MR ALJOSJA ROGIERS (Orcid ID : 0000-0002-3807-9696)

Article type : Research Letter

Dabrafenib plus trametinib in *BRAF* K601E-mutant melanoma

A. Rogiers[1,2], D. Thomas[3], S. Vander Borght[4,5], J. J. van den Oord[5]

O. Bechter[6]

M. Dewaele[1,2]

F. Rambow[1,2]

J.-C. Marine[1,2]

P. Wolter[7]

[1]Laboratory for Molecular Cancer Biology, Center for Cancer Biology, VIB, Belgium

[2]Laboratory for Molecular Cancer Biology, Department of Oncology, KU Leuven, Belgium

[3]Lab for Therapeutic and Diagnostic Antibodies/PharmAbs, KU Leuven, Belgium

[4]Department of Human Genetics, Center for Human Genetics – KU Leuven, Belgium

[5]Department of Pathology, University Hospitals KU Leuven, Belgium

[6]Department of General Medical Oncology, University Hospitals KU Leuven, Belgium

[7]Department of Hematology and Medical Oncology, CHR East Belgium, Verviers, Belgium

Correspondence

Aljosja Rogiers

E-mail:aljosja.rogiers@kuleuven.vib.be

Funding resources

A.R. acknowledges support from Kom op tegen Kanker.

Conflict of interest

None to declare.

DEAR EDITOR, About 40 – 50% of cutaneous melanomas have activating *BRAF* mutations that are reachable with targeted therapy and combined BRAF-MEK inhibition improves clinical outcomes in advanced *BRAF* V600E/K-mutant melanoma[1]. Three combinations are FDA-approved for this indication: dabrafenib-trametinib, vemurafenib-cobimetinib and encorafenib-binimetinib. The K601E mutation comprises approximately 3% of *BRAF* mutations in patients with melanoma[2]. This c.1801A>G mutation results in an amino acid substitution from lysine to glutamic acid in the activation segment of the kinase domain and patients with metastatic melanoma harbouring this mutation have been successfully treated with MEK inhibitor monotherapy[3]. BRAF inhibitors can partially block ERK phosphorylation in ectopically expressed *BRAF* K601E mutants[4] but it remains unclear whether targeting BRAF in addition to MEK would result in more robust tumour growth inhibition.

To assess whether adding dabrafenib to trametinib would result in additional pERK inhibition in BRAF K601E-expressing mutants, we transfected a triple wild type melanoma cell line (MM001) with an empty vector (mock) or a vector carrying wild type, V600E-mutant or K601E-mutant *BRAF*. BRAF was overexpressed in all three conditions (Fig. 1a). Cells were subsequently treated for 24 hours with DMSO (control), trametinib 10 nM, dabrafenib plus trametinib 50-10 nM or dabrafenib 50 nM (Fig. 1b) – concentrations reported to be within the range of trough plasma in a clinical context[5]: the combination of dabrafenib plus trametinib suppressed ERK1/2 phophorylation more effectively than either inhibitor alone.

We subsequently developed a patient-derived xenograft (PDX) model from a cutaneous metastasis harbouring a *BRAF* K601E mutation. Mice were randomised to be treated with either trametinib (0.3 mg/kg/day) (n = 3) or dabrafenib plus trametinib (30 – 0.3 mg/kg/day) (n = 3) via daily oral gavage. Tumours were measured every other day. Based on tumour growth analysis, dabrafenib plus trametinib was more effective than trametinib alone ($p < 0.001$ for treatment allocation and time, two-way ANOVA, Fig. 1c). There was a higher tumour volume reduction in mice treated with dabrafenib plus trametinib compared with those treated with trametinib alone (mean: -59 versus -22%, $p = 0.100$ Mann-Whitney, Fig. 1d) and a longer median duration of response (32 versus 20 days, $p = 0.0628$ Mantel-Cox, Fig. 1e).

A 67-year-old man had a 1.2 mm thick non-ulcerated lentiginous melanoma resected on the scalp in 2011. In 2015, a resection of a melanoma skin metastasis was carried out. DNA sequencing revealed a *BRAF* c.1801A>G (p.K601E) mutation in 60% of the reads. *NRAS/KRAS* was wild type for codon 12, 13 and 61. The above-described PDX model was developed from this lesion.

In August 2015, the patient developed two brain metastases that were treated with stereotactic radiosurgery. By November 2015, the disease progressed with a new brain, lymph node, muscle and liver metastases necessitating systemic treatment. Based on the unavailability of anti-programmed cell death protein 1 (PD-1) antibodies as a first-line treatment at that point in time, evidence of targeted therapy being effective in this genotype and fast disease kinetics, dabrafenib plus trametinib was commenced. The patient achieved a partial response according to RECIST 1.1: -70% (sum of target lesions) with a decrease in fluorodeoxyglucose avidity in multiple lesions (Fig. 1f-i). He experienced no grade 3 or 4 toxicities. After 4 months of dabrafenib plus trametinib, the patient progressed intracranially and second-line treatment with pembrolizumab was initiated with stable disease as best response during one year. Because of progressive brain metastases in April 2017, the patient was rechallenged with the BRAF-MEK combination resulting in a renewed partial response (-42%) (Fig. 1j-m).

In this study, we found that in a *BRAF* K601E-mutant patient-derived melanoma xenograft, dabrafenib plus trametinib inhibited tumour growth more effectively than trametinib alone. The patient from whom the xenograft was derived responded to the same combination and a recent report demonstrated an objective response to BRAF-MEK inhibition in a patient with advanced melanoma harbouring this mutation[6]. It is also noteworthy that, in terms of therapeutic implications, the K601E mutation does not only occur in melanoma but has also been reported in 4 out of 26 cases (15.4%) of *BRAF*-mutant non-small cell lung cancer[7] and also comprises 5.3% of all *BRAF* mutations found in thyroid tumours[8].

We conclude that when confronted with rapidly progressive *BRAF* K601E-mutant advanced melanoma refractory to anti-PD1-based immunotherapy, targeted therapy with a MEK inhibitor – alone or in combination with a BRAF inhibitor – constitutes a valuable therapeutic option. Our report indicates that preclinical models can provide relevant information in answering drug responsiveness questions which, in this case, could lead to further exploration of the BRAF-MEK combination in advanced *BRAF* K601E-mutant melanoma.

Acknowledgements

We thank the patient and his family for providing informed consent to develop the patient-derived melanoma xenograft and permission to publish this report (institutional review board approvals S54185 and P147/2012). We thank Marguerite Stas and Veerle Boecxstaens for providing the surgical sample and Frédéric Amant and Els Hermans from the Leuven PDX platform (TRACE) for their assistance.

References

1 Long GV, Stroyakovskiy D, Gogas H, *et al.* Combined BRAF and MEK Inhibition versus BRAF Inhibition Alone in Melanoma. *N Engl J Med* 2014; **371**:1877-88.

2 Voskoboynik M, Mar V, Mailer S, *et al.* Clinicopathological characteristics associated with $BRAF^{K601E}$ and $BRAF^{L597}$ mutations in melanoma. *Pigment Cell Melanoma Res* 2016; **29**:222-8.

3 Bowyer SE, Rao AD, Lyle M, *et al.* Activity of trametinib in K601E and L597Q BRAF mutation-positive metastatic melanoma. *Melanoma Res* 2014; **24**:504–8.

4 Karoulia Z, Wu Y, Ahmed TA, *et al.* An Integrated Model of RAF Inhibitor Action Predicts Inhibitor Activity against Oncogenic BRAF Signaling. Cancer Cell. 2016; **30**:485-498.

5 Rousset M, Dutriaux C, Bosco-Lévy P, *et al.* Trough dabrafenib plasma concentrations can predict occurrence of adverse events requiring dose reduction in metastatic melanoma. *Clin Chim Acta* 2017; **472**:26–9.

6 Hassel JC, Menzer C, Menzies AM, *et al*. BRAF/MEK inhibition in melanoma patients with rare BRAF mutations. *J Clin Oncol* 2018; **36**:suppl;abstr 9542.

7 Kinno T, Tsuta K, Shiraishi K, *et al.* Clinicopathological features of nonsmall cell lung carcinomas with BRAF mutations. *Ann Oncol* 2014; **25**:138–42.

8 Afkhami M, Karunamurthy A, Chiosea S, *et al.* Histopathologic and Clinical Characterization of Thyroid Tumors Carrying the BRAF(K601E) Mutation. *Thyroid* 2016; **26**:242–7.

Fig. 1: *In vitro* and *in vivo* assessments as well as radiological images illustrating combined BRAF-MEK inhibition in *BRAF* K601E-mutant melanoma.

(a) Western blot analysis for BRAF expression after transfection with mock, *BRAF* WT, *BRAF* V600E or *BRAF* K601E-carrying vectors. (b) Western blot for ERK1/2 phosphorylation (Thr202/Tyr204) after transfection and subsequent treatment with DMSO (control), trametinib (T) alone, dabrafenib plus trametinib (DT) and dabrafenib (D) alone. (c) Tumour volume relative to baseline (mean with standard deviation) in a *BRAF* K601E-mutant patient derived melanoma xenograft treated with DT versus T alone, (d) mean tumour volume reduction (%) and (e) time to progression (days). Brain MRI before (f) and after 2 months (g) of the first treatment course with DT. PET-scan before (h) and after 1 month (i) of the first treatment course with DT (fluorodeoxyglucose uptake in lung, liver and muscle metastases). MRI before (j) and after 2 months (k) of rechallenge with DT. A chest CT scan before (l) and 2 months after rechallenging with DT (m).

Figure 1