

REVIEW

Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic

T. A. Chan^{1,2*}, M. Yarchoan³, E. Jaffee³, C. Swanton⁴, S. A. Quezada⁵, A. Stenzinger^{6,7} & S. Peters^{8*}

¹Human Oncology and Pathogenesis Program; ²The Immunogenomics and Precision Oncology Program, Memorial Sloan Kettering Cancer Center, New York; ³Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, USA; ⁴Translational Cancer Therapeutics Laboratory, Francis Crick Institute, London; ⁵Cancer Immunology Unit, Research Department of Haematology, University College London Cancer Institute, London, UK; ⁶Institute of Pathology, University Hospital Heidelberg, Heidelberg; ⁷Germany and German Cancer Consortium (DKTK), Heidelberg Partner Site, Heidelberg, Germany; ⁸Department of Oncology, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland

*Correspondence to: Dr Timothy A. Chan, 1275 York Avenue, Memorial Sloan Kettering Cancer Center, NY 10065, USA. Tel: 646-888-2765; E-mail: chant@mskcc.org
Prof. Solange Peters, Centre Hospitalier Universitaire Vaudois (CHUV), 1011 Lausanne, Switzerland. Tel: +41795560192; E-mail: solange.peters@chuv.ch

Background: Treatment with immune checkpoint blockade (ICB) with agents such as anti-programmed cell death protein 1 (PD-1), anti-programmed death-ligand 1 (PD-L1), and/or anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) can result in impressive response rates and durable disease remission but only in a subset of patients with cancer. Expression of PD-L1 has demonstrated utility in selecting patients for response to ICB and has proven to be an important biomarker for patient selection. Tumor mutation burden (TMB) is emerging as a potential biomarker. However, refinement of interpretation and contextualization is required.

Materials and methods: In this review, we outline the evolution of TMB as a biomarker in oncology, delineate how TMB can be applied in the clinic, discuss current limitations as a diagnostic test, and highlight mechanistic insights unveiled by the study of TMB. We review available data to date studying TMB as a biomarker for response to ICB by tumor type, focusing on studies proposing a threshold for TMB as a predictive biomarker for ICB activity.

Results: High TMB consistently selects for benefit with ICB therapy. In lung, bladder and head and neck cancers, the current predictive TMB thresholds proposed approximate 200 non-synonymous somatic mutations by whole exome sequencing (WES). PD-L1 expression influences response to ICB in high TMB tumors with single agent PD-(L)1 antibodies; however, response may not be dependent on PD-L1 expression in the setting of anti-CTLA4 or anti-PD-1/CTLA-4 combination therapy. Disease-specific TMB thresholds for effective prediction of response in various other malignancies are not well established.

Conclusions: TMB, in concert with PD-L1 expression, has been demonstrated to be a useful biomarker for ICB selection across some cancer types; however, further prospective validation studies are required. TMB determination by selected targeted panels has been correlated with WES. Calibration and harmonization will be required for optimal utility and alignment across all platforms currently used internationally. Key challenges will need to be addressed before broader use in different tumor types.

Key words: mutation, immunotherapy, immune checkpoint, cancer

Introduction

Currently, immune checkpoint blockade (ICB) therapy has increased the overall survival (OS) rates of patients with advanced melanoma, non-small-cell lung cancer (NSCLC), urothelial cancer (UC), renal cell carcinoma (RCC), and other cancer types [1–8]. Tumors often upregulate immune checkpoints to avoid

being detected and killed by the host immune system. Activation of checkpoint cascades such as those controlled by programmed cell death protein (PD-1) or CTLA-4 result in inactivation of tumor-specific T cells and immune evasion [9–12]. Treatment with anti-PD-1, anti-programmed death-ligand 1 (anti-PD-L1), or anti-CTLA-4 reinvigorates T cells and allows the adaptive

immune system to target tumor cells [13, 14]. Detection of tumor and/or immune cell PD-L1 by immunohistochemical measurement has been extensively studied as a predictor of response to anti-PD(L)-1 treatment and has been convincingly demonstrated to be a valid biomarker in some settings. PD-L1 expression by immunohistochemistry (IHC) is an Food and Drug Administration (FDA)-approved companion diagnostic test for pembrolizumab in NSCLC, gastric/gastroesophageal junction adenocarcinoma, cervical cancer and UC [15–20], and has shown some predictive ability across several other cancer types including head and neck and small-cell lung carcinoma [21–23].

PD-L1 quantitation for immunotherapy response prediction is imperfect and there is a need for improved biomarkers of response. The presence of tumor-infiltrating lymphocytes (TILs) might confer a prognostic and a predictive impact [24, 25]. The T-cell-inflamed gene expression profile (GEP) [26], immune gene expression signatures [27, 28], as well as description of the microbiome [29–31] also represent emerging predictive biomarkers.

Cancer is a genetic disease. Neoplastic transformation results from the accumulation of somatic mutations in the DNA of affected cells. These genetic alterations include driver mutations, mutations that directly affect tumor growth such as those in *TP53*, *epidermal growth factor receptor (EGFR)* or *RAS*, and passenger mutations, which are alterations that do not directly impact the growth of the cancer cell [32–34]. Genetic changes in tumors can include non-synonymous mutations largely comprised of missense mutations (point mutations that change the amino acid codon), synonymous mutations (silent mutations that do not alter amino acid coding), insertions or deletions (indels, which can cause frameshifts), and copy number gains and losses. There is dramatic variation in the frequency of each type of these genetic alterations between individual tumors and between different tumor types [35–38]. Tumor mutation burden (TMB) can be used to predict ICB efficacy and has since become a useful biomarker across many cancer types for identification of patients that will benefit from immunotherapy [39–42].

TMB and its relationship to neoantigens

A minority of somatic mutations in tumor DNA can give rise to neoantigens, mutation-derived antigens that are recognized and targeted by the immune system, especially after treatment with agents that activate T cells [39, 43–46]. These mutations can be transcribed and translated, and neoantigen-containing peptides can be processed by the antigen-processing machinery and loaded on to major histocompatibility complex (MHC) molecules for presentation on the cell surface. Importantly, however, not all mutations will generate neoantigens. In fact, only a minority of mutations generate peptides that are properly processed and loaded on to MHC complexes, and even fewer are able to be recognized by T cells [47, 48]. Therefore, not all neopeptides presented on the cell surface are immunogenic [48–50]. Importantly, however, the more somatic mutations a tumor has, the more neoantigens it is also likely to form, and TMB can represent a useful estimation of tumor neoantigenic load.

It is important to note that the presence of immunogenic neoantigens is not the only factor that influences the ability of T cells

to recognize and kill tumor cells. Inactivating mutations in the antigen presentation pathway can occur, which can influence the ability of cells to present peptides to the immune system. Some immunologically relevant genes that can become mutated in cancers include *JAK1*, *JAK2*, *B2M*, *STK11*, and others [51, 52]. The presence of these alterations can modulate the overall effect of TMB or neoantigen load on ICB response.

Variation of TMB across tumor types

As TMB evolves as a relevant tool for the identification of patients likely to respond to ICB, a large effort has been made to characterize the type and the extent of TMB variation across tumor types and histologies. Over the last few years, a large number of studies have been able to map and characterize TMB variations across disease pathologies [37, 38, 53], documenting the highest levels of TMB in melanoma followed by NSCLCs and other squamous carcinomas, while leukemias and pediatric tumors show the lowest levels of TMB. Cancers such as those of the breast, kidney, and ovary display intermediate levels of mutational load. In addition to the variation in the levels of TMB across tumor types, a significant TMB range is also observed within the same cancer type. Within NSCLCs, there is a high degree of variation in TMB across patients attributable to smoking status compared with gastric and breast cancers. Mutational signatures characterized by Stratton et al. have shed light on the origins of tumor somatic mutations [53]. For example, UV light and tobacco carcinogens are the dominant mutational processes in melanoma and NSCLC, respectively. Later in tumor evolution, mutations present in some cells but not others (so-called subclonal mutations) occur. In many tumor types, these mutations have been attributed to the APOBEC cytidine deaminase family and can also occur following cytotoxic chemotherapy in resistant emergent subclones [54–57]. The clonal nature of neoantigens has been shown to be relevant for T cell priming and responses to immune checkpoint inhibitors. Considering that intratumoral heterogeneity (ITH) can vary across tumor types [58] and can potentially impact antitumor immunity, it is important to consider ITH when analyzing TMB.

Variables defining TMB

Considering that high TMB correlates with a greater probability of displaying tumor neoantigens on HLA molecules on the surface of tumor cells [59, 60], it is rational to hypothesize that the tumors with the highest TMB are more likely to respond to ICB agents as this greater mutation load would increase the likelihood of recognition by neoantigen-reactive T cells. Consistent with this hypothesis, several studies have demonstrated an association between high TMB and response to anti-CTLA-4 in melanoma [39, 61] and anti-PD-1 in NSCLC [40]. Subsequent trials retrospectively analyzing TMB association with ICB treatment have been conducted in NSCLC, small-cell lung cancer (SCLC), and melanoma and have shown a correlation between TMB and ICB benefit [41, 61–65]. Figure 1 shows the evolution of TMB as an immunotherapy biomarker over the last several years.

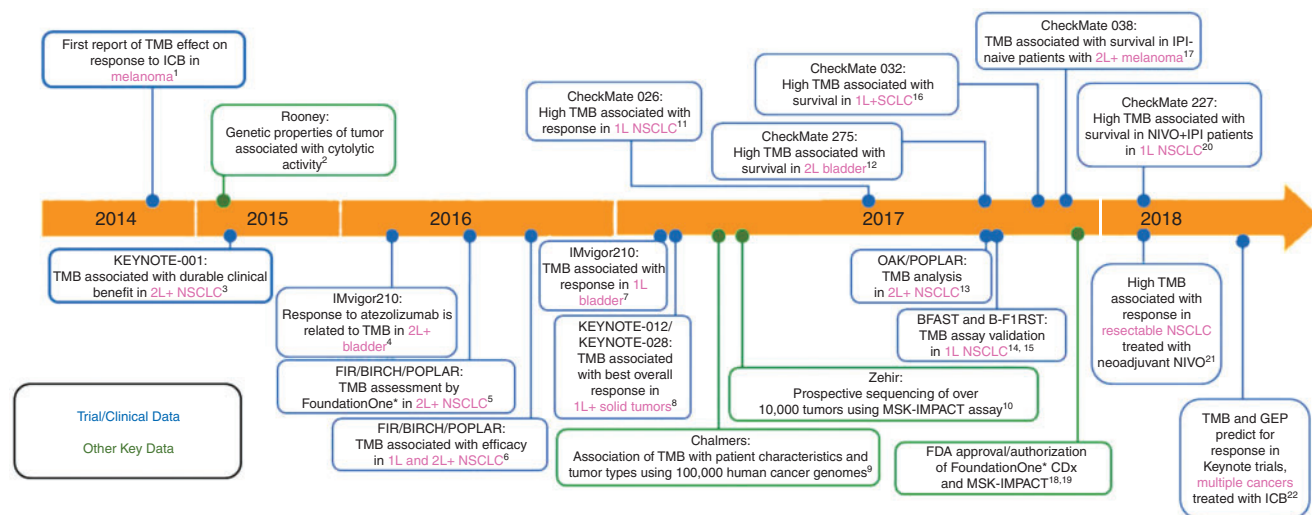


Figure 1. The evolution of tumor mutation burden as an immunotherapy biomarker. Major studies that are important in the development of TMB as a biomarker are shown. Color coding indicates type of study. The studies are ordered as a function of time, with the year indicated in the timeline. ICB, immune checkpoint blockade; 1L, first line; 2L, second line; +, and others; I-O, immune-oncology agent; IPI, ipilimumab; NIVO, nivolumab; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; TMB, tumor mutational burden.

1. Snyder A et al. *N Engl J Med* 2014; 371(23): 2189–2199. 2. Rooney MS et al. *Cell* 2015; 160(1–2): 48–61. 3. Rizvi NA et al. *Science* 2015; 348(6230): 124–128. 4. Rosenberg JE et al. *Lancet* 2016; 387(10031): 1909–1920. 5. Kowanzet M et al. Poster presentation at ESMO 2016. Abstract 77P. 6. Kowanzet M et al. Oral presentation at WCLC 2016. Abstract 6149. 7. Balar AV et al. *Lancet* 2017; 389(10064): 67–76. 8. Seiwer TW et al. *J Clin Oncol* 2018; 36(suppl 5S; abstract 25). 9. Chalmers ZR et al. *Genome Med* 2017; 9(1): 34. 10. Zehir A et al. *Nat Med* 2017; 23(6): 703–713. 11. Carbone DP et al. *N Engl J Med* 2017; 376(25): 2415–2426. 12. Galsky MD et al. Poster presentation at ESMO 2017. Abstract 848PD. 13. Gandara DR et al. Oral presentation at ESMO 2017. Abstract 1295O. 14. Fabrizio DA et al. Poster presentation at ESMO 2017. Abstract 102P. 15. Mok T et al. Poster presentation at ESMO 2017. Abstract 1383TIP. 16. Antonia SJ et al. Oral presentation at WCLC 2017. Abstract 11063. 17. Riaz N et al. *Cell* 2017; 171(4): 934–949. 18. Foundation Medicine. <http://investors.foundationmedicine.com/releasedetail.cfm?ReleaseID=1050380> (11 December 2017, date last accessed). 19. US Food and Drug Administration. <https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm585347.htm> (1 December 2017, date last accessed). 20. Hellmann MD et al. *N Engl J Med* 2018, doi: 10.1056/NEJMoa1801946. 21. Forde PM et al. *N Engl J Med* 2018, doi: 10.1056/NEJMoa1716078. 22. Cristescu et al. *Science* 2018; 362(6411).

TMB-based assays are currently being considered by the FDA for approval as companion diagnostics for ICB agents.

For the initial studies, TMB was determined by whole exome sequencing (WES) carried out on tumor DNA and matching normal DNA. Normal germline variations in DNA sequence between individuals must be identified and removed from consideration in order to tabulate only the somatic alterations, a process that has been well-established [66, 67]. TMB is usually reported as the total number of coding and somatic mutations, but in some cases, can also include insertions and deletions (indels). Exonic TMB is theoretically best measured by WES because this technique samples the entire exome. However, TMB by WES is not yet routinely used as a clinical tool for predicting response to ICB and is used for research only at this time largely due to its greater cost and complexity.

Clinical WES is offered in Clinical Laboratory Improvement Amendments (CLIA)-approved settings and active development to bring these tests into the clinic is ongoing. Recent efforts have begun to validate targeted NGS panels against WES data as these panels are already being used routinely in clinic for oncogenic mutation detection [37, 68]. With the Foundation Medicine (FM) NGS approach (FICDx), TMB was defined as the number of base substitutions (including synonymous mutations) in the coding region of targeted genes. Germline DNA was not sequenced but filtering for both oncogenic driver events and germline status was carried out using public and private variant

databases. The total mutations/megabase (mut/Mb) calculation included both synonymous and non-synonymous mutations requiring a bridging formula for conversion to number of missense mutations as determined by WES. The MSKCC NGS approach (MSK-IMPACT) tabulated non-synonymous mutations using sequencing data from both tumor and germline DNA (for variant calling). The most recent version of this panel sequences 468 genes covering 1.22 Mb. It has been shown that large targeted panels are sufficiently accurate for TMB estimation [37, 69] and panels tested to date (FICDx and MSK-IMPACT), have demonstrated their predictive ability for ICB response [4, 37, 70–73] (Table 1). Both FICDx and MSK-IMPACT have been approved by the US FDA.

Several key variables need to be considered across platforms: depth of sequencing, length of sequencing reads, choice of aligners, variant callers, and filters used. They will all influence TMB measurement (overview provided in Table 1). Preanalytical factors—including sample collection and processing, input material quality and quantity, fixation methodology, and library preparation—affect the quantity and quality of DNA and thus shape TMB estimation values. For example, fixatives and fixation time are preanalytical factors that influence the degree of formaldehyde fixed-paraffin embedded (FFPE)-induced deamination artifacts, which impact analysis of TMB counts. Also, low tumor purity, which can result from sampling errors or a dense tumor microenvironment, may lead to reduced TMB assay sensitivity.

Table 1. Key parameters for some TMB assays

Parameter	WES	FM NGS (F1CDx)	MSKCC NGS (MSK-IMPACT)
No. of genes	~22 000 gene coding regions	324 cancer-related genes	468 cancer-related genes
Types of mutations captured	Coding missense mutations in tumor genome	Coding, missense, and indel mutations per Mb of tumor genome	Coding missense mutations per Mb of tumor genome
Germline mutations	Subtracted using patient-matched normal samples	Estimated via bioinformatics algorithms and subtracted	Subtracted using patient-matched blood samples
Capture region (tumor DNA)	~30 Mb	0.8 Mb	1.22 Mb
TMB definition	No. of somatic, missense mutations in the sequenced tumor genome	No. of somatic, coding mutations (synonymous and non-synonymous), short indels per Mb of tumor genome	No. of somatic, missense mutations per Mb of tumor genome

WES, whole exome sequencing; FM, Foundation Medicine; NGS, next generation sequencing; Mb, megabase.

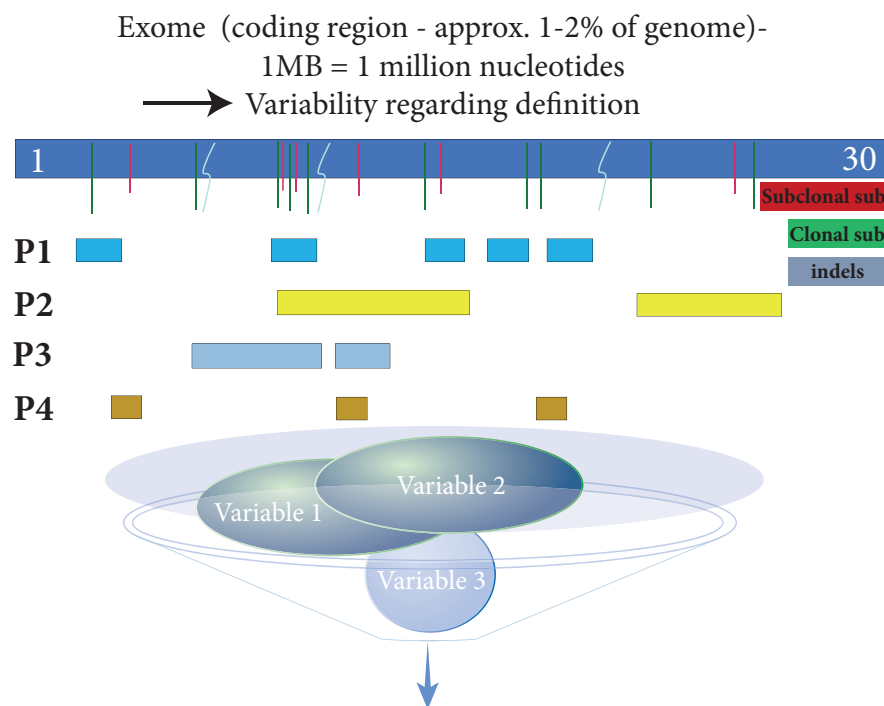


Figure 2. Target regions and sizes of four different hypothetical gene panels (P1–P4). Depending on the size and territory of the exome that is captured by P1–P4, respectively, TMB counts will differ. Other parameters, e.g. filtering of germline variants and cut points for allelic frequencies (blue circles), discussed in this review will influence TMB measurement further.

Sequence coverage and read depth differ between WES and targeted gene panel assays, with WES covering the entire exome coding region and targeted gene panels variably covering pre-specified territories of the exome or genome. Hence, the size and location of the interrogated region differs between targeted gene panel assays and requires careful consideration for accurate TMB assessment (Figure 2). For example, confidence intervals for TMB estimation increase with the use of gene panels that cover only a small region of the genome/exome compared with those that assess a larger area, suggesting that the use of small gene panels can considerably over- or underestimate TMB [69, 74]. While paired germline sequencing would reduce overall false positive mutation calls, a dual analysis is not always carried out. In the

absence of such a comparison, large germline databases are needed to reduce false positive mutation calling and identify germline variants. Depth of sequencing also differs between WES and targeted gene panel assays; sequencing depth is much higher for targeted gene panels than for WES. Both coverage and sequencing depth can determine assay sensitivity and specificity, and therefore, influence TMB estimation output.

Bioinformatic algorithms strongly influence TMB estimation and reporting. As they differ widely across gene panels platforms, it is important that these specific procedures are transparent and open to the scientific and medical community. For example, the mutation types considered for TMB assessment can vary from one assay to another. These may include or exclude short

insertions and deletions (indels) and/or synonymous and nonsynonymous base substitutions/single nucleotide variants. For most retrospective analyses that employed WES, TMB was calculated from missense mutations only, leaving out indels and other mutations, whereas some targeted approaches can include these variant types. Moreover, cut points and filtering algorithms for putative germline variants, variant allele frequency, and FFPE-induced deamination artifacts may vary between assays and can strongly impact TMB values. For example, variant allele frequencies cut-offs can vary from 0.5% to 10%, with lower thresholds increasing the risk of including false positives arising from sequencing artifacts such as C to T transitions introduced by formalin fixation.

TMB definition and correlation with response to ICB

Before exploration of TMB as a biomarker, the expression of PD-L1 in the tumor microenvironment was also being actively investigated as a biomarker and demonstrated some success in identifying patients most likely to benefit. PD-L1 quantitation and has been approved as a companion diagnostic for pembrolizumab in NSCLC [75]. PD-L1 expression as a biomarker has demonstrated an inconsistent record of enriching for response to ICB, which is related to the dynamic and heterogeneous expression of PD-L1 in the tumor microenvironment, assay interpretation, and a lack of standardization across PD-L1 platforms [76], warranting the exploration of new biomarkers for patient selection. TMB will also raise similar practical points, as mentioned previously for PD-L1, possibly with greater complexity and some concerns for accessibility. Additionally, data suggest that TMB will not replace other biomarkers such as IHC-based PD-L1 assessment, but possibly complement them. Importantly, PD-L1 and TMB have consistently been shown to represent independent, not correlated, predictive variables [42, 68, 77–79].

The Checkmate 026 trial in first-line NSCLC comparing nivolumab with standard of care (SOC) demonstrated no improvement in the primary end point of progression-free survival (PFS) in patients with PD-L1 expression $\geq 5\%$ [42, 73]. Based on the emerging phase II TMB data, DNA sequencing of tumors from Checkmate 026 was carried out post hoc and yielded compelling results. Patients with high TMB defined as those with tumors with at least 243 missense mutations (the upper TMB tertile) were found to have significantly improved PFS with nivolumab treatment over SOC chemotherapy [hazard ratio (HR) 0.62]. A caveat to this result is that all patients that underwent this TMB analysis also had PD-L1 expression of $\geq 1\%$. Importantly, patients with mutations in the lower 2/3 (less than 243 mutations) had a worse PFS with nivolumab treatment compared with SOC (HR 1.82), demonstrating the importance of low TMB as a negative predictor of benefit in the context of effective SOC options. Similarly, in UC, a phase III trial in platinum-treated patients, IMvigor211, failed to meet its primary end point of OS improvement with atezolizumab compared with SOC chemotherapy. Even with pre-specified PD-L1 selection, no improvement in OS was demonstrated. Again, based on compelling phase II TMB data in UC, a post hoc analysis of TMB employing a threshold of 9.65 mut/Mb was conducted and showed a non-

significant but numerically improved OS (HR 0.68). Strikingly, the subgroup of patients with both a high TMB and increased PD-L1 (IC2/3) had an HR of 0.50 with atezolizumab treatment [21].

Although TMB and PD-L1 do not co-associate in multiple trials [42, 80, 81], greater benefit with single agent anti-PD-1 and anti-PD-L1 is consistently observed with high TMB and PD-L1 expression, suggesting these independent biomarkers can be used in concert [82, 83]. Given the high response rates when combining PD-L1 with CTLA-4 blockade in initial solid tumor studies, there was speculation that patients with lower TMB tumors could benefit by the addition of anti-CTLA-4 to anti-PD-1 but this was not the case. In NSCLC, using a similar TMB threshold equivalent to ~ 200 missense mutations (10 mut/Mb), the benefit with combination ICB was independent of PD-L1 expression in the ≥ 10 mut/Mb patient population [62]. A similar benefit that was dependent on TMB but independent of PD-L1 expression was observed in SCLC with nivolumab and ipilimumab in combination [62, 63]. These data are further supported in a phase III NSCLC trial (Checkmate 227) comparing first-line nivolumab + ipilimumab with SOC chemotherapy [84]. This study showed a significantly improved PFS in high TMB versus SOC in both PD-L1 positive (HR 0.62) and negative (HR 0.48) patients. The above observations suggesting that TMB, and not necessarily PD-L1 status, associates with response to combination ICB (ipilimumab plus nivolumab) and is consistent with a scenario where tumors with high TMB are potentially immunogenic but T-cell infiltration and/or activation is controlled in a CTLA-4-dependent manner. This control can be either cell intrinsic by upregulation of CTLA-4 on effector T cells (Teff), or by trans-regulation via CTLA-4^{hi} regulatory T cells (Treg). Lack of infiltration or T-cell activation would result in reduced IFN- γ within tumors, correlating with low or negative PD-L1 status at diagnosis. Upon CTLA-4-blockade or anti-CTLA-4-driven Treg depletion [85], re-activated effector T cells would upregulate PD-1 and promote PD-L1 upregulation, hence explaining the synergy with PD-1-blockade. Altogether, the previous supposition that PD-L1 positive, inflamed tumors respond to ICB but PD-L1 negative, non-inflamed tumors do not require adjudication based on the emerging data with TMB and combination immunotherapy (Figure 3).

Interestingly, TMB has also shown predictive value in immunotherapy modalities other than immune checkpoint blockade therapy. Lauss et al. observed that tumor mutation and neoantigen load predicted improved PFS and OS for melanoma patients who were treated with adoptive T cell transfer therapy. This finding suggests that greater numbers of potential neoantigens in tumors may promote better clinical response to expanded and reinfused TILs [86].

Is TMB ready to enter the clinic?

The first FDA approval based on the concept of mutation burden was anti-PD1 therapy for patients with microsatellite instability-high (MSI-H) cancers. MSI is one of a number of defects in DNA repair that results in the accumulation of very high levels of TMB. In the initial reports of anti-PD1 therapy in which multiple different types of tumors were treated, only one of the 33 patients with

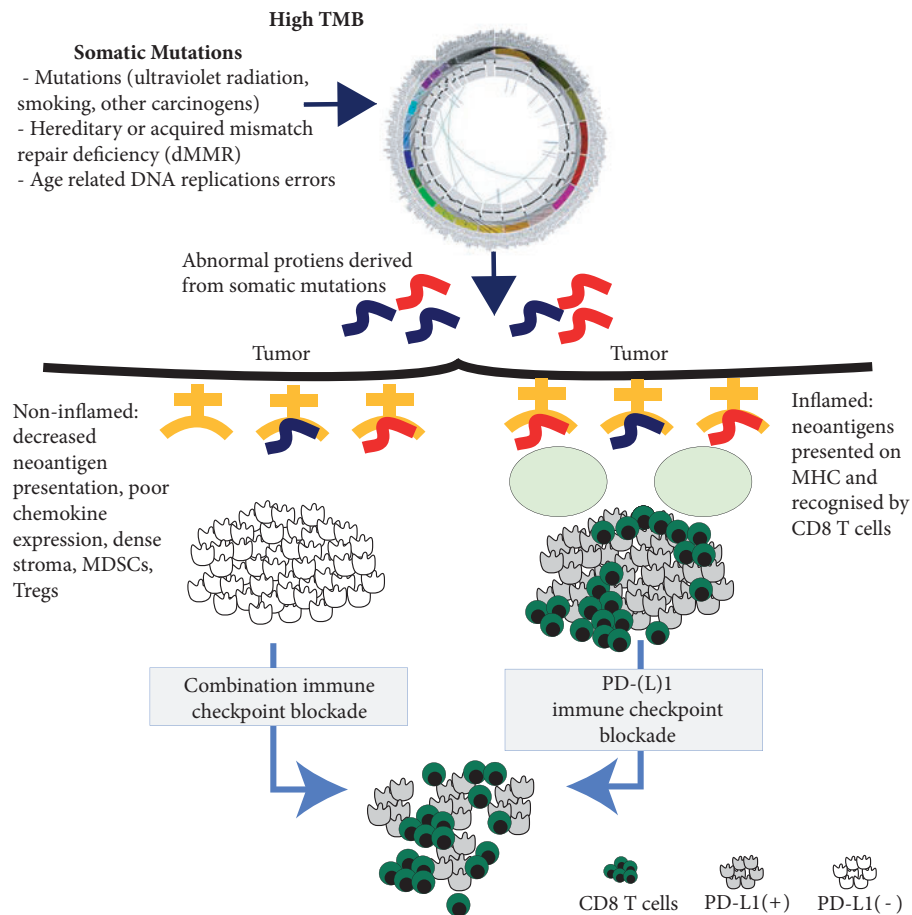


Figure 3. Mutations, neoantigens, and immune checkpoint blockade. Somatic mutations can generate neopeptides that are presented by MHC molecules. Both inflamed and non-inflamed tumors, as well as PD-L1 positive or negative tumors, can respond to immune checkpoint blockade therapy. TMB, tumor mutation burden; MMR, mismatch repair.

colorectal cancer had an objective response to treatment [87, 88]. The discovery that this single responder had MSI-H colorectal cancer led to successful prospective clinical trials of pembrolizumab in adult and pediatric patients with MSI-H or DNA mismatch repair-deficient (dMMR) solid tumors, and the rapid approval of pembrolizumab in this biomarker-defined group of patients [89, 90]. Importantly, this was the first tissue-agnostic drug approval and the first approved companion biomarker assay for any cancer therapy by the FDA [91, 92], and the approval foretells a future in which tumor genomic analyses can be used to personalize cancer immunotherapy. Although the majority of patients with MSI-H solid tumors also have a high TMB, only 16% of patients with high TMB tumors are MSI-H [37]. Whereas NSCLC might become one of the first indications for TMB application as a biomarker (as a variable itself rather than via a surrogate-like MMR deficiency), MSI-H is extremely rare in this entity.

Can the experience with MSI-H tumors be applied to MSI stable tumors? The median number of mutations in MSI-H tumors is often in the thousands [89, 91], whereas in microsatellite stable NSCLC, e.g. it is ~200 mutations. Furthermore, the aggregate data from multiple studies in SCLC, NSCLC, and UC approximate the TMB threshold required to enrich for benefit with ICB (at least in high TMB tumors) to reside at ~200 missense

mutations, which is equivalent to 10 mut/Mb by FoundationOne testing or ~7 mut/Mb by MSK-IMPACT testing [63, 70, 71, 73]. Employing higher thresholds of selection to 16.2 mut/Mb with atezolizumab [80] and 15 mut/Mb with ipilimumab and nivolumab [71] in NSCLC did not improve efficacy. Given the consistent findings for these tumor types, one could envision the use of TMB selection in the clinic for patient decision-making. Checkmate 227 in first-line NSCLC was the first study to prospectively include PFS in high TMB (≥ 10 mut/Mb) patients as a co-primary end point. This trial demonstrated in TMB high patients randomized to ipilimumab and nivolumab versus chemotherapy a significant improvement in PFS (7.1 versus 3.2 months) and response rate (45.3% versus 24.6%) [84]. A summary of the clinical data defining a TMB threshold with ICB treatment is presented in Table 2. Several ongoing clinical trials are using TMB as a key stratification factor or a landmark end point (Table 3). They will help decipher the role of TMB in the treatment decision process across cancer types and, by using distinct TMB thresholds and definitions, support a more refined definition of TMB utility.

Some NGS assay providers have already nominated TMB thresholds for certain applications. For example, the 10 mut/Mb threshold (via FICDx NGS assay) captures a significant fraction of cancer patients and may be particularly useful to identify

Table 2. Key trials defining a TMB threshold for ICB benefit

Cancer	Trial and treatment	Method	Threshold defined	RR	PFS	OS	Ref.
Melanoma	Anti-CTLA-4	WES	100 mutations			OS advantage	[39]
Melanoma	CM 038 Phase II nivolumab	WES	100 mutations			OS advantage in ipilimumab naive	[64]
NSCLC	KN 001 phase I/II Pembrolizumab	WES	200 mutations	59% versus 12%	NR versus 3.4 months		[40]
NSCLC	BIRCH, FIR phase II Atezolizumab	FM NGS	9.9 mut/Mb	25% versus 14%	HR 0.64	HR 0.87	[70]
NSCLC	POPLAR randomized phase II atezolizumab versus docetaxel	FM NGS	9.9 mut/Mb	20% versus 4%	7.3 versus 2.8 months	16.2 versus 8.3 months	[70]
NSCLC	MSKCC: various immunotherapies	MSKCC NGS	7.4 mut/Mb	38.6% versus 25%			[68]
NSCLC	CM 012 Nivolumab/ipilimumab	WES	158 mutations	51% versus 13%	17.1 versus 3.7 months		[62]
NSCLC	CM 568 Nivolumab/ipilimumab	FM NGS	10 mut/Mb	44% versus 12%	7.1 versus 2.6 months		[71]
SCLC	CM 032 phase II nivolumab versus nivolumab/ipilimumab	WES	248 mutations	46.2% versus 21.3%	7.8 versus 1.4 months	22 versus 5.4 months	[63]
NSCLC	CM 026 randomized phase III nivolumab versus chemotherapy	WES	>243 mutations	47% versus 23%	HR 0.62	HR 1.10	[42]
NSCLC	CM 227 randomized phase III nivolumab/ipilimumab versus chemotherapy	FM NGS	>10 mut/Mb	45.3% versus 24.6%	7.1 versus 3.2 months	NA	[77]
UC	CM 275 phase II Nivolumab	WES	≥170 versus <85 mutations	31.9% versus 10.9%	3 versus 2 months	11.63 versus 5.72 months	[78]
UC	IMVigor210 phase II Atezolizumab	FM NGS	16 mut/Mb			OS advantage	[72]
UC	IMVigor211 phase III Atezolizumab versus chemotherapy	FM NGS	>9.65 mut/Mb			HR 0.68	[73]
Solid tumor	Various immunotherapies	FM NGS	20 mut/Mb	58% versus 20%	12.8 versus 3.3 months	NR versus 16.3 months	[79]
Solid tumor	KN 028 and KN 012 Pembrolizumab	WES	102 mutations	30% versus 7%	109 versus 59 days		[81]
HNSCC	KN 012 and KN 055 pembrolizumab	WES	175 mutations		HR 0.64	HR 0.98	[83]

CM, checkmate; KN, keynote; NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer; UC, urothelial cancer; HNSCC, head and neck squamous cell carcinoma; WES, whole exome sequencing; NGS, next generation sequencing; HR, hazard ratio; NA, not applicable; mut, mutation; FM, Foundation Medicine.

cancer types with lower proportion of high TMB tumors, such as MSI testing can currently accomplish (Figure 4). However, applications to additional other tumor types will also require their own threshold validation. This must be examined before application to different malignancies. Furthermore, there is a need for harmonization of TMB reporting across different NGS assays currently in use and new NGS targeted assay platforms will also require their own validation.

TMB limitations and perspectives

TMB is not without limitations. It is a relatively new type of biomarker, and defining standards for determination and reporting of TMB are not well established. Proteins generated from gene fusions and post-translational modifications of non-mutated

proteins are not accounted for in current iterations of TMB, but nonetheless may contribute to neoantigenic load. More critically, current iterations of the TMB assign an equal weight to each tumor mutation, but it is increasingly clear that not all mutations are created equal [53, 56]. Some mutations result in the formation of higher 'quality' antigens, which are more readily identified as 'non-self' by the immune system and are more likely to induce a robust antitumor immune response. Antigens resulting from viral open reading frames in a cancer's genome are an example of a high-quality antigen. This may be the reason the subset of Merkel-cell carcinoma that is associated with the Merkel-cell polyomavirus has a moderate TMB [93] but amongst the highest response rates of any tumor type with anti-PD1 therapy [94].

Another example of a tumor type with intermediate levels of TMB but a high response rate to ICB is RCC [3, 95, 96]. Recent work by Turajlic et al. shows that in addition to single nucleotide

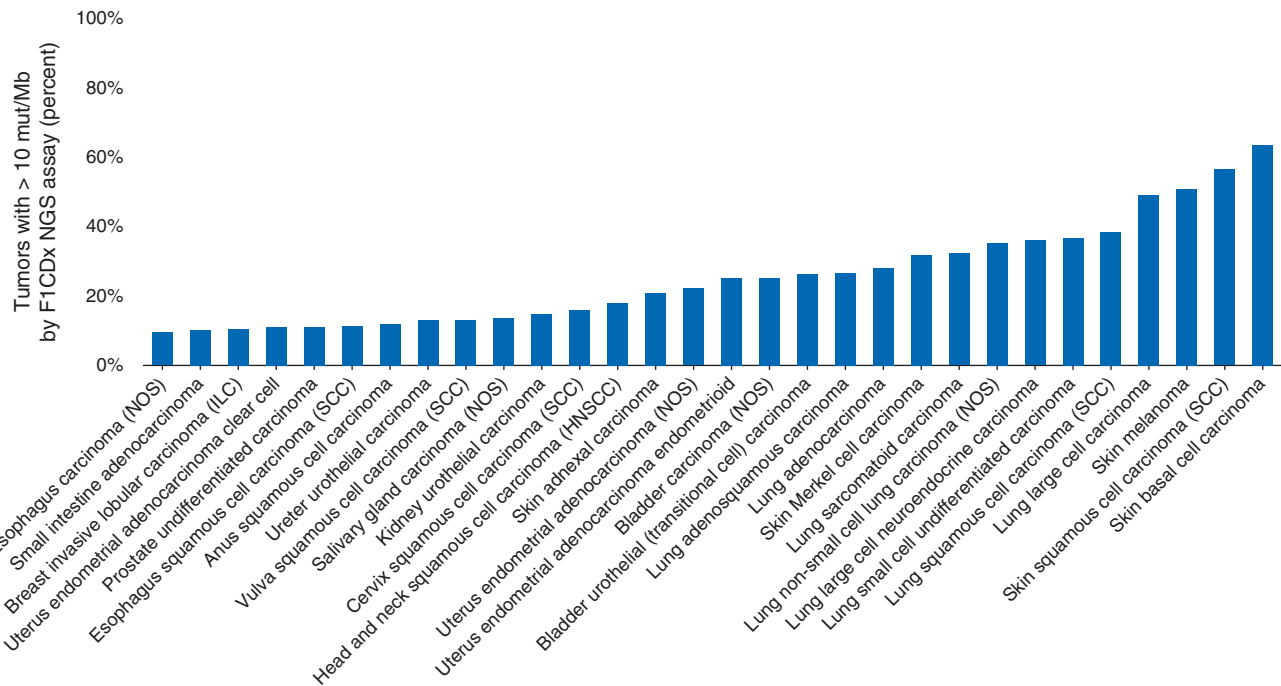


Figure 4. Impact of TMB pan-cancer: percent of solid tumors with TMB ≥ 10 mut/Mb. Analysis of top 30 solid tumor types selected from 104,814 total cases sorted by percent of cases with TMB ≥ 10 mut/Mb according to the Foundation Medicine database. TMB is defined as the number of somatic synonymous and non-synonymous base substitutions and indels divided by the region over which it was counted. Only cancer types with at least 100 total cases are reported. The average across all solid tumor types was 13.3%.

variants, frameshift mutations generated by insertion and deletions that result in the generation of an entirely new peptide amino acid chain before a stop codon being reached, also contribute to the generation of potent tumor neoantigens and the overall TMB of cancers [97]. Interestingly, they demonstrated that RCCs have the highest frequency and number of indel mutations across cancer types. In MSI tumors, genetic instability manifests as short indels resulting from lack of repair of slippages during replication. This, in MMR deficient tumors, indels may also need to be considered in defining total TMB.

Another challenge is to understand how to use TMB while taking into account specific mutations that have been shown to influence response to ICB treatment. For example, mutations in genes such as *JAK1*, *JAK2*, *$\beta 2M$* , *SKT11*, *SERPINB3*, and *SERPINB4* have been shown to affect ICB response [51, 98]. Some mutations such as those in *JAK1* and *JAK2* are rare and do not validate in all patient cohorts [64, 99]. Similarly, some immune evasion mechanisms such as transforming growth factor β (TGF- β) signaling [100] or indoleamine 2,3-dioxygenase (IDO) activity may influence ICB response [101]. The importance of these alterations will have to be tested in prospective trials. For the variables that are currently validated as most useful, a model taking into consideration TMB, individual mutations or pathways that affect ICB outcomes, and PD-L1 levels—perhaps in the form of a nonogram—could be developed to further improve predictive models. Similar models are in use for predicting the likelihood of disease control in patients with prostate cancer and for quantifying benefit from chemotherapy for breast cancer patients [102–104]. It should be noted that the use of expression signatures have had a checkered past in the cancer biomarker field. Despite thousands of expression signatures nominated for use as biomarkers, very few

have found reliable use in the clinic, especially when the expression signatures do not correlate with reproducible genetic alterations. Therefore, use of expression signatures in the immuno-oncology setting needs to be carefully vetted. Indeed, the history of cancer biomarker development suggests that genetic alterations and not simply altered expression of a given target or pathway of interest, which can often be reversible, are more robust predictors of response to a therapy targeting that pathway. Despite expression of IDO1 in tumors, genetic evidence that IDO is a cancer driver is lacking. It is perhaps not surprising, then, that a recent large phase III trial testing an IDO inhibitor in combination with anti-PD-1 did not show benefit [105], leading to the widespread discontinuation of IDO inhibitor development. However, some expression signatures appear to be promising for detection of successful anti-cancer immunity. Interestingly, Cristescu et al. show that TMB and a T-cell inflamed gene expression signature can both provide predictive value for clinical response in patients treated on four Keynote trials [26]. Furthermore, the utility of TMB and other biomarkers noted above in patients treated with ICB plus chemotherapy is unclear and will need to be studied. If TMB is predictive in these settings, it is likely that new thresholds may need to be established.

Regardless, building future algorithms for identifying patients that will benefit from ICB will likely require assessment of tumor and immune cells qualitatively and quantitatively. TMB, specific mutations in oncogenes, as well as PD-L1 expression will describe the tumor component while immune cell PD-L1 expression, HLA genotype, TCR repertoire, and possibly immune signatures (as determined, e.g. by gene expression analysis) might be taken into account for the immune component of response.

Table 3. Ongoing clinical trials registered in ClinicalTrials.gov investigating immune checkpoint blockade in the context of TMB assessment

Trial name (NCT number)	Phase	Tumor type	Therapy
1 MK-3475-016 (NCT01876511)	II	MSI-positive or MSI-negative CRC or other cancers	Pembrolizumab
2 PRO 02 (NCT02091141)	II	Advanced solid tumors	Multiple targeted therapies, including atezolizumab
3 IMpower110 (NCT02409342)	III	NSCLC	Atezolizumab versus chemotherapy
4 OpACIN (NCT02437279)	I	Melanoma	Adjuvant ipilimumab+nivolumab
5 CA209-260 (NCT02553642)	II	Melanoma or UC	Nivolumab±ipilimumab
6 TAPUR (NCT02693535)	II	Advanced solid tumors	Multiple targeted therapies; including pembrolizumab and nivolumab+ipilimumab
7 AAAQ5450 (NCT02710396)	II	NSCLC	Pembrolizumab±chemotherapy
8 NCI-2016-00666 (NCT02775851)	II	Desmoplastic melanoma	Pembrolizumab
9 CheckMate 714 (NCT02823574)	II	SCCHN	Ipilimumab+nivolumab
10 MultiVir Ad-p53-001 (NCT02842125)	I/II	Advanced solid tumors	Adenoviral p53+pembrolizumab/nivolumab or chemotherapy
11 B-F1RST (NCT02848651)	II	NSCLC	Atezolizumab
12 NCI-2016-01589 (NCT02949843)	II	NSCLC (EGFR-mutated)	Multiple, including nivolumab and pembrolizumab
13 OpACIN-neo (NCT02977052)	II	Melanoma	Neoadjuvant ipilimumab+nivolumab
14 NCI-2016-01698 (NCT02965716)	II	Melanoma	Pembrolizumab+talinogene laherparepvec (virus therapy)
15 PEER (NCT02990845)	I/II	Breast	Pembrolizumab+exemestane (aromatase inhibitor)+leuprolide (anti-GnRH)
16 ULTIMATE (NCT02997995)	II	Breast	Tremelimumab+durvalumab+exemestane (aromatase inhibitor)
17 CL-PTL-126 (NCT03073525)	II	Gynecological cancers	Atezolizumab+vigil (immuno-stimulatory autologous cellular therapy)
18 CA209-777 (NCT03091491)	II	NSCLC (EGFR mutant positive)	Nivolumab±ipilimumab
19 ISABR (NCT03148327)	I/II	NSCLC	Durvalumab+radiation
20 CMIW815X2102J (NCT03172936)	I	Advanced solid tumors and lymphomas	PDR001 (anti-PD-1) + MIW815/ADU-S100 (IFN genes stimulator)
21 B-FAST (NCT03178552)	II/III	NSCLC	Atezolizumab versus chemotherapy
22 KELLY (NCT03222856)	II	Breast (HR+/HER2- subtype)	Pembrolizumab+chemotherapy
23 RESPONDER (NCT03263039)	II	UC	Pembrolizumab
24 IFG-NIB-01 (NCT03289819)	II	Breast (triple negative subtype)	Pembrolizumab+chemotherapy
25 NET-002 (NCT03278379)	II	Neuroendocrine	Avelumab
26 B9991023 (NCT03317496)	II	NSCLC, UC	Avelumab+chemotherapy
27 CA209-929 (NCT03342417)	II	Breast, ovarian, gastric	Ipilimumab+nivolumab
28 Javelin Parp Medley (NCT03330405)	Ib/II	Advanced solid tumors	Avelumab+talazoparib (anti-PARP)
29 R2810-ONC-1763 (NCT03430063)	II	NSCLC	Cemiplimab (anti-PD-1)±ipilimumab
30 NIVES (NCT03469713)	II	RCC	Nivolumab+radiotherapy
31 Javelin Medley VEGF (NCT03472560)	II	NSCLC, UC	Avelumab+axitinib (TKI)

Continued

Table 3. *Continued*

Trial name (NCT number)	Phase	Tumor type	Therapy
32 PERSEUS1 (NCT03506997)	II	Prostate	Pembrolizumab
33 ARETHUSA (NCT03519412)	II	CRC	Pembrolizumab, temozolomide
34 KEYNOTE-495 (NCT03516981)	II	NSCLC	Pembrolizumab+lenvatinib (anti-VEGF) or MK-4280 (anti-LAG-3)
35 MOVIE (NCT03518606)	I/II	Advanced solid tumors	Durvalumab+tremelimumab+chemotherapy
36 CIBI308A102 (NCT03568539)	I/II	Advanced solid tumors	Sintilimab (anti-PD-1)
37 Ad-p53-002 (NCT03544723)	II	SCCHN	Ad-p53+nivolumab

Discussion

Conclusions

The relationship between TMB and response to immune checkpoint inhibitors is paving the way towards a precision immunogenetics approach to cancer treatment. From the initial clinical observations associating tumors with genetic damage from environmental factors, we have begun a journey of discovery that will greatly broaden the scope and practice of precision oncology. TMB and other genetic determinants of response to immunotherapy have already provided exciting new avenues to make cancer treatment more precise. Nevertheless, challenges remain. Our knowledge of how genetics shapes immune response is unclear and this gap in knowledge must be bridged in order to build even better predictive models. How TMB can be used in combination with PD-L1 quantitation or measures of tumor inflammation needs to be improved. Moreover, the impact of how HLA genotype and other germline variations influences the effect of TMB and response to ICB needs to be explored further [106]. Lastly, as discussed above, we highlight the need for cross-assay standardization of NGS methods and solidification of interpretation of TMB levels in order to ensure reliable treatment decisions in the clinic based on tumor genetics.

Acknowledgements

We thank Naiyer Rizvi and the Chan laboratory for excellent discussions. We thank Dr Vincent Miller at Foundation Medicine for helpful discussions.

Funding

TAC is supported in part by NIH/NCI Cancer Center Support Grant P30 CA008748, NIH R35 CA232097, and the Mellnikoff Precision Immunotherapy Kidney Cancer Fund. MY acknowledges research grants from Exelixis, Merck, and Bristol-Myers Squibb. AS reports research funding from Chugai and BMS. SP has received grants/research support from the following: (sub)investigator in trials sponsored by Amgen, AstraZeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Clovis, F. Hoffmann-La Roche, Illumina,

Merck Sharp and Dohme, Merck Serono, Novartis, and Pfizer. No additional grant funding is applicable.

Disclosure

EJ has licensed GVAX/Listeria vaccines to AduroBiotech with potential to receive royalties. EJ is on the Genocea, Adaptive Biotech, DragonFly and CSTONE SABs. EJ acknowledges grants from Aduro Biotech, BMS, Amgen and Hertig. TAC is a co-founder of Gritstone Oncology and holds equity. TAC holds equity in An2H. TAC acknowledges grant funding from Bristol-Myers Squibb, AstraZeneca, Illumina, Pfizer, An2H, and Eisai. TAC has served as a paid advisor for Bristol-Myers Squibb, Illumina, Eisai, and An2H. MSK has licensed the use of TMB for the identification of patients that benefit from immune checkpoint therapy to PGDx. MSK, RMS, DC, LGTM, and TAC receive royalties as part of this licensing agreement. MY is a paid advisor for Eisai and received research support from Genentech/Roche. AS acknowledges advisory board honoraria from Bayer, BMS, AstraZeneca, Novartis, ThermoFisher, Illumina. AS reports speaker's honoraria from BMS, Bayer, MSD, Roche, Illumina, AstraZeneca, Novartis, ThermoFisher, Takeda. SP acknowledges receipt of honoraria or consultation fees from: Abbvie, Amgen, AstraZeneca, Bayer, Biocartis, Boehringer-Ingelheim, Bristol-Myers Squibb, Clovis, Daiichi Sankyo, Debiopharm, Eli Lilly, F. Hoffmann-La Roche, Foundation Medicine, Illumina, Janssen, Merck Sharp and Dohme, Merck Serono, Merrimack, Novartis, Pharma Mar, Pfizer, Regeneron, Sanofi, Seattle Genetics and Takeda. SP has given lectures in the following company's organized public events: AstraZeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Eli Lilly, F. Hoffmann-La Roche, Merck Sharp and Dohme, Novartis, Pfizer, Takeda. SP has been (sub)investigator in trials (institutional financial support for clinical trials) sponsored by Amgen, AstraZeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Clovis, F. Hoffmann-La Roche, Illumina, Merck Sharp and Dohme, Merck Serono, Novartis, and Pfizer. SAQ is a scientific founder and paid scientific advisor of Achilles Therapeutics. SAQ is also a scientific advisor to F. Hoffmann-La Roche, BBT, Bicycle therapeutics, Morphosys and Ioncturas. CS discloses receipt of grants/research support from Pfizer, AstraZeneca, BMS, Ventana; receipt of honoraria or consultancy fees by Boehringer Ingelheim, Eli Lilly,

Servier, Novartis, Roche-Genentech, GlaxoSmithKline, Pfizer, BMS, Celgene, AstraZeneca, Illumina, Sarah Cannon Research Institute; stock options/shareholders of Apogen Biotechnologies, Epic Bioscience, GRAIL and has stock options and is co-founder of Achilles therapeutics and patents for method for treating cancer: PCT/EP2016/059401, immune checkpoint intervention in cancer: PCT/EP2016/071471; method: PCT/GB2018/051893; method: PCT/GB2018/051892; method: PCT/GB2018/052004.

References

- Hodi FS, O'Day SJ, McDermott DF et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010; 363: 711–723.
- Borghaei H, Paz-Ares L, Horn L et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med* 2015; 373(17): 1627–1639.
- Motzer RJ, Escudier B, McDermott DF et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. *N Engl J Med* 2015; 373(19): 1803–1813.
- Rosenberg JE, Hoffman-Censits J, Powles T et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet* 2016; 387(10031): 1909–1920.
- Wolchok JD, Kluger H, Callahan MK et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med* 2013; 369(2): 122–133.
- Motzer RJ, Tannir NM, McDermott DF et al. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. *N Engl J Med* 2018; 378(14): 1277–1290.
- Antonia SJ, Villegas A, Daniel D et al. Durvalumab after chemoradiotherapy in stage III non-small-cell lung cancer. *N Engl J Med* 2017; 377(20): 1919–1929.
- Nghiem PT, Bhatia S, Lipson EJ et al. PD-1 blockade with pembrolizumab in advanced Merkel-cell carcinoma. *N Engl J Med* 2016; 374(26): 2542–2552.
- Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J Exp Med* 1995; 182(2): 459–465.
- Brown KE, Freeman GJ, Wherry EJ, Sharpe AH. Role of PD-1 in regulating acute infections. *Curr Opin Immunol* 2010; 22(3): 397–401.
- Iwai Y, Ishida M, Tanaka Y et al. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc Natl Acad Sci USA* 2002; 99(19): 12293–12297.
- Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity* 2004; 21(2): 137–148.
- Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science* 1996; 271(5256): 1734–1736.
- Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012; 12(4): 252–264.
- Reck M, Rodriguez-Abreu D, Robinson AG et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med* 2016; 375(19): 1823–1833.
- Herbst RS, Baas P, Kim DW et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016; 387(10027): 1540–1550.
- Bellmunt J, de Wit R, Vaughn DJ et al. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. *N Engl J Med* 2017; 376(11): 1015–1026.
- Muro K, Chung HC, Shankaran V et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. *Lancet Oncol* 2016; 17(6): 717–726.
- Fuchs CS, Doi T, Jang RW et al. Safety and efficacy of pembrolizumab monotherapy in patients with previously treated advanced gastric and gastroesophageal junction cancer: phase 2 Clinical KEYNOTE-059 Trial. *JAMA Oncol* 2018; 4(5): e180013.
- Chung HC, Schellens JHM, Delord JP et al. Pembrolizumab treatment of advanced cervical cancer: updated results from the phase 2 KEYNOTE-158 study. *JCO* 2018; 36(Suppl 15): 5522–5522.
- Powles T, Duran I, van der Heijden MS et al. Atezolizumab versus chemotherapy in patients with platinum-treated locally advanced or metastatic urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* 2018; 391(10122): 748–757.
- Ferris RL, Blumenschein G Jr, Fayette J et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. *N Engl J Med* 2016; 375(19): 1856–1867.
- Ott PA, Elez E, Hiet S et al. Pembrolizumab in patients with extensive-stage small-cell lung cancer: results from the phase Ib KEYNOTE-028 Study. *J Clin Oncol* 2017; 35(34): 3823–3829.
- Tumeh PC, Harview CL, Yearley JH et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014; 515(7528): 568–571.
- Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer* 2012; 12(4): 298–306.
- Cristescu R, Mogg R, Ayers M et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science* 2018; 362(6411): doi: 10.1126/science.aar3593.
- Auslander N, Zhang G, Lee JS et al. Robust prediction of response to immune checkpoint blockade therapy in metastatic melanoma. *Nat Med* 2018; 24(10): 1545–1549.
- Jiang P, Gu S, Pan D et al. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. *Nat Med* 2018; 24(10): 1550–1558.
- Roy S, Trinchieri G. Microbiota: a key orchestrator of cancer therapy. *Nat Rev Cancer* 2017; 17(5): 271–285.
- Routy B, Le Chatelier E, Derosa L et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 2018; 359(6371): 91–97.
- Gopalakrishnan V, Spencer CN, Nezi L et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* 2018; 359(6371): 97–103.
- Sjoberg T, Jones S, Wood LD et al. The consensus coding sequences of human breast and colorectal cancers. *Science* 2006; 314(5797): 268–274.
- Wood LD, Parsons DW, Jones S et al. The genomic landscapes of human breast and colorectal cancers. *Science* 2007; 318(5853): 1108–1113.
- Kinzler KW, Vogelstein B. Landscaping the cancer terrain. *Science* 1998; 280(5366): 1036–1037.
- Lawrence MS, Stojanov P, Polak P et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* 2013; 499(7457): 214–218.
- Ciriello G, Miller ML, Aksoy BA et al. Emerging landscape of oncogenic signatures across human cancers. *Nat Genet* 2013; 45(10): 1127–1133.
- Chalmers ZR, Connelly CF, Fabrizio D et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med* 2017; 9(1): 34.
- Zehir A, Benayed R, Shah RH et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med* 2017; 23(6): 703–713.
- Snyder A, Makarov V, Merghoub T et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med* 2014; 371(23): 2189–2199.
- Rizvi NA, Hellmann MD, Snyder A et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015; 348(6230): 124–128.
- Hugo W, Zaretsky JM, Sun L et al. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell* 2016; 165(1): 35–44.
- Carbone DP, Reck M, Paz-Ares L et al. First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. *N Engl J Med* 2017; 376(25): 2415–2426.

43. Matsushita H, Vesely MD, Koboldt DC et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoeediting. *Nature* 2012; 482(7385): 400–404.
44. Riaz N, Morris L, Havel JJ et al. The role of neoantigens in response to immune checkpoint blockade. *Int Immunol* 2016; 28(8): 411–419.
45. Ott PA, Hu Z, Keskin DB et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* 2017; 547(7662): 217–221.
46. Cohen CJ, Gartner JJ, Horovitz-Fried M et al. Isolation of neoantigen-specific T cells from tumor and peripheral lymphocytes. *J Clin Invest* 2015; 125(10): 3981–3991.
47. Coulie PG, Van den Eynde BJ, van der Bruggen P, Boon T. Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy. *Nat Rev Cancer* 2014; 14(2): 135–146.
48. Carreno BM, Magrini V, Becker-Hapak M et al. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science* 2015; 348(6236): 803–808.
49. Bassani-Sternberg M, Braunlein E, Klar R et al. Direct identification of clinically relevant neopeptides presented on native human melanoma tissue by mass spectrometry. *Nat Commun* 2016; 7: 13404.
50. Snyder A, Chan TA. Immunogenic peptide discovery in cancer genomes. *Curr Opin Genet Dev* 2015; 30: 7–16.
51. Zaretsky JM, Garcia-Diaz A, Shin DS et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. *N Engl J Med* 2016; 375(9): 819–829.
52. Skoulidis F, Goldberg ME, Greenawalt DM et al. STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma. *Cancer Discov* 2018; 8(7): 822–835.
53. Alexandrov LB, Nik-Zainal S, Wedge DC et al. Signatures of mutational processes in human cancer. *Nature* 2013; 500(7463): 415–421.
54. Jamal-Hanjani M, Wilson GA, McGranahan N et al. Tracking the evolution of non-small-cell lung cancer. *N Engl J Med* 2017; 376(22): 2109–2121.
55. McGranahan N, Favero F, de Bruin EC et al. Clonal status of actionable driver events and the timing of mutational processes in cancer evolution. *Sci Transl Med* 2015; 7: 283ra254.
56. McGranahan N, Furness AJ, Rosenthal R et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* 2016; 351(6280): 1463–1469.
57. Murugaesu N, Wilson GA, Birkbak NJ et al. Tracking the genomic evolution of esophageal adenocarcinoma through neoadjuvant chemotherapy. *Cancer Discov* 2015; 5(8): 821–831.
58. Morris LG, Riaz N, Desrichard A et al. Pan-cancer analysis of intratumor heterogeneity as a prognostic determinant of survival. *Oncotarget* 2016; 7: 10051–10063.
59. Peggs KS, Segal NH, Allison JP. Targeting immunosuppressive cancer therapies: accentuate the positive, eliminate the negative. *Cancer Cell* 2007; 12(3): 192–199.
60. Segal NH, Parsons DW, Peggs KS et al. Epitope landscape in breast and colorectal cancer. *Cancer Res* 2008; 68(3): 889–892.
61. Van Allen EM, Miao D, Schilling B et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* 2015; 350(6257): 207–211.
62. Hellmann MD, Nathanson T, Rizvi H et al. Genomic features of response to combination immunotherapy in patients with advanced non-small-cell lung cancer. *Cancer Cell* 2018; 33(5): 843–852.
63. Antonia SC, Callahan MK, Awad MM et al. Impact of tumor mutation burden on the efficacy of nivolumab or nivolumab+ipilimumab in small cell lung cancer: an exploratory analysis of CheckMate 032. In World Conference on Lung Cancer, 2017; Abstract OA 07.03a.
64. Riaz N, Havel JJ, Makarov V et al. Tumor and microenvironment evolution during immunotherapy with nivolumab. *Cell* 2017; 171(4): 934–949.e915.
65. Weber J, Horak C, Hodi FS et al. Baseline tumor T cell receptor (TCR) sequencing analysis and neo antigen load is associated with benefit in melanoma patients receiving sequential nivolumab and ipilimumab. *Ann Oncol* 2016; 27 (Suppl 6). doi: 10.1093/annonc/mdw378.01.
66. Li MM, Datto M, Duncavage EJ et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the association for molecular pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn* 2017; 19(1): 4–23.
67. McKenna A, Hanna M, Banks E et al. The genome analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 2010; 20(9): 1297–1303.
68. Rizvi H, Sanchez-Vega F, La K et al. Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing. *JCO* 2018; 36(7): 633–641.
69. Garofalo A, Sholl L, Reardon B et al. The impact of tumor profiling approaches and genomic data strategies for cancer precision medicine. *Genome Med* 2016; 8: 79.
70. Kowanetz M, Zou W, Shames DS et al. Tumor mutation load assessed by FoundationOne (FM1) is associated with improved efficacy of atezolizumab (atezo) in patients with advanced NSCLC. *Ann Oncol* 2016; 27 (Suppl 6). doi: 10.1093/annonc/mdw363.25.
71. Ramalingam S, Hellmann MD, Awad MM et al. Tumor mutational burden (TMB) as a biomarker for clinical benefit from dual immune checkpoint blockade with nivolumab+ipilimumab in first-line non-small cell lung cancer: identification of TMB cutoff from Checkmate 568. In AACR Annual Meeting 2018; Abstract #11317.
72. Balar AV, Galsky MD, Rosenberg JE et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. *Lancet* 2017; 389(10064): 67–76.
73. Powles T, Loriot Y, Ravaud A et al. Atezolizumab vs chemotherapy in platinum-treated locally advanced or metastatic urothelial carcinoma: immune biomarkers, tumor mutational burden and clinical outcomes from the phase III IMvigor211 Study. In 2018 Genitourinary Cancer Symposium.
74. Buchhalter I, Rempel E, Endris V et al. Size matters: dissecting key parameters for panel-based tumor mutational burden (TMB) analysis. *Int J Cancer* 2018 Sep 21 [Epub ahead of print], doi: 10.1002/ijc.31878.
75. Garon EB, Rizvi NA, Hui R et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* 2015; 372(21): 2018–2028.
76. McLaughlin J, Han G, Schalper KA et al. Quantitative assessment of the heterogeneity of PD-L1 expression in non-small-cell lung cancer. *JAMA Oncol* 2016; 2(1): 46–54.
77. Hellmann MD, Ciuleanu TE, Pluzanski A et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. *N Engl J Med* 2018; 378(22): 2093–2104.
78. Galsky M, Saci A, Szabo P et al. Impact of tumor mutation burden on nivolumab efficacy in secondline urothelial carcinoma patients: exploratory analysis of the phase ii checkmate 275 study. *Ann Oncol* 2017; 28: ESMO Annual Meeting Abstract.
79. Goodman AM, Kato S, Bazhenova L et al. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. *Mol Cancer Ther* 2017; 16(11): 2598–2608.
80. Kowanetz M, Zou W, Shames D et al. Tumor mutation burden (TMB) is associated with improved efficacy of atezolizumab in 1L and 2L+ NSCLC patients. *J Thoracic Oncol* 2017; 12(1): S321–S322.
81. Cristescu R, Mogg R, Ayers M et al. Mutational load (ML) and T-cell-inflamed microenvironment as predictors of response to pembrolizumab. *J Clin Oncol* 2017; 35, Abstract 1.
82. Peters S, Gettinger S, Johnson ML et al. Phase II trial of atezolizumab as first-line or subsequent therapy for patients with programmed death-ligand 1-selected advanced non-small-cell lung cancer (BIRCH). *J Clin Oncol* 2017; 35(24): 2781.
83. Seiwert T, Haddad R, Bauml J. Biomarkers predictive of response to pembrolizumab in head and neck cancer (HNSCC). In AACR Annual Meeting Oral Presentation 2018; Minisymposium: Late-Breaking Research.
84. Hellmann MD, Ciuleanu TE, Pluzanski A et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. *N Engl J Med* 2018; 378(22): 2093–2104.

85. Arce Vargas F, Furness AJS, Litchfield K et al. Fc effector function contributes to the activity of human anti-CTLA-4 antibodies. *Cancer Cell* 2018; 33(4): 649–663.e644.
86. Lauss M, Donia M, Harbst K et al. Mutational and putative neoantigen load predict clinical benefit of adoptive T cell therapy in melanoma. *Nat Commun* 2017; 8(1): 1738.
87. Topalian SL, Hodi FS, Brahmer JR et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012; 366(26): 2443–2454.
88. Brahmer JR, Drake CG, Wollner I et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *JCO* 2010; 28(19): 3167–3175.
89. Le DT, Uram JN, Wang H et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 2015; 372(26): 2509–2520.
90. Le DT, Durham JN, Smith KN et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 2017; 357(6349): 409–413.
91. Middha S, Zhang L, Nafa K et al. Reliable Pan-cancer microsatellite instability assessment by using targeted next-generation sequencing data. *JCO Precis Oncol* 2017; 2017(1): 1.
92. Lemery S, Keegan P, Pazdur R. First FDA approval agnostic of cancer site—when a biomarker defines the indication. *N Engl J Med* 2017; 377(15): 1409–1412.
93. Goh G, Walradt T, Markarov V et al. Mutational landscape of MCPyV-positive and MCPyV-negative Merkel cell carcinomas with implications for immunotherapy. *Oncotarget* 2016; 7: 3403–3415.
94. Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. *N Engl J Med* 2017; 377(25): 2500–2501.
95. Hammers HJ, Plimack ER, Infante JR et al. Safety and Efficacy of nivolumab in combination with ipilimumab in metastatic renal cell carcinoma: the CheckMate 016 Study. *J Clin Oncol* 2017; 35(34): 3851–3858.
96. Gunjur A. Nivolumab plus ipilimumab in advanced renal-cell carcinoma. *Lancet Oncol* 2018; 19(5): e232.
97. Turajlic S, Litchfield K, Xu H et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. *Lancet Oncol* 2017; 18(8): 1009–1021.
98. Riaz N, Havel JJ, Kendall SM et al. Recurrent SERPINB3 and SERPINB4 mutations in patients who respond to anti-CTLA4 immunotherapy. *Nat Genet* 2016; 48(11): 1327–1329.
99. Forde PM, Chaft JE, Smith KN et al. Neoadjuvant PD-1 blockade in resectable lung cancer. *N Engl J Med* 2018; 378(21): 1976–1986.
100. Mariathasan S, Turley SJ, Nickles D et al. attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature* 2018; 554(7693): 544–548. TGFbeta
101. Banerjee T, Duhadaway JB, Gaspari P et al. A key in vivo antitumor mechanism of action of natural product-based Brassinins is inhibition of indoleamine 2,3-dioxygenase. *Oncogene* 2008; 27(20): 2851–2857.
102. Shariat SF, Kattan MW, Vickers AJ et al. Critical review of prostate cancer predictive tools. *Future Oncol* 2009; 5(10): 1555–1584.
103. Oakman C, Santarpia L, Di Leo A. Breast cancer assessment tools and optimizing adjuvant therapy. *Nat Rev Clin Oncol* 2010; 7(12): 725–732.
104. Olivetto IA, Bajdik CD, Ravdin PM et al. Population-based validation of the prognostic model ADJUVANT! for early breast cancer. *J Clin Oncol* 2005; 23(12): 2716–2725.
105. Long GV, Dummer R, Hamid O et al. Epcadostat (E) plus pembrolizumab (P) versus pembrolizumab alone in patients (pts) with unresectable or metastatic melanoma: results of the phase 3 ECHO-301/KEYNOTE-252 study. *JCO* 2018; 36(Suppl 15): 108; Abstr 2018.
106. Chowell D, Morris LGT, Grigg CM et al. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. *Science* 2018; 359(6375): 582–587.