Supplementary Appendix

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

Supplement to: Wang ML, Rule S, Martin P, et al. Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. N Engl J Med 2013;369:507-16. DOI: 10.1056/NEJMoa1306220

SUPPLEMENTARY APPENDIX

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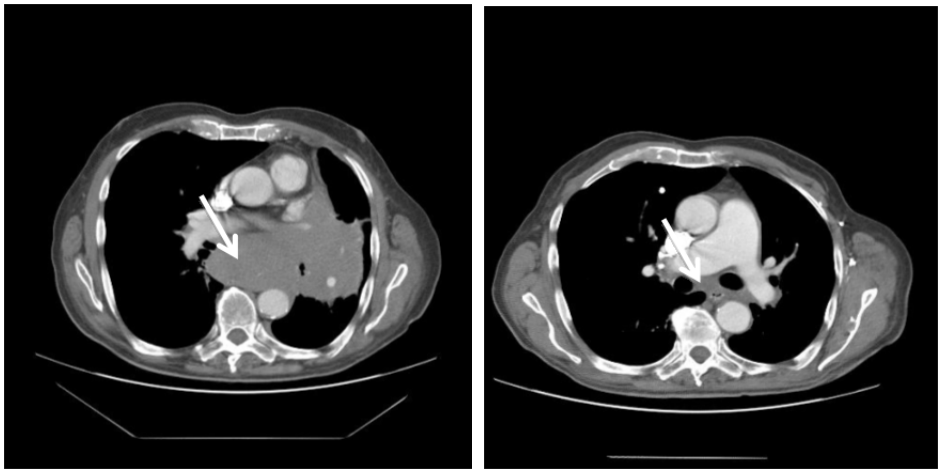
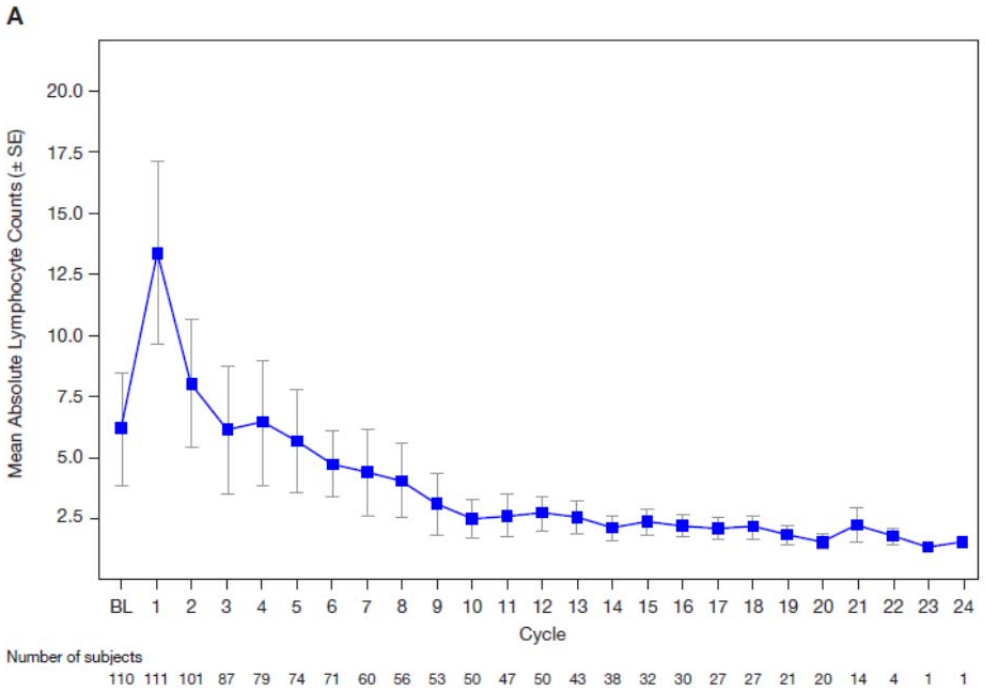
Figure S1: PET-CT scan showing tumor response after 2 cycles of ibrutinib.



Left, subcarinal LAD mass (83 x 54 mm; arrow) before treatment. Right, after 2 cycles of ibrutinib, subcarinal LAD mass was reduced by 75% (21 x 13 mm; arrow).

Figure S2: Ibrutinib induces compartmental shift of mantle cells into the peripheral blood

(A) Mean absolute lymphocyte counts over time. Mean absolute lymphocyte counts over time from patients treated with ibrutinib.



(B) BCR-controlled cytokines are inhibited following ibrutinib treatment. Plasma cytokines/chemokines before (day 1) and after ibrutinib treatment (day 8) were measured with multiplexed immunoassays (n=31, * p<0.05, ** p< 0.01, 1-way ANOVA with Tukey correction).

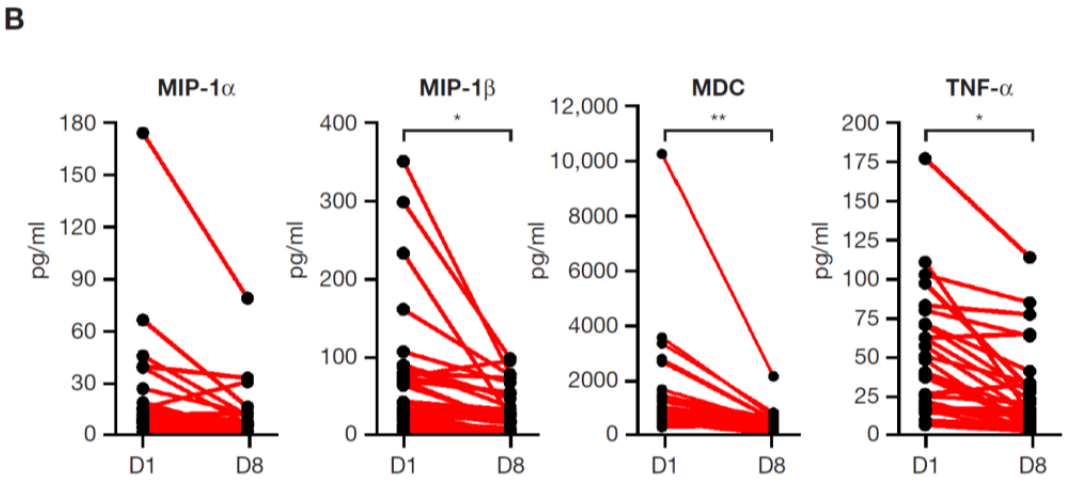


Table S1: Incidence of AEs Leading to Treatment Discontinuation

	Bortezomib-naïve (N=63)	**Bortezomib-exposed (N=48)**	**All patients (N=111)**
Total no. patients with treatment-emergent adverse events leading to treatment discontinuation	5 (7.9%)	5 (10.4%)	10 (9.0%)
Preferred term			
Subdural haematoma	1 (1.6%)	1 (2.1%)	2 (1.8%)
Blood bilirubin abnormal	1 (1.6%)	0	1 (0.9%)
Cardiac arrest	0	1 (2.1%)	1 (0.9%)
Mantle cell lymphoma*	1 (1.6%)	0	1 (0.9%)
Metastatic neoplasm	0	1 (2.1%)	1 (0.9%)
Neck mass*	1 (1.6%)	0	1 (0.9%)
Pneumonia	0	1 (2.1%)	1 (0.9%)
Respiratory failure	1 (1.6%)	0	1 (0.9%)
Sepsis	0	1 (2.1%)	1 (0.9%)

*Due to disease progression
Note: Percentages calculated with the number of subjects in safety population as denominator
Note: Adverse Events were coded using MedDRA version 15.1.

Table S2: Bleeding and Infectious Adverse Events (≥Grade 3)

	Grade 3	Grade 4	Grade 5	Overall
	n (%)			
Bleeding Event	**5 (5)**	**0 (0)**	**0 (0)**	**5 (5)**
Subdural hematoma	2 (2)	0 (0)	0 (0)	2 (2)
Hematuria	2 (2)	0 (0)	0 (0)	2 (2)
Lower gastrointestinal hemorrhage	1 (1)	0 (0)	0 (0)	1 (1)
Infections and infestations	**24 (22)**	**1 (1)**	**3 (3)**	**28 (25)**
Pneumonia	6 (5)	0 (0)	1 (1)	7 (6)
Cellulitis	3 (3)	0 (0)	0 (0)	3 (3)
Urinary tract infection	3(3)	0 (0)	0 (0)	3(3)
Bronchitis	2 (2)	0 (0)	0 (0)	2 (2)
Clostridium difficile colitis	2 (2)	0 (0)	0 (0)	2 (2)
Lower respiratory tract infection	2 (2)	0 (0)	0 (0)	2 (2)
Sepsis	0 (0)	1 (1)	1 (1)	2 (2)
Bacteremia	1 (1)	0 (0)	0 (0)	1 (1)
Cellulitis orbital	1 (1)	0 (0)	0 (0)	1 (1)
Citrobacter infection	1 (1)	0 (0)	0 (0)	1 (1)
Clostridial infection	1 (1)	0 (0)	0 (0)	1 (1)
Enterocolitis infectious	1 (1)	0 (0)	0 (0)	1 (1)
Gastroenteritis	1 (1)	0 (0)	0 (0)	1 (1)
Herpes zoster ophthalmic	1 (1)	0 (0)	0 (0)	1 (1)
Klebsiella infection	1 (1)	0 (0)	0 (0)	1 (1)
Lower respiratory tract infection bacterial	1 (1)	0 (0)	0 (0)	1 (1)
Pneumocystis jiroveci pneumonia	0 (0)	0 (0)	1 (1)	1 (1)
Pneumonia bacterial	1 (1)	0 (0)	0 (0)	1 (1)
Pneumonia klebsiella	1 (1)	0 (0)	0 (0)	1 (1)
Respiratory tract infection	1 (1)	0 (0)	0 (0)	1 (1)
Sinusitis	1 (1)	0 (0)	0 (0)	1 (1)
Staphylococcal bacteremia	1 (1)	0 (0)	0 (0)	1 (1)
Streptococcal infection	1 (1)	0 (0)	0 (0)	1 (1)
Wound infection	1 (1)	0 (0)	0 (0)	1 (1)

Table S3: Serious Adverse Events in at Least 2% of Patients

	All patients (N=111)
Total no. of patients with treatment-emergent serious adverse events	62 (55.9%)
System organ class	
Preferred term	
Infections and infestations	22 (19.8%)
Pneumonia	6 (5.4%)
Urinary tract infection	4 (3.6%)
General disorders and administration site conditions	11 (9.9%)
Oedema peripheral	3 (2.7%)
Pyrexia	3 (2.7%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	10 (9.0%)
Mantle cell lymphoma	8 (7.2%)
Blood and lymphatic system disorders	9 (8.1%)
Febrile neutropenia	3 (2.7%)
Cardiac disorders	8 (7.2%)
Atrial fibrillation	5 (4.5%)
Gastrointestinal disorders	8 (7.2%)
Abdominal pain	3 (2.7%)
Renal and urinary disorders	6 (5.4%)
Renal failure acute	3 (2.7%)
Injury, poisoning and procedural complications	5 (4.5%)
Subdural haematoma	3 (2.7%)

Compartmental shift studies

Flow Cytometry: Peripheral blood mononuclear cells (PBMCs) were purified from whole blood of patients before and after ibrutinib treatment through Ficoll gradients and then stained with CD19-APCCy7, CD5-PerCPCy5.5, and CD3-V500. Stained cells were analyzed with Canto II (BD, San Jose, CA) and FlowJo 7.6 (Treestar, Ashland, OR) software.

Cytokine/Chemokine Analysis

Plasma Cytokine/Chemokine Analysis: Cytokine and chemokine concentrations in plasma samples from patients were determined using a Milliplex multiplexed immunoassay kit (HCYTMAG-60K-PX42, EMD Millipore) following the manufacturer's instructions.

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