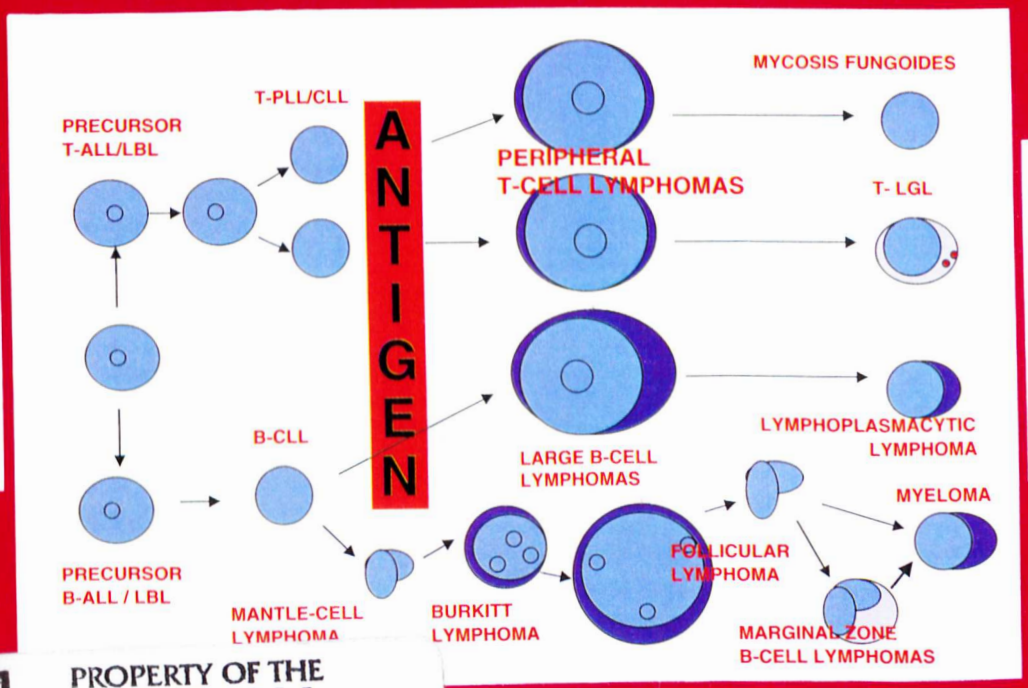
HEMATOLOGY

2001

American Society of Hematology Education Program Book



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Cover: Dr. Nancy Lee Harris. Figure 10D. Hypothetical scheme of lymphocyte differentiation: Many lymphoid neoplasms can be associated with a normal stage of T or B-cell differentiation. (See Appendix for full figure.)

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Rational Approaches to Design of Therapeutics Targeting Molecular Markers

Richard J. Klasa, Alan F. List, and Bruce D. Cheson

This paper introduces novel therapeutic strategies focusing on a molecular marker relevant to a particular hematologic malignancy. Four different approaches targeting specific molecules in unique pathways will be presented. The common theme will be rational target selection in a strategy that has reached the early phase of human clinical trial in one malignancy, but with a much broader potential applicability to the technology.

In Section I Dr. Richard Klasa presents preclinical data on the use of antisense oligonucleotides directed at the *bcl-2* gene message to specifically downregulate Bcl-2 protein expression in non-Hodgkin's lymphomas and render the cells more susceptible to the induction of apoptosis.

In Section II Dr. Alan List reviews the targeting of vascular endothelial growth factor (VEGF) and its receptor in anti-angiogenesis strategies for acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS).

In Section III Dr. Bruce Cheson describes recent progress in inhibiting cell cycle progression by selectively disrupting cyclin D1 with structurally unique compounds such as flavopiridol in mantle cell lymphoma as well as describing a new class of agents that affect proteasome degradation pathways.

I. Antisense Oligonucleotides Directed at the *Bcl-2* Gene Message in Non-Hodgkin's Lymphoma

*Richard J. Klasa, MD**

Hematologic malignancies in general and non-Hodgkin's lymphomas (NHLs) in particular are frequently associated with gain of function mutations, many characterized by balanced chromosomal translocations. Genome-wide surveys of gene expression are identifying both known and new transcripts that are overexpressed in different histological subtypes of lymphoma.[1] As we develop molecular classifications of these diseases it is assumed that a few key genes will account for the particular survival advantage conferred on malignant lymphocytes as compared to their normal counterparts. These genes and their protein products would provide rational targets for the development of therapeutic strategies to reverse this upregulation associated with the malignant phenotype.

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Dr. Klasa is on the international advisory boards of Schering AG and Hoffmann-LaRoche, and is on the speakers' bureau and advisory board for Berlex Canada.

Non-Hodgkin's Lymphoma and Bcl-2

Over the past quarter century cytogenetic analysis has identified a number of reciprocal translocations that frequently occur in histologically identifiable subtypes of NHL.[2] The transposition of the *bcl-2* gene to the immunoglobulin heavy chain promoter region in the t(14;18) translocation is associated with > 90% of follicular lymphomas (FL) at diagnosis and 10% of diffuse large B-cell lymphomas (DLBCL), making it the most frequent event identified in NHL. Additionally, 50% of DLBCL overexpress the BCL-2 protein through other mechanisms, such as gene duplication, and are associated with a poorer prognosis after anthracycline-based combination chemotherapy.[3] BCL-2 is also overexpressed in mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL), multiple myeloma (MM) and acute myelogenous leukemia (AML).[4] This same widespread pattern of distribution is also seen in a variety of solid tumors including melanoma, small cell lung carcinoma, and colon carcinoma as well as prostate and breast carcinoma, especially once the last two are hormone independent. The obvious conclusion is that BCL-2 overexpression, by whatever means, confers a fundamental advantage to malignant cells and that disruption of this overexpression might have therapeutic potential.

Bcl-2 is an anti-apoptotic member of a large family of genes involved in the regulation of programmed cell death.[5,6] Pro-apoptotic (BAX and BCL-Xs) and anti-

apoptotic (BCL-2, BCL-X_L) molecules reside within the inner mitochondrial membrane and can homo- and heterodimerize upon appropriate stimulus. These interactions control the release of substances such as cytochrome C from the mitochondria into the cytosol through the opening or closing of specific pores in the membrane, with permeability determined by the relative abundance of the different molecules. Cytochrome C is central to the activation of caspases that initiate the apoptotic process. Thus, an overabundance of BCL-2 can prevent or retard activation of the apoptotic machinery and allow survival under conditions that might otherwise be lethal to a cell (**Table 1**).

Antisense Oligonucleotide

Reverse complementary or "antisense" oligonucleotides (ASOs) are short sequences of single stranded deoxyribonucleotides complementary to the coding regions of a gene that are designed to hybridize by Watson-Crick base pairing to messenger-RNA (m-RNA) sequences and thus facilitate their degradation.[7,8] Naturally occurring antisense sequences have been identified as regulators of gene expression in a number of systems, supporting their potential for therapeutic development.[9,10] The formation of a heteroduplex of m-RNA with the DNA of the ASO engages RNaseH, an enzyme that proceeds to specifically cleave off the m-RNA moiety, destroying the message and putatively leaving the therapeutic ASO molecule able to hybridize to another message sequence.[11] This results in a reduction in the target m-RNA pool, which subsequently leads to reduction in the specific protein encoded (**Figure 1; see color page 551**). The presence of the ASO may also prevent the m-RNA from appropriately docking with the ribosomal machinery that would allow translation into a functional protein. The end result is loss of expression of that protein in the cell.

ASOs of 16-24 bases in length provide target specificity while shorter or longer sequences can result in random hybridization within the transcript repertoire. Selecting the target areas within a messenger RNA must ultimately take into account its tertiary structure, which will determine the accessibility of an area for hybridization. These target areas are defined in oligonucleotide arrays where the entire antisense sequence to an m-RNA is displayed in overlapping segments on a slide. The intensity of hybridization of the labeled message determines the candidate therapeutic ASOs.[12] Screening of oligonucleotide libraries has also identified RNA sites that are most accessible to hybridization and correlated these sites with protein downregulation and biological function.[13,14] More empirically, the first 6 codons of the open reading frame downstream of the AUG start site have repeatedly been found to be accessible to hybridization and have been chosen for initial development of ASOs against a number of genes.

As organisms have developed a sophisticated system for dealing with rogue strands of DNA both inside and outside the cell, the development of therapeutic molecules required chemical modifications to confer nuclease resistance and a favorable pharmacokinetic profile.[15,16] Substitutions in the phosphodiester linkage of the bases in the ASO backbone has yielded a number of molecules now in clinical development with phosphorothioates being the most widely studied first generation molecules (**Figure 2**). The sulfur substitution yields an ASO that is nuclease resistant and capable of entering the cell. It demonstrates good hybridization kinetics and has little in the way of non-sequence-dependent effects or toxicities at concentrations required to downregulate the target message. Additionally, although in tissue culture a delivery system such as cationic lipid is required for efficient intracellular penetration of these highly charged molecules, in vivo ASOs have been shown to be active in free form, possibly due to interaction with blood lipoproteins.[17,18]

The correlation of a biologic effect with the specific downregulation of target message and protein in vivo has been a major focus of the development of ASOs. However, ASOs can be very potent immune stimulators, by virtue of unmethylated CpG motifs presented in the context of certain flanking sequences, and therapeutic

Table 1. Properties of BCL-2.

- Oncogenic protein
- Anti-apoptotic
- Mitochondrial, endoplasmic reticulum, nuclear membrane localizations
- Homotypic and heterotypic dimerization within family
- Membrane channel/pore function
- Cytochrome C release from mitochondria via BCL-2 family channels regulates cell fate under stress

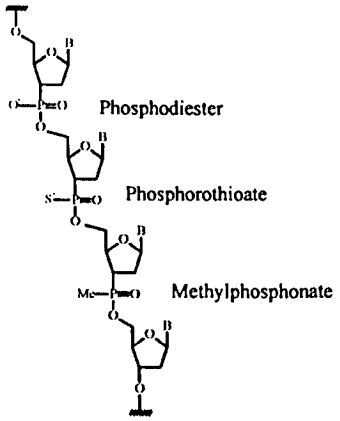

Figure 2. Phosphodiester substitutions in first generation antisense oligonucleotides.

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