 1 recruiting, 2026 completion)	Truvax and University of Connecticut	neoantigen peptides	Epi-Seq pipeline and differential agretopic index to rank epitopes by MHC score differences	not specified	not specified; six injections monthly for 6 months	ovarian	none	immune responses, CD8⁺ T cell proliferation and phenotyping	AEs, immune responses	82,83

Information was obtained from public documents (scientific literature, posters, patents, and/or corporate presentations); ^ indicates the same finding as the prior row. AE, adverse event; AI, artificial intelligence; BC, breast cancer; CBR, clinical benefit rate; CFU, colony-forming units; ChAdV chimpanzee adenoviral vector; CRC, colorectal cancer; DCR, disease control rate; DFS, disease-free survival; DLT, dose-limiting toxicity; DMFS, distant metastasis-free survival; DOR, duration of response; EC, endometrial cancer; ELISPOT, enzyme-linked immunospot; HLA, human leukocyte antigen; HNC, head and neck cancer; IEDB, Immune Epitope Database and Analysis Resource; IFN-γ, interferon γ; imAE, immune-mediated adverse event; i.v., intravenous; MHC, major histocompatibility complex; mRNA, messenger RNA; MSS, microsatellite stable; NSCLC, non-small-cell lung cancer; ORR, objective response rate; OS, overall survival; poly(ICLC), carboxymethylcellulose polyinosinic-polycytidylic acid, and poly-L-lysine double-stranded RNA; PFS, progression-free survival; PSA, prostate-specific antigen; PRO, patient reported outcomes; qw, once weekly; q2w, every 2 weeks; q3w, every three weeks; RCC, renal cell carcinoma; RCR, radiologic complete response; RFS relapse-free survival; RP2D, recommended phase 2 dose; RR, relapse rate; SAE, serious adverse event; SAM self-amplifying; SCCHN, squamous cell carcinoma of the head and neck; TAA, tumor-associated antigen; TCR, T cell receptor; TNBC, triple-negative breast cancer; TTP, time to progression; UC, urothelial cancer; VP, viral particles.

approach to CTL detection, tetramer analysis, uses MHC multimers loaded with antigen peptides to detect and quantitatively measure the number of T cells specific to a particular peptide-MHC complex in response to a personalized TCV. Together, these tools offer high specificity; however, they are considered exploratory, and obstacles remain to their robust clinical use.[98] Immune monitoring requires careful evaluation of stability and assay conditions with the use of proper controls to accurately evaluate induced cellular immune responses. Efforts must be taken to reduce potential sources of variability and improve reliability, as immune-monitoring relationships in response to personalized TCVs are routinely used to inform clinical decisions. More recently, the National Cancer Institute has supported an initiative to fund four Cancer Immune Monitoring and Analysis Centers in support of researchers conducting state-of-the-art analyses such as immune profiling characterization of patient responses in selected immunotherapy trials.

Vaccination Strategy: Dosing and Administration

A first-in-human trial evaluates safety, dose, frequency, immune monitoring, and clinical activity under a suitable schedule of vaccinations to identify the optimal dose, number, and interval of doses required to achieve a peak immune response and anti-tumor activity.[84,88,99] Lower doses of antigen may not be effective, while higher doses may have practical limitations and safety issues, such as strong activation of the innate immune response and cytokine release.[97] To the extent that tolerability allows, the vaccination dose should be increased until the cellular immune response plateaus.[85] Only high-avidity T cells are presumed capable of being stimulated by extremely low concentrations of antigen, whereas higher concentrations have the potential to stimulate lower avidity T cells.[100] Mouse models are commonly used to inform fundamental understanding around the most effective route of administration, adjuvant, carrier, dose, and schedule, providing insight into the kinetics of antigenic stimulation to be further refined in clinical trials.[101] These studies may administer multiple vaccine doses that are 2 weeks apart to evaluate the kinetics of immune responses. Additional studies acquire knowledge on timing of administrations, total number of doses, and induction (priming) and booster (maintenance) intervals to establish a rationale for an initial clinical schedule.[84,102] A lack of widely accepted allometric scaling approaches to calculate an equivalent dose across species limits translatability, providing only a rough estimate to support clinical trial designs.[103–105] The optimal dose in a personalized TCV will likely vary across antigens, and the total antigen dose must ensure delivery of adequate amounts of the less immunogenic antigens.

To address this gap and optimize clinical vaccination strategies, quantitative modeling methodologies, such as immunostimulation/immunodynamic (IS/ID) modeling, have used novel statistical and mechanistic approaches parameterized using relevant preclinical and clinical observations.[105–107] Implementing quantitative modeling methodologies may overcome some of the challenges in establishing an optimal dosing strategy in humans (Table 2). Models using IFN-γ dose-response relationships in mice and humans to
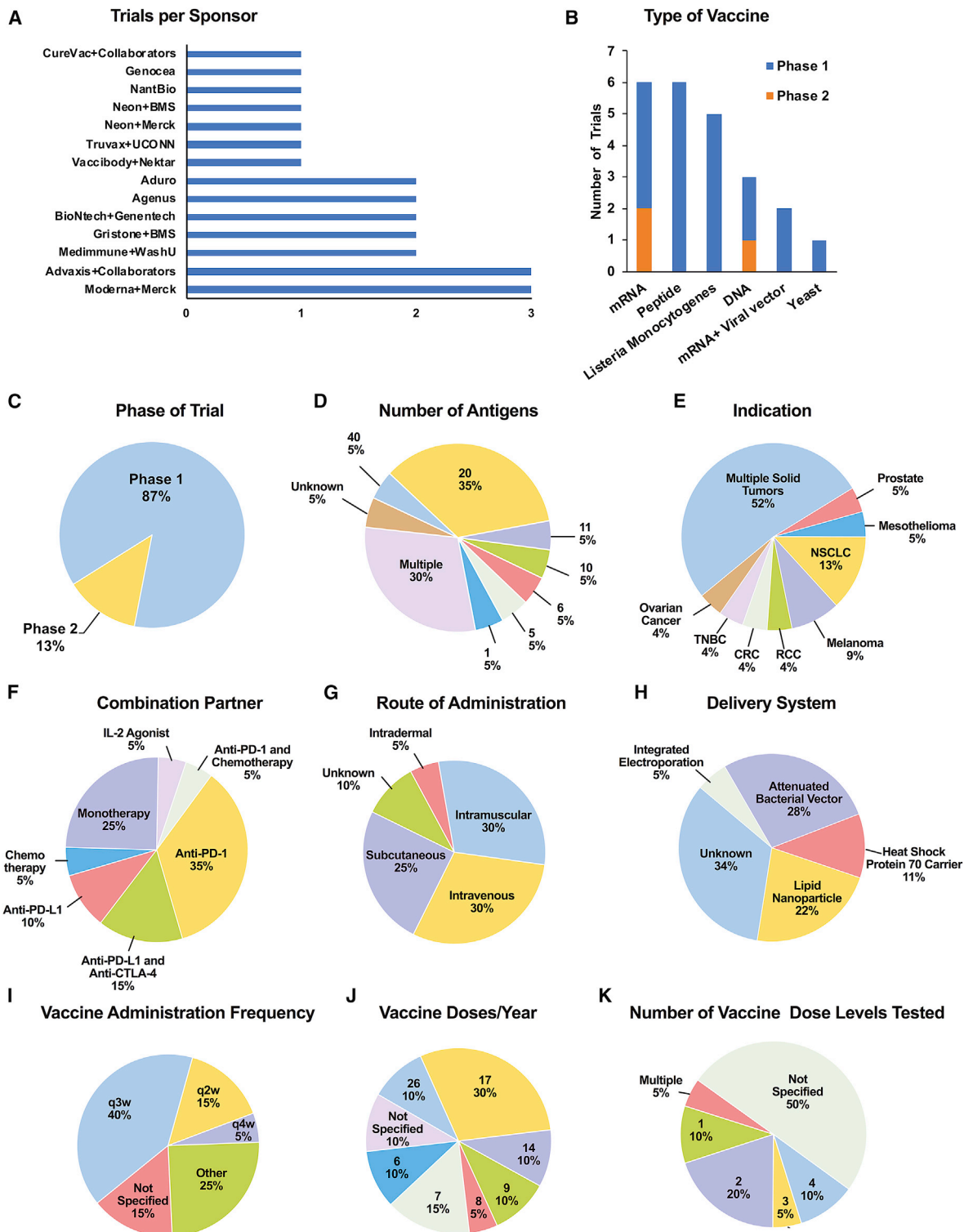
Figure 2. Clinical Trial Landscape for Personalized TCVs

(A–K) Twenty-three personalized TCVs currently in phase 1 or 2 from 13 major sponsors: (A) trials per sponsor; (B) type of vaccine; (C) phase of trial; (D) number of antigens; (E) indication; (F) combination partner; (G) route of administration; (H) delivery system; (I) vaccine administration frequency; (J) vaccine doses per year; and (K) number of vaccine dose levels tested.
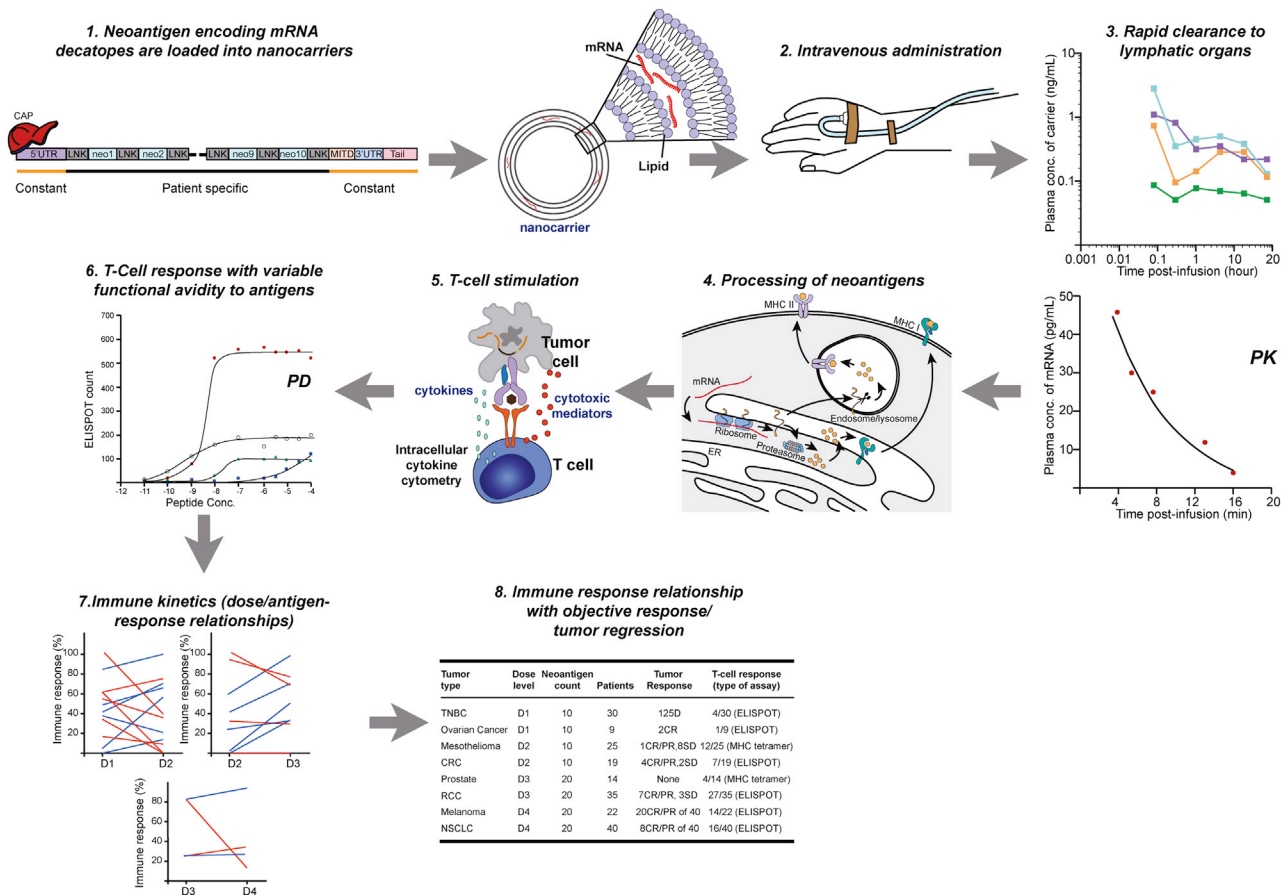
Figure 3. Complexity of Dosing Strategy and Dose-Response for a Personalized Neoantigen-Based TCV

Favorable absorption and distribution of antigen-encoder/antigen to lymphoid organs are precursors to enable successful immune activation, anti-tumor CTL activity, and effective tumor killing. After neoantigen encoding mRNA is packaged into nanocarriers and infused (steps 1 and 2), mRNA and carrier component concentrations are measured in systemic circulation, which may relate to uptake by lymphoid organs for processing of neoantigens (steps 3 and 4). After processing of neoantigens, immune monitoring of antigen-specific T cell responses is evaluated (step 5); however, due to heterogeneity in dose-response relationships across multiple antigens or epitopes in a personalized TCV (steps 6 and 7), it can be difficult to determine specific associations between various personalized TCV components and any resulting clinical response (step 8). Illustrated findings are hypothetical and do not represent actual clinical trial data. CR, complete response; D, dose level; ELISPOT, enzyme-linked immunospot assay; LNK, linker; MHC, major histocompatibility complex; Neo, neoantigen; PD, pharmacodynamics; PK, pharmacokinetics; PR, partial response; SD, stable disease; UTR, untranslated region.

identify ideal regimens have been credited with avoiding suboptimal dosing in late-stage development of certain vaccines.[108,109] Other recent modeling efforts focus on optimizing cancer vaccine dosage and delivery rates to maximize recruitment of high-avidity CTLs to the tumor.[110]

Combination Immunotherapy

Appreciation of the minimal toxicity observed with many personalized TCV platforms has encouraged investigators to combine these agents with immune checkpoint inhibitors (ICIs), chemotherapies, radiation, targeted therapies, hormone therapies, or other immunomodulators that have overlapping effects on immune cells.[1,114] Co-administered therapies that may offer unique synergistic immuno-potentiation with personalized TCVs include (1) cytokines, such as IL-2, IFN, and transforming growth factor (TGF)-β, which may promote differentiation of immature T cells into effector T cells; (2) radiotherapy to release tumor neoantigens, increase inflammation and secretion of immunomodulatory cytokines, and sensitize tumor cells to immune-mediated killing; (3) ICIs, such as anti-CTLA-4 and/or anti-PD-1/PD-L1, to activate different T cell populations; (4) small molecules, such as tyrosine kinase or histone deacetylase inhibitors, to promote immune cell function by decreasing regulatory T cells and myeloid-derived suppressor cells; (5) endocrine therapy to increase production of naive T cells and decrease regulatory T cells; and, lastly, (6) chemotherapy to increase immune-supportive M1 macrophages and induce tumor immuno-surveillance by natural killer (NK) cells.[114–116] Table 1 lists agents currently being tested in combination with personalized TCVs.
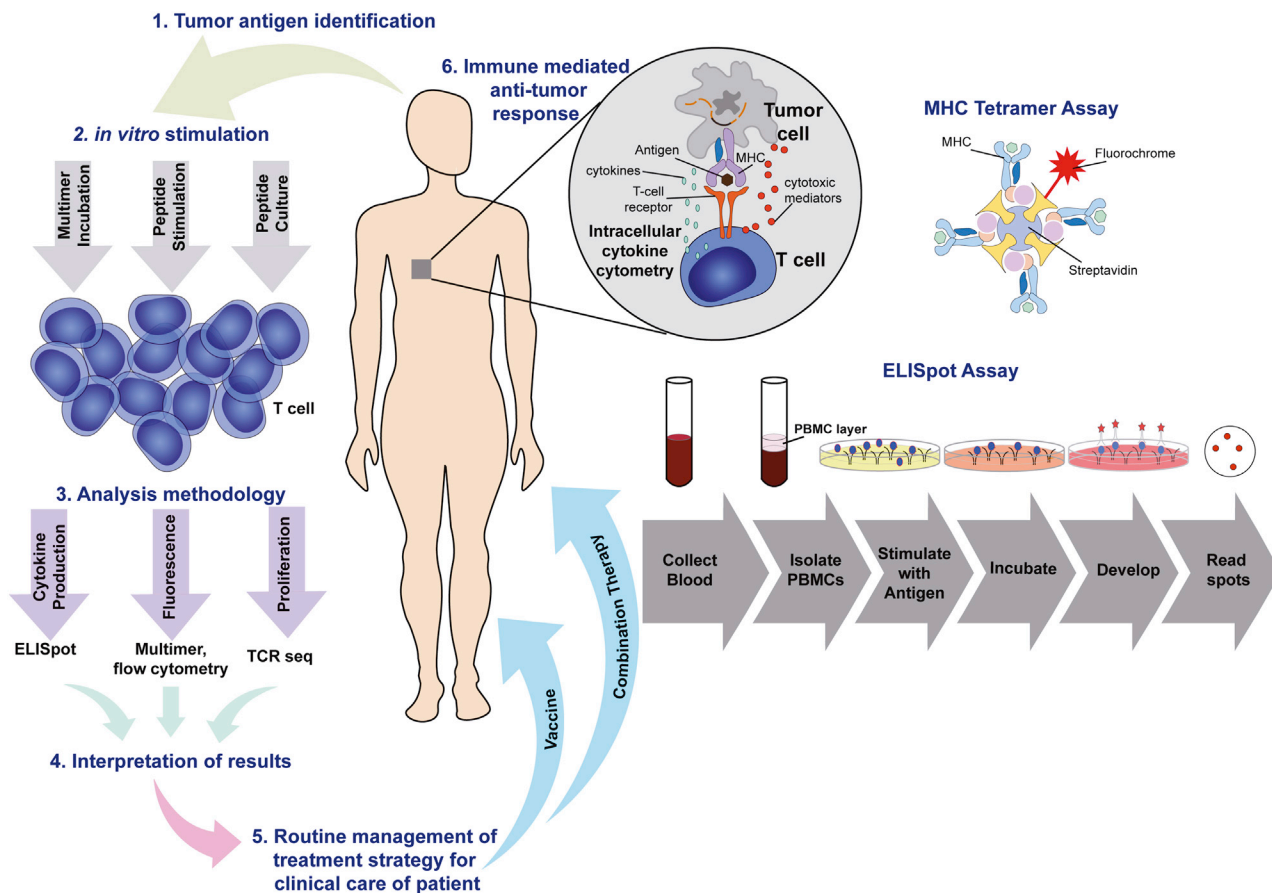
Figure 4. Immune Cells in PBMCs Acquired from Patients Detect and Inform on Antigen-Specific T Lymphocyte Response

ELISPOT identifies CD8^{+} T cell responses to a given antigen after PBMCs are treated with an antigen of interest and stimulated *ex vivo*, leading to CD8^{+} T cell activation in response to a tumor-specific antigen and secretion of IFN-γ captured on an immobilized surface as insoluble spots that are enumerated. Tetramer analysis using MHC multimers loaded with antigen peptides detects T cells specific to a particular peptide-MHC complex in response to a TCV. Immune-monitoring relationships in response to TCVs may be used to help inform clinical decisions. Adapted from presentation by Caushi and Smith.[95]

The biological rationale for combination, and individual contribution of each individual agent, should be established.[117] Active combinations will be influenced by the patient population, and dose/schedule, sequencing, and safety evaluation of combination therapies in relationship to a personalized TCV should be considered.[118] One such example highlighting the importance of proper sequencing for combinations was recently revealed in anti-PD-1-resistant models, which indicated that simultaneous anti-PD-1 and vaccine therapy reversed resistance, while PD-1 blockade before antigen priming abolished therapeutic outcomes.[119]

Common Obstacles in Development

Cell-mediated immunity directing T cells toward tumor-specific antigens has proven difficult. Many completed TCV trials have yielded disappointing results. Provenge (sipuleucel-T) has demonstrated statistically significant associations of antigen-specific CD8^{+} T cell responses with overall survival in patients with metastatic castration-resistant prostate cancer and is the only US Food and Drug

Administration (FDA)-approved TCV.[120,121] A quantitative analysis across 451 clinical trials of TCVs from 1999 to 2014 indicated a lack of consistency in TCV approaches with no clear benefit of any particular adjuvant or platform to induce immune or objective responses.[5] Despite evidence of immunological activity, many TCVs tested failed to reveal correlations between vaccine-induced immunity and clinical benefit. Interpatient heterogeneity affects the immunogenicity of TCVs, and true clinical responders in trials may be too few to allow for robust statistical assessment of TCV-induced T cell responses.[113]

More potent and versatile TCV platforms are needed, and testing of new approaches such as the ongoing trials of personalized TCVs to enhance efficacy are underway.[122] There is also a recent shift to testing TCVs in patients with early stage diseases in tumor types presumed more responsive to immunotherapy.[99,112] The tailor-made approaches to vaccination described herein will advance the field; however, there is little clarity as to which antigen selection

Table 2. Obstacles to Determining an Optimal Dose and Regimen for a Personalized Therapeutic Cancer Vaccine

Key Issues
● Preclinical models used to inform on the best route of administration, adjuvant, carrier, dose, and schedule do not scale allometrically and may not translate across species[103,105]
● Limited understanding of vaccine ADME to allow optimization of biopharmaceutical properties[90]
● Lack of systematic approaches for testing various platforms to induce immune responses, e.g., approaches are scattered across various competing commercial entities[5,59]
● Poor methods for selecting highly immunogenic antigens and lack of adjuvants able to overcome substantial immunosuppression[111]
● Antigens with relatively low avidity for MHC must be given at higher doses to achieve adequate lymphatic exposure, which may not be feasible[97]
● Platform-specific toxicity issues requiring use of short-acting prophylaxis may limit dose escalation[4]
● Lack of knowledge of the optimal interval between immunizations for induction and maintenance phases[84,88]
● Selecting a non-ideal study population, e.g., metastatic population with rapidly progressive disease, non-immunogenic cancers, presence of immune suppression, and/or insufficient time for immune response[99,112]
● Lack of clear dose-response relationships, due to small trial populations and disease heterogeneity, makes it difficult to use surrogate markers of immunity to identify clinical responders, and induced T cell responses in true clinical responders may be too low for statistical assessment[4,113]
● Clinical anti-tumor efficacy not observed despite evidence of immunological activity[4,113]
● Inadequate characterization of the shape of the immune response curve due to insufficient dose levels/patients tested[105]
● Lack of implementation of quantitative modeling approaches to provide insight on optimal dose-response relationships[106,107]
● Speed of development and commercial pressure leading to trial design with inadequate testing of dosing strategies and, during late-stage development dose selection, bias to favor safety over efficacy, jeopardizing future licensure[84–86]

algorithm, adjuvant, or delivery approach is ideal to elicit potent tumor-specific T cell responses. More systematic approaches to testing could help improve the likelihood of better outcomes. Recently, efforts to launch an international, multidisciplinary human vaccines consortium to create a roadmap for systematic testing to assist with reducing the complexity of personalized TCV development have been discussed. Suggested collaborations include a large multi-center pilot clinical study, or iterative studies, to compare delivery platforms, materials, antigens, doses, adjuvants, prime/boost schedules, frequency, and route of administration via selected cohorts of approximately 10 individuals to provide clues as to the potency of various approaches.[19]

Regulatory Framework

Stringent regulations aim to minimize risks and protect patients. To fulfill approval standards a personalized TCV must indicate that the therapy is safe, of sufficient quality, and clinically effective. Understanding the regulatory framework, including classification and implementation of suitable and specific guidance documents to sup-

port investigation and marketing of personalized TCVs, is essential. The neoantigen-based personalized TCV approaches reviewed herein are regulated as gene therapies. In the US, these therapies are regulated by the Center for Biologics Evaluation and Research (CBER) within the FDA, while in EU the Committee for Advanced Therapies (CAT) of the European Medicines Agency (EMA) is responsible for reviewing individualized TCV regulatory submissions, and their decisions are ratified by the Committee for Medicinal Products for Human Use (CHMP).[123] CBER's Office of Cellular, Tissue, and Gene Therapies and other global agencies have published guidance documents to inform development of TCVs. These documents cover critical quality attributes, manufacturing process controls, potency, nonclinical safety, pharmacology, and clinical development. However, these are not all-inclusive and do not account for the broad range of potential issues impacting the varied personalized and state-of-the-art TCV modalities in development. In the long-term, advances in scaling out manufacturing of these agents are needed to (1) enhance production lines and equipment utilization, (2) reduce the cost of goods and increase purchasing volume for raw materials, and (3) increase automation, leading to more efficient quality control. Additionally, earlier optimization of formulation and manufacturing processes will lessen the need for comparability and extensive bridging studies to the proposed commercial product.

Input from regulatory bodies should be sought and obtained as soon as a clear plan and rationale are established for the development strategy.[124] As the science progresses along with advancement in technologies to produce personalized TCVs, developers and regulators need to collaborate to evolve the regulatory landscape for these modalities. Additionally, fostering and implementing policies that support the application of model-informed drug development (MIDD) to personalized TCVs may facilitate dose optimization and clinical trial design.[125–127] Overall, given the current lack of established clinical pharmacology programs to assist with the clinical development of this novel class of medicines, new approaches, solutions, and adaptive practices to address important technical, clinical, and regulatory questions should be considered to bring these innovative therapies to patients faster.

Conclusions

With the success of ICI-based immunotherapy, there has been an explosion of renewed interest in cancer vaccines and a rapidly expanding repertoire of tailored approaches. However, to date TCVs have delivered only modest clinical benefit and have not yet matured as a major pillar of cancer treatment. Many early clinical trials and combination studies of personalized TCVs are now underway. The field is crowded, with a flurry of recent collaborations indicating excitement about the potential of personalized TCVs, yet considerable uncertainty remains as to which platform will perform best. The next decade is expected to bring significant advances in high-throughput sequencing, antigen prediction algorithms, modeling and simulation efforts, manufacturing, and regulatory guidance. As part of this effort, implementing traditional quantitative clinical

Review

pharmacology techniques may have a role to play in more rational personalized TCV study designs, and dose/schedule selection. Methodical testing of complex platforms and quantitative modeling are forward-thinking approaches emerging to assist clinical development. Overcoming key limitations unique to vaccine-based treatment compared to other immunotherapies remains a significant hurdle. In addition to challenges detailed in this review, production and associated development costs leading to affordability and patient access issues must be addressed. Nonetheless, personalized TCVs are certainly worth pursuing to further explore their potential to combat cancer.

AUTHOR CONTRIBUTIONS
C.S.S. wrote the manuscript. J.C.H., I.H., B.-Q.S., A.R., P.T., S.G., and B.W. provided strategic input and helped review and revise the article. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

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