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New biologic and therapeutic insights in Hodgkin's lymphoma

Introduction

Since the technology for the isolation of Hodgkin/Reed-Sternberg cells (H/RS) was developed, there has been a great improvement in the understanding of the pathogenesis of the disease. Hodgkin cell lines have been used extensively in basic research, but there may be intrinsic biologic differences between the *in situ* H/RS cells in primary tumors and the few cell lines derived from such tissues. Nevertheless, new and unique biologic information has also been derived from cell lines which has only partially correlated with *in situ* disease in the design of new therapies.[1] This paper will outline some of the newer biologic investigations that have generated potential therapeutic agents and some in early clinical trials. None have reached the point of approval by regulatory agencies but do offer the hope that targeted agents could emerge that do not possess overlapping toxicity to currently effective myelotoxic agents and may be combined with active regimens or given in sequence to them.

There are several investigative areas which have generated new agents. They include (1) immunomodulation and immunotherapy, (2) specific inhibitors of histone deacetylase, and (3) potential new agents based on gene microarray studies in H/RS cells including inhibitors, of NFκB, galectin 1 and Bcl-2.

Immunotherapy

The evolution of immunotherapeutic efforts against H/RS cells is based on some of the known phenotypic characteristics associated with them. The major focus has been on the presence of CD30, a 120kiloDalton transmembrane protein of the tumor necrosis receptor family on almost all H/RS cells and thus affording a target for the generation monoclonal antibodies against this antigen. The clinical therapeutics of antiCD30 has a relatively long history beginning in the late 1980's and extending to the present. Initial studies with a Ber-H2 antibody showed no direct cytotoxic effort on cell lines, thus generating an effort in immunotoxins linking the monoclonal antibody such as saporin, or ricin A a chain.[2] Alternatively, bispecific complexes with antiCD30 linked to anti-CD16 as a result of a CD30/CD16 hybridoma construct was tested in advanced, refractory HL. Several responses were noted in the later circumstance but the approach was not pursued further.[3]

The results were considered

promising enough so that another hybrid was constructed between murine antiCD30 and humanized CD64 specific for triggering cytotoxic effector cells with FcγRI. Clinical trials in 10 heavily-treated patients showed 4 responses.[4] Further linkage studies which included Iodine[131] demonstrated six responses (ICR, 5PR) in 22 patients.[5] Hematotoxicity was an issue. MDX-060 is a human anti-CD30 monoclonal antibody which, however, was lacking in objective responses in a Phase I/II trial (6/72 patients).[6] The drug was well tolerated with minimal toxicity. Similar modest effects were noted with a chimeric monoclonal anti-CD30 SGN-30.[7]

The most recent rendition is the linkage to antiCD30 with a toxic auristatin E.[8] At a spectrum of dose levels 0.1 mg/kg to 3.6 mg/kg in a phase I trial, there were 17/45 (38%) objective responses including 9 CRs. This may be a promising new agent since the level of response was comparable to that seen in the early experience with gemcitabine in relapsed/refractory HL.[9]

The presence of CD25 on the H/RS cell has also prompted clinical trials of an antiCD25 ricin A-chain immunotoxin.[10] The response in 15 patients with considerable toxicity limited this agent. A recombinant fusion toxin (DAB485 IL-2) with the diphtheria toxin linked by IL-2, now in clinical use for cutaneous T-cell lymphoma, has been tested in only 4 patients with HL with a single CR.[11]

Although few patients have been treated, cellular immunotherapy with autologous EBV (Epstein-Barr virus) antigen (LMP2a) specific cytotoxic T-lymphocytes has been a protracted clinical research effort.[12] *Ex vivo* sensitization of autologous lymphocytes is required and thus restricted to patients who have EBV-positive Hodgkin lymphoma cells. Responses have been noted but this approach has, thus far, had limited applicability.[13,14] Immunomodulation and possibly other mechanisms contribute to the anti-tumor effect of Lenalidomide in the treatment of multiple myeloma and myelodysplasia. These could include direct cellular effects, changes in surrounding cellular environment and alteration of cytokine biology.[15] A single Phase II trial of continuous oral Lenalidomide 25 mg/day resulted in objective responses in 4/12 (33%) patients with advanced HL refractory to prior autologous transplantation. Cytopenias were the major side effect in these heavily-treated patients.[16]

Histone deacetylase inhibitors

The organization of chromatin into a condensed or open status can influence molecular processes such as recombination, replication, DNA repair, and transcription. Gene expression controlled by the status of chromatin formation has a key role in the genesis and progression of cancer. Acetylation of histones (H3, H4) is associated with an open chromatin status and thus increased transcription. Deacetylation of histone residues leads to condensing of chromatin and repression of transcription. Malignant cells are associated with an imbalance between acetylation and deacetylation.[17] Inhibitors of histone deacetylases are a new class of chemotherapeutic agents. The enzyme occurs in various isoforms contained in 4 classes – Class I has 1,2,3,8 isoforms; Class II 5-10; Class III a group not inhibited by HDAC inhibitors; and Class IV isoforms. In experimental systems, HDAC inhibitors have shown: (1) induction of tumor cell apoptosis, (2) decreased proliferation, (3) induction of differentiation, (4) anti-angiogenesis, and (5) increased host immune surveillance.[17] There are a variety of inhibitors with differing chemical structure with varying pharmacologic properties. The inhibitors have several subclasses, such as hydroxyamic acids and aminophenylbenzamides. There have been few trials of HDAC inhibitors in the HL perhaps more in non-Hodgkin lymphoma, especially

the suberoylanilide hydroxyamic acid (SAHA) which has been approved for cutaneous T-cell lymphoma.[18] There have been a number of preliminary phase I phase II trials of different inhibitors in HL presented in abstract form. MGCD 0103, an orally available isotype-specific aminopheylbenzamide targets isoforms 1,2,3 and 11. Initial trials with this agent had a 38% (8/21) response rate when given three times a week for four cycles.[19] Of greater interest is the HDAC inhibitor, panobinostat (LBH 589), an oral agent given three times a week or every other week in a phase I trial which showed a high response rate in 9/13 patients evaluated by PET and/or CT.[20] Another inhibitor, ITF 2357, was evaluated in refractory patients by the Insituto Nazionale Tumori (Milan) group (hydroxamic acid) 7/13 had stabilization of disease with decrease in PET activity.[21] These agents are numerous and of the differing pharmacologic properties. It would appear that one or more will emerge. LBH589 does have an order of thrombocytopenia associated with its administration, which may limit its combination with myelotoxic drugs.

Other potential molecular targets NF-κB

H/RS cell lines have shown constitutive activation of NF-κB as well as nuclear localization. Transcription factor NF-κB plays a key role in the regulation of immune/inflammatory responses and can function as a potent inhibitor of apoptosis. Normal inhibitors are I-κB proteins which retain NF-κB in the cytoplasm. Activation by any number of mechanisms, including receptor/ligand interaction of the cell surface activates I-κB kinase which, in turn, phosphorylates the I-κB proteins with the I-κB kinase complex leading to subsequent proteosomal degradations of the inhibitors, such that NF-κB can then be translocated to the nucleus.[22] Proteosomal inhibition is the presumed mechanism of action of bortezomid which is active in the treatment of multiple myeloma.[23] Bortezomid has undergone two phase II trials in refractory/relapsed HD and appeared to be inactive with one response in 39 patients.[24,25] Despite the data that NF-κB is "activated" in H/R-S cell lines, it is not the sole transcription factor activated in HL. STAT3 and STAT5 are also contribute to activation in H/RS cell lines.[26,27] Whether inhibition of JAK/STAT system will be useful in HL management remains unanswered. JAK2 has been noted to be highly expressed, especially in lymphocyte predominant HL (85% of cases), but the activating mutation in exon 12 characteristic of myeloproliferative disease was not observed.[28] The latter might have allowed for the testing of the new JAK-2 inhibitors.

Gene expression profiles have identified signatures which correlate with specificity for HL[29-32] and for patterns of resistance.[33] The array analyses showed 27 distinct genes upregulated in H/RS cells. Five genes are uniquely specific for HL (PRAME, IPL, Fer, RAB12, EAR3). These analyses have also demonstrated overexpression of GAL1 (galectin-1) in H/RS cell. It is a T-cell inhibitory molecule which is expressed via an Activation Protein 1-dependent (AP-1) enhancer, suggesting that GAL-1 encourages an immunosuppressive microenvironment surrounding the H/RS cell.[34] This has led to a program to structure an anti-galectin-1 antibody inhibitor at the Dana-Farber.

Micro RNA(s) are negative regulators of gene expression. A study of 157 microRNAs in HL tissues and normal lymph nodes revealed a unique signature of 25 microRNAs in classic HL. The important role of these entities in the biology of HL may be therapeutically exploitable in the future.[35]

Other potential areas which could be explored therapeutically include the use of mTOR inhibitors, such as Temsirolimus, either alone or in combination. Among biologic fac-

tors, overproduced related to gene overexpression include cyclinE, CDK2, CDK6, and bcl-2 among others.[30] Bcl-2 is known to be expressed in 26-65% of patients and has been associated with a poorer prognosis in treated patients.[36,37] Bcl-2 as a target is being explored in CLL and non-Hodgkin lymphoma. There are a wide variety of chemical structures involved in Bcl-2 antagonism.[38] A number of these agents in current trial can bind and inhibit with high affinity the Bcl-2 family proteins, thus potentially enhancing apoptosis. These inhibitors have not been clinically tested in HL. One of these compounds ABT-263 is an orally active inhibitor which has shown some activity in non-Hodgkin lymphoma in preliminary trials.[39] The value of rituximab in the treatment of lymphocyte-predominant nodular HL is well established with a high response rate >90% and a prolonged failure-free survival.[40,41] However, the therapeutic value in classic HL is uncertain. There are only 20-25% of patients who express CD20 to some extent as opposed to lymphocyte predominant nodular where there is almost 100% expressed. Despite one study showing an adverse prognostic effect of CD20,[42] several other studies showed either no differences or superior failure-free survival to CD20 positivity.[43,44] The impact of rituximab in classic HL is unclear. Reduction of infiltrating normal B lymphocytes and other non-H/RS directed effects could give an "apparent" response. Only a randomized trial in CD20 positive and CD20 negative classic HL of the same chemotherapy with or without rituximab will give a valid answer.

The moving field of tumor cell molecular biology may allow for new therapies whose toxicity is not additive with chemotherapy. The principle area of investigation up to now is appropriately uncontrolled phase II trials looking for activity. Given the very gratifying effect of modern chemotherapy in newly diagnosed HL, the additive benefit, if any, of new therapies will require considerable numbers of patients, therefore, the best approach is to focus on the salvage/second-line treatment either prior to autologous transplantation or in those who fail or are unable to have an autologous transplant.

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