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Intermittent Treatment of BRAFV600E Melanoma Cells Delays Resistance

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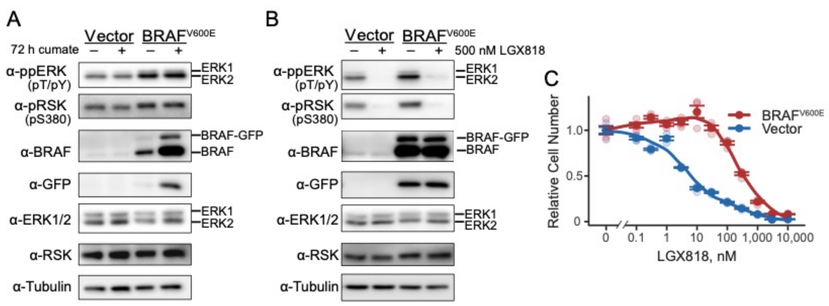
Background

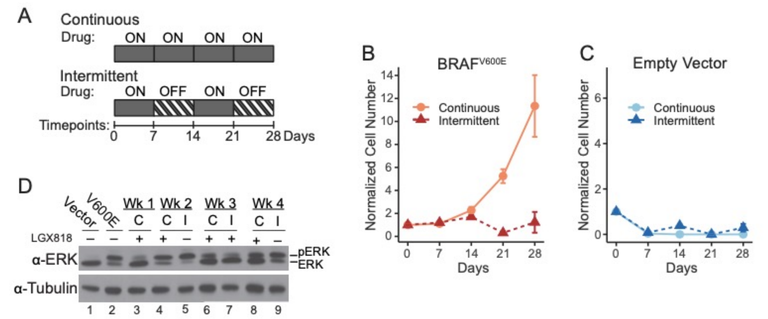
Melanoma patients receiving drugs targeting BRAFV600E and MEK1/2 invariably develop resistance and continue progression. Based on preclinical studies, intermittent treatment involving alternating periods of drug challenge and withdrawl has been proposed as a method to delay the onset of resistance. The beneficial effect of intermittent treatment has been attributed to drug addiction, where drug withdrawal reduces the viability of resistant cells due to MAP kinase pathway hyperactivation. However, the mechanistic basis of the intermittent effect is incompletely understood. We show that intermittent treatment with the BRAFV600E inhibitor, LGX818/encorafenib, suppresses growth compared to continuous treatment in human melanoma cells engineered to express BRAFV600E. Analysis of the BRAFV600E-overexpressing cells shows that growth suppression in an intermittent treatment schedule is best explained by resensitization of cells during periods of drug removal rather than drug addiction. Cells treated intermittently reveal a subset of transcripts that exhibit reversible transcriptional profiles, and include mediators of cell invasiveness and the epithelial to mesenchymal transition. These transcripts change during periods of drug removal by adaptive switching, rather than selection pressure. Resensitization occurs against a background of sustained expression of melanoma resistance genes, producing a transcriptome distinct from that of the initial drug-naïve cell state. We conclude that phenotypic plasticity leading to drug resensitization can underlie the beneficial effect of intermittent treatment.

Modeling Tumor Resistance In Vitro



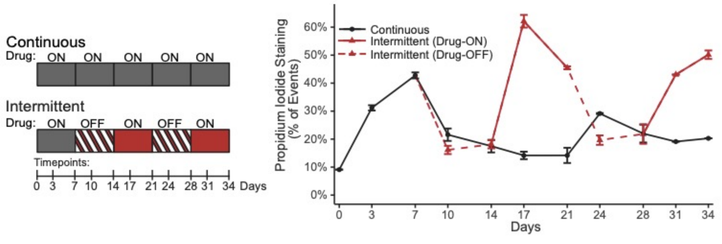
Characterization of melanoma cells with BRAFV600E amplification. (A) ERK, phopho-ERK, RSK, phospho-RSK expression in WM239A metastatic melanoma induced to express BRAFV600E or empty vector. **(B)** WM239A cells induced to express BRAFV600E or empty vector treated with 500 nM LGX818 or DMSO for 2 h and analyzed by Western blotting as in panel A. **(C)** WM239A-BRAFV600E cells were induced with cumate for 72 h and treated for 72 h with varying concentrations of LGX818. Cell numbers were plotted as mean ± s.e.m. (n=4) in dark symbols and individual measurements in light symbols.

Intermittent treatment inhibits cell expansion



Intermittent treatment inhibits cell expansion compared to continuous treatment. (A) WM239A cells induced to express BRAFV600E or empty vector were cultured for 28 days under continuous or intermittent treatment conditions with 500 nM LGX818. Intermittently treated cells followed a schedule of 7 days on LGX818 and 7 days off. Cell numbers (mean ± s.e.m) were quantified at the end of each week. **(B)** Cell numbers for BRAFV600E cells and **(C)** empty vector cells are shown for continuous and intermittent treatments. **(D)** Western blots of lysates in cells expressing empty vector (lane 1); BRAFV600E (lane 2); and BRAFV600E cells treated continuously "C" or intermittently "I".

Intermittent Treatment Resensitizes Cells



Intermittent treatment resensitizes cells to LGX818. Cell death across continuous and intermittent schedules was measured on days 3 and 7 of each week for five weeks. Adherent and nonadherent cells were collected together, stained with propidium iodide (PI), and analyzed by flow cytometry. The media was changed on days 3, 5, and 7 of each week. During the media change on day 5, nonadherent cells were collected by centrifugation of the conditioned media and returned to the dish for flow cytometry analysis on day 7. Measurements report average percentage of PI-positive cells (n=2 independent experiments), and error bars represent the range.

Transcriptomic Profiling of Cells

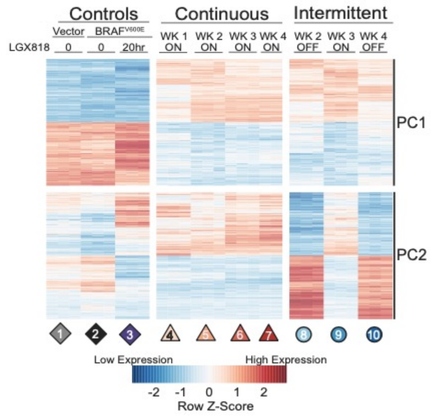
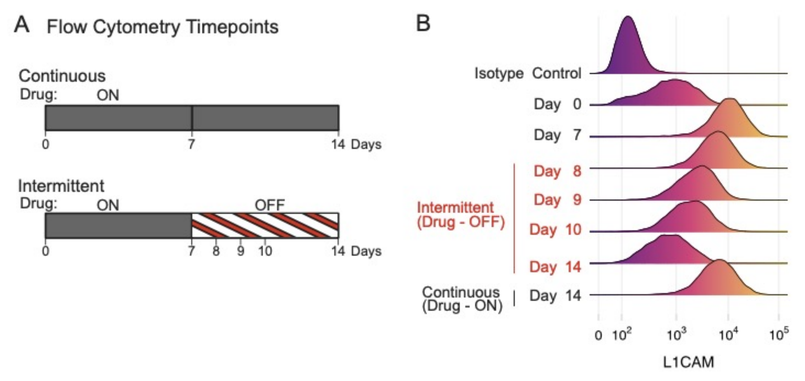


Figure 4. Transcriptomic profiling of continuous and intermittently treated cells. Heatmap of the 400 genes most positively or negatively associated with PC1 or with PC2. Each row is mean centered and scaled to unit variance. RNA samples collected from WM239A-BRAFV600E cells comparing continuous and intermittent drug treatment with 500 nM LGX818. Each condition was collected in biological triplicates Principal component analysis (PCA) of the 6000 highest variance genes. PC1 and PC2 account for almost 70% of the variance of in these genes.

Expression Changes Are Adaptive



Cell resensitization is accompanied by adaptive gene expression changes in single cells, not by counterselection. (A) WM239A-BRAFV600E cells were cumate-induced for 72 h and cultured under continuous or intermittent treatment with 500 nM LGX818. At the indicated time points, cells were fixed and stained with a fluorescently labeled primary antibody for L1CAM and then analyzed by flow cytometry. **(B)** Smoothed histograms of single cell L1CAM expression by flow cytometry at the different timepoints.

Impact and Future Directions

- Previously described benefits of intermittent drug treatment in melanoma can be modeled in vitro
- Intermittent drug treatment produces a unique transcriptional profile
- Transcriptional adaptation can may account for the growth inhibition observed with intermittent drug treatment
- Identifying genes involved in resensitization of cells may help identify therapeutic targets to combat resistance
- Further use of an in vitro system to model optimal schedules of intermittent treatment may identify additional targets

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