Seeking an unbiased method to discover therapeutic targets in cancer, we developed a loss-of-function genetic screen using genomic-scale libraries of small hairpin RNAs that mediate RNA interference. These “Achilles heel” screens are designed to reveal genes essential for cancer cell proliferation and survival. In a parallel structural genomics approach, we are using RNA-seq to globally identify somatic mutations and other structural abnormalities in cancer. The intersection of these two data sets has helped us to discover novel pathogenetic pathways in the most common type of non-Hodgkin lymphoma, diffuse large B cell lymphoma (DLBCL)\(^1\). The ABC DLBCL subtype has constitutive activation of the NF-kB pathway, which we traced to the signaling adapter CARD11 using the Achilles heel screen\(^2\). In ~10% of ABC DLBCL tumor biopsies, we discovered recurrent CARD11 mutations that spontaneously activate NF-kB signaling\(^3\).

We also defined a “chronic active” form of B cell receptor (BCR) signaling that activates NF-kB in ABC DLBCLs with wild type CARD11\(^4\). Such ABC DLBCLs are killed by knockdown of BCR signaling components, such as the kinase BTK, or components of the BCR itself. Over one fifth of ABC DLBCLs have mutations in the CD79B or CD79A subunits of the BCR. In 18% of cases, mutations occur in a single tyrosine residue in the critical “ITAM” signaling motif, generating BCRs that avoid negative autoregulation by the LYN tyrosine kinase. Importantly, the BCR pathway offers a wealth of targets that can be exploited therapeutically. We have initiated a clinical trial in relapsed/refractory ABC DLBCL of an irreversible small molecule inhibitor of BCR signaling components, such as the kinase BTK, or components of the BCR itself. Over one fifth of ABC DLBCLs have mutations in the CD79B or CD79A subunits of the BCR. In 18% of cases, mutations occur in a single tyrosine residue in the critical “ITAM” signaling motif, generating BCRs that avoid negative autoregulation by the LYN tyrosine kinase. Importantly, the BCR pathway offers a wealth of targets that can be exploited therapeutically. We have initiated a clinical trial in relapsed/refractory ABC DLBCL of an irreversible small molecule inhibitor of BTK and have observed complete responses in several patients thus far. Of note, responses have occurred in patients with and without CD79B mutations, suggesting that BCR pathway addiction may be a prevalent feature in this lymphoma subtype.

Our Achilles heel screens revealed that ABC DLBCLs depend upon MYD88, a key adapter in Toll receptor signaling\(^5\). RNA-Seq uncovered somatic mutations in the MYD88 TIR domain in 39% of ABC DLBCLs, with a single point mutation (L265P) accounting for 29% of cases. This mutation was rare or absent in other DLBCL subtypes but is also present in 3-10% of chronic lymphocytic leukemia cases. Interestingly, MYD88 L265P mutations overlap significantly with CD79A/B mutations in ABC DLBCL tumors, suggesting a functional relationship of the MYD88 and BCR pathways. Knockdown of MYD88 or its downstream kinases IRAK1 and IRAK4 is selectively toxic for ABC DLBCL lines, decreasing engagement of the pro-survival NF-kB and JAK/STAT3 pathways. Biochemical mechanisms underlying oncogenic MYD88 signaling will be discussed as will therapeutic strategies to exploit this pathway.