Review Article

Defining precision cellular immunotherapy—seeking biomarkers to predict and optimize outcomes of T cell therapies in cancer

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Abstract: Cellular immunotherapy, harnessing the patient's own and donor immune cells, has emerged as a form of highly personalized treatment in cancer. Adoptive transfer of genetically engineered T cells modified to express chimeric antigen receptor (CAR) has demonstrated remarkable anti-tumor response and long-term remissions in selected hematologic malignancies. However, cancer relapse is still a challenge in some of these patients, and CAR T cell therapy is still investigational and of limited efficacy against solid tumors. On the other hand, tumor infiltrating lymphocyte (TIL) therapy, genetically modified T cell receptor (GM-TCR) therapy and viral-specific T cell therapy are emerging treatment options in solid organ cancers. There is a great interest and increasing effort towards studying biomarkers that can predict for improved outcomes of patients undergoing cellular therapy, still a nascent, emerging field. Identification of such biomarkers may allow enrichment strategies to improve clinical trial design aimed at accelerating clinical development and approvals of such therapies. Given the current high financial costs of cellular therapy, biomarkers can potentially better identify patients that derive maximum benefit as well as potentially reducing pressure on reimbursement-based healthcare systems. Here, we review the current understanding and body of literature on biomarkers for cellular therapy, with a specific focus on T cell therapies. Biomarkers predictive for cellular therapy-based adverse events have been reviewed elsewhere and is not discussed herein.

Keywords: Biomarker; chimeric antigen receptor T cell (CAR-T); tumor infiltrating lymphocyte (TIL); Genetically modified T cell receptor (GM-TCR); cell therapy

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Introduction

Adoptive cellular immunotherapy had its early beginnings in the 1980s with the clinical use of lymphokine activated killer cells, tumor infiltrating lymphocytes (TILs) and other adoptive T cell strategies against human cancers (1). Its scientific and clinical roots originated from the field of bone marrow transplantation, where the graft-versus-leukemia effect is in fact a form of allogeneic anti-tumor immune response.

Subsequent decades saw the development of tumor-specific TILs and genetically-modified T-cell receptor (GM-TCR) therapy predominantly in solid organ tumors, though with modest clinical efficacy (2-4). Virus-specific cytotoxic T lymphocytes (CTLs) infusions have also yielded clinical responses in virus-related malignancies including post-transplant lymphoproliferative disease, Epstein-Barr virus (EBV) related, hepatitis-B related and human papillomavirus (HPV) related cancers (5-8).

However, the true potential of T cell therapy in cancer treatment was only realized in the 21st century, led by the success of chimeric antigen receptor T cell (CAR-T)

therapy against refractory hematologic malignancies (9-11). Following several positive clinical trials and two landmark US Food and Drug Administration (FDA) approvals in quick successions in 2017, CAR-T was recently named "The Advance of the Year" in 2018 by the American Society of Clinical Oncology (ASCO).

Advances in T cell therapies

Genetically engineered CAR contains an antigen recognition single-chain variable fragments (scFv) domain, a hinge region, a transmembrane domain, and a signaling domain which can activate the cytotoxic function of the lymphocytes. Newer generation CAR-T cells also include co-stimulatory endodomains that allow better expansion and survival *in vivo*. Currently, CD19 directed CAR-T cell therapy has established itself in the treatment of various relapsed B cell malignancies including diffuse large B cell lymphoma (DLBCL) (9), B-acute lymphoblastic leukemia (B-ALL) (10), chronic lymphoblastic leukemia (CLL) (11) as well as multiple myeloma (MM) (12) , with complete response rates ranging between 30–90%.

Despite high response rates of CD19 CAR-T in pediatric B-ALL, relapses occur in the form of emergent CD19-negative leukemia, prompting the development of strategies such as CD22 directed and CD19/CD22 bivalent CAR-Ts to improve disease control (13). A plethora of other CAR-T cell targets are in development, including CD20, CD30, CD38, CD44, BCMA, CD123, CD138 and CD171 (14-17). Efforts are also made to accelerate the development of donor-derived (autologous) and universal (allogeneic) "off the shelf" CAR-T. There are currently over a hundred CAR-T trials ongoing—necessitating a greater understanding of biomarkers that may better predict the probability of benefit for these novel therapies (17).

Unfortunately, the CAR-T breakthrough in hematological malignancies has yet to be reproduced in solid cancers. Case series of CAR-T against colon cancer, renal cancer, ovarian cancer, sarcoma and central nervous system (CNS) tumors (18-21) have been reported but often with significant toxicity and mortality outcomes. Efforts are also ongoing to engineer novel therapeutic and homing CAR-T cells to enable better trafficking and survival in the immune-suppressive tumor microenvironment (TME) of solid tumors. Some positive clinical signals are now seen—in phase I clinical trial investigating GD2-specific CAR-T cells for the treatment of pediatric neuroblastoma, 3 out of 11 patients who had active disease achieved complete remission (20). A case of recurrent glioblastoma with spinal metastases achieved durable and significant clinical response with IL13Rα2 directed CAR-T given both through intra-ventricular route and also locally to the intra-parenchymal tumor (21).

Other potential therapeutic targets such as HER2, PMSA, CEA, EGFR, mesothelin, MUC1 and PSM (22-28) are not unique to tumor cells and can result in off-site toxicities, as evidenced by a fatal case of cytokine release syndrome (CRS) in a colorectal patient who received HER2 directed CAR-T (22). In contrast, another trial involving HER2 directed CAR-T against sarcomas did not report similar complications (24), highlighting the variability in clinical response despite the common therapeutic target.

Outside of CAR-T therapy, the major area of cell therapy research against solid organ tumors include autologous TILs infusion and GM-TCR Cell therapy. An advantage of TILs and GM-TCR cell therapy is that they are able to recognize intracellular antigens as opposed to CAR-Ts, which only binds surface antigens. GM-TCRs specific for a variety of somatic tumor antigens (MART-1, gp100, p53, NY-ESO-1, MAGE-A3, MAGE-A4, etc.) (3,4,29) have been engineered into T cells but with variable responses in cancers. Reactive TILs have been increasingly pre-selected by means of *ex vivo* stimulation with pulsed tumor antigen or co-culture with primed antigen presenting cells. Antigens used to be derived from bulk tumor digests, but with improved sequencing and neo-antigen prediction techniques, designer antigens can be manufactured for more potent stimulation of TILs (30).

In fact, adoptive transfer of lymphocytes that target proteins encoded by somatically mutated genes has resulted in objective clinical regression in patients with solid organ tumors. A recent breakthrough is seen with Dr. Rosenberg's breakthrough T cell therapy study, in a patient with chemo-refractory metastatic breast cancer, who achieved a complete response that had lasted more than 22 months at the time of report after receiving somatic mutation specific TILs (30).

For virus-associated tumors, adoptive transfer of *in vitro* activated and expanded autologous T cells that target virus antigens had also demonstrated potential. Infusions of EBV-specific T cells demonstrated promising clinical and survival benefits for EBV-positive nasopharyngeal carcinomas (NPC) patients with little or no immediate nor long-term toxicity (6,31). A similar strategy is undergoing development for HPV-associated malignancies such as cervical cancer. Targeting the tumor-associated viral antigens also brings an added technical benefit. Viral protein targets are thought to be more immunogenic than non-viral antigens, it is

therefore less challenging to expand the T cells to a scale necessary for infusion into patients.

During the development of adoptive lymphocyte therapy, one of the major challenges lies in differentiating and identifying the potential responders from non-responders (NRs). A subset of patients who have received CAR-T therapy may develop life-threatening side effects including CRS, macrophage activation syndrome, tumor lysis syndrome, hepatotoxicity and even neurotoxicity (17,32-35). The variable efficacy, high cost of treatment and the production time required for adoptive cell therapy also remain a barrier for its widespread use. It is therefore imperative to identify predictive biomarkers that can improve the therapeutic efficacy of adoptive T lymphocyte therapy.

Definition of biomarker

The term "biomarker" can be defined loosely as: a prognostic marker that correlates with patients' overall survival and disease trajectory; a predictive marker that correlates with treatment response or toxicity; or even a therapeutic target. A prognostic marker may also be a predictive marker or a combination of the above, i.e., high expression of HER-2 in breast cancer is at the same time a predictive, prognostic and therapeutic biomarker.

An ideal and widely usable biomarker should be simple, easily accessible and reliable. To identify a predictive biomarker, there should ideally be a comparison of the effectiveness of treatment in patients in the context of a clinical trial, via quantitative treatment-by-biomarker interaction (36). However, there are circumstances in which preclinical and early clinical data provide such compelling evidence that definitive clinical trials are performed only in populations enriched for the predictive biomarker.

Biomarkers may be derived from (I) clinical data (e.g., demographics, treatment data, clinical parameters); (II) serum-based (e.g., cytokines and baseline blood tests); (III) tissue-based [e.g., immunohistochemistry (IHC) of tumor or immune cells]; (IV) genomic-based [e.g., whole exome sequencing (WES), polymerase chain reaction (PCR)]; (V) other special tests such as flow cytometry, enzyme-linked immunosorbent assay (ELISA), and functional assays.

Biomarkers of immune-checkpoint inhibitors (ICIs)

Biomarker studies for cytotoxic chemotherapy and small molecule agents have focused on host characteristics and the impact of the treatment on the target tissue. Unlike the discovery and utility of biomarkers in cellular immunotherapy which is only just growing, predictive biomarkers in ICIs have been increasingly well defined in the last decade (37).

The most widely used biomarker of ICI is PD-L1 expression, tumor proportion score (TPS), combined positive score (CPS) or other composite immune scores (38). Other ICI biomarkers include serum neutrophil to lymphocyte ratio (NLR), tumor mutation burden (TMB), microsatellite instability (MSI) and deficient mismatch repair protein (dMMR), which commonly correlate with the neoantigen load and response to ICIs (37,38). Other emerging biomarkers of ICIs include other genomic assays (including RNA sequencing), cytokine analysis, peripheral circulating immune cells, TCR diversity/repertoire, HLA status and even the gut microbiome. Eventually, a validated cancer immunogram may best represent a set of distinct characteristics that could predict for optimal efficacy to ICIs (39). The use of imaging especially multiplex immunofluorescence could also increasingly help to elucidate the TME and the immune state within it.

Biomarkers of adoptive T cell therapies

Adoptive cellular therapy differs from conventional pharmaceuticals in that the product is a "living" biological entity whose therapeutic effect requires functional and specific immune cells that can potentially expand exponentially *in vivo* after infusion. Therefore, cell therapy requires the development of additional biomarkers that describe the biological properties of the complex cell product—including phenotype and functional properties. As summarized by Kalos *et al.* (40), cell therapy parameters can be broadly described as (I) T cell presence; (II) T cell phenotype or functional competence; (III) systemic impact on patient biology (bioactivity); (IV) patient immune responses to the infused product.

Table 1 summarizes these broad principles, distinguishing classes of biomarkers as host versus cell product characteristics. We note that it is inherently difficult to identify unifying predictive biomarkers for T cell therapy due to the limited studies and heterogeneity of cellular products. In this review, we will discuss the positive predictive biomarkers that have been evaluated and reported in adoptive T cell therapy against both hematological malignancies (*Table 2*) and solid tumors (*Table 3*).

In the phase II ZUMA-1 trial investigating axicabtagene

Table 1 T cell predictive biomarkers

T cell product characteristics
Biomarker to detect T cell presence/quality
Biomarker to measure biologically relevant phenotype and function of T cells
Biomarker to evaluation T cell bio-reactivity (overlaps with therapeutic biomarker)
Other biomarkers in T cell products (i.e., cytokines)
Host/tumor factors
Biomarker to assess pre-treatment host characteristics and immune characteristics
Biomarker to assess host immune response to cell therapy
Tumor characteristics and TME

TME, tumor microenvironment.

ciloleucel in relapsed DLBCL, *in vivo* persistence of CAR-T was observed in patients with a more sustained response (in three patients at 24 months), and *in vivo* expansion was significantly associated with objective responses (44), quantified as 5.4 times that of NRs. On a separate note, persistent B cell aplasia was correlated with response in phase I study of CAR-T against relapsed CLL (45). These findings suggest that, though not a pre-emptive "predictive marker", the persistent detection of CAR-T *in vivo* correlates with treatment effect as well as side effects.

Up till recently, there has been a paucity of predictive biomarkers identified for CAR-T therapy. On-treatment cytokine profiles at various time points—before/after lympho-depleting chemotherapy and infusion of CAR-Ts—have been well described and correlates with clinical outcomes (55). Though clinically useful for monitoring, these markers generally reflect a host immune response to the therapy and have limited pre-emptive value in guiding the use or omission or CAR-T currently.

Biomarkers of CRS and other toxicities (cytokine profiles and serum laboratory markers) have also been reported. Pre-infusion markers such as ferritin, CRP and post-infusion rise in cytokines such as IL-6, IFNγ and soluble p130 were shown to be often associated with CRS and may help clinicians in predicting treatment trajectory (17,31,33,56).

Although response rate of B-ALL to CAR-T therapy has been impressive, the complete responders (CRs) in CLL, on the other hand, remain a minority. The CAR-T study group at the University of Pennsylvania reported recently the phenotypic, functional and genomic characteristics of CD19 directed CAR-T in 41 relapsed CLL patients (41,42). They did not find host or disease-related factors that predicted response to CAR-T; treatment response in CAR-T in CLL was not related to patients' age, prior therapy, genetic risk profile, disease burden, cell dose or other clinical characteristics including TMB. Hence, efforts were made to focus on T cell product and pre-modification T cell characteristics in an attempt to identify populations of CAR-T that might be related to better clinical outcomes.

An important finding in the above study was the identification of a subgroup of early memory CAR-T cells that correlated with better outcomes. CAR-T cells from CR patients were found to be enriched in memory-related genes, including IL-6 and STAT3 signatures (IL-6, IL-17, IL-22, IL-31 and CCL20). T cells from NRs had upregulated genes of end effector differentiation, glycolysis, exhaustion and apoptosis, especially PD1, LAG3 and TIM3 (exhaustion markers) expression. Apart from transcriptome studies, functional CAR-T cells from patients produced STAT3-related cytokines, and serum IL-6 correlated with CAR T cell expansion. Also, sustained remission was associated with an elevated frequency of $CD27^+CD45RO^-CD8^+$ lymphocytes, and these cells also possessed memory-like characteristics (41,42). The interrogation of 1,696 phenotypes by flow cytometry identified a $PD1^+CD27^+$ parental population of $CD8^+$ T cells (with high levels of IL-6R) that strongly segregated CR from NR patients—which could be a mechanistically relevant population that was able to drive therapeutic responses. These findings may allow fine-tuning in the future development of CAR-Ts and also improve patient selection based on favorable host lymphocyte phenotypes prior to the CAR-T production.

In another phase I/IIa clinical trial investigating CAR-T and CLL (43), high plasma levels of immunostimulatory markers, including IL-12, DC-LAMP, TRAIL, and Fas ligand, before administering CAR T-cell therapy, was associated with longer overall survival. In contrast, high levels of soluble PDL1 (sPDL1; P=0.0023) and PDL2 (*sPDL2*; P=0.0002) detected *in vivo* post-CAR infusion, correlated with poor survival; as did high levels of IL-6 (P=0.03), IL-8 (P=0.03), and NAP3 (P=0.004). Responding patients also had low monocytic myeloid-derived suppressor cells (MDSCs), defined as $CD14^+CD33^+HLA\text{-}DR^-$ cells.

Presence and persistence of infused TILs after adoptive transfer were reported to correlate with objective clinical response in an initial cohort (n=13) of melanoma patients

Table 2 Positive predictive markers in CAR-T therapy of hematological malignancies clinical trials

Marker	Description	Sample size	Results	Phase	Type of biomarker	Reference
Memory-related genes, including IL-6/STAT3 signatures	Advanced, heavily pretreated and high-risk CLL	41	(I) CAR T cells from complete-responding patients with CLL were enriched in memory-related genes, including IL-6/STAT3 signatures; (II) sustained remission was associated with an elevated frequency of CD27+CD45RO-CD8+ T cells before CAR T cell generation; (III) highly functional CAR T cells from patients produced STAT3-related cytokines, and serum IL-6 correlated with CAR T cell expansion; (IV) $CD27^+PD1^+CD8^+$ CAR-T cells expressing high levels of the IL-6 receptor predicts therapeutic response and is responsible for tumor control	II	Genomic assay	Fraietta *et al.* (41)
T-cell subset: $CD27^+CD45RO^-CD8^+$ early memory T cells					Flow cytometry	
CCL20, IL21, IL 22, IL17, CD27, CD45RO, PD1			(I) CAR-T therapy in responders express STAT3 signaling mediators and targets, including IL-6, IL-17, IL-22, IL-31 and CCL20, a finding consistent with IL-6/STAT3-pathway upregulation; (II) evaluation of 1,696 phenotypes identified the $PD1^+CD27^+$ parental population of $CD8^+$ T cells as a phenotype that strongly segregated CRs from NRs		Flow cytometry	Fraietta *et al.* (42)
IL-12, DC-LAMP, TRAIL, and Fas ligand, low MDSCs	Relapsed CLL	15	Patient blood immuno-phenotype associated with response to CAR-T	I/II	Flow cytometry	Enblad *et al.* (43)
Persistence of CAR-T *in vivo*	Relapsed/ refractory DLBCL	111	Persistence of CAR-T in blood were associated with on-going clinical response (P<0.001)	II	Serum, Flow cytometry	Neelapu *et al.* (44)
B cell aplasia	Relapsed/ refractory CLL	14	All responding patients (8/14, 57%) experienced B cell aplasia which was sustained for 4 years	I	Flow cytometry	Porter *et al.* (45)

CAR-T, chimeric antigen receptor T cell; CLL, chronic lymphoblastic leukemia; CRs, complete responders; NRs, non-responders; DLBCL, diffuse large B cell lymphoma.

Table 3 Positive predictive biomarkers in cell therapy of solid organ tumors clinical trials

Marker	Description	Sample size	Results	Phase	Type of biomarker	Reference
TILs						
CD4, CD8, CD3	Metastatic melanoma and autologous TIL infusion	48	Higher staining in tumor of TIL growers *vs.* non-growers (P<0.0001)	II	IHC	Chen *et al.* (46)
TCR Vβ gene signal	Metastatic Melanoma and autologous TIL infusion	48	Higher level of TCR V*β* gene in TIL predicts TIL growth (P=0.008)	II	Genomic	Chen *et al.* (47)
CD8β and *CD3δ*, *CD45RA, ICOS, PD-1, STAT4*			Higher expression in tumors predicted TIL expansion			
IRAK1			Higher expression on IHC and PCR predicts lower survival			
$PD1^+CD8^+$ T cells	Melanoma cell lines	NA	$PD1^+CD8^+$ TILs noted to have higher response/tumor reactivity (P=0.0007)	Pre-clinical	Flow cytometry	Inozume *et al.* (48)
$CD137^+$ T cells	Melanoma and ovarian cancer cell lines	NA	$CD137^+$ TILs noted to have higher tumor reactivity	Pre-clinical		Ye *et al.* (49)
IL-9	Metastatic melanoma (exposed to previous anti-PD1, CTLA-4) given autologous TIL infusion	72	Higher level of IL-9 predicts better response (7/9, P=0.009)	II	Serum	Forget MA *et al.* (50)
ULBP-9			Higher level noted post infusion in responders (P=0.0232)			
NKG2D			Higher level noted post infusion in responders (P=0.0232)			
$CD8^+$ T cells of effector phenotype	Metastatic melanoma and TIL infusion	31	Responding patients had higher percentage of T effector cells than non-responders (P=0.0004)	II	Flow cytometry	Radvanyi *et al.* (51)
$CD8^+BTLA^+$ cell			Responding patients had higher percentage of $CD8^+BTLA^+$ T cells (P=0.0006) and $CD8^+BTLA^+$ T effector cells (P=0.0002)			
Total amount of TILs infused			Level of persistence observed in responding patients *vs.* non responding patients (P=0.001)		Clinical	
Persistence of infused TILs post adoptive transfer	Metastatic melanoma and TILs infusion	13	Associated with objective clinical response	II	Flow cytometry	Dudley *et al.* (52)
IFNγ	CTLs therapy in NPC	35	Associated with longer survival	II	Serum	Toh *et al.* (53)
TILs with higher $CD137^+$ T cells and IFNγ production	TIL therapy in HPV-associated epithelial cancers	29	Pre-infusion TILs with higher $CD137^+$ T cells (P=0.0091) and IFNγ production (P=0.0026) following stimulation with HPV E6 and E7 peptides are associated in responders (n=7) compared to non-responders (n=20)	II	Flow cytometry	Stevanović *et al.* (8)
CAR-T						
IL-6, IFN-γ	CAR-T in renal cell carcinoma	12	Correlates with persistence of CAR-T	I	Serum	Lamers *et al.* (18) and Klaver *et al.* (54)

TILs, tumor infiltrating lymphocytes; IHC, immunohistochemistry; PCR, polymerase chain reaction; CTLs, cytotoxic T lymphocytes; NPC, nasopharyngeal carcinomas; CAR-T, chimeric antigen receptor T cell.

(P=0.002) (52). Similar conclusions were reported in another study: that higher numbers of TILs within the infusion product correlated with clinical response (n=31) (51). However, other studies did not find significant correlation between persistence of TILs in host and clinical outcome (50).

In a recently published clinical trial by Forget *et al.* (50), seventy-two melanoma patients, some of whom had received previous lines of ICIs (anti-CTLA-4 and anti-PD1 therapies), were given TILs infusion with an overall response rate of 42%. Baseline serum levels of IL-9 appeared to predict response to TILs. Functionally, IL-9 plays a key role in the maturation of $CD4^+$ T-helper cells and is also known to increase Th17 response. However, the role of Th17 T cells is still not completely clear and is paradoxical, as they have both anti-tumor as well as pro-inflammatory functions. It was also interesting that serum ULBP-9 and its ligand NKG2D, were found at higher levels among responders 3 months post-infusion. In the same study, clinical factors such as TILs persistence in host, TMB, and autologous tumor recognition *in vitro* were not found to stratify patient outcomes. In terms of host characteristics, patients with prior exposure to anti-CTLA-4 therapy and shorter TILs infused were noted to be associated with a shorter duration of response to therapy.

In another study of metastatic melanoma patients treated with autologous TILs infusion (n=48) (46), Chen *et al.* noted initially that CD8-positive IHC staining in original tumor correlated with $CD8^+$ T cell content of the final expanded TILs product of these patients. They also found that there were higher intra-tumoral CD8, CD4 and CD3 IHC staining in the TIL growers compared to the non-growers. Although CD8, PD-1 and FoxP3 staining did not predict tumor response, there was suggestion that peri-tumoral and total CD4 staining was a negative predictor of CR and partial response (PR) (P=0.0067 and P=0.082 respectively). A year later, it was reported by the same group that *CD8*β, *CD3*δ, *CD45RA*, *ICOS*, *PD-1* and *STAT4* gene expression in tumor, and a higher level of TCR Vβ gene in the cell product, predicted favorable TIL expansion (47). These results point to the possibility of adopting these signatures as guiding biomarker for selecting metastases sites with higher presence of TILs that are favorable for expansion.

In terms of phenotype of the T cells, Inozume *et al.* found that percentage of $CD8^+PD1^+$ TILs was higher in tumor digests that generated reactive TILs (48). Apart from *PD1*, a few other T cell markers had been found to be clinically relevant in predicting response. CD137 (a co-stimulatory receptor and an activation marker of T cell) expressing T cells in melanoma and ovarian cancer cell lines were found to have higher tumor reactivity by Ye *et al.* (49). Radvanyi *et al.* reported that the TILs product with higher percentage of $CD8^+BTLA^+$ T cells and especially $CD8^+BTLA^+$ T effector cells correlated with improved response in metastatic melanoma (51). Forget *et al.* went on to highlight that while this observation was true in *CTLA-4* ICI naive patients, it was not observed in CTLA-4 refractory melanoma patients.

Separately, Lamers *et al.* 2013 (18) and Klaver *et al.* 2016 (54) reported results and potential predictive biomarkers from an early phase study of 12 renal cell carcinoma patients given CAR-T against carbonic anhydrase IX (CAIX). There was no clinical response in this study and a few cases of Grade 2–4 liver toxicities was seen initially until a monoclonal antibody (anti-CAIX) was used pre-infusion. The number of CAR T-cells in the patients' blood correlated with plasma levels of IFN-γ and IL-6, but not with any of the other cytokines tested (54). Thus, out of the 27 cytokines tested, the authors suggested that IFNγ and IL-6 levels in plasma were potential surrogate markers for CAR T-cell persistence.

Monocytic MDSCs are generally associated with worse clinical outcomes and they are commonly associated with immune suppressive and evasion mechanisms favoring cancer survival. In our center, we completed a Phase II trial in 35 advanced incurable stage 4 NPC patients with six cycles of adoptive transfer of autologous EBV-specific CTLs following first line gemcitabine and carboplatin chemotherapy (6). To identify biomarkers of therapeutic outcomes, we performed multiple deep immune phenotyping analyses including flow cytometry, NanoString and multiplex ELISA assays. Patients with lower median survival had higher levels of MDSCs and cytokines associated with MDSCs such as IL-10 and CCL22 following chemotherapy. This transient burst of MDSCs was followed by a persistent increase in circulating activated regulatory T cells (Tregs). Conversely, serum IFNγ levels were associated with long term survival in these advanced NPC patients who received the CTLs (53).

From a recently reported phase II trial investigating virus-specific TILs in HPV-related epithelial cancers by Stevanović *et al.*, it was found that pre-infusion TILs with higher $CD137^+$ T cells and IFNγ production following stimulation with HPV E6 and E7 peptides were associated with responders compared to NRs (8). This echoed the abovementioned findings of Ye *et al.* (49), highlighting the

importance of *CD137* in T cell activation.

Conclusions

The race to improve efficacy in CAR-T therapy hinges on the identification of promising specific therapeutic targets and the subsequent development of new generation CARs (including incorporating co-stimulatory proteins) to improve CAR-T function, and to circumvent a prohibitive microenvironment especially when combatting solid tumors. With regard to viral-specific T cells, TILs and GM-TCR cell therapies, one of the major predictors of response remains to be the specificity of their TCR repertoires against tumor (or oncovirus) antigens. The target characteristics are important—since with better selected TCRs or CARs, the higher the chance of achieving antigenic activation and thereby achieving tumor killing.

Yet, even with increasingly more specific TCRs or CARs, there exist many variables which may influence the clinical efficacy of cell therapy. The identification of predictive biomarkers of T cell therapy is made challenging by the complexities of such 'living therapy'. The immune-fitness of the patient and the overall efficacy of the cell therapy have many determinants, coupled with the fluidity of the host immune response and interaction with the cell therapy. Furthermore, some of the findings seem contradictory in different platforms. TIL expressing either co-stimulatory (*CD137*) or co-inhibitory signals (PD1, BTLA) predicted tumor reactivity/response (49). In contrast, PD-1, LAG3 and TIM3 exhaustion markers were found on CAR-Ts of NRs (41,42). This example highlights cell product heterogeneity and individualized accompanying biomarkers should be developed.

The rapid growth and establishment of a whole constellation of validated and potential biomarkers in ICI therapy provide hope for finding reliable predictive and prognostic biomarkers for T cell therapy. These advances should be taken in the context that over the last many decades, there have been sparse biomarkers to predict cytotoxic chemotherapy responses and even targeted therapy outside of oncogenic addiction in the latter. The role of predictive biomarkers of cell therapy remains an emerging field of active research, compelled by the current high cost of such therapies, and the quest for identifying and enriching for potentially responding patients.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/pcm.2019.07.02). WWW reports personal fees from Tessa Therapeutics Ltd., Singapore, outside the submitted work. HCT serves as an unpaid editorial board member of *Precision Cancer Medicine* from Jul 2018 to Jun 2020 and held the appointment of Chief Medical Officer at Tessa Therapeutics Ltd (now consultant) but there is no conflict of interest at all with this review article. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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