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ORIGINAL ARTICLE

Lesion-level heterogeneity of radiologic progression in patients treated with pembrolizumab

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Background: Disease progression is often considered a binary state reflecting presence or absence of response. Meaningful heterogeneity between metastatic sites of a given patient may exist, however, and may impact therapeutic outcomes. To characterize the heterogeneity of progression with immunotherapy, we evaluated lesion-level dynamics of pembrolizumab-treated patients across three tumor types.

Patients and methods: Individual metastatic lesion dynamics were analyzed retrospectively in patients with advanced melanoma, non-small-cell lung cancer (NSCLC), and gastric or gastroesophageal junction (G/GEJ) cancer who received pembrolizumab in KEYNOTE-001 or KEYNOTE-059. Primary progression was defined as radiologic progression as per RECIST v1.1 occurring at the first on-treatment study scan (~9-12 weeks, +2-week window) and secondary progression as progression occurring beyond the first scan (~14 weeks and beyond). The change in sum of target lesions and of individual lesions was examined, as were patterns and timing of progression.

Results: 9239 individual lesions from 1194 patients were analyzed. Among patients with primary progression [39% (200/511) of patients with melanoma, 41% (179/432) with NSCLC, 61% (154/251) with G/GEJ cancer], most patients (51%-63%) had a mixture of growing, stable, and shrinking lesions. Despite overall primary progression, a minority of patients (19%-25%) had tumor growth at every metastatic site and 17%-32% had ≥1 shrinking lesion. Among patients with secondary progression [22% (113/511) of patients with melanoma, 27% (117/432) with NSCLC, 18% (44/251) with G/GEJ cancer], few patients had rebound growth (>20% increase in diameter from nadir) in all lesions whereas the majority (74%-84%) had sustained regression in ≥1 lesion.

Conclusions: Lesion-level heterogeneity at the time of disease progression was common in pembrolizumab-treated patients, with many patients demonstrating ongoing disease control in a subset of tumor sites. These results may inform clinical decision-making, trial design, and tumor sampling in the future.

Key words: melanoma, non-small-cell lung cancer, gastric cancer, gastroesophageal junction cancer, progression, intertumoral heterogeneity

INTRODUCTION

A crucial but underappreciated characteristic of disease progression in oncology is the intertumoral heterogeneity that is observed clinically.[1] In clinical trials, radiologic progression is usually determined by a summary approach across target lesions, non-target lesions, and new metastatic lesions.[2] In particular, RECIST defines progressive disease as ≥20% increase in the sum of target lesion diameters, unequivocal progression in existing non-target lesions, or the appearance of ≥1 new lesion. This summary approach to determining progressive disease, however, may obscure clinically and biologically relevant changes that occur at individual metastatic sites. This potential for lesion-level variation in progression aligns with emerging characterization of intra- and intertumoral heterogeneity in the molecular landscape, immunophenotype, and response to treatment.[3-10] Understanding the nature of disease progression in patients receiving immune checkpoint inhibitors may inform the development of successful strategies to prevent or overcome progression.

We hypothesized that evaluating progression at the individual lesion level would provide insight into intertumoral variation that would not be captured when using a summary

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approach such as RECIST v1.1. To address this hypothesis, we characterized lesion-level features of progression in patients with advanced melanoma, non-small-cell lung cancer (NSCLC), and gastric or gastroesophageal junction (G/GEJ) cancer who received pembrolizumab monotherapy in the KEYNOTE-001 and KEYNOTE-059 studies (Clinicaltrials.gov NCT01295827 and NCT02335411), focusing specifically on patients who developed radiologic progression.

METHODS

Study design and participants

This was a retrospective analysis of the dynamics of tumoral response or progression in patients who received pembrolizumab monotherapy in the phase 1 KEYNOTE-001 (melanoma, NSCLC; NCT01295827) and the phase 2 KEYNOTE-059 trials (G/GEJ cancer; cohorts 1 and 3; NCT02335411). Detailed methods from these trials have been published previously.[11-16]

These trials were conducted in accordance with their protocols, good clinical practice guidelines, and the principles of the Declaration of Helsinki. The protocols were approved by the institutional review board or ethics committee at each study site. All patients provided written informed consent. All radiographic data presented here were assessed via central review.

Assessments

Imaging in the KEYNOTE-001 trial was carried out every 12 weeks for the melanoma cohort; for the NSCLC cohort it was every 9 weeks for the first 2 years, every 16 weeks in year 3, and every 6 months thereafter. Imaging in the KEYNOTE-059 trial was carried out at 9 weeks, then every 6 weeks for the first year, and then every 9 weeks thereafter. Primary progression was defined as radiologic progression as per RECIST v1.1 occurring at the first on-treatment scan (∼week 9-12), and secondary progression as progression occurring any time thereafter (∼week 14 and beyond). For clarity, we do not intend to propose secondary progression as a new clinical entity or term *per se*, nor conflate secondary progression with acquired resistance (which has been discussed elsewhere). Instead, we have distinguished these as primary and secondary progression to reflect differences in the methods for the determination of progression and change in lesion size: primary progression is determined by comparison with the baseline scan, whereas secondary progression is determined by comparison with the nadir scan. Furthermore, we report the patterns of radiologic progression among patients with "acquired resistance," using the definition proposed by SITC (Society for Immunotherapy of Cancer), as complete response, partial response, or stable disease lasting at least 6 months followed by progressive disease.[17]

Data analyses

Patients included in this analysis had measurable disease at baseline and $\geq$2 sets of available imaging (one at baseline and $\geq$1 on-treatment radiologic assessment); only patients who received pembrolizumab monotherapy were included.

Primary progression was defined as radiologic progression that occurred at or before the first radiologic assessment in KEYNOTE-001 and KEYNOTE-059 (weeks 9-12, +2-week window). Secondary progression was defined as radiologic progression that occurred beyond the first scan (e.g. week 14 and beyond). The overall percent change in the sum of diameters of target lesions and percent change in diameters of individual target lesions were examined, as were patterns and timing of progression of target, non-target, and new lesions. For patients with primary progression, lesions were defined as growing ($>$20% increase in individual tumor diameter from baseline), stable ($\leq$20% increase and $<$30% decrease in tumor diameter from baseline), or shrinking ($\geq$30% decrease in tumor diameter from baseline). For patients with secondary progression, lesions were defined as rebounding if a $>$20% increase in tumor diameter from nadir occurred, and not rebounding if a $\leq$20% increase in tumor diameter from nadir occurred. The Kaplan-Meier method was used to estimate progression-free survival (PFS). For data analysis, raw clinical datasets in comma-separated values (csv) format were imported into commercially available software, MATLAB (version R2018b, MathWorks).

RESULTS

Patients

For this analysis, the data cut-off was September 12 016, for the melanoma cohort of KEYNOTE-001, November 18 2018, for the NSCLC cohort of KEYNOTE-001, and April 21 2017, for the pembrolizumab monotherapy cohorts (1 and 3) in KEYNOTE-059. From these studies, 511 patients with melanoma, 432 patients with NSCLC, and 251 patients with G/GEJ cancer had measurable disease and $\geq$1 on-treatment radiologic assessment and were included in this analysis. Baseline characteristics for patients included in this analysis are provided in Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2021.09.006.

In total, 9239 individual lesions from 1194 pembrolizumab-treated patients were examined and tracked, including 4433 target lesions (2111 melanoma, 1490 NSCLC, 832 G/GEJ cancer), 2508 non-target lesions (1032 melanoma, 996 NSCLC, 480 G/GEJ cancer) and 2298 new metastatic lesions (1166 melanoma, 919 NSCLC, 213 G/GEJ cancer) (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2021.09.006).

Primary and secondary progression

Radiologic progression occurred at some point during treatment in 61% (313/511) of patients with melanoma, 69% (296/432) with NSCLC, and 79% (198/251) with G/GEJ

cancer. Primary progression occurred in 39% (200/511) of patients with melanoma, 41% (179/432) with NSCLC, and 61% (154/251) with G/GEJ cancer (Supplementary Figure S1A-C, available at https://doi.org/10.1016/j.annonc.2021.09.006). Secondary progression occurred in 22% (113/511) of patients with melanoma, 27% (117/432) with NSCLC, and 18% (44/251) with G/GEJ cancer (Supplementary Figure S1A-C, available at https://doi.org/10.1016/j.annonc.2021.09.006). The remaining patients [38.7% (198/511) with melanoma, 31.5% (136/432) with NSCLC, and 21.1% (53/251) with G/GEJ cancer] had not experienced radiologic progression at the time of their last scan (this categorization includes patients who had continued stable disease/partial response/complete response at the time of last data cut and those who discontinued because of clinical progression/other reasons but without radiologic progression). PFS varied by tumor type, as previously described (Supplementary Figure S1D-F, available at https://doi.org/10.1016/j.annonc.2021.09.006).[12,14-16]

Intertumoral heterogeneity in patients with primary progression

Perhaps surprisingly, the least prevalent reason for primary progression in all three tumor types was growth in target lesions (Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2021.09.006). The most prevalent reason for primary progression in melanoma and NSCLC was new metastatic lesions, whereas in G/GEJ cancer the most prevalent reason was growth in non-target lesions.

Among patients with primary progression, 49% (98/200) with melanoma, 49% (87/179) with NSCLC, and 51% (79/154) with G/GEJ cancer had a >20% increase in the sum of diameters of target lesions; 9% (17/200) of patients with melanoma, 5% (9/179) with NSCLC, and 5% (7/154) with gastric cancer had a reduction in tumor burden from baseline (≥30% decrease in the sum of diameters of target lesions) (Figure 1A-C). At the individual lesion level, 14% (123/889) of lesions from patients with melanoma, 12% (83/715) from patients with NSCLC, and 8% (46/560) from patients with G/GEJ cancer had response to therapy (≥30% decrease in the individual lesion diameter).

Most patients displayed a mixture of growing, stable, and shrinking lesions; 63% (126/200) of patients with melanoma, 60% (107/179) with NSCLC, and 51% (79/154) with G/GEJ cancer had lesions in >1 response category (Figure 1D-F). Only a minority of patients [19% (38/200) melanoma, 22% (39/179) NSCLC, 25% (39/154) gastric cancer] had tumor growth at every metastatic site, and 32% (63/200) of patients with melanoma, 26% (46/179) with NSCLC, and 17% (26/154) with G/GEJ cancer had ≥1 shrinking target lesion (≥30% decrease in diameter). For patients with primary progression, the proportion of each patient's lesions that are progressing is shown, thereby highlighting the heterogeneity of lesion-level response/progression in a given patient (Figure 1E-G).

Intertumoral heterogeneity in patients with secondary progression

The most prevalent reason for secondary progression in melanoma and G/GEJ cancer was new metastatic lesions whereas the most prevalent reason in NSCLC was progression in target lesions (Supplementary Figure S3, available at https://doi.org/10.1016/j.annonc.2021.09.006). A similar pattern of reasons for progression was seen in patients with "acquired resistance," defined as stable disease, partial response, or complete response lasting 6 months followed by progressive disease (Supplementary Figure S4, available at https://doi.org/10.1016/j.annonc.2021.09.006).

Among patients with secondary progression, 38% (43/113) of patients with melanoma, 44% (51/117) with NSCLC, and 27% (12/44) with gastric of GEJ cancer had >20% increase in the sum of diameters of target lesions from nadir; 62% (70/113) patients with melanoma, 56% (66/117) with NSCLC, and 73% (32/44) with G/GEJ cancer had a reduction in tumor burden from nadir (≥20% decrease in the sum of diameters of target lesions) (Figure 2A-C). At the individual lesion level, 29% (141/484) of lesions from patients with melanoma, 39% (135/343) from patients with NSCLC, and 32% (43/134) from patients with G/GEJ cancer had a rebound in lesion growth of >20%.

Few patients had rebound growth (>20% increase in diameter from nadir) in all lesions [16% (18/113) melanoma, 26% (31/117) NSCLC, 16% (7/44) G/GEJ cancer], and most patients had sustained regression (≥20% decrease in diameter) in ≥1 lesion [84% (95/113) melanoma, 74% (86/117) NSCLC, 84% (37/44) G/GEJ cancer] (Figure 2D-F).

Implications of intertumoral heterogeneity on tissue sample collection

Overall, we found substantial heterogeneity in patients with radiological disease progression, both primary and secondary. When progression occurs, it is generally driven by a subset of growing lesions or by the appearance of a new metastatic lesion. Given the lack of uniformity in the lesion-level dynamics, it is possible that a biomarker assessment of a single tumor lesion may not correlate with the response status of the patient. For example, the sample may be derived from a shrinking tumor in a patient with progressive disease due to a new metastatic lesion (example scenarios of variable lesion-level dynamics within patients with progression are shown in Supplementary Figure S5, available at https://doi.org/10.1016/j.annonc.2021.09.006). This divergence between the lesion-level response and patient-level response (Supplementary Figure S6, available at https://doi.org/10.1016/j.annonc.2021.09.006) is problematic when interrogating tissue-based biomarkers and may be a source of imprecision or inaccuracy when trying to understand response and resistance as they relate to biomarker expression. We found that among patients labeled as having primary progression (across all tumor types), the majority of individual target lesions were not themselves progressive [53% (1142/2164) were stable or regressing]. Therefore,

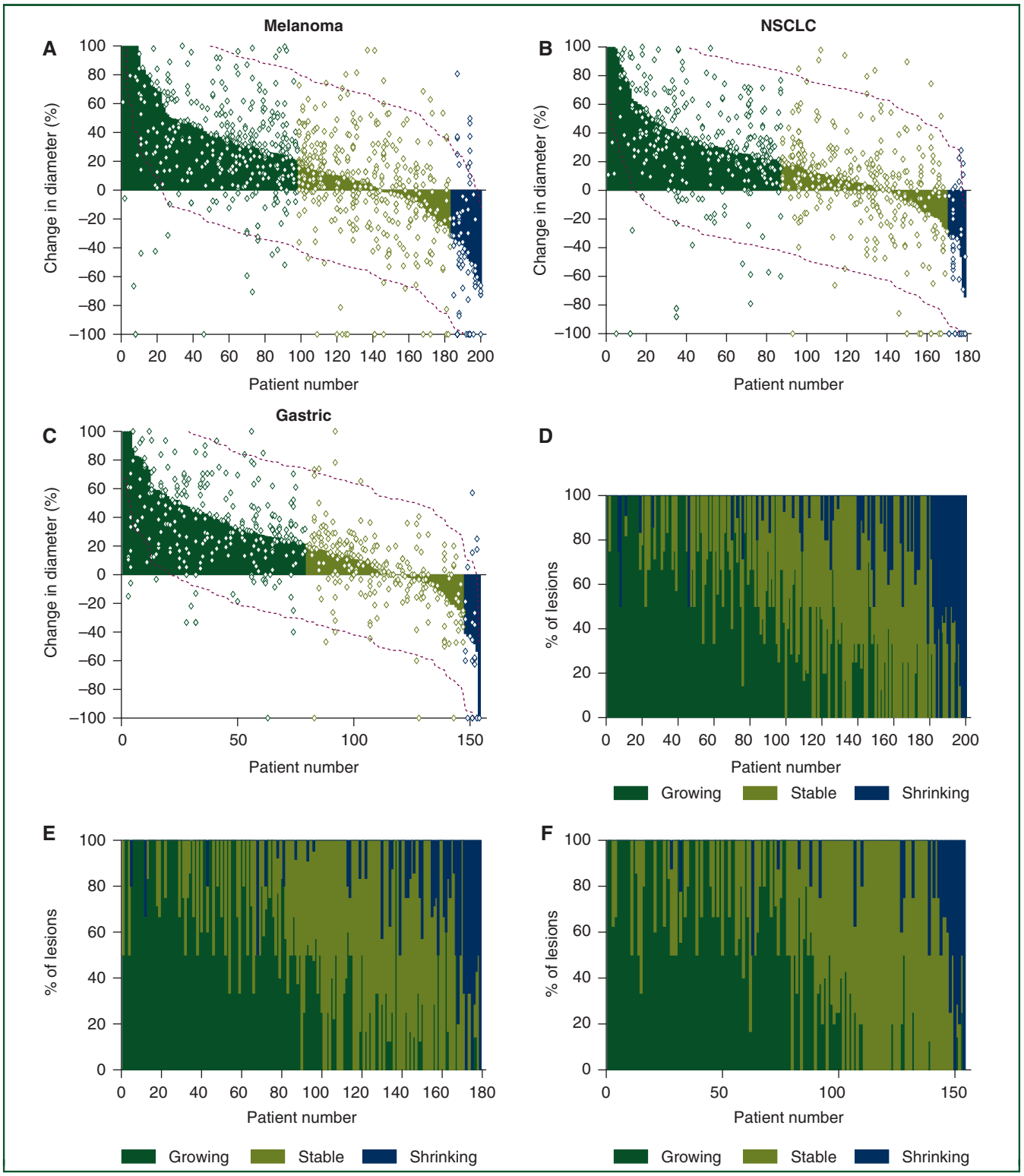

Figure 1. **Percent change in sum of diameters (SoD) of target lesions at the time of primary progression as vertical bars for patients with (A) melanoma, (B) non-small-cell lung cancer (NSCLC), and (C) gastric or gastroesophageal junction (G/GEJ) cancer; dots represent the percent change in individual target lesions at the time of progression for all patients. Change in tumor burden from baseline (growing, ΔSoD >20% increase; stable, ΔSoD ≤20% increase and <30% decrease; shrinking, ΔSoD ≥30% decrease) is shown with variation in colors (dark green, mid green, dark blue, respectively). Percentage of lesions that exhibited growth, stability, and shrinkage at the time of progression in patients with (D) melanoma, (E) NSCLC, and (F) G/GEJ cancer. Percentage of progressing lesions at time of primary progression in patients with (G) melanoma, (H) NSCLC, and (I) G/GEJ cancer. Patients in the heat map and scatter plots are represented in the same order as in the corresponding waterfall plot for melanoma, NSCLC, and G/GEJ cancer, respectively.**

NSCLC, non-small-cell lung cancer.

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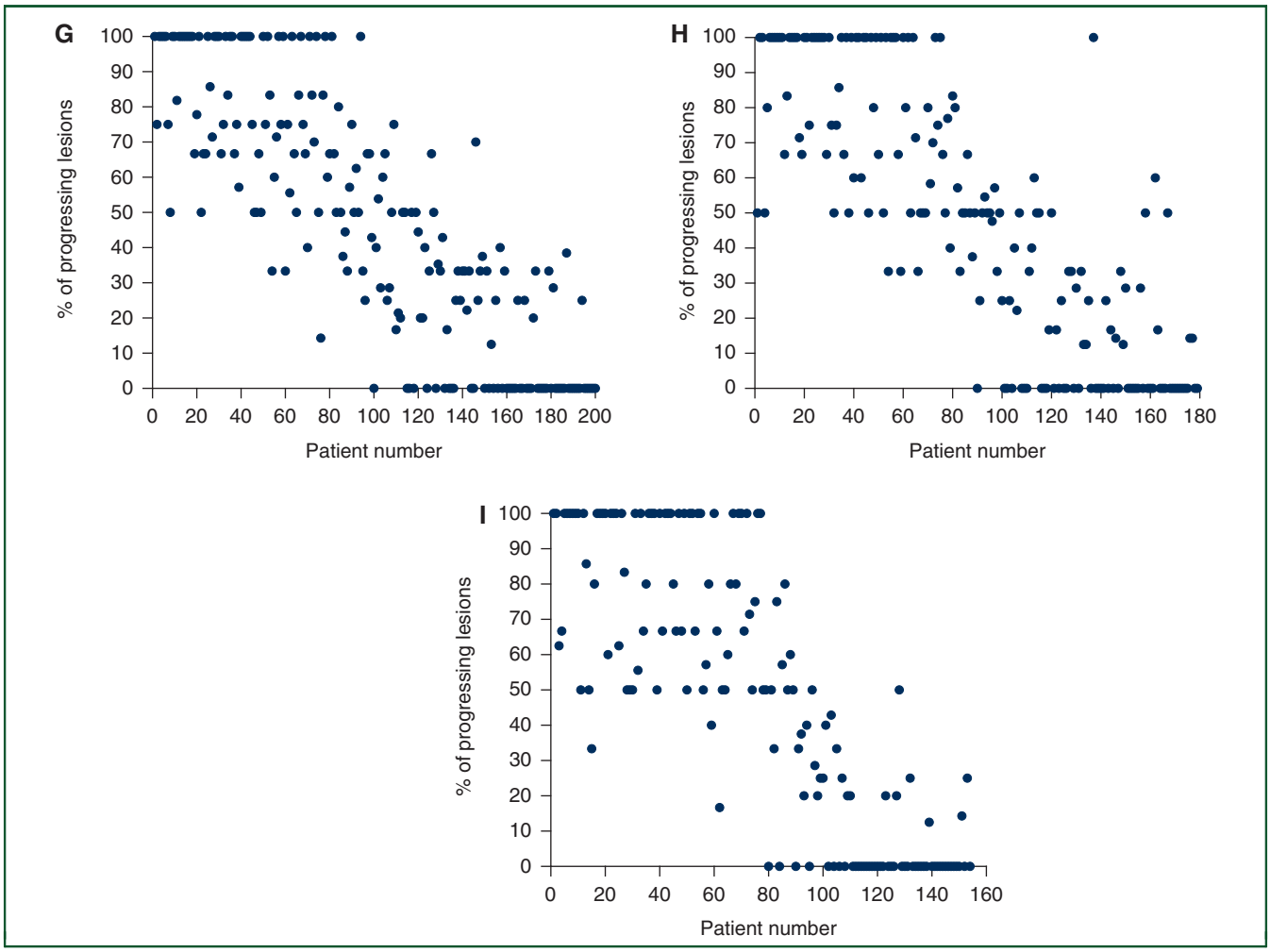

Figure 1. Continued.

many tissue samples used for biomarker analysis may be derived from individual lesions that are discordant with the overall response status of the patient (Figure 1G-I).

DISCUSSION

In this analysis, we quantified the intra- and interpatient heterogeneity of lesion-level radiologic response in patients with advanced melanoma, NSCLC, and G/GEJ cancer treated with pembrolizumab monotherapy. Overall, we found substantial heterogeneity in lesion-level progression between and within patients with radiologic disease progression, both primary and secondary. Although the tumor types included are distinct in their genomic profiles and relative responsiveness to checkpoint blockade,[18] this analysis revealed common themes related to lesion-level heterogeneity progression. Firstly, intra-patient heterogeneity at the time of progression was common, with most patients demonstrating a mix of growing, shrinking, and stable tumors. Secondly, many patients with progression, either primary or secondary, have evidence of benefit in $\geq$1 metastatic site. This observation could have implications for patient selection and/or trial design for studies involving patients who have or may develop progression on anti—PD-1 (programmed cell death protein 1) therapy, potentially favoring continuation of PD-1 blockade as a component of therapy in patients with some sites demonstrating ongoing benefit to checkpoint blockade. Thirdly, the reliability of predictive tumor-based biomarkers is likely affected by lesion-level heterogeneity, wherein the response status of an individual sampled lesion does not necessarily match patient-level response status.

Our results suggest that characterization of the lesion-level nature of progression may be an important refinement in our assessment of both patients and clinical trial results. Such an effort to formalize characterization of heterogeneity and quantification of lesion-level outcomes may improve clarity about the relative benefit of emerging therapies.

Furthermore, accurate characterization of lesion-level responses is necessary for the productive conduct of translational research toward discovery of novel targets and combinations. The relative lack of consensus around biological mechanisms of resistance to checkpoint blockade is multifactorial;[19] however, the contribution of intra-patient heterogeneity may be underappreciated. In the future, detailed work to link biomarker status with the response of the sampled lesion would potentially generate greater precision and insight into the immunobiology of PD-1 blockade.

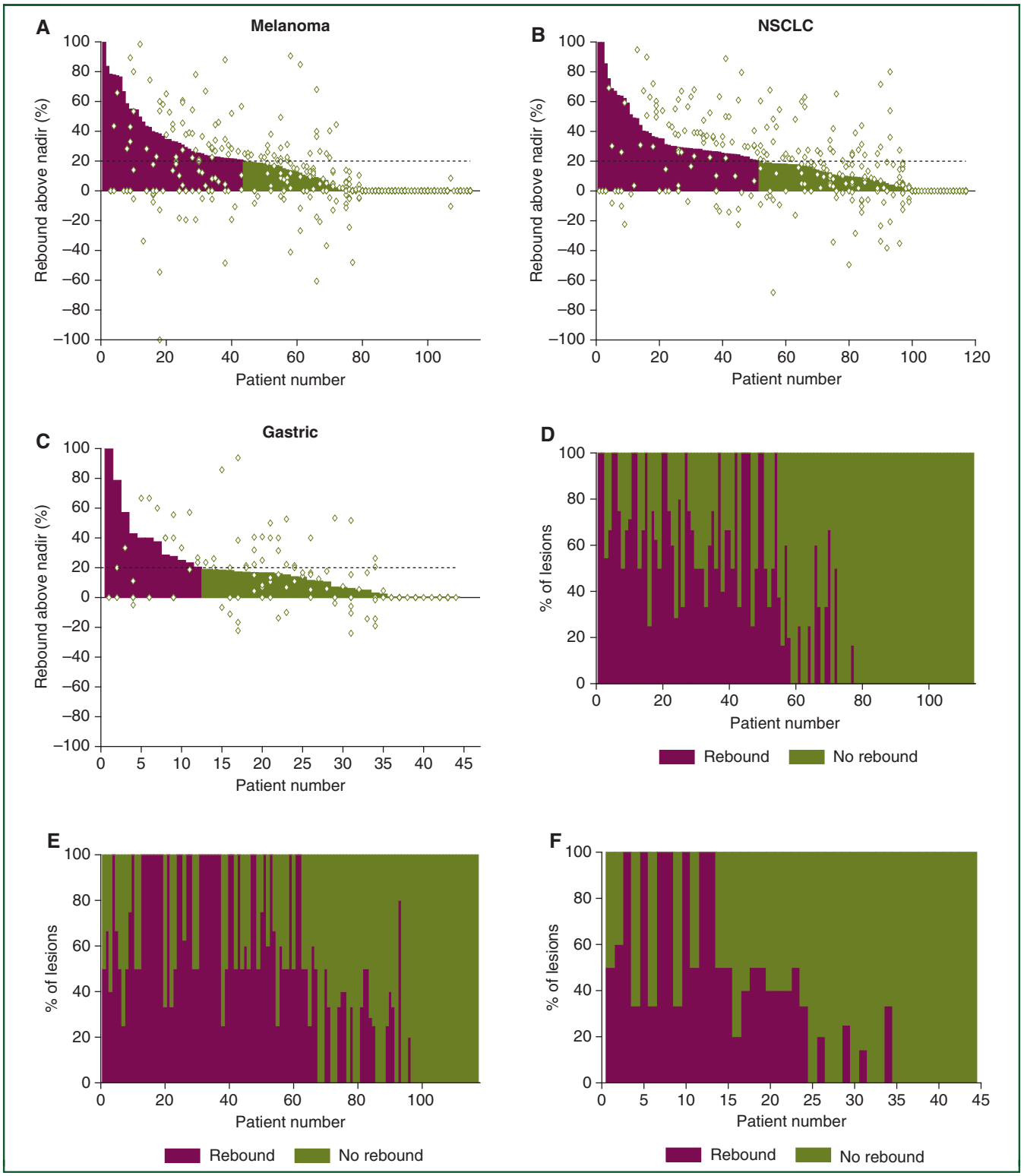

Figure 2. Percent change in sum of diameters (SoD) of target lesions from nadir at the time of secondary progression as vertical bars in patients with (A) melanoma, (B) non-small-cell lung cancer (NSCLC), and (C) gastric or gastroesophageal junction (G/GEJ) cancer; dots represent the percent change in individual lesions at the time of secondary progression. Change in tumor size from nadir (rebound, ΔSoD >20% increase; no rebound, ΔSoD ≤20% increase) is shown with variation in colors (burgundy and mid green, respectively). Percentage of lesions that exhibited rebound or no-rebound from nadir at the time of progression in patients with (D) melanoma, (E) NSCLC, and (F) G/GEJ cancer; patients are represented in the same order as in the corresponding waterfall plot for melanoma, NSCLC, and G/GEJ cancer, respectively.

NSCLC, non-small-cell lung cancer.

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There are several limitations to this study. This analysis is limited to pembrolizumab monotherapy and future work is needed to examine the effect of combination therapy on lesion-level heterogeneity. We were unable to carry out lesion-level biomarker analysis evaluating performance of biomarkers derived from a given lesion with the response of that specific lesion. It was not feasible with the available data to track biopsy source back to imaging to make such links; however, we believe this will be crucial for future progress.

The present study shows that, among pembrolizumab-treated patients with advanced melanoma, NSCLC, and G/GEJ cancers, the majority experience heterogeneous behavior of individual tumor sites at the time of progression, with ongoing disease control at a subset of tumor sites. The partiality versus uniformity of progression could have implications for guiding optimal therapeutic interventions after progression and improving biomarker performance. From a biological perspective, there is also growing evidence showing heterogeneity in immunophenotypes both within and between tumor metastases.[3,20,21] Our report quantifies the radiologic manifestation of that underlying heterogeneity. Dissecting this immunobiology will be crucial to inform therapeutic strategies to successfully achieve the ultimate goal of lasting cancer control for as many patients as possible.

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DISCLOSURE

BGT reports employment at Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, and is a shareholder in Merck & Co., Inc., Kenilworth, NJ, USA. KT has declared no conflicts of interest. DPDA reports employment at Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, and is a shareholder in Merck & Co., Inc., Kenilworth, NJ, USA. AS reports employment at Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, and is a shareholder in Merck & Co., Inc., Kenilworth, NJ, USA. MDH reports grants from Bristol Myers Squibb; nonfinancial support from AstraZeneca and Bristol Myers Squibb; and personal fees from Achilles; Arcus AstraZeneca; Blueprint Medicines; Bristol Myers Squibb; Genentech/Roche; Immunai; Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA; Mirati; Nektar; Pact Pharma; Shattuck Labs; Syndax; Janssen; and Regeneron; as well as equity options from Immunai, Shattuck Labs, and Arcus. A patent filed by Memorial Sloan Kettering related to the use of tumor mutational burden to predict response to immunotherapy (PCT/US2015/062208) is pending and licensed by PGDx.

DATA SHARING

Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA (MSD) is committed to providing qualified scientific researchers access to anonymized data and clinical study reports from the company's clinical trials for the purpose of conducting legitimate scientific research. MSD is also obligated to protect the rights and privacy of trial participants and, as such, has a procedure in place for evaluating and fulfilling requests for sharing company clinical trial data with qualified external scientific researchers. The MSD data sharing website (available at: http://engagezone.msd.com/ds_documentation.php) outlines the process and requirements for submitting a data request. Applications will be promptly assessed for completeness and policy compliance. Feasible requests will be reviewed by a committee of MSD subject matter experts to assess the scientific validity of the request and the qualifications of the requestors. In line with data privacy legislation, submitters of approved requests must enter into a standard data-sharing agreement with MSD before data access is granted. Data will be made available for request after product approval in the US and EU or after product development is discontinued. There are circumstances that may prevent MSD from sharing requested data, including country- or region-specific regulations. If the request is declined, it will be communicated to the investigator. Access to genetic or exploratory biomarker data requires a detailed, hypothesis-driven statistical analysis plan that is collaboratively developed by the requestor and MSD subject matter experts; after approval of the statistical analysis plan and execution of a data-sharing agreement, MSD will either perform the proposed analyses and share the results with the requestor or will construct biomarker covariates and add them to a file with clinical data that is uploaded to an analysis portal so that the requestor can perform the proposed analyses.

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