Review

From chemotherapy to signal therapy (1909-2009): A century pioneered by Paul Ehrlich

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ABSTRACT: Paul Ehrlich (1854-1915), a German microbiologist who was awarded a 1908 Nobel Prize in Physiology/Medicine for his pioneer work on the antibody production, pioneered the modern chemotherapy by discovering his magic bullet for syphilis, called "606" or "Salvarsan" in 1909 with a Japanese young scientist, Sahachiro Hata (1873-1938) from "Denken" (Institute for Infectious Diseases, now called IMS for Institute for Medical Sciences) in Tokyo. His magic bullet was used to eradicate syphilis for more than a half century until a more safe and effective antibiotic called "Penicillin" was introduced to this world towards the end of WWII by Howard Florey (1898-1968).

Celebrating this year the 100th anniversary of his discovery, this brief review will discuss how Ehrlich, now known as the Father of Chemotherapy, managed to design the first effective therapeutic for this then formidable sexually transmitted disease, which is equivalent to AIDS, HIV-infection, in the present century, and how so many new chemotherapeutics have been successfully developed during the past 100 years for other formidable diseases such as cancers and AIDS by his followers (microbe hunters and oncogene hunters) such as Alexander Fleming (1881-1955), Hamao Umezawa (1914-1986) and Brian Druker, culminating in the first signal therapeutics of cancers such as "Gleevec" that block the oncogenic signaling, around the turn of this century.

Keywords: Paul Ehrlich, chemotherapy, signal therapy

1. Ehrlich's Magic Bullet

More than a century ago there were three giants in modern medicine who fought against infectious diseases in Europe. Louis Pasteur in France, and Robert Koch (1843-1910) and Paul Ehrlich in Germany. Pasteur developed a vaccine against rabies, and Koch discovered a bacteria which causes TB (tuberculosis), and Ehrlich developed the first therapeutic for syphilis. These three giants are the major figures in the best-selling book "Microbe Hunters" published by Paul de Kruif in 1926 (*1*). Ehrlich was the anchor of 14 microbe hunters in this book. He developed not only an effective antiserum against diphtheria with Emil von Behring (1854-1917), but also pioneered the chemotherapy by developing the first modern chemical medicine, "Salvarsan" for syphilis (*2*). His scientific life was featured in 1940 MGM film "Dr. Ehrlich's Magic Bullet" which I have treasured since my youth (Figure 1).

He was born on March 14, 1854, between Jewish parents in Silesia then a part of Germany, but now in the territory of Poland. In terms of science, the most influential member of his family was his elder cousin Carl Weigert who introduced the young Ehrlich to histochemistry, staining of tissue specimens by aniline dyes. Through this histochemistry, Ehrlich learned that each chemical shows a specific affinity for a certain tissue or bacteria. Since then he became obsessed with dye-staining for the rest of his life. Soon he developed a new concept, no reaction without binding. Based on

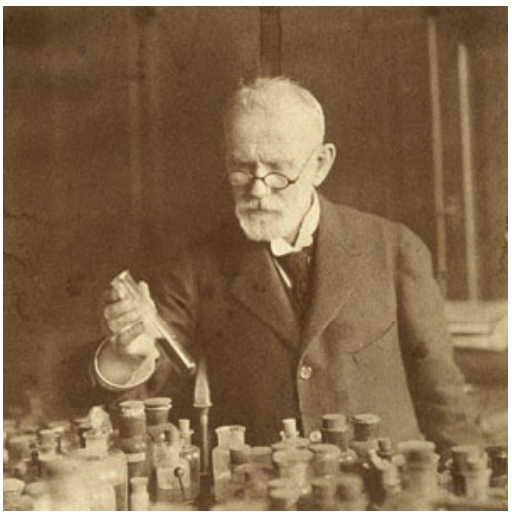

Figure 1. Dr. Paul Ehrlich (1854-1915). Father of Chemotherapy discovering "Salvarsan".

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the specific binding of a given dye to a certain tissue or bacteria, he began cherishing an idea that if a given dye having a specific binding site for a certain pathogen is linked to a toxic side chain, this chimeric dye could kill this pathogen selectively without harming any tissues of our body. He called such a dye "magic bullet", and making such a dye became his life dream.

Shortly after he developed a new staining method for identifying TB bacillus for Robert Koch, he realized that he was contracted with TB himself, and spent a few years with his beloved wife Hedwig in Southern Europe and Egypt for TB therapy. Around 1892, he joined a newly founded Koch's Institute of Infectious Diseases in Berlin, and helped his old friend Emil von Behring develop the effective antiserum from houses against diphtheria. In 1899, a few years after their great success in anti-diphtheria serum therapy, Ehrlich was awarded a new institute in Frankfurt, and later the 1908 Nobel Prize in Medicine, while Behring was given another institute in Marburg, and the 1901 Nobel Prize in Medicine.

At Ehrlich Institute in Frankfurt, he started developing the magic bullet from aniline dyes for sleeping sickness and syphilis. With a young Japanese scientist Kiyoshi Shiga (1871-1957), Ehrlich discovered "Trypan Red" as an effective therapeutic for sleeping sickness caused by a trypanosome in 1903. Then his team started focusing their effort on syphilis which is caused by a bacterium called spirochete. In this team, two young scientists played the major role. The German organic chemist Alfred Bertheim (1879-1914) who synthesized a series of aniline dye-arsenate compounds, and the Japanese microbiologist Sahachiro Hata who assayed the anti-syphilis potential of each dye-arsenate compound, using animal models. In 1909, they discovered that their 606th compound turned out to be the most potent anti-syphilis drug in animal models. It was a hydroxyl aniline-arsenate compound, later called "Salvarsan" which was mass-produced by the pharmaceutical company "Hoechst" in Frankfurt for clinical trials and therapy worldwide. The dye selectively binds the pathogen "Spirochete" and the arsenate kills this parasite.

2. Beyond "Salvarsan"

After the death of Paul Ehrlich, the father of chemotherapy, several scientists followed his foot steps. One of them was Gerhard Domagk (1895-1964), a German pathologist and bacteriologist who developed "Sulfonamidochrysoidine" (KI-730), the first commercially available antibacterial drug (the brand name "Prontosil Red"), for which he received the 1939 Nobel Prize in Medicine (*3*).

Domagk studied medicine at the University of Kiel, but volunteered to serve as a soldier in World War I, where he was wounded in December 1914, working the rest of the war as medic. After the war, he started working at the University of Greifswald, where he studied infections caused by bacteria. In 1925, he followed his professor Walter Gross to the University of Muenster, and became professor there. He also started working at the Bayer laboratories at Wuppertal, as the director of Bayer's Institute of Pathology and Bacteriology, where he continued the studies of Josef Klarer and Fritz Mietzsch, based on works by Paul Ehrlich, to use dyes, products of IG Farben, as antibiotics. He found the sulfonamide "Prontosil" to be effective against streptococcus, and treated his own daughter with it, saving her the amputation of an arm.

In 1939, Domagk was announced to receive the Nobel Prize in Medicine for this discovery. However, he was forced by the Nazi regime to refuse the prize and was arrested by the Gestapo for a week. Sulfonamides became a revolutionary weapon at the time, surpassing phage therapy, but were later replaced by "Penicillin", which showed both better effects and fewer side effects (sulfonamides can cause kidney stones and changes in bone marrow). Domagk's work on sulfonamides eventually led to the development of the anti-TB drugs thiosemicarbazone and isoniazid, which helped to curb the epidemic of TB which swept Europe after World War II. Eventually, after the war, in 1947, Domagk was able to receive his 1939 Nobel Prize.

3. The first natural antibiotics

After Domagk's work, the majority of anti-bacterial drugs were developed from natural (bacterial/fungal) products called antibiotics. One of the first effective antibiotics was "Penicillin" which was discovered by Alexander Fleming (1881-1955) and further developed by Howard Florey (Figure 2) and Ernst Chain (1906-1979). These three scientists shared the 1945 Nobel Prize in Medicine (*4*).

Figure 2. Dr. Howard Florey (1898-1968). Developing "Penicillin" (Australian $50 Note).

In 1928, Fleming was working in London on the bacteria "Staphylococcus", and noticed a bacteria-free circle around a blue fungus colony which was a contaminant in this bacterial plate. During his further study, he found that an extract from this mold kills the bacteria, and he called this substance "Penicillin". However, he did not expect this antibiotic to be developed as the major therapeutic for bacterial infection later.

It was Howard Florey, an Australian scientist working at Oxford University, who realized the great potential of "Penicillin" and developed it for clinical application during the WWII. In 1939, when the war broke in Europe, he started focusing his effort on the mass-production of "Penicillin" by fermentation of this blue fungus, in an attempt to treat so many wounded British soldiers during the war. Ernst Chain, a Jewish-German scientist born in Berlin, who left Nazi Germany for England, joined Florey's team at Oxford for the mass-production and purification of "Penicillin", in collaboration with three American companies. This "miracle drug" eventually would replace both "Salvarsan" and "Prontosil Red", because of its less side-effects and more potency against syphilis and other bacterial infection in general after the end of WWII.

The great success of Penicillin was followed by a flood of new anti-bacterial antibiotics, such as Streptomycin (1943), Chloramphenicol (1945), Tetracyclin (1947), *etc*. during 1940s. However, no anti-viral antibiotics were discovered, and instead a few vaccines such as anti-polio (1955) were developed by Jonas Salk (1914-1995), the founder of Salk Institute in San Diego (*5*).

4. Anti-cancer antibiotics: DNA/MT poisons

A success by a series of anti-bacterial antibiotics would be followed by the discovery of several anti-cancer antibiotics in 1950s-1960s. One of them was Mitomycin C, which was discovered in 1955 by Toju Hata (1908-2004) of Kitasato Institute, a son of Sahachiro Hata. Hamao Umezawa of Tokyo University, who discovered the anti-bacterial antibiotic Kanamycin in 1956, also discovered another anti-cancer antibiotic called "Bleomycin" in 1965 (*5*). These anti-cancer antibiotics as well as chemically synthesized anti-cancer drugs such as Cisplatin (1978) and 5FU (1950s) are so-called DNA/RNA poisons which block DNA/RNA synthesis. Another series of anti-cancer antibiotics such as Vinblastin (1958) and Taxol (1977) are so-called MT (microtubule) poisons which block spindle formation during cell division. These conventional DNA/MT poisons are effective to inhibit the growth of fast-glowing cancers such as leukemias, but not slow-growing tumors such as NF (neurofibromatosis) as well as melanomas, gliomas, pancreatic and lung cancers, and are quite toxic even for normal fast-growing cells such as bone marrow, hair follicle, and digestive duct cells, causing a series of side effects such as suppression of immune system, hair loss and intestinal inflammation.

5. Signal therapeutics (STs) as new anti-cancer drugs

These serious side effects could be avoided or minimized if anti-cancer drugs are developed on the basis of blocking the very cause of cancers, instead of DNA/MT inhibition which would affect the growth of both normal and cancer cells. These conventional anti-cancer drugs (DNA/MT poisons) were screened by their ability to inhibit the growth of Ehrlich's ascites tumor (a mammary gland tumor in mice developed by Paul Ehrlich) and other cancer models in which the genetic cause of carcinogenesis is totally unknown. In 1976, however, a group of oncogene hunters led by Mike Bishop and Harold Varmus at UCSF revealed for the first time that an oncogene (vSRC) in chicken retrovirus (oncogenic RNA virus) called Rouse sarcoma virus is a mutant of a normal cellular (proto-onco) gene (cSRC). This mutation causes an abnormal (constitutive) activation of the Tyr-kinase SRC which phosphorylates Tyr residues of its target proteins. In other words if one can develop a specific inhibitor for vSRC, one could treat cancers caused by vSRC, without affecting the normal cell growth. This discovery opened the entirely new avenue to both understanding of carcinogenesis and therapy of cancers. For this discovery both Bishop and Varmus shared the 1989 Nobel Prize in Physiology/Medicine. I should point it out that a Japanese virologist Hidesaburo Hanafusa (1928-2009) at Rockefeller University contributed greatly to the epoch-making discovery of the first oncogene SRC, and was awarded the 1982 Lasker Award.

Since then more than 100 oncogenes such as RAS and ABL and more than 50 tumor suppressor genes such as p53, RB, NF1 and NF2 were hunted down (cloned). It is now generally accepted that cancers (malignant tumors) are caused by either gain-of-function mutation of proto-oncogenes or loss-of-function of tumor suppressor genes or by a combination of some of these mutations. Interestingly, the majority of these proto-oncogene or tumor suppressor gene products are signal transducers which control the growth of normal cells, positively or negatively, respectively. Among these oncogene products Tyr-kinases such as SRC, ABL and ErbB1 (EGF receptor) kept drawing the first attention from a new generation of anti-cancer developers (so-called "signal therapeutic hunters").

So far the most successful signal therapeutic (ST) was a Tyr-kinase inhibitor called STI-571 or "Gleevec" which was created in 1996 by Novartis' team and further developed by Brian Druker (Figure 3) of OHSU, Oregon Health & Science University (*6*). Gleevec inhibits selectively three Tyr-kinases, ABL, PDGFR

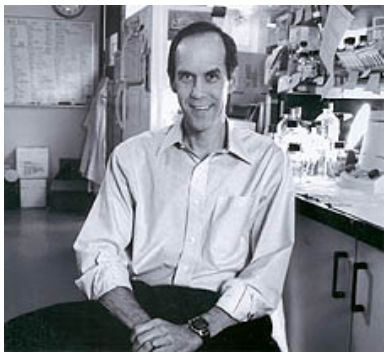

Figure 3. Dr. Brian Druker at OHSU. Developing "Gleevec" for CML & GIST.

(PDGF receptor) and KIT. Gleevec turned out to be the most effective in the treatment of at least two rare cancers CML (Chronic myelogenous leukemia) and GIST (gastrointestinal stromal tumor). CML is caused by an accidental fusion of two genes, ABL and BCR, by a chromosomal translocation that abnormally activates the Tyr-kinase ABL. GIST is caused by abnormal activation of another Tyr-kinase KIT. Since Gleevec inhibits both ABL and KIT, it was approved by FDA in 2001 as the first ST for the treatment of cancers, namely CML and GIST. Unlike the conventional anti-cancer drugs such as DNA/MT poisons, Gleevec causes no serious side effects such as immuno-suppression and hair loss.

6. Future perspective for anti-cancer STs

Unfortunately, however, Gleevec can be used only for these very rare cancers which represent less than 0.1% of all human cancers. The remaining vast majority of cancers should be treated by a much more general ST which would block the major oncogenic signal transducer(s) essential for the growth of majority of cancers such as breast, prostate, colon, lung, ovarian, cervical, and pancreatic cancers as well as melanomas, gliomas, MM (multi-myeloma), and NF tumors. We and others found recently that the kinase PAK1, a Rac/CDC42-dependent Ser/Thr-kinase, is essential for the growth of these cancers/tumors which represent more than 70% of all human cancers, but not for the normal cell growth (*7,8*). Furthermore, this kinase is required for both metastasis of cancers and angiogenesis (blood vessel formation) which is essential for the growth of solid tumors (*8*). In other words anti-PAK1 drugs (synthetic chemicals or natural products) would be a much more general ST, a magic bullet, that would be useful for the treatment of these PAK1-dependent solid cancers/tumors in the future.

During past several years we have identified and developed a series of anti-PAK1 drugs such as FK228 and Bio 30. Among them FK228, a ring peptide antibitotic developed by a Japanese pharmaceutical company called "Asteras" (formerly "Fujisawa") in 1994, is the most potent. However, it is still in clinical trials (phase 2) for only CTCL (cutaneous T-cell lymphoma), and is not available on the market as yet (*7,8*). Bio 30 is a water-miscible CAPE-based extract of NZ (New Zealand) propolis which is inexpensively available on the market (*8,9*). CAPE (caffeic acid phenethyl ester) is its major anti-cancer/anti-PAK1 ingredient and works synergestically with a few other anti-cancer ingredients of Bio 30 (*8,9*), and originally found in propolis sample from Israel in 1988 by Dezider Grunberger of Columbia University (*10*). NZ propolis is the richest in CAPE (6-7% of dry weight). Propolis is a honey bee product which has been used as an antibiotic for the treatment of various infection and inflammation, and preparation of mummies since the ancient Egypt for several thousand years. Thus, propolis is a unique ST for cancers and NF as well as several other diseases including bacterial/viral infection such as AIDS, inflammation such as arthritis and asthma, and neurodegenerative diseases such as Alzheimer (AD) and Huntington's (HD), epilepsy, and malaria (*8,9*). It causes no side effect.

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