Cooperative Networks Between MYC and XPO1 Associated with Decreased T-Cell Presence and a Depleted Tumor Microenvironment may be Addressed by the Synergistic Combination of AZD4573 and Selinexor

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Background

- Diffuse Large B-cell Lymphoma (DLBCL) is the most commonly diagnosed lymphoma, accounting for ~90,000 cases in the USA each year.
- Frontline R-CHOP immunochemotherapy leads to long-term remission in ~50% of cases.
- Patients with refractory tumors or those that eventually relapse from treatment face a dismal outlook but have found improved response rates since the advent of CAR-T.
- Emerging studies indicate that alterations to the *MYC* oncogene portend an inferior outcome when treated with CAR-T, specifically citing the facilitation of a "Cold" or "Depleted" immune microenvironment alongside a myriad of cell survival and proliferation pathways.
- Alterations to the XPO1 exportin oncogene have showcased a similar profile, additionally facilitating MYC RNA transition from the nucleus for expression.
- Two emerging precision treatments include inhibition of the negative CDK9 oncogene, a downstream target of MYC, via AZD4375 and XPO1 inhibition via Selinexor.

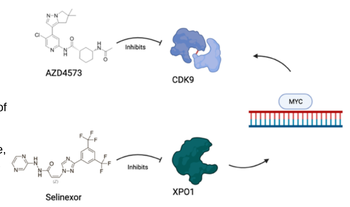

Figure 1

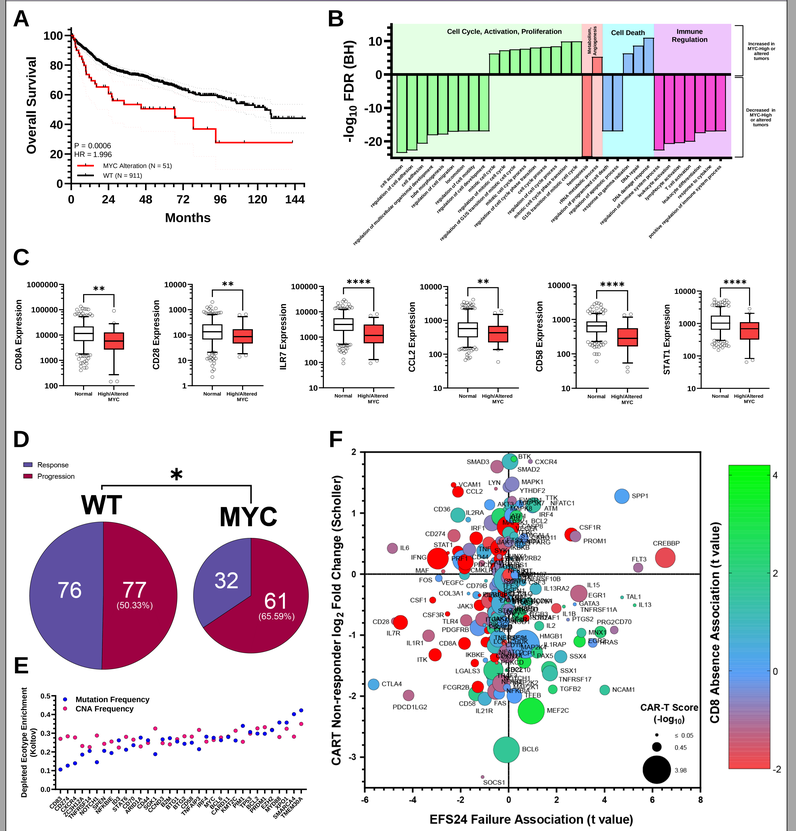

Figure 1: *MYC* alterations are associated with inferior de-novo survival, loss of immune regulation, and inferior CAR-T response. (A) The presence of *MYC* alterations is associated with inferior responses to frontline R-CHOP treatment in the Reddy et. al. 2017 analysis. Dotted lines represent 95% confidence intervals. (B) High expression or alteration of MYC is associated with intracellular and microenvironment changes. Patients with DNA alterations to *MYC* or which expression 1.0 standard deviations above the average within the Xu-Monette et. al. 2020 were analyzed. The study consisted of 418 patients, 49 of which bore High/Altered MYC, across 1397 genes. Genes with FDR < 0 were analyzed for gains (94 genes) and losses (178 genes). Gene pathway ontology analysis was completed using ToppFunn functional enrichment tools from Cincinnati Children's Hospital. $-\log_{10}$ B&H FDR values were oriented as a negative value if the pathway was lost or positive if gained and were restricted to Biological Function ontologies with a minimum 5.0 value. (C) Greater MYC is associated with the loss of key immunoregulation genes. The top 5% values in either direction are displayed individually. Significance was determined using a Welch's t test. (D) The presence of *MYC* alterations or translocation is associated with inferior responses to CD19-directed CAR-T treatments. Pooled data from the Shouval, Swordler, Scholler, and Jain cohorts is analyzed. Patients were categorized by response vs. non-response and by the presence of alterations to *MYC*. Significance was determined using Fisher's test contingency analysis. (E) MYC and XPO1 gene alterations are among those enriched in the Depleted tumor microenvironment. Data from the Koltov et. al. 2021 analysis are compared on the differential basis of their DPE subtype enrichment for both mutation of Copy Number Alteration (CNA) of the gene. (F) Genes promoting CD8 T-cell responses are lost in patients that fail to respond to R-CHOP and to CAR-T. Data from Scholler et. al. 2022 are integrated alongside those from Xu-Monette et. al. 2020 and Dufva et. al. 2020. CD8 absence was calculated after GEDIT deconvolution.

Figure 2

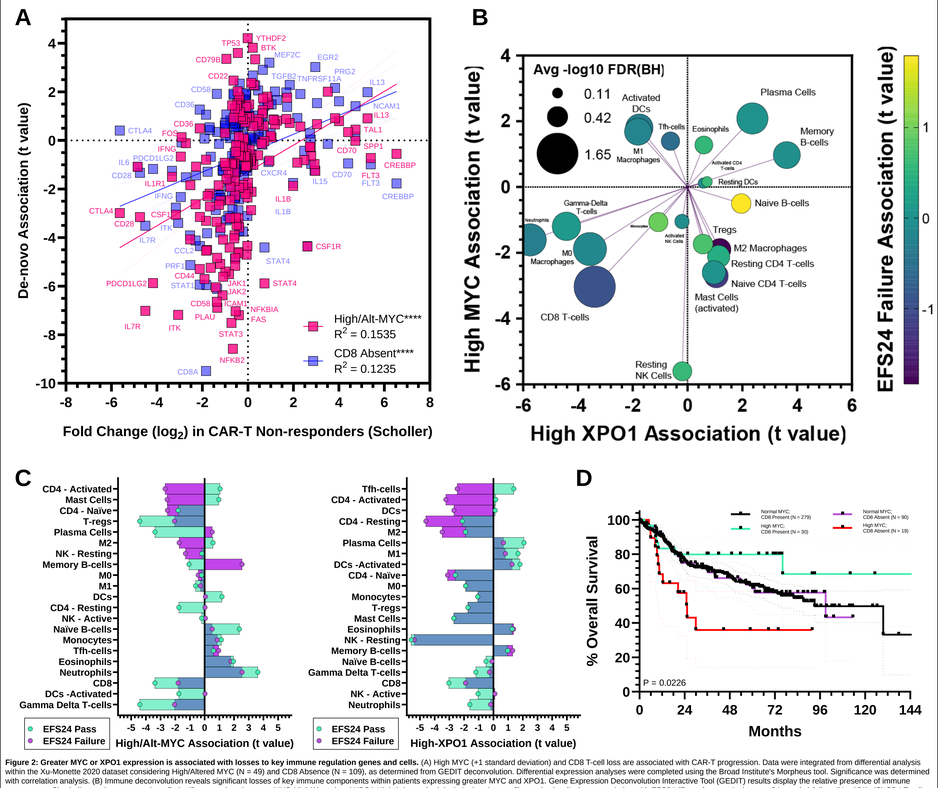

Figure 2: Greater MYC or XPO1 expression is associated with losses to key immune regulation genes and cells. (A) High MYC (+1 standard deviation) and CD8 T-cell loss are associated with CAR-T progression. Data were integrated from differential analysis within the Xu-Monette 2020 dataset considering High/Altered MYC (N = 49) and CD8 Absence (N = 109), as determined from GEDIT deconvolution. Differential expression analyses were completed using the Broad Institute's Morpheus tool. Significance was determined with correlation analysis. (B) Immune deconvolution reveals significant losses of key immune components within patients expressing greater MYC and XPO1. Gene Expression Deconvolution Interactive Tool (GEDIT) results display the relative presence of immune components. Size indicates the average $-\log_{10}$ P significance values between MYC-High/Altered and XPO1-High (+1 standard deviation) patient profiles and color displays association with EFS24 (Event free survival across 24 months) failure (N = 121). (C) CD4 T-cell losses are associated with High MYC and XPO1 patients that experience an event before 24 months (EFS24). Differential GEDIT immune deconvolution values between patients that either pass or fail EFS24 are compared. (D) Patients with High MYC and the absence of CD8 T-cells face an inferior de-novo survival prognosis. Significance was determined using Logrank Kaplan-Meier survival analysis. Dotted lines represent 95% confidence intervals. P values for High-MYC/CD8-Absent are as follows: 0.0064 vs. all other patients and 0.0110 vs. High-MYC/CD8-Present patients, and Logrank Hazard Ratios were 2.287 and 3.156, respectively.

Figure 3

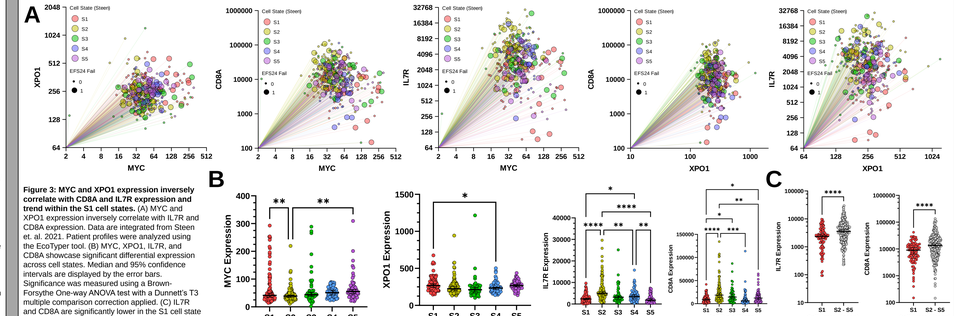

Figure 3: MYC and XPO1 expression inversely correlate with CD8A and IL7R expression and trend within the S1 cell states. (A) MYC and XPO1 expression inversely correlate with IL7R and CD8A expression. Data are integrated from Steen et. al. 2021. Patient profiles were analyzed using the EcoTyper tool. (B) MYC, XPO1, IL7R, and CD8A showcase significant differential expression across cell states. Median and 95% confidence intervals are displayed by the error bars. Significance was measured using a Brown-Forsythe One-way ANOVA test with a Dunnett's T3 multiple comparison correction applied. (C) IL7R and CD8A are significantly lower in the S1 cell state vs. combined S2-S5 patients. Significance was determined using a Welch's t test.

Figure 4

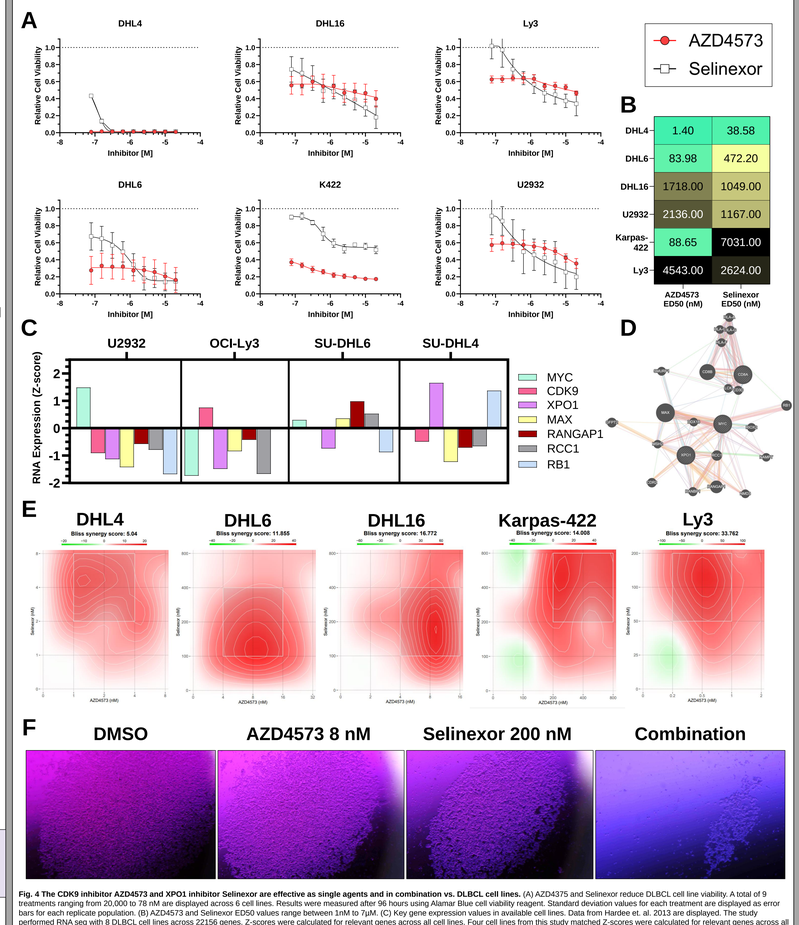

Fig. 4 The CDK9 inhibitor AZD4573 and XPO1 inhibitor Selinexor are effective as single agents and in combination vs. DLBCL cell lines. (A) AZD4375 and Selinexor reduce DLBCL cell line viability. A total of 9 treatments ranging from 20,000 to 78 nM are displayed across 6 cell lines. Results were measured after 96 hours using Alamar Blue cell viability reagent. Standard deviation values for each treatment are displayed as error bars for each replicate population. (B) AZD4573 and Selinexor ED50 values range between 1nM to 7μM. (C) Key gene expression values in available cell lines. Data from Hardee et. al. 2013 are displayed. The study performed RNA seq with 8 DLBCL cell lines across 22156 genes. Z-scores were calculated for relevant genes across all cell lines. Four cell lines from this study matched Z-scores were calculated for relevant genes across all 8 cell lines in the study. (D) MYC, XPO1, and CD8A interaction network. The GeneMANIA tool was used to created the network, Physical Interactions (red), co-expression (purple), predicted interactions (orange), Co-localization (blue), Genetic Interactions (green), and Pathways (light blue) are displayed. (E) Combined treatment with AZD4375 and Selinexor results in synergistic losses of cell viability within 5 DLBCL cell lines. Bliss synergy scores were calculated using the Synergy Finder tool. (F) Combined treatment of the DHL6 cell line displays synergistic rates of cellular disruption. Treatments were read on a Leica stereo brightfield microscope. Z-layered images were combined into a single visualization

Conclusions

- Increased MYC and XPO1 expression, a Cold/Depleted Immune microenvironment, and inferior patient prognoses are significantly associated and inform an emerging, inferior CAR-T DLBCL phenotype
- AZD4573 and Selinexor are effective as single agents and in combination

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Acknowledgements

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