SELECTED OPPORTUNITIES IN ONCOLOGY

Inhibition of proteasome-associated deubiquitinating enzyme USP14 impairs melanoma cell survival and overcomes resistance to MAPK-targeting therapies (BIO16432)

Inhibition of proteasome-associated deubiquitinating enzyme USP14 impairs melanoma cell survival and overcomes resistance to MAPK-targeting therapies (BIO16432)

Product factsheet

POC vivo

- **Target:** proteasome–associate deubiquitinating enzyme USP14
- **Product :** USP14 inhibitor such as as b-AP15 or VLX1710
- **Rational / POC:**
  - An increased activity of the USP14 is observed in melanoma cells compared to melanocytes.
  - High expression of USP14 correlates with melanoma progression and with a poorer survival rate in metastatic melanoma patients
  - Knockdown or pharmacological inhibition of USP14 dramatically impairs viability of melanoma cells irrespective of the mutational status of BRAF, NRAS or TP53 and their transcriptional cell state, and overcomes resistance to MAPK-targeting therapies both in vitro and in human melanoma xenografted mice.
- **Patent :** METHODS AND COMPOSITIONS FOR TREATING MELANOMA EP17305339.8
- **Publication:** Targeting the proteasome-associated deubiquitinating enzyme USP14 induces melanoma cell death and overcomes resistance to MAPK-targeting therapies Didier R. et al. in press in Molecular Cancer Therapeutics

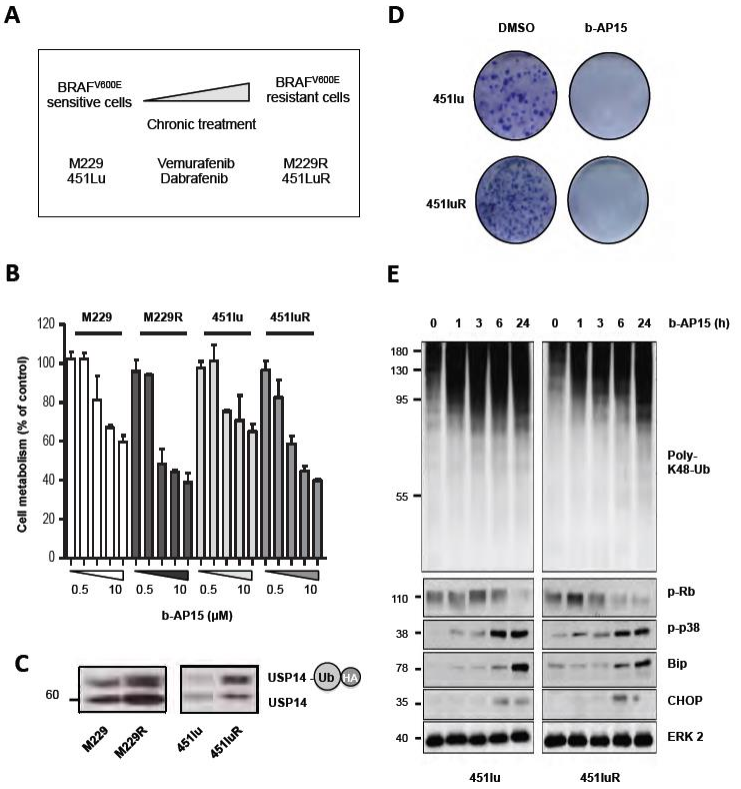
Inhibition of proteasome-associated deubiquitinating enzyme USP14 impairs melanoma cell survival and overcomes resistance to MAPK-targeting therapies (BIO16432)

Proof of concept

Treatment with b-AP15 overcomes resistance to BRAFV600E inhibitors

Treatment with b-AP15 overcomes resistance to BRAFV600E inhibitors. (A) Schematic description of the isogenic pairs of naive and BRAFi-resistant melanoma cells used in this study. (B) USP14 inhibition reduces the proliferation of m229 and 451lu BRAFi-sensitive cells and of their BRAFi-resistant derivatives m229R and 451luR, respectively. MTS assays on cells treated for 24h with increasing doses of b-AP15 (0.1 to 10 µM). (C) Analysis of USP14 activity in m229 / m229R and 451lu / 451luR pairs of melanoma cells using DUB trap assay. Lysates of the indicated cells were incubated at 37°C with the HA-Ub-VS probe and analyzed by anti-HA and anti-USP14 immunoblots. The active form of USP14 is indicated (USP14-Ub-HA). (D) Clonogenicity assay on 451lu and 451luR cells treated with or without b-AP15 (2 µM) and seeded at 2,000 cells/well. After 7 days of culture, colonies were stained with crystal violet. (E) Western blot analysis of lysates from 451lu and 451luR cells treated with 2 µM b-AP15 for the indicated times. Membranes were probed with antibodies against K48-linked poly-ubiquitinated proteins, phosphorylated Rb and p38, BIP/GRP78 and CHOP. Anti-ERK2 was used as a loading control.

Similar results were obtained with VLX1570 another USP14 inhibitor.



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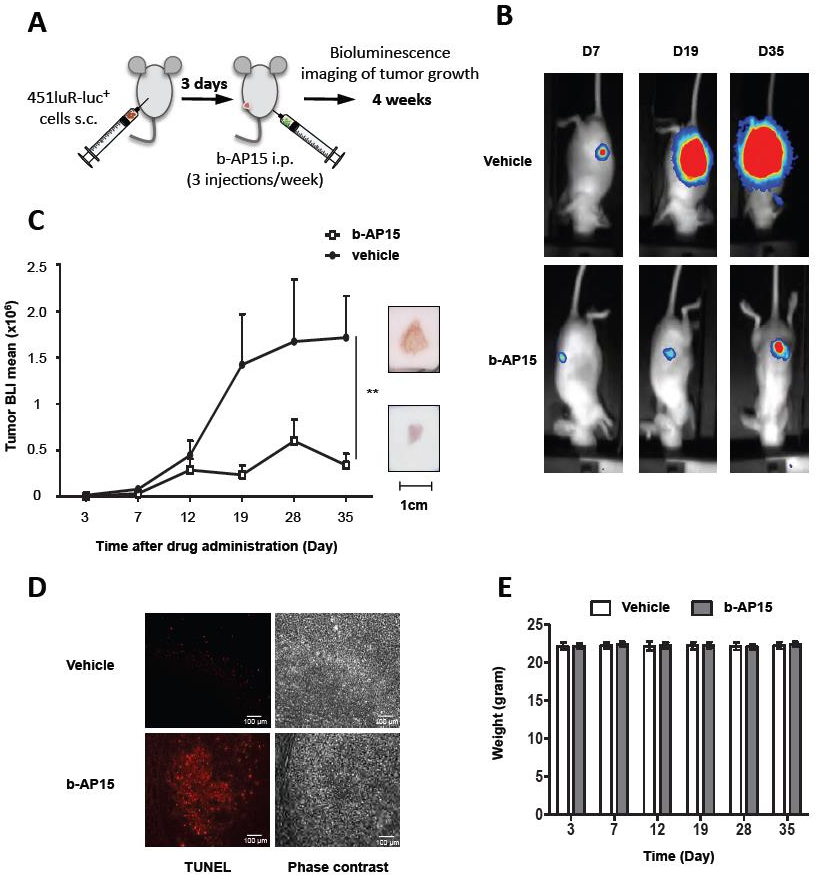
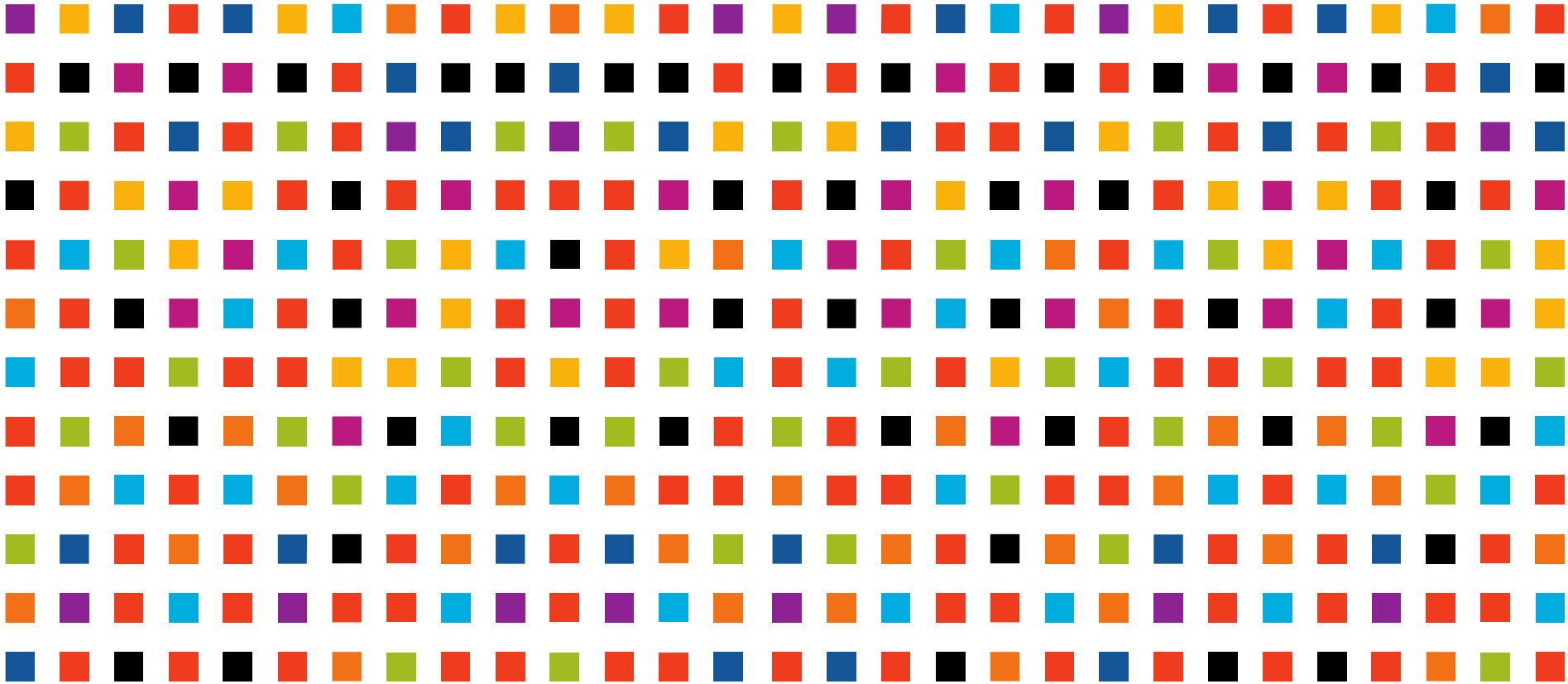
Proof of concept

b-AP15 inhibits tumor growth in melanoma xenographed mouse

b-AP15 inhibits tumor growth in melanoma xenografted mouse. (A) Schematic representation of the experimental procedure used in this study. (B) 451LuR-luc+ cells (1 x 106) were injected subcutaneously into nude mice. After 3 days, mice were injected i.p 3 times per week either with b-AP15 (10 mg/kg) or vehicle. Bioluminescence images were acquired at the indicated times using a Photon Imager system (Biospace Lab). Representative images of tumor bioluminescence at day 7, 19 and 35 of treatment are shown. (C) Quantification of tumor growth inhibition by b-AP15. Tumor BLIs of b-AP15- or vehicle-treated mice were recorded as described above and data analyzed with the M3Vision software (Biospace Lab). Data shown are mean ± SD of tumor BLI (n=12; **, *p*=0.009, 2way ANOVA). Representative micrographs of end-point analysis of b-AP15- or vehicle-treated tumor volume are shown (right). (D) Apoptotic cells were detected in situ on b-AP15- or vehicle-treated tumors by an indirect TUNEL method. Scale bar, 100 µM. (E) Mouse body weight was measured at the indicated days. Data shown are mean ± SD (n=12).





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