Human Vaccines & Immunotherapeutics 11:7, 1569–1572; July 2015; © 2015 Taylor & Francis Group, LLC

My passion is to drive innovation in vaccine development

Manon Cox

My fascination with science started long time ago. I had always been curious about many things, but it was my biology teacher, Dr. Postmus, who triggered my true interest. His enthusiasm and energy stimulated a remarkable 25% of his students to go on to either study biology or medicine. So did I, and it was an exciting time especially in the field of molecular biology where an enormous acceleration in knowledge generation was happening. The characterization of many new genes, restriction enzymes, the invention of the polymerase chain reaction (PCR) provided great opportunities with so many new things to learn and discover. I was also carefully following the literature around the discovery of the then called AIDS (Acquired Immuno Deficiency Syndrome) virus as this virus was killing young people around us and I - like many around me - was terrified about becoming infected. Similarly, I was fascinated by a new expression technology called the baculovirus expression system as it seemed to provide the "ease" of a bacteriophage but enabled the production of complex biologically active proteins.

After graduating from the University of Nijmegen (the Netherlands), I landed my first "job" at the University of Amsterdam in the late 1980s exploring the usefulness of PCR-based human papilloma virus (HPV) screening for early detection of cervical cancer. The work I did was boring, but important, as our findings from analyzing 3,000 cervical scrape specimens supported the hypothesis that HPV 16 and 18 were associated with early stage cervical cancer. Luckily we only found those viruses in approximately 0.5% of the samples analyzed – a striking difference with incidence numbers now (30 years later) that report the virus being present in close to 50% of young women. The good news is that there are two modern vaccines for the prevention of cervical cancer on the market today.

Shortly thereafter I had the opportunity to join Gist-brocades as a research scientist, suiting my attraction to applied research. Gist-brocades was in those days the place to be for molecular biologists in the Netherlands as the Company was doing frontline biotechnology research. This was demonstrated by their many successes including the development and commercialization of the first recombinant enzyme, chymozyme, used for cheese ripening in the food industry. During my six years in Research & Development at Gist-brocades I experienced the power of working in multi-disciplinary teams. Teamwork enabled us to accomplish results that I never could have imagined to be possible. We were able to improve lipase expression levels of our production organism *Pseudomonas alcaligenes* by 10,000-fold by combining classical genetics, molecular biology, microbiology, fermentation and down-stream technology. With a commercially viable process at hand, the company built a factory for the production of this detergent enzyme that dissolves greasy stains in clothing. The performance of the enzyme during the washing process was dramatically improved by changing a methionine amino-acid in the enzyme and thereby preventing oxidation. Never would I have thought that 20 years later we would be applying the same genetic engineering principle to produce a temperature stable influenza vaccine.

During this time I traveled to Africa and was confronted with the dramatic effects of the Human Immunodefiency Virus (HIV/AIDS) throughout small villages in East Africa. Entire populations between the ages of 15 and 40 years old

http://dx.doi.org/10.1080/21645515.2015.1057090

were wiped out in some of the places we visited. It was evident that we desperately needed a vaccine as that would be the only way to take control of the devastating impact of the disease caused by this virus. Unfortunately, more than 25 years later we still haven't made much progress. But it wasn't until much later that I realized that I could play a role in vaccine development.

I first embarked on a management development program and, as part of that, I had the opportunity to go to business school and experience the American education system at the Simon School of Rochester University. This experience made me realize that bioscience was still my passion and what would be a better place to get involved in biotech than the United States (U.S.) where this field was booming. I joined Protein Sciences in the late nineties for the people, the products, and their technology. Gale Smith was one of the inventors of the baculovirus technology and Dan Adams one of the founders of the biotech industry. The company was actively involved in vaccine development and the baculovirus expression system was their technology platform. What would be a better place for learning and making things happen?

Soon we realized that we needed to bring our technology to maturation by taking a product forward through approval by the Food and Drug Administration (FDA) as none of the partners for whom we were developing vaccines using our technology was going to do this for us. An influenza vaccine seemed to be a perfect target as the company had already generated preliminary human clinical data in the mid nineties supporting the hypothesis that a recombinant hemagglutinin (rHA) protein could prevent influenza. Our technology was perfectly suited to support the annual updates required for the influenza vaccine as only the baculovirus would need to be modified. Finally, the 1998 H5N1 bird flu outbreak in Hong Kong had clearly revealed the limitations of the egg-based manufacturing process used for the production of influenza vaccines. The National Institutes of Health (NIH) told us that we were the only company in the world that could develop a vaccine "in time" and we met their expectations delivering doses in just eight weeks. However, what seemed to be pretty simple and straightforward project, became a 14-year trajectory that ultimately lead to FDA approval of the first recombinant influenza vaccine, named Flublok®.

About Dr. Cox

Dr. Manon Cox did her undergraduate and graduate studies in Molecular Biology, Genetics and Biochemistry at University of Nijmegen, Netherlands in 1981–7. She received an MBA with distinction from University of Nijenrode and University of Rochester in 1997, and Doctorate from University of Wageningen in 2009. Dr. Cox worked for one year at University of Amsterdam on the development of a PCR screening test for cervical cancer. She then joined Gist-brocades, a biotechnology company specializing in fermentation and food production, first as a research scientist and later as a manager in R&D Process Development (Production) and New Business Development, where she and her team worked on the optimization of recombinant protein production and then prepared the company's entry into the pharmaceutical market.

Dr. Cox joined Protein Sciences Corporation based in Connecticut, U.S. as Director of Business Development (1998–2001), then Chief Operating Officer (2002–10), and Chief Executive Officer. She led the development of a new method for influenza vaccine production using genetically modified baculovirus to express hemagglutinin protein. Since the process was rapid and the expressed protein was easy to modify, the new technology was suitable for the influenza virus whose antigen properties change annually. The novel vaccine was approved as Flublok by FDA in 2013.

Dr. Cox serves on the Scientific Advisory Boards of Pall BioPharmaceuticals and iCubed, and the Board of Directors of United Way, Meriden & Wallingford, and the Netherlands-America Foundation and its Education Committee. She has received numerous honors and awards including Woman of Innovation award from the Connecticut Technology Council and Doctorate in Humane Letters honoris causa from St. Joseph University.

Influenza (or flu) is a highly contagious, acute, viral, respiratory vaccine-preventable disease that occurs seasonally in most parts of the world and is caused by influenza viruses. Epidemics cause significant morbidity and mortality worldwide. Influenza affects all age groups and in the U.S. alone 25 to 50 million people contract influenza each year and an annual average of 36,000 deaths and 226,000 hospitalizations has been associated with influenza epidemics. Over 90% of the deaths related to annual influenza epidemics occur in people over age 65. Influenza vaccination - now recommended for all ages in the U.S. - is an effective way to reduce the spread of the virus and complications of influenza.

Influenza vaccines are unlike any other vaccine adjusted annually based on global surveillance data as the virus (an RNA virus) constantly evolves to evade the immune system. This annual adjustment poses challenges for vaccine manufacturing as the time available to make adjustments is extremely short- a matter of months. The vast majority of the ~150 million doses of influenza vaccines administered in the U.S. are manufactured by growing the influenza virus in embryonated chicken eggs. Therefore, the manufacture is limited to influenza virus strains that replicate well in eggs and, at times, resulting in vaccines that are less effective. Vaccine strains also need to be selected well in advance due to the time it takes to

develop a high-growth reassortant (a minimum of 3–6 months is needed) and this may lead to mismatch between the virus contained in the vaccine and circulating viruses.

The viral surface protein hemagglutinin (HA) is the key antigen in the host response to influenza virus in both natural infection and vaccination since neutralizing antibodies directed against HA can mitigate or prevent infection. Manufacturing recombinant HA proteins had to be versatile and robust, guaranteeing timely delivery of new antigens that match the circulating strains.

We were able to develop a universal manufacturing process for the production of many different rHA proteins. Various trivalent versions of Flublok were tested in five randomized controlled clinical trials conducted in populations of varying ages. The first pivotal study - a randomized, double-blind, placebo-controlled clinical trial during the 2004–2005 influenza season among 460 healthy adults - determined the dose-related safety, immunogenicity, and protective efficacy. This study demonstrated that Flublok was safe, well-tolerated and immunogenic. None of the recipients of the high dose vaccine, containing 45 μg of each rHA antigen instead of the 15 μg of each HA antigen present in standard inactivated vaccines, developed cell culture-confirmed influenza whereas in unvaccinated individuals 4.6% developed influenza. This suggested that absence of the influenza neuraminidase (NA) in the vaccine and differences in glycosylation in insect versus vertebrate cells did not interfere with vaccine performance. Based on the success of this study a dose selection was made for the "45 μg" vaccine, having three-fold greater rHA content than the traditional inactivated influenza vaccines. Subsequently, three additional clinical studies were performed in a total of more than 3,000 human subjects to support FDA licensure of Flublok in January 2013 initially for adults 18–49 years. These studies demonstrated that the highly purified rHA protein was well tolerated and resulted in a strong immune response. In addition, the higher content of HA in Flublok provided better cross-protection against drifted influenza viruses than the standard inactivated influenza vaccines. The FDA requested additional safety data for the population over 50 years old as the vaccine had only been tested in ~1,000 individuals in this age group. Data generated in a fifth randomized safety study in 2,500 adults 50 and older resulted in approval for adults over 50 years old in October 2014.

The baculovirus technology provided a new influenza vaccine production technology with distinct advantages. Not only could it be used for the expedited production of a safe and efficacious vaccine, the proteins would also be an exact match to the circulating viruses as no egg-adaptations are required to support the manufacturing process. The very short time needed to update the vaccine could even lead to later selection of the vaccine composition, thus providing an opportunity to combat newly emerging influenza strains and/or address pandemics.

The main challenges we encountered on our journey through FDA approval were diverse and varied in nature from scientific to economical. Let's begin with the "valley of death": vaccine development is expensive and investors want a quick return on investment and are not necessarily motivated by "doing the right thing". So the challenge of finding the right funding was enormous. I am grateful for the financial support we received from initially the NIH, our development partners Diamyd and UMN, a few highly motivated private investors – who believed in our cause and the importance of prevention of disease versus treatment – and, later the enormous commitment by BARDA (Biomedical Advanced Research and Development Authority), and finally all researchers who purchased research antigens from the Company, thereby fueling our development work. The scientific challenge of designing a universal process was daunting, particularly for the purification of different HA proteins, but we knew we could overcome that with time and a strong team effort. The FDA presented another significant challenge, as there seemed to be great reluctance to adapt new technology and approve a novel production technology. Many times we considered turning to other regulatory bodies like the Australian or European regulators but the U.S. was our home base and we did have fantastic support from former regulators and a number of progressive FDA employees that helped us through the regulatory mine field. Our Biologics License Application for Flublok was under review for five years! Serious questions were only raised after filing and it certainly felt as if only at that moment they realized we seriously wanted to obtain a license to commercialize Flublok. The responsibility of an FDA reviewer is enormous; however, it can never be an excuse to prevent innovation. The Agency could benefit from taking a more progressive approach and provide exposure to the real-world to its employees, possibly by setting up active employee exchange programs with the industry, public health organizations and academia.

We need to recognize that change is needed in the vaccine industry: most vaccines today (90%) are still made using 60 year old technology when modern technology is available to improve the quality of vaccines. Unfortunately, the economics do not look favorable for vaccine development and we see more and more pharmaceutical companies withdrawing from the field. Vaccines are given to healthy people resulting in a complex risk/benefit evaluation. Large expensive clinical studies are required to demonstrate efficacy and safety of a vaccine candidate. The public and/or the public health sector are not necessarily willing or able to pay for the vaccines and this has stagnated innovation in vaccine development. Recent efforts to expand the size of group purchasers in Europe and thereby further reduce prices threaten to eliminate vaccines from that market – a development that will have disastrous consequences to public health.

Vaccines are critically important but, unfortunately, the general public does not broadly recognize the importance of vaccines. That is only human because once diseases are not around anymore we tend to quickly forget how devastating their

effects were. We only need to think about polio, or small pox. Vaccines are the most cost-effective way to eradicate diseases and we have many challenges ahead of us HIV, Ebola, malaria and so many others.

I am proud that I have been able to help bring a modern vaccine to market and to drive innovation in vaccine development. I am hopeful that our next product will be easier and that many more new vaccines will follow. Because, if we truly want to contain healthcare costs and make this world a better place for all, vaccines are the way to go.