Deciphering root causes of intrinsic BRAF inhibitor resistance in melanoma: ushering in a new genomics case reports feature for Annals of Oncology

Great strides have been made in the management of melanoma over the last 12 years. From the original discovery of common BRAF V600E mutations in melanoma by Futreal and colleagues in 2002 [1] to the first randomised trial demonstrating impressive efficacy in this patient population through BRAF inhibitor targeting [2], clinical management of this disease has been transformed.

However, despite the impressive efficacy of BRAF signalling axis targeting, approximately one in five patients with BRAF V600 mutant melanoma have disease that is intrinsically resistant to BRAF targeting, progressing on therapy at the first scan assessment. Understanding the root causes of primary drug-resistant disease is an important research area in this era of targeted therapy. Whether the spectrum of genetic aberrations that contribute to intrinsic targeted therapy resistance is similar to that conferring acquired therapy resistance through selection of resistant sub-clones is unclear in the majority of cases.

Increasing evidence suggests intra-tumour heterogeneity and branched evolution results in the selection of drug-resistant sub-clones contributing to acquired resistance to targeted therapy [3]. Acquired resistance to BRAF inhibition occurs through reactivation of the MAPK pathway through which BRAF normally signals in the majority of cases [4]. In a minority of cases, resistance is driven by MAPK pathway-independent mechanisms. Mechanisms of intrinsic resistance remain less well studied. Van Allen et al. recently reported melanomas subjected to exome sequencing in 14 patients with early resistance to BRAF inhibitor therapy (progression within 12 weeks) [5]. The authors reported RAC1 P29S mutation driving either intrinsic resistance or early acquired resistance to BRAF inhibitor therapy in three patients and a nonsense mutation in a gene previously shown to confer resistance to RAF inhibition from an in vitro RNAi screen, HOXD8, in one patient [5, 6].

However, what is also becoming increasingly clear in solid tumour oncology is the heterogeneous nature of targeted therapy resistance within individual patients; resistance to therapy may be mediated by more than one mechanism in the same tumour. For example several recent reports from Van Allen et al., Shi et al. and Romano et al. have elegantly described multiple mechanisms of BRAF inhibitor resistance in individual melanoma biopsies or individual patients, illustrating the emerging complexity of targeted therapy drug resistance [5, 7, 8].

Detailed drug resistance studies require a complex multidisciplinary team and are often hampered by the depth and breadth of sequencing, the number of metastatic sites biopsied or studied and/or functional genomics evidence that the identified genomic aberrations confer drug resistance in relevant model systems. Definitive evidence that drug resistance is mediated across all sites of progressive disease by the single genetic event detected is often lacking. Clearly, this remains an intractable problem, given the intrinsic difficulties of sampling many metastatic disease sites simultaneously and the scale of genomics analysis required to satisfy these criteria.

With this background, Turajlic et al. provide a comprehensive analysis of one 26-year-old man with primary refractory BRAF V600E mutant melanoma treated with vemurafenib, sampling five sites of disease with whole-genome sequencing followed by functional validation of the proposed drug resistance mechanisms, in order to address the important question of drivers of intrinsic drug resistance. Spatially and temporally separated biopsies were subject to whole-genome sequencing and SNP array analysis to decipher somatic mutation and focal DNA copy-number changes that might contribute to intrinsic vemurafenib resistance. Notably, the authors provide functional insight into their genomics findings through the analysis of a melanoma cell line derived from a site of metastatic disease. Their work, published in this edition of Annals of Oncology [9], provides important insights into the underlying cause of intrinsic vemurafenib resistance in this case.

The authors identify V600E BRAF mutations at all sampled disease sites, consistent with the founding, truncal nature of this driver event in melanoma. In addition, a Q209P mutation in GNAQ was identified at all sampled disease sites, including the pre-treatment formalin fixed paraffin embedded tumour. This result is again consistent with a founder role for this somatic event, present ubiquitously throughout all tumours. The authors demonstrate that this GNAQ mutation renders BRAF V600E melanoma cells resistant to BRAF inhibitor treatment with sustained ERK activation. By RNAi depletion of the GNAQ Q209P in the melanoma cell line derived from the patient, the authors elegantly demonstrate sensitisation to BRAF inhibitor treatment—firmly implicating this somatic GNAQ mutation in intrinsic drug resistance. However, the authors did not stop there. Further analysis of the genome sequencing datasets revealed a phosphatase and tensin homolog (PTEN) 4 bp deletion, introducing a premature stop codon with loss of chromosome 10, encoding the other allele, at all disease sites. These data are again consistent with PTEN loss being an early founder event present in the trunk of
the tumour’s evolutionary tree. Concordant with these analyses, PTEN was not expressed in the cell line derived from the 26-year-old patient and AKT appeared activated, rendering the cells more sensitive to AKT inhibition with MK2206 than was a melanoma cell line without PTEN loss. The authors conclude that following BRAF inhibition, ERK and AKT signalling are maintained by virtue of GNAQ Q209P and PTEN loss, respectively. Finally, they demonstrate that combined MEK and AKT inhibition synergise to inhibit the growth of the patient-derived melanoma cell line.

This study elegantly illustrates the root cause and likely clonal nature of intrinsic drug resistance mechanisms; Chromosome 10 loss, PTEN deletion and the GNAQ mutation were detected at all disease sites, consistent with all three events occurring relatively early before tumour dissemination. Their presence at all sites of disease and in all cancer sub-clones, in the trunk of the tumour’s evolutionary tree, renders BRAF inhibition ineffective. In contrast, acquired resistance mechanisms may be sub-clonal, present in the tumour evolutionary branches and selected through therapy [10, 11].

This study emphasises the depth of analysis that may be required to realise precision medicine for the individual patient. The analyses are so complex that the laboratory and informatics challenges required to comprehensively decipher drug-response mechanisms surpass the median survival times of the disease itself. Increasing evidence from such cases could establish mechanisms of resistance to therapy and provide the community with deeper insights into how a heterogeneous sub-clonal landscape contributes to acquired drug resistance. These fundamental discoveries may help to anticipate drug-resistant mechanisms and stall their evolution through therapeutic intervention, as well as provide facile clinical assays that permit screening for emerging resistance.

The editorial team of Annals of Oncology appreciates these challenges and the extraordinary value of published molecular analyses of both acquired and intrinsic resistance mechanisms, even in individual case reports, as there is much to be learned from in-depth genomics reports such as these. We anticipate that by having extensive genomics experience on a case-by-case basis, the mechanisms of treatment failure and drug response will be elucidated. Hence, clinicians will be able to design the next cohort of genomics-based clinical studies to prolong drug response and prioritise patients with intrinsic drug resistance early in the disease course on to alternative therapeutic approaches or active surveillance. We welcome additional such case reports for submission to Annals, not only for acquired and intrinsic resistance descriptions but also for exceptional responses to targeted therapies. These case reports can feature molecular analyses of individual patients or cohorts, and should provide detailed information regarding the assays, the associated clinical data for each patient, the computational and confirmatory mechanisms, and the clinical implications for patient care.

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