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RESEARCH REPORT

Successful BRAF/MEK inhibition in a patient with *BRAF*^{V600E}-mutated extrapancreatic acinar cell carcinoma

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Abstract Pancreatic acinar cell carcinoma (PAC) is a rare disease with a poor prognosis. Treatment options for metastatic PAC are limited and often follow chemotherapeutic regimens for pancreatic ductal adenocarcinoma. Although recurrent genomic alterations, such as *BRAF* fusions and defects in genes involved in homologous recombination DNA repair, have been described in PAC, data on the clinical efficacy of molecularly guided, targeted treatment are scarce. Here we describe the case of a 27-yr-old patient with *BRAF*^{V600E}-mutated PAC who was successfully treated with a combination of BRAF and MEK inhibitors. The patient presented to our clinic with abdominal pain and weight loss. Imaging showed extensive retroperitoneal disease as well as mediastinal lymphadenopathy. Because of elevated α-fetoprotein (AFP) levels and inconclusive histologic findings, a germ cell tumor was suspected; however, PEI chemotherapy was unsuccessful. A repeat biopsy yielded the diagnosis of PAC and treatment with FOLFIRINOX was initiated. Comprehensive molecular profiling within the MASTER (Molecularly Aided Stratification for Tumor Eradication Research) precision oncology program revealed a somatic *BRAF*^{V600E} mutation and a germline *PALB2* stop-gain mutation. Therapy was therefore switched to BRAF/MEK inhibition, resulting in almost complete remission and disease control for 12 mo and a remarkable improvement in the patient's general condition. These results indicate that *BRAF* alterations are a valid therapeutic target in PAC that should be routinely assessed in this patient population.

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INTRODUCTION

Pancreatic acinar cell carcinomas (PACs) constitute a rare but distinct and aggressive group of neoplasms that differ from pancreatic ductal adenocarcinomas (PDACs) and neuroendocrine tumors. They differentiate similar to pancreatic acinar cells and, hence, display abundant eosinophilic cytoplasm and enlarged irregular nuclei with prominent nucleoli and positive immunostaining for the enzymes trypsin, chymotrypsin, and lipase (Stelow et al. 2010). Clinical symptoms upon diagnosis are usually nonspecific; however, up to 16% of patients describe systemic manifestations due to liberation of lipase, such as panniculitis and polyarthralgia (Klimstra et al. 1992). PACs most commonly affect adults in the sixth and seventh decade of life, although a wide age span has been described (Schmidt et al. 2008; Wisnoski et al. 2008). Similarly to other pancreatic neoplasms, PACs are often diagnosed at advanced disease stages with metastases present in ~50% of patients. Rarely, PAC-type neoplasms may originate outside the boundaries of the pancreas including in the retroperitoneum, liver, and the gastrointestinal tract. Distinguishing this uncommon presentation from metastatic PAC is mandatory for appropriate therapy and prognostification (Agaimy et al. 2011). Although the clinical course tends to be more favorable in comparison to PDACs, unresectable or metastatic disease is still associated with a poor prognosis and overall survival ranges between 18 and 47 mo (Al-Hader et al. 2017). Despite recent work on the molecular pathogenesis, these tumors are still poorly understood. Comparative genomic hybridization analysis of 57 PAC samples demonstrated considerable chromosomal instability —that is, recurrent losses of Chromosome 1p, 3p, 4q, 5q, 6q, 8p, 9p, 11q, 13q, 16q, and 18 as well as gains of 1q, 7, 8q, 12, 17q, and 20q (Hoorens et al. 1993; Bergmann et al. 2014). Immunohistochemistry revealed DCC reduction or loss, *MYC* amplification, and increased epidermal growth factor receptor (EGFR) expression in major subgroups of 57 tumor samples investigated (Bergmann et al. 2014). Sequencing analyses identified mutations in *TP53, ARID1A, BRAF, SMAD4, BRCA1, BRCA2, CDKN2A, CTNNB1, RB1, MEN1, MYC, JAK1, APC, GNAS*, and *FAT* (Furlan et al. 2014; Jiao et al. 2014; Al-Hader et al. 2017; Jakel et al. 2017; La Rosa et al. 2018) as well as enrichment of mutational signatures linked to tobacco exposition or defective DNA repair mechanisms in some cases (Jakel et al. 2017). Mismatch repair deficiency has been reported in up to 14% of cases (Al-Hader et al. 2017). No randomized controlled trials are available for chemotherapeutic and or radiotherapeutic treatment for locally advanced or metastatic PACs. Across different regimens, an overall response rate of 23% and a median progression-free survival (PFS) of 5.6 mo were reported (Al-Hader et al. 2017); however, platinum-based regimens appear to be more effective because of inherent defects in DNA repair enzymes (BRCA1, BRCA2, ATM, PALB2) (Al-Hader et al. 2017; Yoo et al. 2017). Because of the rarity of the disease, no trials investigating targeted therapy options for the aforementioned mutations are currently underway.

We present the case of a young man with extrapancreatic acinar cell carcinoma with retroperitoneal lymphadenopathy and high α-fetoprotein (AFP) serum levels, leading to initial misdiagnosis as metastatic germ cell tumor, who reached almost complete remission upon targeted therapy with BRAF/MEK inhibition based on a $BRAF^{V600E}$ driver mutation.

RESULTS

A 27-yr-old male patient presented to our outpatient department with abdominal pain, weight loss, nausea, and fatigue. Imaging workup showed massive retroperitoneal bulky disease and mediastinal lymphadenopathy. Tumor marker analysis revealed an elevated AFP (1358.9 IU/mL), whereas others, including beta human chorionic gonadotropin (β-HCG), soluble interleukin-2 receptor (sCD25), lactic acid dehydrogenase (LDH), CA19-9, and

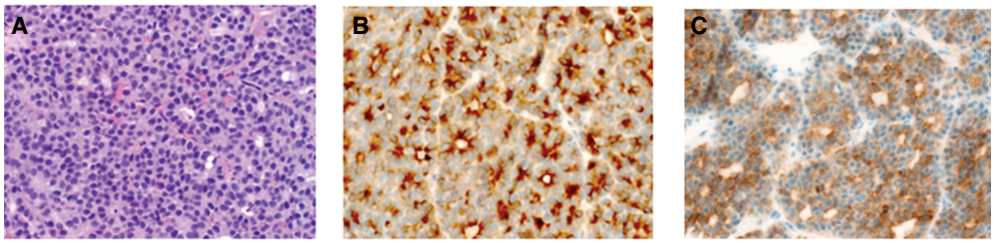

Figure 1. (*A*) Hematoxylin and eosin (H&E) staining and immunohistochemical stainings of (*B*) trypsin and (*C*) α-fetoprotein (AFP), confirming AFP-producing acinar cell carcinoma of pancreatic-type (magnification, 400×).

carcinoembryonic antigen (CEA), were within normal range. Initial core needle biopsy was inconclusive; hence, with respect to the high tumor burden, chemotherapy with cisplatin, etoposide, and ifosfamid (PEI) under the assumption of metastatic germ cell tumor was initiated. Short-term imaging follow-up and AFP levels showed progressive disease. Repeated biopsy findings (Fig. 1) were consistent with PAC, presumably of peripancreatic origin. Treatment with 5-fluoruracil, oxaliplatin, and irinotecan (FOLFIRINOX) was initiated in analogy to PDAC, leading to a decline in AFP levels after two cycles (1038 IU/mL) and stable disease on magnetic resonance imaging (MRI) scans. Because of adverse events (nausea [Common Terminology Criteria for Adverse Events (CTCAE) 3°], loss of appetite [CTCAE 2°], and weight loss [CTCAE 3°]), chemotherapy was deescalated to the FOLFIRI regimen for eight cycles, yielding formally stable disease with an AFP of 760 IU/mL.

To identify additional treatment options, the patient was enrolled in the MASTER precision oncology program of NCT Heidelberg and the German Cancer Consortium (Horak et al. 2017). Whole-genome and transcriptome sequencing revealed a low mutational burden (14 nonsynonymous single-nucleotide variants [SNVs] and two insertions/deletions [indels] within coding regions) and no signs of microsatellite instability (MSIsensor score of 1.65), which was in line with immunohistochemical assessment. The genome-wide copy-number assessment revealed a number of chromosomal and subchromosomal gains and losses (see Fig. 2). Further analysis identified an activating mutation (NM_004333.4, rs113488022, c.T1799A, p.V600E, allele frequency 30%) in *BRAF* exon 15 and a germline heterozygous *PALB2* stop-gain mutation (NM_024675, rs180177100, c.C1240T, p.R414X, allele frequency 47%) (Table 1), which is considered pathogenic according to the current American College of Medical Genetics (ACMG) criteria (Richards et al. 2015) and which has previously been linked to familial pancreatic and breast cancer (Slater et al. 2010).

The patient's family history revealed a case of breast cancer in a first-degree relative but was otherwise unsuspicious. The patient was therefore recommended to receive genetic counseling. Functional indicators of defective homologous recombination DNA repair were inconclusive with very low genomic rearrangement scores (homologous repair deficiency [HRD], 1; large scale transition [LST], 0; telomeric allelic imbalance [TAI], 3) and mutational signature 3 supported by 23% of all somatic SNVs (Alexandrov et al. 2013).

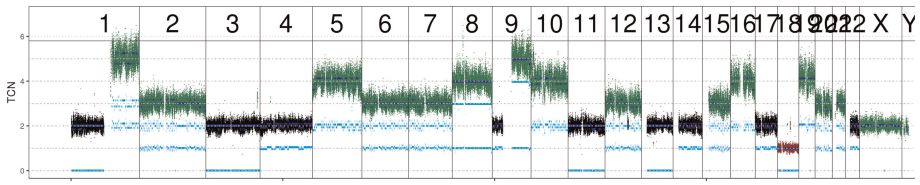

Figure 2. DNA copy number plot displaying chromosomal gains and losses in the tumor sample. Dark and light blue lines show total and allele-specific copy numbers, respectively. (TCN) Total copy number.

Table 1. Variant table

Gene	Chromosome	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect (substitution, deletion, etc.)	dbSNP/ dbVar ID	Genotype (heterozygous/ homozygous)	ClinVar ID	Parent of origin
BRAF	7	NM_004333: c.T1799A	NP_004324: p.V600E	SNV	Substitution	rs113488022	Heterozygous (somatic)	VCV000013961	—
PALB2	16	NM_024675: c.C1240T	NP_078951: p.R414X	Stop-gain-SNV	Substitution	rs180177100	Heterozygous (germline)	VCV000128117	Unknown

(HGVS) Human Genome Variation Society, (SNV) single-nucleotide variant.

Based on the genetic findings and in accordance with the recommendation by the molecular tumor board of NCT Heidelberg, combined BRAF and MEK inhibition with dabrafenib and trametinib was initiated. Follow-up imaging at 3 mo demonstrated almost complete remission with only residual abdominal lymph nodes and lung nodules (Fig. 3). Only pyrexia CTCAE 1° was observed as side effect. AFP levels had dropped to normal range. At the patient's 6-mo follow-up, the remaining lymph nodes further decreased in size and the initial lung lesions were no longer detectable. Hence, he underwent systematic abdominal lymphadenectomy with pancreatectomy and splenectomy with the aim of resecting all macroscopic tumor lesions. Histopathological workup revealed vital tumor within the head of the pancreas, surrounded by a dense chronic inflammatory wall, as well as vital tumor in

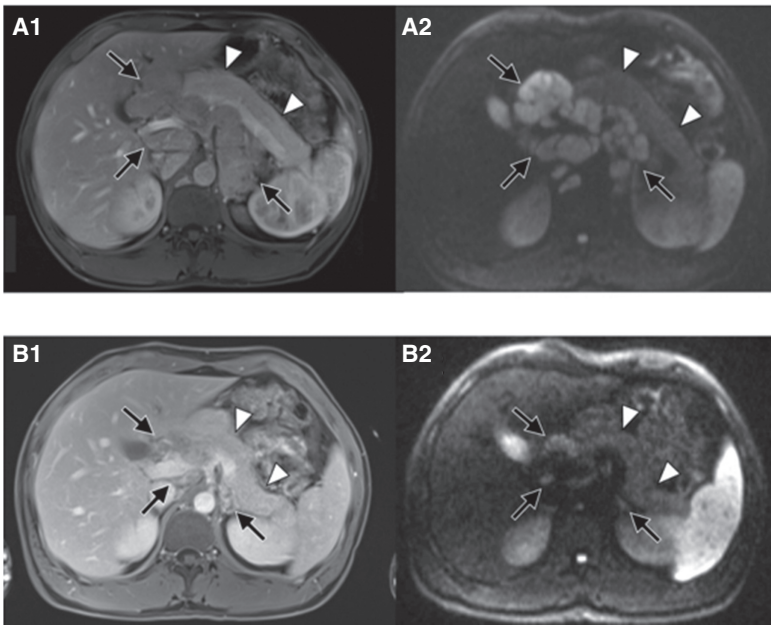

Figure 3. Almost complete remission at 3-mo follow-up after initiation of trametinib/dabrafenib. Baseline imaging: axial early venous phase MR image (A1) and axial diffusion weighted MR image with b-value of 800 sec/mm^2 (A2) show extensive lymphadenopathy in the retroperitoneum and liver hilum (black arrows). There is no lesion in the pancreas (white arrowheads). Follow-up imaging: Axial early venous phase MR image (B1) and axial diffusion weighted MR image with b-value of 800 sec/mm^2 (B2) show marked reduction of lymphadenopathy (black arrows). Still there is no lesion in the pancreas (white arrowheads).

37 of 62 resected lymph nodes. Postoperatively, treatment with dabrafenib/trametinib had to be halted because of dysphagy and malabsorption, leading to a 2-mo treatment break. AFP levels showed a marked increase after discontinuation of treatment (249 IU/mL). Follow-up imaging unfortunately revealed progressive disease within the abdominal lymph nodes. After reinitiation of targeted therapy, the patient reported good general health; however, follow-up imaging showed progressive disease with increasing lymph nodes in both the abdominal and mediastinal compartments. BRAF/MEK inhibition was continued beyond progression because it was well-tolerated, and the young patient refused systemic chemotherapy because of the experienced side effects. After 12 mo of dabrafenib/trametinib the patient developed rapidly progressive disease with enlarging mediastinal and abdominal lymph nodes. He declined any further salvage treatment and succumbed to the disease 21 mo after diagnosis.

DISCUSSION

Chemotherapeutic treatment is currently the mainstay of palliative therapy for PAC patients. Small cohort studies and case reports list a number of regimens with an overall response rate of 23% and a median PFS of 5.6 mo (Al-Hader et al. 2017; Yoo et al. 2017). Chemotherapeutic substances include gemcitabine, capecitabine, 5-fluoruracil, irinotecan, cisplatin/oxaliplatin, and taxanes (Lowery et al. 2011; Al-Hader et al. 2017; Yoo et al. 2017; Brunetti et al. 2018) and also targeted therapies such as erlotinib or panitumumab in *KRAS* wild-type tumors (Morales et al. 2013; Kruger et al. 2016). Regarding predictors of treatment response, defects in DNA repair genes such as *BRCA1*, *BRCA2*, and *PALB2* have been linked to prolonged survival following platinum-based regimens in some patients (Al-Hader et al. 2017; Yoo et al. 2017). Interestingly, the patient reported here had a germline heterozygous stop-gain mutation in *PALB2*, which has previously been reported in the context of familial pancreatic and breast cancer (Slater et al. 2010). However, he did not benefit from cisplatin or oxaliplatin because he developed new lung lesions under the first cycle of PEI treatment and only demonstrated stable disease with oxaliplatin-containing FOLFIRINOX. Other molecular predictors of platinum response, such as the very low HRD, LST, and TAI scores (Waddell et al. 2015) and the rather low level of the mutational signature 3 (Telli et al. 2016), did not point to a major defect in homologous recombination DNA repair mechanisms and were therefore in line with the insensitivity to platinum-containing treatment regimens. The pathologic and clinical significance of the identified *PALB2* mutation for the pathogenesis of the patient's disease therefore remains uncertain.

Until today, targeted treatment options in PAC are not well established. However, there are a number of reports of *RAF* alterations in PACs, especially *SND1-BRAF* and *HERPUD1-BRAF* fusions (Wang et al. 2018), and sensitivity of BRAF-fused PAC cell lines to MEK inhibitors has been demonstrated previously (Chmielecki et al. 2014). It has even been proposed that this *BRAF*-altered cohort of PACs form a unique patient cohort distinctive from other PAC patients characterized by frequent alterations in HR genes and clinical high sensitivity to platinum-based treatment (Chmielecki et al. 2014). In contrast to fusions, *BRAF* point mutations have been found only rarely (Chmielecki et al. 2014; Jiao et al. 2014) or not at all (Bergmann et al. 2014) in PACs. *BRAF* mutations occur in up to 15% across all cancers, predominantly as point mutations with substitution of glutamic acid (E) for valine (V) at position 600 (70%–90%) alongside other *BRAF* mutations, amplifications, and fusions (Turski et al. 2016). Combination therapy using BRAF and MEK inhibition has proven to be superior over monotherapy, both for reasons of efficacy and reduced side effects (Eroglu and Ribas 2016), and has led to approval—for example, $BRAF^{V600E}$-mutated melanoma and lung cancer. Effectiveness of BRAF and/or MEK inhibition has also been observed

for other activating mutations (Dahlman et al. 2012) and even BRAF fusions (Ross et al. 2016). A recent basket trial for *BRAF*^{V600E}-mutated cancer patients demonstrated efficacy in 13 unique cancer types, including cholangiocarcinoma, sarcoma, glioma, neuroendocrine carcinoma, and salivary gland carcinoma (Subbiah et al. 2020). Schreck et al. report on two patients with high-grade glioma who were successfully treated with BRAF/MEK inhibition (Schreck et al. 2018). In *BRAF*^{V600E}-mutated pancreatic cancer, within early basket trials, one patient with PDAC responded to vemurafenib (Hyman et al. 2015) and another PDAC patient showed prolonged survival under dabrafenib/trametinib combination treatment (Guan et al. 2018). In contrast to PDAC, to our knowledge, there have been no reports of BRAF-targeted treatments in PAC patients until today. We present a PAC patient carrying a *BRAF*^{V600E} mutation who responded well to combined BRAF/MEK inhibitor treatment with an almost complete response. This allowed for extensive surgery aiming at complete resection of all visible tumor burden. However, the debulking surgery did not translate into long-term tumor control because postoperative complications delayed the reuptake of targeted therapy. After reinitiation, dabrafenib/trametinib did no longer halt disease progression, possibly because of the outgrowth of *BRAF*^{V600E}-negative and/or -resistant clones.

The transient response to BRAF/MEK inhibitors in many patients remains a significant therapeutic challenge as our case also documents. Acquired mechanisms of BRAF resistance can be divided in upstream reactivation of the MAPK/ERK pathway through, for example, overexpression of the RAF isoforms ARAF and CRAF or activating *RAS* mutations and downstream activation through either BRAF overexpression and dimerization or activation of the PI3K/AKT pathway (Griffin et al. 2017). Although combination treatment with BRAF/MEK inhibition may slow down the development of BRAF inhibitor resistance because tumors cannot exploit the MEK pathway, most patients do eventually progress under combination treatment. Additionally, as demonstrated in our case, resection of all macroscopic tumor burden does not seem to prevent relapse and does not deter the development of drug resistance. Hence, current research focuses on other new possible combination therapies outside the MAP/ERK pathway (Griffin et al. 2017). Interestingly, intermittent dosing, both in vitro and in vivo models, seemed to delay the onset of BRAF inhibitor resistance (Griffin et al. 2017), with one case report describing an ongoing complete remission with intermittent vemurafenib dosing (Dooley et al. 2016). Also, in *BRAF*^{V600E}-mutated colorectal cancer, in which feedback activation of EGFR forms a preexisting resistance mechanism to BRAF/MEK inhibition, a triple combination of cetuximab, encorafenib, and binimetinib has recently shown to successfully overcome this resistance leading to an impressive improvement in response and overall survival within the BEACON trial (Kopetz et al. 2019). Because of the rapid progression of disease and denial of further diagnostics, analysis of possible resistance mechanisms and their targetability was not possible in our case. Still, in this young patient with metastatic PAC, BRAF/MEK-targeted therapy allowed for prolonged disease control with minimal side effects.

CONCLUSION

Here we present the case of a young patient with *BRAF*^{V600E}-mutated PAC who achieved almost complete response upon initiation of combined BRAF/MEK inhibition.

This case underscores the value of comprehensive genomic assessment in patients with rare cancers whose treatment options are often limited. Hence, for PAC patients we recommend at least extended next-generation sequencing (NGS) panel sequencing for druggable targets including *BRAF* point mutations and fusions. BRAF/MEK inhibition is an efficient and well-tolerated treatment option in the *BRAF*-mutated subcohort of PAC patients.

METHODS

Microsatellite Instability Analysis

Genomic DNA was extracted from tissue samples after manual microdissection using the QIAGEN DNA Blood and Tissue Kit (QIAGEN) according to the manufacturer's recommendation. Measurements of DNA content were performed using a NanoDrop (Thermo Scientific). Microsatellite instability typing was carried out using the Bethesda marker panel (Boland et al. 1998) and CAT25 as described previously (Findeisen et al. 2005). Two or more MSI markers were scored as high-level MSI (MSI-H). MSISensor (Niu et al. 2014) was applied with a minimum required coverage of 15 reads in both tumor and control. The MSI score for a sample is the percentage of somatic, instable microsatellites, relative to the total number of microsatellites found in the control sample. According to the paper, a score of >3.5 implies microsatellite instability.

Whole-Genome Sequencing

Tissue samples were provided by the NCT Heidelberg Tissue Bank in accordance with its regulations and after approval by the Ethics Committee of Heidelberg University. DNA isolation from the tumor specimen and the blood sample was performed using the AllPrep DNA/RNA/Protein Mini Kit (QIAGEN), followed by quality control and quantification using a Qubit 2.0 Fluorometer (Life Technologies), a 2200 TapeStation system (Agilent), and a 2100 Bioanalyzer system (Agilent).

For genome sequencing on an Illumina HiSeq X instrument, 100 ng of genomic DNA were fragmented to an insert size of 450 base pairs (bp) with a Covaris LE220 or E220 device, and libraries were prepared using the TruSeq Nano Kit (Illumina). Paired-end sequencing was carried out according to the manufacturer's recommendations, yielding read lengths of 151 bp (HiSeq X). MSIsensor, HRD, LST, and TAI scores were calculated as previously described (Abkevich et al. 2012; Birkbak et al. 2012; Popova et al. 2012; Niu et al. 2014).

RNA Sequencing

RNA sequencing libraries were prepared using the TruSeq RNA Sample Preparation Kit v2 (Illumina). Briefly, mRNA was purified from 1 µg total RNA using oligo(dT) beads, poly(A)$^+$ RNA was fragmented to 150 bp and converted into cDNA, and cDNA fragments were end-repaired, adenylated on the 3' end, adapter-ligated, and amplified with 12 cycles of polymerase chain reaction. The final libraries were validated using a Qubit 2.0 Fluorometer (Life Technologies) and a Bioanalyzer 2100 system (Agilent). Libraries were sequenced on an Illumina-patterned flowcell v2.5.

Mapping and Analysis of Whole-Genome Sequencing Data

Mapping and analysis of whole-genome sequencing data were performed as previously reported (Heining et al. 2018; Groschel et al. 2019). Sequencing coverages are provided in Supplemental Table 1.

ADDITONAL INFORMATION

Data Deposition and Access

Sequencing data were deposited in the European Genome-phenome Archive (https://www.ebi.ac.uk/ega/) under accession EGAS00001004282.

Ethics Statement

Tissue samples were provided by the NCT Heidelberg Tissue Bank after written informed consent was obtained under the NCT MASTER protocol (S-206/2011, approved by the Ethics Committee of Heidelberg University), which covers all aspects relevant to clinical cancer genome sequencing as well as further research and publication. This study was conducted in accordance with the Declaration of Helsinki.

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Author Contributions

E.B., S.K., S.Z., S.F., P.M., D.J., A.A., G.M., and C.S. collected and interpreted patient data and were involved in clinical management. E.B., S.K., S.U., P.H., B.B., B.H., and M.F. were involved in genomic profiling and data analysis. S.F., H.G., E.S., and A.S. supervised data interpretation. All authors have approved the current version of the manuscript and its submission to *Cold Spring Harbor Molecular Case Studies*.

Competing Interest Statement

The authors have declared no competing interest.

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