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Association of Vemurafenib and Pipobroman Enhances BRAF-CRAF Dimerization in Squamous Cell Carcinoma

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TO THE EDITOR

BRAF selective inhibitors (BRAFi) have changed the prognosis of advanced melanoma with BRAF mutations. However, they are responsible for increased risk of cutaneous squamous cell carcinoma (cSCC) in relation with paradoxical activation of the mitogen-activated protein kinase (MAPK) pathway in cells with wild-type BRAF but activated RAS (Lito et al., 2013). Other drugs are known to increase the risk of cSCC such as hydroxyurea and pipobroman. Such antimetabolites inhibit nucleotide excision repair after DNA damage (Francis et al., 1979). We present a patient with multiple and rapidly progressive cSCC after synergistic association of vemurafenib and pipobroman.

A 76-year-old woman had melanoma of the trunk Breslow 1.1 mm, ulcerated Clark IV excised in 2004, and history of essential thrombocythemia (JAK2V617F) in 1999 for which she received hydroxyurea during 10 months, relayed in 2000 by pipobroman. She was type II phototype with limited sun exposure. In 2012, radiological evaluation showed one lung metastasis chirurgicaly removed (BRAF V600K). In 2013, two asymptomatic brain metastases (5 and 10 mm) and two lung metastases were discovered. Vemurafenib, as BRAFi, was administered 960 mg bid with stereotactic radiotherapy on brain metastasis.

Tumoral evaluation at week 8 showed partial response according to RECIST (response evaluation criteria in solid tumors) (Spiro et al., 2015). However, 15 cSCCs were reported in the tegument (Figure 1a). The cSCCs were on sun-exposed and non–sun-exposed skin with no mucosal lesions (size between 2 mm and 30 mm). Lesions appeared within 1 month and were treated surgically. Six were well-differentiated invasive cSCCs and three were micro invasive cSCCs without perineural or vascular invasion. Vemurafenib was switched to ipilimumab (Figure 1b). Pipobroman was continued without occurrence of other cSCCs after 12 months' follow-up. This case illustrates an unusual form of a frequent cutaneous side effect of vemurafenib potentiated by piproboman.

To gain further insight into the mechanism of cSCCs developed by this patient, we investigated BRAF-CRAF dimerization in the patient's cSCC tumor specimens and compare results with cSCCs developed with BRAFi alone or induced by UV without drug. Patient consent was not required because French laws consider human tissue left over from surgery as discarded material. Using a proximity ligation assay to detect BRAF-CRAF dimerization in situ, we found more dimers in our three patient's cSCCs as compared with three SCCs developed in patients treated with BRAFi only although the difference was not significant ($P = 0.0671$). No dimers were present in two cSCCs developed independently of any drug (Figure 2a and b).

The most frequent cutaneous adverse events reported with vemurafenib and other BRAFi are benign or malignant keratotic lesions, exanthematous rash, and photosensitivity (Lacouture et al., 2013). Keratocanthomas and cSCC has been reported in studies in 4–31% of patients treated by vemurafenib (Anforth et al., 2013). They occur within 8 weeks (mean 51 days, median 32 days) but do not require interruption

Abbreviations: BRAFi, BRAF selective inhibitors; cSCC, cutaneous squamous cell carcinoma; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase

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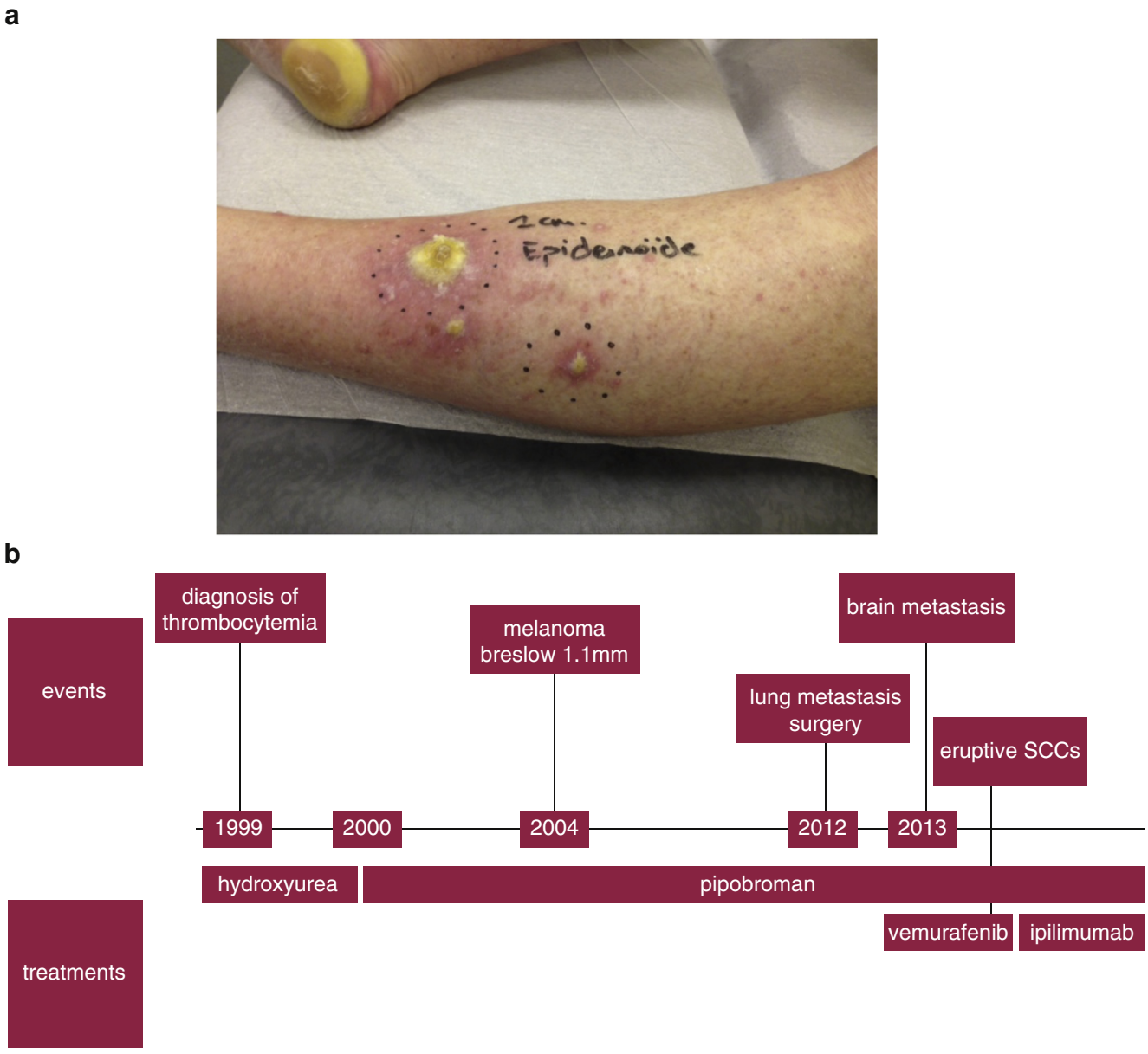

Figure 1. Illustration of lesions and course of the treatment. (**a**) Preoperative view of the squamous cell carcinoma of the left leg. (**b**) Diagram explaining the course of the treatment over time.

of treatment. A retrospective analysis of 25 keratocanthomas and cSCC concluded that they have an average size of 8.7 mm, and that they are located predominantly on the lower extremities (48%) and scalp (20%) whereas 12% occur on the trunk (Belum et al., 2015).

BRAFi-induced cSCC are the result of paradoxal activation of the MAPK pathway. We know from preclinical experiments that BRAFi induces BRAF/CRAF dimerization from direct binding of the BRAFi to the RAF kinase domain, with relief of autoinhibitory P-loop phosphorylation, stimulation of CRAF kinase activity, and downstream MAPK/ERK kinase/extracellular signal-regulated kinase (ERK) activation (Poulikakos et al., 2010). This paradoxical activation of the MAPK in cells without BRAF mutation requires RAS activation. Such mechanism is supported in patients treated by BRAFi by the occurrence of activating mutations of KRAS or HRAS in approximately 21–60% of cases and activation of the MAPK pathway shown by expression of phosphorylated ERK in human BRAFi-induced cSCC biopsies (Anforth et al., 2012; Oberholzer et al., 2011; Su et al., 2012).

Pipobroman is both an antimetabolite and an alkylating agent. Pipobroman-induced cSCCs are reported to be aggressive, appearing rapidly on unusual sites (Tchen et al., 2007). cSCCs are probably related to DNA damage and peroxidation of cell membrane lipids in concert with UV (Svobodova et al., 2006). In our case, the early occurrence, the unusual size and number of lesions, and the presence of BRAF/CRAF dimers favor the synergistic effect between vemurafenib and pipobroman.

We hypothesized that piproboman is responsible for a high rate of cytogenic alterations in the epidermis leading to RAS activation. In accordance, we sequenced all three RAS genes in all