Published OnlineFirst October 6, 2020; DOI: 10.1158/1078-0432.CCR-20-3389

A Space–Time Continuum for Immunotherapy Biomarkers in Gastroesophageal Cancer?

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SUMMARY

In an annotated series of gastroesophageal adenocarcinomas differences in PD-L1 expression and tumor mutation burden occur between both paired contemporaneous primary and metastatic biopsies and pre/posttreatment samples. This work has implications for optimizing patient selection, serial testing, need for mechanistic understanding, and may underlie variable responses to checkpoint inhibitors in gastroesophageal cancers.

See related article by Zhou et al., p. 6453

In this issue of *Clinical Cancer Research*, Zhou and colleagues describe a clinically annotated cohort of 211 patients with gastroesophageal adenocarcinoma ($n = 407$ tumor samples) and examine PD-L1 and tumor mutation burden (TMB) biomarker heterogeneity over both space and time (1). Among their cohort of Western patients, the authors observed marked spatial and temporal heterogeneity using common methods of PD-L1 evaluation [combined positive score (CPS) scoring] and TMB determination (next-generation sequencing derived). Their data are timely and provocative in itself and also raise multiple future avenues for investigation in immuno-oncology beyond gastroesophageal adenocarcinomas.

In examining their patient cohort, several relevant findings clearly emerged. First, the cohort appears largely representative of real-world Western patients and highlights the relative rarity of gastroesophageal adenocarcinoma biomarker subsets associated with the greatest immune checkpoint inhibitor (ICI) benefit. Specifically, the authors observed a PD-L1 positivity rate of 44%, among which 15% had a CPS score ≥10. Similarly, only 14% of patients had a TMB ≥10 mutations/Mb, and PD-L1 status was not significantly associated with TMB. Among treatment-naïve patients with contemporaneous (median 14.5 days separating biopsies) primary and metastatic tumor samples ($n = 62$ patients), PD-L1 concordance was 61% with greater agreement between PD-L1–negative (PD-L1$^-$) tumors, and higher PD-L1–positive (PD-L1$^+$) rates in primary tumor samples versus metastatic sites. Similar concordance was observed for TMB (69% concordant), with all TMB-low/intermediate pairs in agreement. Having observed clear spatial heterogeneity, the authors then turned to exploring changes over time. The authors defined pretreatment samples as prior to or up to 10 days after initiation of first-line therapy and posttreatment as samples obtained anytime greater than 10 days after treatment initiation. By this definition pre/post-PD-L1 concordance was 63% among 83 patients with paired samples, and 75% for samples with CPS score ≥ 10. Interestingly, no PD-L1$^+$ pretreatment samples were PD-L1$^-$ on posttreatment sampling, but three PD-L1$^-$ samples scored as PD-L1$^+$ posttherapy, a phenomenon that has been observed postradiation and after some small-molecule tyrosine kinase inhibitor treatment. The overall TMB concordance was 73% among pre- and posttreatment samples. The temporal agreement among the 61 TMB-low/intermediate pre- and posttherapy samples was 100%, however, one of the initial TMB-high tumors was no longer TMB high at subsequent repeat sampling. In *post hoc* analysis using a dichotomous TMB cutoff of 10 mutations/Mb to define high versus low, the overall TMB concordance was 90%. Overall, using methodologies that are widely used in routine patient care, the authors demonstrated a substantial portion of patients with gastroesophageal adenocarcinoma had discordant PD-L1 and TMB values over both space and time.

What are the translational and clinical implications of this work and where should we go from here? Gastroesophageal adenocarcinomas are well known to contain significant heterogeneity among molecular features, particularly the overexpression of multiple actionable receptor tyrosine kinases including *HER2, MET, EGFR*, and *FGFR2* (2). This observation has implications for response to targeted agents as well as prognosis in nonmetastatic patients (2, 3). Interestingly, the genomic features of gastroesophageal adenocarcinoma itself may inform the tumor microenvironment composition, several components of which are known to influence PD-L1 expression, which is dynamic (3, 4). Baseline spatial genomic heterogeneity and temporal genomic changes may be more pronounced in chromosomally instable tumors, which are expected to comprise the majority of the authors' cohort, and it is unknown whether concordance/discordance rates would differ in cohorts comprised primarily of genomically stable tumors, for example. Among the cohort analyzed by Zhou and colleagues, it may be of interest to explore the details of the PD-L1 CPS score. For example, are discordant patients showing differences in PD-L1 expression in tumor cells or in surrounding mononuclear cells, both of which are incorporated in the CPS scoring method. Here, there is an opportunity for future work incorporating multiplex IHC and/or spatial transcriptomics coupled to single-cell analyses to more deeply define cell types and states driving PD-L1 expression. In addition, spatial and temporal heterogeneity should be queried on novel immune checkpoint targets (e.g., LAG-3, TIM-3, CD39, and TIGIT), for which agents are in clinical development. Importantly, immunocompetent model systems in gastroesophageal adenocarcinoma to help further the mechanistic underpinnings and possible organ-specific influence on PD-L1 expression are needed to study the implications of spatial heterogeneity on

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Clin Cancer Res 2020;26:6401–3

doi: 10.1158/1078-0432.CCR-20-3389

Published OnlineFirst October 6, 2020; DOI: 10.1158/1078-0432.CCR-20-3389

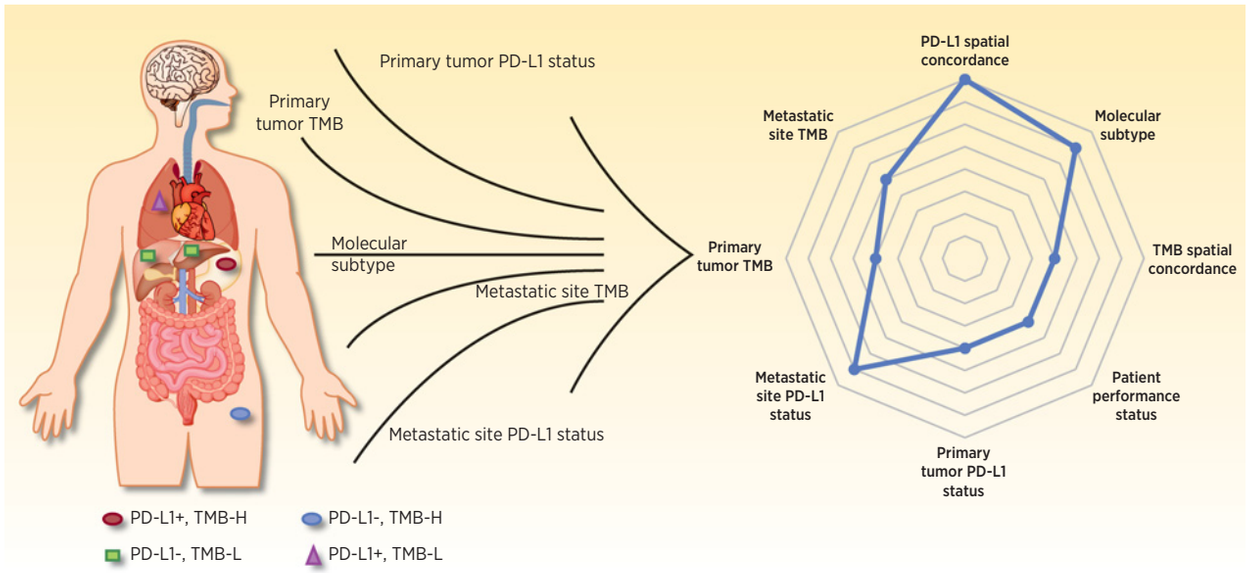

Figure 1.

Conceptual framework for incorporating spatial determinations into multiparametric immunotherapy biomarker assessment in gastroesophageal cancers. The radar plot represents a selection of features available in clinical care and their potential relative contribution to PD-1 response in a hypothetical patient and is not a comprehensive outline of all parameters that influence immune checkpoint response. TMB-H, TMB high; TMB-L, TMB low.

lesion-specific response. Thoughtful sample collection to optimize downstream analyses will be important in ongoing and future trials to ensure scientific and clinical questions can be answered in parallel.

Of equal interest are the multiple clinical questions raised from this dataset. What is the clinical behavior of a patient with gastroesophageal adenocarcinoma with negative initial PD-L1 testing (CPS < 1) but subsequent positive testing (temporal heterogeneity)? What about patients with contemporaneous primary and metastatic samples that are concordant versus discordant (spatial heterogeneity)? Owing to limited subsets and low numbers of ICI-treated patients, the current work lacks statistical power to address these clinical questions. There are several lines of evidence supporting the clinical relevance of serial biomarker testing in molecularly targeted therapies and rationale to support using the most recent chronologic sample to inform therapy. Patients with gastroesophageal adenocarcinoma with concordant HER2 overexpression/amplification between tissue and blood derive the greatest benefit from HER2-directed therapies, including in combination with ICIs. Conversely, patients with intratumoral and intertumoral spatial heterogeneity in molecular targets derive lesser benefit. Perhaps, we need to start considering PD-1/L1–directed therapies similarly in gastroesophageal adenocarcinoma and add a "space" variable to multiparametric biomarker considerations to optimally select patients for treatment approaches (**Fig. 1**). Just as circulating tumor DNA (ctDNA) studies have yielded insight into gastroesophageal adenocarcinoma genomic heterogeneity, efforts querying circulating immune cell populations and plasma proteomics may assist in completing the composite of intrapatient spatial dynamics of the immune response. While large datasets will be needed to answer some of the nuanced clinical questions raised by this work, we do envision the gastroesophageal adenocarcinoma field moving in this direction.

The majority of patients with gastroesophageal adenocarcinoma, including stage IV, are diagnosed by upper endoscopy with primary tumor samples serving as the main source for tissue biomarker determinations. Obtaining contemporaneous tissue sampling from metastatic disease sites may be difficult outside academic institutions and is associated with a small additional patient risk. Certainly, ctDNA serves a role here to assess genomic heterogeneity, but cannot assist in interrogating PD-L1 expression differences. The work from Zhou and colleagues raises interest in noninvasive methods to further explore PD-L1 heterogeneity testing and implications. The use of both ^{Zr}Nivolumab and ^{18}F-BMS-986192 molecular imaging has already demonstrated safety, feasibility, and early correlation with PD-L1 IHC (5). If optimized and further validated, this noninvasive method may prove useful in identifying optimal responders and potentially identifying lesions that may benefit from complementary approaches, including ablative stereotactic radiotherapy and intralesional immunotherapies. Significant additional work is needed to implement this technology and several studies are currently ongoing.

We applaud the authors for extending their prior work on genomic heterogeneity in gastroesophageal adenocarcinoma and applying heterogeneity assessment to common immuno-oncology biomarkers. We hope the generation of larger clinically annotated biomarker datasets developed with thoughtful consideration for both spatial (inter- and intratumoral) and temporal heterogeneity will ultimately improve patient treatment selection in this difficult-to-treat disease.

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Disclosure of Potential Conflicts of Interest

S.J. Klempner reports personal fees from Eli Lilly, Merck, Bristol-Myers Squibb, Pieris, Foundation Medicine, Inc., and Astellas outside the submitted work, as well as stock/equity in Turning Point Therapeutics. V. Upadhyay reports personal fees from EQRx outside the submitted work. J. Chao reports grants and personal fees from Merck and personal fees from Amgen, MacroGenics, Ono Pharmaceuticals, Daiichi Sankyo, and Foundation Medicine, Inc. outside the submitted work. No other potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by AGA Research Foundation's AGA-Gastric Cancer Foundation Ben Feinstein Memorial Research Scholar Award in Gastric Cancer, AGA2020–13–02 (to S.J. Klempner).

Received September 13, 2020; revised September 22, 2020; accepted October 2, 2020; published first October 6, 2020.

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