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DLBCL cells thus require the continued presence and function of BCL6 for their survival. Cells identified as BCL-2 dependent by this method are found to be sensitive to small molecule BCL-2 antagonists like ABT-737. This suggests that response to many conventional agents may often be determined by whether a tumor is dependent on BCL-2 or BCL-XL. To test this idea in a more rigorous manner, we used a recently developed, genetically-encoded FRAP system to evaluate drug effects in real-time. We found that the BCL-2 dependency phenotype is not fixed, but is instead dynamic, and that one can engineer tumor cells to become dependent on BCL-2 by using chemical or viral agents to induce BCL-2 expression. We are currently testing this approach for the treatment of BCL-2 dependent tumors in vivo.

We are also exploring the potential of targeting BCL6 in DLBCL. The BCL6 lateral groove residues that contact N-CoR and SMRT are unique to their function as transcriptional repressors. The BCL6 lateral groove. Moreover, 57-6 induced an allosteric conformational change in the entire BCL6 molecule as shown in fluorescence polarization experiments. 57-6 potently inhibited the growth of already established human DLBCL cell lines but had no effect on BCL6-negative DLBCL cells. A dose escalation experiment in mice revealed no toxic effects. In xenotransplantation experiments, 57-6 potently inhibited the growth of already established human DLBCL tumors in mice, again without toxicity to other organs. In summary, we used a rational approach to design specific and potent peptidomimetic and small molecule inhibitors of BCL6, which could serve as targeted agents for DLBCL in clinical trials. Our data show that transcription factors like BCL6 are druggable targets that can be harnessed to potentially improve cancer therapy.

046 THERAPEUTIC TARGETING OF THE BCL6 ONCOGENE IN DIFFUSE LARGE B-CELL LYMPHOMAS

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Expression of the BCL6 (B-cell lymphoma 6) transcriptional repressor is required for B-cells to form germinal centers (GC) and undergo immunoglobulin affinity maturation. BCL6 contributes to the GC B-cell phenotype of clonal expansion and genetic recombination by repressing target genes involved in DNA damage responses such as ATR, CHEK1, TP53 and CDKN1A. BCL6 can also repress the PIM1 gene and thus inhibit plasma cell differentiation of GC-B-cells. Translocations or mutations of negative regulatory elements that occur by byproducts of class switch recombination or somatic hypermutation can lead to constitutive expression of BCL6. Such events are among the most common genetic lesions found in human diffuse large B-cell lymphomas (DLBCL), and can contribute to malignant transformation. Animals engineered to recapitulate deregulated expression of BCL6 in germinal center B-cells develop DLBCL, similar to the human disease. Oncogenic transcription factors like BCL6 are ideal targets for development of therapeutic inhibitors since they exert a profound influence on cellular phenotype. Directly targeting such factors could transcriptionally reprogram tumor cells to either revert to a normal phenotype or escape from aberrant survival programs. One of the main barriers thus far to development of such inhibitors is that most transcription factors mediate their effects through protein-protein interactions, which are often quite complex and may not be suited to inhibition by small molecules. In recent years this limitation has been overcome by the harnessing of protein transduction domains (PTDs) including the nine residue cationic HIV-TAT motif. PTDs allow even full-length proteins to be effectively transduced into virtually all cell types both in vitro and in vivo. The TAT PTD penetrates cells via macropinocytosis, and enters the cytoplasm by leaking through the macropinosome membrane as the pH drops within. Co-administration of a fusogenic motif from the influenza virus hemagglutinin protein can greatly facilitate escape of PTDs from macropinosomes. Since TAT also functions as a nuclear localization signal it is well suited for the delivery of transcription factor inhibitors. BCL6 is a member of the BTB-POZ (BRIC A BRAC, TRAMTRACK, BROAD COMPLEX – POX VIRUS AND ARROW) family of proteins. Our crystallography studies showed that homo-dimerization of the BCL6 BTB domain forms an extended lateral groove motif along the BTB interface, which is required to recruit the SMRT and N-CoR corepressor complexes. Our crystallography studies showed that homo-dimerization of the BCL6 BTB domain forms an extended lateral groove motif along the BTB interface, which is required to recruit the SMRT and N-CoR corepressor complexes. Among the top-scoring 100 molecules from this screen, we identified 10 compounds that could specifically inhibit the repressor activity of the BCL6 BTB domain in reporter assays and that displayed direct binding to purified BCL6 BTB domains. Using these leads as molecular scaffolds we generated small libraries of molecules derived from each parental compound. The most active of these families was called the 57 series. Series 57 compounds could all specifically block BCL6 repression in reporter assays, and disrupt co-repressor/BCL6 complexes at low micromolar concentrations as shown in fluorescence polarization assays. The BCL6 lateral groove. Moreover, 57-6 induced an allosteric conformational change in the entire BCL6 molecule as shown in fluorescence polarization experiments. 57-6 potently inhibited the growth of already established human DLBCL cell lines but had no effect on normal control cells nor in BCL6-negative DLBCL cells. The mechanism of action was confirmed in ChIP assays showed that 57-6 abrogated BCL6 mediated corepressor recruitment to BCL6 target genes but had no effect on negative control genes. Most importantly, 57-6 specifically killed BCL6-positive DLBCL cells but had no effect on BCL6-negative DLBCL cells. A dose escalation experiment in mice revealed no toxic effects. In xenotransplantation experiments, 57-6 potently inhibited the growth of already established human DLBCL tumors in mice, again without toxicity to other organs. In summary, we used a rational approach to design specific and potent peptidomimetic and small molecule inhibitors of BCL6, which could serve as targeted agents for DLBCL in clinical trials. Our data show that transcription factors like BCL6 are druggable targets that can be harnessed to potentially improve cancer therapy.

047 MOLECULAR HETEROGENEITY AND RATIONAL THERAPEUTIC TARGETS IN DLBCL

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Diffuse large B-cell lymphomas (DLBCLs) are the most common lymphoid malignancies in adults. Although more than 60% of patients with DLBCL are cured with empiric combination chemotherapy and rituximab, the remission rate and many ultimately die of their disease. The striking clinical, genetic and morphologic heterogeneity in DLBCL, it would be useful to have comprehensive molecular signatures of tumors that share similar features. The combination of transcriptional profiling and additional detailed genetic and functional analyses identified three groups of DLBCLs with significantly different molecular signatures and underlying genetic abnormalities. BCR DLBCLs have increased expression of B-cell transcription factors including BCL6 and more frequent BCL6 translocations; these tumors also have more abundant expression of multiple components of the BCR signaling cascade. Previous functional analyses indicate that BCL6 target genes are differentially regulated in BCR DLBCLs and that these tumors are more sensitive to targeted inhibition of BCL6. Since BCR tumors also have increased expression of multiple components of the BCR signaling cascade, we postulated that these DLBCLs might exhibit increased activity of and reliance upon tonic BCR-mediated survival signals. In recent studies, we found that BCR DLBCL cell lines and primary tumors exhibited tonic and ligand-induced BCR signaling. In addition, these DLBCLs were selectively sensitive to targeted inhibition of the spleen tyrosine kinase (SYK), which initiates downstream events and amplifies the initial BCR signal. Taken together, these data suggest that SYK-dependent tonic BCR signaling is an important and potentially targetable survival pathway in BCR DLBCL. These studies prompted the clinical evaluation of an oral SYK inhibitor in DLBCL and other B-cell lymphomas. More generally, these studies highlight the potential clinical utility of identifying DLBCL subtypes reliant upon specific survival pathways.

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**048 DEREGULATION OF THE NFkB PATHWAY BY ONCOGENIC LESIONS IN ABC-DLBCL**

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**Introduction:** Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease comprising biologically and clinically distinct subgroups. A key feature of the activated B cell (ABC)-type is the constitutive activation of the NFkB transcription complex. However, the underlying mechanisms remain to be elucidated. The aim of this study was to investigate whether genetic lesions in NFkB pathway components are responsible for deregulated NFkB expression in this disease.

**Material and Methods:** NFkB activity was first assessed in 85 DLBCL (32 ABC, 38 GCB, 15 unclassified) by immunohistochemical / immunofluorescence staining of p50 and p52, WB analysis of p100/p52, and gene set enrichment analysis (GSEA) for NFkB target genes. The complete coding sequence of 30 NFkB pathway components was analyzed by direct sequencing in 15 ABC-DLBCL (6 cell lines and 9 biopsies); genes found mutated were further analyzed in additional 10 ABC-DLBCL and in a panel of 15 GCB-DLBCL. Copy number changes were detected by FISH.

**Results:** Nuclear localization of p50 and/or p52, indicative of constitutive NFkB activity, was observed in 20/32 (63%) ABC- and 10/38 (26%) GCB-DLBCL. The more sensitive GSEA showed significant enrichment for NFkB target genes in 95% ABC-DLBCL. The complete coding sequence of 30 NFkB pathway components was analyzed by direct sequencing in 15 ABC-DLBCL. Copy number changes were detected by FISH.

**Conclusions:** Multiple genetic lesions in this key signaling pathway, including inactivating mutations of A20, may contribute to ABC-DLBCL pathogenesis by causing abnormally prolonged NFkB responses. These findings may provide new targets for therapeutic intervention.

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**049 SUBSETS OF NHL POPULATIONS MEET CRITERIA FOR LYMPHOMA STEM CELLS**

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**Introduction:** Emerging studies suggest that cancer stem cells appear to play an important role in human leukemias, multiple myeloma, and cancers of the brain, breast and colon. No such tumor-initiating population has been identified in non-Hodgkin lymphoma (NHL). Cancer stem cells are defined phenotypically and functionally as relatively rare cells with self-renewal, differentiative, and tumorigenic capacity. Using these criteria, we looked for putative LySC in cultured and primary human NHL populations.

**Materials and Methods:** Two experimental sources of NHL cells were used: a) primary human DLBCL or Burkitt lymphoma cells derived from malignant effusions obtained after therapeutic procedures, and, b) continuously cultured DLBCL lines. Standard techniques for multiparameter FACS analysis, cell sorting, methylcellulose culture for CFU/progenitor cell enumeration, serial in vitro passage, and a NOD/SCID murine xenograft model were used to identify candidate LySC subpopulations.

**Results:** In both primary tumor populations and cell lines derived from aggressive lymphoma, we readily identified subsets with differential surface expression of CD22, CD27, CD45R/B220, CD184, and CD38. Enriched CFU potential segregated with phenotypically distinct subpopulations, and a purified subpopulation of lymphoma cells, when expanded in culture, could recapitulate the phenotypic heterogeneity found in the original, unfractionated population. In-vivo tumor-initiating cells were confined to a subset of all specimens tested. These data are consistent with the phenotypic and functional heterogeneity expected in a stem cell based hierarchy of malignant populations.

**Conclusions:** Our studies provide evidence that NHL tumor-initiating cells may comprise a unique population amenable to prospective immunophenotypic and/or functional identification. Implicit in the concept of all cancer stem cells such as the putative LySC is that the control, and ultimately the eradication of these unique populations is critical for both clinical remission induction and for cure.