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Combinatorial therapies to overcome B-RAF inhibitor resistance in melanomas

“Successful combinatorial targeting of melanoma and other cancers is arguably the next frontier in accelerating personalized cancer medicine.”

KEYWORDS: B-RAF ■ melanoma ■ N-RAS ■ targeted therapy ■ vemurafenib ■ whole-exome sequencing

The ability of B-RAF inhibitors to induce unprecedented response rates in *B-RAF* mutant melanoma represents a watershed moment in the treatment of this dreadful disease. However, the efficacy of B-RAF inhibitors is short-lived as a result of acquired drug resistance. Augmenting the efficacy of B-RAF inhibitors to effect prolonged survival requires its combination with other targeted agents that block escape or compensatory tumor-survival pathways. Combinatorial targeted strategies based on B-RAF inhibitors require a thorough knowledge of resistance mechanisms. Successful combinatorial targeting of melanoma and other cancers is arguably the next frontier in accelerating personalized cancer medicine.

Optimism & opportunities

2010 has been called the ‘Year of Melanoma’ by cancer scientists and physicians. In 2011, we witnessed the US FDA approval of a type I B-RAF inhibitor (B-RAFi; i.e., PLX4032/vemurafenib/Zelboraf™, Roche, Basel, Switzerland) and an immunomodulatory agent (ipilimumab/Yervoy™, Bristol-Meyers Squibb, NY, USA) for the treatment of advanced melanoma. Although B-RAFi can induce unprecedented response rates in excess of 50% among patients with *V600E/K B-RAF* melanomas, virtually all of those patients who initially respond later suffer from disease progression (i.e., acquired drug resistance) [1–4]. Thus, overcoming B-RAFi resistance promises to significantly advance melanoma survivability.

The *V600E B-RAF* mutation is the most common single-nucleotide mutation in cancer described to date, with an estimated incidence of 7–8% of all cancers. The fact that it is more prevalent in melanoma (50–60% of metastatic melanoma patients), and that the early clinical experience was highly positive in this indication, argues for

studies of B-RAFi resistance in melanoma as being potentially instructive for B-RAFi-based treatment of other cancer types. *V600 B-RAF* mutations can also be found in relatively high frequency in thyroid cancer, and less commonly in colorectal, ovarian, lung and other epithelial cancers. Full appreciation of the impact of this mutation and targeted inhibitors may still be premature. For example, *B-RAF* mutations were recently found in 100% of hairy-cell leukemias [5].

Understanding the mechanisms of B-RAFi resistance (primary or *de novo* vs secondary or acquired) operative in patients will require a rigorous and collaborative effort to bank, annotate and analyze patient-derived tissues. With FDA approval of the first B-RAFi and the likely increasing availability of tissues sampled from patients, mechanisms inferred from model systems, such as melanoma cell lines primarily resistant to B-RAFi or those artificially derived to be B-RAFi resistant by continuous drug treatment and selection, can be validated in patient-derived tissues at an ever faster pace. In fact, direct application of discovery platforms (e.g., whole-genome sequencing, whole-exome sequencing, RNA-sequencing) on patient-derived tissues is already feasible and ongoing.

Progress

Few mechanisms of primary resistance to B-RAFi melanoma have been validated to be recurrent and significant in patients. Lack of melanoma tissues, which fail to respond to B-RAFi (defined by not reaching 30% tumor reduction by RECIST 1.1 criteria or tumor size increase at the specified time point of tumor assessment), is no doubt an impediment to progress in this area. In addition, it is yet to be validated that *V600E B-RAF* melanoma cell lines regarded as primarily resistant to B-RAFi

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in vitro, as determined typically by a short-course (e.g., 3-day) treatment protocol and an IC₅₀ not reached at low micromolar drug concentrations, truly represent primary melanoma resistance to B-RAFi in the clinic.

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Among the proposed mechanisms of acquired B-RAFi resistance, *N-RAS* mutations (restricted to hotspots) [6], ^{V600E}B-RAF truncation (via alternative splicing creating novel exon–exon boundaries) [7], and RTK (PDGFRβ, IGF1-R) [6,8] overexpression have been identified at different study sites from multiple patients who have relapsed on either B-RAFi, vemurafenib/Zelboraf or GSK2118436 (GlaxoSmithKline, London, UK). *N-RAS* mutations and ^{V600E}B-RAF truncation, with the latter resulting in RAS-independent RAF dimerization, reactivate the MAPK pathway in the face of B-RAFi. Preclinical drug treatments predict restoration of B-RAFi sensitivity with MEK inhibitors (MEKi). Alternatively, RTK overexpression activates the PI3K-AKT pathway, serving to provide MAPK-redundant survival signaling. Here, preclinical drug treatments showed restoration of B-RAFi sensitivity with inhibitors of the PI3K-AKT pathway. In addition, although MEKi treatment failed to restore B-RAFi sensitivity in melanoma cell lines with acquired B-RAFi resistance driven by RTK overexpression, MEK inhibition synergized with dual PI3K-mTOR inhibition in growth inhibition and apoptosis induction [9]. Thus, varied mechanisms of acquired B-RAFi resistance shown to recur in clinical samples appear to suggest two general modes of drug escape, namely those that reactivate the MAPK pathway and those that create MAPK-redundant signaling (e.g., RTK-PI3K-AKT signaling). Notably, secondary mutations in ^{V600E}B-RAF have not been identified in drug-resistant tumors (as well as in resistant cell lines derived in the laboratory) [6], arguing for the importance of combinatorial targeted strategies to overcome B-RAFi resistance in melanoma.

Collectively, these mechanisms of acquired B-RAFi resistance (*N-RAS* mutations, ^{V600E}B-RAF truncation and RTK overexpression) are estimated to account for approximately 50% of clinical relapses on B-RAFi, with

several caveats. In addition to the fact that the total number of tumors (some from the same patients), and especially the number of patients examined, is quite limited, most drug-resistant tumor biopsies have come from patients heavily pretreated with other agents (some mutagenic) prior to B-RAFi therapy. Moreover, most tissues for resistance studies are derived from cutaneous or subcutaneous lesions, raising a potential concern about how representative the spectra of mechanisms may be for visceral progressive melanoma tumors on B-RAFi therapy.

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Understanding the full compendium of resistance mechanisms is thus key to designing combinatorial targeted treatments designed to prevent and/or overcome B-RAFi resistance in *B-RAF* mutant melanomas. Clinical trials designed to prevent (upfront combination) or overcome resistance (after development of acquired B-RAFi resistance) are both needed, the latter especially after the FDA approval of vemurafenib as a monotherapy and the expectation that most vemurafenib-treated patients will develop acquired drug resistance. There is theoretical concern for targeted monotherapy yielding tumors with a potentially more aggressive genetic or epigenetic constitution [10]. Histologic evidence of this has been supported by a recent study in a different cancer. In melanomas with acquired B-RAFi resistance, this concern may be theoretically valid with certain adaptive mechanisms that lead to global gene-expression alterations [6] or with switching of driver oncogenic genes (mutant *B-RAF* to mutant *N-RAS*).

Patients who have progressed on B-RAFi and treated subsequently with targeted combination agents (such as B-RAFi with MEKi or MEKi with dual PI3K inhibitors) have provided anecdotal evidence for altered molecular dependencies predicted by preclinical studies. In addition to combinations based on altered tumor dependencies, combinations based further on dynamic signal compensations and recoveries (via transcriptional, translational or post-translational feedbacks) to a matrix of targeted agents should be factored into combinatorial designs. This is an area where proteomic and its variant (e.g., phosphoproteomic, acetyproteomic) profiling could further highlight unexpected B-RAFi-based combinatorial targets.

A combinatorial approach may, in specific settings, even increase drug tolerability by reducing on-target related toxicities. Approximately 20–25% of melanoma patients treated with type I B-RAFi developed cutaneous squamous cell carcinomas (typically of the keratoacanthoma subtype). This is thought to occur via B-RAFi inducing a so-called paradoxical MAPK activation in the context of RAF dimerization driven by upstream RTK or RAS hyperactivity [11]. In a striking parallel to overcoming mutant *N-RAS*-driven, acquired B-RAFi resistance in melanoma using the B-RAFi and MEKi combination, the same combination may in fact tilt the therapeutic index in favor of efficacy and concomitantly against toxicity [12].

Challenges

Melanoma tumor heterogeneity lies at the heart of the challenge to overcome acquired B-RAFi resistance. This is exemplified at the genomic level by the relatively large number of somatic genetic alterations in metastatic melanomas [13] and at the clinical level by the relatively short time to progression on B-RAFi treatment and the heterogeneous landscape of acquired resistance mechanisms that is emerging. Furthermore, tumors at distinct sites from the same patient may circumvent B-RAF inhibitors via distinct routes. But just as the genome is finite, mechanisms of resistance, *de novo* or acquired, should also be finite. Failures on targeted B-RAF inhibition afford us the opportunities to understand such finite events.

High-depth, whole-exome sequencing represents a currently cost-permissive approach to dissect the genetic complexity of melanoma tumors with acquired B-RAFi resistance. Such detailed genetic characterization of patient-matched baseline tumors (prior to B-RAFi therapy) may potential pinpoint pre-existing genetic factors that are predictive of specific type of genetic alterations (e.g., copy number gains vs single nucleotide variants) accounting for acquired drug resistance. Recent work with whole-exome sequence highlighted its potential to dissect the temporal relationship of oncogenic events [14]. This type of analysis may help to provide insights into how potentially distinct pathways of tumor progression, up to the point of B-RAFi therapy, may predispose to or even predetermine the specific mechanism of acquired drug resistance under B-RAFi selection. Moreover, whole-exome sequencing can also provide data on genomic DNA copy

number variations [15], likely a causative source of acquired drug resistance.

Melanoma tumor heterogeneity, amplified by B-RAFi selective pressure, points to next-generation clinical diagnostics as a key to fully realizing personalized combinatorial therapies based on B-RAFi. Efforts to innovate combinatorial therapeutic strategies must be coupled to an equal effort to innovate diagnostic tools. Here, there are several facets of technologic development worth noting. One involves diagnostic platforms that can maximize the predictive power of baseline tumor biopsies. For instance, single-cell analysis of tumors or other clinical samples (e.g., blood) carrying traces of existing tumors promises to parse tumor heterogeneity and uncover minor genetic subpopulations that represent seeds of eventual resistance mechanisms. Another facet of innovation relates to reliable detection of resistance mechanisms from minute sampling of tumor cells (few thousand tumor cells). Ultimately, minute (and repeated) sampling from minimally invasive procedures to acquire tissues from easily accessible sites (e.g., peripheral blood for capture of circulating tumor cells) will enable real-time monitoring of tumor response (or lack thereof) to regimens of combined targeted agents. Pushing these technologic boundaries and their successful implementation in the clinical setting promise to maximize knowledge gained from clinical trials and advance the full potential of targeted cancer therapy.

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