Differential Molecular Expression Patterns Associated with Metastasis in Cutaneous Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis

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The majority of cutaneous squamous cell carcinomas are treated by surgical removal; however, approximately 4% of tumors will metastasize. Molecular expression testing may improve accuracy in estimating the prognosis and defining the mechanisms important in the disease progression, which may impact response to therapy. Using PubMed (MEDLINE) and EMBASE, a systematic review was performed to evaluate studies published from January 2005 to August 2019 reporting tumor protein or RNA expression along with either outcomes (metastasis or death) or a comparison of primary with metastatic tumor samples. Inclusion criteria were met by 45 studies containing 81 comparisons of 44 distinct proteins and 25 microRNAs. On meta-analysis of studies analyzing primary tumor samples in terms of later outcomes, high primary tumor expression of PD-L1 (OR = 2.34, 95% confidence interval = 1.09–5.02, P = 0.030), EGFR (OR = 2.57, 95% confidence interval = 1.24–5.33, P = 0.011), and podoplanin (OR = 2.33, 95% confidence interval = 1.00–5.41, P = 0.049) conferred increased odds for metastasis. In comparison, metastatic tissue was more likely to have a high expression of PD-L1 than primary tissue (OR = 3.13, 95% confidence interval = 1.00–9.75, P = 0.049). Further studies are needed to confirm whether testing for PD-L1, EGFR, and podoplanin expression aids in cutaneous squamous cell carcinomas prognostic estimation of metastasis or death or predicts response to therapy.

INTRODUCTION
Cutaneous squamous cell carcinoma (cSCC) is a common malignancy with an estimated incidence of 1,000,000 cases per year in the United States (Rogers et al., 2015). The vast majority of tumors are cured with surgical excision; however, up to 4% of cSCCs will go on to develop metastasis to regional lymph nodes or distant sites (Brantsch et al., 2008; Schmults et al., 2013). Currently, risk stratification of poor outcomes (i.e., nodal and/or distant metastasis, disease-specific death) is performed using clinical and histopathologic tumor information such as preoperative tumor diameter, degree of cellular dedifferentiation, anatomic depth of invasion, and perineural invasion (Jambusaria-Pahlajani, 2013). The role of molecular expression testing is poorly established but could improve accuracy in estimating prognosis, identify pharmacologic targets for advanced disease, and predict response to various therapeutics.

At an estimated 50 somatic mutations per megabase pair, the mutational burden in cSCC is one of the highest of all human malignancies (South et al., 2014). GWASs in cSCC have identified numerous susceptibility loci across a wide range of biologic domains, including cellular proliferation, apoptosis, pigmentation, and immune regulation (Chahal et al., 2016). Recent work has also found differential mutational frequencies of the key genes involved in tumor suppression (TP53), cell-cycle regulation (NOTCH1, CDKN2A, PI3KCG), and epigenetic regulation (KMT2D) between metastasizing and nonmetastasizing primary cSCCs (Ashford et al., 2017).

Protein expression may also provide important insight into tumor behavior. Proteins are both downstream products of genetic and epigenetic regulation and effectors of cellular function. Expression levels can be measured by clinical laboratory techniques, such as immunohistochemistry and immunoblotting, which are used regularly as means to confirm suspected histologic diagnoses. Systemic treatments for locally advanced and metastatic tumors inhibit proteins important in tumor formation and progression, most notably PD-1 and EGFR. Their success suggests that expression levels of such proteins may predict response to targeted therapies. However, such correlations have not yet been demonstrated in cSCC, suggesting that multiple factors may influence treatment response versus failure. Epigenetic regulation of cSCC tumor cells by microRNAs (miRNAs) is less well-studied in humans, but their role in transcriptional regulation makes them another important area to explore.

Numerous observational studies have investigated the role of individual proteomic and RNA differences as they relate to cSCC tumor behavior. However, results are variable, and unified information is lacking with regards to risk prediction.
for poor outcomes and differences in expression between primary and metastatic tissue. This study systemically reviews the recent literature to consolidate the current understanding and further analyzes molecular features that may characterize or influence the risk for metastasis or death in cSCC.

RESULTS
Using the search process described in Materials and Methods, 335 unique abstracts were identified, and 45 studies met predefined inclusion criteria (Figure 1). Of these 45 studies, 41 reported outcomes with respect to protein expression, and four described comparisons of RNA. All studies were performed using a retrospective or cross-sectional design with the exception of one prospective cohort (Zhang et al., 2015).

Protein expression

Primary cSCC resulting in metastasis or death compared with cases without these outcomes. A total of 37 retrospective cohort and case control studies evaluating the differential protein expression between tissues from primary tumors resulting in metastasis or death versus those with no such events were identified (Supplementary Table S1) (Ahmed Haji Omar et al., 2015; Amoils et al., 2019; Beadle et al., 2013; Cañueto et al., 2016; Ch’ng et al., 2008; Chen et al., 2015, 2008; Cumsky et al., 2019; Föll et al., 2018; Fundyler et al., 2004; García-Díez et al., 2018; García-Pedroso et al., 2017; Hesse et al., 2016; Huang et al., 2012; Jiao et al., 2017; Kamiya et al., 2020; Kang et al., 2009; Kato et al., 2018; Khandelwal et al., 2016; Kreppel et al., 2013; Lee et al., 2013; Meier et al., 2016; Mungúa-Calzada et al., 2019; Roper et al., 2017; Salehi et al., 2007; Santos-Juanes et al., 2019; Satgunaseelan et al., 2017; Schaper et al., 2017; Suiqing et al., 2005; Sweeny et al., 2012a, 2012b; Tanemura et al., 2005; Toll et al., 2013, 2012; Venza et al., 2017; Vinicius et al., 2011; Xu et al., 2016). A total of 57 comparisons of 40 unique proteins were reported in 1,937 subjects, including 1,068 primary tumors resulting in metastasis or death, and 624 tumors not resulting in metastasis or death. Proteins appearing in more than one study included PD-L1 (n = 7), podoplanin (n = 5), EGFR (n = 4), E-cadherin (n = 3), and p16 (n = 2). Assessment of protein expression was performed using immunohistochemistry with (n = 11) or without (n = 23) tissue microarrays, immunoblotting (n = 2), or protein extraction with direct trypsinization (n = 1). Follow-up time was reported in 36 comparisons, with 29 providing a median duration of at least 2 years. The mean and median time to metastasis across samples of primary tumors that reported time to event ranged from 7.2 to 22.8 and 0 to 13.1 months, respectively. Treatment of the primary tumor was reported in 32 comparisons and consisted of either excision alone (n = 18) or a mixed cohort of excision plus lymph node dissection and adjuvant radiation and/or systemic chemotherapy (n = 14). Established clinical risk factors for metastasis and death were not reported consistently between the studies. Sample composition ranged from 10% to 100% with regard to the percentage of tumors at elevated risk for metastasis by the American Joint Committee on Cancer/Union for International Cancer Control or Brigham and Women’s Hospital staging systems (T stage 2 or 2b, respectively).

Significant results (P < 0.05) were found in 52% (26 of 50) of comparisons reporting metastasis and 35% (9 of 26) reporting death. On pooled analysis, high primary tumor expression of PD-L1 (OR = 2.34, 95% confidence interval [CI] = 1.09–5.02, P = 0.030), EGFR (OR = 2.57, 95% CI = 1.24–5.33, P = 0.011), and podoplanin (OR = 2.33, 95% CI = 1.00–5.41, P = 0.049) conferred increased odds for metastasis (Figure 2), whereas no difference was observed for p16 (OR = 0.65, 95% CI = 0.30–1.40, P = 0.271), cytoplasmic E-cadherin (OR = 1.43, 95% CI = 0.71–2.90, P = 0.317), or membranous E-cadherin (OR = 1.20, 95% CI = 0.55–2.63, P = 0.644).

Metastatic cSCC tissue compared with primary cSCC tissue. A total of 13 cross-sectional studies of protein expression between tissue from primary (332 subjects) and metastatic (225 subjects) tumors containing 18 comparisons were identified (Supplementary Table S2) (Amoils et al., 2019; Beadle et al., 2013; Ch’ng et al., 2008; Fundyler et al., 2004; García-Díez et al., 2018; Hesse et al., 2016; Lai et al., 2005; Rokunohe et al., 2010; Roper et al., 2017; Sweeny et al., 2012a, 2012b; Slater et al., 2016; Wang et al., 2012). A total of 14 unique proteins were evaluated. Both PD-L1 (n = 4) and EGFR (n = 2) appeared in more than one comparison. Information regarding high-risk features of the primary tumor cohort was available in 8 of 13 studies.

Significant differences in protein expression were detected in 28% (5 of 18) of comparisons. On pooled analysis, metastatic tumors were more likely to have high expression of PD-L1 than primary tumors (OR = 3.13, 95% CI = 1.00–9.75, P = 0.049) (Figure 3), whereas no difference was observed between these groups with regards to the expression of EGFR (OR = 0.95, 95% CI = 0.40–2.26, P = 0.903).

MiRNA and lincRNA
The risk for metastasis in primary cSCC with regard to RNA expression was evaluated in 358 subjects in six comparisons from four studies (Cañueto et al., 2017; Chen et al., 2019; Gillespie et al., 2016; Zhang et al., 2015). One study also included a comparison of differential expression between primary and metastatic tissue (Gillespie et al., 2016). With the exception of one study examining long intergenic non-coding RNA (LINC01048), all comparisons examined miRNA levels. RNA expression was quantified using PCR (n = 2), tissue microarray (n = 1), or expression analysis panel (n = 1). Significant results included associations between low miR-20a with higher Union for International Cancer Control stage and shorter overall survival (Zhang et al., 2015), high miR-205 with poor clinical evolution (composite outcome of local recurrence and nodal and distant metastasis) (Cañueto et al., 2017), and high LINC01048 with shorter disease-free survival and overall survival (Chen et al., 2019). Null results included miR-203 expression with poor clinical evolution (Cañueto et al., 2017). In addition, one study found significant differences in the expression of 14 miRNAs (e.g., miR-4286, miR-421, miR-4516, miR-574-5p) in primary cSCCs that metastasized compared with primary tumors that did not, along with seven miRNAs that were differentially expressed in metastatic versus that in primary tumor samples.
(upregulated in metastases: miR-4286, miR-200a-3p, miR-148-3p; downregulated in metastases: miR-1915-3p, miR-205-5p, miR-4516, miR-150-5p) (Gillespie et al., 2016).

DISCUSSION
Numerous recent studies have investigated the relationship between cSCC molecular expression profiles and clinical outcomes. The protein and RNA alterations summarized in this review represent the most comprehensively studied molecular features predictive of advanced disease in cSCC accumulated over the past 15 years. A significant proportion of comparisons reported positive findings regarding the risk for poor outcomes (52% of studies examining metastasis, 35% of studies examining death), with differential molecular expression supporting the role of molecular testing in predicting prognosis. Similarly, molecular expression differed between primary and metastatic tissues in nearly one third of comparisons, suggesting a change in molecular phenotype as tumors progress from primary to metastatic disease. Our pooled analysis demonstrates that high expression of PD-L1, EGFR, and podoplanin confer increased odds for subsequent metastasis in primary tumors with high PD-L1 expression as well as heightened expression in metastatic tissue. These findings suggest that upregulation of PD-L1 may both promote and sustain cSCC progression by aiding in host immune evasion through the inhibition of T-lymphocyte activation.

However, technical and biological characteristics of PD-L1 may limit its use as a reliable biomarker. These issues include oncogenic versus induced expression, variability in expression across tumor regions, and limited and distinct binding sites for immunohistochemical antibodies (Patel and Kurzrock, 2015). Furthermore, although the most commonly studied cut-off value of significance was ≥1% of cells, there was significant variation across studies. Given that multiple cells in the tumor microenvironment may display PD-L1 expression, including tumor-infiltrating lymphocytes that have been shown to correlate with improved disease-free survival, examining these patterns of expression is another important component of studying this marker (Jiao et al., 2017; Roper et al., 2017). It is critical that future study designs acknowledge and navigate these considerations.

Immune regulation
Impaired immune response plays a key role in cSCC pathogenesis, which is illustrated by the 65-fold greater risk organ-transplant recipients incur for developing cSCC than the general population (Jensen et al., 1999). Therapeutic augmentation of the host immune response through PD-1 checkpoint inhibition has proven effective in the treatment of advanced disease (Choi et al., 2020; Migden et al., 2018). PD-L1, a ligand whose interaction with T lymphocytes is blocked by PD-1 inhibitors, was the most commonly studied protein in this review. In this paper, we have demonstrated both increased odds for subsequent metastasis in primary tumors with high PD-L1 expression as well as heightened expression in metastatic tissue. These findings suggest that upregulation of PD-L1 may both promote and sustain cSCC progression by aiding in host immune evasion through the inhibition of T-lymphocyte activation.

Figure 1. PRISMA flow diagram.

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cSCC, cutaneous squamous cell carcinoma; miRNA, microRNA; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-analysis; SCC, squamous cell carcinoma.
Previous reports have not demonstrated an association between cSCC PD-L1 protein expression levels and response to PD-1 inhibitors (Migden et al., 2020). In other solid tumors, such as melanoma, renal cell carcinoma, and nonsmall cell lung cancer, an association has been demonstrated in clinical trials, albeit with varying patterns across studies (Teng et al., 2018). Given that several other factors (e.g., burden of neoantigens, genetic mutations, immune cell infiltrate) influence immune-mediated PD-1 inhibitor response (Liebl et al., 2019), the risk for tumor spread and the response to targeted therapy in cSCC are not necessarily correlated and require further investigation.

**Figure 3.** Pooled analysis of PD-L1 expression between primary cSCC and metastatic cSCC tissue. CI, confidence interval; cSCC, cutaneous squamous cell carcinoma.
Apart from PD-L1, several other immune regulators were identified in this review (Fas, Fas-L, PD-L2, LLT1, ASC). High expression of LLT1, which deactivates NK cells, was associated with poor outcomes in a single study with increased nodal metastasis and reduced disease-specific survival (Santos-Juanes et al., 2019). The remaining proteins identified were not found to have an influence on poor outcomes.

Cell cycle and signaling

The role of cell-cycle regulation in cSCC behavior was investigated in several studies assessed in this review. Only the tumor suppressor p16 appeared in more than one comparison of primary tumors, and on pooled analysis, no difference in the risk for metastasis was observed with regard to expression levels. The lack of association may result from the fact that altered expression of p16 is detectable early in cSCC carcinogenesis and therefore does not distinguish high-risk from low-risk lesions (Hodges and Smoller, 2002).

Several less well-known DNA regulators and tumor suppressors (INPP5A, DSSG1, LRIG-1, WOX1, p68, elf4E, cyclin D1) each appeared in a single study that met the inclusion criteria and were associated with poor outcomes. Using receiver operator curve analysis, increased expression of CD133, a marker of cancer stem cells, correlated with poor differentiation and high tumor stage and was shown to confer increased risk for metastasis even after controlling for these other high-risk features in a single study (Xu et al., 2016). It is unknown whether these alterations are direct drivers of aggressive disease or byproducts of previous regulatory loss, but evidence continues to accumulate that markers of disrupted gene control are a common phenomenon in cSCC pathogenesis.

The outcomes based on EGFR expression, a transmembrane protein involved in cellular signaling and proliferation, appeared in four studies. On pooled analysis, increased expression was associated with higher odds for the development of metastasis in primary cSCCs. No differences in expression were detected between primary and metastatic tissues. Together, these data suggest that although elevated EGFR may predict tumor behavior, perhaps as a result of increased growth potential and tumor burden, it does not represent a finding specific for advanced cSCC. This is consistent with modest control rates observed in advanced tumors treated through targeted EGFR inhibition (Dereure et al., 2016; Gold et al., 2016; William et al., 2017;). EGFR inhibitors, which are used off label in the treatment of cSCC, are Food and Drug Administration approved for the treatment of nonsmall cell lung cancer. Data regarding the correlation of EGFR expression and treatment response in nonsmall cell lung cancer are mixed, possibly owing to the variable presence of EGFR mutations, which can alter drug binding site affinity or provide an activating mutation to target (Bethune et al., 2010; Lynch et al., 2004).

Of the other cell signaling molecules in this review (pSTAT3, HER, pERK, pS6, RKIP), only pS6, a downstream product of the mTOR signaling pathway, was associated with metastasis (Khandelwal et al., 2016). Although these results were obtained from a single small retrospective study, the findings are of interest because mTOR inhibitors are known to have antitumor effects and reduce cSCC burden (Euvrard et al., 2012).

Tissue invasion

Progression of primary cSCC to metastatic disease requires changes in cellular phenotypes to facilitate local invasion and transit through lymphovascular channels. Podoplanin, a membrane-associated glycoprotein that normally functions to assist in cardiopulmonary and lymphatic development, has been correlated with increased risk for metastasis in squamous cell carcinomas, including those with noncutaneous origins (e.g., oral, esophagus, lung, uterine cervix) (Ugorski et al., 2016). Podoplanin is highly expressed in cellular protrusions where other promigratory molecules are found, and its role in carcinogenesis may include facilitating adhesion to endothelium and neighboring cancer cells as well as induction of epithelial–mesenchymal transition (EMT). In support of its role in promoting metastatic disease, the pooled analysis presented in this study demonstrates increased odds for metastasis with high expression. This analysis includes data from two studies that demonstrated an association that was maintained on multivariable analysis after controlling for other high-risk tumor features (Hesse et al., 2016; Toll et al., 2012). In addition, Toll et al. (2012) reported an association between podoplanin expression and infiltrative growth patterns, noting that the most common pattern of staining was at the leading edge of the tumor where biologically, this interaction would have the greatest impact.

Another set of markers for cellular invasion are cadherins, which are transmembrane molecules that primarily function as components of adherens junctions connecting neighboring cells. Preserved expression of membranous E-cadherins in invasive or metastatic disease supports the theory of collective cancer invasion in cSCC, whereby adjoining cells maintain their connections in the process of tumor migration; conversely, loss of membranous but increased cytoplasmic E-cadherin suggests EMT as the driving force, whereby malignant epithelial cells transform phenotype to that of mesenchyme that more easily invades tissues.

Combined results of two included studies showed no difference in the pattern of E-cadherin expression between metastatic and nonmetastatic primary cSCCs with both groups showing a loss of membranous protein and moderate cytoplasmic expression (Toll et al., 2012; Vinicius et al., 2011). One study included in this analysis also reported an upregulation of E-cadherin transcription repressors involved in EMT (Twist, Zeb1) and markers of EMT (vinimentin) in tumors that metastasized (Toll et al., 2012). Together, these results suggest that dysregulated E-cadherin could signify early local invasion, but progression to systemic disease is more likely dependent on EMT.

MiRNAs

MicroRNAs are a class of molecules with a diverse range of actions and influence. They primarily function to regulate post-transcriptional mRNA, representing another control system in the promotion or repression of carcinogenesis. Accordingly, there is interest in evaluating their expression levels in tumors to inform prognosis and identify therapeutic targets.
The available literature and the understanding of miRNA's role in cSCC pathogenesis are primarily in vitro, with only four human studies meeting study inclusion criteria. Two studies of note included a prospective cohort following 152 cases that demonstrated an association of decreased miR-20a (tumor suppressor) with higher tumor stage and shorter overall survival as well as a retrospective cohort of 79 tumors that found that increased miR-205 (oncogenic) was associated with a composite endpoint of poor outcomes (Cañeteo et al., 2017; Zhang et al., 2015). Overexpression of miR-20a is correlated with decreased motility in oral squamous cell carcinoma cell lines through interaction with integrins (Chang et al., 2013) and negative repression of cyclin D1 in hepatocellular carcinoma cell lines (Karimkhanoeloo et al., 2017). Whereas in other tumors such as ovarian and colorectal carcinoma, overexpression has also been shown to correlate with invasive features and poor outcomes, suggesting a pleiotropic and potential site-specific influence of miR-20a on tumorigenesis (Fan et al., 2010; Zhu et al., 2019).

As a regulator of the link between genetic data and protein functioning, miRNA expression has both prognostic and therapeutic implications; however, more work is needed to validate findings in human subjects. Circulating miRNAs are detectable in the serum, which can theoretically be used as a noninvasive biomarker, but no studies in human cSCC have been performed to date.

Limitations and/or bias assessment

This study is subject to several biases and limitations, both in itself and as a result of the available literature.

The limited number and often heterogeneous nature of studies examining the same protein allowed for only a few combined analyses. In an effort to quantitatively synthesize results across studies and provide effective measures using a broader population, meta-analyses were performed, but some cases required the use of both cohort and case-control studies. Only one prospective study met inclusion criteria, so results are subject to biases inherent to retrospective study design. However, because metastasis and death were the subjects of study, these were likely accurately recorded. Finally, conclusions from these expression-level results represent associations with poor outcomes but do not prove causality. Nonetheless, observations made regarding the expression levels and tumor behavior reflect biologically reasonable theory and make use of available laboratory tools used in prognostic discrimination in other cancers.

Regarding the general issues with the quality of studies included in this review, a large majority of published literature in this domain utilized grading criteria of molecular expression that were neither clearly predefined nor consistent across the literature. Methods to rectify these issues include the use of receiver operator curve analysis to determine performance characteristics and thresholds as demonstrated by Xu et al. (2016) and García-Pedrero et al. (2017) as well as efforts to improve our understanding between in vitro expression and biologic function with functional assays. Only two studies utilized techniques to identify markers from the entire proteomic or RNA profile where more rigorous statistical methods are required to account for potential false-positive results resulting from multiple comparisons (Föll et al., 2018; Gillespie et al., 2016).

In conclusion, as the overall incidence of cSCC remains high with poor outcomes occurring infrequently, additional efforts to improve the prognostication and likelihood of treatment response using reliable molecular diagnostic techniques are increasingly important. In particular, further studies are needed to determine whether the expression of PD-L1, EGFR, and podoplanin aids in cSCC prognostic estimations or predicts response to therapy.

MATERIALS AND METHODS

Literature search

This unregistered study was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines (Supplementary Figure S1) (Moher et al., 2009). A search of PubMed and/or Medline and EMBASE was performed for articles published between January 1, 2005 and September 1, 2019 using the following search terms: “cutaneous squamous cell carcinoma,” “expression” or “mutation.” Duplicates were removed, and each abstract was screened for eligibility by assessing for potential fulfillment of inclusion criteria. Predetermined criteria were: English language, inclusion of at least 10 human subjects, and comparison of protein or RNA expression levels between either primary and metastatic cSCC tissue or between primary cSCCs that resulted in metastasis or death and primary cSCCs that did not. A full-text review was performed for any article that may have fulfilled these inclusion criteria. Two authors (PMM and JED) independently performed the screening and full-text review for eligible articles, and a third author (PRM) served as an arbitrator for all discordant results. Risk of bias at the study level was performed using the Newcastle–Ottawa Scale, which was used for cohort and case-control studies, and the Appraisal tool for Cross-Sectional Studies, which was used for cross-sectional studies (Supplementary Figures S1 and S2 and Supplementary Tables S3 and S4).

Data extraction

The following information was collected from each included article: number of subjects, number of high-risk tumors, treatments, study design, follow-up duration, method of molecular quantification and grading, and clinical outcomes of metastasis or death. For analysis, studies were divided into two categories: analysis of primary cSCC tissue tracked for later metastasis or death and comparison of tissue samples from primary versus those from metastatic cSCCs (not necessarily from the same individuals). Individual tumor molecular profiles were extracted and tabulated in accordance with each predefined study protocol when available. When a study included more than one protein, each of these comparisons was collected, analyzed, and reported separately (referred to as comparisons in this article) but only contributed once to the total number of subjects. Results from one study were presented, but the 2,101 proteins identified using global proteomic analysis were not counted toward the total protein count owing to this distinct methodology (Föll et al., 2018).

Data analysis

In instances where individual tumor molecular characteristics were available, independent statistical testing using chi-square or Fisher exact test for binary outcomes, chi-square test for trend for ordinal variables, and McNemar’s test for paired data was performed using Stata IC, version16.0 (StataCorp, College Station, TX), with a
predefined significance level of 0.05. Protein expression outcomes appearing in more than one study were extracted when measures of grading were similar (e.g., 0–3+, high vs. low, etc.), and meta-analyses using random-effects models were performed utilizing the grading system of the original authors to limit bias when dichotomizing expression into high and low groups. Given infrequent reporting on similar exposures and outcomes in series containing primary cSCCs tracked for metastasis and death, cohort and case-control study designs were consolidated and analyzed jointly with the ORs as the reported effect measure. For comparisons where individual tumor data were not available, results of statistical testing described by the original authors were reported as originally presented.

Data availability statement

The dataset for this article can be found at [https://data.mendeley.com/datasets/ngd9x6g9c5/1](https://data.mendeley.com/datasets/ngd9x6g9c5/1), hosted at Mendeley (Mulvaney, 2020).

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CONFLICT OF INTEREST

CDS is a steering committee member for Castle Biosciences; is a steering committee member and consultant for Regeneron Pharmaceuticals; is a consultant for Sanofi; has received research funding from Castle Biosciences, Regeneron Pharmaceuticals, Novartis, Genentech, and Merck; and is a chair for National Comprehensive Cancer Network. The remaining authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: PMM, KKY, PRM, JED, CDS; Data Curation: PMM, JED, PRM; Formal Analysis: PMM, PRM; Investigation: PMM, JED, PRM; Methodology: PMM, JED, PRM; Project Administration: CDS; Resources: CDS; Software: PMM, PRM, CDS; Supervision: PMM, PRM, CDS; Visualization: PMM, JED, PRM, CDS; Writing - Original Draft Preparation: PMM; Writing - Review and Editing: PMM, KKY, PRM, CDS

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2021.02.741.

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