Ibrutinib, Single Agent or in Combination with Dexamethasone, in Patients with Relapsed or Relapsed/Refractory Multiple Myeloma (MM): Preliminary Phase 2 Results

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Introduction: Multiple myeloma (MM) remains an incurable disease in need of new therapies with unique targets. Ibrutinib is a first-in-class, once-daily, oral, covalent inhibitor of Bruton's tyrosine kinase (BTK), an essential enzyme in the B-cell receptor signaling pathway. While BTK is essential for the development and function of B cells and is down-regulated in plasma cells, the expression of BTK in malignant plasma cells is increased 4-fold and comparable to BTK expression levels in chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). In addition, pre-clinical models show that BTK inhibition with ibrutinib led to direct inhibition of both osteoclast bone resorption and the release of osteoclast-derived tumor growth factors (Tai et al, Blood 2012). Taken together these data suggest that ibrutinib may have a role in the treatment of MM.

Methods: This open label phase 2 dose escalation study was designed to enroll patients in 4 cohorts (Figure) to evaluate efficacy (≥MR) and secondary endpoints of safety, PK, ORR and DOR. Patients must have had documented non-responsive/progressive disease at the time of study entry following at least 2 prior lines of therapy including at least one immunomodulatory agent. Efficacy and safety were assessed at 4 weeks intervals using the IMWG response criteria for efficacy assessments (Rajkumar et al, Blood 2011), while safety was assessed according to CTCAE v4.0 criteria.

Results: As of 15 May 2014 and a median follow up of 15.2 months, 69 patients with a median age of 64 years (range 43-81) were dosed, of which 20% had either a del 17p or p53 deletion. The number of median prior therapies was 4 (range, 2-14), 41% had ≥ 5 prior therapies and 80% had undergone autologous stem cell transplant. Sixty-two percent of patients were refractory to their last line of therapy and of the 65 patients that had received prior therapy with both an immunomodulatory agent and a proteasome inhibitor, 44% were refractory to both.

Anti-tumor activity was noted across all cohorts. The highest activity with a clinical benefit rate (CBR) of 25% including 1 PR, 4 MR and 5 sustained (>4 cycles) SD was observed in Cohort 4. (Table) This led to expansion of Cohort 4 per protocol design. In Cohorts 1 and 3, 14 patients had dex added following PD, resulting in 1 PR and 9 SD.

Overall, 57% experienced a Grade 3 or higher adverse event. The most commonly reported non-hematologic toxicities (any grade) were diarrhea (51%), fatigue (41%), nausea (35%), dizziness (25%), and muscle spasms (23%). The majority were Grade 1 and 2. Myelosuppression had a reported overall incidence of any grade anemia (29%), thrombocytopenia (23%), and neutropenia (7%) with 16%, 9% and 4% being ≥Grade 3, respectively. There were no clinically meaningful differences among dose levels. Twenty-three patients experienced a SAE for a total of 47 reported events with 16 assessed as possibly/definitely related to ibrutinib per investigator. At least one dose modification occurred in 22% of patients, with 6 discontinuing due to an adverse event.

At the time of the data cut-off 7 patients remain on study treatment. The most common reason for treatment discontinuation was PD in 47% of patients, with additional patients discontinuing due to investigator discretion (18%), patient decision (7%) and non-compliance (3%).

Conclusions: In this heavily pre-treated patient population ibrutinib, as a single agent and in combination with dex, demonstrated evidence of anti-tumor activity. There was a trend toward improved efficacy (≥MR) in Cohort 4 and treatment was well tolerated with manageable toxicities. Ongoing correlative studies are being conducted to determine changes in cytokines, chemokines and indices of bone metabolism and to determine the effect of dex, a known CYP3A4/5 inducer, on the pharmacokinetic profile of ibrutinib. In addition, ibrutinib is currently being evaluated in combination with carfilzomib in an ongoing Phase1/2b study. (NCT01962792)

Figure. Study Schema

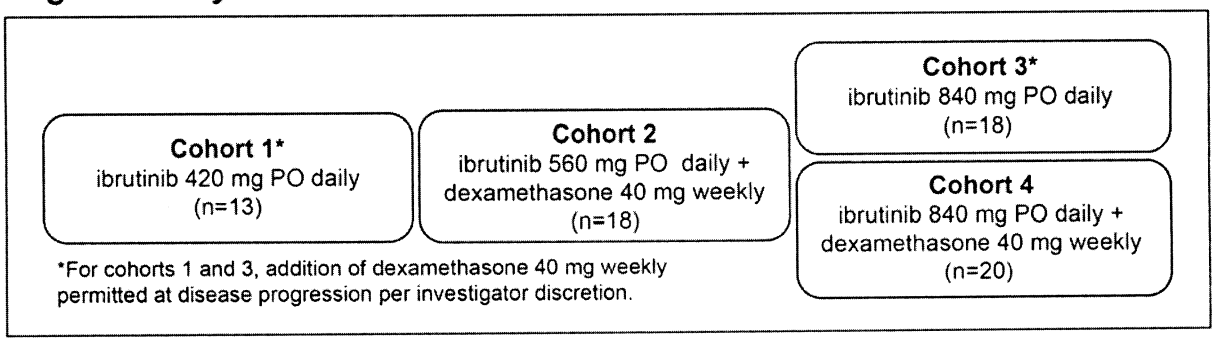

Table Confirmed Response by Assigned Treatment

	Cohort			
Response, n (%)	1 (n=13)	2 (n=18)	3 (n=18)	4 (n=20)
PR	1	1	-	1
MR	1	-	-	4
SD ≥ 4 cycles	2	4	6	5
SD < 4 cycles	5	6	4	1
PD	4	5	7	5
Not evaluable	-	2	1	4
Not evaluable – no post-baseline assessments				

Complex Karyotype, Rather Than del(17p) Is Associated with Inferior Outcomes in Relapsed or Refractory CLL Patients Treated with Ibrutinib-Based Regimens

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Background.
Ibrutinib is active in relapsed/refractory (R/R) CLL, including patients (pts) with del(17p); pts with del(17p) have similar response rate to pts without, but have shorter progression-free survival and a pattern of continuous relapses. (Byrd *NEJM* 2013). It is unknown whether subpopulations of pts with del(17p) with distinct clinical courses can be identified. Del(17p) is frequently associated with a complex metaphase karyotype (CKT), defined as ≥3 distinct chromosomal abnormalities. CKT has been associated with inferior outcomes in treatment-naïve and R/R CLL, but its prognostic significance in ibrutinib-treated pts is unknown.

Methods.
We reviewed 100 pts treated for R/R CLL at MD Anderson Cancer Center with investigational ibrutinib-based regimens from 2010-2013. 50 pts received ibrutinib (Ib) monotherapy, 36 Ib plus rituximab and 14 Ib + bendamustine and rituximab (BR). All pts provided informed consent and studies were conducted according to the declaration of Helsinki. Pre-treatment FISH and CpG-stimulated metaphase cytogenetic analysis was performed on bone marrow.

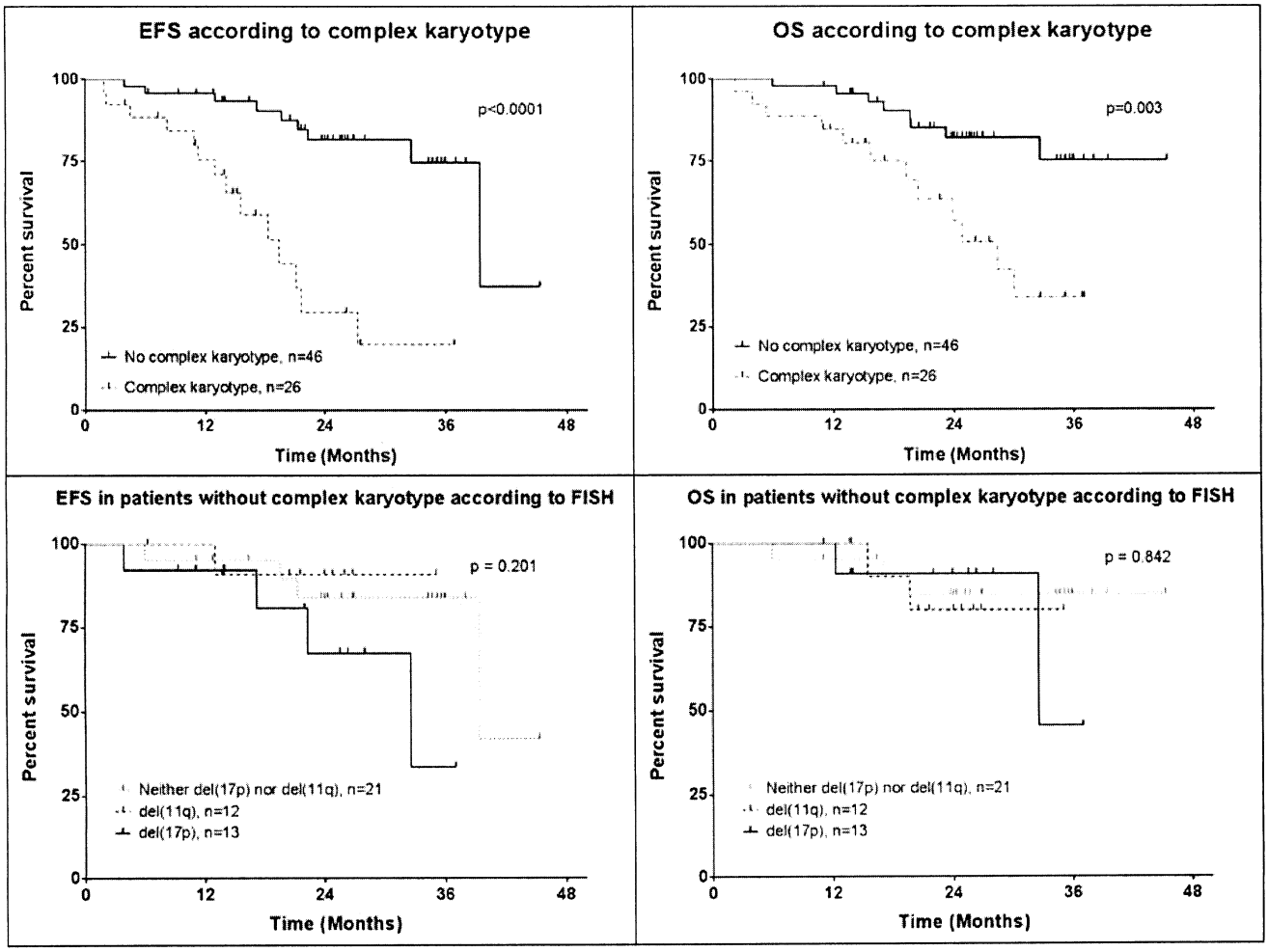
Results.
Pt characteristics are shown below:

Characteristic	
Age, median (range)	65 (35-83)
# prior therapies, median (range)	2 (1-12)
FISH hierarchy, n (%) (n=95)	
del(11q)	26 (28)
del(17p)	46 (49)
Complex karyotype, n (%) (n=72)	26 (36)
Unmutated *IGHV* gene, n (%) (n=98)	80 (81)
Fludarabine-refractory, n	19
β2-microglobulin ≥4.0, n (%)	48 (56)

22/26 pts with CKT had del(17p), [OR 19.2 (4.9-76.4) compared to non-del(17p) pts, p<0.001]; 3 had del(11q). 1 did not have FISH results available. There was no association between CKT and other baseline characteristics. 35/46 pts with del(17p) had metaphase cytogenetics performed. Karyotype was abnormal in 33/35. Mechanisms of *TP53* loss were: unbalanced translocations, -17, del(17p) and i(17q) in association with CKT (n=22), del(17p) in the absence of CKT (n=3) and *TP53* loss identified by FISH only (n=10).

The overall response rate (ORR) for the entire population was 95%, with 16% complete remission (CR). ORR was not different according to baseline characteristics. CR rate was 50% among pts who received Ib + BR versus 10.7% in those combined who received Ib or Ib + rituximab [OR 40.1 (3.0-538.5), p=0.005]. There was a trend toward lower CR rate on multivariable analysis (MVA) for pts with B2M ≥4.0 (p=0.055).

Median follow-up in surviving pts was 27mo. On univariable analyses (UVA), the following were significantly associated with event-free survival (EFS): fludarabine-refractory CLL [median EFS 27mo vs. not reached (NR), p=0.025], del(17p) (27mo vs. NR, p=0.008) and CKT (16 vs. 39mo, p<0.0001). There was no association between del(17p) and EFS when pts with CKT were excluded. On MVA, only CKT was significantly associated with EFS [HR 4.1 (1.3-13.4), p=0.018]. On UVA, the following were significantly associated with survival (OS): fludarabine-refractory CLL (28mo vs. NR, p=0.009), del(17p) (33mo vs. NR, p=0.024) and CKT (24mo vs. NR, p=0.003), with no association between del(17p) and OS when pts with CKT were excluded (Figure). There was a trend toward inferior OS in pts with baseline B2M ≥4.0 (42mo vs. NR, p=0.07). On MVA, fludarabine-refractory CLL [HR 5.6 (1.7-17.9), p=0.004], CKT [HR 5.1(1.5-17.5), p=0.009] and B2M ≥4.0 [HR 3.3 (1.0-10.4), p=0.044] were all significantly associated with inferior OS. There was no association between del(17p) and OS.



EFS and OS were significantly longer in pts who achieved CR compared with PR ($p<0.001$). Pts with primary refractory disease had poor outcomes. 5 pts developed Richter Syndrome, of whom 4 had CKT.

Discussion.

The presence of CKT is independently associated with inferior EFS and OS in pts with relapsed/refractory CLL treated with Ib, while del(17p) is not. CKT is strongly associated with del(17p) and may be a key determinant of biological behavior in del(17p) CLL. This has important implications for treatment of pts with del(17p); those without CKT appear to have equivalent outcomes with Ib compared to pts without del(17p) and could potentially be managed with long-term Ib and close monitoring. In contrast, the inferior outcomes after initial response in pts with CKT make them ideal candidates for treatment-intensification strategies after initial Ib-based treatment, either with novel drug combinations or with allogeneic stem cell transplant, ideally in the context of well-designed clinical trials.

Mutational Analysis of Patients with Primary Resistance to Single-Agent Ibrutinib in Relapsed or Refractory Mantle Cell Lymphoma (MCL)

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Introduction: Ibrutinib, a first-in-class, once-daily, oral covalent inhibitor of Bruton's tyrosine kinase is highly effective in relapsed or refractory mantle cell lymphoma (MCL) patients with an ORR of 68% (Wang M, et al. *N Engl J Med.* 2013;369:507-516). Similar efficacy results were observed in a recent phase 2 study in MCL patients who progressed after rituximab containing chemotherapy and bortezomib therapy (SPARK study, NCT01599949). However, despite the very promising efficacy data, some patients with MCL do not respond to ibrutinib. Here, we report analyses of potential mechanisms associated with primary resistance to ibrutinib therapy in the SPARK study.

Methods: In this phase 2, international, multicenter, single-arm study, patients with MCL received 560 mg/day oral ibrutinib continuously until progressive disease (PD) or unacceptable toxicity. Patients who had PD at the first disease evaluation were considered to have primary resistant disease. DNA was extracted from baseline/pretreatment tumor samples (biopsy or CD19-enriched cells from PBMC) and enriched libraries were constructed with probesets specific for the coding region of 97 genes possibly involved in ibrutinib response and resistance, using the Ovation Target Enrichment system (NUGEN). Deep sequencing (150bp, single end reads) was performed on an Illumina HiSeq instrument . Sequences were aligned to the hg19 reference genome, variants were called using samtools and filters were applied to identify possible somatic mutations (minor allele frequency < 1% in dbSNP, > 5% and < 95% variant allele, > = 10 total reads).

Results: Twenty five (22.7%) of the 120 patients enrolled in this study had Independent Review Committee (IRC)-confirmed disease progression. In these primary treatment resistant patients, the median number of prior lines of systemic therapy was 3 (range 1-5 lines); 37% of patients had high risk MIPI score; 64% had bulky disease (longest diameter ≥ 5 cm), 52% had extranodal disease; 32% had bone marrow involvement and 20% had blastoid subtype. None of these baseline clinical parameters were found to be predictive for primary treatment resistance. The median duration of treatment was 1.54 months. Sequence data could be collected from 23 of the 25 patients, with an average of 9 million reads. After data filtering as described above, 27 genes were found with nonsynonymous variants in at least 2 or more patients. The majority of these variants were previously unreported in dbSNP or COSMIC databases. No mutations previously described in chronic lymphocytic leukemia (CLL) patients with acquired resistance to ibrutinib (BTK C481S, PLCg2 R665W) were seen in these patients, although one patient had a different mutation in PLCg2. Genes previously implicated in diffuse large B-cell lymphoma (DLBCL) pathogenesis (Pasqualucci L, et al. *Nature Genetics.* 2011;43:830-837) such as MLL2 and CREBBP were also found to be mutated in these patients. In addition, mutations in PIM1 and ERBB4 kinase genes were more frequent in patients with PD compared with those non-resistant to therapy. PIM1 kinase has been associated with increased proliferation, survival, and migration of tumor cells, and somatic mutations in this gene have been described in several cancers including hematologic malignancies. Interestingly, several of the mutations detected affect NF-kB signalling inhibition which is thought to contribute to ibrutinib's mechanism of action. These results will be updated at the time of final presentation.

Conclusion: Sequence analyses were conducted on tumor DNA from the fraction of patients with primary resistance to ibrutinib treatment in this study. These studies have revealed a number of known and novel mutations, including genes involved in NF-kB signalling. Among others, the mutational status of PIM1 kinase and ERBB4 kinase genes may be of interest with respect to primary resistance to ibrutinib therapy in MCL.

Functional evidence using deuterated water labeling that the Bruton tyrosine kinase inhibitor ibrutinib inhibits leukemia cell proliferation and trafficking and promotes leukemia cell death in patients with chronic lymphocytic leukemia and small lymphocytic lymphoma

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BACKGROUND: In patients with chronic lymphocytic leukemia (CLL), the Bruton tyrosine kinase (BTK) inhibitor ibrutinib induces a rapid reduction in lymphadenopathy, accompanied by a transient lymphocytosis. Continuation of therapy leads to a reduction in peripheral blood disease burden with achievement of objective responses in a majority of patients over time. Inhibition of B cell receptor (BCR) and chemokine receptor signaling are considered the mechanisms for redistribution lymphocytosis and CLL cell death. However, these mechanisms of action have not been formally documented directly in patients. To address this, before initiating therapy with ibrutinib, we labeled the DNA of proliferating CLL cells with deuterium (^2H) by asking patients to drink deuterated water (^2H_2O) and then determined the effects of ibrutinib on leukemia cell kinetics (proliferation and death rates) and mobilization of cells from lymphoid tissues (trafficking) after ibrutinib administration.

METHODS: 30 previously untreated CLL/SLL patients were enrolled between December 2012 and June 2013 at MD Anderson Cancer Center. Patients required treatment per iwCLL guidelines, had adequate hematopoietic (platelets > 50,000/μL, ANC > 750/μL) and organ functions. Patients drank 50 mL of 70% ^2H_2O 3 times a day for 5 days, followed by 60 mL daily

for a total of 4 weeks ("labeling phase"). After a 6-12 week "washout phase", patients started once-daily ibrutinib 420 mg continuously on 28 day cycles. Study objectives were to determine the impact on CLL cell proliferation and death rates, before and after ibrutinib, and on CLL cell trafficking after ibrutinib.

PATIENT CHARACTERISTICS: Median age of the 30 patients was 64 years (range 48–78) with 19 males. 16 patients exhibited early stage (Rai stage 0-2), and 14 advanced stage (Rai stage 3-4) disease. 17 patients expressed unmutated *IGHV*s, 11 patients had mutated *IGHV*s, and 2 had inconclusive *IGHV* results. 8 were $CD38^+$ and 15 ZAP-70^+ by immunohistochemistry. FISH cytogenetics revealed 11 patients with del13q, 6 with trisomy 12, 3 with del17p or *TP53* mutation, 4 with del11q, and 6 without abnormalities. Median β2 microglobulin level was 2.8 mg/L (1.6 - 7.4).

RESPONSE TO THERAPY: At a median follow up of 13 months, 28 of 30 patients continued on therapy without disease progression. All patients were evaluable for response assessment per 2008 IWCLL guidelines: 28 (93%) achieved partial remission, 1 (3%) complete remission, and 1 (3%) had stable disease, yielding an ORR of 97%. Two patients came off study at 184 and 480 days, respectively.

CLL CELL KINETICS AND TRAFFICKING: The average CLL cell birth rate determined before ibrutinib therapy was 0.42% per day (range 0.32 – 1.42%). After initiating ibrutinib, there was a rapid increase in absolute lymphocyte count in the blood in 26 of 30 patients; this contained previously labeled CLL cells rather than newly-divided unlabeled cells. Over time, the proportion of circulating CLL cells that were labeled did not decrease appreciably despite a concomitant fall in circulating lymphocytes, indicating that newly divided cells were not entering the circulation and strongly suggesting that ibrutinib had a major inhibitory effect on leukemia

cell proliferation. In 3 of the 4 patients in whom there was a rapid influx of unlabeled cells with the onset of ibrutinib treatment, maintenance of circulating labeled cells concomitant with a significant fall in circulating lymphocytes suggested proliferation of CLL cells was inhibited with extended treatment. Prior to ibrutinib therapy, the measured proliferation rate of CLL cells was 0.42 % per day. Because after ibrutinib therapy both cell proliferation and exiting from the blood were markedly reduced, the elimination rate should equal the death rate. Notably, with treatment the elimination rate of CLL cells was much faster than before ibrutinib, revealing a "true" death rate of 1.48 % of the clone per day.

CONCLUSIONS: Using ^{2}H-labelling of dividing leukemic cells, we have shown for the first time directly in CLL patients that ibrutinib significantly inhibits leukemia cell proliferation, leads to an efflux of CLL cells from tissues into the blood, and promotes a remarkably high death rate of CLL cells in an apparently indirect manner, likely due to the interruption of survival signals from the BCR and other receptors that are engaged in lymphoid tissues.

Efficacy and Safety of Ibrutinib in Patients with Relapsed or Refractory Chronic Lymphocytic Leukemia or Small Lymphocytic Leukemia with 17p Deletion: Results from the Phase II RESONATE™-17 Trial

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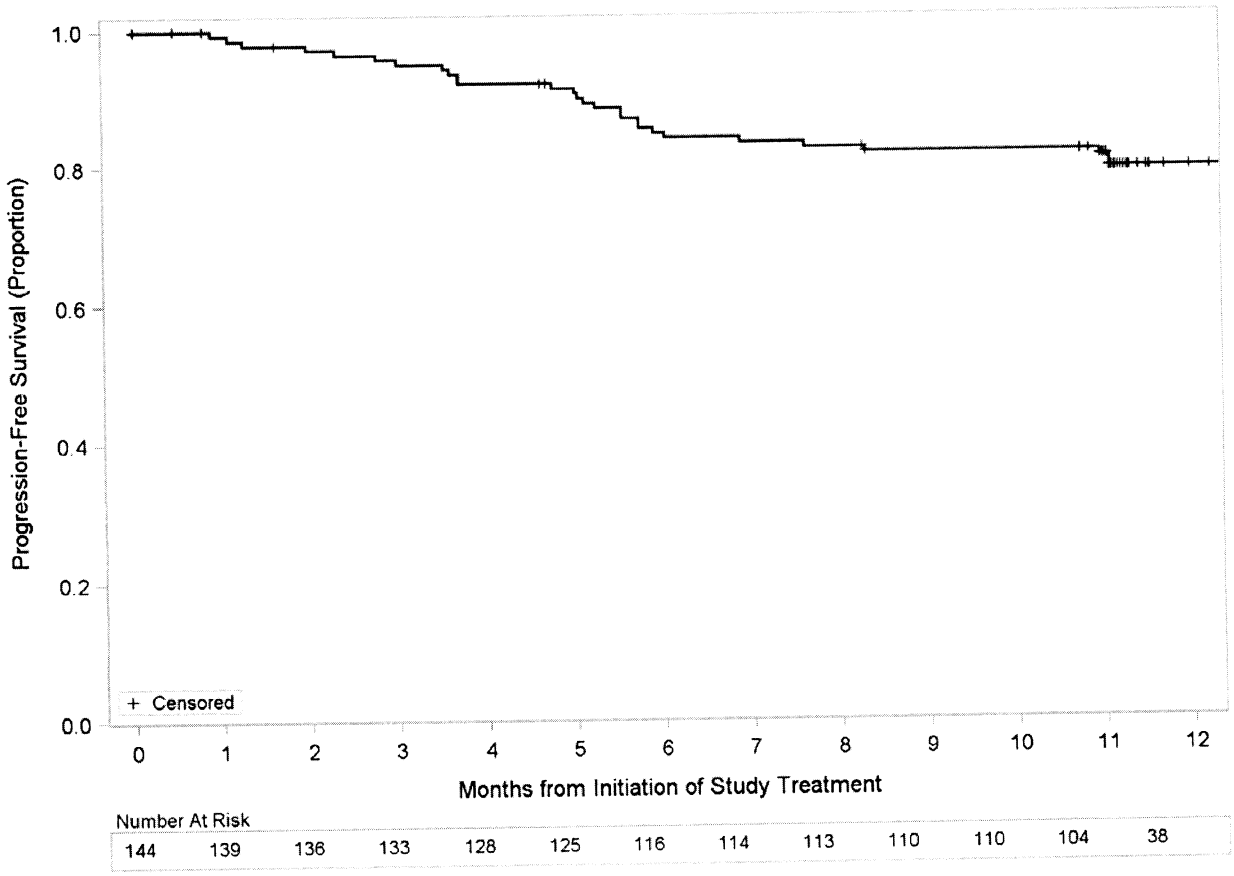
Background: Patients with chronic lymphocytic leukemia (CLL) with deletion of the short arm of chromosome 17 (del 17p) follow an aggressive clinical course and demonstrate a median survival of less than 2 years in the relapsed/refractory (R/R) setting. Ibrutinib (Imbruvica™), a first-in-class Bruton's tyrosine kinase (BTK) inhibitor, has been approved for previously treated patients with CLL and for patients with del 17p CLL. We report results from the primary analysis of the Phase II RESONATE™-17 (PCYC-1117-CA) study, designed to evaluate the efficacy and safety of single-agent ibrutinib for treatment of patients with R/R del 17p CLL or small lymphocytic leukemia (SLL).

Methods: Patients with del 17p CLL or SLL who failed at least one therapy were enrolled to receive 420 mg oral ibrutinib once daily until progression. All patients receiving at least one dose of ibrutinib were included in the analysis. The primary endpoint was overall response rate (ORR) per an independent review committee (IRC). Other endpoints included duration of response (DOR), progression-free survival (PFS), and safety of ibrutinib.

Results: Among 144 treated patients (137 with CLL, 7 with SLL), the median age was 64 (48% 65 years or older) and all had del 17p. Baseline characteristics included 63% of patients with Rai Stage III or IV disease, 49% with bulky lymphadenopathy of at least 5 cm, and 10% with lymphadenopathy of least 10 cm. The median baseline absolute lymphocyte count (ALC) was 32.9 x 10^9/L with 57% of patients with a baseline ALC at least 25.0 x 10^9/L. Baseline beta-2 microglobulin levels were at least 3.5 mg/L in 78% of patients (range 1.8-19.8 mg/L), and lactate dehydrogenase levels were at least 350 U/L in 24% of patients (range 127-1979 U/L). A median of 2 prior therapies (range 1-7) was reported. Investigator-assessed ORR was 82.6% including 17.4% partial response with lymphocytosis (PR-L). Complete response (CR)/complete response with incomplete bone marrow recovery (CRi) were reported in 3 patients. IRC-assessed ORR is pending. At a median follow up of 13.0 months (range 0.5-16.7 months), the median PFS (Figure 1) and DOR by investigator determination had not been reached. At 12 months, 79.3% were alive and progression-free, and 88.3% of responders were progression-free. Progressive disease was reported in 20 patients (13.9%). Richter transformation was reported in 11 of these patients (7.6%), 7 of the cases occurring within the first 24 weeks of treatment. Prolymphocytic leukemia was reported in 1 patient. The most frequently reported adverse events (AE) of any grade were diarrhea (36%; 2% Grade 3-4), fatigue (30%; 1% Grade 3-4), cough (24%; 1% Grade 3-4), and arthralgia (22%; 1% Grade 3-4). Atrial fibrillation of any grade was reported in 11 patients (7.6%; 3.5% Grade 3-4). Seven patients reported basal or squamous cell skin cancer and 1 patient had plasma cell myeloma. Most frequently reported Grade 3-4 AEs were neutropenia (14%), anemia (8%), pneumonia (8%), and hypertension (8%). Major hemorrhage was reported in 7 patients (4.9%, all Grade 2 or 3). Study treatment was discontinued in 16 patients (11.1%) due to AEs with 8 eventually having fatal events (pneumonia, sepsis, myocardial or renal infarction, health deterioration). At the time of data cut, the median treatment duration was 11.1 months, and 101 of 144 patients (70%) continued treatment with ibrutinib.

Conclusions: In the largest prospective trial dedicated to the study of del 17p CLL/SLL, ibrutinib demonstrated marked efficacy in terms of ORR, DOR, and PFS, with a favorable risk-benefit profile. At a median follow up of 13 months, the median DOR had not yet been reached; 79.3% of patients remained progression-free at 12 months, consistent with efficacy observed in earlier studies (Byrd, NEJM 2013;369:32-42). The PFS in this previously treated population compares favorably to that of treatment-naïve del 17p CLL patients receiving fludarabine, cyclophosphamide, and rituximab (FCR) (Hallek, Lancet 2010;376:1164-74) or alemtuzumab (Hillmen, J Clin Oncol 2007;10:5616-23) with median PFS of 11 months. The AEs are consistent with those previously reported for ibrutinib (Byrd, NEJM 2014;371:213-23). These results support ibrutinib as an effective therapy for patients with del 17p CLL/SLL.

Figure 1: Kaplan-Meier Curve for Progression-free Survival



Combination Of Ibrutinib and BCL-2 or SYK Inhibitors In Ibrutinib Resistant ABC-Subtype Of Diffuse Large B-Cell Lymphoma

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin's lymphoma (NHL), accounting for roughly 30% of newly diagnosed cases in the US. DLBCL can progress quickly, and in advanced cases is inconsistently cured with current therapies. Ibrutinib, a first-in-class Bruton's tyrosine kinase (BTK) inhibitor, is approved as a treatment for patients (pts) with mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL) who have had one prior treatment. The ABC subtype of DLBCL is considered especially high risk and characterized by chronic active B-cell receptor (BCR) signaling, which is blocked by ibrutinib. Recent phase II clinical trial results of ibrutinib as a single agent in DLBCL pts show an overall response rate of 41% in the ABC subtype (Wilson et al. ASH 2012). Responses of various cancers to single kinase targeted therapies are often limited by the cell's ability to bypass the target via alternative pathways or acquired mutations in the target or its pathway. It has been shown that a small number of CLL pts acquire resistance to ibrutinib through mutations in BTK and its substrate phospholipase C gamma 2 (PLCG2) in the B lymphoma cells following prolonged treatments (Woyach et al. NEJM 2014). Such mechanisms may be overcome by combinations of targeted agents. Through screening of wild-type and acquired ibrutinib-resistant ABC-DLBCL cell lines (e.g. expressing BTK C481S), we identify and report herein B-cell lymphoma-2 (BCL-2) and spleen tyrosine kinase (SYK) inhibitors that synergize with ibrutinib *in vitro* and *in vivo*.

Methods: Gene expression was analyzed by RT-qPCR using TaqMan Gene Expression Master Mix. Human DLBCL cell lines were treated with drugs for 3 days and

the effect on cell growth was determined by CellTiter-Glo luminescent cell viability assay. SCID mice were treated when the TMD8 tumors reached 100-150 mm^3. Annexin-V-positive and PI-negative population was detected as apoptotic cells in tumor cells at sacrifice. Cell adhesion and migration assays were performed as previously described (Chang et al. Blood 2013). Analysis of clinical samples used for BCL-2 gene expression profiling was performed using Affymetrix microarrays on FFPE specimens from the phase 2 PCYC-1106 trial (NCT01325701) and a rank based statistic (RankProd) was used to determine the significance of gene expression changes.

Results: DLBCL cell lines with higher BCL-2 expression were more sensitive to single agent ABT-199 than those with lower expression. Treatment of DLBCL cells with ibrutinib alone increased BCL-2 expression as well as their sensitivity to BCL-2 inhibitors. Combination treatment with BCL-2 inhibitors and ibrutinib completely inhibited tumor growth in murine models of ABC-DLBCL (Figure). Increased apoptotic cell populations were detected in the combination treated tumors compared to either treatment alone. Clinically, pretreatment tissue samples (n=28) from ABC-DLBCL pts who experienced objective responses to ibrutinib (CR+PR) had lower BCL-2 gene expression. A high BCL-2 mutation rate was observed in pts with poor response to ibrutinib (SD+PD). However, none of these mutations occurred in the BH3 or BH1 domains, both of which appear to interact with ABT-199 based on a 3-dimensional co-crystal structure of the inhibitor with BCL-2 (PDB code 4MAN) and further molecular simulation results. These findings suggest the potential benefit from combination therapy.

SYK is another downstream mediator of BCR signaling. Pretreatment of DLBCL cells with SYK inhibitors (e.g. R406) increased their sensitivity to ibrutinib. Ibrutinib resistant B-lymphoma cells with either C481S BTK or R665W PLCG2 mutations were re-sensitized to ibrutinib in combination with BCL-2 or SYK inhibitors, inhibiting cell growth, IgM-induced calcium flux, cell adhesion or migration in mutant containing cells.

Conclusions: Consistent with previous results from high-throughput combinatorial screenings of drugs interact favorably with ibrutinib (Mathews Griner, et al. PNAS 2013), we found BCL-2 and SYK may function in alternative survival pathways in DLBCL cells

upon BTK inhibition. Human B lymphomas harboring ibrutinib-resistant C481S BTK or R665W PLCG2 may be re-sensitized by BCL-2 or SYK inhibitors, both of which provide a rationale for the design of combination therapies.

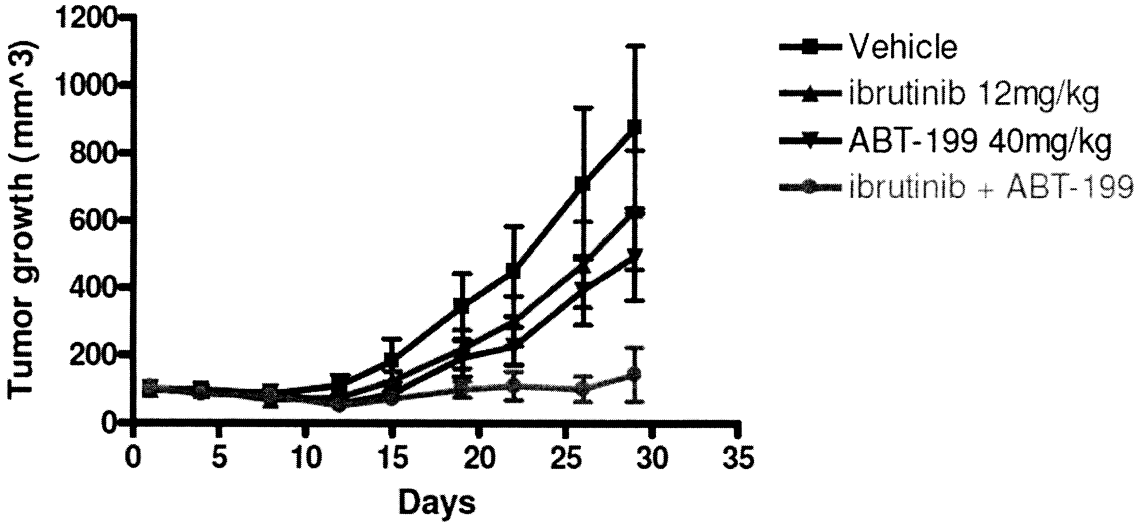

Figure Combination of ibrutinib and ABT-199 on the effect of TMD-8 tumor growth.

Ibrutinib and Rituximab Are an Efficacious and Safe Combination in Relapsed Mantle Cell Lymphoma: Preliminary Results from a Phase II Clinical Trial

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Single-agent ibrutinib has been approved by the FDA for patients with mantle cell lymphoma (MCL) who received at least one prior therapy based on a phase II clinical trial in which ibrutinib elicited a response rate of 68% (Wang et al, NEJM, 2013). In this clinical study we found a transient increase in circulating MCL lymphocytes during the initial phase of tumor reduction. We hypothesized that targeting the circulating MCL cells with intravenous rituximab will further improve the efficacy of ibrutinib. We conducted a single-center phase II clinical trial with ibrutinib in combination with rituximab for relapsed MCL with no upper limit for prior lines of therapy. Among 50 patients with MCL, 100% received prior rituximab, 77% received prior Hyper-CVAD, 75% received prior bortezomib, and 20% received prior lenalidomide. Rituximab was dosed at 375 mg/m2 iv weekly X 4 during cycle 1 (cycle = 28 days), then on day 1 of every cycle from 3-8, and thereafter once every other cycle up to 2 years. Ibrutinib was dosed at 560 mg orally daily continuously. With a median follow up time of 6.5 months (range 1-10), 45 patients are evaluable for toxicity and efficacy as of July 21, 2014. Thirty three patients (73% of evaluable patients) have Ki-67 < 50%. Seventeen (17) patients are now off study including 2 patients with secondary malignancies (AML and lung cancer). One (1) patient in CR withdrew consent due to social issues and continued on commercial ibrutinib. Two (2) patients in remission withdrew consent due to their concerns that rituximab-ibrutinib might worsen their atrial fibrillation and both continued on single-agent commercial ibrutinib. One patient was off study due to bleeding. Three (3) patients in remission went off to stem cell transplantation. Eight (8) patients are off study due to progressive MCL (4 never responded: 4 responded then progressed), all of them had Ki-67 greater than 50% (range 50-100%). There were no toxic deaths due to therapy. Grade 3 hematologic toxicity events included neutropenia (1) and thrombocytopenia (1). The most common (≥ 20%) grade 1-2 non-hematologic toxicity events regardless of its relationship with study therapy included fatigue (18), diarrhea (11), myalgia (11), dyspnea (11), blurred vision (10), nausea (9),dry eye (9) and atrial filbrillation (6). The efficacy data is listed in Table 1. The ORR to date is 87% with CR in 17 patients (38%) and PR in 22 patients (49%). The CR rate is high in this study in the context of historical data (21% by single-agent ibrutinib). Median duration of response and PFS has not been reached. Notably, all 10 patients with SD (2) and PD (8) have Ki-67's ≥ 50%. Excluding the 12 out of 45 evaluable patients with Ki-67 ≥ 50%, **the ORR for 33 patients with lower Ki-67 (< 50%) is 100% (48% for CR and 52% for PR)** in patients with relapsed/refractory MCL. While this trial is ongoing, preliminary data indicated that Ibrutinib-rituximab combination is well-tolerated and is efficacious, especially in patients with Ki-67 less than 50%.

Table 1. The best response related to Ki-67

	All n (%)	Ki-67 < 50%	Ki-67 ≥ 50%
Evaluable patients	45	33	12

ORR	39 (87%)	33 (100%)	6 (50%)
CR	17 (38%)	16 (48%)	1 (8%)
PR	22 (49%)	17 (52%)	5 (42%)
SD	2 (4%)	0	2 (17%)
PD	4 (9%)	0	4 (33%)
Duration of response	NR	NR	NR
PFS	NR	NR	NR

Ibrutinib Monotherapy in Relapsed/Refractory Follicular Lymphoma (FL): Preliminary Results of a Phase 2 Consortium (P2C) Trial

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Background: Bruton's tyrosine kinase (BTK) is an essential component of the B-cell receptor signaling pathway, a pathway critical to the survival and proliferation of malignant B cells. Ibrutinib, a small molecule inhibitor of BTK, has shown significant activity in several B-cell malignancies and is currently FDA approved for the treatment of patients (pts) with CLL or mantle cell lymphoma who have received at least one prior treatment. In a Phase 1 dose-escalation study, ibrutinib induced a response in 6 (38%) of 16 patients (pts) with relapsed/refractory FL and 6/11 (54%) of pts treated at doses ≥ 2.5 mg/kg (Advani JCO 2013, Fowler ASH abstr 156:2012). 560 mg daily was identified as a well-tolerated dose in lymphoma resulting in full-target occupancy. On the basis of these results, the Mayo Clinic and Princess Margaret P2Cs conducted a CTEP-sponsored Phase 2 trial of single-agent ibrutinib in relapsed/refractory FL (NCI 9271).

Methods: Eligible pts had relapsed or refractory FL (Gr 1, 2, or 3a) that had progressed during or after 1 or more prior chemotherapy regimens. Therapy consisted of ibrutinib, 560 mg daily, until progression or unacceptable toxicity. Response was assessed by CT at 8 weeks and then every 12 weeks. All pts were required to have an on-study biopsy and a second biopsy at progression after response with fresh tissue collected for correlative studies. The primary endpoint was overall response rate (ORR) and secondary endpoints were safety and tolerability, overall survival, time to response, time to treatment failure, duration of response, and progression-free survival (PFS). Planned exploratory correlative studies included C1D8 and C3D1 FDG-PET response evaluation in a subset of 20 patients, and whole-transcriptome sequencing for correlation of mutations with response and resistance.

Results: 40 patients (38 evaluable for response) were accrued from April 2013 to April 2014. Median age was 64 years (range 46-82), 70% were men, 55% had FLIPI ≥ 3, median number of prior regimens was 3 (range 1-11), 20% had a prior stem cell transplant, 45% were rituximab refractory, and 36% were refractory to their most recent treatment. At a median follow-up of 6.5 months (mo) (range 1.8-14.6+), the ORR for the 40 intention-to-treat pts is 30% (95% CI: 17-47%) with 1 CR and 11 PRs by CT criteria (4/12 responders had a negative restaging PET/CT). 26 (65%) patients have exhibited tumor size reduction. Only 2/18 (11%) pts with rituximab-refractory disease responded,

compared to 8/19 (42%) pts with rituximab-sensitive disease (P=0.06) and 2/3 who were rituximab-naïve. There was no correlation between response and number of prior regimens. Median time to response was 2.4 mo (range 1.8-12.9 mo). Median PFS is 9.9 mo (95% CI: 6 mo, not reached). One of 12 responders has progressed after 9.9 mo on treatment. Median treatment duration was 5 mo (range 1-15+ mo). Dose delays (2 pts) and reductions were infrequent (4 pts). 17 pts have discontinued treatment due to PD (12), refusal (2), AEs (2), and death (1). Gr 3-4 AEs occurred in 30% of pts, including the following AEs in more than 1 pt: anemia (2), neutropenia (3), and infection (2). 3 pts have died (1 PD, 1 pneumonia, 1 gastric hemorrhage). Of the 20 pts with C1D8 PET scans, 19 also had C3D1 PET scans and were evaluable for response. Six of these 19 pts had objective responses by CT criteria at week 8 or beyond. Of the 6 responders, C1D8 PET/CT showed qualitative (visual) improvement in 3 pts, no visual change in 1 pt, and qualitative worsening in 2 pts. On the C3D1 PET/CT, 4 of 6 had qualitative improvement and 2 had no change. Evaluation of quantitative PET data including SUVs and total lesion glycolysis is ongoing and will be presented.

Conclusions: Single agent ibrutinib is well tolerated with a modest ORR in relapsed/refractory FL at this early assessment. Continued follow-up is warranted to capture late responders and to establish response duration. Ibrutinib appears less active in FL than in MCL and CLL. Early PET scans (C1D8) do not reliably predict for response.

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