Unravelling similarities and differences in the role of circular and linear PVT1 in cancer and human disease

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The plasmacytoma variant translocation 1 (PVT1) is a long non-coding RNA gene involved in human disease, mainly in cancer onset/progression. Although widely analysed, its biological roles need to be further clarified. Notably, functional studies on PVT1 are complicated by the occurrence of multiple transcript variants, linear and circular, which generate technical issues in the experimental procedures used to evaluate its impact on human disease. Among the many PVT1 transcripts, the linear PVT1 (IncPVT1) and the circular hsa_circ_0001821 (circPVT1) are frequently reported to perform similar pathologic and pro-tumorigenic functions when overexpressed. The stimulation of cell proliferation, invasion and drug resistance, cell metabolism regulation, and apoptosis inhibition is controlled through multiple targets, including MYC, p21, STAT3, vimentin, cadherins, the PI3K/AKT, HK2, BCL2, and CASP3. However, some of this evidence may originate from an incorrect evaluation of these transcripts as two separate molecules, as they share the IncPVT1 exon-2 sequence. We here summarise IncPVT1/circPVT1 functions by mainly focusing on shared pathways, pointing out the potential bias that may exist when the biological role of each transcript is analysed. These considerations may improve the knowledge about IncPVT1/circPVT1 and their specific targets, which deserve further studies due to their diagnostic, prognostic, and therapeutic potential.

British Journal of Cancer (2022) 126:835–850; https://doi.org/10.1038/s41416-021-01584-7

BACKGROUND

The one-way DNA→RNA→protein paradigm has been outdated since the discovery of non-coding RNA (ncRNA) genes, which account for two-thirds of the total number of human genes [1]. ncRNAs are involved in critical biological processes. They can regulate gene expression at the transcriptional and post-transcriptional levels and are often deregulated in a variety of human diseases [2]. Thus, they may represent potential keystones for the new targeted therapies of incurable diseases, including cancer.

ncRNAs are classified into two subcategories: small and long ncRNAs (lncRNAs), of less and more than 200 nt, respectively [3]. To date, despite the identification of many lncRNAs, most of them still need to be functionally characterised.

Circular RNAs (circRNAs) represent a particular subtype of ncRNAs originating from back-splicing events. Discovered 40 years ago, they initially were considered as splicing by-products with unknown functions [4]. Their roles have recently been re-evaluated due to the discovery of thousands of circRNA entities. Some of them are highly abundant, evolutionary conserved, and involved in cellular differentiation and tissue homeostasis, as well as in the development of multiple diseases [4, 5]. Notably, the majority of circRNAs originate from genes that show oncogenic effects [6].

Great interest has recently been devoted to the “plasmacytoma variant translocation 1” (PVT1) lncRNA gene, which produces both linear and circular transcripts that have been reported to be overexpressed in several cancer types [7]. Interestingly, positive correlations between PVT1 overexpression and tumour progression are frequently observed [8–10] (see the section “Clinical impact of IncPVT1 and circPVT1”).

PVT1 maps at the 8q24 chromosomal band, reported as a gene desert, harbouring two fragile sites (FRA8C and FRA8D) [11]. It is an exceptionally complex locus, which gives rise to 176 linear splicing variants (according to the Ensembl Genome Browser [https://genome.ucsc.edu/index.html], Fig. 1), as well as to 29 circular RNAs, as reported in the CircInteractome (https://circinteractome.nia.nih.gov/) [12], and circBase (http://www.circbase.org/) [13] databases (Table 1). In addition, according to the UCSC Genome Browser, the PVT1 locus harbours five highly conserved microRNAs (miRNAs) (Fig. 1). Some linear transcripts were detected by exon-specific RT-qPCR [14], 5’RACE PCR [8], and lncRNA microarrays [15]; others resulted from transcript predictions.

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Received: 16 April 2021 Revised: 27 August 2021 Accepted: 4 October 2021 Published online: 9 November 2021
Table 1). Functional data are limited to the hsa_circ_0001821 circular RNA (herein referred to as circPVT1), which shares the exon-2 full-length sequence (410 nt) with lncPVT1, and the hsa_circ_0009143 [24]. The latter is overexpressed in cervical cancer metastases and is involved in epithelial-mesenchymal transition (EMT), which normal polarised epithelial cells transform their phenotype and acquire mesenchymal characteristics and metastasis [24]. circPVT1 derives from a back-splicing event, prompted by a loop structure generated by the presence of Alu repeats flanking exon 2 of PVT1 [21]. The circular structure makes circPVT1 resistant to exonuclease cleavage, and therefore, highly stable. Indeed, its half-life exceeds 24 h, while lncPVT1 shows a half-life of fewer than 4 h [25].

Although lncPVT1 and circPVT1 are different entities, they are often reported in the literature as involved in the same cellular pathways. This review will introduce their specificities and focus on their shared pathways, downstream molecular targets and the technical issues encountered to study them as separate entities.

The PVT1 locus is frequently amplified and rearranged in human cancer

Multiple PVT1 genetic variants are described as associated with cancer susceptibility [26–28]. Previous studies mostly documented its involvement in genomic aberrations, e.g., translocations and high copy number amplification, in different malignancies. Translocations affecting the 8q24 locus are well-documented in multiple myeloma [29], lymphoma [30] and chronic lymphocytic leukaemia [31] (Table 1). Functional data are limited to the hsa_circ_0001821 circular RNA (herein referred to as circPVT1), which shares the exon-2 full-length sequence (410 nt) with lncPVT1, and the hsa_circ_0009143 [24]. The latter is overexpressed in cervical cancer metastases and is involved in epithelial-mesenchymal transition (EMT), which normal polarised epithelial cells transform their phenotype and acquire mesenchymal characteristics and metastasis [24]. circPVT1 derives from a back-splicing event, prompted by a loop structure generated by the presence of Alu repeats flanking exon 2 of PVT1 [21]. The circular structure makes circPVT1 resistant to exonuclease cleavage, and therefore, highly stable. Indeed, its half-life exceeds 24 h, while lncPVT1 shows a half-life of fewer than 4 h [25].

Although lncPVT1 and circPVT1 are different entities, they are often reported in the literature as involved in the same cellular pathways. This review will introduce their specificities and focus on their shared pathways, downstream molecular targets and the technical issues encountered to study them as separate entities.
| Name | Transcript ID | Exon no. | Size (bp) | Position (GRCh38/hg38) | Reference (identification) | Reference (function) | Name | Transcript ID | Exon no. | Size (bp) | Position (GRCh38/hg38) | Reference (identification) | Reference (function) |
|------|---------------|----------|----------|-------------------------|-----------------------------|-----------------------|------|---------------|----------|----------|-------------------------|-----------------------------|-----------------------|
| lncPVT1 | ENST00000512617.7 | 6 | 1109 chr8 | 127,794,512–127,890,628 | – | – | lncPVT1 | ENST00000512617.7 | 6 | 1109 chr8 | 127,794,512–127,890,628 | – | – |
| PVT1-304 | ENST00000561830.1 | 7 | 1460 chr8 | 127,794,533–127,940,454 | – | – | PVT1-304 | ENST00000561830.1 | 7 | 1460 chr8 | 127,794,533–127,940,454 | – | – |
| PVT1-305 | ENST00000561988.1 | 6 | 1560 chr8 | 127,794,533–127,940,454 | – | – | PVT1-305 | ENST00000561988.1 | 6 | 1560 chr8 | 127,794,533–127,940,454 | – | – |

**Table 1.** lncPVT1 and circPVT1 transcript variants.
| Name                | Transcript ID        | Exon no. | Size (bp) | Position (GRCh38/hg38) | Reference (identification) | Reference (function) |
|---------------------|----------------------|----------|-----------|------------------------|---------------------------|---------------------|
| PVT1-231            | ENST00000522993.1    | 4        | 865       | chr8                   | 128,046,371−128,101,256   | –                   |
| PVT1-232            | ENST00000534061.1    | 2        | 619       | chr8                   | 127,891,039−127,952,708   | –                   |
| PVT1-233            | ENST00000534497.1    | 7        | 128       | chr8                   | 127,945,494−127,101,256   | –                   |
| PVT1-234            | ENST00000535321.6    | 6        | 127       | chr8                   | 127,794,527−127,101,256   | –                   |
| PVT1-235            | ENST00000536081.1    | 4        | 906       | chr8                   | 127,945,367−127,101,256   | –                   |
| PVT1-236            | ENST00000536851.1    | 5        | 1490      | chr8                   | 127,945,024−127,101,256   | –                   |
| PVT1-237            | ENST00000539091.1    | 7        | 1329      | chr8                   | 127,794,538−127,101,256   | –                   |
| PVT1-238            | ENST00000539122.1    | 12       | 1312      | chr8                   | 127,794,527−127,101,256   | –                   |
| PVT1-239            | ENST00000540911.1    | 5        | 1311      | chr8                   | 127,794,565−127,101,256   | –                   |
| PVT1-240            | ENST00000541051.1    | 9        | 1460      | chr8                   | 127,794,663−127,101,256   | –                   |
| PVT1-241            | ENST00000542341.1    | 7        | 1411      | chr8                   | 127,794,500−127,101,252   | –                   |
| PVT1-242            | ENST00000543061.1    | 5        | 1482      | chr8                   | 127,795,346−127,101,267   | –                   |
| PVT1-243            | ENST00000550991.1    | 5        | 855       | chr8                   | 127,945,094−127,101,256   | –                   |
| PVT1-244            | ENST00000551481.1    | 4        | 724       | chr8                   | 127,945,403−127,101,256   | –                   |
| PVT1-245            | ENST00000559411.1    | 2        | 978       | chr8                   | 127,945,054−127,101,256   | –                   |
| PVT1-246            | ENST00000570831.1    | 7        | 1118      | chr8                   | 127,945,000−127,101,256   | –                   |
| PVT1-247            | ENST00000560771.1    | 6        | 1109      | chr8                   | 127,945,064−127,101,256   | –                   |
| PVT1-248            | ENST00000561601.1    | 4        | 935       | chr8                   | 127,945,403−127,102,382   | –                   |
| PVT1-249            | ENST00000563961.1    | 5        | 918       | chr8                   | 127,795,967−127,102,701   | –                   |
| PVT1-250            | ENST00000564021.1    | 8        | 1585      | chr8                   | 127,945,590−127,101,256   | –                   |
| PVT1-251            | ENST00000564111.1    | 9        | 1281      | chr8                   | 127,945,094−127,101,256   | –                   |
| PVT1-252            | ENST00000564911.1    | 7        | 1167      | chr8                   | 127,945,060−127,101,256   | –                   |
| PVT1-253            | ENST00000565321.1    | 7        | 1909      | chr8                   | 127,945,357−127,101,256   | –                   |
| PVT1-254            | ENST00000566931.1    | 4        | 1900      | chr8                   | 127,795,802−127,101,365   | –                   |
| PVT1-255            | ENST00000566680.1    | 5        | 1017      | chr8                   | 127,945,537−127,101,267   | –                   |
| PVT1-256            | ENST00000569481.3    | 3        | 904       | chr8                   | 127,845,470−127,102,706   | –                   |
| PVT1-257            | ENST00000569991.6    | 6        | 1314      | chr8                   | 127,945,026−127,101,255   | –                   |
| PVT1-258            | ENST00000571121.5    | 5        | 1489      | chr8                   | 127,945,599−127,103,964   | –                   |
| PVT1-259            | ENST00000571831.6    | 6        | 1172      | chr8                   | 127,945,452−127,103,896   | –                   |
| PVT1-260            | ENST00000572111.6    | 6        | 1553      | chr8                   | 127,945,339−127,101,256   | –                   |

**Table 1 continued**
| Name   | Transcript ID | Exon no. | Size (bp) | Position (GRCh38/hg38) | Reference (identification) | Reference (function) |
|--------|---------------|----------|-----------|-------------------------|---------------------------|---------------------|
| PVT1-1 | ENST0000065463.1 | 8 | 1388 | chr8:127,984,331–127,992,319 | – | – |
| PVT1-2 | ENST0000065463.1 | 7 | 1168 | chr8:127,976,151–127,984,021 | – | – |
| PVT1-3 | ENST0000065463.1 | 6 | 938 | chr8:127,967,971–127,975,841 | – | – |
| PVT1-4 | ENST0000065463.1 | 5 | 674 | chr8:127,959,821–127,967,691 | – | – |
| PVT1-5 | ENST0000065463.1 | 4 | 476 | chr8:127,951,671–127,959,541 | – | – |
| PVT1-6 | ENST0000065463.1 | 3 | 278 | chr8:127,943,521–127,951,391 | – | – |
| PVT1-7 | ENST0000065463.1 | 2 | 178 | chr8:127,935,371–127,943,241 | – | – |
| PVT1-8 | ENST0000065463.1 | 1 | 96 | chr8:127,927,221–127,935,091 | – | – |

**Table 1 continued**
| Name     | Transcript ID | Exon no. | Size (bp) | Position (GRCh38/hg38)     | Reference (identification) | Reference (function) |
|----------|---------------|----------|-----------|----------------------------|-----------------------------|----------------------|
| PVT1-284 | ENST00000659326.1 | 5        | 1706      | chr8:127,795,196–127,996,681 | -                           | hsa_circ_0085540      |
| PVT1-285 | ENST00000659425.1 | 6        | 1363      | chr8:127,795,802–128,101,025 | -                           | hsa_circ_0085541      |
| PVT1-286 | ENST00000659661.1 | 9        | 1533      | chr8:127,795,820–128,101,256 | -                           | hsa_circ_0085542      |
| PVT1-287 | ENST00000659691.2 | 5        | 782       | chr8:127,795,181–128,101,025 | -                           | hsa_circ_0085543      |
| PVT1-288 | ENST00000659791.2 | 9        | 1744      | chr8:127,795,820–128,101,256 | -                           | hsa_circ_0085544      |
| PVT1-289 | ENST00000659892.1 | 5        | 782       | chr8:127,795,181–128,101,025 | -                           | hsa_circ_0085545      |
| PVT1-290 | ENST00000659912.1 | 9        | 1744      | chr8:127,795,820–128,101,256 | -                           | hsa_circ_0085546      |
| PVT1-291 | ENST00000659912.1 | 4        | 1139      | chr8:127,794,537–127,996,670 | -                           | hsa_circ_0085547      |
| PVT1-292 | ENST00000659912.1 | 6        | 1199      | chr8:127,794,537–127,996,670 | -                           | hsa_circ_0085548      |
| PVT1-293 | ENST00000660061.1 | 4        | 870       | chr8:120,948,256–120,950,256 | -                           | hsa_circ_0085549      |
| PVT1-294 | ENST00000660122.1 | 8        | 1199      | chr8:127,795,820–128,101,256 | -                           | hsa_circ_0085550      |
| PVT1-295 | ENST00000660146.1 | 4        | 764       | chr8:120,945,285–128,101,025 | -                           | hsa_circ_0085551      |
| PVT1-296 | ENST00000660200.1 | 6        | 1199      | chr8:127,794,537–127,996,670 | -                           | hsa_circ_0085552      |
| PVT1-297 | ENST00000660381.1 | 13       | 2450      | chr8:127,795,155–128,017,217 | -                           | hsa_circ_0085553      |
| PVT1-298 | ENST00000660456.1 | 9        | 1814      | chr8:127,795,773–128,101,025 | -                           | hsa_circ_0085554      |
| PVT1-299 | ENST00000660511.1 | 12       | 2161      | chr8:127,795,820–128,101,256 | -                           | hsa_circ_0085555      |
| PVT1-300 | ENST00000660591.1 | 4        | 854       | chr8:120,945,282–128,101,025 | -                           | hsa_circ_0085556      |
| PVT1-301 | ENST00000660781.1 | 4        | 854       | chr8:120,945,193–128,101,025 | -                           | hsa_circ_0085557      |
| PVT1-302 | ENST00000660891.1 | 6        | 1139      | chr8:127,795,181–128,101,025 | -                           | hsa_circ_0085558      |
| PVT1-303 | ENST00000661012.1 | 4        | 754       | chr8:120,945,204–128,101,025 | -                           | hsa_circ_0085559      |
| PVT1-304 | ENST00000661160.1 | 3        | 1540      | chr8:127,795,180–127,932,708 | -                           | hsa_circ_0085560      |
| PVT1-305 | ENST00000661205.1 | 3        | 1519      | chr8:127,795,346–127,932,701 | -                           | hsa_circ_0085561      |

Reference (identification): hsa_circ_0085540–0085559
Reference (function): hsa_circ_0085540–0085559
leukaemia [31, 32], and generally result in MYC (located 53 Kb upstream of PVT1 (Fig. 1)) and PVT1 overexpression; these events are associated with poor prognosis.

Moreover, IncPVT1 has also been reported to be part of fusion transcripts either due to a genomic rearrangement or through trans-splicing events [33, 34]. However, the potential oncopgenic roles of these chimeras have not been investigated yet.

8q24 high copy number amplification, in the form of double minute chromosomes or homogeneously staining regions, is described in a series of cancers, from haematological malignancies, such as acute myeloid leukaemia [33, 35] and lymphoma [36], to solid tumours, including gastric cancer (GC) [37], small-cell lung cancer (SCLC) [38], breast cancer [39], medulloblastoma [40], ovarian and endometrial cancers [8, 41, 42], and CRC [43].

The 8q24 genomic amplifications usually cause an increased expression of the embedded oncogenes, particularly MYC, even though some exceptions to the amplification-overexpression paradigm are observed [35, 44]. Interestingly, Takahashi et al. demonstrated a stronger correlation between 8q24 copy number gain and PVT1 expression than the one reported between the genomic amplification and MYC [45]. Indeed, a significant amplification of PVT1 alone was found in some tumour types, suggesting that increased PVT1 expression may be sufficient to increase MYC levels, which is crucial in tumorigenesis [46, 47].

Increasing literature documented interactions between MYC and PVT1 at both genomic and transcripational levels, as also discussed in the section “Cell proliferation”. Recent evidence highlighted, in some cancer models (e.g., breast cancer), the role of the PVT1 promoter in the transcripational regulation of MYC. In detail, Cho et al. identified four PVT1-intergenic enhancers increasing MYC expression when the PVT1 promoter is inactive. The latter acts as a DNA boundary element, modulating enhancer–promoter interactions and displaying a tumour-suppressive role [48]. Although the regulatory action of the PVT1 promoter seems to overcome that of its RNA products, both these elements could contribute to modulating MYC protein levels in a tissue-specific manner. Future studies are needed to clarify the interplay between PVT1-mediated transcriptional and post-transcripational regulation of MYC.

**IncPVT1 upregulation in human disease and cancer**

Independently from genomic events, IncPVT1 is upregulated in tumours relative to normal cells in various cancer types, thus representing a good candidate for targeted therapies [49–51].

Interestingly, You et al. reported the hypomethylation of the PVT1 promoter in several cancer types compared with normal counterparts, suggesting epigenetics as a significant mechanism behind IncPVT1 upregulation [52].

In addition to multiple cellular functions shared between IncPVT1 and circPVT1, which will be discussed later in the review (see the section “Two molecules, same function?”), IncPVT1 is also known to promote angiogenesis, likely by enhancing the expression and secretion of vascular endothelial growth factor (VEGF) [53], and regulating the Wnt/β-catenin axis. IncPVT1 is associated with high cytoplasmic and nuclear β-catenin levels and expression of its CyclinD1 target [54–56]. The upregulation of the Wnt/β-catenin pathway leads to dysregulation of numerous cellular processes, such as cell viability, adhesion, migration, and invasion [57]. Several studies investigated the relationship between PVT1 and Wnt/β-catenin, all of which focused on the linear isoform [58–60].

Notably, p53 positively regulates the expression level of the PVT1b isoform through its binding to a p53-responsive element, located about 1200 bp downstream the PVT1 transcriptional start site, between exon 1a and exon 1b, also conserved in mice [18]. The activation of this isoform is stress-dependent, as it is heavily induced after treatment of mouse embryonic fibroblasts and murine lung adenocarcinoma KPR cells with genotoxic or oncogenic stress, respectively [19]. Interestingly, PVT1b activation is accompanied by the specific downregulation of Myc transcription, indicating its role as a downstream effector of p53 [19].

This evidence is striking because it underlines the dual behaviour of PVT1 in cancer, either as an oncogene or as a tumour suppressor gene.

In addition to cancer, the aberrant expression of IncPVT1 has been reported in other pathological conditions. For example, Zhang et al. described IncPVT1 as a therapeutic target for obesity treatment due to its role in adipocyte differentiation and adipogenesis. Interestingly, they found a significant upregulation of this linear transcript in mature adipocytes compared with preadipocytes, impacting the expression of genes involved in the fatty acid synthesis, transportation and lipogenic transcription [61].

Despite these initial reports, there is still a missing link between the upregulation of IncPVT1 and its causative role in human diseases and cancer development and progression. Moreover, the heterogeneity due to the occurrence of many PVT1 linear isoforms, which hamper specific gene silencing and quantification experiments, represents an issue for investigating the roles of each particular transcript.

**circPVT1 upregulation in cancer and innate immunity**

circPVT1 was first described in GC [25], where its expression is upregulated compared with normal gastric tissue. It was subsequently reported as upregulated in several other tumours. Still, its role in carcinogenesis and potential relevance as a diagnostic or prognostic biomarker and as a drug target in cancer remains to be clarified.

Interestingly, circPVT1 expression can be regulated by the interaction between the YAP1 transcriptional cofactor, belonging to the Hippo pathway, and the mutated p53 protein (mut-p53) [62]. YAP1 exerts oncogenic effects by increasing cell proliferation and inhibiting apoptosis. Verducci et al. found a higher expression of circPVT1 in head and neck squamous cell carcinoma patients harbouring TP53 mutations than in controls [63]. Using siRNA against mut-p53, they observed a downregulation of circPVT1 expression by ~60% 24 h after the transfection. Conversely, no effect on IncPVT1 expression was observed. The authors showed that YAP1 increases circPVT1 expression, acting at both transcriptional (by binding circPVT1 promoter and enhancing its activity) and post-transcriptional (by binding and stabilising circPVT1) levels. This effect is enhanced by mut-p53, which can bind YAP1 and reinforce its interaction with circPVT1. This event, in turn, results in an increased proportion of cells in the cell cycle S and G2 phases and elevated cell proliferation [63].

Finally, many circRNAs, including circPVT1, have been associated with the regulation of innate immunity [64]. Indeed, through the formation of imperfect 16–26-bp RNA duplexes, these highly stable molecules may function as inhibitors of the double-stranded RNA (dsRNA)-activated protein kinase (PKR), which is involved in the innate immune response. Upon viral or bacterial infection, circRNAs are degraded by the endonuclease, RNase L, resulting in a release and subsequent activation of PKR through autophosphorylation [64]. In addition, individuals affected by systemic lupus erythematosus showed lower levels of many circRNAs in their peripheral blood mononuclear cells, including circPVT1, compared with healthy donors, potentially resulting in an aberrant PKR activation [64].

**Two molecules, same function?**

Despite the frequent upregulation of IncPVT1 and circPVT1 in solid tumours and haematological malignancies, their expression levels are poorly correlated [25]. These PVT1 isoforms are transcribed by different promoters [63], therefore, they have to be considered separate transcriptional entities although possibly interconnected. IncPVT1 is enriched in the nucleus versus the cytosol [19, 65, 66], as observed by subcellular fractionation and...
subsequent RT-qPCR [19, 65–68], and RNA fluorescence in situ hybridisation [69, 70] in several cancer cell lines. Interestingly, IncPVT1 is described as a chromatin modifier [71]. It has been demonstrated to bind the histone methyltransferase Enhancer of Zeste Homolog-2 (EZH2), a catalytic subunit of polycomb-repressive complex 2 (PRC2), leading to the direct histone methylation of several gene promoters, including the angiopeitin-like 4 (ANGPTL4) in cholangiocarcinoma [70] and trophoblast cells [72], the thyroid-stimulating hormone receptor (TSHR) in thyroid carcinoma [73], the forkhead box f1 (FOXF1) in breast cancer [74], the large tumour suppressor kinase 2 (LATS2) in non-small-cell lung cancer (NSCLC) [49], the tumour suppressors p15 and p16 in GC [66], the miR-146a in prostate cancer [75], the miR-200c in melanoma [76], and the miR-200b in cervical cancer [77]. IncPVT1 could also recruite DMNT1 via EZH2 and promote DNA methylation of the miR-18b-5p promoter in gallbladder cancer (GBC) [68]. In liver cancer, instead, IncPVT1 interferes with the recruitment of EZH2 to the MYC promoter, thus altering the methylation status and, hence, enhancing its expression [68, 78].

Moreover, IncPVT1 may act as a scaffold for the histone acetyltransferase KAT2A, leading to the final HIF-1α stability increase in nasopharyngeal tumours [71].

Conversely, circPVT1 shows a prevalent cytoplasmic localisation [63, 79–81]. Both IncPVT1 and circPVT1 have been proposed to function as competing endogenous RNAs (ceRNAs) [37, 49, 57, 61]. IncPVT1 functions as a ceRNA by sponging several miRNAs, including miR-186 in GC [82], and miR-186-5p in hepatocellular carcinoma [83]. A similar miRNA-sponging role is described for circPVT1, as for miR-497 in NSCLC [84] and head and neck cancer [63], miR-204-5p in breast cancer [85], miR-125b in NSCLC [79] and GC [25], and miR-145 in CRC [86].

Furthermore, IncPVT1 can directly bind the FOXM1 [87] and MYC [88] proteins to stabilise them post-translationally as well as restrict STAT3 [89] and Lin28 protein degradation by the proteasome machinery [15].

circPVT1 and IncPVT1 are largely thought to be involved in the same cellular processes. The main pathways and targets commonly regulated by circPVT1 and IncPVT1 are summarised in the sections “Cell proliferation”, “Oncogenesis and tumour progression”, “Apoptosis”, “Drug resistance”, “Cancer metabolism” and “Clinical impact of IncPVT1 and circPVT1”, and Fig. 2.

However, some of these observations may result from technical issues related to difficulties in discerning the two as separate entities in particular experimental analyses, as discussed in the section “Technical issues for PVT1 quantification and experimental knockdown”.

Cell proliferation

IncPVT1 and circPVT1 control cell proliferation by regulating target genes such as MYC and CDKN1A (cyclin-dependent kinase inhibitor 1A).

The potential interaction between MYC and PVT1 genes, although widely discussed in the literature, remains controversial. For instance, it is unclear whether these genes may act synergistically, how they are regulated and if PVT1 linear and/or circular isoforms impact MYC transcription and/or translation. In acute lymphoblastic leukaemia (ALL), IncPVT1 increases MYC protein levels with the resulting driver effects on primary tumours [90]. In GC, both circPVT1 and IncPVT1 were described to increase MYC protein levels. circPVT1 facilitates its translation by sequestering let-7b miRNA, whereas IncPVT1 directly stabilises MYC [25]. IncPVT1, indeed, blocks the phosphorylation of MYC at threonine 58 and prevents its degradation through the ubiquitin–proteasome pathway [47, 88]. Therefore, enhanced IncPVT1 level may increase MYC activity in cancer cells by impairing its turnover. In turn, MYC can act as a PVT1 transcriptional activator by binding to two E-box elements located at the PVT1 promoter [9].

Both circPVT1 and IncPVT1 impact the expression of the p21 senescence marker, which is encoded by the CDKN1A transcript. By sponging let-7 miRNAs, circPVT1 decreases the level of CDKN1A in fibroblast cells [91]. In pancreatic cancer cells, the silencing of IncPVT1 significantly increases the expression level of this tumour suppressor gene, influencing proliferation and migration [92]. Moreover, in NSCLC, IncPVT1 promotes cell proliferation by downregulating p21. This effect was demonstrated by using specific siRNA against IncPVT1 [93]. Similarly, in the Raji Burkitt lymphoma cell line, after IncPVT1 silencing, an increased level of p21 was observed, with a subsequent cell cycle block in G0/G1 phases [94].

Oncogenesis and tumour progression

In glioblastoma multiforme (GBM), the upregulation of circPVT1 activates, through miR-199a-5p downregulation, the PIK3/AKT pathway, which promotes tumour progression [95]. Interestingly, IncPVT1 in CRC acts as a ceRNA for the tumour suppressor miR-214-3p, leading to increased PIK3/AKT levels, which may cause cancer development [96]. The same effect was observed in human endometrial carcinoma, where IncPVT1 acts through the PVT1/ miR-195-5p/FGFR1–FGF2 axis, whose main downstream targets are PIK3/AKT [97].

Moreover, in hepatoblastoma, IncPVT1 overexpression is associated with high levels of p-STAT3, thus promoting proliferation and cancer progression [98]. Accordingly, in oral squamous cell carcinoma (OSCC), circPVT1 sponges miR-125b, which targets the STAT3 transcript. Therefore, increased circPVT1 levels cause an accumulation of STAT3, leading to tumour growth [80].

STAT3 has a well-defined role in cancer development, acting in the VEGFA transcriptional activation, promoting angiogenesis. In GC, a positive feedback loop has been demonstrated between STAT3 and the IncPVT1 expression: STAT3 overexpression leads to increased transcription of IncPVT1, which stabilises both STAT3 mRNA and protein in the nucleus. IncPVT1 prevents ubiquitin–proteasomal degradation of phosphorylated STAT3 (p-STAT3), resulting in protein accumulation in the nucleus and activation of the STAT3 signalling pathway [89].

Both IncPVT1 and circPVT1 seem to facilitate cell invasion and metastasis by promoting EMT, losing the adhesion–inhibition capabilities [99]. This phenomenon is mediated by the deregulated expression of key EMT regulators (E-cadherin, N-cadherin and Vimentin), as reported in osteosarcoma, hepatocellular carcinoma, pancreatic cancer, melanoma, oesophageal cancer and cervical cancer [54, 76, 100–103]. Overexpression of IncPVT1 or circPVT1 results in decreased E-cadherin levels (responsible for cell adhesion) and increased expression of N-cadherin and Vimentin (forcing an adhesion-independent phenotype).

Apoptosis

One of the hallmarks of cancer cells is their capability to escape programmed cell death (apoptosis). Failures in the control of apoptosis may cause tumour initiation, progression and metastasis [104]. Some IncRNAs are negative regulators of apoptosis in tumours [105]. It has been reported that IncPVT1 could inhibit apoptosis in GC tissues through the BCL2 anti-apoptotic factor, having the apoptosis effector CASP3 as a downstream target. When IncPVT1 is upregulated, a simultaneous expression increase of BCL2 [105].

In osteosarcoma cells, IncPVT1 regulates BCL2 through miR-195: when IncPVT1 is upregulated, the miR-195 level decreases and BCL2 transcript increases, resulting in inhibition of apoptosis [106]. circPVT1 also regulates BCL2 via miRNAs. In NSCLC patients, circPVT1 regulates the miR-497/BCL2 axis. Indeed, miR-497 shows a binding site at the 3’UTR of the BCL2 transcript [84].

Finally, in ALL, high levels of circPVT1 sustain BCL2 protein levels, potentially through miR-125 regulation, thus resulting in
inhibition of apoptosis. circPVT1 may also force BCL2 expression to inhibit MYC-mediated apoptosis [90].

Drug resistance
Both lncPVT1 and circPVT1 were shown to promote drug resistance in several cancer types by affecting, in some cases, the exact molecular targets. For instance, in osteosarcoma, lncPVT1 modulates miR-152 and prevents its binding to the c-mesenchymal–epithelial transition factor (c-MET). This event, in turn, promotes PI3K activation, inducing drug resistance [107]. The PI3K/AKT pathway is also involved in circPVT1-mediated cisplatin (DDP) resistance in GC. Wang et al. demonstrated that circPVT1 silencing could downregulate the PI3K/AKT signalling through the miR-152-3p/HDGF axis, resulting in decreased DDP resistance and malignancy in GC cells [108].

It has been highlighted that apoptosis and drug resistance are two closely related phenomena in cancer. In GC patients, circPVT1-mediated upregulation of BCL2 seems to enhance drug resistance to the 5-fluorouracil (5-FU), leading to a worse prognosis and shorter overall survival (OS) [105]. Involvement in drug response has also been reported in CRC cells, where lncPVT1 upregulation is associated with 5-FU and DDP resistance. This phenomenon is mediated by BCL2 expression and negative regulation of the apoptotic pathway, influencing BAX and CASP3 pro-apoptotic proteins [109, 110]. In osteosarcoma, circPVT1 upregulation is reported as contributing to doxorubicin (DXR) resistance [111]. Its downregulation in DXR-resistant cell lines resulted in decreased levels of the xenobiotic transmembrane transporters ABCB1 and MRP-1 as well as of BCL2, and increased expression of CASP3 [111].

Cancer metabolism
Both linear and circular PVT1 can sponge specific miRNAs that modulate HK2 levels. HK2 is involved in cellular metabolism, promoting glucose uptake in cancer cells as a carbon source for aerobic glycolysis [112]. High levels of HK2 are observed in several tumour types and are associated with advanced tumour stage, poor prognosis and metastasis occurrence [113]. A positive correlation between HK2 and circPVT1 was detected in osteosarcoma, resulting in a high glucose-uptake rate and subsequent lactate production [114]. HK2 is a direct target of miR-497, harbouring a candidate-binding site in its 3’UTR. circPVT1 promotes tumour development by binding miR-497 and blocking its anticancer effects. Moreover, in GBC, circPVT1 overexpression causes an increase of both HK2 mRNA and protein by suppressing the miR-143-mediated inhibitory effect [115]. The PVT1/miR-143/ HK2 axis represents the leading target candidate for therapies to regulate cancer metabolism and block tumour progression in GBC.

In OSCC, circPVT1 controls HK2 levels by sponging miR-106a-5p, contributing to cell growth, metastasis and glycolytic metabolism. Interestingly, the 3’-UTR of HK2 mRNA displays a binding site for miR-106a-5p that can directly suppress the protein translation. When circPVT1 is upregulated, miR-106a-5p activity is inhibited, leading to increased expression of HK2, which promotes cancer development [116].

Clinical impact of lncPVT1 and circPVT1
The altered expression of lncPVT1 and/or circPVT1 has been associated with tumour progression and poor prognosis in several cancer types (Table 2).

In particular, elevated expression levels of lncPVT1 predict poor prognosis and worse clinicopathological characteristics in both solid and haematological malignancies, resulting in a decrease of OS, progression-free survival (PFS) and/or disease-free survival (DFS). Indeed, as reported in Table 2, the circPVT1 upregulation in tumour tissues is associated with an advanced clinical stage and the presence of lymph node and distant metastases. Similar results were obtained when analysing the clinical impact of circPVT1 overexpression in different solid tumours: it predicted a poor OS and was related with an advanced clinical stage and, when analysed, with the occurrence of lymph node and distant metastases (Table 2). The only exception is GC. In this malignancy, high circPVT1 expression was associated with a good prognosis, likely due to its positive correlation with the tumour suppressor miR-125, which blocks the cell cycle at the G0/G1 phase, seeming to promote apoptosis, and inhibits tumour growth and invasion [25].
| Tumour type                | Patient no. | Follow-up (months) | Overall survival (OS) | Progression-free survival (PFS)/disease-free survival (DFS) | Clinical stage | Lymph node metastasis | Distant metastases | Reference (DOI)               |
|---------------------------|-------------|--------------------|-----------------------|--------------------------------------------------------------|---------------|-----------------------|---------------------|--------------------------|
| IncPVT1                   | Nasopharyngeal cancer 100 | 125 | Poor ($P < 0.001$) | Poor DFS ($P < 0.010$) | na | na | na | 10.1038/s41418-019-0381-y |
| Nasopharyngeal cancer 94  | 125 | Poor ($P = 0.003$) | Poor DFS ($P = 0.001$) | na | na | na | 10.1038/s41419-018-0265-y |
| Nasopharyngeal cancer 20  | 40 | Poor ($P = 0.040$) | Poor DFS ($P = 0.026$) | na | na | na | 10.1007/s12253-018-0453-1 |
| Gastric cancer 80         | 36 | Poor ($P = 0.001$) | Poor DFS ($P = 0.002$) | Advanced ($P = 0.015$) | ns | ns | na | 10.1186/12943-015-0355-8 |
| Gastric cancer 190        | 85 | na | Poor DFS ($P = 0.002$) | ns | ns | Increased ($P = 0.025$) | na | 10.1158/1078-0432.CCR-16-0742 |
| Gastric cancer 111        | 48 | Poor ($P < 0.001$) | Poor DFS ($P < 0.001$) | Advanced ($P = 0.002$) | Increased ($P = 0.029$) | ns | na | 10.4149/314_150825N45 |
| Gastric cancer 200;300*   | 150;110* | Poor ($P = 0.008$; $P = 0.042$) | na | na | na | na | na | 10.1038/s41388-018-0250-z |
| Gastric cancer 42         | 150 | Poor ($P < 0.001$) | na | na | na | na | na | 10.1002/jcp.29881 |
| Gastric cancer 17         | 150 | Poor ($P = 0.032$) | na | ns | ns | ns | ns | 10.390/cancers12102995 |
| Gallbladder cancer 55     | 30 | Poor ($P = 0.001$) | na | Advanced ($P = 0.011$) | Increased ($P = 0.032$) | ns | na | 10.1038/s41419-020-03080-x |
| Gallbladder cancer 66     | 80 | Poor ($P = 0.002$) | na | Advanced ($P = 0.026$) | na | ns | na | 10.1186/12943-019-0947-9 |
| Non-small-cell lung cancer | nr 120 | Poor ($P = 0.001$) | na | na | na | na | na | 10.3892/ol.2019.11237 |
| Non-small-cell lung cancer | 105 | 40 | Poor ($P < 0.001$) | Poor PFS ($P < 0.001$) | Advanced ($P < 0.001$) | Increased ($P = 0.011$) | na | 10.1158/1535-7163.MCT-15-0707 |
| Non-small-cell lung cancer | 108 | 40 | Poor ($P < 0.001$) | Poor PFS ($P < 0.001$) | Advanced ($P = 0.003$) | na | ns | 10.1007/s13277-015-4261-1x |
| Non-small-cell lung cancer | 31 80 | Poor ($P$ value nr) | Advanced ($P = 0.017$) | Increased ($P = 0.018$) | na | na | na | 10.1159/000480209 |
| Non-small-cell lung cancer | 25 230 | Poor ($P = 0.003$) | na | na | na | na | na | 10.2147/OTT.S22898 |
| Non-small-cell lung cancer | 82 60 | Poor ($P < 0.050$) | na | na | Increased ($P = 0.001$) | na | Yang et al. [117] (PMC4230094) |
| Small-cell lung cancer 120 | 60 | Poor ($P = 0.024$) | na | Advanced ($P < 0.001$) | Increased ($P = 0.001$) | Increased ($P < 0.001$) | na | Huang et al. [118] (PMC5126345) |
| Epithelial ovarian cancer 231 | 90 | Poor ($P = 0.020$) | Poor PFS ($P = 0.002$) | Advanced ($P < 0.001$) | ns | na | 10.2089/2/j.issn.2095-3941.2017.0174 |
| Epithelial ovarian cancer 73;129* | 200 | Poor ($P = 0.0012$; $P < 0.001$) | Poor PFS ($P < 0.001$; $P < 0.001$) | na | na | na | 10.1158/1078-0432.CCR-16-1402 |
| Ovarian cancer 40         | 60 | Poor ($P$ value nr) | na | na | na | na | 10.1016/j.biophoton.2018.06.112 |
| Colorectal cancer 112     | 60 | Poor ($P = 0.019$) | na | Advanced ($P = 0.001$) | Increased ($P = 0.015$) | Increased ($P = 0.007$) | Ping et al. [109] (PMCS801353) |
| Tumour type                      | Patient no. | Follow-up (months) | overall survival (OS) | progression-free survival (PFS)/disease-free survival (DFS) | Clinical stage | Lymph node metastasis | Distant metastases | Reference (DOI)          |
|---------------------------------|-------------|--------------------|-----------------------|--------------------------------------------------------------|---------------|-----------------------|---------------------|------------------------|
| Colorectal cancer               | 210         | 72                 | Poor ($P < 0.001$)    | Poor DFS ($P < 0.001$)                                        | Advanced      | Increased             | na                  | 10.1177/1724600818777242 |
| Colorectal cancer               | 62          | 60                 | Poor ($P = 0.040$)    | na                                                            | Advanced      | Increased             | Increased           | 10.2147/CMAR5260537    |
| Colorectal cancer               | 239;75*     | 60                 | Poor ($P = 0.007$; $P = 0.039$) | na                                                            | na            | na                    | na                  | 10.1186/s12943-020-0127-4 |
| Colorectal cancer               | 164         | 180                | Poor ($P = 0.0101$)   | na                                                            | Advanced      | Increased             | ns                  | 10.1038/bjc.2013.698   |
| Osteosarcoma                    | 26          | 60                 | Poor ($P < 0.050$)    | na                                                            | na            | na                    | na                  | 10.18632/oncotarget.13012 |
| Uveal melanoma                  | 80          | 80                 | Poor ($P = 0.009$)    | na                                                            | ns            | na                    | na                  | 10.1371/journal.pone.0189675 |
| Renal cell carcinoma            | 528         | 120                | Poor ($P = 0.001$)    | Poor DFS ($P = 0.001$)                                        | Advanced      | Increased             | na                  | 10.18632/oncotarget.19743 |
| Oesophageal squamous cell cancer| 52          | 100                | Poor ($P < 0.001$)    | Poor DFS ($P = 0.011$)                                        | Advanced      | na                    | na                  | 10.18632/oncotarget.15878 |
| Oesophageal squamous cell cancer| 156         | 120                | Poor ($P = 0.004$)    | na                                                            | Advanced      | na                    | na                  | 10.1186/s12943-019-1064-5 |
| Oesophageal carcinoma           | 50          | 40                 | Poor ($P < 0.050$)    | Poor DFS ($P < 0.050$)                                        | na            | na                    | na                  | 10.1002/1878-0261.12555 |
| Cervical cancer                 | 127         | nr                 | Poor ($P = 0.030$)    | na                                                            | na            | na                    | na                  | 10.1371/journal.pone.0156274 |
| Cervical cancer                 | 90          | 60                 | Poor ($P = 0.015$)    | na                                                            | Advanced      | na                    | na                  | 10.1111/apm.12555     |
| Pancreatic cancer               | 30          | 100                | Poor ($P = 0.008$)    | na                                                            | ns            | Increased             | na                  | 10.7150/jca.37959     |
| Breast cancer                   | 209         | 300                | Poor ($P < 0.050$)    | na                                                            | na            | na                    | ns                  | 10.1038/s41388-018-0310-4 |
| Breast cancer                   | 110         | 60                 | Poor ($P = 0.003$)    | na                                                            | Advanced      | Increased             | Increased           | 10.1016/j.bbr.2017.09.005 |
| Hepatocellular cancer           | 214         | 124                | ns                    | Poor DFS ($P = 0.021$)                                        | Advanced      | na                    | na                  | 10.3892/ol.2014.2730  |
| Hepatocellular cancer           | 89          | 50                 | Poor ($P = 0.0104$)   | Poor DFS ($P = 0.004$)                                        | Advanced      | na                    | na                  | 10.1002/hep.27239     |
| Tumour type                        | Patient no. | Follow-up (months) | overall survival (OS) | progression-free survival (PFS)/disease-free survival (DFS) | Clinical stage | Lymph node metastasis | Distant metastases | Reference (DOI) |
|-----------------------------------|-------------|--------------------|----------------------|-------------------------------------------------------------|---------------|----------------------|--------------------|------------------|
| **circPVT1**                      |             |                    |                      |                                                             |               |                      |                    |                  |
| Gastric cancer                    | 187         | 85                 | Good ($P < 0.001$)   | Good DFS ($P = 0.002$)                                       | ns            | ns                   | ns                 | 10.1016/j.canlet.2016.12.006 |
| Head and neck squamous cell cancer| 106;263*    | 72;210*            | Poor ($P = 0.050$)   | na                                                           | na            | na                   | na                 | 10.1186/s13059-017-1368-y |
| Osteosarcoma                      | 80          | 60                 | Poor ($P = 0.002$)   | Advanced ($P = 0.044$)                                       | na            | Increased ($P = 0.038$) | na                 | 10.7150/jibs.24360   |
| Osteosarcoma                      | 48          | 60                 | Poor ($P = 0.005$)   | Advanced ($P = 0.008$)                                       | na            | Increased ($P = 0.009$) | na                 | 10.1111/jcmm.15215 |
| Osteosarcoma                      | 36          | 50                 | Poor ($P = 0.028$)   | na                                                           | na            | na                   | na                 | 10.1111/cas.14787  |
| Non-small-cell lung cancer        | 90          | 60                 | Poor ($P < 0.050$)   | na                                                           | Advanced ($P = 0.007$) | ns | na | 10.1016/j.biopha.2018.12.007 |
| Non-small-cell lung cancer        | 96          | 100                | Poor ($P = 0.020$)   | na                                                           | Advanced ($P = 0.003$) | ns | na | 10.1177/0300891620941940 |
| Non-small-cell lung cancer        | 8           | 60                 | Poor ($P = 0.002$)   | Advanced ($P < 0.001$)                                       | Increased ($P = 0.001$) | na | na | 10.1186/s13046-021-01976-w |
| Non-small-cell lung cancer        | 104         | 60                 | Poor ($P = 0.011$)   | na                                                           | Advanced ($P = 0.027$) | na | na | 10.1016/j.biopha.2020.109828 |
| Colorectal cancer                 | 64          | 60                 | Poor ($P < 0.001$)   | na                                                           | Advanced ($P = 0.002$) | na | na | 10.1016/j.bbrc.2019.03.121 |
| Hepatocellular carcinoma          | 70          | 60                 | Poor ($P = 0.024$)   | na                                                           | Advanced ($P = 0.029$) | Increased ($P = 0.004$) | na | 10.1242/bio.043687  |
| Ovarian cancer                    | nr          | 200                | na                   | Poor DFS ($P = 0.005$)                                       | na            | na                   | na                 | 10.7150/jca.52234  |
| Breast cancer                     | 99          | 60                 | Poor ($P = 0.022$)   | na                                                           | Advanced ($P = 0.012$) | ns | na | 10.2147/OTT.S180850 |
| Medullary thyroid cancer           | 28          | 48                 | Poor ($P < 0.050$)   | na                                                           | na            | na                   | na                 | 10.1186/s13046-021-01964-0 |

*nr not reported, na not analyzed, ns not significant.

*Two patient cohorts investigated.
In summary, both IncPVT1 and circPVT1 might serve as effective prognostic biomarkers for multiple tumour entities.

Technical issues for PVT1 quantification and experimental knockdown
As IncPVT1 and circPVT1 share the same genomic sequence corresponding to IncPVT1 exon 2, technical approaches capable of discriminating between them are required to understand their individual biological roles.

In this context, we analysed the sequences of primers and siRNAs used to quantify and silence either IncPVT1 or circPVT1 across the literature.

Primers for RT-qPCR assays are often designed within exon 2 of PVT1 with a convergent orientation. If not preceded by RNase R digestion of the linear transcripts, this approach introduces a bias in quantification due to the primer pair annealing to both the circular and linear isoforms, as shown in Fig. 3a. Conversely, divergent primers on exon 2 allow the selective amplification of circPVT1, not requiring preventive digestion of the linear isoform and avoiding issues due to a partial efficiency of this step (Fig. 3a).

Similarly, in knockdown experiments, siRNAs specifically designed on PVT1 exon 2 will not allow a specific inhibition of one of the two isoforms, introducing a bias in evaluating the results, as shown in Fig. 3b. Thus, in a significant fraction of the published papers, it is not clear if the knockdown-related effects are attributable to IncPVT1 or circPVT1 or the result of both being simultaneously silenced. This problem can be overcome by placing the siRNA on the circPVT1 back-splicing junction (Fig. 3b) and a linear splicing junction for IncPVT1.

These technical issues question many published studies claiming specific functions for one of the two isoforms. In particular, the results suggest that both isoforms are involved in the same cellular processes. Thus, more studies are needed to clarify whether the observed effects result from a synergistic action of the two PVT1 isoforms or from technical artefacts.

CONCLUSIONS
The PVT1 gene has been widely investigated for its roles in cancer. However, the discovery of multiple linear and circular isoforms disclosed its multifaceted activity, with several aspects still to be clarified.

IncPVT1 and circPVT1 have to be considered two distinct entities, possibly sharing certain biological functions and having separate roles in cancer.

The molecular mechanisms behind their involvement in cancer initiation and progression have started to be disentangled. Of note, both transcripts might serve as prognostic biomarkers, and their possible connection with MYC highlights their possible role as targets of future therapies. More work is needed to clarify their potential interactions and roles as distinct transcript entities in cancer, mainly due to technical issues on the distinction between linear and circular isoforms in many published studies.

DATA AVAILABILITY
Not applicable.

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AUTHOR CONTRIBUTIONS
DTr, GS and LSK designed the concept of the paper. DTr and GS performed literature research and wrote the first draft of the paper. CTS and DTo supervised the work. DTo, DTr and CTS design the figures. CTS, DTo, LSK, MG, GV, GMac and GMar critically revised the paper. All authors read and approved the final paper.

FUNDING INFORMATION
None.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Not applicable.

CONSENT TO PUBLISH
Not applicable.

COMPETING INTERESTS
The authors declare no competing interests.