Genomic Analysis of Ibrutinib Resistance in Waldenström’s Macroglobulinemia


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Abstract

The Brouton tyrosine kinase (BTK) inhibitor ibrutinib is the first approved therapy for Waldenström’s macroglobulinemia (WM), and is highly active in both treatment-naive and relapsing or refractory patients. Although ibrutinib is highly active in WM patients, disease progression can occur. Acquired mutations in BTK at the binding site of ibrutinib (Cys481), or in the protein immediately downstream of BTK, the phospholipase PLCG2, have been identified in half of progressing WM patients on ibrutinib (Xu et al, Blood 2017). However, not all ibrutinib resistant patients harbor these alterations, suggesting that there are other causes of disease progression on ibrutinib.

The aim of this study was to identify alternative molecular mechanisms that can drive ibrutinib resistance.

Methods

Background

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Small variants and indels

Progression samples showed a high proportion of acquired mutations (median 85%, range 79%–88%), compared to persistent mutations, i.e., conserved from the baseline (median 15%, range 12%–21%). In addition, most persistent alterations maintained the same allele frequency (median 12%), almost double that of the acquired mutations that were more subclonal (median 6.5%, p<0.01).

We found BTK mutations in three out of the five patients (two had the p.C481S in the kinase domain and the other a p.T62A in the PH domain). These patients also harbored alterations in other genes related to the B-cell receptor pathway, such as PLCG2 p.Y495H; CD79B p.D33Y; LYN p.A252Stop and p.A139T.

In patients without BTK mutations, we identified several mutations with a putative role in the emergence of ibrutinib resistance including ITCH (p.A646S, n=2), an E3 protein-ubiquitin ligase whose substrates are CXCR4, LYN or SYK; RNF19B (p.R30G, n=2), another E3 protein-ubiquitin ligase involved in the cytoplolytic activity of natural killer cells and cytotoxic T-cells; FCRL3 (p.E694Q, n=1), an Fc receptor-like 3 that modulates innate immune signaling in B cells; negative regulators of Toll-like receptors signaling such as DOK2 (p.Y345Stop, n=1), and TOLLIP (p.M242R and p.R228H, n=1 for each mutation); and BIRC2 (p.A505E, n=1), an apoptosis inhibitor that regulates the activation of the alternative NF-kB and MAPK signaling pathways. The loss of a truncating SYK mutation (*) in a phosphorylation site (p.Y525Stop) that was present at baseline was also observed in one patient (Figure 1).

Copy number alterations

Copy number analysis identified deletions in chromosome 6q in all patients, becoming homozygous in two of them (IBR2 and IBR4) at progression. In another patient (IBR3), the homozygous deletion was already present at baseline in a third of the tumor population, and increased at relapse. No other recurrent copy number alterations were detected, though in two patients multiple deletions (IBR4) or gains (IBR5) involving large chromosomal regions were observed (Figure 2).

Conclusions

Our findings depict uniform deletion of 6q, including homozygous loss of 6q, which encompasses key regulators of BTK, BCL2, and NFkB; as well as emergence of novel gene mutations, including recurring mutations in E3 ubiquitin ligases, innate immune signaling, and Toll receptor/MYD88 pathway regulators as significant genomic alterations that accompany disease progression on ibrutinib in WM patients.

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Figure 1. Summary of alterations potentially involved in ibrutinib resistance in Waldenström's macroglobulinemia. Patients (represented by lines) are displayed according to time to progression (TTP). For IBR1, IBR2 and IBR3, we can see the proportion of persistent mutations (horizontal bars) respect to the mutations present at only one of the time points (vertical bars). Next to the lines, most remarkable alterations are shown for each patient. BTK mutations on the left, and other alterations that could be involved in the emergence of ibrutinib resistance.

Figure 2. Copy number abnormalities of ibrutinib resistant Waldenström's macroglobulinemia. Left panels represent the copy number profile of each patient at baseline and progression. Panels on the right show the corresponding B-cell allele frequency. Losses are colored in blue, gains in red, and green corresponds to normal copy number. Biallelic deletion of chromosome 6q (highlighted in orange) was present in 3/5 (60%) patients at progression. Del6q was also frequent in this cohort of patients (4/5 cases), compared to conventional WM (7.1%).

**Data were analyzed following the Broad Institute's GATK Best Practice Guidelines.

Small variants → Strelka and MuTect2

Copy number alterations → Control-FREEC

** Five WM patients who progressed on ibrutinib (IBR1-IBR5):

- IBR1-IBR3 → Tumor DNA samples at diagnosis, relapse, and germline DNA
- IBR4-IBR5 → Relapse and germline samples

** Tumor DNA: CD19-selected bone marrow mononuclear cells

** Germline DNA: Non-CD19 cells from peripheral blood

** Whole exome sequencing

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