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An adaptive, biomarker-directed platform study of durvalumab in combination with targeted therapies in advanced urothelial cancer

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Summary paragraph (217)

Durvalumab is a programmed death-ligand 1 (PD-L1) inhibitor with clinical activity in advanced urothelial cancer (AUC)¹. AUC is characterized by several recurrent targetable genomic alterations²⁻⁵. This study (NCT02546661, BISCAY) combined durvalumab with relevant targeted therapies in biomarker-selected chemotherapy refractory AUC populations including: (i) fibroblast growth factor receptor (FGFR) inhibitors in tumors with FGFR DNA alterations (FGFRm); (ii) pharmacological inhibitor of the enzyme poly ADP ribose polymerase (PARP) in tumors with and without DNA homologous recombination repair deficiency (HRRm); (iii) TORC1/2 inhibitors in tumors with DNA alteration to mTOR/PI3K pathway³⁻⁵. This trial adopted a novel, biomarker-driven, multi-arm adaptive design. Safety, efficacy and relevant biomarkers were evaluated. Overall, 391 patients were screened and 135 were allocated to one of six study arms. Response rates (RR) ranged from 9–36% across the study arms, which did not meet efficacy criteria for further development. Overall survival (OS) and progression-free survival (PFS) were similar in the combination arms and durvalumab monotherapy arm. Biomarker analysis showed a correlation between circulating plasma-based DNA (ctDNA) and tissue for FGFRm. Sequential ctDNA analysis showed changes to FGFRm correlated with clinical outcome. These data support the clinical activity of FGFR inhibition and durvalumab monotherapy but did not show increased activity for any of the combinations. These findings question the targeted/immune therapy approach in AUC.

Main text

Platinum-based chemotherapy remains the most widely used systemic therapy for treatment-naïve AUC⁶. However, at progression, immune checkpoint inhibitors, such as PD-L1 inhibitors, are standard therapy⁷. Targeted therapies have had less impact, with a series of negative trials as single agents in unselected patients^{2,8}. However, recent approaches with FGFR inhibitors in selected populations have yielded RRs of 30–40% and US Food and Drug Administration approval⁹. Other potential targeted therapies, such as PARP inhibition and TORC1/2 inhibition, also hold promise, although the biomarkers are not well described^{10,11}. Theoretically, a combination of targeted/immune therapy could increase the RR and better maintain the durability of response. This may be particularly relevant to biomarker selected populations, but trials to test such hypotheses are logistically complex and not widely reported. Therefore, there are very limited combination data with targeted therapy and PD-(L)1 inhibitors in biomarker-selected individuals¹².

Here we test the hypothesis that the PD-(L)1 inhibitor, durvalumab in combination with one of three targeted agents in AUC is safe and may confer improved clinical activity. These therapies include the FGFR tyrosine kinase inhibitor AZD4547 in tumors with FGFRm, the PARP inhibitor olaparib in tumors with or without HRRm and the TORC1/2 inhibitor vistusertib in tumors with *TSC1/2* and *RICTOR* gene alterations (TRm; partial enrichment). AZD4547 monotherapy and durvalumab monotherapy were also explored as single-agent therapies as references within the study design (**Fig. 1a**), as both have established single agent activity and can act as a benchmark for activity.

Results

Patients with measurable AUC, who had progression of disease despite prior platinum-based chemotherapy, were potentially eligible for the study (see methods for detail). Patient characteristics are given in **Supplementary Data Table 1**. The different treatment arms were relatively well balanced for key prognostic factors, despite modest numbers¹³. A high proportion of patients' tumors had poor prognostic features.

An overview of the trial design is shown in **Fig. 1a**. The design is novel for solid tumor oncology trials, in that it pursued a multi-arm, personalized approach with an adaptive design, with the aim of rapidly identifying active combinations within a single tumor type (AUC). There was the opportunity to add new arms or new biomarker strategies in existing arms.

The primary endpoint of the study was to establish the safety of the combinations. Adverse events (AEs) were assessed using Common Terminology Criteria for Adverse Events (CTCAE) v4. There were limited prior safety data on the combinations, therefore this was a phase Ib design. The study was monitored by a Safety Review Committee and had appropriate Institutional Review Board and ethical approval. The predefined efficacy indicators (secondary endpoint) included objective response rate (ORR) (confirmed response, Response Evaluation Criteria in Solid Tumors [RECIST] 1.1) and PFS/OS (Kaplan–Meier). Here we present the results of the first six arms.

The study was designed to efficiently identify a clinically meaningful efficacy signal beyond benchmark levels seen with single-agent immune therapy (15–25%)¹⁴. Personalized combination therapy is complex to deliver and can result in increased toxicity and cost. Therefore, only highly efficacious combinations would be worth pursuing for further development, with responses superseding monotherapy

expectations and being supported by biomarker data. For this reason, the study lacked predefined efficacy objectives and reported 80% confidence interval (CI) for RRs. The study was designed to enroll approximately 20 patients per arm with the potential to expand the cohort or move directly to randomized trials if the combinations were impressive. Efficacy endpoints (RR, PFS, OS) were informally compared with the durvalumab arm and previous monotherapy data. The adaptive design also allowed for the enrollment of biomarker-negative cohorts to test the potential relevance of the biomarkers. The biomarker component of the study was included to support decision making around efficacy signals, and included DNA, RNA, sequential ctDNA and protein analysis^{15,16,17}.

This pragmatic and resource-intensive adaptive approach has advantages, by asking multiple logistically complex questions in parallel and potentially identifying highly efficacious regimens. However, it lacks the accuracy of randomized phase II studies with regards to statistical significance. The feasibility of addressing the questions asked in BISCAY with this more orthodox approach is questionable and would require large numbers of screened patients across multiple parallel trials. The adaptive design allowed interrogation of multiple questions concurrently, some of which were not specifically defined at the start of the study to improve its relevance.

A consort diagram for screened and enrolled participants is in **Extended Data Fig. 1**.

Treatment was assigned according to predefined tumor genomic alterations from archived tissue (Foundation Medicine analysis). DNA alterations were explored in 391 screened patients with platinum-refractory AUC who participated in the study, and 135 patients who received one of five study treatments. All patients also had ctDNA analysis at baseline (**Fig. 1b, Extended Data Fig. 2**). DNA alterations in this cohort were in line with other AUC series^{18,19}, including alterations in TERT, p53, CDKN2A. Predefined biomarker signatures for FGFRm, HRRm and TRm from

archived tumors were identified in 21%, 14%, and 15% of the screened population, respectively (**Fig. 1c, Supplementary Data Table. 4**). Eight of the 135 patients had more than one targetable mutation and were allocated to the arm with the least prevalent alterations. Tumors with FGFR alterations had a low prevalence of high tumor mutational burden (TMB) and PD-L1 expression (16% and 33%, respectively). These biomarkers were present in >50% of tumors in the olaparib + durvalumab HRR biomarker-positive arm (**Supplementary Data Table 1**), which may have influenced efficacy.

Overall ctDNA was identified in 89% of treated patients (gene panel analysis)¹⁶. There was a positive correlation between tumor and ctDNA-based alterations (**Fig. 1c, d, Extended Data Fig. 2**), suggesting plasma-based biomarker screening may be a viable selection approach for some therapies. Future prospective circulating biomarker-based trials in AUC are warranted.

A positive correlation was observed between tumor TMB (tTMB) and blood TMB (bTMB) values (Spearman correlation: 0.527, $p < 0.0001$) (**Fig. 1e**). The age of the archived tumor samples was not relevant in determining concordance between tTMB and bTMB (**Fig. 1f**). Gene expression analysis correlated FGFR3 gene expression with the predefined FGFR DNA alteration signature (**Fig. 1g**). Together these data support approaches beyond tissue-based DNA analysis to select patients for targeted therapy.

Transcriptomic analysis for established molecular subgroups^{20,21} showed an increased proportion of luminal papillary subtype in tumors with FGFR alterations, as described previously²² (**Extended Data Fig. 3**). However, FGFRm tumors did not have higher expression of immune active T cell signatures²². (**Extended Data Fig. 4**).

One hundred and thirty-five patients were enrolled in the treatment arms between October 3, 2016 and March 14, 2019. The median follow-up of the arms ranged between 4.8 months for the vistusertib + durvalumab and 6.1 months for durvalumab monotherapy. Confirmed responses included for the six arms are shown in **Fig. 2a–c**. Two patients in the HRR unselected cohort had HRR/ DNA damage repair (DDR) alterations as defined by our panel. One of these patients responded to therapy and one had disease progression, resulting in the HRR positive and negative RR of 35.3% and 5.0%, respectively, for patients treated with olaparib + durvalumab. A lack of deep durable responses or complete response for immune targeted combinations in this study was notable.

Duration of response and PFS did not appear to be enhanced by the addition of targeted therapy to durvalumab (**Fig. 2d**). Six-month PFS rates ranged between 20% and 43% across the study arms (**Fig. 2e**). Median OS for durvalumab monotherapy was 10.3 months (80% CI, 3.1–not applicable), which was in line with expectations. One-year OS rates ranged between 42% and 56% across the study arms. While direct comparison of the allocated arms was not possible, similar OS outcomes were observed across treatment arms (**Fig. 2f**). In randomized patients, AZD4547 + durvalumab was not clearly associated with a higher level of clinical activity versus AZD4547 monotherapy. The lack of robust clinical activity with the combination of AZD4547 + durvalumab may have been influenced by the low prevalence of immune biomarkers among patients enrolled in that arm (**Supplementary Data Table 1**).

Direct comparisons of the olaparib + durvalumab HRR selected and unselected arms require caution as these enrolled sequentially, and the sample size was small. While RRs in the unselected arm appeared low, overall efficacy (disease control rate, PFS, OS) in the two arms were similar. Neither arm exceeded expectations, halting further development in this study.

Grade 3 and 4 therapy-related AEs occurred in: 31% of patients for AZD4547; 48% for AZD4547 + durvalumab; 27% and 36% for olaparib + durvalumab HRR selected and unselected cohorts, respectively; 24% for vistusertib + durvalumab; and 10% for durvalumab monotherapy (**Supplementary Data Tables 2 and 3**). Discontinuation of both therapies or discontinuation of the targeted therapy for AEs occurred in 25% for AZD4547 monotherapy, 33% for AZD4547 + durvalumab, 40% and 0% for olaparib + durvalumab HRR selected and unselected cohorts, respectively, and 34% for vistusertib + durvalumab. Discontinuation for toxicity is given in **Extended Data Fig. 1**. There were three deaths, which occurred in the olaparib + durvalumab arm (sepsis and pulmonary emboli) and the vistusertib + durvalumab arm (pneumonia). Overall, it did not appear that toxicity, via dose reductions or discontinuations were responsible for the lack of efficacy of the combinations.

Baseline ctDNA levels of the respective inclusion biomarkers (e.g., FGFRm, HRRm, mRT) were analyzed and correlated with clinical outcomes¹⁷. Using an allele frequency (AF) cut-off to >1% the RRs were 3/12 (25%), 5/19(26%), 6/31 (19%), and 7/28 (25%) for the AZD4547 monotherapy, AZD4547 + durvalumab, olaparib + durvalumab, and vistusertib + durvalumab arms, respectively. Together with the concordance between tumor and ctDNA biomarker correlation data, these data suggest plasma-based biomarker screening may become an alternative for patient selection in the future.

Specific HRR alterations were rare and not consistently associated with outcomes for olaparib + durvalumab (**Fig. 1d**). The biomarker(s) predictive of response to olaparib in UC remain uncertain. The lack of dynamic changes to tracked DNA alteration with therapy in **Fig. 3** supports this further. It is likely that durvalumab is driving many of the responses with this combination in view of the immune active environment (high PD-L1/TMB). Olaparib does not appear to increase durvalumab

efficacy in this setting. Previous data shows other single-agent PARP inhibitors have shown limited activity in UC²³.

We did not show a strong correlation between tTMB and outcomes. tTMB has shown promise as a biomarker in other settings, but results are also inconsistent in UC¹⁵.

Whether our findings are driven by the addition of the targeted therapy or the inaccuracy of tTMB as a biomarker is unclear (**Extended Data Fig. 5–9**). Circulating bTMB has been explored as a surrogate marker of outcome in other cancers with inconsistent results^{24,25}. Data on bTMB in AUC is limited. Our data shows a correlation with tumor TMB, but it does not appear to more accurately select responders²⁵. There was no overall change in bTMB expression between baseline and progression and no consistent correlation with initial response or study arm (**Fig. 4d**). Changes to immune biomarkers with therapy has been reported and may represent a changing immune phenotype²⁶.

Higher levels (above median) of ctDNA (mutation allele frequency) at baseline correlated with shorter OS (**Fig. 4a**) and are therefore a likely surrogate for tumor burden²⁷. ctDNA may become a relevant prognostic biomarker, supported by increasing data²⁸. Tumor-specific FGFR and HRR and TR ctDNA alterations were tracked within the respective therapy. Results for the FGFR arm showed a correlation between expression of these mutations and radiological response to therapy (**Fig. 4b, Extended Data Fig. 10, Supplementary Data Fig. 1**). Increasing expression occurred at progression. Landmark analysis showed extended OS in patients treated with AZD4547 therapy who experienced a decrease in tracked FGFR DNA alterations (**Fig. 4c**). This approach opens new avenues for monitoring of treatment effects of targeted therapy with immune therapy. Dynamic changes occurred for tracked FGFR DNA alterations during AZD4547 therapy (**Fig. 3a–c**). Clearance and/or reduction of the tracked mutation with therapy were observed.

Development of new clones occurred and may have a role in the development of resistance²⁴⁻²⁶ (**Supplementary Data Fig. 2**).

Results from tracked HRR mutations treated with the olaparib + durvalumab combination were complicated by germline mutations and mutations that could not be tracked in ctDNA (rearrangements and deletions), resulting in small numbers of somatic mutations for interpretation (5/15). HRR mutations did not change significantly with therapy (**Fig. 3d-f**). The lack of dynamic changes in the HRR arm potentially reinforces the modest efficacy signal for olaparib in this combination. Efficacy and biomarker data from the durvalumab and vistusertib arm did not support the hypothesis that targeting TORC1/2 in combination with durvalumab improved efficacy, dynamic changes occurred in some responding patients (**Fig. 4g-i**). We explored the relationship between molecular subgroup and response in the FGFR targeted arm and durvalumab alone (**Extended Data Fig. 4**). Results showed no specific subgroup associated with response. The role of the molecular classifications in UC remains to be defined.

Discussion

Testing drug combinations with a personalized approach in advanced cancer can be challenging. The prevalence of the biomarker, time taken to assess the biomarker and frequency in the clinical setting is important. Running multiple more traditional randomized phase II studies to address drug activity is not always feasible. This study pursued an alternative approach, with an adaptive design, a modest number of patients in each arm, and the potential to expand or change arms. Safety of the novel combination and efficacy were assessed concurrently. A strong biomarker platform facilitated potential reinforcement of efficacy signals and decision making. We were able to show this approach was feasible and were able to investigate the presence of increased clinical activity, worthy of rapid further development. The

efficacy endpoints of the trial focused on ambitious RRs, PFS and OS, using the monotherapy arms as indirect benchmark comparators. This lacked accuracy, but highly efficacious interactions should be identified with this approach. None of the combination arms showed adequate efficacy or compelling biomarker data to move into randomized trials. Our findings raise broad questions regarding the efficacy of targeted/immune combinations. Although the hypothesis was sound, personalized combination approaches with immune checkpoint inhibitors as the backbone have not been widely reported in UC or other cancers¹². This is perhaps the most robust study to address this question to date and raised concern about the approach.

AZD4547 (FGFR monotherapy) showed activity, which was supported by biomarker analysis, but the addition of durvalumab did not enhance this activity. This is a concern for future, small, randomized trials in this area. Different FGFR inhibitors and different biomarkers of FGFR signaling are under investigation. Our efficacy results do not necessarily apply to other treatments.

Data on PARP inhibition in HRR altered UC are lacking and the frequency and prognostic relevance of such alterations is unclear. Clinical activity was modest and HRR biomarker signal was unsupportive in our study. Strong efficacy/biomarker signals for PARP inhibition in UC have not been forthcoming²³. Future data from platinum therapy-naïve populations will likely be important for the olaparib + durvalumab combination (NCT03459846).

Targeting TORC1 and 2 in combination with durvalumab was driven by limited clinical data in UC^{10,11}. We showed ctDNA mTOR/PI3K alterations were more common than those seen in the tumor, but they did not correlate with outcome. Further pursuit of this combination is not warranted.

A parallel biomarker-driven approach to clinical development of immunotherapy-based combinations has attractive features and shortcomings. By adapting the study

to bring in new study arms and expanding current research questions, BISCAY has the advantage of addressing multiple research questions concurrently. However, it lacks the accuracy and benefit of direct comparisons and formal statistical analysis. Subtle benefits over standard of care will be missed with this approach. Also, the effect of sequencing of these agents was not explored. Other studies with a similar design to BISCAY are ongoing (NCT03193190).

The biomarker work was extensive and robust, supporting efficacy signals in some arms. The ctDNA analysis has the potential to supersede tissue-based analysis^{17,27,29,30}. However, there are also shortcomings. The prognostic effect of the biomarkers cannot be easily determined with this single-arm approach. Selection of biomarkers for one arm may lead to an imbalance of such features in the other arms. Finally, the use of exploratory biomarkers and unproven combinations means different biomarker panels may have different results.

This biomarker-directed multi-arm study, combining durvalumab with three promising molecular targets, shows that this novel design is feasible. The strong biomarker platform, including circulating biomarkers, helped reinforce efficacy signals, which further adds to the utility of this approach. However, the combination of immune therapy and targeted therapy in biomarker driven populations does not appear to improve efficacy having implications for further drug development in UC and beyond.

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Author Contributions Statement

T.P., S.C., D.P., A.B., P.G., D.H., R.M., R.McE., D.L. contributed to conception and design of the study.

T.P., A.F., D.P., S.S.S., M.G., R.J., I.K., R.M. acquired the data.

T.P., S.C., D.P., A.B. S.S.S., M.G., P.G., R.J., D.H., I.K., R.M., R.McE., F.M., D.L. contributed to data analysis and interpretation.

T.P., S.C., J.Ca., A.F., P.M., D.P., G.G., F.R., N.C., A.B., S.S.S., M.G., P.G., A.R., R.J., J.Co., R.M. contributed to clinical management, patient recruitment and data collection.

T.P., A.F., D.P., D.H., R.M. contributed administrative, technical or material support.

T.P., S.C., D.P., R.M., R.McE., F.M. contributed to the drafting of the manuscript.

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All authors reviewed and approved the final version of the manuscript.

Competing Interests Statement

T.P. has received research funding from AstraZeneca, Astellas, Bristol-Myers Squibb, Roche, and Merck; and received honoraria for lectures or advisory boards from AstraZeneca, Astellas, Bristol-Myers Squibb, Roche, Merck, Johnson & Johnson, Ipsen, Exelixis, Pfizer, Novartis, and Seattle Genetics.

D.C. is a full-time employee at AstraZeneca and owns stocks/shares in AstraZeneca.

S.C. has held advisory roles for Astellas Pharma, Bayer, Beigene, Clovis Oncology, Janssen-Cilag, and Pfizer; participated in speakers' bureaus for Pfizer; received honoraria from Clovis Oncology and Novartis; received research funding from Clovis Oncology and Sanofi/Aventis; and received travel expenses from Beigene and Clovis Oncology.

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F.J. has provided consultancy for Roche, Ipsen, Astra Zeneca, Janssen, Tesaro, Bristol-Myers Squibb, Pfizer, Novartis, Sanofi, Astellas. F.J has received

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A.F. has received honoraria and travel expenses from AstraZeneca, MSD, Roche, Janssen, and Astellas.

P.M. declares no competing interests.

D.P. consults for Ada Cap (Advanced Accelerator Applications), Amgen, Astellas, AstraZeneca, Bayer, Bicycle Therapeutics, Boehringer Ingelheim, Bristol-Myers Squibb, Clovis Oncology, Eli Lilly, Exelixis, Incyte, Janssen, Mirati, Monopteros, Pfizer, Pharmacyclics, Roche, Seattle Genetics, and Urogen; has received research funding from Ada Cap (Advanced Accelerator Applications), Agensys, Astellas, AstraZeneca, Bayer, BioXcel Therapeutics, Bristol-Myers Squibb, Clovis Oncology, Eisai, Eli Lilly, Endocyte, Genentech, Innocrin, MedImmune, Medivation, Merck, Mirati, Novartis, Pfizer, Progenics, and Replimune; and is a stockholder in Bellicum.

F.R. has received honoraria from Merck KGaA and MSD.

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S.S.S. has consulted for or served on advisory boards for AstraZeneca, Pfizer, Roche, Merck, Bristol-Myers Squibb, Astellas, Janssen, and Bayer.

M.G. has held a consultancy or advisory role for Aileron Therapeutics and is a stockholder in Rappta Therapeutics.

P.G. has consulted for AstraZeneca, Bayer, Biocept, Bristol-Myers Squibb, Clovis Oncology, Driver, EMD Serono, Exelixis, Foundation Medicine, Genentech/Roche, Genzyme, Heron Therapeutics, Janssen, Merck, Mirati Therapeutics, Pfizer, Seattle Genetics, QED Therapeutics, and GlaxoSmithKline; has delivered educational programmes to GlaxoSmithKline; and received research funding from AstraZeneca, Bayer, Genentech/Roche, Merck, Mirati Therapeutics, Oncogenex, Pfizer, Clovis Oncology, Bavarian Nordic, Immunomedics, Debiopharm, and Bristol-Myers Squibb.

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J.Co. is an AstraZeneca employee.

D.H., I.K., R.M., and R.McE., are AstraZeneca employees and stockholders.

F.M. is a contract employee of AstraZeneca and shareholder in StatProcess and Health Data Process.

D.L. is a former employee of AstraZeneca, as Senior Director Physician on this study; consults for AstraZeneca; and has received research funding from Decision Science.

Figure legends

Figure 1. Biomarker landscape of enrolled patients in tumor and plasma.

A. Overview of trial design. See **Extended Data Fig. 1** for CONSORT diagram.

*Cohort unselected, but RICTOR, TSC1 and TSC2 directed to this cohort

B. Oncoprint of next-generation sequencing tumor tissue profiling (Foundation Medicine) for all enrolled patients (n=135) across the different treatment arms.

Genomic alteration landscape for 135 enrolled patients' primary tumors. Top tumor mutational burden derived from FoundationOne panel (Foundation Medicine)

mutations per megabase of DNA. Somatic mutations for all BISCAY patient selection

biomarkers are shown along with the top five additional significantly mutated genes, percentage prevalence is shown on the right. Copy number alterations for selected genes are also shown across the treatment arms.

C. Venn diagrams depicting the prevalence and overlap between BISCAY enrollment biomarkers in tumor tissue utilizing the FoundationOne panel (Foundation Medicine) screened population, n=391 (i) and all enrolled treated patients, n=135 (ii) and the prevalence and overlap between BISCAY enrollment biomarkers in plasma ctDNA utilizing the Guardant OMNI panel (Guardant Health) for treated patients, n=132 (cut off 1% allele frequency) (iii).

D. Concordance between tissue and plasma next-generation sequencing DNA alteration results for BISCAY across the four biomarker selected arms. Upper triangle represents tumor data (Foundation One analysis) and lower triangle represents the plasma ctDNA data (Guardant OMNI panel).

E. Mutational distribution of archived tissues tTMB and bTMB. Spearman correlation: 0.527 for tTMB and bTMB shows a positive correlation. Red and green color-coded points represent radiological progression and response respectively (RECIST v1.1). Red lines represent median values.

F. Analysis to assess the impact of age of archived tissue from time of starting drug (> or <1yr) on TMB compared to ctDNA taken at the time of study entry. (<1 year tTMB n=38; <1 year bTMB n=36; >1 year tTMB n=92; >1 year bTMB n=86). More contemporary tissue did not appear to correlate more closely with ctDNA (two-sided Wilcoxon test unadj. p=0.1 and 0.06, for < and >1yr, respectively). Whiskers extend from the minima to maxima. The center line represents the median and the box spans the 25th to 75th percentiles.

G. Comparison of FGFR3 expression between patients on arms A, B and D. Screening tumor tissue samples (FFPE material) were subjected to bulk RNA

sequencing using Illumina technology. An unadjusted p-value was calculated using a two-sided Wilcoxon test (**** $p \leq 0.0001$).

bTMB, blood-derived tumor mutational burden; CR, complete response; DNA, deoxyribonucleic acid; DDR, DNA damage response; FGFR, fibroblast growth factor receptor; FGFRm, FGFR alterations; FMI, Foundation Medicine Innovation; HHRm, homologous recombination repair mutant; NE, not evaluable; PD, progressive disease; PR, partial response; R, randomized; RECIST, Response Evaluation Criteria in Solid Tumours; SD, stable disease; TMB, tumor mutational burden; TPM, transcripts per million; tTMB, tissues tumor mutational burden; TRm, *TSC1/2* and *RICTOR* gene alterations

Figure 2: Efficacy data for different arms of the BISCAY study.

A. Confirmed response rate by RECIST v1.1. 80% confidence intervals given.

B. Waterfall plots assessing maximum reduction in target lesion for the study arms.

HRR = DNA homologous recombination repair signature, FGFRm = alterations for FGFR 1–3 genes, TH = DNA alterations to RICTOR, TSC1, TSC2. None of the plots display outstanding efficacy compared with durvalumab monotherapy. Not all patients had response scan due to early discontinuation accounting for the inconsistent number.

C. Summary of best radiological outcomes with treatment (confirmed, RECISTv1.1).

None of the combination arms demonstrated meaningful levels of CR. PD most prominent in durvalumab monotherapy arm (as seen with previous data). PD was least frequent in the durvalumab + AZD4547 arm (24%).

D. Swimlane plot showing duration of therapy and timing of confirmed response and progression. Responses in the combination arms tended to occur soon after starting

therapy, potentially driven by the targeted therapy. Duration of response was comparable in the durvalumab combination and monotherapy arms.

E. PFS for the study arms using Kaplan–Meier method. 80% CI given. No study arm showed outstanding PFS compared to monotherapy. Median PFS for durvalumab is short as seen in previous studies³¹. No formal statistical comparisons made.

F. Overall survival (OS) for the study arms using Kaplan–Meier method. 80% CI given. No study arm shows outstanding OS compared with monotherapy. OS for durvalumab monotherapy was in line with results seen previously in this setting³¹. No formal statistical comparisons made.

CI, confidence interval; CR, complete response; DDR, deoxyribonucleic acid damage response; FGFR, fibroblast growth factor receptor; FGFRm, alterations for FGFR 1–3 genes; NA, not available; OS, overall survival; PD, progression of disease; PFS, progression-free survival; PR, partial response; RECIST, response evaluation criteria in solid tumors; SD, stable disease; TH, DNA alterations to RICTOR, TSC1, TSC2

Figure 3: Longitudinal mutation tracking on treatment

Tracking effects of therapy using bespoke panel of 10 genes (Resolution Bioscience) to monitor dynamic changes in ctDNA FGFR mutant allele fraction. Results showed no consistent changes associated with response or resistance to therapy.

A. No development of new clones during response and subsequent progression of disease on FGFR monotherapy.

B. No development of new clones during response and subsequent progression on FGFR/immune combination therapy.

C. Response followed by progression on combination therapy showing appearance of new FGFR3 clones. Several of the base changes detected led to the same single

amino acid change. We chose to represent the change with higher AF or detected at more timepoints.

D. No change in qualifying BCRA1 allelic frequency on olaparib + durvalumab treatment.

E. No change in qualifying ATM allelic frequency prior to progression on olaparib + durvalumab treatment.

F. Minor change in AFs of two ctDNA-specific missense HRR mutations prior to response on olaparib + durvalumab.

G–I. Mutation AF reduction in responding patients on vistusertib + durvalumab.

Absence of mutation increase in AFs or new resistance mutations prior to resistance to therapy. Nine of 30 patients receiving vistusertib + durvalumab had RICTOR amplification in baseline ctDNA v.s only 2 in the tissue.

AF, allelic frequency; ctDNA, circulating tumor DNA; DNA, deoxyribonucleic acid; FGFR, fibroblast growth factor receptor; PD, progressive disease; PR, partial response; uPR, unconfirmed PR (PR reported only once without a second scan confirmation)

Figure 4: Correlation of FGFR mutant allele fraction ctDNA and response to therapy

A. Comparison of average somatic allelic frequencies (in percentage) for baseline ctDNA showed that higher baseline allelic frequencies are associated with worse outcome. Above median level vs below median *HR: 2.14[1.22; 3.75], p=0.006*.

Logrank test and HR obtained by Cox model.

B. Tracking patient specific FGFR mutations and fusions. Plasma from patients on AZD4547 monotherapy (first panel) or AZD4547 + durvalumab combination (second panel) are shown. A bespoke panel of 10 genes (Resolution Bioscience: see

methods) was used to monitor dynamic changes in ctDNA FGFR mutant allele fraction during early cycles of therapy (cycles 1–3). Results show significant increase in FGFR AF in relapsing tumors. (Each boxplot displays the median, first and third quartiles (lower and upper hinges), the largest value smaller and the smallest value larger than 1.5 interquartile (upper and lower whiskers). P-values were calculated for each timepoint from a Kruskal–Wallis rank-sum test; p=0.44, 0.18, 0.049 and 0.0026 for the SCREEN, C1, C2 and C3 timepoints, respectively. For each timepoint; PR n=5, SD n=9 and PD n=4).

C. Landmark OS analysis comparing patients that have a decrease (above median) in tracked FGF alterations and those that did not. Analysis performed: Kaplan–Meier analysis according to the ratio of the frequency (range 0 to 1) of FGFR mutations or fusions at cycle 3 to baseline (median=0.34). Inclusion of patients receiving AZD4547 ± durvalumab (n=21). p=0.05 using log rank method, HR=4.48 [0.85; 23.7] using Cox model.

D. Comparison of bTMB (Mutations / Mb) between baseline and progression ctDNA samples.

AF, allelic frequency, BOR, best overall response; CR, complete response; ctDNA, circulating tumor deoxyribonucleic acid; FGFR, fibroblast growth factor receptor; HR, hazard ratio; OS, overall survival; PD, progressive disease; PR, partial response; SD, stable disease; TMB, tumor mutational burden

Methods

Study design

This was an open label, multi-drug, biomarker-directed, multi-arm phase Ib study. Patients with advanced/metastatic UC who had progressed on prior platinum-based chemotherapy treatment were included. The study had a modular design, allowing evaluation of the safety, tolerability, pharmacokinetics and anti-tumor activity of multiple agents in combinations in parallel (**Fig. 1a**). Multiple arms were open concurrently. New arms were added during the study in accordance with the adaptive design. All the agents had monotherapy safety data but only olaparib and durvalumab were licenced. There was limited safety data on any of the combinations, giving this a phase Ib design. The study was monitored by a Safety Review Committee. The dose of the agent used in combination with durvalumab was identified from the safety run-in part of the study (first 6 patients) and was based upon a review of all available safety and tolerability data. All participants consented appropriately to the study, which followed appropriate international ethical and governance standards (NCT02546661).

The study consisted of a number of study arms, six of which are mature and are reported here. Each arm evaluated the safety and efficacy of a specific regimen. The allocation of patients to specific arms depended on the presence of predefined genomic alterations as specified in the protocol (**Supplementary Data Table 4**). Genetic alterations were measured by Foundation One central analysis performed on archived tissue (tissue taken <2 years before study participation). It was planned to enrol approximately 20 to 26 patients per arm in North America and Europe. Higher numbers were permitted in the durvalumab + vistusertib combination as it was only partially enriched for *TSC1/2* and *RICTOR* gene alterations.

The arm exploring the fibroblast growth factor receptor (FGFR) inhibitor AZD4547 had a randomized component to explore the activity of FGFR inhibitor monotherapy vs the combination with durvalumab (1:2 randomization). It investigated AZD4547 (80mg orally twice daily) monotherapy (an FGFR 1-3 inhibitor) versus durvalumab (1500mg IV given every 4 weeks) with AZD4547 (80mg orally twice daily) in combination. Tumors with FGFR mutations or fusions were included (FGFR3 mutations and FGFR1-3 fusions).

The arm exploring the poly ADP ribose polymerase (PARP) inhibitor olaparib, investigated the safety and tolerability of durvalumab (1500mg IV infusions every 4 weeks) with olaparib (300mg orally twice daily). Patients were selected based on the presence of alterations in a homologous recombination repair gene panel which is used across the olaparib program (HRR) (**Supplementary Data Table 4**). A separate arm subsequently explored olaparib and durvalumab at the same doses in biomarker unselected patients. This arm was included as an amendment to explore the relevance of the HRR biomarker.

the arm exploring the TORC1/2 inhibitor vistusertib investigated the safety and tolerability of durvalumab (1500mg IV infusions every 4 weeks), in combination vistusertib (50mg orally twice daily). Patients with RICTOR amplification, or TSC1/2 mutations were included in this arm. This biomarker panel has not been investigated previously, therefore the arm also permitted recruitment of biomarker unselected patients, which occurred after the durvalumab alone arm completed enrolment.

If a tumor had >1 targetable mutation the patient was allocated to the cohort with the least common mutation. The exception to this was the FGFRm arm. Tumors with these alterations were prioritized to the FGFR targeted arm.

A further arm investigated durvalumab monotherapy (1500mg IV infusions every 4 weeks) in tumors without eligible selection alterations. As not all the arms were open concurrently, some patients receiving durvalumab had targetable mutations (FGFRm). Treatment continued until lack of clinical benefit, or intolerable due to adverse events. Cross-sectional imaging was performed every 8 weeks to assess confirmed response. The data monitoring committee assessed dosing and safety. The trial has appropriate ethical and regulatory approval.

Key inclusion criteria were histologically confirmed radiologically progressive advanced/metastatic (Stage IV) UC (with a component of transitional cell histology), patients were required to have received at least one prior platinum-containing regimen in a metastatic setting and/or must have progressed after an adjuvant or neo-adjuvant platinum-containing regimen within a period of 1 year prior to the commencement of screening.

Patients were required to have measurable disease at baseline (Response Evaluation Criteria in Solid Tumours [RECIST] v1.1), a World Health Organization performance status of 0 or 1, and a minimum life expectancy of 12 weeks. It was necessary to have archived tissue within 2 years of study entry for biomarker analysis. Key exclusion criteria included inadequate major organ function (including a creatinine clearance below 30ml/min) or prior exposure to drugs targeting the same genetic alterations under investigation in the study (after screening). Patients with prior auto-immune related disease, ongoing immune suppressive therapy or active infection were also excluded. Each arm had specific exclusion criteria related to the targeted therapy under investigation described previously in other monotherapy studies.

The primary objective was to assess the safety and tolerability of study treatments. Adverse events were assessed using Common Terminology Criteria for Adverse Events (CTCAE) v4. Data on related and unrelated events was collected. Data on dose modification, interruption and termination due to toxicity were collected. Secondary objectives, and predefined efficacy endpoint was confirmed overall response rates (based on RECIST v1.1). Efficacy would also be considered within the context of established immune biomarkers such as programmed death-ligand 1 (PD-L1) and tumor mutational burden (TMB). This was in part why the trial lacked formal pre-planned efficacy parameters as immune biomarker expression within selected arms was unknown at the start of the study.

Other secondary efficacy endpoints included progression free survival (PFS) and overall survival (OS) which were calculated using the Kaplan Meier method. Subset analysis of outcome data was performed with respect to PD-L1 status (SP263 immune or tumor cell staining $\geq 10\%$ defining positivity¹) and TMB status (≥ 10 mutation/MB defining positivity). A TMB of 10 mutations/MB was selected as this has previously been shown to be discriminatory for immune checkpoint inhibitors in platinum refractory UC¹⁵.

Circulating tumor DNA was measured at baseline in all participants (ctDNA: Guardant Health OMNI panel at baseline)^{13,23}; detected DNA alterations and TMB were correlated with tissue-based DNA alterations (Foundation One)¹⁵. Tracking patient specific FGFR3 mutations and fusions occurred for those subjects in Arm A. ctDNA from plasma from these patients was sequenced using a bespoke panel of 10 genes (Resolution Bioscience) to monitor dynamic changes in ctDNA FGFR mutant allele fraction (MAF) during early cycles of therapy (Cycles 2-4)²⁴. These data were correlated with outcome. Dynamic changes to DNA alterations were assessed.

Formalin-fixed-paraffin-embedded tumor tissues collected at screening from patients were analysed using paired-end RNA sequencing technology (Almac). The RNA sequencing included enrichment for transcripts from coding genes (RNA Exome) and minimum of 25 million paired reads per sample.

Statistics. Statistical analyses for biomarkers were mainly descriptive. Eighty percent confidence intervals of percentages were provided by use of the Clopper-Pearson method. OS and PFS from the first study dose were analysed by Kaplan Meier analyses. The log-rank test and Cox models were used for subgroups comparisons. In addition, a landmark analysis was performed for the FGFRm ratio at cycle 3 from the cycle 3 date. The boxplots show the first and third quartiles (boxes) and the median (middle line); the whiskers extend up to 1.5× the IQR.

Ethics approval. Ethical approval was granted by all study sites and institutions.

Life Sciences Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The authors declare that access to the clinical and biomarker data analysed here may be obtained in accordance with AstraZeneca's data sharing policy as part of an external collaborative request (<https://astrazenecagroup->

dt.pharmacm.com//DT/Home/Index/) or an external data access request
(<https://vivli.org/ourmember/astrazeneca/>).