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Discovery and Development of Zidovudine as the Cornerstone of Therapy to Control Human Immunodeficiency Virus Infection

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Background of Antiviral Research at Wellcome

From the time Dr. George Hitchings began the research program at Wellcome Research Laboratories in 1942 to search for antagonists of nucleic acid bases, viruses were among the potential chemotherapeutic targets. A number of 5-substituted uracil derivatives and 2,6-diaminopurine, which had been identified as inhibitors of bacterial nucleic acid synthesis in *Lactobacillus casei,* were found to also interfere in tissue culture with the multiplication of vaccinia virus, a DNA virus. Simultaneously, the laboratory had discovered a new antiparasitic drug, pyrimethamine, a pyrimidine, to treat malaria and toxoplasmosis, and a new antileukemic drug, 6-mercaptopurine, in the purine series. As a result, extensive efforts were devoted to bringing these to clinical use. Unfortunately, the antiviral agents had to take a back seat, particularly because the toxicity of 2,6-diaminopurine to bone marrow discouraged further pursuit of this lead at levels required for antiviral activity. It was over ten years later that the deoxyribosides of 5-iodouracil, 5-chlorouracil, and 5-trifluoromethyluracil were synthesized by others and found to have activity against the herpes viruses. While two of these deoxynucleosides (5-iodo- and 5-trifluoromethyluracil) were clinically useful topically for herpes keratitis, they were too toxic to be employed systemically.

Research on nucleosides at Wellcome was concentrated during the 1950s and 1960s on purine analogues in the search for new anticancer drugs. The discovery of the antiviral activity of adenine arabinoside reawakened interest in searching for antiviral agents among the nucleosides. This initiative stimulated a close collaboration between the

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Wellcome Research Laboratories in the United States and at Beckenham in the United Kingdom, where antiviral testing was conducted. The first successes came with the finding that 2,6-diaminopurine arabinoside and guanine arabinoside had good activity against the herpes simplex viruses and vaccinia virus, in vivo as well as in vitro. There was, however, no firm commitment to pursuing the search for antiviral nucleosides at that time.

Whereas the early studies in other laboratories had been confined to 2′-deoxyribosides of 5-substituted pyrimidines, the 1970s saw a flurry of synthetic activity at Wellcome in the modification of the structure of the sugar moiety. One of the most exciting of these modifications was the substitution of an acyclic side chain for the deoxyribose. In particular, 9-[(2-hydroxyethoxy)methyl]guanine, or acyclovir, synthesized by Schaeffer and his colleagues created great excitement because of its high selectivity for the herpes viruses and its low human host toxicity. This led to a major effort at Wellcome not only to elucidate the mechanism of action and selectivity of acyclovir but also to expand the facilities and staff for all antiviral research. This research brought into sharp focus the exploitable differences between virally induced enzymes and normal cellular enzymes and made it clear that selective interference with viral nucleic acid synthesis was an achievable goal. With the launching of acyclovir (Zovirax®) in the early 1980s as a successful chemotherapeutic agent for the treatment of a variety of herpesvirus infections, the highly trained group of chemists, biologists, and clinicians at Wellcome began to turn attention to other viruses and to other nucleoside analogues. The human retroviruses were emerging as a particular challenge as medical pathogens, and the time was ripe since a major epidemic was pending.

Discovery of the Antiviral Zidovudine and Its Early Evaluation

Chemistry

In her research department at Wellcome Research Laboratories, Dr. Gertrude B. Elion established a multidisciplinary team approach to antiviral drug development.

The 3′-azido-2′,3′-deoxyribonucleosides and 2′-azido-2′-deoxyribonucleosides were targeted (Krenitsky et al., 1981), and two series of novel compounds resulted. The azide moiety was chemically reduced to the corresponding amine. Peptide derivatives attached through the nucleoside amino functionality were also synthesized; the 2′-series paled in light of what ensued with the 3′-series. Early in 1981 two 3′-azido-3′-deoxythymidines were prepared at Wellcome and entered

into compound screening. Compound 22U81 was the *threo*-analogue with the 3′-azido substituent above the plane of the sugar ring, and compound 509U81 (later named zidovudine; also known as azidothymidine or AZT) was the *erythro*-analogue with the 3-azido below the plane of the sugar ring where thymidine would have a hydroxyl group (Figure 1). Both compounds were synthesized by published procedures (Lin and Prusoff, 1978; Glinski et al., 1973) and were tested at Wellcome Research Laboratories in the United States and the United Kingdom. Although zidovudine had initially been synthesized by Dr. Jerome Horwitz and associates in 1964 at the Michigan Cancer Foundation as a potential anticancer agent, studies with the compound were abandoned shortly thereafter because of a lack of activity against animal cancers.

Early Evaluation for Microbiological Activity

Both compounds were active in microbiological screens, but the minimum inhibitory concentrations (MICs) obtained with bacterial strains sensitive to zidovudine were less than 10- to greater than 100-fold lower than those obtained for 22U81. Extensive in vitro studies of zidovudine showed a limited spectrum of activity, with inhibition against a variety of gram-negative enteric bacteria, but the gram-positive bacteria were naturally resistant. The MICs in vitro were in the range of 0.1–4 g/mL for *Escherichia coli* B, *Salmonella typhimurium, Shigella flexneri, Klebsiella pneumoniae,* and *Enterobacter aerogenes,* but gram-positive bacteria such as *Streptococcus pyogenes* and *Pseudomonas aeruginosa,* as well as anaerobic bacteria, mycobacteria, and various fungi, were not inhibited. Some of these organisms found to be naturally resistant have low levels of thymidine kinase or lack it. In fact, cultures of *Escherichia* and *Salmonella* could grow out from the inhibitory effects of zidovudine with time, with the resistant mutants demonstrating much reduced levels of thymidine kinase. In addition, *Escherichia* grown in the presence of zidovudine was found to contain the mono-, di-, and triphosphates of the drug. Purified

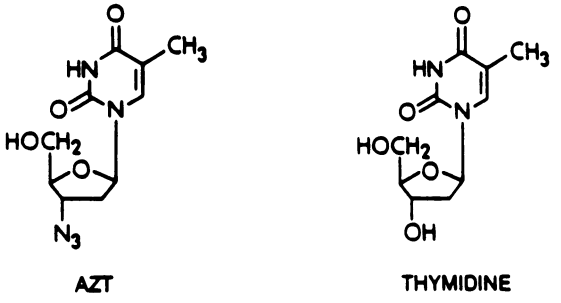

FIGURE 1. Zidovudine (AZT) and thymidine.

thymidine kinase from *Escherichia* converts the nucleoside to its monophosphate with the same efficiency as thymidine. Zidovudine binds to the enzyme and is an alternative substrate, and zidovudine triphosphate is a DNA chain terminator in the in vitro DNA polymerization catalyzed by the Klenow fragment of *E. coli* DNA polymerase 1 (Elwell et al., 1987). Therefore, incorporation into DNA II and chain termination could explain the bactericidal action of zidovudine.

In vivo, antibacterial studies with zidovudine at the US facilities and at Wellcome, Berkhamsted, in the United Kingdom demonstrated activity in experimental models. Mice were protected from life-threatening septicemic infections of *Escherichia* and acute ascending pyelonephritis. Veterinary models showed that calves were protected from fatal infections of *Salmonella dublin.* Other experimental models utilized chickens and weaning pigs. No toxicity to the compound was noted during these short-term experiments.

Other Bioactivity Assessments

Zidovudine, based upon its microbiological activity and lack of toxicity, was further assessed. Inactivity against a wide variety of DNA and RNA viruses was demonstrated: herpes simplex virus types 1 and 2, varicella virus, adenovirus type 5, influenza A, respiratory syncytial virus, rhinovirus B, yellow fever virus, measles virus, coronavirus, and bovine rotavirus. Zidovudine was also inactive against vaccina virus, vesicular stomatitis virus, and the murine leukemia, L1210, in vitro. Ultimately, tests showed it was inactive against human cytomegalovirus. Some activity is reported for zidovudine against Epstein–Barr virus (EBV), for the drug inhibits viral growth by 50% in vitro (ED_{50}) at a concentration of 3 M.

Discovery of Antiretroviral Activity

Sandra Nusinoff-Lehrman, M.D., a senior clinical research scientist, David W. Barry, M.D., the director of clinical investigation, Phil Furman, Ph.D., a senior virologist, and Marty St. Clair, a junior virologist, formed a task force early in 1984 to review the literature and to determine what approaches Wellcome should take to most quickly and efficiently direct their research efforts since the human immunodeficiency virus (HIV), a retrovirus, had been identified as the causative agent of AIDS. In October 1984, the newly discovered retrovirus HIV, then termed HTLV III or LAV, was the subject of a series of seminars given at Wellcome Research Laboratories in the United States by Drs. Françoise Barre-Sinoussi, Robert Gallo, and Samuel Broder. These discussions served as a catalyst to ensure Wellcome's involvement in the discovery and development of therapies for the new disease syndrome

devastating thousands of patients, mainly in the prime of their lives. An early decision was to further develop a plaque reduction assay already in place since 1980 for an animal retrovirus, which then would be used to screen potential anti-HIV compounds.

Friend leukemia virus (F-MuLV, a murine retrovirus), Harvey sarcoma virus (HaSV, another murine retrovirus), and FG-10 (murine) cells were acquired from Kent Weinhold at Duke University. Since both F-MuLV and HaSV form plaques in FG-10 cells, the basis for the initial Wellcome assay was that if a compound inhibits the ability of the virus to grow, the number of plaques formed will be reduced. Once the murine plaque reduction assay was functioning, known antiviral compounds, such as Wellcome's acyclovir, were screened. When no exciting activities were observed with these initial candidates, the focus shifted to the dideoxynucleoside group of compounds, such as dideoxycytosine, dideoxyadenosine, dideoxyguanosine, and dideoxythymidine. While activities in the 10–20 M range were demonstrated with these compounds in the assay, the Wellcome scientists were optimistic that a compound could be identified with even greater anti-HIV activity.

Wellcome's senior organic chemists supplied 10–20 compounds representative of their synthetic expertise and established drug development programs for testing in the new plaque reduction assay. Among 12 such compounds furnished on November 2, 1984, was zidovudine. Not knowing what excitement would befall the laboratory, the compounds were analyzed at the next available opportunity.

When counting the plaques from that assay, it became obvious that none of the 18 plates with zidovudine had plaques. None at all! The virus used was apparently completely incapable of replicating in any of the concentrations tested. Notwithstanding the modest activity seen in previous assays with other compounds, no plaque formation was a rare event. The virologist, incredibly excited but also somewhat apprehensive, postulated that inoculation of those plates might not have occurred. The result was discussed by the task force team, but there were no plans to share the news widely until there was a chance to repeat the assay. But little was it realized how interested the company scientists were in the budding HIV program, for rumors began circulating almost immediately, causing feverish activity.

In quickly developing the plaque reduction assay, Wellcome virologists were fully aware that the surrogate viruses they had chosen for their retrovirus screen at this stage of the program were just that, surrogate viruses. Arrangements to have active compounds analyzed in other relevant screens, such as feline leukemia virus and HIV (known then as HTLV III), had already been made and numerous coded compounds had been sent to William Hardy (at Sloan-Kettering Cancer Center) for feline leukemia virus testing, and Sam Broder (National Cancer Institute), Kent Weinhold (Duke University), and Gerald

Quinnan (U.S. Food and Drug Administration) for analysis with HIV. The field of HIV laboratory research, very young at that time, lacked standardization, and few groups possessed the expertise sufficient to provide Wellcome with the testing required to evaluate its active compounds. As a result, several scientists using different assays were sought to confirm the antiviral activity seen and better quantitate it.

The initial indication of antiviral activity with zidovudine in each of these assays caused continued excitement. After discussions with the highest management, the largest mobilization of the human and monetary resources necessary to bring a drug to patients in the most efficient and timely manner occurred in Wellcome's history and has set the standard for the development of any new drug for the treatment of a life-threatening disease.

Medicinal Chemistry

Many of the studies described above required the continual support from chemists who furnished the compound initially and who developed methods for making the labeled compound necessary for mechanistic studies. Analogues of zidovudine, as well as compounds where the 3′-azido moiety was the *threo* (above the plane of the sugar) configuration, were synthesized. Compounding of the *threo*-analogues continued for some time, ceasing only when it became clear that this series produced weak antibacterials and antivirals. Numerous batches of zidovudine were produced for in vivo and toxicity studies. In addition, a number of compounds were synthesized or purchased to extend the structure–activity relationship (SAR) work ongoing at Wellcome. Various analogues with a 3′-substituent different from the azido, such as hydrogen unsaturation (2′,3′-didehydro-), and others, like F, Cl, Br, NCS, NH_2, CN, NHC(O)R, were made with thymine, uracil, cytosine, and *S*-methylcytosine as the heterocycle. Few analogues retained potent activity. Keeping the sugar moiety as 3′-azido-2′,3′-dideoxyribose, changes at the 5-position (H, C_2H_5, C_3H_7, $CH_2CH{=}BCH_2$, [*E*-&*Z*-]$CH{=}CHBr$, $C{=}CH$, CF_3, N_3), were investigated. Keeping thymine and the sugar moiety constant, substituents at the 2-, 3-, and 4-positions were varied: 2-(OR, 5, SR, NHR, NR_2); 3-[C(O)Ar, CH_3]; and 4-(OR, OAr, NHR, NR_2, SH, SR).

At the 5′-position, prodrugs to alter the pharmacokinetics of zidovudine were synthesized. The first produced were the mono-, di-, and triphosphates, which serve as metabolic markers since most nucleosides are activated by anabolism. Other 5′-derivatives were the deoxy derivatives and esters (sulfonyl, acyl, arylacyl, and alkoxymethylacyl), homologated and methyl- (and phenyl-)substituted hydroxymethylene and the phosphonate. In the antibacterial mechanistic work, the 5′-esters were labile in the presence of serum in the medium and hence could not

be ranked as in the absence of serum. The 5′-deoxy and *N*-3-methyl derivatives were not active antibacterials or antivirals; this is likely since 5′-phosphate cannot form in the first case, and the *N*-3-methyl group either interferes with necessary hydrogen bonding in a growing DNA chain or is not a substrate for bacterial or host cell thymidine kinase.

The early HIV tests were limited and difficult to use in the SAR studies at Wellcome. Since each laboratory employed a different assay measuring different parameters (inhibition of HIV-reverse transcriptase, inhibition of p24 viral antigen and syncytia formation), only those samples with distinct activity were tested. Because at that time the development of standardized tests controlling for host cell, infection level, and quantitative measures of activity had not been completed, the activity of a compound was obtained but its potency could not be ranked except relative to zidovudine, which was eventually tested in each system, though not as a standard. Thus, using a general indication of positive or negative activity, the SAR program at Wellcome was extended among the *erythro* and *threo* series using zidovudine as the benchmark compound. Purine analogues were also synthesized and investigated and, while some purine analogues gave interesting results, the risk of cross-resistance deterred further development. When an advantage over zidovudine was discovered, such as longer serum half-life, decreased toxicity, or broader spectrum of antiviral activity, for any one of these compounds, research on that compound would continue. That work is ongoing.

The public revelation of zidovudine's activity in October 1985 placed intense pressure on Wellcome scientists because many groups around the world had precursors to this type of compound and would undoubtedly work to find active analogues. In fact, other investigators worldwide had published results on compounds in the broad category of 2′,3′-dideoxynucleosides, including the 3′-azido-2′,3′-dideoxynucleosides. At Wellcome, analogues were synthesized and extensive study of zidovudine continued with the primary objectives to make, test, and rank the most important compounds.

New synthetic routes to zidovudine were also investigated. An X-ray structure of the compound was obtained, and nuclear magnetic resonance (NMR) studies determined its solution conformation. Its substrate activities with thymidine kinase from bacterial and human host cells were studied. While some analogues were found to be substrates for cellular thymidine kinase, they were not potent against HIV, indicating that the substrate activity for other phosphorylation steps can be rate limiting.

Zidovudine is metabolized to its 5′-*O*-β-D-glucuronide (GAZT). Although the glucuronide was successfully synthesized in the laboratory, its isolation from the urine of monkeys was less difficult. GAZT is used as an HPLC (high-pressure liquid chromatography) marker in assays of

clinical trial samples. The continual need for relatively large amounts of compound in 1985 and 1986 for preclinical and clinical development allowed the chemists to evaluate each of the three synthetic routes referenced in the literature, in order to determine the most productive route, initiate optimization studies, and provide initial recommendations to the development chemists.

The need for large amounts of thymidine, very apparent by March 1985, forced an intense search for producers of thymidine around the world and called for creative measures from all parties concerned with HIV therapies. Compound already produced could be reworked, and the National Institutes of Health (NIH) donated 40 kg of thymidine to allay the crisis. In July 1985 the first pilot plant scale preparation of zidovudine was run, producing usable compound. Since the last two steps of the synthesis required a reaction with azide, which caused some safety concerns at the pilot plant in Greenville, North Carolina, these steps were handled at the Wellcome laboratories at Research Triangle Park in that state. Ultimately (based on some work in 1975 at Wellcome's facilities in Dartford, United Kingdom) safe monitoring for unreacted azide was developed so that the chemical manufacturing department in Greenville has produced the compound since November 1985 without difficulty.

On October 24, 1985 a celebration was held in the chemical development laboratories (CDL) featuring a huge cookie frosted with the zidovudine structure, its melting point, and "Over 15 kg!!!!" The group had completed the final reaction steps on many batches to produce that much clinical-grade material. In January 1986, CDL ran its last development work on zidovudine.

Toxicology Perspective

During its evaluation as a potential antibacterial agent in the early 1980s, the Division of Toxicology and Pathology at Wellcome Research Laboratories was asked by the Department of Microbiology to conduct a preliminary toxicological evaluation of zidovudine. Any "red flags" from a toxicological standpoint that might preclude further work and/or development of the compound as an antibacterial was the main objective at this time. The usual approach at Wellcome is to evaluate the drug in preliminary dose-range-finding (DRF) studies in both rodents and nonrodents. The latter may be purpose-bred beagle dogs or monkeys. With zidovudine, however, there was only enough compound available for a DRF study in rodents. With that in mind, a 2-week oral study in rats in which a variety of antemortem and postmortem parameters were monitored was conducted. The dose levels chosen in this experiment allowed drug conservation but at the same time were significant multiples of the known antibacterial inhibitory concentrations. The

results of this study were encouraging in that there were no "red flags" to cause initial concern, but this was not surprising given Wellcome's experience with nucleosides of this class of compounds. So the message given to the chemists and microbiologists based upon completion of this experiment in the early 1980s was that, while there were no significant toxicological findings in this study to preclude the drug's development as an antibacterial, a "final" preliminary assessment would require evaluation in a second species.

By late 1984, the emphasis of the project had changed. The immediate task in support of the development of zidovudine was to conduct the studies considered necessary to support the initial clinical testing of the drug as an antiviral in humans. Beyond that short-term goal, a plan was required for those experiments that would be necessary to support continuing clinical development of the drug and eventually the filing of appropriate regulatory applications. Discussions with clinicians at Wellcome suggested that the initial experience in humans would encompass an intravenous dosing trial in AIDS (acquired immune deficiency syndrome) patients. There were plans to begin oral dosing soon thereafter and, if the drug appeared to be of benefit, patients would be given zidovudine chronically, since it was not likely to be a cure for HIV. With this information in hand the studies thereby needed for the initial Investigational New Drug Application (IND) would include acute (single-dose) intravenous toxicity studies in rats and mice, multiple-dose intravenous toxicity studies in a rodent and a nonrodent species, and an in vitro hemolysis and protein flocculation study in human type O blood. The latter would ensure that the proposed intravenous formulation was compatible with human blood. It was also recognized, however, that oral toxicology studies should be initiated as quickly as possible in order to support that route of dosing clinically.

If these studies were to be conducted in a timely manner, however, there was a major hurdle to be overcome. That hurdle was compound supply. In the Division of Toxicology and Pathology, this required adoption of a much more flexible approach to the design of the supportive toxicology studies. For most drugs, DRFs are conducted to assist in the selection of the dose levels to be employed in formal toxicology studies conducted under Good Laboratory Practices (GLP). The available supply of zidovudine would not support both DRFs and formal toxicology studies. Therefore, the multidose GLP experiments were initiated in the rat and dog without the benefit of preliminary experiments. In the interest of drug conservation, the dose levels for these studies would simply be severalfold multiples of the projected human dose. In consultation with scientists in the Medical and Virology Divisions, the projected human dose was estimated. Based on this projection and the amount of drug available, a 4-week intravenous study in rats at a 10-fold multiple of the projected human dose and a

2-week intravenous study in beagle in which the high dose level was 6-fold greater than the projected human dose could be conducted. Only later was it learned that, unlike the dog, the metabolism of zidovudine in monkeys was identical to that in humans, and for that reason all later nonrodent toxicology studies were carried out in monkeys. In summary, these experiments would provide for an acceptable toxicological evaluation of intravenous zidovudine prior to study of the drug in patients.

These toxicology experiments of zidovudine were mounted and completed in record time. The fact that signed reports for these studies were available 2.5 months after the first animal was dosed was symbolic of the speed with which all scientists and clinicians contributing to the zidovudine effort were approaching their task. Within a few days of the signing of the preclinical toxicology reports, the Phase I clinical trial got under way. This was, however, the last point in the development of zidovudine in which the nonclinical toxicology studies were ahead of the clinical development program.

While the Phase I and Phase II clinical trials proceeded, the Division of Toxicology and Pathology took steps to carry out the remaining studies that were thought to be required for eventual registration. Since the oral route of administration would be the primary route employed in humans, in the remainder of these experiments the drug was given by gavage. One of the primary considerations in determining what studies should be done was the nature of the target population. If zidovudine were to be given to severely ill AIDS patients only, it was felt that very little in terms of additional studies would have been required using an approach for the nonclinical development of the drug which was the same as that employed for the development of potential anticancer drugs. If, however, as suspected, the drug was shown to be efficacious in AIDS patients, clinicians would in all probability eventually target it toward less ill individuals with HIV, and perhaps even HIV-positive asymptomatic persons. So it seemed certain that the risk–benefit equation would likely change over time, and for this reason the decision was made to approach the development of zidovudine as for any compound given to patients chronically. The studies that we considered necessary for a thorough evaluation of the toxicologic potential of zidovudine included the following:

3-month toxicity study in rats
3-month toxicity study in rats
6-month toxicity study in monkeys
1-year toxicity study in rats
1-year toxicity study in monkeys
carcinogenicity study in rats
carcinogenicity study in mice
reproduction–fertility study in rats
teratology study in rats
perinatal–postnatal study in rats
neonatal toxicity study in rats
Ames mutagenicity assay
mouse lymphoma cell assay
in vitro cytogenetics assay
in vitro cytogenetics assay
cell transformation study

Normally, these studies are done in a relatively orderly fashion and in such a way as to support the phase development of the drug under study. Additionally, all of these experiments would usually have been completed prior to submission of the New Drug Application (NDA) for regulatory review. The toxicological development program for zidovudine was a different story altogether. The clinical development of the drug proceeded at such a pace that, whereas the pivotal Phase II trial was halted in September 1986 when it was shown that zidovudine significantly lowered the mortality rate for AIDS patients compared to those receiving placebo, the 6-month toxicology studies in rats and monkeys were still being conducted. The 1-year toxicity studies in rats and monkeys, the oral carcinogenicity studies in rats and mice, and the reproduction-fertility or perinatal-postnatal studies in rats had not yet begun. Those experiments got under way shortly before or near the marketing of the product in March 1987.

Clinical Development of Zidovudine for Treatment of HIV Infection

Wellcome clinicians in the United States first became directly involved in efforts to discover and develop therapy for the treatment of AIDS and associated infections in 1980, a year before the illness was described as a syndrome by the U.S. Public Health Service Centers for Disease Control. Initial involvement was based upon the fact that many of the drugs used to treat the opportunistic infections associated with AIDS are manufactured by Wellcome. In that year, Burroughs Wellcome Co. began to receive a number of calls requesting intravenous Septra® for the treatment of adult patients with *Pneumocystis carinii* pneumonia (PCP). At that time the intravenous preparation of Septra was not marketed in the United States but was available under a treatment IND program. Initially, there was skepticism of the increase in requests because, until that time, episodes of PCP generally occurred primarily in children who had received intensive chemotherapy for leukemia. Approximately a year later, epidemiological studies demonstrated PCP as one of the prime manifestations of AIDS. Wellcome scientists have also studied pyrimethamine (Daraprim®), leucovorin (Wellcovorin®), acyclovir (Zovirax®), DHPG (BW 759U, ganciclovir), and interferon (Wellferon®) to treat various opportunistic infections or tumors occurring in AIDS patients. Because of this involvement, clinicians at Wellcome became familiar with the disease during the early 1980s. In addition Wellcome's long history in the prior two decades of development of antiviral therapy included the development of Marboran® for the prevention and treatment of smallpox and complications of its

vaccination in the 1960s, trifluorothymidine (Viroptic®) for ocular herpes infections in the 1970s, and then acyclovir (Zovirax®) for herpes infections in the 1980s.

A Phase I study began in July 1985 and was a collaboration between the National Cancer Institute and Duke University sponsored by Burroughs Wellcome Co. This study was conducted in patients infected with HIV who had been diagnosed as having AIDS or ARC (AIDS-related complex). The results indicated zidovudine was well absorbed orally, with dose-dependent kinetics observed over a fairly wide dosing range. Zidovudine was shown to be 65% bioavailable, but in reality it may be as high as 100% bioavailable. This discrepancy results because there is a first-pass metabolism effect in which a portion of the zidovudine is converted to its 5'-glucuronide as the result of glucuronidation in the liver. Both peak and trough levels that were above the in vitro sensitivity of the virus were achieved in these studies, suggesting that anti-HIV effects might be possible in humans. In addition, it was found that zidovudine penetrated the blood-brain barrier quite well, suggesting that viral infections in brain could be treated. The significant glucuronidation of zidovudine may be an important factor, since other drugs that are glucuronidated may have some effect on its metabolism.

When the Phase I studies were completed in January 1986, a very difficult decision as to how to proceed was required. Traditionally, early clinical studies of new drugs proceed in a very regimented way. New drugs are typically tested in normal, healthy volunteers to evaluate safety and tolerance, and then the new drug is examined in a larger number of patients to evaluate its efficacy and safety profile—usually in patients with milder stages of the disease in question. There are many reasons for this approach. The first is that any toxicity seen is likely to be milder in patients whose baseline physical status is relatively good. More importantly, the likelihood of therapeutic success in less ill patients is often greater in these patients than in those who are at a more severe stage of their disease. In the case of zidovudine, however, Wellcome clinicians believed that there were two counterbalancing elements which required that a less classical approach be taken. The first was that there were a large number of people, possibly hundreds per week, dying of AIDS at the time the Phase I study was completed in January 1986. Wellcome also believed that testing zidovudine in patients with advanced manifestations of HIV infection was the most vigorous test to determine its therapeutic index. If it proved to be effective in the most severely ill patients while exhibiting manageable adverse effects, then it might be more beneficial in patients with milder forms of disease. A difficult decision was therefore made to conduct a double-blind, placebo-controlled study in advanced AIDS and ARC patients in February 1986.

Another key issue in the study was the decision to administer placebo

to half of the patients enrolled. This study was initiated at a time when the Phase I study had given only hints that the drug might be effective. Yet with hundreds of people dying and the publication of the Phase I study that described potentially beneficial therapeutic effects in humans, there arose a number of ethical and scientific questions concerning the conduct of a placebo-controlled study. Nevertheless, this drug had to be proven, by classical clinical research methodology, to be both safe and effective, or otherwise many patients might be put at risk without knowledge of the actual benefits of the drug. In order to ensure that the risk and benefits of zidovudine were evaluated adequately without withholding, for any longer than necessary, a promising therapy, it was agreed to appoint a Data Safety and Monitoring Board whose members would examine data from the ongoing study every 2 months and make recommendations about how to proceed. After analysis of various safety and efficacy parameters, this board of medical, ethical, and statistical experts was to advise Wellcome whether one group was experiencing significantly greater side effects or greater benefit from therapy than the other group and recommend whether it would be ethical or not to proceed.

In this study, 282 AIDS and ARC patients were entered at 12 university-associated medical centers in the United States between January and June 1986. In order to have as uniform and comparable groups as possible between patients given drug and those given placebo, narrow categories of disease progression were studied. For the AIDS component, only those patients who had experienced their first episode of PCP within the prior 4 months were entered in the study. ARC patients were enrolled if they had a number of symptoms including, among others, weight loss, sustained fever for over a month, and/or extensive oral candidiasis. All patients were required to have fewer than 500 CD4 cells and to have complete cutaneous anergy to four common antigens. The vast majority of patients had fewer than 200 CD4 cells. Patients with Kaposi's sarcoma, intravenous drug abusers, and children were excluded from this study. The drug and placebo groups were quite comparable in a variety of baseline characteristics that were examined.

On September 19, 1986, the Data Safety and Monitoring Board recommended to Wellcome that the study should be terminated because a significantly higher mortality rate in the placebo group compared to the therapy group was demonstrated. Since patients were enrolled at different times, the length of time on drug ranged from 10 to 28 weeks, with an average of 17 weeks. Analysis of the data at that time indicated that, when compared to placebo, zidovudine recipients had significant improvements in the number of CD4 cells, delayed cutaneous hypersensitivity, weight gain, activities of daily living, and neurological function. In addition, zidovudine recipients had significant decreases (in many cases to an undetectable level) of previously circulating p24

antigen and significant decreases in the frequency and severity of opportunistic infections. Most importantly, the probability of death within 6 months of initiating therapy was 22% for the placebo group and 2% for the drug-treated group.

Symptomatic adverse reactions were extremely common in both groups. This was likely the result of the complicated nature of the underlying disease. Nausea, myalgias, insomnia, and headache, however, were significantly more common in the drug-treated group. The most significant toxicity was myelosuppression, which was dependent upon dose and duration of therapy, as well as upon preexistent bone marrow reserve. Up to 45% of patients with poor bone marrow reserve had significant decreases in either red cell and/or white cell numbers during the observation period. The incidence of such decreases in patients with better but still compromised marrow reserve was only slightly higher than in the same subset of individuals in the placebo group. Such myelosuppression could generally be managed by dose reduction, dose interruption, transfusion, or a combination of these approaches.

At the time the placebo-controlled portion of the study was terminated, all patients, including those originally randomized to receive placebo, were offered the opportunity to receive zidovudine in an unblinded fashion provided they agreed to continued follow-up by the original investigator. While most of the patients agreed to continue taking zidovudine, a small number elected to leave the study for a variety of reasons. Because of these factors, continued follow-up and comparison of the two groups was particularly difficult. Patients can be classified a number of ways depending upon their diagnosis at entry and/or their management after the code was broken. Although, as shown in Table 1, these analyses yield slightly different survival values, there is great consistency in the fact that zidovudine recipients always had a significantly higher survival rate than placebo recipients. Too few patients in the original placebo group remained after 9 months to provide meaningful comparisons. In fact, only 4 of the 28 patients originally assigned to placebo who did not elect to receive zidovudine after unblinding of the study or who received it for less than 3 weeks were alive 1 year after initiation of the study, and all are now dead. Thus, comparison of survival of patients on zidovudine for greater than 9 months must be compared to historical controls. These comparisons are, however, less than ideal because historical groups may represent a significantly different patient cohort and because of the very incomplete follow-up of individual patients in most epidemiological studies used for this comparison. Also, most epidemiological studies use spontaneous reporting of death or registration of death certificates specifying death from AIDS within a particular locale to make their projection of survival rates. Both of these factors lead to significant underreporting of deaths.

TABLE 1. Extended survival of patients in original double-blind, placebo-controlled study

Patient category	Months				
	6	9	12	15	18
1. All original Retrovir recipients	96.5	91.5	84.3	78.6	68.3
2. Same as 1, but excluding patients off drug >60 days before death	97.7	93.7	88.7	85.2	77.7
3. Same as 1, but prophylaxed at any time for PCP	100.0	97.7	90.7	90.7	83.5
4. Same as 1, but never prophylaxed for PCP	94.9	88.8	81.5	74.2	61.5
5. Same as 1, but AIDS only	95.3	90.2	78.8	74.0	61.8
6. Same as 5, but calculated since date of PCP rather than date of entry into study	100.0	92.9	87.1	83.5	72.8
7. Same as 1, but ARC only	98.2	96.4	92.8	85.5	78.2
8. Placebo recipients who received no AZT or <3 weeks AZT	75.8	51.5	–	–	4.1[a]
9. Same as 8, but AIDS only	69.2	38.8	22.2	–	0–4.1[a]
10. Same as 8, but ARC only	83.2	67.8	–	–	0–4.1[a]

[a]Only one patient alive at 18 months in "true placebo" group. Unclear at this time whether AIDS or ARC. This patient died 2 months later. Follow-up between 9 and 18 months unclear because patients dropped out of study.

For example, a study that made extensive efforts to track down purported "longer term survivors" of AIDS found that at least 58% of such patients were in fact, dead.

With these caveats in mind, the best historical comparison to the original cohort of patients who were randomized to zidovudine in the study is a cohort of AIDS patients in New York City in 1985 who had their diagnosis made exclusively on the basis of PCP. Their minimum 1-year mortality from the date of PCP diagnosis was 51%. Mortality was probably significantly greater because only those patients who had AIDS listed as their cause of death on a death certificate registered in New York City were considered to be dead for purposes of the study. Because AIDS patients in the original double-blind, placebo-controlled study began taking zidovudine about $2\frac{1}{2}$ months after their original episode of PCP, their 13% mortality at 1 year after diagnosis of PCP and 21% after entry into the study is one-fourth to one-half the minimal mortality reported in the New York study.

The denouement of the original double-blind, placebo-controlled study in September 1986, provided another opportunity to study a large cohort of patients. Wellcome set up a program, in conjunction with the National Institutes of Health, to dispense zidovudine free of charge to any AIDS patient in the United States who had had PCP at any time in the past and who fulfilled minimum entry criteria. ARC patients were

not included in this program. Approximately 4800 AIDS patients received AZT under this "treatment IND," "compassionate plea," or "parallel track" program between October 1986 and March 1987, when the drug became available by prescription. The characteristics of the patients in this study were similar to those of AIDS patients in the general population, with the vast majority being homosexual or bisexual men. Nevertheless, a number of patient categories not well represented in the Phase II double-blind, placebo-controlled study did participate in this uncontrolled study. There were nearly 150 women and more than 250 intravenous drug abusers. In addition, 424 patients were Hispanic and more than 500 were black. Although zidovudine was approved for general use in March 1987, the survival of these patients could be monitored until September 15, 1987, when the controlled distribution program that was in place during that interval was dismantled. The amount of data that can be obtained during "treatment IND" studies is generally limited, but sufficient controls were instituted so that mortality statistics are reasonably reliable, at least for a 9-month period. After adjusting for the fact that significantly sicker patients could participate, overall survival data were very similar to those observed in the original placebo-controlled study.

The incidence of adverse reactions was somewhat less than noted in the original placebo-controlled study and may have been the result of less intensive observation and management of the patients or less aggressive reporting of such reactions. Although significantly higher rates of death have been reported in untreated women and drug addicts with AIDS when compared to male homosexuals with AIDS, no such differences were noted if these patients were receiving zidovudine. Likewise, no differences in mortality were noted between black and white AIDS patients receiving zidovudine. The highest mortality rates were recorded during the first 8 weeks of study, indicating the very advanced state of illness of many of the participants. The mortality of people who had acquired disease through blood transfusion was somewhat higher than those who had acquired infection by other means. This observation may have been the result of the more advanced age of such patients, as well as their poorer general state of health.

Certain prognostic factors of survival were noted in this study (Table 2). Better survival was associated with higher hemoglobin and performance levels (Karnofsky Score) at enrollment, as well as the brevity of the period between the first episode of PCP and the initiation of zidovudine therapy. These data point to the importance of beginning therapy as soon as possible after the diagnosis of AIDS or advanced ARC is made.

Although a great deal of information about the usefulness of zidovudine was gathered in a relatively short period of time, a very aggressive worldwide program of clinical research was mounted to address many

TABLE 2. Treatment IND study

Factors not associated with difference in survival while on AZT:	*Factors associated with difference in survival while on AZT:*
Sex	Baseline hemoglobin
Race	Baseline performance level of activities of daily living (Karnofsky Score)
Method of virus acquisition[a]	Duration since diagnosis of PCP

[a]With the exception of blood transfusion recipients.

unanswered questions. In the United States this program was conducted, in part, in conjunction with the AIDS Treatment and Evaluation Units (ATEUs), now the AIDS Clinical Trials Group (ACTG), of the National Institutes of Health. The largest group of studies involved patients with different degrees of severity of HIV infection, including patients with advanced disease (AIDS), milder forms of ARC, lymphadenopathy syndrome and those who are infected but who do not have obvious signs or symptoms of disease. The results of these landmark clinical trials have defined the role of zidovudine in treating all stages of HIV infection and disease. Consequently, zidovudine has emerged as the cornerstone of therapy for HIV infection and the most commonly prescribed initial treatment, as well as the agent with which new therapies are usually compared.

Studies have been conducted in special patient populations such as hemophiliacs, intravenous drug users, and children. Data from children indicate that their absorption, distribution, metabolism, and excretion of zidovudine is similar to those of adults, as are the benefits and adverse reactions to the drug. Particularly striking improvement in neurological function have been noted in pediatric patients. Additional studies concerning the effect of zidovudine on abnormal neurological function in HIV-infected adults have been conducted. Although significant improvements in neurological function in adults with AIDS and ARC have already been noted in controlled as well as uncontrolled studies, additional research is required to define precisely the degree of benefit and to understand the occasional neurological adverse experiences associated with the use of zidovudine. Likewise, the role of zidovudine in improving the thrombocytopenia often seen in AIDS patients has been evaluated.

The use of zidovudine in conjunction with a variety of other medications is being examined for two distinct reasons. Certain drugs such as other nucleoside analogues like ddC (2′,3′-dideoxycitidine), ddI (2′,3′-dideoxyinosine), acyclovir, and interferon have been shown to be synergistic in vitro with zidovudine in inhibiting HIV replication (Hartshorn et al., 1987; Mitchell et al., 1987; Ruprecht et al., 1987), but additional studies are required to determine whether an additive or

synergistic effect can be observed in people. Additionally, some compounds such as G-CSF and erythropoietin counteract the marrow suppressive effects of zidovudine. Some immunomodulators may enhance immune function at the same time that zidovudine inhibits viral replication, and such combinations are currently under study.

Studies have been conducted to determine the safety and tolerance of zidovudine when used in conjunction with drugs employed in the therapy of opportunistic infections. In addition, studies of the appropriate dosage adjustment in patients with renal and/or hepatic failure have been included as part of an extensive postmarketing surveillance program. Finally, intensive viral sensitivity studies are continuing to determine the significance of resistance development. Although years may pass before the results of some of these studies enable us to have a more complete knowledge of the full therapeutic profile of zidovudine, sufficient data already exist to indicate that the drug is a valuable weapon in the physician's armamentarium to improve and lengthen the life of patients with HIV infection.

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