

RESEARCH HIGHLIGHT

Receptor tyrosine kinases in cancer escape from BRAF inhibitors

Roger S Lo^{1,2}

¹Division of Dermatology, Department of Medicine, ²Department of Molecular and Medical Pharmacology, Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California, Los Angeles, 52-121 CHS, 10833 Le Conte Ave, Los Angeles, CA 90095-1750, USA

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The BRAF inhibitors (BRAFi) induce anti-tumor responses in nearly 60% of patients with advanced *V600*BRAF-mutant melanomas but only 5% of patients with *V600*BRAF-mutant colorectal carcinomas. Earlier studies of how a subset of melanoma that initially responds to BRAFi but later acquires drug resistance pointed to the importance of receptor tyrosine kinases (RTKs) in drug escape. In a pair of recent reports, this RTK-mediated mechanism of acquired BRAFi resistance in melanoma is re-surfacing in the context of innate or primary BRAFi resistance in *V600*BRAF-mutant colorectal carcinomas, suggesting potential upfront therapeutic strategies to prevent BRAFi resistance.

*V600*BRAF mutations are found in > 50% of melanomas, nearly 100% of hairy cell leukemias but smaller subsets of more common human malignancies (e.g., colorectal, thyroid) [1]. The in-human “druggability” of mutant BRAF has been best demonstrated in metastatic BRAF mutant melanomas using the novel small-molecule BRAF inhibitor (BRAFi) PLX4032/vemurafenib, producing survival benefits [2]. Early clinical results of BRAFi in

colorectal carcinoma, however, were disappointing, with only 5% of patients (1 of 21 patients) experiencing a partial response and 19% of patients (4 of 21 patients) experiencing minor responses [3]. This difference in the clinical results (melanoma vs. colorectal carcinoma) may relate less to their ontological origins but more to alternative states of a dynamic and plastic survival signaling network.

The majority of BRAF mutant melanomas responds to BRAFi rapidly but acquires drug resistance within a median time of 6-7 months. The specific mechanisms of acquired BRAFi resistance are variegated but fall under two core pathways: 1) reactivation of RAF-MEK-ERK MAPK signaling, and 2) activation of MAPK-redundant signaling via the receptor tyrosine kinase (RTK)-PI3K-AKT pathway, which is parallel but interconnected to the MAPK pathway. MAPK reactivation can occur via *NRAS* activating mutations [4], *COT* overexpression [5], *V600E*BRAF alternative splicing [6], *V600E*BRAF amplification [7], and *MEK1* activating mutation [8, 9]. MAPK-redundant signaling via RTK overexpression has been shown to result in AKT activation and RAS-CRAF-MEK signaling, bypassing mutant BRAF [4, 10, 11]. The repertoire of RTK overexpressed appears restricted but shares a common pattern of PDGFR β and EGFR overexpression,

at least in melanoma cell lines with acquired resistance to vemurafenib [4]. It is unclear at present how this overexpression of a select number of wild-type RTKs contributes to the molecular details of survival pathway redundancy and cooperativity. Nevertheless, understanding how melanomas acquire BRAFi resistance via core pathways may shed key insights into mechanisms of innate BRAFi resistance in multiple malignancies. Hence, it came as not a complete surprise that a pair of papers published recently implicated RTKs in innate BRAFi resistance in colorectal cancer cell lines [12, 13]. Both studies pointed to EGFR activation and downstream signaling as a key component to innate BRAFi resistance, at least in a majority of colorectal carcinoma (CRC) cell lines examined.

Corcoran *et al.* [12] showed that BRAF mutant CRC cell lines, in contrast to BRAF mutant melanoma cell lines, displayed innate resistance to growth inhibition by vemurafenib. An important clue implicating RTK involvement in innate vemurafenib resistance of BRAF mutant CRC cell lines came from the observation that p-ERK recovery occurred soon (hours to days) after vemurafenib treatment, unlike the kinetics of p-ERK recovery in BRAF mutant melanoma cell lines. This relatively rapid recovery of p-ERK post vemurafenib treatment in CRC cell lines

is akin to that in melanoma cell lines with acquired BRAFi resistance driven by RTK overexpression [10]. Corcoran *et al.* then traced this propensity for early p-ERK recovery to vemurafenib treatment (24 h)-dependent enhancement of (activated) RAS-GTP levels and MEK activity, parallel to elevated RAS-GTP levels in melanoma cell lines with RTK-driven, acquired BRAFi resistance [4]. In phospho-RTK arrays, they determined that the p-EGFR level (among others such as p-c-MET and p-IGF1R levels) was elevated in CRC cell lines relative to those in melanoma cells. Vemurafenib treatment (24 h) did not significantly enhance the p-EGFR level (but did elevate the p-IGFR1 level). Elevated p-EGFR levels in *BRAF* mutant CRC cell lines were correlated with elevated total EGFR levels (i.e., overexpressed compared with *BRAF* mutant melanoma cell lines). Thus, several observations correlated with innate BRAFi resistance in CRC cell lines: RTK (mostly consistently EGFR) overexpression (at baseline); upregulation of activation-associated phosphorylation of RTKs (at baseline); and upregulation of RAS-GTP levels (in response to BRAFi treatment). Curiously, although EGFR is highly phosphorylated at baseline, the RAS-GTP levels only rose in response to vemurafenib treatment.

Corcoran *et al.* further showed that small-molecule EGFR inhibitors (EGFRi) could downregulate, partially or completely, the RAS-GTP level induced by vemurafenib treatment. The combination of vemurafenib (BRAFi) and gefitinib (EGFRi) could synergistically reduce p-ERK levels and the net growth inhibition of most but not all CRC cell lines studied, suggesting that survival in some CRC cell lines may also depend on other RTKs and downstream signaling (e.g., AKT). Consistently, the combination of vemurafenib and erlotinib (EGFRi) stabilized the growth of, but did not cause significant regression of, CRC xenografts. Simultaneous inhibition or genetic knockdown of

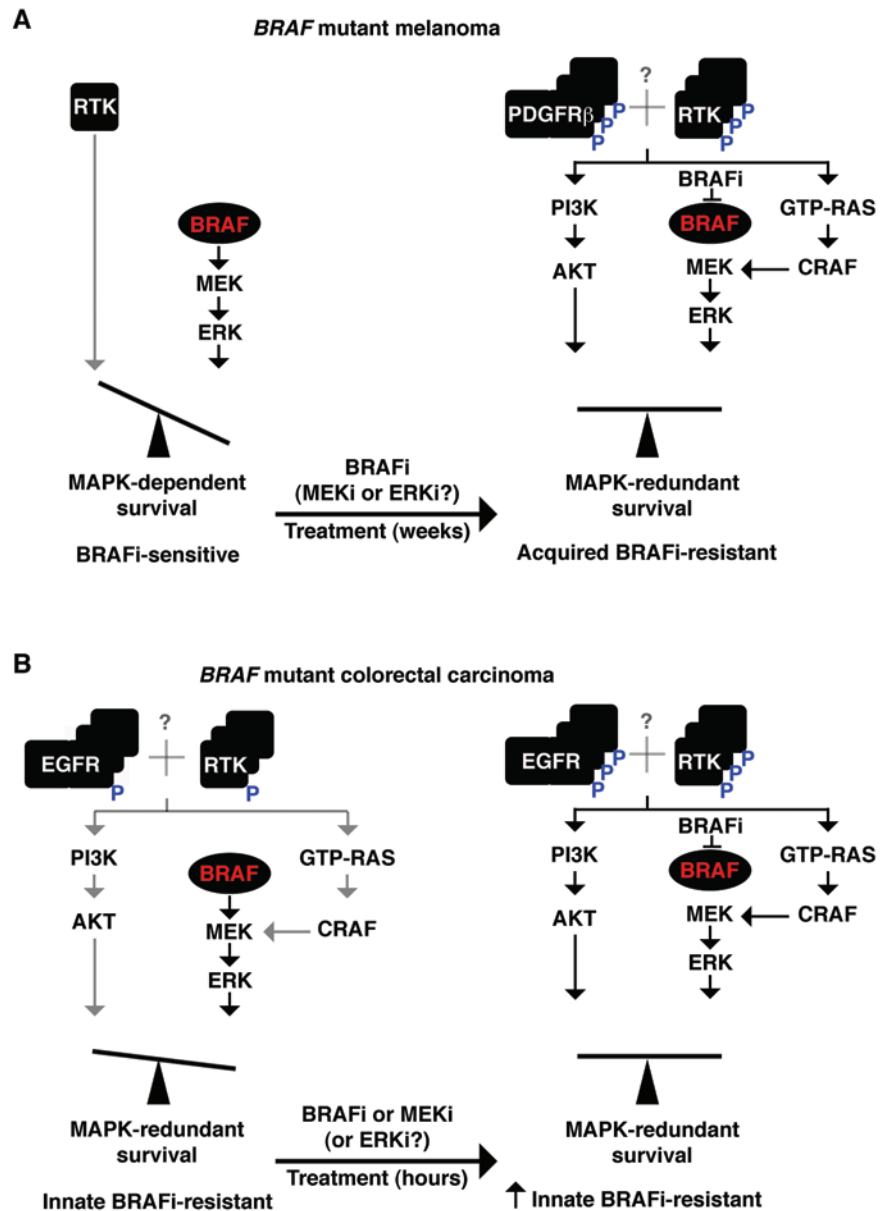


Figure 1 Upregulation of receptor tyrosine kinase(s) (RTKs) as a key sensitivity determinant of BRAFi resistance in *BRAF* mutant cancer cell lines. **(A)** In *BRAF* mutant melanoma cell lines, RTKs are generally expressed at very low levels and contribute minimally to survival signaling, resulting in a strong addiction to mutant BRAF signaling and sensitivity to BRAFi. When *BRAF* mutant melanoma cell lines acquire BRAFi resistance, they upregulate the expression and activity of PDGFRβ and other RTKs, resulting in reactivation of MEK-ERK as well as MAPK-redundant PI3K-AKT survival signaling. **(B)** In *BRAF* mutant colorectal carcinoma (CRC) cell lines, EGFR and other RTKs are upregulated by overexpression and some level of activation, resulting in MAPK-redundant survival signaling and conferring innate or primary BRAFi resistance. Treatment of CRC cell lines with a BRAF or a MEK inhibitor can further activate EGFR and potentially other RTKs and stimulate GTP-RAS levels, consolidating innate BRAFi resistance. Red denotes mutated protein (e.g., BRAF); gray symbols denote weak signaling or interactions; multiplicity of protein symbols denotes overexpression; P in blue denotes activation-associated phosphorylation.

multiple RTKs was not explored, leaving unresolved the issue of how multiple RTKs may potentially play cooperative survival roles at baseline or in response to kinase inhibitor therapy.

Prahallad *et al.* [13] also compared CRC and melanoma cell lines and showed that EGFR expression is generally higher in CRC cell lines. Vemurafenib treatment (6 h) of the WiDr CRC cell line led to an induction in p-EGFR and p-AKT levels, concomitant with the expected suppression of p-MEK and p-ERK. MEK inhibition, by AZD6244 treatment, similarly led to the rebound phosphorylation of EGFR. Based on earlier literature showing that the ERK kinase phosphorylates Cdc25c, activating its phosphatase activity, and that Cdc25c can dephosphorylate EGFR, Prahallad *et al.* went on to show that Cdc25c knockdown mimicked vemurafenib treatment in inducing p-EGFR levels. As predicted, vemurafenib treatment of CRC cell line inhibited Cdc25c phosphorylation at a key threonine (Thr 48), which was previously demonstrated to be a key event for its phosphatase activity. Addition of an EGFRi (cetuximab or gefitinib) to the BRAFi vemurafenib treatment downregulated the baseline level of p-ERK and the BRAFi-induced p-AKT level (but not the baseline p-AKT level). Moreover, addition of an EGFRi sensitized CRC cell lines to growth inhibition by vemurafenib *in vitro* but did not induce tumor regression *in vivo*, again suggesting incomplete survival signaling blockade. Accordingly, it has been shown that the effect of vemurafenib in shrinking CRC tumor xenografts was enhanced by combining with an AKT inhibitor (MK-2206) [14]. Moreover, in this study, the addition of vemurafenib to erlotinib treatment also resulted in increased anti-tumor activity and improved survival in xenograft models. It should be pointed out that Prahallad *et al.* did not formally assess BRAFi and EGFRi synergy, nor did they examine the diversity of RTK overexpression/activity and its contribu-

tion to downstream survival signaling (e.g., AKT).

These works, along with prior studies [4, 10], highlight the importance of expression and activity level of RTKs as a key sensitivity determinant of BRAFi resistance in *BRAF* mutant cancer cell lines (Figure 1). An important question remains as to whether the diversity of RTK overexpression and/or upregulation participates in and contributes to the full BRAFi resistance phenotype. A recent study afforded us a systems-wide view of the RTKinome reprogramming in response to MEK inhibition in the so-called triple-negative breast cancer cell lines [15]. The balance of the MAPK vs. RTK network signaling may be dynamically influenced by kinase inhibitors targeting RAF or MEK. This daunting diversity of RTK expression/activity may corner us into abandoning a combination of RTK inhibitors (already approved for clinical usage) with a BRAF inhibitor. Instead, we might need to resort to downstream pathway inhibitors not yet approved for clinical usage (e.g., an inhibitor of MEK with an inhibitor of the PI3K-AKT-mTORC1/2 axis) before we have a chance to corner *BRAF* mutant cancers into death.

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