

Forum for Collaborative HIV Research

**Immune-based therapies: A review of
clinical endpoints used in trials of
selected immunologic agents**

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While the use of highly active antiretroviral therapy (HAART) has lowered the level of virus in the bloodstream, immune reconstitution may offer additional benefits in maintaining viral suppression and enhancing immune function. New approaches in immune-based therapy (IBT) must be designed to capture endpoints that will allow approval of these agents by regulatory agencies. The challenge is to identify, develop, and validate biomarkers that can serve as endpoints in evaluating the efficacy of immunologic agents in HIV disease.

On December 7-8, 2000, the Forum for Collaborative HIV Research will sponsor a workshop, "Immune-Based Therapies and HIV Disease." Some of the questions for discussion at that meeting will include:

- **What are the appropriate indices to measure immune competence?**
- **What are the appropriate research designs and endpoints for trials evaluating IBTs?**
- **What can we learn from the research on IBTs in diseases other than HIV?**
- **What are the requirements of regulatory agencies for the approval of IBTs?**

This report prepared as background information for participants attending the December workshop, addresses issues related to these discussion questions. The report provides a review of study designs and endpoints used in clinical trials of selected immunologic agents. Eight of these agents have been approved by the U.S. Food and Drug Administration (FDA) for use in disease conditions other than HIV and five agents are under study for use in HIV-infected individuals but not FDA-approved for use in HIV disease.

This report was written by Karen Eddleman, who has done a remarkable job in identifying and reviewing many difficult references and producing a very reader-friendly report. Alan Landay has provided valuable input and assistance in the creation of this report. Houtan Movafagh provided much needed assistance in finding and copying the many references for this report.

June Bray, Ph.D – Deputy Director

The Forum for Collaborative HIV Research, a project of the Center for Health Services Research and Policy at the George Washington University School of Public Health and Health Services, was founded in 1997. The goal of the Forum is to facilitate discussion regarding emerging issues in HIV clinical research and the transfer of research results into care.

The Forum is a coalition of government agencies, clinical researchers, health care providers, pharmaceutical companies, and patient advocates. An Executive Committee made up of representatives from each of the above named constituency groups governs the Forum. The Executive Committee determines the subject and scope of the Forum projects. The Forum brings these constituencies together to identify gaps and impediments in the understanding of the medical management of HIV disease and develops recommendations to fill those gaps. The Forum is a public/private partnership, which receives financial support from its governmental and industry members and with in-kind support from its membership within the academic research, patient care, and advocacy communities.

For more information about the Forum or to download this report or prior ones, visit the Website at

www.hivforum.org

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Introduction

Immune-based therapy for HIV treatment and prevention:

A review of clinical endpoints for trials of immunologic agents

Early in the HIV epidemic, clinical trials of AIDS drugs followed the model of cancer-drug trials, which were commonly based on the endpoint of 5-year survival. This endpoint, however, in the era before highly active antiretroviral therapy, was impracticable given the rapid time course of disease progression to AIDS and eventual death. On December 11, 1992, the U.S. Food and Drug Administration issued a final rule whereby approval for certain new drugs and biological products for serious or life-threatening illnesses could receive accelerated approval with provisions for any necessary continued study of the drugs' clinical benefits after approval or with restrictions on use, if necessary.¹ The FDA will consider accelerated approval for a drug or biological product in two situations, the first of which is relevant here:

When approval can be reliably based on evidence from adequate and well-controlled studies of the drug's effect on a *surrogate endpoint* that reasonably suggests clinical benefit or on evidence of the drug's effect on a clinical endpoint other than survival or irreversible morbidity, pending completion of studies to establish and define the degree of clinical benefits to patients [emphasis added].¹

One comment received on the rule noted that:

Approval based on surrogate endpoints is not new, although the issue has not previously been considered in regulations. The agency has, in a number of instances, approved drugs based on surrogate endpoints. For example...drugs for hypercholesterolemia have been approved based on effects on serum cholesterol rather than on coronary heart disease (angina, heart attacks)...But, there was very good evidence from clinical trials...that improving the surrogate would lead to or is associated with the desired effects on morbidity and mortality. Even so, there is still today considerable debate about who will benefit from lowering cholesterol. Controlled trials assessing effects on clinical endpoints of morbidity and mortality from use of cholesterol-lowering drugs have been, and are being, conducted.¹

The general standard for approval of AIDS drugs evolved to be improved survival or delayed progression to an AIDS-defining opportunistic condition. In a clinical endpoint trial, the new drug must be shown to be at least as good as some standard treatment in reducing death or major disease progression in some group of patients.² Clinical endpoint trials present a spectrum of problems, though, including the following:

- need for a thousand or more volunteers
- requirement for at least one year of actual time on treatment
- questionable ethics of asking volunteers to remain in suboptimal treatment arms.²

In light of these problems, the trend in trials of HIV therapies has been toward surrogate markers of clinical efficacy. In current studies, primary endpoints are often virologic and clinical, with secondary endpoints being immunologic or assay-dependent. Study sample sizes are more often based on the need to detect differences in the magnitude or duration of the virologic effect, rather than clinically significant differences. It is important to note, however, that virologic failure and clinical failure are not equivalent.

¹ U.S. Food and Drug Administration, HHS. Final rule. 21 CFR Parts 314 and 601. December 11, 1992.

² James J. 1997. "Clinical trials and viral load: Statement for FDA community meeting." *AIDS Treatment News Archive*. www.immunet.org/immunet.atn.nsf/page/a-271-02.

Virologic failure may precede immunologic and clinical failure by months or years. No one yet knows how long it will take for a person experiencing a viral load rebound to progress to a clinical endpoint—an AIDS-defining illness or death.³

In lieu of a clinical endpoint, clinical trials of HIV therapies have moved away from the cancer-trial model and now rely more upon CD4+ cell count and viral load as surrogate markers. This shift is reflected in trends in antiretroviral study designs:

- Clinical endpoints, such as progression to AIDS and death, have become less practical, whereas laboratory measurements and treatment history have become important factors in trial design and analysis.
- Studies that once used CD4+ cell count thresholds for entry or exclusion criteria are relying increasingly on viral load thresholds.
- Many studies have restrictions related to prior therapy (e.g., protease-inhibitor naïve)
- As the use of laboratory measurements has increased and the incidence of clinical endpoints has declined, studies are becoming smaller and their duration shorter.
- Studies appear to be attempting to answer several questions at once. For example, they are simultaneously attempting to validate the use of new doses, new treatment combinations, and new diagnostic tests (such as genotypic or phenotypic resistance assays).³

Because the human immune response to HIV infection is effective at keeping the virus suppressed for a number of years, a focus of current HIV research has been immune-based therapies that attempt to bolster the patient's immune system,⁴ sometimes in tandem with antiretroviral therapy to suppress viral load. With the introduction of immune-based therapies, new, diverse surrogate markers are needed to monitor immune status and reflect viral burden. To widen the search for endpoints and surrogates that may be applicable to trials of immune-based therapies in HIV infection, the Forum for Collaborative HIV Research reviewed phase II and phase III trials of a dozen or so immune-based therapies. The results of that review have been summarized on fact sheets, which highlight relevant immune-based therapies, scrutinizing each study's design, as well as primary and secondary clinical endpoints. The first 8 immune-based therapies have been approved by the FDA for treatment of conditions other than HIV infection. The remaining fact sheets summarize immune-based therapies that have been evaluated or are currently being evaluated in phase II or phase III trials with HIV-infected individuals. These trials, too, are examined with an eye to study design and endpoints.

The endpoints, outcomes, surrogates, and assessments used in the supporting clinical trials involve a spectrum of clinical and immunological surrogates because they are addressing a wide variety of conditions. Some of them—tumor size, transplant rejection—clearly do not apply to the clinical setting of HIV infection, but others—quality of life assessment, gene rearrangement, C-reactive protein concentration, delayed hypersensitivity to recall antigens—may be exploited to gauge potential effects on immune reconstitution, viral load reduction, or development of protective immunity. By looking across the phase II and phase III clinical trials of the immune-based therapies scrutinized for this review, one can see that the study endpoints tend to fall into several broad categories. The table below shows the results of this “analysis.”

³ Forum for Collaborative HIV Research. 1998 *HIV treatment failure: A review of current clinical research*. www.gwumc.edu/chrp/treat.htm.

⁴ Piscitelli SC et al. 1996 *Ann Pharmacother* 30(1):62-76.

Endpoints for efficacy trials of immune-based therapies.

Endpoint category	Endpoints
Immunologic parameters	Immunoglobulin level Complement level C-reactive protein level Plasma levels of interleukins and interferons Change in serum neopterin level TNF bioactivity in plasma Production of neutralizing antibodies Production of cytotoxic lymphocytes Western blot immunoreactivity Delayed-type hypersensitivity Markers of cellular activation
Molecular markers	<i>bcl-2</i> gene rearrangement
Virologic parameters	Viral nucleic acid level Evidence of virologic resistance Onset of viral suppression Rate of clearance of HIV from long-lived cells and tissue macrophages by enhanced clearance of virions from plasma and clearance of proviral DNA and MS/US RNA from PBMC Half-life of cell-free virus
Hematologic parameters	Numbers of circulating B-cells Erythrocyte sedimentation rate White blood cell count Platelet count CD4+, CD8+ cell count, percentage Lymphoproliferative responses
Therapeutic parameters	Change in antiretroviral therapy Change in opportunistic infection prophylaxis
Major clinical endpoints	Death Time to AIDS-defining event Time to disease progression Response duration Length of time to response Response (complete, partial) Occurrence of severe systemic or severe localized reactions Toxicity Progression-free survival Graft/transplant rejection
Quality of life assessments	Inflammatory bowel disease questionnaire American College of Rheumatology (ACR) criteria for rheumatoid arthritis Kurtzke expanded disability status scale Childhood health assessment questionnaire SF-36 health outcome measure Health assessment questionnaire (HAQ) Paulus assessment of joint morphology and function
Musculoskeletal parameters	Limb function tests Sharp score for radiographic evaluation of joint damage Change in number of tender and swollen joints
Tumor-related indices	Tumor measurements Histology (histological activity index, Crohn's disease activity index, perianal disease activity index) Number of tumors/fistulae/lesions Molecular markers of lymphomagenesis

Other	Pain assessment via Likert scale or visual analog scale Physician global assessment Patient global assessment Body temperature Body weight
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When a repertoire of appropriate surrogate markers is available to correlate with increased resistance to infections, reduced disease progression, and clinical survival,⁵ additional protocols for clinical trials of immune-based therapies can be developed and implemented to rapidly and accurately assess how effectively these therapies prolong survival, protect against infection, and improve quality of life.

⁵ Treatment Action Group. 1998 *TAGline* 5(2). www.aidsinfonyc.org/tag/taglines/9803.html.

FDA-approved immune-based therapies

Interferon-beta-1a

On May 17, 1996, the U.S. Food and Drug Administration approved use of interferon-beta-1a (Avonex) for treating relapsing forms of multiple sclerosis in order to slow the accumulation of physical disability and decrease the frequency of clinical exacerbations.⁶ The amino acid sequence of Avonex is identical to that of natural human interferon beta. It is produced by mammalian cells (Chinese hamster ovary cells) into which the human interferon beta gene has been introduced.⁷

The basis for approval was a *multicenter, phase III study* conducted by Jacobs et al.⁸ The study involved 301 patients who were randomized into a *double-blinded, placebo-controlled trial*. Sample size calculation was based on a Kaplan-Meier analysis and an intent-to-treat design. The expected placebo progression rate was based on the median time to progression (104 weeks) in the placebo arm of another clinical trial. The study was designed to have a statistical power of 80% to detect a group difference of this magnitude with an α level of .05. Interferon-beta-1a was administered intramuscularly at a dosage of 6.0 million units (30 μ g) weekly for up to 104 weeks.

The *primary clinical endpoint* was time to sustained disability progression of at least 1.0 point on the Kurtzke expanded disability status scale (EDSS)⁹. The EDSS is a quantitative clinical rating scale of neurological impairment

Interferon-beta-1a: Summary of clinical trial upon which FDA approval was based.	
	Jacobs et al. 1996 ³
Study design	Multicenter, phase III, double-blind, placebo-controlled trial
N	301
Primary clinical endpoint	Time to sustained disability progression of at least 1.0 point on the Kurtzke expanded disability status scale (EDSS) ⁴
Secondary endpoints	1. Frequency of exacerbations 2. Decreasing gadolinium enhancement on MRIs 3. Two upper limb (both arms) and three lower limb function tests

with scores ranging from 0 to 10; increasing numbers reflect increasing disability. Only objectively verifiable defects due to multiple sclerosis as elicited upon neurologic examination are included. This endpoint was used to ensure that progression reflected a permanent increase in disability rather than a transient effect due to an exacerbation.⁸

The scale evaluates 8 functional systems (FSs): pyramidal functions, cerebellar functions, brain stem function, sensory functions, bowel & bladder functions, visual (or optic) functions, cerebral (or mental)

functions, and other functions. All FSs save the last are graded from 0 (normal) to maximal impairment (grade 5 or 6). For example, within the brain stem FS, the grades are:

0. Normal
1. Signs only
2. Moderate nystagmus or other mild disability
3. Severe nystagmus, marked extraocular weakness, or moderate disability of other cranial nerves
4. Marked dysarthria or other marked disability
5. Inability to swallow or speak
- V. Unknown.

The grades for the individual functional systems then roll up into the EDSS steps, which are briefly summarized below (see Kurtzke 1983⁹ for the full scale):

- 0 = Normal neurologic exam (grade 0 for all FSs).
- 1.0 = No disability, minimal signs in one FS.
- 2.0 = Minimal disability in one FS.

⁶ Center for Biologics Evaluation and Research. www.fda.gov/cber/products/ifnbbio051796.htm.

⁷ Biogen, Inc. Avonex prescribing information.

⁸ Jacobs DL et al. 1996 *Annals of Neurology* 39(3):285-294.

⁹ Kurtzke JF. 1983 *Neurology* 33:1444-1452.

- 3.0 = Moderate disability in one FS.
- 4.0= Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest some 500 meters.
- 5.0 = Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities. (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combination of lesser grades usually exceeding specifications for step 4.0).
- 6.0 = Intermittent or unilateral constant assistance (cane, crutch, or brace) required to walk about 100 meters with or without resting. (Usual FS equivalents are combinations with more than two FS grade 3+).
- 7.0 = Unable to walk beyond about 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair some 12 hours a day. (Usual FS equivalents are combinations with more than one FS grade 4+; very rarely, pyramidal grade 5 alone.)
- 8.0 = Essentially restricted to bed or chair or perambulated in wheel chair, but may be out of bed much of the day; retains many self-care functions; generally has effective use of arms. (Usual FS equivalents are combinations, generally grade 4+ in several systems.)
- 9.0 = Helpless bed patient; can communicate and eat. (Usual FS equivalents are combinations, mostly grade 4+).
- 10.0 = Death due to multiple sclerosis.⁸

Interferon-beta-1a was found to produce a significant delay in time to sustained EDSS progression ($P = .02$).

Two major *secondary endpoints* were also evaluated: exacerbation frequency and results of magnetic resonance imaging (MRI) scans to evaluate lesion number and volume. Additional secondary endpoints included two upper limb (tested in both arms) and three lower limb function tests. Patients treated with interferon-beta-1a had significantly fewer exacerbations ($P = .03$), which were defined by the appearance of new neurological symptoms or worsening of preexisting neurological symptoms lasting at least 48 hours in a patient who had been neurologically stable or improving of the previous 30 days accompanied by objective change on neurological examination (worsening of 0.5 point on the EDSS or a worsening by a point or more within one of the FS scores).⁸

Furthermore, the patients treated with interferon-beta-1a had a significantly lower number and volume of gadolinium-enhanced brain lesions on magnetic resonance images (P values ranging between .02 and .05). T2 lesion volume measurements were based on a modification of a thresholding approach using the long-repetition time/short-echo time images.⁸ Of the limb function tests, only one demonstrated a statistically significant difference between treatment groups (favoring interferon-beta-1a).

An additional multicenter, phase II study is underway to determine the tolerability and efficacy of 3 doses of interferon-beta-1a versus placebo in the treatment of idiopathic pulmonary fibrosis. In the laboratory, interferons have been shown to produce changes favoring reduction in collagen deposition and fibrosis. Treatment is aimed at minimizing the disease progression from inflammation to fibrosis.¹⁰

An ongoing phase II trial is designed to determine the effectiveness of interferon-beta-1a to shrink recurring gliomas.¹¹ A related phase I trial used as *primary endpoints* fall in natural killer cell number, radiographic response, and prolonged survival.¹²

¹⁰ The Pulmonary Fibrosis Foundation. www.pulmonaryfibrosis.org/biogen.htm

¹¹ The Brain Tumor Society. www.tbts.org/pII_interf.htm.

¹² Fine HA et al. 1997. *Clin Cancer Res* 3(3):381-387.

Interferon alfa-n1, Lymphoblastoid

Interferon alfa-n1 (Wellferon) is a mixture of alpha-interferons isolated from a human lymphoblastoid cell line following induction with murine parainfluenza virus type 1 (Sendai strain) and purified by immuno-affinity chromatography. The lymphoblastoid cell and virus banks have been qualified and the production process validated to inactivate or remove known adventitious viruses and microorganisms.¹³ On March 25, 1999, the U.S. Food and Drug Administration approved the use of interferon alfa-n1 for the treatment of chronic hepatitis C in patients 18 years of age or older without decompensated liver disease.¹⁴

Glaxo-Wellcome has since ceased production of Wellferon. Heretofore, Wellferon had been available abroad and licensed in various countries for such indications as chronic hepatitis B, chronic hepatitis C, condylomata acuminata, juvenile laryngeal papillomatosis, chronic myeloid leukemia, hairy cell leukemia, Kaposi's sarcoma, and renal cell carcinoma.¹⁵

The safety and efficacy of interferon alfa-n1 in the treatment of adult patients with chronic hepatitis C were established in 2 large trials that enrolled a total of 1,511 patients. The first¹⁶ of the 2 trials, a *multicenter, phase III, randomized trial* (N = 440) compared 4 different regimens of interferon alfa-n1 administered subcutaneously: 3MU thrice weekly for 6 months, 5 MU thrice weekly for 6 months, 3 MU thrice weekly for 12 months, and 5 MU thrice weekly for 12 months. The *primary clinical endpoint* was the normalization of serum alanine transaminase (ALT) at the end of treatment and at 12 months post-treatment. Normalization of ALT was defined as the presence of 2 consecutive ALT values less than or equal to the upper limit of normal, at least 7 days apart.¹⁶ A higher number of sustained responses was observed in patients treated with 3 MU of interferon alfa-n1 for 12 months than those treated for 6 months. Patients receiving 5 MU did not have significantly higher sustained response rates than those receiving 3 MU of interferon alfa-n1.^{13,16}

The second major *multicenter, phase III, open-label, randomized trial*¹⁷ (N= 1,071) compared treatment with interferon alfa-n1 to treatment with recombinant interferon alfa-2b, both given at 3 MU subcutaneously thrice weekly

Interferon alfa-n1: Summary of clinical trials upon which FDA approval was based.		
	Benhamou et al. 1995¹⁶	Farrell et al. 1998¹⁷
Study design	Multicenter, phase III, randomized trial comparing two doses of interferon alfa-n1 administered for either 6 or 12 months	Multicenter, phase III, open-label, randomized trial comparing short-term and long-term treatment with either interferon alfa-n1 with recombinant interferon-alfa in a 24-week course
N	440	1,071
Primary clinical endpoint	Normalization of ALT at the end of treatment and at 12 months post-treatment	Normalization of serum ALT level with the principal treatment comparison occurring at the end of treatment (week 24)
Secondary endpoints	<ol style="list-style-type: none"> 1. Clearance of serum hepatitis C virus RNA as demonstrated by RT-PCR 2. Histologic improvement from baseline to end of treatment as evidenced by liver biopsies evaluated using Knodell's HAI score¹⁸ 	<ol style="list-style-type: none"> 1. Clearance of serum hepatitis C virus RNA as demonstrated by RT-PCR 2. Histologic improvement from baseline to end of treatment as evidenced by liver biopsies evaluated using Knodell's HAI score¹⁸

¹³ Glaxo Wellcome. Wellferon prescribing information.

¹⁴ Center for Biologics Evaluation and Research. www.fda.gov/cber/products/ifnawel032599.htm.

¹⁵ Glaxo Wellcome Website. www.glaxowellcome.co.uk/fighting/medicines/mn_wellferon.html.

¹⁶ Benhamou JP et al. 1995. *Hepatology* 22(4, part 2):151A.

for 6 months). The *primary clinical endpoint* was the normalization of serum ALT level with the principal treatment comparison occurring at the end of treatment (week 24). An end-of-treatment response in ALT levels was defined as the presence of 2 consecutive ALT values less than or equal to the upper limit of normal, at least 7 days apart and with a normal value at week 24. A second comparison on the primary treatment endpoint was sustained response, defined as an end-of-treatment response without relapse between weeks 24 and 48 and weeks 24 and 72.⁵ Interferon alfa-n1 and interferon alfa-2b had similar end-of-treatment response rates, but the sustained response rate was higher with interferon alfa-n1.¹⁷

Secondary endpoints for both studies were assessment of clearance of serum hepatitis C virus (HCV) RNA and histological improvement as evidenced by the liver biopsies post-treatment. The presence and level of HCV RNA in serum was determined by an unvalidated quantitative reverse transcription polymerase chain reaction (RT-PCR) targeted to the 5' noncoding region of the HCV genome. The assay cutoffs were <100 HCV RNA copies per milliliter. Subjects who received 3 MU thrice weekly of interferon alfa-n1 for 6 months had a virologic response rate of 17.3% 1 year after therapy. The study results suggested a correlation between sustained normalization of serum ALT levels and clearance of detectable serum HCV RNA at the end of post-treatment observation.¹⁷

Baseline liver biopsies were performed within 12 months before initiation of treatment. Follow-up liver biopsies were obtained at weeks 24 and 72.^{16,17} Blinded liver biopsies were assessed and scored separately for total (portal, periportal plus lobular) necroinflammatory activity (grade) and fibrosis (stage) using the components of Knodell's histological activity index (HAI) score.¹⁸ The HAI generates a numerical score for asymptomatic chronic active hepatitis liver biopsy specimens. Biopsy specimens are graded in 4 categories:

- periportal +/- bridging hepatocellular necrosis, graded from 0 (none) to 10 (multilobular necrosis)
- intralobular degeneration and focal hepatocellular necrosis, graded from 0 (none) to 4 (marked, involving more than 2/3 of lobules or nodules)
- portal inflammation, graded from 0 (no portal inflammation) to 4 (marked, with dense packing of inflammatory cells in more than 2/3 of portal tracts)
- fibrosis, graded from 0 (no fibrosis) to 4 (cirrhosis).¹⁸

Note that periportal necrosis +/- bridging necrosis is weighted more heavily than other parameters because it appears to be more influential in determining the activity and severity of severe chronic active hepatitis. A numerical score for each category is assigned to each biopsy, and the combined score of the four categories forms the HAI score for that biopsy specimen.⁶

In Farrell's study, liver histology in patients who had an end-of-treatment response was improved in 57% of patients, as indicated by a 2-point or greater reduction in the total necroinflammatory activity score of the HAI compared with 38% in nonresponders. There were no differences in liver histology between the groups treated with interferon alfa-n1 or with interferon alfa-2b.¹⁷

A review of the literature revealed an additional phase III study of interferon alfa-n1 in combination chemotherapy for treatment of advanced malignant melanoma,¹⁹ although no details were available about study design or clinical endpoints.

¹⁷ Farrell GC et al. 1998. *Hepatology* 27(4):1121-1127.

¹⁸ Knodell et al. 1981. *Hepatology* 1(5):431-435.

¹⁹ Gorbonova VA et al. 2000. *Gan To Kagaku Ryoho* 27(Suppl 2):310-314.

Interferon alfacon-1

On October 16, 1997, the U.S. Food and Drug Administration (FDA) approved the use of interferon alfacon-1 (Infergen), a recombinant, non-naturally occurring, type-I interferon, for treatment of chronic hepatitis C infection in patients 18 years or older with compensated liver disease who have anti-HCV serum antibodies and/or the presence of HCV RNA.²⁰ Interferon alfacon-1, also called consensus interferon, was derived by scanning the sequences of several natural interferon alpha subtypes and assigning the most frequently observed amino acid in each corresponding position.²¹

Interferon alfacon-1: Summary of clinical trials upon which FDA approval was based.		
	Tong et al. 1997²²	Keeffe et al. 1997²³
Study design	Multicenter, randomized, double-blind, parallel-group, phase III study comparing treatment with interferon alfacon-1 to treatment with recombinant interferon alfa-2b	Multicenter, randomized, open-label, retreatment study
N	704	337*
Primary clinical endpoint	Normalization of serum ALT (below 48 U/L) from baseline (average of four ALT measurements determined during the screening and pretreatment observation periods)	Normalization of serum ALT (below 48 U/L) from baseline (average of four ALT measurements determined during the screening and pretreatment observation periods)
Secondary endpoints	<ol style="list-style-type: none"> 3. Decrease in serum hepatitis C virus RNA concentration below the limit of detection by RT-PCR (100 copies/mL) 4. Liver histology based on HAI score²⁴ 5. Differences in response rates between the two interferons 6. ALT and HCV RNA responses by HCV genotype 	<ol style="list-style-type: none"> 1. Decrease in serum hepatitis C virus RNA concentration below the limit of detection by RT-PCR (100 copies/mL) 2. Liver histology based on HAI score²⁴ 3. Differences in response rates between the two interferons 4. ALT and HCV RNA responses by HCV genotype

* Only the results of the 24 weeks' additional retreatment (107 patients) were used as the basis for FDA approval.

FDA approval was granted on the basis of two published clinical trials.^{22,23} Tong et al.²² compared interferon alfacon-1 treatment to a standard regimen of recombinant interferon alfa-2b. This study was a *multicenter, double-blind, parallel-group, phase III clinical trial involving 704 patients* randomized to receive interferon alfacon-1 at doses of 3 or 9 μ g, or interferon alfa-2b at 15 μ g (3 million units) subcutaneously thrice weekly for 24 weeks, followed by a 24-week posttreatment observation period.²²

Tong et al. assessed the efficacy of interferon alfacon-1 on an intent-to-treat basis using a *primary clinical endpoint* of normalization of serum alanine transaminase (ALT) concentration. Baseline ALT was the average of four ALT measurements determined during the screening and pretreatment observation periods. At baseline, the mean serum ALT concentrations were 140 \pm 61, 147 \pm 63, and 151 \pm 70 U/L in the 3- μ g interferon alfacon-1 group, the 9- μ g interferon alfacon-1 group, and the 15- μ g interferon alfa-2b group, respectively. By the end of the 24-week treatment period, the mean serum ALT concentrations decreased to (respectively) 118 \pm 106, 84 \pm 82, and 84 \pm 73 U/L. The mean serum ALT levels increased by the end of the posttreatment 24-week observation period to 124 \pm 81, 107 \pm 74, and

²⁰ Center for Biological Evaluation and Research. www.fda.gov/cber/products/ifnamg100697.htm.

²¹ Amgen, Inc. Infergen prescribing information.

²² Tong MJ et al. 1997. *Hepatology* 26(3):747-754.

²³ Keeffe EB et al. 1997. *Hepatology* 26(3, Suppl. 1):101S-107S.

108/176, respectively. Qualitatively, 16.8%, 42.2%, and 36.7% of the patients in the 3- μ g interferon alfacon-1 group, the 9- μ g interferon alfacon-1 group, and the 15- μ g interferon alfa-2b group, respectively, experienced normalization of their serum ALT concentrations at the end of the 24-week treatment period. By the end of the 24-week posttreatment observation period, however, 6.5%, 20.3%, and 19.6% of the 3- μ g interferon alfacon-1 group, the 9- μ g interferon alfacon-1 group, and the 15- μ g interferon alfa-2b group, respectively, had serum ALT concentrations that were within the normal range.²²

The study of Tong et al. was designed around 4 *secondary endpoints*: (1) decrease in serum hepatitis C virus RNA concentration below the limit of detection by reverse-transcription polymerase chain reaction (100 copies/mL); (2) liver histology; (3) differences in response rates between the two interferons; and (4) ALT and HCV RNA responses by HCV genotype.²²

This clinical trial was the first one to document the impact of interferon therapy on HCV RNA concentrations in a large, prospective, randomized, double-blind study. The baseline serum HCV RNA concentration for each patient was calculated as the mean of the HCV RNA concentrations determined 12 weeks before the study and at time 0. Serum HCV RNA concentrations were determined at weeks 12, 20, 24, 36, 44, and 48 using a quantitative, multicycle RT-PCR method (National Genetics Institute, Culver City, CA). Response to therapy was determined both quantitatively (reduction in serum HCV RNA) and qualitatively (proportion of patients with serum HCV RNA below the limits of detection).²²

Liver biopsy specimens were obtained within the 12-month period before enrollment and at the end of the posttreatment observation period (week 48). The biopsies were evaluated using Knodell's histological activity index (HAI),²⁴ a numerical score for asymptomatic chronic active hepatitis liver biopsy specimens. Biopsy specimens are graded in four categories:

- periportal +/- bridging hepatocellular necrosis, graded from 0 (none) to 10 (multilobular necrosis)
- intralobular degeneration and focal hepatocellular necrosis, graded from 0 (none) to 4 (marked, involving more than two-thirds of lobules or nodules)
- portal inflammation, graded from 0 (no portal inflammation) to 4 (marked, with dense packing of inflammatory cells in more than two-thirds of portal tracts)
- fibrosis, graded from 0 (no fibrosis) to 4 (cirrhosis).²⁴

Note that periportal necrosis +/- bridging hepatocellular necrosis is weighted more heavily than other parameters because it appears to be more influential in determining the activity and severity of severe chronic active hepatitis. A numerical score for each category is assigned to each biopsy, and the combined score of the four categories of the four categories forms the HAI score for that biopsy specimen.⁵

The mean changes in the HAI scores were -1.73, -2.01, and -2.03 units for the 3- μ g interferon alfacon-1 group, the 9- μ g interferon alfacon-1 group, and the 15- μ g interferon alfa-2b group, respectively. The changes were attributed primarily to reductions in inflammation. There was at least a 2-unit improvement in the HAI score at the end of the posttreatment period in 52% to 55% of the patients in the three cohorts, although there were no statistically significant differences among the three treatment groups. Patients who were responders by the ALT or HCV RNA criteria had statistically significantly greater improvement in HAI scores compared to patients who were not ALT or HCV RNA responders ($P < .001$ for both comparisons).²² Patients infected with HCV genotypes 2 (39%) and 3 (48%) exhibited significantly higher response rates compared to those infected with genotype 1 (14%) at the end of the treatment period ($P < .001$), independent of treatment regimen. The differences persisted through the end of the observation period as well.²²

A second study by Keeffe et al.²³ was instituted, drawing on 337 patients from the initial phase III study who had relapsed or had not responded to 24 weeks of therapy with interferon alfacon-1 or interferon alfa-2b. In this *double-blind, open-label, retreatment study*, patients were *randomized* to receive 15 μ g of interferon alfacon-1 administered thrice weekly for either 24 or 48 weeks. Keeffe et al. relied upon the same primary and secondary clinical endpoints as Tong et al.²² Retreatment of nonresponders with a higher dose of interferon alfacon-1 (15 μ g) produced end-of-retreatment response rates of 19% and 17% in the 24- and 48-week retreatment cohorts, respectively, and serum HCV RNA sustained response rates of 5% and 13%, respectively. Relapsers were more likely to respond to retreatment than nonresponders. The serum HCV RNA end-of-retreatment response rates in relapsers were 72% and 76% in the 24- and 48-week retreatment cohorts, respectively. By comparison, the serum HCV RNA sustained response rates, as determined at the end of the posttreatment observation period, in relapsers were 28% and 58% in the 24- and 48-week retreatment cohorts, respectively.²³

²⁴ Knodell et al. 1981. *Hepatology* 1(5):431-435.

Rituximab

On November 26, 1997, the U.S. Food and Drug Administration approved use of rituximab (Rituxan) for treating patients with relapsed or refractory low-grade or follicular, CD20-positive, B-cell non-Hodgkin's lymphoma.²⁵ Rituximab, the first monoclonal antibody approved to treat cancer,²⁶ is a chimeric murine-human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes.²⁷

The clinical efficacy of rituximab was demonstrated in 4 clinical trials, which served as the basis for FDA approval. The pivotal clinical trial, a *phase III multicenter, open-label, single-arm study*,²⁸ showed that 48% of the intent-to-treat group responded to treatment, with 6% achieving a complete response and 42% achieving a partial response.²⁶ The study involved 166 patients who received antibody doses of 375 mg/m², administered intravenously once weekly for a total of four infusions (days 1, 8, 15, 22). Time to progression was analyzed by the Kaplan-Meier method.²⁸

For this pivotal trial, *clinical endpoints* were defined in terms of complete or partial response and time to progression. Complete response (CR) required the resolution of all symptoms and signs of lymphoma, including bone marrow clearing, for at least 28 days as evidenced by computed tomography (CT) scans. Partial response (PR) required a decrease of at least 50% in the sum of the products of perpendicular measurements of lesions, without

Interferon-beta-1a: Summary of clinical trials upon which FDA approval was based.				
	McLaughlin et al. 1998²⁸	Maloney et al. 1997²⁹	Davis et al. 1999³⁰	Davis et al. 2000³¹
Study design	Phase III, multicenter, open-label, single-arm I	Multicenter, phase II, open-label, single-arm	Phase II, open-label, single-arm, multicenter trial	Multicenter, phase II, open-label, single-arm
N	166	37	31	58
Primary clinical endpoints	<ol style="list-style-type: none"> Complete response: resolution of all symptoms and signs of lymphoma, including bone marrow clearing as evidenced by CT scans Partial response: decrease of at least 50% in the sum of the products of perpendicular measurements of lesions, without evidence of progressive disease for at least 28 days. Time to progression 	<ol style="list-style-type: none"> Complete response: disappearance of all disease Partial response: decrease of at least 50% in the sum of the products of perpendicular measurements of lesions, without any evidence of progressive disease for at least 28 days Stable disease: no significant change in tumor measurements without progression over the period of observation. Time to progression 	<ol style="list-style-type: none"> Complete response: all lymph nodes less than 1 cm x 1 cm; nodes not palpable nodes or negative on biopsy or fine-needle aspirate; bone marrow histologically negative for lymphoma; liver and spleen returned to normal size Partial response: ≥ 50% decrease from baseline in the sum of the products of the greatest perpendicular diameters of all the measured lesions Time to progression Response duration 	Same as Davis et al. (1999)
Immunologic parameters monitored	<ol style="list-style-type: none"> Numbers of circulating B-cells Immunoglobulin levels <i>bcl-2</i> gene rearrangement 	<ol style="list-style-type: none"> Numbers of circulating B-cells Immunoglobulin levels Complement levels 	<ol style="list-style-type: none"> Numbers of circulating B-cells 	None listed

²⁵ Center for Biologics Evaluation and Research. www.fda.gov/cber/products/ritugen112697.htm.

²⁶ U.S. Department of Health and Human Services. November 26, 1997. Press release. www.fda.gov/bbs/topics/NEWS/NEW00601.html.

²⁷ Genentech, Inc. Rituxan prescribing information.

²⁸ McLaughlin P et al. 1998 *J Clin Oncol* 16(8):2825-2833.

any evidence of progressive disease for at least 28 days. Patients who did not achieve a CR or PR were considered nonresponders, even if there was a net decrease (<50%) of measurable disease. Nonetheless, among those who did not achieve a CR or PR, the majority (56 of 75) had a net decrease of measurable disease (mean decrease, 32%). Time to progression was measured from the first infusion until progression. The projected median time to progression for responders was 13.0 months for the intent-to-treat group and 12.5 months for the assessable group. Within a median follow-up duration of 11.8 months, 53 of the 76 responders had not yet relapsed.²⁸

Several immunologic parameters were followed during the study, although they were not specifically identified as secondary endpoints. The median B-cell count declined with treatment to undetectable levels after the first dose for the majority. A minority of patients (16/166) did not deplete circulating B cells. The mean serum IgM level had decreased to 541.5 mg/dL (normal range, 45 to 145 mg/dL) at 6 months posttreatment. Twenty-three of the 166 patients had reductions in immunoglobulin levels by $\geq 50\%$ to subnormal levels. A $\geq 20\%$ decrease from baseline in serum complement (C3) was noted in 18/166 patients. Cells with *bcl-2* gene rearrangement were detected pretreatment by polymerase chain reaction in the peripheral blood of 66 patients and in the bone marrow of 52 patients. For those who had serial monitoring, reversion to negative status (no detectable rearranged cells by PCR) occurred in the peripheral blood in 19% following the first infusion, 50% before the fourth infusion, and 62% by 3 months. In the bone marrow, reversion to negative was seen in 56% at 3 months. Median serum levels of rituximab were higher for responders than nonresponders. Attainable serum rituximab concentrations correlated negatively with the number of circulating B cells, with the size of the largest pretreatment measurable tumor, and with the baseline sum of the products of the diameters of the 6 largest lesions.²⁸

A second, *phase II, open-label, single-arm, multicenter trial* evaluated efficacy of 4 intravenous infusions of rituximab at a dose of 375 mg/m² in 37 patients with relapsed, low-grade non-Hodgkin's lymphoma.²⁹ Patients received a single infusion weekly and completed the 4 antibody infusions in 22 days. *Clinical endpoints* were defined in terms of CR, PR, or stable disease. CR was defined as the disappearance of all disease. PR required a decrease of at least 50% in the sum of the products of perpendicular measurements of lesions, without any evidence of progressive disease for at least 28 days. Stable disease was defined by no significant change in tumor measurements without progression over the period of observation. Clinical responses were observed in 17 of the 37 patients (46%), with 9% showing CR and 41% showing PR. Thirty-two percent had stable disease, and 18% were judged to have progressive disease. The median time to onset of clinical response for the 17 responders was 50 days. The median duration of response was 8.6 months.²⁹

Again, *several other immunologic parameters* were monitored. B-cells were rapidly and effectively depleted from peripheral blood circulation. Circulating B-cells remained nearly undetectable until approximately 6 months posttreatment, followed by slow gradual recovery. A significant correlation was observed between clinical response and median levels of circulating rituximab before the second infusion, with responders having a median of serum level of 83 μ g/mL versus nonresponders with a median of 22 μ g/mL. Mean serum immunoglobulin levels remained stable. No clinically significant change in serum complement levels was observed.²⁹

A third, *phase II, open-label, single-arm, multicenter trial* demonstrated the safety and efficacy of rituximab in 31 patients with bulky (> 10-cm lesion) relapsed or refractory low-grade or follicular non-Hodgkin's lymphoma.³⁰ *Clinical endpoints* included overall response rate (CR and PR), time to progression, and response duration. Response categories consisted of CR, PR, stable disease, and progressive disease. CR required that all lymph nodes visible on CT scans of neck, chest, abdomen, and pelvis be less than 1 cm x 1 cm; any node that was palpable on physical examination be no longer palpable or negative on biopsy or fine-needle aspirate; bone marrow must have been histologically negative for lymphoma; and the liver and spleen (if abnormal at baseline) must have returned to normal size. PR was defined as $\geq 50\%$ decrease from baseline in the sum of the products of the greatest perpendicular diameters of all the measured lesions. The overall response rate in 28 assessable patients was 43% with a median time to progression of 8.1 months. As in the other trials, median serum rituximab concentrations were higher in responders than in nonresponders, with higher concentrations being associated with lower tumor bulk. In contrast to the pivotal trial, however, there was no correlation between serum rituximab concentration and the number of circulating B cells.³⁰

Finally, a fourth, *multicenter, phase II, open-label, single-arm clinical trial* investigated the safety and efficacy of retreatment with rituximab in 58 patients with low-grade or follicular non-Hodgkin's lymphoma who had relapsed after a previous response to rituximab therapy.³¹ Clinical endpoints and response criteria were similar to those used

²⁹ Maloney DG et al. 1997 *Blood* 90(6):2188-2195.

³⁰ Davis TA et al. 1999 *J Clin Oncol* 17(6):1851-1857.

³¹ Davis TA et al. 2000 *J Clin Oncol* 18(17):3135-3143.

in the study by Davis et al. (1999). The overall response rate in 57 assessable patients was 40% (11% CR and 30% PR). Safety and efficacy were not significantly different from those found in trials of initial rituximab exposure.

Rituximab is being tested in Europe in patients with other B-cell malignancies, including mantle cell lymphoma, Waldenström's macroglobulinemia, and lymphoplasmacytoid lymphoma.³² Early results indicate that single-agent rituximab has efficacy in mantle cell lymphoma and large-cell lymphoma. Mantle cell lymphoma is a particularly frustrating entity for which new treatment approaches are needed.³³

³² McLaughlin P et al. 1998 *Oncology* 12(12):1763-1769.

³³ Coiffier B et al. 1998 *Blood* 92:1927-1932.

Infliximab

Infliximab (Remicade), a chimeric monoclonal antibody that binds specifically to human tumor necrosis factor alpha (TNF-alpha),³⁴ has been approved by the U.S. Food and Drug Administration (FDA) for two indications:

- On August 24, 1998, infliximab was approved for the treatment of moderately to severely active Crohn's disease for the reduction of the signs and symptoms in patients who have an inadequate response to conventional therapies; and treatment of patients with fistulizing Crohn's disease for the reduction in the number of draining enterocutaneous fistula(s).³⁵
- On November 10, 1999, FDA approved the use of infliximab for reducing signs and symptoms of rheumatoid arthritis in patients who have had an inadequate response to methotrexate.³⁶

The clinical efficacy of infliximab for treating active Crohn's disease was demonstrated in a *multicenter, randomized, double-blind, placebo-controlled, dose-ranging study of 108 patients* with moderate to severe active

Infliximab: Summary of clinical trials upon which FDA approval was based.			
	Targan et al. 1997³⁷ (for active Crohn's disease)	Present et al. 1999³⁸ (for fistulizing Crohn's disease)	Maini et al. 1999³⁹ (for rheumatoid arthritis)
Study design	Multicenter, randomized, double-blind, placebo-controlled, dose-ranging study	Randomized, double-blind, placebo-controlled study	Multicenter, randomized, double-blind, placebo-controlled, phase III trial
N	108	94	428 patients
Primary clinical endpoint	Reduction of 70 points or more in the score on the Crohn's Disease Activity Index ³⁸ (CDAI) at the 4-week evaluation that was not accompanied by a change in any concomitant medications	Reduction of 50% or more from baseline in the number of draining fistulas observed at two or more consecutive study visits	Proportion of patients at week 30 who attained an improvement in signs and symptoms as measured by the American College of Rheumatology criteria (ACR20). ⁴² An ACR 20 response is defined as at least a 20% improvement in both the tender joint count and the swollen joint count (assessments of 28 or more joints) in addition to $\geq 20\%$ improvement in 3 of 5 core set measures.
Secondary endpoints	<ol style="list-style-type: none"> 1. Change in the score for the Inflammatory Bowel Disease questionnaire³⁹ 2. Decrease in C-reactive protein concentration 	<ol style="list-style-type: none"> 1. Number of patients with a complete response (defined as the absence of any draining fistulas at two consecutive visits) 2. Length of time to the beginning of a response 3. Duration of the response 4. Changes in scores on the CDAI and the Perianal Disease Activity Index 	<ol style="list-style-type: none"> 1. Documentation of 50% and 70% improvement in ACR criteria 2. Reduction in individual measurements of disease activity 3. Improvement in general health assessment

Crohn's disease.³⁷ Patients were randomly assigned to receive a single 2-hour intravenous infusion of either placebo or infliximab in a dose of 5, 10, or 20 mg per kilogram of body weight. The *primary clinical endpoint* was defined as a reduction of 70 points or more in the score on the Crohn's Disease Activity Index³⁸ (CDAI) at the 4-week evaluation

³⁴ Centocor, Inc. Remicade prescribing information.

³⁵ Center for Biologics Evaluation and Research. www.fda.gov/cber/products/inflcen082498.htm.

³⁶ Center for Biologics Evaluation and Research. www.fda.gov/cber/products/inflcen111099.htm.

³⁷ Targan SR et al. 1997 *New Engl J Med* 337(15):1029-1035.

³⁸ Best WR et al. 1979 *Gastroenterology* 77:843-846.

that was not accompanied by a change in any concomitant medications.³⁷ The CDAI incorporates 8 disease-related variables: the number of liquid or very soft stools, the severity of abdominal pain or cramping, general well-being, the presence of extraintestinal manifestations, abdominal mass, use of antidiarrheal drugs, hematocrit, and body weight. These items yield a composite score ranging from 0 to approximately 600. Higher scores indicate greater disease activity. Scores below 150 indicate remission, whereas scores above 450 indicate severe illness.³⁸ At week 4, 16% of the placebo patients achieved a clinical response compared to 82% of patients receiving 5 mg/kg infliximab. Four percent of placebo patients and 48% of patients receiving 5 mg/kg infliximab achieved a CDAI <150 at week 4.³⁴

Secondary clinical endpoints were increased score for the Inflammatory Bowel Disease questionnaire³⁹ and decreased C-reactive protein concentration.³⁷ The Inflammatory Bowel Disease questionnaire consists of 32 items that evaluate quality of life with respect to bowel function (e.g., loose stools and abdominal pain), systemic symptoms (e.g., fatigue, altered sleep pattern), social function (work attendance and the need to cancel social events), and emotional status (angry, depressed, or irritable). The score ranges from 32 to 224, with higher scores indicating a better quality of life. Patients in remission usually score between 170 and 190.³⁹ During the 12-week period following infusion, patients treated with infliximab compared to placebo demonstrated improvement in outcomes as measured by the questionnaire.³⁴ C-reactive protein concentration decreased by an average of 14.3 mg/L for all infliximab-treatment groups, whereas C-reactive protein concentration increased by 2.0 mg/L for the placebo group.³⁷

Safety and efficacy of infliximab for treating fistulizing Crohn's disease were assessed in a *randomized, double-blind, placebo-controlled study of 94 patients* who had draining abdominal or perianal fistulas of at least 3 months' duration.⁴⁰ The *primary clinical endpoint* was defined as a reduction of 50% or more from baseline in the number of draining fistulas observed at two or more consecutive study visits. With respect to the primary efficacy endpoint, response rates were significantly greater among the patients receiving infliximab (68%) than in the placebo group (26%). *Secondary clinical endpoints* evaluated the number of patients with a complete response (defined as the absence of any draining fistulas at two consecutive visits), the length of time to the beginning of a response, the duration of the response, and changes in scores on the CDAI and the Perianal Disease Activity Index. Complete responses were achieved in 55% of patients treated with 5 mg infliximab per kilogram, in 38% of those treated with 10 mg/kg, and in 13% of patients receiving placebo. The median time to the onset of a response was shorter among patients treated with infliximab (2 weeks) than among those given placebo (6 weeks). The median duration of response was approximately 3 months in patients who reached the primary endpoint.

Infliximab was shown to be safe and efficacious for treatment of rheumatoid arthritis³⁴ in a *multicenter, randomized, double-blind, placebo-controlled, phase III trial* involving 428 patients with active rheumatoid arthritis despite treatment with methotrexate.⁴¹ The patients were randomized to receive placebo, 3 mg/kg or 10 mg/kg of infliximab by intravenous infusion at weeks 0, 2, and 6 followed by additional infusions every 4 or 8 weeks thereafter. *The primary clinical endpoint* was the proportion of patients at week 30 who attained an improvement in signs and symptoms as measured by the American College of Rheumatology criteria (ACR20).⁴² An ACR 20 response is defined as at least a 20% improvement in both the tender joint count and the swollen joint count (assessments of 28 or more joints) in addition to $\geq 20\%$ improvement in 3 of the following 5 core set measures:

- physician global assessment
- patient global assessment
- patient pain assessment (using a horizontal visual analog scale or Likert-scale assessment)
- patient-assessed disability (based upon any validated, reliable, sensitive instrument, which measures physical function in rheumatoid arthritis patients, e.g., the Health Assessment Questionnaire⁴³)
- an acute-phase reactant (erythrocyte sedimentation rate (ESR) or C-reactive protein).⁴¹

Secondary endpoints included documentation of 50% and 70% improvement in ACR criteria, reduction in individual measurements of disease activity, and a general health assessment. Infliximab proved efficacious as judged by all the response criteria used. More patients ($P < .001$) treated with infliximab, achieved the intention-to-treat primary clinical endpoint than in the placebo group.⁴¹ The response was rapid; more than half the ultimate responders attained an ACR20 response by the 2-week evaluation and about 90% at the 6-week evaluation.⁴¹

³⁹ Irvine EJ et al. 1994 *Gastroenterology* 106:287-296.

⁴⁰ Present DH et al. 1999 *New Engl J Med* 340(18):1398-1405.

⁴¹ Maini R et al. 1999 *Lancet* 354(9194):1932-1939.

⁴² Felson DT et al. 1995 *Arthritis Rheum* 38(6):727-735.

⁴³ Ramey DR et al. 1996 "The Health Assessment Questionnaire 1995—Status and Review." In: Spilker B (editor) *Quality of Life and Pharmacoeconomics in Clinical Trials* 2d edition. New York: Raven Press.

Basiliximab

Basiliximab (Simulect) is a murine-human chimeric monoclonal antibody that specifically binds to and blocks the interleukin-2 receptor alpha-chain (also called CD25 antigen) on the surface of activated T-lymphocytes⁴⁴ On 5/12/98, the U.S. Food and Drug Administration (FDA) approved its use as prophylaxis of acute organ rejection in

Basiliximab: Summary of clinical trials upon which FDA approval was based.		
	Nashan et al. 1997 ³	Kahan et al. 1999 ⁴
Study design	Phase III, multicenter, randomized, double-blind, placebo-controlled	Phase III, multicenter, randomized, double-blind, placebo-controlled
N	375	346
Primary clinical endpoint	Incidence of death, graft loss, or an episode of acute rejection during the first 6 month post-transplantation	Incidence of death, graft loss, or an episode of acute rejection during the first 6 month post-transplantation
Secondary endpoints	<ol style="list-style-type: none"> 1. Death, graft loss, or acute rejection episode within 12 months post-transplantation 2. Biopsy-confirmed rejection episode within the first 6 or 12 months post-transplantation 3. Patient survival within 12 months post-transplantation 4. Patients with functioning grafts 12 months post-transplantation 	<ol style="list-style-type: none"> 1. Death, graft loss, or acute rejection episode within 12 months post-transplantation 2. Biopsy-confirmed rejection episode within the first 6 or 12 months post-transplantation 3. Patient survival within 12 months post-transplantation 4. Patients with functioning grafts 12 months post-transplantation

patients receiving renal transplantation when used as part of an immunosuppressive regimen that includes cyclosporine and corticosteroids.⁴⁵

Approval was granted on the basis of 2 *phase III, multicenter, randomized, double-blind, placebo-controlled trials*. The EU/CAN study⁴⁶ involved 375 patients eligible for intention-to-treat analysis. The U.S. study⁴⁷ involved 346 patients. In both trials, patients were randomized to receive either 2 doses of basiliximab or placebo. Basiliximab (20 mg) was administered 2 hours before and then 4 days after transplantation. Both patient groups also received baseline dual immunosuppression with cyclosporine and corticosteroids.⁴⁶

The *primary clinical endpoint* for both trials was the incidence of death, graft loss, or an episode of acute rejection during the first 6 months post-

transplantation.⁴⁴

Secondary endpoints included the primary endpoint measured during the first 12 months post-transplantation, the incidence of biopsy-confirmed acute rejection during the first 6 and 12 months post-transplantation, and patient survival and graft survival, each measured at 12 months post-transplantation. Patients in both studies receiving basiliximab experienced a significantly lower incidence of biopsy-confirmed rejection episodes at both 6 and 12 months post-transplantation. There were no differences in the rate of delayed graft function, patient survival, or graft survival between basiliximab-treated patients and placebo-treated patients in either study.^{46,47} The incidence of infections (including active cytomegalovirus infection) and post-transplant lymphoproliferative disorders was similar with basiliximab and placebo. Cytokine release syndrome was not observed in patients who received basiliximab.⁴⁸

⁴⁴ Novartis. Prescribing information for Simulect.

⁴⁵ Center for Biologics Evaluation and Research. www.fda.gov/cber/products/basnov051298.htm.

⁴⁶ Nashan B et al. 1997 *Lancet* 350(9086):1193-1198.

⁴⁷ Kahan BD et al. 1999 *Transplantation* 67(2):276-284.

⁴⁸ Onrust SV et al. 1999 *Drugs* 57(2):207-213.

Denileukin diftitox

On February 5, 1999, the U.S. Food and Drug Administration approved use of denileukin diftitox (Ontak⁴⁹, DAB₃₈₉IL-2⁵⁰) for treating patients with persistent or recurrent cutaneous T-cell lymphoma whose malignant cells express the CD25 component of the interleukin-2 receptor.⁵¹ Denileukin diftitox is an interleukin-2-receptor-specific ligand fusion protein.⁵⁰ It consists of the amino acid sequences for diphtheria toxin fragments *A* and *B* followed by the sequences for interleukin-2.⁴⁹

Two studies provided the basis for approval. In a *phase I/II, multicenter, open-label, dose-ranging study*, 35 patients with cutaneous T-cell lymphoma (CTCL) were treated at doses ranging from 3 to 31 mcg/kg/day, daily for 5

days every 3 weeks.² Subsequently, a *multicenter, randomized, double-blind, phase III study* involving 72 patients with recurrent or persistent CTCL were randomized to either 9 or 18 mcg/kg/day of denileukin diftitox administered as an IV infusion daily for 5 days every 3 weeks.⁵² Patients received a median of 6 courses. Prior to entry in the studies, patients were screened to ensure expression of the CD25 antigen on at least 20% of the cells in any relevant tumor tissue or circulating cells.

All sites of disease were documented at baseline radiographically and by physical examination. Disease assessments were scheduled at 6-week intervals.⁵⁰ For both studies, the *primary clinical endpoint* was based upon attainment of a complete response (no evidence of active disease for 4 or more weeks with no evidence of new disease) or a partial response (reduction of

measurable disease greater than or equal to 50% for 4 or more weeks with no evidence of new disease).² *Secondary clinical endpoints* included time to response, duration of response, results of a quality-of-life questionnaire, and the physician's global assessment of CTCL severity.⁵²

In the phase I/II study, the overall response rate in patients with CTCL who expressed CD25 was 38%; the complete response rate was 16%, and the partial response rate was 22%. The median time to response was 2 months, and the median duration of response was 10 months.⁵⁰ In the phase III study, 30% of patients treated with denileukin diftitox experienced an objective tumor response (>50% reduction in tumor burden sustained for ≥ 6 weeks). Seven patients (10%) achieved a complete response, and 14 patients (20%) achieved a partial response. The median duration of response, measured from the first day of response was 4 months with a median duration for complete response of 9 months and for partial response of 4 months.⁴⁹

⁴⁹ Seragen, Incorporated. Ontak prescribing information.

⁵⁰ LeMaistre CF et al. 1998 *Blood* 91(2):399-405.

⁵¹ Center for Biologics Evaluation and Research. www.fda.gov/cber/products/denseer020599.htm.

⁵² CancerNet Website. Protocol ID: Seragen-93-04-10, NCI-V95-0738. www.cancernet.nci.nih.gov/cgi-bin/srchcgj.exe.

Etanercept

Etanercept (Enbrel), a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human p75 tumor necrosis factor (TNF) receptor linked to the Fc portion of human IgG1,⁵³ has been approved by the U.S. Food and Drug Administration for the following indications:

- On November 2, 1998, etanercept was approved for the reduction in signs and symptoms of moderately to severely active rheumatoid arthritis in patients who have had an inadequate response to one or more disease-modifying antirheumatic drugs.
- On May 27, 1999, etanercept's biologics license was supplemented to include as an indication for use polyarticular-course juvenile rheumatoid arthritis.
- On June 6, 2000, etanercept was approved for reducing the signs and symptoms and delaying structural damage in patients with moderately to severely active rheumatoid arthritis, including those who have not previously failed treatment with disease-modifying antirheumatic drugs.⁵⁴

The safety and clinical efficacy of etanercept for treatment of adult rheumatoid arthritis were assessed in 2 published clinical trials. The study of Moreland et al.⁵⁵ examined 234 patients in a *phase II, multicenter, randomized, double-blind, placebo-controlled trial*. Patients received twice-weekly subcutaneous injections of etanercept, 10 or 25 mg, or placebo for 6 months. The *primary clinical endpoints* were 20% and 50% improvement in disease activity according to American College of Rheumatology (ACR) responses⁵⁶ at 3 and 6 months. An ACR 20 response is defined as at least a 20% improvement in both the tender joint count and the swollen joint count (assessments of 28 or more joints) in addition to $\geq 20\%$ improvement in 3 of the following 5 core set measures:

- physician global assessment
- patient global assessment
- patient pain assessment (using a horizontal visual analog scale or Likert-scale assessment)
- patient-assessed disability (based upon any validated, reliable, sensitive instrument, which measures physical function in rheumatoid arthritis patients, e.g., the Health Assessment Questionnaire⁵⁷)
- an acute-phase reactant (erythrocyte sedimentation rate or C-reactive protein).⁵⁶

At 3 months, 62% of patients receiving 25 mg of etanercept and 23% of the placebo recipients achieved an ACR 20 response ($P = .001$). At 6 months, 59% of the 25-mg group and 11% of the placebo group achieved an ACR 20 response ($P = .001$); 40% and 5%, respectively, achieved an ACR 50 response ($P = .01$).⁵⁵

Secondary endpoints were ACR 70 responses at 3 and 6 months and percentage change in the following: tender joint count, swollen joint count, duration of morning stiffness, patients' global assessment, physician's global assessment, patient's assessment of pain, quality of life, erythrocyte sedimentation rate (ESR), and C-reactive protein level. Response was also evaluated according to the Paulus index, defined as a 20% or 50% improvement in at least 4 of the following variables: tender joint scores, swollen joint scores, duration of morning stiffness, ESR, patient's global assessment, and physician's global assessment.⁵⁸ Significantly, more etanercept recipients achieved an ACR 70 response, minimal disease status (0 to 5 affected joints), and improved quality of life. Other measures of disease activity, such as C-reactive protein levels, ESR, and duration of morning stiffness were also significantly improved with etanercept treatment. Improvement was observed as early as 2 weeks after initiation of etanercept therapy.⁵⁵

A second clinical trial, that of Weinblatt et al.,⁵⁹ *randomized 89 patients*, who had additionally received methotrexate for at least 6 months, to etanercept or placebo in addition to methotrexate. The study was conducted in *double-blind* fashion at *multiple centers*. The *primary clinical endpoint* was the proportion of patients meeting the ACR 20 criteria at 24 weeks. *Secondary endpoints* were the proportion of patients who reached the ACR 20 at 12 weeks, proportions who met the ACR 50 and ACR 70 at 12 and 24 weeks, numbers of swollen and tender joints,

⁵³ Immunex Corporation. Enbrel prescribing information.

⁵⁴ Center for Biologics Evaluation and Research. www.fda.gov/cber/products/etanimm060600.htm.

⁵⁵ Moreland LW et al. 1999 *Ann Intern Med* 130(6):478-486.

⁵⁶ Felson DT et al. 1995 *Arthritis Rheum* 38(6):727-735.

⁵⁷ Ramey DR et al. 1996 "The Health Assessment Questionnaire 1995—Status and Review." In: Spilker B (editor) *Quality of Life and Pharmacoeconomics in Clinical Trials* 2d edition. New York: Raven Press.

⁵⁸ Paulus HE et al. 1990 *Arthritis Rheum* 33:477-484.

⁵⁹ Weinblatt ME et al. 1999 *New Engl J Med* 340(4):253-259.

improvement in Health Assessment Questionnaire score, decreased ESR, and decreased C-reactive protein level. The combination of etanercept and methotrexate was superior to the combination of placebo and methotrexate regardless of the dose of methotrexate, the duration of methotrexate therapy, or background use of corticosteroids or nonsteroidal anti-inflammatory drugs.

A third, unpublished study involved 632 patients randomized to 10 or 25 mg etanercept, methotrexate, or

Etanercept: Summary of clinical trials upon which FDA approval was based.				
	Moreland et al. 1999⁵⁵	Weinblatt et al. 1999⁵⁹	Unpublished study⁵³	Lovell et al. 2000⁶²
Study design	Phase II, multicenter, randomized, double-blind, placebo-controlled trial	Phase II, multicenter, randomized, double-blind, placebo-controlled trial	Randomized, placebo-controlled trial	<i>Part 1:</i> open-label, multicenter study <i>Part 2:</i> randomized, double-blind trial
N	234	89	632	69
Primary endpoints	Proportion of patients at 3 and 6 months who attained a 20% or 50% improvement in signs and symptoms as measured by American College of Rheumatology criteria. ⁵⁶	Proportion of patients at 24 weeks who attained a 20% improvement in signs and symptoms as measured by American College of Rheumatology criteria. ⁵⁶	Proportion of patients at 3, 6, and 12 months who attained a 20%, 50%, or 70% improvement in signs and symptoms as measured by American College of Rheumatology criteria. ⁵⁶	<i>Part 1:</i> Response assessment via a core set of 6 response variables: global assessment of the severity of disease by the physician, global assessment of overall well-being by the patient or parents, number of "active" joints, number of joints with limitation of motion, functional ability, and ESR. <i>Part 2:</i> number of patients with disease flare, defined as worsening of 30% or more in 3 of the 6 response variables and a minimum of 2 active joints.
Secondary endpoints	<ol style="list-style-type: none"> 1. ACR 70 responses at 3 and 6 months 2. Change in the number of tender and swollen joints 3. Duration of morning stiffness 4. Global assessments of patient and physician 5. Patient's assessment of pain using a visual analog scale 6. Quality of life as measured by Health Assessment Questionnaire 7. ESR 8. C-reactive protein level 9. 20% or 50% improvement on Paulus index⁵⁸ 	<ol style="list-style-type: none"> 1. Proportion of patients who reached the ACR 20 at 12 weeks 2. Proportion of patients who reached the ACR 50 and ACR 70 at 12 and 24 weeks 3. Change in the number of tender and swollen joints 4. Quality of life as measured by Health Assessment Questionnaire 5. ESR 6. C-reactive protein level 	<ol style="list-style-type: none"> 1. Results of a health outcome measure, termed the SF-36 questionnaire⁶⁰ 2. Radiographic evaluation of structural joint damage using the Sharp score⁶¹ 	<ol style="list-style-type: none"> 1. Score in the disability domain of the Childhood Health Assessment Questionnaire^{64,65} 2. C-reactive protein level 3. ESR 4. White blood cell and platelet counts

placebo for 12 months.⁵³ Clinical endpoints were again based upon the ACR response criteria with the addition of two additional secondary clinical endpoints: (1) results of a health outcome measure, termed the SF-36 questionnaire; and (2) radiographic evaluation of structural joint damage. The SF-36 is a multipurpose, short-form health survey that consists of 36 questions aggregated into 8 scales of 2-10 items each. The SF-36 is a generic measure of health status as opposed to one that targets a specific age, disease, or treatment group. The 8 scales are physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional, and emotional health. The first 4 scales roll up into the physical health summary measure, and the latter 4 roll up into the mental health summary measure.⁶⁰ Patients treated with 25 mg etanercept showed significantly more improvement in the physical health summary score than patients in the 10-mg etanercept group. No significant changes were detected in the mental health summary measure.⁵³ Structural joint damage was assessed radiographically and expressed as change in total Sharp score.⁵³ The Sharp score derives from a simplified scheme for assessing abnormalities of rheumatoid arthritis in hand and wrist radiographs. The scheme uses a combination of 17 joints to score erosions and 18 to score joint space narrowing.⁶¹ At 6 and 12 months, patients treated with 25 mg etanercept showed a significant decrease in the erosion score (but not for total Sharp score or joint-space narrowing score) compared to patients treated with methotrexate.⁵³

The safety and efficacy of etanercept for treating polyarticular course juvenile rheumatoid arthritis was evaluated in a 2-part study of 69 pediatric patients.⁶² The first part of the study was an open-label, multicenter study. Those who responded to treatment were then randomized in a double-blind study to receive placebo or etanercept. A response was assessed via a core set of 6 response variables: global assessment of the severity of disease by the physician, global assessment of overall well-being by the patient or parents, number of "active" joints, number of joints with limitation of motion, functional ability, and ESR.⁶³ The primary clinical endpoint for the double-blind portion of the study was the number of patients with disease flare. Disease flare, as defined for this study, was based on the change in the core set of response variables from the beginning of the double-blind study. Criteria for disease flare were worsening of 30% or more in 3 of the 6 response variables and a minimum of 2 active joints. They could also have improvement of 30% or more in no more than 1 of the 6 response variables. Global assessments, if used to define flare, had to change by at least 2 units on a scale from 0 to 10. Secondary endpoints included score in the disability domain of the Childhood Health Assessment Questionnaire,^{64,65} C-reactive protein level, ESR, and white blood cell and platelet counts. In the first part of the study, 74% of patients demonstrated a clinical response and entered part 2. Twenty-four percent of patients remaining on etanercept in part 2 experienced a disease flare, compared to 77% of patients receiving placebo.⁶²

Etanercept (TNFR:Fc) has also been studied in the context of HIV infection. Both interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) are substances naturally produced by the body's immune system. Elevated TNF-alpha levels can result in increased IL-6 production and possibly increased HIV replication. It has been postulated that exogenous administration of TNF receptors, which bind to and block the action of TNF-alpha, may result in decreased activity of TNF-alpha.⁶⁶ Collectively, several laboratory and pilot studies have demonstrated that:

- TNF-alpha production may be excessive or inappropriate in HIV-infected patients⁶⁷ and may contribute to declines in CD4+ cell counts.⁶⁸
- By decreasing the amount of IL-6 in the body and by decreasing the action of TNF-alpha in the body, TNFR:Fc may have a role in the treatment of HIV disease or in alleviating some of the symptoms related to IL-2 administration.⁶⁶
- In vitro, TNFR:Fc inhibits TNF-alpha induced expression of HIV-1 and limits the activation of the HIV-long terminal repeat transcription in chronically infected human cell lines⁶⁹
- Soluble TNF receptors can inhibit stimuli-induced HIV-1 expression and NK - kappa B DNA-binding activity in promonocytic U1 cells chronically infected with HIV-1.⁷⁰

⁶⁰ Ware JE et al. 1998 *J Clin Epidemiol* 51(11): 903-912.

⁶¹ Sharp JT et al. 1985 *Arthritis Rheum* 28(12): 1326-1335.

⁶² Lovell DJ et al. 2000 *New Engl J Med* 342(11):763-769.

⁶³ Giannini EH et al. 1997 *Arthritis Rheum* 40(7):1202.

⁶⁴ Singh G et al. 1994 *Arthritis Rheum* 37:1761-1769.

⁶⁵ Ramey DR et al. 1992 *Arthritis Care Res* 5:119-129.

⁶⁶ www.actis.org/rwscripts/rwisapi.dll/@actis.env?CQ_USER_NAME=NLM_JUMP&CQ_PASSWORD=hf924hm&CQ_LOGIN=Yes&CQ_CUR_LI
BRARY=protocol&CQ_JUMP=yes&CQ_DO_QUERY=yes&CQ_CD=1&CQ_QUERY_STRING=document_id=985.

⁶⁷ Rizzardi GP et al. 1996 *AIDS* 10(13):F45-50.

⁶⁸ Zangerle R et al. 1994 *Clin Immunol Immunopathol* 72(3):328-34.

⁶⁹ Howard OM et al. 1993 *Proc Natl Acad Sci USA* 90(6):2335-9.

A phase I/II study (ACTG 928) was conducted to:⁶⁶

- determine whether soluble TNFR:Fc can decrease plasma levels of IL-6 in HIV-infected patients receiving treatment with recombinant human IL-2
- explore whether soluble TNFR:Fc can inhibit plasma TNF bioactivity in HIV-infected individuals
- discern effects of soluble TNFR:Fc receptor on C-reactive protein, body temperature, plasma IL-10, plasma IL-12, plasma IL-4, interferon-gamma and plasma HIV-1 RNA levels in HIV-infected individuals
- explore whether pretreatment levels of TNFR Type I or II are associated with cytokine, C-reactive protein, plasma HIV-RNA, or body temperature changes in HIV-infected individuals
- determine whether soluble TNFR:Fc can decrease plasma levels of IL-6 in HIV-infected patients receiving highly active antiretroviral therapy (HAART).

Etanercept in HIV: Summary of a phase I/II trial.	
ACTG 928⁶⁶	
Study design	Phase I/II, cohort study with 3 treatment arms: HAART + I.V. IL-2 + TNFR:Fc HAART + S.Q. IL-2 + TNFR:Fc HAART + TNFR:Fc
N	18
Outcomes	1. Plasma levels of IL-6 2. TNF bioactivity in plasma 3. C-reactive protein level 4. Body temperature 5. Plasma IL-10, plasma IL-12, plasma IL-4, and interferon-gamma levels 6. Plasma HIV-1 RNA levels

Six patients from each of the 3 treatment arms of ACTG 328 (HAART alone, HAART plus intravenous recombinant human IL-2, and HAART plus subcutaneous IL-2) of a prior study (ACTG 328) participated in this prospective, nested substudy. The patients in all three arms received TNFR:Fc by infusion over 30 minutes at week 16 of ACTG 928 (Course 3, Week 28 of ACTG 328); for patients in the I.V. IL-2 treatment arm, TNFR:Fc

was infused immediately prior to IL-2.⁶⁶ No findings have been published from this study.

⁷⁰ Granowitz EV et al. 1996 *J Acquir Immune Defic Syndr Hum Retrovirol* 11(5):430-7.

Immune-based therapies in HIV

Interleukin-2 (aldesleukin)

Aldesleukin (Proleukin⁷¹), a human recombinant interleukin-2 (IL-2) product has been approved by the U.S. Food and Drug Administration for two indications:

- On May 5, 1992, it was approved for the treatment of adults 18 years of age or older with metastatic renal cell carcinoma.
- On January 9, 1998, the biologics license was supplemented to include a new indication for use in adults with metastatic melanoma.⁷²

Over the last 17 years or so, investigators have conducted at least 25 trials of IL-2 in HIV-infected individuals, making it the most extensively studied immune-based therapy in the history of the epidemic.⁷³ Interleukin-2, in fact, has undergone more thorough evaluation than most antiretroviral therapies, which are now often approved on the basis of surrogate marker data and without clinical endpoint data. Collectively, clinical studies of IL-2 in an HIV setting suggest that:⁷³

- IL-2, administered intravenously or subcutaneously, produces significant, polyclonal increases in peripheral CD4+ cell counts.^{74,75,76,77,78}
- Dramatic rises in CD4+ cell numbers are more likely to be attained in those with less advanced disease;^{79,80} however, patients with a baseline CD4+ count of less than 200 cells may still benefit when IL-2 is used in conjunction with highly active antiretroviral therapy (HAART).
- CD4+ cells produced as a result of IL-2 therapy are functional.⁷⁵
- Despite transient increases in plasma viremia post-infusion, treatment with IL-2 does not cause sustained rises in viral load;^{74,75,76,77,78} viral loads are suppressed from baseline levels in those receiving IL-2 compared to those receiving antiretroviral therapy alone.^{81,82}
- Clinical disease progression may occur more slowly among patients receiving IL-2 plus antiviral therapy compared to those receiving antiviral therapy alone.⁸⁰

These findings have driven the initiation of large clinical trials. The CPCRA 059 trial⁸³ was the largest randomized study of IL-2 treatment of early-stage HIV-infected individuals completed to date. The study involved 511 patients with CD4+ counts greater than 300 cells/mm³ who were already receiving or about to begin antiretroviral therapy. Reports about the effects of IL-2 on viral replication have been inconsistent, with early data suggesting that IL-2 may increase viral loads at least transiently by activating CD4+ cells and a more recent report suggesting that patients responding to aggressive HAART experiencing decreases in viral load with IL-2 treatment. The CPCRA 059 trial addressed this question by using a primary endpoint of proportion of patients with plasma HIV RNA below 50 copies/mL after 1 year on study.⁸⁴ After 1 year of follow-up, the proportion of patients with HIV RNA less than 50 copies/mL was 64.4% in the IL-2 arm and 61.7% in the control arm ($P = .63$). No increase in viral replication with IL-2 treatment was seen during the course of study. As expected, however, the mean CD4+ count increased significantly among patients receiving IL-2 with a mean increase in CD4+ count over the course of the study of 22 cells/mm³ and 276 cells/mm³ in the control and IL-2 study arms, respectively ($P < .0001$).⁸⁵

⁷¹ Chiron Corporation. Proleukin prescribing information.

⁷² Center for Biologics Evaluation and Research. www.fda.gov/cber/products/aldechi010998.htm.

⁷³ Simmons P. 1999 *Research Initiative Treatment Action!* 5(2): www.centerforaids.org/rita/0499/interleuk.htm.

⁷⁴ Kovacs JA et al. 1996 *N Engl J Med* 335(18):1350-1356.

⁷⁵ Jacobson EL et al. 1996 *Proc Natl Acad Sci* 93: 10405-10410.

⁷⁶ Hengge UR et al. 1998 *AIDS* 12(17):F225-234.

⁷⁷ Losso MH et al. 2000 *J Infect Dis* 181(5):1614-1621.

⁷⁸ Larsen CS et al. 2000 *Scan J Infect Dis* 32(2):153-160.

⁷⁹ Kovacs JA et al. 1995 *N Engl J Med* 332(9):567-75.

⁸⁰ Davey RT Jr et al. 1997 *J Inf Dis* 175(4):781-89.

⁸¹ Davey RT Jr et al. 2000 *JAMA* 283(2):183-189.

⁸² Emery S et al. 2000 *J Infect Dis* 182(2):428-434.

⁸³ The Terry Beinr Community Programs for Clinical Research on AIDS. IL-2 VL/Dose Protocol (CPCRA 059). www.cpcra.org/studies.

⁸⁴ Abrams D. 2000 in "Immune reconstitution in HIV: Looking for a better way." www.medscape.com/medscape/cno/2000/ICAAC/Story.cfm?story_id=1650.

⁸⁵ Abrams DI et al. 2000. "IL-2 therapy produces no change in HIV RNA after one year." Abstract L-11. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy Addendum; September 17-20.

Having elucidated the effects of IL-2 on CD4+ cell counts and HIV RNA levels, all that remains now is to translate these results into clinical outcomes. Presently, 2 large, phase III, clinical-endpoint trials—the ESPRIT⁸⁶ and SILCAAT⁸⁷ trials—are underway to evaluate IL-2 as an adjunct to antiretroviral therapy for HIV and determine if surrogate markers for HIV disease will lead to long-term clinical benefits for patients. These studies will determine if IL-2 administered with combination antiretroviral therapy will affect progression of AIDS-defining conditions or death, thus providing the first evidence that immune-based therapy can alter the clinical course of HIV infection.

Interleukin 2: Summary of major clinical trials.			
	CPCRA 059⁸⁴	SILCAAT⁸⁷	ESPRIT⁸⁶
Study design	Phase III, open-label trial with patients randomized to one of two subcutaneous doses of IL-2 therapy or no IL-2 over an enrollment period of 12 months	Phase III, multicenter, open-label trial with patients randomized to IL-2 plus triple-drug antiretroviral therapy or triple-drug antiretroviral therapy only (patients with CD4+ cell counts < 300/mm ³)	Phase III, international, open-label, 5-year trial with patients randomized to IL-2 plus combination antiretroviral therapy or to antiretroviral therapy only (patients with CD4+ cell counts ≥ 300/mm ³)
N	511	1400	4000
Primary clinical endpoint	Change in log ₁₀ HIV RNA after 12 months as determined by the Quantiplex HIV RNA 3.0 Assay (Chiron Corp.), an ultrasensitive, sandwich nucleic-acid hybridization procedure for quantification of HIV RNA in human plasma (beta version)	Time of appearance of first AIDS-defining event or death in patients treated with (IL-2 + triple-drug therapy) versus (triple-drug therapy alone)	Disease progression as evidenced by AIDS-defining illnesses or other conditions considered to be evidence of disease progression (Appendix B of ESPRIT protocol), including death
Secondary clinical endpoints	<ol style="list-style-type: none"> 1. Change in absolute CD4+ cell counts after 12 months 2. Area under the curve for viral load and CD4+ cell count during the first 12 months of the study. 3. Grade 4 events 4. Changes in antiretroviral therapy and opportunistic infection prophylaxes 5. Change in quality of life after 12 months 6. Cirrhosis, hepatic steatosis and hepatitis 7. Viral load ≥ 1,000 copies/mL associated with resistance 	<ol style="list-style-type: none"> 1. Change from baseline in CD4+ cell count 2. Proportion of patients with at least a 50% increase in CD4+ cell counts and an absolute increase of at least 75 cells 3. Change in HIV viral load 4. Proportion of IL-2 treated participants with an undetectable viral load 5. Time to first change in antiretroviral therapy 6. Time to first change in antiretroviral therapy due to virologic or clinical failure 7. Incidence of adverse events and the incidence of IL-2-related adverse events that require a change in IL-2 dose 	<ol style="list-style-type: none"> 1. Serious disease progression events, including death 2. Survival 3. Absolute CD4+ cell counts and percent CD4+ of lymphocytes 4. Plasma HIV RNA levels 5. Changes in antiretroviral treatment 6. Grade 4 signs and symptoms 7. Pattern of use of prophylaxes for opportunistic infections 8. Hepatic, metabolic, and cardiac conditions

⁸⁶ www.espritstudy.org.

Interleukin-12

Interleukin-12 (IL-12), a heterodimeric cytokine that enhances the lytic activity of natural killer cells, induces interferon-gamma production, and stimulates the proliferation of activated T-cells and natural killer cells,⁸⁸ has been evaluated in several clinical trials. The conditions addressed in these trials run the gamut from melanoma, hepatitis C, and chronic hepatitis B to metastatic renal cell cancer. It has been investigated as a treatment for HIV disease because it stimulates the TH₁ subset of CD4⁺ cells undergoing primary activation.^{89,90} Collectively, laboratory and initial human studies have led to the following conclusions:⁹¹

- IL-12 produced similar increases in natural killer cell activity in peripheral blood mononuclear cells in uninfected children and in HIV⁺ children.⁹²
- IL-12 augmented the in vitro production of cytokines associated with TH₁ responses (IL-2 and interferon-gamma) in HIV⁺ individuals.

Interleukin 12: Summary of major clinical trials.		
	Phase II trial of EPOCH chemotherapy ± IL-12 for patients with AIDS-associated lymphoma ^{100,101}	ACTG 325 ^{102,103,104}
Study design	Phase II	Phase I, double-blind, randomized, dose-escalation, placebo-controlled
N	Not available	65
Primary endpoints	<ol style="list-style-type: none"> 1. Toxicity 2. Response 3. Progression-free survival 4. Overall survival 	Grade 3 or Grade 4 toxicity
Secondary endpoints	<ol style="list-style-type: none"> 1. Dynamics of HIV plasma load 2. CD4⁺ and CD8⁺ cell numbers 3. Functionally defined TH₁ and TH₂ CD4⁺ subtypes 4. Molecular markers of drug resistance 5. Molecular markers of lymphomagenesis 	Change from baseline to posttreatment in: <ol style="list-style-type: none"> 1. Serum interferon-gamma level 2. Serum neopterin level 3. Lymphoproliferative responses of PBMCs to MAC antigen 4. Type 1 Th responses (i.e., inducible interferon-gamma) to mitogens and alloantigens 5. Absolute count of CD4⁺, CD8⁺, CD3⁻/CD16⁺/CD56⁺ 6. Lymphoproliferative responses to tetanus toxoid, <i>Candida</i>, streptokinase, and HIV antigens with PHA as a control 7. Delayed-type hypersensitivity skin tests 8. Plasma HIV-1 RNA copy number

- PBMC from HIV⁺ people were stimulated in vitro with non-HIV antigens; IL-12 increased the T-helper cell response to these antigens. Elevated responses to these antigens were not observed in cells from uninfected individuals.⁹³
- 80% of patients (8/10) with Kaposi's sarcoma who received high doses of IL-12 achieved sustained partial responses.⁹⁴

⁸⁷ www.silcaat.com.

⁸⁸ Lackie J. "The dictionary of cell and molecular biology—online!" www.mblab.gla.ac.uk/~julian/dict2.cgi?3299.

⁸⁹ Schearer G et al. 1993 Presentation at the Roundtable for the Development of Drugs and Vaccines Against AIDS, Institute of Medicine.

⁹⁰ Hsieh C et al. 1993 Science 260:547-549.

⁹¹ amFAR's HIV/AIDS Treatment Directory. <http://199.105.91.6/treatment/Drug/ID268.ASP>.

⁹² McFarland E et al. 1995. 2d National Conference on Human Retroviruses. Abstract 309.

⁹³ Clerici M et al. 1993. Science 262:1721-1724.

⁹⁴ Little RF et al. 2000. 7th Conference on Retroviruses and Opportunistic Infections. Abstract 5.

- PBMC from children at a less advanced HIV disease stage appeared to be more likely to have increased HIV-specific cytotoxic lymphocyte (CTL) activity in response to IL-12 than children with more advanced disease⁹⁵
- One phase I study showed no marked effects on viral load levels or CD4+ counts in patients receiving single-dose treatment.⁹⁶
- Repeated subcutaneous injections of IL-12 in patients with cancer resulted in the selective expansion of a subset of peripheral blood CD8+ T-cells. This T-cell subset expressed high levels of CD18 and upregulated IL-12 receptor expression after IL-12 treatment in vivo.⁹⁷
- IL-12 therapy of cutaneous T-cell lymphoma induces lesion regression and augments antitumor cytotoxic T-cell responses.⁹⁸
- Impaired T-lymphocyte recognition of foreign antigen, including HIV, can be reconstituted in part by supplementation with IL-12 for selected HIV-seropositive individuals.⁹⁹

Building upon these initial findings, one phase I study and one phase II study have been undertaken. The goal of the ongoing phase II study^{100,101} is to determine the efficacy and toxicity of EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin) chemotherapy in previously untreated AIDS-related lymphoma and to study the effects of IL-12 on immune reconstitution and virologic control in such patients after completion of chemotherapy. *Clinical endpoints* for the trial include toxicity, response, progression-free survival, and overall survival. *Translational endpoints* include dynamics of HIV plasma load, CD4+ and CD8+ cell numbers, and functionally defined TH₁ and TH₂ CD4+ subtypes. Also being monitored are molecular markers of drug resistance (i.e., p53, p16, bcl-2, and MIB-1) and lymphomagenesis (i.e., c-myc, EBV, and HHV-8) in tumor tissue. No details on the number of patients or study design are available.

A completed *multicenter, double-blind, randomized, placebo-controlled phase I trial*^{102,103,104} evaluated the tolerability of a range of chronic subcutaneous IL-12 dosing regimens in 65 HIV-infected patients with fewer than 50 CD4+ cells/mm³, who have no evidence of serious ongoing opportunistic infections and who maintain concomitant antiretroviral therapy. Patients were randomized within one of the three sequential dose cohorts and received either IL-12 or placebo. The *primary endpoint* of the study was the occurrence, 8 weeks or less after the initiation of study drug treatment, of either: (a) Grade 3 toxicity judged to be definitely related or possibility related to study medication, or (b) a Grade 4 toxicity or death judged to be definitely related, possibly related, or whose relationship to study medication is unable to be judged by the local investigator. *Secondary endpoints* were the change from baseline to posttreatment in serum interferon-gamma level; serum neopterin level; lymphoproliferative responses of PBMCs to MAC antigen; type 1 Th responses (i.e., inducible interferon-gamma) to mitogens and alloantigens; absolute count of CD4+, CD8+, CD3-/CD16+/CD56+ cells; lymphoproliferative responses to tetanus toxoid, *Candida*, streptokinase, and HIV antigens with PHA as a control; delayed-type hypersensitivity skin tests; and plasma HIV-1 RNA copy number. This study has closed enrollment, and the results are being analyzed.

⁹⁵ McFarland EJ et al. 1996. 3d Conference on Retroviruses and Opportunistic Infections.

⁹⁶ Jacobson M et al. 1996. 3d Conference on Retroviruses and Opportunistic Infections. Abstract 309.

⁹⁷ Gollob JA et al. 1998 *J Clin Invest* 102(3):561-575.

⁹⁸ Rook AH et al. 1999 *Blood* 94(3):902-908.

⁹⁹ Nagy-Agren SE et al. 1999 179:493-496.

¹⁰⁰ AIDSTRIALS listing of clinical trials. <http://www.actis.org/index.html>.

¹⁰¹ www.clinicaltrials.gov/ct/gui/c/a2r/action/GetStudy/screen/ResultScreen?order=1&xml.

¹⁰² www.clinicaltrials.gov/ct/gui/c/a1b/action/GetStudy/screen/ResultScreen?order=1&xml.

¹⁰³ Record 813 "ACTG 325" AIDSTRIALS listing of clinical trials. <http://www.actis.org/index.html>.

¹⁰⁴ ACTG 325 protocol version 3.0. <http://aactg.s-3.com>.

GM-CSF (sargramostim)

Recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF, Leukine, Prokine), stimulates proliferation and differentiation of hematopoietic progenitor cells¹⁰⁵. It has been approved by the U.S. Food and Drug Administration for five indications:

- following induction chemotherapy in acute myelogenous leukemia
- in mobilization and following transplantation of autologous peripheral blood progenitor cells
- in myeloid reconstitution after autologous bone marrow transplantation
- in myeloid reconstitution after allogeneic bone marrow transplantation
- in bone marrow transplantation failure or engraftment delay.¹⁰⁶

The clinical studies that served as the basis for FDA approval used as *efficacy endpoints* median time to

GM-CSF: Summary of major clinical trials.			
	ACTG A5041^{124,125}	Brites et al.¹²⁶	Angel et al.¹¹²
Study design	Phase II, randomized, multicenter, placebo-controlled trial with patients receiving potent antiretroviral therapy plus either GM-CSF or placebo for 16 weeks	Phase II, placebo-controlled, double-blind trial with patients randomized to GM-CSF or placebo in addition to nucleoside analog therapy	Phase III, multicenter, double-blind, placebo-controlled trial with patients receiving antiretroviral therapy randomized to GM-CSF or placebo
N	108	105	309
Primary endpoint	Change in HIV RNA copy number	Change in mean virus load	Incidence of clinical events, specifically CDC-defined opportunistic infections, bacterial pneumonia, or death
Secondary endpoints	1. Change from baseline in CD4+ cell counts	1. Change from baseline in CD4+ cell count 2. Proportion of patients achieving HIV-RNA levels < 500 copies/mL at ≥ 2 evaluations 3. Frequency of zidovudine-resistant mutations detected by genotypic analysis 4. Incidence of opportunistic infections	1. Change from baseline in CD4+ cell count 2. Change in HIV-RNA copy number as determined using Amplicor (lower limit of detection 400 copies/mL) 3. Change in absolute neutrophil count 4. Time to clinical event 5. Maximum intensity of infection

neutrophil, erythrocyte, and platelet recovery, time to relapse, proportion of patients who attained complete remission, and overall survival.^{107,108,109,110}

GM-CSF boosts number and function of a wide range of immune cells, including lymphocytes, macrophages, neutrophils, and dendritic cells through augmentation of cytokine secretion and upregulation of class II

¹⁰⁵ Immunex Corporation. Leukine prescribing information.

¹⁰⁶ Center for Biologics Evaluation and Research. www.fda.gov/cber/products/sargimm110796.htm.

¹⁰⁷ Rowe JM et al. 1995 *Blood* 86(2):457-462.

¹⁰⁸ Nemunaitis J et al. 1991 *Bone Marrow Transplant* 7:49-52.

¹⁰⁹ Nemunaitis J et al. 1991 *N Engl J Med* 324(25):1773-1778.

¹¹⁰ Nemunaitis J et al. 1988 *Blood* 72(2):834-836.

major histocompatibility complexes and accessory receptors involved in the immune response.¹¹¹ Several laboratory and pilot studies have investigated the possibility that GM-CSF may be useful in the treatment of HIV infection. Collectively, these studies suggest that GM-CSF:^{111,112}

- enhances number and function of myeloid-derived and lymphoid-derived cells^{113,114,115,116,117,118}
- downregulates HIV co-receptor expression by monocyte-derived macrophages in vitro, rendering these cells resistant to infection with HIV-1^{119,120,121}
- enhances the antiretroviral activity of zidovudine and stavudine in macrophages and ameliorates the hematologic side effects of these agents¹¹¹
- enhances clearance of HIV from macrophage reservoirs^{115,119}
- increases resistance of CD4+ cells to HIV infection^{119,120,121}
- may improve surrogate markers of HIV disease in individuals also receiving reverse transcriptase inhibitors¹²² or protease inhibitors,¹²³ including increases in CD4+ cell counts and decreases in HIV RNA.⁷

These findings have driven the initiation of *several phase II and phase III clinical trials*. One phase II study (ACTG A5041)^{124,125} has recently closed enrollment and no findings have been reported yet. Another phase II study¹²⁶ randomized 105 individuals with AIDS who were receiving nucleoside analog therapy to either GM-CSF or to placebo. A significant decrease in mean virus load was observed for the GM-CSF treatment group at 6 months. Genotypic analysis demonstrated a lower frequency of zidovudine-resistant mutations among those receiving GM-CSF. No difference was observed in the incidence of opportunistic infections through 6 months of survival.

A phase III trial¹¹² enrolled HIV-infected individuals with CD4+ cell counts $\leq 50 \times 10^6/L$ or $\leq 100 \times 10^6/L$ with a prior AIDS-defining illness on stable antiretroviral therapy. Subjects were stratified by baseline HIV-RNA level and randomized to either GM-CSF or placebo. Significant increases in CD4+ and neutrophil counts were observed at 1, 3, and 6 months in the GM-CSF group. GM-CSF treatment decreased virologic breakthrough. No statistical difference in cumulative opportunistic infections was observed between groups.

¹¹¹ Skowron et al. 1999 *J Infect Dis* 180:1064-1071.

¹¹² Angel JB et al. 2000 *AIDS* 14:387-395.

¹¹³ Chun TW et al. 1999 *Nature Med* 5:651-655.

¹¹⁴ Morrissey PJ et al. 1987 *J Immunol* 139:1113-1119.

¹¹⁵ Fischer HG et al. 1988 *J Immunol* 141:3882-3888.

¹¹⁶ Reid CD et al. 1992 *J Immunol* 149:2681-2688.

¹¹⁷ Stewart-Akers AM et al. 1994 *Blood* 83:713-723.

¹¹⁸ Lopez AF et al. 1986 *J Clin Invest* 78:1220-1228.

¹¹⁹ Matsuda S et al. 1996 *J Acquir Immune Defic Syndr Hum Retrovirol* 11:1031-1038.

¹²⁰ Di Marzio P et al. 1998 Fifth Conference on Retroviruses and Opportunistic Infections. Abstract 37.

¹²¹ Di Marzio P et al. 1998 *J Acquir Immune Defic Syndr Hum Retrovirol* 14:129-138.

¹²² Barbaro G et al. 1997 *AIDS* 11:1453-1461.

¹²³ Bernstein ZP et al. 1997 *Blood* 90:133a.

¹²⁴ www.actis.org

¹²⁵ http://clinicaltrials.gov/ct/gui/c/a1r/action/SearchAction/screen/OpeningScreen?JServSessionIdcs_current=jp2b7qthms&Term=GM-CSF%2C+Hiv%2C+phase+II&submit=Search

¹²⁶ Brites et al. 2000 *J Infect Dis* 182(5):1531-1535.

Remune

Remune (HIV-1 immunogen), is a therapeutic vaccine made from whole HIV particles stripped of the envelope layer and sterilized. Several possible mechanisms of action have been proposed:¹²⁷

- It may stimulate the immune system to mount a greater immune response to virus particles and HIV-infected cells.
- It may stimulate the production of chemokines in the body, which may help protect cells against infection with HIV.
- It may activate latently infected cells by generally activating the immune system or by triggering pools of memory cells that recognize HIV antigens, but which become quiescent when no longer stimulated by HIV in patients with undetectable viral loads on HAART.

Remune was shown in early studies to be safe and to have effects on certain immunologic parameters, but these effects did not correlate with changes in CD4+ cell count or viral load. However, it was postulated that with viral suppression by HAART that HIV antigenic stimulation of the immune system could help control HIV. Study 806,¹²⁸ a *phase III, multicenter, double-blind, placebo-controlled, randomized trial* involving 2527 patients had as its *primary efficacy endpoint* AIDS-free survival—the time to development of AIDS-defining opportunistic infections or death. The primary endpoint was subsequently modified to include several additional conditions and infections. The list of *secondary efficacy endpoints* included overall survival, changes in HIV-RNA, CD4+ cell count and percentage, p24 antibody titers, body weight, immunogenicity, and virologic failure (in a substudy). The trial, however, was halted when an interim analysis failed to detect a difference in the clinical endpoint between study arms.¹²⁹ (In a separate analysis of a cohort of 250 patients, surrogate marker analysis showed a significantly greater reduction in viral load after 48 and 96 weeks of treatment and significantly greater increases in lymphocyte proliferation in those who added Remune to their underlying antiretroviral therapy compared to those who did not.¹³⁰) It is believed that the number of HIV-associated clinical endpoints observed in the trial was far less than originally anticipated because of the increasing use of highly active antiretroviral therapy. This study is being revamped to rely upon a viral load endpoint instead of a clinical endpoint, as is the case with other new and ongoing trials (Trial numbers AG1661-201, AG1661-202) of Remune.¹³¹

A *phase II, placebo-controlled, randomized trial*¹³² in Thailand involved 297 patients who received either Remune or placebo as monotherapy. The *primary endpoint* of the study was a change in CD4+ cell count as determined by a validated method. *Secondary endpoints* included changes in CD8+ count, body weight, changes in HIV-1 RNA levels (subset study), Western blot immunoreactivity (subset study), and HIV-1 delayed-type hypersensitivity skin test reactivity. It was found that HIV-RNA levels remained stable in both groups, but CD4+ cell counts increased by 84 cells/mm³ in the Remune group compared to 38 cells/mm³ in the placebo group.

¹²⁷ AmfAR's HIV/AIDS Treatment Directory. <http://199.105.91.6/treatment/drug/ID294.asp>.

¹²⁸ Kahn JO et al. 2000 *JAMA* 284(17):2193-2202.

¹²⁹ amfAR's HIV/AIDS Treatment Directory. <http://199.105.91.6/treatment/drug/ID294.asp>.

¹³⁰ *PRNewswire*. May 17, 1999. www.aegis.com/news/pr/1999/PR990513.html.

¹³¹ *PRNewswire*. May 17, 1999. www.aegis.com/news/pr/1999/PR990513.html.

¹³² Churdboonchart V et al. 1998 *AIDS* 12:1521-1527.

Remune: Summary of major clinical trials.				
	Study 806 (Kahn 2000)	Churdboonchart 1998	AG1661-201 (B008)	AG1661-202 (B009)
Study design	Phase III, multicenter, double-blind, placebo-controlled, randomized trial	Phase II, placebo-controlled, randomized trial	Phase II, multicenter, double-blind trial	Phase III, double-blind, multicenter, randomized control trial
N	2527	297	40	688
Primary efficacy endpoint	AIDS-free survival, i.e., time to development of AIDS-defining opportunistic infections or death (endpoint definition was expanded to include other infections and conditions)	Changes in CD4+ cell count as determined by a validated method	Change in pre-HAART plasma HIV-1 RNA set point to the new plasma HIV-1 RNA steady state after HAART has been discontinued for 6 weeks in patients who received Remune or placebo prior to HAART initiation	Time to virologic failure
Secondary efficacy endpoints	<ol style="list-style-type: none"> 1. Overall survival 2. Changes in HIV-RNA 3. CD4+ cell count and percentage 4. p24 antibody titers 5. Body weight 6. Immunogenicity 7. Virologic failure (substudy) 	<ol style="list-style-type: none"> 1. Changes in CD8+ count 2. Body weight 3. Changes in HIV-1 RNA levels (subset study) 4. Western blot immunoreactivity (subset study) 5. HIV-1 delayed-type hypersensitivity skin test reactivity 	Not available	Not available

Canarypox-vectored vaccine

Live-attenuated vaccines are among the most effective viral vaccines developed, although safety concerns persist about using live-attenuated HIV-1 vaccines. One alternative is to introduce HIV-1 antigens through other vectors. Such recombinant vectors seem to result in antigen processing that induces cellular immune responses, including CD8+ cytotoxic T-lymphocytes (CTL) and humoral responses. CTL responses are believed to be important in controlling acute infection with HIV. Previous studies have explored the safety and immunogenicity of vaccinia virus as a vector for carrying HIV antigens, but this approach is hampered by widespread pre-existing immunity in the global population due to routine immunization programs before 1977. Canarypox-vectored HIV vaccines¹³³ can carry HIV antigens and offer another advantage, too: Avipox viruses are unable to replicate in mammals,¹³⁴ eliminating safety issues in normal and immunocompromised individuals.¹³⁵

Collectively, several initial trials in humans have demonstrated that canarypox vectors are able to elicit humoral and cell-mediated immune responses.¹³⁶ Specifically:

- Detectable CTL responses have been observed in up to 70% of volunteers.
- CTL responses have persisted up to 2 years following initial vaccination.
- CTL responses in some patients have cross-clade killing activity.¹³⁷
- ADCC responses and HIV-specific CD4+ T-cell responses have been observed in approximately 50% of participants.¹³⁸

Based on these promising initial results and data that emerged from basic research, in 1997, vCP205 canarypox vaccine, in combination with a gp 120 construct, entered a *phase II, multicenter, double-blind, randomized study* in the United States. The study involved 435 volunteers. The vCP205 recombinant canarypox expresses the gp120 envelope protein and 2 HIV internal proteins, gag and protease. The booster, gp120, consists of incomplete recombinant envelope glycoprotein.¹³⁹ The main objective of the study was to evaluate safety, hence the *primary endpoint* was occurrence of severe systemic or severe localized reactions. To evaluate the immunologic responses elicited by vaccine administration, several *secondary outcomes* were tracked.

Humoral immune responses to HIV-1 were tested using an enzyme linked immunosorbent assay for antibody binding to V3 peptides, each containing the central 24-residue sequence of HIV-1_{MF} or HIV-1_{SF2}. An assay for neutralization of HIV-1 laboratory strains was used.¹⁴⁰ Primary isolate neutralization was assessed in

Canarypox vector vaccine: Summary of a phase II trial.	
AVEG 202/ HIVNET 014 (Belshe 1999 ¹⁴³)	
Study design	Phase II, multicenter (U.S.), double-blind, with patients randomized to 3 treatment arms: vCP205 alone, or vCP205 + gp120 booster, or 2 placebos
N	435
Primary endpoint	Occurrence of severe systemic or severe localized reactions
Secondary outcomes	1. Production of neutralizing antibodies 2. Production of cytotoxic lymphocytes

phytohemagglutinin (PHA) stimulated peripheral blood mononuclear cells (PBMC).¹⁴¹ Serum samples were considered positive for neutralization if they caused > 80% reduction in viral p24 relative to the corresponding pre-immunization sample for each volunteer. This cutoff corresponds to an approximate 5- to 10-fold reduction in infectious virus. A variety of primary isolates from chronically infected individuals and from primary infections were used to assess the breadth of neutralizing serum reactivity. Syncytium-inducing and non-syncytium-inducing phenotypes were determined in MT-2 cells and in co-receptor utilization assays.

PBMCs were collected from the volunteers and assays were performed to assess HIV-1 specific CTL activity. PBMC cultures were stimulated in vitro with autologous lymphocytes infected with a recombinant vaccinia virus vector

¹³³ Belshe RB et al. 1998 *AIDS* 12(18):2407-2415.

¹³⁴ Plotkin SA et al. 1995 *Dev Biol Stand* 84:165-170.

¹³⁵ Piloux G et al. 1995 *AIDS Res Human Retroviruses* 11(3):373-381.

¹³⁶ The AIDS Knowledge Base. "AIDS vaccines." www.hivinsite.ucsf.edu/akb/current/03vacc/index.html.

¹³⁷ Verrier F et al. 2000. *J Virol* 74(21):L10025-10033.

¹³⁸ NIAID. 1999 "Canarypox-vectored vaccines: Progress and plans." www.niaid.nih.gov/director/conference/1998/0722/newmilf.htm.

¹³⁹ Aventis Pasteur Website. www.aventispasteur.com/us/media/kit_aids5.html.

¹⁴⁰ Pilgrim AK et al. 1997 *J Infect Dis* 176:924-932.

¹⁴¹ Montefiori DC et al. 1996 *J Infect Dis* 173: 60-67.

matching the canarypox/HIV-1 immunogen and gp-120. The assay was considered to be positive when the percent lysis of infected targets was greater than 10% above that of control vaccinia.¹⁴²

This phase II trial found that the vCP205 prime-boost combination was safe and capable of inducing neutralizing antibodies in half of volunteers receiving vCP205 alone and more than 90% of those receiving the prime-boost combination. Furthermore, measurable cellular immune responses were elicited in approximately one-third of those immunized.¹⁴³

Additional phase I/II trials are underway to compare additional canarypox vector vaccines to assess which may be best for evaluation in larger trials.¹⁴⁴

A recent report¹⁴⁵ notes the lack of a validated correlate of protective immunity against HIV infection, and, consequently, no validated criterion is available for moving experimental HIV vaccines forward into phase II trials. Induction of neutralizing antibodies and induction of specific cytotoxic T-lymphocyte precursors are the consensus goals of current vaccine development initiatives sponsored by the National Institutes of Health, but these goals have not been consistently shown to correlate with protection. Low or undetectable plasma viremia is the current "gold standard" for predicting AIDS-free survival, but it is not a useful parameter for assessing immune status in uninfected vaccine recipients. Described in the article is an in vitro assay developed as a correlate of vaccine-induced protection from HIV. The assay uses cultures of PBMC, which are challenged with two strains of HIV to gauge resistance. Among 34 HIV vaccine recipients of canarypox vaccine, PBMC from postvaccination samples were significantly resistant to both strains, and cytotoxic T-lymphocyte precursor-positive samples were significantly more resistant than were precursor-negative samples. The assay was validated in populations with relative resistance to HIV-1 as well as in HIV vaccine recipients. This study provided the first evidence of the induction by vaccination of a validated correlate of protection.¹⁴⁵

Another recently reported development that may have relevance to the search for surrogate markers of protective immunity against HIV is a report on cytokine profiles of individuals receiving the vCP205 prime-boost vaccine regimen.¹⁴⁶ Cytokines were measured by enzyme linked immunosorbent assay in culture supernatants of PBMC collected from the vaccine recipients at multiple time points. Peak responses varied by individual and by specific cytokine. It was noted, however, that all prime-boost recipients experienced peak responses at a time point following the boost administration. The PBMC of all prime-boost vaccinated individuals produced detectable interferon-gamma and interleukin-10 in response to stimulation with HIV-1 envelope glycoprotein antigens; 83% also had detectable levels of IL-2 and IL-6, 71% had detectable levels of IL-4, and 86% had detectable levels of IL-5.

¹⁴² Belshe RB et al. 1998 *AIDS* 12:2407-2415.

¹⁴³ Belshe RB. 1999. Data presented at ISSTD Meeting, July 14. www.iavi.org/reports/71/sep-oct-1999-2.html.

¹⁴⁴ "Nine years and counting: Will we have an AIDS vaccine by 2007?" www.thebody.com/avac/9years/9years.html.

¹⁴⁵ Castillo et al. 2000 *J Infect Dis* 181:897-903.

¹⁴⁶ Sabbaj S et al. 2000 *AIDS* 14:1365-1374.