First-in-Human Study of the Ataxia Telangiectasia and Rad3-Related (ATR) Inhibitor Tuvusertib (M1774) as Monotherapy in Patients with Solid Tumors

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ABSTRACT

Purpose: Tuvusertib (M1774) is a potent, selective, orally administered ataxia telangiectasia and Rad3-related (ATR) protein kinase inhibitor. This first-in-human study (NCT04170153) evaluated safety, tolerability, maximum tolerated dose (MTD), recommended dose for expansion (RDE), pharmacokinetics (PK), pharmacodynamics (PD), and preliminary efficacy of tuvusertib monotherapy.

Patients and Methods: Ascending tuvusertib doses were evaluated in 55 patients with metastatic or locally advanced unresectable solid tumors. A safety monitoring committee determined dose escalation based on PK, PD, and safety data guided by a Bayesian 2-parameter logistic regression model. Molecular responses (MR) were assessed in circulating tumor DNA samples.

Results: Most common grade ≥3 treatment-emergent adverse events were anemia (36%), neutropenia, and lymphopenia (both 7%). Eleven patients experienced dose-limiting toxicities, most commonly grade 2 ($n = 2$) or 3 ($n = 8$) anemia. No persistent effects on blood immune cell populations were observed. The RDE was 180 mg tuvusertib QD (once daily), 2 weeks on/1 week off treatment, which was better tolerated than the MTD (180 mg QD continuously). Tuvusertib median time to peak plasma concentration ranged from 0.5 to 3.5 hours and mean elimination half-life from 1.2 to 5.6 hours. Exposure-related PD analysis suggested maximum target engagement at ≥130 mg tuvusertib QD. Tuvusertib induced frequent MRs in the predicted efficacious dose range; MRs were enriched in patients with radiological disease stabilization, and complete MRs were detected for mutations in *ARID1A*, *ATRX*, and *DAXX*. One patient with platinum- and PARP inhibitor–resistant *BRCA* wild-type ovarian cancer achieved an unconfirmed RECIST v1.1 partial response.

Conclusions: Tuvusertib demonstrated manageable safety and exposure-related target engagement. Further clinical evaluation of tuvusertib is ongoing.

Introduction

The DNA-damage response (DDR) is a complex network of signaling pathways that monitors and maintains genomic integrity, which is activated in response to all intrinsic and extrinsic DNA damage. Ataxia telangiectasia and Rad3-related (ATR) protein kinase is activated by exposed sections of single-stranded DNA (ssDNA) and is an essential regulator of the replication stress response in actively dividing cells (1, 2).

Replication stress is the slowing or stalling of DNA replication fork progression, one cause of which is endogenous DNA damage resulting from errors during DNA replication (3). When this occurs, ATR is activated and recruited to the resulting extended stretches of ssDNA. Subsequently, activated ATR phosphorylates multiple substrates involved in cell-cycle regulation, including checkpoint kinase 1 (CHK1; ref. 4). Phosphorylated CHK1 then induces transient cell-cycle arrest at the S–G_2 checkpoint through degradation of cell division cycle 25A phosphatase and promotes DNA repair. This stabilizes stalled replication forks, prevents premature mitotic entry, and ultimately restores replication fork progression (5, 6).

Replication stress is a hallmark of pre-cancerous and cancerous cells due to their high levels of DNA damage (7). Consequently, ATR inhibition (ATRi) holds promise as a potential anticancer therapy because it leads to unhindered cell-cycle progression and accumulation of unrepaired DNA damage, resulting in further genomic instability and ultimately cancer cell death (4). In addition, the therapeutic effects of DNA-damaging interventions may be enhanced through combination with ATR and other DDR inhibitors that prevent DNA repair (1).

If ATR is inhibited, alternative pathways within the DDR may be used to repair DNA. Therefore, sensitivity to ATRi depends on the presence of tumor mutations in genes that encode other key components of DDR pathways. Preliminary signs of clinical activity have been reported for several such predictive biomarkers with potential utility for patient stratification based on this synthetic lethality mechanism, for example, loss-of-function (LOF) mutations in *ATM* and *ARID1A* (8–10). ATR also mediates alternative lengthening of telomeres (ALT), a telomerase-independent mechanism for maintaining telomeres that is activated in 10% to 15% of all cancers, enabling them to bypass replicative senescence (11). Tumors that rely primarily on the ALT pathway have been found to be sensitive to ATRi in preclinical

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Clin Cancer Res 2024;30:2057–67

doi: 10.1158/1078-0432.CCR-23-2409

Translational Relevance

This first-in-human study demonstrates that ataxia telangiectasia and Rad3-related inhibitor tuvusertib as monotherapy is well tolerated, has a manageable safety profile, and shows exposure-related target engagement in adult patients with advanced solid tumors. We also establish the recommended dose for expansion (RDE). No sustained detrimental impact was detected on peripheral T and B immune cells in immunophenotyping analyses, whereas an expected pharmacodynamic (PD) effect was seen in monocytes. Tuvusertib exposure was well above the *in vitro* pCHK1 IC90 at the RDE, and exposure-related PD analyses suggested target engagement at the RDE. Therefore, tuvusertib has potential to be used as a combination partner, including with other DNA-damage response inhibitors and immunotherapies, in future trials.

studies; hence, indicators of ALT upregulation such as *ATRX or DAXX* mutations may also predict ATRi sensitivity (12–14).

Tuvusertib (M1774) is a potent, selective, orally administered ATR inhibitor with antitumor activity as monotherapy in preclinical models with DDR pathway gene mutations, including an ATM^{mut} non–small cell lung cancer (NSCLC) xenograft model and an $ARID1A^{mut}$ gastric cancer xenograft model (Supplementary Fig. S1; ref. 15). Tuvusertib also showed antitumor activity as monotherapy and in combination with the PARP inhibitor niraparib in xenograft models of $BRCA^{mut}$ high-grade serous ovarian cancer (16).

In part A1 of this first-in-human (FIH) study, we evaluated safety, tolerability [including definition of the maximum tolerated dose (MTD) and recommended dose for expansion (RDE)], pharmacokinetics (PK), and preliminary efficacy of tuvusertib as monotherapy in patients with advanced solid tumors. We also investigated the pharmacodynamics (PD) of tuvusertib, its potential effects on the immunophenotype, as well as the molecular profile and evolution of the underlying disease in patients treated with tuvusertib.

Patients and Methods

Study design and treatment

This trial was a part of the multicenter, open-label, non-randomized Phase 1 study DDRiver Solid Tumors 301 (NCT04170153; ref. 17). The primary objectives of this dose-escalation study were to determine safety, tolerability, MTD, and RDE of tuvusertib as monotherapy in patients with advanced solid tumors. The secondary objective was to investigate the PK profile of tuvusertib as monotherapy. Exploratory objectives included assessing the change in PD markers in peripheral blood mononuclear cells (PBMC), evaluating molecular biomarkers of response to tuvusertib, preliminary clinical efficacy of tuvusertib in patients with advanced solid tumors, and exploring the potential impact of tuvusertib on the immune system.

The primary endpoints investigated were the occurrence of dose-limiting toxicities (DLT), treatment-emergent adverse events (TEAE), treatment-related adverse events (AE) and deaths, abnormalities (grade ≥3) in laboratory test values, markedly abnormal vital sign measurements, clinically significant abnormal ECGs, and establishing the RDE using PK and PD data in addition to the safety profile. Secondary endpoints included PK parameters of tuvusertib in plasma after a single dose and at steady state as calculated by noncompartmental analysis. Additional exploratory endpoints were relative changes in γ-H2AX expression in serial blood samples after first tuvusertib administration, correlation of baseline and on-treatment genetic alterations in tumor and plasma circulating tumor DNA (ctDNA) in tumors with clinical efficacy, relative changes of total blood cell count and immune cells (e.g., T cells, B cells, NK cells) after tuvusertib administrations, and objective response according to RECIST Version 1.1 as assessed by the investigator.

This study was performed in compliance with the International Council for Harmonization Good Clinical Practice guideline and in accordance with the Declaration of Helsinki. The study protocol and other relevant documents were reviewed and approved by an Institutional Review Board/Independent Ethics Committee before study activation and all patients provided their written informed consent.

Patients were enrolled sequentially in cohorts at different dose levels, between December 20, 2019 and April 21, 2022. The doses tested, the number of patients treated in each cohort, and occurrence of any DLTs are summarized in **Fig. 1**. Tuvusertib was administered once daily (QD) as a single agent under fasting conditions, with a starting dose of 5 mg. Justification for the starting dose, predicted efficacious dose, and details on the preclinical risk assessment are provided in the Supplementary Methods. Treatment breaks (tuvusertib 180 or 220 mg QD 2 weeks on/1 week off treatment) and a more dose-dense regimen of tuvusertib 150 mg twice daily (BID) with 4 days on treatment/3 days off treatment were also explored. A Bayesian 2-parameter logistic regression model with overdose control was applied to assist the safety monitoring committee (SMC) in dose recommendations during tuvusertib dose escalation (18). Details on the methods used to guide dose escalation are provided in the Supplementary Methods. The DLT observation period was 21 days, and the target toxicity rate of DLTs to establish the MTD was 30%. Holistic integration of the clinical PK, PD, and safety data was used to select the tuvusertib RDE (19).

Patients

Eligible patients were males or females of ≥18 years of age with locally advanced or metastatic solid tumors refractory to standard therapy, or for which no standard therapy was judged appropriate, Eastern Cooperative Oncology Group performance status ≤1 and adequate hematologic, hepatic, and renal function.

Adequate hematologic function was indicated by platelet count ≥100,000/mm^3; hemoglobin ≥9.0 g/dL, and absolute neutrophil count ≥1,500/μL with no growth factor treatment in the 14 days preceding tuvusertib administration. Adequate hepatic function was defined by a total bilirubin level ≤1.5 × upper limit of normal (ULN; total bilirubin >1.5 × ULN in case of Gilbert's Syndrome was allowed); an aspartate aminotransferase (AST) level ≤3 × ULN, and an alanine aminotransferase level ≤3 × ULN or ≤5 × ULN in presence of liver metastases. Adequate renal function was defined as serum creatinine ≤1.5 × ULN. If serum creatinine was >1.5 × ULN, creatinine clearance needed to be ≥50 mL/min by Cockcroft–Gault calculation or by a measured 24-hour urine collection.

Key exclusion criteria included the presence of persistent toxicities due to prior anticancer therapies; any active and/or uncontrolled infection; unstable angina, myocardial infarction, congestive heart failure ≥ stage II or a coronary revascularization procedure within 180 days of study entry; calculated corrected QT interval average (using the Fridericia correction calculation) of >450 ms for males and >470 ms for females that did not resolve with correction of electrolyte abnormalities; prior treatment with an ATR inhibitor and/or CHK1 inhibitor; receiving hematopoietic growth factor in the 14 days before

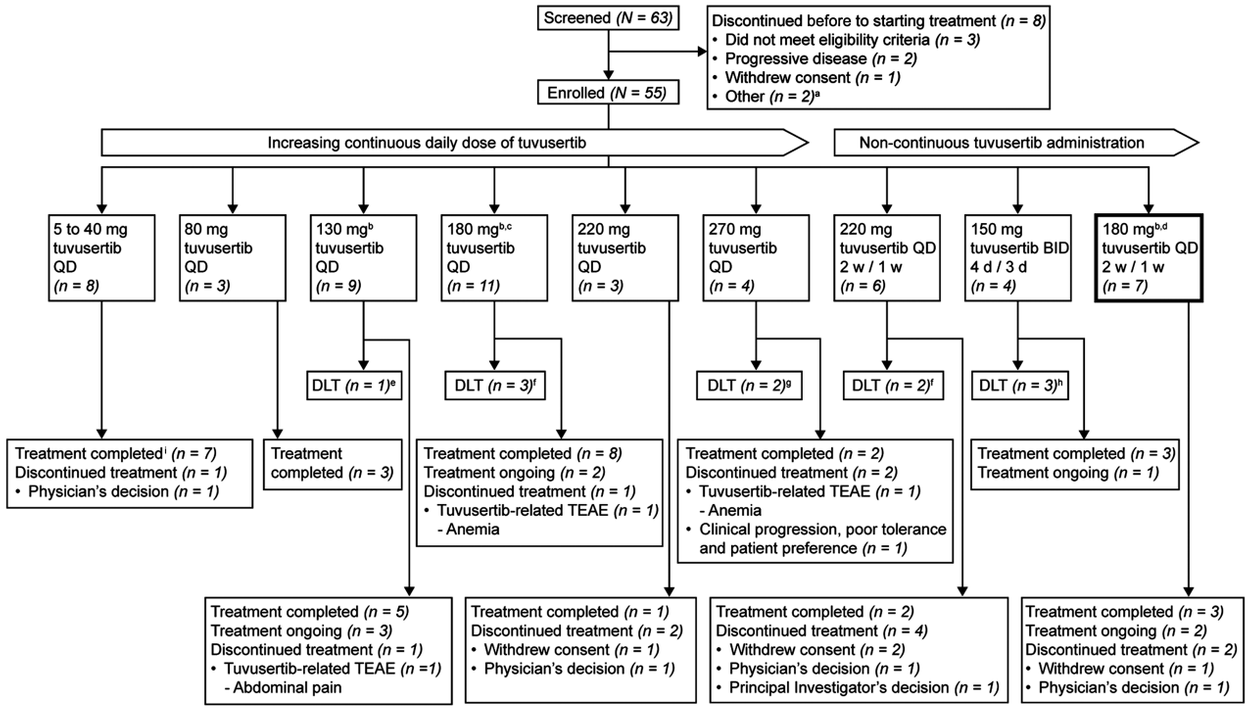

Figure 1.

Patient disposition. Bold box indicates recommended dose for expansion (RDE). [a]One patient had a small bowel obstruction requiring surgery, and another initially met all eligibility criteria, but low hemoglobin later made them ineligible; [b]One patient in dose cohort not evaluable for DLTs; [c]MTD declared; [d]RDE declared; [e]Anemia grade 2 requiring transfusion; [f]Anemia grade 3 requiring transfusion; [g]Anemia grade 3 requiring transfusion ($n = 1$), upper gastrointestinal hemorrhage grade 3 and platelet count decreased ($n = 1$); [h]Anemia grade 2 ($n = 1$), Anemia grade 3 ($n = 2$), all requiring transfusion; [i]Patients were treated until disease progression or death; BID, twice daily; DLT, dose-limiting toxicity; MTD, maximum-tolerated dose; QD, once daily; RDE, recommended dose for expansion; TEAE, treatment emergent adverse event; 2 w/1 w, 2 weeks on treatment/1 week off treatment; 4 d/3 d, 4 days on treatment/3 days off treatment.

the first dose of tuvusertib; receiving medications known to be strong inhibitors or inducers of CYP3A4 or CYP1A2 enzymes; and receiving treatment with proton pump inhibitors that could not be discontinued at least one week before the start of treatment with tuvusertib and for the duration of the study. Complete details of all exclusion and inclusion criteria are provided in the Supplementary Methods.

PK analysis

The PK of tuvusertib were characterized during dose escalation using an intensive PK sample collection schedule. Blood samples were collected during Cycle 1 on day 1 and steady-state PK on day 8 at the following times: Day 1, pre-dose at 0 hours, post-dose at 0.5, 1, 2, 3, 4, 6, 8, 12 hours; Day 2, pre-dose at 0 hours (24 hours after previous dose on day 1); Day 8, pre-dose at 0 hours, post dose at 0.5, 1, 2, 3, 4, 6, 8, 12 hours; Day 9, pre-dose at 0 hours (24 hours after previous dose on Day 8) to assess steady-state PK; Day 15, pre-dose at 0 hours. PK parameters were calculated from individual plasma concentration–time data using noncompartmental methods (WinNonlin Version 8.3.0.5005). Bioanalytical assays were validated for the quantification of tuvusertib in K2EDTA human plasma using a LC/MS-MS assay. Plasma concentrations below the lower limit of quantification of the bioanalytical assay were set to zero for the purpose of noncompartmental analysis.

In addition to PK analysis, the PK/PD relationship was established between the area under the plasma concentration–time curve (AUC_{0-3h}) and γ-H2AX 3 hours after a single-dose of tuvusertib.

PD profiling

The PD of tuvusertib was explored by assessing the level of phosphorylation of the Ser-139 residue of the histone variant H2AX (γ-H2AX), a molecular marker for monitoring DNA-damage initiation and resolution (20) and an established biomarker of ATRi (21, 22), in circulating lymphocytes as tumor surrogate tissue. Whole-blood samples were collected before and 3 hours after the first tuvusertib dose. The samples were stimulated with either 4NQO (4-Nitroquinoline N-oxide, Sigma) or dimethyl sulfoxide, as control. Following treatment with Lyse and Fix Solution and permeabilization, cells were stained with CD45 and Phospho-Histone H2A.X antibodies. Analysis was performed using a FacsCanto II (Becton Dickinson). The PK/PD relationship was established between the area under the plasma concentration–time curve (AUC_{0-3h}) and γ-H2AX 3 hours after a single-dose of tuvusertib.

The percentage of γ-H2AX–positive lymphocytes was used as main read-out and calculated as:

$$\left[\frac{\text{4NQO } \textit{post treatment}}{\text{4NQO } \textit{baseline}} \times 100\right] - 100^{*}$$

(*baseline γ-H2AX expression was fixed at 100 as maximum expression within the dose level under evaluation).

Immunophenotyping assay

For immunophenotyping, flow cytometry panels were used to detect 43 immune cell subsets, including T, B, and NK cells, myeloid-derived

suppressor cells (MDSC) and monocytes, in human whole-blood samples. Whole-blood samples were collected at visits on days 1 and 15 of cycles 1 and 2 before tuvusertib intake (cycle duration, 21 days). Analyses were performed at Q2 Solutions Global Central Laboratories, in agreement and following Q2 Solution validated methods. Relative changes to the baseline of each measurement were computed as follows:

$$\%RC = \left(\frac{measurement\ at\ each\ visit - measurement\ at\ baseline}{measurement\ at\ baseline}\right) \times 100$$

Translational analyses

Blood samples for plasma ctDNA analyses were collected from patients on day 1 of each treatment cycle (21 days) and at disease progression (23). A total of 173 ctDNA samples from 55 patients were analyzed by next-generation sequencing at Guardant Health using the OMNI panel. These included 55 baseline samples from 55 patients and 118 on-treatment samples from 47 of 55 patients. 172/173 (99%) samples were processed successfully, and 171/172 (99%) samples sequenced had detectable ctDNA after filtering for putative clonal hematopoiesis.

Molecular response (MR) was defined as best delta-mean variant allele fraction (VAF) across all variants and visits <50%, calculated as follows:

$$Best\ delta\ mean\ VAF = \min(treati)\frac{VAFtreati - VAFbaseline}{VAFbaseline}$$

Somatic putative clonal hematopoiesis of indeterminate potential mutations were excluded, as were samples with VAF at baseline <0.3%. Putative "baseline variants" not detected at treatment received VAF = 0.0001. High-functional impact of a mutation was given when it belonged to one of the following categories: frameshift, nonsense, splice_donor, splice_acceptor or pathogenic according to Clinvar (24).

Statistical analyses

The full and safety analysis sets included all patients who received at least one dose of tuvusertib ($N = 55$). The DLT analysis set included all patients who received at least 75% of the cumulative planned dose during Cycle 1 and who completed Cycle 1, or experienced a DLT as defined by the SMC during Cycle 1 and received any amount of tuvusertib ($N = 52$). The PK analysis set included all patients who received at least one dose of tuvusertib and had at least one measurable post-dose PK sample ($N = 55$).

A Bayesian 2-parameter logistic regression model with overdose control was used to assist the SMC in dose recommendations. The model has been extended by including a binary covariate to account for the different drug dosing holiday schedules. Posterior probabilities for DLT were produced from the model, displaying the mean and percentiles for the posterior probability of a participant experiencing a DLT at each of the dose levels. A dose was suggested as MTD by the model for each drug-dosing holiday schedule separately, if the upper bound of the one-sided 95% credible interval of the estimate for DLT probability did not exceed 40%, whereas the median estimated DLT probability was between 20% and 30%.

Baseline was defined as the last value before the first administration of tuvusertib, and the study intervention period was defined as the period from the start of tuvusertib administration to 30 days after its last administration.

Data availability statement

Any requests for data by qualified scientific and medical researchers for legitimate research purposes will be subject to the healthcare business of Merck KGaA, Darmstadt, Germany's (CrossRef Funder ID: 10.13039/100009945) Data Sharing Policy. All requests should be submitted in writing to the healthcare business of Merck KGaA, Darmstadt, Germany's data sharing portal (https://www.emdgroup.com/en/research/our-approach-to-research-and-development/healthcare/clinical-trials/commitment-responsible-data-sharing.html). When the healthcare business of Merck KGaA, Darmstadt, Germany has a co-research, co-development, or comarketing or co-promotion agreement, or when the product has been out-licensed, the responsibility for disclosure might be dependent on the agreement between parties. Under these circumstances, the healthcare business of Merck KGaA, Darmstadt, Germany, will endeavor to gain agreement to share data in response to requests.

Results

Patient demographics

A total of 55 patients with locally advanced or metastatic unresectable solid tumors were enrolled in this study at four centers across the UK (16 patients, 2 sites) and US (39 patients, 2 sites), all of whom received ≥1 dose of tuvusertib ranging from 5 to 270 mg. The median age was 62 years (range, 33–82) and 58% of patients were female. The most common primary tumor types were prostate (26%), ovary (16%), breast (6%), and pancreas (6%; **Table 1**). Representativeness of study participants is shown in Supplementary Table S1.

Table 1. Patient demographics and baseline characteristics.

Characteristic	Total $N = 55$
Sex, *n* (%)	
Male	23 (42)
Female	32 (58)
Ethnicity, *n* (%)	
Asian	5 (9)
Black or African American	2 (4)
White	42 (76)
Other	6 (11)
Median (range) age (y)	62 (33, 82)
<65 years	34 (62)
65 to <75 years	14 (26)
75 to <85 years	7 (13)
ECOG PS, *n* (%)	
0	6 (11)
1	49 (89)
M Stage at study entry, *n* (%)	
M0	1 (2)
M1	52 (95)
MX	1 (2)
Missing	1 (2)
Primary tumor location, *n* (%)	
Prostate gland	14 (26)
Ovary	9 (16)
Pancreas	3 (6)
Breast	3 (6)
Other	26 (47)

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group Performance Status.

Dose-escalation outcomes and DLTs

Key results obtained in dose-escalation cohorts to the RDE and DLTs by dose are summarized in **Fig. 1**. Dosing started at tuvusertib 5 mg QD and was escalated to 10, 20, 40, 80, 130, 180, 220, and 270 mg QD. Eleven patients experienced DLTs (as assessed by the SMC, see Supplementary Table S2) during the predefined observation period of 21 days. Of these, 10 were events of anemia requiring blood transfusion, occurring in the dosing cohorts tuvusertib 130 mg QD (1/8 patients), 180 mg QD (3/10), 270 mg QD (1/4), 220 mg QD 2 weeks on/ 1 week off treatment (2/6), and 150 mg BID 4 days on treatment /3 days off treatment (3/4). Upper gastrointestinal hemorrhage and decreased platelet counts (one each) were observed in the tuvusertib 270 mg QD dosing cohort (2/4). Given the frequent observation of anemia at tuvusertib doses ≥130 mg, treatment breaks (180 or 220 mg QD 2 weeks on/1 week off treatment) were explored. A more dose-dense regimen of tuvusertib 150 mg BID, 4 days on treatment/3 days off treatment was also explored, but resulted in increased toxicity and further BID regimens were not evaluated.

The Bayesian model suggested the MTD of tuvusertib was 180 mg QD when given continuously because the median estimated DLT probability of 23% was within the range of 20% to 30%, and the upper boundary of the one-sided 95% credible interval of 37% was below the 40% threshold. The median estimated DLT probability for the intermittent 2 weeks on/1 week off schedule was below the threshold of 20%, and therefore, no MTD was formally established as per the statistical model requirements.

A 67% less intense dose than the MTD, that is, tuvusertib 180 mg QD 2 weeks on/1 week off treatment, was defined as well-tolerable dosing regimen, given the 1 week break in the dosing cycle led to a lower incidence of anemia compared with tuvusertib 180 mg QD, as described in the safety outcomes below.

On the basis of the PK/PD relationship of γ-H2AX (see PD results below), >80% γ-H2AX inhibition was observed at doses of tuvusertib 130 mg and above. Selecting the dosing regimen of tuvusertib 180 mg QD 2 weeks on/1 week off treatment allowed for >80% γ-H2AX inhibition while also allowing to reduce the dose to tuvusertib 130 mg QD if needed, which is still in the efficacious range.

Based on the holistic integration of safety, PK and PD data, the dose of tuvusertib 180 mg QD at an intermittent schedule of 2 weeks on/1 week off treatment was declared as the RDE.

Safety outcomes

Most patients (95%) experienced at least one TEAE, the majority of which (87%) were assessed as related to tuvusertib. Overall, the most frequently reported TEAEs of any grade were anemia (71%), nausea (62%), and fatigue (40%). The most common grade ≥3 TEAEs were anemia (36%; the majority of these were seen at doses ≥ MTD, with none seen at the RDE), neutrophil count decreased (7%), and lymphocyte count decreased (7%). Grade ≥4 platelet count decreased (4%) was reported at dose levels exceeding the MTD. The most common (≥30%) TEAEs of any grade seen at the RDE ($n = 7$) were nausea (71%) and anemia (57%), whereas those at the MTD ($n = 11$) were anemia (100%), nausea (55%), constipation, and vomiting (both 36%). The most common treatment-related AEs across all dose levels were anemia (67%), with nausea (56%), fatigue (38%), and vomiting (33%) also reported. No clinically significant abnormal ECG readings, vital signs or high-grade laboratory abnormalities were observed. A summary of all TEAEs is provided in **Table 2**. Grade ≥3 laboratory abnormalities are shown in Supplementary Table S3. Six deaths occurred during the study, none of which were considered related to tuvusertib. Overall, tuvusertib was well tolerated and demonstrated a manageable safety profile.

Table 2. Overview of TEAEs and TEAEs occurring in 15% of patients by preferred term (safety analysis set).

Patients with	Tuvusertib (all doses), $N = 55$, n (%)	
Any TEAE	52 (95)	
Any serious TEAE	15 (27)	
Any grade ≥3 TEAE	26 (47)	
TEAEs related to tuvusertib	48 (87)	
Serious TEAE related to tuvusertib	3 (6)	
Grade ≥3 TEAE related to tuvusertib	19 (35)	
TEAE leading to temporary tuvusertib discontinuation	26 (47)	
TEAE leading to permanent tuvusertib discontinuation	3 (6)	
TEAEs occurring in ≥15% of patients	**Any grade**	**Grade ≥3**
Anemia	39 (71)	20 (36)
Nausea	34 (62)	0
Fatigue	22 (40.0)	1 (2)
Vomiting	21 (38)	3 (6)
Constipation	13 (24)	0

Abbreviations: QD, once daily; TEAE, treatment-emergent adverse event.

Clinical responses

This study was conducted in patients with advanced solid tumors, without protocol-mandated biomarker selection. Best overall responses to tuvusertib treatment are summarized in **Fig. 2A**. One patient with platinum- and PARP inhibitor–resistant $BRCA_{wt}$ ovarian cancer, receiving a dose of tuvusertib 150 mg BID 4 days on treatment /3 days off treatment, derived significant clinical benefit from treatment with 9 months of disease stabilization (unconfirmed partial response per RECIST v1.1, as revealed by CT scans; Supplementary Figs. S2 and S3). Furthermore, 15 (27%) patients had stable disease (RECIST v1.1).

MRs

MRs were observed in 14/37 (38%) patients treated with more than 130 mg tuvusertib QD; conversely, none were observed in the 10 patients who received lower doses of tuvusertib (**Fig. 2B**). MRs were enriched in patients with radiological disease stabilization, as 7/14 (50%) of patients with MRs showed a reduction in sum of tumor diameter, compared with 8/33 (24%) of those without MRs. Finally, MRs were enriched in patients with ovarian (5/10, 50%), prostate (4/13, 31%), and breast cancer (1/3, 33%), whereas less frequent (4/21, 19%) in other tumor types.

PK

Data from all 55 treated patients were used for noncompartmental PK analysis. In general, plasma concentration–time profiles showed fast absorption with median t_{max} ranging from approximately 0.5 to 3.5 hours after oral administration of tuvusertib. Tuvusertib exposure was approximately dose proportional up to 180 mg QD and slightly more than proportional at higher doses. PK steady-state conditions were achieved by day 8. Steady-state mean plasma concentration time profiles across the evaluated doses are shown in **Fig. 3**, and mean steady-state AUC accumulation ratio ranged from 1.0 to 2.5 across the dose groups evaluated. Terminal mean elimination half-life ($t_{1/2}$) across the dose groups was estimated ranging from approximately 1.2 to 5.6 hours. At tuvusertib 180 mg QD, the average steady-state plasma concentration (C_{av}) was approximately 30-fold higher than the

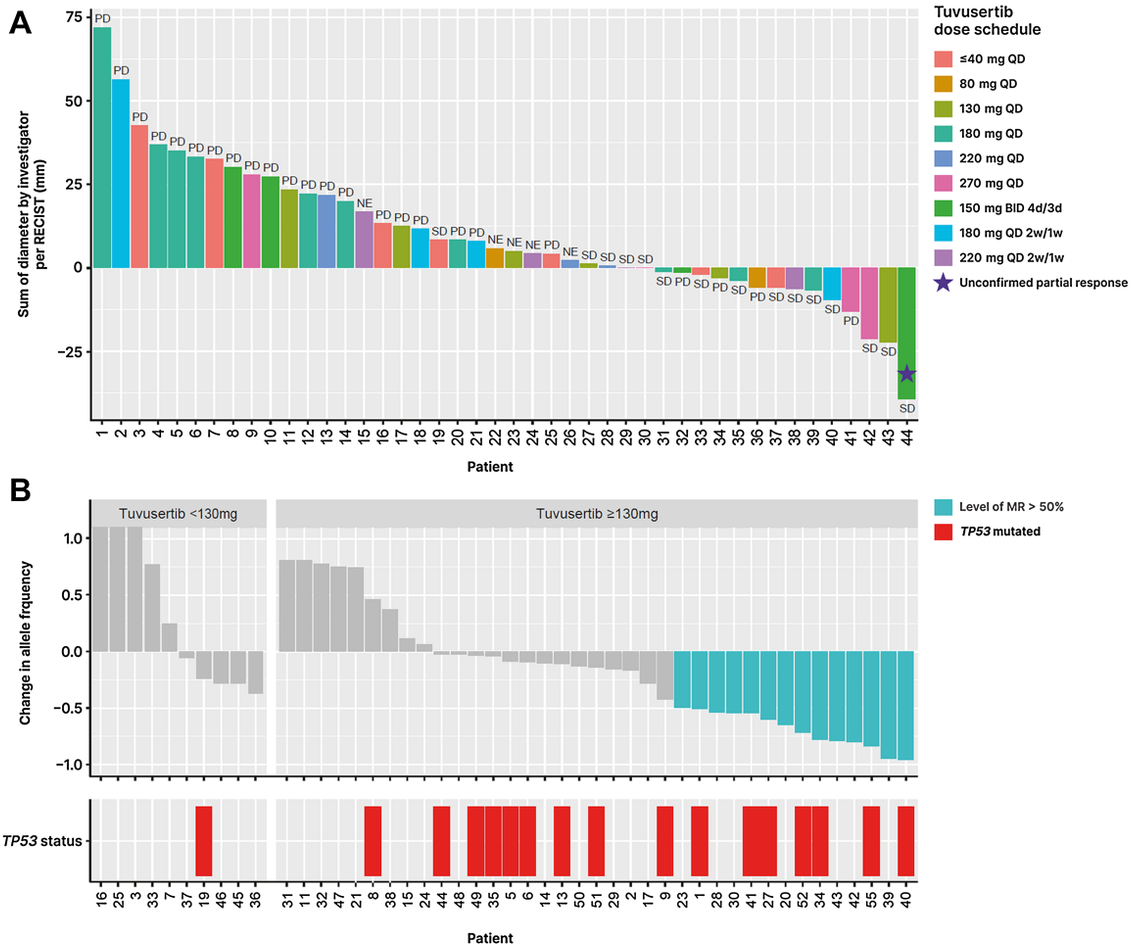

Figure 2.

A, Clinical responses and tuvusertib dose levels, and molecular responses in patients treated with tuvusertib doses <130 and ≥130 mg (**B**). **A,** Change in target lesion sum of longest diameters and best response (RECIST v1.1) is shown per patient. **B,** MRs are enriched in patients treated with biologically active doses of ≥130 mg tuvusertib. Reduction of somatic allele frequencies from baseline by more than 50% is highlighted in blue. *TP53* mutation status (red) is shown below. BID, twice daily; MR, molecular response; NE, not evaluable; PD, progressive disease; QD, once daily; RECIST, response evaluation criteria in solid tumours; SD, stable disease.

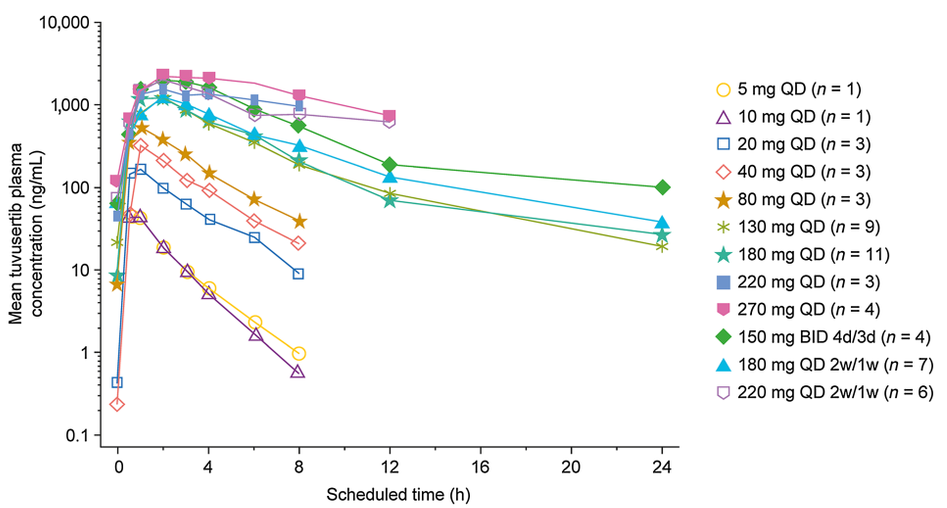

Figure 3.

Mean steady-state tuvusertib plasma concentration–time profiles during cycle 1. 2 w/1 w, 2 weeks on treatment/1 week off treatment; 4 d/3 d, 4 days on treatment/3 days off treatment; BID, twice daily; QD, once daily.

in vitro pCHK1 IC_{90} and remained higher for the whole 24 hours dosing period, supporting QD as dosing regimen.

PD

With tuvusertib doses of 130 mg QD or higher, levels of γ-H2AX in *ex vivo* 4-NQO–treated patient PBMCs were reduced by >80% 3 hours after the first dose, showing target engagement based on PK/PD analyses (Supplementary Fig. S4; ref. 19).

Immunophenotyping outcomes

Tuvusertib treatment did not cause any significant or persistent changes in the levels of most explored immune cell subsets at the tested dose levels, including MDSCs, T and B lymphocytes, including proliferating (Ki67+) subsets. Transient decreases in monocytes and NK cell subsets were observed at tuvusertib doses ≥130 mg during the on-treatment period, with full recovery during treatment breaks (ref. 25; **Fig. 4A** and **B**).

Translational analyses

The molecular characteristics and disease evolution in patients treated with tuvusertib were explored using archival biopsies collected from 33 of 55 patients and ctDNA samples from 55 of 55 patients. In ctDNA samples, high-impact mutations were detected in *ARID1A* ($n = 10$), *ATM* ($n = 5$), *ATRX* ($n = 3$), *BRCA1/2* ($n = 13$), and other HR-related genes ($n = 5$). *TP53* mutations were significantly associated with MR, independently of tumor type (Wilcoxon rank-sum test; $P < 0.03$). Complete MRs were achieved for any mutations in *ARID1A* (2/8), *ATRX* (2/5), *DAXX* (2/3), and *BRCA1/2* (2/11; ref. 23).

Discussion

Tuvusertib was well tolerated at doses up to 180 mg QD continuously (MTD). The RDE for tuvusertib was established as 180 mg QD administered as a regimen of 2 weeks on/1 week off treatment, because this schedule was better tolerated than the MTD schedule due to the decreased likelihood of anemia. However, as only 7 patients were treated at the RDE, the safety of the RDE is under further evaluation in other ongoing parts of the DDRiver Solid Tumors 301 study. For tuvusertib doses up to and including 180 mg QD, anemia was the most frequently reported AE, with no significant frequency of neutropenia or thrombocytopenia. This contrasts with prior data on other ATRis, which identified neutropenia and thrombocytopenia as DLTs (9, 10, 26, 27). Anemia is a commonly reported class effect of ATR inhibitors (10, 27–29); in a single-center analysis of 141 patients receiving several different ATR inhibitors in multiple trials, grade ≥3 anemia was reported in 48% of patients (30) and grade 3 anemia within the first 6 months of ATRi was associated with improved progression-free survival (31). Other common TEAEs reported in our study were generally consistent with those observed in previous studies of ATR inhibitors (9, 10, 27–29, 32–34).

Immunophenotyping analyses demonstrated no significant or persistent impact of tuvusertib on MDSCs, T and B immune cells, including proliferating (Ki67+) subsets. Decreases in monocytes and NK cell subsets seen at tuvusertib doses ≥130 mg fully recovered during treatment breaks. Monocytes have been reported to have defects in DDR (35) and ATR suppression of monocyte proliferation has been used as a PD marker (26).

Contrary to other ATR inhibitors in clinical development (36), tuvusertib had no effect on other immune cell populations, including proliferating T and B cells.

Tuvusertib was rapidly absorbed, with a median time to peak plasma concentration (T_{max}) ranging from approximately 0.5 to 3.5 hours and a $t_{1/2}$ ranging from approximately 1.2 to 5.6 hours. This is in line with PK characteristics reported for other ATR inhibitors, where T_{max} ranged from 1 to 2 hours and $t_{1/2}$ from 5.8 to 11.5 hours (10, 27). At tuvusertib 180 mg QD, an average steady-state plasma concentration approximately 30-fold higher than the *in vitro* pCHK1 IC_{90} value was achieved, which remained higher for the whole 24 hours dosing period and supported QD as dosing regimen. Furthermore, exposure-related PD analyses suggested >80% target inhibition at ≥130 mg tuvusertib (19, 25). Consistent with the mechanism of action, the PD analysis of γ-H2AX as proximal ATR biomarker in PBMCs as a surrogate tissue allowed to quantitatively estimate the tuvusertib target engagement at the RDE. In other ATR inhibitor studies, a PD analysis based on γ-H2AX and p-KAP1 measurement as distal biomarkers of DNA damage in paired biopsies indicated a qualitative assessment of biologic activity (10, 27).

In this unselected patient population, clinical benefit was seen in one patient with an unconfirmed partial response. Other ATR inhibitors demonstrated clinical response rates of 4% to 13% in patients receiving biologically active doses and selected for ATRi-sensitizing biomarkers, where higher activity would be expected (8, 10).

The clinical utility of MR analyses for disease monitoring is being explored in prospective clinical trials (37). Exploratory retrospective translational analyses showed that tuvusertib induced frequent MRs in patients treated with doses in the predicted efficacious concentration range. The 38% MR rate seen in these unselected patients is encouraging, particularly given the 43% MR rate reported for another oral ATR inhibitor in patients with solid tumors harboring ATRi-sensitizing mutations in DDR genes, and the observed correlation of MR with outcomes (10). Importantly, the MRs seen in patients treated with tuvusertib were enriched in those with prior radiological disease stabilization and longest treatment duration. Furthermore, *TP53* alterations, which have been shown to sensitize cells to ATRi (38), were significantly associated with MRs. Complete MRs were detected for mutations in the genes *ARID1A*, *ATRX*, *DAXX*, which predict ATRi sensitivity and are being used for patient selection in the biomarker expansion cohorts in part A3 of the present study. One patient with platinum- and PARP inhibitor–resistant $BRCA_{wt}$ ovarian cancer achieved an unconfirmed RECIST v1.1 PR.

Mutations in *ATRX* and *DAXX* are biomarkers for ALT positivity, which has been found to confer sensitivity to ATRi. For example, a recent study of pancreatic neuroendocrine tumors found that 43% of the samples (29/68) assessed harbored inactivating mutations in *ATRX* or *DAXX* genes, resulting in dependency on the ALT mechanism of telomere maintenance (14, 39). Overall, the study suggested that around 5% to 15% of all human cancers rely on ALT, including brain and neuroendocrine tumors (e.g., glioblastoma) and sarcomas (e.g., osteosarcoma; ref. 12). Therefore, LOF mutations in *ATRX* and *DAXX* might act as biomarkers for ATRi sensitivity in these tumors (12). LOF mutations in *ARID1A* alter the function of the SWI/SNF chromatin remodeling complex and ultimately reduce the cellular ability to repair DNA. SWI/SNF comprises several subunits, including the DNA-targeting ARID1A protein. Such changes may also increase sensitivity to ATRi (40). Our preliminary MR results are promising, warranting further exploration of the molecular profile and evolution of the underlying disease to better understand which patients may benefit most from treatment with tuvusertib.

Combining ATR inhibitors with other therapies that damage DNA, induce replication stress or hinder effective DNA-damage repair, may both enhance the efficacy of, and overcome acquired or inherent resistance to, existing treatments (41, 42). Preclinical evidence suggests

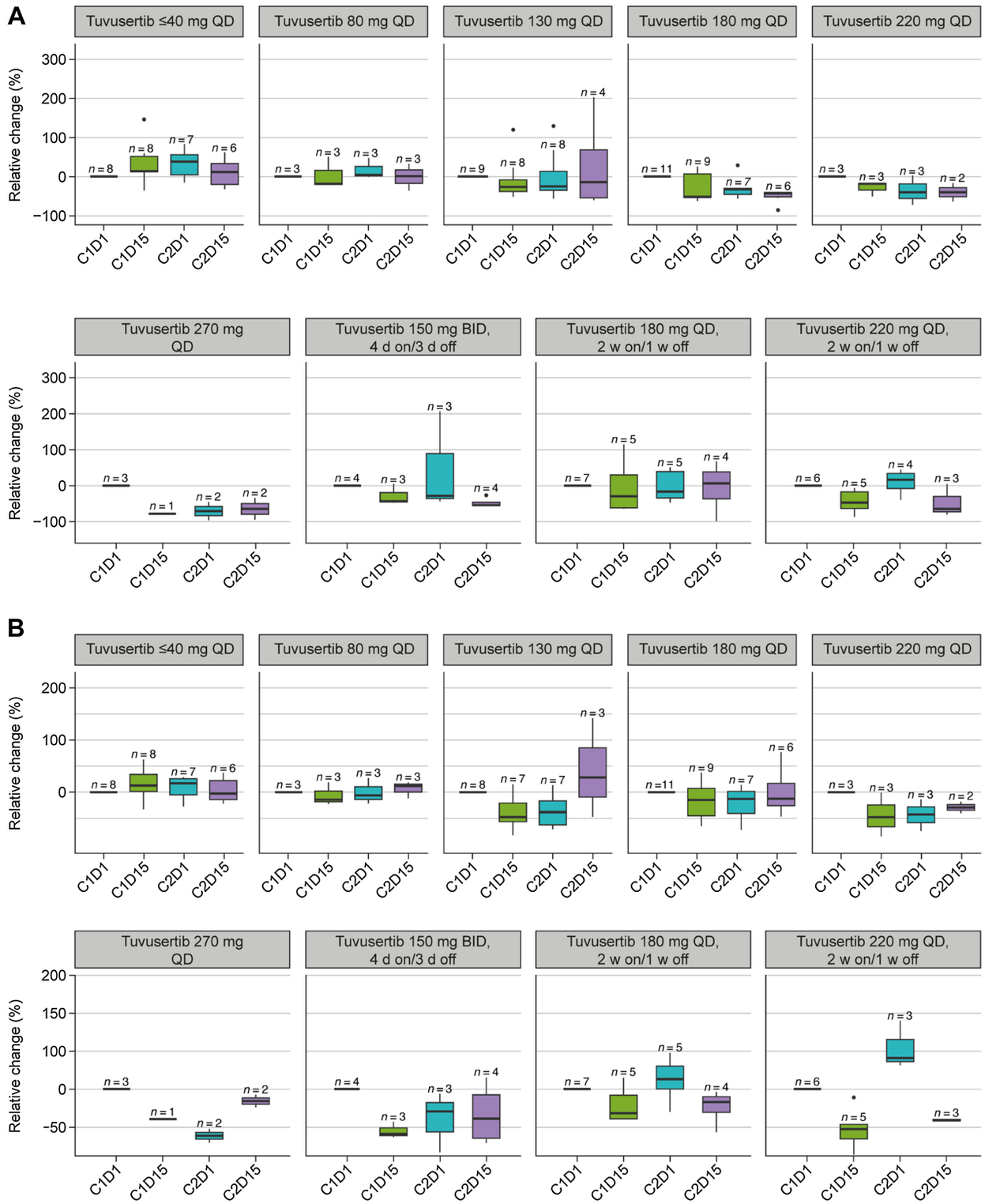

Figure 4.

Aggregated data grouped by single tuvusertib dose levels for NK cells (**A**) and monocytes (**B**). For each parameter, measurements obtained at each visit are grouped by dose level. Each graph visualizes the relative change (% RC) from the baseline of each measurement. C, cycle; D, day; MDSC, myeloid-derived suppressor cells; NK, natural killer; QD, once daily.

pronounced synergy of tuvusertib with other treatments that target the DDR, including the ATM inhibitor lartesertib (M4076) and the PARP inhibitor niraparib, and DNA-damaging agents such as cisplatin and gemcitabine, and topoisomerase inhibitors, including irinotecan (15, 16, 43, 44). The DNA damage caused by DDR inhibition results in DNA fragmentation and micronucleation, increasing the expression of neoantigens on the cell surface (45). Micronucleation activates a type I IFN response via the cyclic GMP AMP synthase–stimulator of IFN genes (cGAS–STING) pathway. Thus, immune responses mediated by ATRi in cancer cells may also enhance the efficacy of immune checkpoint inhibitors (ICI; refs. 45, 46). The absence of a significant or persistent influence of tuvusertib on CD8+ T cells, including proliferating Ki67+ subsets, may optimize the synergistic effects achieved by combining an ATR inhibitor with ICIs.

This study is ongoing, with a separate part evaluating tuvusertib in combination with the PARP inhibitor niraparib open and enrolling patients. Additional studies are currently investigating tuvusertib-based combinations in patients with advanced solid tumors, including the PD-L1 inhibitor avelumab or the ATM inhibitor lartesertib (47), the alkylating agent temozolomide (48), and the DNA-PK inhibitor peposertib (49). Furthermore, a phase 2 study is investigating tuvusertib in combination with the PD-1 inhibitor cemiplimab in patients with non-squamous NSCLC that has progressed on prior anti–PD-(L)1 and platinum-based therapies (50).

In conclusion, this FIH study demonstrated a manageable safety profile and exposure-related target engagement for tuvusertib as monotherapy in adult patients with advanced solid tumors. Furthermore, in contrast with other ATR inhibitors in development, it found no significant or persistent effect of tuvusertib on immunophenotype in patients with advanced solid tumors. These findings show potential for using tuvusertib as a combination partner in further clinical studies, which are ongoing.

Authors' Disclosures

T.A. Yap reports other support from University of Texas MD Anderson Cancer Center, as a Vice President, Head of Clinical Development in the Therapeutics Discovery Division, which has a commercial interest in DDR and other inhibitors (IACS30380/ART0380 was licensed to Artios); personal fees from AbbVie Adagene, Almac, Amgen, Inc., Amphista, Astex, Athena, Atrin, Avenzo, Avoro, Axiom, Baptist Health Systems, Biocity Pharma, Boxer, Bristol Myers Squibb, C4 Therapeutics, Calithera, Cancer Research UK, Carrick Therapeutics, Circle Pharma, Cybrexa, Daiichi Sankyo, Dark Blue Therapeutics, Diffusion, Duke Street Bio, 858 Therapeutics, EcoR1 Capital, Ellipses Pharma, Entos, FoRx Therapeutics AG, Genesis Therapeutics, Genmab, Glenmark, GLG, Globe Life Sciences, GSK, Guidepoint, Idience, Ignyta, I-Mab, Impact Therapeutics, Institut Gustave Roussy, Intellisphere, Jansen, Kyn, MEI Pharma, Merck, Merit, Monte Rosa Therapeutics, Natera, Nested Therapeutics, Nexys, Nimbus, Novocure, Odyssey, OHSU, OncoSec, Ono Pharma, Onxeo, PanAngium Therapeutics, Pegascy, PER, Piper-Sandler, Pliant Therapeutics, Prolynx, Protai Bio, Radiopharma Theranostics, resTORbio, Roche, Ryvu Therapeutics, SAKK, Schrodinger, Servier, Synnovation, Synthis Therapeutics, TCG Crossover, TD2, Terremoto Biosciences, Tessellate Bio, Theragnostics, Terns Pharmaceuticals, Tolremo, Tome, Thryv Therapeutics, Trevarx Biomedical, Varian, Veeva, Versant, Vibliome, Voronoi, Inc., Xinthera, Zai Labs, and ZielBio; grants and personal fees from Acrivon, Artios, AstraZeneca, Bayer and Beigene, Blueprint, Clovis, EMD Serono, F-Star, Ideaya Biosciences, ImmuneSensor, Mereo, Pfizer, Repare, Sanofi, and Tango; grants from BioNTech, BMS, Boundless Bio, Constellation, Cyteir, Eli Lilly, Forbius, Glaxosmithkline, Genentech, Haihe, Insilico Medicine, Ionis, Ipsen, Jounce, Karyopharm, KSQ, Kyowa, Mirati, Novartis, Ribon Therapeutics, Regeneron, Rubius, Scholar Rock, Seattle Genetics, Tesaro, Vivace, and Zenith; and other support from Seagen outside the submitted work. A.W. Tolcher reports other support from Aclaris Therapeutics, Affinia Therapeutics, Inc., Agenus, Inc., Asana Biosciences, Ascentage, Astex Pharmaceuticals, Axlmmune, Bayer, Blu Print Oncology, Daiichi Sankyo, Inc., Exelixis, Inc., Gilde Healthcare Partners, HBM Partners, IDEA Pharma, Ikena Oncology, Immuneering, Immunomet Therapeutics, Inc., Impact Therapeutics US, Inc., Karma Oncology B.V., Kirilys Therapeutics, Inc., Lengo Therapeutics, Inc., Link Immunotherapeutics, Medicxi, Mekanistic Therapeutics, Menarini Ricerche, Mersana, Nanobiotix, Nerviano Medical Sciences S.r.I. (NMS), Novo Nordisk, Inc., Nurix Therapeutics, Ocellaris Pharma, Inc. & Eli Lilly, Partner Therapeutics, Pfizer, Inc., Pierre Fabre, Pyramid Biosciences, Qualigen Therapeutics, Roche, Ryvu Therapeutics, Seattle Genetics, Singzyme Pte Ltd., SK Life Science, SOTIO Biotechnology Co., Senti Biosciences, Sun Pharma Advanced Research Company (SPARC), TheraTechnologies, Transcenta Therapeutics, Inc., Transgene, Trillium Therapeutics, Inc., Tubulis, Venus Oncology, Verastem Oncology, Adagene, Inc., Bioinvent, Boeringer Ingelheim International GmbH, Bright Peak Therapeutics, Cullinan Oncology, Elucida Oncology, EMD Serono, Immunome, Jazz, Mirati, NBE Therapeutics, Nested Therapeutics, Inc., Pheon Therapeutics, PYXIS Oncology, Roche, Spirea Limited, Inc., Vincerx, VRISE Therapeutics, Inc., Zentalis Pharmaceuticals, and ZielBio, Inc., during the conduct of the study; and other support from ABBVIE, Inc., Aclaris Therapeutics, Affinia Therapeutics, Inc., AGENUS, Inc., ASANA BIOSCIENCES, ASCENTAGE, Astex Pharmaceuticals, Axlmmune, Bayer, Blu Print Oncology, Daiichi Sankyo, Inc., Exelixis, Inc., GILDE HEALTHCARE PARTNERS, HBM PARTNERS, IDEA Pharma, Ikena Oncology, Immuneering, Immunomet Therapeutics, Inc., Impact Therapeutics US, Inc., Karma Oncology B.V., Kirilys Therapeutics, Inc., Lengo Therapeutics, Inc., Link Immunotherapeutics, Medicxi, Mekanistic Therapeutics, Menarini Ricerche, Mersana, Nanobiotix, Nerviano Medical Sciences S.r.I. (NMS), Novo Nordisk, Inc., Nurix Therapeutics, Ocellaris Pharma, Inc. & Eli Lilly, Partner Therapeutics, Pfizer, Inc., Pierre Fabre, Pyramid Biosciences, Qualigen Therapeutics, Roche, Ryvu Therapeutics, Seattle Genetics, Singzyme Pte Ltd., SK Life Science, SOTIO Biotechnology Co., Senti Biosciences, Sun Pharma Advanced Research Company (SPARC), TheraTechnologies, Transcenta Therapeutics Inc., Transgene, Trillium Therapeutics Inc., Tubulis, Venus Oncology, Verastem Oncology, Adagene, Inc., BIOINVENT, Boeringer Ingelheim International GmbH, Bright Peak Therapeutics, Cullinan Oncology, Elucida Oncology, EMD Serono, Immunome, JAZZ, Mirati, NBE Therapeutics, Nested Therapeutics, Inc., Pheon Therapeutics, PYXIS Oncology, Roche, Spirea Limited, Inc., Vincerx, Vrise Therapeutics, Inc., Zentalis Pharmaceuticals, and ZielBio, Inc., outside the submitted work; as well as reports patents for Ascentage Pharma and Zentails issued. R. Plummer reports other support from the healthcare business of Merck KGaA, Darmstadt, Germany during the conduct of the study; and personal fees from Pierre Faber, Bayer, Novartis, BMS, Cybrexa, Ellipses, CV6 Therapeutics, Astex Pharmaceuticals, Medivir, Sanofi Aventis, AstraZeneca, Merck & Co., Kenilworth, NJ, Onexo, Genmab, Immunocore, Sotio Biotech AG, Alliagator Biosciences, GSK, and Incyte outside the submitted work. T. Grombacher reports personal fees from the healthcare business of Merck KGaA, Darmstadt, Germany during the conduct of the study. Z. Szucs reports other support from Merck Serono Ltd., Feltham, UK, an affiliate of Merck KGaA, Darmstadt, Germany during the conduct of the study. I. Gounaris reports other support from Merck Serono Ltd., Feltham, UK, an affiliate of Merck KGaA, Darmstadt, Germany during the conduct of the study; other support from Merck Serono Ltd., Feltham, UK, an affiliate of Merck KGaA, Darmstadt, Germany outside the submitted work. J.S. de Bono reports personal fees from Bioxcel Therapeutics, Boehringer Ingelheim, ImCheck Therapeutics, Immunic Therapeutics, Qiagen, and Terumo; grants and personal fees from Amgen, AstraZeneca, Astellas, Bayer, Cellcentric, Crescendo, Daiichi, Genentech/Roche, Genmab, Harpoon, Janssen, Merck Serono, Merck Sharp & Dohme, Menarini/Silicon Biosystems, MetaCurUm, Myricx, Nurix Therapeutics, Oncternal, Orion, Sanofi Aventis, Sierra Oncology, Taiho, and Vertex Pharmaceuticals outside the submitted work; grants and other support from Pfizer; other support from Acai Therapeutics, Dark Blue Therapeutics, and Eisai Therapeutics, GSK, Takeda, and Tango Therapeutics; personal fees and other support from Novartis; and patents for DNA-damage repair inhibitors for treatment of cancer licensed to AstraZeneca and 17-substituted steroids useful in cancer treatment licensed to Janssen. No disclosures were reported by the other authors.

Authors' Contributions

T.A. Yap: Resources, validation, investigation, writing–review and editing. **A.W. Tolcher:** Resources, validation, investigation, writing–review and editing. **R. Plummer:** Resources, validation, investigation, writing–review and editing. **J.K. Mukker:** Conceptualization, resources, validation, investigation, methodology, writing–review and editing. **M. Enderlin:** Resources, validation, investigation, writing–original draft. **C. Hicking:** Resources, data curation, formal analysis, validation, investigation, writing–review and editing. **T. Grombacher:** Resources, validation, investigation, writing–review and editing. **G. Locatelli:** Conceptualization, resources, validation, investigation, methodology, writing–review and editing. **Z. Szucs:** Conceptualization, resources, validation, investigation, methodology, writing–review and editing. **I. Gounaris:** Conceptualization, resources, validation,

investigation, methodology, writing–review and editing. **J.S. de Bono:** Conceptualization, resources, validation, investigation, methodology, writing–original draft.

Acknowledgments

This study was sponsored by the healthcare business of Merck KGaA, Darmstadt, Germany (CrossRef Funder ID: 10.13039/100009945). Medical writing support was provided by Jess Buxton of Bioscript Group, Macclesfield, UK, and funded by the healthcare business of Merck KGaA, Darmstadt, Germany. T.A. Yap was supported by NCI Cancer Center Support Grant CA016672 to The University of Texas MD Anderson Cancer Center, Department of Defense grants W81XWH2210504_BC211174, W81XWH-21–1-0282_OC200482, and NIH R01 grant 1R01CA255074. Study sites in the UK received support from Cancer Research UK and the Department of Health for England as Experimental Cancer Medicine Centers. The authors would like to thank patients, investigators, co-investigators, and the study teams at each of the participating centers and at the healthcare business of Merck KGaA, Darmstadt, Germany. Corinna Schaffroth of the healthcare business of Merck KGaA, Darmstadt, Germany, performed medical data review and analysis. Annick Seithel-Keuth of the healthcare business of Merck KGaA, Darmstadt, Germany, contributed to the analysis and interpretation of PK data. Giuseppe Sessa of the healthcare business of Merck KGaA, Darmstadt, Germany, led the immunophenotyping and pharmacodynamic sample analysis. Danyi Wang of EMD Serono led the molecular sample analysis. Angela Manginelli and Aslihan Gerhold-Ay, both of the healthcare business of Merck KGaA, Darmstadt, Germany, conducted the immunophenotyping data analysis.

Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Received August 10, 2023; revised October 26, 2023; accepted February 21, 2024; published first February 26, 2024.

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