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622.LYMPHOMAS: TRANSLATIONAL-NON-GENETIC

JAK1/2 Inhibition Modulates Key Markers of Lymphomagenesis in 9p24.1 Amplified Lymphoma, Priming for Enhanced Antigenicity

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Background

Primary Mediastinal B-cell Lymphoma (PMBCL) and Hodgkin Lymphoma (HL) are frequently defined by chromosome 9p24.1 amplification, leading to upregulation of **PDL-1/-2** and **JAK2.** JAK2, in addition to its known role in the JAK/STAT pathway, has also been shown to act as an epigenetic modifier by phosphorylation of H3Y41, enabling an euchromatin state and expression of MYC. When these epigenetic modifications are coupled with upregulation of PDL-1/-2, the 9p amplicon leads to immune escape, unchecked cell proliferation, and tumorigenesis. **We hypothesize that if deranged histone modification and amplified expression of PD-1 ligands cooperate to drive cell growth in 9p amplified PMBCL and HL, then dual targeting with JAK inhibitor and immune checkpoint blockade will lead to altered gene expression and enhanced immunogenicity inducing synergistic cell death.** The following abstract demonstrates preliminary data using ruxolintib monotherapy in cell lines, with goal of combining immunotherapy in mouse models.

Methods

In order to understand how ruxolitinib modulates key drivers of lymphomagenesis in 9p24.1 amplified lymphoma, human lymphoma cell lines **with 9p amplification**, (n=2): Karpas1106p (PMBCL) and L540 (HL)were compared to cell lines **lacking 9p24.1 amplification**, (n=2): Farage (PMBCL) and U2940 (PMBCL). All samples were treated for 3- and 6-day intervals. The IC50 concentrations were established for ruxolintib using Cell TiterGlo assay: Karpas1106p 5.72 μM, L540 22 μM, Farage 29.46 μM, and U2940 32.99 μM. All cells were treated for 48 hours with control or ruxolitnib and probed by western blot, flow cytometry, and RNA-sequencing. The expression of key determinants of survival including JAK/STAT, pro- and anti-apoptotic proteins (SOCS1 and MYC, respectively) and MHCII were measured by western blot. PD-1/-2 ligands were measured by flow cytometry. Transcriptomic changes were evaluated by RNA-seq and data was presented as Log $_2$ fold change compared to vehicle controls.

Results

Pharmacodynamic effects were most prominent in the **9p amplified Karpas1106p** cell line demonstrating increased markers of immunogenicity by protein quantification: **increased CIITA** by 3.5-fold (SEM ± 1.15) and **increased MHCII** by 2.67-fold (SEM ± 0.33). Expression of tumor suppressor **SOCS1 was increased** by 2.67-fold (SEM ± 0.68) and expression of oncogene **MYC was decreased** by 6.06-fold (SEM ± 4.66). The 9p amplified L540 line similarly demonstrated increased CIITA by 3.22-fold (SEM ± 1.59) and decreased MYC by 2.69-fold (SEM ± 0.65), however no significant change in MHCII or SOCS1 was observed. Significant reduction of STAT3p and/or STAT6p was demonstrated across all cell lines ($p < 0.01$). **Cell lines without 9p amplification**, Farage and U2940, **did not demonstrate consistent increase nor decrease** in markers of immunogenicity or oncogenes, suggesting ruxolitinib preferentially modulates downstream markers of survival and immune recognition driven by the 9p amplicon.

Flow cytometry demonstrates significantly reduced expression of PDL-1 ($p = 0.01$) and PDL-2 ($p = 0.001$) in 9p amplified cell lines (Karpas1106p and L540), and non-amplified cell lines (Farage and U2940), as shown in **Figure 1**. Down-regulation of PDL-1/-2 was more prominent in the 9p amplified cell lines compared to those without 9p amplification ($p = 0.055$ and $p =$

0.18, respectively), suggesting that **ruxolitinib may prime for increased immunogenicity by reducing PDL-1/-2, especially in 9p amplified cell lines.**

RNA-seq of the 9p amplified Karpas1106p cell line demonstrated increased MHCII: HLA-DQ (1.12, $p < 0.001$), HLA-DO (0.70, $p < 0.001$), HLA DM (0.14, $p = 0.001$), and decreased PDL-1 (-1.04, $p < 0.009$), PDL-2 (-1.70, $p < 0.001$), C-MYC (-0.95, $p < 0.001$), and STAT1/3/4 (all $p < 0.001$). Gene-ontology analysis demonstrated increased lymphocyte differentiation ($p < 0.001$) and immune response activating pathways ($p = 0.003$) (**Fig. 2).**

Conclusion

Altogether, data suggests that ruxolitinib enhances immunogenicity primarily in 9p amplified cell lines and supports the hypothesis that ruxolitinib may prime for lymphoma cell death when combined with immunotherapy. These findings provide a foundation for in vivo assessment in humanized mouse models treated with combination anti-PDL-1 and JAK1/2 inhibitor, targeting tumorigenesis driven by 9p amplification.

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Figure 1: Ruxolitnib Suppresses PDL-1/2 Expression In Lymphoma Cell Lines

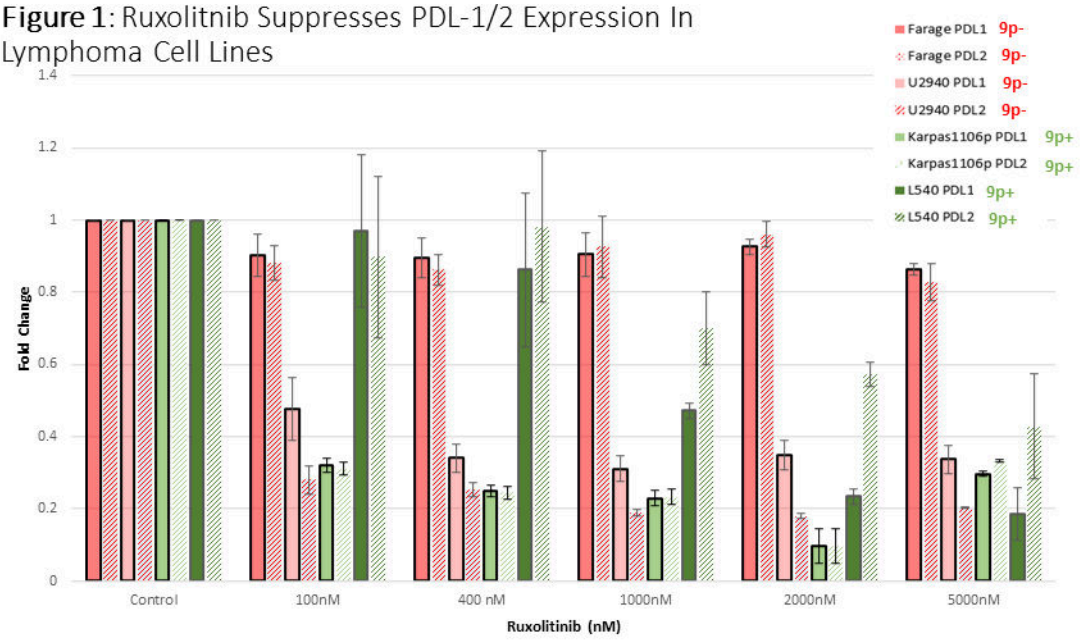

Figure 1: Expression of PDL1/2 ligands is significantly reduced in 9p amplified cell lines. Cells treated at concentration of [500k] per flask using increasing doses of ruxolintib: 0, 100, 400, 1000, 2000, 5000nM. The L540 cell line required higher doses of ruxolitinib: 0, 1000, 2000, 5000, 8000, 1000nM. Data shown represents treatment for 72 hours.

Figure 2: Ruxolitnib Modulates Key Determinants of Lymphomagenesis

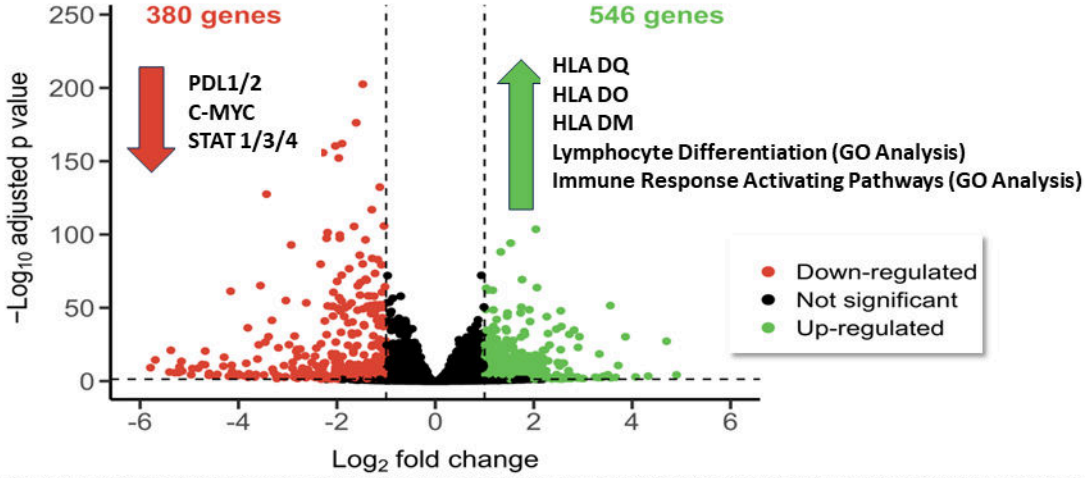

Figure 2: RNA-sequencing performed on Karpas1106p (9p+) cells treated with ruxolintib 1000nm for 48-hours. Volcano plot depicting differentially expressed genes (adjusted p value < 0.05), with down-regulated genes designated as having log2 fold change < -1, and up-regulated genes with a log2 fold change > 1.

Figure 1

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