Parallel in-vivo assessment of drug phenotypes at various time points during systemic BRAF inhibition reveals tumor adaptation and altered treatment vulnerabilities

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Running title: Parallel examination of adaptive tumor responses during BRAF inhibition

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TRANSLATIONAL RELEVANCE:

Cancer is increasingly viewed as an adaptive disease in which response to drugs can vary as tumors evolve during therapy and over time. In BRAF-mutant melanoma, many potential adaptive signaling mechanisms have been reported which are suspected to contribute to widespread disease recurrence. We demonstrate repeated use of microdevices in live tumors to measure in situ phenotypic responses to many potential escape pathways in parallel.

In clinical practice, these microdevices may be placed inside the tumor in a minimally invasive manner and provide parallel phenotypic readouts of tumor vulnerability to additional targeted or cytotoxic inhibitors at various time points during standard-of-care treatment. This may provide a new personalized treatment paradigm in which physicians rationally manage drug therapy by identifying optimal combinations throughout the treatment cycle. Importantly, the microdevices provide actionable phenotypic drug readouts from within the tumor microenvironment and may expand clinical biomarker-based selection beyond genomic testing.
ABSTRACT:

**Purpose:** Treatment of BRAF-mutated melanoma tumors with BRAF inhibitor-based therapy produces high response rates, but of limited duration in the vast majority of patients. Published investigations of resistance mechanisms suggest numerous examples of tumor adaptation and signal transduction bypass mechanisms, but without insight into biomarkers that would predict which mechanism will predominate. Monitoring phenotypic response of multiple adaptive mechanisms simultaneously within the same tumor as it adapts during treatment has been elusive.

**Experimental Design:** This study reports on a method to provide a more complete understanding of adaptive tumor responses. We simultaneously measured *in vivo* anti-tumor activity of 12 classes of inhibitors which are suspected of enabling adaptive escape mechanisms, at various time points during systemic BRAF inhibition. We used implantable microdevices to release multiple compounds into distinct regions of a tumor to measure the efficacy of each compound independently, and repeated these measurements as tumors progressed on systemic BRAF treatment.

**Results:** We observed varying phenotypic responses to specific inhibitors before, during and after prolonged systemic treatment with BRAF inhibitors. Our results specifically identify PI3K, PDGFR, EGFR and HDAC inhibitors as becoming significantly more efficacious during systemic BRAF inhibition. The sensitivity to other targeted inhibitors remained mostly unchanged, while local incremental sensitivity to PLX4720 declined sharply.

**Conclusions:** These findings suggest redundancy of several resistance mechanisms and may help identify optimal constituents of more effective combination therapy in BRAF-mutant melanoma. They also represent a new paradigm for dynamic measurement of adaptive signaling mechanisms within the same tumor during therapy.
INTRODUCTION:

Mutations in the BRAF gene occur with significant frequency in melanoma and several other cancers, and have been targeted successfully by multiple drugs in the clinical setting. High initial response rates are achieved by BRAF inhibition in melanoma(1) but a majority of patients relapse within 9-12 months with more aggressive tumors that are increasingly resistant to a range of other therapies. (2) Tumor recurrence is thought to be the result of adaptive mechanisms by which tumor cells respond to inhibition of their preferred oncogenic signaling pathway with an upregulation of alternative pathways for survival and proliferation. (3,4) In BRAF-mutated melanomas significant adaptive responses (and thus potential targets for therapeutic combinations) have been reported, for instance involving MEK inhibitors.(3,5) The clinical implications are far-reaching. Though systemic treatment with one agent can significantly affect the response to other potential subsequent therapies, such systematic, parallel investigations of multiple agents or combinations are usually performed only for small numbers of compounds in vivo due primarily to the rapidly increasing cohort size required to assess combinations between multiple agents. Insights are often restricted to in vitro studies, but tumor responses to drugs are not only determined by an individual tumor's genome, but also by the complex interaction of tumor cells with their microenvironment, including immune and stromal cells, and many known and yet unknown factors that can dramatically alter phenotypic drug response. (6–8) We demonstrate in this study parallel assessment of phenotypic in situ drug response to inhibitors of the majority of signaling pathways known to be relevant and druggable in this cancer type. The approach uses an intratumor implantable microdevice for the simultaneous delivery of 18 drug compounds into isolated non-overlapping regions of tumor (adapted from (9)), including targeted inhibitors of BRAF, Erk, CDK4/6, PI3K, EGFR, C-Met, MDM2, PDGFR, FGFR1, HDAC and HSP-90. Through appropriate spacing and sizing of reservoirs and formulation of drug compounds, it can be ensured that the drug contents from a given reservoir do not disseminate to the vicinity of an adjacent reservoir within the time course of the experiment. (9) The anti-tumor effect of the compounds is assessed by targeting
each of these signaling nodes at multiple treatment time points in a given tumor: before, during and after systemic inhibition of BRAF. Our results show great diversity in how targeted BRAF inhibition affected the intratumor response to the various agents very differently. While the response to many agents remained virtually constant, prolonged BRAF inhibition induced a significantly increased sensitivity of tumors to agents targeting PI3K, PDGFR, EGFR and HDAC.
METHODS:

Study design
The objective of the studies in Figures 2 and 3 is to show biological response to release of drugs, and to test whether this response was significantly different between different treatment time points. Sample sizes were chosen to demonstrate statistical significance by Student’s t-test between biologically distinct conditions or outcomes. Tissue sections were scored by an ImageJ image analysis algorithm in a blinded manner (see below). Only biological replicates were used in data analysis. Average values and standard deviations are from 8 samples for all studies. Data from tissue sections was only excluded in the rare event that the tissue section was damaged during retrieval or was found to be entirely necrotic by IHC.

Device preparation & implantation
Microdose drug delivery devices were manufactured as described in Jonas et al.9. In short, cylindrical, micro-scale devices with 0.82 (diameter) × 4 mm (length) were manufactured from medical-grade Delrin acetyl resin blocks (DuPont) by micromachining (CNC Micromachining Center, Cameron). Circular reservoirs (8-12 per device) were shaped on the outer surface of devices in dimensions of 200 μm (diameter) × 250 μm (depth). Drug-polymer mixtures were packed into device reservoirs using a tapered, metal needle (Electron Microscopy Sciences) until the reservoirs were completely filled. Each drug reservoir in the microdevice contained approximately 1 μg of the compound. Devices were implanted directly into the mouse tumor using a 19-gauge spinal biopsy needle (Angiotech) and a retractable needle obturator to push the device into the tissue. Devices containing the drug remained in situ for 24 hours. All drug compounds were purchased from Selleck Chemicals, Inc.

The flank tumor was excised and the tissue containing the device was fixed for 24 hours in 10% formalin and perfused with paraffin. This specimen was sectioned using a standard microtome and tissue sections were collected from each reservoir. Sections were antibody-stained by standard immunohistochemistry using Cleaved-caspase-3 antibody (Cell Signaling) and scored using an ImageJ image (v1.48) analysis algorithm in a blinded manner.

Scoring of IHC sections:
Percent area of DAB stained cells was calculated for 400 μm × 400 μm regions in tissue directly perpendicular to the reservoirs. The analysis was performed in a semi-automated fashion using ImageJ Color Deconvolution with the vector “H DAB”. A threshold was set to eliminate background staining and all outliers smaller than 2 pixels in diameter were removed. The Smooth function was also used to fill in cells that did not have completely homogenous staining. Error bars represent one standard deviation. In a small number of samples (<10%), device reservoir was located in a region of tumor that was highly necrotic. These sections were not included in the analysis because the differential effect caused by the presence of drug in this tissue region cannot be determined independently.

Statistical analysis
Apoptotic index (AI) was calculated as AI= % cleaved-caspase-3 positive cells / total cells within 400 μm from reservoir-tissue interface for device sections. AI = % cleaved-caspase-3 positive cells / total cells for entire tumor section for systemic studies. For graph 3, curves shown are averaged over 8 samples for each time point. All error bars represent one
standard deviation. Coefficient of variation is defined as standard deviation divided by mean. Significance tests and P-values were calculated using Student's t-test, using normal-based 95% confidence intervals. Two-sided testing was used. The data sets were verified to be normally distributed.

Cell lines, mice, xenograft tumors & systemic dosing

Human cancer cell lines A375 were passaged in DMEM with 10% Fetal Bovine Serum, 1% Pen-Strep (10,000 units/mL penicillin & 10,000 ug/mL streptomycin), and 1% of 200 mM L-glutamine. All supplies were purchased from Life Technologies. Cells were maintained in conditions of 37°C and 5% CO₂ and passaged 1-3 times per week on reaching ~70% confluence. Tumor cells (~3×10^6) in 150μL PBS were injected subcutaneously into the flanks above the hind limbs of 6-8-week-old female athymic nude mice (Crl:NU(NCr)-Foxn1<sup>nu</sup>) purchased from Charles River Laboratories. Tumors grew for approximately 2 weeks, to a size of 5-9 mm, before the first device implantation. Animals were chosen randomly for experiments when tumor size was between 5 and 9 mm. No specific method was chosen for randomization.

Tumor bearing mice were dosed daily for 15 days with PLX4720 at 45mg/kg by oral gavage. At 3 distinct time points (Before treatment began: Day 0; during early treatment: Day 4; and after treatment: Day 16) microdevices were implanted into the tumors and remained in situ 24 hours. After the final device retrieval, mice were euthanized in order to collect the tumors. In some cases, mouse tumors were biopsied with a 11-G Cassi biopsy gun (Scion) to retrieve the tissue immediately surrounding the device. All animal studies were conducted in accordance with protocols approved by the Committee on Animal Care (CAC) at the Massachusetts Institute of Technology. (Protocol 0415-038-18)

**In vitro survival assay**

For cell growth assays, A375 cells were treated with various concentrations of PLX4720 (0.1 or 1 μM) for 7 days. Then, 10,000 cells were plated per well in a 96-well plate and treated with Erlotinib, Vorinostat, Imatinib or BYL719 (at 0.8, 4 or 20 μM). Cells were counted at 24h according to a rezasurin assay (PrestoBlue; Life Tech.) using manufacturer's guidelines.

**Western Blot**

Standard procedures were used for protein electrophoresis and western blotting. A375 cells were treated with 1 μM PLX4720 for 7 days and then with a second drug for 24h (Erlotinib, Imatinib, Vorinostat or BYL719 at 2 μM). Cells were then lysed in 25 mM Tris, 150 mM NaCl, 10% glycerol, 1% NP 40 and 0.5M EDTA with a protease Mini-complete protease inhibitors (Roche) and a phosphatase inhibitor cocktail (PhosSTOP, Roche) at 4°C. Protein lysates were separated by SDS-PAGE, transferred to a nitrocellulose membrane, blocked with Odyssey Blocking Buffer (LiCor), incubated in primary antibody overnight at 4°C. Proteins were detected using Licor secondary antibodies. Protein level intensity was measured with Image J and data were pooled from at least 3 different experiments. Antibodies used: pAkt5473 (CST #9271), pRb S807/811 (#8516, CST), pERK (E4) sc-7383 (Santa Cruz), GAPDH (Sigma, G9545).
RESULTS:

Animals harboring A375 tumors were dosed systemically for 15 days with the BRAF inhibitor PLX4720. At 3 distinct time points (Before treatment began: Day 0; during early treatment: Day 4; and after treatment: Day 16) microdevices were implanted into the tumors and remained in situ 24 hours. These microdevices carried spatially distinct reservoirs, loaded with doxorubicin, PLX4720, erlotinib, crizotinib, BYL719, lapatinib, palbociclib, imatinib, 17-DMAG, vorinostat, PD173074, YH239-EE and SCH772984. Drugs were released from reservoirs during the incubation time into confined regions of tumor tissue, and interacted with the local tissue in its native microenvironment. The devices were then excised with surrounding tissue in a minimally invasive manner and analyzed. Phenotypic responses for each drug were obtained in this manner before BRAF inhibition, during initial treatment regimen of 4 days, and after a total of 15 consecutive daily treatments with PLX4720. The experimental approach is shown schematically in Figure 1. The 13 drugs tested on each microdevice are listed in Table 1 with their respective molecular targets. The microdevice provides a unique way to test drug sensitivity of a tumor within its native microenvironment and without systemic exposure or toxicities. It is possible to examine the effect of multiple drugs independent of one another, and all within a single tumor because drug microdoses are only released over a small and confined region of tumor. Formulation of therapies in a (poly)ethylene-glycol polymer matrix ensures that diffusion distances of drugs are limited to 300-400 μm within 24h,(9) which is significantly lower than the separation distances between nearest adjacent reservoirs of 900 μm.

By performing biopsies of the device with surrounding tissue, we assay a single tumor’s response to many drug compounds individually at multiple time points, without the need for whole tumor resection. Device/tissue specimens are sectioned layer-by-layer and analysis is performed on tissue sections that contain regions of tumor which are exposed to only one drug. Immunohistochemical staining (IHC) provides a phenotypic readout of each drug’s activity in the tumor. The preferred metric of a drug’s local intratumor efficacy is the apoptotic index, defined as the percentage of apoptotic cells vs. total cells within the exposed tumor region.
Initial drug sensitivities:
Prior to any systemic treatments, we implanted the microdevices into tumors and tested initial sensitivities to all agents listed in Table 1. These melanoma tumors are initially highly sensitive to local microdose treatment with PLX4720, as exhibited by an apoptotic index (AI) of 40%. This sensitivity is likely due to the BRAF V600E mutation that is reported for this model.10,11 We also determined the tumors to be highly sensitive to doxorubicin (AI=52%) in concordance with previously reported results, as well as in vitro and whole animal studies.9,10 The a priori sensitivities to all other targeted agents listed in table 1 were comparatively low. These ranged from AI = 8% to 22%, with HDAC, MDM2 and MEK1/2 inhibitors among the more potent agents. (Fig.2, 3)

Rapid changes during initial phase of systemic BRAF inhibition:
Local microdose effect of PLX4720 decreases significantly (from AI = 40% to 24%) on day 4 of systemic BRAF inhibition indicating that the tumor rapidly becomes less sensitive to the drug during early treatment. Concurrently, there is a strong rise in efficacy of the EGFR inhibitor erlotinib and PDGFR inhibitor imatinib, as indicated by a near doubling of the apoptotic response. Palbociclib (Cdk4/6 inhibitor) and BYL719 (PI3K inhibitor) also induce significantly increased apoptotic responses in the treated tumors. The efficacies of the other 7 targeted inhibitors tested do not change appreciably during the first 4 days of systemic BRAF inhibition. (Fig.2, 3)

Adaptive responses during later stages of prolonged BRAF-inhibition:
We observe a further decrease in sensitivity to PLX4720 following 15 days of continuous BRAF inhibition. PLX4720 is mostly ineffective at this time point in inducing apoptosis (AI= 8%). Tumor sensitivity to doxorubicin appears to be largely unaffected by BRAF inhibition, showing only a slight decrease at high apoptosis levels compared to before BRAF treatment. (AI= 49% vs. 52%). (Fig.3)
In contrast, tumors become significantly more sensitive to vorinostat and Byl719 on Day 16, with both increasing in apoptosis induction by over 50% compared to treatment at Day 4. Erlotinib and imatinib remain at high levels of potency but the remaining targeted agents stagnate or exhibit decreasing potency, such as palbociclib, crizotinib and PD173074. (Fig.3)

Identification of the most prominent bypass mechanisms:

Systemic inhibition of BRAF by PLX4720 is observed to drastically increase susceptibility to several other targeted inhibitors. The most prominent increases occur in erlotinib targeting EGFR and imatinib targeting PDGFR, BYL719 targeting PI3K, and vorinostat targeting HDAC. Each of these compounds becomes 50-100% more efficacious in inducing intratumor apoptosis during systemic BRAF inhibition. (Fig. 3B)

The tumor’s sensitivity to local treatment with PLX4720 decreases greatly during systemic BRAF inhibition from AI = 40% prior to treatment to AI = 9% at 16 days. This is likely explained by the systemic BRAF inhibition rendering the remaining tumor cells increasingly resistant to this drug. Interestingly, however, the cytotoxic DNA-damaging agent doxorubicin exhibits similarly high apoptosis induction both before and after systemic BRAF inhibition. Cytotoxic DNA-damaging chemotherapy, such as doxorubicin, may be more agnostic to adaptive in signaling pathways induced by BRAF inhibition.

Investigation into the mechanisms regulating drug sensitivity after long-term Braf inhibition:

In vitro tests were performed to better understand the mechanistic basis for the efficacy enhancements observed in vivo. First, we performed survival assays on A375 cells treated for 7 days with increasing doses of PLX4720 and then challenged with an additional drug for 24h, focusing on four drugs which showed the highest efficacy enhancement effects in vivo: Imatinib, Byl719, Erlotinib and Vorinostat. Treatment with all 4 drugs subsequent to PLX 4720 pre-treatment lead to decreases
in cell survival that were significant relative to treatment with vehicle, or PLX4720 alone (Fig 4A-D). The highest degree of efficacy enhancement is observed for Vorinostat and Byl719 (Fig 4A,B). Imatinib and Erlotinib induced comparatively lower overall cell death, but nonetheless exhibited an increased effect with PLX4720 (Fig 4C,D). In sum, PLX4720 treatment sensitized cells \textit{in vitro} to each of the four therapies identified \textit{in vivo} as exhibiting the greatest increase in potency, leading to enhanced inhibitory effects on cell growth.

We next investigated the mechanism by which these drugs may combine for greater efficacy with PLX4720, focusing on three signaling pathways known to be involved in driving tumor cell proliferation in BRAF-mutant cancers: MAPK (via ERK phosphorylation), Akt (e.g. by PI3K activation) and Rb. Following long-term PLX4720 treatment, Imatinib leads to a further decrease in pERK (Fig 4E,F) an effect not observed for the other agents. Analogously, AKT signaling is further decreased significantly by PI3K inhibition via BYL719 (Fig 4G,H). Lastly, after PLX4720, the HDAC inhibitor Vorinostat led to a significant decrease in RB phosphorylation relative to each treatment alone (Fig 4I,J).

**DISCUSSION**

It is now well established that resistance to targeted therapies can emerge through the activation of secondary bypass signaling pathways, which can be further targeted by the use of second-line combination therapies. Given the complex nature and heterogeneity of resistance mechanisms, such studies are generally carried \textit{out in vitro}, with single combinations carried out \textit{in vivo}. Here, for the first time, we demonstrate the ability to study multiple drug combinations \textit{in vivo} simultaneously to measure how tumors adapt functionally during systemic standard-of-care treatment, and to rapidly assess the potential for more effective combinations in
inducing tumor cell death. The examination of adaptive phenotypic drug responses using microdevices allows testing of several molecular hypotheses directly for each individual tumor, which broadens its potential clinical applicability as a tool for personalized medicine.

The current study revealed that long-term PLX4720 treatment sensitized tumors specifically to BYL719, Erlotinib, Vorinostat and Imatinib. Further in vitro studies confirmed these results, and the signaling pathways driving the enhanced efficacy were investigated. Reactivation of the ERK pathway after Braf inhibitor treatment is a well established resistance pathway. We find decreased ERK phosphorylation in cells treated with PLX4720 and Imatinib, suggesting this combination may be effective in bypassing resistance to Braf inhibition. Imatinib has been shown to cause decreased pERK and pAkt in combination with BRAF inhibitors due to suspected adaptive responses in PDGFR signaling, leading to Imatinib now being tested clinically in melanoma. Similarly, PI3K activation has also previously been suspected as a major potential bypass mechanism in BRAF-treated tumors. We show that pre-treatment with PLX4270 can sensitize cells to BYL719, leading to a significant decrease in pAkt, relative to BYL719 treatment alone. Recent studies have shown that resistance to MAPK inhibition can arise through decreased shedding of several RTKs including AXL, leading to increased activity via Akt and c-Jun. Given the enhanced effect of Braf and PI3K/Akt inhibitors, clinical trials with combinations of BRAF and PI3K inhibition are currently underway for metastatic melanoma. Finally, we show that pre-treatment with PLX4720 can sensitize melanoma cells to the HDAC inhibitor Vorinostat via decreased phosphorylation of Rb. HDAC inhibitors have been shown to promote cell death by activation of the pro-apoptotic protein Bim and the oncogenic Rb-E2F1 pathway. Upregulation of MITF has been implicated as a consequence of BRAF inhibitor treatment in some tumors with resistance being driven by BCL2A1 expression and a switch to oxidative phosphorylation. HDAC inhibition has been suggested a strategy for decreasing MITF expression, and could further help explain the increased combined effect of these two drugs. Among other agents
tested, YH239-EE and 17-DMAG each exhibit a moderate increase in sensitivity but the levels of apoptosis remain below 30% at all treatment time points. Several drug compounds exhibit a low and stable intratumor apoptotic responses that does not vary significantly throughout systemic BRAF inhibition. These compounds include crizotinib, PD173074, palbociclib and lapatinib. SCH772984 has relatively high effect (AI=25%) but it remains stagnant throughout treatment.

It is noteworthy that adaptive sensitizations to targeted therapies emerge at different time points during systemic treatment. For example, all of the increased sensitivity to erlotinib is observed within 4 days of treatment start, with no additional increase in the remaining 12 days. For imatinib and Byl719, greater than 50% of the total increase in drug efficacy also occurs within the first 4 treatment days. Sensitivity to vorinostat does not, however, change significantly during the initial 4 days of treatment. Here, ~90% of the observed sensitization occurs during the later stages of systemic BRAF therapy. Such information could be used clinically not only to choose which drugs should comprise a combination regimen, but also at which time points they should be combined for optimal efficacy.

To our knowledge, this is the first in-vivo study that examines phenotypic drug response to a large number of targeted agents over the course of systemic treatment and is capable of observing parallel drug phenotypes during tumor evolution. Rather than waiting for resistance mechanisms to manifest at the time of disease progression, when the primary targeted therapy is no longer inhibiting the primary targeted pathway, this approach enables deployment of combination therapy at a time when synergistic interactions can be leveraged. The presented technology enables a novel understanding of emerging tumor drug resistance by mapping how a given tumor responds to a range of other agents while the most prominent and actionable aberration is targeted. Clinically, this assay could be used in patients to discover which drugs should be added at a given time point to the standard-of-care treatment to achieve optimal anti-tumor effect of the combination therapy.
REFERENCES:


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TABLES:

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Table 1: List of drugs tested during systemic BRAF-inhibition, with respective molecular targets.
**Figure 1:** (A) Schematic of tumor growth vs. days of systemic treatment with a BRAF inhibitor. The 3 arrows show when microdevice tests were performed on the tumor: before treatment began; during early treatment; and at the end of the 15-day systemic treatment. (B) Concept for in-vivo drug sensitivity assay: Device is implanted directly into tissue where drugs diffuse into confined regions of tumor. Each region is assayed independently to assess the tumor-specific response to a given drug. Following incubation, a second biopsy procedure is administered in which a coring needle selectively retrieves a small column of tissue that immediately surrounds the device. This tissue contains the regions of drug diffusion and is sufficient for determination of efficacy of drugs.

**Figure 2:** Representative tumor sections for each drug and time point tested via the microdevice. Tumor sections are stained for Cleaved-caspase-3 (brown cells) indicating apoptosis. The ratio of apoptotic vs. all cells (=apoptotic index) is used as a measure of drug efficacy.

**Figure 3:** A. Quantitative analysis for each drug, showing changes in intratumor treatment response versus PLX4720 systemic treatment time. For each drug, its apoptotic response is shown at 3 time points: day 0 = before systemic PLX treatment has begun; day=4 is after 4 days of systemic treatment; day 16= one day after the 15th and final day of systemic treatment. N=8 for each time point and drug. Error bars represent one standard deviation. B. Ranking of absolute changes in apoptotic index for each of the drugs tested on the microdevice during systemic BRAF inhibition from day 0 to day 16.

**Figure 4:** *In vitro* validation and investigation into mechanisms driving enhanced combined drug responses: Survival curve for A375 cells treated with multiple concentrations of PLX4270 (0.1 or 1 μM) for 7 days and then treated with Vorinostat (A), Byl719 (B), Imatinib (C) or Erlotinib (D) for 24hrs. Representative Western Blots and quantifications for pERK Y204 (E,F), pAkt473 (G,H) and pRB S807/811 (I,J) using the same drug treatment paradigm. Data pooled from 3 separate experiments, Results show mean ± SEM, significance determined by one way ANOVA, * p<0.5, ** p<0.01, *** 0.005.
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AUTHOR CONTRIBUTIONS:
O.J. & K.T.F. conceived of and designed the research. O.J., T.K., M.W. & M.J.O. conducted experiments and acquired data. O.J. & M.J.O. wrote the manuscript. R.L., M.J.C., F.B.G. & K.T.F. supervised the research and reviewed and edited the manuscript.
Figure 2

The figure shows the expression levels of different drugs in cell cultures over time. The drugs included are:

- DOXORUBICIN
- PLX4720
- ERLOTINIB
- CRIZOTINIB
- BYL719
- LAPATINIB
- PALBOCLICLIB
- IMATINIB
- YH239-EE
- SCH772984
- PD173074
- VORINOSTAT
- 17-DMAG
- CONTROL (syst. PLX4720)

The images represent the expression levels on days 0, 4, and 16 for each drug.
Figure 4
Parallel in-vivo assessment of drug phenotypes at various time points during systemic BRAF inhibition reveals tumor adaptation and altered treatment vulnerabilities

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