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Review Article

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A Review on Magic Bullets

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Abstract

Magic bullets are the agents that cures or remedies by attacking a specific disease without causing harmful side effects. German medical scientist Paul Ehrlich (1854–1915) used the expression "magic bullet" to describe a chemical substance with the potential to cure a wide range of diseases. Magic bullet is a therapeutic agent, which is to be very effective in treating a condition, by specifically targeting the diseased tissue. Magic bullets can be used in the treatment of various diseases like syphilis, cancer. Earlier magic bullets of salvarsan was used to treat syphilis, which has selective affinity to eliminate the germ causing disease without body injury, meant the glory and led to further medicine development such as sulfonamide, antibiotics and more recently, anti-tumor medicines including recombinant peptides, as well as cytotoxic or radioactive agents which may be marked on a selective way with monoclonal antibodies on targeted approach. It is evident that among the findings made by Ehrlich in the therapeutic field we can find the seed that inspired the design of synthetic pharmacological agents used in current research and medical therapy. This review mainly discussed about the history, importance and applications of magic bullets.

Key words: Magic bullets, anti- tumour, syphilis, salvarsan, monoclonal antibodies.

Introduction

History and Importance of Magic Bullets

Magic bullets are drug substances which directly kills disease causing microbes present in host cells (or) magic bullet combined with chemotherapy drugs directly kills tumour cells without effecting (harming) other normal cells. Paul Ehrlich (1854–1915) is the man behind the 'magic bullet'. He is the 'Father of Chemotherapy' made lasting contributions to immunology and hematology. He was awarded the Nobel Prize in Physiology and Medicine in 1908 for his contributions to immunology. He coined the terms 'chemotherapy' and 'magic bullet'. His first investigations on therapy obtained special importance since Ehrlich showed interest in elucidating the relationship between pharmacological activity and the chemical structure of certain medicines [2]. He wanted to identify changes in the

therapeutic activity as a result of changes in the chemical structure of certain compounds such as chemical dyes that had been used so far only as diagnostic elements.

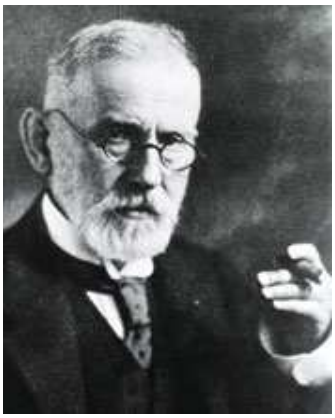


Fig: I. Paul Ehrlich

In the late 19th century, Paul Ehrlich developed an early interest in the specific staining of tissues with dyes, first with methylene blue and then with trypan red and atoxyl. Working from the observation that the uptake of different dyes varied in different tissues, Ehrlich proposed that a true chemical reaction occurred in the staining of cells. After it had been shown in 1905 that atoxyl, an arsenical, had some activity against trypanosomes. He reasoned this might allow the detection of a substance that would specifically bind to and kill microbes without harming human cells. During his graduate work with aniline dyes he discovered Salvarsan, which is one of the first consistently effective treatments for syphilis [3]. In 1897 Paul Ehrlich published his revolutionary side-chain theory. He described the process as being similar to key fitting a lock i.e., in modern terms, it would involve cells having receptors for antigens. Upon contact with the antigen, the receptors are shed into the bloodstream as antitoxins. The theory included Ehrlich's first use of the term "magic bullet" with the concept that chemicals could be designed to bind to and kill specific microbes or tumor cells. Later in his career, he applied the same theory to the study of tumor cells and effectively invented the field of chemotherapy, a word which he coined. His 'magic bullet' theory, which was known as the 'side-chain' theory at the time, led to contributions in immunology that resonate today. Penicillin was one of the first magic bullets. It was capable of curing a broad spectrum of bacterial diseases, and its success during World War II suggested that similar agents were yet to be discovered. A magic bullet might even be found for cancer.

Applications

Magic Bullets; An Early Approach to Chemotherapy:

a) A more targeted approach in cancer therapy:

It was within this context of hopeful expectations that cancer drug discovery got its start in the 1940s to find the magic bullet to kill cancer cells. In the late 20th century, fueled by the "War on Cancer" and made possible by dramatic advances in molecular biology, researchers began to think differently about cancer drug discovery, leading to today's focus on "targeted therapies." This approach focuses on a single gene or protein for which there is a good to fair understanding of how it works to cause cancer to develop or spread. A targeted cancer therapy is a drug which is designed to work directly on a single protein or enzyme, with the fewest possible side effects. The expectations on such targeted therapies [4] are successful. They kill the cancer and they do so with fewer toxic side effects. In the 21st century, the era of 'molecularly targeted' anti-cancer therapy, which brings to mind Paul Ehrlich's concept of the Zauberkugel, the 'magic bullet' for cancer cells [5,6] . Ehrlich's concept was developed into the 'selective toxicity'. A concept by Albert, which was particularly appropriate for the cytotoxic anti-cancer drugs. The current focus on molecularly targeted agents that alter cell growth but do not necessarily kill cells directly, points us to more subtle forms of bullet. The first major success of molecularly targeted therapy was imatinib mesylate (Glivec, Gleevec). Imatinib is an ATP mimetic and competitive inhibitor of several cellular ABL-kinases, which is the single molecular mutation that drives cellular proliferation in Philadelphia chromosome-positive chronic myeloid leukemia (CML)[6]. The success brings new challenges in imatinib treatment, it selects surviving leukemic clones, ultimately producing resistant disease in a number of patients with CML. With the current large oncology drug pipeline of an era during which 'magic bullets' may be developed and effectively used to treat a wider spectrum of cancers. Only by undertaking thoughtful studies of the clinical pharmacology of these agents, molecularly targeted anti-cancer therapy change the natural course of cancer. Many 2, 4-diaminopyrimidines

(Antifolates, Methotrexate)[7] have been prepared and examined by Dr. George Hitchings and Paul Ehrlich colleagues, including Drs. Gertrude Elion and Barbara Roth (56, 57). From these studies, they have developed trimethoprim as an antibacterial agent, and pyrimethamine as an antimalarial drug (57). These compounds function as inhibitors of dihydrofolate reductase. Trimethoprim has a high specificity for bacterial dihydrofolicreductases, some of which are several thousand times more sensitive to trimethoprim than is the corresponding mammalian enzyme. Similarly, pyrimethamine has a high specific affinity for the corresponding plasmodial enzymes, and Roth commented that "this was indeed the discovery of a magic bullet (56)". It was possible to obtain therapeutic activity at levels that are too low to produce symptoms of folate deficiency in the host.

b) Crizotinib magic bullets:

Crizotinib, which was approved for the treatment of some lung cancers, is one of the good examples for magic bullets understanding the complex nature of cancer, the rigors of drug development and testing interact and eventually lead to a successful treatment. Crizotinib targets an enzyme made by the Anaplastic Lymphoma Kinase gene (ALK). This gene was first discovered back in 1994 by scientists studying a rare form of non-Hodgkin lymphoma that affects both adults and children. As scientists learned more about ALK, it became apparent that a drug targeting ALK might also be useful against neuroblastoma and a subset of non-small cell lung cancers, which have similar molecular defects. Only a small fraction of lung cancer patients have the ALK gene mutation targeted by crizotinib, but the drug is very exciting because it helps a type of cancer which is typically very hard to treat. And now that the drug is approved and on the market, it is being tested in other types of cancer with this ALK mutation. The first clinical trials of Crizotinib began in 2006, with FDA approval granted in 2011, nearly 2 decades after the original gene discovery in 1994, which itself built upon work first done in the 1980s.

c) P53 Magic bullets:

Still, more than half of all human cancers carry mutations that affect p53, a protein shown in Fig: II. That has been studied intensely for more than three decades but scientists recently discovered molecular treatments such as magic p53-targeted drugs [4]. The p53 protein prevents cells with damaged DNA from dividing or, when damage is too great, promotes cell death. The relative pervasiveness of mutated p53 across various types of cancer has made the protein a major target of interest in cancer drug discovery. But while several p53-targeted drugs[8] have been developed, progress has been hindered by the protein's ability to adopt different shapes seemingly at will. As it morphs, it alternately exposes and conceals potential binding sites for drugs. When those agents successfully latch onto specific sites on mutated forms of p53, they often are able to restore normal function to the proteins. The newly identified nuance in p53′s structure is a binding pocket. The scientists used a computational (computer-based) approach to screen nearly 3,000 different compounds for their ability to slip inside the pocket. They eventually found one stictic acid, was not only a perfect fit but also reactivated the tumor-suppressing activity of a mutated p53 protein. The compound may be a suitable lead for the development of a new p53-targeted drug. The generation of stictic acid like molecules need to be identified, screened for activity, and developed into preparations appropriate for use in humans.

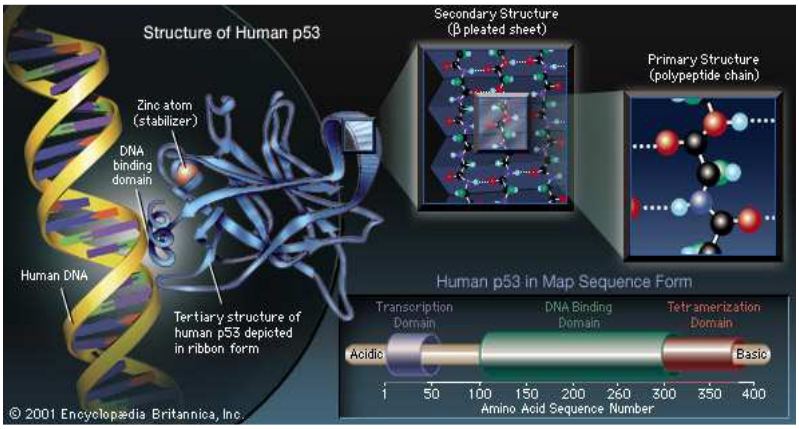


Fig: 2. Structure of p53 protein in human DNA

In the latter part of the 20th century, the hunt for a magic bullet against cancer has evolved into a search for many magic bullet so called targeted therapies. These agents are designed to seek out specific binding sites on mutated proteins expressed by cancer cells, such as those found on p53. The development of drugs with such high precision is time-consuming, but those that have been developed and approved for clinical use have brought significant benefits for patients, including improved survival and prognosis.

d) ORNL's Magic bullets targeting health:

Magic bullets are a group of chemical compounds that have an uncanny ability to home in on particular targets within the body. Their "magic" is provided by chemically attached radioactive isotopes, labels made of small quantities of radioactive material that enable physicians to obtain detailed images of internal organs, deliver doses of radiation to specific destinations, and trace the movement of medications all without picking up a scalpel."*Magic* bullets enable physicians to trace the movement of medication or obtain detailed images of internal organs all without lifting a scapel."In recent years, a barrage of magic bullets has been fired from laboratories around the country, but because of their long and involved development process, relatively few have been tested in human patients, few of them still have found commercial applications. Despite these odds, the researchers of ORNL's Nuclear Medicine Group have gained reputations as sharpshooters i.e., four new magic bullets now in clinical testing: 1) A radio-labeled antibody that targets colon cancer cells 2) A test agent for pancreatic problems 3) Magic bullets in Tracking Communication in the Brain 4) Imaging agents for monitoring blood flow in the heart and 5) Detecting early signs of heart disease. A fifth agent that promises to help track the changes in brain chemistry resulting from Alzheimer's and related diseases is undergoing preclinical studies.

i) Radio-labeled antibody magic bullets in treating colo-rectal cancer:

Colorectal cancer is expected to claim 57,000 lives this year in the United States, making it among the most deadly forms of the disease. This grim distinction places it high on the "hit lists" of scientists who work to make magic bullets ever more effective. One of the obstacles facing these researchers is the need for radio-labeled compounds that not only do the diagnostic or therapeutic job they were designed for, but also are easy and convenient to use. "The rhenium-188 labeled antibody homes in on colon cancer cells, delivering a precisely targeted dose of radiation." A new radioisotope generator system developed at ORNL promises to make the versatile radioisotope, rhenium-188, readily available for the treatment of colon cancer and arthritis. "Rhenium-188 is expected to have several applications," said by Russ Knapp, head of ORNL's Nuclear Medicine Group. "It can be attached to therapeutic agents and used as a tracer to monitor their movements through the body, or it can deliver a dose of radiation to shrink or kill an inoperable tumor. It may also be attached to small ceramic particles and injected into patients to treat uterine cancer. Members of the group who have made important contributions to the development of this generator system include Al Callahan, Ed Lisic, Saed Mirzadeh, and Arnold Beets. David Goldenberg and his colleagues at the Center for Molecular Medicine and Immunology at the University of New Jersey, researchers have developed a quick and easy procedure for chemically linking rhenium-188 to an antibody that homes in on colon cancer cells. This combination, known as an immune-conjugate, delivers a precisely targeted dose of radiation to colorectal tumors. Although the half-life of radioactive rhenium is only 16.9 hours, the radiation it releases can penetrate nearly a centimeter into tumor tissue, suggesting this technique could be useful for treating larger tumors. Full-scale clinical trials for the compound are scheduled to begin in the near future. In addition, the rhenium isotope can be used to treat rheumatoid arthritis in knees and other, fluid-filled joints. In this treatment the isotope is bonded to compounds that are injected into the fluid of the joint; the energy released as the rhenium decays helps relieve the painful swelling and inflammation of joint membranes. Similar applications have been suggested for reducing the pain associated with bone cancer.

ii) Magic bullets in Targeting Pancreatic Disease:

The pancreas is a gland located behind the stomach that secretes insulin, a hormone that enables the body to absorb sugar, and enzymes that help digest food, including fat. The role of the pancreas in digestion begins after food is eaten and partially digested in the stomach. When food enters the upper intestinal tract, it stimulates the pancreas to secrete its digestive enzymes. Most fat can't be absorbed by the small intestine. It must first be broken apart by digestive enzymes, then absorbed by intestinal cells, and finally reassembled and transported to the liver and other tissues for storage or use shown in Fig:3.

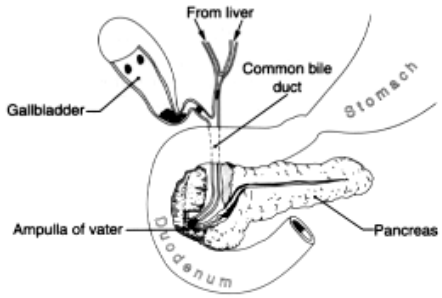
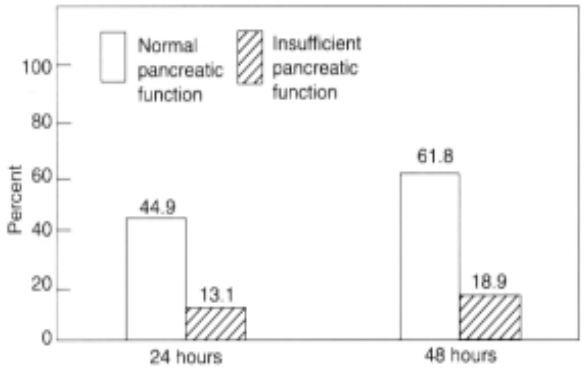


Fig: 3. Structure of pancreas

The failure of the pancreas to produce enough of these enzymes often signals serious problems, such as pancreatic cancer or inflammation of the pancreas [6]. Traditional tests for measuring the performance of the fat-digesting enzymes produced by the pancreas are impractical and unpleasant because they usually require a chemical analysis of fecal samples to determine how much fat has passed through the digestive system without being absorbed. Recently, ORNL's Nuclear Medicine Group has overcome the problems that plagued earlier research efforts and developed a test that produces enough radioactive by-products in the urine to provide a direct measure of the metabolism of fat by pancreatic enzymes. This technique, which has been proven successful in both animal tests and initial human studies, was designed by Knapp while he was conducting research as a Senior American Scientist of the Alexander von Humboldt Foundation at the University of Bonn in Germany.

Knapp e.tal., decided that, if they could synthesize a fat whose radio-labeled by-products would probably be excreted in the urine, we could develop an effective test. While Bonn, Knapp synthesized a new triglyceride fat containing the iodine-131-labeled fatty acid residue. This radio-labeled test agent is stable, can be stored for several weeks, and most importantly, its radio-labeled component is released in the urine. The amount of radioactivity in urine samples is then analyzed and compared to the amount administered to determine the rate at which the fatty acid residue is being metabolized by the pancreas. Initial studies in laboratory rats were conducted at ORNL with an iodine-125-labeled compound by Nuclear Medicine Group members Kathleen Ambrose, Al Callahan, Carla Lambert, and Dan McPherson. The iodine-125 tag has a longer half-life than iodine-131 (60 days versus 8), making it more convenient to use in animal experiments. The results of these studies were very promising 18.9% of the radioactivity from the orally administered test agent was released in the urine during the first 24 hours as shown in Fig: 4.



In the first round of clinical tests, patients with no pancreas problems released 51.8% of the test agent in the first 48 hours. Patients with impaired pancreas function released only 18.9%.

Fig: 4. Laboratory studies of ORNL iodine-125-labeled compound in rats.

Knapp then developed a test procedure and synthesized the iodine-131-labeled agent for initial tests in humans conducted by Joachim Kropp, M.D., at the Clinic for Nuclear Medicine in Bonn. Of the 23 individuals participating, 20 had normal pancreatic function and 3 had previously documented pancreatic insufficiency. The results of these studies showed that participants with normal function released an average of 61.8% of the iodine-131 in their urine after 48 hours. The patients with impaired function released only 18.9% significantly less than the control group.

iii) Magic bullets in Tracking Communication in the Brain:

To aid in the diagnosis and treatment of these disorders, such as Alzheimer's disease, Parkinson's disease, and many other neurological disorders which are characterized by abnormalities in the central nervous system ORNL's Nuclear Medicine Group develop new radiopharmaceutical agents. Normal brain cells have many receptors that receive chemical messages from other cells; in contrast, the brain cells of Alzheimer's patients often possess fewer receptors or many of their receptors are "turned off." Some of the brain-centered disorders involve the message-carrying chemical compounds that interact with receptors, known as neurotransmitters. A neurotransmitter of critical importance to normal brain function is dopamine. Its absence in the brains of patients with Parkinson's disease leads to a debilitating loss of muscle control; on the other hand, high levels of dopamine are often associated with schizophrenic behavior.

ORNL's new radiopharmaceutical agents attach themselves chemically to the receptors involved in neurological diseases, such as Alzheimer's. Researchers have developed a simple method of producing large quantities of an iodine-123-labeled imaging agent, designated IQNP. Because iodine-123 produces photons as it decays, its concentration and activity in the brain can be determined using photosensitive imaging techniques. A fluorine-18-labeled version of INQP is also on the drawing board will be available. The advantage of using fluorine-18 as a

radioactive tag for INQP is that it emits positrons as it decays and, therefore, can be used in conjunction with higher-resolution positron emission tomography (PET) imaging systems. With the help of these agents, researchers can track changes in the concentration and activity of receptors and neurotransmitters in the brain. These changes mark the onset and progress of Alzheimer's and similar diseases of the brain. Initial studies were done in rats have demonstrated that INQP concentrates almost exclusively in the receptor-rich areas of rat brains. Further tests of INQP in primates are planned as a prelude to seeking approval for testing in human patients.

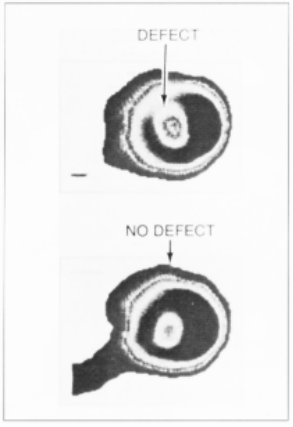
iv) Magic bullets in Tracing Blood Flow in the Heart:

Heart function tests have critical importance in diagnosing and treating both congenital defects and diseases of the heart. In a typical heart function test, a photon-emitting isotope is injected into the patient's bloodstream and a photon-sensitive camera then captures an image of blood flow through the heart's chambers [8, 9] and within the heart muscle itself. One of ORNL's contributions to cardiac imaging has been the development of a generator system for producing iridium-191*m*. This ephemeral test agent is the product of the decay of osmium-191, which has a 15-day half-life and is produced at ORNL's High Flux Isotope Reactor. Because iridium-191*m* has a half-life of less than 5 seconds and emits photons, it provides a safe, fast method of obtaining high-quality cardiac images. In fact, the isotope's short half-life enables tests to be repeated almost immediately to monitor the effects of exercise and drug therapy on the heart's pumping efficiency. In European tests, the iridium generator has been successfully used in evaluating heart performance in more than 200 patients.

v) Magic bullets in Spotting Early Signs of Heart Disease:

Early stages of several cardiac disorders, such as hypertensive heart disease, may have none of the symptoms traditionally associated with heart trouble clogged arteries, restricted blood flow, or oxygen-deprived heart muscle. ORNL researchers have determined a habit of altering the way affected areas of the heart muscle metabolize and absorb fatty acids. To detect these subtle changes, ORNL researchers developed a blood-borne fatty acid tagged with radioactive iodine-123, which emits photons as it decays as shown in Fig: V. The usual first step in deciding whether a problem with fatty acid metabolism exists is to use a radioisotope to produce an image of blood flow to the patient's heart muscle through the coronary arteries. If blood flow is normal, the radio-labeled fatty acids are administered to the patient. An uneven distribution of these fatty acids throughout the heart can be detected by a photon-sensitive camera, suggests that the ability of some areas of the heart muscle to metabolize fatty acids which are impaired, perhaps as a result of the early stages of heart disease.

The image may also enable a physician to determine which regions of a damaged heart muscle could be salvaged through treatment [9]. The clearest indication that the ORNLs-developed agent has helped shed new light on the subtleties of cardiac metabolism is its worldwide acceptance. Studies of iodine-123 are in progress at several European clinics and at Brookhaven National Laboratory. The agent has already won approval from the Japanese Food and Drug Administration and is being marketed in the Far East by Nihon Medi-Physics Co., Inc., under the name Cardiodine ™. Studies of more than 600 patients at 30 Japanese institutions were completed before the agent was approved for use .Two symposia recently held in Japan, the Third International Symposium on Radio-iodinated Free Fatty Acids in Cardiac Imaging and the Thirteenth New Town Conference on Nuclear Cardiology, had a single focus on the clinical use of ORNL's iodine-123-labeled fatty acids as a gauge of the heart's metabolism.



The top image shows an area of decreased concentration of iodine-123 following exercise. After rest, the bottom image shows the concentration has increased, indicating the patient is a good candidate for corrective surgery or other treatment. (Courtesy Clinic for Nuclear Medicine, University of Bonn, Germany.)

Fig: 5. Fatty acid tagged with radioactive Iodine-123

The isotope's short half-life enables tests to be repeated almost immediately to monitor the effects of exercise & drug therapy on the heart.

Magic bullets in reducing Low Density Level (LDL) cholesterol:

The quick "magic bullet" jab offers new hope to patients who fail to control their soaring cholesterol levels despite taking wonder drugs such as statins. Scientists have created a potent jab that can dramatically slash blood levels of the "bad" LDL cholesterol while significantly increasing levels of the "good" HDL cholesterol. Risk of heart disease is particularly high in those with a high level of LDL cholesterol and a low level of HDL. According to the first preliminary human tests, the medicine in this new jab has been shown to lower the LDL in healthy volunteers on the highest dose by an average 64 per cent more than those on an inactive placebo injection. There was an injected treatment, called AMG145, is a "monoclonal" antibody, a laboratory-made human protein that targets a recently-identified cholesterol regulator [8]. Monoclonal antibodies are already used to treat certain cancers as they use the body's own immune system to kill tumour cells.

The study, which was presented at the American Heart Association's Scientific Sessions, involved 54 men and two women, aged between 18 and 45, who were healthy and not on other medications. In this study, scientists have created AMG145 to "turn off" a cholesterol regulator which interferes with the liver's ability to remove bad cholesterol from the blood. Participants have received a single injection that contained one of five levels of doses of AMG145 or a placebo. Sixteen received those injections intravenously. The others had simple injections that delivered the drug just beneath the skin. After the injections, bad cholesterol was measured frequently for 85 to 113 days, along with other laboratory measures related to heart disease. With increasing doses of AMG145, blood tests revealed that lower levels of bad cholesterol, total cholesterol and apolipoprotein-B, which "delivers" bad - cholesterol to the tissue, causing fatty deposits to clog the arteries. Dr Clapton Dias, lead researcher and medical sciences director of clinical pharmacology and early development at Amgen, Inc., in California, said that: "It appears to be a promising way to lower bad cholesterol". "With higher doses, bad cholesterol stayed lower for a longer period." The injections were well tolerated and volunteers receiving AMG145 experienced no more side effects than those on the placebo. The company is now conducting a similar study that gives multiple doses of AMG145 to adults who take statins to control their cholesterol. If AMG145 proves safe and effective in further clinical trials, it could help people unable to control their cholesterol with current medications that work in different ways.

Conclusion

Discovery of these magic bullets inspired the design of synthetic pharmacological agents used in current research and medical therapy. Magic bullet combined with chemotherapy drugs directly kills tumour cells without harming other normal cells. Paul Ehrlich's 'magic bullet' theory, which was known as the 'side-chain' theory at the time, led to contributions in immunology that resonate today. The hunt for a magic bullet against cancer has evolved into a search for many magic bullets so called targeted therapies. Scientists recently discovered molecular treatments such as magic p53 bullets where these agents are designed to seek out specific binding sites on mutated proteins expressed by cancer cells, such as those found on p53. In the 21st century novel paradigms for anti-cancer drug treatment are evolving for different cancers with regimens in which initially classical cytotoxic drugs either alone or in combination with molecularly targeted drugs which are first used to reduce tumour burden, followed by maintenance treatment with molecularly targeted drugs either alone or in scientifically individualized combinations. The implication of such therapeutic regimens is that there will always be new therapeutic challenges for clinical pharmacologists in collaboration with oncologists. ORNL's Magic bullets now in clinical testing process which can be used to treat various heart, brain diseases. We are all impatient for drugs that can cure cancer no matter when and where we find it. But the reality is we have to balance that wish with a desire for the safest and most effective drugs possible. Fighting the war against cancer inevitably requires time, money, risk, and innovation to discover the magic bullets needed to save more lives.

References

1. Ehrlich P. Experimental researches on specific therapy. On immunity with special relationship between distribution and action of antigens, Harben Lecture. London: Lewis; 1908.
2. Witkop B. Paul Ehrlich and his magic bullets–revisited.Lecture given at the Paul Ehrlich Foundation, Frankfurt,13 November 1998. See http://www.aps-pub.com/proceedings/1434/Witkop.
3. Abraham JJ. Some account of the history of the treatment of syphilis. *Br J Venereal Diseases* 1948;24:153–61.

4. Kara rogers., New cancer therapies: Magic Bullets at the Guardian of genome ., Britannica.com Feb 4,2013.
5. KJ Williams., The introduction of 'chemotherapy' using arsphenamine –the first magic bullet, Journal of the Royal Society of Medicine ,*2009: 102: 343–348.*
6. L D Lewis., Editor's view Cancer pharmacotherapy: 21st century 'magic bullets' and changing paradigms British Journal of Clinical Pharmacology., 2006 July; 62(1): 1–4.
7. Thomas H. Jukes., Searching for Magic Bullets: Early Approaches to Chemotherapy-Antifolates, Methotrexate, The Bruce F. Cain Memorial Award Lecture1., CANCER RESEARCH 47, 1987; November 1, 5528-5536.
8. Jo Willey., Magic bullet' jab to fight heart disease : Tue, November 15, 2011.
9. From Nobel Lectures, Physiology, Medicine, Elsevier Publishing Company. Amsterdam, 1967. Nobelprize.org. Accessed March 11, 2009; 1901-1921.