ONLINE EDITION
JERUSALEM POST

Fat chance

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Liposomes - microscopic fat globules covered with one or more bi-layered fat membranes - were first photographed in the middle of the 19th century, but what they do was described and characterized only in 1961 by British hematologist Dr. Alec Bangham.

With the name liposome derived from two Greek words, lipid (meaning fat) and some (meaning body), these polymeric nanoparticles may not look like much. However, one of Israel's leading biochemists, Prof. Yechezkel Barenholz of the Hebrew University-Hadassah Medical School, has used them to design better drug delivery systems that extend and improve the lives of cancer patients around the world, and is working on superior vaccines with the potential to better protect people against possibly deadly diseases from influenza to hepatitis B and the biological warfare agent anthrax.

DOXIL, THE liposome-based medication modelled on the drug doxorubicin that he developed with Prof. Alberto Gabizon (formerly of Hadassah and currently head of oncology at Jerusalem's Shaare Zedek Medical Center), is the only Israeli-designed cancer medication; it's not yet included in the Israeli basket of health services, but has annual sales around the world of over $400 million.

The Doxil liposomes were designed to have a natural ability to target cancer. The endothelial wall of all healthy human blood vessels are encapsulated by endothelial cells bound together by tight junctions, preventing any large particle in the blood from leaking out. However, tumor blood vessels don't have the same sealing ability between cells and are leaky. Liposomes of certain sizes can slowly leave these special blood vessels and permeate to the tumor sites selectively from the blood. However, they are not kept in the bloodstream and do not leak out through the endothelial wall in healthy blood vessels.

These special nano liposomes are referred to as "stealth liposomes" due to the coating of polyethylene glycol that helps them avoid detection and clearance by the body's immune system. Therefore, they can circulate in the blood for a long time, during which they can leak out to the tumor tissue. These passively targeted liposomes can zoom in and reach tumors and inflammation sites to deliver drugs, making naturally toxic chemotherapy drugs much less so by delivering them only to diseased tissue.

BARENHOLZ, BORN in Rehov Sheinkin in Tel Aviv, says in his medical school office that he liked high-school science - especially biology - but never wanted to be a physician. "I wanted to be a chemist, but my grades weren't high enough," says Barenholz - described by colleagues as both "brilliant and modest." So he studied biology, which today requires higher grades than chemistry. "I am not sorry, as I learned a lot of chemistry on my own that has helped in my career. I learned from life that there is no reason to look back and regret." He earned his bachelor's (natural sciences, microbiology and biochemistry), master's and doctoral degrees (biochemistry) from The Hebrew University in Jerusalem. Pointing to a corner in the medical school's biochemistry department, he says: "This is where I did the work for my doctorate."

As a young student in 1964 needing money to support himself, Barenholz took a lab job making

enzyme substrates to study enzymes involved in lipid metabolism and lipid-related diseases. His love affair with lipids began there, and he did his second degree in lipid metabolism, becoming one of the first to discover how sphingolipids (often found in neural tissue; they play an important role in membranes, signal transmission and cell recognition) break down.

At the University of Cambridge in the UK, he worked under Bangham, the hematologist who had described liposome functioning a few years before and was knighted for his discovery. "Alec was chief pathologist for the British Army here in 1948. Nine years ago, when he was 76, I invited him to Israel to see all the places he remembered. We had a lot of interaction, and today he is 85."

Bangham used liposomes as a model for cell membranes rather than for applications. "I fell in love with liposomes already in 1969 in Cambridge, and my intense interest in this wonderful system has continued since then. I started with liposomes as a model for cell membranes, but by 1980, I went into applications. Today they are entities of their own."

WORKING WITH Gabizon, Barenholz focused on liposomes as drug delivery systems for cancer therapy, with Yissum - the Hebrew University's R&D arm - selling the technology to LTI (Liposome Technology of Menlo Park, California, which later changed its name to Sequus, which was bought by ALZA, now part of Johnson & Johnson). He and Gabizon aimed at using liposomes to deliver doxorubicin to tumor cells in patients with ovarian and breast cancer, multiple myeloma and Kaposi's sarcoma (a tumor that may appear in AIDS patients).

"It works better than doxorubicin and can extend life by 25 percent to 33% compared to the next-best treatment. Patients have a higher quality of life and survive longer, as it targets the cancer cells," says Barenholz. It isn't a cure, but patients suffer from many fewer side effects: They don't feel nauseous or vomit and don't lose their hair. Approved by the US Food and Drug Administration 12 years ago, it is taken by almost every American woman with ovarian cancer, and can also be given with chemotherapy drugs like cisplatin. Doxil is now approved for clinical use in most countries.

As Doxil, given by infusion, will lose its patent protection in two or three years, Barenholz and colleagues are working on replacements. In most cases, a single chemotherapic drug does not cure. Another mode of treatment is immunotherapy, which aims at making use of the immune system to fight cancer. But it also does not work well on its own, as it can't deal with a large number of cancer cells. However, when both are combined, the two may work synergistically." But this, explains Barenholz, is difficult to do, as "chemotherapy harms immune cells, so you have no such cells to use. Doxil doesn't hurt the immune system, so you can start immunotherapy and get better results. We have already demonstrated this in mice studies performed with Gabizon and HU medical faculty colleague Prof. Eli Kedar."

Barenholz is certain that eventually there will be cures for many types of cancer. In the meantime, breast cancer - which used to kill most victims in a few years - is becoming more a chronic disease. Besides cancer drugs, Barenholz and colleagues are working in several other directions, including treatments for inflammatory autoimmune diseases such as rheumatoid arthritis and multiple sclerosis, and a liposome-based long-lasting local anesthetic with New York University anesthesiologist Dr. Gilbert Grant. Barenholz and his team are inventors of 29 approved patent "families" owned by Yissum, and most of these are already licensed, with patent applications for 12 more. Barenholz has written or participated in 330 articles in scientific journals, some of which have been cited by as many as 1,500 authors.

ELEVEN YEARS AGO, Barenholz, Kedar and their teams started to develop improved influenza vaccines. Conventional vaccines don't fully protect the elderly - who are the most common victims of flu complications.

"Flu takes a tremendous human toll and has an immense economic impact," he says. Influenza is one of the major causes of death (estimated at 70,000 to 125,000 cases per year) in the developed world, especially among the old and immune-compromised. The biggest challenge for vaccine companies is the development of improved flu vaccines and increasing the number of people who develop an immune response to it. Other challenges include cutting the dosage of vaccine to extend the world's supply and provide an alternative, needle-free and more "friendly" method of administration via a nasal spray.

Thus a startup named NasVax, of which Barenholz is a co-founder, was established three years ago under the "umbrella" of the Meytav Technological Incubator in Kiryat Shmona with two workers, and then moved to the Kiryat Weizmann Science Park in Nes Ziona. It now has 13 staffers and is listed on the Tel Aviv Stock Exchange. Barenholz notes that there are often shortages of conventional flu vaccine, and many people avoid getting vaccinated because they are afraid of injections. NasVax is thus working on an approved delivery system, a nasal-spray vaccine and improved injectible vaccines that will offer more protection, especially in the elderly.

AN INTRA-NASAL vaccine has inherent benefits, as it enters the body through the nose - the same entry (along with the mouth) used by the flu virus, says Barenholz. "The active vaccine-activated immunity will be right there in the nasal and lung mucosa waiting to neutralize the virus when it arrives. The digestive system doesn't cause it to break down.

At the same time, the attenuated virus vaccine can seep out of the nose, while an injected vaccine remains in the body." An oral flu vaccine was developed in Russia, he adds, but was hard to get onto the market because it could harm asthmatics and couldn't be given to the elderly. "Many young people are petrified of needles, while old people don't care and just worry about getting a taxi to and from the clinic," says Barenholz.

To improve the immune response to vaccines, adjuvants (helpers) were developed. "Until a decade ago, there was only one adjuvant. Alum (for aluminum salts) acts as an antigen depot by releasing the antigen slowly, and mostly activates the production of antibodies but not the cellular responses of the immune system. As it became evident that the adjuvant may have a major effect on the intensity and type of immune responses, researchers have been looking for new, more effective helpers. Only recently, a few more adjuvants were approved and are still in limited use."

NaxVax is aiming to develop such a superior adjuvants for a broad spectrum of applications. "Researchers tried for years to use liposomes as adjuvants, but the simple, standard ones were not very effective. So we looked for new things and created new molecules not existent in nature. They activate the immune system and create antibodies," notes Barenholz. "Unlike other adjuvants that don't work intra-nasally, we found that we can administer our molecules in the form of a nasal spray." NasVax workers take a bottle with liposome adjuvant, squirt it on existing flu vaccine and make it more effective. In animal models, it was shown that an immune response is obtained with only one-third of the conventional dose.

A PERFECT model for testing flu vaccine is in those popular rodent pets called ferrets, which sneeze, get muscular pain and fever and shake when they are intentionally infected with the flu.

NasVax's vaccine has been tested at Retroscreen, a British lab with much experience testing ferrets, and now it has been given to about 100 healthy young people aged 25 to 60, plus some 60 healthy older people in Phase I/IIa clinical trails at Hadassah. In addition, NasVax has launched preclinical studies with anthrax, avian flu and hepatitis B vaccines.

Because liposome-based vaccines have high potency, they can increase the efficacy of various existing antigens, so vaccines may require lower and less-frequent antigen doses. The flu vaccine market by the end of 2007 is expected to be more than $2.5 billion dollars, as the recommended

ages for vaccination are expanding to include children up to five.

Barenholz is also in the early stages of working on a vaccine against cancer. "We want to bring an approach of rational design against tumors. There have been such failures, especially in the US, but we are working differently, in cooperation with the Weizmann Institute of Science. We are also starting to think about an improved pneumococcal pneumonia vaccine. It's a giant market."

The HU medical school biochemist naturally enjoys the royalties he earns from Doxil, but declares that there are more important things than just money. "It takes a phenomenal amount of motivation to travel the uphill path that leads to something that works. I really enjoy helping people and creating something useful and effective."

In a highly unusual initiative, he created the Barenholz Fund with $1.25 million of the proceeds from his research. The fund allocates prizes for three HU students each year. "I didn't have to, but I want to encourage applied scientific research. While it's hard to know at this stage what practical results will emerge from the program, we have gotten very good feedback."

There is no doubt, Barenholz concludes, that "the state doesn't invest enough money in science, just as it fails to spend enough on health, education and other things. I can't complain, as I have plenty of money for research, but I see what's happening around me. Israelis' cleverness is not declining, and neither is our originality or entrepreneurial spirit, but our ability to compete technologically is.

"We're spread too thin. Any bright young person who graduates from university can get the starting salary of a professor by taking a job in hi-tech or business administration. The Israeli brain drain is not due to salaries but rather to the shortage in opportunities. The state must invest more in infrastructure and equipment."