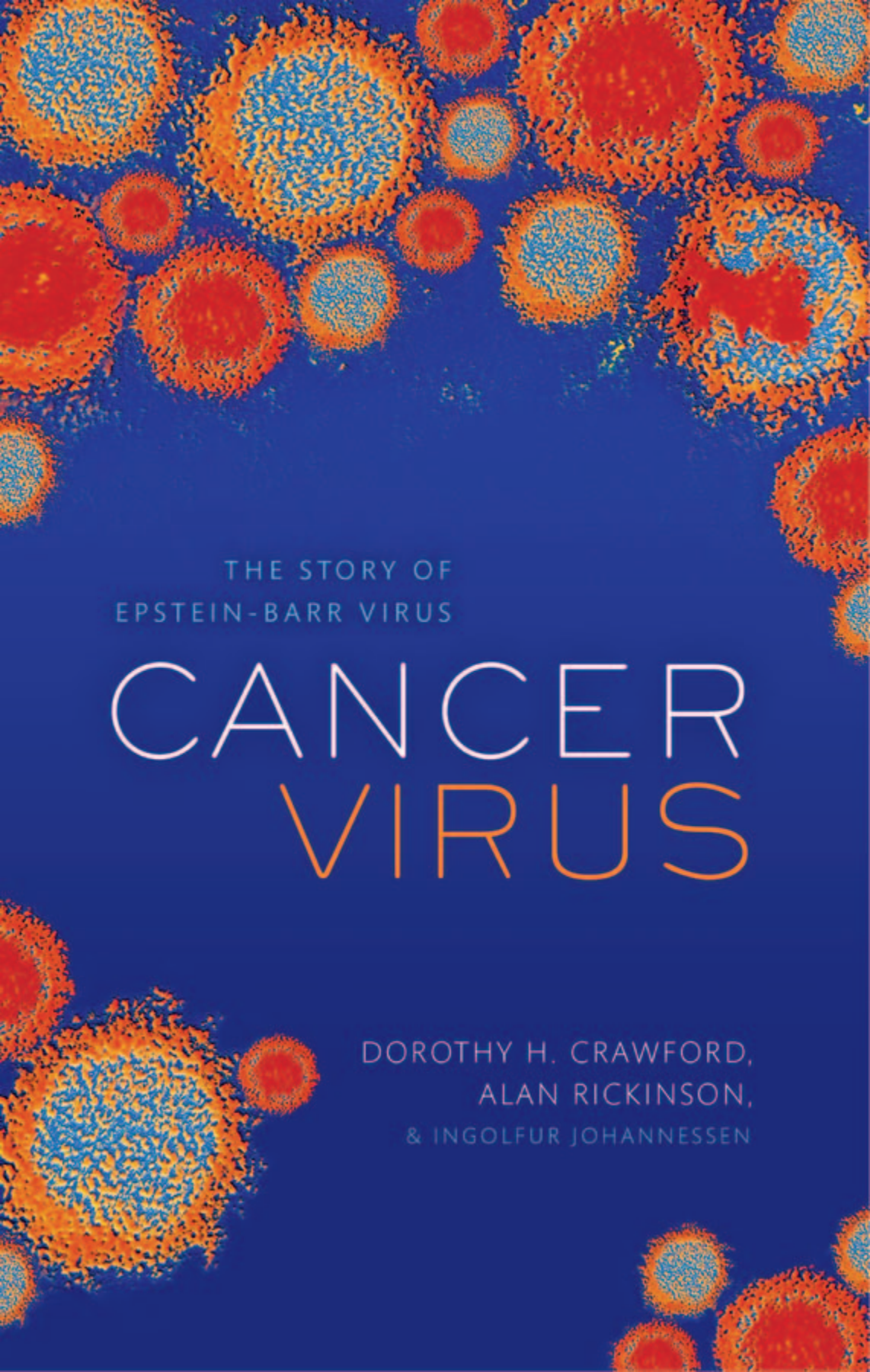

THE STORY OF
EPSTEIN-BARR VIRUS

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Cancer Virus records 50 years of scientific research on EBV since its discovery in 1964. Many thousands of scientists have played their part in this journey of discovery—too many to name here but we thank them all. We are particularly indebted to those who contributed interesting stories and quotes for the book, and to the following who gave their time to reminisce about their work on EBV: Arthur Ammann, George Ball, Guy de Thé, Volker Diehl, Anthony Epstein, Paul Farrell, Jim Jones, Hideaki Kikuta, Eva Klein, George Klein, Ian Magrath, Denis Moss, Dennis Wright, Zeng Yi, John Ziegler, and Harald zur Hausen.

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PREFACE

With the fiftieth anniversary of the discovery of the first human tumour virus looming, we realized that the story of the discovery of Epstein-Barr virus (EBV) and the contents of Pandora's box that this revealed have never been related to a general audience. Aimed at the interested lay person, *Cancer Virus* takes the reader on a journey from the first key observation of a unique type of tumour in a Ugandan hospital in the 1950s to the landmark discovery of a virus in this tumour in a research laboratory in London in the 1960s. We then travel on to the US, Europe, Australia, China, and Japan, making more discoveries relating to this virus. Along the way we meet the key players involved, starting with Denis Burkitt, the Irish surgeon who was working in Uganda when he noticed a previously unrecognized but extremely common childhood tumour now called Burkitt Lymphoma. It was from the cells of this tumour that Anthony Epstein, a research virologist, and Yvonne Barr, a research assistant at the Middlesex Hospital in London, isolated the cancer virus of our title that now bears their names.

Since then the quest to prove that EBV is indeed a cancer virus, and to understand how those cancers arise, has involved many fascinating characters and several twists of fate. En route, even more EBV-related diseases, including several different types of cancer, have come to light. The discovery of EBV and the work that it inspired opened the door to a new science—the link between viruses and human tumours. Today this is a hugely important area of medical research involving thousands of clinicians and scientists across the fields of cell and molecular biology, genetics, epidemiology, immunology, and vaccine design.

All three authors of *Cancer Virus* are, or have been, EBV researchers who have watched its history unfold. The story in the book is our interpretation of the key events in that history. Inevitably many essential findings, the building blocks that set the stage for these events and the individual researchers who made those findings, have been glossed over in the interests of brevity; we apologize if this causes offence. In the division of labour, Dorothy Crawford and Alan Rickinson wrote the text while Ingólfur Johannessen carried out extensive interviews with key EBV researchers.

As far as possible we have avoided using technical terms but inevitably some have crept in that were essential for a clear understanding of the research that generated certain important discoveries. These terms are explained in the text, and there is also a glossary of terms at the end of the book that contains more detailed information. There is a simple timeline of key discoveries at the end of the book.

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Introduction

London in the early 1960s: change was in the air. As the decade unfolded, the young embraced a future seemingly full of possibilities. Artists, musicians, and designers flocked to the city and 'The Swinging Sixties' was born.

Less trumpeted, yet just as important, a scientific revolution was also under way, with London as an exciting and innovative international centre for medical research. Among the many scientific breakthroughs of the time, there was one that epitomizes the spirit of the age. An idea that was original, iconoclastic, and *so* contrary to conventional wisdom that it had to fight for acceptance all the way. Yet it had a kernel of truth that, 50 years later, has come to full fruition.

The idea was that certain types of human cancer, one of the most feared diseases, might be caused by a virus infection. Everyone was familiar with viruses causing acute diseases like influenza or measles from which most people soon recover. But it seemed fanciful that a virus, just a tiny, inert particle, described by Sir Peter Medawar as 'a piece of bad news wrapped up in protein',[1] could also cause a chronic, progressive, life-threatening illness like cancer—a disease that everyone knew was not infectious.

That kind of conventional wisdom did not deter Anthony Epstein, a medical researcher working at the Middlesex Hospital Medical

School. He was fascinated by earlier reports of 'infectious agents' causing cancer in certain laboratory animals, reports that many dismissed as rare curiosities with no general relevance to humans. But if under special circumstances viruses could cause cancer in animals, then why *not* in humans?

Epstein had trained at the Rockefeller Institute in New York, where scientists were beginning to look at cells with the electron microscope. With this instrument, much more powerful than a conventional light microscope, researchers could see a whole new world of sub-cellular structures, including viruses. Epstein reasoned that if some human cancers were caused by viruses he might be able to see them in the cancer cells using an electron microscope.

That was all well and good, but where to start? There are many types of cancer, probably all caused by different combinations of factors, so how to find one that might, just might, involve a virus?

At this point one of those coincidences occurred that lie at the heart of so many scientific breakthroughs. A doctor from Mulago Hospital in Uganda, Denis Burkitt, was visiting London in 1961, lecturing about a childhood cancer, later to become known as Burkitt Lymphoma. He had first seen a child with this tumour during his daily work in Uganda, but at the time it was unknown in the West. Burkitt then went on to show that this cancer was common in children throughout sub-Saharan Africa, but only in areas with high rainfall and high temperature. When Epstein heard Burkitt's lecture at the Middlesex Hospital he immediately thought 'A cancer which is restricted by temperature and rainfall: that's where to look for a cancer virus!'

From that chance encounter came the discovery, by Epstein and his research assistant, Yvonne Barr, of the virus that is now called the Epstein-Barr virus (EBV). They did indeed find the virus in Burkitt Lymphoma cells by electron microscopy—the first human virus ever to be discovered by that technique, and the first human cancer virus.

Today we know that EBV is not just linked to Burkitt Lymphoma but plays an important part in the development of at least five other

types of human cancer. Many of these occur worldwide but, like Burkitt Lymphoma, with varying frequencies in different human populations. Interestingly, as well as being a cancer virus, EBV also causes the infectious disease, infectious mononucleosis (or glandular fever), which is common in the affluent Western world yet rare elsewhere. The same virus is now increasingly being linked to another disease with a similar preponderance for affluent societies, namely multiple sclerosis.

Who would have thought that a virus first seen under the electron microscope in a cancer from an African child would have such worldwide significance? *Cancer Virus* traces the story of EBV from those early days in sixties London to the present time, a journey full of surprises, setbacks, and personal drama, and a real insight into how circumstances and chance can shape the world of scientific discovery.

1

Out of Africa

The trail that led to the discovery of the first human tumour virus began in 1957 when Denis Burkitt, an Irish surgeon working in Uganda, identified the tumour that now bears his name—Burkitt Lymphoma. His meticulous descriptions of the tumour's remarkable clinical aspects and his tenacious pursuit of its geographical distribution made for a fascinating story that caught the attention of the researchers who eventually found the virus. This discovery heralded a profound change in thinking about the causes of cancer worldwide. Since 1964 several other human cancer-associated viruses have been identified, so that we now know that viruses are involved in the causation of around 10 per cent of all cases of human cancer.

At the height of his fame in the 1970s and 1980s, Burkitt (Figure 1) was feted the world over. Showered with prestigious prizes and medals, awarded numerous honorary degrees and fellowships, he was in constant demand to give lectures—always delivered with humour, panache and the timing of a natural actor. Yet Burkitt came from a very different background; a small town in rural Ireland. He was born in Enniskillen, County Fermanagh, Ireland, in 1911, the elder son of James Burkitt, from Donegal, and Gwendoline Hill from County Cork. James Burkitt was the County Surveyor for Fermanagh, but obviously had a natural interest in biology, and in the end was better known for his hobby—bird watching. He was the first to ring birds so that each

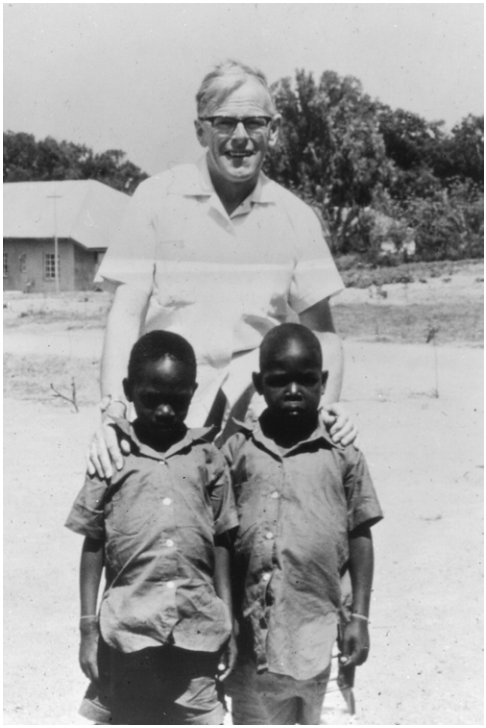

FIGURE 1 Denis Burkitt with two young patients

could be identified and studied in detail. He spent years observing the goings-on of individual robins in his 10-acre garden: mapping their territories, tracking their movements, and spotting their nest sites. In a lifetime of close scrutiny he discovered that each robin has its own territory that is so jealously guarded that fights against imposters are not unusual, sometimes even ending in death for one of the combatants. Burkitt senior was the first to show that these colourful and supposedly friendly little birds are in fact the bullies of the garden's avian community, and in doing so he became a legend in the world of ornithology.[2] It was this same attention to detail that led his son Denis to make his own ground-breaking discoveries.

Within the family home two strong influences clearly laid the foundations for the young Burkitt's future life. The first of these was service to the British Empire where Burkitt had three paternal uncles living and working: one was a surgeon in East Africa, while the other two were in India, where one became Governor of Madras, the other Chief Engineer to the Punjab. While on leave back home these uncles must have regaled the family with stories from far-flung lands that inspired Burkitt as he grew up. On one occasion the surgeon uncle removed Denis' and his brother Robin's tonsils and adenoids on the kitchen table—clearly not an event that put them off medicine as both boys later became doctors.

The second enduring influence was that of religion. Both Burkitt's parents had a strong Christian faith and this influence obviously took root in the young Burkitt. To quote from his obituary: 'An unshakable belief in divine guidance and the efficacy of prayer lasted from these early days throughout Denis Burkitt's life and resulted in daily bible reading, the display of biblical texts in each of his successive offices, and abstinence from alcohol.'[3]

In 1929, Burkitt entered Trinity College Dublin to study engineering. But during his first year he believed that he had received a divine calling to become a doctor and so switched to studying medicine. After qualifying in 1935 he went to England to train and practise as a surgeon. Soon after war broke out in 1939 Burkitt was similarly moved to join the British army. So in 1940 he applied to join the Royal Army Medical Corp, but at that time they did not need surgeons and so his application was rejected. However, in early 1941 Burkitt again applied but was again rejected, this time because an accident at school at the age of 11 had left him with only one eye—a handicap that he overcame so effectively that few even suspected it. He was eventually accepted by the Medical Corp in May 1941 and spent the next two years training at army hospitals in the south of England.

Then in 1943 Burkitt was posted to East Africa where he served with African troops in Kenya and Somaliland. While on leave he

visited friends in Uganda and here he decided that his life's vocation was 'in helping the people of Uganda both medically and spiritually'.[4] So it was that after demobilization in 1946 Burkitt joined the Colonial Service and six months later he was on his way to Lira Hospital in Lango District, Uganda, leaving his wife, Olive, at home in England, pregnant with their first child.

Burkitt boarded a ship bound for the port of Mombasa in Kenya, and then took the train to Kampala, capital of Uganda. From there he travelled by pickup truck to Lira, a journey of some 220 miles. Six months later Olive undertook this arduous journey with their baby daughter. When she arrived at Lira she had to adapt to the primitive living conditions—no piped water, a bucket served as a toilet, and light was provided by hurricane lamps. But she apparently made the best of it and soon made friends. Even in this remote place the British had all the usual traditional expatriate entertainments—golf, tennis, coffee mornings, and evenings at 'the club'.[5]

Burkitt, describing himself as a 'simple bush surgeon', ran a 100-bedded bush hospital in Lira assisted by just one African doctor. The workload was enormous: in the first year alone he increased the number of surgical operations from 17 to over 600. He and his family were happy in Lira, but had been there for less than two years when, in 1948, he received a telegram informing him that he had been posted to Mulago Hospital, the Makerere University College teaching hospital in Kampala. At the time, this hospital was expanding rapidly and the workload was even heavier than at Lira. Burkitt headed one of three surgical units and during his first 10 years at Mulago Hospital he made many improvements to the surgical services. However, it was in 1957 that a chance observation changed the direction of his career from 'simple bush surgeon' to medical superstar.

This transformation began when Hugh Trowell, an eminent physician and head of medical services at Mulago Hospital, asked Burkitt, as the duty surgeon at the time, to look at a patient who was puzzling him. This was a 5-year-old boy with swellings of his jaws. Many years later Burkitt recalled: 'His face was massively swollen, with bizarre lesions

involving both sides of his upper and lower jaws. I had never seen anything like it. The teeth were loose and the features grossly distorted.'[6] Burkitt knew that single jaw tumours were common in Ugandan children (Figure 2), and thought to be some kind of sarcoma—that is a tumour of soft tissue such as muscle or nerve. Indeed he had operated on several of these jaw tumours, although he had never followed them up to find out how they fared. But he could shed no light on the diagnosis in this case because of the involvement of all four quadrants of the jaw. Thinking that it must either be an infection or a very unusual tumour, he took a biopsy, but unfortunately this too proved inconclusive. So, having made detailed notes on the case and taken photographs of the child's swollen face, Burkitt considered it to be 'another of the curiosities one had to become accustomed to seeing from time to time in Africa'.[7]

And this is where the story might have rested had Burkitt not visited the district hospital at Jinja on the shores of Lake Victoria a few weeks later. Here, while doing a ward round, he happened to glance

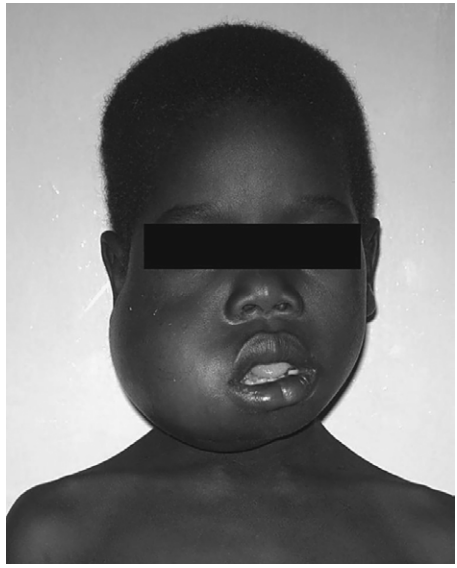

FIGURE 2 A typical case of Burkitt Lymphoma

out of the window and saw a small boy with a similar grossly swollen face sitting on the grass beside his mother. 'My interest was rivetted immediately. A curiosity can occur once, but two cases indicate more than a curiosity.'[8]

Now Burkitt's attention was truly aroused. Perhaps like his father with the robins, he would not rest until he had got to the bottom of the puzzle. He examined the second child thoroughly and found that, in addition to the jaw lesions, he had a lump in his abdomen. Burkitt immediately asked himself—could this be linked to the jaw swellings? To find the answer Burkitt pored over old hospital case notes and autopsy reports. Surprisingly, this revealed that the jaw swellings were frequently associated with tumours in a wide variety of organs, commonly the kidneys, ovaries, adrenal glands, and less often, the liver. Other tumour sites included the eyes, orbits, testes, thyroid gland, and long bones. On occasions the spinal cord was involved, causing paraplegia. The link between all these tumours had previously been missed because, while the jaw tumours were labelled sarcomas, the others were diagnosed as tumours common to the organs in which they arose. For example those in the eye were labelled retinoblastomas, those in the bones osteosarcomas and so on.

Burkitt's first breakthrough was the hunch that these disparate tumours were all part of the same disease. With this realization, he wrote his first scientific paper on the subject, describing 38 patients seen at Mulago and surrounding district hospitals. The report entitled *A sarcoma involving the jaws in African children* was published in the *British Journal of Surgery* in 1958.[9] In the publication he described cases with single or multiple tumours in the jaw and co-existing abdominal tumours, and discussed their bizarre presenting symptoms. With regard to the multiple jaw tumours, he thought it unlikely that one of the masses represented the primary tumour that had subsequently spread to the other affected sites because no known mode of spread existed between the four quadrants of the jaw. Equally, spread from a primary tumour in the jaw or abdomen to multiple abdominal

organs was very unusual. In fact most other tumours first spread to local lymph glands, but with this tumour these glands were very rarely involved. So the question of how this clinical presentation came about left Burkitt mystified.

Burkitt's report attracted very little attention at the time, probably because it was published in a specialist journal read mainly by surgeons. However, several years later it became a citation classic. His discovery generated a host of new questions in Burkitt's mind: what was the nature of the disease and where was the primary tumour? How common was it? Was it a new disease or just newly recognized? With the help of colleagues, Burkitt wasted no time in finding answers to these questions.

First, Gregory O'Conor and Jack Davies, both pathologists at Mulago Hospital, re-examined tumours from all the different sites microscopically, including those in the jaw. They concluded that the tumours were all identical and classified them as lymphomas, in other words tumours that arise from white blood cells called lymphocytes. O'Conor and Davies described tumour tissue composed of malignant lymphocytes interspersed with larger, paler staining cells called histiocytes. Under the microscope this pattern resembled a 'starry sky'—a term that is still used to describe the unique appearance of the tumour today.[10] Thereafter the disease became known as the 'lymphoma syndrome' or the 'African lymphoma'. O'Conor and Davies thought that the jaw tumours started growing in bone marrow lymphoid cells inside the jaw bone near developing teeth and spread to other sites from there.

In 1963 another British pathologist, Dennis Wright, began to study the lymphoma syndrome. Wright had been training as a pathologist in England when, in the early 1960s, he knew that he was about to be called up for military service. This he was not keen to do as it would not only separate him from his wife and family but also stall his career in pathology for several years. So when a colleague suggested that he apply for the post of lecturer in pathology at Makerere University, and the army agreed to a stint in Uganda in lieu of National Service, he

jumped at the chance. He and his family arrived in Kampala in 1960. At the hospital he found O'Conor enthusiastically working on the lymphoma syndrome while he himself was supposed to be working with Davies on quite a different disease common in Africa at the time—a heart condition called endomyocardial fibrosis. However, the funding for the fibrosis study never materialized and soon after, when both O'Conor and Davies left the hospital, Wright became the resident expert. At this point the lymphoma was thought to be an odd manifestation of other known lymphomas but Wright's work changed all that. He decided to apply some special techniques to pin down the true nature of the tumour cells. For this he had to collect fresh tumour biopsies directly from the operating theatre where Burkitt was working. Then, instead of dropping the material into formalin for permanent preservation as was the usual practice, he made imprints, or touch preparations. These involve gently touching the biopsy on a glass slide thereby depositing some tumour cells onto the slide. The cells could then be stained with dyes, so avoiding the usual fixing, wax embedding, and cutting of the tissue, all of which distort the cell architecture. Using this technique, Wright was able to report that the cell structure of the lymphoma, along with its starry sky appearance, differed from all other lymphomas known at the time.[11] Shortly afterwards, in recognition of its unique characteristics, the tumour was renamed 'Burkitt Lymphoma'.

Burkitt answered the question of how frequently the tumour occurred by scrutinizing hospital case records. He found that the lymphoma was surprisingly common, with an incidence of up to 18 per 100,000 children per year. This made it the most common tumour in children attending the Mulago Hospital, comprising over half of all childhood cancers seen there. It occurred between the ages of 6 months and 14 years, with a peak incidence of between 5 and 6 years. The tumour was seen two to three times more often in boys than girls, was remarkably fast growing, and rapidly fatal. Indeed, Wright and colleagues showed that the number of cells doubled every one to two days,[12] an incredible rate that makes Burkitt Lymphoma the fastest-growing human tumour known.