Reverse transcriptase inhibition potentiates target therapy in BRAF-mutant melanomas: an in vitro study

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Luigi Fattore [1,2], Gianluca Sbardella[3], Paolo A. Ascierto[2], Rita Mancini [4] and Gennaro Ciliberto[5]

[1] SAFU Laboratory, Department of Research, Advanced Diagnostics and Technological Innovation, Translational Research Area, IRCCS Regina Elena National Cancer Institute, 00144 Rome, Italy;
[2] Istituto Nazionale Tumori IRCCS, "Fondazione G. Pascale", Naples, Italy; [3] Department of Pharmacy, Epigenetic Med Chem Lab, University of Salerno, Fisciano, SA, Italy;
[4] Department of Molecular and Clinical Medicine, University of Roma "Sapienza", Rome, Italy; [5] IRCCS, Istituto Nazionale Tumori "Regina Elena", Scientific Directorate, Via Elio Chianesi 53, 00144 Rome.

Objective

BRAF+MEK inhibitors have become the standard of care for BRAF-mutated melanoma patients. However, drug resistance remains a major clinical hurdle. From here, the need to identify additional therapeutics capable to tackle the onset of drug resistant clones. Our group has been involved in this topic during last years. Thereby, we reported that anti-ErbB3 receptor monoclonal antibodies are able to delay the emergence of resistance to target therapy *in vitro* and *in vivo* (1). More recently, we have demonstrated that microRNAs are key players of resistance to BRAFi and MEKi in melanoma and that their targeting is able to restore drug sensitivity (2,3). Here, we have started to investigate whether reverse transcriptase inhibitors (RTIs) frequently used in the treatment of AIDS can act in combination with target therapy to fight the development of drug resistance.

Methods and Materials

Human melanoma cells M14 and A375 have been treated with different concentrations of BRAFi, MEKi and/or the non-nucleoside RTI, i.e. SPV122. MTT and colony formation assays have been used to determine cell proliferation. Annexin V assay, cell cycle and mitochondrial membrane depolarization have been tested through FACS analyses. DNA damage have been determined through Western Blot and Immunofluorescence analyses

Results

Our present work has reported for the first time the capability of RTIs to potentiate target therapy in BRAF-mutant melanomas *in vitro*. We show that SPV122 synergizes with BRAFi+MEKi to: 1) impair BRAF-mutant melanoma cell growth; 2) induce apoptosis; 3) block cell cycle progression; 4) delay the emergence of resistance *in vitro;* 5) provoke DNA double-strand breaks, mitochondrial membrane depolarization and increased ROS (4).

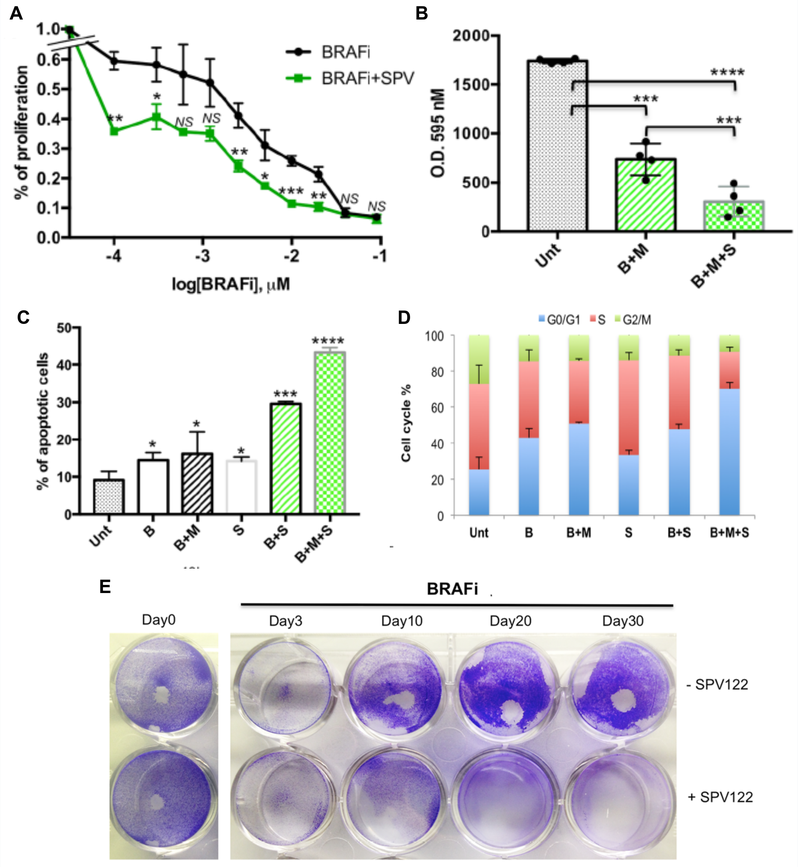
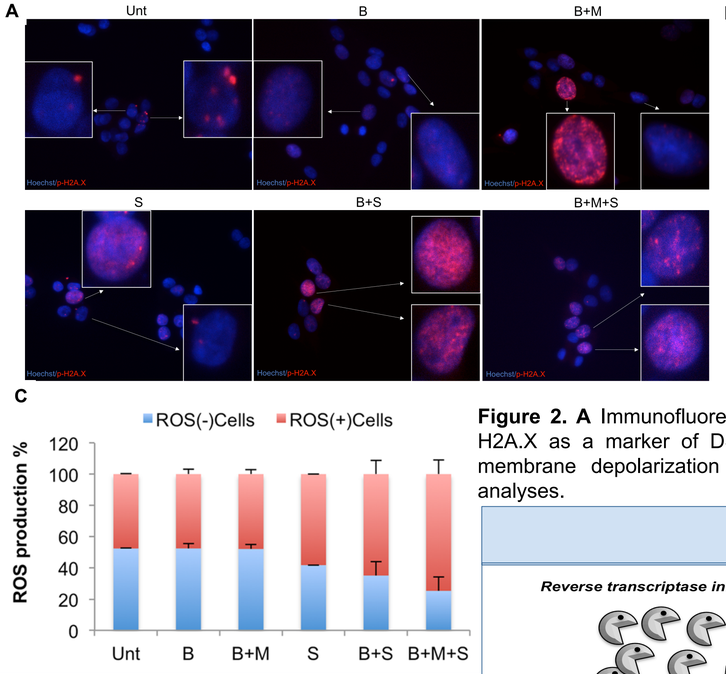


Figure 1. **A** M14 melanoma cells have been exposed to BRAFi starting from 5 **μ**M and then diluted 1:2 for 10 times in the presence or not of SPV122 at fixed dose of 1.25 **μ**M to measure cell viability. **B** Crystal violet (CV) staining assessed the growth inhibitory effects of BRAFi (150 nM) and MEKi (75 nM) +/- SPV122. The same drugs alone or in combination have been tested for apoptosis induction (**C**) and cell cycle (**D**). **E** M14 cells have been exposed two times a week to a BRAFi +/- SPV122 and then stained with CV at the indicated time points



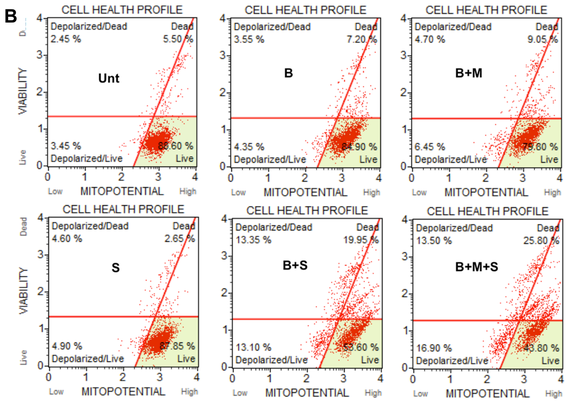


Figure 2. A Immunofluorescence analyses have been performed to quantify nuclear p-H2A.X as a marker of DSBs upon exposure to the indicated drugs. **B** Mitochondrial membrane depolarization and ROS production (**C**) have been assessed by FACS analyses.

References

1. Fattore L, et al. Oncotarget. 2015. doi: 10.18632/oncotarget.4485.
2. Fattore L, et al. Cell Death Differ. 2019. doi: 10.1038 s41418-018-0205-5.
3. Fattore L, et al. Int J Mol Sci. 2020 doi: 10.3390/ijms21061930
4. Fattore L, et al. Cell Commun Signal. 2020 Sep. doi: 10.1186/s12964-020-00633-7.

Conclusion