The Oncologist®

Precision Medicine Clinic: Molecular Tumor Board

CIC Mutation as a Molecular Mechanism of Acquired Resistance to Combined BRAF-MEK Inhibition in Extramedullary Multiple Myeloma with Central Nervous System Involvement

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Multiple myeloma • Extramedullary disease • Capicua transcriptional repressor • Drug resistance • BRAF mutation

Abstract

Combined MEK-BRAF inhibition is a well-established treatment strategy in *BRAF*-mutated cancer, most prominently in malignant melanoma with durable responses being achieved through this targeted therapy. However, a subset of patients face primary unresponsiveness despite presence of the activating mutation at position V600E, and others acquire resistance under treatment. Underlying resistance mechanisms are largely unknown, and diagnostic tests to predict tumor response to BRAF-MEK inhibitor treatment are unavailable.

Multiple myeloma represents the second most common hematologic malignancy, and point mutations in BRAF are detectable in about 10% of patients. Targeted inhibition has been successfully applied, with mixed responses observed in a substantial subset of patients mirroring the widespread spatial heterogeneity in this genomically complex disease. Central nervous system (CNS) involvement is an extremely rare, extramedullary form of multiple myeloma that can be diagnosed in less than 1% of patients. It is considered an ultimate high-risk feature, associated with unfavorable cytogenetics, and, even with intense treatment applied, survival is short, reaching less than 12 months in most cases. Here we not only describe the first patient with an extramedullary CNS relapse responding to targeted dabrafenib and trametinib treatment, we furthermore provide evidence that a point mutation within the capicua transcriptional repressor (CIC) gene mediated the acquired resistance in this patient. ***The Oncologist*** 2020;25:112–118

Key Points

- BRAF mutations constitute an attractive druggable target in multiple myeloma. This is the first genomic dissection of the central nervous system involvement in a multiple myeloma patient harboring a druggable $BRAF^{V600E}$ mutation. Deep genomic characterization of the extramedullary lesion prompted a personalized therapeutic approach.
- Acquisition of *CIC* mutation confers a mechanism of BRAF-MEK inhibitor drug resistance in multiple myeloma.
- The in silico interrogation of the CoMMpass clinical study revealed 10 patients with somatic mutations of *CIC* and its downregulation at gene expression level in multiple myeloma.
- *CIC* gene silencing decreases the sensitivity of multiple myeloma cells to BRAF-MEK inhibition in vitro. The correlation between CIC downregulation and ETV4/5 nuclear factor expression in multiple myeloma *BRAF*-mutant cells is shown for the first time.
- *CIC* mutation, its downregulation, and the related downstream effect on MMP24 support disseminative potential providing new clues in the extramedullary biology definition.

Patient History

An 81-year-old patient with κ light chain multiple myeloma (MM) was referred to our center after having a seizure and increasing M-proteins. MM had been diagnosed 2 years before and the patient had undergone nine cycles of bortezomib-

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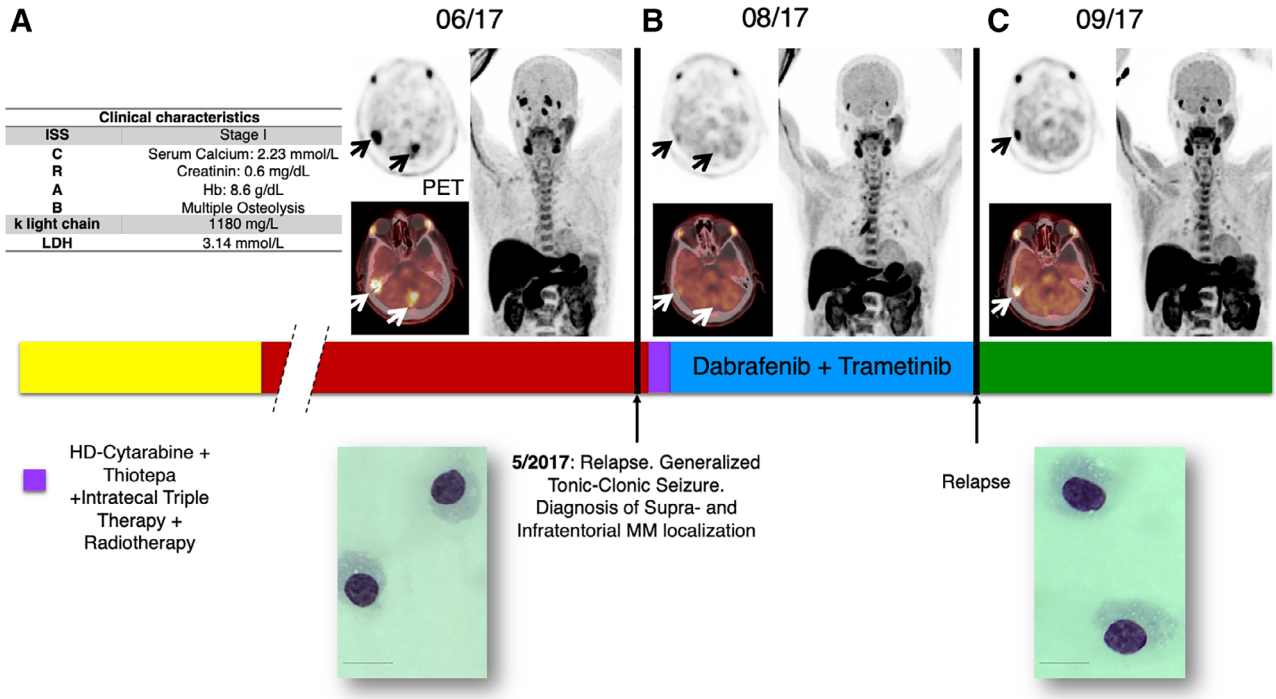


Figure 1. Patient disease history schematic view. **(A):** Clinical characteristics (left panel). ^{11}C-methionine positron emission tomography (PET)-computed tomography central nervous system showing disease manifestation at first relapse (right panel): remission **(B)**, and second brain recurrence **(C)**.
Abbreviation: MM, multiple myeloma.

based combination therapy (VMP) resulting in an initial good disease control. Magnetic resonance imaging of the brain and additional experimental whole body ^{11}C-methionine positron emission tomography (PET)-computed tomography (CT) scan, which increased MM imaging sensitivity [1], demonstrated metabolic active disease supra- and infratentorial in the clivus, as well as in the right femur as the underlying cause of the clinical scenario (Fig. 1A). A high load of clonal plasma cells (PCs) was detected in his cerebrospinal fluid (CSF), whereas in the bone marrow (BM), only a few CD138^{pos} MM cells could be detected. Thus, we diagnosed extramedullary central nervous system (CNS) relapse, and intrathecal triple therapy (methotrexate, cytarabine, and dexamethasone) along with age-adjusted systemic chemotherapy (cytarabine and thiotepa) was initiated.

Molecular Tumor Board 1

Genotyping Results and Interpretation of the Molecular Results

Given the peculiar clinical course and the poor prognosis associated with a CNS localization, with limited effective therapeutic options available, we performed a deep molecular characterization of CSF and BM tumoral plasma cells.

DNA extracted from CD138^{pos} purified cells obtained from CSF and BM paired samples was analyzed by next-generation sequencing (NGS). We applied the M^{3}P (v3.0) panel [2, 3], a disease-specific in-house customized, NGS-targeted deep sequencing panel for MM (Ion torrent platform) that includes an 88-gene selection of most commonly mutated genes such as *TP53*, *DIS3*, *FAM46C*, *CYLD*, *MAF*, *XBP1*, *MYC*, *MAX* [4, 5], actionable drug targets (i.e., *NRAS*, *KRAS*, *BRAF*) [6], and genes being associated with drug resistance [7, 8] (e.g., *CRBN*, *IKZF1/3*, *NR3C1*, *PSMB5*). The average sequencing depth increased 700×. The CSF cells harbored a clonal *BRAF*^{V600E} mutation (allele frequency variance 52%) that was absent in the BM, highlighting spatial genomic heterogeneity [9]; no other somatic point mutations were detected within the M^{3}P genes and no circulating PCs were identified by peripheral blood flow-cytometry analysis.

Functional and Clinical Significance

The *BRAF*^{V600E} mutation in exon 15 of BRAF gene is present in between 4% and 10% of patients with MM at diagnosis [4–6, 10], rising to almost 20% at relapse [7], displaying a role in the extramedullary disease and exerting a negative impact overall survival (45 vs. 105 months, p = .04) [11, 12]. Applying the M^{3}P gene panel, we sequenced 608 MM patients at different disease stages. Concerning BRAF, we have identified 59 (9.7%) mutated patients with a total of 25 distinctive mutations. Among our patient cohort, 21 of 59 (35%) harbored the *BRAF*^{V600E} mutation; within the remaining 38 patients with 24 *BRAF*$^{non\text{-}V600E}$ mutations, we found 12 alterations conferring a kinase domain activation, comprising also a rare K601 mutation, and 12 leading to BRAF functional impairment (supplemental online Fig. 1) [13]. *BRAF* exon 15 mutations confer sensitivity to target therapies such as vemurafenib, dabrafenib, and trametinib [14, 15]. Heuck et al. reported BRAF-MEK targeted therapy approach in 58 patients with MM with either *BRAF*, *NRAS*, and *KRAS* mutations or high-risk gene expression profiling; out of

Table 1. Clinically relevant single nucleotide variations

Chr	h19 position	SNV base change	Gene	SIFT	Polyphen2	Mut. taster	Effect	Amino acid	dbSNP
15	40662125	c.3812 C>T	*DISP2*	Damaging	Damaging	Damaging	Missense	p.P1271L	
16	3842027	c.1285 C>A	*CREBBP*	Damaging	Damaging	Damaging	Missense	p.V429F	
19	42796301	c.2950 G>C	*CIC*	Damaging	Damaging	Damaging	Missense	p.A984P	
X	69478534	c.941 C>T	*P2RY4*	Tolerated	Damaging	Damaging	Missense	p.R314Q	rs775064288

Abbreviations: Chr, chromosome, dbSNP, Short Genetic Variations Database; Mut. taster, Mutation taster tool; Polyphen2, Polymorphism Phenotyping v2 tool; SIFT, Sorting Intolerant From Tolerant tool; SNV, single nucleotide variant.

58 patients, 11 displayed an extramedullary localization. Ten patients received a combination therapy with trametinib, and two of them were additionally treated with dabrafenib or vemurafenib. Interestingly, within the patients with a measurable disease (40 patients), 16 patients achieved a reduction of at least 50% of the MM protein, and 9 achieved a complete remission evaluated by PET-CT. Raab et al. described a case of a patient with MM with a disseminated disease harbor a *BRAF*^{V600E} mutation. The patient was successfully treated with vemurafenib upfront and with a subsequent combination therapy with bortezomib at disease relapse owing to a clonal selection of NRAS mutants' resistant subclones. [16, 17]. These published real-life based evidences and ongoing clinical trials (i.e., BIRMA trial) combining BRAF and MEK inhibitors, highlight the clinical relevance of circumventing the paradoxical RAS pathway activation upon BRAF inhibition already described in melanoma [18, 19]. Lohr et al. tested in vitro the combination of trametinib and dabrafenib in several MM cell lines harboring distinct *BRAF* or *RAS* mutations; the U266 *BRAF*^{K601N} proved most sensitive and displayed a similar paradoxical feedback loop of RAS-activation [5].

Recently a combination of dabrafenib and trametinib effectively eliminated in *BRAF*^{V600E} mutant melanoma brain metastases, demonstrating that the drug can cross the blood brain barrier [20, 21].

Patient Update

Although neutropenic, because of the cytarabine-based chemotherapy, the patient developed a Gram-positive septicemia. Taking into account the risk profile, the therapy-related infectious episode, and the sequencing results, and according to German law and ethical approval (Einzelheilversuch), the patient started a combinational targeted therapy with continuous BRAF-MEK inhibitor (dabrafenib 150 mg twice daily and trametinib 2 mg daily). Neurological examination revealed a significant clinical improvement on the basis of the absence of pathological signs and symptoms, which was confirmed by ^{11}C-methionine PET subtotal tumor shrinkage (Fig. 1B).

Regrettably, only 3 months after the treatment initiation, ^{11}C-methionine PET revealed local MM recurrence and disseminated bone while on continuous therapy (Fig. 1C). To confirm the disease relapse, we repeated the CSF assessment, revealing, as expected, a high mononucleated tumoral plasma cells load. The patient underwent palliation with hyperfractionated radiotherapy of the cerebrum (cumulative irradiation dose: 30 Gy); because of compromised performance status of the patient, no further systemic therapy could be applied, and best supportive care was adopted until patient exitus occurred at the end of October 2017.

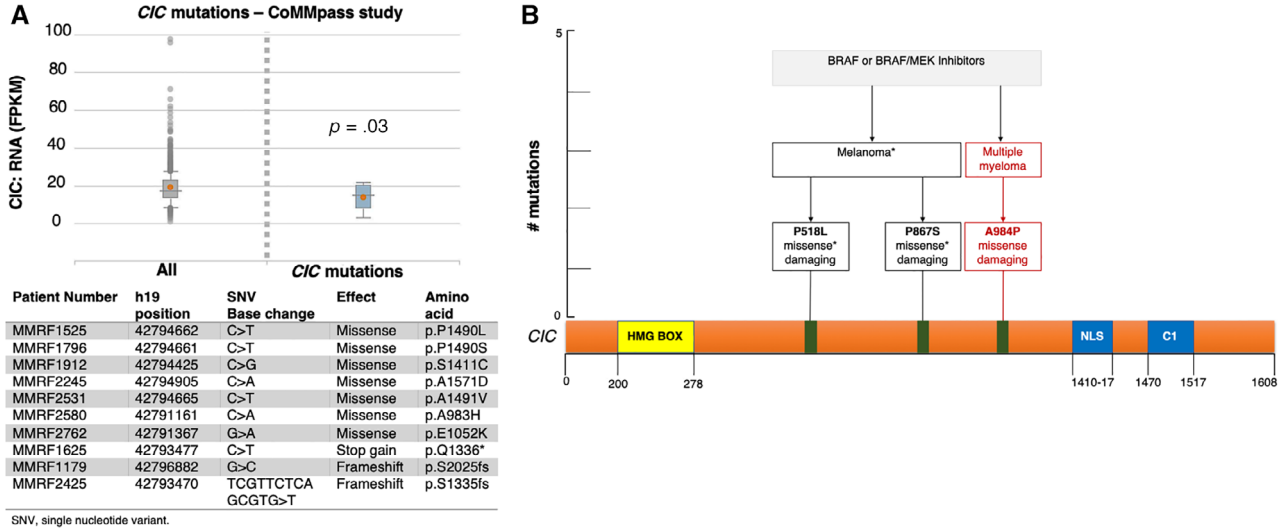
Molecular Tumor Board 2

Genotyping Results and Interpretation of the Molecular Results

To investigate the underlying mechanisms of resistance development upon targeted MEK-BRAF inhibitor therapy, we performed a whole exome DNA sequencing (Illumina platform) on the pretherapy sample and on CD138^{pos} purified MM cells obtained from the CSF after confirmed disease relapse. Sequencing depth of 115× was applied. A total number of 97 nonsilent coding variants (missense, nonsense, indels, splice) with an allele frequency higher than 5% were identified, of which 74 were shared between the timepoints. Sequencing revealed 19 additional point mutations acquired at relapse. According to published guidelines, we performed an extensive literature revision, and we systematically selected four potential clinically relevant nonsynonymous point mutations (Table 1) [22]. Dispatched RND transporter family member 2 (*DISP2*; p.P1271L) is a key regulator of the hedgehog signaling pathway [23, 24] and has been associated with the development of bortezomib resistance in MM [25]. It further impacts fibroblast growth factor receptor 3 signaling to RAS pathway, thus potentially mediating the paradoxical activation of the downstream pathway in a BRAF-independent manner [26]. CREB binding protein (*CREBBP*; p.V429F) represents an epigenetic modulator able to control the TP53 apoptosis machinery activation [27] and the downstream regulation of the RAS-RAF pathway [28]. The pyrimidinergic receptor P2Y4 (*P2RY4*, p.R314Q) is an upstream regulator of PLCβ/PI3K pathway able to cross-talk with the EGFR-RAS pathway [29], a well-known mechanism of resistance described in *BRAF*^{V600E} mutated melanoma [30]. We also identify a missense mutation in capicua transcriptional repressor (*CIC*; p.A984P) mapped on chromosome 19 with an allelic variance of 17% (Table 1). CIC represents a transcriptional repressor gene directly involved in the downstream regulation of the RAS-RAF pathway able to drive the development of BRAF-MEK inhibitor resistance [31]. Based on this correlation, we hypothesized that the acquisition of *CIC* mutation may mechanistically underlie the BRAF-MEK resistance in our patient.

Functional and Clinical Significance of CIC in Cancer

Next, we aimed to functionally validate the molecular significance of CIC alteration in mediating resistance to BRAF and MEK inhibitors. Wang et al. demonstrated in lung, colon, pancreatic [31, 32], and melanoma [31, 33] human cancer models the pivotal role of low CIC expression in inducing resistance to vemurafenib and trametinib; however, evidences of its role of resistance induction in MM or other hematological malignancies



Patient Number	h19 position	SNV Base change	Effect	Amino acid
MMRF1525	42794662	C>T	Missense	p.P1490L
MMRF1796	42794661	C>T	Missense	p.P1490S
MMRF1912	42794425	C>G	Missense	p.S1411C
MMRF2245	42794905	C>A	Missense	p.A1571D
MMRF2531	42794665	C>T	Missense	p.A1491V
MMRF2580	42791161	C>A	Missense	p.A983H
MMRF2762	42791367	G>A	Missense	p.E1052K
MMRF1625	42793477	C>T	Stop gain	p.Q1336*
MMRF1179	42796882	G>C	Frameshift	p.S2025fs
MMRF2425	42793470	TCGTTCTCA GCGTG>T	Frameshift	p.S1335fs

SNV, single nucleotide variant.

Figure 2. Capicua transcriptional repressor (CIC) is altered in multiple myeloma and is mutated after BRAF target treatment in cancer. **(A):** Report from in silico interrogation on CoMMpass study data set: comparison between RNA expression levels of CIC wild-type and mutated patients (upper panel); list of single nucleotide variations among patients enrolled in the CoMMpass study (lower panel, *t* test performed); **(B):** *CIC* somatic mutations acquired in melanoma and central nervous system multiple myeloma after BRAF inhibition therapy. Abbreviations: *, stop codon; FPKM, fragments per kilobase of exon model per million reads mapped.

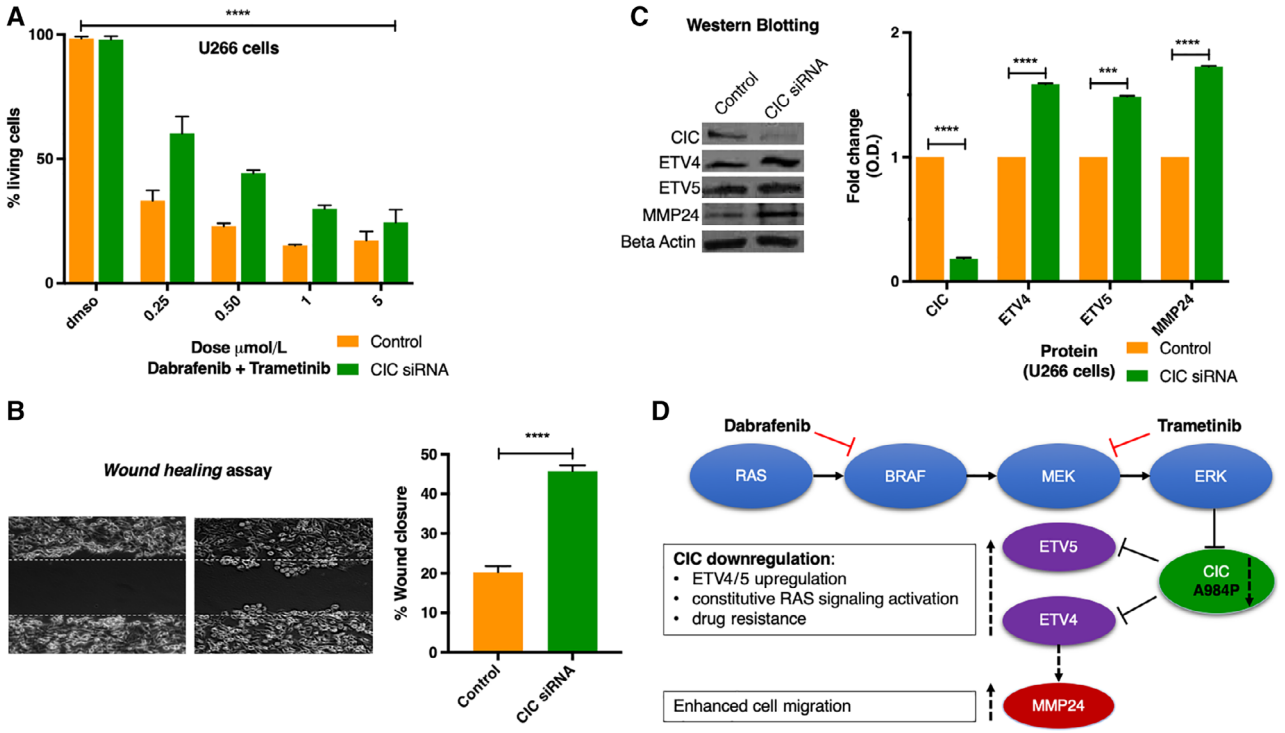


Figure 3. Functional validation of capicua transcriptional repressor (CIC) downregulation biological effect in multiple myeloma. **(A):** Cell viability assay measured with bioluminescence upon drug treatment with escalating doses of trametinib and dabrafenib in U266 human multiple myeloma cell line: scrambled versus CIC small interfering RNA (siRNA) transduced percentage of living cells are compared by ANOVA test. Experiments were conducted in three biological and technical replicates following manufacturer's instructions (CellTiter-Glo Luminescent Cell Viability Assay; Promega, Madison, WI). **(B):** Scratch-wound healing assay was performed as previously described [44, 45]. Briefly, wound areas were analyzed with ImageJ Lab 1.51 software and quantified as percentage of total surface. **(C):** Western-Blot analysis after CIC knockdown on U266 cells and corresponding densitometric quantification. **(D):** Dabrafenib and trametinib targets on RAS-RAF pathway overview and CIC axis schematic interaction with the RAS-RAF downstream signaling. ***$p < .001$; ****$p < .0001$.

are lacking. Le Blanc et al. reported that CIC missense mutations result in a gene expression downregulation [34]. Interrogating the Multiple Myeloma Research Foundation CoMMpass study we found a similar correlation: the 10 patients that harbored a *CIC* mutation (Fig. 2A) had a significant gene downregulation compared with the unmutated ones ($p = .03$). Mutations in the proline-rich (Fig. 2B) region are reported to impair the protein expression [35]. Therefore, we established a CIC knockdown

in vitro model, using a small interfering RNA as specific gene silencing technique. We employed the U266 MM cell line harboring an activating $BRAF^{K601N}$ mutation, usually sensitive to BRAF-MEK inhibition [5], as a commercially available MM model harboring a BRAF activating mutation. Of note, upon CIC gene silencing, we observed drug resistance induction to BRAF-MEK inhibition (Fig. 3A); in detail, we cultured the silenced and not-silenced MM cells with trametinib and dabrafenib, either as single agents or in combination, and we observed resistance induction to the combination of the two drugs (row factor, 91.16%; $p < .0001$, two-way ANOVA test). Next, we investigated whether this drug-resistance phenotype also coincided with a more invasive behavior. Thus, we performed a motility and migration assay. CIC knockdown in U266 $BRAF^{K601N}$ cells significantly enhanced MM migration in a scratch wound healing assay (Fig. 3B). These findings prompted us to investigate potential mechanism able to explain the CIC-impairment-related biological effects. In particular, CIC is the direct master regulator of several transcription factors such as ETV4 and ETV5 two oncogenes able to modulate the RAS downstream pathway [31–33]. Moreover, indirectly CIC induces an invasiveness related protein namely MMP24 [32, 33]. Consequently, we confirmed by Western blotting an upregulation of ETV4, ETV5, and MMP24 protein expression in the CIC-knockdown U266, as mediators of drug resistance and MM invasiveness (Fig. 3C). The upregulation of these transcription factors can activate the MAPK signaling, in an independent p-MEK manner [31], providing an escape mechanism from BRAF-MEK inhibition (Fig. 3D) [36].

Potential Strategies to Target the Pathway and Implications for Clinical Practice

CIC has recently been identified as a candidate gene related to MEK-BRAF resistance development [31, 33]. Our clinical observations, the subsequent in vitro MM model, and the public datasets interrogations support that the acquisition of *CIC* mutation and its subsequent downregulation confers MEK-BRAF inhibitors resistance for the first time in MM.

As large BRAF-RAS treated cohorts in MM are not available, we screened for published datasets to answer the question of whether mutation acquisition in CIC under BRAF-RAS targeted therapy can be observed in a significant number of patients being resistant to BRAF inhibitors. Remarkably, Van Allen et al. recently published a comprehensive genomic characterization of 45 patients with metastatic melanoma resistant to BRAF inhibitors; 5 of them (11%) harbored a somatic *CIC* mutation (4 missense and 1 frame shift). Intriguingly, two of the single nucleotide variations out of these five were acquired at time of relapse (Fig. 2B). One out of these five patients harboring a pretherapy *CIC* mutation experienced a very early disease relapse under dabrafenib therapy [37]. Given that almost 30% of patients with MM harbor mutations affecting the BRAF-RAS pathway, this may represent a potential biomarker to predict therapy response. Based on prior published findings [31, 33, 34], public available datasets [37] and previous [31–34] and original in vitro validations pinpoint *CIC* mutation as one of the mechanisms of drug resistance of BRAF-MEK inhibition therapy. Extramedullary (EMD) dissemination in MM typically correlates with very poor prognosis, especially when the clinical onset manifests at disease relapse [12, 38–42]. Furthermore, given the scanty evidences available about the disease biology behind the EMD [43], CIC oncosuppressive functions might be also expressed as ancillary mechanism that sustain the EMD phenotype in MM [39].

Prospective clinical trials including the BRAF and MEK inhibition are ongoing in multiple myeloma in Europe (NCT02834364) and in the U.S. (NCT03091257) as well as in different solid cancers such as melanoma and colon cancer (NCT02974803, NCT03668431); these ongoing studies represent the ideal opportunity to determine and validate the role of *CIC* mutations as potential disease biomarker in a large clinical and controlled-prospective setting.

Glossary of Genomic Terms and Nomenclature

Allelic frequency: percentage of reads referred to the mutated allele
Average sequencing depth: mean number of unique reads for each single nucleotide aligned to a reference sequence
Spatial genomic heterogeneity: presence of distinctive genomic alterations in different anatomical sites
BRAF: B-Raf Proto-Oncogene, Serine/Threonine Kinase
MEK: Mitogen-Activated Protein Kinase Kinase 1
CIC: Capicua Transcriptional Repressor
DISP2: Dispatched RND Transporter Family Member 2
CREBBP: CREB Binding Protein
P2RY4: Pyrimidinergic receptor P2Y4
ETV4: ETS Variant 4
ETV5: ETS Variant 5
MMP24: Matrix Metallopeptidase 24
TP53: Tumor Protein P53
DIS3: DIS3 Homolog, Exosome Endoribonuclease And 3′-5′ Exoribonuclease
FAM46C: Terminal Nucleotidyltransferase 5C
CYLD: CYLD Lysine 63 Deubiquitinase
MAF: MAF BZIP Transcription Factor
XBP1: X-Box Binding Protein 1
MYC: MYC Proto-Oncogene, BHLH Transcription Factor
MAX: MYC Associated Factor X
KRAS: Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
NRAS: Neuroblastoma Ras viral oncogene homolog
CRBN: Cereblon
IKZF1: IKAROS Family Zinc Finger 1
IKZF3: IKAROS Family Zinc Finger 3
NR3C1: Nuclear Receptor Subfamily 3 Group C Member 1
PSMB5: Proteasome Subunit Beta 5
PLCβ/PI3K: Phospholipase C beta/Phosphatidil-Inositol-3-Kinase pathway
EGFR: Epidermal grow factor receptor

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Disclosures

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See http://www.TheOncologist.com for supplemental material available online.

For Further Reading:
Winnie S. Liang, Jo-Anne Vergilio, Bodour Salhia et al. Comprehensive Genomic Profiling of Hodgkin Lymphoma Reveals Recurrently Mutated Genes and Increased Mutation Burden. ***The Oncologist*** 2018;24:219–228; first published August 14, 2018.

Implications for Practice:
This study provides the first evidence that comprehensive genomic profiling can be performed to map the genomic landscape of Hodgkin lymphoma and that a subpopulation of patients has mutations in *TP53, B2M, XPO1,* and other genes. It was found that 15% of patients have high mutation burden, which, in cancers such as melanoma, may indicate sensitivity to immune checkpoint inhibitors, and may thus be explored for Hodgkin lymphoma. Lastly, this work demonstrates that changes in the mutant allele frequency of *XPO1* in serially collected plasma cell-free DNA samples correspond with treatment outcomes measured with conventional radiographic imaging.