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Identification of robust predictors for ibrutinib response by multi-omics in MYD88 mutated Waldenstrom Macroglobulinemia

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Abstract:

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Blood Advances – Research Letter

Identification of robust predictors for ibrutinib response by multi-omics in MYD88 mutated Waldenstrom Macroglobulinemia

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De-identified data from this study will be available to investigators through email to the corresponding author.

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Waldenstrom's Macroglobulinemia (WM) is characterized by recurring MYD88 (95-97%) and CXCR4 (40%) mutations.[1] Mutated MYD88 ($MYD88^{Mut}$) triggers HCK transcription which enables BTK activation.[2,3] BTK triggers NFKB pro-survival signaling, as well as ERK1/2 activation leading to inflammatory cytokine release supporting autocrine and paracrine mediated growth and survival signaling.[3,4] Over 40 frameshift and nonsense variants have been reported in $CXCR4^{Mut}$ patients.[1,5] $CXCR4^{Mut}$ are sub-clonal to $MYD88^{Mut}$ and show heterogeneous clonality in WM patients.[5] WM cells expressing $CXCR4^{Mut}$ show enhanced BTK, AKT and ERK1/2 signaling in response to the CXCR4 ligand CXCL12.[6,7] These findings supported the development of BTK-inhibitors, and regulatory approval for ibrutinib and zanubrutinib for treating WM.

Differences in treatment outcomes have been reported in WM patients receiving ibrutinib and zanubrutinib by $CXCR4^{Mut}$ status.[8-12] $CXCR4^{Mut}$ WM patients can show a longer time to major response, fewer major and VGPR responses, and/or shorter progression-free survival (PFS) in response to BTK-inhibitors.[8-13] The effect is particularly noteworthy in WM patients treated with ibrutinib who carry nonsense CXCR4 variants.[14] Clonality of $CXCR4^{Mut}$ can also contribute to inferior responses and shorter PFS in WM patients receiving ibrutinib.[15] Detecting $CXCR4^{Mut}$ in WM patients can be problematic, particularly by next generation sequencing (NGS).[15] $CXCR4^{Mut}$ were missed in two-thirds of WM patients using NGS, particularly in those with lower bone marrow (BM) disease involvement and clonality.[16] While presence of $CXCR4^{Mut}$ can predict inferior outcomes with BTK-inhibitors, many wild-type CXCR4 ($CXCR4^{WT}$) patients can also have inferior outcomes. Conversely, some patients with $CXCR4^{Mut}$ disease can have robust responses to BTK-inhibitors. Thus, we sought to identify more robust biomarker(s) to better predict BTK-inhibitor response activity using a multi-omic approach. Such biomarkers may help position BTK-inhibitors relative to other available therapeutics such as bendamustine or proteasome-inhibitor based regimens. For these

studies, we focused on ibrutinib utilizing BM samples obtained in a prospective clinical trial with long-term follow-up in symptomatic, treatment-naïve WM patients.

We used CD19-selected BM samples obtained at baseline from a prospective clinical trial of ibrutinib monotherapy (NCT02604511). Thirty symptomatic, treatment-naïve patients were enrolled whose *MYD88* and *CXCR4* mutation status and treatment outcomes are published.[9] Samples were available for whole exome (WES), RNA (RNA-Seq), and ATAC (ATAC-Seq) sequencing for 23, 27, and 20 patients, respectively. Methods for WES, RNA-Seq, and ATAC-Seq appear in the Supplement. We utilized a previous validated model for PFS prediction in WM patients treated with ibrutinib.[17] ElasticNet regression using the glmnet package was utilized for feature selection with the response variable defined as responders (PR, or better) vs non-responderswith predictors from the relevant combined features of the WES, RNA-Seq and ATAC-Seq analyses.[18] To determine the most robust predictors, this analysis was bootstrapped 500 times and only features identified in > 95% of the bootstraps were considered further. Raw counts were used to run the default DESeq2 pipeline to obtain differentially expressed (DE) ATAC regions and transcripts between response groups using a l2fc > |1| and adjusted p-value (adj p) of < 0.1. Adjusted p-values for FDR (Benjamini-Hochberg) corrected p-values determined by DESeq2 are reported.[19] Survival and survminer R modules were used to perform Kaplan-Meier time to response (TTR) and PFS analyses. The study was approved by our IRB, and written consent was obtained from all patients for their sample use.

The baseline characteristics for the 27 patients appear in **Supplemental Table 1**. All 27 were $MYD88^{Mut}$; 13 were $CXCR4^{Mut}$ of whom 12 had nonsense variants, and one a frameshift mutation. Their median follow-up was 50.1 months. At best response, the overall (minor response or better), major (partial response or better), and very good partial response rates were 100%, 87%, and 30%, respectively. The median time to major response was significantly longer in $CXCR4^{Mut}$ versus $CXCR4^{WT}$ patients (7.3 vs. 1.8 months; p=0.01). The median PFS was not reached. At 4 years, the PFS rate was 76% for all study patients, and was shorter for $CXCR4^{Mut}$ versus $CXCR4^{WT}$ patients

(59% vs. 92%; $p = 0.06$). Among the 27 WM patients included in this study, PFS showed a trend for being longer among those who attained a major versus less than a major response at 6 months (median not reached vs 64.5 months; p=0.10).

We next compared multi-omic findings between the two response groups to identify factors influencing Ibrutinib response. By WES, the only somatic mutation that showed significant association with major response attainment at 6 months was CXCR4. $CXCR4^{Mut}$ patients showed fewer major responses at 6 months (16.7% vs. 93%; p<0.0002) and shorter PFS (36.4 months vs. not reached; p=0.046) versus $CXCR4^{WT}$ patients. While the presence of $CXCR4^{Mut}$ associated with non-response status at 6 months and shorter PFS, 3 of 13 (23%) $CXCR4^{Mut}$ patients were major responders at 6 months. By RNA-Seq, 64 differentially expressed (DE) genes were identified that included WNK2 (adj p=0.00005), DUSP22 (adj p=0.0008), GPER1 (adj p=0.0008) as the top hits expressed in $CXCR4^{WT}$ and $CXCR4^{Mut}$ patients who attained a major response at 6 months (**Supplemental Table 2**). Other top DE genes identified by RNA-Seq in major responders included OSBPL3, PRDM15, GPLD1, GPR18, NEB, and DDR1 (**Supplemental Table 2**). ATAC-Seq revealed one differentially open genomic region at chr12 which mapped to the 5' region of the KIF21A locus. This region was found to be more open or accessible in the 16 WM patients who attained a major response at 6 months. KIF21A was also a significant gene in the DE RNA-Seq data as being upregulated for those who attained a major response at 6 months (**Supplemental Tables 2,3)**.

Elasticnet analysis, using the significant hits from WES, RNA and ATAC-Seq analysis, identified a set of nineteen RNA transcripts that best distinguished major and non-major 6-month responders. Notably, many of these are known regulators of ERK1/2 signaling including WNK2, DUSP22, GPR18, GPER1, and PRDM15 (**Figure 1**).[20-24] The suppression of ERK1/2 signaling has emerged as an important signaling pathway for ibrutinib response, as well as acquired resistance related to its role in inflammatory cytokine release and microenvironmental support.[4,6] A time to response analysis revealed that the attainment of a major response at 6 months was most strongly

associated with baseline expression of DUSP22, GPER1, CHST15, GPR18, ACVR2B, KIF21A and WNK2 (**Supplemental Table 4**). To identify which of these candidates might also influence long term response, a PFS analysis was performed. At baseline, only GPR18 (p=0.013) significantly impacted PFS, with low GPR18 transcript levels predicting longer PFS. A trend for longer PFS was also notable in those with high versus low levels of WNK2 expression (p=0.097). Differential gene expression of CHST15, KIF21A, PRDM15, DUSP22, KIF21A also showed an impact on PFS, but were not statistically significant. The small sample size of our study may have contributed to these findings (**Figure 2**).

In summary, by use of comprehensive multi-omics, we identified putative biomarkers for clinical investigation to predict ibrutinib major responders at 6 months, TTR and PFS outcomes in $MYD88^{Mut}$ WM patients. Low baseline expression of GPR18, as well as high levels of WNK2 showed the strongest associations with major response attainment at 6 months, shorter TTR and longer PFS in this study. In addition, differential gene expression of CHST15, KIF21A, PRDM15, DUSP22, S100A6 and KIF21A also associated with major response attainment at 6 months and TTR but showed more marginal association with predicting PFS outcomes to ibrutinib. Many of these biomarkers including GPR18 and WNK2 are known modulators of ERK1/2 signaling.[20-24] Further validation of these findings in a larger cohort will be required, as well as their potential use as predictors with other covalent and non-covalent BTK-inhibitors. The applied use of such validated biomarkers may better BTK-inhibitors over other available treatment options in WM. The findings may also be relevant for other B-cell malignancies in which BTK-inhibitors are used. Our studies thereby provide a framework for the clinical investigation of novel, multi-omic identified genes as predictive biomarkers for BTK-inhibitors in WM.

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Author Contributions

SPT, KR, and ZRH designed the study. JJC, SS, ARB, CAF and SPT provided patient care. KM, CJP provided study administrative support. JNG coordinated clinical trial and sample collection. XL, AK, SL, NT, MLG performed sample processing, genomic and translational studies. KR and ZRH performed the genomic analyses. KR, ZRH, SPT, and JJC analyzed the data. KR and SPT drafted the initial manuscript. KK and JLW performed statistical analyses. All the authors critically reviewed the manuscript and approved the final version for submission.

Declaration of Interests

JJC received research funds from Abbvie, AstraZeneca, Beigene, Cellectar, LOXO, Pharmacyclics, TG Therapeutics, and honoraria from Abbvie, Beigene, Cellectar, Kite, LOXO, Janssen, Pharmacyclics, and Roche Pharmaceuticals. SS received research funding and/or consulting fees from Beigene, Cellectar Biosciences, and ADC

Therapeutics. ARB received research funds and/or honoraria from Adaptive, Beigene, CSL Behring, Genzyme, Karyopharm, Pharmacyclics, and Sanofi. SPT received research funds and/or consulting fees from Abbvie/Pharmacyclics, Janssen Pharmaceuticals, Beigene, BMS, and Eli Lilly. All other authors have no conflicts of interest to disclose.

References

1. Hunter ZR, Xu L, Yang G, et al. The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. Blood 2014; 123(11):1637-46.

2. Yang G, Zhou Y, Liu X, et al. A mutation in MYD88 (L265P) supports the survival of lymphoplasmacytic cells by activation of Bruton tyrosine kinase in Waldenström macroglobulinemia. Blood. 2013 Aug 15;122(7):1222-32.

3. Yang G, Buhrlage S, Tan L, et al. HCK is a survival determinant transactivated by mutated MYD88, and a direct target of ibrutinib. Blood 2016; 127(25): 3237–3252.

4. Chen JG, Liu X, Munshi M, Xu L, et al. $BTK^{Cys481Ser}$ drives ibrutinib resistance via ERK1/2 and protects $BTK^{wild\text{-}type}$ MYD88-mutated cells by a paracrine mechanism. Blood. 2018;131(18):2047-2059.

5. Xu L, Hunter ZR, Tsakmaklis N, et al. Clonal architecture of CXCR4 WHIM-like mutations in Waldenström Macroglobulinaemia. Br J Haematol 2016;172(5):735-44.

6. Cao Y, Hunter ZR, Liu X, et al. The WHIM-like CXCR4(S338X) somatic mutation activates AKT and ERK, and promotes resistance to ibrutinib and other agents used in the treatment of Waldenstrom's Macroglobulinemia. Leukemia. 2015; 29(1):169-76.

7. Cao Y, Hunter ZR, Liu X, et al. CXCR4 WHIM-like frameshift and nonsense mutations promote ibrutinib resistance but do not supplant MYD88(L265P) -directed survival signalling in Waldenström macroglobulinaemia cells. Br J Haematol. 2015;168(5):701-7.

8. Treon SP, Meid K, Gustine J, et al. Long-Term Follow-Up of Ibrutinib Monotherapy in Symptomatic, Previously Treated Patients With Waldenström Macroglobulinemia. J Clin Oncol. 2021; 39(6):565-575.

9. Castillo JJ, Meid K, Gustine JN, et al. Long-term follow-up of ibrutinib monotherapy in treatment-naive patients with Waldenstrom macroglobulinemia..Leukemia 2022;36(2):532-539.

10. Trotman J, Buske C, Tedeschi A, et al. Single-Agent Ibrutinib for Rituximab-Refractory Waldenström Macroglobulinemia: Final Analysis of the Substudy of the Phase III InnovateTM Trial. Clin. Cancer Res. 2021; 27(21):5793-5800.

11. Trotman J, Opat S, Gottlieb D, et al. Zanubrutinib for the treatment of patients with Waldenström macroglobulinemia: 3 years of follow-up. Blood 2020; 136(18):2027-2037.

12. Dimopoulos MA, Opat S, D'Sa, et al. ASPEN biomarker analysis: Response to BTK inhibitor treatment in patients with Waldenström Macroglobulinemia harboring CXCR4, TP53, and TERT mutations. Proc. 11th Internation Workshop on Waldenstrom's Macroglobulinemia 2020; Abstract WM041.

13. Dimopoulos MA, Opat S, D'Sa S, et al. Zanubrutinib Versus Ibrutinib in Symptomatic Waldenstrom¨Macroglobulinemia: Final Analysis From the Randomized Phase III ASPEN Study. J. Clin. Oncol. 2023; ;JCO2202830. doi: 10.1200/JCO.22.02830. Online ahead of print.

14. Castillo JJ, Xu L, Gustine JN, et al. CXCR4 mutation subtypes impact response and survival outcomes in patients with Waldenström macroglobulinaemia treated with ibrutinib. Br J Haematol. 2019;187(3):356-363.

15. Gustine JN, Xu L, Yang G, et al. Bone marrow involvement and subclonal diversity impairs detection of mutated CXCR4 by diagnostic next-generation sequencing in Waldenström macroglobulinaemia. Br J Haematol. 2021;194(4):730-733.

16. Gustine JN, Xu L, Tsakmaklis N, et al. $CXCR4^{S338X}$ clonality is an important determinant of ibrutinib outcomes in patients with Waldenström macroglobulinemia. Blood Adv. 2019; 3(19):2800-2803.

17. Castillo JJ, Abeykoon JP, Gustine JN, et al. Partial response or better at six months is prognostic of superior progression-free survival in Waldenström macroglobulinaemia patients treated with ibrutinib. Br J Haematol. 2021;192(3):542-550.

18. Friedman J, Hastie T, Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent [Internet]. Vol. 33, Journal of Statistical Software. 2010. p. 1–22.

19. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**, 550 (2014). https://doi.org/10.1186/s13059-014-0550-8.

20. Finlay DB, Joseph WR, Grimsley NL, Glass M. GPR18 undergoes a high degree of constitutive trafficking but is unresponsive to N-Arachidonoyl Glycine. PeerJ. 2016; 21:4:e1835.

21. Guerrera ML, Hunter ZR, Richardson K, et al. Aberrant Expression of Spliced WNK2 Is an Early Event in MYD88 Mutated WM That Activates ERK1/2 and Supports Tumor Growth. Proc. Am. Soc. Hematol. 2023; Abstract 3303.

22. Patterson KI, Brummer T, O'Brien PM, Daly RJ. Dual-specificity phosphatases: critical regulators with diverse cellular targets. Biochem J. 2009; 418(3):475-489.

23. De Valdivia G, Broselid S, Kahn R, et al. G protein-coupled estrogen receptor 1 (GPER1)/GPR30 increases ERK1/2 activity through PDZ motif-dependent and -independent mechanisms. J. Biol. Chem. 2017; 292(24):9932-9943.

24. Mzoughi S, Zhang J, Hequet D, et al. PRDM15 safeguards naive pluripotency by transcriptionally regulating WNT and MAPK-ERK signaling. Nat. Genet. 2017; 49(9):1354-1363.

Legends

Figure 1. Top gene candidates from ElasticNet analysis distinguishing response groups. **ElasticNet** heatmap for baseline expressed genes in MYD88 mutated WM patients who received ibrutinib monotherapy on a clinical trial, and who attained a major response (green) or no major response (gray) at 6 months. The heatmap shows the scaled VST gene expression levels selected by ElasticNet which best predicts differences observed in response between the two groups. Each patient's CXCR4 mutation status is shown above the heatmap; WT – Wild Type, FS – Frameshift mutation, NS – Nonsense mutation**.**

Figure 2. Kaplan-Meier curves for progression-free survival and risk tables for A) GPR18, B) WNK2, C) CHST15, D) PRDM15, E) GPER1, F) DUSP22 and G) KIF21A. Tables show progression for high and low expressors of each candidate. Risk tables show progression data for each group.

Figure 1

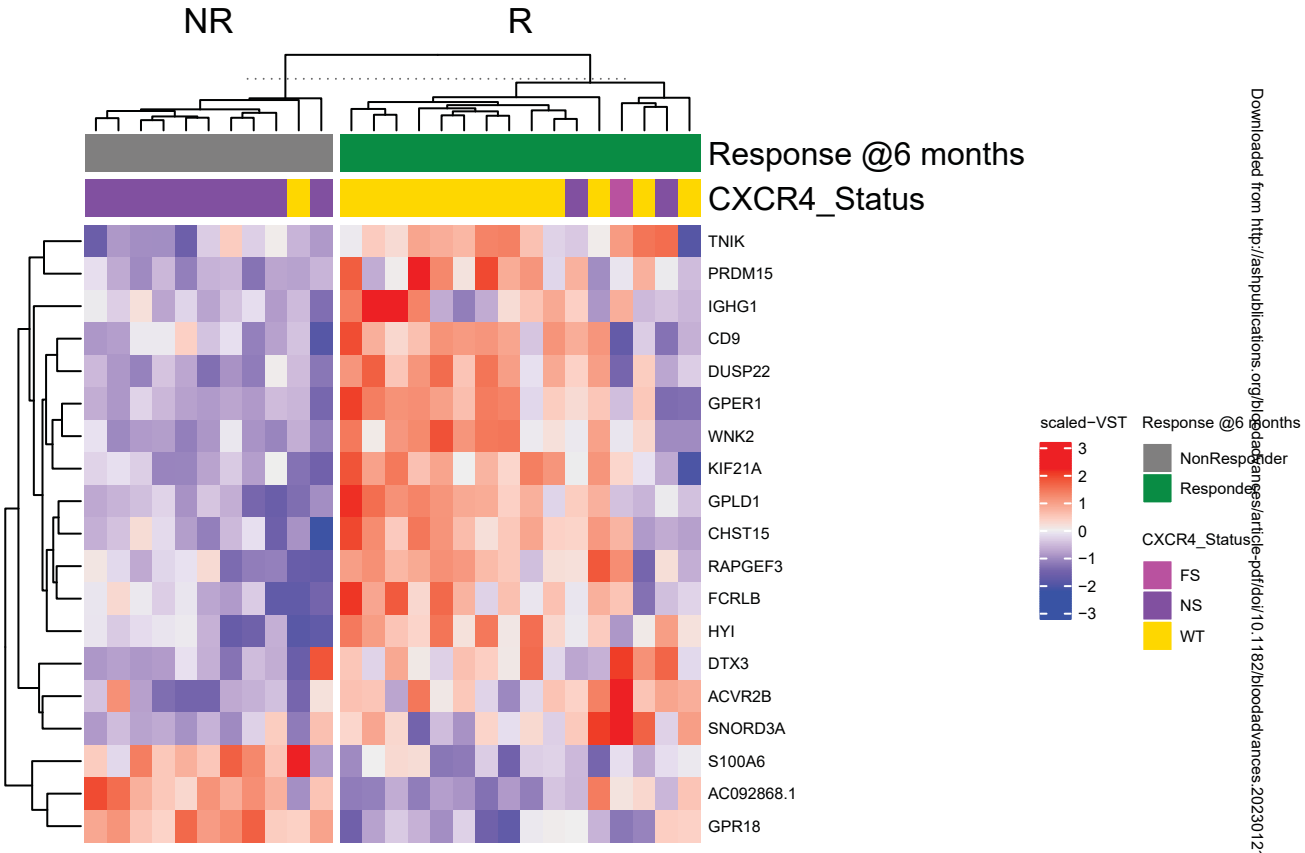

Figure 2