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Original Research

Tankyrase-selective inhibitor STP1002 shows preclinical antitumour efficacy without on-target toxicity in the gastrointestinal tract

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KEYWORDS
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Abstract ***Background:*** Tankyrase inhibition stabilises AXINs and antagonises the Wnt/β-catenin pathway in adenomatous polyposis coli (*APC*)-mutated colorectal cancer (CRC), suggesting that tankyrase is a potential therapeutic target for *APC*-mutated CRC. However, clinical trials on reported tankyrase inhibitors have been severely limited by on-target toxicity in the gastrointestinal (GI) tract. Herein, we report a new tankyrase-selective inhibitor, STP1002, having preclinical antitumour efficacy without on-target toxicity in *APC*-mutated CRC models.

Methods: STP1002 was developed and characterised using *in vitro* and *in vivo* functional studies; its pharmacokinetics, antitumour efficacy and toxicity were evaluated *in vivo*.

Abbreviations: ADME, absorption, distribution, metabolism, and excretion; ANOVA, analysis of variance; APC, adenomatous polyposis coli; APCDD1, adenomatous polyposis coli down-regulated 1; ASCL2, achaete-scute family bHLH transcription factor 2; AUC, area under the plasma concentration–time curve; BID, bis in die (twice a day); β-NAD^+, β-nicotinamide adenine dinucleotide; C_{max}, maximum plasma concentration; CCND1, cyclin D1; CK1α, casein kinase 1 α; CRC, colorectal cancer; FABP2, fatty acid binding protein 2; GI, gastro intestinal; GSK3β, glycogen synthase kinase 3β; IP, intraperitoneal; KRT20, keratin 20; LGR5, leucine rich repeat containing G protein-coupled receptor 5; NKD1, naked cuticle 1; PARP, members of the poly (ADP-ribose) polymerase; PK, pharmacokinetic; PD, pharmacodynamic; PDX, Patient-Derived Xenograft; PO, per os (by mouth); PP2A, protein phosphatase 2A; QD, queque die (every day); T_{max}, time of maximum concentration; TNKS, tankyrase; TGI, tumour growth inhibition; TNFRSF19, TNF receptor superfamily member 19; TFF3, trefoil factor 3.

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Results: STP1002 showed potent, selective inhibition of tankyrase 1/2 but not of members of the poly (ADP-ribose) polymerase 1/2 (PARP1/2). STP1002 exerted antitumour activity by stabilising AXINs and antagonising the Wnt/β-catenin pathway in a subset of *APC*-mutated CRC cell lines but not in inhibitor-resistant cells and *APC*-wild-type CRC cell lines. STP1002 showed favourable pharmacokinetic profiles for oral administration once daily. STP1002 inhibited tumour growth of *APC*-mutated CRC xenograft animal models but not of *APC*-wild type models in a dose-dependent manner. The antitumour efficacy of STP1002 was confirmed using *APC*-mutated CRC patient–derived tumour xenograft models. STP1002 showed no significant on-target toxicity in the GI tract compared to G007-LK, which shows severe ileum toxicity in preclinical animal models.

Conclusions: These results demonstrate that STP1002, a novel, orally active tankyrase inhibitor, shows preclinical antitumour efficacy without on-target toxicity in the GI tract. Our data provide a rationale for a clinical trial on STP1002 as a potential tankyrase-targeted drug in patients with *APC*-mutated CRC.

1. Introduction

The Wnt/β-catenin signalling pathway plays a key role in many biological processes, including cell proliferation, stem cell renewal and tissue development [1–3]. The hyperactivation of the Wnt/β-catenin pathway is often observed in many cancers, especially in colorectal cancer (CRC) [4,5]. In the presence of Wnt ligands, a key downstream factor, β-catenin, localises to the nucleus and forms a transcription complex with T-cell factor (TCF), and the β-catenin/TCF complex turns on the target genes such as MYC, cyclin D1 (CCND1), adenomatous polyposis coli down-regulated 1 (APCDD1), achaete-scute family bHLH transcription factor 2 (ASCL2), naked cuticle 1 (NKD1) and AXIN2 [1,3]. In the absence of Wnt ligands, accumulated β-catenin is degraded by phosphorylation-mediated proteolysis [1,3]. This phosphorylation is regulated by a destruction complex comprising adenomatous polyposis coli (*APC*), protein phosphatase 2A (PP2A), glycogen synthase kinase 3β (GSK3β) and casein kinase 1α (CK1α), and AXIN [1,3]. Loss-of-function mutations in the tumour suppressor gene *APC*, a negative regulator of Wnt/β-catenin signalling, promote Wnt/β-catenin-driven tumorigenesis of CRC, in which the mutation of *APC* is found in approximately 90% of cases [5]. Thus, the development of therapeutic agents that inhibit the Wnt/β-catenin pathway is an important approach for treating Wnt/β-catenin-driven tumours such as CRC [6]. Although a number of small molecules have been reported to inhibit the Wnt/β-catenin pathway, no drugs have been approved for usage in the market [7].

Tankyrase (TNKS) 1 and 2, members of the poly (ADP-ribose) polymerase (PARP) family protein, are poly-ADP-ribosyltransferases that catalyse the modification of proteins using β-nicotinamide adenine dinucleotide as a substrate [8,9]. In the Wnt/β-catenin pathway, TNKS act as an activator of the Wnt/β-catenin pathway through PARylation of AXIN1/2, which leads to AXIN degradation and activates the β-catenin/TCF complex [8,10]. Thus, TNKS inhibition stabilises AXIN and antagonises the Wnt/β-catenin pathway [10], suggesting TNKS to be a suitable target in *APC*-mutated CRC. TNKS has three domains: a catalytic PARP domain, an ankyrin domain, and a sterile alpha module domain. TNKS inhibitors, including XAV939 [10], IWR-1 [11], K-756 [12], AZ1366 [13], G-631 [14], JW55 [15], RK-287107 [16], MSC2504877 [17], NVP-TNKS656 [18] and G007-LK [19] have been developed by identifying compounds that interfere with the catalytic PARP domain either the nicotinamide pocket or the induced pocket (adenosine), or both [8]. These TNKS inhibitors showed antitumour efficacy by upregulating AXINs and inhibiting the Wnt/β-catenin pathway in *APC*-mutated CRC cell line and mouse models [15–19]. However, several TNKS inhibitors have not progressed beyond clinical trials due to their on-target toxicity in the gastrointestinal (GI) tract [14,19]. Therefore, it is essential to develop a next-generation TNKS inhibitor with better safety and pharmacokinetic (PK) profiles.

Here, we report that the novel TNKS-selective inhibitor STP1002 exhibits antitumour efficacy by stabilising AXINs and inhibiting the Wnt/β-catenin pathway in *APC*-mutated CRC cell line and mouse models. Notably, our data showed that STP1002 have notable potential as a drug, with orally available PK profiles that lack on-target toxicity in the GI tract in preclinical models.

2. Materials and methods

Detailed experimental procedures and a list of materials used are provided in Supplementary Materials and Methods.

2.1. Compound formulation

STP1002 is tentatively classified as Biopharmaceutics Classification System class II, which has low solubility and high permeability. Amorphous solid dispersion technology was used to increase the solubility and bioavailability of STP1002 [20]. STP1002M−1 and STP1002M−2 were formulated with different ratios of STP1002 and excipients. STP1002 was used for DLD-1, RKO and HCT-116 xenograft models. STP1002M−1 was used for the patient-derived xenograft model and STP1002M−2 was used for the Colo320DM xenograft model.

2.2. Statistical analysis

A two-tailed Student's t-test was performed to analyse the statistical differences between the groups. Data were statistically analysed by one-way ANOVA, with post hoc Bonferroni's multiple comparison tests. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using Excel and XLSTAT software. To identify whether we had sufficient subjects to detect with statistics, a post-hoc power analysis was performed after the study using G-Power 3.1 software (https://download.cnet.com/s/g-power).

3. Results

3.1. STP1002 is a potent TNKS1/2-selective inhibitor

STP1002 [5-(4-(2,6-difluoro-4-(2-methoxyethoxy)phenyl) piperazin-1-yl)-3-methyl-3,6-dihydro-7H-[1,2,3]triazolo [4,5-d]pyrimidin-7-one] is a novel analogue of nicotinamide adenine dinucleotide site inhibitors of TNKS1/2 (Fig. 1A). It was designed using an information-driven approach followed by a structure−property relationship study [21]. The crystal structures of TNKS1 and TNKS2 in complex with STP1002 showed that the compound binds strongly to the nicotinamide pocket and weakly with the induced pocket (adenosine) (Fig. 1A). STP1002 showed potent inhibition activities towards TNKS1/2 (IC_{50} = 5.8 nM for TNKS1 and 3.2 nM for TNKS2, respectively) and excellent selectivity against PARP1/2 compared to XAV939 (Figs. 1B and C) [10]. In addition, STP1002 did not substantially modulate receptor binding for 68 receptors, inhibit enzyme activity or have cellular agonist or antagonist function for 468 panels in a battery of radioligand binding assays (Lead profiling screen by Eurofins) at 2 μM and 468 human kinases at 1 μM (Supplementary Fig. 1). Furthermore, we also observed that STP1002 significantly inhibited Wnt/β-catenin signalling (IC_{50} = 8.3 nM) compared to XAV939 and IWR-

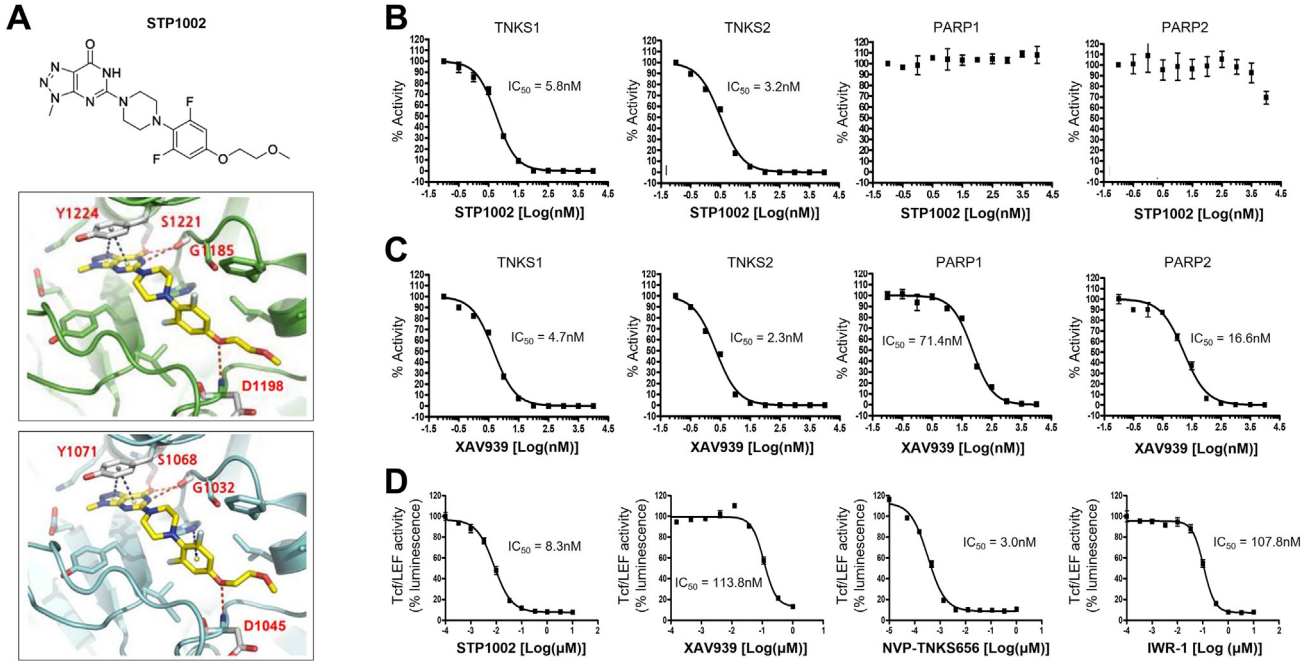

Fig. 1. STP1002 is a novel tankyrase1/2-selective inhibitor. (A) Chemical structure of STP1002 (*upper panel*). Crystal structures of TNKS1 (*middle panel*) and TNKS2 (*lower panel*) in complex with STP1002. (B), (C) Dose−response curves for the inhibition of TNKS1/2 or PARP1/2 activities with STP1002 (B) or XAV939 (C), respectively. Dose ranges were 0.3 nM−10 μM in the assay. (D) Dose−response curves for Tcf/LEF activities with STP1002, XAV939, IWR-1 or NVP-TNKS656. Value of each concentration is presented as the mean ± standard deviation of three biological replicates (n = 3).

1 (Fig. 1D). Thus, our data indicate that STP1002 is a potent and selective inhibitor of TNKS1/2.

3.2. STP1002 inhibits Wnt/β-catenin signalling in APC-mutated CRC cell lines

It is well established that TNKS inhibitors block the growth of Wnt/β-catenin-dependent *APC*-mutated CRC cells with the cytostatic mode of growth inhibition [8,19,22], therefore, the inhibitory effect on cell growth of STP1002 was evaluated using *APC*-mutant or *APC* wild-type CRC cell lines. Colo320DM, Colo320 H SR, DLD-1, HT-29, LS-1034, SW620, SW480 and HCT-15 cells were *APC*-mutant, while HCT116 and RKO cells were *APC* wild-type. XAV939 and NVP-TNKS656 were used as positive controls in our experiments [10,18]. Similar to the effect of positive control, STP1002 inhibited colony formation in *APC*-mutated CRC cell lines but not in inhibitor-resistant HCT-15 cells [22] and *APC*-wild-type cells (Table 1 andSupplementary Fig. 2). Next, the protein levels of Wnt/β-catenin signalling components were evaluated. STP1002 upregulated AXINs (AXIN2, especially) and phosphorylated β-catenin (i.e. inactive β-catenin) and downregulated non-phosphorylated β-catenin (i.e. active β-catenin) in *APC*-mutated CRC cells (Fig. 2A). In contrast, in the HCT-15 cells and *APC* wild-type cells, STP1002 upregulated AXINs but failed to downregulate active β-catenin (Fig. 2A). The TCF/LEF reporter assay and immunofluorescence analysis for the accumulation of nuclear active β-catenin also showed the similar results as Fig. 2A (Fig. 2B–E andSupplementary Fig. 3). Since Wnt regulates colon cancer stem cells and is regulated by the microenvironment [22–25], the antitumour activity of STP1002 was further investigated by performing 3D culture. Consistently, STP1002 inhibited the growth of Colo320DM cells under both Wnt3a stimulation and control (Fig. 2F). However, RKO cells did not respond to STP1002 (Fig. 2G). Collectively, these results demonstrate that STP1002 inhibits Wnt/β-catenin signalling in *APC*-mutated CRC cells.

3.3. STP1002 has favourable PK profiles with good in vitro absorption, distribution, metabolism and excretion properties

The *in vitro* absorption, distribution, metabolism and excretion (ADME) properties of STP1002 were analysed next. STP1002 showed high permeability across the Caco-2 cell monolayer; stable metabolism profiles for microsome and plasma in humans, monkeys, dogs and rats; no inhibition activities for cytochrome P450 enzymes (Supplementary Fig. 4A). In addition, STP1002 administration did not lead to any noticeable inhibition of hERG channels, indicating that STP1002 has excellent *in vitro* ADME properties. PK analysis of the single administration of STP1002 showed that STP1002 was rapidly absorbed and maintained moderately well in rats and dogs. STP1002 also showed intermediate oral bioavailability, indicating that STP1002 showed a favourable PK profile in both rodent and non-rodent models (Supplementary Fig. 4B). Therefore, STP1002 proved to possess a favourable PK profile with good *in vitro* ADME properties.

3.4. STP1002 exhibits antitumour efficacy in APC-mutated CRC preclinical tumour models

The *in vivo* efficacy of STP1002 was further evaluated in both *APC*-mutated and *APC*-wild-type tumour xenograft models and in *APC*-mutated CRC patient–derived tumour xenograft (PDX) models. STP1002 inhibited the tumour growth of the *APC*-mutated Colo320DM xenograft model in a dose-dependent manner (5 mg/kg orally (PO) once daily (QD); TGI: 30% or 10 mg/kg PO, QD; TGI: 51%) (Fig. 3A). Increased levels of AXINs and phosphorylated β-catenin with a decreased expression of active β-catenin (Fig. 3C) and downregulated

Table 1
Tankyrase inhibitors block APC-driven CRC colony formation.

APC mutant	APC mutation	β-catenin	XAV939	NVP-TNKS656	STP1002
			Colony forming assay (%)	Colony forming assay (%)	Colony forming assay (%)
Colo320DM	S811X	WT	39.7	22.4	18.9
Colo320HSR	S811X	WT	72.0	88.0	73.0
DLD-1	R727M, K993N, G1417fs*2, R2166X	WT	54.5	57.4	65.1
LS-1034	E1309fs*4	WT	29.1	37.5	46.3
HT-29	E853X, T1556fs*3	WT	50.3	46.4	59.1
SW620	Q1338X	WT	49.1	46.7	40.4
SW480	Q1338X	WT	54.7	34.5	35.7
HCT-15 APC WT	R727M, K993N, I1417fs*2, R2166X	WT	NR	NR	NR
HCT116	WT	MT	NR	NR	NR
RKO	WT	WT	NR	NR	NR

NR: no response. Detailed results are provided in Supplementary Figure 2.

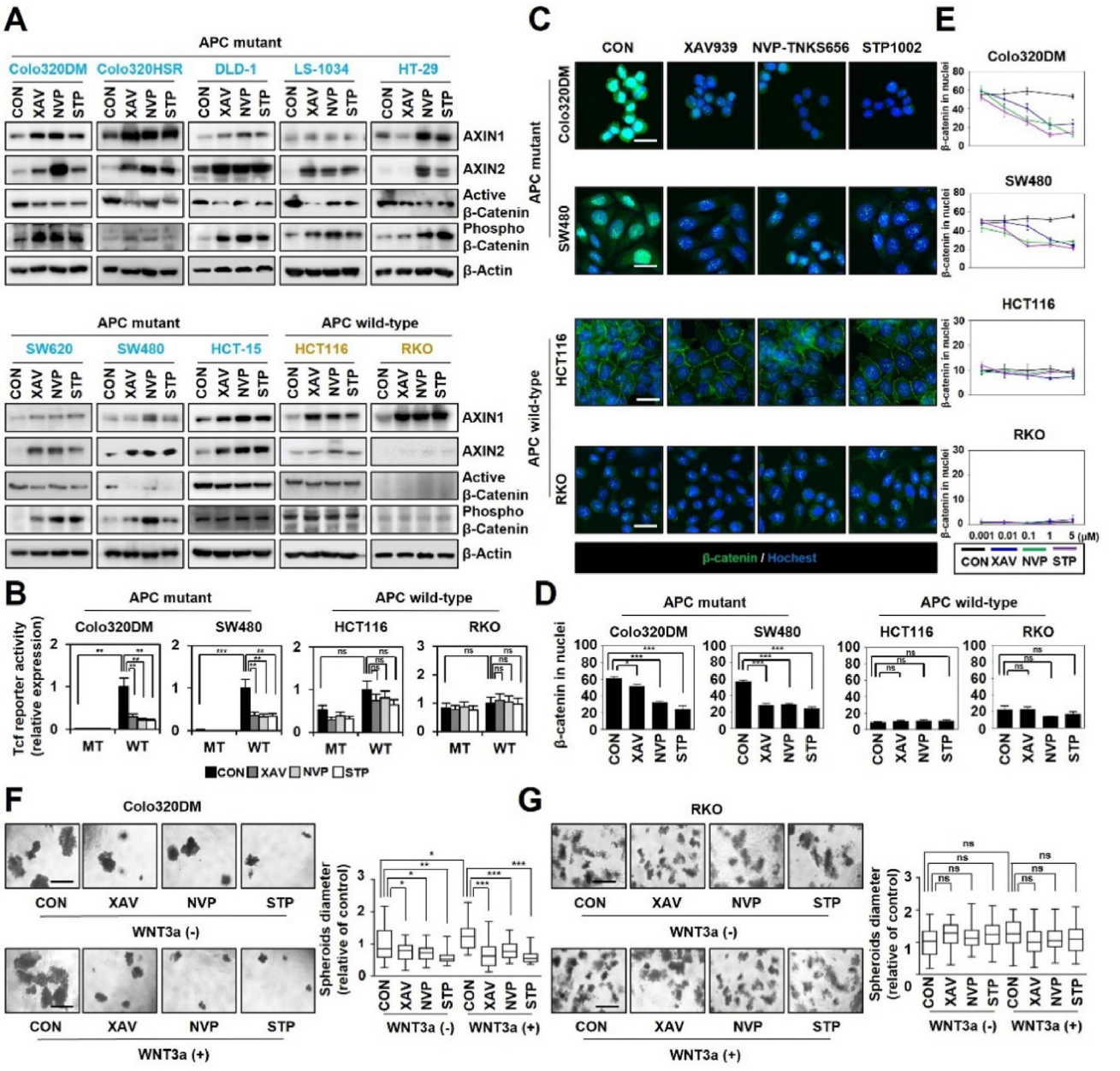

Fig. 2. STP1002 inhibits Wnt/β-catenin signalling in *APC*-mutated CRC cell lines. (A) APC-mutated and APC-wild-type CRC cell lines were treated with 5 μM XAV939, NVP-TNKS656 and STP1002 for 48 h. Cells were analysed by immunoblotting with the indicated antibodies. (B) Cells were treated with 5 μM XAV939, NVP-TNKS656 or STP1002 for 48 h after transfection with the reporter vectors. Tcf reporter activity was determined using a luciferase reporter assay. (C) Immunofluorescence staining of active-(non-phosphorylated) β-catenin after 5 μM XAV939, NVP-TNKS656 and STP1002 treatment of cells for 48 h. (D) Nuclear active-β-catenin levels were quantified using the INCELL analyser. (E) Nuclear active-β-catenin levels were quantified using the INCELL analyser with the indicated doses of XAV939, NVP-TNKS656 and STP1002 treatment for 48 h. (F) 3D spheroid images (*left panel*) and diameter (*right panel*) of Colo320DM after 7 days of 5 μM XAV939, NVP-TNKS656 and STP1002 with Wnt3a (200 ng/μl) treatments. (G) 3D spheroid images (left panel) and diameter (right panel) of RKO after 7 days of treatment with 5 μM XAV939, NVP-TNKS656 and STP1002 with Wnt3a (200 ng/μl) treatments. All data represent typical results and are presented as the mean ± standard deviation of three biological replicates (n = 3). We used t-tests for two-group comparisons and ANOVA for multi-group comparisons. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Ns: not significant. Scale bars: 30 μm. WT, pTcf7wt-luc; MT, pTcf7mt-luc. CON: control, XAV: XAV939, NVP: NVP-TNKS656, STP: STP1002. APC, adenomatous polyposis coli; CRC, colorectal cancer.

Wnt/β-catenin-activated genes, including *APCDD1*, *NKD1*, *AXIN2*, TNF receptor superfamily member 19 (*TNFRSF19*), *ASCL2* and leucine rich repeat containing G protein-coupled receptor 5 (*LGR5*) (Fig. 3D), were clearly observed in STP1002-treated tumour tissues. Consistently, Wnt signalling–targeted genes were also downregulated (Fig. 3E). The expression level differences of some Wnt signalling-regulated genes between

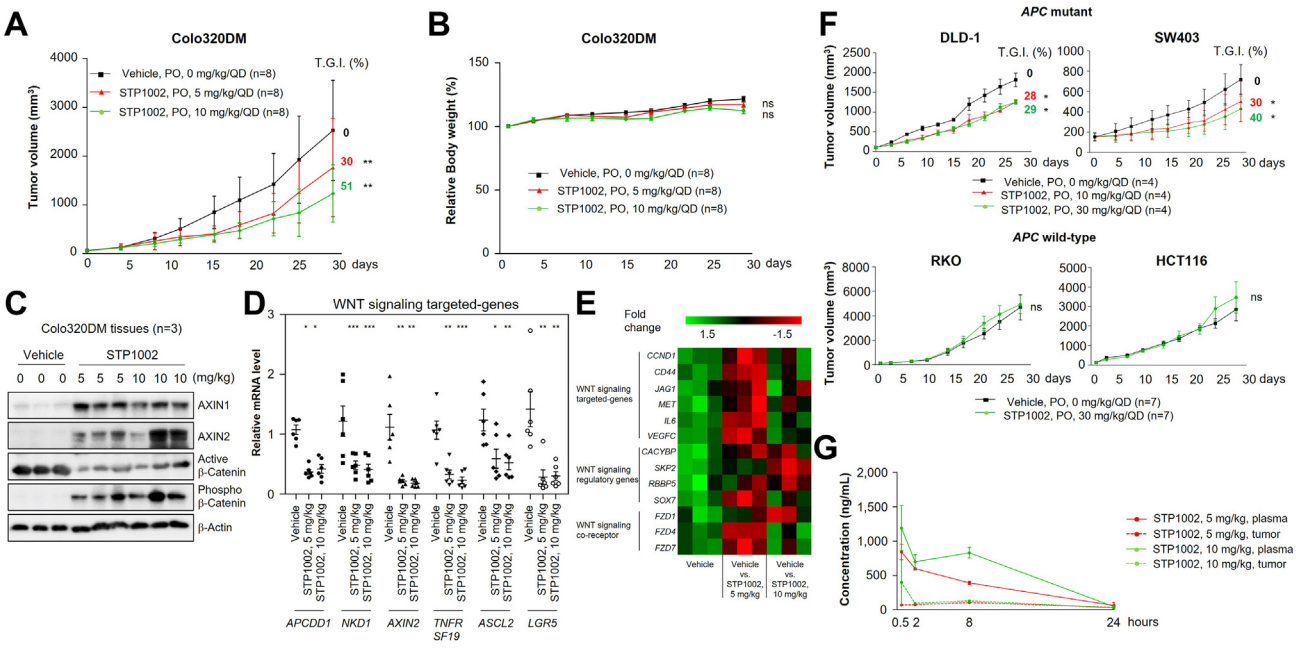

Fig. 3. STP1002 reduces the growth of *APC*-mutated CRC xenograft models by inhibiting Wnt/β-catenin signalling. (A), (B) Colo320DM xenograft tumour volume (A) and relative body weight (B) after STP1002 PO/QD dosing for the duration of the study. Eight mice were used in each group. (C) Vehicle- and STP1002-treated tumour samples were analysed by immunoblotting with the indicated antibodies. Three mice were randomly selected from eight mice in each group (n = 3). (D) qPCR gene expression analysis of Wnt/β-catenin genes in vehicle- and STP1002-treated tumour samples. Six mice were randomly selected from eight mice in each group for mRNA isolation. (E) nCounter analysis of Wnt/β-catenin genes in vehicle- and STP1002-treated tumour samples. Heatmap shows the differentially expressed mRNAs found in vehicle-versus STP1002-treated (5 mg/kg or 10 mg/kg) tumour samples. The colour pattern is log2-transformed normalised mRNA count, including 13 Wnt signalling-related genes. Three mice were randomly selected from eight mice in each group (n = 3). Green indicates high expression, and red indicates low expression. (F) DLD-1 and SW403 (APC-mutated; upper panel) or RKO and HCT116 (APC wild-type: lower panel) xenograft tumour volume after STP1002 PO/QD dosing for the duration of the study. (G) Pharmacokinetic parameters of STP1002 (mouse oral administration 5 or 10 mg/kg) in plasma and tumours. We used t-tests for two-group comparisons and ANOVA for multi-group comparisons. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Ns: not significant. TGI: tumour growth inhibition. PO: oral administration. QD: daily administration. APC, adenomatous polyposis coli; CRC, colorectal cancer.

the 5 and 10 mg/kg group may be due to the individual difference. DLD-1 and SW403 xenograft models also showed the similar effects as Fig. 3a (Fig. 3F). In contrast to *APC*-mutated xenograft models, HCT116 and RKO xenograft models were insensitive to STP1002 treatment (Fig. 3F). Notably, we observed no changes in body weight (Fig. 3B andSupplementary Fig. 5A and B).

PK parameters were also evaluated in mouse xenograft models. The exposure of STP1002 in plasma was 18.75 μM and 31.62 μM at 5 mg/kg and 10 mg/kg, respectively, and in both peaks, occurred at 0.5 h following administration of STP1002 (Fig. 3G and-Supplementary Table 1). The concentration ratios of tumour to plasma (T/P ratios) of STP1002 based on area under the plasma concentration−time curve (AUC) were 0.22 and 0.18 at 5 mg/kg and 10 mg/kg, respectively. In addition, following single oral administration of STP1002, 30 mg/kg was absorbed with a median T_{max} of 0.5 h. After reaching the maximum concentration (C_{max}), the plasma concentration of STP1002 decreased with a terminal half-life ($t_{1/2}$) of 4.87 h (Supplementary Table 2). STP1002 was rapidly absorbed in the plasma and tumour and was maintained for up to 24 h at detectable levels, indicating that STP1002 shows a favourable PK profile in mice.

The *in vivo* efficacy was further tested in *APC*-mutated (R1450X) CRC PDX models, a mutation cluster region known to be sensitive to TNKS inhibition [26]. STP1002 reduced tumour growth in a CRC PDX model (Fig. 4A and C). The increased AXIN2 and decreased active β-catenin levels were determined via immunohistochemistry in STP1002-treated tumour tissues (Fig. 4D). We also observed no noticeable changes in body weight (Fig. 4B). Collectively, our results indicate that STP1002 exhibits antitumour efficacy in *APC*-mutated CRC preclinical tumour models without significant toxicity.

3.5. STP1002 shows no on-target toxicity in the GI tract

As therapeutic agents targeting the Wnt pathway, including TNKS inhibitors, are often associated with

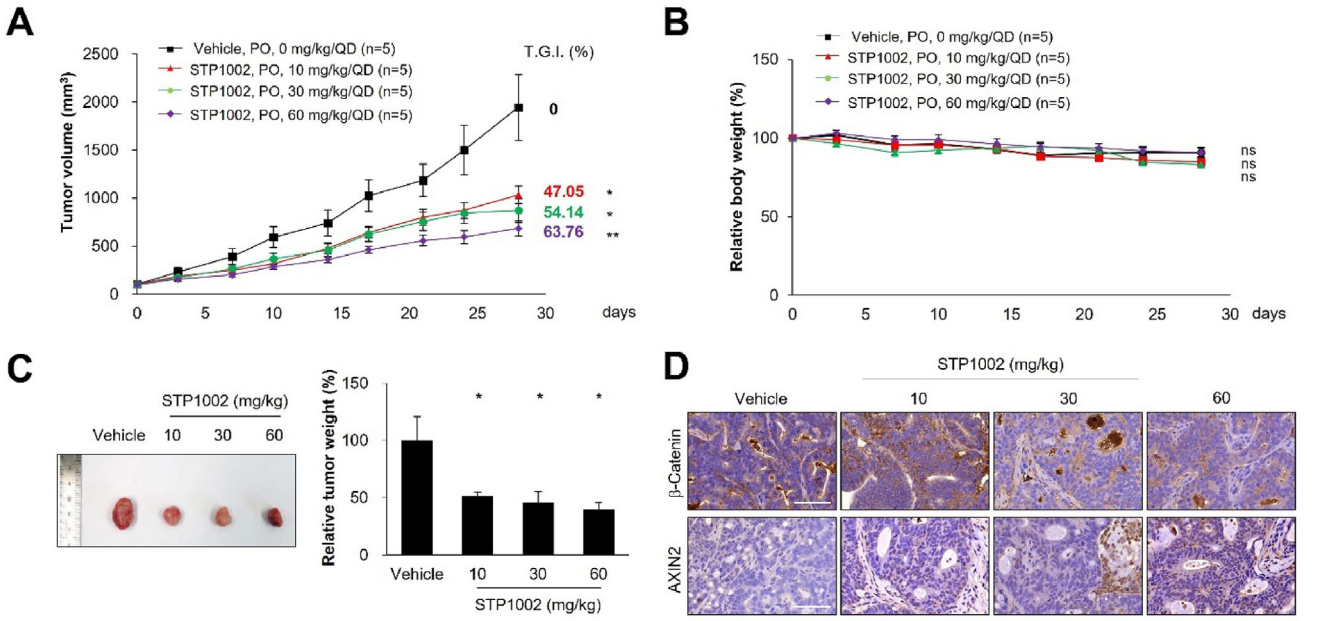

Fig. 4. STP1002 reduces the growth of *APC*-mutated PDX tumour models. (A), (B) CRC PDX tumour volume (A) and relative body weight (B) after STP1002 PO/QD dosing for the duration of the study. (C) PDX tumour images (*left panel*) and relative tumour weights (*right panel*). (D) Immunohistochemical analysis of β-catenin and AXIN2 in the indicated tumour samples. Five mice per group were used. Indicated levels of protein expression were quantified and confirmed using ImageJ. We used t-tests for two-group comparisons and ANOVA for multi-group comparisons. $*P < 0.05$, $**P < 0.01$. Scale bars: 20 μm. APC, adenomatous polyposis coli; CRC, colorectal cancer; ns: not significant; PDX, patient-derived tumour xenograft; PO: oral administration. QD: daily administration.

several toxicities [27], the toxicity of STP1002 was evaluated through four-week repeat-dose good laboratory practice toxicology studies in rats and dogs. The histopathological analysis indicated that STP1002 did not elicit any morphological indicator of toxicity, especially in the small intestine, in STP1002-treated rats and dogs (Fig. 5A and C). In addition, there were no gender differences of body weight (Fig. 5B and D) suggesting that the therapeutic dose ranges of STP1002 did not induce any toxicity in rats and dogs.

Since TNKS inhibitors such as G007-LK show toxicities [14,19], we used G007-LK as a reference compound in a Colo320DM xenograft model to further evaluate the on-target toxicity effects of STP1002 [19]. Indeed, G007-LK significantly reduced body weight within 8 days and killed all mice at 15 days (Fig. 6A). In contrast, STP1002 did not change the body weight of mice during treatment for 28 days (Fig. 6A). Histopathological analysis of the STP1002- or G007-LK-treated ileums showed that STP1002 did not change the morphology of the ileum compared to G007-LK (Fig. 6B). The intestinal Wnt-activated genes (*Axin2* and *Tnfrsf19*), intestinal stemness genes (*Lgr5, olfactomedin 4 (Olfm4),* and *Ascl2*), intestinal differentiated genes (fatty acid binding protein 2 (*Fabp2*), trefoil factor 3 (*Tff3*) and keratin 20 (*Krt20*)) and inflammatory genes (interleukin-1β (*Il-1β*), interferon-γ (*Ifn-γ*), and *Il-10*) were further analysed in both STP1002- and G007-LK-treated ileums. Transcript analysis indicated that STP1002 inhibited the intestinal Wnt-activated genes and intestinal stemness genes (Fig. 6C). Interestingly, STP1002 neiher inhibit intestinal proliferation marker *MKI67* [19] nor induce a pro-inflammatory response [28], compared to G007-LK (Fig. 6C). In addition, STP1002 or G007-LK did not affect intestinal differentiated genes (Supplementary Fig. 6). Notably, STP1002 inhibited the Wnt and Hedgehog signalling pathways, while G007-LK significantly inhibited global pathways (Fig. 6D), suggesting that STP1002 selectively inhibited the Wnt pathway in ileum tissue. Therefore, our data demonstrated that STP1002 shows no on-target toxicity in the GI tract, especially the ileum.

4. Discussion

Although TNKSs are validated druggable targets for Wnt/β-catenin-driven cancers such as CRC, the reported on-target toxicity of previously designed TNKS inhibitors in the GI tract is a major barrier to the development of clinically available TNKS inhibitors. Thus, new TNKS inhibitors with better efficacy and improved toxicity profiles are required for clinical trials. Our data showed that STP1002 (a novel, potent and selective TNKS1/2 inhibitor) effectively stabilised AXINs, leading to the inhibition of the WNT/β-catenin pathway in *APC*-mutated CRC cells. In addition, STP1002 showed favourable PK profiles following 5–30 mg/kg once-daily oral administration and antitumour efficacy in *APC*-

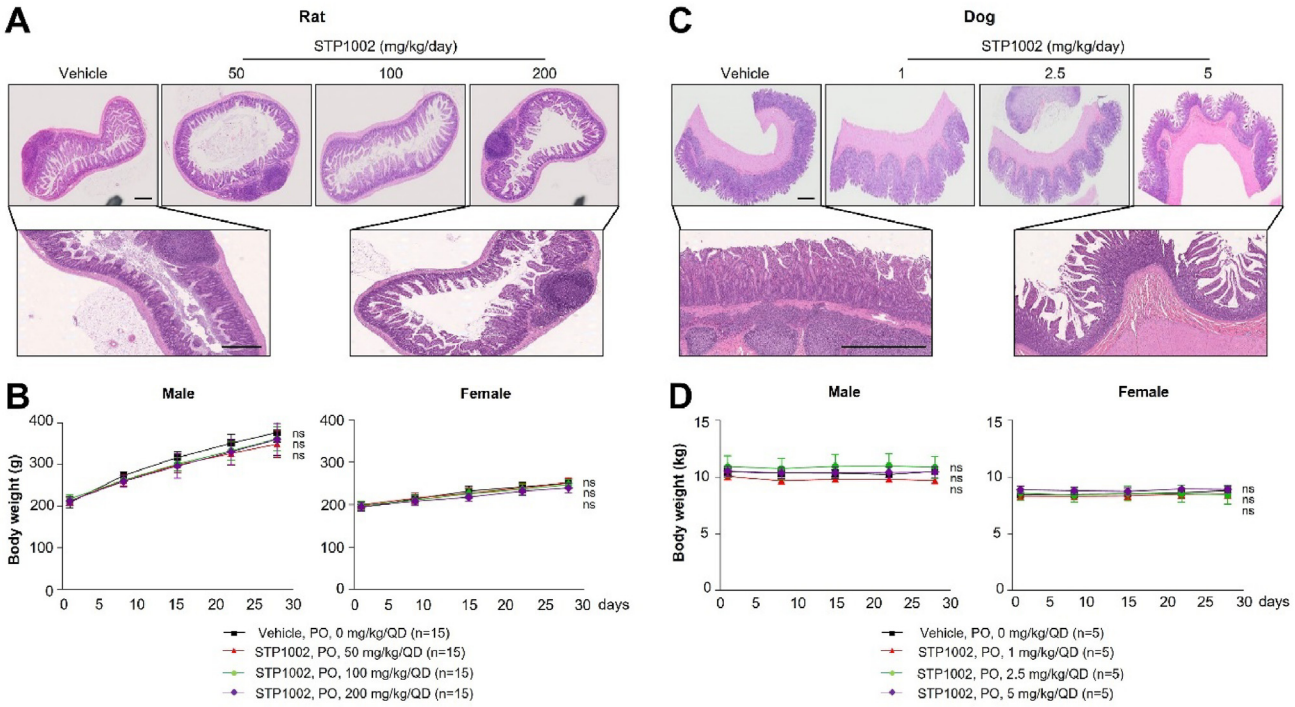

Fig. 5. STP1002 does not induce intestinal toxicity in rat and dog. (A), (B) Haematoxylin and eosin staining of intestinal sections (A, *upper panel*) enlarged images of vehicle and 200 mg/kg sections (A, *lower panel*), and body weight (B) from the rat after STP1002 PO/QD dosing for the duration of the study. Fifteen rats were used per group (C), (D) Haematoxylin and eosin staining of intestine sections (C, *upper panel*), enlarged images of vehicle-treated and 5 mg/kg STP1002-treated sections (C, *lower panel*) and body weight (D) from the dog after treatments PO/QD dosing for the duration of the study. Five dogs were used per group. We used t-tests for two-group comparisons and ANOVA for multi-group comparisons. Ns: not significant. PO: oral administration. QD: daily administration. Scale bars: 5 μm.

mutated CRC preclinical models. Importantly, we found that STP1002 demonstrated a good safety profile with no on-target toxicity in the GI tract. Therefore, our data provide PK and pharmacodynamic evidence of STP1002 as a strong candidate in clinical trials for TNKS-targeted drugs.

Since TNKS1/2 are members of the PARP protein superfamily that share a catalytic PARP domain, it is important to design a small molecule that inhibits only the catalytic PARP domain of TNKS but does not inhibit other PARP subfamily proteins. Compared with several TNKS inhibitors that show cross−inhibitory activity against PARP1 and/or PARP2 [10,18,29], STP1002 was a highly selective and potent TNKS1 and TNKS2 inhibitor.

In vivo preclinical studies have shown the antitumour activity of various TNKS inhibitors [15,16,19,30–32]. Compared to these studies, our preclinical data showed that STP1002 inhibited tumour growth in Colo320DM, SW403, DLD1 xenograft models and CRC PDX models. It is noteworthy that approximately 30% of TGI was observed in the 5 mg/kg STP1002-treated mice, suggesting a superior therapeutic efficacy compared to that of other TNKS inhibitors. Thus, our data suggest that STP1002 has potent antitumour efficacy at a relatively low therapeutic dose.

On-target toxicity in the GI tract is a major challenge for many Wnt/β-catenin pathway inhibitors, including TNKS inhibitor [27]. Previously reported TNKS inhibitors showed several toxicities, such as intestinal toxicity, body weight loss and even death in rodents [14,19]. For instance, G007-LK induced severe necrosis, inflammation and crypt loss in the small intestine, especially in the ileum, resulting in weight loss and morbidity in mice [19]. Similarly, mice treated with G-631 also demonstrated body weight loss with reddened, oedematous and/or fluid-filled intestinal segments [14]. In contrast, JW74 and RK-287107 did not induce noticeable body weight changes at therapeutic doses. However, it remains unclear whether these inhibitors have no toxicity as there is insufficient toxicological data, including long-term toxicity and intestinal histopathological analysis [16,30]. Compared to these observations, our preclinical data clearly showed that STP1002 did not induce body weight loss and intestinal toxicity in rats, mice and dogs. Furthermore, our pathology and molecular analysis of STP1002-treated tissues in mice also showed that STP1002 did not inhibit intestinal cell proliferation, inflammation and intestinal stem cells compared to G007-LK. As the selectivity of a new drug is important for safety [33], the absence of intestinal toxicity of STP1002 could be associated with

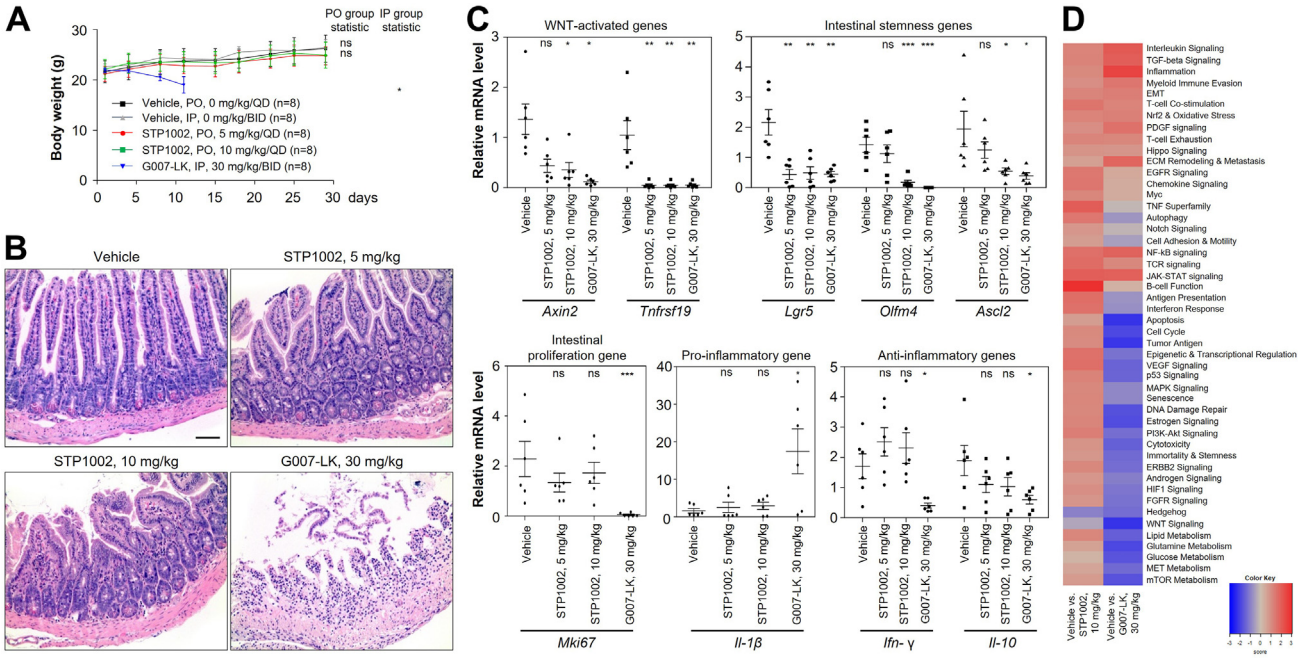

Fig. 6. STP1002 does not induce ileum toxicity in mouse. (A) Body weight changes after STP1002 PO/QD or G007-LK IP/BID dosing for the duration of the study. Eight mice were used per group. (B) Haematoxylin and eosin staining of ileum sections from mice after STP1002 or G007-LK treatment. (C) qPCR gene expression analysis for Wnt-activated genes, intestinal stemness genes, intestinal proliferation genes, and inflammatory genes in vehicle-, STP1002- and G007-LK-treated ileum samples. Six mice were randomly selected from eight mice in each group for mRNA isolation. (D) nCounter analysis of pan signalling pathways in vehicle- and STP1002-treated ileum samples. The heatmap shows each group's global significant pathway scores in vehicle versus STP1002- or G007-LK-treated ileum samples. Three mice per group were used. Red and blue denote sets of genes displaying upregulated and downregulated expression, respectively. We used t-tests for two-group comparisons and ANOVA for multi-group comparisons. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Ns: not significant. IP: intraperitoneal. PO: oral administration. QD: daily administration. BID: twice a day. Scale bar: 10 μm.

its selectivity to the Wnt pathway. Indeed, our transcript analysis clearly showed that STP1002 selectively inhibited Wnt and Hedgehog pathways compared to G007-LK, which significantly inhibited global pathways [34]. In addition, STP1002 showed lower concentrations in the tumour than in the blood compared to other TNKS inhibitors such as G-631 and RK-287107, and C_{max} values of STP1002 at the therapeutic doses were relatively lower than that of G-631 and RK-287107 [14,35]. Thus, our data suggest that the selective TNKS1/2-mediated inhibition of the Wnt pathway and/or the optimum PK profile of STP1002 may be associated with the absence of intestinal toxicity.

In conclusion, STP1002, a new orally active TNKS inhibitor, shows preclinical antitumor efficacy without on-target toxicity in the GI tract. Our data provide preclinical evidence that STP1002 could be used as a potential TNKS-targeted drug in patients with *APC*-mutated CRC. STP1002 has recently been used in phase I clinical trials (NCT04505839).

Authors contributions

D.Y.Kim: Conceptualisation, resources, data curation, formal analysis, validation, investigation, methodology, visualisation, writing—original draft, writing—review and editing. Y-J. Kwon: Data curation, formal analysis, validation, investigation, visualisation. W.Y.Seo: Data curation, formal analysis, investigation, methodology, writing—original draft. S. Ahn: data curation, resources, methodology, writing—original draft. U—I. Kim: Investigation, methodology, writing—original draft. S.M. Choi: Data curation, methodology. H.T. Bang: Investigation, methodology. K. Kim: Conceptualisation, resources, data curation, formal analysis, supervision, funding acquisition, project administration. J-S. Kim: Conceptualisation, resources, data curation, formal analysis, supervision, funding acquisition, validation, investigation, visualisation, methodology, writing—original draft, project administration, writing—review and editing

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Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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