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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection.
Data analysis	All statistical analyses were performed in R (https://www.R-project.org/), version 3.5.2. Other softwares used include bcl2fastq2 (v2.20), FastQC (v0.11.8), Burrows–Wheeler Alignment tool (v.0.7.12), Picard (v2.14), MultiQC (v1.7), GSNAP (version 2013-10-10), GenomicAlignments (version 1.22.0.), and voom (v3.44.3). Code is deposited in public repository and the link is in the Code Availability statement of the manuscript.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Public datasets that were used during data processing included 1000 Genomes project (https://www.internationalgenome.org/), ExAC (http://exac.broadinstitute.org/), ESP (https://esp.gs.washington.edu/drupal/), dbSNP (https://www.ncbi.nlm.nih.gov/snp/), and Molecular Signature Database (http://www.gsea-msigdb.org/gsea/msigdb/)
The data required to reproduce results, including Figures and Tables, will be made available upon request. Qualified researchers may request access to individual patient level data through the clinical study data request platform (https://vivli.org/). Further details on Roche's criteria for eligible studies are available here

(https://vivli.org/members/ourmembers/). For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here (https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Details on the IMvigor010 study plan are published elsewhere. No sample size calculation was performed for this ctDNA substudy. The sample size was determined by the number of patients in IMvigor010 (n=809) which had evaluable tissue and plasma samples at C1D1 (n=581), and at a combination of C1D1 and C3D1 (n=485). RNA sequence data was available for n=728 patients (will be made available according to data sharing agreement), and all RNA analyses in the paper use n=573 patients which also have ctDNA available. For ABACUS study, the sample size was also determined by the number of patients which had pre-treatment plasma (n=40).
Data exclusions	Patients were only excluded from this ctDNA substudy if they did not have evaluable tissue and plasma. Please also see Consort diagrams in primary Figure 1 and ED Fig 7.
Replication	Biomarkers measured in patient plasma samples were not replicated, and were unable to be replicated due to the limited patient samples available. Note that the ctDNA assay run on the patient samples has been analytically validated previously.
Randomization	Details on the IMvigor010 study plan, including randomization, has been published elsewhere. The ctDNA substudy is a biomarker study of patient tissue and plasma samples. Covariates controlled for in our multivariate Cox regression analyses include patient nodal status (positive or negative), PD-L1 status (IC0/1 or IC2/3), tumor stage (<T3 or T3/4), prior neoadjuvant chemotherapy (Yes or No), and number of lymph nodes resected at surgery (<10 or ≥10). ABACUS was not a randomized trial.
Blinding	IMvigor010 was an open-label study. The investigator and patient were not blinded to treatment assignment. Given the concerns of giving mock infusion every 3 weeks intravenously for up to one year to patients in the control arm, this study elected not to employ a placebo-controlled design. In ABACUS, no blinding was performed as it is not ethical to give neoadjuvant placebo therapy and thereby postponing active surgical treatment. Hence, all subjects received the same treatment. All investigators and collaborators were blinded to clinical results when performing measurements and assays.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	The VENTANA PD-L1 (SP142) rabbit monoclonal primary antibody (RPA; Ventana Medical Systems Inc.) (diluted to 3 mg/mL total protein concentration) was optimized for use as a fully automated IHC assay on the BenchMark ULTRA (Ventana Medical Systems Inc., Tucson, AZ) staining platform using the OptiView DAB IHC Detection Kit and OptiView Amplification Kit (Ventana Medical Systems Inc., Tucson, AZ). The antibody supplier was Spring Biosciences (catalog number, NA); the clone used was SP142 (19H3L2).
Validation	VENTANA PD-L1 (SP142) assay validation can be found at the following link: https://www.accessdata.fda.gov/cdrh_docs/pdf16/p160002c.pdf

Human research participants

Policy information about studies involving human research participants

Population characteristics	Patient metadata are included in the manuscript. Briefly, IMvigor010 patients had muscle-invasive urothelial carcinoma of the bladder or upper tract (UC of the renal pelvis or ureters) who had undergone radical surgical resection (cystectomy for patients with bladder cancer or nephroureterectomy for patients with upper tract UC) with bilateral lymph node dissection. Please refer to Extended Data Tables 1-2 for covariate-relevant population characteristics of the human research participants in the IMvigor010 ctDNA study, including Race, Sex, BECOG, Primary tumor site, tumor stage, prior neoadjuvant, PD-L1 status, TMB, and lymph node status, and number of lymph nodes resected. Patients in the ABACUS trial had histologically confirmed T2-T4a urothelial bladder cancer awaiting planned cystectomy, and covariate-relevant baseline population characteristics included smoking status (77.5% yes), tumor stage (74% T2, 18% T3, 8% T4), gender (87% male), and age (mean 71).
Recruitment	In the IMvigor010 study, a multicentre, open-label, randomised, phase 3 trial, we enrolled patients from 192 hospitals, academic centres, and community oncology practices across 24 countries or regions (appendix pp 3–6). Eligible patients were aged 18 years or older and had histologically confirmed muscle-invasive urothelial carcinoma of either the bladder or, following a protocol amendment (on July 14, 2016), the upper tract (ie, urothelial carcinoma of the renal pelvis or ureters). Patients with mixed histology were required to have a dominant urothelial carcinoma pattern. Enrolment of patients with upper tract urothelial carcinoma was capped at 10% of the study population to reflect the real-world distribution of patients. Patients who did not receive previous neoadjuvant chemotherapy (or only received one cycle of a platinum-based regimen) must have declined or been ineligible for cisplatin-based adjuvant chemotherapy based on impaired renal function (glomerular filtration rate <60 mL/min), grade 2 or worse hearing loss, grade 2 or worse peripheral neuropathy, or Eastern Cooperative Oncology Group (ECOG) performance status of 2. Patients were required to have undergone radical surgical resection (cystectomy for patients with bladder cancer or nephroureterectomy for patients with upper tract urothelial carcinoma) with lymph node dissection, to the extent deemed necessary by the treating surgeon, with negative surgical margins 14 weeks or less before enrolment (≤12 weeks per earlier protocol version from July 14, 2016). For patients with upper tract disease, excision of the bladder cuff was required. Based on pathological stage per tumour–node–metastasis classification (Union for International Cancer Control–American Joint Committee on Cancer, seventh edition), the disease must have been M0 and either ypT2–4a or ypN+ (ypT2-4 or ypN+ for upper tract urothelial cancer) in patients treated with previous neoadjuvant chemotherapy, or pT3–4a or pN+ (pT3–4 or pN+ for upper tract disease) in patients without previous neoadjuvant chemotherapy. These criteria for patient selection aimed to focus on a population at high risk of recurrence (with an expected 5-year recurrence risk of approximately 50%). The absence of pathological residual disease and the absence of metastasis—as confirmed by negative postoperative radiological imaging, an ECOG performance status of 2 or less, and adequate haematological and end-organ function—were required for enrolment. Patients with a history of autoimmune disease, ongoing infection, or clinically significant cardiovascular disease (New York Heart Association Class II or worse) were excluded. Patients with a history of autoimmune-related hypothyroidism or type 1 diabetes were eligible provided that they were on a stable dose of thyroid replacement hormone or a stable dose of insulin, respectively. Central evaluation of a surgical resection or lymph node sample to test for PD-L1 expression using the VENTANA SP142 immunohistochemical assay (Ventana Medical Systems, Oro Valley, AZ, USA) was also required. Tissue samples were required at the time of cystectomy or nephroureterectomy (after neoadjuvant chemotherapy for applicable patients); PD-L1 status was determined from this sample. Adequate haematological and end-organ function was determined by laboratory test results (absolute neutrophil count, white blood cell count, lymphocyte count, platelet count, haemoglobin concentration, aspartate aminotransferase concentration, alanine aminotransferase concentration, alkaline phosphatase concentration, serum bilirubin concentration, prothrombin time, partial thromboplastin time, and calculated creatinine clearance [Cockcroft-Gault formula]) obtained within 14 days before the first study treatment. In IMvigor010, enrolment was initially restricted to patients whose tumours expressed PD-L1 (immunohistochemistry IC2 or IC3 status, defined as PD-L1-expressing tumour-infiltrating immune cells covering ≥5% of the tumour area); however, on the basis of results from IMvigor210 cohort 120 that showed that clinical activity was not dependent on PD-L1 status, we amended the protocol (on July 14, 2016) to expand enrolment to patients regardless of PD-L1 status. Adjuvant chemotherapy or radiotherapy for urothelial carcinoma following surgical resection was not allowed, and for patients with upper tract urothelial carcinoma, post-surgical intravesical chemotherapy or BCG was not allowed based on treatment patterns in clinical practice at the time of study design. ABACUS was a phase II, investigator-initiated clinical trial (NCT02662309) sponsored by the Queen Mary University of London. Between May 2016 and June 2018, 121 patients were screened in 21 sites from 4 European countries and 95 patients were recruited and received atezolizumab. Patients with histologically confirmed (T2-T4aN0M0) transitional cell UC of the bladder, awaiting planned radical cystectomy were eligible to the study. Additional eligibility criteria included residual disease after transurethral resection of the bladder (TURBT), adequate fitness for planned cystectomy (according to local guidelines), ineligible for or refusal of cisplatin based neoadjuvant chemotherapy, no evidence of nodal or metastatic disease on cross sectional imaging, Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 or 1 and adequate hematologic and end-organ function within 4 weeks prior to the first study treatment. Major exclusion criteria included evidence of significant uncontrolled concomitant disease that could affect compliance with the protocol or interpretation of results, previous autoimmune disease, ongoing active infections or prior use of immune checkpoint inhibitors. All patients provided written informed consent. The relevant institutional review board or ethics committee for each participating centre approved the study, which was conducted in accordance with the principles of Good Clinical Practice, the provisions of the Declaration of Helsinki, and other applicable local regulations. The study was sponsored by Queen Mary University of London.
Ethics oversight	For IMvigor010: The trial was done according to Good Clinical Practice and the Declaration of Helsinki. Protocol approval was obtained from independent review boards or ethics committees at each site. All patients provided written, informed consent. The study protocol is in the appendix of the primary clinical manuscript for IMvigor010 (Bellmunt 2021). For ABACUS: All patients provided written informed consent, which included the exploratory biomarker endpoints described here. The study was approved by the relevant institutional review board and ethics committee for each participating centre and was conducted in accordance with the principles of Good Clinical Practice, the provisions of the Declaration of Helsinki,

and other applicable local regulations (NCT02662309). The study was sponsored by Queen Mary University of London. The Barts Experimental Cancer Centre Clinical Trials Group had overall responsibility for trial management and day-to-day running of the trial, and the trial was overseen by an independent data monitoring committee (IDMC). Emerging safety data was reviewed regularly by the IDMC.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT02450331 IMvigor010, and NCT02662309 ABACUS
Study protocol	Full trial protocols can be found in primary clinical manuscript for IMvigor010 (Bellmunt 2021 Lancet Oncology) and previously published manuscript for ABACUS (Powles 2019 Nature Medicine)
Data collection	Details of clinical data collection can be found in primary clinical manuscripts for IMvigor010 and ABACUS. Briefly, IMvigor010 data was collected between October 5, 2015, and 30 November 2019, patients from 24 countries or regions were enrolled in 192 centres. Patient's who had evaluable tumor tissue, matched normal, and plasma had samples shipped to Natera for ctDNA evaluation.
Outcomes	The primary efficacy endpoint of IMvigor010 was DFS, which was defined by local (pelvic) or urinary tract recurrence, distant UC metastasis or death from any cause. OS, defined by the time from randomization to death from any cause, was a secondary efficacy endpoint. The clinical cutoff date was November 30, 2019. The design endpoints and inclusion criteria have been published previously for ABACUS, and pathological complete response was the primary endpoint.