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How we use Genomics and BTK-Inhibitors in the Treatment of Waldenstrom Macroglobulinemia.

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Abstract:
Mutations in MYD88 (95-97%) and CXCR4 (30-40%) are common in Waldenstrom macroglobulinemia (WM). TP53 is also altered in 20-30% of WM patients, particularly those previously treated. Mutated MYD88 upregulates and activates HCK that drives BTK pro-survival signaling. Both nonsense and frameshift CXCR4 mutations occur in WM. Nonsense variants such as CXCR4S338X show greater resistance to BTK-inhibitors. Covalent BTK-inhibitors (cBTK-i) produce major responses in 70-80% of WM patients. MYD88 and CXCR4 mutation status can impact time to major response, depth of response and/or progression-free survival (PFS) in WM patients treated with cBTK-i. The cBTK-i zanubrutinib shows greater response activity and/or improved PFS in wild-type MYD88, mutated CXCR4, or altered TP53 patients. Marked differences in adverse events have been observed between BTK-inhibitors in WM patients, including atrial fibrillation, bleeding diathesis and neutropenia. Intolerance is also common with c-BTKi, and dose reduction or switchover to another c-BTKi can be considered. For patients with acquired resistance to c-BTKi, newer options include the non-covalent BTK-inhibitor pirtobrutinib or the BCL2 antagonist venetoclax. Combinations of BTK-inhibitors with chemoimmunotherapy, CXCR4 and BCL2 antagonists have advanced and are discussed. Algorithms for positioning BTK-inhibitors in treatment-naïve and previously treated WM patients based on genomics, disease characteristics, and co-morbidities are presented.

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ABSTRACT

Mutations in MYD88 (95-97%) and CXCR4 (30-40%) are common in Waldenstrom macroglobulinemia (WM). TP53 is also altered in 20-30% of WM patients, particularly those previously treated. Mutated MYD88 upregulates and activates HCK that drives BTK pro-survival signaling. Both nonsense and frameshift CXCR4 mutations occur in WM. Nonsense variants such as CXCR4^{S338X} show greater resistance to BTK-inhibitors. Covalent BTK-inhibitors (cBTK-i) produce major responses in 70-80% of WM patients. MYD88 and CXCR4 mutation status can impact time to major response, depth of response and/or progression-free survival (PFS) in WM patients treated with cBTK-i. The cBTK-i zanubrutinib shows greater response activity and/or improved PFS in wild-type MYD88, mutated CXCR4, or altered TP53 patients. Marked differences in adverse events have been observed between BTK-inhibitors in WM patients, including atrial fibrillation, bleeding diathesis and neutropenia. Intolerance is also common with c-BTKi, and dose reduction or switchover to another c-BTKi can be considered. For patients with acquired resistance to c-BTKi, newer options include the non-covalent BTK-inhibitor pirtobrutinib or the BCL2 antagonist venetoclax. Combinations of BTK-inhibitors with chemoimmunotherapy, CXCR4 and BCL2 antagonists have advanced and are discussed. Algorithms for positioning BTK-inhibitors in treatment-naïve and previously treated WM patients based on genomics, disease characteristics, and co-morbidities are presented.

INTRODUCTION

GENOMICS OF WM AND BTK-INHIBITORS

MYD88 MUTATIONS

Mutated MYD88 ($MYD88^{Mut}$) is found in 95-97% of WM patients.[1-6] Nearly all cases carry the $MYD88^{L265P}$ variant, though other $MYD88^{Mut}$ variants occur.[7,8] Next-generation sequencing (NGS) may miss $MYD88^{L265P}$ in 35% of WM patients, particularly in patients with a bone marrow (BM) disease burden of <10%.[9] AS-PCR is therefore recommended for $MYD88^{L265P}$ detection.[10] The signaling cascades for $MYD88^{Mut}$ are shown in **Figure 1**.[11-17] Five percent of WM patients are MYD88 wild-type ($MYD88^{WT}$), many of whom carry NFKB activating mutations distal to BTK signaling.[18] $MYD88^{WT}$ patients have a higher risk for disease transformation, and/or show shorter overall survival (OS).[19,20] $MYD88^{Mut}$ status can differentially impact outcomes with BTK-inhibitors (discussed below). *We therefore recommend determination of $MYD88^{Mut}$ status as part of the diagnostic workup by AS-PCR for the $MYD88^{L265P}$ variant, and if negative, consider NGS to identify any non-$MYD88^{L265P}$ variants.*

CXCR4 MUTATIONS

$CXCR4^{Mut}$ are found in 30-40% of WM patients.[2,19] Over 40 CXCR4 nonsense and frameshift variants in the C-terminal domain have been identified in WM.[2,5,21-24] Nonsense ($CXCR4^{Mut/NS}$) variants, the most common being $CXCR4^{S338X}$, cause truncation of the C-terminal regulatory domain.[2,21,23] Frameshift ($CXCR4^{Mut/FS}$) variants result from insertions or deletions. $CXCR4^{Mut}$ clones are usually sub-clonal.[2,5,21] Despite heterogeneous clonality, the transcriptional profile for $CXCR4^{Mut}$ WM patients shows great uniformity.[25] In response to CXCL12, $CXCR4^{Mut}$ triggers BTK, AKT and ERK signaling which promotes BM chemotaxis and/or ibrutinib-resistance.[26-28] $CXCR4^{Mut/NS}$ expressing WM cells exhibit more robust AKT and ERK signaling versus those expressing CXCR4^{WT} or CXCR4$^{Mut/FS}$.[28] CXCR4 antagonists can sensitize CXCR4^{Mut} expressing WM cells to ibrutinib.[26-28]

CXCR4$^{Mut/NS}$ WM patients have higher BM disease burden; higher serum IgM (sIgM) levels and incidence of symptomatic hyperviscosity; and/or shorter time to initial treatment versus those with CXCR4^{WT} or CXCR4$^{Mut/FS}$ mutation status.[5,22-24,29] One study also showed shorter OS in patients with CXCR4$^{Mut/NS}$ versus CXCR4^{WT} or CXCR4$^{Mut/FS}$.[29] CXCR4$^{Mut/NS}$ may also more adversely impact treatment outcomes with BTK-inhibitors versus CXCR4^{WT} or CXCR4$^{Mut/FS}$ (discussed below).[30] NGS may miss two-thirds of patients with CXCR4^{Mut}, particularly those with lower BM disease burden and clonality.[31] Newer NGS platforms may improve detection.[29] Given the significance of CXCR4$^{Mut/NS}$, AS-PCR may be used to evaluate this variant.[10] *We therefore recommend CXCR4^{Mut} status as part of the diagnostic workup by NGS, and if negative consider AS-PCR for CXCR4^{S338X} variants.*

TP53 ALTERATIONS

Alterations in TP53 (TP53^{Alt}) occur in 5-10% of treatment-naïve WM patients that include TP53^{Mut} and 17p deletions.[5,32,33] The incidence of TP53^{Alt} appears higher (25-30%) in previously treated WM patients, most of whom received alkylators (85%) and/or nucleoside analogues (22%).[34,35] TP53^{Alt} WM patients show shorter OS and/or PFS with BTK-inhibitors.[32-34] *We recommend evaluating for TP53^{Alt} at diagnosis, and at relapse using NGS as their presence may guide treatment considerations (discussed below). As studies to date have been qualitative, no cutoffs for TP53^{Alt} have been established.*

BTK MUTATIONS

BTK^{Cys481} is the binding site for the covalent BTK inhibitors (cBTK-i) that include ibrutinib, zanubrutinib, acalabrutinib, and tirabrutinib. BTK^{Cys481} variants are the most common mutations in WM patients with acquired ibrutinib-resistance.[36] Importantly, multiple clones bearing different BTK^{Cys481} mutations can occur within individual WM patients progressing on ibrutinib.[36,37] Like CLL, BTK^{Cys481} mutations in WM patients progressing on BTK-inhibitors are typically sub-clonal.[36,38] WM cells expressing the $BTK^{Cys481Ser}$ mutation show ibrutinib-resistance and re-activation of BTK-PLCγ2-ERK1/2 signaling.[17] Use of ERK1/2 inhibitors triggers apoptosis in $BTK^{Cys481Ser}$ expressing cells, and re-sensitization to ibrutinib.[17] Moreover, ERK1/2 re-activation is accompanied by IL-6 and IL-10 release which protects co-cultured wild-type BTK^{Cys481} WM cells from ibrutinib, demonstrating a paracrine means for propagating cBTK-i resistance.[17] Deletions in chromosomes 6q or 8p which encompass regulators of BTK, MYD88/NF-κB, and apoptotic signaling also occur in WM patients progressing on ibrutinib.[39] *We continue to hold off making recommendations for routine BTK^{Cys481} variant testing in WM patients progressing on cBTK-inhibitors since salvage treatment approaches (discussed below) did not evaluate outcomes based on BTK^{Cys481} mutation status.*

CLINICAL EXPERIENCE WITH BTK-INHIBITORS IN WM

Table 1 summarizes studies with BTK-inhibitors in WM with regulatory approvals in any indication and jurisdiction. Clinicians should consult local guidelines on approval status and/or use of the discussed BTK-inhibitors. **Table 2** summarizes data for $CXCR4^{Mut}$ status on ibrutinib and zanubrutinib outcomes.

IBRUTINIB

Monotherapy

Ibrutinib was the first BTK-inhibitor approved for WM following a pivotal trial in 63 relapsed/refractory WM patients.[40] The median number of therapies was 2, and 40% were refractory. With a median follow-up of 59 months, the overall (ORR) and major (MRR) response rates were 90% and 79%, respectively. Thirty-percent achieved a very good partial response (VGPR). The 5-year median PFS and OS rates were 54% and

87%, respectively. In MYD88^{WT} patients, no major responses occurred. MYD88^{Mut}CXCR4^{Mut} patients showed longer time to major response, and lower major and VGPR rates versus MYD88^{Mut} only patients (**Table 2**). The 5-year PFS rate for MYD88^{Mut} only and MYD88^{Mut}CXCR4^{Mut} patients were 70% and 38%, respectively. For MYD88^{WT} patients, the median PFS was 0.4 months. Atrial fibrillation (all grades) occurred in 8 (13%) patients, though most continued ibrutinib with medical management.

A sub-study of the INNOVATE trial (discussed below) evaluated ibrutinib monotherapy in 31 heavily pretreated, rituximab-refractory WM patients.[41] Their median prior therapies were 4, and 40% had ≥5 prior therapies. With a median follow-up of 58 months, the ORR and MRR were 87% and 77%, respectively. The 5-year PFS and OS rates were 40% and 73%, respectively. MYD88^{Mut}CXCR4^{Mut} patients had fewer VGPRs, and longer time to major response versus MYD88^{Mut} only patients (**Table 2**). The median PFS was 18 months for MYD88^{Mut}CXCR4^{Mut} patients and was not reached for MYD88^{Mut} only patients. A single MYD88^{WT} patient progressed at 6 months. No atrial fibrillation events observed.

Ibrutinib monotherapy was also evaluated in 30 MYD88^{Mut} treatment-naive WM patients.[42] Half were CXCR4^{Mut}, and nearly all with CXCR4$^{Mut/NS}$. With a median follow-up of 50 months, the ORR, MRR, and VGPR response rates were 100%, 87%, and 30%, respectively. The 4-year PFS rate was 76% for all patients. Major and VGPR response rates were lower, and time to major response longer in CXCR4^{Mut} patients (**Table 2**). The 4-year PFS was lower (59% vs. 92%) in CXCR4^{Mut} patients. Atrial fibrillation (all grades) occurred in 20% of patients.

The importance of CXCR4^{Mut} subtype on ibrutinib outcomes was also evaluated in 180 symptomatic WM patients receiving ibrutinib. CXCR4$^{Mut/NS}$ was associated with lower MRR and shorter PFS versus those with CXCR4$^{Mut/FS}$ or CXCR4^{WT}.[30]

Ibrutinib in combination therapy

The Phase III (INNOVATE) study randomized 150 treatment-naïve and relapsed/refractory WM patients to placebo/rituximab or ibrutinib/rituximab.[43] With a median follow-up of 50 months, the MRR was 76% for ibrutinib/rituximab versus 31% for placebo/rituximab cohorts. Among ibrutinib/rituximab treated patients, MRRs were similar across the genomic subtypes. Fewer VGPRs occurred in MYD88^{Mut}CXCR4^{Mut}, as well as MYD88^{WT} patients on ibrutinib/rituximab versus MYD88^{Mut}/CXCR4^{WT} patients. Time to major responses were 2, 3 and 7 months for MYD88^{Mut}/CXCR4^{WT}, MYD88^{Mut}CXCR4^{Mut} and MYD88^{WT}/CXCR4^{WT} patients, respectively. The estimated 54-month PFS rate was 68% for ibrutinib/rituximab and 25% for placebo/rituximab cohorts. Among ibrutinib/rituximab treated patients, the 54-month estimated PFS was 72%, 63%, and 70% for MYD88^{Mut}/CXCR4^{WT}, MYD88^{Mut}CXCR4^{Mut} and MYD88^{WT}/CXCR4^{WT} patients, respectively. The high activity level for ibrutinib/rituximab in MYD88^{WT} patients may have reflected MYD88^{Mut} patients classified as MYD88^{WT} since NGS was used to determine MYD88^{Mut} status. Indeed, four-times more patients were MYD88^{WT} in this study compared to findings in symptomatic WM patients with AS-PCR.[44] Atrial fibrillation (any grade) occurred in 19% of patients on ibrutinib/rituximab, and most continued protocol therapy with medical management.

Ibrutinib is active in WM patients with symptomatic CNS involvement (Bing Neel Syndrome; BNS).[45,46] In a multicenter, retrospective study, 85% and 60% of BNS patients experienced symptomatic and radiological improvements within 3 months of ibrutinib initiation, respectively.[46] The 2-year event free survival (EFS) rate for BNS patients on ibrutinib was 80%. Ibrutinib also showed activity in WM patients with IgM-related morbidities.[40,42]

CXCR4 antagonists have been investigated with ibrutinib. In a Phase I trial, ulocuplumab was combined with ibrutinib to treat symptomatic MYD88^{Mut}CXCR4^{Mut} WM patients.[47] All achieved a major response, and 33% a VGPR. Major responses occurred at a median of 1.2 months, and the 24-month PFS rate was 90%. Reversible thrombocytopenia, rash and skin infections were the most common adverse events. Unfortunately, ulocuplumab was discontinued by the manufacturer. A study of the oral CXCR4 antagonist mavorixafor with ibrutinib is currently underway (NCT 04274738).

Ibrutinib was also investigated with venetoclax in a fixed duration study that treated 45 symptomatic, treatment-naïve WM patients.[48] All were MYD88^{Mut}, and 17 (38%) CXCR4^{Mut}. The ORR and MRR were 100% and 96%, respectively. Notably, 42% of patients attained a VGPR, though CXCR4^{Mut} patients had a lower VGPR rate (29% vs. 50%). The PFS rate at 24-months was 76% and was not impacted by CXCR4^{Mut} status. The study ended early due to ventricular arrythmias that occurred in 4 (9%) patients, including two Grade 5 events. *Therefore, the combination of ibrutinib and venetoclax should be avoided.*

ZANUBRUTINIB

Monotherapy

In a Phase 1/2 study, 73 treatment-naïve and relapsed/refractory WM patients received zanubrutinib at 160-mg twice or 320-mg once daily.[49] The median follow-up was 24 and 36 months for treatment-naïve and relapsed/refractory patients, respectively. The ORR, MRR, and VGPR responses were 96%, 78% and 45%, respectively. The MRRs were similar among MYD88^{Mut} patients regardless of CXCR4^{Mut} status. VGPR/CR attainment was lower among MYD88^{Mut}CXCR4^{Mut} patients (**Table 2**). The ORR and MRR were similar across dosing schedules, though fewer VGPR/CRs (32% vs. 49%) occurred in those receiving 320-mg once versus 160-mg twice daily, respectively.

The Phase III (ASPEN) study of zanubrutinib versus ibrutinib was recently updated.[50] In Cohort 1, 201 MYD88^{Mut} treatment-naïve and relapsed/refractory patients were randomized to zanubrutinib or ibrutinib. Of these patients, 20% and 32% were CXCR4^{Mut} in the ibrutinib and zanubrutinib arms, respectively. In Cohort 2, 28 MYD88^{WT} patients received zanubrutinib. MYD88^{Mut} status was determined by an AS-PCR assay. The median follow-up was 44.4 and 42.9 months for Cohorts 1 and 2, respectively. In Cohort 1, the best ORR (94% and 95%) and MRR (80% and 81%) were similar for ibrutinib and zanubrutinib, respectively. However, more VGPRs occurred for zanubrutinib (36%) versus ibrutinib (25%). The median PFS has not been reached for either arm in Cohort 1, with estimated 42-month PFS rates of 70% and 78% for ibrutinib and zanubrutinib, respectively. OS was similar at 85% and 88% for ibrutinib and zanubrutinib, respectively.

CXCR4^{Mut} status differentially impacted the two arms of Cohort 1. The median time to major response was longer for CXCR4^{Mut} patients on ibrutinib versus zanubrutinib (**Table 2**). More VGPRs also occurred in CXCR4^{Mut} patients who received zanubrutinib (**Table 2**). At 42-months, the PFS rates for CXCR4^{Mut} patients were 49% and 73% for ibrutinib and zanubrutinib treated patients, respectively.

Of particular interest was the differential impact of zanubrutinib and ibrutinib in TP53^{Alt} patients on Cohort 1. Major (64% vs. 86%) and VGPR (14% vs. 30%) responses were lower among ibrutinib treated patients with TP53^{Alt} versus TP53^{WT} disease, respectively.[35] Among zanubrutinib treated patients, the MRR were similar regardless of TP53^{Alt} status, i.e. 81% and 82% for TP53^{Alt} and TP53^{WT} patients, respectively. VGPR responses were also higher for zanubrutinib (35%) versus ibrutinib (14%) treated patients with TP53^{Alt} disease.[35] PFS rates were adversely impacted by TP53^{Alt} status in both arms of Cohort 1, though fewer progression events occurred among TP53^{Alt} patients on zanubrutinib versus ibrutinib.[35]

There were notable differences for adverse events in Cohort 1. A higher incidence (>10% difference) of diarrhea (35% vs. 23%), muscle spasms (29% vs. 12%), hypertension (24% vs. 12%), atrial fibrillation (21% vs. 7%) and pneumonia (18% vs. 5%) occurred at any grade for patients on ibrutinib versus zanubrutinib, respectively. Conversely, more patients experienced neutropenia at any grade (29% vs. 16%) on zanubrutinib versus ibrutinib, respectively. Grade 3/4 hypertension (19% vs 10%) and pneumonia (10% vs. 1%) were more pronounced for patients on ibrutinib, while neutropenia (19% vs. 9%) and anemia (12% vs. 6%) were more frequent for those on zanubrutinib.

In Cohort 2, zanubrutinib showed marked activity in $MYD88^{WT}$ patients. The ORR and MRR were 81% and 65%, respectively; VGPR was attained in 30% of $MYD88^{WT}$ patients. The 42-month EFS rate was 54%, which greatly contrasted to the median PFS of 4 months with ibrutinib monotherapy in the pivotal trial. Efficacy data on specific morbidities in WM for zanubrutinib are limited. Response to zanubrutinib was documented in a single case report of a BNS patient.[51]

Zanubrutinib in combination therapy

The combination of zanubrutinib, ixazomib and dexamethasone was evaluated in 20 symptomatic, treatment-naïve WM patients and showed an ORR, MRR and VGPR attainment in 100%, 95%, and 42%, respectively.[52] Therapy was well tolerated with grade $\geq$ 3 neutropenia or rash in 2 patients (one each).

ACALABRUTINIB

Monotherapy

Acalabrutinib was evaluated in a multicenter study of 106 treatment-naïve and relapsed/refractory WM patients. An update was recently presented with a median follow-up of 64 months.[53] Treatment duration was longer for relapsed/refractory (61 months) versus treatment-naïve (42 months) patients. The ORR and MRR were 94% and 83%, respectively. A VGPR was attained in 25% of patients. The 66-month PFS rate was 84% for treatment-naïve, and 52% for relapsed/refractory patients. OS rates at 66-months were 91% and 71% for treatment-naïve and relapsed/refractory patients, respectively. With longer follow-up, the incidence of any grade atrial fibrillation was 11%. Grade 3/4 toxicities for all patients also included bleeding (6%), hypertension (4%), and infections (30%). Data on the impact of $MYD88^{Mut}$ and $CXCR4^{Mut}$ status on acalabrutinib response are limited.

Acalabrutinib in combination therapy

Preliminary results were recently presented for the BRAWM study that investigated fixed duration treatment with acalabrutinib, bendamustine and rituximab (Benda-R) in symptomatic, treatment naïve WM patients.[54] A pre-planned analysis for the first 30 enrolled patient showed an MRR of 88%, and VGPR attainment for 70% for 17 evaluable WM patients who completed 7 cycles. Grade 3/4 toxicities included neutropenia (27%) with febrile neutropenia (6%). 1 patient had atrial fibrillation. A dedicated study of acalabrutinib with rituximab is ongoing in patients with MAG-positive demyelinating neuropathy (NCT05065554).

TIRABRUTINIB

Tirabrutinib is approved in Japan. In a Phase 2 study, 18 treatment-naïve and 9 relapsed/refractory patients received tirabrutinib.[55] With a median follow-up of 25 months, the ORR and MRR were 96% and 93%, respectively. The VGPR rate was 30%. $CXCR4^{Mut}$ status impacted time to major response and VGPR attainment, though all $CXCR4^{Mut}$ patients achieved a major response. One $MYD88^{WT}$ patient achieved a major response. The 24-month PFS and OS rates were 92.6% and 100%, respectively. Rash (all grades) occurred in 12 (44%) patients. Grade 3/4 neutropenia occurred in 6 (22%) patients, and 2 (7%) experienced atrial fibrillation. Tirabrutinib activity has also been reported in multiple BNS case reports.[56-58]

PIRTOBRUTINIB

Pirtobrutinib is a non-covalent BTK inhibitor that binds to non-BTK^{Cys481} sites and inhibits both wild-type and Cys^{481} mutated BTK. Pirtobrutinib also blocks $MYD88^{Mut}$ driven ERK signaling in ibrutinib-resistant $BTK^{Cys481Ser}$ expressing WM cells.[59,60] Twenty-six WM patients were included in a Phase 1/2 (BRUIN) study.[61] An expansion of the WM cohort was recently reported with 80 evaluable WM patients.[62] Sixty-three of these patients were cBTK-i-exposed. The MRR (66% vs. 88%) was lower in cBTK-i-exposed versus cBTK-i-naïve patients, respectively. VGPRs occurred in 24% and 29% of cBTK-i-exposed and c-BTK-i-naïve patients, respectively. The median PFS for those receiving prior cBTK-i therapy was 19.4 months.

POSITIONING OF BTK-INHIBITORS IN WM

Case 1. Newly Diagnosed WM Patient with Symptomatic Hyperviscosity.

A 42-year old male presented with blurry vision and nosebleeds. Physical exam revealed retinal hemorrhages, marked axillary and inguinal adenopathy, and splenomegaly. He was pancytopenic. Serum protein electrophoresis (SPEP) revealed an IgMλ monoclonal protein, and sIgM of 12,400 mg/dL. CT scans showed diffuse bulky adenopathy. BM biopsy demonstrated 80% lymphoplasmacytic (LPL) cell involvement, and molecular diagnostics showed $MYD88^{L265P}$ *and* $CXCR4^{S338X}$ *mutations.*

Figure 2 provides an algorithm for symptomatic, treatment-naïve WM patients. The recommendations presented below considered recent consensus panel guidance.[63] For symptomatic treatment-naïve patients, chemoimmunotherapy with Benda-R, Dexamethasone, Rituximab and Cyclophosphamide (DRC), as well as BTK-inhibitors can be considered. For chemoimmunotherapy, we favor Benda-R over DRC since the former may offer deeper responses and longer PFS.[64,65] We favor using 4 cycles when possible to minimize short and long term adverse events given findings from two retrospective studies including a dedicated study in symptomatic treatment-naïve WM patients that showed no difference in outcomes versus 6 cycles.[64,65] We do favor use of up to 6 cycles of Benda-R when treating bulky disease or symptomatic amyloidosis. As part of these recommendations, we considered a multicenter, retrospective study, wherein start dosing depended on local practice and showed a benefit in major response attainment and PFS for front-line patients who received ≥1000 mg/m2.[66] No such benefit was seen with relapsed/refractory patients.

In patients >70 years or a low EGFR, reduced dose bendamustine should be considered. Data on the relative activity of BTK-inhibitors versus chemoimmunotherapy is minimal. A multicenter, retrospective study of 246 treatment-naïve MYD88^{Mut} WM patients compared outcomes for Benda-R versus ibrutinib.[67] Seventy-seven percent of patients received >4 cycles of Benda-R. The median follow-up was 4.2 years. Major (92% vs. 83%), VGPR (50% vs. 33%) and CR (20% vs. 2%) rates were greater for Benda-R versus ibrutinib treated patients, respectively. PFS and OS were similar supporting that BTK-inhibitors offer comparable long-term disease control as Benda-R.

For MYD88^{Mut} only patients, we favor cBTK-i to minimize risk for acquired TP53^{Alt}. As all cBTK-i exhibit similar activity in MYD88^{Mut} only patients (**Table 1**), the choice should consider accessibility and guidance offered in **Table 3.** For CXCR4^{Mut} patients requiring a rapid response such as **Case 1**, Benda-R or zanubrutinib may be reasonable. Plasmapheresis should be performed in those with symptomatic hyperviscosity. Rituximab should be held if Benda-R is chosen until the sIgM levels <4,000 mg/dL to avoid triggering a hyperviscosity crisis.[63] Zanubrutinib may also be considered in CXCR4^{Mut} patients needing a rapid response. The median time to a major response was 2.8 months in CXCR4^{Mut} WM patients receiving zanubrutinib in the ASPEN study, which is comparable to Benda-R.[35,68] In **Case 1**, Benda-R is more suitable since data with BTK-inhibitors in patients with bulky disease is lacking.

Zanubrutinib can be considered in CXCR4^{Mut} patients not needing rapid disease control since a shorter time to major response, deeper responses, and longer PFS were observed versus ibrutinib.[35,50] For MYD88^{WT} patients, zanubrutinib is favored for symptomatic, treatment-naïve patients since high levels of response and long-term disease control can be achieved.[50] Benda-R and proteasome-inhibitor (PI) based therapy are reasonable alternatives in CXCR4^{Mut} or MYD88^{WT} patients.[68,69] TP53^{Alt} status can be considered in positioning BTK-inhibitors. Zanubrutinib is our preference for TP53^{Alt} WM patients given the ASPEN study findings showing higher levels of activity and long-term disease control versus ibrutinib.[35]

Case 2. Previously treated WM patient with high-risk disease requiring therapy.

A 42-year old female presented with headaches, blurry vision. Workup showed a sIgM monoclonal protein; sIgM level >10,000 mg/dL; and 85-90% BM LPL involvement. Molecular studies revealed MYD88^{L265P}, CXCR4^{V340FS} and TP53^{Y163C} mutations. Her hemoglobin was 9.9 g/dL and platelet count was 72,000 K/uL. She received Bortezomib, Dexamethasone and Rituximab (BDR) with a partial response but progressed 1.5 years later. She then received venetoclax (on a clinical trial) with no response after three months of therapy.

Figure 3 provides an algorithm for symptomatic, previously treated WM patients. The recommendations considered recent consensus panel guidance.[70] The panel noted that biological age, co-morbidities and fitness; nature of the relapse; patient preferences; hematopoietic reserve; and mutation status (MYD88, CXCR4, TP53) should be considered in treatment selection. For MYD88^{Mut} only patients who are refractory or in first relapse following initial chemoimmunotherapy, cBTK-i should be considered. As all cBTK-i exhibit similar response activity in MYD88^{Mut} only patients (**Table 1**), the choice of agent should consider accessibility and guidance offered in **Table 3**. For MYD88^{Mut}CXCR4^{Mut} patients who are refractory or in first relapse after initial chemoimmunotherapy, zanubrutinib would be our preference.[50] In MYD88^{WT} WM patients, zanubrutinib would also be our recommended treatment after initial chemoimmunotherapy.[50] Zanubrutinib is also preferred for $TP53^{Alt}$ WM patients such as **Case 2** with $MYD88^{Mut}CXCR4^{Mut}TP53^{Alt}$.

In patients discontinuing ibrutinib, chemoimmunotherapy showed an ORR of 73%.[71] Response rates were higher in patients who began chemoimmunotherapy within 2 weeks of ibrutinib discontinuation.[71] As sIgM rebound is common following cessation of BTK-inhibitors, a bridging strategy for continuing BTK-inhibitor until disease control with chemoimmunotherapy is achieved can be considered in patients with high sIgM levels.[41,71]

For patients refractory to initial cBTK-i therapy, Benda-R is recommended regardless of genomic subtype. Rituximab should be held if Benda-R is chosen until the sIgM is <4,000 mg/dL to avoid triggering a hyperviscosity crisis. For those progressing after initial cBTK-I response, options include Benda-R, PI-based therapy, venetoclax, or pirtobrutinib. We prefer to minimize alkylator exposure whenever possible, particularly in patients <70 years or with $TP53^{Alt}$. For these patients, venetoclax is preferable since it is highly active in WM patients previously exposed to cBTK-i or with $CXCR4^{Mut}$ disease.[72,73] The activity of venetoclax in $MYD88^{WT}$ or $TP53^{Alt}$ WM patients remains to be clarified. Pirtobrutinib is an option post-cBTK-i therapy though its activity in $MYD88^{WT}$ or $MYD88^{Mut}CXCR4^{Mut}$ patients is not known.[61,62] Benda-R or PI-based regimens can also be considered in those progressing on a cBTK-i as these are active across all genomic subtypes.[68,69] Additional options in second or later relapse include re-use of chemotherapy if a response lasted for >3 years, alternative chemoimmunotherapy, nucleoside analogues or everolimus.[70] Clinical trials should be prioritized in relapsing patients.[73]

For those with bulky extramedullary disease such as **Case 1**, Benda-R is more suitable since data with BTK-inhibitors in patients with bulky disease is lacking. For WM patients with symptomatic amyloidosis, consensus recommendations favor Benda-R or PI-based therapy followed by consolidation with high dose chemotherapy and autologous stem cell transplant in suitable patients.[75] Findings in a small number of cases treated with ibrutinib or acalabrutinib showed a short PFS as well as more pronounced bleeding and atrial fibrillation.[40,76,77] BTK-inhibitors, particularly ibrutinib or tirabrutinib (in Japan) can be prioritized for BNS patients given more extensive reports showing activity and durability for these BTK-inhibitors.[45,46,56-58]

MANAGEMENT OF BTK-INHIBITOR INTOLERANT OR RESISTANT WM DISEASE

Case 3. Patient on ibrutinib who is in a VGPR and develops atrial fibrillation.

A 73-year old man presented with fatigue and a hemoglobin of 9.1 g/dL. Work-up showed an sIgM monoclonal protein; sIgM level of 2,301 mg/dL. BM biopsy showed 50% LPL involvement, and only the MYD88^{L265} mutation was detected. CT scans showed diffuse adenopathy (largest node 2.3 cm) and mild splenomegaly. He began ibrutinib and attained a VGPR. One year after starting ibrutinib, he had symptomatic atrial fibrillation with a rapid ventricular response. Cardiac workup for amyloid deposition was unremarkable. He was started on metoprolol and anti-coagulated with apixaban. He was cardioverted but had multiple additional episodes of atrial fibrillation. He also had hematuria and ecchymosis on anti-coagulation.

The discontinuation rate for rituximab intolerance is about 30%.[78,79] Dose reduction with ibrutinib was evaluated in 353 WM patients followed for a median of 64 months.[79] Ninety-six (27%) required a dose reduction due to adverse events.[79] Dose reductions were more common in those > 65 years and in women. Following dose reduction, most patients (65%) had improvement or resolution of adverse effects. Hematologic responses were sustained or deepened in most (79%) patients. Of 10 patients with atrial fibrillation, only one had no recurrence of arrhythmia after dose reduction. For WM patients such as **Case 3** with ibrutinib related atrial fibrillation, switching to another BTK-inhibitor is more appropriate. The use of zanubrutinib to treat patients with ibrutinib or acalabrutinib intolerance was investigated in a study that included WM patients.[80] Most intolerance events for ibrutinib (70%) and acalabrutnib (83%) did not recur after zanubrutinib switchover. Of 10 patients switched over for atrial fibrillation (all from ibrutinib), 9 had no recurrence. The long-term disease control rate was 94% following switchover to zanubrutinib. A switchover should be initiated as soon as possible to prevent an sIgM rebound.[42] Data for switchover from ibrutinib to acalabrutinib are limited, with recurrence of atrial fibrillation occurring in 1 of 2 CLL patients.[81]

Case 4. Heavily pre-treated WM patient who is progressing on ibrutinib.

A 55-year old man was diagnosed 13 years earlier with WM after presenting with anemia and fatigue. At diagnosis, his hemoglobin was 9.6 g/dL, sIgM was 2,800 mg/dL, and BM biopsy showed 90% involvement. CT scans were unremarkable. He had minimal or no response to Cyclophosphamide, Prednisone and Rituximab; Benda-R; Bortezomib/Dexamethasone; and Everolimus. He then started ibrutinib at 420 mg/dL and attained a VGPR. Eleven years later he presented with fatigue and a hemoglobin of 7.1 g/dL. BM biopsy showed 95% LPL involvement, and two distinct BTK^{Cys481} (c.1442G>C; c1441 T>A) mutations.

In patients with BTK^{Cys481} mutation related acquired c-BTK-I resistance such as **Case 4**, a switchover to another c-BTK-i is not recommended. Pirtobrutinib can be considered for patients previously exposed to a cBTK-i in whom a MRR of 66% and PFS of 19.4 months was reported, though specific data for patients harboring BTK^{Cys481} mutations is awaited.[61,62] Venetoclax also is an option, and is highly active in WM patients previously exposed to BTK-inhibitors or with CXCR4^{Mut} disease.[72,73] Lower ORR (76% vs. 93%) and MRR (76% vs. 87%) were observed among previously cBTK-i exposed patients in comparison to c-BTK-I exposed patients, though no significant difference in PFS was observed.[73] As discussed above, chemoimmunotherapy may also be considered in those intolerant or progressing on c-BTK-i.[70] Additional salvage options are presented in **Figure 3**. Our preference in WM patients with acquired resistance to c-BTK-i is to alternate to a non-covalent BTK-inhibitor such as pirtobrutinib, if available, then class switch to a BCL2 inhibitor such as venetoclax. Chemoimmunotherapy or clinical trial options may subsequently be considered for patients who progressed on both BTK and BCL2 inhibitors.

CONSIDERATIONS FOR THE CHOICE OF BTK-INHIBITORS IN WM

In selecting BTK-inhibitors, compliance, nature of disease manifestation, co-morbidities, mutational status (MYD88, CXCR4, TP53, BTK^{Cys481}), as well as tolerance and presence of acquired resistance to cBTK-i should be considered. **Table 3** summarizes patient and disease characteristics including genomics in the choice of BTK-inhibitors for WM.

WITHDRAWAL SYMPTOMS RELATED TO BTK-INHIBITORS

Withdrawal symptoms can develop in 20% of WM patients holding ibrutinib.[82] Symptoms usually ensue within 2 days of ibrutinib hold and resolve rapidly following reinitiation of therapy. In two-thirds of patients experiencing withdrawal, there is no evidence of disease progression during ibrutinib hold. Withdrawal symptoms include fever, body aches, night sweats, arthralgias, chills, headaches and fatigue. An sIgM rebound can also occur when ibrutinib is withheld.[83] Reports regarding withdrawal or sIgM rebound with other BTK-inhibitors are limited.

RESPONSE ASSESSMENT IN WM

Consensus criteria were recently updated that utilize a more simplified sIgM based response assessment for most categorical responses and help distinguish progressive disease versus sIgM rebound in patients whose BTK-inhibitor is held.[82]

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LEGENDS

Figure 1. Pro-survival signaling driven by mutated MYD88 signaling. Mutated MYD88 triggers gene expression of HCK, a SRC family member through PAX5 mediated signaling. HCK is activated through IL-6/IL-6R/gp130/JAK2/STAT3 mediated signaling triggered by autocrine and paracrine release from the surrounding microenvironment. HCK triggers BTK, SYK and ERK1/2 signaling. BTK also triggers ERK1/2 as well as NFKB p65 mediated pro-survival signaling. IRAK4/IRAK1 are also activated by mutated MYD88 through HCK independent pathway, and trigger along with BTK NFKB p65 mediated pro-survival signaling.

Figure 2. Genomic Based Treatment Algorithm for Symptomatic, Treatment Naïve patients with Waldenstrom Macroglobulinemia. Clinicians should consult local regulatory approvals and guidelines for BTK-inhibitor status and use in WM. Algorithm represents the recommendations of the authors based on clinical trial data summarized in the text, consensus recommendations (see Ref. 63) and their practice experiences in WM patients. Recommendations are intended for educational purposes. Rituximab should be held if chemoimmunotherapy is chosen until the sIgM levels <4,000 mg/dL to avoid triggering or exacerbating a hyperviscosity crisis. Benda-R can be considered for patients with bulky adenopathy or extramedullary disease. PI based therapy or Benda-R can be considered for symptomatic amyloidosis with autologous stem cell transplantation as consolidation in select patients (see ref. 75). Rituximab alone, or with ibrutinib if $MYD88^{Mut}$ or Benda-R are options for patients with IgM demyelinating peripheral neuropathy depending on severity and pace of progression. Maintenance rituximab may be considered in patients >65 years responding to chemoimmunotherapy with rituximab (see ref. 63). Rituximab, Cyclophosphamide, and Dexamethasone (RCD) is an option for chemoimmunotherapy if Benda-R is not accessible (see ref. 63). [1]Zanubrutinib may also be prioritized for those with TP53 alterations (see ref. 35). Clinical trial options should always be considered. Benda, bendamustine; R, rituximab,

PI, proteasome-inhibitor; HV, hyperviscosity; CAGG, cold agglutinins; CRYOS, cryoglobulinemia; PN, peripheral neuropathy.

Figure 3. Genomic Based Treatment Algorithm for Symptomatic, Previously Treated patients with Waldenstrom Macroglobulinemia. Clinicians should consult local regulatory approvals and guidelines for BTK-inhibitor status and use in WM. Algorithm represents the recommendations of the authors based on clinical trial data summarized in the text, consensus recommendations (see ref. 68) and their practice experiences in WM patients. Recommendations are intended for educational purposes. See also notations for **Figure 2.** Nucleoside analogues should be avoided in younger patients, and candidates for autologous stem cell transplantation (ASCT). ASCT may be considered in patients with multiple relapses and chemosensitive disease, and those with amyloidosis for consolidation after PI or Benda-R therapy (see ref. 75). [1]Zanubrutinib may also be prioritized for those with TP53 alterations (see ref. 35). Clinical trial options should always be considered.

Table 1. Summary of approved BTK-inhibitors and their activity in Waldenstrom Macroglobulinemia. Listed BTK-inhibitors are approved for WM and/or other indications in any jurisdiction. Clinicians should consult local regulatory approvals and guidelines for their status and use in WM. TN, treatment-naïve; R/R, relapsed or refractory; N/A, data not available; VGPR, very good partial response rate; PFS, progression-free survival; cBTKi, covalent BTK-inhibitor.

Table 2. Impact of mutated CXCR4 on BTK-inhibitor activity for Ibrutinib and Zanubrutinib in Waldenstrom Macroglobulinemia. Comparisons listed are for MYD88 mutated patients. TN, treatment-naïve; R/R, relapsed or refractory; N/A, data not available; VGPR, very good partial response rate; PFS, progression free survival.

Table 3. Guide for Selecting BTK-Inhibitors for the Treatment of Waldenstrom Macroglobulinemia. Preferences are based on availability and weight of clinical data supporting the use of a particular BTK-inhibitor for a select patient and represent the viewpoint and experience of the authors. Supporting data for these recommendations are presented in the text. Listed BTK-inhibitors are approved for WM and/or other indications in any jurisdiction. Clinicians should consult local regulatory approvals and guidelines for their status and use in WM. [1]Zanubrutinib is approved for use as single (320-mg) daily or twice daily (160-mg) administration. [2]ASPEN data demonstrating a higher VGPR rate for zanubrutinib versus ibrutinib is based on twice daily administration of zanubrutinib. [3]Tirabrutinib is only approved in Japan. No randomized data against any other covalent BTK-inhibitor. [4]ASPEN data demonstrating a lower risk of atrial fibrillation and bleeding diathesis for zanubrutinib versus ibrutinib is based on twice daily administration of zanubrutinib. [5]Switchover data supported by a study in CLL patients (see ref.80). [6]Safety data for use in WM remains limited. *Alternatives to BTK-inhibitors for intolerant patients or those with acquired resistance to BTK-inhibitors are presented in the text.*

Table 1.

Study	Patient Population	Agent (s)	N=	Time to Minor/Major Response	ORR/ Major RR	≥VGPR	PFS
Pivotal Study[40]	R/R	Ibrutinib	63	0.9 mo. / 2.0 mos.	91% / 79%	30%	54% (@ 60 months)
INNOVATE Arm C[41]	R/R	Ibrutinib	31	1 mo. / 2 mos.	87% / 77%	29%	40% (@ 60 months)
Phase 2[42]	TN	Ibrutinib	30	0.9 mo. / 1.9 mos.	100% / 87%	30%	76% (@ 48 months)
INNOVATE Arms A, B[43]	TN, R/R	Ibrutinib Rituximab	150	1 mo. / 3 mos.	92% / 76%	31%	68% (@ 54 months)
Phase 2[48]	TN, R/R	Zanubrutinib	77	N/A/ 2.8 mos.	96% / 82%	45%	76% (@ 36 months)
ASPEN Cohort 1 (MYD88^{Mut})[50]	TN, R/R	Ibrutinib	99	1 mo./ 2.9 mos.	94% / 80%	25%	85% (@ 42 months)
	TN, R/R	Zanubrutinib	102	1 mo./ 2.8 mos.	95% / 81%	36%	88% (@ 42 months)
ASPEN Cohort 2 (MYD88^{WT})[50]	TN, R/R	Zanubrutinib	28	1 mo./ 3.0 mos.	78% / 63%	27%	84% (@ 42 months)
Phase 2[52]	TN, R/R	Acalabrutinib	106	1 mo./ N/A	94% / 81%	39%	84% TN / 52% R/R (@ 66 months)
Phase 2[55]	TN, R/R	Tirabrutinib	27	N/A 1.9 (TN) 2.1 (R/R)	96% / 93%	33%	93% (@ 24 months)
Phase 2[61,62]	R/R	Pirtobrutinib	80	N/A / N/A	81% and 67% (prior cBTKi) 88% and 88% (cBTKi naïve)	24% (prior cBTKi) 29% (cBTKi naïve)	57% (@18months for prior cBTKi) N/A for cBTKi naïve.

Table 2.

Study	Patient Population	Agent (s)	Time to Major Response (CXCR^{Mut} vs. CXCR4^{WT})	Major Response Rate (CXCR^{Mut} vs. CXCR4^{WT})	>VGPR (CXCR^{Mut} vs. CXCR4^{WT})	PFS (CXCR^{Mut} vs. CXCR4^{WT})
Pivotal Study[40]	R/R	Ibrutinib	4.7 vs.1.8 mos.	68% vs. 97%	9% vs. 47%	38% vs. 70% (@ 60 months)
INNOVATE Arm C[41]	R/R	Ibrutinib	3.6 vs. 1.0 mos.	71% vs. 88%	14% vs. 41%	18 mo. vs. NR (@ 60 months)
Phase 2[42]	TN	Ibrutinib	7.3 vs. 1.8 mos.	78% vs. 94%	14% vs. 44%	59% vs. 92% (@ 48 months)
INNOVATE Arms A, B[43]	TN, R/R	Ibrutinib Rituximab	3 vs. 2 mos.	77% vs. 81%	23% vs. 41%	63% vs. 72% (@ 54 months)
Phase 2[49]	R/R	Zanubrutinib	N/A	91% vs. 87%	27% vs. 59%	~90% vs.~78% (@ 42 months)
ASPEN Cohort 1[50]	TN, R/R	Ibrutinib	6.6 vs. 2.8 mos.	65% vs. 85%	10% vs. 31%	49% vs. 75% (@ 42 months)
	TN, R/R	Zanubrutinib	3.4 vs. 2.8 mos.	79% vs. 83%	21% vs. 45%	73% vs. 81% (@ 42 months)

Table 3.

	Preference	Alternative
BTK-Inhibitor Options for Initial Therapy		
Convenience/Compliance	Ibrutinib Zanubrutinib[1] Tirabrutinib[1]	Acalabrutinib
Deep IgM Response Needed (i.e. IgM demyelinating neuropathy, cryoglobulinemia, cold agglutinemia)	Zanubrutinib[2]	Ibrutinib Acalabrutinib Tirabrutinib[3]
Bing Neel Syndrome	Ibrutinib Tirabrutinib[3]	Zanubrutinib
History or predisposition to arrythmia	Zanubrutinib[4]	
History or predisposition to bleeding	Zanubrutinib[4]	
Neutropenic or Pancytopenic	Ibrutinib	
$MYD88^{WT}$	Zanubrutinib	
$CXCR4^{Mut}$	Zanubrutinib	Ibrutinib plus rituximab
TP53 Alteration	Zanubrutinib	Ibrutinib
BTK-Inhibitor options for Switchover		
Intolerant to ibrutinib for adverse events other than Atrial Fibrillation	Dose-reduction of Ibrutinib Zanubrutinib Acalabrutinib[5]	Pirtobrutinib[6]
Intolerant to ibrutinib due to Atrial Fibrillation	Zanubrutinib	Pirtobrutinib[6]
Acquired resistance to a covalent BTK-inhibitor	Pirtobrutinib	

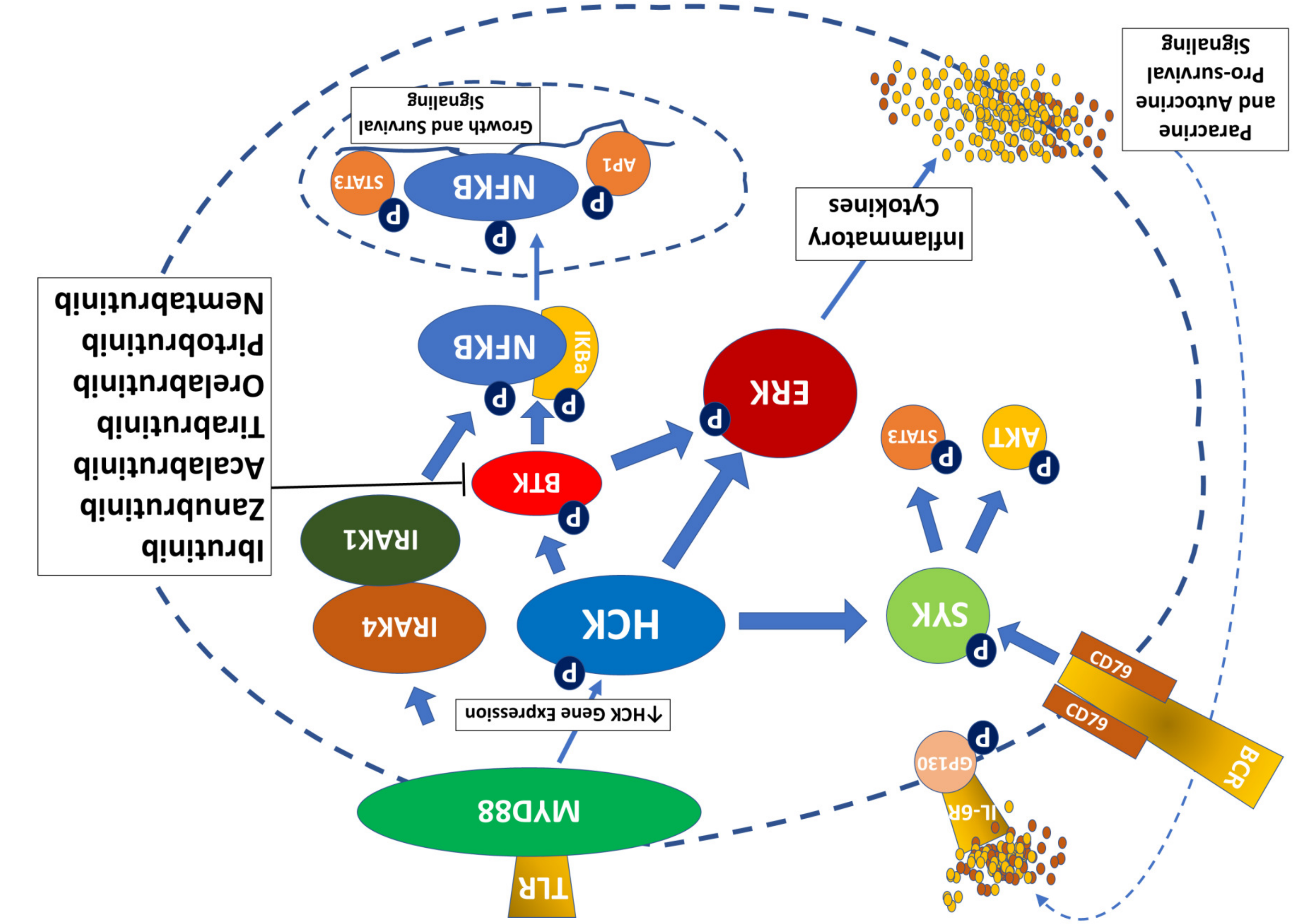

Ibrutinib
Zanubrutinib
Acalabrutinib
Tirabrutinib
Orelabrutinib
Pirtobrutinib
Nemtabrutinib
TLR
MYD88
↑HCK Gene Expression
IRAK4
IRAK1
HCK
BTK
NFKB
IKBa
NFKB
STAT3
AP1
Growth and Survival Signaling
ERK
SYK
STAT3
AKT
Inflammatory Cytokines
GP130
IL-6R
CD79
CD79
BCR
Paracrine and Autocrine Pro-survival Signaling
P

Figure 2.

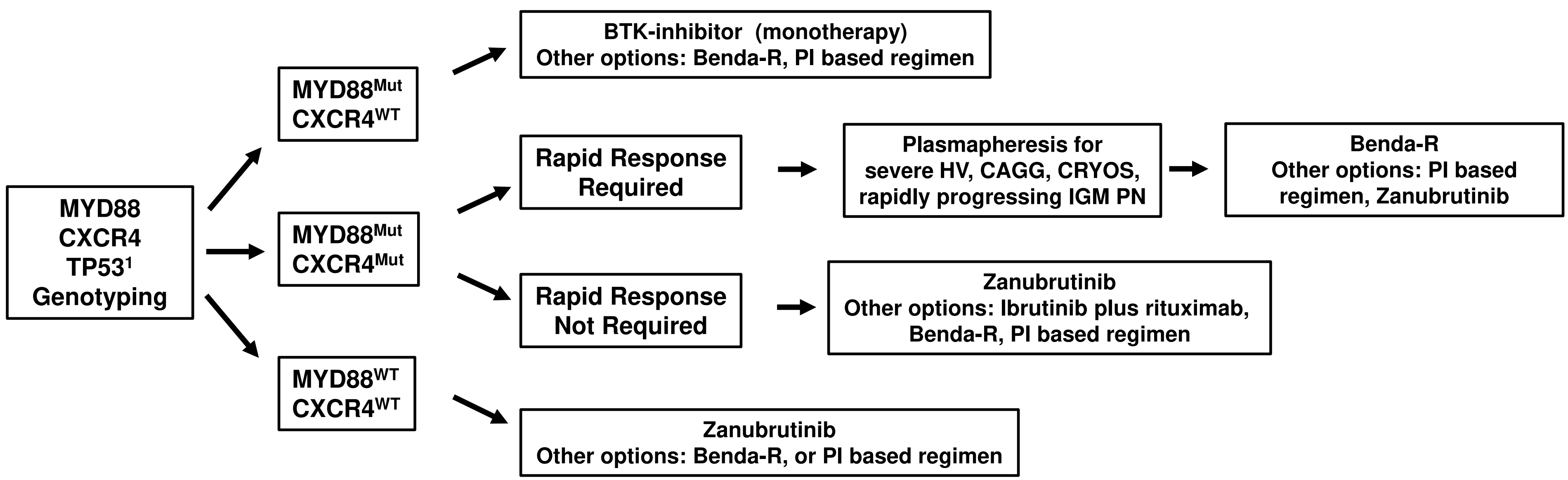
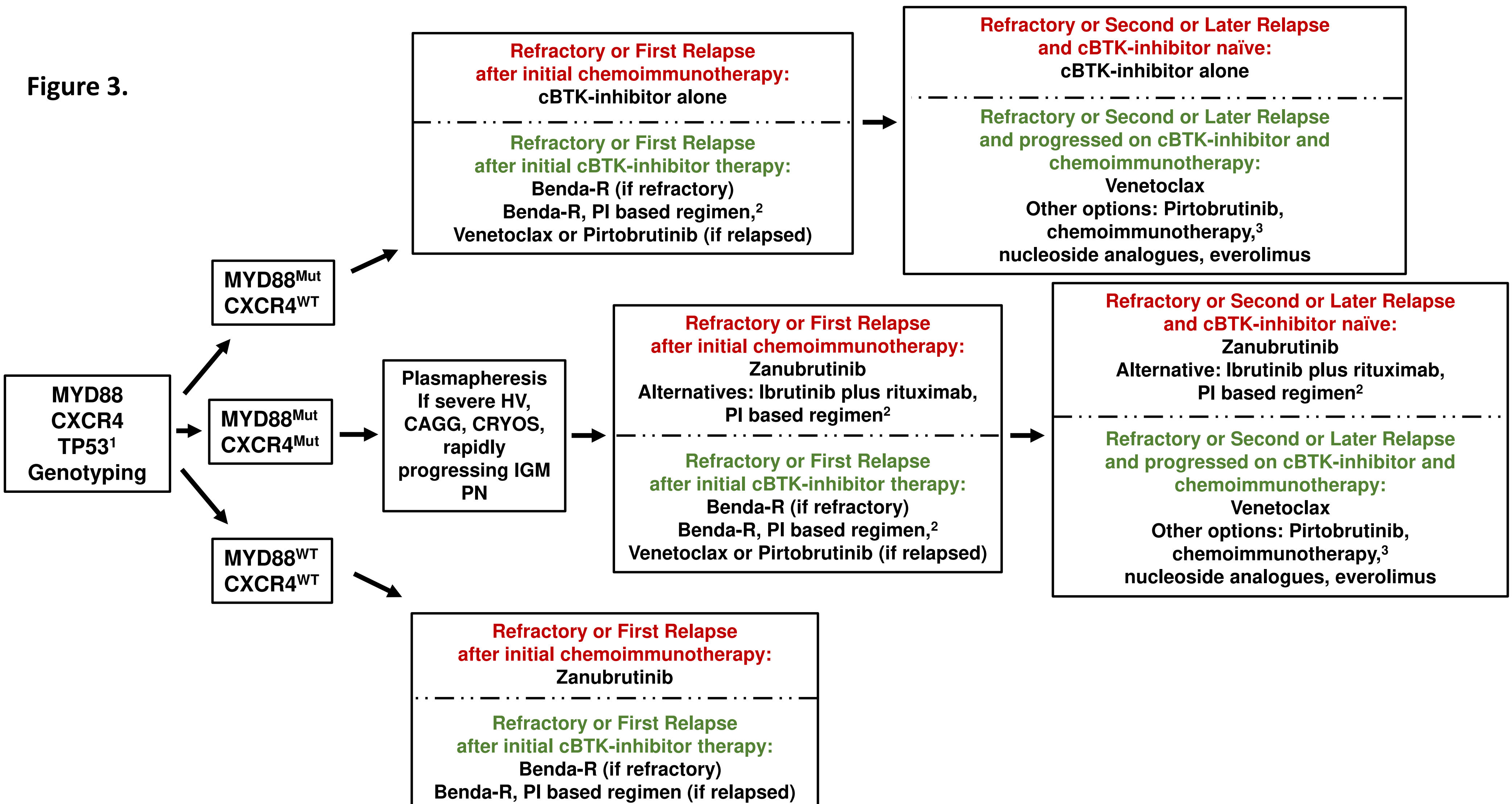
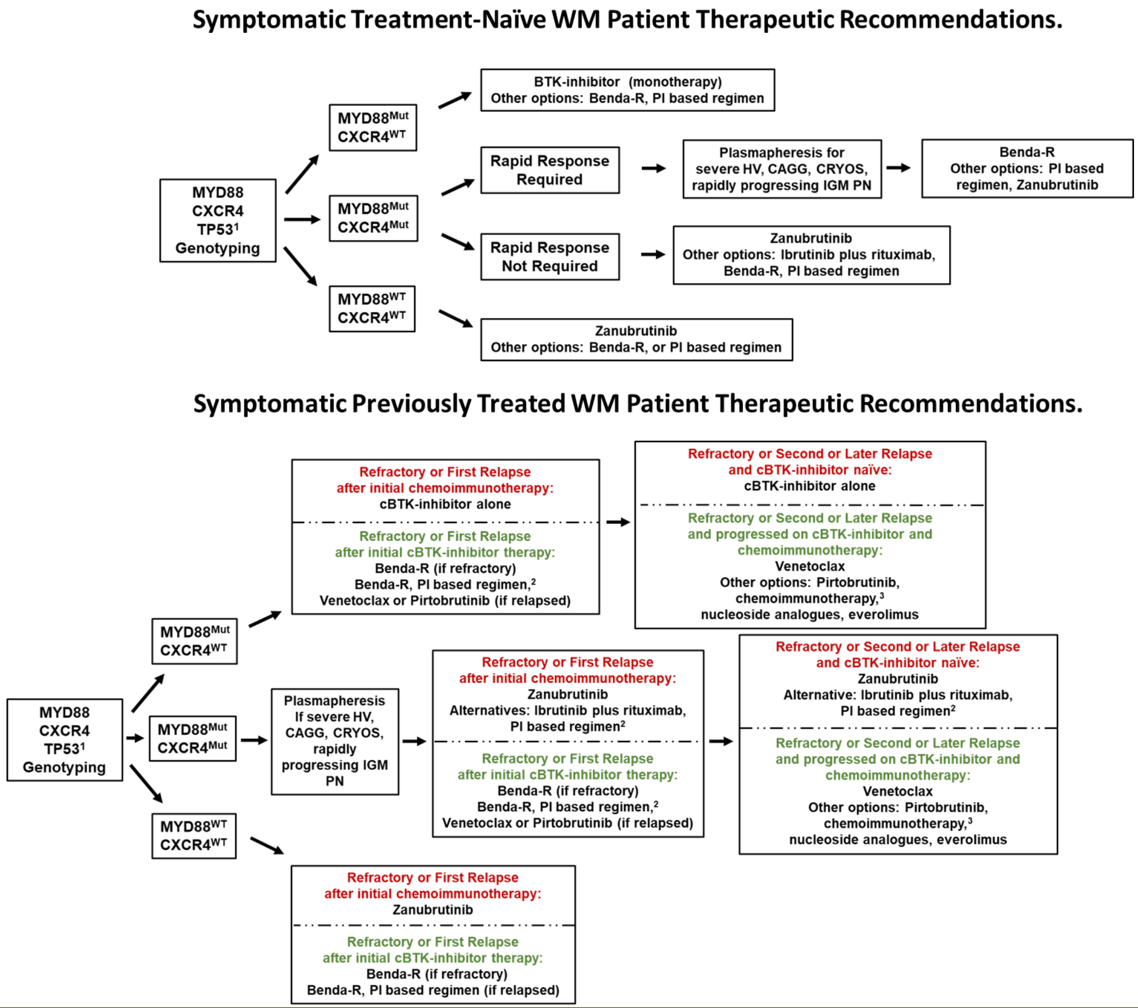

MYD88
CXCR4
TP53¹
Genotyping
MYD88Mut CXCR4WT
MYD88Mut CXCR4Mut
MYD88WT CXCR4WT
BTK-inhibitor (monotherapy)
Other options: Benda-R, PI based regimen
Rapid Response Required
Plasmapheresis for severe HV, CAGG, CRYOS, rapidly progressing IGM PN
Benda-R
Other options: PI based regimen, Zanubrutinib
Rapid Response Not Required
Zanubrutinib
Other options: Ibrutinib plus rituximab, Benda-R, PI based regimen
Zanubrutinib
Other options: Benda-R, or PI based regimen

Figure 3.


MYD88
CXCR4
TP53[1]
Genotyping
MYD88Mut CXCR4WT
MYD88Mut CXCR4Mut
MYD88WT CXCR4WT
Refractory or First Relapse after initial chemoimmunotherapy:
cBTK-inhibitor alone
Refractory or First Relapse after initial cBTK-inhibitor therapy:
Benda-R (if refractory)
Benda-R, PI based regimen,[2]
Venetoclax or Pirtobrutinib (if relapsed)
Refractory or Second or Later Relapse and cBTK-inhibitor naïve:
cBTK-inhibitor alone
Refractory or Second or Later Relapse and progressed on cBTK-inhibitor and chemoimmunotherapy:
Venetoclax
Other options: Pirtobrutinib,
chemoimmunotherapy,[3]
nucleoside analogues, everolimus
Plasmapheresis
If severe HV, CAGG, CRYOS, rapidly progressing IGM PN
Refractory or First Relapse after initial chemoimmunotherapy:
Zanubrutinib
Alternatives: Ibrutinib plus rituximab,
PI based regimen[2]
Refractory or First Relapse after initial cBTK-inhibitor therapy:
Benda-R (if refractory)
Benda-R, PI based regimen,[2]
Venetoclax or Pirtobrutinib (if relapsed)
Refractory or Second or Later Relapse and cBTK-inhibitor naïve:
Zanubrutinib
Alternative: Ibrutinib plus rituximab,
PI based regimen[2]
Refractory or Second or Later Relapse and progressed on cBTK-inhibitor and chemoimmunotherapy:
Venetoclax
Other options: Pirtobrutinib,
chemoimmunotherapy,[3]
nucleoside analogues, everolimus
Refractory or First Relapse after initial chemoimmunotherapy:
Zanubrutinib
Refractory or First Relapse after initial cBTK-inhibitor therapy:
Benda-R (if refractory)
Benda-R, PI based regimen (if relapsed)

How We Use Genomics and BTK-Inhibitors in the Treatment of Waldenström's Macroglobulinemia (WM)



Conclusions: Genomics, disease characteristics, and co-morbidities can be considered in the positioning of BTK-inhibitors in treatment-naïve and previously treated WM patients.

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Blood Visual Abstract