

# Treatment of HIV-1 infection with combination therapy: Antiretroviral agents and biological response modifiers

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**SCHNITTMAN SM. Treatment of HIV-1 infection with combination therapy: Antiretroviral agents and biological response modifiers. Can J Infect Dis 1994;5(Suppl A):42A-46A.** While nucleoside antiretroviral agents are effective in delaying disease progression in human immunodeficiency virus (HIV)-infected individuals, their activity is limited in magnitude and duration. Therefore, approaches to attacking HIV via combination therapies have recently been under investigation. In particular, since HIV infection dysregulates and destroys the immune system, it is logical to develop therapeutic approaches that would both restore the immune response and have direct antiviral activity. Preliminary evaluations of the combination of zidovudine with interferon alpha (IFN- $\alpha$ ) have demonstrated enhanced antiviral, antitumour and immunomodulatory activity. Other promising approaches include antiretroviral therapy with interleukin (IL)-2, and IFN- $\alpha$  with IL-2. The clinical research pertaining to these combinations of antiretrovirals and biological response modifiers is reviewed.

**Key Words:** AIDS, Combination therapy, Human immunodeficiency virus type 1, Interferon alpha, Interleukin-2

## Traitement d'association dans l'infection au VIH-1: antirétroviraux et modificateurs de la réponse biologique

**RÉSUMÉ :** Bien que les antirétroviraux de type nucléoside soient efficaces à retarder la progression de la maladie chez les sujets infectés par le virus de l'immunodéficience humaine (VIH), leurs actions restent limitées en ampleur et en durée. C'est pourquoi les approches qui visent à lutter contre le VIH au moyen d'associations pharmacologiques ont récemment fait l'objet de recherche. Particulièrement, étant donné que l'infection au VIH dérègle et détruit le système immunitaire, il est logique de mettre au point des approches thérapeutiques qui restaureraient la réponse immunitaire et auraient une action antivirale directe. Les évaluations préliminaires des associations de zidovudine et d'interféron alpha ont montré une activité antivirale, antitumorale et immunomodulatrice accrue. D'autres approches prometteuses sont, entre autres, le traitement antirétroviral avec l'interleukine (IL)-2 et l'interféron alpha avec IL-2. La recherche clinique sur ces deux associations d'antirétroviraux et de modificateurs de la réponse biologique est passée en revue.

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THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) infects the CD4+ T helper lymphocyte population, leading to a progressive decline in immunological function and ultimately to the development of AIDS (1). While both the humoral and cellular arms of the immune system respond to the presence of HIV-1 infection, the relative contribution of each component in controlling HIV-1 infection and disease progression remains unclear.

Despite the considerable progress made in understanding the pathogenesis of AIDS, the treatment of HIV-1 infection is still in its infancy. Zidovudine (ZDV, AZT) remains the current standard therapy (2); however ZDV has toxicities, is only partially effective, and its duration of benefit is limited by the development of resistance. These observations support the concept that combination therapy can be used to extend clinical benefits in HIV-infected patients and to prevent the selection of drug-resistant viral variants.

Since HIV-1 infection is a viral disease that destroys the immune system, it is logical to look for combination therapeutic approaches that would bolster the immune response in general and against HIV-1 specifically. A useful strategy for developing combination drug therapies would be to combine agents that do not share cross-resistance, have different mechanisms of action and have different dose-limiting toxicities.

#### ANTIRETROVIRAL THERAPY IN COMBINATION WITH INTERFERON FOR PATIENTS WITH HIV-1 INFECTION

Interferon alpha (IFN- $\alpha$ ) has demonstrated antiviral, antitumour and immunomodulating activities. Recombinant (r) IFN- $\alpha$  and ZDV have been shown to inhibit synergistically the replication of HIV-1 in peripheral blood mononuclear cells (PBMC) *in vitro* at concentrations readily achieved in patients (3).

ZDV and IFN- $\alpha$  inhibit HIV replication by different mechanisms. ZDV acts at an early stage of replication by inhibiting HIV reverse transcriptase. IFN- $\alpha$  acts on later stages of viral replication by inhibiting budding of viral particles (and possibly earlier stages as well).

Many reports have shown that IFN- $\alpha$  can cause lesions of Kaposi's sarcoma to regress in patients with AIDS (4-11). Patient characteristics likely to be associated with a response of Kaposi's sarcoma lesions to IFN- $\alpha$  include higher CD4+ T cells and no prior opportunistic infections or AIDS-related complex (ARC)-like symptoms. The most common side effects encountered in IFN- $\alpha$  therapy include 'flu-like symptoms (fever, chills, myalgias, fatigue), leukopenia, thrombocytopenia and elevated liver enzyme levels.

ZDV prolongs survival and delays the progression of AIDS and ARC (2). The rationale for the use of ZDV in patients with AIDS has to do with the mechanism of HIV replication rather than any specific effect on AIDS-related Kaposi's sarcoma. Patients treated with ZDV show decreases in serum HIV p24 antigen, an increase in CD4+ T cell number and partial reversal of skin test

anergy. The most common side effects encountered in ZDV therapy include nausea, fatigue, anemia and neutropenia (dose-dependent) and peripheral neuropathy.

#### PHASE 1 TRIALS IN PATIENTS WITH AIDS-RELATED KAPOSI'S SARCOMA

Kovacs et al (12) were the first to describe the safety, tolerance, therapeutic efficacy and antiviral activity of IFN- $\alpha$  and ZDV combination therapy in patients with AIDS-related Kaposi's sarcoma, and in HIV-1 infection in general. The authors reported the results of a clinical trial of combination therapy with IFN- $\alpha$  and ZDV in 39 patients with AIDS-related Kaposi's sarcoma. Patients received 250, 100 or 50 mg of ZDV every 4 h, six weeks after IFN- $\alpha$  therapy was initiated at 5 million U/day. IFN- $\alpha$  dose was then increased every two weeks until a maximal tolerated dose (MTD) was reached, and then both agents were continued for 12 weeks.

The most common side effects were neutropenia, thrombocytopenia, hepatic dysfunction and weight loss. Twenty-two of 39 patients tolerated 12 weeks of therapy without dose reduction - of these, one had a complete response, and 10 had a partial response. The antiviral responses included six of 22 patients who became culture-negative, and three of six patients with p24 antigenemia who became p24 antigen-negative. The CD4+ T cell counts decreased significantly during treatment: 552 to 450 cells/mm<sup>3</sup> over 12 weeks; however, the CD4 remained stable at 35%.

In a phase 1 trial, Krown et al (13) gave IFN- $\alpha$  in combination with ZDV to 41 patients with AIDS-related Kaposi's sarcoma. Patients received 100 or 200 mg ZDV every 4 h and 4.5, 9 or 18 million U/day of IFN- $\alpha$  for eight weeks. Thirty-four of 38 evaluable patients completed the therapy.

The major dose-limiting toxicity was neutropenia. MTDs for IFN- $\alpha$  were 4.5 million U/day with 200 mg ZDV, or 9 million U/day with 100 mg ZDV. Antitumour effects were seen in 17 of 37 patients who had complete or partial regressions, with antitumour responses more common in patients with CD4 counts over 200. Eleven of 17 responding patients had sustained tumour regressions for weeks 31 to 101 during extended treatment, while six of 17 patients relapsed.

Antiviral effects seen included a progressive increase in the proportion of negative cultures over time. In addition, eight of nine p24 antigenemic patients became p24 antigen-negative during the study. Immunological effects described included 16 of 21 anergic patients who developed skin test reactivity to at least one antigen during the study. In addition, CD4 counts decreased over eight weeks of study.

The authors concluded that combination therapy with IFN- $\alpha$  and ZDV can be safely administered to patients with AIDS-related Kaposi's sarcoma, and that the antitumour and antiviral effects were such that further study was warranted.

In a phase 1 trial, Fischl et al (14) administered IFN- $\alpha$  and ZDV to 56 patients with AIDS-related Kaposi's sarcoma. Patients received IFN- $\alpha$  at 9, 18 or 27 million U/day and ZDV at 100 or 200 mg every 4 h for eight weeks, followed by a 48-week maintenance period. The mean CD4 count at entry was 185 cells/mm<sup>3</sup>. The major toxicities were hematological, with anemia (2.9 to 3.8 g/dL fall in hemoglobin levels over eight weeks) and neutropenia (44% of patients fell to less than 750 cells/mm<sup>3</sup> [worse with high dose ZDV]), as well as hepatotoxicity (3.5-fold rise in transaminase activity in all groups, 10 patients with more than a 10-fold increase at the highest combination dose).

Immunologically, an overall gradual increase in CD4 cell counts was seen. Antiviral effects noted were a p24 antigenemia decrease in 12 of 17 p24 antigen-positive patients. Tumour regressions were seen in 42 to 55% of patients, a higher percentage than that seen in previous studies with IFN- $\alpha$  alone. Resistance to ZDV was studied in four patients, and HIV susceptibility showed no change in these patients during 12 months of treatment.

The authors concluded that the study showed that ZDV enhances the beneficial effects of IFN- $\alpha$  in the treatment of AIDS-related Kaposi's sarcoma. In addition, IFN- $\alpha$  may delay the development of ZDV resistance.

### PHASE 1 TRIALS IN ADVANCED HIV-1 INFECTION

In another phase 1 trial, Berglund et al (15) studied 18 patients with symptomatic HIV infection (Centers for Disease Control and Prevention group IV). All patients were on ZDV (four to 28 months) with p24 antigenemia and CD4 mean cell count of approximately 150 cells/mm<sup>3</sup>. In this study, patients received 3 million U/day of native IFN- $\alpha$  for three months, in addition to continuing ZDV (400 to 1200 mg daily). The toxicities seen included: transient myalgias/arthralgias, persistent fatigue, hemoglobin decrease from 12.9 to 12.0 g/dL, white blood cell count decrease from 3400 to 2800 cells/mm<sup>3</sup>, platelet decrease from 198,000 to 157,000 cells/mm<sup>3</sup> and weight loss from 68 to 65 kg.

The antiviral effects observed in the study included at least a 70% decrease in HIV culture titres in completely treated patients (8), and significant decrease in p24 antigen in six of eight completely treated patients, but in only one of nine incompletely treated patients. A significant increase in p24 antigenemia in serum was seen after IFN- $\alpha$  treatment was stopped. During IFN- $\alpha$  treatment, CD4 cell counts showed a tendency toward an increased rate of decline.

The conclusions from this study were that low dose native IFN- $\alpha$  can exert an antiviral effect even in the absence of a potentiating effect of ZDV in some severely immunocompromised HIV-infected patients. Native IFN- $\alpha$  appears to be more likely to induce side effects in combination with ZDV than is rIFN- $\alpha$ .

Combination ZDV/IFN- $\alpha$  therapy in patients with ad-

vanced HIV-1 infection was studied by Edlin et al (16). Thirteen homosexual men with p24 antigenemia despite six weeks of ZDV monotherapy were enrolled in an open label dose-ranging pilot trial. Patients continued on ZDV and were given IFN- $\alpha$  1.25 to 7.5x10<sup>6</sup> U/m<sup>2</sup> subcutaneously three times a week.

In this study, plasma p24 antigenemia levels demonstrated a biphasic response, falling initially by a mean of 50% (P=0.001) in 11 patients by 11 weeks, but rising steadily thereafter (P=0.001). CD4+ T cell counts fell by a mean of 7.1 cells/mm<sup>3</sup>/week. Higher initial CD4 counts predicted greater p24 antigenemia reductions. Higher IFN- $\alpha$  doses were associated with more severe side effects and greater falls in CD4, but no greater reductions in p24 antigenemia. Quantitative polymerase chain reaction for HIV-1 DNA in three patients showed a biphasic pattern paralleling the p24 antigenemia response. One can conclude from this study that, although some evidence of short term effects was seen, combination ZDV/IFN- $\alpha$  showed no lasting antiviral activity beyond that achieved with ZDV alone in patients with advanced HIV-1 infection.

### LONG TERM FOLLOW-UP IN CLINICAL TRIALS

Long term follow-up of 21 patients with HIV-associated Kaposi's sarcoma treated with ZDV and IFN- $\alpha$  was reported by Stadler et al (17). Patients received rIFN- $\alpha$  18 million IU every other day with ZDV 800 to 1200 mg/day. Fifteen patients tolerated therapy for an average of 10 months (range two to 20), with dose reductions as needed.

In this study, complete remission of Kaposi's sarcoma lesions was seen in four patients, partial remission in three patients, stable disease in two patients and progression in six patients, for an overall response rate of 46%. Even patients with CD4+ T cell counts under 100 cells/mm<sup>3</sup> responded. In contrast to results of monotherapy with IFN- $\alpha$ , patients with severely impaired immune systems benefitted from combined treatment with IFN- $\alpha$  and ZDV.

In summary, the phase 1/2 clinical trials of ZDV in combination with IFN- $\alpha$  have demonstrated safety and tolerability as well as antitumour and antiviral effects. In general, the best responses are seen in individuals with CD4 cell counts greater than 200 cells/mm<sup>3</sup>. Randomized, controlled phase 3 trials are underway to determine this combination's clinical efficacy (18).

### ANTIRETROVIRAL THERAPY IN COMBINATION WITH INTERLEUKIN-2 FOR PATIENTS WITH HIV-1 INFECTION

Interleukin (IL)-2 is a lymphokine produced by T cells that has a number of immunomodulating effects, which include:

- activation and blast transformation of T cells;
- stimulation of cytolytic activity against a variety of target cells;

- enhanced production of the macrophage activating factor IFN- $\gamma$ ;
- stimulation of B cells to proliferate, differentiate and secrete immunoglobulins;
- increased depressed natural killer activity and cytomegalovirus-specific cytotoxicity of lymphocytes from patients with AIDS;
- increased circulating lymphocytes in patients with HIV infection.

Previous trials of IL-2 alone in patients with AIDS or ARC have given results ranging from no detectable immune enhancement (19,20) to encouraging transient improvements in a number of parameters (21-26). These preliminary trials have suggested further studies of IL-2 in the presence of antiviral drugs and/or less advanced HIV disease.

In a recent study by Schwartz et al (27), the safety and preliminary efficacy of continuous intravenous IL-2 in conjunction with ZDV was assessed in 27 asymptomatic patients infected with HIV-1. All patients had CD4 cell counts greater than 400 cells/mm<sup>3</sup>. Patients received ZDV 200 mg orally every 4 h for eight weeks, before beginning IL-2 infusions of 0, 1.5, 3.0, 6.0 or 12.0x10<sup>6</sup> IU/m<sup>2</sup>/day given Monday through Friday for eight weeks.

In this study, all doses of IL-2 in combination with ZDV were reasonably tolerated (only two of 27 failed to complete therapy), although virtually all patients experienced headache, fever, myalgia, malaise, fatigue, dry skin and gastrointestinal disturbances to varying degrees. Immunologically, significant increases in CD4 cell counts were seen after 23 weeks of receiving the IL-2 infusions with ZDV, however, the effect was transient and was gone by week 4. CD8 cells followed the same trend. With respect to delayed type hypersensitivity, mild decreases in skin test responses during high dose IL-2 infusion were seen, but returned to baseline after IL-2 was discontinued. This was in contrast to previous reports of low dose IL-2 enhancing skin test reactivity. Significant increases in natural killer and lymphokine-activated killer activity were seen at the higher IL-2 doses. Circulating hypodense eosinophils and soluble IL-2 receptors increased more than 10-fold during combination therapy. Virologically, no patient who was p24 antigen-negative developed p24 antigenemia.

The authors concluded that the relatively good tolerance, absence of newly detectable p24 antigenemia and CD4 elevations are encouraging for combination therapy with IL-2 and ZDV.

### COMBINATION THERAPY WITH IFN- $\alpha$ AND IL-2 FOR PATIENTS WITH HIV-1 INFECTION

In a phase 1 trial, Schnittman et al (28) evaluated the safety/toxicity of combination treatment with IFN- $\alpha$  and rIL-2 in HIV-infected patients having CD4+ T cell counts of more than 200 cells/mm<sup>3</sup>. MTD of between 5 and 20 million U/day of IFN- $\alpha$  was determined for 16 patients;

after a minimum of two weeks at the MTD, four patients each received in combination one of the following doses of IL-2, given by continuous intravenous infusion over a three-week period: 0.5, 1.0, 2.0 or 3.0 million IU/day. Of the first 15 patients enrolled, 10 have completed the study as planned, and five went off study before receiving IL-2 (four due to toxicity of IFN- $\alpha$ ).

Toxicities of combination therapy with IFN- $\alpha$ /IL-2 included fatigue/malaise, anemia, transaminemia and fever. The immunological responses observed included transient increases in CD4+ T cell counts and spontaneous lymphocyte blast transformation, particularly with the higher doses of IL-2.

Antiviral effects seen included four of five p24 antigenemic patients who became serum p24 antigen-negative while receiving IFN- $\alpha$ . No patient developed serum p24 antigenemia while on study. PBMC quantitative microcultures demonstrated decreasing titres with IFN- $\alpha$  alone, with further decrements seen with the combination of IFN- $\alpha$ /IL-2. Two of two patients with Kaposi's sarcoma had significant tumour regressions.

It was concluded that an MTD of between 1 and 2 million IU/day of IL-2 is reasonably tolerated in combination with an MTD of IFN- $\alpha$  for three weeks. The antiviral responses seen appeared to be enhanced by the addition of IL-2.

In conclusion, the need for more effective therapies for HIV infection is self-evident. Therapies that combine agents with antiretroviral activity with those that can reverse the progressive immunosuppression of HIV-1 infection appear to be the most logical approach to treatment. Promising combination therapies of this type include ZDV/IFN- $\alpha$ , ZDV/IL-2 and IFN- $\alpha$ /IL-2. Larger controlled trials of these combinations in various staged populations of patients are required to determine the clinical utility of such regimens. The future of combination therapy lies in several directions. Preliminary studies are underway looking at adoptive immunotherapy with expansion and re-infusion of CD8+ cytotoxic T cells specific for HIV with concomitant IL-2. In addition, studies are underway exploring inhibitors of so-called 'bad' cytokines in HIV-1 infection, namely tumour necrosis factor alpha and IL-6. Finally, considerable interest in therapeutic vaccination has led to development of several clinical trials that may suggest that this approach is essentially one of biological response modifier.

### REFERENCES

1. Fauci AS. The human immunodeficiency virus: Infectivity and mechanisms of pathogenesis. *Science* 1988;239:617-22.
2. McLeod GX, Hammer SM. Zidovudine: Five years later. *Ann Intern Med* 1992;117:487-501.
3. Hartshorn KL, Vogt MW, Chou TC, et al. Synergistic inhibition of human immunodeficiency virus in vitro by azidothymidine and recombinant alpha A interferon. *Antimicrob Agents Chemother* 1987;31:168-72.
4. Krown SE, Real FX, Cunningham-Rundles S, et al.

- Preliminary observations on the effect of recombinant leukocyte A interferon in homosexual men with Kaposi's sarcoma. *N Engl J Med* 1983;305:1071-6.
5. Groopman JE, Gottlieb JS, Goodman J, et al. Recombinant alpha-2 interferon therapy for Kaposi sarcoma associated with the acquired immunodeficiency syndrome. *Ann Intern Med* 1984;100:671-6.
  6. Volberding P, Valero R, Rothman J, Gee G. Alpha interferon therapy of Kaposi's sarcoma in AIDS. *Ann N Y Acad Sci* 1984;437:439-45.
  7. Rios A, Mansell PW, Newell GA, Reuben JM, Hersh EM, Gutterman JV. Treatment of acquired immunodeficiency syndrome-related Kaposi's sarcoma with lymphoblastoid interferon. *J Clin Oncol* 1985;3:506-12.
  8. Gelman EP, Preble OT, Steis R, et al. Human lymphoblastoid interferon treatment of Kaposi's sarcoma in the acquired immunodeficiency syndrome: Clinical response and prognostic parameters. *Am J Med* 1985;78:737-41.
  9. Real FX, Oettgen HF, Krown SE. Kaposi's sarcoma and the acquired immunodeficiency syndrome: Treatment with high and low doses of recombinant leukocyte A interferon. *J Clin Oncol* 1986;4:544-51.
  10. Krown SE. The role of interferon in the therapy of epidemic Kaposi's sarcoma. *Semin Oncol* 1987;14(Suppl 3):27-33.
  11. Abrams DI, Volberding PA. Alpha interferon therapy of AIDS-associated Kaposi's sarcoma. *Semin Oncol* 1987;14(Suppl 2):43-7.
  12. Kovacs JA, Deyton L, Davey R, et al. Combined zidovudine and interferon- $\alpha$  in patients with Kaposi sarcoma and the acquired immunodeficiency syndrome (AIDS). *Ann Intern Med* 1989;111:280-7.
  13. Krown SE, Gold JWM, Niedzwiecki D, et al. Interferon- $\alpha$  with zidovudine: Safety, tolerance, and clinical and virology effects in patients with Kaposi sarcoma associated with the acquired immunodeficiency syndrome (AIDS). *Ann Intern Med* 1990;112:812-21.
  14. Fischl MA, Uttamchandani RB, Resnick L, et al. A phase I study of recombinant human interferon- $\alpha$ 2a or human lymphoblastoid interferon- $\alpha$ n1 and concomitant zidovudine in patients with AIDS-related Kaposi's sarcoma. *J AIDS* 1991;4:1-10.
  15. Berglund O, Engman K, Ehrnst A, et al. Combined treatment of symptomatic human immunodeficiency virus type-1 infection with native interferon-A and zidovudine. *J Infect Dis* 1991;163:710-5.
  16. Edlin BR, Weinstein RA, Whaling SM, et al. Zidovudine-interferon- $\alpha$  combination therapy in patients with advanced human immunodeficiency virus type-1 infection: Biphasic response of p24 antigen and quantitative polymerase chain reaction. *J Infect Dis* 1992;165:793-8.
  17. Stadler R, Bratzke B, Schaart F, Orfanos CE. Long-term combined rIFN-alpha-2A and zidovudine therapy for HIV-associated Kaposi' sarcoma: Clinical consequences and side effects. *J Invest Dermatol* 1990;95:170S-5S.
  18. Lane HC, Herpin B, Banks S, et al. Zidovudine vs alpha interferon vs the combination in patients with early HIV infection. Program and abstracts of the 8th International Conference on AIDS. Amsterdam, 1992:MoB 0052.
  19. Lotze MT, Robb RJ, Sharrow SO, Frana LW, Rosenberg SA. Systemic administration of interleukin-2 in humans. *J Biol Response Mod* 1984;3:475-82.
  20. Volberding P, Moody DJ, Beardslee D, Bradley EC, Wofsy CB. Therapy of acquired immunodeficiency syndrome with recombinant interleukin-2. *AIDS Res Hum Retroviruses* 1987;3:115-24.
  21. Lane HC, Siegal JP, Rook AH, et al. Use of interleukin-2 in patients with acquired immunodeficiency syndrome. *J Biol Response Mod* 1984;3:512-6.
  22. Mertelsmann R, Welte K, Sternberg C, et al. Treatment of immunodeficiency with interleukin-2: Initial exploration. *J Biol Response Mod* 1984;4:483-90.
  23. Lane HC, Fauci AS. Immunologic reconstitution in the acquired immunodeficiency syndrome. *Ann Intern Med* 1985;103:714-8.
  24. Kern P, Toy J, Dietrich M. Preliminary clinical observations with recombinant interleukin-2 in patients with AIDS or LAS. *Blut* 1985;50:1-6.
  25. Ernst M, Kern P, Flad HD, Ulmer AJ. Effects of systemic in vivo interleukin-2 (IL-2) reconstruction in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC) on phenotypes and functions of peripheral blood mononuclear cells (PBMC). *J Clin Immunol* 1986;6:170-81.
  26. Fauci AS, Rosenberg SA, Sherwin SA, Dinarello CA, Longo DL, Lane HC. Immunomodulators in clinical medicine. *Ann Intern Med* 1987;106:421-33.
  27. Schwartz DH, Skowron G, Merigan TC. Safety and effects of interleukin-2 plus zidovudine in asymptomatic individuals infected with human immunodeficiency virus. *J Acquir Immune Defic Syndr* 1991;4:11-23.
  28. Schnittman SM, Davey RT, Haneiwich S, Fauci AS, Lane HC. A phase I study of alpha interferon in combination with IL-2 in patients with HIV infection. *Clin Res* 1992;246A. (Abst)



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