[haematologica reports]
2006;2(13):83-86

Rationale for the design of combination therapies that are active in T-cell lymphomas?

W. PLUNKETT

Department of Experimental Therapeutics, The University of Texas M. D. Anderson Cancer Center, Houston, Tx, USA

Supported by grants CA28596, CA32839, CA 81534, and CA100632, from the National Cancer Institute, Department of Health and Human Service.

The lymphomas are the most diverse group of diseases within any single class of malignancy. More than thirty different clinicopathologic diseases have been identified and classified. Such a variety of diseases of the lymphoid system likely are the result of the unique etiology of each individual tumor type. On the basis of function, T-cells and B-cells have distinctly different functions, and undoubtedly activate genes to drive the different specialized pathways to achieve these purposes. Factors that clearly have a role in lymphomagenesis include illegitimate gene recombination, infection by oncogenic viruses, impaired host immunity, and persistent proliferation driven by inflammation. Indeed, major subtypes of lymphomas have now been identified with unique gene expression profiles. Each of these factors, and other more specialize characteristics, directly and uniquely affect the response of these cancers to therapies. As more is known of the etiology and underlying defects of each disease, of the unique molecular signatures of genes expressed and pathways that are involved, therapies will be developed that are increasingly more individualized toward such tumor-specific characteristics.

Nevertheless, as inevitable as this outcome is, the reality that we do not yet have adequate knowledge about the essential driving characteristics of this family of diseases. Obtaining adequate understanding to support and guide the development of disease-specific therapeutic strategies will require substantial efforts. Although these endeavors are ongoing, we would do well to take advantage of existing leads to develop mechanism-based hypotheses for the design of trials that will put our postulates to the test in the clinic. In this respect, this article will examine the molecular actions of some established therapeutic approaches to the current treatment of lymphomas as well as emerging strategies. The former will address classes of agents such as alkylating agents, platinum derivatives, and topoisomerase II inhibitors that alter the structure of DNA or frankly damage DNA to an extent that induces excision DNA repair processes. The latter will be represented by the nucleoside analogues gemcitabine, nelarabine and clofarabine, which are relatively new to the treatment of lymphoma. The goal of this discussion is consider combinations of these agents with regard to their mechanisms of action, and the responses elicited from the tumor with respect their susceptibility to inhibition of DNA repair, with a view toward the development of new therapeutic strategies.

New Nucleoside Analogues

While fludarabine has substantial activity in B-cell malignancies, including in combinations with other agents in lymphomas, it has not been effective in T-cell diseases. However, early results indicate that other nucleoside analogues have activities as single agents in T-cell malignancies. For instance, as preclinical studies indicated that nelarabine could be selectively toxic to T-cells, these patients were preferentially recruited for participation in early trials. Results of a phase I trial demonstrated impressive activity in both adult and pediatric T-lymphoblastic lymphoma (Kurtzberg, 2005). Recently, a phase II trial of pediatric patients with T-cell diseases demonstrated activity in T-cell lymphomas (Berg, 2005). The results of nelarabine treatment of adult patients with peripheral T-cell lymphoma indicated promising activity an phase II trials (Goy, 2003; Thompson, 2005). Although gemcitabine has be used mainly for treatment of solid

tumors, two recent studies in of gemcitabine patients with CTCL have generated promising results (Marchi, 2005; Duvic, 2006). As with nelarabine, clofarabine has recently been approved for use against pediatric acute lymphocytic leukemias. Although reports of the evaluation of clofarabine in T-cell malignancies are presently lacking, it has an action mechanism similar to nelarabine and gemcitabine, and the active triphosphate metabolites of all three nucleoside analogues are long lived in leukemia cells (Plunkett, 1996; Gandhi, 2006).

DNA damaging agents

Alkylating agents, and platinum derivatives have clinical activity against a wide variety of hematopoietic neoplasms, some solid tumors, and in the context of bone marrow transplantation. The cytotoxicity of these agents is thought to be due to adducts formed on DNA that cause difficulties for the cell to conduct DNA replication and transcription activities. Generally, the interstrand cross-links, which are the minor component of the adducts, are associated with therapeutic activity (Chaney, 1996). Nevertheless, it is likely that monoadducts may also be of therapeutic value. When adducts are formed on DNA, cells respond by activating one or several DNA repair processes that include a step that excises the damaged nucleotides. These include base excision repair, which generally replaces a single damaged nucleotide, although as many as eight adjacent nucleotides may be removed. Subsequently the gap is filled by DNA polymerase, and sealed by DNA ligase. Generally this mechanism is activated to repair relatively small alkyl groups that result from the actions of triazine compounds that are frequently used in lymphoma therapy, dacarbazine and procarbazine.

Larger DNA adducts, such as those generated by nitrogen mustards (mustargen) and chloroethylnitrosoureas (cyclophosphamide, chlorambucil, BCNU, busulfan) and platinum derivatives (cisplatin, carboplatin, oxaliplatin) are repaired by the nucleotide excision repair mechanism. Adducts caused by UV light are also removed by this treatment. Following recognition and endonucleolytic incision on either side of the lesion, a single strand of 27 to 29 nucleotides is removed intact. Thereafter, a replicative DNA polymerase (delta or epsilon) re-synthesizes the DNA in the gap, and DNA ligase completes the process. Generally speaking, this is a widely prevalent process, particularly in lymphoid tissues, and is highly efficient.

These same agents may be bi-functional, that is they are capable of making adducts to two separate nucleophiles. Practically, this means that they are capable of forming crosslinks within or between DNA strands, or between DNA and RNA or protein. Interstrand crosslinks in DNA are thought to be potentially lethal lesions, as they block essential functions such as DNA replication and interfere with RNA transcription. Although less well characterized then other damage repair mechanisms, DNA interstrand crosslinks are thought to be repaired by a combination of nucleotide excision repair and recombination events.

Activation of cell death signaling pathways in response to inhibition of DNA repair processes

Lymphoid tissues are known to be capable of highly efficient repair of damaged DNA. Our work investigated a mechanistic basis for combining agents that damage DNA and thereby initiate excision DNA repair processes with a nucleotide analog that could inhibit this action. This strategy, using UVC (Sandoval, 1996), cisplatin (Li, 1997, 2000), or cyclophosphamide (4-hydroxycyclophosphamide) (Yamauchi, 2001, 2002) to induce excision DNA repair and several nucleotide analogs to block these processes, was synergistically cytotoxic to cells in culture. When translated into clinical trials as the fludarabine and cytoxan combination, this treatment produced responses including complete remissions in patients with advanced CLL who had failed prior therapy with each agent (O'Brien, 2001). Further, this treatment extended event-free survival by nearly 50% in previously untreated CLL (Wierda, 2005). These clinical results supported the hypothesis that the combination was acting by a mechanism that was different from those activated by nucleosides or alkylating agents administered alone.

Recent work has been directed at understanding how cells sense and respond to inhibition of DNA repair (Rao, 2003). Investigations in model systems using quiescent human lymphocytes demonstrated that non-toxic UVC irradiation (2 J/m^2 UVC) initiated nucleotide excision repair. Incubation with fludarabine nucleoside was also not toxic, but when the two agents were combined, there was greater than additive apoptosis after 24 hr. Blocking the incorporation of fludarabine nucleotide into repairing DNA using with aphidicolin lowered the apoptotic response to the low level observed with aphidicolin and UV. Phosphorylation of p53 and p53 protein

accumulation were detected 2 hr after combination treatment. Importantly, Fas and Fas ligand mRNA expression and protein levels increased significantly after repair inhibition. Neutralizing antibodies against Fas or Fas ligand significantly reduced apoptosis. Caspase-8 was activated by the combination. These results suggest that inhibition of excision DNA repair by a nucleoside analogue is critical for cytotoxicity, and that induction of apoptosis may be conducted by a p53-mediated signaling mechanism to the Fas death pathway.

These investigations have been extended to primary CLL lymphocytes, which respond to DNA alkylation by excision repair, with the extent of repair increasing as the cells acquire resistance to alkylating agents. Because incorporation of nucleotide analogues into the repair patches elicits death signals in quiescent cells, the increased capacity for excision repair in alkylator-resistant cells could facilitate incorporation of nucleotide analogues. It was hypothesized that the mechanism-based interaction of nucleoside analogues with alkylating agents could elicit greater than additive killing of CLL cells (Yamauchi, 2001). Lymphocytes from patients with CLL that were not refractory to alkylators were treated *in vitro* with cyclophosphamide pro-drug 4-hydroperoxycyclophosphamide (4-HC) with or without prior incubation with fludarabine nucleoside or clofarabine. CLL lymphocytes promptly initiated and completed excision repair in response to 4-HC. A pre-incubation with either nucleoside analogue inhibited the repair initiated by 4-HC. Combining 4-HC with either nucleoside produced more than additive apoptotic cell death than the sum of each alone. The increase in cytotoxicity was proportional to the initial magnitude of the DNA incision and to the extent of repair inhibition by the nucleoside analogues, suggesting close correlation between the repair inhibition and induction of cell death. These results indicate that DNA repair, which is active in CLL lymphocytes, may be a biologic target for facilitating the incorporation of nucleoside analogues and increasing their cytotoxicity. Thus, the increased repair capacity associated with resistant disease may be manipulated to therapeutic advantage in T-cell malignancies as well.

Oxaliplatin and nucleoside analogues have different but potentially complementary mechanisms of action. We hypothesized that potentiation of oxaliplatin toxicity by nucleoside analogues maybe due to the inhibition of the DNA excision repair pathways activated by oxaliplatin adducts. To test this, the cytotoxic interactions between the oxaliplatin and fludarabine, clofarabine and cytarabine were investigated in normal and CLL lymphocytes (Moufarij, 2006). In each population, the combination resulted in greater than additive killing. Analysis of oxaliplatin damage revealed thatnucleosides enhanced accumulation of interstrand crosslinks in specific regions of the genome in both populations, but to a lesser extent in normal lymphocytes. The action of nucleosides on the removal of oxaliplatin crosslinks was explored to investigate the mechanism by which oxaliplatin toxicity was increased. Lymphocytes from CLL patients have a greater capacity for crosslink unhooking compared to normal lymphocytes. In the presence of the nucleosides, the extent of repair was significantly reduced in both populations, more so in CLL. These findings support a role of nucleoside analogue-mediated DNA repair inhibition as a mechanism critical for the cytotoxic synergy of the two drugs.

In conclusion, clinical results of single agent studies with newer nucleoside analogues indicate promising activities in T-cell malignancies. The challenge now is to combine these agents with other drugs to which these diseases respond. The mechanism-based interactions of nucleoside analogs with DNA damaging agents that initiate excision DNA repair processes would generate a new mechanism of action, one that perhaps prior treatment has not generated resistance. The agents are at hand to test this rationale for the rationale design of combination therapies in T-cell malignancies.

References

Berg SL, Blaney SM, Devidas M, et al. Phase II study of nelarabine (compound 506U78) in children and young adults with refractory T-cell malignancies: a report from the children's Oncology Group. J. Clin. Oncol 2005;23:3376-82.

Chaney SG, Sancar A. DNA repair: enzymatic mechanisms and relevance to drug response. J Natl Cancer Inst 1996; 88: 1346-60.

Duvic M, Talpur R, Wen S. et al. Phase II evaluation of gemcitabine monotherapy for cutaneous T-cell lymphoma. Clin Lymphoma Myeloma 2006;7:51-8.

Gandhi V, Plunkett W.Clofarabine and nelarabine: two new purine nucleoside analogues. Curr Opin Oncol 2006;18: 584-90.

Goy A, Bleyer A, Hagemeister F, Fayad L, Pro B, Romaguera J, et al. Phase II study of Compound GW506U78 (ara-G) for patients with indolent B-cell or peripheral T-cell lymphoma previously treated with chemotherapy. Blood 2003;102: 639a.

Kurtzberg J, Ernst TJ, Keating MJ. et al. Phase I study of 506U78 administered on a consecutive 5-day schedule in children and adults with refractory hematologic malignancies. J. Colin Oncol 2005;23:3396-403.

Li L, Keating MJ, Plunkett W, Yang L-Y. Fludarabine-mediated repair inhibition of cisplatin-induced DNA lesions in human chronic myelogenous leukemia-blast crisis K562 cells: induction of synergistic cytotoxicity independent of

reversal of apoptosis resistance. Mol Pharmacol 1997;52: 798-806.
Li L, Liu X, Glassman AB, Keating MJ, Stros M, Plunkett Wet al. Fludarabine triphosphate inhibits nucleotide excision repair of cisplatin-induced DNA adducts in vitro. Cancer Res 1997;57:1487-94.
Marchi E, Alinari L, Tani M, et al. Gemcitabine as frontline treatment for cutaneous T-cell lymphoma. Phase II study of 32 patients. Cancer 2005;104:2437-441.
Moufarij M, Sampath D, Keating MJ, Plunkett W. Fludarabine increases oxaliplatin cytotoxicity in normal and chronic lymphocytic leukemia lymphocytes by suppressing interstrand DNA crosslink removal, Blood, In press, 2006.
O'Brien SM, Kantarjian HM, Cortes J, Beran M, Koller CA, Giles FJ, et al. Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia. J Clin Oncol 2001;19:1414-20.
Plunkett W, Searcy C, Huang P, Gandhi V. Gemcitabine: preclinical pharmacology and mechanisms of action. Semin Oncol. 23(Suppl 10):3-15, 1996.
Rao VA, Plunkett W. Activation of a p53-mediated apoptotic pathway in quiescent lymphocytes after the inhibition of DNA repair by fludarabine. Clin Cancer Res 2003;9: 3204-12.
Sandoval A, Consoli U, Plunkett W. Fludarabine-mediated inhibition of nucleotide excision repair induces apoptosis in quiescent human lymphocytes. Clin Cancer Res 1996;2: 731-1741.
Thompson MA, Pro B, Sarris A, Hagemeister FB, Goy A, Bleyer A, et al. Results of a Phase II study of 506U78 (Nelarabine) in refractory indolent B-Cell or peripheral T-Cell lymphoma. Blood, 106:753a, 2005 (abstract).
Wierda W, O'Brien S, Wen S, et al. Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab for relapsed and refractory chronic lymphocytic leukemia. J Clin Oncol 2005;23:4070-8.
Yamauchi T, Keating MJ, Plunkett. W. UCN-01 (7-hydroxystaurosporine) inhibits DNA repair and increases cytotoxicity in normal lymphocytes and chronic lymphocytic leukemia lymphocytes. Mol Cancer Ther 2002;1:287-94.
Yamauchi T, Nowak BJ, Keating MJ, Plunkett W. DNA repair initiated in chronic lymphocytic leukemia lymphocytes by 4-hydroperoxycyclophosphamide is inhibited by fludarabine and clofarabine. Clin Cancer Res 2001; 7:3580-9.
Yang LY. Li L, Jiang H, Shen Y, Plunkett W. Expression of ERCC1 antisense RNA abrogates gemcitabine-mediated cytotoxic synergism with cisplatin in human colon tumor cells defective in mismatch repair but proficient in nucleotide excision repair. Clin Cancer Res 2000;6: 773-81.