Abstract #37 | Poster

Defining Functional Subgroups and Novel Oncogenic Addictions in Burkitt Lymphoma via Genome-wide CRISPR/Cas9 Screening

A. K.P. Götze[1], S. P. Chillappagari[1], S. Becker[1], R. Kumar[2], M. Braun[3], A. Bräuninger[1], D. Körholz[3], S. Gattenlöhner[1], R. Schmitz[1]

[1] *Justus-Liebig University, Institute for Pathology, Gießen, Germany*
[2] *Indian Institute of Technology, Department of Biotechnology, Hyderabad, India*
[3] *Justus-Liebig University, Pediatric Hematology and Oncology Department, Gießen, Germany*

Background: Recent clinical trials demonstrate improved outcomes using tailored therapies based on genetic stratification in diffuse large B-cell lymphoma (DLBCL), indicating that biologically defined subgroups are essential for precision therapy. Phelan et al. (Nature 2018) established that CRISPR/Cas9-derived gene dependency signatures can define functional subgroups and reveal therapeutic vulnerabilities in DLBCL. Expanding upon this framework, we developed a CRISPR-based analytical pipeline combining a dependency seed generator (CRIMPACT) with a classification algorithm (CRISPRclass) to identify novel functional subsets in aggressive lymphoma cell lines.

Methods: Genome-wide pooled CRISPR/Cas9 screens were performed in T-cell lymphoma, DLBCL, and Burkitt lymphoma (BL) cell lines. Gene-level dependency scores were calculated using the JACKS algorithm. CRIMPACT identified candidate dependency seeds, allowing CRISPRclass to assign functional subgroups. Oncogenic addiction was validated by knockout rescue experiments. Mechanistic characterization of functional subgroups was performed via overexpression of mutant gene isoforms, RNA-seq, reporter assays, and Western blotting.

Results: Our pipeline recovered both known and novel functional gene dependency signatures across various lymphoma categories, including ABC and GCB DLBCL. BL clustered with GCB cell lines, suggesting a shared dark zone functional signature (Figure 1.). However, our approach identified a distinct BL subset with strong dependency on MYD88 and pathway genes leading to NFκB transcriptional activation. Whole-exome sequencing established a MYD88 S219C mutation exclusively in this subset. Remarkably, MYD88 knockout-induced viability loss was rescued by overexpression of MYD88-S219C, but not WT, exemplifying oncogenic addiction (Figure 2.). In BL41 cells, MYD88-S219C induced potent NFκB activation and upregulated IRF4 and PRDM1, with BACH2 downregulation, consistent with plasmablast-like reprogramming.

Conclusions: We identified MYD88 mutations as key pathogenic events driving specific gene dependencies in an NFκB-addicted, biological subset of dark zone lymphoma demonstrating that our seed-based classification tool can recover biologically meaningful functional subgroups from pooled CRISPR screen data, both with and without prior knowledge of structural and biological annotation.

This research was supported by an research grant (SCHM 2372/3-1) from the German Research Foundation (DFG).

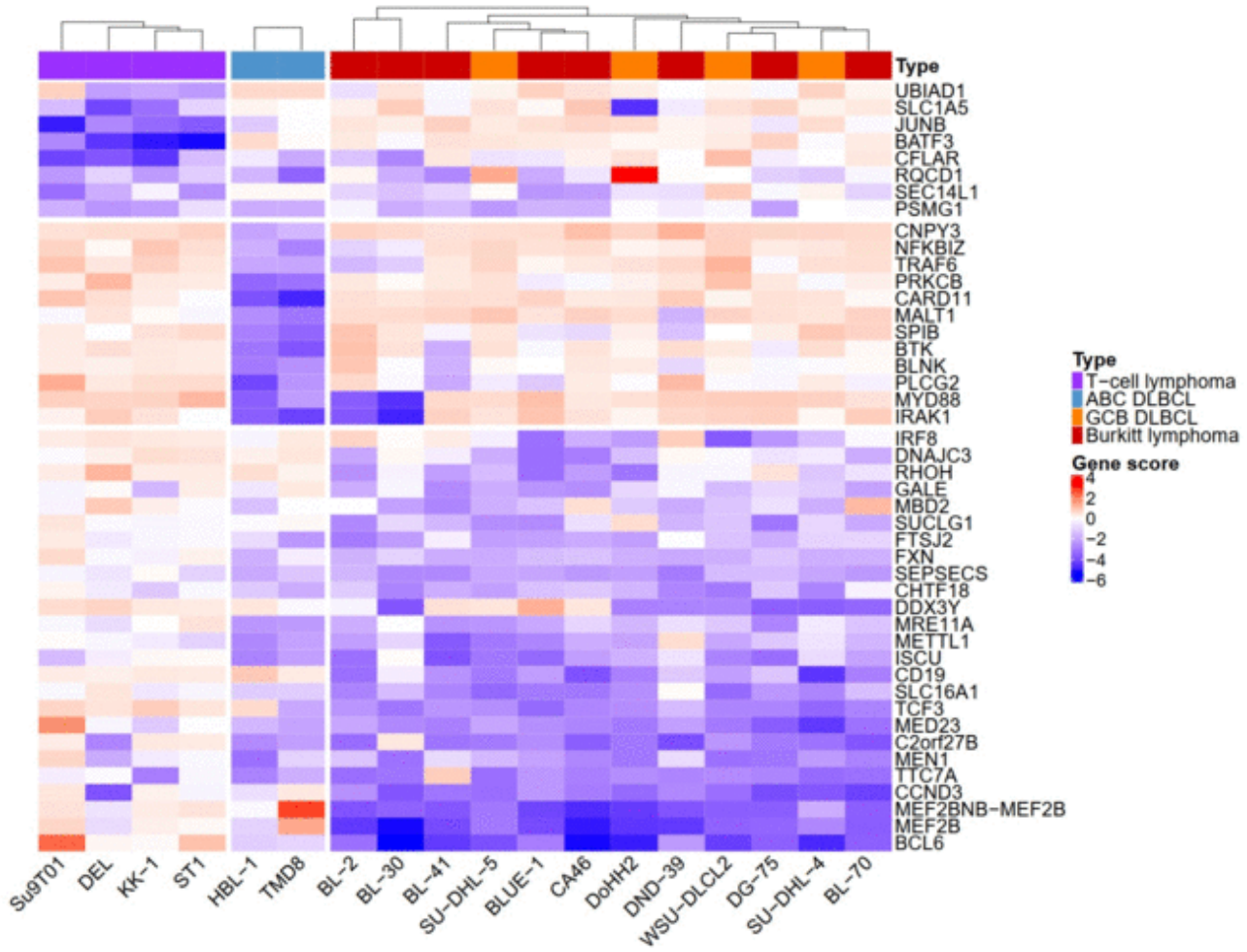

Figure 1: CRISPRclass algorithm identifies functional dependency subsets across lymphoma lineages. Heatmap displaying k-means clustering of gene dependency profiles in T-cell, diffuse large B-cell, and Burkitt lymphoma cell lines. The algorithm recovered distinct functional signatures that correlate with specific lymphoma categories.

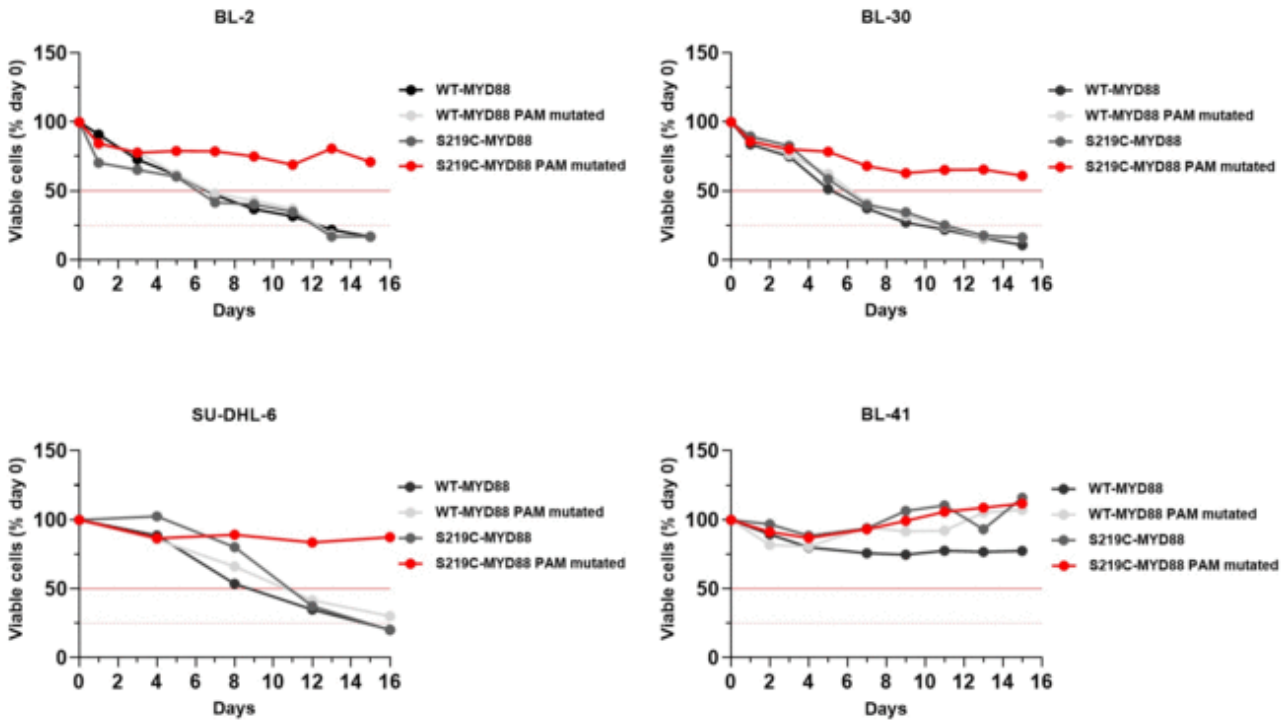

Figure 2: Oncogenic addiction of dark zone lymphoma to MYD88 S219C.
MYD88-mutant (BL2, BL30, SU-DHL-6) and MYD88 wild-type (BL-41) cell lines were transduced with sgRNAs targeting MYD88. To assess knockout-rescue, cells were subsequently transduced with MYD88 constructs (wild-type or S219C) containing either modified PAM sites (sgRNA-resistant) or unmodified PAM sites (sgRNA-sensitive). Data show the fraction of GFP+ (sgRNA-expressing) cells relative to the GFP– population over time, normalized to day 0.