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# Phase Ib Trial of an Ibrutinib-Based Combination Therapy in Recurrent/Refractory CNS Lymphoma

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## KEY POINTS

- Ibrutinib/methotrexate/rituximab combination treatment is safe and shows promising clinical activity in CNS lymphoma
- Circulating tumor DNA analysis of cerebrospinal fluid may be useful to monitor disease burden in patients with CNS lymphoma

## ABSTRACT

Ibrutinib is a first-in-class inhibitor of Bruton tyrosine kinase (BTK) and has shown single-agent activity in recurrent/refractory (r/r) central nervous system lymphoma (CNSL). Clinical responses are often transient or incomplete, suggesting a need for a combination therapy approach. We conducted a Phase Ib clinical trial to explore the sequential combination of ibrutinib at 560 or 840 mg daily dosing with methotrexate (HD-MTX) and rituximab in patients with CNSL. HD-MTX was given at 3.5g/m2 every 2 weeks for a total of 8 doses (4 cycles; cycle = 28 days). Ibrutinib was held on days of HD-MTX infusion and resumed 5 days after HD-MTX infusion or after HD-MTX-clearance. Single-agent daily ibrutinib was administered continuously after completion of induction therapy until disease progression, intolerable toxicity, or death. We also explored next-generation sequencing of circulating tumor DNA (ctDNA) in cerebrospinal fluid (CSF) before and during treatment. The combination of ibrutinib, HD-MTX and rituximab was tolerated with acceptable safety profile (no grade 5 events, 3 grade 4 events). No dose-limiting toxicity was observed. 11/15 patients proceeded to maintenance ibrutinib after completing four cycles of the ibrutinib/HD-MTX/rituximab combination. Clinical responses occurred in 12/15 patients (80%). Sustained tumor responses were associated with clearance of ctDNA from the CSF. The study is registered to https://clinicaltrials.gov as NCT02315326.

## Introduction

Primary Central Nervous System Lymphoma (PCNSL) is a rare and aggressive subtype of diffuse large B-cell lymphoma (DLBCL) that manifests exclusively in the CNS. The incidence of this disease has been increasing over the last decade[1]. Standard induction treatment of PCNSL in most reported single-arm or randomized trials includes high-dose methotrexate (HD-MTX)-based therapy, an alkylating agent, with or without cytarabine and the anti-CD20 antibody rituximab. Treatment is associated with considerable morbidity and disease recurrences with a five-year survival of approximately 40%[2].

Compared to DLBCL outside the CNS, the B-cell receptor (BCR) signaling pathway is more frequently mutated in PCNSL. The most common alterations include gain-of-function mutations in *MYD88* and *CD79B* [3-5]. Bruton Tyrosine Kinase (BTK) mediates signals downstream of MYD88 and CD79B and therefore represents an attractive drug target in PCNSL. The first-in-class BTK inhibitor ibrutinib has shown antitumor activity in pre-clinical PCNSL models[3] and in patients with recurrent or refractory (r/r) PCNSL[3,6], pointing toward an important role of BTK for maintenance of the malignant phenotype in PCNSL.

Tumor responses to single-agent ibrutinib in central nervous system lymphoma (CNSL) are often incomplete or transient. We therefore investigated the safety and efficacy of ibrutinib in combination with HD-MTX and rituximab. Both methotrexate and rituximab form the backbone of many combination chemotherapy regimens in first-line therapy for CNSL. In a prior study, ibrutinib was combined with a non-HD-MTX containing polychemotherapy regimen and considerable treatment-associated toxicity was observed, including aspergillosis involving lung and brain[6]. To minimize the risk of adverse events, we held ibrutinib on days of HD-MTX infusion and resumed 5 days after HD-MTX infusion or after MTX-clearance. Daily ibrutinib was

administered continuously after completion of induction therapy until disease progression, intolerable toxicity, or death.

## Methods

### Study Design and Treatment

This was an open-label, non-randomized, single center, dose escalation study of rituximab, HD-MTX, and ibrutinib in r/r PCNSL/SCNSL (NCT02315326). The primary objective was to determine the maximum tolerated dose (MTD) of ibrutinib in combination with high-dose HD-MTX alone and ibrutinib in combination with HD-MTX and rituximab. Adverse events were graded using the NCI Common Terminology Criteria for Adverse Events (4.0). Dose limiting toxicities (DLTs) were defined as any grade 4 hematologic toxicity, grade 3 febrile neutropenia and grade 3 thrombocytopenia associated with bleeding or any grade 3 non-hematologic toxicity that did not respond to supportive therapy, occurring during the first 28 days of therapy and at least possibly related to ibrutinib. The secondary objectives were overall response rate (ORR) defined as proportion of subjects with complete or partial response, progression-free survival (PFS), overall survival and pharmacokinetics in the blood and CSF. The study was approved by the Institutional Review Board of Memorial Sloan Kettering Cancer Center (MSKCC).

The treatment regimen consisted of a combined induction therapy followed by ibrutinib maintenance (Supplemental Figure S1). Following the National Comprehensive Cancer Network (NCCN) guidelines for recurrent/refractory PCNSL (https://www.nccn.org/professionals/physician_gls/default.aspx), HD-MTX was given at 3.5g/m$^2$ every 2 weeks for a total of 8 doses (4 cycles; cycle = 28 days). Ibrutinib dose escalation among cohorts followed the "3+3" design and was allowed if, after 28 days of therapy, none of three or not more than one of six patients had a DLT during the first cycle. The ibrutinib starting dose was 560mg/day and was escalated to 840mg/day in the next cohort. After no DLTs were

observed in patients treated with the ibrutinib/MTX combination, Rituximab was added at 500mg/m$^2$ every 2 weeks during the induction phase for a total of 8 doses. To minimize potential adverse events, ibrutinib was given sequentially and held on days of HD-MTX infusion and resumed 5 days after HD-MTX infusion or after MTX-clearance. Daily ibrutinib was administered continuously after completion of induction therapy until disease progression, intolerable toxicity, or death.

Plasma samples were collected at 1, 2, 3, 4, and 6 hours and CSF samples were collected through lumbar puncture two hours after ibrutinib dosing on day 28 of cycle 2 (before initiation of cycle 3) for pharmacokinetic studies. Additional CSF was collected at day 28 of cycle 4 (before initiation of cycle 5) to assess treatment response in the CSF in patients with leptomeningeal involvement.

Baseline staging assessments to assess disease burden followed the Primary CNS Lymphoma Collaborative Group guidelines[7] and included MRI brain, MRI total spine, CSF collection, ophthalmologic examination and PET body. A bone marrow biopsy was performed if the PET body demonstrated an abnormal bone marrow signal.

**Eligibility**

The trial population comprised patients with r/r PCNSL/SCNSL. Moreover, patients with systemic DLBCL who had completed systemic therapy without further signs of systemic disease and then developed CNS involvement for the first time, were eligible to receiving the study therapy as their first CNS-directed therapy. All subjects had histopathologic confirmation of DLBCL at initial diagnosis. Patients met the following criteria: age ≥18; disease on imaging or in CSF; Eastern Cooperative Oncology Group (ECOG) performance status score of 0-2; adequate bone marrow and organ function; recovery to grade 1 toxicity from prior therapy. Patients with active non-CNS disease, prior ibrutinib therapy, or requiring >8mg of dexamethasone daily for neurologic disability were excluded.

### Treatment Response Assessments

Evaluation of treatment response followed the International Primary CNS Lymphoma Collaborative Group guidelines[7]. Response to treatment was assessed in all CNS compartments using MRI imaging and CSF cytology as well as ophthalmologic examination in case of eye involvement.

### Statistical Analysis

Descriptive statistics, including means, standard deviations, and medians for continuous variables and proportions for discrete variables, were used to summarize the findings in each of the combination cohorts. The Kaplan–Meier method was used for time-to-event analysis. PFS was calculated from trial registration until disease progression, last clinical assessment, or death, whichever came first. Progressions and deaths were considered events in the PFS analysis. Overall survival (OS) was calculated from trial registration until death. Deaths were considered events in the OS analysis.

### Genomic Analysis

Archival tumor biopsy samples were obtained from patients who participated in the clinical trial. DLBCL subtype (ABC or GCB) was determined using immunohistochemical staining for CD10, BCL-6, and MUM-1 following the Hans Classification[8]. Up to 4 ml of CSF was collected for genomic analysis if sufficient material was available at each CSF collection (baseline staging and after completion of cycles 2 and 4) and sequenced using the MSK-HemePACT targeted panel including 585 cancer genes specifically targeting genes associated with hematologic malignancies. All samples were studied in accordance with a protocol approved by the Memorial Sloan Kettering Cancer Center Institutional Review Board. Genomic analysis followed methods and algorithm used in prior studies[3,9,10].

## Results

### Patient population

Fifteen eligible patients (9 PCNSL and 6 SCNSL) were enrolled. Median age was 62 years (range 23-74) and median ECOG 1 (range 0-2); seven were women. Thirteen patients had parenchymal brain lesions; five isolated brain lesions, 7 brain and CSF, 1 brain and eye involvement, and two had isolated leptomeningeal disease confirmed on CSF cytology. Nine patients had recurrent disease (8 PCNSL; 1 SCNSL), three HD-MTX-based chemotherapy refractory disease (1 PCNSL, 2 SCNSL) and three newly diagnosed SCNSL. (Table 1 and Supplemental Table S1). For patients with recurrent or refractory disease (n=12), the median time from the last CNS directed treatment was 8.55 months (range: 0.5-43.8). All patients with recurrent/refractory disease had received HD-MTX chemotherapy in combination with rituximab. In 9/12 (75%) rituximab/HD-MTX was combined with an alkylating agent (procarbazine in 7 and temozolomide in 2). One patient received prior cranial radiotherapy and one hematopoietic cell transplantation. Three/12 (25%) also received HD-MTX as salvage. Five patients (33%) required corticosteroid treatment to control neurologic symptoms at enrollment (Supplemental Table S2). Each patient received methotrexate (dosed at 3.5g/m$^2$). Using the '3+3' design, ibrutinib was first combined with methotrexate. Next, rituximab (dosed at 500mg/m$^2$) was combined with methotrexate (dosed 3.5g/m$^2$) and ibrutinib. Ibrutinib was dose-escalated from 560mg daily to 840mg daily. In summary, HD-MTX and 560mg ibrutinib was given to 3 patients, HD-MTX and 840mg ibrutinib to 3 patients, HD-MTX, rituximab and 560mg ibrutinib to 3 patients and HD-MTX, rituximab and 840mg ibrutinib to 6 patients. Six patients received HD-MTX with ibrutinib (560mg: n=3; 840mg: n=3); 9 patients received HD-MTX, rituximab, and ibrutinib (560mg: n=3; 840mg: n=6).

### Safety and Adverse Events

No dose limiting (DLT) toxicities were observed during the DLT period. No treatment discontinuation occurred due to adverse events with ibrutinib treatment. Rituximab and HD-MTX were not dose reduced in any of the patients. Ibrutinib was given on a median of 18 days (range 15-20) per cycle. Ibrutinib dosing was delayed by HD-MTX clearing and minor surgical procedures (tooth extraction, bone marrow biopsy, MediPort placement). There were three non-DLT grade 4 adverse events (lung infection, lymphopenia, neutropenia) (Table 2; Supplementary Table S3). Of those events, two occurred during the single-ibrutinib treatment phase (Supplemental Table S3). All grade 4 adverse events were seen in patients treated with rituximab, HD-MTX, and ibrutinib (two receiving 840mg and one receiving 560mg) (Supplemental Table S3). We observed twenty-nine grade 3 events (most frequent: 8 lymphopenia, 6 ALT/AST elevation, 3 anemia, 2 lung infections). Of those grade 3 adverse events, 8 were seen in patients receiving HD-MTX and ibrutinib at 560mg, 3 were observed in patients treated with HD-MTX and ibrutinib at 840mg, 5 in those receiving HD-MTX, rituximab and ibrutinib at 460mg, and 13 in the cohort receiving HD-MTX, rituximab and ibrutinib at 840mg. The most common adverse events were anemia, thrombocytopenia, ALT/AST elevation and lymphopenia. No fungal infections were observed. Single-agent ibrutinib treatment was dose-reduced in three patients for diarrhea, recurrent bacterial infection (skin, lung), and drug interaction (CYP3A inhibitor amlodipine was started to control atrial fibrillation).

**Treatment Duration and Response**

12/15 patients completed the induction phase of ibrutinib-based combination therapy (47 delivered out of 48 cycles planned). Three patients did not complete the assigned combined induction regimen due to progression after cycle 1 (patients #3, #10) or withdrawal after cycle 2 due to personal choice (patient #2). One patient (#11) completed the induction phase of ibrutinib-based combination therapy but did not continue to single agent ibrutinib maintenance

because of progression found after completion of cycle 4. 11/15 patients started the maintenance stage of our regimen with single-agent ibrutinib (Supplemental Figure S2).

At a median follow-up of 19.7 months (range:12.7-27.1) for the entire cohort, all 15 patients were evaluated for response. Best responses included 8 CRs, 4 PRs, 1 SD, and 2 PDs with an overall response rate of 12/15 (80%; 95% CI: 52%, 96%) (Figure 1A). CRs were seen in patients receiving HD-MTX/ibrutinib as well as in those receiving HD-MTX/rituximab/ibrutinib. None of the patients who achieved a CR received corticosteroids (Supplemental Table S2). The response rate in r/r PCNSL was (8/9) 89% (95% CI: 52%-100%) and (4/6) 67% (95% CI: 22%-96%) in SCNSL.

The median PFS for all 15 patients was 9.2 months (95% CI 3.39-no upper limit). The median PFS for the subset of PCNSL patients has not been reached yet. The median OS was not reached (11/15 subjects alive) (Figure 1B, Supplemental Figure S3). The 1-year OS is 71.1% (95% CI: 46.7-95.5). Responses were observed in PCNSL and SCNSL and in both subtypes (ABC, GCB). No CR or PR was seen in the three patients with refractory CNS disease, all of which were of the GCB subtype.

Five patients have remained disease-free on ibrutinib maintenance but received high-dose chemotherapy with stem cell rescue off study (#1, #7, #9, #13, #15). None of these patients encountered difficulties mobilizing stem cells while on ibrutinib monotherapy and none have developed recurrent disease. Four patients continued ibrutinib (#5, #8, #12, #14) and two (#4, #6) developed disease progression while receiving single-agent ibrutinib.

The median duration of response was 12.8 months in all patients (range 0.53-25.63) and 14.3 months (range 3.5-23.03) for those 6 patients that continued ibrutinib maintenance (#4, #5, #6, #8, #12, #14).

**Ibrutinib concentration in the CSF**

We measured CSF ibrutinib concentrations two hours post-dose on day 28 of cycle 2 in 11/15 patients (Supplemental Figure S4). Mean CSF ibrutinib concentration was 3.105 ng/mL (equivalent to 7.05 nM; range: 0.305-9.22ng/ml). In patients receiving 560mg ibrutinib (n=4), the mean CSF concentration was 1.553 ng/mL (range: 0.991-2.62 ng/ml). The mean CSF levels in patients receiving 840mg ibrutinib (n=7) was 3.992 ng/mL (range: 0.305-9.22ng/mL). These ibrutinib concentrations are similar to the reported ibrutinib CSF concentrations observed in patients receiving single agent ibrutinib[3,6]. CSF was not collected in two patients due to disease progression and two patients declined CSF collection.

**Detection of circulating tumor DNA (ctDNA) in CSF in CNS Lymphoma**

Disease burden in CNSL is typically assessed by magnetic resonance imaging (MRI), CSF cytology, and CSF flow cytometry. We examined whether patients with r/r CNSL might harbor tumor-derived DNA in cerebrospinal fluid (CSF). For 8/15 patients, we had sufficient pretreatment CSF volume for this exploratory analysis (Fig. 2A). All samples were analyzed using MSK-HemPACT, a custom FDA-authorized next-generation sequencing based tumor sequencing assay[9,10]. We detected at least one tumor-derived genetic alteration in CSF from all eight patients (Supplemental Figure S5A). For six of these patients, we were able to compare the genetic profile in CSF to the genetic profile of a previous tumor biopsy, collected prior to CSF collection (median interval between tumor and CSF collection: 31 months) (Supplemental Figure S5B). Between 11% and 37% of identified single nucleotide variants (SNVs) were shared between the archival tumor tissue and CSF ctDNA at recurrence (Supplemental Figure S5C). It is currently unclear how frequently genomic alterations are shared between tumor and ctDNA in the CSF of patients with brain tumors due to a paucity of data. Nonetheless, it is noteworthy that the frequency of shared mutations was considerably higher (60%) for mutations in BCR pathway members (*MYD88_L265P*, *CD79B_Y196*, *CARD11*, *MALT1*, *PLCG2*, *TNFAIP3*)

(Supplemental Table S3; Supplemental Figure S5D), pointing toward a fitness advantage conferred by maintenance of these mutations.

**Clinical response and pre-treatment tumor genotype**

The molecular basis of *de-novo* and acquired resistance to ibrutinib in CNSL remains poorly understood. Clinical responses to single agent ibrutinib are more frequent in PCNSL (overall response rate ORR: 77%)[3] and Secondary CNS Lymphoma (SCNSL; ORR: 71%)[3] than in DLBCL outside the CNS (ORR: 25%)[11] and complete responses have been observed even in tumors without activating mutations in *MYD88* or *CD79B*. In DLBCL outside the CNS, clinical responses to ibrutinib are more common in tumors of the activated B-cell (ABC) DLBCL subtype than in patients with the germinal center B-cell (GCB) DLBCL subtype[11]. Activating mutations in *PLCγ2* and *CARD11*, downstream members of the BCR pathway, have been associated with resistance to single-agent ibrutinib in several human B cell malignancies[11-14 12,15].

We therefore examined the relationship between clinical response to the ibrutinib-based combination therapy and pre-treatment tumor genotype, ascertained in either tumor or CSF (whichever was closer to treatment begin). Twelve/15 (80%) tumor samples had mutations in at least one BCR pathway member, including *MYD88* (8/15; 53%), *CD79B* (7/15; 47%), *CARD11* (6/15; 40%), *TNFAIP3* (1/15; 7%), *MALT1* (1/15; 7%), and *PLCG2* (1/15; 7%) (Table 3). Consistent with our prior data, we observed responses to ibrutinib-based combination therapies even in tumors without mutations in the examined BCR pathway members. Interestingly, we also observed responses in patients whose tumors harbored mutations that might be expected to restore BCR pathway activity in the presence of ibrutinib, for example *CARD11* mutations at F97Y[16] and the coiled-coil domain mutations[17] at M166T, K215M, and R418K as well as the *TNFAIP3* mutation at C483W[18].

### Monitoring of CSF ctDNA during therapy

We evaluated the effects of ibrutinib-based combination therapy on the presence of ctDNA in the CSF. We collected sequential CSF samples at study begin ("baseline"), before treatment Cycle 3 [C3], and before Cycle 5 [C5]). For 9/15 patients in our study, we were able to obtain multiple CSF samples (Fig. 2A). The remaining six patients declined repeated CSF collection, suffered disease progression with clinical deterioration preventing serial sample collection, or had insufficient CSF volume to complete sequencing (Figure 2A). 7/9 patients with repeated CSF collections had a complete or near-complete (PR >90%) radiographic response of their measurable disease to the ibrutinib-based combination treatment (Fig. 2B) and this response was accompanied by the disappearance of CSF ctDNA (Figure 2C; Supplemental Figure S6). One patient with repeated CSF collections experienced rapid disease progression after an initial tumor response (#11) and showed persistence (Fig 2D) of tumor specific alterations in the CSF. One patient (#6) with non-measurable leptomeningeal involvement had a complete response on imaging and CSF assessments (Fig. 2E). The genomic alterations cleared with therapy (C3) but re-occurred (C5) even before conventional CSF studies (cytology, flow cytometry) suggested disease recurrence. A summary of our integrated treatment response analysis (including MR imaging, CSF cytology, and CSF ctDNA evaluation) is shown in Figure 3.

### Discussion

Our study demonstrates that the sequential combination of ibrutinib with HD-MTX-based chemotherapy had acceptable toxicity in the setting of our single center phase I trial. 11/15 patients proceeded to maintenance ibrutinib after completing four cycles of the ibrutinib/HD-MTX/rituximab combination and we observed no dose-limiting toxicities, treatment related deaths, or aspergillosis. For future studies, we propose to use an ibrutinib dose of 840mg because CSF drug concentrations achieved at this dose level are consistently above the IC50 needed to induce cell death *in-vitro*[3,6]. The tolerability of the current regimen (4 grade 4 events,

29 grade 3 events) contrasts with the considerable toxicity reported for the combination of ibrutinib with dose-adjusted temozolomide, etoposide, liposomal doxorubicin, dexamethasone, and rituximab (TEDDi-R)[6] (27 grade 4 events, 51 grade 3 events). We cannot exclude the possibility that our patients were healthier or less heavily pretreated, contributing to the better tolerability of the ibrutinib/HD-MTX/rituximab combination. However, this seems less likely as many patients in our trial (9/12 patients with r/r CNS lymphoma) had received intensive prior therapy (HD-MTX, rituximab, and alkylating agent) and had aggressive disease with only a short relapse-free interval since receiving front-line therapy.

The ibrutinib/HD-MTX/rituximab combination regimen showed promising antitumor activity, but there are several caveats in interpreting these results, including the overall small study size, the phase Ib design, exclusion of patients receiving >8mg dexamethasone daily and the heterogeneous patient population with inclusion of both PCNSL and SCNSL. Given the non-randomized design, we are also unable to determine to what extent the addition of ibrutinib increased the activity of high-dose MTX. At first glance, the response rates with salvage high-dose methotrexate plus ibrutinib and rituximab in our cohort may seem similar to those described for salvage with high-dose methotrexate in relapsed PCNSL. However, response rates to MTX-based chemotherapy have been obtained retrospectively[19,20] and the longer median time to first relapse in these retrospective studies (>2 years) suggests an enrichment for patients with MTX-responsive disease[21] compared to the patients in our current study. In comparison to our prior study with single-agent ibrutinib[3], the radiographic response of r/r PCNSL was higher with the ibrutinib/HD-MTX/rituximab combination regimen (89% versus 77%) and PFS was longer with the combination therapy. However, this finding will require longer follow up as 5/15 patients on our current study proceeded to high-dose chemotherapy with autologous stem cell rescue (ASCT) after responding to the ibrutinib/HD-MTX/ibrutinib combination therapy. Lastly, we observed complete responses even in patients with tumors that

would be predicted to respond less favorably to single-agent ibrutinib due to mutations in the distal BCR pathway members *CARD11* or *TNFAIP3*. Future evaluation of the ibrutinib-based combination therapy regimen seems therefore warranted. Recently, the role of rituximab in PCNSL has become questionable. In the HOVON 105/ALLG NHL 24 phase III study[22], including 200 newly diagnosed PCNSL patients, the addition of rituximab to a methotrexate-based polychemotherapy regimen (HD-MTX, BCNU, teniposide, prednisone) did not demonstrate a significant benefit on clinical outcome parameter. Of note, 5/9 (56%) patients in our study receiving rituximab had a CR in contrast to only 2/6 (33%) in those not receiving rituximab.

Lastly, our exploratory biomarker analysis suggests that CSF liquid biopsies, obtained through office-based lumbar puncture and examined with an FDA-authorized next-generation sequencing assay, may be useful to monitor disease burden and evaluate treatment response in CNS lymphoma. While not all patients in our study participated in this exploratory biomarker analysis, our preliminary data suggests that a considerable fraction of patients with r/r CNSL harbor tumor DNA in CSF, even if CSF involvement is undetectable by conventional techniques (MRI, CSF cytology, CSF flow cytometry). Longer follow-up and larger studies are needed to extend and validate these observations and their impact on our understanding of acquired drug resistance, currently a major roadblock in the treatment of brain tumors.

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## Authorship Contributions

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## Disclosures of Conflicts of Interest

CG reports consulting for BTH plc and Kite. EIP reports advisory roles with AstraZeneca. AL received research funding from Nantomics and Bristol-Myers Squibb. KSP reports stock ownership in Pfizer. KSP reports stock ownership in Johnson & Johnson, Pfizer, Viking Therapeutics, and Catalyst Biotech. LMD reports advisory roles for Sapience Therapeutics, Tocagen, BTG International, Roche, and Syndax. MFB reports advisory roles with Roche and research funding from Illumina. IKM reports research funding from General Electric, Amgen, and Lilly; advisory roles with Agios, Puma Biotechnology, and Debiopharm Group; and honoraria from Roche for a presentation.

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**Table 1. Baseline Characteristics of Patients**

| Characteristics | |
|---|---|
| **Age - years** | **No.** |
| Median | 62 |
| Range | 23-74 |
| **Sex** | **No. (%)** |
| Male | 8 (53) |
| Female | 7 (47) |
| **ECOG** | **No.** |
| Median | 1 |
| Range | 0-2 |
| **CNS Lymphoma** | **No. (%)** |
| Primary (PCNSL) | 9 (60) |
| Secondary (SCNSL) | 6 (40) |
| **Disease Status** | **No. (%)** |
| Recurrent PCNSL or SCNSL | 9 (60) |
| Refractory PCNSL or SCNSL | 3 (20) |
| Newly diagnosed SCNSL | 3 (20) |
| Newly diagnosed PCNSL | 0 (0) |
| **CNS Involvement** | **No. (%)** |
| Brain | 5 (33) |
| Cerebrospinal fluid (CSF) | 2 (13) |
| Brain and CSF | 7 (47) |
| Brain and eye | 1 (7) |
| **Prior Treatment in r/r [n=12]** | **No. (%)** |
| Chemotherapy | 12 (100) |
| - HD-MTX Chemotherapy | 12 (100) |
| - HD-MTX + alkylator | 9 (75) |
| - Rituximab | 12 (100) |
| Radiation | 1 (8) |
| Stem cell transplant | 1 (8) |
| HD-MTX at recurrence | 3 (25) |
| **Number of Prior Regimens** | **No. (%)** |
| Median | 1 |
| Range | 0-2 |
| **Corticosteroids at enrollment** | **5 (33)** |

ECOG: Eastern Cooperative Oncology Group Performance Status; CNS: Central Nervous System; PCNSL: Primary Central Nervous System Lymphoma; SCNSL: Secondary Central Nervous System Lymphoma; HD-MTX: methotrexate; CSF: cerebrospinal fluid.

**Table 2. Adverse Events, most common events (>10% of patients) and all Grade 3/4 toxicities**

| Adverse Event | Grade 1-2 | Grade 3 | Grade 4 | Total |
|---|---|---|---|---|
| | Number of patients (percent) | | | |
| Anemia | 12 (80%) | 3 (20%) | - | 15 (100%) |
| AST increased | 10 (67%) | 5 (33%) | - | 13 (87%) |
| Platelet count decreased | 11 (73%) | 1 (7%) | - | 12 (80%) |
| ALT increased | 11 (73%) | 1 (7%) | - | 12 (80%) |
| Lymphocyte count decreased | - | 8 (53%) | 1 (7%) | 9 (60%) |
| White blood cell decreased | 8 (53%) | 1 (7%) | - | 9 (60%) |
| Hyperglycemia | 7 (47%) | 1 (7%) | - | 8 (53%) |
| Neutrophil count decreased | 4 (27%) | 1 (7%) | 1 (7%) | 6 (40%) |
| Alk. phos. increased | 7 (47%) | - | - | 7 (47%) |
| Blood bilirubin increased | 7 (47%) | - | - | 7 (47%) |
| Cholesterol high | 7 (47%) | - | - | 7 (47%) |
| Hypokalemia | 6 (40%) | 1 (7%) | - | 7 (47%) |
| Hypocalcemia | 4 (27%) | 1 (7%) | - | 5 (33%) |
| Fatigue | 5 (33%) | - | - | 5 (33%) |
| Creatinine increased | 4 (27%) | - | - | 4 (27%) |
| Nausea | 4 (27%) | - | - | 4 (27%) |
| Musculoskeletal & conn tissue disorder (cramps) | 4 (27%) | - | - | 4 (27%) |
| Lung Infection | - | 2 (13%) | 1 (7%) | 3 (20%) |
| Hyponatremia | 2 (13%) | 1 (7%) | - | 3 (20%) |
| Activated partial thromboplastin time prolonged | 3 (20%) | - | - | 3 (20%) |
| Hypertriglyceridemia | 3 (20%) | - | - | 3 (20%) |
| Hypoalbuminemia | 3 (20%) | - | - | 3 (20%) |
| Diarrhea | 1 (7%) | 1 (7%) | - | 2 (13%) |
| Acute kidney injury | 2 (13%) | - | - | 2 (13%) |
| Arthralgia | 2 (13%) | - | - | 2 (13%) |
| Headache | 2 (13%) | - | - | 2 (13%) |
| Infections and infestations - Other (infection of unknown origin) | - | 1 (7%) | - | 1 (7%) |
| Hyperkalemia | - | 1 (7%) | - | 1 (7%) |

**Table 3. Mutations in BCR pathway members in 'pre-treatment' archival tumor tissue or CSF**

| ID | Disease | COO/ Status | Best Response (Duration) | MYD88 | CD79B | CARD11 | MALT1 | TNFAIP3 | PLCG2 |
|---|---|---|---|---|---|---|---|---|---|
| #5 | PCNSL | ABC/recu | CR (24m)* | **L265P (C)** | **Y196H (C)** | WT (C) | WT (C) | WT (C) | WT (C) |
| #8 | PCNSL | ABC/recu | CR (19.4m)* | **L265P (C)** | **Y196H (C)** | WT (C) | WT (C) | WT (C) | WT (C) |
| #12 | PCNSL | ABC/recu | CR (16.7m)* | WT (C) | **Y196H (T)** | WT (C) | WT (C) | WT (C) | WT (C) |
| #4 | PCNSL | ABC/recu | CR (9.2m) | **L265P (T)** | **X185splice/D185N (T)** | **F97Y (T)** | WT (T) | WT (T) | WT (T) |
| #9 | PCNSL | ABC/recu | CR (4.3m)# | WT (C) | WT (C) | WT (C) | WT (C) | WT (C) | WT (C) |
| #7 | PCNSL | ABC/recu | CR (4m)# | WT (C) | WT (C) | **M166T (C)** | WT (C) | WT (C) | WT (C) |
| #13 | PCNSL | ABC/recu | PR (4.3m)# | **L265P (T)** | **Y196S (T)** | **R418K (T)** | WT (T) | WT (T) | WT (T) |
| #15 | PCNSL | GCB/recu | PR (3.8m)# | **L265P (C)** | **Y196S/D201G (C)** | WT (C) | WT (C) | WT (C) | WT (C) |
| #10 | PCNSL | GCB/refr | PD (0.9m) | WT (T) | WT (T) | **T128M/K252E (T)** | WT (T) | WT (T) | WT (T) |
| #14 | SCNSL | GCB/new | CR (13.8m)* | **L265P (C)** | **Y196C (C)** | WT (C) | WT (C) | WT (C) | WT (C) |
| #6 | SCNSL | ABC/new | PR (4.47m) | **L265P (T)** | WT (T) | WT (T) | **AMP (T)** | WT (T) | WT (T) |
| #1 | SCNSL | GCB/recu | PR (4m)# | **D288_F298del (T)** | **Y196F/M164I (T)** | **K215M (T)** | WT (T) | **C483W (T)** | WT (T) |
| #11 | SCNSL | GCB/new | PR (3.4m) | **A272P (C)** | WT (C) | **S66A/L251P/R418S (C)** | WT (C) | WT (C) | WT (C) |
| #2 | SCNSL | GCB/refr | SD (1.5m) | WT (T) | WT (T) | WT (T) | WT (T) | WT (T) | **R268W (T)** |
| #3 | SCNSL | GCB/refr | PD (0.9m) | WT (C) | WT (C) | WT (C) | WT (C) | WT (C) | WT (C) |

PCNSL: Primary CNS Lymphoma; SCNSL: Secondary CNS Lymphoma; COO; cell-of-origin; ABC: activate B-cell type; GBC: germinal center; recu: recurrent tumor; refr: refractory tumor; (C): cerebrospinal fluid; (T): archival formalin-fixed, paraffin-embedded tissue; WT: wild-type; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; m: months; #: treated with high-dose chemotherapy with autologous stem cell rescue; * ongoing treatment with study drug.

## FIGURE LEGENDS

**Figure 1. Clinical Response to ibrutinib-based combination therapy in CNSL**

(A) Best response to ibrutinib-based combination therapy. Displayed is the change in target lesion diameter from baseline (%) by magnetic resonance imaging or clearance of malignant cells in cerebrospinal fluid (CSF); negative values indicate tumor shrinkage; 8 of 9 (89%) Primary CNS Lymphoma (PCNSL) (89%) and 4 of 6 (67%) Secondary CNS Lymphoma (SCNSL) responded to ibrutinib-based combination therapy. Black, progression of disease (PD); orange, stable disease (SD); blue, partial response (PR); green, complete response (CR). (B) Progression-free survival in patients with PCNSL (top) and SCNSL (bottom). ➔, patient still receiving treatment; *, hematopoietic stem cell transplantation; ♦ , progression; #, discontinued. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

**Figure 2. Molecular Response to Ibrutinib-based Therapy**

(A) Shown is a biospecimen collection for all patients enrolled in our study. Diamond represent the primary tumor and circle the collected cerebrospinal fluid (CSF). Included is the time (in months) between the tumor and CSF collection (T/C interval). Red diamonds indicate primary tumor tissue that was collected and sequenced (BM: diagnosed by bone marrow biopsy; C: diagnosed by CSF cytology). X: Patient refused CSF collection. P: Patient off study for disease progression. Blue circles represent sequenced CSF samples whereas white circles represent samples with insufficient volume to perform sequencing. (B) Imaging was performed at baseline and before initiation of Cycle 3 (C3) and Cycle 5 (C5) in nine patients. Displayed is the spider plot of patients with measurable disease, of whom one had disease progression after an initial response to therapy (#11) and 7 patients (#5, #7, #9, #12, #13, #14, #15) had either a partial response >90% or complete responses on MR imaging. (C) Shown are heatmaps of the variant allelic frequencies of all the mutations present in CSF collected before treatment initiation

(Baseline), during ibrutinib-based combination therapy (C3, C5) and at progression (PD) in representative patients with sustained response demonstrating a 'clearance' of tumor DNA (for all CSF profiles see Supplemental Figure S6). Variant Allelic Frequency Scale = 0-1 (0=white; 1=dark blue). (D) Heatmap of the variant allelic frequencies (Baseline, C3, C5 and at progression (PD)) and early progression, demonstrating a persistent clone (#11). (E) Patient with non-measurable leptomeningeal disease on magnetic resonance imaging (MRI; T1 post contrast sequences) and CSF (cytology and flow cytometry) at baseline. After two cycles of study therapy the MRI changes resolved. No malignant cells and no ctDNA were detectable in the CSF (C3). After completion of the induction therapy (C5), the MRI brain and CSF (cytology and flow cytometry) continued to show a response, whereas ctDNA was detectable in the CSF. Ultimately, the patient developed progression of disease on MRI, CSF cytology and CSF flow cytometry after one month of maintenance ibrutinib. White arrow heads: leptomeningeal involvement in the cerebellar folia; white arrows: leptomeningeal involvement of both trigeminal nerves; red arrows: recurrent leptomeningeal disease affecting both trigeminal nerves. (F) Heatmap of the variant allelic frequencies in a case of early progression with reemergence of genetic alterations (#6). Variant Allelic Frequency Scale = 0-1 (0=white; 1=dark blue).

**Figure 3: Integration of Clinical and Molecular Response Assessment**

Conventional treatment response assessment using Magnetic Resonance Imagining (MRI) and cytology is combined with genomic testing of circulating tumor DNA (ctDNA) in cerebrospinal fluid (CSF). CSF and imaging were performed at baseline prior to treatment initiation, before start of Cycle 3 (C3), and before start of Cycle 5 (C5). Displayed are the patients with serial CSF collections and their response to study treatment using MRI, cytology and ctDNA. Patient #6 had radiographic progression of disease at cycle 7.

**Figure 1: Clinical response**



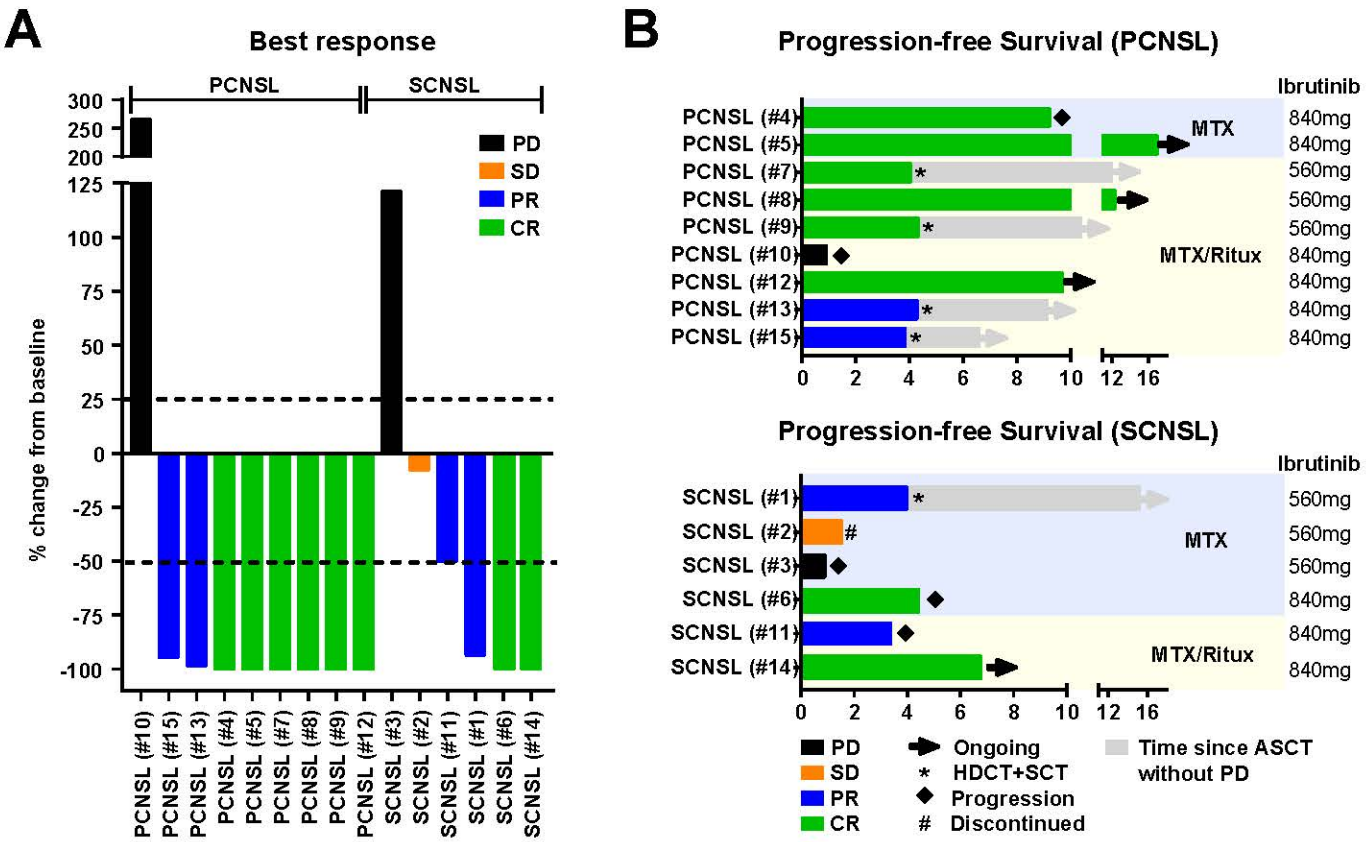


**Figure 2: Genomic Interrogation of the CSF in CNS Lymphoma Patients**

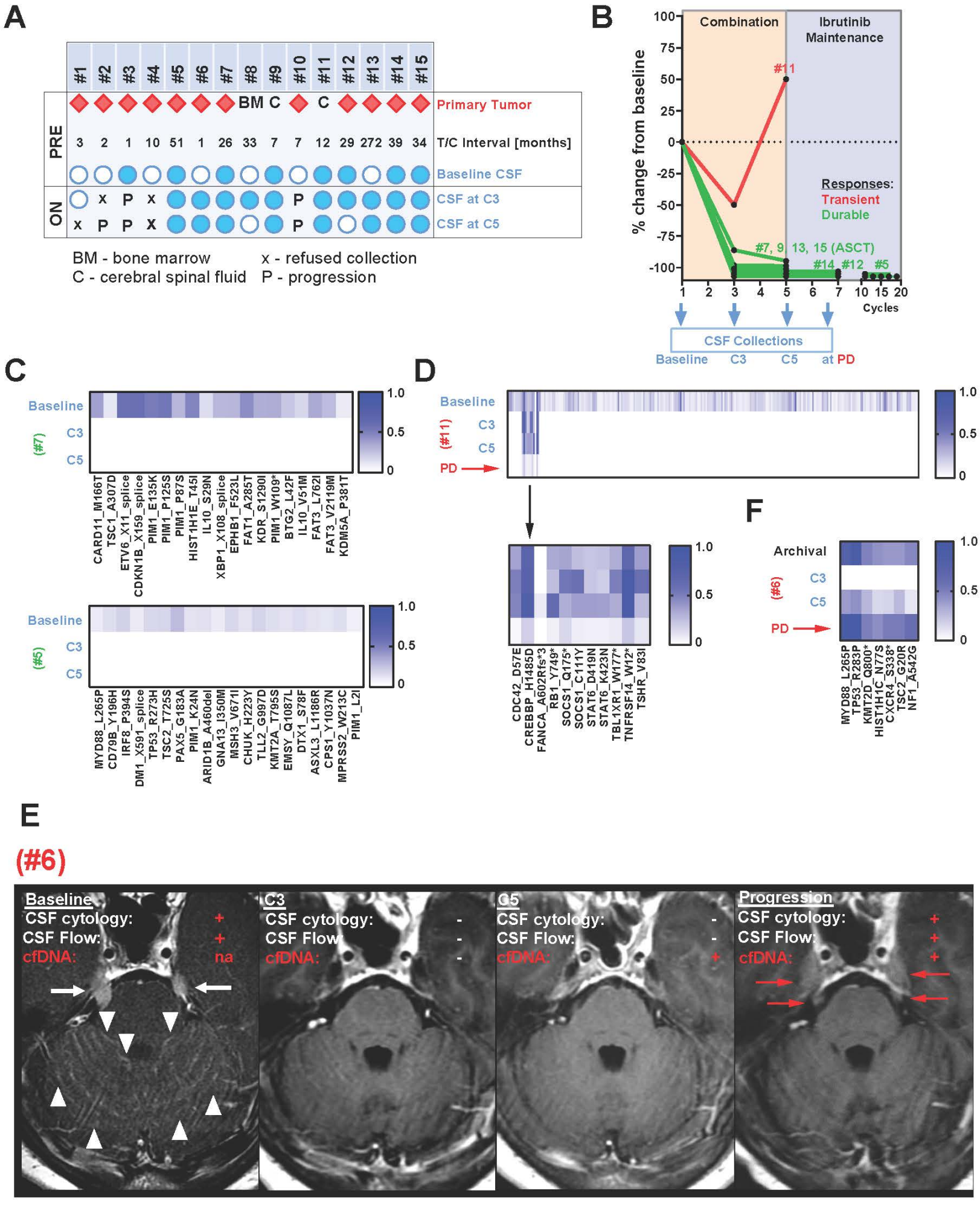
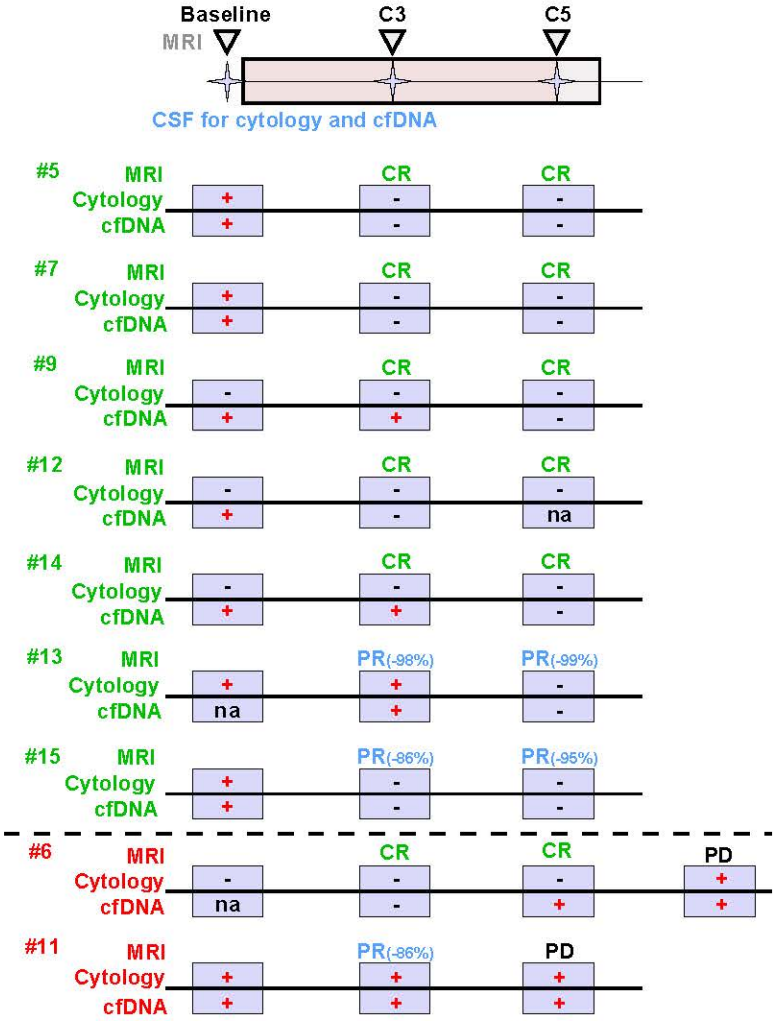


**Figure 3: Integration of Clinical and Molecular Response Assessment**





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# Phase Ib trial of an ibrutinib-based combination therapy in recurrent/refractory CNS lymphoma

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