HOW CLOSE ARE WE TO CURING CLL?

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As in any malignant disease, an essential path to cure is to achieve a complete remission (CR) of the disease. Criteria for CR in CLL have evolved over the last 20 years. In the initial version of the National Cancer Institute Working Group (NCIWG) criteria for response, nodules were allowed to be present in the bone marrow and the patients could still be classified as a CR. When it became obvious that most of these patients had residual CLL nodules, the most recent NCIWG guidelines required no nodules to be present so that the 3-tiered system of CR, nodular PR, and PR have been established. Several patients who achieve a PR will have no measurable disease in the blood, bone marrow, or clinically but will be classified as a PR because of persistent cytopenias. The evolution of more sophisticated measures of minimal residual disease (MRD) such as residual cells on bone marrow, flow cytometry using 4-parameter flow criteria and PCR for the IVGH gene have led to a further level of sophistication. The development of new chemo-immunotherapy protocols with rituximab being combined with fludarabine by itself (FR)[1] or fludarabine and cyclophosphamide (FCR)[2] has markedly improved the CR rate which is noted with these regimens. We have recently conducted a study of FCR in 300 previously untreated patients. The CR rate is 72%. The median duration of CR and NPR patients has not been reached at 7+ years. The NCIWG criteria predict for remission duration and this is confirmed by the impact of flow cytometry and PCR testing. Following FCR, 40% of patients in CR, NPR, or PR will be PCR negative. When multivariate analysis is conducted to predict for the likelihood of patients remaining in remission, the NCI-WG criteria and flow cytometry residual disease measurements appear to be the best combination. The study was commenced before ZAP70, mutation status, and FISH cytogenetics were in place. Strategies are now in place to use antibodies such as alemtuzumab (Campath-1H) to eradicate these residual cells. A number of studies have now been conducted demonstrating that the use of alemtuzumab to eradicate MRD is effective in achieving flow and PCR negativity in blood and bone marrow cells.[3,4] In addition, the evolution of non-ablative stem cell transplants (NST) in CLL has enabled us to offer this modality to older patients. NST relies on the graft-versus-leukemia effect of the transplant to the immune system.[5] Thus 3 modalities are in place to achieve PCR negativity. New paradigms for treatment are in place to test the curative approach to CLL. Definition of cure in a disease such as CLL does not necessarily mean that patients should never have recurrence of CLL cells. If the patient dies of coincidental illness without any contribution of the CLL to their death, these patients have effectively been cured of the CLL as a threat to their life. Optimism is present at the continued development of newer, effective modalities will increase the probability of patients with CLL who require therapy living a normal life expectancy and good health.

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TARGETING TRANSCRIPTIONAL REPRESSION IN DIFFUSE LARGE B-CELL LYMPHOMA

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Recent classification studies utilizing microarrays demonstrate that diffuse large-B cell lymphomas (DLBCL) can be classified into distinct subtypes with specific biological features. DLBCLs with B-cell receptor or germinal center signatures strongly express the BCL6 oncoprotein. The BCL6 gene is required for normal development of the germinal center B cell. Work from many groups including ours suggests that BCL6 is required for survival of DLBCL cells and can limit their ability to respond to DNA damaging agents. We have studied the BCL6-repression complex and found that BCL6 binds to the SMRT co-repressor through a tight and unique interaction mediated by the N-terminal BTB/POZ domain of BCL6. We designed a peptide (BPI-BCL6 peptide inhibitor) that mimics the SMRT interface that docks with BCL6, displaces SMRT from BCL6 and de-represses BCL6 target genes *in vitro* and *in vivo.* As a result B-cell lymphomas expressing BCL6 are killed while other cell lines and normal cells are unaffected. We identified a BCL6 dependent gene expression signature in patients with DLBCL, that is predictive for tumors that are BCL6 dependent, all of which are highly sensitive to BPI. By targeting waves of transcriptional regulation downstream of BCL6 the therapeutic effects of BPI could be enhanced. Targeting transcription factors that underlie the pathogenesis of DLBCL is thus a promising approach for translation into clinical trials.

GENOMIC-SCALE RNA INTERFERENCE SCREENS TO REVEAL THE ACHILLES HEEL OF CANCER

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To define functionally critical signaling pathways in lymphoid malignancies using RNA interference, our laboratory has embarked on a major new initiative to create a retroviral expression library of small hairpin RNAs (shRNAs). We have created an shRNA library targeting 2,500 genes, with three shRNA constructs per gene. The retroviral vector that we created for this purpose makes use of the tetracycline repressor system to allow for inducible shRNA expression. In this vector, the polIII promoter that is used to drive expression of the shRNA contains two tetracycline repressor binding sites. In cell lines expressing the tetracycline repressor, shRNA is not expressed from this vector unless doxycycline is added. Each shRNA construct is tagged with a unique 60 base pair *bar code* to allow us to monitor the abundance of each shRNA vector in a cell population. We determined the association of a bar code sequence with each shRNA sequence by DNA sequencing.

In a typical screen, the retroviral library is introduced into a lymphoma cell line and puromycin is used to select stable integrants. The cell population is then divided in two, with one half receiving doxycycline to induce shRNA expression and the other half used as a control cell population. Any shRNA that knocks down the expression of a gene that is critical for proliferation or survival of the lymphoma cells will be selectively eliminated from the doxycycline-induced culture. After 3 weeks, genomic DNA is harvested from the two populations and PCR is used to amplify the bar code sequences present in the genomic DNA. Amplified DNAs from the doxycycline-induced and control cultures are fluorescently labeled with different dyes and co-hybridized to a DNA microarray consisting of the bar code

oligonucleotides. The microarray is scanned to reveal the relative abundance of each bar code in the two populations and hence the relative depletion or enrichment of cells expressing a given shRNA.

An shRNA library screen in cell lines belonging to the activated B cell-like (ABC) subgroup of diffuse large B cell lymphoma (DLBCL) revealed that this lymphoma subgroup depends on CARD11, MALT1 and BCL10 for survival. Previously, our laboratory discovered that constitutive activity of the NF-kB signaling pathway is required for the survival of ABC DLBCLs. We demonstrated that the CARD11/MALT1/BCL10 complex activates IkB kinase in ABC DLBCL, thereby causing constitutive NF-kB signaling. In contrast to ABC DLBCL, interference with the CARD11 pathway had no effect on DLBCL cell lines belonging to the germinal center B cell-like (GCB) subgroup, in keeping with the fact that this DLBCL subgroup does not rely upon NF-kB signaling for survival. Another DLBCL subgroup, known as primary mediastinal B cell lymphoma (PMBL), is also characterized by constitutive NF-kB signaling, but the CARD pathway was not required for IkB kinase activity in this subgroup. Thus, different DLBCL subgroups activate the NF-kB pathway by distinct molecular mechanisms. Since the CARD11 pathway is not required for the function or survival of normal cells outside of the lymphoid compartment, therapeutic targeting of this pathway in ABC DLBCL should be explored.

LRF/POKEMON PLAYS A CRITICAL ROLE IN B VERSUS T LYMPHOID LINEAGE FATE DECISION

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Differentiation is a critical process for the maintenance of proper lymphoid cell fate and its perturbation can result in malignant tumor formation as well as immunodeficiency. Hematopoietic stem cells in the bone marrow give rise to lymphoid progenitors, which subsequently differentiate into B and T lymphocytes. Consequently, lymphoid progenitors must critically decide whether to adopt a B- or T-cell and Notch1 signaling is crucial for T-cell fate specification. We demonstrate, by taking advantage of both conventional and conditional knockout mutant mice, that the proto-oncogene LRF (previously known as Pokemon) plays an essential role in B versus T lymphoid cell fate decision. LRF loss in early lymphoid progenitors causes aberrant de-repression of T cell lineage specific genes, thereby blocking B cell development and allowing extrathymic T cell production in the bone marrow. The mechanism of by which Pokemon exerts its critical role will be discussed. We propose a new model for lymphoid lineage commitment, in which LRF acts as a master regulator of T versus B lineage fate decision.

ABERRANT SOMATIC HYPERMUTATION IN THE PATHOGENESIS OF DIFFUSE LARGE B CELL LYMPHOMA

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Diffuse large B cell Lymphoma (DLBCL), the most common type of B-cell non-Hodgkin lymphoma in adults, is a heterogenous disease comprising several biologically and clinically distinct subgroups. The genetic lesions associated with this malignancy are also diverse, and include alterations common to other cancer types, such as gene amplifications and deletions, but especially lesions that seem to result from mistakes in two germinal center (GC) specific DNA remodeling processes: class switch recombination (CSR) and somatic hypermutation (SHM). These lesions comprise balanced, reciprocal chromosomal translocations and a more recently identified mechanism of genomic instability, termed aberrant somatic hypermutation (ASHM) and presumably due to a malfunction (i.e., a loss of target specificity) of the physiologic SHM process that normally diversifies the antibody genes in GC B cells1.

This aberrant mechanism, observed in >50% of DLBCL cases as well as in few other lymphoma types (among them, AIDS-lymphomas and primary central nervous system lymphoma)1-3, leads to the accumulation of multiple somatic mutations in the 5' sequences (~1.5 Kb downstream the transcription initiation site) of a number of genes that are not physiologic targets, including well-known proto-oncogenes such as cMYC or PIM1. Indeed, high-throughput amplification and sequence analysis of >150 genes, selected based on their expression in normal or transformed GC B cells, has recently led to the identification of an increasing number of target loci with possible pathogenetic significance in DLBCL (~8% of all genes screened). Of these, ten were further analyzed in a panel of 100 primary DLBCL biopsies, confirming their frequent involvement in a significant fraction of cases (range: 25-50%, depending on the gene). While the mutations found display features reminiscent of the SHM process, supporting their derivation from a common mechanism, none of these genes appears to be mutated in normal GC B cells, pointing to a tumor-specific malfunction of SHM. Among over 100 DLBCL samples analyzed to date, ASHM was independent of cell-of-origin based tumor subtype, being observed at similar frequencies in both GC B cell type, activated B cell type and unclassified DLBCL; however, a significant correlation was found between targeting of individual genes and disease phenotype, which in turn was coupled with the expression levels of the affected gene in a given subtype.

Since both regulatory elements and coding exons of the target gene can fall within the hypermutation domain, ASHM represents a powerful mechanism of transformation, which may act by altering gene expression programs as well as structural/functional properties of the target gene. Recent studies aimed at evaluating the functional consequences of ASHM-introduced mutations have shown that some of them lead to the oncogenic activation of the target gene, as in the case of cMYC, where specific changes at critical residues were associated with loss of phosphorylation, increase in protein stability and enhanced transforming activity. Finally, studies addressing the role of AID–the protein essential for both SHM and CSR–in the generation of DLBCL-associated lesions will be discussed.

By targeting regulatory and coding sequences of a significant number of genes, frequently within the same tumor case and in variable combinations, ASHM may represent a major contributor to the pathogenesis of DLBCL and explain in part the unique heterogeneity of this disease.

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