THE LANCET

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: El-Khoueiry AB,Sangro B, Yau T, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017; published online April 20. http://dx.doi.org/10.1016/S0140-6736(17)31046-2.

SUPPLEMENTARY APPENDIX

Additional eligibility criteria

The uninfected cohorts allowed the enrolment of patients who had prior infection with HCV or HBV but no active viral replication (ie, negative for HCV RNA and HBV DNA and/or surface antigen). Patients in the HCV-infected cohort must have had detectable HCV RNA and could have had evidence of prior, but not active, HBV infection. Additional inclusion criteria included adequate organ and bone marrow function (white blood cell counts ≥2000/uL, neutrophils ≥1000/uL, platelets ≥60 × 10^3/uL, haemoglobin ≥9·0 g/dL, creatinine clearance >40 mL/min, ALT and AST ≤5× upper limit of normal each, bilirubin ≤3 mg/dL, international normalised ratio (INR) ≤2·3 or prothrombin time ≤6 seconds above control, and albumin ≥2·8 g/dL). Additional exclusion criteria included active coinfection with HBV and HCV, active coinfection with HBV and hepatitis D virus, HIV infection, brain metastases, history of hepatic encephalopathy, any prior or current clinically significant ascites as measured by physical examination and that requires active paracentesis for control (patients who had ascites only on radiographic imaging were eligible), malignancies occurring within the previous 3 years, active or history of autoimmune disease, and active drug or alcohol abuse.

Tumour assessments

Tumour assessments were conducted with computed tomography scans of the chest, abdomen, and pelvis, including contrast-enhanced triphasic computed tomography scans of the abdomen, at baseline, every six weeks for one year, and then every 12 weeks thereafter until disease progression. If a patient was found to have disease progression but was tolerating and benefiting from treatment per investigator assessment, the patient could continue study therapy until disease progression was confirmed. Evaluation of target lesions was performed according to RECIST v1.1. A complete response was defined as disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have had a reduction in short axis to <10 mm. A partial response was defined as a decrease of ≥30% in the sum of diameters of target lesions, with the sum of baseline diameters as the reference. Progressive disease was defined as an increase of ≥20% in the sum of diameters of target lesions, with the smallest sum on study as the reference (this included the baseline sum if that was the smallest on study). In addition to the relative increase of 20%, the sum must have also demonstrated an absolute increase of ≥5 mm (the appearance of ≥1 new lesions was also considered progressive disease). Stable disease was defined as neither sufficient target lesion shrinkage from baseline to qualify for partial response nor sufficient increase to qualify for progressive disease, with the smallest sum of diameters while on study as the reference.

Additional study endpoints

Secondary and exploratory endpoints of CheckMate 040 that were not included in this report are time to progression rate (secondary), nivolumab pharmacokinetics (secondary; dose-escalation phase), nivolumab immunogenicity (secondary; dose-escalation phase), and biomarker measurements, including α-fetoprotein and viral load (exploratory).

Treatment beyond progression

Patients were allowed to continue study therapy after initial investigator-assessed RECIST v1.1 disease progression as long as the investigator assessed there was clinical benefit, the patient was tolerant of nivolumab, treatment beyond progression did not delay an imminent intervention to prevent serious complications of disease progression (eg, central nervous system metastases), and the patient provided written informed consent prior to receiving any additional nivolumab treatment (using an informed consent form describing any reasonably foreseeable risks or discomforts, or other alternative treatment options). These criteria were to ensure that the risk versus benefit of continuing treatment continued to favour the patient. The assessment of clinical benefit took into account whether the patient was clinically deteriorating and unlikely to receive further benefit from continued treatment. In the dose-escalation phase, 26 of 48 patients were treated beyond progression with a median of 3 doses (range, 1 to 34 doses), and in the dose-expansion phase, 95 of 214 patients were treated beyond progression with a median of 3 doses (range, 1 to 30 doses).

Assessment of select adverse events

Select adverse events were defined as those with a potential inflammatory mechanism and therefore characterised as potentially immune-mediated, and included adverse events that may differ in type, frequency, or severity from adverse events caused by non-immunotherapies; adverse events that may require immunosuppression (eg, corticosteroids) as part of their management; adverse events whose early recognition and management may mitigate

severe toxicity; and adverse events for which multiple event terms may be used to describe a single type of adverse event, thereby necessitating the pooling of terms for full characterisation.

Measurement of tumour PD-L1 expression
Expression of PD-L1 prior to nivolumab treatment was measured by means of immunohistochemical testing in formalin-fixed, paraffin-embedded tumour specimens with the use of a rabbit monoclonal anti-human PD-L1 antibody (clone 28-8) and an automated assay (PD-L1 IHC 28-8 pharmDx) developed by Dako North America (Carpinteria, CA, USA). The staining controls for the PD-L1 assay consisted of tonsillar tissue (with strong plasma membrane staining of crypt epithelium) and cell line controls provided by the manufacturer, including an NCI-H226 cell line (positive; with plasma membrane staining of at least moderate intensity in >80% of cells) and a MCF-7 cell line (negative). A species-matched negative-control antibody (rabbit immunoglobulin G) was also used. Automated staining was performed using an anti–PD-L1 primary antibody, a horseradish peroxidase–conjugated polymer backbone with secondary antibodies (EnVision FLEX detection, Dako/Agilent), and 3,3'-diaminobenzidine chromogen. Samples were also counterstained with hematoxylin. A sample was defined as PD-L1 positive if at least 1% of tumour cells exhibited membrane PD-L1 staining of any intensity in a section containing at least 100 evaluable cells. Representative images of positive and negative PD-L1 staining are shown in Figure S1.

Patient-reported outcomes methodology
The EQ-5D-3L has two components, the EQ-5D descriptive system and the EQ-5D-VAS. The EQ 5D-3L descriptive system consists of five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has three levels: no problems, some problems, and extreme problems. An index score is generated from the descriptive system by combining responses from each dimension and is calculated on a scale of 0 to 1, where 0 corresponds with death and 1 corresponds to full health. The EQ-5D-3L also includes a visual analogue scale (VAS), which asks patients to rate their current health on a 100-point scale ranging from 0 for "worst imaginable health state" to 100 for "best imaginable health state."[1] A score difference of 0·08 for the EQ-5D utility score and of 7 for the EQ-5D-VAS will be used as minimally important difference estimates for the EQ-5D-3L.[2]

In Checkmate 040, the EQ-5D-3L questionnaire was used to obtain patient health outcomes information at each site visit prior to study treatment or any clinical activities. Patients completed a paper and pen–based form in the patient's preferred language. The paper forms were then incorporated into electronic case report forms for the study. Patients completed the EQ-5D-3L at baseline and every 6 weeks thereafter through week 25. EQ-5D index scores and EQ-5D-VAS were summarized at each assessment time point using descriptive statistics (ie, n, mean, standard deviation, median, first and third quartiles, minimum, maximum). No adjustment was made for missing data when scoring the EQ-5D index or the EQ-5D-VAS.

Treatment summary
Overall, patients in the dose-escalation phase received a median of 6 doses (range, 1–55) of nivolumab. Patients in the HCV-infected cohort had a median of 32 doses compared with a median of 6 doses in both the uninfected and HBV-infected cohorts. The median duration of therapy was 2·6 months (range, 0–31). In the dose-expansion phase, patients received a median of 11 doses (range, 1–36) of nivolumab, with a median duration of therapy of 5·1 months (range, 0–18).

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2. Pickard AS, Neary MP, Cella D. Estimation of minimally important differences in EQ-5D utility and VAS scores in cancer. *Health Qual Life Outcomes* 2007; **5**: 70.

Table S1. Off-treatment therapies after nivolumab discontinuation

	Escalation phase				Expansion phase				
Patients, n (%)	**Uninfected (n=23)**	**HCV infected (n=10)**	**HBV infected (n=15)**	**All patients (n=48)**	**Uninfected untreated/intolerant (n=56)**	**Uninfected progressor (n=57)**	**HCV infected (n=50)**	**HBV infected (n=51)**	**All patients (n=214)**
Any subsequent therapy*	11 (48)	5 (50)	7 (47)	23 (48)	17 (30)	17 (30)	11 (22)	24 (47)	69 (32)
Surgery	3 (13)	1 (10)	1 (7)	5 (10)	0	2 (4)	1 (2)	4 (8)	7 (3)
Radiotherapy	3 (13)	2 (20)	2 (13)	7 (15)	6 (11)	8 (14)	2 (4)	8 (16)	24 (11)
Systemic therapy	7 (30)	2 (20)	6 (40)	15 (31)	11 (20)	8 (14)	3 (6)	16 (31)	38 (18)
Localised therapy	1 (4)	2 (20)	1 (7)	4 (8)	4 (7)	5 (9)	7 (14)	10 (20)	26 (12)

HCV, hepatitis C virus; HBV, hepatitis B virus.
*Therapies that were initiated after the last nivolumab dose date.

Table S2. Select adverse events occurring in patients in the dose-escalation phase

	0·1 mg/kg (n=6)		0·3 mg/kg (n=9)		1 mg/kg (n=10)		3 mg/kg (n=10)		10 mg/kg (n=13)		All patients (n=48)	
Select AEs, n (%)*	**Any grade**	**Grade 3/4**	**Any grade**	**Grade 3/4**	**Any grade**	**Grade 3/4**	**Any grade**	**Grade 3/4**	**Any grade**	**Grade 3/4**	**Any grade**	**Grade 3/4**
Endocrinopathy												
Hypothyroidism	1 (17)	0	0	0	0	0	1 (10)	0	0	0	2 (4)	0
Adrenal insufficiency	0	0	1 (11)	1 (11)	0	0	0	0	0	0	1 (2)	1 (2)
Diabetes mellitus	0	0	0	0	1 (10)	0	0	0	0	0	1 (2)	0
Gastrointestinal												
Diarrhoea	2 (33)	0	5 (56)	0	3 (30)	0	2 (20)	0	3 (23)	1 (8)	15 (31)	1 (2)
Hepatic												
AST increase	4 (67)	2 (33)	2 (22)	2 (22)	4 (40)	4 (40)	2 (20)	2 (20)	4 (31)	0	16 (33)	10 (21)
ALT increase	2 (33)	1 (17)	3 (33)	2 (22)	1 (10)	1 (10)	3 (30)	1 (10)	3 (23)	0	12 (25)	5 (10)
Blood bilirubin increase	0	0	0	0	3 (30)	3 (30)	3 (30)	2 (20)	2 (15)	1 (8)	8 (17)	6 (13)
Blood ALP increase	3 (50)	0	0	0	1 (10)	1 (10)	1 (10)	1 (10)	1 (8)	0	6 (13)	2 (4)
Hepatitis	0	0	1 (11)	1 (11)	0	0	1 (10)	1 (10)	0	0	2 (4)	2 (4)
Liver disorder	0	0	0	0	0	0	0	0	1 (8)	0	1 (2)	0
Hyperbilirubinemia	0	0	0	0	1 (10)	0	0	0	0	0	1 (2)	0
Infusion												
Hypersensitivity	0	0	0	0	0	0	0	0	2 (15)	1 (8)	2 (4)	1 (2)
Infusion-related reaction	0	0	0	0	0	0	1 (10)	0	1 (8)	0	2 (4)	0
Pulmonary												
Pneumonitis	0	0	0	0	0	0	0	0	0	0	0	0
Renal												
Blood creatinine increase	1 (17)	0	1 (11)	0	1 (10)	0	0	0	0	0	3 (6)	0
Acute kidney injury	0	0	0	0	1 (10)	1 (10)	0	0	1 (8)	0	2 (4)	1 (2)
Skin												
Rash	1 (17)	0	2 (22)	0	3 (30)	0	2 (20)	0	7 (54)	0	15 (31)	0
Pruritus	2 (33)	0	3 (33)	0	1 (10)	0	1 (10)	0	6 (46)	0	13 (27)	0
Rash pruritic	1 (17)	0	1 (11)	0	0	0	0	0	1 (8)	0	3 (6)	0
Rash maculopapular	0	0	0	0	1 (10)	0	1 (10)	0	1 (8)	0	3 (6)	0

Eczema	0	0	0	0	0	0	1 (10)	0	0	0	1 (2)	0
Erythema	0	0	1 (11)	0	0	0	0	0	0	0	1 (2)	0
Urticaria	0	0	0	0	1 (10)	0	0	0	0	0	1 (2)	0
Dermatitis	0	0	0	0	0	0	1 (10)	0	0	0	1 (2)	0
Dermatitis allergic	0	0	1 (11)	0	0	0	0	0	0	0	1 (2)	0
Skin hypopigmentation	0	0	0	0	0	0	1 (10)	0	0	0	1 (2)	0
Blister	0	0	1 (11)	0	0	0	0	0	0	0	1 (2)	0
PPE	1 (17)	0	0	0	0	0	0	0	0	0	1 (2)	0

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; PPE, palmar-plantar erythrodysaesthesia.
*Defined as AEs that may differ in type, frequency, or severity from AEs caused by non-immunotherapies; AEs that may require immunosuppression (eg, corticosteroids) as part of their management; or AEs where early recognition and management may mitigate severe toxicity.

Table S3. Tumour response assessment of nivolumab in the dose-escalation phase

	Uninfected (n=23)	HCV infected (n=10)	HBV infected (n=15)	All patients (n=48)
Objective response, n (%) [95% CI]*	3 (13) [3 to 34]	3 (30) [7 to 65]	1 (7) [0 to 32]	7 (15) [6 to 28]
Complete response, n (%)	2 (9)	1 (10)	0	3 (6)
Partial response, n (%)	1 (4)	2 (20)	1 (7)	4 (8)
Stable disease, n (%)	11 (48)	5 (50)	5 (33)	21 (44)
Progressive disease, n (%)	6 (26)	2 (20)	8 (53)	16 (33)
Not evaluable, n (%)	3 (13)	0	1 (7)	4 (8)
Duration of response*				
KM median, months [95% CI]	24 [15 to NE]	17 [6 to 18]	7 [NE to NE]	17 [6 to 24]
Ongoing response, n/N (%)	1/3 (33)	0	0	1/7 (14)
Disease control, n (%) [95% CI]*	14 (61) [39 to 80]	8 (80) [44 to 98]	6 (40) [16 to 68]	28 (58) [43 to 72]
Disease control with stable disease for ≥6 months	6 (26) [10 to 48]	7 (70) [35 to 93]	3 (20) [4 to 48]	16 (33) [20 to 48]

HCV, hepatitis C virus; HBV, hepatitis B virus; KM, Kaplan-Meier estimate; NE, not estimable; RECIST, Response Evaluation Criteria In Solid Tumours.
*Determined by investigator assessment using RECIST v1.1.

Table S4. Safety and tolerability of nivolumab in the dose-expansion phase

	Uninfected untreated/intolerant (n=56)		Uninfected progressor (n=57)		HCV infected (n=50)		HBV infected (n=51)		All patients (n=214)	
Patients, n (%)	**Any grade**	**Grade 3/4**	**Any grade**	**Grade 3/4**	**Any grade**	**Grade 3/4**	**Any grade**	**Grade 3/4**	**Any grade**	**Grade 3/4**
Treatment-related serious AEs	4 (7)	2 (4)	5 (9)	2 (4)	5 (10)	4 (8)	2 (4)	1 (2)	16 (7)	9 (4)
AEs leading to discontinuation	5 (9)	1 (2)	7 (12)	3 (5)	9 (18)	8 (16)	3 (6)*	2 (4)	24 (11)*	14 (7)
Treatment-related deaths	0	0	0	0	0	0	0	0	0	0
Patients with a treatment-related AE	44 (79)	15 (27)	40 (70)	7 (12)	40 (80)	15 (30)	35 (69)	3 (6)	159 (74)	40 (19)
Treatment-related AEs†										
Rash	6 (11)	1 (2)	10 (18)	1 (2)	9 (18)	0	8 (16)	0	33 (15)	2 (1)
Pruritus	11 (20)	0	7 (12)	0	14 (28)	1 (2)	13 (25)	0	45 (21)	1 (<1)
Diarrhoea	10 (18)	1 (2)	9 (16)	1 (2)	5 (10)	0	3 (6)	1 (2)	27 (13)	3 (1)
Decreased appetite	4 (7)	0	2 (4)	0	2 (4)	1 (2)	3 (6)	0	11 (5)	1 (<1)
Fatigue	14 (25)	1 (2)	20 (35)	1 (2)	8 (16)	1 (2)	7 (14)	0	49 (23)	3 (1)
Nausea	3 (5)	0	7 (12)	0	6 (12)	0	1 (2)	0	17 (8)	0
Dry mouth	4 (7)	0	5 (9)	0	2 (4)	0	2 (4)	0	13 (6)	0
Laboratory treatment-related AEs†										
AST increase	6 (11)	2 (4)	3 (5)	2 (4)	6 (12)	5 (10)	1 (2)	0	16 (7)	9 (4)
ALT increase	4 (7)	0	3 (5)	2 (4)	7 (14)	3 (6)	3 (6)	0	17 (8)	5 (2)

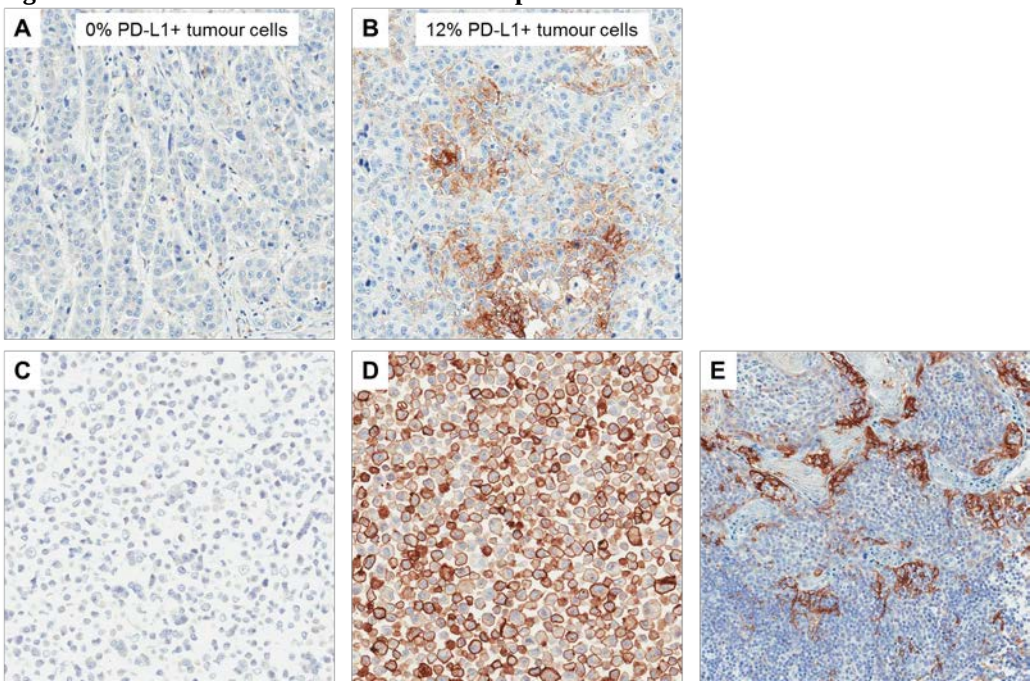
HCV, hepatitis C virus; HBV, hepatitis B virus; AE, adverse event; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
*Includes 1 patient who discontinued due to a grade 5 malignant neoplasm progression event.
†Treatment-related AEs reported in ≥5% of all patients, any grade.

Table S5. Tumour response assessment by mRECIST and RECIST v1.1 in patients with prior sorafenib treatment in the dose-expansion phase

	mRECIST (n=145)	RECIST v1.1 (n=145)
Objective response, n (%) [95% CI]*	27 (19) [13, 26]	21 (14) [9, 21]
Complete response, n (%)	5 (3)	1 (1)
Partial response, n (%)	22 (15)	20 (14)
Stable disease, n (%)	52 (36)	59 (41)
Progressive disease, n (%)	58 (40)	56 (39)
Not evaluable, n (%)	8 (6)	9 (6)

RECIST, Response Evaluation Criteria In Solid Tumours.
*Determined by blinded independent central review.

Figure S1. Measurement of tumour PD-L1 expression



PD-L1 immunohistochemical staining of tumour and control tissues. Representative photomicrographs show PD-L1 staining in hepatocellular carcinoma samples either negative (A) or positive (>1%) (B) for PD-L1. Appropriate staining is observed in control tissues including a negative control cell line (C), a positive control cell line (D), and crypt epithelium in tonsil (E). All images are shown at 100×.