

Cracking the system: melanoma complexity demands new therapeutic approaches

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The melanoma field remains rich with discovery opportunities and stands poised for significant therapeutic advances. Although melanoma is notoriously resistant to chemotherapeutic and immunotherapeutic strategies, recent reports of complete clinical responses following the use of small molecule inhibitors in acral and mucosal metastatic melanomas harboring *KIT* alterations provide hope that melanomas can be managed clinically (Hodi et al., 2008). Given such significant patient responses, we have a hint that successful therapies do exist after all against advanced melanoma and teasing out more positive outcomes should not be an impossible feat. 'Magic bullets' however, are not presently available for most melanomas and the poor efficacy of single-agent chemotherapeutic approaches warrants the investigation of novel therapeutic strategies and inhibitors.

The path to target and drug discovery is now facilitated by increased access

to powerful tools such as high-throughput sequencing machines, extensive compound libraries, and expanding panels of genetically diverse melanoma cell lines or samples. While a number of melanoma targets have already been flagged and await proper validation and inhibition in the clinical setting (mutant BRAF inhibitors come to mind), gene-specific mutational analysis, and gene expression profiling are constantly exposing novel melanoma effectors and pathways that may prove just as valuable for melanoma research as those presently available, if not more. Using genomewide genetic analysis, Jones et al. (2008) most recently showed that 24 pancreatic cancers contain an average of 63 genetic alterations, which define a smaller key set of 12 signaling pathways and processes. In addition, these core pathways and processes are found altered in 67–100% of tumors analyzed. This novel and powerful approach to cancer research, which will be applied to melanoma shortly, is physiologically and therapeutically relevant given that it focuses the great genetic variations found across patients into fewer pathways that can be more easily studied and targeted.

On a smaller scale, a study by Smalley et al. (2008a), showed that mutational analysis and use of a diverse panel of melanoma cell lines provide insights into melanoma resistance to BRAF inhibition, namely through the involvement of cyclin-dependent kinase 4 (CDK4) and/or cyclin D1 (CCND1). Unlike cell lines harboring a *CDK4* mutation alone, cell lines with both a *CDK4* mutation and *CCND1* amplification were able to display resistance to BRAF inhibition. In addition, overexpression of cyclin D1 increased resistance to BRAF inhibition which was further potentiated by CDK4 overexpression. These findings hold clinical relevance since array comparative genomic hybridization analyses (aCGH) show *CCND1* amplifica-

tions in 17% of BRAF V600E-mutated metastatic melanoma samples (Smalley et al., 2008a). CDK small molecule inhibitors are presently being developed and may prove powerful in combination with BRAF inhibitors.

The observation that a great proportion of melanomas display constitutive mitogen-activated protein kinase (MAPK) pathway activity led to the eager development of a number of RAF and MEK inhibitors; however, these compounds in early clinical studies led to disappointing results despite encouraging preclinical findings. Current opinion now points to the need to target several pathways concomitantly in order to control melanoma growth, survival, and metastasis due to the presence of redundant protein functions and compensatory/adaptable signaling pathways. In fact, melanoma researchers are increasingly adopting therapeutic strategies from a new paradigm emerging in drug discovery: 'network pharmacology', which integrates systems biology, network analysis, connectivity, redundancy and pleiotropy. Such strategies include the use of rational multidrug combinations, exploiting the properties of approved drugs in combination, and using single compounds specifically binding to two or more diverse molecular targets in order to improve treatment efficacy (Hopkins, 2008). Exemplifying the use of the rational multidrug combination approach to potentiate a drug response, a recent study by Cragg et al. showed that MEK inhibition caused the upregulation of the proapoptotic Bcl-2 family member Bim in BRAF mutant melanoma and colon cancer cell lines. Given that apoptosis was minimal owing to the neutralizing effects of Bcl-2 and Bcl-x_L, the Bcl-2 antagonist ABT-737 was employed causing the MEK inhibition-induced cytostatic effect to shift to a cytotoxic effect with long-term tumor regression in a mouse xenograft model (Cragg et al., 2008).

Coverage on: Jones et al. (2008). Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 321: 1801–1806.

Smalley et al. (2008a). Increased cyclin D1 expression can mediate BRAF inhibitor resistance in BRAF V600E-mutated melanomas. *Mol. Cancer Ther.* 7: 2876–2883.

Cragg et al. (2008). Treatment of B-RAF mutant human tumor cells with a MEK inhibitor requires Bim and is enhanced by a BH3 mimetic. *J. Clin. Invest.* 118: 3651–3659.

Smalley et al. (2008b). CRAF inhibition induces apoptosis in melanoma cells with non-V600E BRAF mutations. *Oncogene* [Epub ahead of print].

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Another explanation for the lack of effective single-agent therapies is melanoma heterogeneity. Melanomas can be effectively subdivided based on distinct mutation profiles; therefore, interrogating as genetically diverse a panel of melanoma samples as possible will allow the identification of a potentially rare group of responders that may have been overlooked through a random sample selection. Validating this approach, Smalley et al. recently identified a small panel of melanoma cell lines with low activity non-V600E BRAF mutations (K601E, G469E and D594G) which are sensitive to the CRAF inhibitor sorafenib. Treatment of these cell lines with sorafenib caused apoptosis and decreased tumor growth in xenograft models while V600E BRAF mutated cell lines were less responsive. Further analysis established that these rare cell lines signal through CRAF which modulates Bcl-2 and BAD activity to control apoptosis. These findings suggest that melanomas with low-activity BRAF mutations are sensitive to sorafenib treatment as they rely on CRAF activity for survival (Smalley et al., 2008b).

The task of handling multiple targets and redundant signaling networks with

non-fully characterized compounds may appear daunting. However, we have already accumulated a significant amount of knowledge on small molecule inhibitors and their activity on melanoma biology which can help fine tune therapeutic strategies. For example, the MAPK and the PI3K-AKT-mTOR signaling pathways are often constitutively and coordinately activated in many melanomas and targeting both well-studied pathways abrogates melanoma cell growth in preclinical studies (Lasithiotakis et al., 2008). Given that MAPK (sorafenib) and mTOR (CCI-779) inhibitors are available for clinical use, results from clinical trials involving parallel inhibition of the MAPK and PI3K pathways are highly anticipated and are expected to shape the next major effort in combination therapies (ClinicalTrials.gov Identifiers: NCT00349206 and NCT00281957). The integration of knowledge acquired through genetic, biological, and compound screens is likely to provide many more translational leads in the future, and hopefully significant therapeutic successes.

In sum, several complete responses have now been reported for advanced melanoma patients following small mol-

ecule inhibitor treatment, thus providing optimism that melanomas can be managed clinically. The 'one target, one drug' approach however, is likely to be efficient in only a small proportion of patients; therefore, our increased efforts to understand melanoma circuitry and combine therapies are expected to generate the most positive clinical outcomes in the future.

References

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