

IN THE SPOTLIGHT

Unpicking the Combination Lock for Mutant *BRAF* and *RAS* Melanomas

Bissan Al-Lazikani and Paul Workman

Summary: Large-scale, unbiased combinatorial drug screening has been used to identify effective genotype-selective therapeutic combinations that show promising activity in preclinical models of mutant *BRAF* and *RAS* melanoma that are resistant to the clinical *BRAF* inhibitor vemurafenib. *Cancer Discov*; 3(1); 14-9. ©2012 AACR.

See related article by Held et al., p. 52 (5).

Cancer is the disease in which the promise of drug and companion biomarker outputs from the human genome sequence has been best exemplified so far (1). Remarkable progress has been made in “drugging the cancer genome” and developing personalized or precision cancer medicine in which treatment is increasingly based on the genetic makeup of the individual tumor (2). Yet, we have arguably now reached a major inflection point. Thus, notwithstanding a veritable deluge of data from massively parallel sequencing and other large-scale omics initiatives, we remain far from achieving a complete systems biology understanding of human cancers (3). Furthermore, although many molecularly targeted cancer drugs have been approved, median survival is generally enhanced only by a matter of months and drug resistance is just as problematic as with traditional cytotoxic drugs—attributable to tumor heterogeneity, Darwinian selection of resistant clones, and biochemical feedback loops (4). New approaches are urgently needed to tackle drug resistance. A report in this issue of *Cancer Discovery* describes the first successful use of systematic, high-throughput, unbiased combinatorial screening of small-molecule agents to discover new and unexpected genotype-selective 2- and 3- drug cocktails with encouraging therapeutic activity in models of mutant *BRAF* and *RAS* melanoma resistant to the *BRAF* inhibitor vemurafenib and other treatments (5).

Despite initial hopes for single-agent therapies targeting addictions to unique driver oncogenes, best exemplified by the BCR-ABL inhibitor imatinib in chronic myeloid leukemia, it is increasingly clear that the challenges posed by genetic and phenotypic heterogeneity within individual tumors mean that the use of intelligent targeted drug combinations,

longitudinally adapted to meet the changing biology of the cancer, will be the only way to circumvent drug resistance and hence achieve long-term remission and potential cure (4, 6). But how do we identify the best drug combination for a particular cancer genotype?

The classical pragmatic approach to clinical cytotoxic drug cocktails was to combine agents with different molecular mechanisms, aiming to enhance anticancer effects and reduce cross-resistance while avoiding overlapping toxicity (4). Commercial factors and regulatory approval strategies also have an impact. These considerations can lead to relatively *ad hoc* combinations that fail to exploit our growing molecular knowledge. However, the high number of potential drug combinations precludes their thorough evaluation in preclinical models, let alone clinically.

The common 40-digit combination lock generates 64,000 permutations, which is why deciphering the code is essentially impossible if it is forgotten. For the approximately 250 approved cancer drugs, 31,250 2-way combinations and 2,573,000 3-way combinations are possible, whereas for the approximately 1,200 developmental oncology drugs, the respective numbers are 719,000 and 287,280,400 (4). Viewed in another way, 7,626 2-way and 310,124 3-way potential combinations are possible for our estimate of 124 conventionally druggable targets among the 479 genes reported to have causative genetic alterations in cancer (4). How can we reduce these prohibitively large numbers to actionable clinical combinations? The main options currently are as follows: (i) hypothesis-driven approaches based on our increasing knowledge of the molecular basis of drug resistance; or (ii) large-scale strategies centered on *in silico* modeling, genetic technologies such as synthetic lethal screens, and unbiased combinatorial drug screens (4).

In the new work, Held and colleagues (5) used large-scale unbiased combinatorial drug screening to identify cocktails active against melanomas with activating *BRAF* or *RAS* mutations, including those with intrinsic or acquired resistance to the approved breakthrough *BRAF* inhibitor vemurafenib—a major problem that is now seen routinely in the clinic (7, 8). Their workflow is shown in Fig. 1A.

First, to establish an unbiased chemical library for the initial single-agent screen, Held and colleagues (5) curated

Authors' Affiliation: Cancer Research UK Cancer Therapeutics Unit, The Institute of Cancer Research, London, United Kingdom

Corresponding Authors: Paul Workman and Bissan Al-Lazikani, Division of Cancer Therapeutics, Cancer Research UK Cancer Therapeutics Unit, The Institute of Cancer Research, Sutton SM2 5NG, United Kingdom. Phone: 44-20-8722-4301; Fax: 44-20-8722-4324; E-mail: Paul.Workman@icr.ac.uk; Bissan.Al-Lazikani@icr.ac.uk

doi: 10.1158/2159-8290.CD-12-0520

©2012 American Association for Cancer Research.

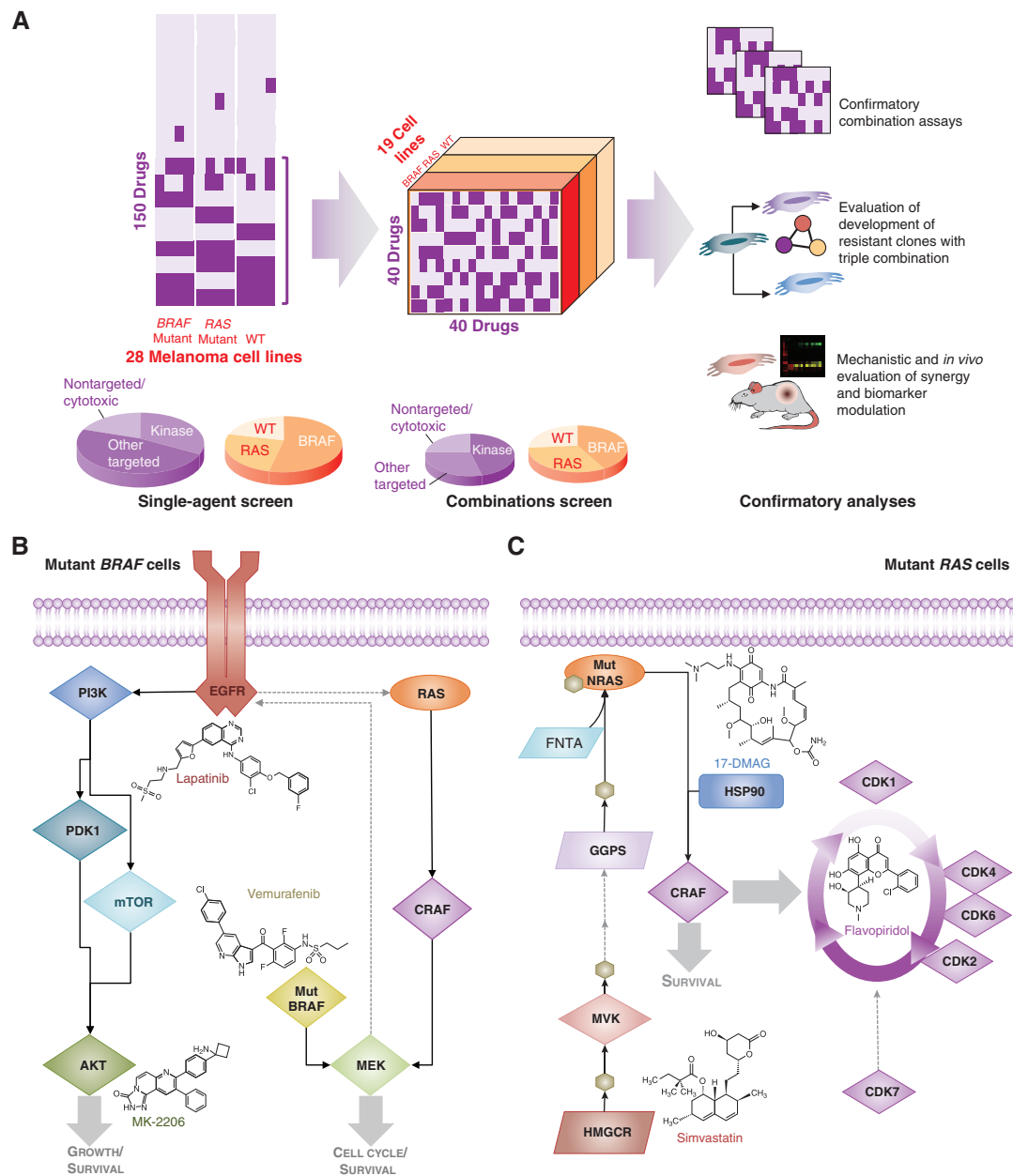


Figure 1. Study workflow for unbiased combinatorial screening, leading to effective genotype-selective combinations discovered by Held and colleagues (5), and the emerging pathways and interaction network derived from their data. **A**, a mechanistically broad library of 150 small-molecule compounds was screened against 28 early-passage human melanoma cell lines, including mutant *BRAF* or *RAS* and wild type (WT). A representative set of 40 compounds that were broadly or selectively active against the melanoma panel were chosen for the combinatorial screen. The most synergistic combinations were carried through for further confirmatory analyses, including elucidation of target engagement and pathway modulation; confirmation of on-target activity through the use of alternative chemotype compounds against the same target; identification of a promising triple combination in *BRAF*-mutant cells; exploration of avoidance of combination-resistant clones arising; and finally, *in vivo* demonstration of the promising therapeutic effect of a selected 2-drug combination in a mutant *NRAS* melanoma xenograft in nude mice. **B**, signaling pathways involved in the effective triple combination active in vemurafenib-resistant *BRAF*-mutant cells. The combination of the *BRAF*, *EGFR/ERBB2*, and *AKT* inhibitors was found to be highly synergistic and most effective in overcoming resistance when compared with 2-agent combinations. Resistant clones could not be generated when melanoma cells were exposed to the triple-drug regimen. Biomarker data suggest that the triple combination is very effective at reducing signaling output in the phosphoinositide 3-kinase (*PI3K*)–*AKT* pathway and the mutant *BRAF*–*MEK*–*ERK* MAPK pathway. Dotted lines represent omitted pathway components. *RAF* homo- and heterodimers are not shown for simplicity. **C**, 2-drug combinations found to be effective in *NRAS*-mutant cells exploit both signaling and metabolic pathways. *HMGCR* is a key component of the mevalonate pathway, leading to the production of geranyl-geranyl pyrophosphate, required for the prenylation of *NRAS*, and hence for localizing *NRAS* proximally to the plasma membrane. Inhibition of prenylation through the inhibition of *HMGCR* alone did not have a major growth inhibition effect on *RAS*-mutant cells; however, combining it with either an *HSP90* molecular chaperone inhibitor or a pan-cyclin-dependent kinase (*CDK*) inhibitor gave significant synergy. It is likely that the synergy seen within *NRAS*-mutant melanomas involves the cooperative effects of blocking the essential *NRAS* prenylation through *HMGCR* inhibition, thus preventing membrane localization and reducing *NRAS*–*RAF*–*MEK* MAPK signaling output, coupled with depletion of *RAF* isoforms, *CDK4* and other relevant *HSP90* clients in the case of 17-DMAG, or the inhibition of cell cycle and transcriptional kinases for flavopiridol. Dotted lines represent omitted pathway components; brown hexagons represent metabolites. (continued on following page)

D

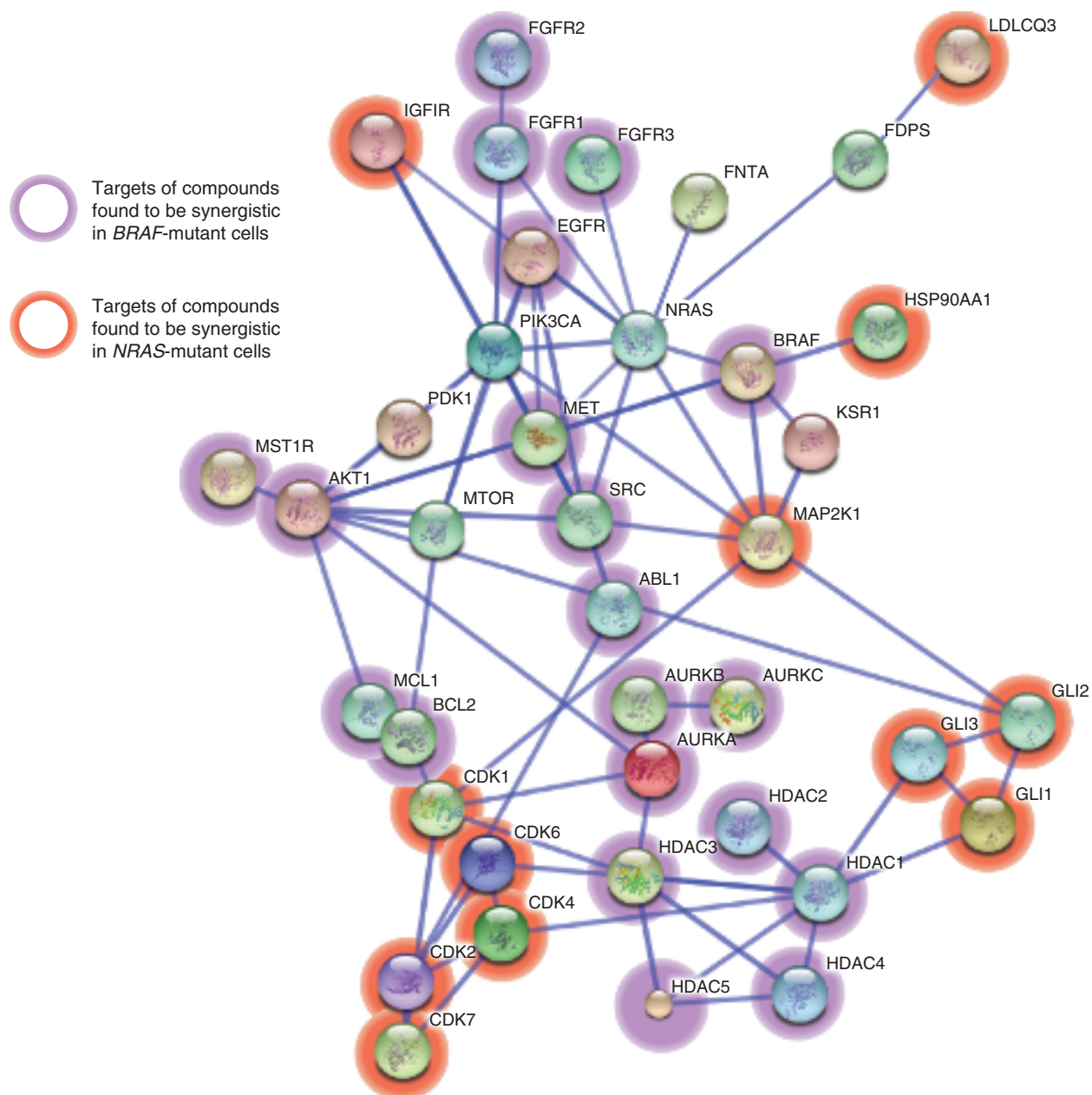


Figure 1. (Continued) D, network diagram of targets of agents showing synergy in the 2-drug combination screen. Proteins with orange outline are targets of compounds showing efficacy and synergy selectively for *NRAS*-mutant melanoma cells. Proteins with purple outline are targets of compounds showing efficacy and synergy selectively for *BRAF*-mutant melanoma lines. Whereas synergistic combinations effective in *BRAF*-mutant cells primarily act on receptor tyrosine kinase signaling pathways through *BRAF* and *PI3K*, effective combinations in *NRAS*-mutant melanoma cells involve deactivation of *NRAS* together with inhibition of less predictable and more broadly acting targets, exemplified by multiple *CDKs* and *HSP90*. Interestingly, the 2 sets of effective combinations, selective for *BRAF*-mutant vs. *NRAS*-mutant genotypes, exploit distinct yet highly interconnected subnetworks. The network diagram was generated semiautomatically using the *STRING* database incorporating targets of compounds listed in Table 1 in the publication by Held and colleagues (5). *FGRF*, fibroblast growth factor receptor; *IGF1R*, insulin-like growth factor 1 receptor. *MAPK*, mitogen-activated protein kinase; *ERK*, extracellular-regulated kinase; *HMGCR*, 3-hydroxy-3-methylglutaryl-CoA reductase.

150 small molecules selected to represent diverse biologic mechanisms. One-third comprised a wide range of kinase inhibitors, whereas 20% were nontargeted or broadly cytotoxic agents. The screening library contained approved drugs plus compounds under clinical investigation and chemical tools. High-throughput screening (HTS) was carried out with cell-

growth inhibition as the end point, testing a range of concentrations against a panel of 28 patient-derived early-passage melanoma cell lines harboring either *BRAF*- or *RAS*-activating mutations or WT for both oncogenes (Fig. 1A, left).

To identify whether any tested agents exhibited selective effects in a genotype-specific pattern, Held and colleagues (5)

clustered the compounds based on their growth inhibition profiles and classified them into 3 major categories: those with little effect across the whole melanoma panel, those active across the majority of the panel, and those showing clear differential effects.

Of obvious interest, certain single-agent compounds were selective toward *BRAF*-mutant cells. As expected (9), these included the *BRAF* inhibitors vemurafenib, PLX4720, and GDC-0879. However, growth inhibition was often incomplete, and Held and colleagues (5) also confirmed previous observations that a significant fraction (25%) of *BRAF*-mutant cell lines exhibited innate resistance to vemurafenib, whereas growth stimulation was seen in some mutant *RAS* and WT lines (10). In addition, loss of *PTEN* or *RB1*, known vemurafenib resistance mechanisms, was not necessary for reduced sensitivity, consistent with a role for additional resistance factors such as *MET*/*HGFR* (6, 8). These various results emphasize the need for new therapies or combinations in melanomas of all genotypes.

Additional compounds showing selectivity toward mutant *BRAF* melanomas included the *SRC*/*ABL* inhibitor bosutinib, the *FGFR* inhibitor dovitinib, and the *EGFR* inhibitor gefitinib—all effectively inactive against *BRAF*, indicating that sensitivity does not derive from off-target *BRAF* inhibition. Two *MEK* inhibitors were active in both mutant *BRAF* and *RAS* melanomas, especially the former. Interestingly, melanoma lines resistant to *BRAF* inhibitors (including the 25% of mutant *BRAF* cultures with intrinsic resistance) were also resistant to *MEK* inhibitors. Most intriguingly, the approved 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*) inhibitor simvastatin, a cholesterol-lowering agent which indirectly blocks prenylation and essential membrane localization of *RAS*, was the only single agent showing a trend to greater growth inhibition in *RAS*-mutant melanomas, albeit at high concentrations. Of note, no compounds selective for the WT cells were found, likely due to the genetic heterogeneity of this group.

The rather lackluster performance of compounds in the single-agent screen, and especially the relatively poor activity of compounds against the *RAS*-mutant and WT melanomas, further highlighted the need to identify effective combinations. In the second phase of their workflow, Held and colleagues (5) selected 40 representative compounds, chosen because of their broad growth-inhibitory effect or genotype-specific profiles, as well as representing the diverse molecular and biologic mechanisms of the full compound library, and carried these through to the combinatorial HTS (cHTS) campaign (Fig. 1A, middle). In total, they examined more than 7,000 pairwise combinations of the 40 agents, with 3 concentrations per drug, against 19 representative melanoma cell lines, comprising 8 *BRAF*-mutant, 5 *NRAS*-mutant, one *HRAS*-mutant, and 5 WT lines.

The cHTS identified many new, highly effective, and biologically interesting genotype-selective 2-drug combinations. Surprisingly, the selective combinations revealed for *BRAF*-mutant melanomas (1,021) massively outnumbered those selective for either *RAS*-mutant (75) or WT cells (22). Combinations found to be selective for *BRAF*-mutant cells involved a range of primary targets, albeit dominated by protein kinase inhibitors such as vemurafenib, the *SRC*/*ABL* inhibitor bosutinib, the *FGFR* inhibitor PD-173074, the *MET* inhibitor PHA-665752, the *AKT* inhibitor MK-2206, the *ERBB2*/*EGFR* inhibitor lapatinib, and the relatively unselective vascular endothelial growth

factor receptor (*VEGFR*) inhibitor sunitinib, together with the proposed pan-*BCL2* inhibitor obitoclax.

Remarkably, the *HMGCR* inhibitor simvastatin was involved in half of the 2-drug combinations selective against *RAS*-mutant melanomas. In contrast to the combination pairs in *BRAF*-mutant-selective combinations that were dominated by protein kinase inhibitors, simvastatin was effective against mutant *RAS* melanomas in combination with a far wider mechanistic range of compounds, including, particularly, inhibitors of the molecular chaperone *HSP90* and the ubiquitin ligase *XIAP*, as well as those for *MEK*, pan-cyclin-dependent kinases (*CDK*), and *IGF1R*. No combinations were found to be selective for WT melanomas, but some combinations were effective across all 3 melanoma genotypes, the strongest of which was the combination of a *STAT* inhibitor (stattic) with *FAK* inhibitor 14.

Moving on to the third phase of the study, Held and colleagues (5) characterized promising 2-way combinations in greater biologic detail, carried out mechanistic biomarker studies to show target engagement and pathway inhibition, tested additional compounds acting on highlighted targets, progressed promising members of effective 2-drug combinations into triple combination studies for mutant *BRAF* melanoma lines, and assessed ability to suppress development of resistant clones (Fig. 1A, right).

To identify the 2-way combinations with the most added value, Held and colleagues (5) looked for additive, synergistic, or antagonistic interactions according to the Bliss independence method. Intriguingly, most combinations selective toward *BRAF*-mutant cells showed additivity or synergy, whereas most combinations in *RAS*-mutant melanomas were formally antagonistic; that is, although effective, they were less active than predicted from the sum of single-agent sensitivities.

Among the many effective combinations in *BRAF*-mutant melanomas, the strongest 2-drug efficacies and synergies observed were between the allosteric *AKT* inhibitor MK-2206 combined with either the *ERBB2*/*EGFR* inhibitor lapatinib or the *SRC*/*ABL* inhibitor bosutinib. *RAS*-mutant (and WT) melanomas were generally less sensitive and the most efficacious and synergistic 2-way combinations for *RAS*-mutant melanomas involved the *HMGCR* inhibitor simvastatin plus either the *HSP90* molecular chaperone inhibitor 17-DMAG or the pan-*CDK* inhibitor flavopiridol. It was reassuring that combinations using alternative compounds acting against the same targets gave similar synergistic effects, increasing confidence that the synergy derives from on-target, not off-target, modulation.

Importantly, Held and colleagues (5) identified a key triple combination, involving *BRAF*+*EGFR*+*AKT* inhibition, which was highly effective in *BRAF*-mutant melanomas and overcame vemurafenib resistance. Vemurafenib sensitivity was reestablished by blocking *EGFR* and *AKT*, with biomarker studies indicating that this was most likely because the combination suppressed both the phosphoinositide-3 kinase (*PI3K*) and *RAS*-*RAF*-*MEK*-*ERK* *MAPK* pathways, which makes obvious mechanistic sense (Fig. 1B). The results are consistent with the known effects of *EGFR* inhibition in blocking the feedback activation loop between *MEK* and *EGFR* (potentially via *CDC25C*) which activates *CRAF* and *MEK* following inhibition of mutant *BRAF* in colorectal cancer

(11), although ERBB2 may play an additional role here. Also appealing was that the triple combination was highly effective in preventing the emergence of resistance in cell culture.

As mentioned, in *NRAS*-mutant melanomas, the HMGCR inhibitor simvastatin was a key sensitizer to other agents. Held and colleagues (5) focused their mechanistic work on the interaction of the statin with the pan-CDK inhibitor flavopiridol. Since membrane localization of *NRAS* was almost eliminated by simvastatin, they silenced *NRAS* expression by RNA interference (RNAi) and showed that, in combination with flavopiridol, the *NRAS* knockdown phenocopied the decrease in MAPK signaling seen with simvastatin as well as the cytotoxic effects of combined HMGCR and pan-CDK inhibition. Thus, the synergistic effects of simvastatin in the combination are likely mediated by reduced *NRAS* activity, which may also apply to the other HMGCR-containing combinations, for example, with the HSP90 inhibitor 17-DMAG (Fig. 1C). The beneficial interactions seen with HMGCR inhibition in *NRAS*-mutant melanomas may be hypothesized to involve the cooperative effects of blocking *NRAS*-*RAF*-*MEK* MAPK signaling output coupled to the depletion of *RAF* isoforms, *CDK4*, and other relevant HSP90 clients in the case of 17-DMAG or the inhibition of cell cycle and transcriptional kinases for flavopiridol (Fig. 1C). The potentially interesting 3-drug combination for *NRAS*-mutant melanomas was not investigated.

Finally, Held and colleagues (5) translated one exemplar key finding into an *in vivo* *NRAS*-mutant melanoma xenograft in nude mice. It was encouraging that the 2-drug combination of simvastatin plus flavopiridol was well tolerated and showed greater antitumor activity over the single agents. Further *in vivo* animal model studies are now required, including evaluation of the 3-drug combination of *BRAF*+*EGFR*+*AKT* inhibitors identified *in vitro* for *BRAF*-mutant melanomas. Investigation of the effects of a triple combination of simvastatin, flavopiridol, and 17-DMAG in *RAS*-mutant melanoma would also be especially interesting to pursue *in vivo* (Fig. 1C), as would additional detailed exploration of the mechanisms underlying the observed selective combinations. Our own initial analysis of the network of the targets of drugs for which the combinations showed the highest efficacy and synergy in *BRAF*- or *NRAS*-mutant melanomas illustrates connected but distinct processes involved in the combinatorial treatment of the 2 genotypes (Fig. 1D).

The new work illustrates the power of systematic large-scale combinatorial screening of unbiased 2-way combinations for discovering novel therapeutically actionable genotype-selective combinations that merit progression toward clinical investigation in mutant *BRAF* and *RAS* melanoma, including vemurafenib-resistant disease. Significantly, the effective combinations were not predicted by single-agent screening. Of special note are the 2-drug combination of statins and pan-CDK inhibitors (with additional potential of HSP90 inhibition) for *RAS*-mutant, and the 3-drug regimen of vemurafenib plus *EGFR* and *AKT* inhibition for *BRAF*-mutant melanoma. In another recent large-scale compound screen of pairwise combinations of 300 compounds in 9 established melanoma cell lines, Roller and colleagues (12) did not find synergistic interactions specific for *BRAF*, *RAS*, or WT genotypes but did identify robust synergy across all genotypes between the multikinase inhibitor sorafenib and the nonsteroidal anti-inflammatory drug (NSAID) diclofenac, with drug substitution experiments

indicating combined effects on MAPK and cyclooxygenase (COX). Differences seen may reflect the distinct properties of early-passage versus established melanoma lines. The combinatorial small-molecule screening approach complements the use of genome sequencing of vemurafenib-resistant melanomas, examination of signaling crosstalk and feedback loops, and unbiased genome- or kinome-wide loss-of-function RNAi screening—which have also led to clinically actionable combinations such as *BRAF* plus *MEK* or receptor tyrosine kinase inhibitors (4, 6, 8, 11)—and certainly yielded some unexpected combinations. Especially given the eye-watering number of possible drug-genotype combinations, rapid progress in the search for the best genotype-selective combinations to overcome resistance in multiple cancer types, which will likely reveal many unpredicted results, as here, will require global collaborations and data sharing (13). Furthermore, some combinatorial targets revealed by unbiased screens will not have clinical drugs available, emphasizing the importance of systematic approaches to extending the druggable cancer genome on the way to achieving the reality of combinatorial precision cancer medicine (2, 14).

Disclosure of Potential Conflicts of Interest

P. Workman and B. Al-Lazikani are employees of The Institute of Cancer Research which has a commercial interest in the discovery and development of anticancer drugs, including kinase inhibitors, and operates a Rewards to Inventors scheme. P. Workman is a former employee of AstraZeneca and declares commercial interactions with Yamanouchi (now Astellas), Piramed Pharma (acquired by Roche), Genentech, Vernalis, Novartis, Chroma Therapeutics, Astex Pharmaceuticals, AstraZeneca, Cyclacel, Onyx Pharmaceuticals, Merck Serono, Sareum, Janssen, Willex and Nextech Ventures. B. Al-Lazikani is a former employee of Inpharmatica.

Grant Support

P. Workman and B. Al-Lazikani are supported by a program grant from Cancer Research UK (C309/A8274). P. Workman is a Cancer Research UK Life Fellow (C309/89) and The Institute of Cancer Research/Royal Marsden Hospital receive support as a Cancer Research UK Centre. P. Workman is supported by Experimental Cancer Medicine Centre funding from Cancer Research UK, the National Institute of Health Research (NIHR) and the Department of Health. P. Workman and B. Al-Lazikani benefit from National Health Service funding to the NIHR Biomedical Research Centre.

Published online January 14, 2013.

REFERENCES

- Lander ES. Initial impact of the sequencing of the human genome. *Nature* 2011;470:187–97.
- Yap TA, Workman P. Exploiting the cancer genome: strategies for the discovery and clinical development of targeted molecular therapeutics. *Annu Rev Pharmacol Toxicol* 2012;52:549–73.
- Gatenby R. Finding cancer's first principles. *Nature* 2012;491:S55.
- Al-Lazikani B, Banerji U, Workman P. Combinatorial drug therapy for cancer in the post-genomic era. *Nat Biotechnol* 2012;30:679–92.
- Held MA, Langdon CG, Platt JT, Graham-Steed T, Liu Z, Chakraborty A, et al. Genotype-selective combination therapies for melanoma identified by high-throughput drug screening. *Cancer Discov* 2013;3:52–67.
- Garraway LA, Jänne PA. Circumventing cancer drug resistance in the era of personalized medicine. *Cancer Discov* 2012;2:214–26.

7. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 2011;364:2507-16.
8. Kudchadkar R, Paraiso KH, Smalley KS. Targeting mutant BRAF in melanoma: current status and future development of combination therapy strategies. *Cancer J* 2012;18:124-31.
9. Joseph EW, Pratilas CA, Poulidakos PI, Tadi M, Wang W, Taylor BS, et al. The RAF inhibitor PLX4032 inhibits ERK signaling and tumor cell proliferation in a V600E BRAF-selective manner. *Proc Natl Acad Sci U S A*. 2010;107:14903-8.
10. Heidorn SJ, Milagre C, Whittaker S, Nourry A, Niculescu-Duvas I, Dhomen N. Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. *Cell* 2010;151:209-21.
11. Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, Beijersbergen RL, Bardelli A, Bernards R. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 2012;483:100-3.
12. Roller DG, Axelrod M, Capaldo BJ, Jensen K, Mackay A, Weber MY, et al. Synthetic lethal screening with small-molecule inhibitors provides a pathway to rational combination therapies for melanoma. *Mol Cancer Ther* 2012;11:2505-15.
13. Bernards R. A missing link in genotype-directed cancer therapy. *Cell* 2012;151:465-8.
14. Patel MN, Halling-Brown MD, Tym JE, Workman P, Al-Lazikani A. Analysis: Objective assessment of cancer genes for drug discovery. *Nat Rev Drug Discov* 2013;12:35-50.

CANCER DISCOVERY

Unpicking the Combination Lock for Mutant *BRAF* and *RAS* Melanomas

Bissan Al-Lazikani and Paul Workman

Cancer Discovery 2013;3:14-19.

Updated version Access the most recent version of this article at:
<http://cancerdiscovery.aacrjournals.org/content/3/1/14>

Cited articles This article cites 14 articles, 4 of which you can access for free at:
<http://cancerdiscovery.aacrjournals.org/content/3/1/14.full#ref-list-1>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cancerdiscovery.aacrjournals.org/content/3/1/14>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.