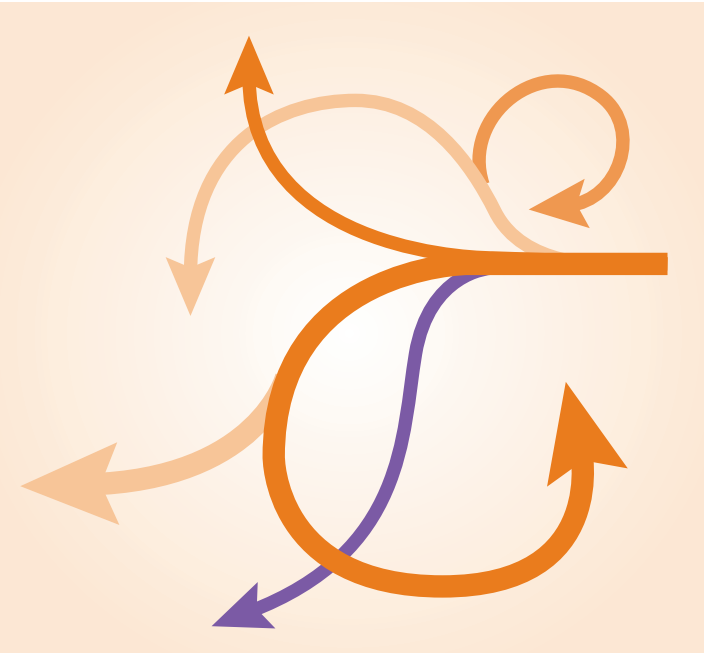
THERAPY

Multiple bypass problem for BRAF inhibition

> Cells that maintain MAPK signalling in the presence of BRAF inhibitors could be sensitive to inhibitors of downstream MAPK components.

Inhibitors of BRAF are showing promise for the treatment of melanomas that express mutant BRAF-V600E, but the emergence of resistant disease is a growing concern. Two recent studies have identified novel resistance mechanisms and have suggested improved treatment strategies.

To identify potential mechanisms of resistance to BRAF inhibitors, Roger Lo and colleagues used chronic BRAF inhibitor treatment *in vitro* to generate resistant derivatives of BRAF-V600E melanoma cell lines. Despite extensive sequencing, no secondary mutations in *BRAF* were found. Instead, a subset of resistant lines maintained MAPK pathway activation during treatment with a BRAF inhibitor, and sequencing of Ras isoforms suggested that acquired *NRAS* mutations, which circumvent the need for BRAF activity, were responsible for this constitutive activation of the MAPK pathway. Another subset of resistant cell lines continued to permit the inactivation of MAPK signalling by BRAF inhibitors, implying the activation of an independent survival pathway. Gene expression and phospho-protein analyses indicated that hyperactivation of platelet-derived growth factor receptor-β (PDGFRβ) is another route to resistance. Manipulation of NRAS or PDGFRβ levels *in vitro* altered the response to BRAF inhibition, implying a direct, causative role for these proteins in resistance. This was further supported by the discovery of *NRAS* mutations and PDGFRβ overexpression in clinical melanoma samples from patients who had relapsed following treatment with a BRAF inhibitor.



In a parallel study, Levi Garraway and colleagues carried out a kinome overexpression screen to search for mediators of BRAF inhibitor resistance. These authors identified multiple resistance-promoting kinases, including CRAF (previously characterized as maintaining MAPK signalling during BRAF inhibition) and MAP3K8. Mirroring the effect of activating *NRAS* mutations, overexpression of MAP3K8 caused hyperactivation of MAPK signalling even in the presence of BRAF inhibition and was observed in biopsy samples from patients who relapsed after treatment with a BRAF inhibitor. Interestingly, two BRAF-V600E cell lines previously untreated with MAPK pathway inhibitors were found to overexpress MAP3K8 and were resistant to BRAF inhibitors, offering a potential explanation for the ~10% of clinical BRAF-V660E melanomas with *de novo* resistance to BRAF inhibitors.

In addition to segregating BRAF inhibitor-refractory melanomas into distinct categories of molecular resistance mechanisms, these studies also suggest tailored therapeutic avenues to overcome such resistance. Cells that maintain MAPK signalling in the presence of BRAF inhibitors could be sensitive to inhibitors of downstream MAPK components. Indeed, *NRAS*-mutant cell lines, but not PDGFRβ-overexpressing cell lines remained sensitive to a MEK inhibitor as a single agent. Intriguingly, MAP3K8-overexpressing cells, despite continued MAPK pathway activation, were only sensitive to MEK inhibitors when in combination with BRAF inhibition.

It remains to be seen to what extent these novel resistance mechanisms will be mutually exclusive events, and the results of ongoing clinical trials of MEK inhibitors in BRAF inhibitor-refractory melanoma are eagerly awaited.

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ORIGINAL RESEARCH PAPERS Nazarian, R. *et al.* Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature* 24 Nov 2010 (doi:10.1038/nature09626) | Johannessen, C. M. *et. al.* COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. *Nature* 24 Nov 2010 (doi:10.1038/nature09627)