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LETTER TO THE EDITOR

Lack of *BRAF* V600E mutation in human myeloma cell lines established from myeloma patients with extramedullary disease

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After whole genome sequencing of samples from 38 patients with multiple myeloma (MM) had identified one patient with an activating mutation of *BRAF* (G469A), *BRAF* mutations have been intensively screened in MM patients.[1] Among 161 samples, 3 K601N and 4 V600E (the most common in melanoma) mutations were found in 4% of the patients.[1] This low incidence is still consistent with a recent study involving 32 MM samples that reported no *BRAF* mutation.[2] In the more specific field of extramedullary disease (EMD) in MM, Andrulis *et al.*[3] recently reported a significant association with *BRAF* exon 15 mutations. Notably, the V600E mutation was found in 8.5% of patients with EMD (4 out of 47) versus 1.5% of patients without EMD (3 out of 204). The EMD of the four patients harbouring *BRAF* V600E mutation (all soft tissue plasmacytomas) was primitive for one patient and secondary after one or several lines of treatments for the three others. This recent work contrasts with an older one in which no BRAF mutation was found in 65 fresh bone marrow samples from 18 patients with PCL and 47 patients with MM at diagnosis.[4] The incidence of EMD in MM is rare at diagnosis but extramedullary involvement increases with disease evolution. Spreading of MM cells out of the bone marrow is commonly associated with a poor outcome and resistance to salvage therapies.[5] In this context, the recent findings of Andrulis *et al.* raise the interest of identifying

Table 1. *BRAF* and *RAS* mutations in human myeloma cell lines

HMCLs	*Disease at diagnosis*	*Sample*	*BRAF exon 15*	*K-RAS codons 12, 13 and 61*	*N-RAS codons 12, 13 and 61*	*Vemurafenib IC_{50} (μM)*
AMO1	PCT	AF	Wt	Wt	Wt	
XG10	PCT	AF	Wt	G13G + R	Wt	
U266	MM	PB	K601K + N	Wt	Wt	10
ANBL6	MM	PB	Wt	Wt	Wt	
LP1	MM	PB	Wt	Wt	Wt	
NAN6	MM	PB	Wt	Wt	Wt	
NAN8	PCL	PB	Wt	Wt	Wt	
NAN9	MM	PB	Wt	Wt	Wt	
OPM2	MM	PB	Wt	Wt	Wt	12
SKMM2	PCL	PB	Wt	Wt	Wt	
XG5	MM	PB	Wt	Wt	Wt	
XG6	MM	PB	Wt	Wt	Wt	
XG11	PCL	PB	Wt	Wt	Wt	
RPMI8226	MM	PB	Wt	G12A	Wt	
XG7	MM	PB	Wt	G12C	Wt	
KARPAS620	PCL	PB	Wt	G12D	Wt	
NAN10	MM	PB	Wt	Wt	G12G + R	
XG1	MM	PB	Wt	Wt	G12R	
MDN	MM	PB	Wt	Wt	G13D	
NAN7	MM	PB	Wt	Wt	Q61H	
JIM3	MM	PE	Wt	Wt	Wt	
KMS11	MM	PE	Wt	Wt	Wt	
KMS12PE	MM	PE	Wt	Wt	Wt	18
NAN1	MM	PE	Wt	Wt	Wt	
SBN	PCT	PE	Wt	Wt	Wt	
XG4	MM	PE	Wt	Wt	Wt	
XG2	MM	PE	Wt	G12A	Wt	
JJN3	MM	PE	Wt	Wt	G12D	
NCI-H929	MM	PE	Wt	Wt	G13D	9.5
L363	PCL	PE	Wt	Wt	Q61H	
NAN3	MM	PE	Wt	Wt	Q61K	
XG3	PCL	PE	Wt	Wt	Q61K	
KMM1	MM	SC	Wt	Wt	G13D	

Abbreviations: AF, ascites fluid; MM, multiple myeloma; PB, peripheral blood; PCL, plasma cell leukemia; PCT, plasmacytoma; PE, pleural effusion; SC, subcutaneous; Wt, wild type. HMCLs were previously reported.[6,7] DNA *BRAF* sequencing was performed using primers located within introns 14 and 15 (forward: 5′-ACTCTTCATAATGCTTGCTCTGA-3′, reverse-5′-AGTAACTCAGCAGCATCTCAGG-3′, respectively). *K-* and *N-RAS* sequencing was previously reported.[6] For NAN8 and NAN10 cell lines, screening of *BRAF* mutation was also performed in the cryopreserved myeloma peripheral cells from which the cell lines were established, and the same results were found, that is, no *BRAF* mutation (data not shown). No BRAF mutation was found in four other PCLs (data not shown). Vemurafenib IC_{50} values were defined as the doses that inhibited 50% of proliferation (see Figure 1b).

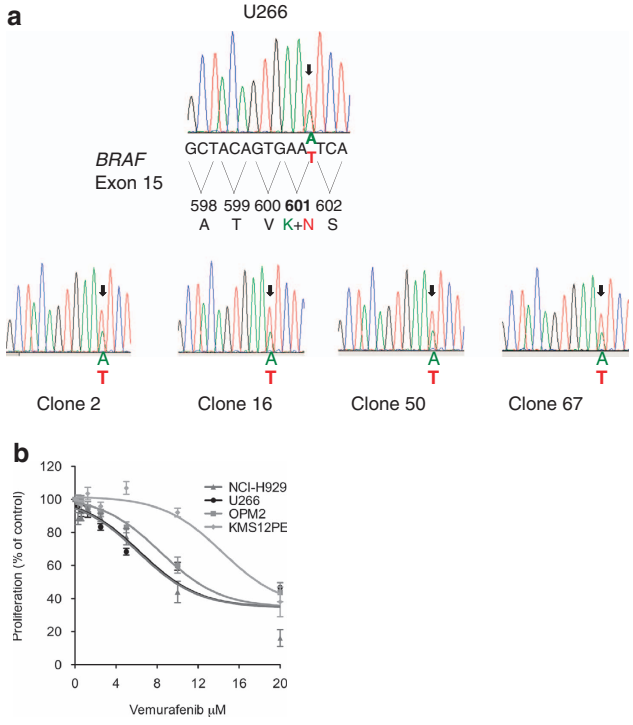

Figure 1. (**a**) *BRAF* exon 15 DNA sequencing was performed in U266 cell line and in 17 clones derived by limiting dilution assay. All sequenced clones harboured the same mutation proportion as illustrated in the figure. (**b**) Cells (30 000 cells per 0.2 ml) were seeded for 3 days in the presence of increasing concentrations of vemurafenib (Selleckchem, www.selleckchem.com). Proliferation was determined with the Alamar Blue Assay according to the manufacturer's instructions (AbD Serotec, Biorad, Marnes La Coquette, France). The data represent the mean ± s.e.m. of three independent experiments performed in triplicate wells.

patients with EMD carrying the *BRAF* V600E mutation, who could benefit from the V600E-mutated BRAF protein targeted therapy, that is, vemurafenib.

The establishment of human myeloma cell lines (HMCLs) remains rare and has mainly been obtained in samples from patients who had massive and/or serous EMD (mostly secondary), whatever the origin of patient's samples, that is, bone marrow, peripheral blood, pleural effusion or ascites fluid.[6,7] Although these HMCLs mostly derived from end-stage disease, they retained the oncogenic abnormalities found at the time of diagnosis.[6,7] A recent study, which assessed the presence of *BRAF* mutation in six HMCLs, reported that U266 harboured the K601N mutation, suggesting that *BRAF* mutation could be frequent in HMCLs.[8] We thus screened 33 HMCLs for V600E *BRAF* mutation by sequencing exon 15 to determine whether vemurafenib could be a common therapeutic approach for patients with massive EMD, especially plasma cell leukaemia. In this collection, 2 HMCLs were derived from ascites fluid, 18 from peripheral blood, 12 from pleural effusion and 1 from subcutaneous sample (Table 1). Unfortunately, none of the HMCLs carried the V600E mutation (Table 1). U266 was retrieved in this screening to harbour the K601N *BRAF* mutation (66% of mutated allele) and no other *BRAF* mutation was found in the collection. U266 was subcloned in order to define whether the mutation was present in each cell. As shown in Figure 1a, all clones evaluated ($n = 17$) harboured the mutation with the same proportion of 66%, as found within the parental cell line. Because U266 is known to carry a duplication of the q32q34 region of chromosome 7 where *BRAF* is located, it is likely that the mutated *BRAF* allele is duplicated.[9] We further determined the sensitivity of U266 and three *BRAF* wild-type cell lines to vemurafenib. All cell lines displayed a very weak sensitivity with IC_{50} values higher than 5 μM (Figure 1b and Table 1). By contrast, in V600E-mutated melanoma cells, IC_{50} values were lower than 100 nM, whereas V600E unmutated cells (including cells carrying other *BRAF* mutations) required more than 1 μM to display any sensitivity.[10] Although the *BRAF*-mutated HMCL did not carry any *RAS* mutation, 45% of HMCLs (15 out of 33) harboured a *K-* or *N-RAS* activating mutation (Table 1). Our findings show that *BRAF* mutation, in contrast to that of *RAS*,[1] is a rare event even in massive and/or serous EMD and that targeting *BRAF* V600E mutation with vemurafenib in MM could unfortunately be of limited value in patients with massive and/or serous EMD, such as pleural effusion or plasma cell leukaemia. Nevertheless, vemurafenib could be of high interest for patients with soft tissue plasmacytomas, in which the *BRAF* V600E mutation has been found, provided the mutation incidence should be significant in that infrequent MM presentation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

LL and CPD designed the study, performed experiments and wrote the paper. PM and MCB participated in writing the paper. AM performed experiments. CG reviewed karyotype. CT, MA and SLG reviewed the manuscript.

L Lodé[1], P Moreau[2,3], A Ménard[1], C Godon[1], C Touzeau[2,3,4,5], M Amiot[3,4,5], S Le Gouill[2,3,4,5], MC Béné[1,3] and C Pellat-Deceunynck[2,3,4,5]

[1]*Laboratoire d'Hématologie, CHU de Nantes, Nantes, France;*
[2]*Service d'Hématologie, CHU Nantes, Nantes, France;*
[3]*Université de Nantes, Nantes, France;*
[4]*INSERM, UMR892, Nantes, France and*
[5]*CNRS, UMR 6299, Nantes, France*
E-mail: catherine.pellat-deceunynck@inserm.fr

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