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The oral VEGF receptor tyrosine kinase inhibitor pazopanib in combination with the MEK inhibitor trametinib in advanced cholangiocarcinoma

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Background: Cholangiocarcinoma is an aggressive malignancy with limited therapeutic options. MEK inhibition and antiangiogenic therapies have individually shown modest activity in advanced cholangiocarcinoma, whereas dual inhibition of these pathways has not been previously evaluated. We evaluated the safety and efficacy of combination therapy with the oral VEGF receptor tyrosine kinase inhibitor pazopanib plus the MEK inhibitor trametinib in patients with advanced cholangiocarcinoma.

Methods: In this open-label, multicentre, single-arm trial, adults with advanced unresectable cholangiocarcinoma received pazopanib 800 mg daily and trametinib 2 mg daily until disease progression or unacceptable toxicity. The primary end point was progression-free survival (PFS) with secondary end points including overall survival (OS), response rate, and disease control rate (DCR).

Results: A total of 25 patients were enrolled and had received a median of 2 prior systemic therapies (range 1–7). Median PFS was 3.6 months (95% CI: 2.7–5.1) and the 4-month PFS was 40% (95% CI: 24.7–64.6%). There was a trend towards increased 4-month PFS as compared with the prespecified null hypothesised 4-month PFS of 25%, but this difference did not reach statistical significance ($P = 0.063$). The median survival was 6.4 months (95% CI: 4.3–10.2). The objective response rate was 5% (95% CI: 0.13–24.9%) and the DCR was 75% (95% CI: 51%, 91%). Grade 3/4 adverse events attributable to study drugs were observed in 14 (56%) and included thrombocytopenia, abnormal liver enzymes, rash, and hypertension.

Conclusions: Although the combination of pazopanib plus trametinib had acceptable toxicity with evidence of clinical activity, it did not achieve a statistically significant improvement in 4-month PFS over the prespecified null hypothesised 4-month PFS.

Cholangiocarcinoma refers to cancers of the bile duct that arise in the intrahepatic, perihilar, or distal (extrahepatic) biliary tree. Cholangiocarcinoma is a relatively rare cancer, accounting for ~3% of all gastrointestinal malignancies, although the incidence of intrahepatic cholangiocarcinoma is increasing globally (Patel, 2001; Khan *et al*, 2008; Siegel *et al*, 2016). The majority of patients with

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cholangiocarcinoma have unresectable disease at the time of presentation, with <5% of all patients surviving to 5 years (Shaib and El-Serag, 2004). Gemcitabine plus cisplatin is the standard of care first-line regimen for locally advanced or metastatic disease (Valle *et al*, 2010). No chemotherapy regimen has conclusively shown benefit in patients progressing after initial chemotherapy, and multiple retrospective studies suggest a progression-free survival (PFS) on second-line chemotherapy of 2–3 months (Lamarca *et al*, 2014; Rogers *et al*, 2014). Recent efforts have focussed on developing novel therapies for this disease.

The RAF/MEK/ERK signalling pathway is involved in the regulation of normal cell proliferation, survival, and differentiation, and this pathway is frequently aberrantly upregulated in a wide number of cancers including cholangiocarcinoma (Yoon *et al*, 2004; Roberts and Der, 2007; Schmitz *et al*, 2007; Wang *et al*, 2009). Alterations in this pathway have been reported in up to 35% and 55% of intrahepatic and extrahepatic cholangiocarcinomas, respectively (Churi *et al*, 2014). The MEK inhibitors have previously shown modest signs of activity in cholangiocarcinoma. In a 28-patient phase 1 clinical trial, the MEK 1/2 inhibitor binimetinib (MEK162, ARRY438162) showed evidence of clinical efficacy with two objective responses (8% of subjects) and a 46% stable disease rate for a median duration of 5 months (Finn *et al*, 2012). Similarly, in a phase 2 clinical trial of the MEK1/2 inhibitor selumetinib (AZD6244, ARRY142886) in cholangiocarcinoma, 3 of 28 patients (12%) had a confirmed objective response and 17 of 28 patients (68%) had stable disease (Bekaii-Saab *et al*, 2011).

The vascular endothelial growth factor (VEGF) pathway is a principal mediator of tumour angiogenesis and is also implicated in the growth and metastasis of many cancers including cholangiocarcinoma (Leung *et al*, 1989; Folkman, 1990; Benckert *et al*, 2003; Park *et al*, 2006; Yoshikawa *et al*, 2008; Goel and Mercurio, 2013). In a retrospective pathologic study of 236 cases of cholangiocarcinoma, overexpression of VEGF was noted in more than half of all cases (Yoshikawa *et al*, 2008). Several small studies have previously been conducted with inhibitors of VEGF signalling in cholangiocarcinoma, with modest signs of activity. A phase 2 study of single agent sorafenib, a multitargeted kinase inhibitor that inhibits VEGF signalling, reported a 32.6% disease control rate at 12 weeks (Bengala *et al*, 2010). Limited antitumour activity was also reported in a separate phase 2 trial of sorafenib in cholangiocarcinoma (El-Khoueiry *et al*, 2012) and with other antiangiogenic agents: cabozantinib (Goyal *et al*, 2015), bevacizumab (Lubner *et al*, 2010), and sunitinib (Dreyer *et al*, 2015).

Although MEK inhibition and antiangiogenic therapies have each individually shown limited activity in cholangiocarcinoma, dual inhibition of these pathways has not been evaluated previously. Trametinib is an orally available highly specific inhibitor of MEK1 and MEK2 that is approved for BRAF V600E or V600K mutation-positive unresectable or metastatic melanoma (Flaherty *et al*, 2012; Infante *et al*, 2012; Robert *et al*, 2015). Pazopanib is an orally available multikinase inhibitor of VEGFR, PDGFR, KIT, FGFR, as well as RAF that is approved for advanced renal cell carcinoma and advanced refractory soft tissue sarcoma (Sternberg *et al*, 2010; Gril *et al*, 2011; van der Graaf *et al*, 2012). Together, pazopanib and trametinib provide vertical inhibition of the RAF/MEK/ERK signalling pathway through combined inhibition of RAF and MEK, as well as potent inhibition of VEGFR and PDGFR for inhibition of angiogenesis. Our group has demonstrated the synergistic effects of trametinib and pazopanib in thyroid cancer cell lines and xenograft models (Ball *et al*, 2015). In an exploratory phase 1 study in advanced solid tumours, we previously reported that pazopanib 800 mg daily and trametinib 2 mg daily was safe and tolerable (Azad *et al*, 2014). Two patients with cholangiocarcinoma were enrolled in our initial dose escalation study, with one patient attaining a prolonged partial response and the other patient with prolonged stable disease. Based on this initial signal of activity, we further evaluated the safety and efficacy of combination pazopanib plus trametinib in an expansion cohort of 25 pretreated patients with advanced cholangiocarcinoma.

MATERIALS AND METHODS

This was an open-label, multicentre (Sidney Kimmel Comprehensive Cancer Center (SKCCC) at John Hopkins University (JHU) and The University of Texas MD Anderson Comprehensive Cancer Center (MDACC)) trial supported by the National Comprehensive Cancer Network (NCCN). Patients >18 years old with advanced cholangiocarcinoma that was refractory to standard of care treatment options (or patients who refused standard of care treatment options) were eligible. Other eligibility criteria included the presence of Response Evaluation Criteria in Solid Tumors (RECIST) criteria 1.1 measurable disease (Eisenhauer *et al*, 2009), an Eastern Cooperative Oncology Group performance status ⩽1 and adequate organ function as defined by absolute neutrophil count ⩾1500 cells per μl, platelet count ⩾100 000 cells per μl, international normalised ratio ⩽1.2 × upper limit of normal (unless stabilised with anticoagulation therapy and within the recommended range for the desired level of anticoagulation), total bilirubin ⩽1.5 × upper limit of normal (or, in patients with Gilbert syndrome, total bilirubin >1.5 × as long as direct bilirubin is normal), and serum creatinine ⩽1.5 × upper limit of normal or creatinine clearance ⩾45 ml min^{-1}, and urine protein to creatinine ratio <1 (or, if >1, 24-h urine protein <1 g).

Evaluation and treatment. The protocol was approved by the institutional review board (IRB) at both study sites, and complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects and the Declaration of Helsinki. The study drugs (trametinib and pazopanib) were provided by GlaxoSmithKline (Brentford, UK) (eventually Novartis). Eligible patients were enrolled centrally at the SKCCC at JHU. The trial was registered under ClinicalTrials.gov as NCT01438554. All patients provided written informed consent before enrolment.

Patients received 800 mg of pazopanib and 2.0 mg of trametinib orally daily every day of a 28-day cycle, a dose that was established in our initial dose escalation study across multiple tumour types. The treatment protocol allowed dose delays or reduction if patients experienced unacceptable side effects and adverse reactions. Patients were evaluated every cycle for trial therapy compliance and monitoring of adverse events. The National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 was implemented for adverse event monitoring (National Institute of Cancer, 2009). Disease assessments (computed tomography or magnetic resonance imaging) were performed every other cycle. Response was evaluated according to the RECIST, version 1.1 (Eisenhauer *et al*, 2009). Upon progression of disease, patients were monitored for long-term adverse events, new primary tumours, and survival.

Statistical methods. The primary outcome measure was 4-month PFS rate. Secondary outcome measures included overall survival (OS) duration and disease control rate (DCR), defined as the percentage of patients with no disease progression (complete response, partial response, or stable disease) by RECIST as a best response to therapy. Proportions are reported with exact 95% binomial confidence intervals (CIs). Event time distributions for OS and PFS were estimated using the Kaplan–Meier method (Kaplan and Meier, 1958) and CIs were calculated using the Brookmeyer–Crowley method. Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) and R version 3.0. To determine the distribution of the follow-up times if no patient had died, we reversed the coding of the OS censoring

indicator and censored deaths, and the previously censored patients were considered events. The median follow-up for the study was calculated from this curve. A null 4-month PFS of 25% was pre-established as a benchmark for the treatment of patients included in our study, based on a prior phase 2 study of sorafenib in unresectable cholangiocarcinoma that reported a median PFS of 2.3 months and a 4-month PFS of 25% (Bengala *et al*, 2010). We aimed to demonstrate a 4-month PFS that was significantly higher than this historical benchmark. For the purposes of monitoring, a nonparametric Kaplan–Meier estimate at 4 months was utilised with a planned interim analysis for futility after 10 of 25 patients were enrolled.

RESULTS

Patients. From September 2013 until September 2014, 25 patients with advanced cholangiocarcinoma were enrolled at MD Anderson Cancer Center ($n=18$ patients) and Johns Hopkins University ($n=7$) in this expansion cohort of pazopanib plus trametinib. Although the original study design included an interim analysis for futility, the interim analysis was not able to be performed due to rapid study accrual. The clinicopathological characteristics of the patients entered into this study are shown in Table 1. Patients had received a median of 2 prior systemic therapies for cholangiocarcinoma (range 1–7). Several patients had received prior antiangiogenesis therapy including sorafenib ($n=1$), sunitinib ($n=1$), or a bevacizumab-containing regimen ($n=4$). No patients had received prior therapy with a MEK inhibitor. All patients were treated according to the study protocol, and no patients remain on study at the time of this analysis.

Efficacy. Of the 25 patients enrolled in the study, 20 (80%) were evaluable for objective response. Five patients were not evaluable for objective response because they did not complete one 28-day cycle of therapy or did not have their disease re-evaluated after starting therapy. Responses ranged from progressive disease to a decrease in target lesions of 30% by RECIST 1.1 (see Figure 1). A partial response occurred in one (5%) of the evaluable patients (95% CI: 0.13–24.9%). This patient had a response to therapy that lasted ~6 months. The DCR was 15 out of 20 (75%) (95% CI: 51–91%). A total of five patients were determined to have progressive disease as a best response to therapy. Of these five patients, three patients had stable target lesions but in one case developed new lesions, and in the other two cases had a significant increase in the size of nontarget lesions. Of the five inevaluable patients, one patient withdrew consent for the study, one patient withdrew for treatment toxicity including thrombocytopenia and fatigue, and three patients were withdrawn from study for adverse events unrelated to therapy.

Figures 2 and 3 show the Kaplan–Meier curves for PFS and OS for all 25 study patients. The PFS and OS data have matured for all patients. The median PFS was 3.6 months (95% CI: 2.7–5.1). The 2-month PFS was 72% (95% CI: 56–92), and the 4-month PFS was 40% (95% CI: 24.7–64.6%). The 4-month PFS did not differ significantly from a prespecified null hypothesised 4-month PFS of 25%, $P=0.063$. The median survival was 6.4 months (95% CI: 4.3–10.2). The 2-, 4-, and 6-month OS rates were 88 (95% CI: 76–100%), 76 (95% CI: 61–95%), and 52 (95% CI: 36–76%), respectively.

Adverse events. The median duration of treatment was 12 weeks. Four patients (16%) discontinued treatment because of treatment toxicity. The median time to treatment discontinuation for these patients was ~8 weeks. The treatment dose of either pazopanib or trametinib was reduced in 9 of 25 (36%) patients due to adverse events. Of those who required a dose reduction, trametinib was reduced from 2 mg daily to 1.5 mg daily in 8 patients. One patient required further dose reduction to 1 mg daily, whereas one patient who initially required a dose reduction of trametinib due to a rash was able to resume full dosing of trametinib after resolution of the adverse event. The most common reason for a dose reduction of trametinib was a rash. Pazopanib was dose reduced from 800 to 600 mg in 6 patients. Two patients necessitated further dose reduction of pazopanib, to 400 mg daily in one patient and to discontinuation of pazopanib in another patient. Thrombocytopenia was the most common reason for dose reduction of pazopanib. Drug interruptions were required as a result of adverse events in 11 (44%) patients. The median duration of dose interruptions was 9 days. The most common reasons for dose interruptions were rash, hypertension, abnormal liver enzymes, thrombocytopenia, and gastrointestinal side effects including nausea, vomiting, or diarrhoea.

Table 1. Baseline patient characteristics

Characteristic	
Age, years	
Median	62
Range	38–80
Sex, number (%)	
Male	14 (56%)
Female	11 (44%)
Race, number (%)	
White	19 (76%)
Black	3 (12%)
Asian	1 (4%)
Unknown or other	2 (8%)
Previous chemotherapy regimens for advanced disease, number	
Median	2
Range	1–7
Prior radiation therapy for cholangiocarcinoma, number (%)	11 (44%)
Treatment site, number (%)	
MD Anderson Cancer Center	18 (72%)
Johns Hopkins University	7 (28%)
ECOG performance status, number (%)	
0	10 (40%)
1	15 (60%)
Cholangiocarcinoma subtype, number (%)	
Intrahepatic	5 (20%)
Perihilar or distal	20 (80%)

Abbreviation: ECOG = Eastern Cooperative Oncology Group.

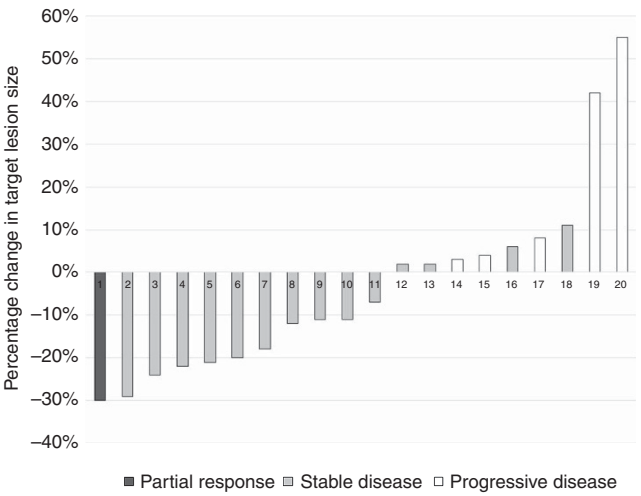

Figure 1. Best response per RECIST 1.1.

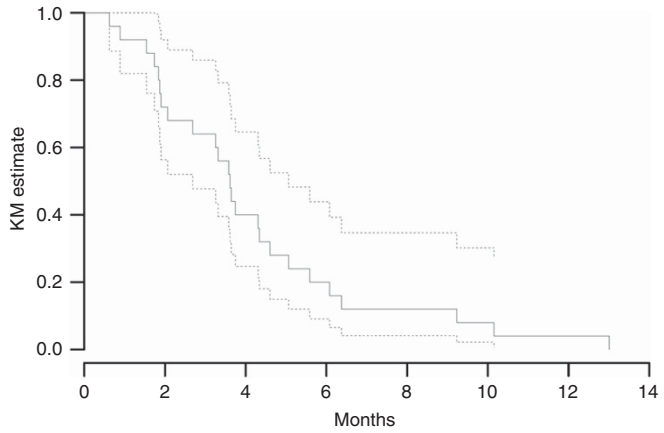

Figure 2. Kaplan–Meier (KM) estimate of progression-free survival (PFS) for patients in the study. The median PFS was 3.6 months. The dotted lines represent 95% confidence intervals.

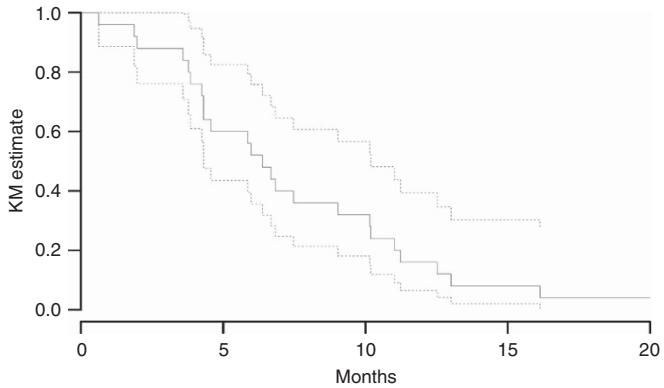

Figure 3. Kaplan–Meier (KM) estimate of overall survival (OS) for patients in the study. The median OS was 6.4 months. The dotted lines represent 95% confidence intervals.

Treatment-related toxicities observed in two or more participants, and all grade 3/4 treatment-related toxicities, are listed in Table 2. Treatment-related toxicities were predominantly of mild or moderate severity, with the most common events including rash (80% of patients), hypertension (64%), nausea or vomiting (64%), fatigue (60%), diarrhoea (52%), and thrombocytopenia (40%). In most cases, rash was treated successfully with topical steroids as well as dosage reductions or interruptions of trametinib. Similarly, hypertension was managed successfully in most cases with antihypertensive agents and dose reduction or interruption of pazopanib and trametinib. Grade 3–4 treatment-related toxicities were observed in 14 (56%) of patients and included hypertension, fatigue, rash, diarrhoea, anaemia, thrombocytopenia, amnesia, and one case of posterior reversible encephalopathy syndrome (PRES) syndrome, also known as reversible posterior leukoencephalopathy syndrome (RPLS), in a patient with refractory hypertension. Thrombocytopenia was the most common severe treatment-related toxicity, and was managed with pazopanib dose interruption and dose reduction. There were no treatment-related deaths.

Table 2. Treatment-related adverse events occurring in two or more participants, and all grade 3/4 treatment-related adverse events

	Grades 1–2		Grades 3–4	
Event	No. of patients	%	No. of patients	%
Cardiorenal				
Oedema, facial	3	12		
Oedema, limbs	4	16		
Elevated creatinine	2	8		
Hypertension	16	64	2	8
Hypomagnesaemia	7	28		
Hyponatraemia	2	8		
Proteinuria	3	12		
Constitutional				
Dehydration	2	8		
Fatigue	15	60	1	4
Dermatologic				
Dry skin	3	12		
Finger or nail changes	3	12		
Rash	20	80	3	12
Gastrointestinal				
Anorexia	9	36		
Constipation	3	12		
Diarrhoea	13	52	1	4
Elevated liver function tests	8	32	3	12
Mucositis	2	8		
Nausea or vomiting	16	64	2	8
HEENT				
Blurry vision	5	20		
Change in taste	6	24		
Congestion or postnasal drip	2	8		
Floaters	2	8		
Haematologic				
Anaemia	3	12	1	4
Bleeding or bruising	7	28		
Neutropenia	3	12		
Thrombocytopenia	10	40	6	24
Neurological				
Amnesia	1	4	1	4
Dizziness	2	8		
Dysgeusia	2	8		
Headache	2	8		
Posterior reversible encephalopathy syndrome (PRES)	1	4	1	4

Abbreviation: HEENT = head, eyes, ears, nose, and throat.

DISCUSSION

Cholangiocarcinoma is an aggressive disease with a poor prognosis and no clear therapy options in the refractory setting. Here, we report the results of a nonrandomised expansion cohort of combination pazopanib and trametinib therapy in 25 patients with highly refractory cholangiocarcinoma who had received a median of 2 prior systemic therapies. Together, pazopanib and trametinib provide inhibition of angiogenesis and RAF/MEK/ERK signalling, two pathways that have been identified as therapeutic targets for cholangiocarcinoma. Although limited activity was previously described with inhibitors of either pathway, to our knowledge dual inhibition of these pathways has not been explored previously in this cancer subtype.

In this trial, our observed median PFS is reflective of the aggressive nature of refractory cholangiocarcinoma. Although cross-trial comparisons in the context of this heavily pretreated subset of patients must be made only with caution, the observed PFS compares favourably with other trials in refractory cholangiocarcinoma (Shaib and El-Serag, 2004; Lamarca *et al*, 2014). Although there was a trend towards increased 4-month PFS as compared with the prespecified null hypothesised 4-month PFS, this difference did not reach statistical significance. On this basis, the trial did not achieve the prespecified target to justify further clinical development of this combination in cholangiocarcinoma.

However, the disease response and the 75% disease control rate suggests that a subset of patients may benefit from this therapy and that further clinical investigation may be warranted, potentially of a biomarker-based approach that can prospectively identify patients to be treated. Mutations in the RAS/RAF/MEK/ERK pathway as well as PI3 Kinase/AKT have been reported in this entity and could rationally be evaluated as predictive biomarkers of this regimen. Additional studies are needed to explore potential molecular phenotype(s) of patients in whom the combination of a MEK inhibitor and antiangiogenic therapy may provide more clinical benefit. Although the clinical activity of MEK inhibitors in cholangiocarcinoma has been presumed to be due to on-targeted inhibition of the RAF/MEK/ERK signalling pathway, it is notable that two of the most dramatic responses previously observed did not have any detectable driver mutations in this signalling pathway (Finn *et al*, 2012). Therefore, the mechanism of action of MEK inhibition in cholangiocarcinoma remains somewhat unclear and may be more complex than previously assumed. Recently, MEK inhibitors have been found to have important immune modulatory properties (Liu *et al*, 2015; Bendell *et al*, 2016; Ebert *et al*, 2016), and the VEGF signalling pathway has also been implicated as a mechanism of tumour immune escape (Goel and Mercurio, 2013). This raises the possibility that some the clinical activity of these agents in cholangiocarcinoma may in part be immunologically mediated, and further investigation of one or more of these agents in combination with novel immunotherapies may be warranted.

The reported adverse events are similar to those observed in a prior dose escalation trial of pazopanib and trametinib in advanced solid tumours, and are also consistent with the known toxic effects of each individual agent. Although this trial was nonrandomised, the observed rate of serious treatment-related adverse events (56%) was somewhat higher than the rate observed in prior registration trials of each agent alone (Sternberg *et al*, 2010; Flaherty *et al*, 2012; van der Graaf *et al*, 2012), suggesting that there is additive toxicity when these agents are combined. As monotherapy, pazopanib and trametinib have overlapping toxicity profiles that include rash, fatigue, and diarrhoea, and therefore additive toxicity with this combination was anticipated. Although rash was the most common adverse events, a severe rash was uncommon and most cases were managed successfully with topical steroids, dose reductions, or treatment interruption. The toxicity of pazopanib plus trametinib remains an important consideration, and close monitoring of patients and optimisation of toxicity management are needed for further development of this treatment combination. The dose reductions seen in this trial should be seen as part of an ongoing dialogue in the drug development community about whether the definition of maximum tolerated dose should include ongoing toxicity and dose adjustments past the usual first cycle of therapy, as has been the standard.

In conclusion, we find that the combination of pazopanib and trametinib has signs of possible cumulative toxicity, requiring frequent dose reductions, and has modest clinical activity in advanced refractory cholangiocarcinoma. Further studies are needed to better characterise the benefit of combination anti-angiogenic therapy with MEK inhibition, and to explore molecular phenotypes in which this combination may provide more significant benefit.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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