Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial

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Summary

Background Patients with metastatic urothelial carcinoma have few treatment options after failure of platinum-based chemotherapy. In this trial, we assessed treatment with atezolizumab, an engineered humanised immunoglobulin G1 monoclonal antibody that binds selectively to programmed death ligand 1 (PD-L1), in this patient population.

Methods For this multicentre, single-arm, two-cohort, phase 2 trial, patients (aged ≥18 years) with inoperable locally advanced or metastatic urothelial carcinoma whose disease had progressed after previous platinum-based chemotherapy were enrolled from 70 major academic medical centres and community oncology practices in Europe and North America. Key inclusion criteria for enrolment were Eastern Cooperative Oncology Group performance status of 0 or 1, measurable disease defined by Response Evaluation Criteria In Solid Tumors version 1.1 (RECIST v1.1), adequate haematological and end-organ function, and no autoimmune disease or active infections. Formalin-fixed paraffin-embedded tumour specimens with sufficient viable tumour content were needed from all patients before enrolment. Patients received treatment with intravenous atezolizumab (1200 mg, given every 3 weeks). PD-L1 expression on tumour-infiltrating immune cells (ICs) was assessed prospectively by immunohistochemistry. The co-primary endpoints were the independent review facility-assessed objective response rate according to RECIST v1.1 and the investigator-assessed objective response rate according to immune-modified RECIST, analysed by intention to treat. A hierarchical testing procedure was used to assess whether the objective response rate was significantly higher than the historical control rate of 10% at an α level of 0·05. This study is registered with ClinicalTrials.gov, number NCT02108652.

Findings Between May 13, 2014, and Nov 19, 2014, 486 patients were screened and 315 patients were enrolled into the study. Of these patients, 310 received atezolizumab treatment (five enrolled patients later did not meet eligibility criteria and were not dosed with study drug). The PD-L1 expression status on infiltrating immune cells (ICs) in the tumour microenvironment was defined by the percentage of PD-L1-positive immune cells: IC0 (<1%), IC1 (≥1% but <5%), and IC2/3 (≥5%). The primary analysis (data cutoff May 5, 2015) showed that compared with a historical control overall response rate of 10%, treatment with atezolizumab resulted in a significantly improved RECIST v1.1 objective response rate for each prespecified immune cell group (IC2/3: 27% [95% CI 19–37], p<0·0001; IC1/2/3: 18% [13–24], p=0·0004) and in all patients (15% [11–20], p=0·0058). With longer follow-up (data cutoff Sept 14, 2015), by independent review, objective response rates were 26% (95% CI 18–36) in the IC2/3 group, 18% (13–24) in the IC1/2/3 group, and 15% (11–19) overall in all 310 patients. With a median follow-up of 11·7 months (95% CI 11·4–12·2), ongoing responses were recorded in 38 (84%) of 45 responders. Exploratory analyses showed The Cancer Genome Atlas (TCGA) subtypes and mutation load to be independently predictive for response to atezolizumab. Grade 3–4 treatment-related adverse events, of which fatigue was the most common (five patients [2%]), occurred in 50 (16%) of 310 treated patients. Grade 3–4 immune-mediated adverse events occurred in 15 (5%) of 310 treated patients, with pneumonitis, increased aspartate aminotransferase, increased alanine aminotransferase, rash, and dyspnoea being the most common. No treatment-related deaths occurred during the study.

Interpretation Atezolizumab showed durable activity and good tolerability in this patient population. Increased levels of PD-L1 expression on immune cells were associated with increased response. This report is the first to show the association of TCGA subtypes with response to immune checkpoint inhibition and to show the importance of mutation load as a biomarker of response to this class of agents in advanced urothelial carcinoma.

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Research in context

Evidence before this study

We searched MEDLINE and PubMed for reports published in English using the search terms "bladder cancer", "metastatic bladder cancer", "transitional cell carcinoma of the urothelium", "metastatic urothelial carcinoma", "urothelial carcinoma" AND "docetaxel", "urothelial carcinoma" AND "paclitaxel", "urothelial carcinoma" AND "vinflunine", "urothelial carcinoma" AND "immunotherapy", "urothelial carcinoma" AND "second-line", "urothelial carcinoma" AND "salvage treatment" and "urothelial carcinoma" AND "PD-L1". We focused on reports published in the 10 years before the start of the trial. We reviewed all evidence for treatments presently being assessed in patients with inoperable locally advanced or metastatic urothelial carcinoma, with a particular emphasis on salvage treatments following progression on platinum-based regimens. We identified strong evidence for an endogenous immune response against urothelial cell carcinoma, and identified an unmet clinical need for approaches that enhance anti-tumour immunity in patients with urothelial cancer. Although platinum-based chemotherapy is associated with high response rates and overall survival benefits in metastatic urothelial carcinoma, few patients have durable responses, and, after progression, treatment options are scarce. The only agent approved anywhere worldwide for second-line therapy is vinflunine in Europe; however, this drug did not improve survival in its pivotal phase 3 trial when compared with best supportive care. No agents are approved by the US Food and Drug Administration, and frequently used cytotoxic chemotherapy agents, such as docetaxel, paclitaxel, or pemetrexed, have response rates of around 10%, are associated with substantial toxicity, and do not improve survival.

Added value of this study

In this trial, atezolizumab, a humanised monoclonal anti-programmed death ligand 1 (PD-L1) antibody, was investigated as a treatment for patients with metastatic urothelial cancer after platinum-based chemotherapy, many of whom had been heavily pretreated. Atezolizumab showed an improvement in the co-primary endpoints of objective response rate by independent assessment according to Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1 and by investigator assessment according to immune-modified RECIST. Responses were durable, with 84% of patients continuing to respond after almost 1 year of follow-up. Higher levels of PD-L1 expression on immune cells were associated with higher response rates and longer survival. Atezolizumab also seemed to be safe and generally well tolerated in this highly comorbid population. Translational analyses done in this study help to bridge the gaps between immunotherapy response and our understanding of molecular and immune biology. These exploratory analyses describe a link between response to PD-L1 inhibition and intrinsic molecular subtypes of bladder cancer described by The Cancer Genome Atlas (TCGA), and show potential clinical phenotypes of the unique immune microenvironments associated within each of these TCGA subtypes. Furthermore, the results support the importance of mutation load as a biomarker of response to this class of agents in urothelial carcinoma.

Implications of all the available evidence

Since the development of combination methotrexate, vinblastine, doxorubicin, and cisplatin chemotherapy for advanced urothelial carcinoma, no major improvements in therapeutic efficacy have been achieved in the past 30 years. The prognosis for patients who relapse after platinum-based chemotherapy is dismal. Based on these results, atezolizumab shows promise as a second-line treatment option for patients with metastatic urothelial carcinoma who have progressed on previous platinum-based therapy. PD-L1 expression on immune cells is a potential biomarker for the selection of patients for treatment with atezolizumab. This report is the first to link intrinsic TCGA subtypes with immunotherapy response, and shows the importance of mutation load in urothelial carcinoma on immune checkpoint therapy outcome.

Introduction

Urothelial carcinoma kills more than 165 000 patients annually worldwide and is the ninth most common cancer overall worldwide.[1,2] The efficacy of immunotherapy in non-muscle-invasive urothelial carcinoma of the bladder was first established in 1976 with BCG, but no immunotherapy has been approved for the treatment of advanced disease.[3] Platinum-based chemotherapy is the standard of care in previously untreated patients with metastatic urothelial carcinoma, and is associated with an overall survival of around 9–15 months.[4,5] The prognosis for patients who relapse after platinum-based chemotherapy is poor, with median survival ranging from 5 to 7 months and no known life-prolonging treatments available.[6] New approaches are needed to break this therapeutic stalemate.

Programmed death ligand 1 (PD-L1) is an immune checkpoint that negatively regulates T-cell function by binding to its receptors programmed death 1 (PD-1) or B7-1 on activated T lymphocytes and other immune cells. Because T lymphocytes have a central role in mediating acquired anti-tumour immunity, expression of PD-L1 in the tumour microenvironment endows tumours with a mechanism to evade eradication by the host immune system.[7–9] PD-L1 is broadly expressed across a wide range of malignancies, including urothelial carcinoma, and blockade of the PD-L1–PD-1 pathway has been shown to produce overall survival benefits in non-small-cell lung cancer, melanoma, and renal cell carcinoma.[7,10–15]

Recent data have suggested that immune checkpoint inhibitors are more active in tumours with high mutation

rates than in those with lower mutation rates.[11,16–22] Emerging data from The Cancer Genome Atlas (TCGA) suggest that urothelial carcinoma carries the third highest mutation rate of all studied cancers and that gene expression signatures could be used to separate the disease into luminal and basal subtypes.[23,24] Additional mechanisms, such as increased prevalence of non-synonymous mutations, higher neoantigen load, higher antigen binding affinity, and some T-effector signatures, have all been identified as factors that might predict for a durable clinical benefit in patients treated with immune checkpoint inhibitors, which is consistent with the hypothesis that mutations might create neoantigens that are recognised by anti-tumour T cells.[25–29] Taken together, these observations provide a rationale for the clinical investigation of anti-PD-L1 immunotherapy in metastatic urothelial cancer.

Atezolizumab is an engineered humanised monoclonal immunoglobulin G1 antibody that binds selectively to PD-L1 and prevents its interaction with PD-1 and B7-1, while sparing the interaction between PD-L2 and PD-1.[30,31] Atezolizumab has shown durable responses in a cohort of patients with metastatic bladder cancer in a phase 1 study, with higher response rates recorded in patients with higher levels of PD-L1 expression on tumour-infiltrating immune cells than in those with lower PD-L1 expression.[32]

To confirm the anti-tumour activity of atezolizumab in patients with advanced urothelial carcinoma whose disease had progressed after previous platinum-based chemotherapy, we conducted a phase 2, global, multicentre, single-arm trial to assess the efficacy and safety of atezolizumab. Prospective assessment of the association of PD-L1 expression with response was a co-primary endpoint. Additionally, exploratory translational studies were done to address the scientific hypotheses associated with checkpoint inhibition in metastatic urothelial carcinoma.

Methods

Study design and participants

For this phase 2, global, multicentre, single-arm two-cohort trial (appendix p 9), patients aged 18 years or older were eligible for enrolment into either cohort 1 or 2 if they had histologically or cytologically documented locally advanced (on the TNM staging system, T4b and any N; or any T and N2–3) or metastatic (M1, stage IV) urothelial carcinoma (including of the renal pelvis, ureter, urinary bladder, or urethra). Cohort 1 comprised patients who had not received previous treatment in the metastatic setting and were judged to be ineligible for cisplatin treatment; these patients are not described in this report because of insufficient follow-up. Cohort 2 consisted of patients with inoperable locally advanced or metastatic urothelial carcinoma whose disease had progressed after previous platinum-based chemotherapy. These patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; measurable disease defined by Response Evaluation Criteria In Solid Tumors version 1.1 (RECIST v1.1); and adequate haematological and end-organ function according to laboratory results obtained within 14 days before the first study treatment (absolute neutrophil count ≥1500 cells/μL [without granulocyte colony-stimulating factor support within 2 weeks before day 1 of cycle 1]; white blood cell count >2500 cells/μL; lymphocyte count ≥300/μL; platelet count ≥100 000/μL [without transfusion within 2 weeks before day 1 of cycle 1]; haemoglobin ≥9·0 g/dL [although patients were allowed to be transfused or receive erythropoiesis-stimulating agents to meet this criterion]; and aspartate transaminase [AST], alanine transaminase [ALT], and alkaline phosphatase ≤2·5×the upper limit of normal [ULN], except for those with documented liver metastases [AST and/or ALT ≤5×ULN], those with

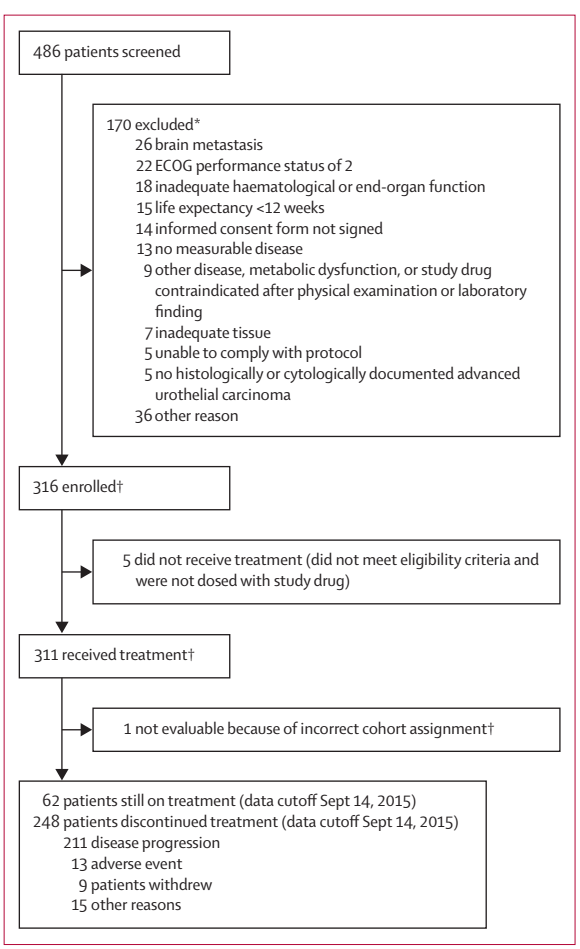

Figure 1: **Trial profile**
This trial profile is only for patients in cohort 2. ECOG=Eastern Cooperative Oncology Group. *Includes rescreened patients. †Based on data cutoff date of May 5, 2015. Two cohort 2 patients and one cohort 1 patient were reassigned to the alternative cohort based on eligibility reassessments between the May 5 and Sept 14, 2015, data cutoffs (enrolled and treated numbers of patients based on the Sept 14 data cutoff are 315 and 310, respectively). ‡Excludes one patient with unknown site.

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See Online for appendix

	IC2/3 (n=100)	IC1/2/3 (n=207)	All patients (n=310)*
Age, years	66 (41–84)	67 (32–91)	66 (32–91)
Male sex	78 (78%)	160 (77%)	241 (78%)
White race	87 (87%)	184 (89%)	282 (91%)
Site of primary tumour			
Bladder	79 (79%)	159 (77%)	230 (74%)
Renal pelvis	11 (11%)	27 (13%)	42 (14%)
Ureter	5 (5%)	12 (6%)	23 (7%)
Urethra	3 (3%)	5 (2%)	5 (2%)
Other	2 (2%)	4 (2%)	10 (3%)
Baseline creatinine clearance <60 mL/min	40 (40%)	69 (33%)	110 (36%)
ECOG performance status			
0	42 (42%)	83 (40%)	117 (38%)
1	58 (58%)	124 (60%)	193 (62%)
Haemoglobin concentration <100 g/L	24 (24%)	50 (24%)	69 (22%)
Tobacco use			
Current	6 (6%)	19 (9%)	35 (11%)
Never	34 (34%)	72 (35%)	107 (35%)
Previous	60 (60%)	116 (56%)	168 (54%)
Number of Bellmunt risk factors			
0	31 (31%)	61 (30%)	83 (27%)
1	35 (35%)	72 (35%)	117 (38%)
2	28 (28%)	59 (29%)	89 (29%)
3	6 (6%)	15 (7%)	21 (7%)
Metastatic sites at baseline			
Visceral†	66 (66%)	152 (73%)	243 (78%)
Liver	27 (27%)	61 (30%)	96 (31%)
Lymph node only	24 (24%)	39 (19%)	43 (14%)
Previous cystectomy	44 (44%)	83 (40%)	115 (37%)
Time since previous chemotherapy ≤3 months	43 (43%)	87 (42%)	121 (39%)
Previous therapy with platinum-based regimen			
Cisplatin-based	83 (83%)	161 (78%)	227 (73%)
Carboplatin-based	17 (17%)	43 (21%)	80 (26%)
Other platinum combination	0	3 (1%)	3 (1%)
Previous neoadjuvant or adjuvant chemotherapy, with first progression within ≤12 months	24 (24%)	42 (20%)	57 (18%)
Number of previous systemic regimens in the metastatic setting			
0	24 (24%)	42 (20%)	59 (19%)
1	36 (36%)	83 (40%)	124 (40%)
2	19 (19%)	41 (20%)	64 (21%)
3	11 (11%)	24 (12%)	39 (13%)
≥4	10 (10%)	17 (8%)	24 (8%)
Intravesical BCG administered, n (%)	15 (15%)	46 (22%)	73 (24%)

IC=immune cell. ECOG=Eastern Cooperative Oncology Group. *Of the total of 310 patients, 103 patients were in the IC0 group (not shown), 107 in the IC1 group (not shown), and 100 in the IC2/3 group. †Visceral metastasis defined as liver, lung, bone, or any non-lymph node or soft tissue metastasis.

Table 1: **Baseline characteristics**

documented liver or bone metastases [alkaline phosphatase ≤5×ULN]; and serum bilirubin ≤1·5×ULN [apart from patients with known Gilbert disease who have serum bilirubin ≤3×ULN]). Patients could not have any autoimmune disease or corticosteroid use.

Formalin-fixed paraffin-embedded tumour specimens with sufficient viable tumour content were required from each patient before study enrolment.

Patients were enrolled at major academic medical centres and community oncology practices. In total, 77 sites from North America and Europe were selected. Patients were enrolled by 70 of the 77 selected sites.

The study was approved by the independent review board at each participating site and was done in full conformance of the provisions of the Declaration of Helsinki and Good Clinical Practice Guidelines. Approval from the Institutional Ethics Committee (IEC) or the Institutional Review Board (IRB) was obtained before study start and was documented in a letter to the investigator specifying the date on which the committee met and granted the approval. Roche also obtained approval from the relevant Competent Authority before starting the study. An independent data monitoring committee reviewed the available safety data every 6 months after the first patient enrolled. All patients provided written informed consent.

Procedures

Patients in cohort 2 received a fixed dose of 1200 mg intravenous atezolizumab administered on day 1 of each 21-day cycle. Dose interruptions were allowed for toxicity, but dose reductions were not permitted. Patients were allowed to continue atezolizumab treatment after RECIST v1.1 criteria for progressive disease if they met prespecified criteria for clinical benefit to allow for identification of non-conventional responses. Patients were informed of the potential for pseudoprogression as part of the consent process, and were advised to discuss treatment beyond progression with their study physician.

Measurable and non-target lesions (which were not measurable) were assessed and documented before treatment was started. Patients underwent tumour assessments with cross-sectional imaging at study sites every 9 weeks for the first 12 months following day 1 of cycle 1. These tumour assessments were done by an Independent Review Facility (BioClinica, NJ, USA) and by the local investigator. After 12 months, tumour assessments were done every 12 weeks. Safety assessments were done according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.0. A sample of archived tumour tissues, in addition to serum and plasma samples, was collected for exploratory biomarker assessments.

Patient tumour samples were assessed prospectively and centrally (by HistoGeneX, Brussels, Belgium) for PD-L1 expression by immunohistochemistry with the SP142 assay (Ventana, AZ, USA).[33] The PD-L1 tumour-infiltrating immune cell (IC) status was defined by the percentage of PD-L1-positive immune cells in the tumour microenvironment: IC0 (<1%), IC1 (≥1% but <5%), and IC2/3 (≥5%). Areas of BCG inflammatory response were excluded from the assessment of PD-L1 immune cell

status. An analysis of PD-L1 expression on tumour cells and CD8+ infiltration by immunohistochemistry was also done (see appendix pp 2–3 for additional details).[30]

Gene expression levels were quantified by TruSeq RNA Access RNA-seq (Illumina, CA, USA).[34–36] Molecular subtypes were assigned according to The Cancer Genome Atlas, with some modifications to adapt for the use of RNA Access RNA-seq platform for formalin-fixed, paraffin-embedded tissues from our study.[23] Mutation detection and mutation load assessment as estimated by targeted genomic profiling were done by Foundation Medicine (Cambridge, MA, USA).[37] These analyses were done on tumour tissue collected during screening (appendix pp 2–3).

Outcomes

The primary endpoint of this study was objective response rate based on two distinct methods: independent review facility-assessed objective response rate according to RECIST v1.1, and investigator-assessed objective response rate according to immune-modified RECIST criteria to better assess atypical response kinetics described with immunotherapy.[38,39] These co-primary endpoints were chosen because of the emerging recognition that RECIST v1.1 might be inadequate to fully capture the benefit of the unique patterns of response from immunotherapeutic agents.[40]

Secondary endpoints included: duration of response and progression-free survival by both independent review facility according to RECIST v1.1 and investigator assessed as per immune-modified RECIST; overall survival; 12-month overall survival; and safety. Exploratory analyses included the association between gene-expression profiling, CD8+ T-cell infiltration, and mutation load with independent review facility-assessed objective response.

Statistical analysis

We performed the efficacy analyses on the intention-to-treat population. We assessed the objective response rate in the objective response-evaluable population, defined as intention-to-treat patients who had measurable disease according to RECIST v1.1 at baseline, and analyses of the duration of response were done on the subset of patients who achieved an objective response. We used the exact binomial test to test the binary endpoints of objective response rate. We estimated time-to-event outcomes, including duration of response, progression-free survival, and overall survival, using the Kaplan-Meier method.[41] We computed the 95% CIs for median duration of response, progression-free survival, and overall survival using a robust non-parametric Brookmeyer and Crowley method.[42]

For the primary endpoint of objective response rate, we used a hierarchical fixed-sequence testing procedure to compare the objective response rate between the treatment group and a historical control for three prespecified populations in the following order: objective response-evaluable patients with a PD-L1 immunohistochemistry score of IC2 or 3 (IC2/3), followed by those with a score of IC1, 2, or 3 (IC1/2/3), followed by all objective response-evaluable patients (appendix p 5). We did the hypothesis tests on these three populations sequentially on the basis of independent review facility-assessed objective response rate according to RECIST v1.1 followed by the investigator-assessed objective response rate according to immune-modified RECIST at a specific two-sided α level of 0·05 for each test, while controlling the overall type I error at the same α level. If no statistical significance was detected at a particular level in the hierarchy, then no further hypothesis testing was done. The study was designed to estimate the objective response rate in patients receiving atezolizumab and to detect an improvement in the objective response rate compared with a historical 10% response rate. No formal alternative objective response rate hypothesis was chosen. The study had a variable range of statistical power at different alternative objective response rates. We planned to enrol a minimum of around 100 patients with an immunohistochemistry score of IC2/3, resulting in an overall sample size of approximately 300 patients based on an estimated 30% prevalence for the IC2/3 population. The 95% CI using the Clopper-Pearson method for an observed objective response rate of 40% was 30–50%, and the study would have 100% power to detect a 30% increase in objective response rate from 10% to 40%.[43] Alternatively, the 95% CI using the Clopper-Pearson method for an observed objective response rate of 20% was 13–29%, and the study would have 85% power to detect a 10% increase in objective response rate from 10% to 20%. The primary analysis (data cutoff May 5, 2015) was triggered by a minimum of 24 weeks of follow-up from the final patient enrolled. This report used a later data cutoff of Sept 14, 2015, to explore duration of response.

	Patients, n	Objective response rate, n (% [95% CI])	Complete response	Partial response	Stable disease	Progressive disease
RECIST version 1.1 criteria by independent review						
IC2/3	100	26 (26% [18–36])	11 (11%)	15 (15%)	16 (16%)	44 (44%)
IC1/2/3	207	37 (18% [13–24])	13 (6%)	24 (12%)	34 (16%)	107 (52%)
All patients	310	45 (15% [11–19])	15 (5%)	30 (10%)	59 (19%)	159 (51%)
IC1*	107	11 (10% [5–18])	2 (2%)	9 (8%)	18 (17%)	63 (59%)
IC0*	103	8 (8% [3–15])	2 (2%)	6 (6%)	25 (24%)	52 (50%)
Modified RECIST criteria by investigator review						
IC2/3	100	27 (27% [19–37])	8 (8%)	19 (19%)	31 (31%)	28 (28%)
IC1/2/3	207	45 (22% [16–28])	14 (7%)	31 (15%)	58 (28%)	74 (36%)
All patients	310	58 (19% [15–24])	16 (5%)	42 (14%)	92 (30%)	110 (35%)
IC1*	107	18 (17% [10–25])	6 (6%)	12 (11%)	27 (25%)	46 (43%)
IC0*	103	13 (13% [7–21])	2 (2%)	11 (11%)	34 (33%)	36 (35%)

These results are for the objective response-evaluable population; data cutoff Sept 14, 2015. PD-L1=programmed death ligand 1. IC=immune cell. RECIST=Response Evaluation Criteria In Solid Tumors. *These patient subgroups were not part of the prespecified analysis (but are part of the "all patients" group) and the data are provided for the sake of completeness.

Table 2: **Objective response rate by PD-L1 immune cell score**

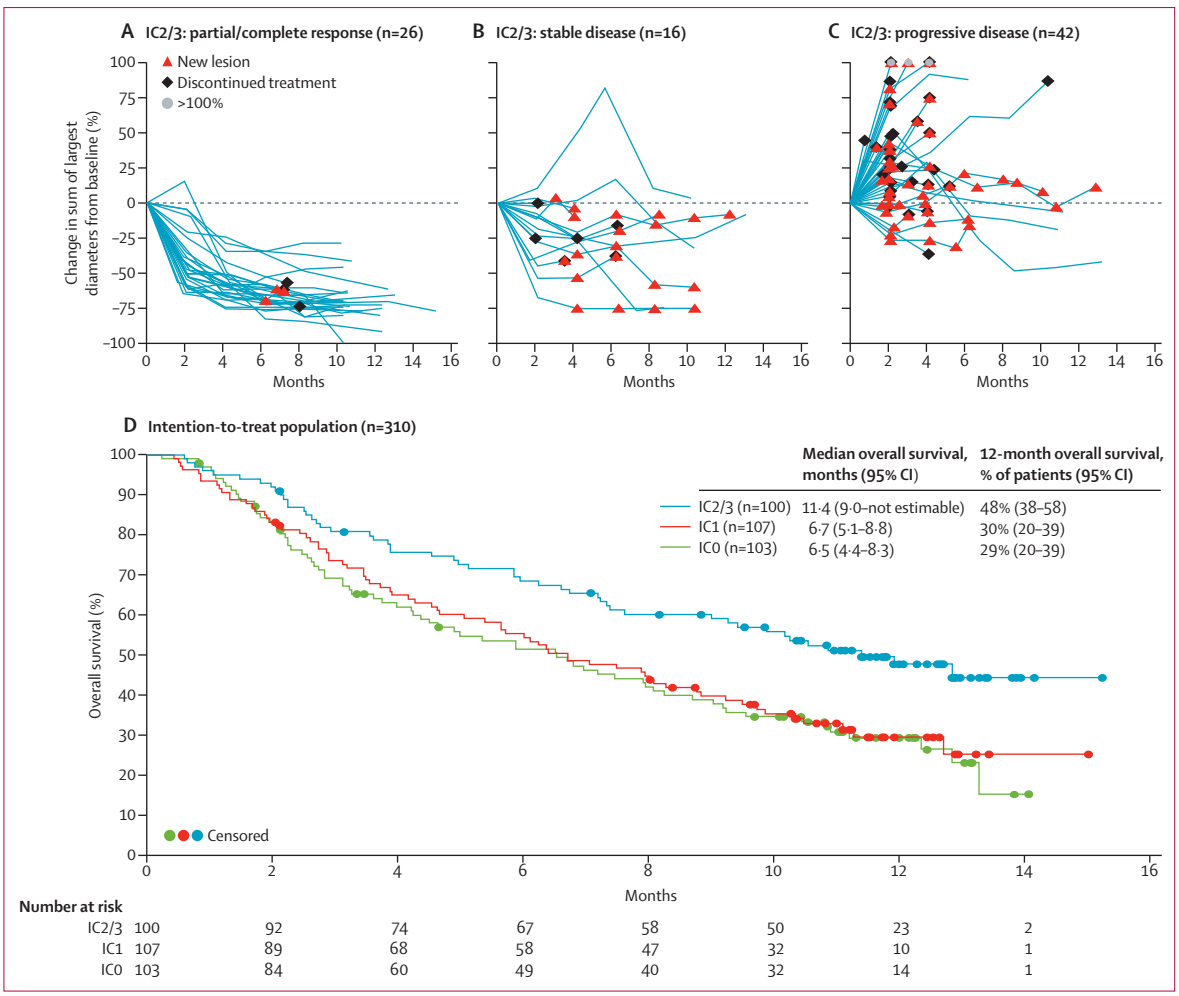

Figure 2: **Change in sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) over time by best response in the PD-L1 IC2/3 group and overall survival in PD-L1 immune cell groups**

Percentage change in the sum of longest diameters by independent review-assessed Response Evaluation Criteria In Solid Tumors version 1.1 in the IC2/3 group by (A) responders, (B) patients with stable disease, and (C) patients with progressive disease. Patients without a measurable baseline tumour assessment or without post-baseline tumour measurements were not included. (D) Kaplan-Meier overall survival curves for the IC0, IC1, and IC2/3 groups. IC=immune cell.

We did safety analyses on all treated patients, defined as all enrolled patients who received any amount of the study drug. We did analyses of objective response rate in prespecified subgroups based on known baseline prognostic factors and reported these results descriptively. No formal hypothesis testing was planned. Additional biomarker analyses beyond PD-L1 immune cells were exploratory only and were not prespecified. The biomarker evaluable population was based upon the objective response-evaluable population who had available associated gene expression and mutational load data.

This study is registered with ClinicalTrials.gov, number NCT02108652.

Role of the funding source

The funder of the study (F Hoffmann-La Roche Ltd) was involved in the study design, data collection, data analysis, data interpretation, and writing of the report, and gave approval to submit for publication. All authors were involved in the study design and data interpretation, had full access to all the data in the study, were involved in the writing of the report, and had final responsibility for the decision to submit for publication.

Results

Between May 13, 2014, and Nov 19, 2014, 486 patients were screened and 315 eligible patients were enrolled into the study in cohort 2 (figure 1 and appendix p 9). 310 patients received at least one dose of atezolizumab and were evaluable for efficacy and safety, whereas the other five enrolled patients later did not meet eligibility criteria and were not dosed with study drug. At the time of the data cutoff on Sept 14, 2015, 202 (65%) of 310 patients had discontinued treatment, of whom 193 patients had died, eight had withdrawn from treatment, and one had discontinued for other reasons.

Table 1 summarises the baseline characteristics of the patients in cohort 2. 127 (41%) of 310 patients had received two or more previous systemic regimens for metastatic disease. Many patients had adverse prognostic risk factors, including visceral or liver metastasis at study entry, and baseline haemoglobin lower than 100 g per L (table 1).

Tissue for PD-L1 immunohistochemistry analysis consisted of surgical resection specimens (n=215), biopsies (eg, core needle or forceps) from primary lesions (n=23) or metastatic sites (n=41), transurethral resection of bladder tumour samples (n=29), and biopsy from an unknown lesion (n=2). PD-L1 IC2/3 prevalence was higher in resection specimens (83/215 [39%]) and transurethral resection of bladder tumour specimens (10/29 [34%]) than in biopsies from primary lesions (4/24 [17%]) or metastatic sites (3/40 [8%]). The prevalence of PD-L1 IC2/3 in primary tumour samples (irrespective of specimen type) was 33% (n=233) whereas PD-L1 IC2/3 prevalence in metastatic tumour samples was 28% (n=78). Patients were evenly distributed between the PD-L1 immune cell groups; IC0 (103 [33%] of 310 patients), IC1 (107 [35%]), and IC2/3 (100 [32%]). Baseline characteristics were well balanced between the IC2/3 group, the IC1/2/3 group, and the overall intention-to-treat population (table 1).

The primary analysis showed that compared with a historical control overall response rate of 10%, treatment with atezolizumab resulted in a significantly improved RECIST v1.1 objective response rate for each prespecified immune cell group (IC2/3: 27% [95% CI 19–37], p<0·0001; IC1/2/3: 18% [13–24], p=0·0004) and in all patients (15% [11–20], p=0·0058; appendix p 6). The updated analysis of efficacy described in this report was later done to assess the durability of response (table 2). According to independent radiological review (RECIST v1.1), the updated analysis of efficacy showed an objective response rate of 26% (95% CI 18–36) in the IC2/3 group, including 11 (11%) patients who achieved a complete response. In the IC1/2/3 group, the objective response rate was 18% (95% CI 13–24), with complete response recorded in 13 (6%) patients (table 2). For all evaluable patients, the objective response rate was 15% (95% CI 11–19), with a complete response recorded in 15 (5%) of 310 patients (figure 2; appendix pp 10–11). Investigator-assessed response rates (according to immune-modified RECIST) were similar to the RECIST v1.1 results (table 2).

After a median follow-up of 11·7 months (95% CI 11·4–12·2), the median duration of response was not yet reached in any of the PD-L1 immunohistochemistry groups (range 2·0–13·7 months [with censored values at these timepoints]; figure 2A–C, appendix pp 12–14). At the time of the updated data cutoff (Sept 14, 2015), ongoing responses were reported in 38 (84%) of the 45 responding patients. The median time to response was 2·1 months (95% CI 2·0–2·2).

	Any grade	Grade 3–4
Any adverse event	215 (69%)	50 (16%)
Fatigue	93 (30%)	5 (2%)
Nausea	42 (14%)	0
Decreased appetite	36 (12%)	2 (1%)
Pruritis	31 (10%)	1 (<1%)
Pyrexia	28 (9%)	1 (<1%)
Diarrhoea	24 (8%)	1 (<1%)
Rash	23 (7%)	1 (<1%)
Arthralgia	21 (7%)	2 (1%)
Vomiting	18 (6%)	1 (<1%)
Dyspnoea	10 (3%)	2 (1%)
Anaemia	9 (3%)	3 (1%)
Aspartate aminotransferase increased	10 (3%)	2 (1%)
Pneumonitis	7 (2%)	2 (1%)
Hypotension	5 (2%)	2 (1%)
Hypertension	3 (1%)	3 (1%)
Colitis	3 (1%)	2 (1%)

Adverse events reported up until data cutoff on Sept 14, 2015.

Table 3: **Treatment-related adverse events in the 310 patients who received atezolizumab**

To account for the occurrence of pseudoprogression, patients were allowed to continue treatment beyond independent review facility-assessed RECIST v1.1 progression. 121 patients were treated beyond progression for a median of 7·8 weeks (range 0–51 weeks), of whom 20 (17%) subsequently experienced target lesion reduction of at least 30% from their baseline scans (appendix pp 15–16).

Durable responses were recorded in patients that included those with upper tract disease and those with poor prognostic features. Although the presence of liver metastasis in patients resulted in a lower objective response rate than in patients with no liver metastases (5% *vs* 19%; appendix p 7), these responses were durable with the median duration of response not reached at the time of the data cutoff (95% CI not estimable). A similar trend was noted in patients with visceral metastases (10% *vs* 31% for patients with no visceral metastases) and ECOG performance status 1 (8% *vs* 25% for patients with ECOG performance status 0). The absence of visceral metastasis (ie, lymph node-only disease) at baseline was associated with the highest complete response rate (presence of visceral metastases 1% *vs* 18% for absence of visceral metastases). The median duration of response was not yet reached across any subgroup analysed (data not shown).

With a median survival follow-up of about 11·7 months (range 0·2 [censored value]–15·2 months) the median progression-free survival (according to RECIST v1.1) was 2·1 months (95% CI 2·1–2·1) in all patients and was similar across all immune cell groups (IC2/3 group: median progression-free survival 2·1 months [2·1–4·1]; IC1/2/3 group: 2·1 months [2·1–2·1]). The

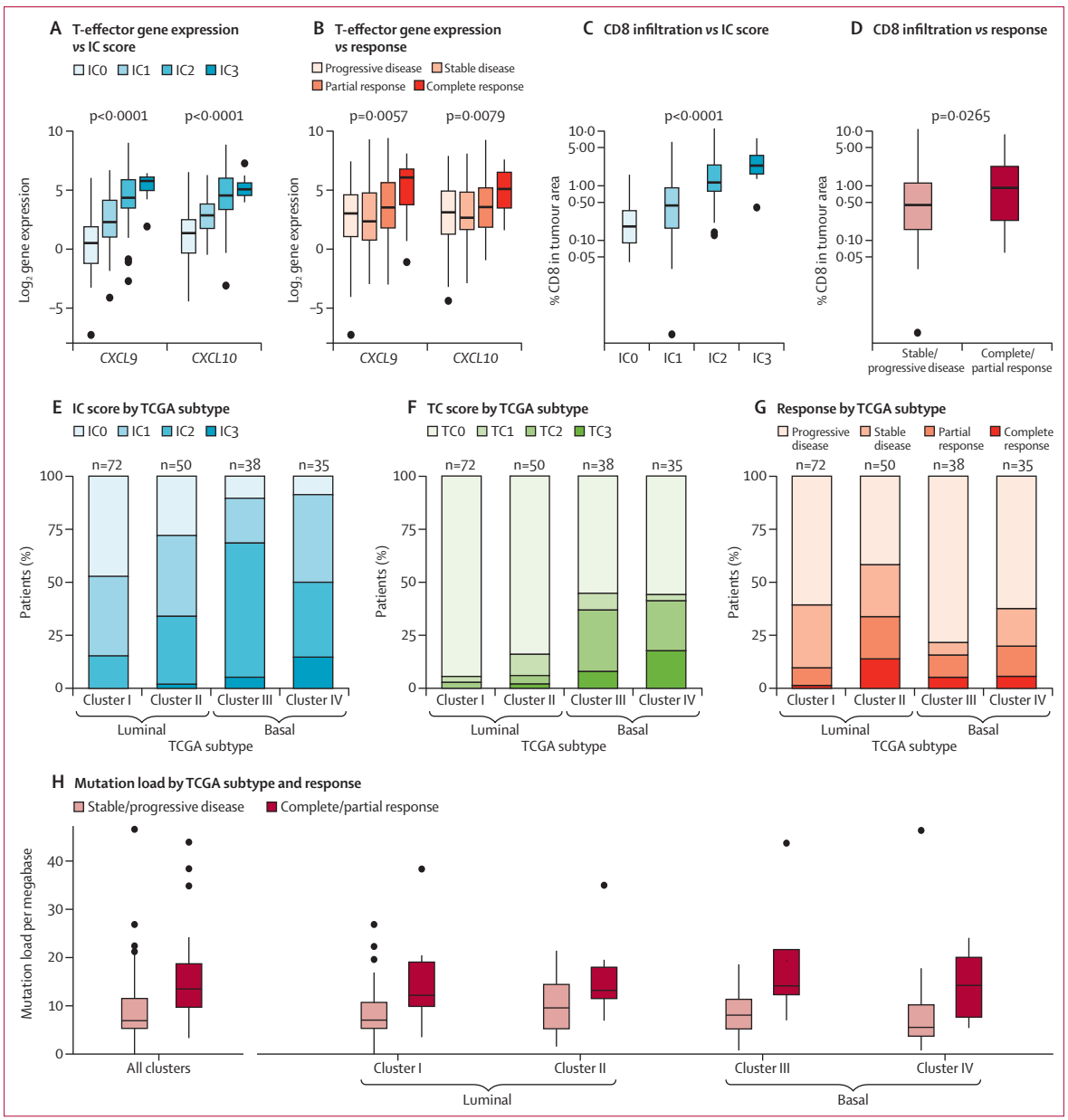

Figure 3: **Association of response and PD-L1 immunohistochemistry status with gene-expression profiling and mutation load**
(A) Association of immune cell PD-L1 immunohistochemistry score with gene expression for *CXCL9* and *CXCL10*, two representatives of a CD8 T-effector gene set. (B) Association of *CXCL9* and *CXCL10* with response to treatment. (C) Association of tumour CD8+ T-cell infiltration in the tumour area and PD-L1 immune cell score. (D) Association of CD8+ immunohistochemistry staining in the tumour area and response to treatment. (E) Immune cell PD-L1 immunohistochemistry score distributions by TCGA subtype. (F) Tumour cell PD-L1 immunohistochemistry score distributions by TCGA subtype. (G) Response distributions by TCGA subtype. (H) Estimated mutation load per megabase versus patient response, overall (n=150) and also disaggregated by TCGA subtype. IC=immune cell. TC=tumour cell. TCGA=The Cancer Genome Atlas.

investigator-assessed median progression-free survival by immune-modified RECIST criteria was 4·0 months (95% CI 2·6–5·9) in the IC2/3 group compared with 2·9 months (2·1–4·1) in the IC1/2/3 group and 2·7 months (2·1–3·9) in all patients.

The median overall survival was 11·4 months (95% CI 9·0–not estimable) in patients in the IC2/3 group, 8·8 months (7·1–10·6) in the IC1/2/3 group, and 7·9 months (6·6–9·3) for the entire cohort of patients (figure 2D). The 12-month landmark overall survival rate was 48% (95% CI 38–58) in the IC2/3 group, 39% (32–46) in the IC1/2/3 group and 36% (30–41) in the intention-to-treat population. In patients who received only one previous line of therapy (n=124) in the metastatic setting

and no previous adjuvant or neoadjuvant therapy, the median overall survival was not estimable (95% CI 9·3–not estimable) for the IC2/3 group, 10·3 months (7·5–12·7) in the IC1/2/3 group, and 9·0 months (7·1–10·9) for the entire second-line population.

The median duration of treatment was 12 weeks (range 0–66 weeks). All-cause, any grade adverse events were reported in 300 (97%) of 310 patients, with 170 (55%) patients experiencing a grade 3–4 adverse event (appendix p 8). 215 (69%) of patients had a treatment-related adverse event of any grade, and 50 (16%) of patients had a grade 3–4 treatment-related adverse event (table 3). Treatment-related serious adverse events were reported in 34 (11%) of 310 patients. No treatment-related deaths occurred during the study. Most treatment-related adverse events were mild to moderate in nature, with fatigue (93 patients [30%]), nausea (42 [14%]), decreased appetite (36 [12%]), pruritus (31 [10%]), pyrexia (28 [9%]), diarrhoea (24 [8%]), rash (23 [7%]), and arthralgia (21 [7%]) among the most common any-grade adverse events (table 3). The incidence of grade 3–4 treatment-related adverse events was low, with fatigue being the most common, occurring in five (2%) patients (table 3). No cases of febrile neutropenia were reported.

23 (7%) patients had an immune-mediated adverse event of any grade, with pneumonitis, increased aspartate aminotransferase, increased alanine aminotransferase, rash, and dyspnoea being the most common, each occurring in two (1%) patients. 15 (5%) patients had a grade 3–4 immune-mediated adverse event of any cause. No immune-mediated renal toxicity was observed. 93 (30%) patients had an adverse event leading to dose interruption. 11 (4%) patients had an adverse event that led to treatment withdrawal. 69 (22%) of 310 patients had an adverse event that necessitated systemic steroid use.

Exploratory translational analyses showed that PD-L1 immunohistochemistry expression on tumour-infiltrating immune cells was associated with expression of genes in a CD8 T-effector set (figure 3A; appendix p 17). Of the genes in the T-effector set, responses to atezolizumab were most closely associated with high expression of two interferon-γ-inducible T-helper-1-type chemokines: CXCL9 (p=0·0057) and CXCL10 (p=0·0079, figure 3B). A similar, although less pronounced, trend was also noted with respect to other genes in the set (appendix p 17)—these other genes in the set did not achieve statistical significance individually, probably because of lower RNA-seq read counts. Consistent with increased T-cell trafficking chemokine expression, tumour area CD8+ T-cell infiltration was also associated with both PD-L1 immune cell prevalence (p<0·0001, figure 3C) and response to atezolizumab (p=0·0265, figure 3D).

After adaptation of The Cancer Genome Atlas (TCGA) classification approach for use with our expression assay, gene expression analysis was used to classify 195 patients into luminal (n=73) and basal (n=122) subtypes as defined by TCGA (appendix p 18). PD-L1 immune cell prevalence was highly enriched in the basal subtype versus the luminal subtype (60% *vs* 23%, p<0·0001, figure 3E) with IC2/3 expression of 15% in the papillary-like luminal cluster I, 34% in cluster II, 68% in the squamous-like basal cluster III, and 50% in the basal cluster IV subtype (figure 3E). Increased PD-L1 tumour cell expression was almost exclusively seen in the basal subtype (39% in basal *vs* 4% in luminal, p<0·0001; figure 3F) and did not correlate with objective response rate. Consistent with PD-L1 IC2/3 expression, CD8 T-effector gene expression was increased in luminal cluster II and basal cluster III or IV and not in luminal cluster I (appendix p 18). Response to atezolizumab occurred in all TCGA subtypes but was significantly higher in the luminal cluster II subtype than in other subtypes, which showed an objective response rate of 34% (p=0·0017, figure 3G), compared with 10% for subtype I, 16% for subtype III, and 20% for subtype IV.

Mutation load was estimated in 150 patients by examination of a representative panel of 315 cancer-related genes. The median mutation load was significantly increased in responders (12·4 per megabase [Mb]) compared with non-responders (6·4 per Mb, p<0·0001; figure 3H). The association between mutation load and response was unrelated to TCGA subtype (p=0·2200, figure 3H) or immune cell subgroup (appendix p 20). A subgroup analysis of only those patients with bladder primary tumours, (appendix pp 21–22), produced essentially equivalent results. Finally, smoking status did not correlate with median mutation load (8·1 per Mb for non-smokers *vs* 9·0 per Mb for current or past smokers; p=0·2454) or with response to atezolizumab (objective response rate 14·7% *vs* 19·3%, p=0·5373).

Discussion

Since the development of combination treatment with methotrexate, vinblastine, doxorubicin, and cisplatin chemotherapy 30 years ago, no major improvements have been made in treatment outcomes for patients with urothelial carcinoma.[44] The results of this large single-arm phase 2 study show that atezolizumab induced durable anti-tumour responses in patients with advanced urothelial carcinoma whose tumours have progressed during or after treatment with platinum-based chemotherapy. This trial included heavily pretreated patients and, notably, the median duration of response had not been reached despite a median follow-up of 11·7 months. The low incidence of clinically relevant treatment-related adverse events makes atezolizumab widely applicable in these patients, who often have renal impairment and/or other comorbidities. This durable efficacy and tolerability is striking in comparison with outcomes recorded with presently available second-line chemotherapy for urothelial carcinoma.[6,45,46]

The overall survival results recorded in this trial compare favourably to a landmark 12-month survival rate of 20% (95% CI 17–24) from a pooled analysis of ten

phase 2 trials that assessed 646 patients who received second-line chemotherapy or biologicals.[47]

At present, the prognostic value of PD-L1 immune cell expression is unknown, with conflicting reports in the published literature, although it does not seem to be associated with validated adverse risk factors in this dataset.[48,49] Therefore, the improved survival in this patient population is probably related to atezolizumab treatment. The results of an ongoing randomised study (NCT02302807) are needed to appropriately assess the prognostic and predictive value of the Ventana SP142 immunohistochemistry assay, and to better understand which patients derive clinical benefit from atezolizumab treatment.

Responses to atezolizumab were associated with both conventional RECIST and atypical response kinetics. 20 (17%) of 121 patients treated beyond progression showed shrinkage (at least 30% reduction) of target lesions following RECIST v1.1 progression. The median progression-free survival was similar across the immunohistochemistry subsets with RECIST v1.1; however, it increased in all subgroups (with the most pronounced effect in the IC2/3 group) when immune-modified RECIST criteria were used to account for the non-classical responses that can be observed with cancer immunotherapy. In this study, a disconnect between progression-free survival and overall survival was recorded, similar to other immune checkpoint agents in other diseases, further suggesting that modifications of RECIST v1.1 are needed to better capture the benefit of immunotherapy treatment.

This study required a tumour specimen to be submitted during screening for prospective PD-L1 testing with the Ventana SP142 assay. In a prespecified analysis, higher levels of PD-L1 immunohistochemistry expression on immune cells were associated with a higher response rate to atezolizumab and longer overall survival. By contrast, the frequency of PD-L1 expression on tumour cells was low and did not show an association with objective response, lending further support to the importance of adaptive immunity in driving clinical benefit to immune checkpoint inhibitors.

Similarly, the association of immune activation gene subsets (eg, *CXCL9* and *CD8A*) and other immune checkpoint genes (*PD-L1*, *CTLA-4*, and *TIGIT*; data not shown) with immune cell PD-L1 expression suggests that the immune cell PD-L1 expression represents adaptive immune regulation and the presence of a pre-existing (but suppressed) immune response in urothelial carcinoma tumours.[30] The presence of other negative regulators (eg, *TIGIT*) further suggests that combination immunotherapeutic approaches could further enhance responses to treatment.

In addition to PD-L1 immunohistochemistry expression on immune cells, response to atezolizumab was strongly related to mutation load. This association was independent of the association between TCGA subtype or PD-L1 immune cell score and response (appendix p 23). This study used a novel approach to interrogating the FoundationOne panel (Foundation Medicine, Cambridge, MA, USA) that covers around 3% of the exome to estimate mutation load. Although this targeted approach interrogated a much smaller fraction of the exome than that typically used for mutation load estimation, a re-analysis of TCGA bladder urothelial carcinoma mutation data showed that whole exome results were well correlated with those obtained from only the FoundationOne regions (appendix p 24). Moreover, the correlation of mutational load and response to atezolizumab is consistent with the pattern recorded in other malignancies, and reinforces the notion that the many mutations that occur in cancer create novel epitopes against which protective T-cell responses are directed.[25]

Notably, the molecular subtypes identified by the TCGA analysis were also associated with response to atezolizumab, suggesting that in addition to PD-L1 expression, subtypes differed in their underlying immune biology. Although responses were recorded across all TCGA subtypes, significantly higher response rates were noted in the luminal cluster II subtype, which was characterised by transcriptional signatures associated with the presence of activated T-effector cells. By contrast, luminal cluster I was associated with low expression of CD8+ effector genes, lower PD-L1 immune cell or tumour cell expression and lower responses to atezolizumab, consistent with a landscape often devoid of pre-existing immune activity. Basal clusters III and IV were also associated with increased PD-L1 immune cell expression and CD8+ effector genes. However, unlike luminal cluster II, basal clusters III and IV also showed high PD-L1 tumour cell expression. The reduced response rates in the basal subtypes compared with luminal cluster II strongly suggest that other immunosuppressive factors exist in the basal subtypes that prevent effective T-cell activation with inhibition of the PD-L1–PD-1 pathway. The differences in the immune landscape of luminal versus basal subtypes draw attention to the need to further understand the underlying immune biology to develop future rational combination or sequential treatment strategies.

Although PD-L1 immune cell status is clearly associated with atezolizumab response, incorporation of TCGA gene expression subtype, mutation load, or both of these novel biomarkers into a model based on PD-L1 immune cell staining significantly improved the association with response (appendix p 23). Thus, disease subtype and mutation load do not simply recapitulate the information already provided by PD-L1 expression in immune cells, but rather, they provide independent and complementary information. Additional data and larger sample sizes are needed to allow the formal construction of a multi-biomarker classifier, and continued consideration of all three biomarkers is warranted in next-generation companion diagnostics.

In conclusion, we report that targeting PD-L1 with atezolizumab is effective in heavily pretreated patients with locally advanced or metastatic urothelial carcinoma, and that responses are more common in patients with higher levels of PD-L1 expression on immune cells than in those with lower expression. The efficacy seems to be driven by underlying genomic, molecular, and immunological factors.

Contributors

JER, TP, DPP, DB, NC, OA, GDF, and RD contributed to study design. JER, JH-C, TP, MSvdH, AN, PHO'D, PG, RWJ, MDG, JLP-G, HAB, JB, DB, GMF, SM, and RD contributed to data collection. DN and RB contributed to preprocessing and analysis of gene expression data (including assignment of subtype) and all statistical analyses related to gene expression, CD8 immunohistochemistry, and mutation load exploratory biomarkers. GMF contributed to analysis method development. SM contributed to data analysis, data interpretation, and writing with regards to biomarkers. OA was the medical monitor for the study and responsible for the database lock, data analysis, and interpretation. JER and GDF contributed to editing the report and oversight of author review of the report. JER, GDF, RB, and SM contributed to the design of the figures. AVB, ND, AB, YL, SS, MMR, MTF, DC, CC, and the other authors were involved in data analysis and interpretation; the drafting, review, and approval of the report; and the decision to submit for publication.

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JER has received non-financial support from Roche Genentech and consulting fees from Agensys, Eli Lilly, Sanofi, and Oncogenex. JH-C has received consulting fees from Roche Genentech. TP has received honoraria from Roche, Bristol-Myers Squibb, and Merck, and research funding from Roche and AstraZeneca. MSvdH has advisory board agreements with Roche Genentech, Astellas, and AstraZeneca, and has received grants from Astellas. AVB has received consulting fees from Roche Genentech. AN has received consulting fees from Roche and consulting fees and grants from Merck Sharp & Dohme. PHO'D has received honoraria from Genentech, Novartis, Algeta ASA, and Bayer, and research support from Boehringer Ingelheim. AB has received consulting fees from Bristol-Myers Squibb, and Merck. YL has received consulting fees from Roche, Sanofi, Astellas, Janssen, Ipsen, and Bristol-Myers Squibb, and has received a grant from Sanofi. PG has consulting agreements with Genentech, Dendreon, Bayer, and Myriad Genetics; participated as a speaker for Genentech for unbranded educational-related programmes; and has received grants from Genmab; fees were paid to his institution of Cleveland Clinic Foundation from Merck, Mirati, and Oncogenex. RWJ has consulting and advisory board agreements for BMS, Merck, Nektar, Eisai, Novartis, and Cerulean. MDG has advisory board agreements for Genentech, Merck, Astellas, and Novartis, and has received consulting fees from BioMotiv, and grants from Novartis, Bristol-Myers Squibb, and Celgene. DPP received grants and consulting fees from Genentech during the conduct of the study, and grants and personal fees from Merck, AstraZeneca, Novartis, Pfizer, and Agensys. JLP-G has received grants from Roche. CC has speaker and advisory board agreements with Sanofi, Janssen, and Astellas; advisory board agreements with Amgen and Bristol-Myers Squibb; and has received congress travel grants from Sanofi and Novartis. JB has received consulting fees from Genentech. DB has an advisory board agreement with Roche Genentech. GMF is an employee and shareholder of Foundation Medicine. DN, RB, NC, SM, OA, and GDF are employees and shareholders of Genentech. RD has received consulting fees from Genentech and Merck. ND, SS, MMR, MTF, HAB, and DC declare no competing interests.

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