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POSTER ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

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: Genomically-targeted Diffuse Large B-cell (DLBCL) treatments for refractory or relapsing patients remain a clinical need alongside the growing success of Chimeric Antigen T-cells (CAR-T), which have ushered in durable responses in 40-70% of patients. However, recent studies have identified factors indicative of an inferior CAR-T response. Recent tumor Ecotype studies from Kotlov and Steen reveal that a Depleted or Cold tumor microenvironment (TME) is associated with poor CAR-T response. Notable features of these environments include a decreased presence of native T-cells alongside oncogenic alterations associated with their exhaustion, namely MYC and XPO1. Few precision treatments exist given the difficulty targeting MYC, but two avenues have shown recent success: inhibition of the downstream oncogene CDK9 via AZD4573 and upstream inhibition of a MYC propagator, the nuclear exportin XPO1 via Selinexor. Herein, we report the first observations of synergy between AZD4573 and Selinexor within DLBCL cell lines and data supporting that these genes may serve as accessible markers for inferior tumor immune microenvironments.

Methods: We combined progression vs. response results from four previous CAR-T genomics studies, noting MYC status (Jaeger 2020, Jain 2021, Sworder 2021, and Shouval 2022). We next analyzed a 418-patient de-novo DLBCL patient cohort (Xu-Monette 2020) and 48-patient TCGA cohort after GEDIT immune deconvolution for differential expression and component enrichment between patients expressing high levels of MYC and XPO1 (+1 Standard Deviation) compared to normal counterparts. Significantly expressed genes were evaluated via gene set enrichment analyses. We next assayed AZD4573 and Selinexor vs. 7 DLBCL cell lines, 5 of which harbor MYC alterations, across a series of 9 concentrations (78nM-20μM) across 3-9 replicates. We followed these results by combining treatments in the *MYC*-altered Ly3, DHL6, and DHL4 cell lines, analyzing results using the BLISS synergy model.

Results: Integrative analyses support that MYC alterations are associated with significantly reduced CAR-T outcomes. Of the 93 MYC-altered patients within the total 246-patient cohort, 65.59% of them experienced progression during CAR-T treatment compared to just 50.33% of WT CAR-T patients ($p = 0.0242$). Differential gene expression analyses between de-novo patients expressing high MYC or XPO1 revealed losses in Cytokine Signaling pathways (FDR = 6.96E-18) and gains in Cell Cycle modulators (FDR = 2.525E-20). Importantly, CD8A expression was significantly lower in these patients (FDR < 0.0001) alongside other key reductions of CDKN1A and TNFAIP3. After immune deconvolution, both High-MYC and High-XPO1 patients displayed significant losses of CD8 T-cell association, respectively (FDR = 0.0005, FDR = 0.0396). Notably, High-MYC patients without a CD8 T-cell presence experienced an inferior survival prognosis compared to High-MYC counterparts with a CD8 presence ($p = 0.0272$). High MYC or XPO1 patients that experienced a failure event before 24-months (EFS24) had significantly lower activated CD4 T-cell presence compared to normal counterparts as well (FDR = 0.0095, FDR = 0.0069). Next, AZD4573 and Selinexor assays vs. DLBCL cell lines demonstrated dose and MYC-dependent cell inhibition, with ED50 values ranging between 58.1 nM to 8.07 μM. Lastly, the molecules were combined vs. Ly3, DHL6 and DHL4 cell lines, resulting in synergistic reduction of cell viability and BLISS synergy scores of 33.76, and 11.86, and 5.04, respectively.

Conclusions: Our results support that the cooperation between MYC and XPO1 is associated with T-cell reductions characteristic of a Depleted or Cold TME, a key issue for CAR-T success. We applied the targeted therapies AZD4573 and Selinexor vs. cell line models to address this pathway, with both displaying anti-tumor effects as single agents. Most importantly, we observed synergistic activity in the MYC-harboring Ly3 and DHL6 cell lines, and in the WT DHL4 cell line when these molecules were combined. These results are supported by integrative analyses that highlight the importance of targeting aberrant MYC signaling via CDK9 and XPO1 inhibition, revealing a potential avenue to address inferior response rates faced by patients harboring MYC-positive, Depleted tumor microenvironments that would otherwise face an inferior CAR-T prognosis.

Disclosures No relevant conflicts of interest to declare.

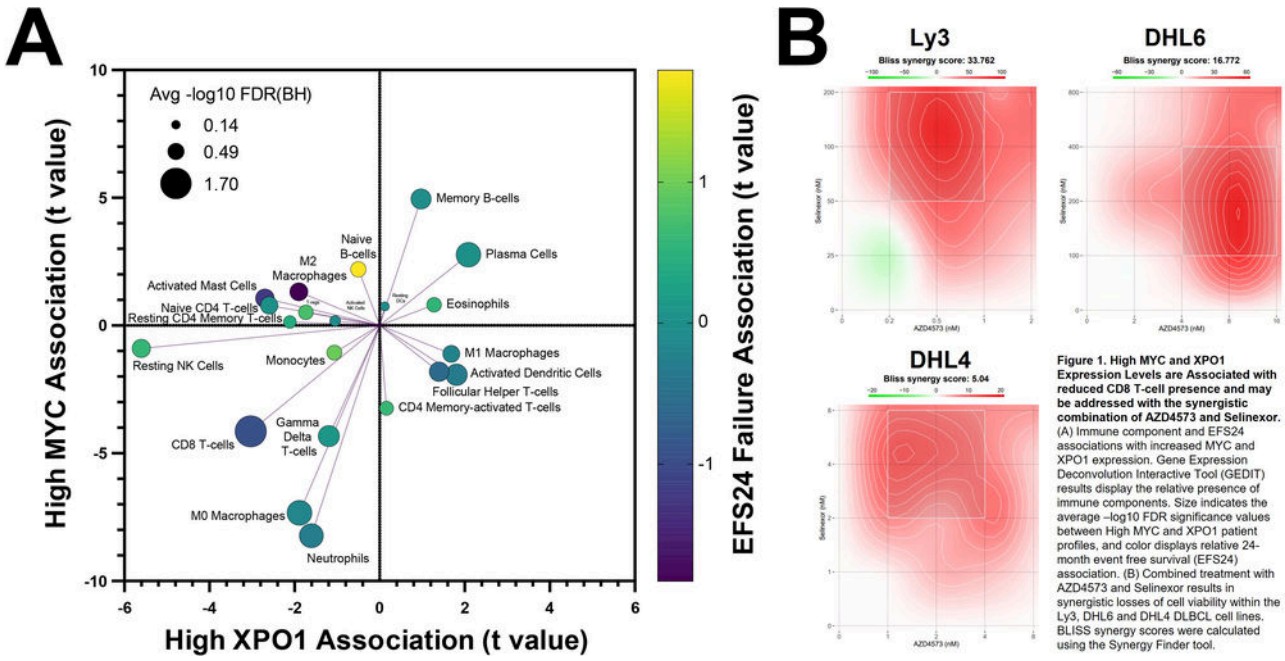

Figure 1

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