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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2016.05.099.

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Low Levels of Genetic Heterogeneity in Matched Lymph Node Metastases from Patients with Melanoma

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TO THE EDITOR

In our previous experience, a high consistency of *BRAF* and *NRAS* mutation patterns was observed between primary tumors and lymph node metastases in patients with advanced melanoma (Colombino et al., 2012). Conversely, increasing rates of discrepancies in *BRAF/NRAS* mutation patterns were found between primary melanomas and metastases in other sites (brain or, mostly, skin) (Colombino et al., 2012). When the distribution of *BRAF/NRAS* mutations was evaluated in a larger cohort, the high rate of consistency in sequence variations of these two genes was further confirmed between primary melanomas and lymph node metastases (142/156; 91%) (Colombino et al., 2013; unpublished data). However, intraindividual heterogeneity of *BRAF* mutations has been

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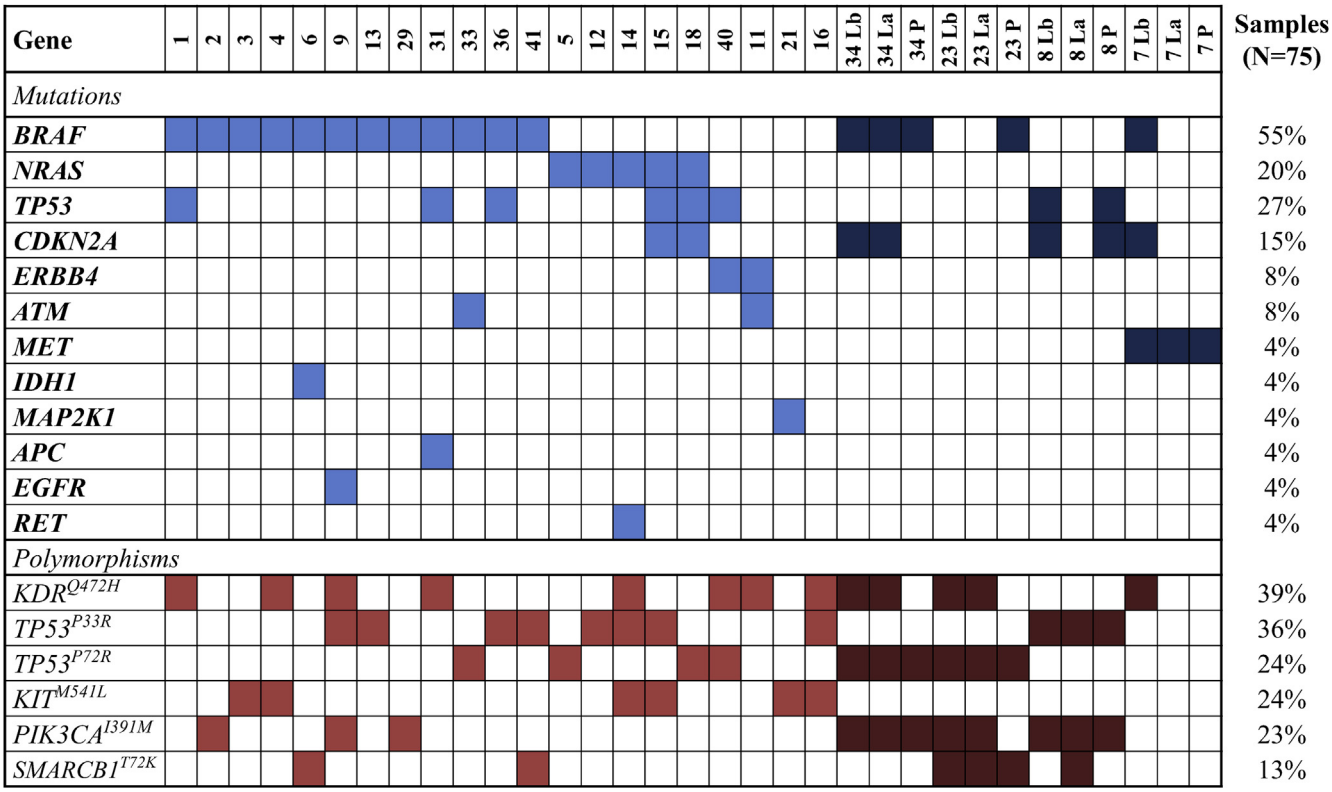

Figure 1. Distribution of nonsilent sequence variations in melanoma samples. Individual gene mutations (blue squares) and polymorphic sequence variants with unknown functional significance (red squares) are shown across all cases. Sample results for the four discrepant cases are indicated with darker colors into the right part of the diagram. La and Lb, the two paired lymph node samples from each patient; P, primary melanoma.

reported in primary and secondary melanomas (Lin et al., 2011; Shi et al., 2014; Yancovitz et al., 2012).

Recently, recurrent or driver mutations have been searched for comparison in different types of primary tumors and matched metastases from the same patients—including melanoma—through next-generation sequencing approaches (The Cancer Genome Atlas Network, 2015; Vakiani et al., 2012; Vignot et al., 2013). Using a next-generation sequencing methodology, we here tried to definitely assess whether clonal expansion to lymph node sites may be associated with consistently low levels of genetic discrepancies in multiple lesions from patients with melanoma.

Genomic DNA was isolated from a total of 75 paired specimens of primary melanomas and synchronous or asynchronous lymph node metastases among consecutively collected patients (N = 25) (see Supplementary Table S1 online), after obtaining their written informed consent for tissue sampling; the study protocol was reviewed and approved by the Bioethics Committee of the Sassari Healthcare District. In particular, the following formalin-fixed, paraffin-embedded tumor tissues from pathological archives were collected: (i) two asynchronous metastatic lymph nodes sequentially excised from the same patients with melanoma (N = 9) (median time period between first and second nodal excision, 7 months; range, 3–15); (ii) two metastatic lymph nodes synchronously excised from the same patients with melanoma (N = 16); and (iii) corresponding primary melanoma tissues.

Genomic DNA was obtained from macrodissected tumor tissues containing at least 80% neoplastic cells and analyzed for approximately 2,800 mutations in 50 most common oncogenes and tumor suppressor genes, using the AmpliSeq Cancer Panel HotSpot V2/CHPv2 on the Ion Torrent platform (Life Technologies-Thermo Fisher Scientific, Waltham, MA). Data from the runs on such a platform were processed to generate multiple sequence reads, by which redundant mutated alleles were identified (Supplementary Materials and Methods and Table S2 online). Significantly recurrent gene mutations were validated by Sanger sequencing (Supplementary Materials and Methods); all detected variants have already been reported in both the Human Gene Mutation Database at http://www.hgmd.cf.ac.uk/ac/index.php and the Catalogue of Somatic Mutations in Cancer at http://www.sanger.ac.uk/genetics/CGP/cosmic/.

Overall, 4 of 25 (16%) analyzed patients presented discrepant mutation patterns between primary melanomas and correspondent lymph nodal metastases (Figure 1; Table 1). Discrepancies were markedly higher among patients with asynchronous (2 of 9; 22%) than among those with synchronous (2 of 16; 12.5%) metastases (Table 1). In nearly all (3 of 4; 75%) discrepant cases, the main differences were observed in driver mutations of the *BRAF* gene: a mutated metastasis in a patient with wild-type primary tumor (case 7 in Table 1), a mutated primary tumor and wild-type metastases (case 23), or two distinct mutations in the two lesion types (case 34).