Supplementary Text

Transition rules

The cohort transition rules shown in the **Supplementary Table 1** were applied when three patients enrolled in the same cohort completed evaluation for the 28-day period after initial tirabrutinib administration (cycle 1).

Prohibited therapies

Prohibited therapies were chemotherapy within 21 days, or administration of nitrosourea within 42 days, radiation therapy within 14 days, invasive surgery within 28 days prior to the first tirabrutinib dose, as well as any unapproved drugs, such as adrenocortical hormone, anticoagulant use, cytochrome CYP3A4 inducer, P-gp inducer, or potent CYP3A4 inhibitor, and hematopoietic factor products. Prophylactic administration of the following drugs was permitted in this study: antibacterial agent such as ciprofloxacin or other fluoroquinolone agent, amoxicillin, or trimethoprim-sulfamethoxazole; acyclovir; antihistamine, and adrenocortical hormone (at lower doses than the prohibited dose).

Main exclusion criteria

The main exclusion criteria were presence of intraocular primary central nervous system lymphoma (PCNSL) without a cerebral lesion; inability to undergo contrast-enhanced magnetic resonance imaging; PCNSL without a B-cell neoplasm; systemic lymphomatous lesions; presence of severe neurological symptoms; recent round of chemotherapy or radiotherapy; use of nitrosurea, antibody drug, or any other unapproved drug prior to the first tirabrutinib dose; medical history of other malignant tumours; presence of severe disease complications that may affect protocol compliance, presence of comorbidities, such as severe cardiac or lung disease; infectious diseases (including HIV and cytomegalovirus); history of severe allergies; pregnant or nursing women; inability to swallow tablets; prior treatment with any Bruton's tyrosine kinase inhibitor; inability to provide consent; or considered ineligible by the investigator.

Kits used for genetic testing

The kits used were kept at room temp, except for QIAamp MinElute® Columns that are kept refrigerated.

The QIAamp® DNA Formalin-Fixed Paraffin-Embedded Tissue Kit (50) (Cat. No. 56404, Lot No. 157042227, 160042934; Qiagen K.K., Tokyo, Japan) was used. For library preparation, polymerase chain reaction (PCR) was run on the GeneAmp® PCR System 9700 using the following programme: 99° C for 2 min for 1 cycle; 99°C for 15 sec followed by 60°C for 4 mins for 23 cycles; and hold the reaction at 10°C. PCR was performed at maximum ramp speed and the reaction volume was 20 μL. The nucleotide sequence primer pool used is shown in **Supplementary Table 3**. The software used for library quantification was 2100 Expert Software (Version B.02.08.S1648, Agilent Technologies, Inc., Tokyo, Japan). Software used for analysis of mutations was Variant Caller (Version 5.0.4.0, Thermo Fisher Scientific Inc., Hudson, NH, USA). For the analysis of coverage of each Amplicon, coverageAnalysis (Version 5.0.4.0, ThermoFisher Scientific Inc., Hudson, NH, USA) was used. For other calculations, Microsoft Excel 2016 (Microsoft Corp.) was used.

Pharmacokinetics

In phase 1, blood samples were obtained immediately before tirabrutinib administration and 30 min and 1, 2, 3, 4, 6, 8, and 12 hours after tirabrutinib administration on day 1 and day 28 of cycle 1; and immediately before tirabrutinib administration on days 2, 8, and 15. In phase 2, blood samples were obtained immediately before tirabrutinib

administration and 2 hours after tirabrutinib administration on day 1 and day 28 of cycle 1; and immediately before tirabrutinib administration on days 8 and 15. To measure the trough concentration of tirabrutinib in cerebrospinal fluid (CSF), samples of CSF were obtained on day 1 of cycle 1 (2–4 hours after tirabrutinib administration), immediately before tirabrutinib administration on days 2 and 28, and 2–4 hours after administration on day 28.

Rational for sample size

For phase 1, the number of subjects required to examine the increase in the next cohort was set according to the Guidelines for Clinical Evaluation Methods of Anti-Cancer Drugs; thus, three or six patients were planned per cohort. The threshold response rate of this study was set at 14·0% for patients with relapsed/refractory PCNSL, and it was assumed that an overall response rate (ORR) of 50·9% or higher would be obtained as an ORR of 75·0% (95% confidence interval [exact method]: 50·9–91·3) was obtained in overseas phase 1/2 ibrutinib studies.[1–4]

Study analysis sets

The safety analysis set (SAF) included all the patients who received the study drug at least once. The full analysis set included all patients in the SAF in whom efficacy could be analysed. The dose-limiting toxicity (DLT) population was defined as the population of patients in phase 1 who presented a DLT in cycle 1, had a study drug compliance ≥80%, and had not discontinued treatment in cycle 1. The pharmacokinetics analysis set was defined as patients who had at least one plasma or CSF tirabrutinib concentration measurement.

Data sharing

Request for deidentified patient data should be made by qualified researchers to Ono Pharmaceutical Co., Ltd. via the following website: Clinical Study Data Request.com (https://clinicalstudydatarequest.com/). Approval for any data request will be considered in accordance with Ono Pharmaceutical Co., Ltd. Policy for the Disclosure of Clinical Study Data as outlined in the following website: https://www.ono.co.jp/eng/rd/policy.html.

References

[1] Grommes C, Pastore A, Palaskas N, et al. Ibrutinib Unmasks Critical Role of Bruton Tyrosine Kinase in Primary CNS Lymphoma. C*ancer Discov* 2017; **7**: 1018–29.
[2] Lionakis MS, Dunleavy K, Roschewski M, et al. Inhibition of B Cell Receptor Signaling by Ibrutinib in Primary CNS Lymphoma*. Cancer Cell* 2017; **31**: 833–43.
[3] Soussain C, Choquet S, Blonski M, et al. Ibrutinib monotherapy for relapse or refractory primary CNS lymphoma and primary vitreoretinal lymphoma: Final analysis of the phase II 'proof-of-concept' iLOC study by the Lymphoma study association (LYSA) and the French oculo-cerebral lymphoma (LOC) network. *Eur J Cancer* 2019; **117**: 121–30.
[4] Chamoun K, Choquet S, Boyle E, et al. Ibrutinib monotherapy in relapsed/refractory CNS lymphoma: A retrospective case series. *Neurology* 2017; **88**: 101–2.

Supplementary Table 1. Cohort transition rules

Number of patients with DLT at each dose	Dose escalation rule
0 of 3 patients, or 1 of 6 patients	Cohort 1: Administer to the next 3 patients in cohort 2. Cohort 2: Completion of phase 1 (deemed tolerable at doses up to 480 mg).
1 of 3 patients	Add 3 patients at the same dose.
≥2 of 3 patients, or ≥2 of 6 patients	Cohort 1: Stop dose escalation and regard the same dose as the maximum dose*.Cohort 2: Completion of phase 1 (regard the same dose as the maximum dose)

*Maximum dose administered

DLT, dose-limiting toxicity

Supplementary Table 2. Tirabrutinib dose reduction steps

Dose	Dose reduction step 1	Dose reduction step 2
320 mg QD	160 mg QD	--
480 mg QD	320 mg QD	160 mg QD

QD, once daily

Supplementary Table 3. Sequencing primer pool

Amplicon ID	Ion AmpliSeq, forward	Ion AmpliSeq, reverse	Gene	Chr	Amplicon Start	Amplicon Stop
AMPL7153023015	GTGGCCTCATGAGGATCATCAC	TGAGTTCCTCAGAAGGCAGAAGA	*CARD11* exon 23	7	2951742	2951914
AMPL7153023032	AGCAGAATATGATTGAAAAATCGGTAGGT	GCAGGGCCTGACTGATTGATAAA	*CARD11* exon 20	7	2956878	2957052
AMPL7153023055	GGGTACCTTTCCAGAGAGAACT	TCCCTCTGCAGATGACAGTCA	*CARD11* exon 15	7	2963861	2964010
AMPL7153036771	GGGATGGCTGTTGTTAACCCT	GGGAACTCTTTCTTCATTGCCTTG	*MYD88* codon 256	3	38182530	38182680
AMPL7153055306	CCAACCACACCAGCAGATAGTG	GGGACACTAACACTCTGATCTCCA	*CD79B* exon 5	17	62006723	62006876
AMPL7153109082	TTTCTGGAAAAGTACTTCCTGGCAT	TCACTCACTTCATAAAGGTTTTCATCTTCA	*CD79A* exon 4	19	42384644	42384813
AMPL7153111770	CTCCAGTGTCTCAGCTCACTTC	GGCAGTTTAGAGGGAAGAAGAGTG	*CD79A* exon 5	19	42385092	42385260
AMPL7153723923	CACTGGACAAAACACTCTGAAGGA	GGAAAGGACTGTGAAATGTACAAGC	*CARD11* exon 8	7	2977447	2977621
AMPL7153723924	TCTAGTCCCTGGAAAGGAGTGTG	TCAACAGGATCTACAACCTGCAG	*CARD11* exon 7	7	2978197	2978371
AMPL7153854474	GTTGAAATAGGGTGAGTCACAGTCT	AGCGCAGGATGAACCTGAA	*CARD11* exon 10	7	2973941	2974115
AMPL7154390036	CAGCTCCGAAGCAGTCACT	GCCACCTATGAGGACATAGTGAC	*CD79B* exon 6	17	62006524	62006663
AMPL7154390082	CTCTTTCCCAGTCACCAGTTTGT	GTATTCTCACAGAGTGATCTGGTCTTTC	*CARD11* exon 4	7	2985481	2985624
AMPL7154390136	TGGGTATGGTGCCCATATCCA	CACCATGTCCCTGAAGTGTGA	*CARD11* exon 18	7	2959016	2959143
AMPL7154390246	ACACCCTGGCAGGTTCATC	GAGCAGGTTCTGGAACTGGA	*CARD11* exon 6	7	2979299	2979457
AMPL7154390249	GGCCGATTTTCAATGTCATTCTTCAG	CCTTTTCTGAGTGTTTCTTGCCA	*CARD11* exon 6	7	2979465	2979622
AMPL7154390254	GCCATGTTCTTCTCCTCACTGA	CAGGAGCGGTACTACAAGATGAAG	*CARD11* exon 5	7	2983877	2984001
AMPL7154390256	CAGGTCCTTGGCCTTCATCTG	TCTGGAACCTCCTTTTTCCACTTC	*CARD11* exon 5	7	2984089	2984233
AMPL7154390380	CCTTCGTGGTTGACCTTGTAGTG	TGTCTCTGCTCTCCAGCTAGAAG	*CARD11* exon 7	7	2962267	2962410
AMPL7154390387	GGCATTAAGCACTTCATCTTCATCC	ACTACATGGAGACGCTGAAGGAT	*CARD11* exon 3	7	2987240	2987400
AMPL7154390389	GACCCTGTCTCTCCATAATATATAGAGAGG	GCCGGAAAGTGTAGAAGTCACAC	*CARD11* exon 2	7	2998076	2998205
AMPL7154393843	TGGAAGAATCGTCAGCTTCTTGAC	CCACTAACGCCAGCTTTTTCTCT	*CARD11* exon 10	7	2974170	2974294
AMPL7154393848	CGCACCATCTCGATCCTCATC	GCCTTCCACTCCCGAGATGA	*CARD11* exon 9	7	2976744	2976868
AMPL7154630246	CTGATGTTCGCTGCCTCATTTC	TCCAGCTGGACATCTCCTATGT	*CD79A* exon 5	19	42384904	42385037

AMPL7155670905	CGATCCATGGAGGCTTGGTG	GACTTGATCAAGTCCAACATCTACCC	*CARD11* exon 24	7	2949615	2949757
AMPL7156929138	CGCTCACCTGACTTGCAGAT	GGCAAGAACCTCAGCCTCAT	*CARD11* exon 22	7	2952914	2953077
AMPL7157916034	TTCAAACAACAGAAGTGCCCACAT	GCCACAGGAACATCATTCCTCTC	*CD79B* exon 6	17	62006028	62006202
AMPL7157916035	GGGACGGATCACCTCATAGC	AGAGCTGCCATTTGTCTCCAG	*CD79B* exon 6	17	62006317	62006462
AMPL7158623679	GGGTAATGAGCCCTTAATCGCTG	TAAGATCCACGACTCCTGCCA	*CD79A* exon 5	19	42385320	42385465
AMPL7159608551	GTCCCAGACACTCTTCTGCAG	ATCTCGTCTCCCGTGAGGA	*CARD11* exon 12	7	2969556	2969730
AMPL7161194764	CCCATGTACCTTCCTGCTTCT	CCCTCTTCTCCTCCAGTTCGT	*CARD11* exon 21	7	2954862	2955022
AMPL7163273168	TCGTCCACCCAGATGGTCT	CTGAGACGGAGGAGGAGTTCCT	*CARD11* exon 25	7	2946285	2946459
AMPL7163274850	GCTTGACCGAGTGCACGA	TGACATTCTGTTTCCTGCTGCA	*CARD11* exon 16	7	2962819	2962990
AMPL7163388233	CCTTTCAGGGTTGCTTCGAGG	CCGGGAAACGACTCCATCG	*CARD11* exon 13	7	2968110	2968284
AMPL7163388238	TTGGCAGACCTACCCGGAGT	CTGAATTTCAGCCTCTGAGGCT	*CARD11* exon 19	7	2958112	2958261
AMPL7153023028	CTCCTCTCCCTTTGGAGGACT	ACGCCACCAAGCTCTTGA	*CARD11* exon 21	7	2954750	2954903
AMPL7153023031	CGCTTATACTTGTTCTCGGACCT	GGAAATGACTGGCCCAGGAAC	*CARD11* exon 21	7	2954975	2955147
AMPL7153023037	CTCCATAGACTCAGGGAAATGGAG	TGAAGTGTGACGATGTTGTGCA	*CARD11* exon 18	7	2958958	2959132
AMPL7153023043	GGTCACTTGTCCAGGTCAGAGA	TTGGACACATGCACCAAAGAGGAAG	*CARD11* exon 17	7	2962197	2962355
AMPL7153023129	GTACCGCTCCTGGAAGGTTAG	CTTCCTGATGAACGAGGTCATCA	*CARD11* exon 5	7	2983990	2984143
AMPL7153055524	CCGGGAGTTAGGTTTGAAGC	CTCCTCCTGGTGAAACTGCA	*CARD11* exon 19	7	2958055	2958211
AMPL7153109091	CGAGAAGCTCGGGTTGGAT	ATGAGGCAGCGAACATCAGCA	*CD79A* exon 4	19	42384751	42384922
AMPL7153111265	ACCTGCTTTATGGGAGAATTGAGC	GACGACAACTACAACTTAGCCATG	*CARD11* exon 5	7	2983762	2983935
AMPL7153111774	GAGGGTAACCTCACTCTTCTCCA	GCAGCGATTAAGGGCTCATTAC	*CD79A* exon 5	19	42385180	42385343
AMPL7153118744	GGGATCCATGGCTGAATGCA	GCTTCGTGCACTCGGTCAA	*CARD11* exon 16	7	2962674	2962839
AMPL7154162014	CCTCGCCGATCTTGTCCTT	TCTCCTCCCAGAAAGCTGCT	*CARD11* exon 25	7	2946313	2946487
AMPL7154390037	CTCACCTACAGACCACTTCACTTC	CCACTATCTGCTGGTGTGGTT	*CD79B* exon 6	17	62006604	62006745
AMPL7154390081	CCCTCCTCTTAGAGTCCAGATGT	GGTCTTCTTGGAGAGCCTAGAATTTTATTA	*CARD11* exon 4	7	2985408	2985544
AMPL7154390095	TGCAGCATGACCGTGTTCA	CGCATATTGATTTCTCTGTGTGGTT	*CARD11* exon 8	7	2977572	2977696

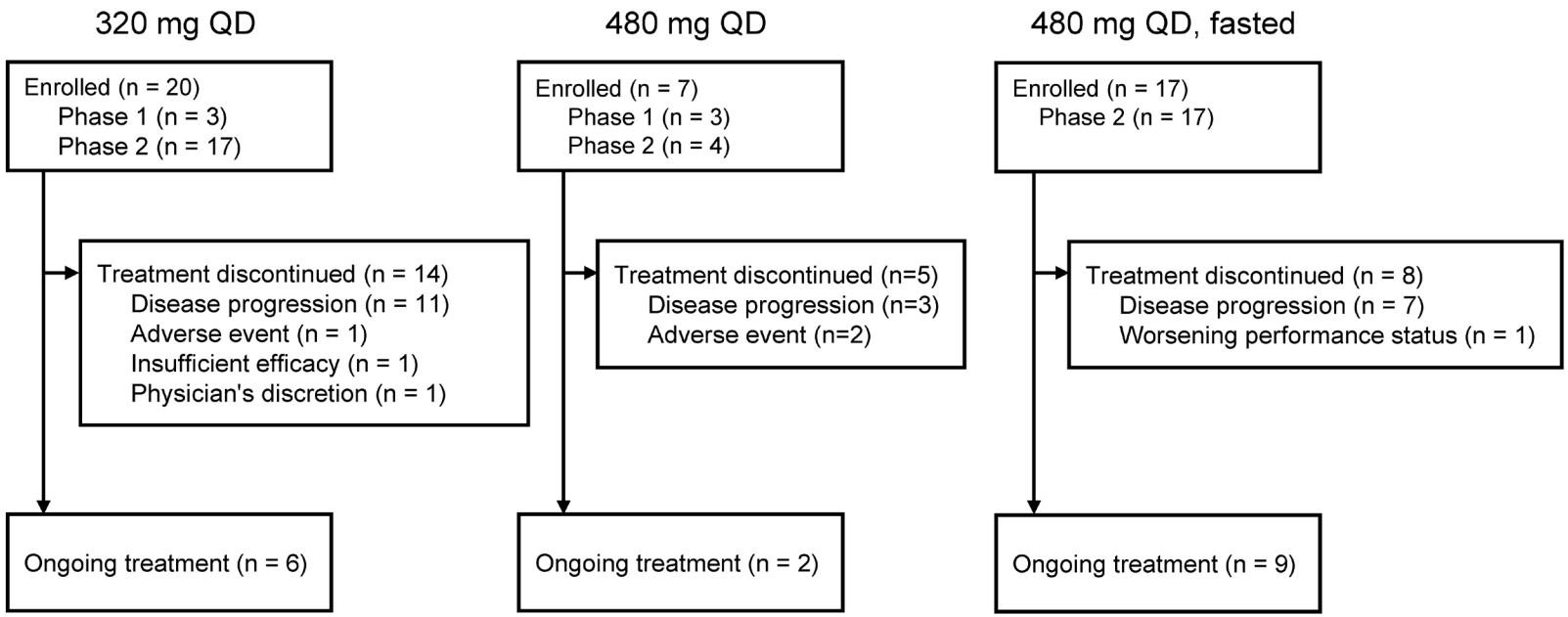
AMPL7154390181	GGGTTCTTCTCTCGGGAGTAGAT	TCGAGTTCTGTAGAGCCCAGAA	*CARD11* exon 23	7	2951858	2952032
AMPL7154390247	CCTGGTTTTTGGTCTTCAGCATTT	GGAGGAATGTAAGCTGGAGAGAAATC	*CARD11* exon 6	7	2979403	2979527
AMPL7154390381	GCTACAATTCTGGTTCTGGCTCTA	GGAGCAGGTCAACCTCATGTTC	*CARD11* exon 15	7	2963737	2963908
AMPL7154390384	GACCCTCCCGGATCTGAGA	ACACACTCACATGCATGCTTTC	*CARD11* exon 14	7	2966345	2966471
AMPL7154390386	ATTTTGGTACAGCTACCAAAGGGT	CTACCTGCGTCAGTGTAAGGTC	*CARD11* exon 3	7	2987124	2987297
AMPL7154390388	GTTACACTCCACATTCTCCCACAA	CAGTGAGACTGCTTTATTAAGCTTGTTG	*CARD11* exon 3	7	2987342	2987466
AMPL7155246262	TCCAGGTTGTTGCTGTCCTT	AAAGGACAAGTACAGGAAGCAGATC	*CARD11* exon 9	7	2976675	2976815
AMPL7155585458	AGGGCACCTACCAGGATGT	CCAGAAGGGCTGAAAGGAGAATG	*CD79A* exon 5	19	42384986	42385143
AMPL7155670937	TTTCGATTAAGCACTGCGAGTACT	CGAGAGCCACAGACGATGTG	*CARD11* exon 9	7	2976814	2976987
AMPL7156929142	CCTCCTCCTGCAGGTTGTAG	CCTGACCCTCTGAAACCTCCT	*CARD11* exon 7	7	2978342	2978499
AMPL7157916033	CGAGAGAGACAGAGTGACAGATGA	TCTAGGTCCCTGTTTGATTTGTGTC	*CARD11* exon 11	7	2972111	2972280
AMPL7160408562	CTTCTCACACACCCGGATGA	CTGCAGGCACCTTCACAGGAG	*CARD11* exon 24	7	2949701	2949866
AMPL7160516146	ACGAGCCTGTGTGTGGTTT	CTCCCAGCAGAGCTTGGGAA	*CD79B* exon 6	17	62006087	62006252
AMPL7163388221	CTGGATGCCCTTCAGGTTCA	CAGTAACCATCATCTCTCAGGACTTTG	*CARD11* exon 10	7	2974087	2974244
AMPL7163388234	CGTCCTCCTTGTAGCGTCT	CCCTGCGTGTGTAATATTTATGCAC	*CARD11* exon 13	7	2968245	2968386
AMPL7163890965	CTTCTACTCCAGGCCCTTTGG	CCTCAGTGACTGCTTCGGAG	*CD79B* exon 6	17	62006401	62006546

Supplementary Table 4. **Relationship between mutations and clinical response**

Usage / dose	Best response	COO	*CARD11*	*CD79A*	*CD79B*	*MYD88*
320 mg	CR	NGC	R235Q/S622del	E145Q	E229K	WT
320 mg	CR	GCB	WT	WT	K187Q/Y196S	L265P
320 mg	CRu	GCB	S622del/H737Y	WT	Y196H	L265P
320 mg	CRu	GCB	WT	WT	Y196S	L265P
320 mg	CRu	GCB	S622del	WT	WT	L265P
320 mg	PR	NGC	S622del	WT	WT	WT
320 mg	PR	NGC	WT	WT	Y196C	WT
320 mg	PR	NGC	WT	WT	WT	L265P
320 mg	PR	NGC	WT	WT	WT	L265P
320 mg	PR	GCB	D200N/A209T	WT	WT	WT
320 mg	PR	GCB	WT	WT	WT	L265P
320 mg	PR	GCB	S622del	WT	WT	L265P
320 mg	SD	NGC	WT	WT	Y196N	L265P
320 mg	SD	NGC	WT	WT	Y196S	L265P
320 mg	SD	NGC	WT	WT	K187R/Y196C	L265P
320 mg	SD	NGC	G540E/A581T	WT	E192K/Y196C	L265P
320 mg	PD	NGC	WT	WT	WT	L265P
320 mg	PD	NGC	WT	WT	Y196H	L265P
320 mg	PD	NGC	W814end	WT	WT	L265P
320 mg	PD	NGC	F115I	WT	WT	WT
480 mg	CR	NGC	S622del	WT	WT	L265P
480 mg	CRu	NGC	WT	WT	WT	L265P
480 mg	CRu	NGC	G123S/S622del	WT	WT	L265P
480 mg	CRu	GCB	WT	WT	WT	L265P
480 mg	PR	NGC	WT	WT	WT	L265P
480 mg	PR	NGC	WT	WT	WT	L265P
480 mg	PR	NGC	F130L	WT	WT	WT

480 mg, fasted	CR	NGC	S622del	WT	WT	WT
480 mg, fasted	CRu	NGC	S622del	WT	Y196S	L265P
480 mg, fasted	CRu	NGC	C49Y/Q288*	WT	E229K	Q262*
480 mg, fasted	CRu	GCB	E185A/S622del	WT	Y196H	L265P
480 mg, fasted	CRu	GCB	E185A/S622del	WT	WT	L265P
480 mg, fasted	CRu	GCB	E185A	WT	Y196D	WT
480 mg, fasted	PR	NGC	S622del	WT	WT	L265P
480 mg, fasted	PR	NGC	N255D/Q395*	WT	WT	WT
480 mg, fasted	PR	NGC	E185A/A581T/S622del	WT	WT	WT
480 mg, fasted	SD	NGC	M275I/G504D/S622del	WT	WT	WT
480 mg, fasted	SD	NGC	S622del	WT	WT	L265P
480 mg, fasted	SD	NGC	E185A/S622del	WT	Y196S	L265P
480 mg, fasted	PD	NGC	E185A	WT	Y196H	L265P
480 mg, fasted	PD	NGC	E185A/S622del	WT	A188G/E224D	L265P
480 mg, fasted	PD	NGC	S622del	WT	WT	L265P
480 mg, fasted	PD	GCB	Q48*/Q288*	G167S/L168F	Y196C/D202N	L265P/K269E
480 mg, fasted	PD	GCB	D224N/D230N/M365I	WT	WT	WT

COO, cell-of-origin (as determined by immunohistochemistry); WT, wild-type; DEL, deletion; GCB, germinal centre subtype; NGC, non-germinal centre subtype; CRu, unconfirmed complete response ; PR, partial response; SD, stable disease; PD, progressive disease



Supplementary Fig. 1. Patient disposition

QD, once daily