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Reflections on the Rega Institute for Medical Research, at the fiftieth anniversary of the Rega Stichting vzw (Rega Instituut vzw, Rega Foundation)

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Abstract

The idea to start the Rega Foundation was conceived in 1971 at an informal meeting organized by Prof. Piet De Somer (where Prof. Alfons Billiau, Prof. André Vlerick and I were also present), before the Foundation was formally created in 1972. From the early years some antiviral compounds, such as BVDU and the aminoacyl esters of acyclovir (from which ultimately valacyclovir evolved) originated. The advent of AIDS in 1981 and the discovery of the etiologic agent (HIV) thereof in 1983 have led to the identification of an avalanche of anti-HIV compounds in which the Rega Institute has played a primordial role. Foremost among these compounds was tenofovir, discovered in collaboration with Antonín Holý from the IOCB (Institute of Organic Chemistry and Biochemistry) in Prague. Tenofovir laid the basis for the treatment of HIV (AIDS) and hepatitis B virus (HBV) infections, and in combination with emtricitabine it was the first chemical ever approved by the US FDA (Food and Drug Administration) for the prophylaxis of HIV infections.

Keywords

Fifty-year anniversary, antiviral, AIDS, HIV, HBV, tenofovir, treatment, prophylaxis

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Foreword

The Rega Institute Stichting was created by Prof. Dr. Piet De Somer (Fig. 1a-c) and incorporated as a vzw ("vereniging zonder winstoogmerk" or non-profit association) on 8 January 1972. The Institute was named after Prof. Henri-Joseph Rega (Fig. 2), who was the rector of the KU Leuven in 1719 and in 1722 (a rectorship then lasted 6 months). The Rega Institute was founded in 1954 (its 50th anniversary was celebrated on 9 October 2004). Figure 3 shows pictures of the old and new Rega Institute buildings. The new building (part of the Gasthuisberg complex) formally dates from 13 September 2017). The original by-laws ("statuten") of the Rega Foundation ("Stichting Rega") are depicted in Fig. 4.

Part A

My first encounter with Prof. Piet De Somer was in 1963 at the end of the exam Bacteriology (4th year of Medicine) when he asked whether I would be interested to come to work in his laboratory at the Rega Institute for Medical Research. Not overly interested in bacteria, I answered "No". I was then working as a laboratory student in the lab of a certain Prof. Raymond Devis. On the recommendation of Prof. Dr Xavier Aubert (Physiology), I had joined Devis's laboratory of Chimie Hormonologique. My real interest was to work on Biochemistry but the Professor of Biochemistry (Flemish section), Paul Putzeys, did not allow medical students to work in his lab. The other option was to contact the Professor of Biochemistry of the French section, Christian de Duve, but when I asked what he was working on, I was told fractionation of cells into subcellular compartments, and this kind of work (dissection of the cells in smaller organelles) did not enthrall me (sorry for missing the opportunity to work with a later Nobel laureate but I was too young to judge). I then stuck with Raymond Devis with the hope that I could get involved in the biosynthesis of steroid hormones, a topic that I had covered as a dissertation

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Figure 1. a. Drawing of Prof. Piet De Somer (Anne Van Herreweghen). b. Drawing of Prof. Piet De Somer (Gerard Thijs). c. Painting of Prof. Piet De Somer (in reception area of the Pieter De Somer auditorium) (Sam Dillemans).

topic for the course of Physiology (Prof. J.J. Bouckaert). Unfortunately, the task entrusted to me by Prof. R. Devis had nothing to do with steroid hormones but concerned the diagnosis of catecholamines (i.e., adrenaline and noradrenaline) by chromatography and spectrometry, and I spent many afternoons in doing spectrometry and thin layer chromatography, from time to time interacting with the technicians (i.e., Lydia Tchernenko) in French, but the final palm was that from three years of lab work in "Chimie hormonologique", I distilled no publications, so that I cannot even prove by any written documents whether I accomplished anything of value during this period (2–5 years of medical education).

June 15th, 1964 marked an unforgettable event in my life. It started as a terrifically sunny day. I had to get up at 5 am to travel from Hamme by bus and train to arrive before 8 am for my exam in Leuven (Propedeutics by Prof. J.V. Joossens). The exam started at 8 am with a patient's examination. I would never forget, the patient was Ms. Liliane Blanpain, daughter of a general practitioner from Neerheylissem. She was French speaking and accompanied by her mother. I had made the diagnosis in one second: obesity. As I had to spend three hours before physical appearance of the Professor, I wrote about 10 pages of all the possible causes of obesity. Then, around 11 am, the Professor arrived, and as it was Monday and very sunny, he was not in a pleasant mood. When he asked my diagnosis, and I replied obesity, he immediately inquired about the reason for it. I diligently gave him 10 pages of possible reasons. He then asked whether I had mentioned (French) fries or (Belgian) chocolates among the possibilities for the obesity. I had written down "diet" without being more specific, and then he threw all my pages in the air, and I felt that I failed the exam because I had not mentioned chocolates or fries. I was terribly shocked and so was the mother of the patient. She withdrew her daughter the same day from the clinic, whereas I went back to the students' dormitory (Pope College) to fall down on my bed. This was the first exam of the 5th year in Medicine, considered to be the heaviest in the curriculum for MD. The beginning for the exam session was dismal, and I found some relief when later that day I could meet my girlfriend Emmy, working

Figure 2. Pictures of Henri-Joseph Rega, Rector of the KU Leuven (1719 and 1722). The picture shown on the right is from a drawing by Gerard Thijs.

for Tintin, in Brussels from where we returned together back home to Hamme.

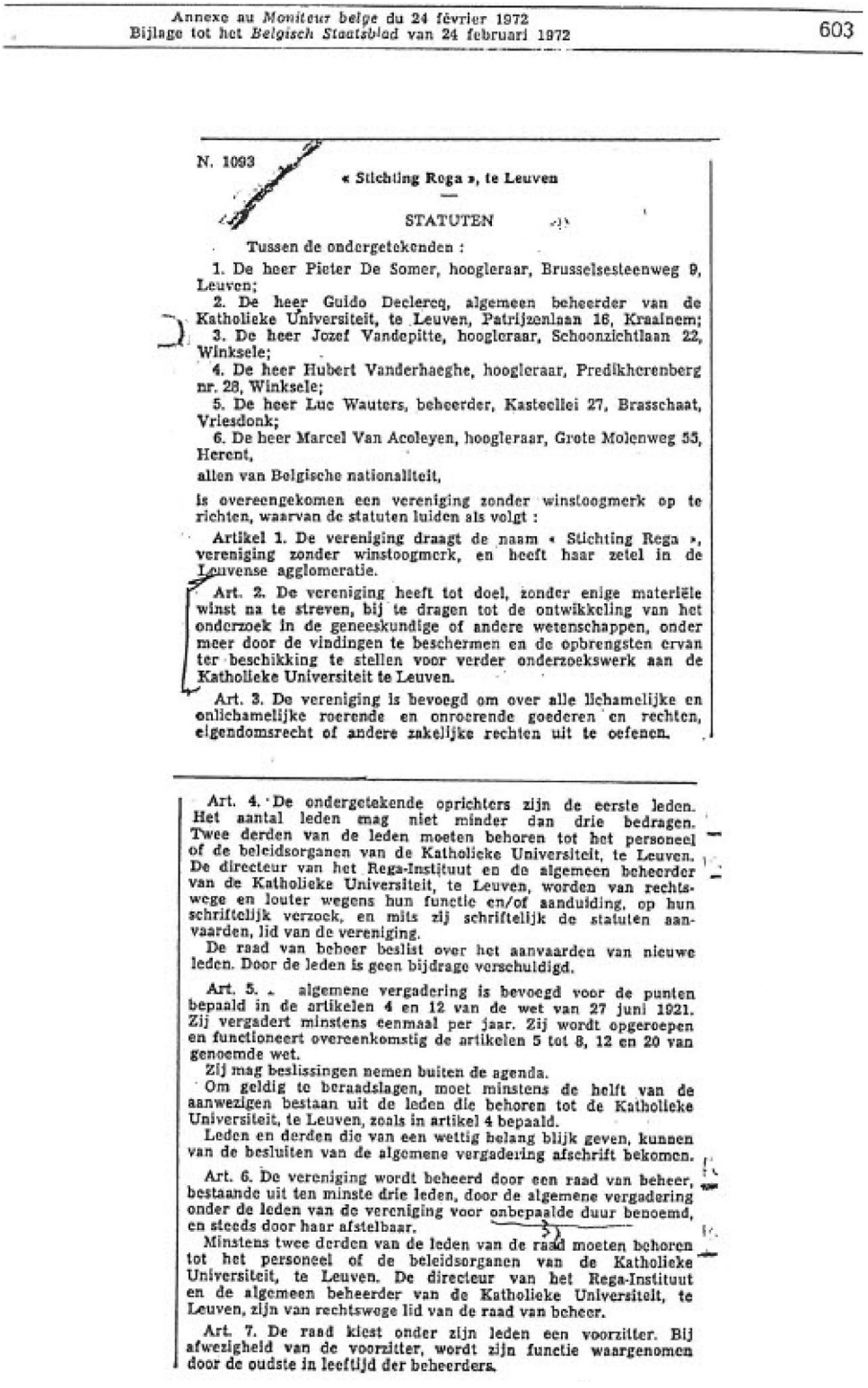
Following the exam of "Propedeutics" on Monday, 15 June 1964, followed the other exams, i.e., Orthopedic Surgery, Pharmacology, and, most importantly, Microbiology (Virology) with Prof. De Somer. I was anxious to know whether the Professor had remembered he had asked me the previous year to work in his laboratory, and when he did, and asked me again to join him in his lab, I did no longer doubt and answered "Yes"! Little did I know this would be Yes for the rest of my life. Meanwhile, the Preses of the Medical Student's Union, Olaf Leuridan, had already pointed out to me that my refusal the previous year to join Prof. Piet De Somer's team was an unforgivable mistake as he was going to be the most important person in Leuven, and refusing his offer was unheard of. So, I felt relieved when I had now said "yes" and looked forward to the consequences. Due to the mishap at the exam of Propedeutics, the final score for my exams at the 5th year of the Medical School decreased from Greatest Distinction (Maxima Cum Laude) to Great Distinction (Magna Cum Laude), a small difference for outsiders, but for me, it gave me the feeling of a failure (I would regain the status of Maxima Cum Laude again in the sixth and seventh year of my medical studies). The sixth year was cramped with internships (Internal Medicine, Surgery, Obstetrics, Neurology, Cancerology) and I had no time for laboratory work. This was again possible in the 7th year, but then I started to think seriously about my future, either to do lab work with Prof. Piet De Somer and to spend my future days with pipets, test tubes, mice and rabbits, or to see patients, and enjoy their daily recognition, in Internal Medicine.

I discussed this dilemma with Prof. De Somer, and he proposed a possible solution: 50% to work with him and 50% to spend time with patients under the guidance of the Professor of Internal Medicine, Prof. Josué Vandenbroucke. And, as I had to start at either site, Piet De Somer suggested I should start with him, and so I did. As I started my lab work with Piet De Somer in August 1966, I made two relatively important findings in this month, (*i*) the fact that interferon appeared in the urine of rabbits (following its induction by Sindbis virus, a finding that Prof. De Somer presented at a Pan American Health Organization (PAHO) meeting (Second International Symposium of Applied Virology and Medicine – December 4–7, 1966), organized by Gray Research Foundation Inc. & Nova University, Fort Lauderdale, Florida, where he met with Prof. T.C. Merigan), and (*ii*) the fact that interferon could be induced by synthetic polyanions such as polyacrylic acid (PAA). Just a few months earlier T.C. Merigan had reported on pyran copolymer as the first synthetic polyanion that induced interferon (in mice and men). From Fort Lauderdale, Piet De Somer came back with the message that I should spend some post-doctoral time at Stanford University with T.C. Merigan. As possible alternatives, he also mentioned Phil Marcus at the Bronx (New York City) and Bob Wagner at Johns Hopkins (Baltimore) but staying at Stanford attracted me more than New York or Baltimore, so that on 3 September 1968 I flew with my newlywed wife Lili, to Palo Alto (from Brussels to London, to Los Angeles, then to San Francisco and finally to Palo Alto). Once at Stanford University Medical School, I quickly adapted to my new life and I thoroughly enjoyed,

Figure 3. Pictures of the old Rega Institute building (Minderbroedersstraat 10, Leuven) and new Rega Institute building (Herestraat 49, Leuven). The picture shown above is from a drawing by Gerard Thijs.

as a free student, the courses given on Biochemistry by the staff members of the Biochemistry Department, particularly Arthur Kornberg and Paul Berg, and where I had obtained a fellowship to stay with Prof. Tom Merigan for one year (Eli Lilly fellowship), I stayed for a second year with a Damon Runyon fellowship, so that after a total stay at Stanford for two years and two months (a stay which my wife and I would lifelong cherish as the most beautiful time of our life), I returned back home in November 1970.

During my stay at Stanford University (September 1968 – November 1970), Professor Piet De Somer came over twice to persuade me to come back home, where a bright future would be waiting for me. A second force inciting my return were my parents, whereas my wife rather wished to stay at Palo Alto (Stanford), and Prof. Merigan



Annexe au *Moniteur belge* du 24 février 1972
Bijlage tot het *Belgisch Staatsblad* van 24 februari 1972 603

N. 1093

« Stichting Rega », te Leuven

STATUTEN

Tussen de ondergetekenden :

1. De heer Pieter De Somer, hoogleraar, Brusselsesteenweg 9, Leuven;
2. De heer Guido Declercq, algemeen beheerder van de Katholieke Universiteit, te Leuven, Patrijzenlaan 16, Kraainem;
3. De heer Jozef Vandepitte, hoogleraar, Schoonzichtlaan 22, Winksele;
4. De heer Hubert Vanderhaeghe, hoogleraar, Predikherenberg nr. 28, Winksele;
5. De heer Luc Wauters, beheerder, Kasteellei 27, Brasschaat, Vriesdonk;
6. De heer Marcel Van Acoleyen, hoogleraar, Grote Molenweg 55, Herent,

allen van Belgische nationaliteit,

is overeengekomen een vereniging zonder winstoogmerk op te richten, waarvan de statuten luiden als volgt :

Artikel 1. De vereniging draagt de naam « Stichting Rega », vereniging zonder winstoogmerk, en heeft haar zetel in de Leuvense agglomeratie.

Art. 2. De vereniging heeft tot doel, zonder enige materiële winst na te streven, bij te dragen tot de ontwikkeling van het onderzoek in de geneeskundige of andere wetenschappen, onder meer door de vindingen te beschermen en de opbrengsten ervan ter beschikking te stellen voor verder onderzoekswerk aan de Katholieke Universiteit te Leuven.

Art. 3. De vereniging is bevoegd om over alle lichamelijke en onlichamelijke roerende en onroerende goederen en rechten, eigendomsrecht of andere zakelijke rechten uit te oefenen.

Art. 4. De ondergetekende oprichters zijn de eerste leden. Het aantal leden mag niet minder dan drie bedragen. Twee derden van de leden moeten behoren tot het personeel of de beleidsorganen van de Katholieke Universiteit, te Leuven. De directeur van het Rega-Instituut en de algemeen beheerder van de Katholieke Universiteit, te Leuven, worden van rechtswege en louter wegens hun functie en/of aanduiding, op hun schriftelijk verzoek, en mits zij schriftelijk de statuten aanvaarden, lid van de vereniging.

De raad van beheer beslist over het aanvaarden van nieuwe leden. Door de leden is geen bijdrage verschuldigd.

Art. 5. De algemene vergadering is bevoegd voor de punten bepaald in de artikelen 4 en 12 van de wet van 27 juni 1921. Zij vergadert minstens eenmaal per jaar. Zij wordt opgeroepen en functioneert overeenkomstig de artikelen 5 tot 8, 12 en 20 van genoemde wet.

Zij mag beslissingen nemen buiten de agenda.

Om geldig te beraadslagen, moet minstens de helft van de aanwezigen bestaan uit de leden die behoren tot de Katholieke Universiteit, te Leuven, zoals in artikel 4 bepaald.

Leden en derden die van een wettig belang blijk geven, kunnen van de besluiten van de algemene vergadering afschrift bekomen.

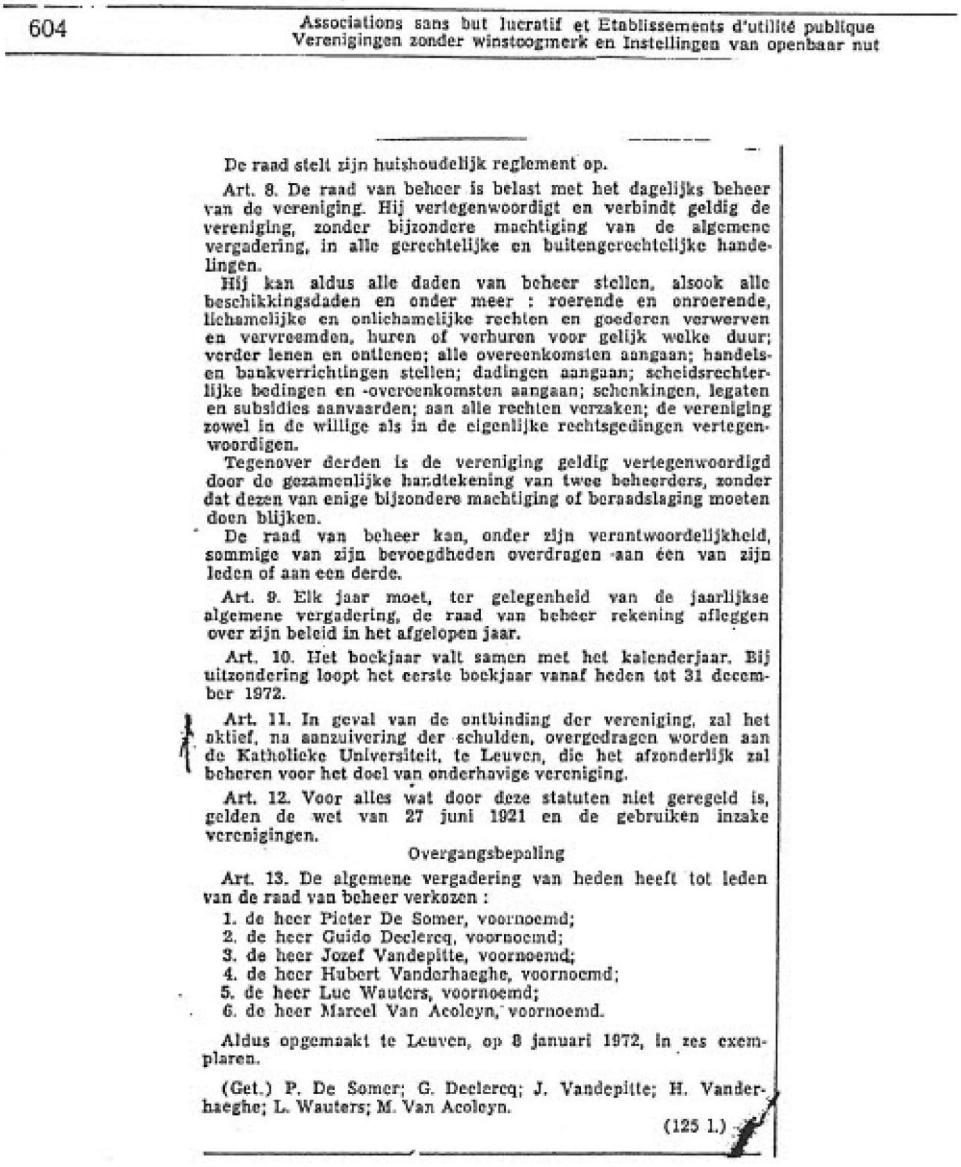
Art. 6. De vereniging wordt beheerd door een raad van beheer, bestaande uit ten minste drie leden, door de algemene vergadering onder de leden van de vereniging voor onbepaalde duur benoemd, en steeds door haar afstelbaar.

Minstens twee derden van de leden van de raad moeten behoren tot het personeel of de beleidsorganen van de Katholieke Universiteit, te Leuven. De directeur van het Rega-Instituut en de algemeen beheerder van de Katholieke Universiteit, te Leuven, zijn van rechtswege lid van de raad van beheer.

Art. 7. De raad kiest onder zijn leden een voorzitter. Bij afwezigheid van de voorzitter, wordt zijn functie waargenomen door de oudste in leeftijd der beheerders.

Figure 4. Rega Foundation (original by-laws, dating from 8 January 1972, published on 24 February 1972). (to be continued)

offered me the opportunity to run the laboratory for clinical virology, but Lili and I finally returned home to the Rega Institute, not without giving some guest lectures at NIH (Robert Friedman), Johns Hopkins (William A. Carter) and DuPont de Nemours (Royce Lockhart Jr.). Once back home, I resumed my work on the induction (and inducers) of interferon, but with the discovery of the reverse transcriptase (RT) by Temin (and Mizutani) and Baltimore



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De raad stelt zijn huishoudelijk reglement op.

Art. 8. De raad van beheer is belast met het dagelijks beheer van de vereniging. Hij vertegenwoordigt en verbindt geldig de vereniging, zonder bijzondere machtiging van de algemene vergadering, in alle gerechtelijke en buitengerechtelijke handelingen.

Hij kan aldus alle daden van beheer stellen, alsook alle beschikkingsdaden en onder meer : roerende en onroerende, lichamelijke en onlichamelijke rechten en goederen verwerven en vervreemden, huren of verhuren voor gelijk welke duur; verder lenen en ontlenen; alle overeenkomsten aangaan; handels- en bankverrichtingen stellen; dadingen aangaan; scheidsrechterlijke bedingen en -overeenkomsten aangaan; schenkingen, legaten en subsidies aanvaarden; aan alle rechten verzaken; de vereniging zowel in de willige als in de eigenlijke rechtsgedingen vertegenwoordigen.

Tegenover derden is de vereniging geldig vertegenwoordigd door de gezamenlijke handtekening van twee beheerders, zonder dat dezen van enige bijzondere machtiging of beraadslaging moeten doen blijken.

De raad van beheer kan, onder zijn verantwoordelijkheid, sommige van zijn bevoegdheden overdragen aan één van zijn leden of aan een derde.

Art. 9. Elk jaar moet, ter gelegenheid van de jaarlijkse algemene vergadering, de raad van beheer rekening afleggen over zijn beleid in het afgelopen jaar.

Art. 10. Het boekjaar valt samen met het kalenderjaar. Bij uitzondering loopt het eerste boekjaar vanaf heden tot 31 december 1972.

Art. 11. In geval van de ontbinding der vereniging, zal het aktief, na aanzuivering der schulden, overgedragen worden aan de Katholieke Universiteit, te Leuven, die het afzonderlijk zal beheren voor het doel van onderhavige vereniging.

Art. 12. Voor alles wat door deze statuten niet geregeld is, gelden de wet van 27 juni 1921 en de gebruiken inzake verenigingen.

Overgangsbepaling

Art. 13. De algemene vergadering van heden heeft tot leden van de raad van beheer verkozen :

1. de heer Pieter De Somer, voornoemd;
2. de heer Guido Declercq, voornoemd;
3. de heer Jozef Vandepitte, voornoemd;
4. de heer Hubert Vanderhaeghe, voornoemd;
5. de heer Luc Wauters, voornoemd;
6. de heer Marcel Van Acoleyn, voornoemd.

Aldus opgemaakt te Leuven, op 8 januari 1972, in zes exemplaren.

(Get.) P. De Somer; G. Declercq; J. Vandepitte; H. Vanderhaeghe; L. Wauters; M. Van Acoleyn.

(125 l.)

Figure 4. Continued.

published in the September issue of Nature 1970, I had acquired a new love, that of RT, which, together with interferon, would occupy a definitive place in my future research projects.

Where in the period 1968–1970 my own life was governed by my married state and stay at Stanford, Prof. De Somer's life was vehemently shaken by two events of the magnitude of an earthquake, (*i*) the imminent undoubling of the (Catholic) University of Louvain in two parts: the Flemish part (of which Piet De Somer would become the new Rector (President)) and the Walloon part (Université Catholique de Louvain) which would even leave the Leuven location to settle in the Walloon region (Louvain-La-Neuve) with the medical school moving to Woluwe-Saint-Pierre, close to Brussels, and (*ii*) the expedition of SKF (Smith Kline French Laboratories from Philadelphia). Their interest had already started in 1968 (before I left for the US), and I remember the SKF delegation (Ferlauto, Pagano, Di Cuollo, …) striking down in Leuven at the Rega Institute, and even the dinner party at La Maison du Boeuf at the top of the Hilton hotel (now renamed as The Hotel) in Brussels. Conspicuously, Prof. De Somer was absent at this party. After my return from the US, I would learn that SKF had decided to take over the company RIT (Recherches Industrielles Thérapeutiques) that Piet De Somer had started in Genval, but they would not take over the Rega Institute, ostensibly because they did not agree with all the projects of the Institute. After RIT had been taken over by SKF, the name of SKF would change a few times, to become Smith Kline Wellcome, Smith

Kline Beecham, and finally GSK (Glaxo Smith Kline) Biologicals. The Rega Institute for Medical Research has, since 1970, become totally independent from GSK Biologicals, and a few years later, it would be fully integrated within the Flemish KU Leuven (Katholieke Universiteit Leuven). At the separation of the Rega Institute from RIT when the latter was incorporated into SKF, Prof. De Somer obtained a financial compensation which laid the basis for the origin of the Rega Foundation, originally called Rega Stichting, which was actually conceived at a private meeting in 1971 at the Faculty Club [(Room) Roelants]; were present at this meeting: Prof. Piet De Somer, Prof. André Vlerick, Prof. Alfons Billiau and myself, having just returned from the US, and at the age of 30, the youngest attendant. At this meeting, the discussions concentrated on how a Foundation should be constructed. This Foundation (Stichting Rega vzw) was eventually materialized in 1972, and in 1997 we celebrated the 25th anniversary (we actually did so on 18 June 1996). On 9 October 2004, we had celebrated the 50th anniversary of the Rega Institute, but for the Foundation we are still looking forward to celebrating its 50th anniversary in 2022.

Although both Alfons Billiau and I had been taken up in the Rega Foundation for some time in the early 1980s, we had not been formally entrusted with any duties. The date of 17 June 1985 would change all this. In 1982, Prof. De Somer had undergone a surgical intervention because of an intestinal obstruction, and it would take several months for him to recover. When he finally did, he reappeared at the Rega Institute and seemed to enjoy his life at the top of the University, as he did before his surgery. But, in May 1985, the intestinal cancer had returned, and despite his worsening health condition, he still wanted to greet Pope John Paul II on his visit to Leuven on 18 May 1985. A few days later, Prof. De Somer was taken to surgery again and shortly after this new surgical intervention, he developed a lung embolism when still being in the hospital, and he died on 17 June 1985, a Sunday afternoon, alone after his family had gone home.

The death of Piet De Somer on 17 June 1985 was sudden but not unexpected given the preceding health problems, but Piet De Somer had not foreseen any instructions for his disciples at the Rega Institute. Prof. Karel Tavernier as the General Manager of the University became the Rector *ad interim* until the elections took place, which indicated Roger Dillemans as the winning candidate, defeating Paul De Meester (I remember I had voted for the latter). More urgently, a new President for the Rega Foundation had to be selected, and here, Prof. Tavernier as "ex officio" member of the Board proposed that I should fulfill this task, and so I became as from July 1985 onwards, President of the Rega Foundation, a function which I would keep till the age of 70 years as foreseen by the rules ("by-laws"). There was no election for this function, it was just proposed by the General Manager and accepted by all the members of the Board. Prof. Jan Desmyter was at that time not a member of the Foundation, and with the advent of AIDS and HIV (at that time still called LAV/HTLV-III) he was eager to assume greater responsibility in the daily life and actions of the Institute. Prof. Desmyter suggested that the Foundation should install a new body (the General Assembly), separate from the Directory Board. I would stay on as President of the Foundation (and Chairman of the Directory Board), whereas Prof. Billiau would become Chairman of the General Assembly. Prof. Billiau then assumed this position and has in the meantime been succeeded as Chairman of the General Assembly, successively by Prof. Jo Van Damme and Prof. Dominique Schols. As Chairman of the Directory Board, I have in the meantime (in 2011, when I turned 70 years old) been succeeded by Prof. Piet Herdewijn. Piet Herdewijn is expected to remain Chairman of the Directory Board till he will reach the age of 70 years in 2025.

Which are the most important scientific realizations achieved within the scope of the Rega Foundation? In the 1970s, Prof. De Somer's major interest was still vested in interferon, till in 1977 at a meeting at the Weizman Institute in Rehovot (Israel), he rather unexpectedly declared no longer to believe in interferon's promise to be the all-round treatment for virus infections. In the period 1970–1975, he was still an adept of interferon. This was also evident from his interest in the findings of a certain W.E. Stewart II, who under my impulse had passed several years in the Institute. W.E. Stewart was originally from Texas and I had met him in the lab of Royce Lockart at DuPont de Nemours. W.E. Stewart had an inborn talent for writing, drawing and … enjoying life. At his last expedition to Poland, he charged the Institute with the incurred expenses, and Prof. Piet De Somer fired him, not without a ("Yankee, go home") party at some place in Tildonk, that was still attended by Piet De Somer. But the Yankee did not go home, he continued his odyssey to Paris (Dr Ion Gresser), before returning to the US. At the Rega Institute he accomplished innovative work with interferon, i.e., regarding reversible denaturation (that is re-activation) which resulted in several publications and at least two patents which were authored by W.E. Stewart and Piet De Somer and assigned to the Rega Foundation.

At Piet De Somer's advice to abandon interferon research "because with interferon I would never get a Nobel Prize" (as if I would ever win the Prize otherwise), I got more actively engaged in the antiviral activity of nucleoside analogues, such as acyclovir aminoacyl esters. These compounds were subjected to a patent application in the name of the Rega Foundation and surprisingly yielded some return in royalties when in 1995, acyclovir was replaced by valacyclovir, the valine ester of acyclovir. Also, another nucleoside analogue, BVDU [(*E*)-5-(2-bromovinyl)-2'-deoxyuridine] was patented

and yielded some relatively minor royalties. The rights for the acyclovir aminoacyl esters and BVDU were transferred respectively to Wellcome (that later would become part of GSK) and Searle (that would later drop his interest in BVDU), which made me to travel by plane, with Prof. De Somer as my companion to Beckenham and High Wycombe, respectively. Both valacyclovir and BVDU would be commercialized for the treatment of HSV (herpes simplex virus) and VZV (varicella-zoster virus) infections.

With the advent of AIDS in 1981, and the identification of HIV (then called HTLV-III/LAV) as its etiological agent in 1983, started the search for anti-HIV compounds. One of the first anti-HIV agents, already discovered in November 1986, was d4T (2′,3′-didehydro-2′,3′-dideoxythymidine), that later would be commercialized by Bristol-Myers Squibb. Within one month after AZT (3′-azido-2′,3′-dideoxythymidine) had been published in PNAS (October 1985), Piet Herdewijn had already (re)synthesized d4T (the first synthesis was done by Jerome Horwitz), which we sent to Jan Balzarini then working in the lab of Sam Broder at NCI (I had originally been invited by Sam Broder to come over to NCI to work in his lab, but as I had preferred my teaching at the KULAK (KU Leuven campus in Kortrijk), I had delegated this mission to Jan Balzarini). The results obtained by Jan Balzarini in Sam Broder's lab were not overwhelming, apparently because they used a cell line ATH8 which was not optimal to demonstrate the anti-HIV activity of d4T. We (Jan Balzarini and I) did not consider the results obtained with d4T worth patenting. Then, in August 1986, Masanori Baba from Fukushima, a student of Prof. Shiro Shigeta, arrived in Leuven, and one of the first tasks I gave to him was to evaluate d4T for its anti-HIV activity in the MT-4 cells, a cell line that originated from Japan (Prof. Dr. Naoki Yamamoto) and then moved through Prof. Luc Montagnier as intermediary to our lab. Masanori Baba found d4T to be highly active against HIV in these cells. Within a few weeks, we had a paper constructed that we submitted to Biochemical and Biophysical Research Communications (BBRC), where the Editor (Prof. A. Sols, Madrid) accepted it within a few days for publication. The date of receipt was 25 November 1986. The paper was published on 17 January 1987. The text was at the same time of his submission to BBRC, sent to Arnold and Siedsma in Den Hague, our patent agency (care of Mr C.W. Bruin), but Mr Bruin did not undertake any action till he learned that the paper had already been published. Then, he submitted (January 1987) the patent application. Meanwhile, Prusoff and Lin (from Yale University) had already sent in their patent application in December 1986. That we, with our BBRC paper received by the Editor on 25 November 1986 were clearly the first, was not recognized by the American patent laws. Although Mr Bruin's negligence in submitting the patent application immediately was the evident cause for the Rega Institute (and KU Leuven) not being recognized as those that had been the first to discover the anti-HIV activity of d4T, he did not fail to repeatedly send invoices to the Rega Foundation, which we always paid diligently, until advised that to enforce our rights, the Rega Foundation should get involved in legally suing Yale University. At our University I felt, without the help of any lawyers, as vulnerable as David fighting against Goliath in Biblical times, with all the expenses that such a legal fight might eventually engender, so that I finally gave in. With Bristol-Myers (BM) I had signed an agreement that whoever had a legal patent on d4T would obtain the license agreement from Bristol-Myers (BM). And so, the agreement was concluded between BM and Yale University, the role of the Rega Institute being dismissed.

The next opportunity arose with the discovery of the NNRTIs (non-nucleoside reverse transcriptase inhibitors). We, at the Rega Institute, laid the basis of this totally new class of anti-HIV agents with two series of compounds, the HEPT and TIBO series; the TIBO series emanated from an intense collaboration that we had started with Paul A. Janssen (Dr Paul): the initiative came from Dr Paul. From his side he could fall back on the Janssen Foundation, whereas on our side, we should have engaged the Rega Foundation to back up the partnership. And I defended this viewpoint in 1987 to the KU Leuven Rector, Prof. Roger Dillemans. I still remember his reaction at that time: "The Rega Stichting, what is this?" From now on, it was evident, all further proceedings towards the Janssen-Rega collaboration would be directed by the KU Leuven. However, before we had discovered the TIBO derivatives (first published on 1 February 1990) we had already described the HEPT derivatives (December 1989). When we first reported on HEPT (again with Masanori Baba as first author) as HIV replication inhibitor, we had no evidence whatsoever for its mode of action. That is perhaps the reason why the paper was originally rejected by Science (it was finally published in BBRC). With the help of Masanori Baba, we would later resolve that the modes of action of HEPT and TIBO were quite similar and that they both acted as NNRTIs. I once pointed out to Dr Paul that HEPT and TIBO could in fact be viewed as structurally similar. Dr Paul then replied that I had too much imagination. I admit that in my approach I was rather artisanal, but several years later more sophisticated structural-biology studies (by K. Das and E. Arnold) would indeed prove my original intuition.

Following the TIBO line, Rilpivirine would later be commercialized, almost 20 years after TIBO itself had been discovered. From HEPT, emivirine was developed which, once upon a time named Co-actinon®, proceeded to phase III clinical trials; it went from Mitsubishi Kasei Corporation (MKC) to Wellcome, Triangle Pharmaceuticals, and finally Gilead Sciences, where its ultimate development was abandoned.

Prof. Piet De Somer himself had signed an agreement with IOCB (Institute of Organic Chemistry and Biochemistry in Prague) to enable my collaboration with Antonín Holý, at a time that it was not evident that such East-West collaboration would ever bear fruits. Except for

a Science paper co-authored by E. De Clercq, A. Holý and P. De Somer, that appeared in 1978, Prof. Piet De Somer never witnessed the consequences of all De Clercq's whereabouts in the Czech (initially Czechoslovak) country. This is because my first paper with Holý on the acyclic nucleoside phosphonates (ANPs) appeared in Nature in 1986, more than one year after the death of Piet De Somer. An almost yearly growing list of ANP compounds, HPMPA, PMEA, PMEG, HPMPC, FPMPA, PMPA (tenofovir) and many others, were synthesized by Holý, evaluated by me and my colleagues, submitted for patent protection (with the rights transferred to the Rega Foundation and IOCB) and publication. Meanwhile, an intimate link grew between A. Holý and myself, so that in 2005, one year before I was inevitably going to be relieved (because of my formal retirement at the age of 65 years) from all my duties, including research and teaching, Holý advised me to come to Prague for the remainder of my career (based on a personal letter of A. Holý to myself). I did not do it, but, nevertheless, I have continued my teaching in the Czech Republic (Česke Budějovice), on "Biochemistry at the service of medicine" till today. Regrettably, Tony Holý passed away on 16 July 2012, but Prof. Libor Grubhoffer has continued and even strengthened the intimate friendship I had initiated with Antonín Holý.

Part B

Erik De Clercq played a crucial role in the (co)discovery of several compounds that were eventually licensed for clinical use by the Rega Foundation:

1. The discovery of patentable compounds started with the search for interferon inducers of synthetic origin. This search was initiated with pyran copolymer by Dr T.C. Merigan (at Stanford U) and polyacrylic acid by Prof. P. De Somer and his co-workers at the Rega Institute.[1,2] It was highly stimulated by the discovery by Maurice Hilleman's group (at Merck) of the interferon inducing capacity of double-stranded RNAs such as poly(I).poly(C). We demonstrated that of the two components of poly(I).poly(C), the hypoxanthine-based strand [poly(I)] played the dominant role [3] in its interferon-inducing potential. At Stanford U, we discovered a new class of thiophosphate-substituted polynucleotides, i.e., poly (AsUs) [4] that was patented and licensed to Wyeth. It was developed for a short while, but then abandoned. At Merck, poly(I).poly(C) was, likewise, not further pursued for its potential medical application.
2. In the period 1972–1975, a post-doctoral investigator, William ("Bill") E. Stewart II, engineered a method to stabilize/regenerate interferon which was patented but not commercialized.[5] In the 1970s patent protection was also obtained for some interferon species, i.e., lymphoblastoid interferon, which were sublicensed to and temporarily investigated by some companies, but eventually discontinued for further development.
3. Through a collaborative study between the University of Leuven (E. De Clercq), the Pasteur Institute of Brabant, Brussels (Jean Content) and the laboratory of Walter Fiers (Ghent U), the molecular cloning and expression of human interferon-β, and, as a spin-off thereof, that of human interferon-β2 [later termed interleukin 6 (IL-6)] were achieved.[6–8] Human interferon-β has been commercialized and further used clinically in the treatment of multiple sclerosis (MS).
4. With the purpose to increase its aqueous solubility aminoacyl (i.e., glycyl, alanyl, …) esters of acyclovir were prepared, so as to improve its applicability, i.e., topically as eye drops (instead of eye ointment) and parenterally for intramuscular injection (instead of intravenous infusion.[9,10] Potential therapeutic applicability of the aminoacyl esters of acyclovir was patented, and this patent (owned by the Rega Foundation) was licensed to Burroughs Wellcome (BW), where they found that one of the aminoacyl esters (i.e., valyl ester) showed superior oral bioavailability compared to acyclovir. Consequently, this valyl ester of acyclovir (valacyclovir, Valtrex®, Zelitrex®) was substituted in 1995 for acyclovir in the oral treatment of HSV (herpes simplex virus) and VZV (varicella-zoster virus) infections, and for the period 1995–2002 the Rega Foundation received some remuneration from Glaxo Smith Kline (GSK) for the discovery of valacyclovir (Zelitrex®). This remuneration was based on the worldwide sales of valacyclovir, excluding the US sales. In 2002, valacyclovir became generically available.
5. In 1976 Erik De Clercq had started a collaboration with the University of Birmingham (UK) (R.T. Walker, P. Barr, A.S. Jones) that led to the discovery of BVDU [(*E*)-5-(2-bromovinyl)-2'-deoxyuridine, later also known as brivudin] as a highly potent and specific inhibitor of HSV-1 and VZV. Its antiviral potential was discovered shortly after that of acyclovir.[11,12] As compared to acyclovir, BVDU was slightly more potent against HSV-1, considerably less potent against HSV-2, but by several orders of magnitude more potent against VZV. This makes BVDU still as of today the drug of choice for the treatment of herpes zoster (shingles). From the beginning (1979), the Rega Foundation obtained patent rights on BVDU, which was first licensed to Searle (UK), and subsequently to Berlin-Chemie (which was then taken over by Menarini), before it became generic. That the compound, in its clinical development followed a meandrous route, involving the DDR (East Germany), may not be surprising as the East Germans working in Berlin-Buch, at a certain time (1975) had described the synthesis of BVDU, which they erroneously characterized as (*E*)-5-(1-bromovinyl)-2'-deoxyuridine.
6. The discovery of the inhibitory activity of AZT (2',3'-dideoxy-3'-azidothymidine) against HIV (then

called LAV/HTLV-III) in 1985, to be extended by the anti-HIV activity of other 2',3'-dideoxynucleoside analogues such as ddC and ddI, brought P. Herdewijn to the synthesis of 2',3'-didehydro-2',3'-dideoxythymidine (d4T, stavudine), which we (M. Baba et al.) found to be highly active against HIV in the MT-4 cells that appeared to be very permissive for HIV replication.[13] The paper had been received by the Editor of BBRC on 25 November 1986 and was published on 17 January 1987. In this interval, T.S. Lin and W.H. Prusoff must have filed a patent application on the anti-HIV activity of d4T, whereas we did so in January 1987. Six months after our publication, the publications of Hamamoto *et al*. (June 1987) in Antimicrobial Agents and Chemotherapy[14] and Lin *et al*. (September 1987) in Biochemical Pharmacology [15] appeared, confirming our earlier observations. Nevertheless, the patent on the anti-HIV activity of d4T was assigned to Yale University (Lin and Prusoff) and based on the agreement they made with Bristol-Myers (BM), the further clinical development of d4T, leading to the commercialization of Zerit®, was taken care of by BM. The involvement of the Rega Institute was hereby dismissed.

7. TS-II-25, the first of the NNRTIs (Non-Nucleoside Reverse Transcriptase Inhibitors) was identified in 1987, serendipitously, for its anti-HIV activity. It was sent to me by R.T. Walker (Birmingham U) with an accompanying letter that the compound (and several others) came from Showa U, where they had been synthesized by Hiromichi Tanaka in the laboratory of Tadashi Miyasaka. These compounds had all been synthesized with the hope that they would behave like acyclovir and might be active against HSV. I already knew that, unlike acyclovir (which is a purine derivative), pyrimidine analogues (like TS-II-25) would not be effective against HSV. Nevertheless, we evaluated them for their activity against HSV, and, surely enough, they were inactive. My task should have ended there, but then I saw M. Baba looking for work and I thought that after he had found d4T to be so active against HIV, it may be appropriate for him to find some negative controls. To our surprise, he found one of these pyrimidine derivatives (i.e., TS-II-25) to be active against HIV (at that time only HIV type 1 was evaluated), and we did not have the slightest clue of what the mechanism of action might be.[16,17] The compounds were baptized 1-[(2-hydroxyethoxy) methyl]-6-(phenylthiol)thymine (HEPT) derivatives. An agreement was concluded with Mitsubishi Kasei Corporation (MKC) which yielded some royalties to the Rega Foundation, and in the meantime, we found that the HEPT derivatives behaved akin to the TIBO derivatives in that they specifically interacted with an allosteric binding site of the HIV-1 reverse transcriptase.[18,19] Further optimization of the HEPT derivatives led to the identification of emivirine (MKC-442),[20] which was sublicensed from MKC to Burroughs-Wellcome (BW) and subsequently to Triangle Pharmaceuticals and, finally, Gilead Sciences. The compound then named Coactinon® had proceeded to phase III clinical trials, when it was discontinued primarily because of a too competitive perspective.
8. Through a collaborative study that was initiated between the Janssen Research Foundation (under impulse of Dr Paul Janssen) and the Rega Institute (not the Rega Foundation at the specific suggestion of Rector Roger Dillemans), a new class of anti-HIV agents were discovered that *ab initio* were identified as NNRTIs, the so-called tetrahydro-imidazo[45,1 -jk][1,4]-benzodiazepine-2(1H)-one and -thione (TIBO-) derivatives.[21] That these TIBO derivatives were specifically targeted at the HIV-1 reverse transcriptase was further ascertained in a subsequent investigation .[22] In attempts to improve the therapeutic applicability of the TIBO derivatives, various new analogues were developed that *via* the α-APAs ,[23] ATAs, DATAs, and DAPYs led to the identification of rilpivirine (TMC278, Edurant®, Rekambys®). Rilpivirine that had (almost) fulfilled Dr Paul Janssen's dream to develop the ideal compound for the treatment of AIDS (HIV infection) finally found its niche, in combination with Tenofovir disoproxil fumarate (TDF) and Emtricitabine, in the treatment of HIV infections: it was marketed in the US (Complera®) and the EU (Eviplera®).[24]
9. With the polyoxometalate HPA-23 as the starting point, in collaboration with Johnson Matthey (JM) Company, various polyoxometalates were examined (and found effective) as inhibitors of HIV replication.[25] In follow-up studies it was envisaged to use the monocyclam moiety as the organic support for binding the metal component, but a monocyclam preparation contained, as impurity, a bicyclam, yielding, unexpectedly, anti-HIV activity.[26] The original bicyclam molecule could not be resynthesized, but derivatives thereof, containing an aliphatic bridge tethering the two cyclam rings, afforded highly potent anti-HIV activity (at non-cytotoxic concentrations).[27] This molecule, originally dubbed JM3100, became known as AMD3100, after AnorMED (AMD) had been split off from JM. Initial mechanistic studies indicated that AMD3100 interfered with the viral entry process ("uncoating"), and its target was identified as the CXCR4, one of the co-receptors responsible for the cellular uptake of HIV.[28] Initial clinical studies then revealed that AMD3100 caused an increase in the white blood cell (WBC) count and, on closer inspection, this increased WBC count appeared to consist primarily of WBCs containing the CD34 marker, thus representing hematopoietic WBC stem cells. Hence, AMD3100, in the meantime also known as Plerixafor, through its

TDF	:	Tenofovir disoproxil fumarate					:	Viread®
TDF	+	Emtricitabine (Emtriva®)					:	Truvada®
TDF	+	Emtricitabine	+	Efavirenz (Sustiva®, Stocrin®)			:	Atripla®
TDF	+	Emtricitabine	+	Rilpivirine (Edurant®)			:	Complera®, Eviplera®
TDF	+	Emtricitabine		Elvitegravir	+	Cobicistat	:	Stribild®
TAF	:	Tenofovir alafenamide					:	Vemlidy®
TAF	+	Emtricitabine					:	Descovy®
TAF	+	Emtricitabine	+	Rilpivirine			:	Odefsey®
TAF	+	Emtricitabine	+	Elvitegravir	+	Cobicistat	:	Genvoya®
TAF	+	Emtricitabine	+	Bictegravir			:	Biktarvy®
TAF	+	Emtricitabine	+	Darunavir	+	Cobicistat	:	Symtuza®
TDF	+	Lamivudine					:	Cimduo™
TDF	+	Lamivudine	+	Efavirenz (600 mg)			:	Symfi™
TDF	+	Lamivudine	+	Efavirenz (400 mg)			:	Symfi Lo™
TDF	+	Lamivudine	+	Doravirine			:	Delstrigo™

Figure 5. Tenofovir formulations as TDF (tenofovir disoproxil fumarate) and TAF (tenofovir alafenamide). (to be continued)

interaction with CXCR4, counteracted the "homing" of hematopoietic WBCs in the bone marrow, allowing these cells to be released from the bone marrow into the blood circulation, where they could be diagnosed as stem cells. In this respect AMD3100 acted as a stem cell mobilizer, thus explaining the name of Mozobil® for Plerixafor. AnorMED has in the meantime been taken over by Genzyme, which is now incorporated into Sanofi. Mozobil® has been marketed for autologous transplantation of hematopoietic bone marrow stem cells, in patients with multiple myeloma (MM) or Non-Hodgkin's Lymphoma (NHL), and this in combination with GSF (Granulocyte Stimulating Factor). AMD3100 has been the subject of several review articles.[29–32]

10. Erik De Clercq met Dr Antonín Holý for the first time in Göttingen during a small conference (3–5 May 1976) organized by Karl-Heinz Scheit at the Max Planck Institut für Biophysikalische Chemie, where we decided to start a collaboration between our laboratory at the Rega Institute and Dr Holý's laboratory at IOCB (Institute of Organic Chemistry and Biochemistry) in Prague (Czechoslovakia). An agreement was signed in 1977 by Dr Karel Martinek for IOCB and Prof. P. De Somer for the Rega Institute. The initial collaboration concerned essentially three compounds, one of which [(S)-9-(2,3-dihydroxypropyl)adenine (DHPA)] turned out to be antivirally active. The antiviral activity of DHPA was patented, published ,[33] and the compound was marketed as Duviragel® by Lachema in Czechoslovakia for the topical treatment of herpes labialis ("cold sores"). The mechanism of action of DHPA was resolved [it appeared to be an inhibitor of S-adenosyl homocysteine (SAH) hydrolase], and several new analogues of DHPA were synthesized, but the major breakthrough followed in 1986 with the discovery of the broad-spectrum anti-DNA virus activity of HPMPA, PMEA [34] and various other acyclic nucleoside phosphonates

Figure 5. Continued - I

(ANPs).[35] HPMPA would never be commercialized for medical use, but PMEA (Adefovir) was marketed as its prodrug (Adefovir dipivoxil, Hepsera®) for the treatment of hepatitis B virus (HBV) infections, and the cytosine counterpart of HPMPA, namely HPMPC (Cidofovir, Vistide®), was approved by the US FDA in 1996 for the treatment of cytomegalovirus (CMV) retinitis in AIDS patients; it is now generically available for the treatment of various DNA virus infections, i.e., human papillomavirus (HPV) infections, against which HPMPC has proven to be effective anecdotally on a compassionate base.[36] While PMEA (Adefovir) was initially pursued for the treatment of HIV infections, it was not approved by the US FDA for this indication because, on the one hand, it was nephrotoxic at the contemplated dosage, and, on the other hand, a new ANP, namely PMPA, had become available, that in its prodrug form, Tenofovir disoproxil fumarate (TDF, Viread®) could be administered at a higher dosage to be active against HIV. The anti-HIV activity of PMPA had first been described in 1993 ,[37] but of crucial importance was the impressive effectiveness

Figure 5. Continued - 2

it had shown against simian immunodeficiency virus (SIV) infection in rhesus macaques, in a study of Tsai and colleagues in 1995 [38]; it would predict its prophylactic value in the prevention of HIV infection for which it would be approved (in combination with Emtricitabine) by the US FDA on 16 July 2012. For all ANPs, including Adefovir, Cidofovir and Tenofovir, an agreement was concluded between Bristol-Myers (BM), Rega and IOCB, that BM should further develop these compounds in compensation for a down payment and future royalties. Down payment was kept exceptionally low as a trade-off for future royalties resulting from prodrugs that may possibly emanate from licensed compounds. This means, in practical terms, that besides TDF, also future Tenofovir prodrugs such as TAF (Tenofovir alafenamide) would fall under the same royalty provisions as Tenofovir itself. Hence, all pharmaceutical combinations of either TDF or TAF with Emtricitabine, Sustiva, Rilpivirine, Elvitegravir, Cobicistat, Darunavir and Bictegravir would also fall under this rule. In 1991 BM merged with Squibb to become Bristol-Myers Squibb (BMS), and BMS decided to relinquish the development of all ANPs. The provisions stipulated in the agreement with BM were taken over without any change(s) by Gilead Sciences. The transition of TDF to TAF has been described previously.[39] There are now on the market, 15 formulations containing either TDF or TAF (Fig. 5).[40]

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