



Review

Adoptive cell therapy with tumour-infiltrating lymphocytes: the emerging importance of clonal neoantigen targets for next-generation products in non-small cell lung cancer

Jane Robertson^{*}, Max Salm, Markus Dangl

Achilles Therapeutics Ltd, Stevenage Bioscience Catalyst, Stevenage, UK

ARTICLE INFO

Keywords:

Clonal neoantigens
Adoptive cell therapy
Non-small cell lung cancer

ABSTRACT

Immune checkpoint blockade has significantly improved clinical outcomes for patients with non-small cell lung cancer (NSCLC) and other solid tumours, but many patients do not respond and acquired resistance is common. Aspects of the tumour microenvironment linked to clinical outcomes include the proportion of tumour-infiltrating lymphocytes (TIL), tumour programmed death ligand 1 (PD-L1) score and tumour mutation burden. Adoptive cell therapy (ACT), a technique that works by infusing *ex vivo* expanded T lymphocytes to increase the effector cell pool in tumours, is anticipated to become a viable therapeutic option for patients with solid tumours, akin to chimeric antigen receptor T cell (CAR-T) therapy in haematological malignancies. TIL therapy has shown durable clinical responses in heavily pre-treated patients with melanoma and other solid tumours. We review the experience of ACT with TILs and the recent evidence that clonal neoantigens might be the most relevant immunotherapeutic targets in heterogeneous solid tumours such as NSCLC. Clonal (or truncal) neoantigens arise from the earliest mutagenic events in tumour evolution, and are retained over time in all tumour cells within a patient, making them the ideal target for T cell therapy. NSCLC has one of the highest clonal mutation burdens of all cancers through exposure to carcinogens in tobacco smoke, providing a strong rationale to develop clonal neoantigen reactive T cells (cNeT) for this indication. The first treatment modality to test this concept clinically is ATL001, a cNeT product that is derived from autologous TILs and enriched for T cells specifically recognizing clonal neoantigenic epitopes by selective expansion. Clinical studies of ATL001 will commence in 2019.

Introduction

The most significant recent advancement in the treatment of non-small cell lung cancer (NSCLC), melanoma, urothelial cancers and other solid tumours has been the development of monoclonal antibodies against immune checkpoint molecules to overcome immune evasion, one of the hallmarks of cancer. Of these, the most successful class of agents has been programmed death receptor 1 (PD-1)/programmed death ligand 1 (PD-L1) inhibitors. Activated cytotoxic T cells express PD-1, which interacts with PD-L1 which is expressed on a proportion of tumour cells and antigen-presenting cells. When PD-1 binds to PD-L1, T-cell antitumour activity is effectively suppressed, but the interaction can

be prevented by using antibodies blocking PD-1 (e.g. nivolumab and pembrolizumab) or PD-L1 (e.g. atezolizumab, avelumab and durvalumab), allowing T cells to recognize and kill tumour cells.

PD-1 and PD-L1 inhibitors have shown clinical activity in a wide variety of solid tumours, and are approved for the treatment of melanoma, NSCLC, small cell lung cancer, head and neck squamous cell carcinoma, urothelial carcinoma, microsatellite instability-high cancer, gastric cancer, cervical cancer, hepatocellular carcinoma, Merkel cell carcinoma and renal cell carcinoma. Initial approvals were in the second-line (or later) treatment setting following disease progression on standard therapies, but recent approvals are as first-line treatments for melanoma, NSCLC, renal and head and neck cancers, and as adjuvant

^{*} Corresponding author. Jane Robertson, MD. Chief Medical Officer, Achilles Therapeutics Ltd, Stevenage Bioscience Catalyst, Stevenage SG1 2FX, UK. Tel.: +44 (0) 1438 906 855.

E-mail address: j.robertson@achillestx.com (J. Robertson).

<https://doi.org/10.1016/j.iotech.2019.09.003>

Available online 9 October 2019

2590-0188/© 2019 Achilles Therapeutics Limited. Published by Elsevier Ltd on behalf of European Society for Medical Oncology. This is an open access article under

the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Table 1

Clinical results with combinations of programmed death receptor 1 (PD-1)/programmed death ligand 1 (PD-L1) inhibitors and platinum-based chemotherapy regimens as first-line therapy for metastatic non-small cell lung cancer (NSCLC)

Clinical study	Investigational agent and subgroup	Progression-free survival		Response rate		Median overall survival		Reference
		Active	Control	Active	Control	Active	Control	
KEYNOTE-189 (n = 616) Cisplatin/carboplatin + pemetrexed ± pembrolizumab	Pembrolizumab Non-squamous NSCLC No <i>EGFR/ALK/ROS-1</i> mutation	8.8 months 35% alive and progression free at 12 months	4.9 months 18% alive and progression free at 12 months	48%	19%	NR	11.3 months	[4]
KEYNOTE-407 (n = 559) Carboplatin + nab paclitaxel/paclitaxel ± pembrolizumab	Pembrolizumab Squamous NSCLC No <i>EGFR/ALK/ROS-1</i> mutation	6.4 months 31% alive and progression free at 12 months	4.8 months 15% alive and progression free at 12 months	57%	36%	15.9 months	11.3 months	[5]
Impower150 (Arm B versus C; n = 800) Carboplatin + paclitaxel + bevacizumab ± atezolizumab	Atezolizumab Non-squamous NSCLC No <i>EGFR/ALK/ROS-1</i> mutation	8.4 months 38% alive and progression free at 12 months	6.8 months 20% alive and progression free at 12 months	56%	40%	19.2 months	14.7 months	[6]
Impower 131 (Arm B versus C; n = 688) Carboplatin + paclitaxel + bevacizumab ± atezolizumab	Atezolizumab Squamous NSCLC No <i>EGFR/ALK/ROS-1</i> mutation	6.3 months 25% alive and progression free at 12 months	5.6 months 12% alive and progression free at 12 months	49%	41%	14 months	13.9 months	[7]

treatments in melanoma.

The PD-1 inhibitor pembrolizumab has greatly improved outcomes for patients with advanced NSCLC. Its approval for first-line treatment of patients with metastatic NSCLC with high PD-L1 expression (tumour proportion score $\geq 50\%$) was based on data from the KEYNOTE-024 study which demonstrated a response rate of 44.8% versus 27.8% on platinum-based chemotherapy, progression-free survival (PFS) of 10.3 versus 6.0 months (hazard ratio 0.5; $P \leq 0.001$) [1,2] and median overall survival (OS) of 30.0 versus 14.2 months [3], with fewer grade 3–5 adverse events than chemotherapy (26.6% versus 53.3%). The addition of pembrolizumab to standard platinum-based chemotherapy regimens has also improved the response rate, PFS and OS compared with chemotherapy alone, with a safety profile that is similar to chemotherapy alone. Similar results have been reported for the PD-L1 inhibitor, atezolizumab (Table 1).

These improvements in clinical outcomes with PD-1/PD-L1 are significant, and some patients experience long-term benefit. However, notwithstanding these encouraging statistics, 45–50% of patients with metastatic NSCLC do not achieve an optimal response with chemotherapy plus PD-1/PD-L1, and ~70% patients experience disease progression, or die, within 12 months of starting treatment.

Resistance to checkpoint inhibitors may be primary or acquired, and several mechanisms may contribute to both phenotypes. These are summarized briefly here and have been the subject of many excellent reviews [8–11].

Primary resistance may be a result of intrinsically low antigenicity of the tumour, resulting from low expression of surface antigens or failure to present them to the immune system via loss of major histocompatibility complex (MHC) or β_2 microglobulin (B2M). Other mechanisms include poor T cell infiltration into the tumour, failure of the T-cell recognition machinery, or functional T cell suppression through other components of the immune microenvironment such as regulatory T cells (Tregs) or myeloid-derived suppressor cells (MDSCs). Mutations in tumour cell signalling pathways such as the $IFN\gamma R$ -JAK-STAT, MAPK, VEGF, PTEN and PI3K pathways may negatively influence the infiltration and function of tumour-infiltrating lymphocytes (TIL), dampen antitumour responses, and reduce the effectiveness of PD-1/PD-L1 inhibitors [12–14].

Acquired resistance may evolve through adaptive loss of antigenicity (e.g. through loss of tumour neoantigen expression or defective surface presentation through loss of MHC) and loss of antigen recognition machinery. The tumour microenvironment changes through treatment; for example, the checkpoint inhibitor molecule TIM-3 is often co-expressed with PD-1 on T cells, and its increased expression has been associated

with the recurrence of lung cancer following PD-1 treatment in both animal models and patients [15].

There are multiple reports of the positive prognostic significance of the degree of lymphocyte infiltration in patients with lung cancer and other solid tumours, and the proportion of intratumoural CD8+ T cells recognizing tumour neoantigens is an important predictor of outcome to checkpoint inhibitors [16]. The high tumour PD-L1 levels that are generally associated with better outcomes for PD-1 inhibitors may also be a surrogate marker of TIL infiltration; tumour cell PD-L1 expression increases as an adaptive response to immune attack, and its presence on antigen-presenting cells also signifies an immune response.

For patients with resistant NSCLC following PD-1/PD-L1 inhibitor plus platinum doublet chemotherapy as first-line treatment, there is currently no standard second-line treatment for their condition. Adoptive cell therapy (ACT) might offer a promising alternative approach for these patients.

Adoptive cell therapy for solid tumours

The genetic modification of blood-derived T cells has been shown to be effective in the treatment of some haematological malignancies, but limited activity has thus far been observed in solid tumours. The most encouraging clinical activity to date has been observed from the use of non-genetically modified, polyclonal TILs which target multiple epitopes.

TILs have been isolated from multiple solid tumours and expanded *ex vivo* for clinical trials since the first reports using this approach in 1986, which described the efficacy of TIL in mouse models when given in combination with cyclophosphamide and interleukin-2 (IL-2) [17]. Most clinical trials were initially conducted in patients with malignant melanoma, a tumour which was known to respond to immune-enhancing treatments such as IL-2 and interferon. In this setting, *ex vivo* expanded TIL achieved clinically meaningful results [overall response rates of 40–50% and complete response (CR) rates of 20%], and patients who achieve CR have demonstrated long-term disease-free survival for many years [18–25]. In 2019, Sarnaik et al. presented data on the safety and efficacy of cryopreserved autologous TIL therapy (LN-144, lifileucel) in 66 patients with advanced metastatic melanoma who progressed on multiple prior therapies including anti-PD-1 [26]. In this heavily pre-treated and PD-1-resistant group, the response rate was 38%, with 3% CR and 80% disease control rate. Durable responses were observed, and some responses were observed in patients with PD-1-negative tumours, suggesting that ACT with TIL may be an effective option for patients with PD-1-resistant cancers or cancers with lower immunogenicity.

There is growing evidence to suggest that the success of TIL therapy is driven by neoantigen-directed T cells and the number of neoantigens that are targeted. Cancer neoantigens arise from somatic tumour-specific mutations and are present in cancer cells but absent from normal cells. The clinical importance of T cell responses against neoantigens has been suggested from a number of studies which have detected T cells in both the TIL and circulating T cell compartments, recognizing neoantigens arising from tumour-specific mutations in *TP53*, *BRAF* and *KRAS* in patients with epithelial solid tumours including NSCLC, ovarian and colorectal cancer [27–32]. The tumour mutational load and predicted neoantigen load have been shown to correlate with clinical outcomes for TIL therapies as well as PD-1 inhibitors in patients with melanoma, suggesting that TIL efficacy is driven through neoantigen-reactive T cells within the product [33].

Recent reports have demonstrated that TIL therapy can deliver a significant reduction in tumour burden in late-stage cancer patients suffering from a diverse set of epithelial tumours as well as melanoma. A response rate of 28% was reported in 18 patients with human papilloma virus (HPV)-related cervical cancer using HPV-targeted T cells [34], two of whom had achieved ongoing durable CRs for over 4 years at the time of the report. In an ongoing study of TIL therapy, a 44% response rate, with two CRs, was observed among 27 patients with advanced recurrent, metastatic or persistent cervical cancer [35]. Case histories of durable remissions have been reported in patients with metastatic colorectal cancer [36], metastatic cholangiocarcinoma [37] and chemotherapy-resistant metastatic breast cancer [38]. In the case report describing the treatment of a patient with cholangiocarcinoma [37], a T cell product that was 95% reactive to a single neoantigen was responsible for a durable remission lasting over 35 months, and in the breast cancer case reported by Zacharakis et al. [38], multiple reactivities against neoantigens were identified that led to CR lasting over 22 months at the time of the report. In this last case, other T cells targeting unknown antigens persisted at a relative high frequency after transfer, so the possibility that they also contributed to the observed effect cannot be excluded.

There have been limited studies of TIL therapies in NSCLC, but its responsiveness to PD-1/PD-L1 inhibitors and its high mutation burden suggest that it would be a good candidate for this approach. In 1996, the first clinical study using TIL therapy in NSCLC was published [39]. TIL cultures were successfully expanded from tumours removed during standard surgical procedures from 113 of 118 patients with stage II and III lung cancer, who were randomized to be treated with TIL in a monotherapy setting (stage II) or in combination with standard chemoradiotherapy compared with standard therapy (stage III). TIL were expanded to large numbers using high IL-2 doses and were subsequently infused without any preconditioning regimen. It was shown that preconditioning significantly improves the efficacy of TIL therapy approaches by giving the TIL space and nutrition to expand and limits the negative influence of Tregs and MDSCs [40]. Despite these limitations, a significant improvement in 3-year OS compared with controls was shown in stage III patients, in whom local relapse was also significantly improved. Interestingly, the authors noted that the most beneficial impact of adding TIL therapy was seen in the first 6 months after treatment, which might indicate limited persistence due to poor engraftment of the transferred cells, or that the infused T cells were exhausted after this period due to the high IL-2 dose during expansion and/or outgrown or suppressed by Tregs and MDSCs. Nonetheless, this study was the initial proof that TIL therapy can be applied successfully to patients with late-stage lung cancer.

In 2017, Ben-Avi et al. used multiple methods to isolate and expand TIL cultures in a good manufacturing practices (GMP) environment from surgically removed tumour samples of five patients with NSCLC [41]. In contrast to the approach of Ratto et al. [39], after isolation from the tumour tissue, the TIL were expanded rapidly with anti-CD3 antibody, IL-2 and irradiated feeder cells resulting in a massive numerical expansion of the TIL producing up to 0.5×10^{10} cells for infusion. The results

obtained using these procedures were compared with data from patients with melanoma who had previously undergone TIL treatment at the same centre, and it was demonstrated that both TIL populations showed comparable expansion rates and similar phenotypes, although the TIL derived from lung cancer contained a higher percentage of CD4+ T cells. Although this work has limitations as it did not specifically compare tumour recognition and reactivities between the TIL populations, it demonstrated that TIL from patients with lung cancer can be expanded to treatment level under GMP conditions using state-of-the-art protocols.

In 2018, Creelan et al. isolated and expanded TIL from metastatic tumour tissue from 13 of 14 heavily pre-treated patients with NSCLC who were enrolled in a phase I clinical trial [42]. The patients were treated with nivolumab for 8 weeks, and nine patients with progressive disease at this time received a lymphodepleting regimen (cyclophosphamide/fludarabine) and were infused with a median of 81×10^9 expanded TIL. Patients then received six doses of IL-2 and nivolumab maintenance treatment. In this initially nivolumab-refractory population, seven of nine patients showed a reduction in tumour size compared with baseline, with three partial responses and one pathological CR. Four patients remained on treatment after 6–15 months of follow-up.

These reports demonstrate that it is feasible to isolate TIL from NSCLC metastatic lesions and expand them *ex vivo* for adoptive T-cell treatment within a clinical trial setting, with some encouraging early signals of clinical activity in heavily pre-treated and PD-1-resistant patients.

Genetic landscape of NSCLC and emerging importance of clonal neoantigens as a therapeutic target

Tumour-specific neoantigens arise from mutations that accumulate in tumours over time, and have been demonstrated to elicit T cell responses within the patient. These neoantigens are thought to be the major contributors to the clinically relevant responses that have been documented following treatment with immune-therapeutic approaches [43]. Tumours with the highest mutational burden present more tumour neoantigens to the host and appear to be more susceptible to immunotherapy [16,44].

Recent data from the TRACERx study suggest that clonal mutations arising from the earliest transforming mutagenic events are retained in all the subclones despite the acquisition of more mutations during the natural history of the tumour due to evolutionary or therapeutic selection pressures [45]. Therefore, the clonal mutations are present in all cancer cells of a patient, whereas subclonal mutations are present in only a proportion of the cancer cells. Theoretically, T cells targeting a single clonal neoantigen could lead to complete tumour regression if all of the cells express and present the antigen targeted.

The lung cancer genome is sculpted by a complex system of exogenous and endogenous mutagenic forces, resulting in intratumour heterogeneity upon which local natural selection pressures can act. This process is commonly initiated and fuelled by the >60 carcinogens found in tobacco smoke, which induce ~150 mutations per year in people who smoke 20 cigarettes per day [46]. The average mutation frequency is > 10-fold higher in smokers than in never-smokers [47].

This accelerated mutagenic rate fuels an evolutionary search that invariably converges on key mutations [48], which themselves significantly alter the evolutionary potential of a tumour [49,50]. The most common early events marking the emergent founding clone are loss-of-function *TP53* mutations (61.7% of 4678 NSCLC samples in 18 studies [51]), and the mutations responsible for this loss of function are frequently attributable to tobacco smoke [52]. Due to the aetiological heterogeneity of NSCLC [53], tumours harbour other early events including mutations of *KRAS* (22%), *STK11* (12.5%), *KEAP1* (15.6%), *EGFR* (14.5%), *BRAF* (6%) and *MET* (3.9%) [45,54–56].

In general, the patterns of genomic alterations are markedly distinct between lung adenocarcinomas and lung squamous cell carcinomas [57]. Of the 58 significantly mutated genes in both tumour types, only six are common to both: *TP53*, *RB1* [58], *ARID1A*, *CDKN2A*, *PIK3CA* and *NF1* [59]. *KRAS* and *EGFR* mutations are essentially restricted to lung

adenocarcinomas, and *EGFR* mutations are enriched in females and non-smokers [59–61]. Notably, the tumour mutation burden is markedly lower in patients with *EGFR*, *ALK*, *RET* or *ROS1* mutations [34].

TP53 mutations are associated with whole-genome duplication [60], a common early event in NSCLC [45,57–61], and aneuploidy in general [62]. This genomic instability may generate a profusion of neoantigens, which in turn fuel an ‘arms race’ of immune predation and evasion [63–65]. Two recent reports demonstrated that T cell responses against oncogenic drivers like *P53* and *BRAF*N581I can be detected in patients with NSCLC [27,28]. Ultimately, this explosion of diversity and ensuing competition commonly leads to punctuated evolution and the emergence of a dominant clone [66], and it is this set of clonal mutations that are passed on to future generations of tumour cells.

The clonal mutation burden has clinical consequences. Patients with NSCLC whose tumours contain large numbers of these original clonal mutations appear to have a survival benefit on treatment with PD-1 inhibitors compared with patients whose tumours are dominated by sub-clonal mutations [44], suggesting that T cell responses are more effective in these cases. This publication also demonstrated that T cells recognizing clonal neoantigens can be successfully isolated from patients with NSCLC. Two further recent publications have suggested that the number of these non-synonymous clonal mutations is the driver of disease-free survival in patients with early-stage NSCLC [67,68]. These data suggest that specifically targeting clonal neoantigens with TIL might result in enhanced clinical benefit. To prove this hypothesis, clinical data are needed in multiple patients with lung cancer. Higher clonal neoantigen burdens are associated with cytotoxic T lymphocyte and Treg infiltration, and a relative paucity of Th2 cells and cancer-associated fibroblasts [69]. The fundamental mechanisms mediating these phenomena remain obscure, although recent evidence suggests that an effective antitumour T cell response requires optimal effector-to-target ratios which are, in part, defined by the clonal neoantigen fraction [70,71].

Rationale to develop a cNeT product for the treatment of NSCLC

A therapeutic approach of identifying clonal neoantigens, priming TILs *ex vivo* to recognize them and treating patients with their own expanded clonal neoantigen-reactive T cell (cNeT) product is expected to effectively enhance the ability of the immune system to attack all of the tumour cells in the body, and overcome the problem of intratumoural heterogeneity, as clonal neoantigens are present in all tumour cells and are the most relevant therapeutic targets for T cells.

There are some challenges to developing a cNeT product, including the identification of clonal neoantigens, and the development of a scalable manufacturing process to provide a product made up of T cells that are capable of expansion and persistence over time, and can maintain their functional effector phenotype *in vivo*. First-generation TIL therapies have demonstrated the ability of the cells to home to and penetrate into tumours in different locations. The resistance mechanisms to immune checkpoint inhibitors may also be relevant to TIL products, including tumour expression of PD-L1, TIM-3 or other immune checkpoint molecules; reduction in interferon signaling; decreased expression of neoantigens over time [67]; and loss of antigen-presenting machinery. An example of this is the case report of a patient with colorectal cancer cited previously, who initially responded to TIL therapy but relapsed some months later due to loss of human leukocyte antigen expression in one lesion [36]. It is currently estimated that <2% of mutations in gastrointestinal cancers are immunogenic [72]. One way to overcome some of the challenges associated, for example, with human leukocyte antigen or B2M loss is to develop a manufacturing process that generates multiple cNeT clones targeting a variety of clonal neoantigens per patient, including CD4⁺ and CD8⁺ T cells in the final product. In addition, thorough analysis of tumour specificity of the clonal mutation targeted must be undertaken to ensure that the mutation is not present in normal cells.

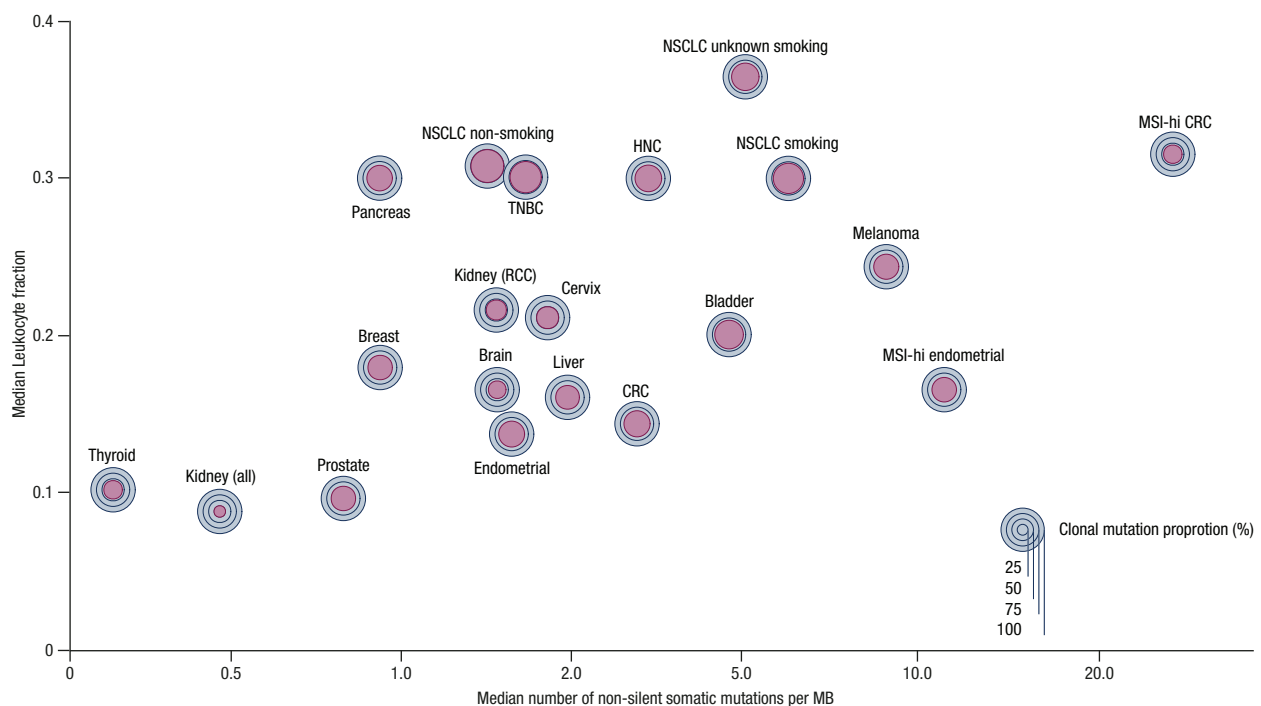


Figure 1. Correlation between tumour mutational burden and tumour immune infiltrate in 20 tumour types. For each tumour type (or subtype), the median number of coding somatic mutations per megabase (MB) of DNA and the corresponding median leukocyte fraction were plotted. Data on the x axis are shown on a logarithmic scale. Each circle is shaded to reflect the median proportion of clonal mutations for that tumour type. In general, leukocyte infiltration correlates with tumour mutation burden. NSCLC, non-small cell lung cancer; TNBC, triple negative breast cancer; HNC, head and neck cancer; MSI-hi CRC, microsatellite instability-high colorectal cancer; CRC, colorectal cancer; RCC, renal cell carcinoma. Sources: Thorsson et al. (Immunity 2018; 48:812–830), Charoentong et al (Cell reports 2017; 18:248–262) and Cortes-Ciriano et al [73,74] (Nature communications 2017; 8:15180)

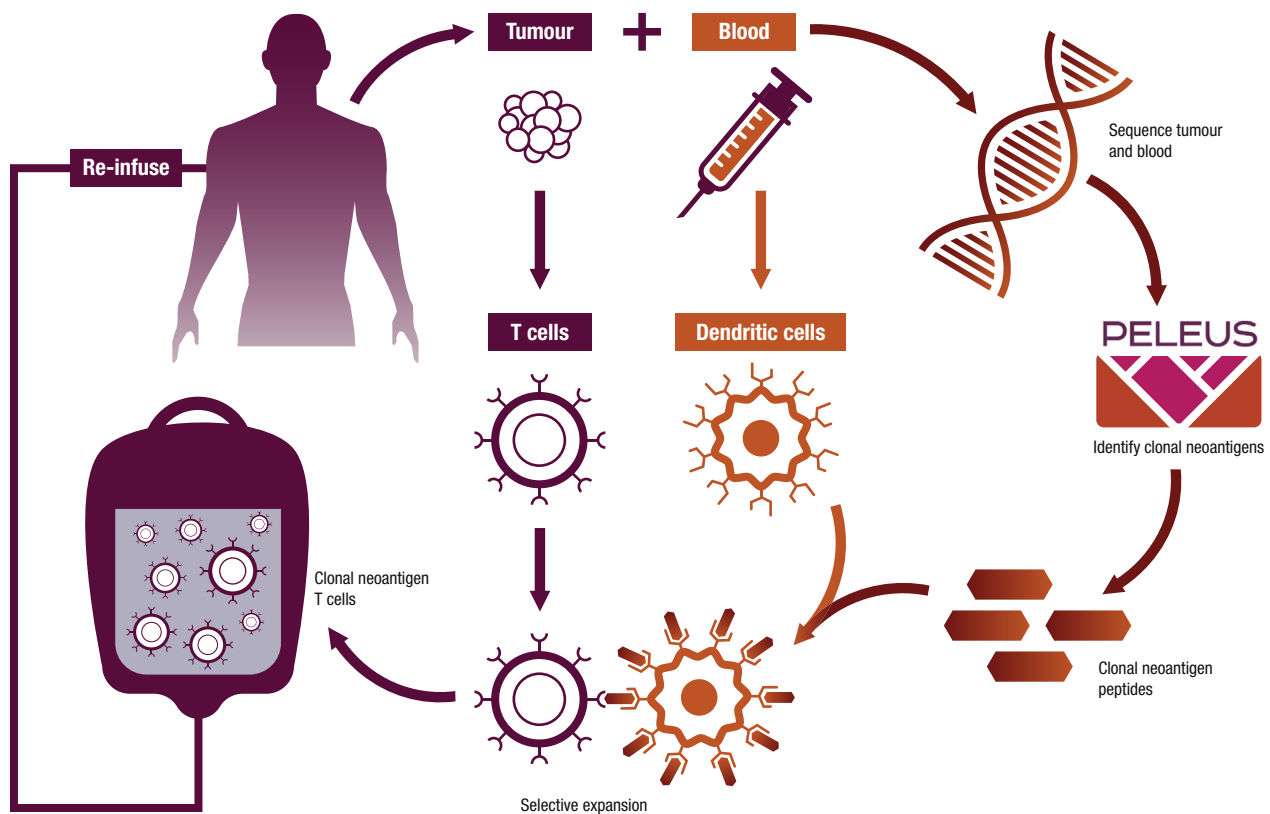


Figure 2. The VELOS™ manufacturing process for ATL001.

NSCLC is an ideal candidate tumour to develop and test a cNeT product, and Creelan et al. have demonstrated that it is possible to manufacture TIL products consistently from tumour tissue from patients with lung cancer. In addition to the observations that NSCLC has a high tumour mutation burden, high T cell infiltrate and high PD-L1 expression, smoking-related NSCLC has a very high clonal mutation burden compared with other tumours and compared with NSCLC that is not caused by smoking (Figure 1).

ATL001 is a cNeT product derived from autologous TIL isolated from tumour tissue, which are then enriched through the selective expansion of T cells specific for clonal neoantigen epitopes expressed by the patient's tumour.

In the ATL001 manufacturing process (VELOS™), autologous TIL are isolated from a sample of the patient's tumour. In addition, samples from the patient's tumour and blood are analysed using whole-exome sequencing and RNA sequencing which enables the identification of candidate clonal neoantigens, potentially presented on MHC class I and class II, using the proprietary PELEUS™ bioinformatics platform. Dendritic cells (DCs) isolated from the patient's blood are loaded with peptides corresponding to the patient-specific clonal neoantigens, and are subsequently cultured with the patient's TIL derived from tumour fragments. By harnessing the basic immunological principles of DC-based specific expansion to activate and expand T cells which recognize their cognate patient-specific clonal neoantigens, this process results in the *ex vivo* expansion of cNeT which can be re-infused to the patient (Figure 2).

ATL001 is designed to target multiple clonal neoantigens expressed on all tumour cells and absent from healthy tissue, on a personalised basis, in contrast to gene-modified approaches which are largely limited to single shared antigens that are not expressed on all cancer cells. As DCs present antigens through both MHC class I and class II, the product contains a mixed population of CD4⁺ and CD8⁺ T cells, both of which have been shown to be important for maintenance of long-term cytotoxic responses.

Two clinical studies of ATL001 have been initiated:

- ATX-NS-001 (CHIRON), a phase I/IIa study to evaluate the safety and clinical activity of cNeT in patients with advanced NSCLC ([ClinicalTrials.gov Identifier: NCT04032847](https://clinicaltrials.gov/ct2/show/study/NCT04032847)); and
- ATX-ME-001 (THETIS), a phase I/IIa study to evaluate the safety and clinical activity of cNeT in patients with metastatic or recurrent melanoma following treatment with a PD-1 inhibitor ([ClinicalTrials.gov Identifier: NCT03997474](https://clinicaltrials.gov/ct2/show/study/NCT03997474)).

The primary objective of these trials is to establish the safety of cNeT therapy; the tolerability and clinical activity will also be explored. In each study, patients will be treated with standard therapies for their condition, which will include a PD-1 inhibitor prior to receiving the product. Patients will undergo a non-myeloablative lymphodepletion regimen of cyclophosphamide and fludarabine, after which they will receive a single infusion of cNeT followed by a short course of IL-2.

Summary

Recent data emerging from longitudinal studies of lung cancer evolution, particularly the TRACERx study [45], have highlighted the potential of neoantigens, specifically clonal neoantigens, as emerging therapeutic targets for NSCLC and other solid tumours. As confidence in identifying clonal neoantigens increases, so does the potential to create a personalized cell therapy product directed against multiple clonal neoantigens, which may overcome the barriers of tumour heterogeneity and immunoediting. As clonal neoantigens may be present on all tumour cells but not normal tissues, and because TIL are not genetically modified, clonal neoantigen-reactive T cells are anticipated to be better tolerated than engineered T cell products. It is hoped that cNeTs may eventually offer a therapeutic option for patients with NSCLC who have become resistant to PD-1/PD-L1 inhibitors.

Funding

None declared.

Disclosure

The authors are all employees of Achilles Therapeutics Ltd.

References

- Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csósz T, Fülöp A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med* 2016;375:1823–33.
- Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csósz T, Fülöp A, et al. Updated analysis of keynote-024: pembrolizumab versus platinum-based chemotherapy for advanced non-small-cell lung cancer with PD-L1 tumor proportion score of 50. *J Clin Oncol* 2019;37:537–46.
- Brahmer JR, Govindan R, Anders RA, Antonia SJ, Sagorsky S, Davies MJ, et al. The Society for Immunotherapy of Cancer consensus statement on immunotherapy for the treatment of non-small cell lung cancer (NSCLC). *J Immunother Cancer* 2018;6:75.
- Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med* 2016;378:2078–92.
- Paz-Ares L, Luft A, Vicente D, Tafreshi A, Günter M, Mazières J, et al. Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. *N Engl J Med* 2018;379:2040–51.
- Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. *N Engl J Med* 2018;378:2288–301.
- Jotte RM, Cappuzzo F, Vynnychenko I, Stroyakovskiy D, Abreu DR, Hussein MA, et al. Primary PFS and safety analysis of a randomized phase III study of atezolizumab + carboplatin + paclitaxel or nab-paclitaxel vs carboplatin + nab-paclitaxel as 1L therapy in advanced squamous NSCLC. *J Clin Oncol* 2018;36(Suppl. 1):LBA9000.
- Kelderman S, Schumacher TN, Haanen J. Acquired and intrinsic resistance in cancer immunotherapy. *Mol Oncol* 2014;8:1132–9.
- Pitt JM, Vétizou M, Daillère R, Roberti MP, Yamazaki T, Routy B, et al. Resistance mechanisms to immune-checkpoint blockade in cancer: tumor-intrinsic and -extrinsic factors. *Immunity* 2016;44:1255–69.
- Sharma P, Lieskovan SH, Wargo JA, Ribas A. Primary, adaptive and acquired resistance to cancer immunotherapy. *Cell* 2017;168:707–23.
- O'Donnell JS, Teng MWL, Smyth MJ. Cancer immunoeediting and resistance to T cell-based immunotherapy. *Nat Rev Clin Oncol* 2019;16:151–67.
- Shin DS, Zaretsky JM, Escuin-Ordinas H, Garcia-Diaz A, Lieskovan H-S, Kalbasi A, et al. Primary resistance to PD-1 blockade mediated by JAK1/2 mutations. *Cancer Discov* 2017;7:188–201.
- Liu S, Matsuzaki J, Wei L, Tsuji T, Battaglia S, Hu Q, et al. Efficient identification of neoantigen specific T-cell responses in advanced human ovarian cancer. *J Immunother Cancer* 2019;7:156.
- Peng W, Chen JQ, Liu C, Malu S, Creasy C, Tetzlaff MT, et al. Loss of PTEN promotes resistance to T cell-mediated immunotherapy. *Cancer Discov* 2016;6:202–16.
- Koyama S, Akbay EA, Li YY, Herter-Sprie GS, Buczkowski KA, Richards WG, et al. Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. *Nat Commun* 2016;7:10501.
- Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015;348:124–8.
- Rosenberg SA, Spiess P, Lafreniere R. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. *Science* 1986;233:1318–21.
- Itzhaki O, Hovav E, Ziporen Y, Levy D, Kubi A, Zikich D, et al. Establishment and large-scale expansion of minimally cultured “young” tumor infiltrating lymphocytes for adoptive transfer therapy. *J Immunother* 2011;34:212–20.
- Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes M, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T cell transfer immunotherapy. *Clin Cancer Res* 2011;17:4550–7.
- Hinrichs CS, Rosenberg SA. Exploiting the curative potential of adoptive T-cell therapy for cancer. *Immunol Rev* 2014;257:56–71.
- Besser MJ, Shapira-Frommer R, Itzhaki O, Treves AJ, Zippel DB, Levy D, et al. Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma: intent-to-treat analysis and efficacy after failure to prior immunotherapies. *Clin Cancer Res* 2013;19:4792–800.
- Dudley ME, Gross CA, Somerville RPT, Hong Y, Schaub NP, Rosati SF, et al. Randomized selection design trial evaluating CD8+ enriched versus unselected tumor-infiltrating lymphocytes for adoptive cell therapy for patients with melanoma. *J Clin Oncol* 2013;31:2152–9.
- Goff SL, Dudley ME, Citrin DE, Somerville RP, Wunderlich JR, Danforth DN, et al. Randomized, prospective evaluation comparing intensity of lymphodepletion before adoptive transfer of tumor-infiltrating lymphocytes for patients with metastatic melanoma. *J Clin Oncol* 2016;34:2389–97.
- Andersen R, Donia M, Ellebaek E, Borch TH, Kongsted P, Iversen TZ, et al. Long-lasting complete responses in patients with metastatic melanoma after adoptive cell therapy with tumor-infiltrating lymphocytes and an attenuated IL-2 regimen. *Clin Cancer Res* 2016;22:3734–45.
- Forget M-A, Haymaker C, Hess KR, Meng YJ, Creasy C, Karpinets T, et al. Prospective analysis of adoptive TIL therapy in patients with metastatic melanoma: response, impact of anti-CTLA4, and biomarkers to predict clinical outcome. *Clin Cancer Res* 2018;24:4416–28.
- Sarnaik A, Khushalani NI, Chesney JA, Kluger HM, Curti BD, Lewis KD, et al. Safety and efficacy of cryopreserved autologous tumor infiltrating lymphocyte therapy (LN-144, lifileucel) in advanced metastatic melanoma patients who progressed on multiple prior therapies including anti-PD-1. *J Clin Oncol* 2019;37:2518–2518.
- Smith KN, Llosa NJ, Cottrell TR, Siegel N, Fan H, Suri P, et al. Persistent mutant oncogene specific T cells in two patients benefitting from anti-PD-1. *J Immunother Cancer* 2019;7:40.
- Malekzadeh P, Pasetto A, Robbins PF, Parkhurst MR, Paria BC, Jia L, et al. Neoantigen screening identifies broad TP53 mutant immunogenicity in patients with epithelial cancers. *J Clin Invest* 2019;129:1109–14.
- Lo W, Parkhurst M, Robbins PF, Tran E, Lu Y-C, Jia L, et al. Immunologic recognition of a shared p53 mutated neoantigen in a patient with metastatic colorectal cancer. *Cancer Immunol Res* 2019;7:534–43.
- Cafri G, Yossef R, Pasetto A, Deniger DC, Lu Y-C, Parkhurst M, et al. Memory T cells targeting oncogenic mutations detected in peripheral blood of epithelial cancer patients. *Nat Commun* 2019;10:449.
- Yossef R, Tran E, Deniger D, Gros A, Pasetto A, Parkhurst MR, et al. Enhanced detection of neoantigen-reactive T cells targeting unique and shared oncogenes for personalized cancer immunotherapy. *JCI Insight* 2018;3:e122467.
- Liu S, Matsuzaki J, Wei L, Tsuji T, Battaglia S, Hu Q, et al. Efficient identification of neoantigen-specific T-cell responses in advanced human ovarian cancer. *J Immunother Cancer* 2019;7:156.
- Lauss M, Donia M, Harbst K, Andersen R, Mitra S, Rosengren F, et al. Mutational and putative neoantigen load predict clinical benefit of adoptive t cell therapy in melanoma. *Nat Commun* 2017;8:1738.
- Stevanović S, Helman SR, Wunderlich JR, Langhan MM, Doran SL, Kwong MLM, et al. A phase II study of tumor-infiltrating lymphocyte therapy for human papillomavirus-associated epithelial cancers. *Clin Cancer Res* 2019;25:1486–93.
- Jazaeri AA, Zsiros E, Amaria RN, Artz AS, Edwards RP, Wenham RM, et al. Safety & efficacy of adoptive cell transfer using autologous tumor infiltrating lymphocytes (LN-145) for treatment of recurrent, metastatic, or persistent cervical carcinoma. *ASCO Annu Meet* 2019. Abstract 2058, May 31–June 4, 2019, Chicago, IL, USA.
- Tran E, Robbins PF, Lu YC, Prickett TD, Gartner JJ, Jia L, et al. T-cell transfer therapy targeting mutant KRAS in cancer. *N Engl J Med* 2016;375:2255–62.
- Tran E, Turcotte S, Gros A, Robbins PF, Lu Y-C, Dudley ME, et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. *Science* 2014;344:641–5.
- Zacharakis N, Chinnsamy H, Black M, Xu H, Lu Y-C, Zheng Z, et al. Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer. *Nat Med* 2018;24:724.
- Ratto GB, Zino P, Mirabelli S, Minuti P, Aquilina R, Fantino G, et al. A randomized trial of adoptive immunotherapy with tumor-infiltrating lymphocytes and interleukin-2 versus standard therapy in the postoperative treatment of resected non-small cell lung cancer. *Cancer* 1996;78:244–51.
- Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, et al. Adoptive cell transfer therapy following non-myceloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol* 2005;23:2346.
- Ben-Avi R, Farhi R, Ben-Nun A, Gorodner M, Greenberg E, Markel G, et al. Establishment of adoptive cell therapy with tumor infiltrating lymphocytes for non-small cell lung cancer patients. *Cancer Immunol Immunother* 2018;67:1221–30.
- Creelan B, Teer J, Toloz E, Mullinax J, Landin A, Gray J, et al. Safety and clinical activity of adoptive cell transfer using tumor infiltrating lymphocytes (TIL) combined with nivolumab in NSCLC. *J Thorac Oncol* 2018;13:S330–1.
- Tran E, Robbins PF, Rosenberg SA. ‘Final common pathway’ of human cancer immunotherapy: targeting random somatic mutations. *Nat Immunol* 2017;18:255–62.
- McGranahan N, Furness AJS, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit t cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* 2016;351:1463–9.
- Jamal-Hanjani M, Wilson GA, McGranahan N, Birkbak NJ, Watkins TBK, Veeriah S, et al. Tracking the evolution of non-small-cell lung cancer. *N Engl J Med* 2017;376:2109–21.
- Alexandrov LB, Ju YS, Haase K, Van Loo P, Martincorena I, Nik-Zainal S, et al. Mutational signatures associated with tobacco smoking in human cancer. *Science* 2016;354:618–22.
- Swanton C, Govindan R. Clinical implications of genomic discoveries in lung cancer. *N Engl J Med* 2016;374:1864–73.
- Bailey MH, Tokheim C, Porta-Pardo E, Sengupta S, Bertrand D, Weerasinghe A, et al. Comprehensive characterization of cancer driver genes and mutations. *Cell* 2018;173:371–385.e18.
- Nowell PC. The clonal evolution of tumor cell populations. *Science* 1976;194:23–8.
- Rogers ZN, McFarland CD, Winters IP, Seoane JA, Brady JJ, Yoon S, et al. Mapping the in vivo fitness landscape of lung adenocarcinoma tumor suppression in mice. *Nat Genet* 2018;50:483–6.

- [51] Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 2013;6:11.
- [52] Giacomelli AO, Yang X, Lintner RE, McFarland JM, Duby M, Kim J, et al. Mutational processes shape the landscape of tp53 mutations in human cancer. *Nat Genet* 2018; 50:1381–7.
- [53] Chen Z, Fillmore CM, Hammerman PS, Kim CF, Wong K-K. Non-small-cell lung cancers: a heterogeneous set of diseases. *Nat Rev Cancer* 2014;14:535–46.
- [54] Govindan R, Ding L, Griffith M, Subramanian J, Dees ND, Kanchi KL, et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. *Cell* 2012; 150:1121–34.
- [55] Shi J, Hua X, Zhu B, Ravichandran S, Wang M, Nguyen C, et al. Somatic genomics and clinical features of lung adenocarcinoma: a retrospective study. *PLoS Med* 2016;13:e1002162.
- [56] Singal G, Miller PG, Agarwala V, Li G, Kaushik G, Backenroth D, et al. Association of patient characteristics and tumor genomics with clinical outcomes among patients with non-small cell lung cancer using a clinicogenomic database. *JAMA* 2019;321: 1391–9.
- [57] Campbell JD, Alexandrov A, Kim J, Wala J, Berger AH, Peadarallu CS, et al. Distinct patterns of somatic genome alterations in lung adenocarcinomas and squamous cell carcinomas. *Nat Genet* 2016;48:607–16.
- [58] Imielinski M, Berger AH, Hammerman PS, Hernandez B, Pugh TJ, Hodis E, et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. *Cell* 2012;150:1107–20.
- [59] Network CGAR. Comprehensive molecular profiling of lung adenocarcinoma. *Nature* 2014;511:543–50.
- [60] Bielski CM, Zehir A, Penson AV, Donoghue MTA, Chatila W, Armenia J, et al. Genome doubling shapes the evolution and prognosis of advanced cancers. *Nat Genet* 2018;50:1189–95.
- [61] Lopez S, Lim E, Huebner A, Dietzen M, Mourikis T, Watkins TB, et al. Whole genome doubling mitigates muller's ratchet in cancer evolution. *bioRxiv* 2019: 513457. <https://doi.org/10.1101/513457>.
- [62] Taylor AM, Shih J, Ha G, Gao GF, Zhang X, Berger AC, et al. Genomic and functional approaches to understanding cancer aneuploidy. *Cancer Cell* 2018;33: 676–689.e3.
- [63] Maley CC, Aktipis A, Graham TA, Sottoriva A, Boddy AM, Janiszewska M, et al. Classifying the evolutionary and ecological features of neoplasms. *Nat Rev Cancer* 2017;17:605–19.
- [64] Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang T-H, et al. The immune landscape of cancer. *Immunity* 2018;48: 812–830.e14.
- [65] Lakatos E, Williams MJ, Schenck RO, Cross WCH, Househam J, Werner B, et al. Evolutionary dynamics of neoantigens in growing tumours. *bioRxiv* 2019.
- [66] McGranahan N, Swanton C. Clonal heterogeneity and tumor evolution: past, present, and the future. *Cell* 2017;168:613–28.
- [67] Rosenthal R, Cadieux EL, Salgado R, Al Bakir M, Moore DA, Hiley CT, et al. Neoantigen-directed immune escape in lung cancer evolution. *Nature* 2019;567: 479.
- [68] Miao D, Margolis CA, Vokes NI, Liu D, Taylor-Weiner A, Wankowicz SM, et al. Genomic correlates of response to immune checkpoint blockade in microsatellite-stable solid tumors. *Nat Genet* 2018;50:1271–81.
- [69] Chakravarthy A, Furness A, Joshi K, Ghorani E, Ford K, Ward MJ, et al. Pan-cancer deconvolution of tumour composition using DNA methylation. *Nat Commun* 2018; 9:3220.
- [70] Gejman RS, Chang AY, Jones HF, DiKun K, Hakimi AA, Schietinger A, et al. Rejection of immunogenic tumor clones is limited by clonal fraction. *Elife* 2018;7: e41090.
- [71] Evgin L, Huff AL, Kottke T, Thompson J, Molan AM, Driscoll CB, et al. Suboptimal T-cell therapy drives a tumor cell mutator phenotype that promotes escape from first-line treatment. *Cancer Immunol Res* 2019;7:828–40.
- [72] Parkhurst MR, Robbins PF, Tran E, Prickett TD, Gartner JJ, Jia L, et al. Unique neoantigens arise from somatic mutations in patients with gastrointestinal cancers. *Cancer Discov* 2019;9:1022–35.
- [73] Charoentong P, et al. Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade. *Cell Rep* 2017;18:248–62. <https://doi.org/10.1016/j.celrep.2016.12.019>.
- [74] Cortes-Ciriano I, et al. A molecular portrait of microsatellite instability across multiple cancers. *Nat Commun* 2017;8. <https://doi.org/10.1038/ncomms15180>. 15180-15180.