SUPPLEMENTARY INFORMATION – Online only

Supplementary methods

Additional inclusion and exclusion criteria for the phase I study

Inclusion criteria for patients with chronic lymphocytic leukemia (CLL) were: diagnosis meeting the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) 2008 criteria, active disease meeting at least 1 of the IWCLL 2008 criteria requiring active therapy, and at least 1 prior therapy. Patients with CLL had to be considered not appropriate for treatment or retreatment with purine analog-based therapy.

Patients with diffuse large B-cell lymphoma (DLBCL) were eligible for inclusion if they had received at least 1 prior therapy, and were either ineligible for or had progressed after high-dose therapy/autologous stem cell transplantation. One protocol amendment allowed for DLBCL patients with an Eastern Cooperative Oncology Group (ECOG) performance status score of 2 to be eligible, but this was revised back to an ECOG performance status score of 0–1 in a subsequent amendment.

Patients with indolent non-Hodgkin lymphoma (iNHL) were included if they had any of the following: follicular lymphoma (FL; grade 1, 2, or 3a), small lymphocytic lymphoma, lymphoplasmacytic lymphoma, or marginal zone lymphoma (splenic, nodal, or extranodal) that was relapsed/refractory after at least 1 prior therapy (excluding radiation). Patients with mantle cell lymphoma (MCL) were included if they had disease that was relapsed/refractory after at least 1 prior therapy (excluding radiation).

Patients with Epstein-Barr virus-positive (EBV+) post-transplant lymphoproliferative disease (PTLD) were included if they: had received a diagnosis of early lesion, polymorphic, monomorphic, classical Hodgkin lymphoma-type, EBV+ DLBCL of the elderly, or DLBCL associated with chronic inflammation; had documented or documentable EBV-encoded RNA status by tissue *in-situ* hybridization; and had failed or were considered ineligible for at least 1 prior therapy.

Patients were excluded if they had brain metastasis, except in patients who had completed definitive therapy, were not on steroids, had stable neurologic status for ≥2 weeks after completion of the definitive therapy and steroids, and did not have neurologic dysfunction that would confound the evaluation of neurologic and other adverse events (AEs). Additional exclusion criteria were: systemic anticancer treatment or radiotherapy <2 weeks before the first dose of study treatment (≤4 weeks for monoclonal antibodies with evidence of disease progression); treatment with inhibitors or inducers of P-glycoprotein, or strong inhibitors or inducers of CYP3A that could not be discontinued or replaced ≥7 days before the first dose of TAK-659 and for the duration of the study; and prior treatment with investigational agents ≤21 days or ≤5 times their half-lives (whichever was shorter) before the first dose of study treatment.

Determining safe starting dose of TAK-659

The safe starting dose of TAK-659 (based on non-clinical Good Laboratory Practice-compliant toxicology data for this first-in-human study) was estimated to be 80 mg once-daily (QD), and was calculated by converting one-tenth the rat STD10 on a body surface-area basis to a human equivalent dose, assuming 70 kg for adult human weight. The dose was selected based on the STD10 in rodents because on a body surface area basis, one tenth the rat STD10 was tolerated in dogs (Millennium Pharmaceuticals, Inc., data on file).

Pharmacokinetic sampling schedule

During dose-escalation, serial or sparse plasma samples were collected on days 1, 2, 8, 15, and 16, and urine samples were collected on days 1 and 15 of cycle 1. In the expansion phase, plasma samples were collected on days 1, 15, and 22 of cycle 1, and day 1 of cycle 2. During the run-in period of the expansion phase, serial collection of plasma samples from patients with iNHL occurred daily for 8 days prior to cycle 1.

Response assessment

An imaging modality (e.g., computed tomography [CT] with contrast or magnetic resonance imaging for solid tumors; CT with contrast or fluoro-2-deoxy-D-glucose-positron emission tomography for lymphoma; and CT with contrast for CLL) was used to follow sites of measurable disease during the study. Patients with non-measurable lesions were eligible for the dose-escalation phase, and investigators assessed overall disease status, relevant tumor markers, and disease symptoms to determine their response status.

Pharmacodynamic modulation of SYK and FLT-3 downstream signaling by flow cytometry.

MV4-11 cells (ATCC) were cultured in Iscove Modified Dulbecco Media (Gibco) supplemented with 10% heat-inactivated Fetal Bovine Serum (Gibco) at 37ºC in a humidified 5% CO_2 incubator. TAK-659 and MLN0518 (FLT3-specific inhibitor) powder were reconstituted in dimethyl sulfoxide (DMSO) as stock solutions. Exponentially growing MV4-11 cells were plated at 5 X 105 cells/mL and cultured overnight. DMSO, TAK-659 or MLN0518 were added to the cultures and incubated for 1 hour, as indicated. The treated MV4-11 cells were collected by centrifugation (600xg, 10 minutes at RT) and washed twice with room temperature phosphate buffered saline (pH 7.5, Gibco). Cells were resuspended in PhosFlow Lyse/Fix Buffer I (BD Bioscience), permeabilized with Permeabilization Buffer (BD Biosciences) and incubated in staining buffer (BSA, BD Bioscience) with either unconjugated or Alexa Fluor® 647 conjugated antibodies to pSYK S525/526 (Mil-81, unconjugated), pFLT3 Y969 (clone C24D9, Cell Signaling, unconjugated), pBLNK Y84 (Cell Signaling, unconjugated), pBTK Y551 (clone EP267Y, Abcam, unconjugated) or pS6 kinase S235/236 (clone A17020B, Biolegend, conjugated) for 1 hour in the dark on ice according to the manufacturer's instructions. Cells were washed twice with stain buffer. Cells incubated with unconjugated antibodies in the prior step were subsequently incubated with Alexa Fluor® 647 secondary antibody (Goat anti-Rabbit IgG (H+L) Cross-Adsorbed,

ThermoFisher) in stain buffer where indicated for an additional 30 minutes in the dark on ice according to the manufacturer's instructions, while the cells previously incubated with conjugated antibodies remained in stain buffer. Cells were washed twice with stain buffer and flow cytometry data was collected on a BD FACS Canto II (10,000 events). Flow cytometry data was analyzed using WinList V8 software on single live cells.

Supplemental Tables and Figures

Supplementary Table S1. Definition of dose-limiting toxicities

Dose-limiting toxicities were defined as any events listed below that were considered by the investigator to be at least possibly related to TAK-659:

- Grade 4 neutropenia (ANC <500 cells/mm^3) unresolved to grade ≤1 (ANC >1500 cells/mm^3) or baseline for more than 7 consecutive days in the absence of growth factor support
- Grade ≥3 neutropenia (ANC <1000 cells/mm^3) with fever and/or infection, where fever is defined as an oral temperature ≥38.5°C
- Grade 3 thrombocytopenia (<50,000/mm^3) with clinically significant bleeding
- Grade 4 thrombocytopenia (<25,000/mm^3) unresolved to grade ≤1 (>75,000/mm^3) or baseline for more than 7 consecutive days or a platelet count <10,000/mm^3 at any time
- Grade 4 anemia
- Any grade ≥3 non-hematologic toxicity, with the following exceptions:
    - Grade 3 nausea and/or vomiting and grade ≥3 diarrhea that has not resolved to grade <3 after 48 hours of optimal antiemetic and/or antidiarrheal treatment.
    - Transient grade 3 fatigue (≤72 hours)
    - Asymptomatic lipase elevation (grade <4) in the absence of significant amylase elevation (grade <3) considered not dose limiting
    - Asymptomatic amylase elevation (grade <4) in the absence of significant lipase elevation (grade <3) considered not dose limiting
    - Asymptomatic grade 3 elevation of a single liver enzyme (AST or ALT) in the absence of significant bilirubin elevation (grade <3) considered not dose limiting

- Inability to administer at least 75% of planned doses of study drug within cycle 1 due to treatment-related toxicity
- Other TAK-659-related non-hematologic toxicities grade ≥2 that, in the opinion of the investigator, require a dose reduction or discontinuation of therapy

Abbreviations: ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase.

Supplementary Table S2. Disease subtypes

Disease subtype	n
Solid tumors	19
Neuroendocrine	3
Endometrial	2
Colon	2
Squamous cell carcinoma	2
Breast	1
Cholangiocarcinoma	1
Head and neck cancer	1
Small cell lung cancer	1
Ovarian	1
Pancreas	1
Thymoma	1
Paranasal sinus	1
Uterine	1
Unknown primary	1

Lymphomas	86
Diffuse large B-cell lymphoma	53
Follicular lymphoma	14
Chronic lymphocytic leukemia	6
Mantle cell lymphoma	5
Mucosa-associated lymphoid tissue	2
Nodal marginal zone B-cell lymphoma	2
B-cell lymphoplasmacytic lymphoma/immunocytoma	1
B-cell small lymphocytic lymphoma	1
Splenic marginal zone lymphoma	1
Post-transplant lymphoproliferative disorder	1

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Supplementary Table S3. Patient disposition

n (%)	Solid tumors[a] n=19	Lymphoma			All patients N=105
		DLBCL n=53	FL n=14	All lymphomas[a] n=86	
Patients discontinuing study treatment	18 (95)	49 (92)	13 (93)	79 (92)	97 (92)
Progressive disease	9 (47)	26 (49)	5 (36)	35 (41)	44 (42)
AE	4 (21)	13 (25)	7 (50)	28 (33)	32 (30)
Withdrawal by patient	0	2 (4)	0	3 (3)	3 (3)
Symptomatic deterioration	2 (11)	5 (9)	0	8 (9)	10 (10)
Protocol violation	1 (5)	0	0	0	1 (<1)
Initiation of HSCT	0	2 (4)	0	2 (2)	2 (2)
Other	2 (11)	1 (2)	1 (7)	3 (3)	5 (5)
On study deaths					
All deaths	4 (21)	18 (34)	1 (7)	25 (29)	29 (28)
Related deaths	0	2 (4)	0	4 (5)	4 (4)

Abbreviations: AE, adverse event; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HSCT, hematopoietic stem cell transplant.

[a]See Supplementary Table 2 for details of specific diseases.

Supplementary Table S4. Dose escalation, dose-limiting toxicities, and maximum tolerated dose

Dose level, n	Solid tumor n=19	Lymphoma n=17	Total n=36	DLTs n=6	Dose-limiting toxicities
60 mg	6	4	10	1	Increased AST (grade 3)[a]
80 mg	1	3	4	0	None
100 mg	1	2	3	0	None
120 mg	6	1	7	4	Stomatitis (grade 3)[b], generalized edema (grade 3)[a], increased lipase (n=2; grade 3, grade 4)[a]
100 mg (MTD)	5	7	12	1	Hypophosphatemia (grade 3)[b]

Abbreviations: AST, aspartate aminotransferase; DLT, dose-limiting toxicity; MTD, maximum tolerated dose.

[a]Solid tumor patient(s). [b]Lymphoma patient(s).

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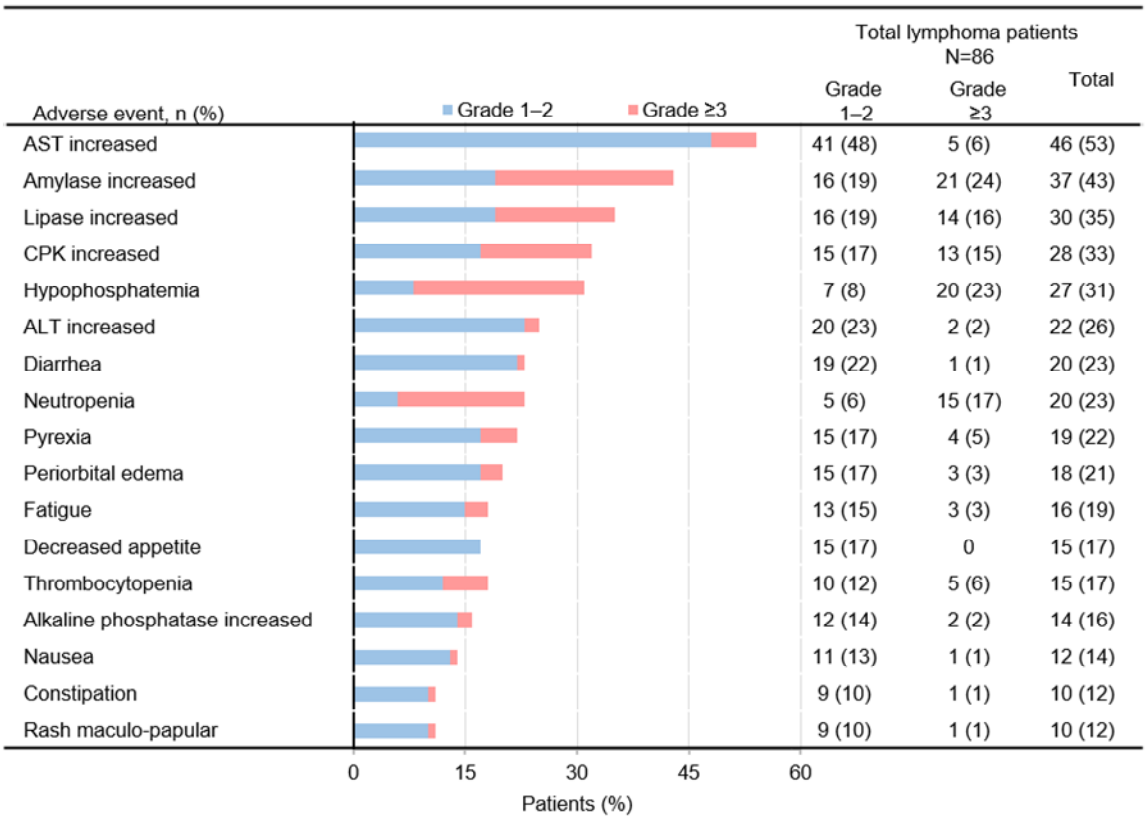
Supplementary Table S5. Most common treatment-related adverse events. Bar lengths may appear different than the corresponding values of frequency due to rounding. AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatine phosphokinase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase.



Adverse event, n (%)	Total lymphoma patients N=86 Grade 1–2	Grade ≥3	Total
AST increased	41 (48)	5 (6)	46 (53)
Amylase increased	16 (19)	21 (24)	37 (43)
Lipase increased	16 (19)	14 (16)	30 (35)
CPK increased	15 (17)	13 (15)	28 (33)
Hypophosphatemia	7 (8)	20 (23)	27 (31)
ALT increased	20 (23)	2 (2)	22 (26)
Diarrhea	19 (22)	1 (1)	20 (23)
Neutropenia	5 (6)	15 (17)	20 (23)
Pyrexia	15 (17)	4 (5)	19 (22)
Periorbital edema	15 (17)	3 (3)	18 (21)
Fatigue	13 (15)	3 (3)	16 (19)
Decreased appetite	15 (17)	0	15 (17)
Thrombocytopenia	10 (12)	5 (6)	15 (17)
Alkaline phosphatase increased	12 (14)	2 (2)	14 (16)
Nausea	11 (13)	1 (1)	12 (14)
Constipation	9 (10)	1 (1)	10 (12)
Rash maculo-papular	9 (10)	1 (1)	10 (12)

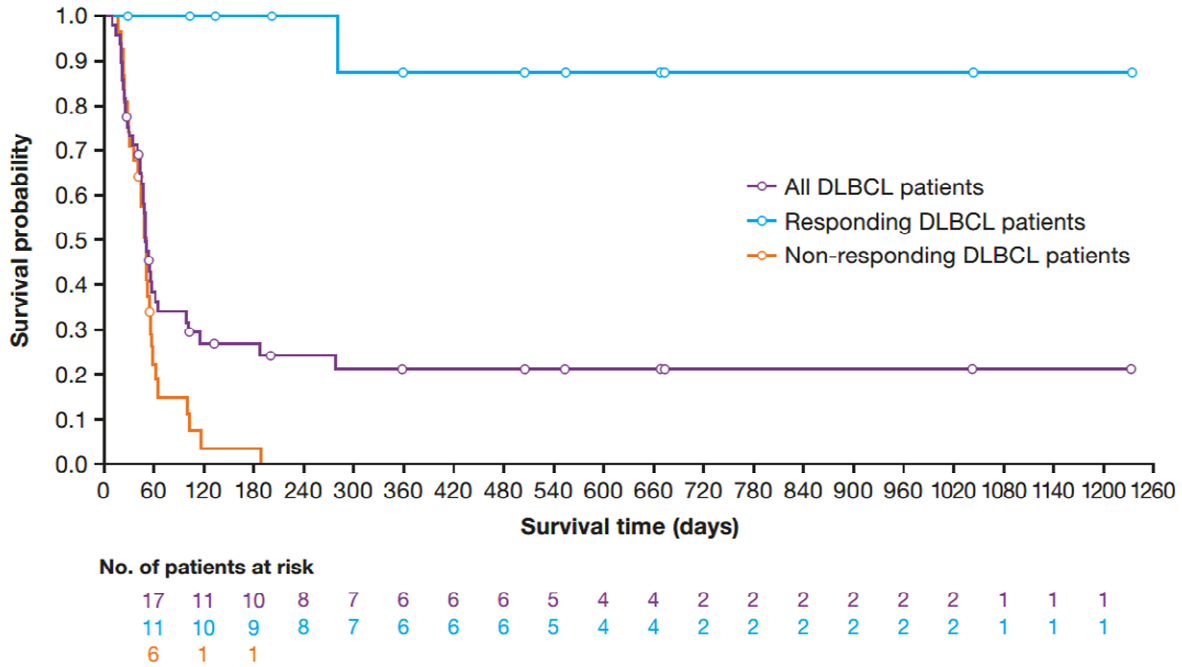
Supplementary Table S6. Best antitumor response by diffuse large B-cell lymphoma molecular classification

DLBCL Type	Evaluable N	CR n (%)	PR n (%)	ORR n (%)
DLBCL	43 (100)	8 (19)	4 (9)	12 (28)
De novo	25 (58)	4 (16)	2 (8)	6 (24)
GCB[a]	10 (23)	3 (30)	0	3 (30)
Non-GCB[a]	4 (9)	0	0	0
Unknown	11 (26)	1 (9)	2 (18)	3 (27)
Transformed	17 (40)	4 (24)	1 (6)	5 (29)
GCB[a]	15 (35)	3 (20)	1 (7)	4 (27)
Non-GCB[a]	1 (2)	1 (100)	0	1 (100)
Unknown	1 (2)	0	0	0
Unknown	1 (2)	0	1 (100)	1 (100)
Non-GCB[a]	1 (2)	0	1 (100)	1 (100)

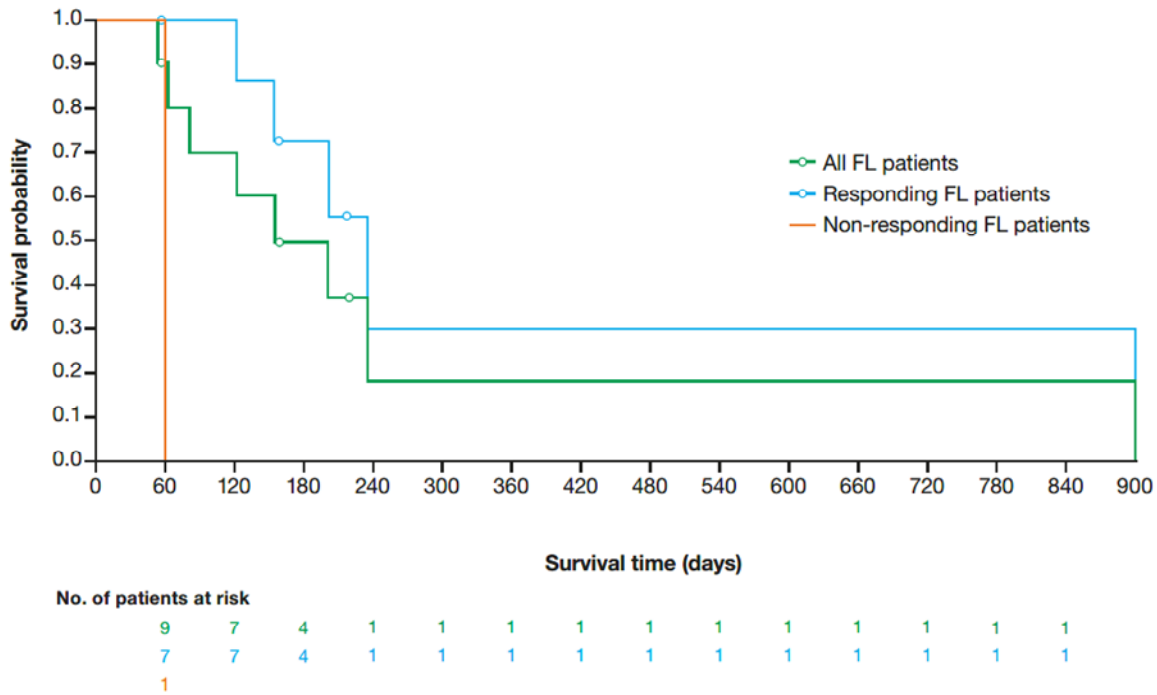
Abbreviations: CR, complete response; GCB, germinal center B-cell; ORR, overall response rate; PR, partial response.

[a]Molecular classifications based on local results, mostly by the Hans method.

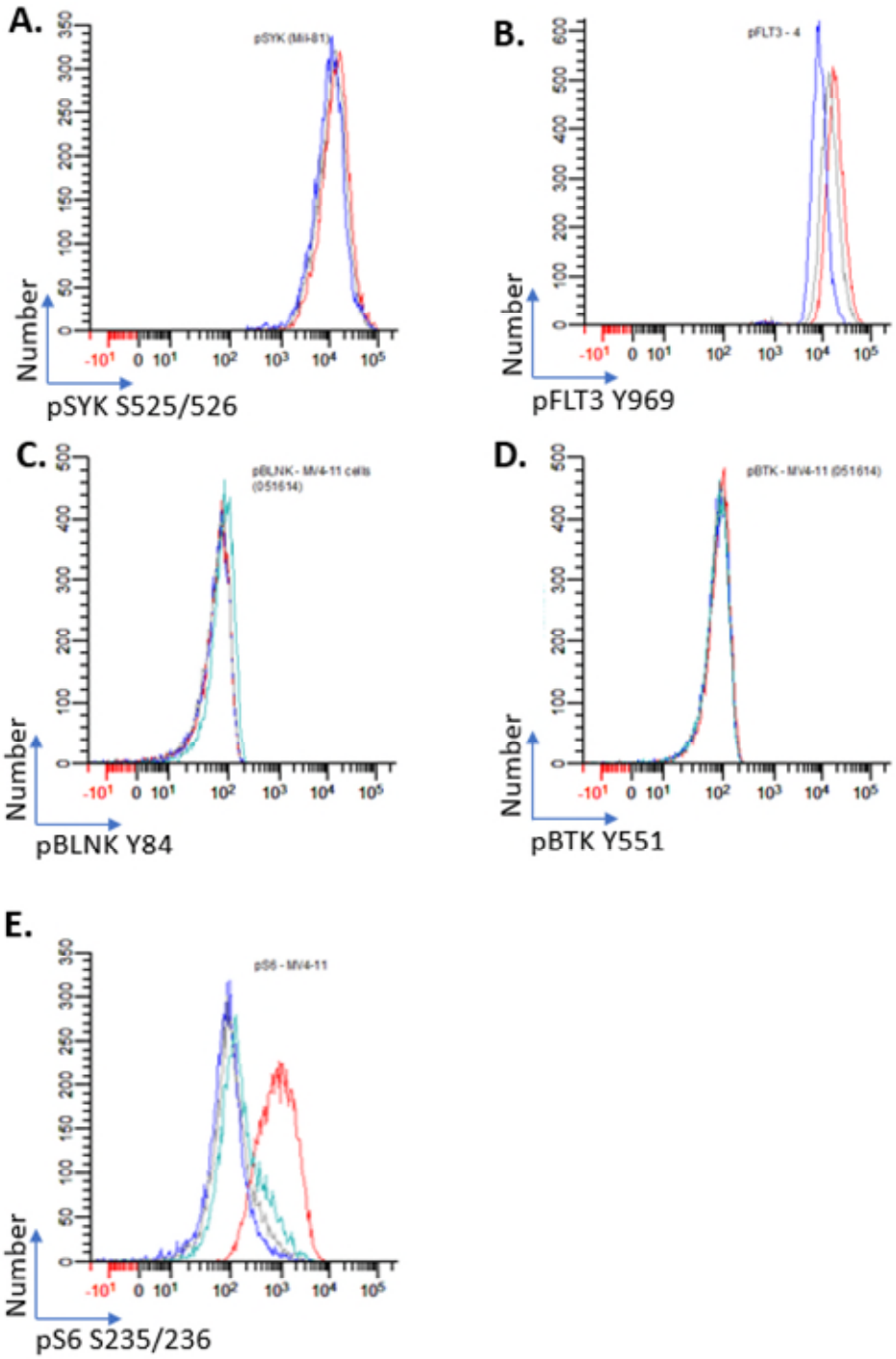
Supplementary Figure S1. Progression-free survival in diffuse large B-cell lymphoma responders and non-responders DLBCL, diffuse large B-cell lymphoma



Supplementary Figure S2. Progression-free survival in follicular lymphoma responders and non-responders FL, follicular lymphoma



Supplementary Figure S3. Evaluation of downstream signaling of SYK and FLT-3 as potential pharmacodynamic markers of TAK-659 activity.



Pharmacodynamic effects of TAK-659 on downstream signaling were evaluated in MV4-11 cells treated for 1 hour with DMSO (red), 1μM TAK-659 (Grey), 5μM TAK-659 (Blue), 1μM MLN0518 (FLT3 specific inhibitor) or IgG (black, panel E only). Flow cytometry was

29 performed on cells stained with antibodies to pSYK S525/526 (A), pFLT3 Y969 (B), pBLNK
30 Y84 (C), pBTK Y551 (D) or pS6 kinase S235/S236 (E).

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