Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Younes A, Santoro A, Shipp M, et al. Nivolumab for classical Hodgkin lymphoma after autologous stem-cell transplantation and brentuximab vedotin failure: a phase 2 study

CONTENTS

Study Sites and Investigators

Site - country	Site number	Investigator	Site - institution	Patients treated (N=80)
Germany	0033	Andreas Engert	University Hospital of Cologne	16
United States	0040	Anas Younes	Memorial Sloan Kettering Cancer Center	10
United States	0008	Voravit Ratanatharathorn	Barbara Ann Karmanos Cancer Institute	9
Italy	0019	Pier Luigi Zinzani	Institute of Hematology “L. e A. Seràgnoli”, University of Bologna	7
United States	0009	John Timmerman	UCLA Medical Center	6
United States	0003	Stephen Ansell	Mayo Clinic	5
Italy	0035	Armando Santoro	Humanitas Cancer Center, Humanitas University	5
United States	002	Philippe Armand	Dana–Farber Cancer Institute	2
United States	0007	Michelle Fanale	University of Texas MD Anderson Cancer Center	4
Canada	042	John Kuruvilla	Princess Margaret Cancer Centre	4
United States	0001	Jonathon Cohen	Winship Cancer Institute, Emory University	3
Canada	0046	Kerry Savage	British Columbia Cancer Agency	2
United Kingdom	0026	Graham Collins	Churchill Hospital	2
Germany	0034	Michaela Feuring-Buske	University Hospital Ulm	1
Netherlands	0016	Jan Paul De Boer	NKI AVL, Amsterdam	1
United Kingdom	0013	David Cunningham	Royal Marsden Hospital	1
United States	0005	Lisa Giulini Roth	Weill Cornell Medical College	1
United States	0006	Suresh Nair	Lehigh Valley Hospital	1

ADDITIONAL METHODS

Eligibility criteria

At study entry, one lesion had to measure greater than 15 mm in the longest diameter on cross-sectional imaging and be measurable in two perpendicular dimensions on computed tomography or magnetic resonance imaging and ^{18}F-fluoro-deoxyglucose avid by positron-emission tomography (FDG-PET). Pathological confirmation of classical Hodgkin lymphoma was required. Screening laboratory values had to meet the following criteria and to be obtained within 14 days before the first treatment dose: absolute neutrophil count ≥750/μL (≥0·75 x 10^9/L; no white blood cell growth factors for prior 14 days); platelets ≥50 x 10^3/μL (≥50 x 10^9/L; no platelet transfusions for prior 14 days); haemoglobin ≥8·5 g/dL (≥85 g/L; no red blood cell transfusions for prior 7 days); serum creatinine ≤1·5 times the upper limit of normal or creatinine clearance ≥40 mL/minute (0·04 L; measured using the Cockcroft-Gault formula); aspartate aminotransferase/alanine aminotransferase levels ≤3 times the upper limit of normal; total bilirubin ≤1·5 times the upper limit of normal.

Exclusion criteria

Key exclusion criteria were known central nervous system lymphoma; nodular lymphocyte-predominant Hodgkin lymphoma, active interstitial pneumonitis, autoimmune disease, known history of testing positive for human immunodeficiency virus or known AIDS, any positive test for hepatitis B or C indicating acute or chronic infection, a condition requiring systemic treatment with corticosteroids (>10 mg daily prednisone equivalent), or other immunosuppressive medications within 14 days of nivolumab administration; autologous stem-cell transplantation no more than 90 days prior to the first dose of nivolumab; radiation therapy within 3 weeks, or chest radiation no more than 24 weeks prior to the first dose of nivolumab; carmustine (BCNU) ≥600 mg/m² received as part of the pretransplant conditioning regimen; and a previous malignancy active within the previous 3 years, except for locally curable cancers that have been cured.

Protocol amendment

Under the original discontinuation criteria, patients stopped the study drug when investigator assessment determined disease progression using the 2007 International Working Group criteria. An amendment permitted patients to continue on the study drug beyond investigator-assessed disease progression in certain cases.

Interventions

For grade 1 nivolumab-related infusion reactions, infusion interruption or intervention was not indicated; however, prophylactic premedications (diphenhydramine 50 mg [or equivalent] and/or paracetamol [acetaminophen] 325–1000 mg) were recommended for future infusions at least 30 minutes before additional nivolumab administrations. For grade 2 symptoms, nivolumab infusion was stopped and an intravenous infusion of normal saline was initiated. The patient was treated with diphenhydramine 50 mg intravenously (or equivalent) and/or paracetamol (acetaminophen) 325–1000 mg, and, if appropriate, corticosteroid or bronchodilator therapy. Nivolumab infusion was stopped immediately and permanently discontinued in the case of grade 3/4 infusion reactions, and an intravenous infusion of normal saline was initiated. The patient was treated with bronchodilators, epinephrine 0·2–1 mg of a 1:1000 solution for subcutaneous administration or 0·1–0·25 mg of a 1:10 000 solution injected slowly for intravenous administration, and/or diphenhydramine 50 mg intravenously with methylprednisolone 100 mg intravenously (or equivalent), as needed.

Assessments

Patients were evaluated for safety if they had received any study drug. Toxicity assessments were continuous during the treatment phase. During the safety follow-up phase, toxicity assessments were done in person. Once patients reached the survival follow-up phase, safety could be done in person or via documented telephone calls.

Patient-reported general health status and quality of life were assessed using the EQ-5D and the European Organization for Research and Treatment of Cancer Quality-of-Life Questionnaire–Core 36 (EORTC QLQ-C30). Quality-of-life assessments were performed on day 1 of cycle 1 and then every four cycles for the first 17 cycles (day 1 [prior to dosing] of cycles 5, 9, 13, and 17), and then every six cycles thereafter (cycles 23, 29, 35+). EQ-5D records the patient's self-rated health state on a 100-point vertical visual analogue scale (VAS; 0=worst imaginable health state; 100=best imaginable health state). EORTC QLQ-C30 is a 30-item questionnaire comprised of five functional scales, three symptom scales, and a global health/quality-of-life scale. Scale score rages from 0–100, with higher scores representing a better response level for functional and global health/quality-of-life scales and lower scores representing a better response level for symptom scales.

Fluorescence *in situ* hybridization was performed using the bacterial artificial chromosome (BAC) clones (CHORI; www.chori.org) RP11-599H20 and RP11-635N21, which both map to 9p24·1 and include CD274 (encoding PD-L1, labelled with Spectrum Orange) and PDCD1LG2 (encoding PD-L2, labelled with Spectrum Green), respectively. As a control, the centromeric probe Spectrum Aqua–labelled CEP9 (Abbott Molecular) that maps to 9p11-q11 was hybridised per manufacturer's recommendations. Immunohistochemical staining was performed using an automated staining system (BOND-III, Leica Biosystems). A double-staining technique for PD-L1 (405·9A11) and PAX5 (24/Pax-5, BD Biosciences), and for PD-L2 (366C·9E5) and phosphorylated STAT3 (pSTAT3; D3A7, Cell Signaling Technology) was used. For PD-L1 immunohistochemical evaluation, 50 Reed-Sternberg cells were scored per case unless <50 Reed-Sternberg cells were present, and then all Reed-Sternberg cells were evaluated for positive and negative staining. The H-score (0–300) was calculated by multiplying the percentage of malignant cells with positive staining (0–100%) and average intensity of positive staining in the malignant cells (1, 2, or 3+).

Statistical analysis

The planned sample size was 60. Eighty patients were enrolled due to high demand from investigators and also to account for the possibility of a high rate of screening failures.

Safety was assessed using National Cancer Institute Common Terminology Criteria for Adverse Events, version 4·0, and adverse events were coded using the *Medical Dictionary for Regulatory Activities* (MedDRA), version 18·0. Descriptive statistics of on-study adverse events were tabulated using worst grade by system organ class and MedDRA preferred term.

Descriptive statistics were used to evaluate mean change in EORTC QLQ-C30 and EQ-5D scores from baseline to week 33. EQ-5D VAS was summarised using descriptive statistics at each assessment time point, and analyses evaluating mean score changes from baseline using the EORTC QLQ-C30 were performed in all treated patients who had an assessment at baseline and at least one subsequent assessment.

Associations between variables were evaluated post-hoc using the Kruskal-Wallis rank-sum tests for continuous data comparing two or more groups. The modified H-score for PD- L1 and PD-L2 protein expression was divided into four equally sized groups (quartiles).

Table S1: Previous systemic cancer therapy

Therapy	Patients (N = 80)
Immunotherapy by monoclonal antibody	80 (100%)
Brentuximab vedotin	80 (100%)
Investigational antineoplastic	4 (5%)
Rituximab	14 (18%)
Steroid	45 (56%)
Dexamethasone	31 (39%)
Methylprednisolone	8 (10%)
Prednisolone	11 (14%)
Prednisone	18 (23%)
Chemotherapy (other than anthracyclines)	80 (100%)
Bendamustine	26 (33%)
Bleomycin	77 (96%)
Busulfan	1 (1%)
Carboplatin	37 (46%)
Carmustine	5 (6%)
Chlorambucil	4 (5%)
Cisplatin	40 (50%)
Cyclophosphamide	22 (28%)
Cytarabine	28 (35%)
Dacarbazine	72 (90%)
Etoposide	65 (81%)
Fludarabine	1 (1%)
Gemcitabine	54 (68%)
Ifosfamide	59 (74%)
Irinotecan	1 (1%)
Lomustine	1 (1%)
Melphalan	10 (13%)
Methotrexate	2 (3%)
Nitrogen mustard	5 (6%)
Oxaliplatin	3 (4%)
Paclitaxel	1 (1%)
Procarbazine	23 (29%)
Thiotepa	2 (3%)
Trofosfamide	1 (1%)
Vinblastine	72 (90%)
Vincristine	24 (30%)
Vinorelbine	39 (49%)
Chemotherapy (anthracyclines)	80 (100%)
Doxorubicin	79 (99%)
Doxorubicin liposomal	12 (15%)
Epirubicin	4 (5%)
Mitoxantrone	1 (1%)
Kinase inhibitors	5 (6%)
Idelalisib	1 (1%)
Investigational antineoplastic	2 (3%)
Sorafenib	2 (3%)

Immunomodulary derivatives	10 (13%)
Arsenic trioxide	1 (1%)
Lenalidomide	7 (9%)
Thalidomide	2 (3%)
Radioimmunotherapy	1 (1%)
Investigational antineoplastic	1 (1%)
Other	21 (26%)
Bortezomib	1 (1%)
Everolimus	15 (19%)
Investigational antineoplastic	8 (10%)
Investigational immunomodulating agent	1 (1%)
Investigational immunotherapy	1 (1%)
Mesna	1 (1%)
Sirolimus	1 (1%)
T-cell infusion	1 (1%)
Vorinostat	2 (3%)

Data are n (%).

***Table S2*: Best overall response by 9p24·1 genetic alterations and PD-L1 H-score**

Best overall response	9p24·1 genetic alteration			PD-L1 H-score*			
	Polysomy	Copy gain	Amplification	Q1	Q2	Q3	Q4
Progressive disease	1	2	0	2	1	0	0
Stable disease†	0	6	2	2	1	3	0
Partial response†	5	16	8	6	8	6	8
Complete response	0	1	2	0	0	1	2

*p=0·013, Kruskal-Wallis test. †Two patients with stable disease and 9p24·1 copy gain and one patient with partial response and 9p24·1 copy gain did not have enough material for assessment of PD-L1 protein expression.

Table S3*: Serious adverse events.

Event	Patients with adverse event (N=80)		
	Any grade	**Grade 3–4**	**Grade 5**
Total patients with an event	20 (25%)	10 (13%)	1 (1%)†
Pyrexia	3 (4%)	1 (1%)	0
Malignant neoplasm progression‡	2 (3%)	2 (3%)	0
Pneumonia	2 (3%)	1 (1%)	0
Arrhythmia	2 (3%)	1 (1%)	0
Meningitis	2 (3%)	1 (1%)	0
Infusion-related reaction	2 (3%)	0	0

Data are number (%).
*Listed are serious adverse events that were reported in at least 2% of patients. Includes events reported between the first dose and 30 days after the last dose of study therapy. †Multi-organ failure. ‡Includes progression of Hodgkin lymphoma.

***Figure S1*: Study design**

*Includes patients who responded to brentuximab vedotin and later experienced disease progression.

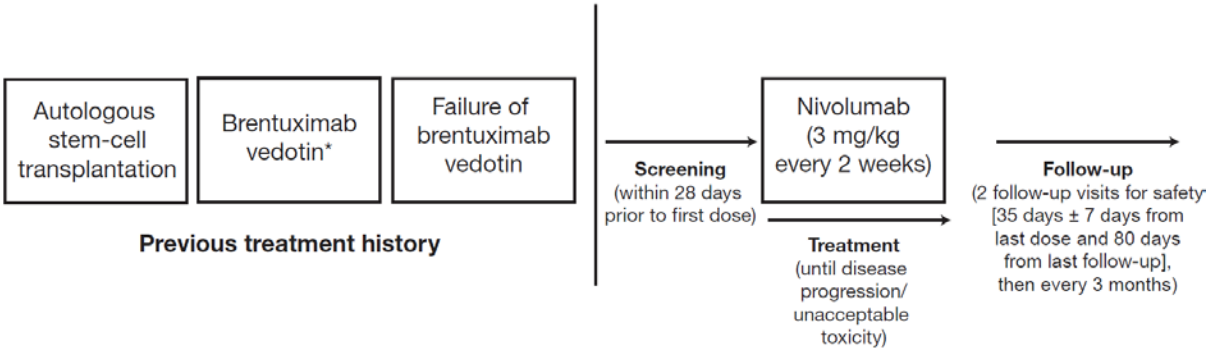

***Figure S2*: CONSORT patient disposition flow diagram**

*Autoimmune hepatitis, increased alanine aminotransferase, increased aspartate aminotransferase, and multi-organ failure. †Six patients proceeded to stem-cell transplantation (one additional patient underwent stem-cell transplantation after data cut-off date); one patient discontinued because of lack of response (investigator's decision). Note: total number of patients with progression, n=23

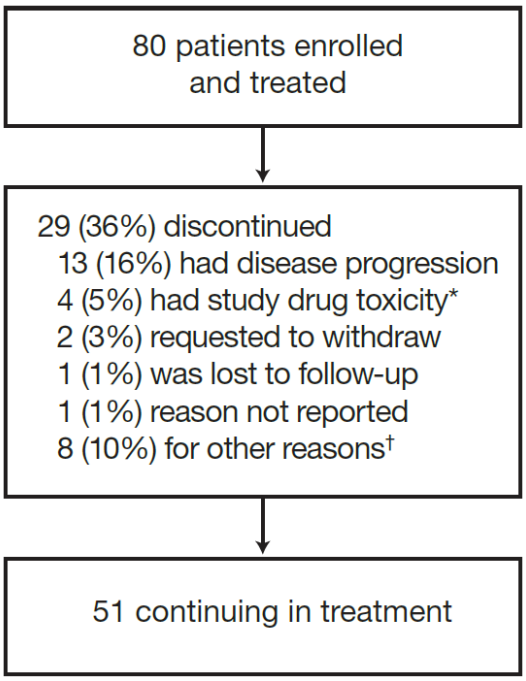

***Figure S3*: Change in tumour burden**
Shown are the results for change in tumour burden in patients treated beyond progression per investigator assessment.

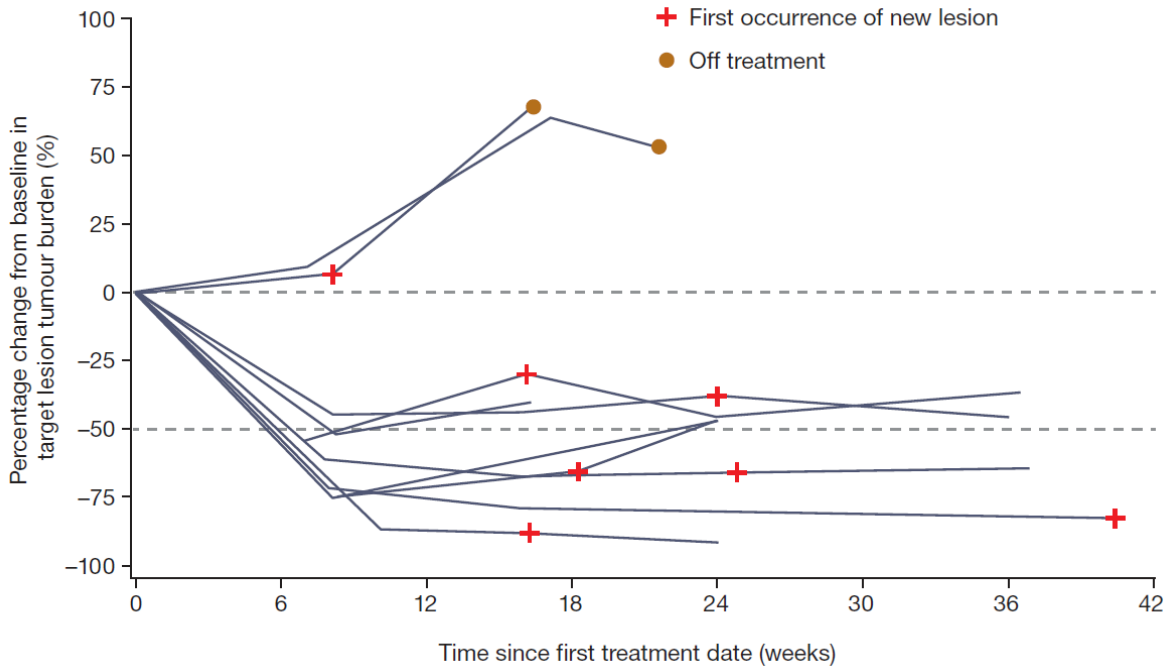

***Figure S4: PD-L1/PD-L2* alterations and PD-1 ligand expression in tumour biopsies from trial patients: representative patient specimens**

Row 1: representative FISH images (*PD-L1*, red; *PD-L2*, green; centromeric probe, aqua) from patients with: polysomy (left), three green–red fusion signals and three centromeric signals; copy gain (middle), five green–red fusion signals and three centromeric signals; and amplification (right), aggregates of multiple red–green fusion signals and two centromeric signals. Row 2: expression of PD-L1 protein by Reed-Sternberg cells for corresponding cases in row 1 (positive staining = brown). PD-L1 was evaluated in conjunction with PAX5 to identify Reed-Sternberg cell nuclei (positive staining = red). Row 3: expression of PD-L2 by Reed-Sternberg cells for corresponding cases shown in Row 1 (positive staining = brown). Phosphorylated signal transducer and activator of transcription 3 (pSTAT3), which reflects Janus kinase-STAT activation in Reed-Sternberg cells, was assessed on the same slide (positive staining = red). Images were acquired with 1000× magnification. Scale bars indicate 50 µm. See appendix p 7.

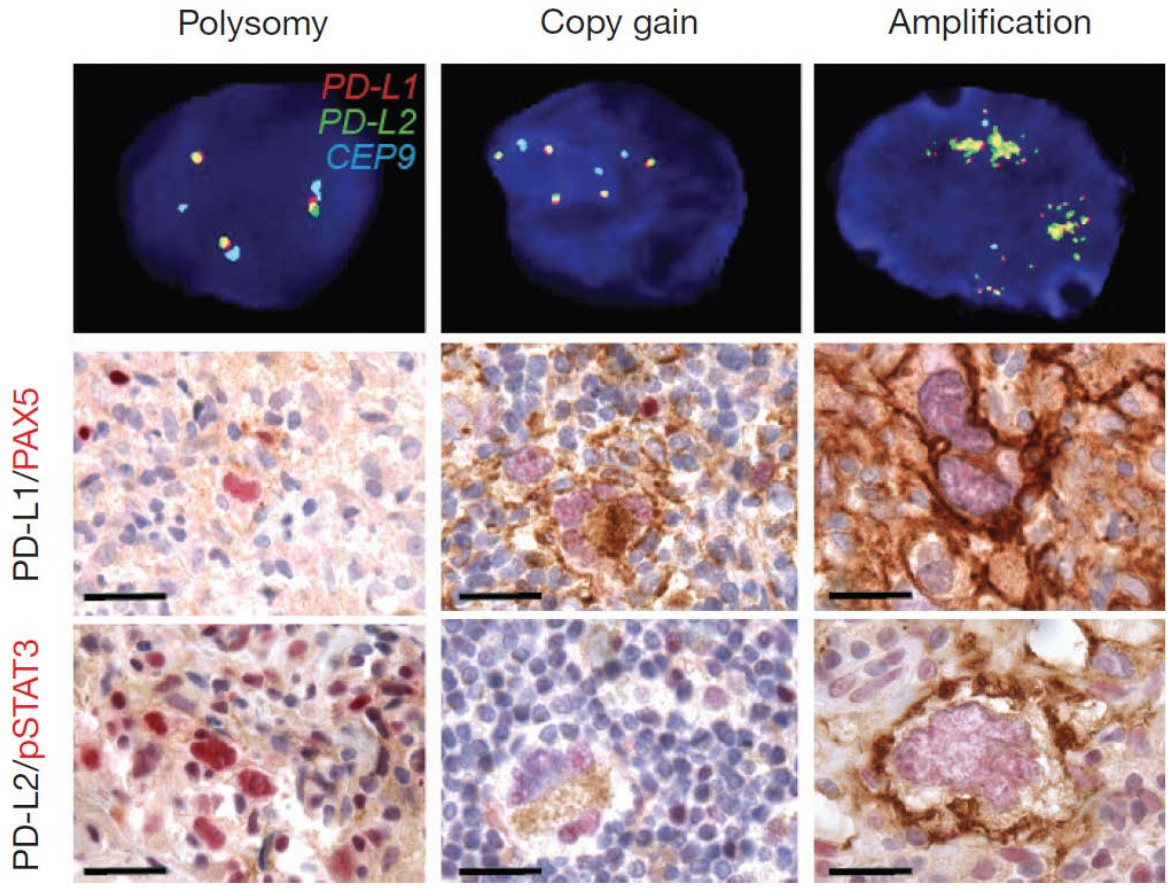

***Figure S5*: Best change from baseline in target lesion**
Shown are the results for best change from baseline in target lesion for all response-evaluable patients per IRRC (panel A) and investigator (panel B), where crosses denote ongoing response. IRRC=independent radiologic review committee.