Identification of CD39 as a Marker for the Circulating Malignant T-Cell Clone of Sézary Syndrome Patients

Journal of Investigative Dermatology (2019) **139,** 725–728; doi:10.1016/j.jid.2018.09.026

TO THE EDITOR

Sézary syndrome (SS) represents a subtype of cutaneous T-cell lymphoma characterized by the presence of a malignant $CD4^+$ T-cell clone that accumulates mainly in the skin and peripheral blood (Jawed et al., 2014a; Ortonne et al., 2006). This disease shows an aggressive evolution and a poor prognosis at advanced stages (Jawed et al., 2014b). Over the last years, flow cytometry appears to be the method of choice for measuring blood tumor burden in SS (Scarisbrick et al., 2018). However, Sézary cell detection still essentially relies on a loss of expression of CD7 and/or CD26 antigen rather than on the aberrant expression of a specific tumor marker (Bernengo et al., 1998; Jones et al., 2001; Kelemen et al., 2008). Some years ago, we identified a unique and specific positive marker for Sézary cells, the natural killer cell receptor KIR3DL2 (Bagot et al., 2001; Ortonne et al., 2006). We established that KIR3DL2 delineates the malignant T cells in both skin and blood and represents a valuable diagnosis and prognosis marker (Hurabielle et al., 2017). However, we also reported that KIR3DL2 expression could not be detected by flow cytometry on the circulating malignant T-cell clone of about 10% of Sézary patients (Marie-Cardine et al., 2014). In an effort to validate tumor markers that could identify the malignant T-cell clone in a patient-independent manner, we performed multiparameter mass cytometry using an antibody panel focused on T lymphocytes and on putative or known positive markers for circulating Sézary cells, including KIR3DL2 (Marie-Cardine et al., 2014), CCR4 (Ferenczi et al., 2002), and CD39. The last of these was selected because, in addition to its involvement in the control of immune responses and, notably, of regulatory T-cell immunosuppressive functions (Deaglio et al., 2007), recent reports have highlighted its expression by tumor cells and its participation in the suppression of antitumor immune responses (Bastid et al., 2013; Bonnefoy et al., 2015).

Immunolabeling and CyTOF phenotyping was conducted on peripheral blood mononuclear cells (PBMCs) isolated from SS patients ($n = 2$) and healthy donors (HDs) ($n = 5$). After gating, live $CD3^+CD4^+CD45^+CD14^-CD8^-$ events were exported for analysis by application of the t-stochastic neighbor embedding algorithm. The resulting plots clearly showed a major evolution of the $CD4^+$ T-cell population phenotype between HD and SS patients, with the detection of totally different cluster segregation (Figure 1a). Thus, for a given SS patient, most $CD4^+$ T cells were found to segregate in a single cluster (Figure 1b) corresponding to the malignant T-cell clone, as confirmed by its high expression levels of KIR3DL2 and CCR4. The expression of these two markers was limited to more restricted $CD4^+$ T-cell subsets in HDs (representative data in Figure 1b). In addition, CD39 was coexpressed by the $KIR3DL2^+CCR4^+CD4^+$ T-cell cluster in SS patients, whereas its expression was restricted to a small number of cells in HDs (Figure 1b). Comparison of the percentage of CD39-, KIR3DL2-, and CCR4-expressing $CD4^+$ T cells in the HD and SS groups supports the idea that CD39 might represent a valuable marker for identification of SS patients' T-cell clone (Figure 1c). To confirm this possibility, classical flow cytometry analysis was performed on blood from HDs ($n = 12$) and SS patients ($n = 20$) whose tumor clone can be detected among the $CD4^+$ T-cell population through the use of an available anti–T-cell receptor (TCR)-Vβ monoclonal antibody (mAb). Results obtained on one representative HD and one SS patient (harboring a malignant TCR-$V\beta8^+$ T-cell clone) are shown in Figure 2a ($CD3^+CD4^+$ gate) and Supplementary Figure S1a online ($V\beta8^+$ vs $V\beta8^-$ gates). Statistical comparison of the data obtained from each group shows a strong and significant enhancement of CD39, KIR3DL2, and CCR4 expressions, readily detectable in the $CD4^+$ T-cell population of SS patients when compared with HDs ($P < 0.0001$) (Figure 2b), with a strong correlation between KIR3DL2 and CD39 expressions on SS patients' $CD4^+$ T cells ($P < 0.0001$) (Figure 1c). This observation results from the strong positivity of the $V\beta^+$ cells for all three markers (Figure 2b, and see Supplementary Figure S1a), with a similar and excellent correlation observed between the percentages of TCR-$V\beta^+$ and $CD39^+$ or $KIR3DL2^+$ cells among total $CD4^+$ T lymphocytes ($P < 0.0001$) (see Supplementary Figure S1b; mean percentage of positive cells ± standard deviation is given in Supplementary Table S1 online).

A T-cell subclone, sharing the same Vβ reactivity as the tumor clone, could be detected in some patients but was excluded from the pool of malignant T cells because of its $KIR3DL2^-CD39^-$ phenotype (see Supplementary Figure S1a). CD39, and to a lesser extent KIR3DL2, expression appears marginally increased in SS patients' nonmalignant $CD4^+$TCR-$V\beta^-$ T cells compared with HDs' $CD4^+$ T cells ($P < 0.01$ and 0.05, respectively), but no significant difference regarding CCR4 expression was observed (Figure 2b, and see Supplementary Table S1). CD127 staining identifies the normal/nonmalignant $CD39^+$ T cells as a mix of regulatory T cells and conventional

Abbreviations: HD, healthy donor; SS, Sézary syndrome; TCR, T-cell receptor

Accepted manuscript published online 25 October 2018; corrected proof published online 28 December 2018

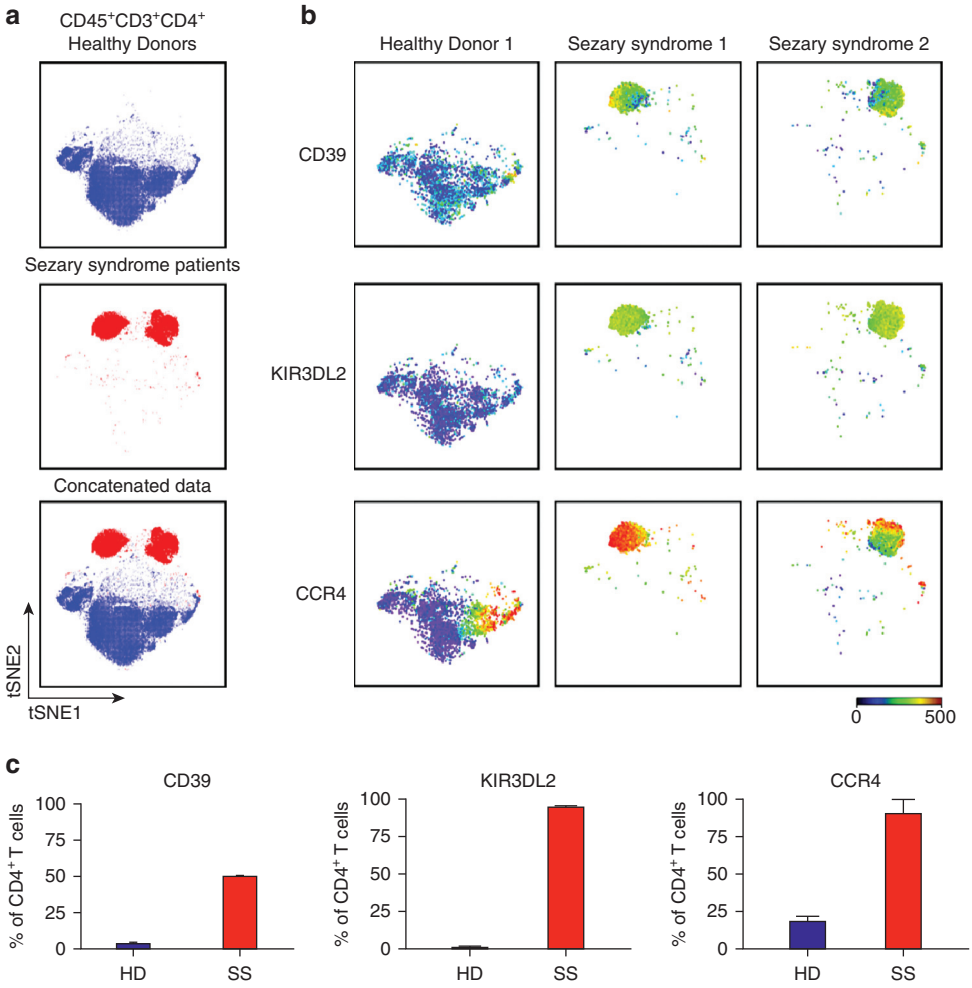

Figure 1. High-dimensional phenotypic analysis of peripheral blood CD4$^+$ T cells of healthy donors or Sézary syndrome patients. Blood was collected after patients gave written informed consent, and the study was approved by the institutional ethics committee (Saint Louis Hospital, Paris). After PBMC preparation and labeling, analysis was carried out on gated live (cisplatin$^-$) CD3$^+$CD45$^+$CD14$^-$CD8$^-$CD4$^+$ T cells. (**a**) t-SNE plots showing the distribution of the CD4$^+$ T cells among PBMCs of healthy donors (blue, n = 5) or Sézary syndrome patients (red, n = 2). (**b**) Heat map representation of the relative expression intensities of CD39, KIR3DL2, and CCR4 on circulating CD4$^+$ T cells from one representative healthy donor and two Sézary syndrome patients. (**c**) Frequency of expression of CD39, KIR3DL2, and CCR4 on CD4$^+$ T cells, extracted from the CyTOF data obtained on the healthy control individuals and Sézary syndrome patients tested. HD, healthy donor; PBMC, peripheral blood mononuclear cell; SS, Sézary syndrome; tSNE, t-stochastic neighbor embedding.

CD4$^+$ T cells (see Supplementary Figure S1c), suggesting that each or both cell types might be expanded in the context of SS. In our cohort, one patient exhibited tumor cells that were not identified by CD39 labeling but were detected through KIR3DL2 expression (Figure 2d). Conversely, a second patient showed a mainly CD39$^+$CCR4$^-$ malignant clone, some of which was negative for KIR3DL2 (Figure 2e). Although additional work on inflammatory cutaneous diseases will be necessary to validate CD39 as a single marker for SS diagnosis, our data suggest that the combined use of anti-CD39 and -KIR3DL2 mAbs might represent a powerful tool for an easy and fast routine evaluation, based on the detection of positive markers, of SS patients' circulating tumor burden.

CD39 is an ectonucleotidase involved in the process of adenosine triphosphate/adenosine diphosphate hydrolysis, leading to the generation of adenosine. Its expression by tumor cells was recently correlated to their ability to inhibit T-cell proliferation and generation of cytotoxic effector CD8$^+$ T-cells in an adenosine-dependent manner. An antagonist anti-CD39 mAb, able to block CD39 enzymatic activity, neutralized this tumor-induced immune suppression (Bastid et al., 2015). It is therefore tempting to speculate that CD39 expression by SS patient malignant clone could be in part involved in the processes leading to tumor escape. These findings support the attractive possibility of using CD39 as a specific marker for the evaluation of SS patients' circulating tumor burden but also as a promising target in the context of SS with the development of CD39-blocking antibodies that may restore efficient antitumor responses.

This study was approved by the institutional ethics committee of Saint Louis Hospital, Paris; patients provided written informed consent.

CONFLICT OF INTEREST

AB is a co-founder of Orega Biotech (Ecully, France) and a co-inventor on CD39 patent (US20130273062A1). The other authors state no conflict of interest.

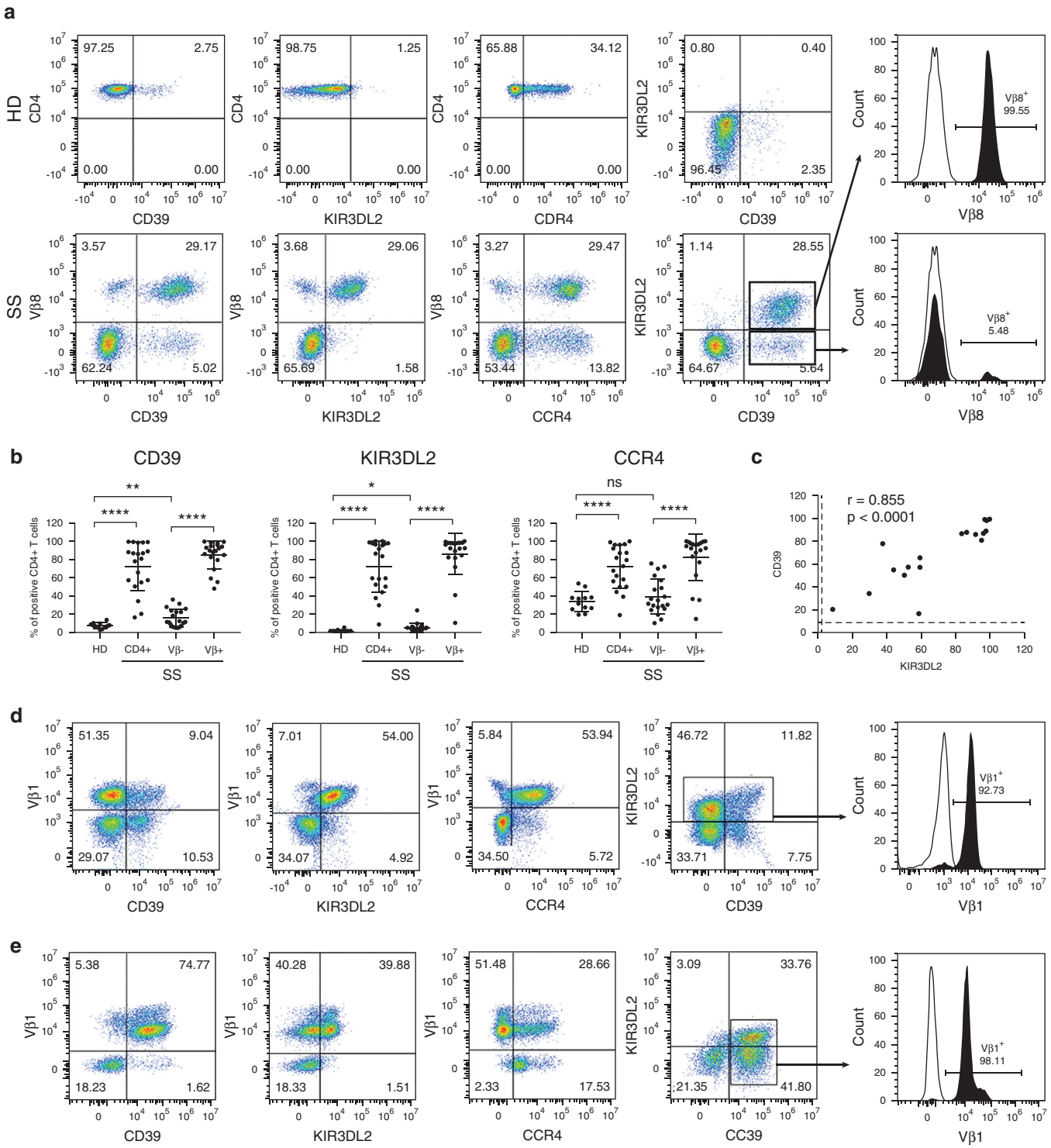

Figure 2. Flow cytometry analysis of CD39 expression on SS patients' malignant T-cell clones. (**a**) Immunolabeling was performed on blood from HDs (n = 12) and SS patients (n = 20) to analyze the expressions of the positive markers CD39 (clone A1), KIR3DL2 (clone 13E4), and CCR4 (clone L291H4) on the gated $CD3^+CD4^+$ T-cell population. For SS patients, an anti-TCR-Vβ mAb was added to distinguish the malignant ($Vβ^+$) from the nonmalignant ($Vβ^-$) $CD4^+$ T cells among the $CD4^+$ T-cell population. Shown are results from one representative HD and one SS patient (harboring a $Vβ8^+$ malignant T-cell clone that co-expressed CD39 and KIR3DL2). (**b**) Scatter dot plots from the data obtained as described in **a** and corresponding to 12 HD and 20 SS patients tested. Statistical analysis was performed using a Mann-Whitney *t* test. $^*P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$. (**c**) Correlation between KIR3DL2 and CD39 expressions on circulating $CD4^+$ T cells from 20 SS patients. Each plot represents the individual percentage of $KIR3DL2^+$ and $CD39^+$ cells within the total $CD4^+$ T-cell population for one SS patient. The correlation between the two factors was analyzed with a Spearman nonparametric correlation test. Dashed lines corresponded to the upper limit of the mean percentage ± standard deviation found for each marker in HD $CD4^+$ T cells (n = 12). (**d, e**) Labeling was performed as in **a**. The malignant clones of these two patients exhibit a mixed KIR3DL2/CD39 phenotype, with the detection of either (**d**) $KIR3DL2^+CD39^{-\text{ or }+}$ or (**e**) $CD39^+KIR3DL2^{-\text{ or }+}$ tumor cells. HD, healthy donor; mAb, monoclonal antibody; ns, not significant; SS, Sézary syndrome.

ACKNOWLEDGMENTS

This work was supported by grants from INSERM and Paris Diderot University. We thank H. Sicard (Innate Pharma, Marseille, France) for the kind gift of the anti-KIR3DL2 antibody.

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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.2018.09.026.

REFERENCES

Bagot M, Moretta A, Sivori S, Biassoni R, Cantoni C, Bottino C, et al. CD4$^+$ cutaneous T-cell lymphoma cells express the p140-killer cell immunoglobulin-like receptor. Blood 2001;97:1388–91.

Bastid J, Cottalorda-Regairaz A, Alberici G, Bonnefoy N, Eliaou JF, Bensussan A. ENTPD1/CD39 is a promising therapeutic target in oncology. Oncogene 2013;32:1743–51.

Bastid J, Regairaz A, Bonnefoy N, Dejou C, Giustiniani J, Laheurte C, et al. Inhibition of CD39 enzymatic function at the surface of tumor cells alleviates their immunosuppressive activity. Cancer Immunol Res 2015;3:254–65.

Bernengo MG, Quaglino P, Novelli M, Cappello N, Doveil GC, Lisa F, et al. Prognostic factors in Sézary syndrome: a multivariate analysis of clinical, haematological and immunological features. Ann Oncol 1998;9:857–63.

Bonnefoy N, Bastid J, Alberici G, Bensussan A, Eliaou JF. CD39: a complementary target to immune checkpoints to counteract tumor-mediated immunosuppression. Oncoimmunology 2015;4(5):e1003015.

Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med 2007;204:1257–65.

Ferenczi K, Fuhlbrigge RC, Pinkus J, Pinkus GS, Kupper TS. Increased CCR4 expression in cutaneous T cell lymphoma. J Invest Dermatol 2002;119:1405–10.

Hurabielle C, Thonnart N, Ram-Wolff C, Sicard H, Bensussan A, Bagot M, et al. Usefulness of KIR3DL2 to diagnose, follow-up, and manage the treatment of patients with Sézary syndrome. Clin Cancer Res 2017;23:3619–27.

Jawed SI, Myskowski PL, Horwitz S, Moskowitz A, Querfeld C. Primary cutaneous T-cell lymphoma (mycosis fungoides and Sézary syndrome): part I. Diagnosis: clinical and histopathologic features and new molecular and biologic markers. J Am Acad Dermatol 2014a;70:205.

Jawed SI, Myskowski PL, Horwitz S, Moskowitz A, Querfeld C. Primary cutaneous T-cell lymphoma (mycosis fungoides and Sézary syndrome): part II. Prognosis, management, and future directions. J Am Acad Dermatol 2014b;70:223.

Jones D, Dang NH, Duvic M, Washington LT, Huh YO. Absence of CD26 expression is a useful marker for diagnosis of T-cell lymphoma in peripheral blood. Am J Clin Pathol 2001;115:885–92.

Kelemen K, Guitart J, Kuzel TM, Goolsby CL, Peterson LC. The usefulness of CD26 in flow cytometric analysis of peripheral blood in Sézary syndrome. Am J Clin Pathol 2008;129:146–56.

Marie-Cardine A, Viaud N, Thonnart N, Joly R, Chanteux S, Gauthier L, et al. IPH4102, a humanized KIR3DL2 antibody with potent activity against cutaneous T-cell lymphoma. Cancer Res 2014;74:6060–70.

Ortonne N, Huet D, Gaudez C, Marie-Cardine A, Schiavon V, Bagot M, et al. Significance of circulating T-cell clones in Sézary syndrome. Blood 2006;107:4030–8.

Scarisbrick JJ, Hodak E, Bagot M, Stranzenbach R, Stadler R, Ortiz-Romero PL, et al. Blood classification and blood response criteria in mycosis fungoides and Sézary syndrome using flow cytometry: recommendations from the EORTC cutaneous lymphoma task force. Eur J Cancer 2018;93:47–56.

Tyrosine Kinase Inhibitor and Immune Checkpoint Inhibitor Responses in *KIT*-Mutant Metastatic Melanoma

Journal of Investigative Dermatology (2019) **139,** 728–731; doi:10.1016/j.jid.2018.10.009

TO THE EDITOR

KIT is a frequently mutated gene identified in acral lentiginous, mucosal, and non-acral cutaneous melanoma (Woodman and Davies, 2010). Targeting oncogenic *KIT* mutations with tyrosine kinase inhibitors (TKIs) in melanoma has demonstrated response rates up to 29%, with progression-free survival (PFS) of 3.5 months (Carvajal et al., 2011). Immune checkpoint inhibitors (ICIs) have shown overall survival (OS) benefit in cutaneous melanoma, but to our knowledge no prior study has evaluated responses of *KIT*-mutant melanomas to ICI (Hodi et al., 2010). This retrospective study investigates TKIs and ICIs in *KIT*-mutant melanoma to determine genetic and clinical factors associated with response, PFS, and OS. All patients were treated at The University of Texas MD Anderson Cancer Center and signed informed consent for collection and analysis of their tumor samples. This protocol was approved by the Institutional Review Board.

Nineteen patients were identified with a *KIT*-activating mutation that received TKI treatment (Supplementary Table S1 online). Although the TKI analysis may be complicated by the grouping of three distinct KIT inhibitor TKIs, we observed an overall response rate of 20% for TKI treatment, similar to prior prospective clinical trials (Carvajal et al., 2011; Guo et al., 2011; Guo et al., 2017; Hodi et al., 2013; Kalinsky et al., 2017; Lee et al., 2015), and 32% when evaluating patients treated with a TKI concurrently with another agent

Abbreviations: CI, confidence interval; ICI, immune checkpoint inhibitor; OS, overall survival; PFS, progression-free survival; TKI, tyrosine kinase inhibitor

Accepted manuscript published online 25 October 2018; corrected proof published online 21 December 2018