











# 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*<sup>V600E</sup> 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.

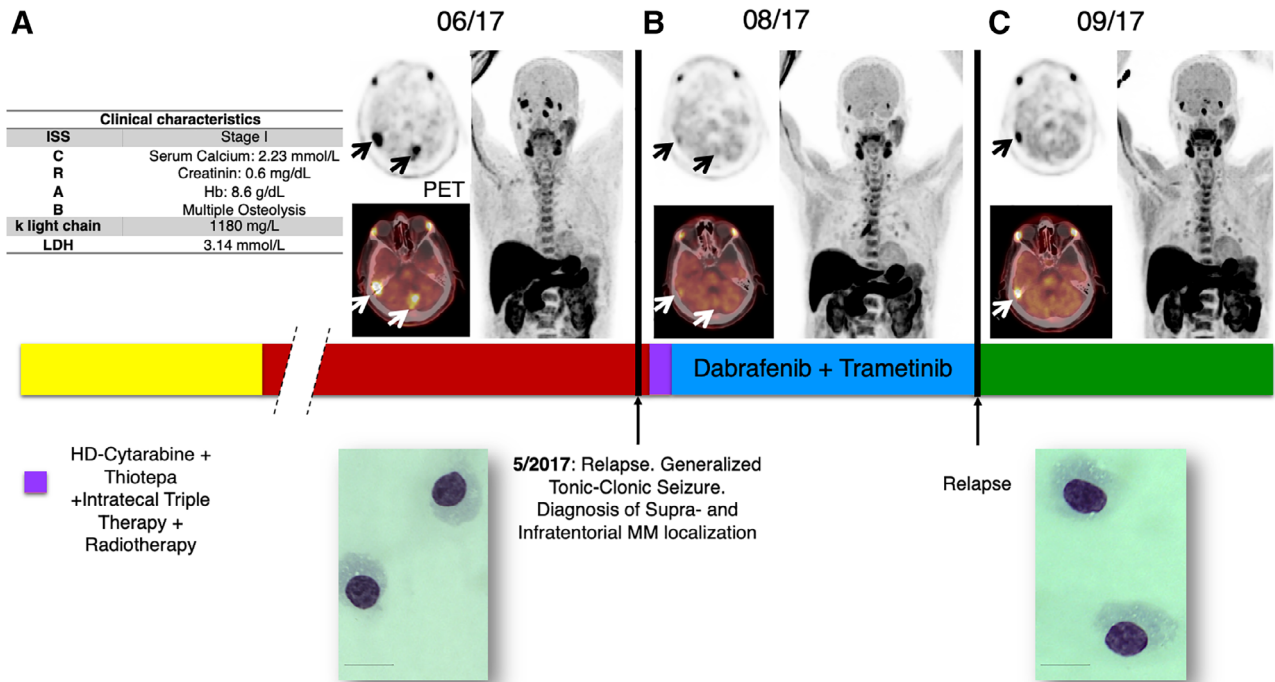
## PATIENT HISTORY

An 81-year-old patient with  $\kappa$  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).  $^{11}\text{C}$ -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  $^{11}\text{C}$ -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  $\text{CD}138^{\text{pos}}$  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  $\text{CD}138^{\text{pos}}$  purified cells obtained from CSF and BM paired samples was analyzed by next-generation sequencing (NGS). We applied the  $\text{M}^3\text{P}$  (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 700x. The CSF cells harbored a clonal *BRAF*<sup>V600E</sup> 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  $\text{M}^3\text{P}$  genes and no circulating PCs were identified by peripheral blood flow-cytometry analysis.

### Functional and Clinical Significance

The *BRAF*<sup>V600E</sup> 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  $\text{M}^3\text{P}$  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*<sup>V600E</sup> mutation; within the remaining 38 patients with 24 *BRAF*<sup>non-V600E</sup> 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	<i>DISP2</i>	Damaging	Damaging	Damaging	Missense	p.P1271L	
16	3842027	c.1285 C>A	<i>CREBBP</i>	Damaging	Damaging	Damaging	Missense	p.V429F	
19	42796301	c.2950 G>C	<i>CIC</i>	Damaging	Damaging	Damaging	Missense	p.A984P	
X	69478534	c.941 C>T	<i>P2RY4</i>	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*<sup>V600E</sup> 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*<sup>K601N</sup> 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*<sup>V600E</sup> 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 <sup>11</sup>C-methionine PET subtotal tumor shrinkage (Fig. 1B).

Regrettably, only 3 months after the treatment initiation, <sup>11</sup>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.

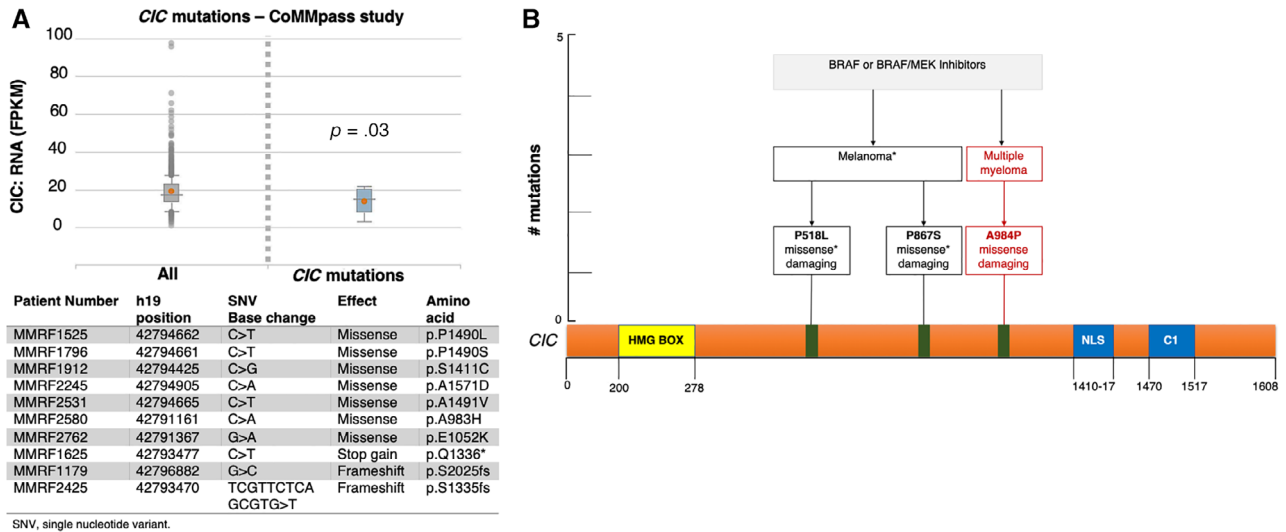
## MOLECULAR TUMOR BOARD 2

### Genotyping Results and Interpretation of the Molecular Results

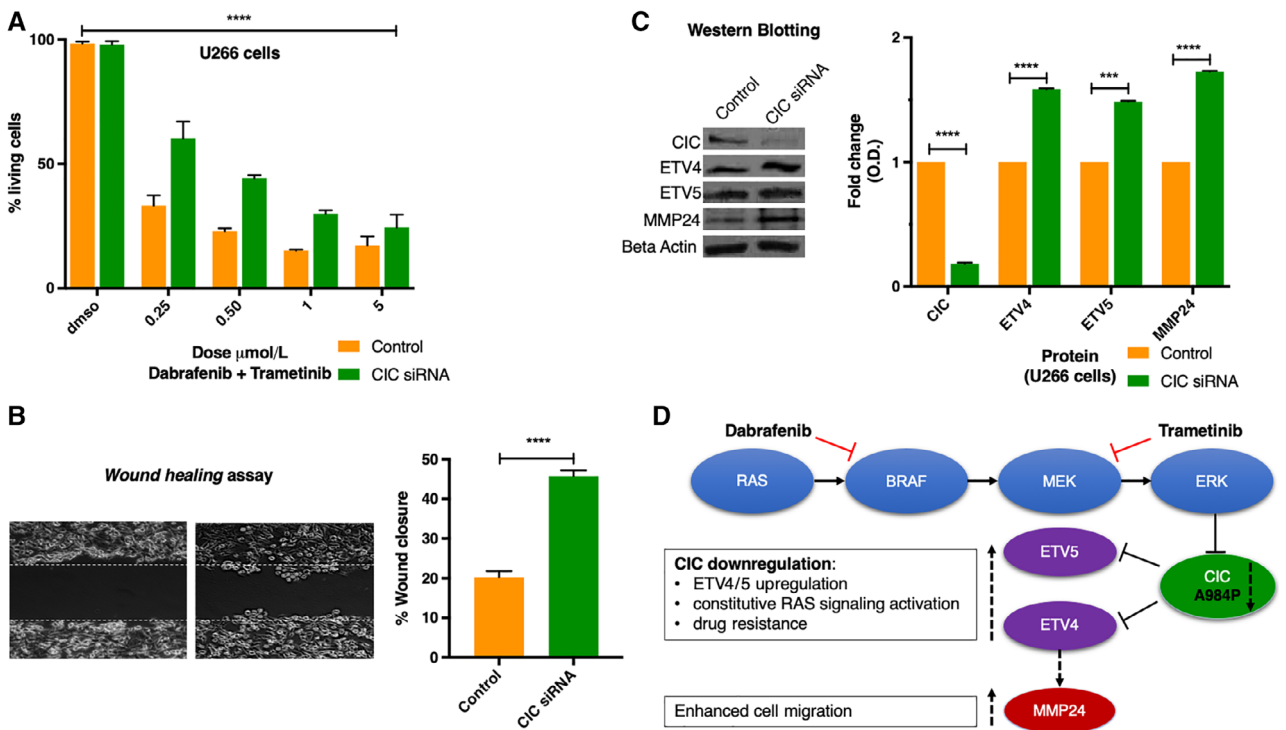
To investigate the underlying mechanisms of resistance development upon targeted MEK-BRAF inhibitor therapy, we performed a whole exome DNA sequencing (Illumina platform) on the pretherapy sample and on CD138<sup>pos</sup> purified MM cells obtained from the CSF after confirmed disease relapse. Sequencing depth of 115x 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 non-synonymous 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*<sup>V600E</sup> 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



**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*<sup>K601N</sup> 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*<sup>K601N</sup> 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* onco-suppressive 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 Metalloproteinase 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:** Cereblin  
**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/Phosphatidylinositol-3-Kinase pathway  
**EGFR:** Epidermal growth factor receptor

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[Correction added on October 23, 2020, after first online publication: Projekt Deal funding statement has been added.]

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**Final approval of manuscript:** Matteo Claudio Da Vià, Antonio Giovanni Solimando, Andoni Garitano-Trojaola, Santiago Barrio, Umair Munawar, Susanne Striffler, Larissa Haertle, Nadine Rhodes, Eva Teufel, Cornelia Vogt, Constantin Lapa, Andreas Beilhack, Leo Rasche, Hermann Einsele, K. Martin Kortüm

## DISCLOSURES

**Hermann Einsele:** Bristol-Myers Squibb, Novartis, Takeda (C/A), Celgene, Janssen (RF, SAB), Celgene, Janssen, Bristol-Myers Squibb, Novartis, Takeda (H). The other authors indicated no financial relationships.

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## REFERENCES

- Lapa C, Garcia-Velloso MJ, Lücknerath K et al. (11)C-Methionine-PET in multiple myeloma: A combined study from two different institutions. *Theranostics* 2017;7:2956–2964.
- Kortüm KM, Langer C, Monge J et al. Longitudinal analysis of 25 sequential sample-pairs using a custom multiple myeloma mutation sequencing panel (M(3)P). *Ann Hematol* 2015;94:1205–1211.
- Barrio S, Dávila M, Bruins L et al. Protocol for M<sup>3</sup>P: A comprehensive and clinical oriented targeted sequencing panel for routine molecular analysis in multiple myeloma. *Methods Mol Biol* 2018;1792:117–128.
- Bolli N, Avet-Loiseau H, Wedge DC et al. Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. *Nat Commun* 2014;5:2997.
- Lohr JG, Stojanov P, Carter SL et al. Widespread genetic heterogeneity in multiple myeloma: Implications for targeted therapy. *Cancer Cell* 2014;25:91–101.
- Walker BA, Boyle EM, Wardell CP et al. Mutational spectrum, copy number changes, and outcome: Results of a sequencing study of patients with newly diagnosed myeloma. *J Clin Oncol* 2015;33:3911–3920.
- Kortüm KM, Mai EK, Hanafiah NH et al. Targeted sequencing of refractory myeloma reveals a high incidence of mutations in CRBN and Ras pathway genes. *Blood* 2016;128:1226–1233.
- Barrio S, Stühmer T, Da-Vià M et al. Spectrum and functional validation of PSMB5 mutations in multiple myeloma. *Leukemia* 2018;33:447–456.
- Rasche L, Chavan SS, Stephens OW et al. Spatial genomic heterogeneity in multiple myeloma revealed by multi-region sequencing. *Nat Commun* 2017;8:268.
- Ruiz-Heredia Y, Sánchez-Vega B, Onecha E et al. Mutational screening of newly diagnosed multiple myeloma patients by deep targeted sequencing. *Haematologica* 2018;103:e544–e548.
- Andrulis M, Lehnert N, Capper D et al. Targeting the BRAF V600E mutation in multiple myeloma. *Cancer Discov* 2013;3:862–869.
- Varettoni M, Corso A, Pica G et al. Incidence, presenting features and outcome of extramedullary disease in multiple myeloma: A longitudinal study on 1003 consecutive patients. *Ann Oncol* 2010;21:325–330.
- Kortüm KM, Braggio E, Bruins L et al. Panel sequencing for clinically oriented variant screening and copy number detection in 142 untreated multiple myeloma patients. *Blood Cancer J* 2016;6:e397.
- Kim KB, Kefford R, Pavlick AC et al. Phase II study of the MEK1/MEK2 inhibitor Trametinib in patients with metastatic BRAF-mutant cutaneous melanoma previously treated with or without a BRAF inhibitor. *J Clin Oncol* 2013;31:482–489.
- Dahlman KB, Xia J, Hutchinson K et al. BRAF (L597) mutations in melanoma are associated with sensitivity to MEK inhibitors. *Cancer Discov* 2012;2:791–797.
- Heuck CJ, Jethava Y, Khan R et al. Inhibiting MEK in MAPK pathway-activated myeloma. *Leukemia* 2016;30:976–980.
- Raab MS, Lehnert N, Xu J et al. Spatially divergent clonal evolution in multiple myeloma: Overcoming resistance to BRAF inhibition. *Blood* 2016;127:2155–2157.
- Flaherty KT, Robert C, Hersey P et al. Improved survival with MEK inhibition in BRAF-mutated melanoma. *N Engl J Med* 2012;367:107–114.
- Poulidakos PI, Zhang C, Bollag G et al. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature* 2010;464:427–430.
- Davies MA, Saiag P, Robert C et al. Dabrafenib plus trametinib in patients with BRAF(V600)-mutant melanoma brain metastases (COMBI-MB): A multi-centre, multicohort, open-label, phase 2 trial. *Lancet Oncol* 2017;18:863–873.
- Puzkiel A, Noé G, Bellesoeur A et al. Clinical pharmacokinetics and pharmacodynamics of dabrafenib. *Clin Pharmacokinet* 2019;58:451–467.
- Services NUSDoHH. PubMed Tutorial. 2018. Available at <https://www.nlm.nih.gov/bsd/disted/pubmedtutorial/cover.html>.
- Shou Y, Robinson DM, Amakye DD et al. A five-gene hedgehog signature developed as a patient preselection tool for hedgehog inhibitor therapy in medulloblastoma. *Clin Cancer Res* 2015;21:585–593.
- Martello M, Remondini D, Borsi E et al. Opposite activation of the Hedgehog pathway in CD138+ plasma cells and CD138-CD19+ B cells identifies two subgroups of patients with multiple myeloma and different prognosis. *Leukemia* 2016;30:1869–1876.
- Alonso S, Hernandez D, Chang YT et al. Hedgehog and retinoid signaling alters multiple myeloma microenvironment and generates bortezomib resistance. *J Clin Invest* 2016;126:4460–4468.
- Katoh M, Nakagama H. FGF receptors: Cancer biology and therapeutics. *Med Res Rev* 2014;34:280–300.
- Lee CW, Ferreone JC, Ferreone AC et al. Graded enhancement of p53 binding to CREB-binding protein (CBP) by multisite phosphorylation. *Proc Natl Acad Sci USA* 2010;107:19290–19295.
- Dixon ZA, Nicholson L, Zeppetbauer M et al. CREBBP knockdown enhances RAS/RAF/MEK/ERK signaling in Ras pathway mutated acute lymphoblastic leukemia but does not modulate chemotherapeutic response. *Haematologica* 2017;102:736–745.
- Erb L, Weisman GA. Coupling of P2Y receptors to G proteins and other signaling pathways. *Wiley Interdiscip Rev Membr Transp Signal* 2012;1:789–803.
- Sun C, Wang L, Huang S et al. Reversible and adaptive resistance to BRAF(V600E) inhibition in melanoma. *Nature* 2014;508:118–122.
- Wang B, Krall EB, Aguirre AJ et al. ATXN1L, CIC, and ETS transcription factors modulate sensitivity to MAPK pathway inhibition. *Cell Rep* 2017;18:1543–1557.
- Okimoto RA, Breitenbuecher F, Olivas VR et al. Inactivation of Capicua drives cancer metastasis. *Nat Genet* 2017;49:87–96.
- Dissanayake K, Toth R, Blakey J et al. ERK/p90(RSK)/14-3-3 signalling has an impact on expression of PEA3 Ets transcription factors via the transcriptional repressor capicua. *Biochem J* 2011;433:515–525.
- LeBlanc VG, Firme M, Song J et al. Comparative transcriptome analysis of isogenic cell line models and primary cancers links capicua (CIC) loss to activation of the MAPK signalling cascade. *J Pathol* 2017;242:206–220.
- Lu HC, Tan Q, Rousseaux MW et al. Disruption of the ATXN1-CIC complex causes a spectrum of neurobehavioral phenotypes in mice and humans. *Nat Genet* 2017;49:527–536.
- Xu J, Pfarr N, Endris V et al. Molecular signaling in multiple myeloma: Association of RAS/RAF mutations and MEK/ERK pathway activation. *Oncogenesis* 2017;6:e337.
- Van Allen EM, Wagle N, Sucker A et al. The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma. *Cancer Discov* 2014;4:94–109.
- Bladé J, Fernández de Larrea C, Rosiñol L et al. Soft-tissue plasmacytomas in multiple myeloma: Incidence, mechanisms of extramedullary spread, and treatment approach. *J Clin Oncol* 2011;29:3805–3812.
- Ghobrial IM. Myeloma as a model for the process of metastasis: Implications for therapy. *Blood* 2012;120:20–30.

40. Rasche L, Bernard C, Topp MS et al. Features of extramedullary myeloma relapse: High proliferation, minimal marrow involvement, adverse cytogenetics: A retrospective single-center study of 24 cases. *Ann Hematol* 2012;91:1031–1037.

41. Varettoni M, Marchioni E, Bonfichi M et al. Successful treatment with Rituximab and Bendamustine in a patient with newly diagnosed Waldenstrom's Macroglobulinemia complicated by Bing-Neel syndrome. *Am J Hematol* 2015;90:E152–153.

42. Jurczyszyn A, Grzasko N, Gozzetti A et al. Central nervous system involvement by multiple myeloma: A multi-institutional retrospective study of 172 patients in daily clinical practice. *Am J Hematol* 2016;91:575–580.

43. Egan JB, Kortuem KM, Kurdoglu A et al. Extramedullary myeloma whole genome sequencing reveals novel mutations in Cereblon, proteasome subunit G2 and the glucocorticoid receptor in multi drug resistant disease. *Br J Haematol* 2013;161:748–751.

44. Solimando AG, Brandl A, Mattenheimer K et al. JAM-A as a prognostic factor and new therapeutic target in multiple myeloma. *Leukemia* 2018;32:736–743.

45. Rao L, De Veirman K, Giannico D et al. Targeting angiogenesis in multiple myeloma by the VEGF and HGF blocking DARPIn<sup>®</sup> protein MPO250: A preclinical study. *Oncotarget* 2018;9:13366–13381.



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.