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Discovery and Development of the Anti-Human Immunodeficiency Virus Drug, Emtricitabine (Emtriva, FTC)

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CONSPECTUS

The HIV/AIDS epidemic, which was first reported on in 1981, progressed in just 10 years to a disease afflicting 10 million people worldwide including 1 million in the US. In 1987, **AZT** was approved for treating HIV/AIDS. Unfortunately, its clinical usefulness was severely limited by associated toxicities and the emergence of resistance. Three other drugs that were approved in the early 1990s suffered from similar liabilities.

In 1990, the Liotta group at Emory University developed a highly diastereoselective synthesis of racemic 3'-thia-2',3'-dideoxycytidine and 3'-thia-2',3'-5-fluorodideoxycytidine and demonstrated that these compounds exhibited excellent anti-HIV activity with no apparent cytotoxicity. Subsequently, the enantiomers of these compounds were separated using enzyme-mediated kinetic resolutions and their (–)-enantiomers (**3TC** and **FTC**, respectively) were found to have exceptionally attractive preclinical profiles. In addition to their anti-HIV activity, **3TC** and **FTC** potently inhibit the replication of hepatitis B virus. The development of **FTC**, which was being carried out by Burroughs Wellcome, had many remarkable starts and stops. For example, passage studies indicated that the compound rapidly selected for a single resistant mutant, M184V, and that this strain was 500–1000-fold less sensitive to **FTC** than was wild-type virus. Fortunately, it was found that combinations of **AZT** with either **3TC** or **FTC** were synergistic. The effectiveness of **AZT–3TC** combination therapy was subsequently demonstrated in four independent clinical trials, and in 1997, the FDA approved Combivir, a fixed dose combination of **AZT** and **3TC**.

In phase I clinical trials, **FTC** was well tolerated by all subjects with no adverse events observed. However, the development of **FTC** was halted by the acquisition of Wellcome PLC by Glaxo PLC in January 1995. In 1996, Triangle Pharmaceuticals licensed **FTC** from Emory and initiated a series of phase I/II clinical studies that demonstrated the safety and efficacy of the drug. In August 1998, **FTC** was granted “Fast Track” status, based primarily on its potential for once daily dosing. While the outcomes of two subsequent phase III trials were positive, a third phase III clinical trial involving combinations of **3TC** or **FTC** with stavudine and nevirapine had to be terminated due to

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Notes

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serious liver-related adverse events. Although analysis of the data suggested that the liver toxicity was due to nevirapine, the FDA decided that the study could not be used for drug registration. Ultimately, in January 2003, Gilead Sciences acquired Triangle Pharmaceuticals and completed the development of **FTC** (emtricitabine), which was approved for once a day, oral administration in July 2003. A year later, Truvada, a once a day, oral, fixed dose combination of emtricitabine and tenofovir disoproxil fumarate received FDA approval and quickly became the accepted first line therapy when used with a third antiretroviral agent. In July 2006, the FDA approved Atripla, a once a day, oral, fixed dose combination of emtricitabine, tenofovir disoproxil fumarate, and efavirenz, which represented the culmination of two decades of research that had transformed AIDS from a death sentence to a manageable chronic disease.

Graphical Abstract

Product	Licensee
Epivir®	GlaxoSmithKline/Shire
Combivir®	GlaxoSmithKline/Shire
Trizivir®	GlaxoSmithKline/Shire
Epizicom®	GlaxoSmithKline/Shire
Epivir-HBV®	GlaxoSmithKline/Shire
Emtriva®	Gilead Sciences, Inc.
Truvada®	Gilead Sciences, Inc.
Atripla®	Gilead Sciences, Inc.
Complera®	Gilead Sciences, Inc.
Stribild®	Gilead Sciences, Inc.
Genvoya®	Gilead Sciences, Inc.
Descovy®	Gilead Sciences, Inc.

Over 90% of HIV infected persons on therapy in the United States take a drug containing either emtricitabine or lamivudine

INTRODUCTION

The first publication alerting the medical community to the emerging AIDS epidemic was in the June 5, 1981 issue of the Centers for Disease Control and Prevention's (CDC) *Morbidity and Mortality Weekly Report (MMWR)* where five cases of a rare form of pneumonia, *Pneumocystis jirovecii* pneumonia (PJP, formerly known as *Pneumocystis carinii* pneumonia (PCP)), usually found only in severely immune-compromised patients, was described in five homosexual men.¹ Three weeks later the MMWR reported 26 cases of Kaposi's sarcoma, a rare cancer, in homosexual men in New York and California.² In 1982, the CDC introduced the term, acquired immunodeficiency syndrome (AIDS) and identified a risk profile for AIDS, which, in addition to male homosexuality, included intravenous drug use, Haitian origin, and hemophilia A.³ By 1984, the etiologic agent for AIDS had been identified as a novel retrovirus, later named human immunodeficiency virus (HIV).^{4,5} The global HIV/AIDS epidemic continued to grow. By the end of 1991, HIV infection had been reported in 51 countries, and the World Health Organization (WHO) reported that 10 million people were infected with the virus worldwide.⁶ That same year, the CDC reported that one million Americans were infected and that HIV/AIDS had become the eighth leading cause of death in the US. Almost 10 years to the day after that first MMWR report, the CDC predicted that AIDS would remain a global pandemic into the 21st century and that by the year 2000 40 million persons could be infected with HIV.⁷ Furthermore, the emerging pattern of infection predicted that 90% of these persons would reside in developing countries in sub-Saharan Africa, South and Southeast Asia, Latin America, and the Caribbean. Sadly today, even with the emergence of over 20 HIV therapies that did not exist in 1991, these predictions proved

to be accurate (in 2015 WHO estimated that 36.7 million people were living with HIV/AIDS).

Early in the HIV epidemic (1984), the then Secretary of Health and Human Services, Margaret Heckler, expressed hope that an AIDS vaccine would be ready for testing within 2 years.⁸ Unfortunately, the anticipated vaccine never appeared, and the ongoing search for effective antiviral agents became the only viable option for providing a desperately needed antidote. This search was carried out against a backdrop of little experience or success. The age of modern antiviral therapy had arguably only started in 1983 with the approval of acyclovir for the treatment of herpes virus infections, and very few companies were involved in the discovery and development of antiviral agents. One company with a commitment to antiviral drug discovery was the Burroughs Wellcome Co. (BW), the wholly owned subsidiary of Wellcome, PLC, that had discovered and developed acyclovir. The company viewed research into HIV/AIDS as an extension of its ongoing antiviral program. In late 1984, BW researchers determined that azidothymidine (**AZT**, zidovudine, **1**), a failed cancer drug, was active against surrogate retroviruses and submitted the compound to the National Cancer Institute to confirm activity against HIV in a cell based assay that had been developed there.⁹ Confirmation of activity was received in February of 1985, and BW immediately submitted an Investigational New Drug (IND) application to the FDA. Zidovudine's development was fast tracked, and the drug was approved for use on March 19, 1987, based on the results of a single clinical study conducted by the company in collaboration with the AZT Collaborative Working Group.¹⁰ Although a great deal of controversy surrounded the initially approved dose and clinical efficacy of zidovudine,¹¹ subsequent trials conducted in early symptomatic and asymptomatic HIV patients reinforced its utility in the treatment of HIV infection.¹² However, the clinical usefulness of the drug was ultimately time limited by associated toxicity (the label contains boxed warnings on the risk of hematologic toxicity including neutropenia, severe anemia, myopathy, and severe hepatomegaly) or the emergence of zidovudine resistant strains of virus.^{13,14} Within BW, it was felt that another antiviral agent would displace the drug within 6 months of approval, and the company initiated an aggressive program to identify other nucleoside analogs with a better safety and tolerability profile.

Outside BW, the development of anti-HIV agents began to gain momentum. The next drug to be approved was didanosine (**DDI**, **2**) in October 1991, which like zidovudine is a nucleoside analog that acts as a competitive, alternative substrate inhibitor of the virally encoded reverse transcriptase (collectively nucleoside analogs with this mechanism of action were termed nucleoside reverse transcriptase inhibitors, NRTIs).¹⁵ Didanosine has a significant adverse event profile and a boxed warning status for life-threatening pancreatitis, lactic acidosis, and severe hepatomegaly.¹⁶ Resistance to didanosine also develops, albeit somewhat more slowly than zidovudine.¹⁷ In June 1992, zalcitabine (**DDC**, **3**) was

approved, but its use was limited by severe nervous system toxicity (primarily sensory peripheral neuropathy).¹⁸ Given the side effect profiles of the approved NRTIs and the rapid development of resistance to them in the clinic, it was clear that additional drugs were needed. Although early stage preclinical work was ongoing at this time on additional NRTIs, as well as on other mechanistic classes of drugs including non-nucleoside reverse transcriptase inhibitors¹⁵ (NNRTIs), protease inhibitors¹⁵ (PIs), and entry and integrase inhibitors,¹⁵ zidovudine, didanosine, and zalcitabine would remain the only clinical options available for the treatment of HIV/AIDS until 1994. Meanwhile, people continued to die (Figure 1). This was the state of affairs when the development of emtricitabine ((-)-**FTC**, (-)-**5**) began in 1990.

RESULTS

Emory University's involvement with 3'-heteronucleoside antivirals began after Dr. Raymond Schinazi, a virologist from Emory's Department of Pediatrics, told Dr. Liotta about a poster he had seen at the Fifth International Conference on AIDS in 1989. This poster described the synthesis of racemic 3'-thia-2',3'-dideoxycytidine (**4**, BCH-189), as well as *in vitro* data showing that it exhibited good anti-HIV activity with no apparent cytotoxicity.¹⁹ Given the need for additional low toxicity NRTIs, it seemed worthwhile to prepare analogs in this series and assess their potential as anti-HIV agents.

Unfortunately, the reported synthesis of **4** was extremely inefficient, thus creating a major obstacle to developing a comprehensive structure–activity relationship (SAR) for this series. Indeed, the most problematic step was the stereorandom glycosylation reaction, which resulted in an inseparable 1:1 mixture of α -(*trans*) and β -(*cis*) diastereomers (Scheme 1, Pathway A). This was not surprising since the presumed intermediate, carbocation **7**, would not be expected to exhibit any facial selectivity.²⁰ Since it had been established that only the β -isomers of nucleoside analogs could act as NRTIs, the Liotta group set out to develop a general, β -selective glycosylation strategy that could be useful for 3'-heteronucleosides and perhaps could be extended to the syntheses of all 2'-deoxynucleoside analogs.

Conceptually, this approach centered on amplifying the stereodifferentiating capability of the protected hydroxymethyl group in **6** by using stannic chloride, a thiophilic Lewis acid, to selectively complex on the α -face of the oxathiolane ring, thereby minimizing the destabilizing its 1,2-steric interactions with the hydroxymethyl group (see Scheme 1, Pathway B). At the very least, this complexation should dramatically hinder the approach of the silylated base to the α -face. Alternatively, intermediate **10** could be formed when the associated metal delivers one of its ligands (presumably chloride) to the α -face of the proximal incipient carbocation, followed by S_N2 attack to form the N-glycoside. Both processes would produce the same result.

To evaluate the viability of this *in situ* complexation strategy, the Liotta group prepared **13** as a mixture of anomers from glycoaldehyde **11** (Scheme 2).²¹ Consistent with the hypothesis, Vorbruggen reaction of **13** with bis-trimethylsilyl-cytosine and virtually any Lewis acid (e.g., TMSOTf, TiCl₄, MgCl₂, MgBr₂, SnCl₂, FeCl₃) resulted in the formation of inseparable mixtures of N-glycosylated anomers. However, use of stannic chloride (2 equiv, CH₂Cl₂) at

ambient temperature led to the exclusive formation of the β -cytosine adduct **14**.²² This level of selectivity, which was estimated by HPLC to be >300:1, was unprecedented for glycosylation reactions in the synthesis of 2'-deoxynucleoside analogs and suggested that this strategy might provide a general approach for controlling the glycosylation stereochemistry of 3'-heteronucleosides.²²

The anti-HIV activity and lack of cytotoxicity of **4**, prepared by the route described in Scheme 2, was confirmed in infected peripheral blood mononuclear cells (PBMCs) by Dr. Schinazi's lab. Because of a long-standing collaboration between Dr. Liotta and Dr. Painter at BW and the aggressive NRTI program ongoing at the company, Dr. Liotta sent the compound to Drs. Painter and Furman who further corroborated its activity and lack of cytotoxicity. In addition, in a collaboration with Dr. Yung-Chi Cheng at Yale, it was determined that **4** potently inhibited the replication of hepatitis B virus (HBV) *in vitro*.²³ Taken together, these observations created a great deal of excitement about the potential of developing **4** as an anti-HIV agent and a sense of urgency to prepare each of the stereoisomers of **4** and to determine whether their activity/ toxicity profiles were superior to those of the racemate. In addition, it appeared sensible to prepare additional 3'-heteronucleosides to determine whether analogs of **4** exhibited equal or superior anti-HIV and anti-HBV activity.

Resolving the enantiomers of **4** (Scheme 3) soon became a highly competitive undertaking. In addition to the efforts of the Liotta group, other groups, including those at Glaxo (Coates),²⁴ Yale University (Y.-C. Cheng), the University of Georgia (C. Chu),²⁵ and Emory University (R. Schinazi),²⁶ developed approaches for obtaining the stereoisomers, either through stereoselective syntheses or by using chiral resolution approaches. In all published reports of the *in vitro* anti-HIV activity, the enantiomers of **4** were found to be approximately equipotent.^{24,26} However, the (–)-enantiomer, (–)-**4**, (which subsequently become known as **3TC** (lamivudine), *vide infra*) was considerably less cytotoxic than its (+)-counterpart.^{24,26} In addition, and to the surprise of many, the (–)-enantiomer possessed the “unnatural” “L-nucleoside” absolute configuration (*vide infra*). Indeed, for a time many researchers hoped that L-nucleosides in general might possess better safety profiles than their “natural” D-counterparts. It was hypothesized that these unnatural L-analogs would not interfere with endogenous nucleosides or nucleotides in essential host (human) transcription processes since they would not bind to host polymerases as well as they would to the HIV encoded reverse transcriptase.²⁷ Unfortunately, this hypothesis failed; there was no magic bullet here!.

Since early in 1990, the development of BCH-189 (and, subsequently, **3TC**) was being handled through a partnership between Glaxo and Biochem Pharma, the Canadian firm that grew out of the discovery of BCH-189. Consistent with the previously reported cellular data, the toxicity of BCH-189 arose primarily from its (+)-enantiomer, leading to the development of its (–)-enantiomer, **3TC**.

During the time period that the BCH-189/**3TC** story had gone from an interesting discovery to a fast moving, multinational clinical program to develop **3TC** for the treatment of HIV infection, the Liotta group had, in parallel, been preparing a variety of 5-substituted

pyrimidine oxathiolane nucleoside analogs and evaluating their anti-HIV profiles. While most of these proved to be inactive, one compound, the racemic 5-fluorocytosine derivative (which came to be known as **FTC**, (–)-**5**), exhibited an anti-HIV activity/toxicity profile that was as good as if not better than that of BCH-189.²⁸ In addition, like BCH-189, racemic **FTC** potently inhibited HBV replication,²³ an observation that was subsequently confirmed by Drs. Painter and Furman at BW.²⁹ As a consequence of this preliminary interaction, a scientific collaboration between Emory (Liotta/Schinazi) and BW (Painter/Furman) focused on the development of **FTC** for treating HIV and HBV was initiated. Within 18 months, this collaboration grew into a license agreement between the two institutions under which BW would develop the compound, now designated as 524W91, for the treatment of HIV and HBV viral infections.

The BW team was anxious to begin development of **FTC**, so, after having boasted about the merits of its synthetic route, the Liotta group was asked to prepare 200 g of racemic **FTC**, which was to be used to begin generating the data necessary to elevate 524W91 to project status within the company. Obtaining this status would trigger the availability of the resources and funding necessary to complete all of the required tasks to file an IND and, if the FDA agreed that the data supported it, to initiate a phase I first time in a human clinical trial. The Liotta group quickly prepared the material, and the BW team carried out rigorous pharmacodynamic and pharmacokinetic assessments. The compound proved to be quite potent, had good oral bioavailability, and, importantly, had a very clean toxicological profile (reviewed in ref 30). One specific concern, that **FTC** would be deaminated by cytidine (or deoxycytidine) deaminase, followed by deglycosylation to produce the cytotoxic agent, 5-fluorouracil (5-FU), was demonstrated by BW to be baseless (see Scheme 4).

The next step involved developing an approach for accessing the enantiomers of **FTC** in multigram quantities. Most of the Emory team's efforts focused on developing either stereoselective syntheses of the enantiomers, or a kinetic resolution approach. The Liotta group was particularly excited about the former since they had access to an enantiomerically enriched version of the lactone used in their racemic synthesis. Unfortunately, when this was attempted, only racemic **FTC** was produced. In retrospect, the reason for the racemization was obvious. Stannic chloride complexation enhanced the nucleofugality of the sulfur, thereby allowing a stereorandom carbon–sulfur bond opening and reclosure that was more rapid than intermolecular glycosylation (see Scheme 5).

The kinetic resolution approach proved to be much more successful. After testing a variety of combinations of proteolytic enzymes and 5'-alkyl esters, a highly enantioselective hydrolysis of the butyrate ester of racemic **FTC** in pH 8 buffer–acetonitrile was achieved using pig liver esterase (Scheme 6). The hydrolysis dramatically slowed once the 50% conversion level was reached. The use of butyrate esters facilitated the separation of the optically enriched, unreacted substrate from the medium by extraction with CHCl₃. This process was scaled to produce multigram quantities of enantiomerically pure (–)-**FTC**, which proved to be approximately 100 times more potent than its positive counterpart. Interestingly, unlike BCH-189, neither enantiomer of **FTC** exhibited any cellular toxicity.³¹

The subsequent preclinical development of 524W91 ((-)-**FTC**) was proceeding at a very brisk pace until the startling observation was made at BW that in HIV passaging studies the compound rapidly selected for a single resistant mutant, M184V, which was 500–1000-fold less sensitive to (-)-**FTC** compared to wild-type virus.^{32–34} The day that George Painter first informed Dennis Liotta about this, he began the telephone conversation with a greeting neither of us would ever forget: “Dennis, **FTC** is dead”! At that moment it seemed that many years of hard work by groups at Emory and BW might all be for naught.

Fortunately, this cloud had a silver lining.^{35,36} Two independent research groups were simultaneously conducting *in vitro* experiments to evaluate the impact of combining the oxathiolane nucleoside analogs, **3TC** and **FTC**, with other NRTIs.^{35,36} Mathes et al. tested combinations of **3TC** and **FTC** with **AZT** in a primary lymphocyte cell culture system with cells isolated from **AZT**-treated and **AZT**-naïve patients.³⁵ Combined inhibitory concentrations of **AZT** plus **3TC** or **FTC** were synergistic and active against virus harboring **AZT**-resistant mutations. Tisdale et al. demonstrated that passage of wild-type virus with a combination of **AZT** and **FTC** appreciably delayed the emergence of resistant virus.³⁶ Based on these data, the authors suggested that treating patients with a combination of **AZT** and an oxathiolane nucleoside analog may delay the emergence of resistant virus and prove beneficial in the treatment of HIV infection and AIDS. The effectiveness of **AZT–3TC** combination therapy was subsequently demonstrated in four independent clinical trials conducted in both antiretroviral untreated and **AZT**-pretreated patients. The FDA approved Combivir, a fixed dose combination form of the approved drugs, **AZT** (300 mg) and **3TC** (150 mg), on September 26, 1997, for the treatment of HIV infection.

An IND application for 524W91 was submitted in September of 1995 by BW (see Figure 2 for an overview of supporting data), and a phase I study was initiated the very next month.³⁰ The study was conducted in 18 HIV infected patients with CD4 counts >200. HIV positive volunteers were randomized to receive six single escalating oral doses of 524W91 between 200 and 1200 mg or placebo with each dose separated by at least a six-day washout interval. The effect of food on pharmacokinetics was investigated with one dose administered in conjunction with a high-fat meal and one dose administered in a fasted state. The drug was well tolerated by all subjects with no adverse events observed and its pharmacokinetics were linear with small intersubject variability. The stage was set to initiate phase 2 of clinical trials.³⁰

However, the development of **FTC** was interrupted by an unsolicited \$14 billion offer to purchase Wellcome PLC by Glaxo PLC on January 23, 1995. When it was completed, the merger of the two companies was part of a growing string of mergers and acquisitions in the pharmaceutical and healthcare sector that would rank among the top three largest deals to date in any industry. In business terms, the purchase of Wellcome was driven by Glaxo's need to maintain sales growth. Wellcome's sales of £2.3 billion were an attractive addition to Glaxo's £5.7 billion, so that, by the time the merger was completed, the new company had £8.3 billion of sales. In addition to an immediate increase in market share, the merger was justified to shareholders by synergies that would reduce operating costs, which was largely achieved by eliminating 7105 jobs, including 1953 jobs in research and development (R&D). While the reasons behind this acquisition were multifaceted, surely part of its attractiveness

included the acquisition of products and intellectual property rights that would make GlaxoWellcome the world's leading antiviral company. Since Glaxo PLC was developing the other oxathiolane nucleoside analog, **3TC**, it is hard to ignore the possibility that the merger also provided an attractive opportunity to block the development of the competitive product, (–)-**FTC**.

In 1996 a group headed by Dr. David Barry that had constituted the core of the BW HIV discovery and development team left GlaxoWellcome to form the startup biotechnology company Triangle Pharmaceuticals, Inc., in Research Triangle Park, North Carolina. A key component of the Triangle strategy was to license and to quickly reinstate the development of **FTC**. Toward that end in April of 1996, Triangle licensed the development and commercialization rights that had been returned to Emory when GlaxoWellcome dropped development of **FTC**. However, the GlaxoWellcome data (data generated by BW prior to the merger), drug substance, and patent rights necessary to jump start the program were withheld from Triangle until several years later (*vide infra*). To get the clinical development program moving, Triangle submitted a new IND in August 1997 and quickly initiated a series of phase I/II clinical studies to determine optimal doses for moving into pivotal efficacy trials for both HIV and HBV. The outcome of these studies further supported the safety and tolerability of the drug observed in the phase I study and established the optimal dose of the drug for the treatment of HIV infection to be 200 mg/day. In the largest of the HIV phase II studies, 80 patients were randomized to receive one of four doses (25, 100, 150, and 200 mg) once a day for 10 days. The once daily 200 mg dose exhibited superior viral suppression and produced a greater overall reduction in plasma HIV-1 than did 150 mg of **3TC** twice daily. Based on these results, the 200 mg/day dose was carried forward into phase III HIV pivotal clinical trials. Extensive studies in HBV patients also identified the 200 mg/day dose as the optimal dose for all future HBV studies. In August, 1998 **FTC** was granted “Fast Track” status, based primarily on its potential for once daily dosing.³⁰

Although the first HIV drugs were given as monotherapies in the late 1980s, the standard of care for the treatment of HIV infection evolved to combination therapy (e.g., Combivir) and then to the administration of a cocktail of drugs in different mechanistic classes, a method of treatment then also known as HAART (highly active antiretroviral therapy). HAART was seminal in reducing the morbidity and mortality associated with HIV infection and AIDS. As a consequence of HAART becoming the standard of care by the time **FTC** entered phase III clinical trials, the pivotal efficacy trials were run with **FTC** in combination with two additional approved antiviral drugs. Ultimately, the FDA's approval of **FTC** would be based on positive data from two 48-week clinical trials, FTC-301 and FTC-303 (a brief overview of these studies is given in ref 37). In both studies, two criteria (primary end points) were used to establish outcome, a decrease in virus in the plasma (expressed as HIV RNA copies/mL) and an increase in immune function expressed as an increase in CD4+ cell count (CD4+ cells, T-cells, are the most important indicator of immune function in HIV infected individuals).

The outcomes of the two phase III trials were positive, that is, the two primary clinical end points were met in both studies, and **FTC** demonstrated equal or superior anti-HIV activity after once daily administration when compared head-to-head with twice-daily administration

of stavudine (**d4T**) and **3TC** in a three drug combination (a brief review of the phase 3 studies is given in ref 37). In the FTC-301 study, the combination of **FTC** with didanosine and efavirenz was compared to the combination of stavudine, didanosine, and efavirenz in 571 antiretroviral naive patients. After 48 weeks of treatment 78% of **FTC** recipients had a viral load under 50 copies/mL versus 59% of stavudine patients. In addition, **FTC** recipients experienced a more robust CD4⁺ cell response, with an increase of 168 cells/ μ L for **FTC** versus 134 cells/ μ L for stavudine. FTC-303 compared the safety and efficacy of switching **3TC**, given twice daily, to **FTC**, given once daily, while maintaining all other background medications in a triple drug regimen. The study demonstrated that patients on twice daily **3TC** could successfully modify their regimen by switching to **FTC**. Based on these data, Triangle submitted a New Drug Application (NDA) to the FDA in September of 2002.

A third phase III clinical trial in antiretroviral treatment naive patients, FTC-302, had been started in the Republic of South Africa in August of 1999 and was to serve as the second pivotal study to support the approval of **FTC** for the treatment of HIV infection. The study was designed as a randomized, double-blind (neither the patient nor the treating physician knew which drug regimen the patient was taking) study in which the efficacy of 200 mg of once daily **FTC** was compared to 150 mg of twice daily **3TC** after 48 weeks of treatment. The two drugs were combined with approved doses of stavudine and either nevirapine for patients with baseline viral loads <100 000 copies/mL of HIV RNA or efavirenz for patients with baseline viral loads >100 000 copies/mL of HIV RNA. In April of 2000, Triangle Pharmaceuticals received a letter from the South African Medicines Control Council (MCC) indicating that study FTC-302 should be terminated due to a number of serious liver related adverse events including the deaths of two women from hepatitis. That same month, the FDA issued a clinical hold on FTC-302 and indicated that, due to the factors responsible for the hold, it was unlikely the study could be used for approval of the drug. After the study was interrupted and unblinded, it became clear that a large number of the liver related adverse events occurred in patients taking drug regimens containing nevirapine. The two deaths were in patients (one randomized to **FTC** and one randomized to **3TC**) who had both received nevirapine. On analysis of the data, it was concluded that the liver toxicity was due to nevirapine. Ultimately, the MCC and Triangle Pharmaceuticals came to an agreement under which all patients enrolled in FTC-302 completed 48 weeks of blinded treatment. Subsequently, the company with the endorsement of the MCC requested that FDA lift the clinical hold. However, the request was denied, and the data from the study, which strongly supported the efficacy of **FTC**, could not be used for approval.

While the clinical development of **FTC** was underway, a critical impediment to its development was simultaneously playing out. On July 23, 1996, the day that the first Emory patent on oxathiolane nucleosides issued, Emory sued GlaxoWellcome and Biochem Pharma for patent infringement, claiming ownership of **3TC**, which GlaxoWellcome had been selling in the United States since late 1995. A second, parallel lawsuit, claiming Emory's ownership of **FTC**, was simultaneously filed. GlaxoWellcome and Biochem Pharma countersued, beginning a complex series of proceedings in the United States District Court for the Northern District of Georgia and the United States Patent and Trademark Office that would not be completely resolved for over six years. As noted in an August 1996 article

about the litigation in the Atlanta Business Chronicle, “Looming over the dispute is the question of whether Emory will seek an injunction to halt sales of **3TC**”.³⁷ Based on ethical concerns for HIV-infected patients, Emory did not seek this injunction, even though it would likely have led to a much quicker resolution of the intellectual property disputes in question.

The first partial resolution of the disputes came on May 7, 1999, when GlaxoWellcome granted a worldwide, exclusive license to their **FTC** development and clinical data, drug substance, and patent property to Emory University and Triangle Pharmaceuticals. The parties also agreed to a settlement that included mutual releases from claims made by Emory in its **FTC** lawsuit. On June 6, 2002, GlaxoSmithKline, Shire Pharmaceuticals, and Emory University announced a global settlement agreement that resolved all disputes relating to lamivudine (**3TC**) and emtricitabine (**FTC**) patent rights. Under the terms of the settlement, Emory University received cash and a license to Shire’s emtricitabine (**FTC**) patents, and Shire and GSK received licenses to Emory’s lamivudine (**3TC**) patents. With the resolution of all intellectual property disputes surrounding **FTC**, it appeared that the clinical development could now finally be completed without the uncertainties associated with pending litigations.

Meanwhile, the impact of mergers and acquisitions within the pharmaceutical and biotechnology sectors continued to impact emtricitabine development. On December 4, 2002, Gilead Sciences (NASDAQ: GILD) announced they had signed an agreement under which they would acquire Triangle Pharmaceuticals, Inc. (NASDAQ: VIRS). The value of the transaction was approximately \$464 M and was completed on January 23, 2003. As a company focused on discovering and developing therapeutics against life-threatening infectious disease, Gilead had a strong scientific and strategic rationale for the acquisition of Triangle. In the year prior to the acquisition, Gilead had launched two new antiviral products, Viread (tenofovir disoproxil fumarate) for the treatment of HIV infection and Hepsera (adefovir dipivoxil) for the treatment of HBV infection. The portfolio of drugs Triangle was developing, particularly emtricitabine, offered considerable opportunity for synergy and market growth. Gilead now took over the development of emtricitabine, and the drug, under the trade name Emtriva, was approved by the FDA for once a day, oral administration (200 mg) on July 2, 2003.

CONCLUSION

Emtricitabine³⁸ was approved by the FDA for once a day, oral administration (200 mg) on July 2, 2003. A year later, Truvada, a once a day, oral, fixed dose combination of emtricitabine (200 mg) and tenofovir disoproxil fumarate (300 mg), received FDA approval and quickly became the accepted first line therapy when used with a third antiretroviral agent. However, the real game changer for HIV therapy came on July 12, 2006, when the FDA approved Atripla, a once a day, oral, fixed dose combination of emtricitabine (200 mg), tenofovir disoproxil fumarate (300 mg), and efavirenz (600 mg). Although other important fixed dose combinations of emtricitabine would follow,³⁹ for those of us who were involved in HIV therapeutics development from its onset, the development of Atripla represented the gamechanger: the culmination of two decades of research that had transformed AIDS from a death sentence to a manageable chronic disease. *We estimate that over 90% of HIV-infected*

patients on therapy in the US (where we have good statistics) take or have taken a drug containing either emtricitabine or lamivudine.

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Biographies

Dennis Liotta, Ph.D., D.Sc. (Samuel Candler Dobbs Professor, Executive Director, The Emory Institute for Drug Development), has focused his research over the past two and a half decades on the discovery and development of novel antiviral, anticancer, and anti-inflammatory therapeutic agents. He is recognized as one of the premier discoverers of novel therapeutics, having been one of the inventors associated with 12 FDA approved therapeutics including Epivir, Combivir, Trizivir, Epzicom, Epivir-HBV, Emtriva, Truvada, Atripla, Complera, Stribid, Genvoya, and Descovy. He is also the lead inventor of Q-122 (formerly known as MSX-122), a safe, orally available clinical agent for controlling hot flashes in postmenopausal women. A company he founded, Pharmasset (acquired by Gilead Sciences), developed sofosbuvir, which has become the first line therapy for treating hepatitis C. He is also a founder of DRIVE (Drug Innovation Ventures at Emory).

George Painter, Ph.D. (Director, The Emory Institute for Drug Development, CEO, Drug Innovation Ventures at Emory (DRIVE)) has over 30 years of experience in the discovery and development of pharmaceutical agents in the biotechnology and large pharmaceutical company sectors. He has been a member of the founding management teams of two highly successful biotechnology companies, Triangle Pharmaceuticals, Inc., where he served as executive vice president of research and development; and Chimerix, Inc., where he served as president and chief executive officer. Prior to his management experience in biotechnology, Dr. Painter held positions of increasing responsibility in the pharmaceutical industry beginning in 1982 including Director of Chemistry and Director of Virology at Burroughs Wellcome Co. After the merger of Glaxo and Wellcome in 1995 to form GlaxoWellcome, he served as its World-Wide Director of Research Process and Deputy Therapeutic Head for Antiviral Research. Dr. Painter is a founder of DRIVE (Drug Innovation Ventures at Emory, a nonprofit drug development company focused on the development of therapies for treating single stranded RNA virus infections, such as dengue fever, hepatitis C, chikungunya, zika virus, respiratory syncytial virus, and various equine encephalitis viruses). DRIVE utilizes an innovative model that seeks to extract maximum

value from therapeutic innovations discovered at Emory or elsewhere by efficiently advancing them into clinical trials.

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Figure 1.
New HIV infections and AIDS deaths increased rapidly worldwide in the 1990s.

- . Potent Inhibitor of HIV-1 and HIV-2 with EC₅₀ values ranging from 0.009 to 0.1 μ M
- . Mechanism of Action: obligate chain terminator
- . Decreases extra- and intra-cellular HBV DNA in a dose dependent fashion with an IC₅₀ of 0.010 μ M
- . Shows a high selectivity for the virally encoded reverse transcriptase over human host polymerases α , β , γ and ϵ
- . Cytotoxicity was evaluated in multiple cell lines. CC₅₀ values were all greater than 200 μ M
- . High oral bioavailability (79%)
- . Long intracellular half life of its triphosphate (39 hrs.) is well suited for once-a-day dosing
- . Thirty day toxicity studies were conducted in rats and monkeys. In both species the NOAEL (No Observed Adverse Effect Level) was greater than 2000 mg/kg/day

Figure 2.
FTC preclinical profile.

Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.

Scheme 5.

Scheme 6.