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What are the mechanisms of resistance to ibrutinib in WM?

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Acquired resistance to ibrutinib is increasingly being recognized with prolonged therapy in chronic lymphocytic leukemia (CLL),[Woyach et.al., *N Engl J Med.* 2014; Furman et.al., *N Engl J Med.* 2014] mantle cell lymphoma (MCL),[Chiron et.al., *Cancer Discov.* 2014] and WM patients[Xu et.al., *Blood.* 2017] due to somatic mutations affecting BTK^{Cys481} that abrogate BTK-ibrutinib binding. Among these are c.1635G>C and c.1634T>A mutations resulting in a p.Cys481Ser ($BTK^{Cys481Ser}$) that is frequently found in CLL, MCL, and WM patients. BTK^{Cys481} mutations are usually sub-clonal, and can be detected up to a year before clinical progression.[Burger et.al., *Nat Commun.* 2016] To clarify how BTK^{Cys481} mutations promote ibrutinib resistance, we engineered $BTK^{Cys481Ser}$ and BTK^{WT} expressing MYD88 mutated WM and ABC DLBCL cells that were previously ibrutinib sensitive. Using these cell model, we observed re-activation of BTK–PLCγ2–ERK1/2 signaling in $BTK^{Cys481Ser}$ but not BTK^{WT} cells, and ibrutinib resistance in the former. Use of ERK1/2 inhibitors triggered apoptosis in $BTK^{Cys481Ser}$ expressing cells, and showed synergistic cytotoxicity with ibrutinib. Furthermore, using a multiplex cytokine assay, we found ERK1/2 re-activation in ibrutinib treated $BTK^{Cys481Ser}$ cells was accompanied by release of many pro-survival cytokines, including IL-6 and IL-10 that were also blocked by ERK1/2 inhibition. To clarify if cytokine release by ibrutinib treated $BTK^{Cys481Ser}$ cells could protect BTK^{WT} MYD88 mutated malignant cells, we used a TranswellTM co-culture system, and showed that non-transduced BTK^{WT} MYD88 mutated WM or ABC DLBCL cells were rescued from ibrutinib induced killing when co-cultured with $BTK^{Cys481Ser}$ but not their BTK^{WT} expressing counterparts. Use of IL-6 and/or IL-10 blocking antibodies abolished the protective effect conferred on non-transduced BTK^{WT} by co-culture with $BTK^{Cys481Ser}$ expressing WM or ABC DLBCL cell counterparts. Rebound of IL-6 and IL-10 serum levels also accompanied disease progression in WM patients with acquired BTK^{Cys481} mutations. Our findings show that the $BTK^{Cys481Ser}$ mutation drives ibrutinib resistance in MYD88 mutated WM and ABC DLBCL cells through re-activation of ERK1/2 activation, and can confer a protective effect on BTK^{WT} cells through a paracrine mechanism. Our findings show that the $BTK^{Cys481Ser}$ mutation drives ibrutinib resistance in MYD88 mutated WM and ABC DLBCL cells through re-activation of ERK1/2 activation, and can confer a protective effect on BTK^{WT} cells through a paracrine mechanism. The findings provide evidence for tumor clonal cooperativity in mediating ibrutinib resistance, and for possible treatment options by using ERK1/2 inhibitors or using IL-6 and/or IL-10 blocking antibodies in combination with ibrutinib to overcome ibrutinib resistance in WM.