ACCADEMIA NAZIONALE DEI LINCEI

Vaccines

A position paper by the Accademia Nazionale dei Lincei

Guido Forni (Linceo, University of Torino, Coordinator)
Alberto Mantovani (Linceo, Università Humanitas, Milano)
Lorenzo Moretta (Linceo, Ospedale Pediatrico Bambino Gesù, Roma)
Giovanni Rezza (Istituto Superiore di Sanità, Roma)

ROMA - PALAZZO CORSINI
Via della Lungara 10
www.lincei.it

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Abbreviations: AIDS, Acquired Human Immunodeficiency Syndrome; Alum, aluminum salts; AMC, Advanced Market Commitment; ART, Anti-Retroviral Therapy; BCG, the Bacille Calmette-Guérin; COMILVA, Coordination of the Italian Movement for Vaccination Liberty; CEPI, Coalition for Epidemic Preparedness Innovations; CIN, Cervical Intraepithelial Neoplasia; CpG, citosine-phosphate-guanine; DCIS, Ductal Carcinoma In Situ; DTP, Diphtheria, Tetanus, Pertussis; EBV, Epstein Barr Virus; GAVI, Global Alliance for Vaccines and Immunisation; HBsAg, B surface antigen or Australia antigen; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HIB, *Heamophilus influenzae* type B; HIV, Human Immunodeficiency Virus; HPV, Human Papilloma Virus; IFFIm, International Finance Facility for Immunization; ILC, Innate Lymphoid Cells; LPS, Lipopolysaccharide; MERS, Middle East Respiratory Syndrome; MPR, Measles, Mumps, Rubella; NIH, National Institutes of Health; WHO: World Health Organization; PPD, Purified Protein Derivative; SARS, Severe Acute Respiratory Syndrome; TLR, Toll-like receptor; TNF, Tumor Necrosis Factor; UNICEF, United Nations International Children's Emergency Fund.

Correspondence: Accademia Nazionale dei Lincei, Via della Lungara 10, 00165 Roma;
E-mail: segreteria@lincei.it

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SUMMARY

The purpose of this position paper is to provide lay persons with the foundations of vaccination, as well as to share some of the challenges of current research in immunology and vaccine research.

Owing to the concurrence of distinct factors, life expectancy in the richest countries has doubled over the past hundred years. Among the factors contributing to this major achievement, vaccines are a low-cost medical intervention allowing defeating diseases causing disastrous epidemics. According to World Health Organization estimates, vaccines save the world 5 lives every minute, i.e. 7,200 every day.

A key concept related to vaccines is *sharing*: for protection to be effective most of the population needs to be vaccinated. The fact that in the poorest nations not everyone has access to vaccines is a serious problem of global health and social inequality.

The immune memory. When microbes (viruses, bacteria, protozoa, parasites ...) overcome the skin and mucosal barriers and resist rapid initial attacks by the immune system, a new defense line based on the combined action of B and T lymphocytes is put into motion. When one of these lymphocytes recognizes its target, the cell starts dividing generating a family (a *clone*) of identical lymphocytes directed against the target initially recognized. Therefore, the microbial invasion is progressively counteracted by enlarging clones of effector B and T lymphocytes. By secreting high levels of antibodies or driving a complex inflammatory response these effector cells kill the intruders with high efficiency. The ensuing long persistence of both the expanded population of the effector lymphocytes and a high antibody titer (*the immune memory*) accounts for elimination of a subsequent invasion by the same intruder microbe with such an efficiency and rapidity that generally goes unnoticed.

Technological evolution. The goal of vaccination is to trigger an effective and persistent immune memory. The progress of technological innovation is allowing preparing more and more effective and well tolerated vaccines. The empirical use of animal-derived vaccines, such as those that were prepared from bovine pustules used to immunize against smallpox, has been replaced by vaccines made of whole microbes, inactivated or attenuated by a series of cell culture steps (for example the oral anti-polio vaccine or the anti-yellow fever vaccine). To make the side effects more unlikely or to induce a more precise immune memory, the trend is to abandon full-microbial vaccines in favor of vaccines based on biomolecules from the surface of microbes. At present, these molecules are often obtained through recombinant DNA technology, such as in the case of Hepatitis B (HBV) or Papilloma Virus (HPV) vaccines.

The challenge of new vaccines. Currently there are vaccines against a little more than 25 species of microbes causing disease in humans. It is conceivable that in the next 20 years many innovative vaccines will be available. The development of vaccines against microbes for which there is still no effective vaccine is not, however, an easy task, as microbes which have evolved ingenious strategies to escape the powerful reactions of immune memory are still being addressed. New vaccines will also have to be able to elicit effective immune memories in fragile people such as newborns, elderly people and persons with immunodeficiency or cancer. Immune memory will also have: *a)* to be effective against parasites and fungi; *b)* to act against microbes capable of modulating their molecular structures; *c)* to persist for long periods of time, possibly for a life time, avoiding the need for boosts; *d)* to protect the mucosal membranes blocking intruder microbes before they spread through. New vaccines will also need to be administered via routes other than needle and syringe injection and to cause negligible side effects.

Efficacy and risks. When a new vaccine is produced, its efficacy is assessed by complex studies based on comparing the incidence of the disease in two groups of people (*cohorts*), one vaccinated and the other non-vaccinated. Epidemiological data make also possible a continuous surveillance of the risks of vaccination. For example, epidemiological data show that the risk of encephalitis following natural measles virus infection is about 1 case per 1,000 affected people compared to less than 1 case per million following vaccination.

The struggle of making vaccines against ancient and new devastating diseases. Despite the many past and ongoing efforts, a fully effective vaccine against tuberculosis has not yet been obtained. This is a major global issue, since two billion people, more than a quarter of humanity, are infected with *Mycobacterium tuberculosis* that kills 4,000 people a day. Every year there are 250 million new cases of malaria, a *Plasmodium* parasitic infection transmitted by a mosquito: over a million people a year, largely children, die of malaria. An effective vaccine has not yet been made, although promising products are currently being tested. In the world more than 35 million people are infected with the Human Immunodeficiency Virus (HIV), with 2.3 million new infections per year and with over 1.6 million people dying of Acquired Immune Deficiency Syndrome (AIDS). Current therapies, based on the combination of anti-retroviral drugs are effective in blocking the virus but economically out of reach in the poorest nations. Despite many discoveries, huge funding and strenuous advocacy by affected people, thirty years were not sufficient to obtain a vaccine against HIV infection. Some scientific problems are very difficult.

Anticancer vaccines. New vaccines aimed at preventing chronic infections by microbes leading to tumor onset are getting an extraordinary social impact. Liver carcinoma accounts for more than 4% of all human cancers and 80% of cases are associated with hepatitis B virus (HBV) infection. Worldwide, more than 300 million people are infected by the HBV. Epidemiological data show that when the vaccination cycle with new HBV vaccines is completed, the protection against liver carcinoma is virtually total. Human Papilloma Virus infection is extremely common; however, only 5-10% of infected women develop intraepithelial cervical lesions with different degrees of potential neoplastic transformation. Even so, cervical carcinoma of the uterus is the most common female tumor after breast cancer. Since 2007, vaccines effectively preventing the HPV infection are available. Unfortunately, these vaccines are ineffective when a woman has already been infected. While all these vaccines are directed against microbes, recent clinical studies suggest that vaccines directed against cell abnormalities occurring during neoplastic transformation are able to slow down, or completely stop, the progression of pre-neoplastic lesions. However, despite numerous studies, the only currently approved curative vaccine is the one against metastatic prostate cancer. The procedures required for its preparation are complex and costly, while the therapeutic efficacy of this vaccine is limited.

Vaccination strategies. Each nation develops vaccine prevention plans that define who should be vaccinated and at which age. The Italian 2017-2019 National Plan for Vaccine Prevention adopts the so-called *Life Calendar*, a vaccine protection scheme designed to include not only the youngest but also older people. In addition to vaccination against chicken pox, rotavirus, HPV and meningococcal B, vaccination against the so-called *cursed triad of the elderly* has also been introduced: influenza, invasive pneumococcal disease and herpes zoster. Unlike these programmed vaccination plans, urgent interventions are triggered when epidemic outbreaks or real pandemics are emerging. In the face of a limited availability of the vaccine, it may be necessary to decide which population groups should be prioritized.

Global Alliance for Vaccines and Immunization. In 1990, 12 million children under the age of 5 died in the world. Twenty years later the number of dead children fell to 7.5 million. The spread of vaccination against diphtheria, tetanus, pertussis (the DTP vaccine) and that against measles has played a fundamental role in this reduction in child mortality. In order to make vaccines more available in the poorest Countries, the Global Alliance for Vaccines and Immunization (GAVI) was created. Thanks to innovative financing systems, GAVI is significantly enhancing the reduction of global mortality, making available vaccines specific for the microbial strains endemic in the poorest Countries. The development of a new vaccine that elicits persistent immune memory against meningococcus has virtually eliminated meningitis epidemics in 15 African nations, liberating 300 million people from a dreadful nightmare. GAVI funding has also allowed big vaccine manufacturers to join producers in developing Countries like India, Brazil, Cuba and others, encouraging the production of vaccines specific for the most unlucky areas of the planet.

The vaccination between technology, costs and policy. The development of a new vaccine - from initial design to market - has a cost that varies from 200 to 900 million euro and requires a scientific and technological effort about 10 years long *vis-à-vis* a probability to enter the market as low as 6%. It is obvious, therefore, that in order to decide whether to develop a new vaccine, companies should carefully consider the required investment, the risks and prospects of gain. The actual efficacy of a vaccine is another quite unpredictable variable. The technology required to produce enormous doses of vaccine, in the order of hundreds of millions, constitutes one more variable that significantly affects the project feasibility. The consequences of decisions based only on technical/financial evaluations are twofold: there is no market and therefore there are no vaccines against microbes that could cause epidemics; there is no gain and therefore no vaccines are available in the poorest nations.

The case of poorest Countries. The inability of poorer governments to respond to health problems, the difficulty of overcoming traditional culture, the lack of vaccine information and the problems of organizing an effective vaccination service in remote areas of the planet is combined with the commercial attitude of manufacturers, which have no incentive to study vaccines suited to the needs of areas of the world inhabited by populations with extremely limited purchasing power. The main current goals of GAVI and other international consortia are: *a)* To foster the development of new vaccines specific for the diseases of the world's poorest areas; *b)* To enable technologies to be available in developing Countries; *c)* To make national vaccination programs compatible with the health systems of poor nations.

Vaccines for epidemics that might emerge. Recent cholera, meningitis, Severe Acute Respiratory Syndrome (SARS) or the Ebola and Zika viruses have dramatically highlighted the absence of vaccines to control the sudden spread of an infectious disease. During the outbreak of the epidemic, there is vocal discussion about how the world needs to be better prepared, but with the attenuation of the media's clamor the problem seems to vanish, even though scientists have long lists of microbes that could cause horrifying epidemics. Faced with this fatalistic attitude, the Coalition for Epidemic Preparedness Innovations (CEPI) was launched in 2017 at the World Economy Forum in Davos, Switzerland, with the aim of promoting the development and storage of vaccines against those microbes that could cause new scary epidemics.

Opinion polls against vaccines: why? Efforts to make vaccines more effective and universally available clash with the passionate anti-vaccination reactions that from 1700 to date slithers in the population. Until the last century, these movements were minorities and vaccination coverage tended to grow. At present, vaccine-opposing groups found the internet an effective vehicle to spread their

positions and thereby we are witnessing a fall in vaccine coverage. Opposition to vaccines is triggered by the inherent characteristics of vaccination, a practice administered to a person (most often a baby) who is healthy, to prevent a hypothetical risk of infection. Vaccination is an individual act which, however, acquires particular protective value when a large percentage of the community is vaccinated (*herd immunity*). Laws that invite or oblige people to be vaccinated, as needed as they are, induce a reaction against excessive public intrusion into the private sphere. The mass media emphasis on hypothetical side effects of vaccines triggers waves of collective fear that mainly concern the accusation of causing autism, adjuvant and preservative toxicity, and the weakening of the immune system caused by too many vaccines. While anti-vaccine movements spread their objections with militant enthusiasm, health authorities often appear unable to convincingly explain the fundamental importance of vaccines. No matter how authoritative the official documents are, it appears extremely difficult to wipe out the suspicion that these documents are the result of a concerned manipulation and global conspiracy.

The journey of vaccines between epidemiological data, political issues and the Internet. While on one hand the opposition to vaccines must be accepted as a widespread social reality, epidemiological data shows the wake of suffering, illness and death created by these campaigns. Where a fall in vaccination coverage occurs, almost forgotten diseases, such as measles, often hit again. It is a serious mistake to think that there is no reason to vaccinate against preventable diseases because they are almost eradicated in Western Countries. Many infectious agents are still in circulation in some parts of the world, and globalization - with travel on the agenda, migration and poverty - makes vaccination a tool more than ever necessary. The two key words about vaccines are *research* and *sharing.* A better elucidation of the mechanisms of the immune memory will lead to more effective vaccines. The challenge that, more than anything else remains current and pressing is that of sharing. We have extraordinarily effective tools to prevent and tackle global scourges, but they are often not accessible in the poorest Countries or are rejected by some of the wellbeing societies. Their sharing is, however, vital to reducing unfair health inequalities among populations across the world.

1. INTRODUCTION

For 100 years, life expectancy has changed dramatically, ranging from 40 to 80 years for men and 40 to 84 for women. At the roots of this remarkable change there are many different factors, from the quality of drinking water to antibiotics, to nutrition, warm houses and others. Among these, the contribution of vaccines has been of utmost importance. According to World Health Organization (WHO) global estimates, vaccines save 5 lives every single minute and 7,200 daily. Thus, vaccination will have avoided over 25 million deaths in the present decade (Rappuoli and Vozza, 2013; Mantovani, 2016).

Along with environmental remediation measures, vaccines are the main public health tool for the prevention and control of infectious diseases. As shown by epidemiological data collected in all Countries and a huge number of scientific publications, vaccines are a low cost medical intervention that is most effective in reducing the burden of disease and death. Thanks to mass vaccination campaigns, to recall what a polio or diphtheria epidemic is, one should read Philip Roth, Mark Twain or Italo Svevo…

Vaccines are a prevention tool useful not only for single individuals but for the community as a whole, as discussed below: immunization of a sufficiently large number of people against a particular disease prevents the microbe from spreading, protecting also unvaccinated people (*herd immunity*). Without vaccines, long ago eradicated microbes (such as polio and diphtheria) would strike again, and we would lose an effective shield against diseases that would sooner or later remerge.

In fact, our relationship with the microbial environment (viruses, bacteria, parasites...) is continuously evolving, and media news constantly reveal emerging diseases, such as the recent serious threats due to Ebola and Zika viruses, or the spread of Dengue due to climate change. Unfortunately, it is not possible to predict the threat associated with newly emerging viruses and bacteria. Especially alarming is the scenario created by the impressive increase of antibiotic-resistant strains of bacteria. In a nutshell, vaccines and immunological interventions can play a key role in addressing old and new microbial threats.

Fundamental are therefore both global commitment to the control and surveillance of infections, and advanced scientific research to unveil the mechanisms whereby microbes cause a disease and the counteractions set into motion by the immune system. The final aim is to develop new weapons against microbes, first of all novel effective vaccines.

Several new challenges emerge on the horizon. Among these, the development of vaccines to be taken orally or by inhalation. By activating specialized alarm cells, vaccine of this kind trigger an immunological response at mucosal surfaces switching the antibody production towards IgA, a class of antibody able to neutralize toxins and microbes before they spread into the body through the fragile mucosal surfaces, their main entrance door. Not to mention that in the poorest Countries, vaccination without injection would be a major practical benefit facilitating vaccine diffusion.

Naturally a major challenge will be the development of vaccines activating effective immune responses against sneaky viruses such as the Human Immunodeficiency Virus (HIV), which resettles within the immune system itself making this task extremely difficult. Furthermore, a most important scientific challenge is the development of vaccines able to cure an ongoing disease, over and above prevention. The development of vaccines able to block the spreading of an existing tumor would be a major therapeutic success.

Last but not least, global sharing is an additional crucial challenge. One of the biggest frustrations is that such effective weapons cannot be exploited by people who need the most e.g. in the poorest nations where infectious diseases are endemic yet many persons do not have access to the most elementary vaccines (Hotez *et al*, 2016).

The purpose of this position paper is to provide lay persons with a few of the pillars on which the development and epidemiology of vaccines are based, as well as to highlight critical challenges of research in immunology and vaccination.

2. VACCINE TECHNOLOGY

2a. The immune memory.

Skin and mucosal epithelia provide a first efficient barrier to microbial invasions. When microbes overcome this barrier, the rapid release of danger signals leads within a few hours to the activation of a powerful *innate immunity* defense reaction. The reaction involves various cell types (innate lymphoid cells, natural killer cells, neutrophil and eosinophil granulocytes, monocytes, macrophages...) and several humoral control systems (cytokines, pre-existing antibodies, the complement system…) (Sonnenberg and Artis, 2015).

Once called into the invasion sites, cells and molecules of innate immunity mediate a wide array of defense mechanisms (the *inflammatory reaction*). In most cases, this rapid and structured reaction leads to an effective containment of the attacking microbes.

In rare occasions, the large numbers of microbes and their peculiar resistance to innate immunity reaction allow a more persistent invasion. In this case, the activation of a subsequent line of immune defense, based on the combined action of B and T lymphocytes, will come into play.

In our body billions of quiescent *precursor* T and B cells circulate. As each of them is intended to react against a distinct target (e.g. microbial molecules), the number of targets recognized by the lymphocyte populations is enormous. When one of these lymphocytes binds to its target in the presence of accessory signals delivered by innate immunity cells, it undergoes activation, begins to divide and generates a family (a *clone*) of new lymphocytes, all reacting against the target initially recognized by the precursor lymphocyte. Progressively, invading microbes are no longer recognized by just one or a few precursor lymphocytes, but by a large family of activated (*effector)* T and B cells.

When effector B cells meet their target, they differentiate and acquire the capacity of secreting in the body fluids very high levels of antibodies. These are proteins attacking their target wherever it may be. In addition, a few of the new effector T-cells become T *killer* lymphocytes able to find and kill the cells invaded by viruses, endocellular bacteria and endocellular parasites… Other effector T-cells differentiate into T *helper* cells that secrete a combination of molecules (*cytokines*) necessary to drive the powerful innate immunity reaction. Moreover, through the localized secretion of cytokines, T helper cells activate and modulate the B cell production of antibodies.

The reaction based on the T and B cells is called *adaptive immunity*[1], since the immune system changes in order to respond more efficiently to the attack of the intruders. Precisely because adaptive immunity is based on the expansion of clones of effector lymphocytes, the process takes time (at least one or two weeks) to become fully operative.

In conclusion, to contain and defeat an invasion, the immune system activates in sequence complementary and interconnected lines of defense:

i) A fast and powerful response of innate immunity, activated a few minutes to a few hours after the invasion;

ii) A later adaptive immune response.

The ensemble of reactions that are activated is complex, articulated and sophisticated and in many cases leads to recovery after a more or less extended hard war (the period of illness).

When the invader is destroyed, the immune system is no longer the same because the T and B cells that target the invading microbe have increased from 100- to 1000-fold. The persistence of these expanded lymphocyte populations (*effector/memory cells*) gives the organism the extraordinary ability to eliminate a subsequent invasion by the same microbe; often the efficacy and rapidity are so

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[1] Rather than offering a superficial description of the intricate mechanisms of *Innate Immunity* and *Adaptive Immunity*, which will often be referred to in this document, those who are interested should refer to Murphy 2016.

extreme that a person does not even notice subsequent infections. These individuals have become *immune*, exempted from the danger of contracting the same illness again.

This extraordinary protection (the *immune memory*) is due to the persistence of numerous effector/memory T and B cells which have fought the initial war. A first vivid account of the effectiveness of immune memory is given by Thucydides when describing the devastating plague epidemic exploded in Athens in 430 BC during the second year of the Peloponnese war: *There were survivors who had compassion on who was dying or sick, because they had already had experience and were in a confident state because the disease did not take the same person twice, at least not in order to kill one* (The Plague of Athens, 1980).

The clones of T and B effector/memory cells (*see Box 1*) persist for long periods, often for decades and in some cases for a lifetime, so much so that about 40% of adult B cells are indeed effector/memory lymphocytes (Seifert and Küppers, 2016). Epigenetic mechanisms also cause cells of innate immunity to acquire a form of immune memory to react more effectively to subsequent invasion by the same microbe (Hamon and Quintin, 2016). This *innate immune memory* probably explains why the vaccines against tuberculosis (BCG, the Bacille Calmette-Guerin[2]) and measles provide protection beyond the original target the vaccine is directed to.

Often the long lasting persistence of T and B effector/memory cells depends on the fact that, without being aware, a person is repeatedly infected by the same microbe, endemic in a particular environment or periodically epidemic. In addition, some microbes are confined to small body areas where they persist for years or reactivate after long time. Re-infections or re-activations that are quickly eradicated by effector/memory T and B cells induce their repeated re-activation and re-expansion, thus enhancing the immune memory. In our minds memories fade away with time; some, however, are better preserved, others are refreshed by stories or images and thereby kept for a lifetime. Something comparable happens for the immune memory (Mantovani, 2016).

***Box 1*. Compared to precursor lymphocytes, *memory* T and B cells:**

- Are 100 to 1,000 times more numerous;
- Display several distinctive features of their own;
- Are those expressing a receptor that binds its target with the highest precision;
- Are re-activated faster by the interaction with their target;
- Once re-activated, they generate intense responses. T cells activate both T killer and T helper reactions. B cells differentiate in plasma cells producing high amounts of antibodies binding to the invading microbes with remarkable precision;
- Live longer, giving rise to a persistent population of ready to fight against the target.

Immune memory not only has an extraordinary importance for our disease-free survival, but it is the basis of vaccination. A vaccine does nothing but create and re-stimulate an artificial immune memory, that is, a memory of a war that has never been fought being replaced by a minor and short skirmish, i.e. precisely the vaccine. Vaccination is nothing more than a procedure to induce an effective and often long-lasting expansion of effector/memory T and B cells and to maintain a high level of antibodies specifically reacting against a particular microbe. It is precisely the study of the cellular and molecular mechanisms that lead to the induction and maintenance of effector/memory T and B cell populations that is guiding the technological evolution of vaccines aimed at increasing their ability of inducing more specific, effective and lasting *immune memories*.

2b. The evolution of vaccines.

2b1. Variolation. The first testimony of a smallpox-like disease in China dates back to about a millennium BC. Smallpox was introduced in Europe probably around 500 AD. There are several testimonies of serious epidemics in London in the 17th and 18th centuries and in the American

colonies somewhat later. These epidemics caused many deaths or left disfiguring scars and blindness in the survivors. To stem the devastation caused by these epidemics, and long before the scientific history of vaccines began, various vaccination practices were empirically used. An ancient method used in China for the prevention of smallpox was to let children to inhale a dust obtained from the smallpox scabs.

Another method, practiced in the East, especially in the Ottoman Empire but also known in Europe, consisted in the introduction into superficial scratches made in the skin of powdered smallpox scabs or fluid from pustules of people affected by light cases of smallpox (Behbehani, 1983). After living for a while in Turkey, Lady Mary Wortley Montagu, an English aristocrat (1689-1762), sent letters to influential European personalities to promote this primitive form of vaccination, known as *variolation* or *inoculation*. Thanks to the influence of Lady Wortley Montagu and other influential Enlightenment thinkers, variolation spread throughout Europe so much so that in 1722 even members of the English royal family had been variolated (Grundy, 1997). In 1777, a smallpox epidemic persuaded George Washington to make the variolation to his soldiers mandatory (Grizzar, 1985).

Variolation caused by-and-large the development of local lesions that healed and protected the person from the systemic illness. According to current interpretation, this procedure introduced in the body killed or attenuated viruses which stimulated an immune response with production of antibodies.

2b2. Vaccines based on killed or attenuated microbes and inactivated toxins. The history of vaccinations begins in 1796 when Edward Jenner showed that the inoculation of purulent material obtained from bovine smallpox pustules protected humans from the infection by the human smallpox virus. Jenner had come to this fundamental discovery because he had noticed that women milking infected cows with breast pustules developed hand lesions that healed in a few days. Over-and-above he realized that these women did not contract the disease during smallpox epidemics. This discovery began the era of vaccinations and gave a fundamental contribution to Immunology (Fisk, 1959).

About a century later, Louis Pasteur marked another crucial milestone in the history of vaccination. In fact, he succeeded in obtaining a drastic reduction in the pathogenicity of the rabies virus by formulating the first anti-rabies vaccine (Debrè, 1994). The Jenner and Pasteur studies in fact started the era of first generation vaccines (*live attenuated vaccines*) based on a living microbe that has been weakened so it can not cause disease. Since attenuated microbes retain the ability to replicate *in vivo*, they are most effective in stimulating the immune system and inducing a strong and lifelong immune memory. In contrast, vaccines based on killed microorganisms (*inactivated vaccines*) are more stable and safer than live attenuated vaccines but their limit is mainly related to the short duration of immune memory which demands inoculation of higher amounts of vaccine.

Another major step was the formulation of vaccines against diphtheria and tetanus, terrible diseases caused by protein toxins. In the twenties of the last century French and British scientists discovered a laboratory procedure to transform diphtheria and tetanus toxins into *toxoids*, i.e. modified proteins that are innocuous but still capable of stimulating the immune response.

Later, based on Pasteur's discoveries and thanks to the development of *in vitro* cell cultures new attenuated microbes could be obtained. These second-generation o*f live attenuated vaccines* include the anti-poliomyelitis vaccine developed by Albert Sabin in 1953, the vaccines against measles, rubella, varicella and mumps up to the recent promising anti-malaria experimental approach based on the inoculation of live and attenuated malaria plasmodium sporozoites (FfSPZ vaccine, *see also 3b2*).

It is well-known that hundreds of millions of people have been protected from disabling and fatal diseases by using vaccines based on killed or attenuated microbes or inactivated toxins. Some fundamental complexities however have still to be tackled by scientists. The above approaches are not

feasible for some microbes that cannot be grown in culture (e.g. *Hepatitis C virus, HCV*). Another serious limitation is related to the hypervariability of certain microbes (e.g. *Human Immunodeficieny Virus*, HIV). In addition, in the case of microbes living within cells, the development of antibody immunity is not protective and defense mechanisms are only mediated by white cells (leukocytes), involving T cells and phagocytic cells. To overcome at least some of these issues and face serious epidemics, such as Ebola, new vaccine technologies based on conjugated polysaccharide, *reverse vaccinology*, and DNA or RNA have been developed.

2b3. Conjugated polysaccharides. The need to develop vaccines against polysaccharide components of bacteria is especially important for children in the first two years of life. However this approach is jeopardized since polysaccharides are very poor antigens. The problem was partially solved by conjugation of polysaccharides of the bacterial capsule with proteins that can elicit a strong immune response, such as inactive mutants of diphtheria and tetanus toxins. This approach made it possible to obtain a more intensive T-cell-dependent response with production of high affinity antibodies of the IgG class.

The first vaccines based on conjugated polysaccharides were made by John Robbins at the National Institutes of Health (NIH), Bethesda, USA. Conjugated vaccines of this type have then been produced against *Neisseria meningitidis* (meningococcus), type B *Haemophilus influenzae* (HIB) and *Streptococcus pneumoniae* (pneumococcus). The meningococcal type A and type C conjugated vaccines have been produced by Sclavo, Siena, Italy. This technique is currently being developed in order to formulate vaccines against multiple targets (BiosYnth, 2017).

2b4. Reverse vaccinology. This strongly innovative technology, has been conceived, developed and commercialized by a group led by Rino Rappuoli, from the Novartis Vaccine, Siena, Italy. The first successful product was the B-type meningococcal vaccine (Rappuoli, 2000). The conceptual approach is based on sequencing the bacterial genome to detect proteins present on the surface of the microbe. Numerous proteins were identified, cloned, and used to immunize mice. After many *in vitro* and *in vivo* tests, three proteins common to several meningococci were selected and employed to formulate a first universal vaccine providing good protection against meningococcus B.

Reverse vaccinology can offer the solution for the development of vaccines that could hardly be obtained by conventional techniques. For example, this approach was used in the development of vaccines against *Staphylococcus aureus*, *Pneumococcus* and *Chlamydia*. In addition, due to the poor capability of some of these technological products to activate a robust immune response, the design and use of new adjuvants, both of bacterial and synthetic origin, has been an important by product of this approach (*see 2c*).

2b5. DNA vaccines. This technological approach is based on the ability to induce cells of the individual to be immunized to synthesize the antigen against which an immune response is desirable. This is possible by injecting intramuscularly cDNA plasmids encoding the protein of interest. The efficacy of DNA vaccines is increased by applying a small and short electric shock at the inoculum site to increases the permeability of cell membranes in order to favors the penetration into the cells of the DNA vaccine (Quaglino *et al*, 2004). DNA vaccination induces the production of antibodies, but it can also favor the development of cell immunity mediated by T killer cells that are more effective in controlling a primary viral infection.

2b6. RNA vaccines. These have been developed recently with the aim of speeding up the formulation of new effective vaccines. As messenger RNA translates the genetic information copied from DNA into protein, its introduction into cells is followed by the synthesis of the protein of interest that is expected

to elicit an immune response. The specific messenger RNA is entrapped into virosomes, liposomes (*see 2cb*) or other nanoparticles that can be easily internalized by the cells. Employing this experimental approach with mice it has been possible to induce abundant production of antibodies against influenza, Ebola, Toxoplasmosis, Zika (Pardi *et al*, 2017), even in the absence of other strong adjuvants. Once confirmed in humans, this technology may allow obtaining an effective vaccine in about a one week, thereby representing an important innovative approach for combatting serious epidemics.

2b7. Development of new vaccines. Several other vaccination strategies are being developed including mucosal vaccines that block microbes before they can enter the body (*see 1*) and vaccines based on Dendritic Cells specialized in the capture of foreign substances and in the activation of the T-lymphocytes (*see for example the prostate cancer vaccine discussed at paragraph 3c3).*

Viral vectors (such as Vaccinia virus and Adenoviruses) genetically modified by insertion of the gene encoding for the target protein are promising approaches for new vaccines. During the infection the release of the target protein triggers a robust and specific immune memory; moreover these recombinant viruses are endowed with natural adjuvants triggering long lasting immunity.

These vaccines in development, though very promising, have not yet been approved for clinical use by regulatory authorities even if many clinical trials are currently implemented to evaluate their efficacy. New formulations to induce an effective and appropriate immune memory are made possible thanks to the availability of fresh technologies. It should be emphasized, however, that integration of new vaccination strategies has not substituted traditional vaccines. The exploitation of the advantages of the new vaccines led to an expansion of the spectrum of diseases protected by vaccination.

2c. Adjuvants.

The keynote lecture by Charles Janeway Jr, held in 1989 at the Cold Spring Harbor Symposium, is a milestone of Immunology: by highlighting the key role of innate immunity in the induction of immune memory he revolutionized our understanding of the immune response (Janeway, 1989). The simple entering of foreign molecules in the body is not enough to activate T and B cells. To induce effective activation one usually resorted to what Janeway called "*the Immunologist's dirty little secret*", that is, the use of raw mycobacterial extracts, of mineral oils or aluminum hydroxide, substances collectively called *adjuvants*. Prior to Janeway's studies it was unclear why adjuvants were needed or how they worked. Thanks to the studies prompted by Janeway, it was understood that adjuvants interact with families of trans-membrane receptors expressed by the cells of innate immunity. Among those, the *Toll-like receptors* (TLR) allow immune cells to perceive traces of microbial invasion. Indeed a prototype of these receptors was identified by Janeway (Medzhitov *et al*, 1997). The discovery of the TLR and the key role of the cells of innate immunity in the activation of T and B lymphocytes and thereby of the immune memory boosted the three Nobel Prizes for Medicine in 2011. Unfortunately, Charles Janeway, who most of all deserved this recognition for his insights and his discoveries, had died in 2003 at only 60 years because of a B lymphoma against which he had struggled with courage for years without never giving up his great passions: research and teaching. This premise, In addition to being a tribute due to a great scientist, is needed this consideration is appropriate because Janeway's research provided, for the first time, a conceptual framework for the formulation of new adjuvants.

Initially vaccines made use of live attenuated or killed and inactivated microbes that naturally express on their surface adjuvant molecules recognized by TLR and other similar receptors activating the cells of innate immunity. On the other hand, most vaccines developed in recent years employ as target molecules or molecular aggregates rather than the whole microbe. These molecules have to be associated with adjuvants in order to trigger an innate immune cells activation. The currently used adjuvants are of various kinds and act with different mechanisms.

2c1. Aluminum salts. The first adjuvants used in the preparation of vaccines were Aluminum salts (*Alum*). It was Alexander Glenny who discovered how effectively Alum acts through a repository effect by trapping the vaccine at the injection site (Glenny, 1921). Another casual observation by Gaston Ramon working on immunized horses to obtain antibodies against tetanus and diphtheria revealed that a clear increase in response was obtained when an inflammation is taking place at the vaccine inoculation site (Ebisawa, 1987).

The slowly released vaccine caused by Alum provides a continuous stimulus to the immune system and triggers a small local inflammation with the recruitment and activation of macrophages, Dendritic Cells and other innate immune cells. Moreover, Alum has also the ability to bind vaccine molecules forming large aggregates that are more easily captured by macrophages and Dendritic Cells essential to activate the immune memory of T and B cells. In this way Alum acts by favoring the production of antibodies (schematically TH2-type immune responses) and is therefore effective as an adjuvant mainly for vaccines against those microbes that are susceptible to antibodies and for vaccines against bacterial toxins.

2c2. Liposomes and virosomes. Liposomes, phospholipids and cholesterol vesicles with a diameter between 25 nm and 1 µm, can be exploited to transport encapsulated substances such as drugs or vaccines. For example, the RTS, S/AS01 malaria vaccine discussed later (*see 3b2*) is carried by a liposome.

The first human vaccine using an adjuvant other than Alum is the vaccine against hepatitis A, in which a virosome is used as an adjuvant. Virosomes are structures that can be assimilated to liposomes, which can carry proteins present on the surface of the virus, either encapsulated inside or stuck outside. By exploiting the virus's ability to bind to specific cellular receptors, virosomes can be used to convey drugs or vaccines to specific target cells. In addition to hepatitis A vaccines, virosomes are also used as adjuvants for anti-influenza vaccines.

2c3. Adjuvants acting on cell receptors. Classical adjuvants favor almost exclusively the production of antibodies (TH2-type immune responses), while one of the main challenges is the development of adjuvants favoring the activation of T killer cells, i.e., the induction of TH1-type immune responses. To achieve this goal, natural or synthetic adjuvants interacting with Toll-like receptors *(*TLR) and other cell membrane receptors expressed by cells of innate immunity have been developed. The activation of these receptors triggers the secretion of molecules (cytokines and chemokines) favoring local inflammation. The combination of adjuvants acting on cell receptors with classic adjuvants (emulsions or Alum) allows the induction of immune responses especially effective against microbes living within cells, such as viruses (endocellular microbes). These new adjuvants have been exploited for the hepatitis and the papilloma virus vaccines.

Often, natural molecules that bind to these receptors have been replaced by less toxic synthetic products. For example, *a)* the lipopolysaccharide of Gram-negative bacteria (LPS) has been replaced by monophosphorylated lipid A, because the first one induces fever; *b)* single receptor-linked RNA adjuvants have been replaced by imidochinols, which effectively activate Dendritic Cells by stimulating the production of cytokines and consequently TH1 type immune responses.

Immune responses of type TH1 are also induced by particular nucleotide base sequences (*CpG sequences*, Cytosine phosphate-Guanine) acting as natural adjuvants in DNA vaccines.

2c4. Modern Adjuvants. New approaches are currently being studied to make existing vaccines more effective and to produce new ones. Big challenges for immunologists not only because of the

complexity of the pathogenic action of some microbes but also for the specific features of the immune response of people at risk.

Aging is accompanied by a gradual weakening of immune responses. This causes increased susceptibility to infections, coupled with a decreased capacity to respond to traditional vaccines. Also newborns and, in general, children under the age of three, who have an immune system still under development, may have inadequate responses to vaccines. Not to mention patients with congenital or acquired immunodeficiencies caused by infections, chemotherapic treatments and immunosuppressive drugs.

To overcome these difficulties, various strategies and new approaches based on modern technologies and increased knowledge of the mechanisms of immune memory are being investigated. Beside the development of new adjuvants, great attention is paid to new ways to elicit immune responses of greater intensity and duration. This goal is of critical importance for vaccines based on proteins or their fragments which are not so effective in activating an immune response. An enhanced ability to elicit an effective immune response would allow to reduce the amount of vaccine to be used and obviously an increase in the number of people that could be vaccinated.

Several adjuvant combinations are currently evaluated in order to obtain more potent and selective activation of innate immunity cells using emulsions (of oil-in-water) of squalene, biodegradable natural oil. These emulsions are administered not only in combination with proteins but also in association with the killed influenza H5N1 virus, responsible for the fearful avian influenza, causing high mortality in humans. The combination of H5N1 virus with squalene emulsion may overcome the poor ability of the virus to induce an effective immune memory.

2d. Challenges for new vaccines.

Currently there are vaccines against a little more than 25 species of microbes causing disease in humans (Smith *et al*, 2011). However, as ever before in the history of humanity, a large number of public and private researchers are involved in studies leading to new scientific and technological knowledge that can be applied to vaccines. It is therefore foreseeable that in the next 20 years many innovative vaccines will be available. It is not an easy task to develop vaccines against microbes for which no vaccine is as yet available since many of them have evolved ingenious strategies to escape the powerful reactions of immune memory.

New vaccines should to induce effective immune responses in:

- Infants, a population group that is crucial to defend, lacking a fully developed immune system;
- Elderly persons, a rapidly increasing immunologically fragile group of people whose immune system poorly responds to new antigenic stimuli;
- Persons with an immunodeficiency, a population group that is numerous in vast geographical areas, particularly in Africa, due to the spread of HIV infection;
- Cancer patients whose immune system is dampened by both antiblastic drugs and cancer progression.

In addition, new vaccines should ideally be able to able to induce an immune memory that is:

- Persistent for long periods, possibly for the rest of life, avoiding the need for repeated boosts;
- Effective against parasites and fungi. There is currently no approved vaccine for human use against diseases caused by parasites despite their dramatic spread;
- Protective against microbes that exhibit a high degree of variability in target molecular structures, and are therefore a mobile target that can slip between the mechanisms of immune memory.
- Based on the selective activation of T helper or T killer cells; alternatively on the induction of particular class of antibodies;

- Present at the mucosal surfaces in order to neutralize intruder microbes before they penetrates the body.
- Against numerous types of the same microbe in order to prevent:
  a) that the vaccine is effective only against microbial types present just in a few areas of the world;
  b) that the herd immunity induced in a population of vaccinated people favors the emergence of microbial types escaping the immune memory reactions induced by that vaccine.

Other technological advances should lead to new vaccines:

- To be administered via routes other than needle and syringe injection, a practice which poses problems of sterility and management;
- To remain effective even when stored for relatively long periods.

In order to increase safety and reduce side effects, the trend is to abandon vaccines based on inactivated or killed microbes, and to develop new vaccines based on molecules from the outer surface of microbe, often obtained by recombinant DNA technology. The immune memory elicited by these new vaccines is precisely directed towards biomolecules of vital importance for microbe's survival. These molecular vaccines lack of the ability to trigger innate immunity signals playing a crucial role in activating an effective immune response. Therefore their efficacy critically rests on combination with suitable adjuvants (as discussed in *2c*).

Finally, a fascinating prospect is to replace mass vaccination with a vaccination "*à la carte*" based on the assessment of genetic features and vulnerability of the individual to be vaccinated. Even if nowadays this perspective appears removed and extremely costly, a personalized vaccination program that maximizes the likelihood of benefits but mitigate mitigating the risks of adverse events would be extraordinarily efficient (Moxon and Siegrist, 2011).

3. VICTORIES, DEFEATS AND BATTLES IN PROGRESS.

3a. Benefits and risks of vaccination: an epidemiological assessment.

Eradication of smallpox was officially declared by the WHO in 1979, three years after identification of the last case in Somalia. This is considered the most striking success in the history of vaccination and a paradigmatic example of the efficacy of vaccines. Vaccination has eradicated poliomyelitis from the Americas, Europe and Australia, but not yet from Africa and Asia because of political reasons and wars, although is well under way. Vaccination also reduced up to 99% the cases of diphtheria and tetanus worldwide.

The methods exploited to evaluate the effectiveness of vaccines have evolved over the last two centuries. The efficacy of smallpox vaccination was allegedly demonstrated by Edward Jenner immunizing just one child: a single *in vivo* test, carried out when ethics committees or controlled clinical trials were not in sight, proved the validity of many concomitant observations. Following that single demonstration of protection, the practice of vaccination became more and more widespread, and the disappearance of smallpox from the face of earth is absolute evidence that vaccination can be very effective. This achievement is all the more convincing given the mode transmission of smallpox: infections transmitted through air can hardly be controlled via alternative interventions. It is therefore concluded that the success achieved against smallpox can only be attributed to vaccination campaigns.

The decline in the number of disease cases following the introduction of a vaccine as evaluated through ecological studies provides a straightforward assessment of efficacy. However, these studies based on an extremely simple design do not take into account the confounding variables that may come into play and cause *natural* fluctuations in the incidence of a disease, regardless of the vaccine efficacy. Ecological studies are indeed capable of producing scientific evidence when the effects are macroscopic, as in the case of smallpox eradication.

3a1. From empirical observation to efficacy studies: controlled randomized clinical trials. Nowadays sophisticated tests are exploited to assess vaccine efficacy and possible collateral effects. The *gold standard* is the randomized controlled clinical trial based on the comparison between vaccinated and non-vaccinated cohorts of people. If the vaccine is clearly protective, the incidence of the disease will be significantly higher in the non-vaccinated cohort. Among many others, anti-polio vaccination was introduced in the 1950s following large-scale, controlled clinical trials, which showed its efficacy and safety. Currently, any new vaccine could hardly be marketed without having passed a rigorous evaluation based on controlled clinical trials. To give an example, the protective efficacy of a vaccine directed against 13 different types of *Pneumococcus* has been shown in elderly people by carrying out a study on approximately 85,000 individuals above age 65, randomly assigned to the vaccine or placebo groups (Weinberger *et al*, 2015).

For low-risk illnesses, however, it is not possible to achieve such efficacy tests, since a much larger number of people should be followed for a very long time. For example, the efficacy of yellow fever vaccine in travelers is virtually impossible to evaluate since it is very difficult to recruit a sufficiently large number of cases given that even in Countries where the yellow fever is endemic, the risk of contracting infection during a trip is very low. Therefore, the use of surrogate protection indicators, such as the evaluation of the titer of antibodies elicited by the vaccine, is often used.

3a2. Efficacy assessment. For regulatory purposes it is necessary to show the protective efficacy of a vaccine by determining the frequency of infections, diseases, deaths, or surrogate markers such as the antibody titer. When a vaccine is available on the market it is important to assess the real impact of the introduction of this vaccine on the inhabitants of a certain area of the word (Weinberg, 2010; Bruhn *et al*, 2017). This assessment can be made:

- By comparing the incidence of the disease in the population before and after mass vaccination;
- By estimating the relative risk of disease, that is the ratio of the incidence of disease in vaccinated and non-vaccinated persons.

The efficacy of a vaccine may be *underestimated* because the disease incidence assessed after its introduction may also suffer from the extent of vaccine coverage (i.e., a number of cases that may occur in not vaccinated people). The efficacy of vaccination may also be *overestimated* because vaccination of a portion of the population may reduce the circulation of the infectious agent, reducing the likelihood of being infected. Thus, an assessment of vaccine efficacy may be affected by several uncontrolled confounding factors (the so-called *ecological fallacy*).

Distortions in the estimate of vaccine efficacy may also be due to incomplete homogeneity between vaccinated vs. non-vaccinated people: unlike in randomized trials where randomization minimizes selection bias, here the two groups may differ in several respects. It should also be taken into account that, with certain vaccines such as the anti-influenza, efficacy may vary from year to year due several causes. For example, genetic mutations occurring during a seasonal outbreak of the virus may cause a change in the viral proteins targeted by the vaccine. In spite of these difficulties observational studies evaluating the real efficacy of a vaccine are of crucial importance to confirm the validity of a given formulation beyond the experimental context.

3a3. Active surveillance of adverse events. Once the efficacy of a vaccine has been shown, it has been approved by the national health authorities and marketed, its effectiveness and tolerability continues to be carefully monitored. To this end, European legislation has developed a set of standards for the communication, collection, analysis and evaluation of adverse events (side effects). At regular intervals, the vaccine manufacturer must submit to the health authorities three types of documents:

- *Periodic Benefit Risk Evaluation Reports* in which the vaccine safety data and the benefits that derive from the vaccine are presented and discussed periodically;
- *Risk Management Plans*, which describe the supervisory activities and interventions implemented to identify, characterize, prevent and minimize risks related to the vaccine;
- *Post-authorization safety studies*, i.e. the collection of studies aimed at identifying, characterizing and quantifying a risk associated with the vaccine, confirming its safety profile, or to assess the effectiveness of the measures implemented to minimize the risks associated with that vaccination.

Surveillance by the national health authority will continue analyzing *spontaneous reports* of adverse events following vaccination. These reports can be sent by physicians, healthcare professionals, patients and parents either online or by submitting properly filled-in forms. From the collection and analysis of these reports, warnings may emerge that deserve further in-depth analysis to deny or confirm the causal link between the reported adverse event and vaccination. In special cases, such as following the introduction of a new vaccine, the regulatory authority actively encourages the submission of reports by healthcare professionals. To manage this complex set of activities, European national health authorities have set up workgroups of professionals dedicated to vaccine surveillance.

The procedure proposed by the Word Health Organization (WHO, 2013) to assess a causality link between a vaccination and adverse events has been adapted to the reality of the various European countries. This assessment is in fact central in determining the safety of a vaccine. To establish the existence of a causal link, a number of factors are considered such as:

- The interval between vaccination and onset of the reaction;
- The presence of predisposing or concurrent factors;
- The presence of other treatments potentially responsible for the adverse event;

- Biological plausibility and information on what happens after the vaccination is suspended or eventually resumed.

Based on these assessments, the WHO document proposes to rank the causality nexus in four categories: unclassifiable, connected, unrelated and indeterminate.

The vaccine supervisory working group also plays an important role in communicating with healthcare professionals and citizens by means of publications that update on vaccine safety issues.

3a4. Benefits and risks of vaccines. Vaccines are a medical intervention and therefore adverse events cannot be excluded *a priori*, even though in the vast majority of cases side effects are of mild to moderate severity. Nevertheless, it is rational to compare any vaccination risks with the benefits that may arise. Epidemiological data often indicates that the benefits far outweigh the risk of side effects. For example, the risk of encephalitis due to natural infection by the measles virus is about 1 in 1,000, while it is 1 in 1 million (1000 times lower) following vaccination (*see Box 6*).

The case of polio well illustrates the care of national health systems to diminish the probability of adverse events. The very efficacious, live-attenuated *Sabin* vaccine has long been used when polio was a widespread disease. It was preferred over the virus-inactivated and therefore totally harmless *Salk vaccine* as it simulates a natural infection, ensuring the circulation of the attenuated virus. In this way, Sabin vaccine contributes to create an efficacious *herd immunity*. However, a live-attenuated virus, especially if administered to an immune-depressed person, can cause illness. Therefore, as soon as polio had been eliminated from Italy, the Italian national health system switched from the attenuated to the inactivated vaccine to minimize the occurrence of adverse events as rare as they are.

3b. The struggle of inventing vaccines against devastating ancient and new diseases.

The traditional application of vaccines is against infectious diseases. Nevertheless not only vaccines protecting against all the major infectious diseases are not yet available but there are no vaccines preventing chronic or degenerative diseases *(see also 2d)*. Difficult scientific conundrums as well as issues connected to market economy and the need for large investments explain why the vaccines currently available respond only partially to global health needs.

Undeniable is the failure to develop effective vaccines against tuberculosis, *Acquired Human Immunodeficiency Syndrome* (AIDS), and malaria, three global scourges. These diseases are still a major challenge for the research in immunology since obstacles of different kind hinder the preparation of an effective vaccine. The scientific difficulties encountered and only partially overcome are spurring scientists to pursue new ways to better understand the functioning of the immune system and trigger an effective immunity, paradoxically learning from the behavior of microbes.

3b1. Tuberculosis: an ongoing challenge. Currently, tuberculosis is causing *only* about 1 million deaths per year (*see Box 2*). In industrialized nations it was possible to control the disease thanks to the enforcement of public health measures (hygiene and cleanness, living conditions and good nutrition, isolation of infected people in sanatoriums), the introduction of the BCG vaccine, and the availability of drugs such as streptomycin, which in the long run may induces the emergence of drug-resistant strains.

Box 2. **Tragic are the figures of tuberculosis infection in the world:**

- 125,000 new infections per day;
- 2 billion people - more than 1/4 of humanity! - infected in a latent way, i.e. people carrying an infection that is kept under control by the immune system
- 25,000 new cases of tuberculosis per day, 9 million a year;
- 4,000 deaths per day, or about 1-1.5 million deaths per year, mostly children.

(Center for Disease Control and Prevention, 2017)

However, owing to the intense migratory flows, the health emergency persistent in the poorest nations is becoming global.

The BCG is a safe and relatively effective vaccine[2] that has been available since the 1920s. Unfortunately it has a limited spectrum of action protecting children against miliary tuberculosis and tuberculous meningitis while it does not adequately protect adults nor prevent lung tuberculosis. It is no surprise therefore, that in 2015 the WHO has set the reduction of tuberculosis mortality by 95% and disease incidence by 90% as goals for 2035. These are ambitious goals, achievable only with targeted efforts in formulating a vaccine able to trigger an effective immune protection with a broad spectrum of action.

Mycobacterium tuberculosis is a bacterium identified by the German physician and bacteriologist Robert Koch at the end of the 19th century. Koch also made a first attempt to formulate a vaccine using a component of mycobacterium. Unfortunately, Koch lacked the scientific know-how that, a hundred years later, allowed to learn to use isolated components of a microbe to generate an effective vaccine (such as the anti-hepatitis B virus). The BCG vaccine, based on the more traditional technology of live and attenuated microbes, was instead developed by the rival institution, the Institut Pasteur of Paris, in 1921.

Mycobacterium tuberculosis is able to survive within the cells of our body and even within the cells of the immune system, avoiding the attack by antibodies that are unable to penetrate cells. However, clinical experience has shown how important the immune system is in controlling infection by this mycobacterium: after all only 10 to 15% of infected people develop the disease during their lifetime generally due to decline in immune defenses. It is the case of people who, owing to congenital defects of some immunity components have serious problems in controlling all bacteria that behave like mycobacterium. It is also the case of patients infected by Human Immunodeficiency Virus or with rheumatoid arthritis, which should be followed with special attention because under these conditions mycobacteria may activate.

3b2. The case of malaria. In some ways similar is the case of malaria, an infection by parasites of the genus *Plasmodium* transmitted by a particular type of mosquito. The *Plasmodium* has an extremely complex life cycle that makes it able to elude the immune response.

> ***BOX 3*. Malaria: current figures:**
>
> - Almost half of the world's population is at risk;
> - 250 million people are infected every year;
> - 1 million people, especially children, die every year.
>
> *(Center for Disease Control and Prevention, 2017)*

Today, malaria mainly affects tropical areas and, especially, sub-Saharan Africa, while in the past it was endemic in Countries that nowadays we do not associate with this disease, such as Italy and even Norway. Contagion and mortality have also decreased in Africa over the last years thanks to the introduction of pharmacological therapies and the use of long-lasting insecticide-impregnated mosquito nets: nevertheless, variants of plasmodium resistant to drugs and mosquitoes surviving insecticides have both emerged.

New hopes, however, have come to light with the introduction into clinical use of *artemisinin*[3] and its derivatives, a major therapeutic progress, and with the likely prospect that a preventive vaccine (RTS, S/AS01) is becoming available. This is a sophisticated vaccine, based on the combination of a protein fragment from the pre-erythrocytic stage of *Plasmodium falciparum* and a fragment of the

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[2] The Bacille Calmette-Guérin (BCG) consists of live attenuated *Mycobacteria tuberculosis:* each year about 100 million children are vaccinated with BCG.

[3] The discovery of artemisinin by the Chinese scientist Tu Youyou was worth the Nobel Prize in 2015, anticipated by the Lasker Award in 2011.

hepatitis virus. The two protein fragments are inserted into liposome (*see 2cb*) together with an adjuvant. A study published in the New England Journal of Medicine reports that vaccination with RTS, S/AS01 induces a protection of about 50% against infection and about 30% towards serious episodes of illness (Olotu *et al*, 2016). If confirmed, these data could shortly lead to the RTS, S/AS01 vaccine approval even if it is only partially effective. The
limited protection elicited probably depends on the fact that the protein targeted by the vaccine is partially different in various strains of plasmodium. Moreover, the protection elicited vanishes after three to four years from the last booster. Therefore, despite the tremendous efforts made - 28 years of research, $ 565 million investment and a 3-5 years pilot study on 1 million children launched in 2016- RTS, S/AS01 seems not to be a decisive vaccine, but only an ameliorative step forward. By itself it cannot be the solution, but combined with new therapies and *ad hoc* environmental strategies, this vaccine could make a significant contribution.

Several other anti-malaria vaccines are being developed. Among these, a promising one is a new approach based on *Plasmodium falciparum* pre-erythrocyte forms (the sporozoites), alive but attenuated by radiation (the FfSPZ vaccine). When administered to people taking an anti-malaria medication (chloroquine) FfSPZ appears capable of inducing a particularly protective immunity (Mordmuller *et al*, 2017).

3b3. AIDS: victories and defeats. The Human Immunodeficiency Virus (HIV) penetrates and reproduces in T helper cells. Their gradual decrease leads to a severe dysfunction in the immune response, the Acquired Immune Deficiency Syndrome (AIDS).

BOX 4. **The numbers of HIV infection speak alone.**

- More than 35 million people infected worldwide;
- 2.3 million new diagnosis each year;
- 1.6 million deaths each year.

(Center for Disease Control and Prevention, 2017)

Isolated cases of AIDS had been first reported in the 1970s in the United States and in other areas of the world such as Haiti, Africa and Europe. The epidemic emerged in the United States after 1980 was quickly recognized as a new the clinical syndrome. In 1983, when the HIV was identified, scientists and politicians promised an HIV vaccine in three years. It still does not exist and this is perhaps the most scorching defeat for modern vaccinology (Rappuoli and Aderem, 2011).

Over 30 years of study and thousands of committed researchers have led to hundreds of small and major scientific breakthroughs: following HIV identification, scientists unveiled the mechanism of cell infection, the defense reaction of the immune system, and eventually the design and synthesis of highly effective anti-retroviral drugs (Anti-Retroviral Therapy, ART, used in combination of three or more). By targeting distinct phases of the natural history of HIV infection, combination ART controls the progress of the infection substantially reducing the mortality in HIV-infected people. In Europe, from 1996 to today, the AIDS mortality rate has dropped by about 80% and the progression of the infection from asymptomatic to full-blown illness has declined proportionally in such a way that life expectancy is approaching that of non-infected individuals. By contrast epidemiological data on AIDS incidence and mortality remain dramatically high in countries where combined ART cannot be implemented for cultural, economic or political-social reasons (*see Box 4*).

One of the difficulties in formulating an effective vaccine is the HIV virus itself, a sly retrovirus that exploit immune system cells as *Trojan horses* to spread throughout the body: the first to be infected during an unprotected sexual relationship with a HIV infected person are Dendritic Cells, namely the sentinels who have the task of alerting the immune system of the presence of an alien agent. Hence the virus passes to T helper cells. Originating in the thymus, these lymphocytes maintain, as directors of the immunological orchestra, the harmonious functioning of all components of the immune system, and become the strategists of the defense immune reaction following the invasion by

microbial agents. Finally, the virus is transmitted to macrophages that patrol the whole organism and are literally able to eat the aggressors. By exploiting macrophages as *Trojan horses*, HIV enters the central nervous system, causing serious damage. Thanks to its genetically unstable structure, the HIV virus evades defense immune reactions by changing continuously and rapidly, acting as a mobile target.

3c. Cancer vaccines.

Vaccination may induce an immune memory that may interfere with the development of tumors by:

- Preventing infections, and in particular chronic infections, by microbes leading to the onset of tumors *(Primary prevention)*;
- Inducing an immune response against the abnormalities associated with neoplastic transformation and thereby slow down or inhibit the progression of pre-neoplastic lesions (*Secondary prevention*) or cure tumors already clinically diagnosed (*Cancer therapy*).

3c1. Primary cancer prevention. Vaccines can be exploited to prevent a cancer caused by a chronic infection, a prevention based on the removal of an essential risk factor. Vaccinations of this type are currently having an extraordinary impact on global health.

3c1a. Liver cancer. The case of liver cancer paradigmatically illustrates both the extraordinary effectiveness of this form of primary prevention and the importance of the technological evolution of vaccines. Hepatocellular carcinoma accounts for more than 4% of all human cancers and 80% of those are associated with chronic *Hepatitis B Virus* (HBV) infection affecting over 300 million people in the world. The first vaccines against HBV produced at the beginning of the 1980s were based on viral proteins purified from human plasma. These vaccines are now replaced by safer and effective vaccines consisting of HBV capsid proteins (HBsAg, *B surface antigen* or Australia antigen) obtained in yeast by recombinant DNA technology. The vaccination of children against HBV protects not only against acute hepatitis, but also against complications that develop in a minority of patients: chronic hepatitis, liver cirrhosis, and liver cancer. Epidemiological data indicate that in children the risk reduction of carcinoma development is directly related to the number of vaccinations and the type of vaccine used. When the vaccination cycle is completed using the recombinant vaccine, the protection against liver cancer is virtually total (Lollini *et al*, 2011).

An effective vaccine against *Hepatitis C Virus* (HCV) would be needed to complete the immune prevention of hepatocellular carcinoma. HCV infection is less prevalent than HBV, but carries a higher risk of chronic infection and cancer. However, the efforts toward HCV vaccine are hampered by the shortage of preclinical *in vitro* and *in vivo* models and by the heterogeneity and mutability of the virus. Nonetheless, some promising candidates are now undergoing early clinical trials (Strickland *et al*, 2008).

3c1b. Cervical carcinoma of the uterus. *Human Papilloma Viruses* (HPV) form a family of over one hundred types of viruses infecting humans. By promoting excessive growth of epithelial cells, HPV infection may cause common benign lesions, such as warts affecting the skin of the hands, feet, or face, and condylomas or papillomas affecting the mucosal surfaces of the genitals and mouth. The most dangerous types of HPV are those that cause lesions that can slowly evolve to upper respiratory tract carcinomas (larynx, pharynx, tongue, tonsils, palate, and nose) or male and female genitals.

Genital infection is transmitted almost exclusively through sexual intercourse, though not necessarily following a full encounter: it is one of the most frequently reported sexually transmitted diseases. Generally, the most dangerous infections of the respiratory tract or the oral cavity are transmitted through oral sex.

HPV genital infection is very common in the population: it is estimated that up to 80% of sexually active women are infected over the course of their lives with an HPV virus, with prevalence in young women up to 25 years of age. In the vast majority of cases, though, infection is spontaneously eliminated within a few years. Only 5-10% of the women who are HPV-positive develop condylomas and warts or intraepithelial wounds of the uterine cervix that may have different degrees of potential carcinogenic transformation.

In the rich nations screening programs based on the molecular diagnosis of HPV infection, on Pap-test and colposcopy allow identification of possible lesions. In the vast majority these are low grade malignancy intra-epithelial lesions that are eliminated by partial removal of the uterus, allowing women to maintain reproductive capacity. In Italy, these check-ups, recommended every three years for women between 25 and 64 years, reduce by more than 70% the risk of developing uterine cervix cancer. However, despite this complex organization for early diagnosis, it is estimated that in Italy about 1,000 women die every year because of uterine cervix cancer.

Unfortunately, in the absence of screening programs, as is the case in many African nations, cancer of the uterine cervix can become the first cause of death. There are over 400,000 new cases per year in the world and more than 250,000 deaths, indicating that it is the most common female tumor after breast cancer.

While the development of therapeutic vaccines is still at an experimental level, currently available vaccines effectively prevent HPV infection but are unable to cure it. These are vaccines made from HPV proteins produced in baculovirus with recombinant DNA technology. The commercial bivalent and tetravalent vaccines are both capable of inducing protection against infection by type 16 and 18 HPVs, the two viral types that most frequently cause cancer lesions. The tetravalent vaccine also protects against HPV types 6 and 11, two viral types associated with the generation of genital condylomas. A more recently released vaccine, in addition to inducing protection against HPV types 16 and18 as well as types 6 and 11, also protects against other 5 HPV oncogenic types.

The worldwide implementation of vaccination programs against HPV has only begun since 2007, thus there is still no reliable data on the duration of induced protection against cancer. Although HPV vaccines are relatively expensive, vaccination programs have been activated almost in all rich nations and even in many poor nations. The sexually transmitted HPV also infects females and males. However, in several countries only girls are included in vaccination programs because tumors mainly affect females. Vaccination programs involving males and females are, however, more rational because males are a reservoir of infection. In addition, vaccination also protects the boys because the oropharyngeal carcinomas and genital warts affect both sexes. Including males in vaccination programs is also the only way to establish herd immunity and realistically target HPV infection eradication (Michels and zur Hausen, 2009).

Although vaccines against HPV do not cause any kind of adverse event worthy of note (the syncope, which sometimes follow vaccination, is most likely associated with the typical emotion of girls of that age), this vaccination has stimulated intense controversy because the vaccine has to be administered before the children run the risk of being infected, that is, before any kind of sexual activity begins. Parental opinions on the correct age for this vaccination often contrast with data available on the true beginning of sexual activity. Additionally, the vaccine can be understood as an official *pass* to begin sexual activity *(see also 4f*).

3c1c. The Epstein-Barr virus (EBV). About 95% of the human population is infected by EBV. In Western countries, EBV infection can cause infectious mononucleosis, nasopharyngeal carcinoma in Asia, Burkitt's lymphoma in Africa. In other patients, especially in those with some kind of immunodeficiency, EBV infection can cause Hodgkin's and non-Hodgkin lymphomas. Despite the numerous ongoing studies, the complexity of this infection has hindered the development of effective vaccines in blocking the EBV infection (Cohen, 2015). A vaccine that effectively prevented EBV

infection could significantly affect global health because EBV-related neoplasias are more than 1% of all human cancers (Lollini, 2011).

3c1d. Helicobacter pylori. Another pathology where there are no vaccines available is the prevention of gastric cancer linked to chronic infection by the bacterium *Helicobacter pylori*, although the incidence of this cancer in the world is not different from that of liver or uterine cervical carcinoma. In the absence of a vaccine that would provide long-term protection against *Helicobacter pylori* infection, pharmacological eradication of the bacterium is a strategy that is not without difficulty and does not prevent re-infection (Xin *et al*, 2016).

3c2. Vaccines in secondary tumor prevention. By secondary prevention of tumors it is meant the management of pre-neoplastic lesions and the inhibition of their progression in full blown tumor. Early diagnosis programs are the essential components of this secondary prevention. Diagnosed pre-neoplastic lesions are generally small and more easily treatable than clinically diagnosed tumors. In most cases surgical intervention leads to the conclusive elimination of the lesion. However, when surgery is not feasible or does not prevent the recurrence of injuries, a persistent immune memory induced by vaccines against *tumor associated antigens* could constitute an effective prevention method. Antigens targeted by these vaccines are no longer microbial molecules, as in the cases so far treated, but instead the abnormalities expressed by a cell during neoplastic transformation.

Clinical evidence of the efficacy of vaccines in preventing the progression of pre-neoplastic lesions begins to emerge. In a pilot study, patients with *in situ* breast ductal carcinoma (DCIS) expressing the Her-2 oncoantigen were vaccinated with Dendritic Cells pulsed with Her-2 peptides one month prior to partial mastectomy. After mastectomy, the anatomo-pathological examination of the excised breast portion revealed that pre-neoplastic lesions were no longer visible in all patients in which the vaccine had elicited immune response to Her-2 (Fracol *et al*, 2013). Only time will tell whether these immune responses effectively reduce the risk that pre-neoplastic lesions will progress to breast cancer.

In another study, patients with a history of colon adenomas, which are pre-neoplastic lesions that over time progresses to carcinoma, have been vaccinated against the MUC1 glycoprotein, another tumor associated antigen (Kimura *et al*, 2013). In the coming years the evaluation of the recurrence of adenomas and their progression in carcinoma will allow to evaluate the protective efficacy of this vaccine.

Finally, while the HPV vaccines discussed in the previous section prevent the infection of the virus but do not cure pre-neoplastic lesions of the uterus, various therapeutic vaccines against HPV-induced neoplastic lesions are undergoing study. Among these, VGX-3100 is a DNA vaccine against two proteins of HPV type 16 and 18. A recent clinical study reports that the electroporation of VGX-3100 vaccine in patients with pre-neoplastic uterine lesions (*Cervical Intraepithelial Neoplasia*, CIN) has led to the regression of lesions in a significant number of cases. These data suggest that the VGX-3100 vaccine can become a new therapeutic option for pre-neoplastic lesions caused by HPV (Trimble, 2015).

3c3. Vaccines in therapy of tumors. The obstacles to be overcome by a vaccine in order to have a real efficacy in the therapy of a clinically diagnosed cancer are many. The two main ones are related to cancer ability to:

- Respond to the immune attack elicited by the vaccine by selecting clones of cancer cells that no longer express the target tumor associated antigens to which the immune reaction is directed;
- Create a microenvironment capable of dampening immune reactivity.

Through these and other strategies tumors escape the immune reactions induced by the vaccines. It is therefore not surprising that very numerous ongoing experimental studies and clinical trials have not as yet led to the formulation of vaccines effective in tumor therapy (Lollini *et al*, 2006). The only vaccine currently approved for cancer therapy in the United States is a prostate metastatic cancer vaccine which is based on obtaining leukocytes from the patient's blood that are matured in Dendritic Cells and exposed to a protein commonly expressed by prostate carcinomas. When the modified Dendritic Cells are re-infused into the patient, they stimulate T lymphocyte reaction against carcinoma cells (Kantoff *et al*, 2010). Unfortunately these procedures are complex and costly while vaccine therapeutic efficacy is limited. The company that produced the vaccine has recently been acquired by another firm and it is unclear whether the production will continue.

Other recent data suggest that the combination of tumor vaccines with maneuvers that counteract the suppression of the immune response can lead to new and effective forms of cancer therapy (Moynihan *et al*, 2016).

3d. Routine vaccination strategies, reactive vaccination, and pandemic preparedness.

The increasing availability of effective and safe vaccines demands clear decisions on how vaccines should be offered to people. In general, vaccination strategies can be either Proactive or Reactive. Proactive strategies include national plans, articulate in the so-called vaccination calendars: for example, childhood vaccinations are an essential part of the vaccine routine of each nation. On the other hand, reactive strategies concern control measures that are activated at national and or international levels during epidemic outbreaks.

3d1. Proactive strategies: national vaccination plans. Childhood vaccination calendars are at the heart of vaccine prevention plans developed by various nations of the world. These national plans go along WHO recommendations even if with relevant local differences.

National vaccination plans define who should be vaccinated (the whole population in the case of general vaccination programs) and at what age. For a few vaccines gender differences may be considered.

For decades the Italian State secured an active and free offer for the so-called *mandatory* vaccinations (*see box 5*). Starting from the Italian 2012-2014 National Prevention Vaccine Plan, vaccination against seasonal influenza has been added for the elderly (> 65 years) and for people with cardiopathies, chronic respiratory pathologies, diabetics, neurological patients, and also women in the third trimester of pregnancy and hyper-obese. A major adjustment is currently ongoing with the Italian 2017-2019 National Plan for Vaccine Prevention since it is now based on the so-called *Life Calendar*, a vaccine protection program designed to cover not only the younger but also the elderly. In addition to vaccination against chickenpox, rotaviruses and meningococcal disease B, as well as the extension of HPV vaccination to male adolescents (*see 3c1b*), vaccination has also been introduced against the so-called *cursed triad of the elderly* which includes, besides influenza, also the invasive pneumococcal disease and herpes zoster.

Another critical node of the Italian 2017-2019 National Prevention Vaccine Plan concerns the retraction of the distinction between *mandatory* and *recommended* vaccinations since this

Box 5. **Italy: Mandatory and Recommended Vaccines**

The four *mandatory* vaccines:

- The anti-tetanus vaccine;
- The anti-diphtheria vaccine;
- The anti-polio vaccine;
- The hepatitis B vaccine

Gradually, to these *mandatory* vaccines have been added several *recommended*" vaccines against:

- Measles;
- Parotitis;
- Rubella;
- Type H Haemophilus influenza (HIB);
- Pertussis;
- Type C meningitis;
- Type B meningitis;
- Pneumococcus;
- Chicken pox;
- Papilloma virus (HPV);
- Rotavirus

distinction seems to set a priority of importance which is absolutely unjustified. Recommended vaccinations are no less important than the mandatory. They were said to be recommended due to the consideration that any effective preventive action should not be based on obligation but rather a free choice of the parents and the candidates for vaccination. Alternatively, the vaccination requisite for enrolling at school is a strategy to be considered, especially when the vaccine coverage is decreasing, in order to shield children and the most fragile persons, particularly the immune depressed, which cannot be otherwise protected. Unfortunately, the efficacy of such an approach has been criticized since it may ignite the opposition of anti-vaccine movements *(see 5).*

3d2. Coverage and control of vaccine preventable diseases: the Basic Reproduction Number (R0). Ninety five percent is the optimal threshold of vaccine coverage for mandatory vaccines and measles. In effect, when 95% of people are vaccinated, it is possible to fully control the spread of the disease or even eliminate it from the country, as shown by the cases of polio or diphtheria (*herd immunity*). By contrast, the strategic framework for the elimination of measles in Europe has failed because of the difficulty to reach 95% vaccination coverage. This high coverage threshold is especially important with measles since its R_0 is very high. The R_0, i.e. the *basic reproduction number* estimates the average number of individuals infected by a single sick person in a totally susceptible population and in the absence of any intervention. For measles, a sick person can infect about 15 other people. The higher is the R_0, the greater is the vaccine coverage needed to obtain a sufficient herd immunity to control the disease (Anderson and May, 2013).

Box 6. **Vaccinate against measles too? What a nonsense! It's a disease we've all done.**

Measles is a very infectious disease, i.e. it is a high R_0 (R_0 = 15). It is commonly considered a minor disease, thus vaccinating newborns may seem superfluous. While not considering the serious complications caused by measles in the poorest areas of the world, epidemiological data points out that, even in countries with good health care, in 30 over 100 cases, children sick of measles develop more or less serious complications.

Frequency of some complications associated with measles:

DIARRHEA	about 1 case per 10 sick children
OTITIS MEDIA	about 1 case per 10 sick children
PNEUMONIA	about 1 case per 20/30 sick children
FEBRILE CONVULSIONS	about 1 case per 100 sick children
THROMBOCYTOPENIA	about 3 cases per 1000 sick children
ACUTE ENCEPHALITIS	about 1 case per 1000/2000 sick children; a permanent neurological damage in 1 case every 4
SCLEROSING PANENCEPHALITIS	5-10 cases every million sick children but much more frequent in children

3d3. Reactive vaccination strategies. Unlike proactive vaccination plans, interventions that are being conducted during epidemic outbreaks or in real pandemics are of urgency, and are not always involving the whole population. Sometimes it may be decided not to vaccinate people belonging to a certain category or age class. For example, one of the strategies successfully used in the campaign that led to smallpox eradication was the so-called *ring vaccination*, consisting of vaccinating only those who are most likely to be infected: when a symptomatic patient is diagnosed, all people who are or may have been exposed to the infection (contacts) are identified and vaccinated (first ring of contacts) or even contact's contacts (second ring). Vaccination of the contacts is now also used in other circumstances, such as in the vaccination of close contacts in the cases of meningococcal invasive diseases, after being subjected to antibiotic prophylaxis. A similar ring strategy has also been used in a vaccination trial against Ebola (Henao-Restrepo *et al*, 2017)

3d4. Pandemic vaccination strategies. A special kind of reactive vaccination should be implemented to counteract a global disease outbreak with mitigation and control actions.

In the wake of the crisis caused by the outbreak of the avian influenza A/H5N1 in China, a pre-pandemic plan was developed in several Western countries to provide the vaccine to essential service personnel as well as to persons belonging to the traditional categories at risk of complications. A similar plan was prepared in 2009 when a swine-derived A/H1N1 virus emerged in Mexico. Following a jump of species the A/H1N1 virus transmitted from person to person.

Facing a limited availability of vaccine doses, the discussion deals with the age group(s) to be protected, such as older people who are at risk of developing the disease in a severe form, and children, to protect them but also to block the spreading of the virus in kindergartens and schools.

Another important point concerns the development of the vaccine and its production on a large scale, possibly in a hurry. Unfortunately, an influenza virus can travel worldwide in 6 months, so technical times often do not allow the production of sufficiently large-scale doses of vaccines (*see also 4h).*

Never as in this field, the epidemiological surveillance to identify emerging viral strains and innovation in the field of vaccination are complementary ingredients of the so-called *foresight*, essential in the controlling epidemic events.

4. VACCINATION AND GLOBAL HEALTH

4a. Vaccination between technology, finance and politics.

The cost of developing a new vaccine from design to market availability amounts up to 900 million euro and requires sophisticated scientific and technological effort for about ten years, with a probability of success not exceeding 6% (Pronker *et al*, 2013). It is no surprise, therefore, that the world's innovative vaccine market, currently around twenty-five billion euro, is dominated by a few large companies (Sanofi-Pasteur, GlaxoSmithKline, Merck Sharp Dohme, and Pfizer), although many smaller companies produce licensed or patent expired vaccines (FiercePharma, 2012). Concentration is not uncommon in the pharmaceutical industry, since vaccine manufacturers, largely state-owned or financially aided, were progressively acquired by large private pharmaceutical companies, starting from the mid-1900s.

In deciding whether to develop a new vaccine, companies need to carefully assess the required investment, risk and prospects for gain. The initial phase consists of *a)* estimating the burden that the disease exerts on world health and *b)* assessing the global economic value and the benefits to the population at risk, prompted by the introduction of the new vaccine.

The following steps to be evaluated before embarking on the risky development of a new vaccine are:

- *Disease*: severity, frequency and number of deaths caused by the disease.
- *Vaccine*: biological and technical difficulties involved and estimates of efficacy and safety.
- *Economic outlook*: cost of the vaccine development in relation to global benefits expected as well as profit and earnings for the manufacturer.

These assessments, however, are complex since countless variables make each step of different weight and significance (Barocchi *et al*, 2016). A given disease could be rare but it may lead to epidemics or pandemics, thus acquiring a very different significance. Two relatively recent examples involved the decision whether or not to develop vaccines against Ebola and Zika. In addition, the actual efficacy of a vaccine is a variable difficult to assess. Over-and-above, a vaccine leading to complete eradication of a disease would acquire a particularly high priceless value.

The fairly crucial cost-benefit assessment is often difficult to evaluate being dependent on very broad parameters such as, for example, the prevention of secondary illnesses and their social cost that may be exorbitant. A case in point is represented by meningococcal infections in which mortality is significantly reduced, while the frequency of serious complications -due to the meningitis (deafness, mental retardation, etc.) or to sepsis and intravascular coagulation (amputations of the extremities or other serious consequences) is high.

In wealthy countries, the sector of the population predominantly affected by the disease of interest (e.g. children or the elderly) and the interest of local health services have to be considered. In the poorest areas of the world it is important to assess whether alternative measures could control the spread of the disease more efficiently. In the cases of infectious diseases transmitted by vector agents, such as malaria and Zika, considerable success may be obtained more efficiently by fighting the vector agent than by producing a vaccine. It is therefore possible that in the time necessary for setting up and marketing a vaccine, the disease may have already been controlled by other means, thus making the new vaccine redundant and without a market (Barocchi *et al*, 2016).

Other risky variables associated with the preparation of a new vaccine are the biological and technical complexities required for vaccine preparation, the competition with existing treatments, and the need for large-scale studies to validate its efficacy and risk profile (*see, for example, the discussion on the RTS, S/AS01 vaccine in 3b2*).

The technologies needed to produce hundreds of millions of affordable vaccine doses are another variable that affects the viability of the project. While a live but attenuated virus, such as that of the Sabin anti-polio vaccine, can be produced in large quantities at low cost, much more complex and expensive technologies are required to fine-tune and scale-up the production of glycoconjugated vaccines *(see 2b3)* (Smith *et al*, 2011). It is expected that revolutionary technologies, multi-component vaccines that can incorporate new targets, DNA or RNA vaccines should allow the production of huge doses of vaccines at much lower costs.

Any new vaccine must then go through several meticulous evaluations required by the regulatory authorities of the various nations. Diversities in registration procedures not only complicate the production and marketing of the vaccine but also imply that, in case of fluctuations in the request, a vaccine intended for a given nation cannot be made available for another.

All of these variables and complexities affect the likelihood that a new vaccine will enter the market within ten years from the start of the project, and that its marketing may lead to a real gain. Despite the many difficulties and risks mentioned above, the vaccine market has been growing steadily since vaccines were sold, going from a total of $ 6 billion in 2000 to an estimated $ 30 billion for 2020 (Moxon and Siegrist, 2011).

For vaccine manufacturers, the most lucrative endeavor is vaccine against seasonal influenza epidemics, requiring over 900 million doses, mostly sold to wealthy countries. Both large-scale production and marketing in countries that can afford relatively high prices diminish the risk profile of the company, although antigenic drift of the influenza virus makes the project complex and feasible only through the collaboration of over 120 national monitoring centers working with sentinel medical professionals distributed in 90 countries. Clinical influenza virus samples isolated in a given country are sent to WHO centers in Atlanta, Tokyo, London and Melbourne, in February (for the northern hemisphere) and in September (for the southern); thereby companies are informed of the features of influenza viruses expected to cause the next epidemic. At this point, there is a struggle against time for scaling-up the production of vaccine doses, their complex validation, the approval by the various national authorities and finally their marketing and distribution (Smith *et al*, 2011). The 2015 Nagoya Protocol will force vaccine-producing companies to make agreements with national governments for a remuneration of the influenza virus isolated. However, it is possible that this fair agreement may slow down the marketing of the new vaccine with serious consequences on health (Cressey, 2017).

Relying solely on technical and financial assessments in deciding whether or not to develop a vaccine, however, would be a short-sighted strategy in the face of serious global health problems, because it is unthinkable to believe that diseases can be permanently confined to certain areas of the planet. The negative consequences of decisions based solely on technical and financial considerations are twofold:

- In the absence of profit, no effective vaccines against diseases would be available in the poorest Countries that currently lack economic resources;
- In the absence of market, no vaccines, against microbes that may cause future epidemics would be developed.

4b. The Global Alliance for Vaccines and Immunization (GAVI).

In the poorest nations of the world vaccination plans are hindered by multiple factors including indifference or inability of governments to address health problems, the difficulty of overcoming traditional culture barriers, the lack of vaccine information, and the problems associated with the organization of an effective vaccination service in remote areas. Additional elements which concur to create a dismal situation include: *a)* the profit-oriented attitude often pursued by vaccine manufacturers mainly with reference to innovative vaccines; and *b)* the shortage of studies on

vaccines that induce immunity to the strains of local microbes, which are often different from those against which the vaccine was originally directed.

Companies have no economic interest in producing vaccines that fit the health needs of the countries with extremely limited purchasing power. In these areas, UNICEF (*United Nations International Children's Emergency Fund*) has been involved in vaccination programs for several decades, providing more than 40% of vaccines, often insufficient to address medical needs.

In order to achieve vital goals that individual institutions could never achieve on their own, the *Global Alliance for Vaccines and Immunization* (GAVI, 2017) was established in 2000 through cooperation between public and private institutions. In addition to the Bill & Melinda Gates Foundation, GAVI was joined by governments of both industrialized and developing nations, UNICEF, WHO, the World Bank, non-governmental organizations, vaccine producers of the industrialized and developing nations and public health and research institutes, as well as eminent personalities who have contributed to philanthropic initiatives such as Jordan's Queen, Graça Michel, Nelson Mandela's wife, and Mary Robinson, former President of the Republic of Ireland.

GAVI has decided to focus its action on 73 of the poorest nations in the world. At the onset of the 2008 economic crisis, GAVI appealed to governments in the hope of inverting the fate of the poorest children who generally pay the highest death tall. Although it is still too early to draw definite conclusions, the trend indicates that the global financial crisis has not compromised the progress made by GAVI in terms of global health.

Another important challenge faced by GAVI is to reduce the time (typically twenty years) between the development of a vaccine and its transfer to the poorest countries where the need is most urgent.

The winning decision made by GAVI of using market economical mechanisms to finance its activities was crucial to achieve positive results: the *International Finance Facility for Immunization* (IFFIm) and *Advanced Market Commitment* (AMC) are important innovative financial instruments that Italy, a generous and creative partner of GAVI, has helped to promote. IFFIm issues bonds by converting long-term public commitments to immediately available cash resources for the production and purchase of vaccines. The AMC initiative provides a market to the company which develops a new vaccine addressing a predefined medical need at a tier price. As an example, the vaccine against pneumococcal strains prevalent in developing countries was the task of the first AMC exercise.

The launch of *GAVI Bonds*, guaranteed by the refunding commitment of donor countries, and whose first signatory was the Pope John Paul II, allowed to raise about $ 5 billion; with Italy contributing 600 million. This strategy led to massive mobilization of resources employed during the period 2006-2015 to purchase vaccines, saving over 5 million children and as many adults from diseases such as measles and polio.

4c. Vaccines for the poorest nations.

The new scenario created by GAVI has allowed producers in developing countries such as India, Brazil and Cuba to emerge and flourish. Today, producers in developing countries provide GAVI with 65% of the requested vaccines. The ensuing reduction of vaccine cost still maintaining the same WHO certifications is a significant benefit for the poorest areas of the planet. The largest vaccine manufacturer is no longer one of the five largest drug companies, but rather the Serum Institute of India which produces and distributes over 1.3 billion vaccine doses, most of which for developing countries (*Serum Institute, 2017*).

This important change is the result of the financial strategy triggered by GAVI. Mechanisms such as GAVI bonds and AMCs (*see 4b*) have laid the ground for the large investments needed to enter the market by producers in developing countries. In this respect it is rewarding that the last meningitis epidemic in Niger and sub-Saharan Africa was met with a million vaccine doses produced in Brazil and

800,000 in Cuba, at a time when worldwide availability of vaccines against meningitis was insufficient (*see also 4h and 4g*).

To foster the development of new vaccines specific for the diseases prevailing in the poorest nations, GAVI and other international organizations have set three key goals:

- Accelerate the purchase and use of new vaccines and the related production technologies in developing countries;
- Enable local health systems to provide vaccinations and other health services;
- Increase the programming and sustainability of long-term funding for national vaccination programs.

The commitment to create a market in the medium to long term for vaccines to be developed against diseases affecting mainly the poor nations, was proposed by Italy in February 2005 to the G8 finance ministers. The pilot project focused on pneumococcus, the cause of about 800,000 victims a year, largely children under the age of five. According to GAVI estimates, the introduction of the anti-pneumococcal vaccine promoted by the AMC initiative could save more than 5 million lives by 2030. The initial clinical testing in Africa of the AMC promoted vaccine yielded positive results.

A lesson to be learned from the anti- pneumococcus AMC initiative is that introduction of a new vaccine not only protects against the targeted bacterium, but it also reduces the emergence of bacterial strains resistant to antibiotics. Along a different line, the testing of a new vaccine against rotavirus, which is currently carried out only in areas of the world troubled by intestinal infections, is yielding positive results. If the initial encouraging results are confirmed, this potential new weapon will be gradually extended to a greater number of nations.

The populations of tropical areas living in extreme poverty are often affected by a diverse group of communicable diseases that currently are not among GAVI's priority objectives, the so called *Neglected Tropical Diseases (see Box 7)*. Some of these (leishmaniosis and trypanosomiasis) are lethal while others have a chronic yet disabling course. The invalidity caused by these easily spreading chronic diseases is a factor contributing to worsening the living conditions of these unlucky populations. At present, a few international consortia coordinated by the WHO are studying the formulation of appropriate new vaccines. However, this is not an easy task because of the chronic evolution of these diseases, the complexity of the reproduction cycle of the pathogens involved and the close contact with infectious vectors of the populations living in poverty, without adequate sanitation. The efficacy of vaccines against Schistosoma, Anchilostoma and Leishmania is currently being tested in human studies.

Box 7. **Some of the most common *Neglected Tropical Diseases***

- The infestation by Ancylostoma hookworms affecting over 440 million people;
- Schistosomiasis, affecting over 250 million people;
- Leishmaniasis, affecting over 10 million people;
- Filariasis, affecting over 36 million people.

4d. The importance of the *last mile.*

Reaching to the furthest village home with vaccines is crucial to global health. Today, also thanks to changes in the production scenario, there is a greater availability of vaccines in the poorest nations and global health initiatives are making it possible to tackle the financial commitments required to produce out-patent vaccines and to develop new vaccines (such as the one for type B meningitis).

The challenges for the future are therefore clear and require:

- To overcome the distrusts on the efficacy and safety of vaccination for the prevention and eradication of fatal infectious illnesses, through increased information to families and medical doctors;

- To continue studies and investments for the development of new vaccines, such as that against diarrhea from *Salmonella*, because the one already available does not confer immune memory and thus no protection against a subsequent infection.;
- To learn how to provide vaccines to the whole population up to the last village in the most unlucky areas of the world.

The stake is global health, which unavoidably goes through global vaccination.

4e. A first global goal: reducing child mortality.

Respiratory infections, diarrhea, malaria, pneumonia, neonatal tetanus are the diseases that kill millions of children each year in the poorest nations of the world. Poverty and malnutrition, along with difficulties in access to care, make preventable diseases lethal: in these countries, for example, about 800,000 people a year die of pneumococcal pneumonia, while diarrhea and intestinal infections, trivial and curable in affluent nations, constitute a real health emergency.

According to WHO, in the south of the world, where hygiene conditions are often precarious and it is more difficult to access rehydration therapy and other medical care, at least 600,000 children die of rotavirus diarrhea every year, i.e. the severe form of viral gastroenteritis that affects children under 5, not forgetting that salmonellosis, an intestinal infection caused by the salmonella, causes 400-600,000 deaths each year, for the vast majority children.

From 1990 to 2010, substantial progress has been made in reducing child mortality rates globally: in 1990, the number of deaths among children under the age of five was 12 million, in 2000 it fell to 9.6 million and in 2010 to 7.6 million (Ministero della Salute, 2016). What made this positive trend in reduced child mortality possible? Although still insufficient, an important role has been played by the spread of vaccinations, in particular against diphtheria, tetanus, pertussis (the DTP vaccine) that are to be considered as the minimum standard for all children, and against measles (*on the worldwide diffusion of these vaccines also see 6*). The measles vaccine, in particular, has a dual action in helping to reduce mortality below five years of age: on the one hand it reduces the incidence of measles itself, and on the other it prevents co-infections responsible for complications and even death, especially in malnourished children (Simons *et al*, 2012).

The dream of vaccinating all children of the poorest nations is a bit less far. The results obtained after the first ten years of GAVI's activity include more than 4 billion euros distributed, 250 million children vaccinated for the various diseases in 70 developing countries, 5.4 million lives saved. Thanks to GAVI, children have been protected against diseases such as diphtheria, pertussis, hepatitis B, type B *Haemophilus influenzae* (HIB), measles, meningitis, yellow fever, tetanus and polio (Clemens *et al*, 2010).

4f. Why global health has to specifically target women?

For biological and social reasons, 80% of the burden of suffering and illness affects women. The management of disease in a young childbearing woman points to issues such as the importance of ensuring the right to pregnancy and to control the effects of pregnancy and post-pregnancy on the disease itself. It is therefore important to support studies which address gender differences and sex-related issues. These should include disease targets such as breast and uterus, and pregnancy, which is risky for both mother and offspring. Autoimmune diseases, particularly lupus and rheumatoid arthritis, affect women with a frequency of about nine times higher than men. Although it is still unclear why the female immune system is more prone to self-attack, it seems conceivable that women have more sophisticated immune regulation mechanisms to allow them to carry a non-self human being during pregnancy, without rejection .

In addition to biological reasons, social factors such as discrimination, weaker attention and minimal access to medical care, underlie the prevalence of the burden of illness on women. For

example, in poorer countries, females are vaccinated less than males, except for those ethnicities who falsely believe that vaccines may affect male sexual performance. In addition, high-risk health practices such as infibulation, i.e. genital mutilation, are for girls and women a cause of suffering in all respects. In many communities, women's illness also has serious adverse effects on children care and family members.

For the first time, women's health has a tool, the HPV vaccine which could improve women global health, in particular in areas of the sub-Saharan Africa. In addition to failing to reach all women in the poorest regions, even in an affluent country like Italy, vaccination coverage has not been as extensive as desirable (*see also the discussion on the resistance to this vaccination in 3c1b)*.

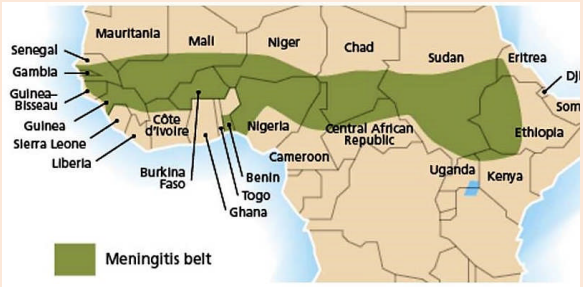
4g. Meningitis, a global problem.

The incidence of meningitis is a real drama in the African Countries of the so-called *Meningitis belt*, but also on this front great stride forward has been made with the winning immunology-solidarity combination (Maurice, 2015). The *Meningitis Vaccine Project* has led to the development of a conjugate vaccine that induces and maintains high levels of antibodies against type A meningococcus, which is responsible for about 80% of cases in the *Meningitis belt*. The technology needed for the development of this vaccine has been granted by the NIH, a United States government institution to the Serum Institute of India (NIH, 2014). Thanks to the international collaboration of WHO and GAVI, this vaccine has been marketed in the world's poorest countries at a cost that is compatible with mass vaccination (0,55 €) and indeed very low considering that conjugated vaccines are usually more expensive.

Epidemiological data of the last 5 years indicate that this vaccine, introduced in 2010, virtually eliminated type A outbreaks in 15 African nations, liberating 300 million people from a real nightmare *(see Box 8)*. This outcome is satisfactory though not completely decisive, as the meningitis belt nations are more than 15 (i.e. 26), people at risk are about 700 million, and other meningococcal strains such as types C, Y, W are lurking .

In Australia, for example, the disappearance of type C meningitis, made possible by extensive vaccination, did not however improve the epidemic due to the type B meningococcus, due to its different structure. The scientific and technological revolution of *reverse vaccinology* (*see also 2b4)* has made it possible to produce an effective and innovative vaccine, starting from the genome of type B meningococcal. The rapid approval of this vaccine in the United States, was fostered by the repeated appearance of limited type B meningitis epidemics in Santa Barbara and in Princeton.

Box 8. The 2015 meningitis epidemic in Africa.



http://www.meningvax.org/epidemics-africa.php

Over 300 million people live in the *Meningitis Belt* area where devastating epidemics occur every 5-12 years. These are due to meningococcus infection spreading through direct contagion among people.

In 2015, a meningococcal C meningitis epidemic begins in Nigeria and Niger during the dry season from January to June. With its cold nights and dust-bearing winds, this is the ideal season for the development of upper respiratory tract infections, particularly related to meningococcus, so that in May, 12,000 cases of meningitis and 800 deaths were estimated, with a track of disability whose magnitude is difficult to assess: for example meningitis is the first cause of deafness.

Medecins Sans Frontieres physicians were at the forefront but vaccines prepared for emergency by WHO were not sufficient: 1.5 million doses were missing.
Thanks to pressure from UNICEF and WHO, 800,000 extra doses were made available, 600,000 of which were produced in Cuba and Brazil (*InterHealth, 2015*).

When is the next devastating emergency?

4h. Preparedness for emerging epidemics.

The 2011 cholera epidemic in Haiti has dramatically highlighted the absence of vaccine depots immediately available to control the sudden outbreak of infectious diseases. The only vaccine approved by the WHO against cholera is Dukoral: in 2011 there were only 400,000 doses of Dukoral available in the world, a number totally inadequate to protect a population of ten million people at risk, considering that two or three vaccine boosters are needed to induce effective protection (Smith *et al*, 2011).

In 2013, when the Ebola epidemic broke out in South Africa, no vaccine was immediately available. However, an experimental anti-Ebola vaccine was at hand, but its development had been abandoned because of the poor prospects of economic return. Starting from this experimental anti-Ebola preparation however an effective and safe vaccine for human use was made available in a year and a half (Butler, 2017).

During the outbreak of an epidemic such as those of meningitis, cholera, SARS, Ebola, or Zika, experts meetings are called in a hurry to discuss on how the world should be better prepared to fight future epidemics; however with the fall of the most critical stage, the media and the politicians forget the *news*. Scientists, however, have a list of microbes that could give rise to dangerous epidemics causing global health emergencies and convinced the World Economy Forum in Davos on January 18, 2017 to launch the *Coalition for Epidemic Preparedness Innovations* (CEPI) with the aim of promoting development and storage of vaccines against those microbes that could cause new alarming epidemics. Large sums were donated to CEPI by the Bill & Melinda Gates Foundation, the Wellcome Trust and the Governments of Norway, Germany and Japan (Nature Editorial, 2017); moreover the major pharmaceutical companies announced their intention to collaborate to this initiative. Among the CEPI priorities is the promotion of the development of vaccines against the Nipah virus, viruses causing the Middle East Respiratory Syndrome (MERS) and the Lassa fever (Butler, 2017). Time will tell if the world will be better prepared in the future thanks to CEPI.

5. ANTI-VACCINE MOVEMENTS: WHY?

Already in the eighteenth century the diffusion in Europe of the practice of variolation elicited numerous reactions of high emotional impact. On a Sunday of 1722, Reverend Edmund Massey pronounced in the church of Saint Andrew's Holborn in London the *Sermon against the dangerous and sinful practice of inoculation*, a sermon printed and spread in England and North America that lit up fiery reactions contrary to vaccination (Massey, 2010).

The chronicle of smallpox epidemics that outreached in the Boston city of Massachusetts from 1720 to 1770 highlights how both public opinion and the various authorities wavered over a short period of time between the refusal or even prohibition of variolation on one hand, and on the other resorting to this practice in the face of growing epidemics. Mass variolation as it was practiced in the 1700s was a dangerous, non-standardized practice, administered under primitive hygienic conditions and in any case associated with a high incidence of side effects. Despite all this, as Benjamin Franklin diligently reported, the protective effect of this primitive anti-smallpox procedure was immediately evident when mortality was assessed by comparing groups of citizens who had undergone variolation to those who had refused it (Blake, 1959).

The main reason for the reactions against variolation, and specifically against the smallpox vaccine was of a religious and naturalistic nature: smallpox epidemics were interpreted as natural events sent by God and, therefore the decision on who should die and who could survive should be left to Him. Vaccination appeared to be a *rebellion attempt to take God's work out of his hand* since the epidemic was seen as an opportunity to repent of ones sins and reconsider ones lives (Massey, 2010). This interpretation was associated with the protest of physicians who considered variolation outside the medical culture of the time, a practice with no scientific basis, imported from culturally different Countries, spreading by exploiting the dupe ignorance of the population and dangerous for those who accepted variolation and those who refused it (Blake, 1959).

Along with the progressive development of modern vaccines, various opinion movements against vaccination have flourished in the western world. Until the last century, these movements were minorities and vaccine coverage continued to grow. Currently, however, we notice with alarm a trend reversal that must be understood. In Italy, the percentage of vaccinated children, stable or slightly increasing until 2012, is diminishing somewhat for the so-called *mandatory* vaccinations (polio, diphtheria, tetanus, hepatitis B), still remaining around 95% (the limit threshold for herd immunity). But a much greater drop is taking place in the so-called *recommended* vaccinations, the percentage of vaccinated children for measles, rubella, mumps (MPR) dropping from 90.3% in 2013 to 86.6% of 2014 (ISS, 2017).

The introduction of new technologies and new medical practices not rarely causes mistrust and refusal. However, it may seem peculiar that opposition to vaccines is so widespread and persistent, capable of permeating large areas of the population. Compared to most commonly accepted medical practices, vaccines are cheap and simple to administer, often very effective and associated with rare side effects. Why, then, this persistent and widespread opposition?

Skepticism against the practice of vaccination is triggered by its inherent features:

- Vaccination is a typical act of preventive medicine, a product administered to a healthy person in order to avoid a hypothetical risk of contagion;
- An altered perception of the risk-benefit ratio can make unacceptable the risk and discomfort associated to vaccination;
- Vaccination is an individual act that gains a global protective value when it becomes a collective act, i.e. when a large majority of the population (between 85 and 95%) is vaccinated (herd immunity);

- To administer a vaccine to most of the population, it is necessary to issue regulations or laws that encourage or impose vaccination; unavoidably, directives of this kind raise refusals related to the feelings of loss of individual freedom and excessive public intrusion into the private sphere;
- To accept vaccination it is necessary to periodically deal with small but significant discomforts in the routine of daily life (work permits, where and when the vaccine is to be administered, waitings, addressing the reaction to the vaccine, etc.);
- Finally, the fact that vaccines are inexpensive or even free of charge and commonly available reduces their perceived value.

The current spread of the opposition to vaccination is the result of complex and multi-faceted cultural changes, questioning the concept of authority, the physician-patient relationships, and easy access to widespread news on Internet. It should be noted, however, that the same arguments against variolation and anti-smallpox vaccination that had been the basis of the eighteenth-century fiery debate continue to be the basis of the current opposition to vaccines while taking different accents and intensities. The main reasons that are leading to the reduction of vaccine coverage in western populations can be assembled in the following five major sets (5a-5e).

5a. The altered perception of the risk-benefit ratio.

In industrialized nations, it has progressively been forgotten what it means to see a baby die of neonatal tetanus because of unclean deliveries, such as the use of non-sterilized instruments to cut the umbilical cord. Only doctors of a certain age remember the deaths due to diphtheria croup, i.e. the lesions caused to the larynx by the diphtheria bacillus creating difficult breathing and eventually suffocation. The latest generation of parents fortunately has no longer any experience of polio, and many do not know the consequences of disease pathologies that have almost disappeared in recent years such as pertussis, measles and mumps. The drastic reduction of infectious diseases in western nations, largely due to effective vaccination campaigns, has made the perception of the importance of mass vaccination impervious: vaccines are *victims of their own efficacy*.

While it is common to dramatize any clinical complication more or less connected with vaccination, complications of infectious diseases are generally accepted as unfortunate *natural* events. A widespread distortion in the perception of risk attaches a higher emotional importance to the hazards created by human technology (damage caused by vaccines) than those caused by natural events (spread of dangerous infectious diseases). Often more importance is ascribed to rare dangerous events directly observed or referred by friends (a child ill after vaccination) rather than to solid epidemiological evidence (data on the incidence of risks associated with vaccinations) (Kahan, 2013).

5b. The belief of the inefficiency of vaccines and the fears of their dangers.

People opposing vaccination believe that vaccines are ineffective: infectious diseases have disappeared not for the diffusion of vaccines but for improved nutrition, living conditions, hygiene, etc. The dangers that are believed to be most frequently associated with vaccination are:

- Induced autism;
- Toxicity of adjuvants and preservatives;
- The weakening of the immune system caused by the large number of vaccines offered today.

For a careful and documented disproof of each of these beliefs, refer to Rappuoli and Vozza (2013); Grignolio (2016); Mantovani (2016).

A vaccination can be compared to a limited training that confers to the immune system the extraordinary ability to fight a subsequent invasion. An exercise that is always extremely limited as

compared to the complex and total war fought by the immune system against any infectious disease, from measles to influenza, not to mention illnesses that are even more serious. The dissemination of fear towards these minor immunological exercises is contributed by the fact that reporting of adverse events more or less related to vaccination has a scary impact on media, at variance with the generally delayed result of institutional rebuttal. There are no doubts risks associated with vaccination, but their actual incidence is very low. In contrast, the history of opinion movements against vaccination is studded with reports on the connection between vaccination and negative events or serious side effects, reports that stick in the popular imagination even when causal connections with vaccination are proven false. Once emotions such as fear and suspicion have been insinuated, they propagate epidemically virally through personal contacts (Christakis and Fowler, 2009).

Over the last hundred and fifty years there have been continuous waves of collective fear triggered by reports of complications and side effects caused by particular vaccines, phobias fueled by television talk shows, newspaper articles or social networks and legal actions against vaccine manufacturers. Especially damaging has been the case of A. Wakefield's discredited study of the relationship between vaccination and the onset of autism. Governments and health authorities from various countries have responded time after time by setting up investigation committees that, with variable impact, have provided reassuring responses, highlighting the inconsistency of the causal connection between vaccination and autism and proving that Wakefield had falsified the data (*see Box 9*). As authoritative as these assessments may be and as obvious as the data provided by the control committees appear, it seems almost impossible to remove the suspicion that these official denials are the result of self-absorbed manipulations and global conspiracies (Grignolio, 2016).

Box 9. **Vaccination against measles, mumps and rubella (MPR) in not the cause of autism.**

In 1998, Andrew Wakefield, a British physician, published on *Lancet* an epidemiological study on a possible relationship between MPR vaccination and autism. Subsequently, the data and conclusions of the Wakefield study were proved to be false and the study was repeatedly refuted as well as withdrawn from the magazine that accepted the initial paper (*The Editors of the Lancet, 2010*).
Also on Wakefield's integrity, serious doubts were raised that led to his expulsion from the Medical Council of his country.

Autism, whose causes are not yet fully known, originates before birth even if, unfortunately, its symptoms become manifest in the early years of life just during the period when the MPR is administered

Neverthelss, recently Wakefield collaborated on the controversial documentary film *VAXXED From cover-up to catastrophe* that illustrate the alleged rogue machinations put in place to hide the dangers of vaccines (*VAXXED, 2016*).
This very controversial film is continuing to stir up intense anti-vaccine emotions.

With the utmost respect and understanding for parents warried by the hypothetical side effects of vaccination against MPR, it must be stressed that there is no evidence at all of a relationship between vaccines and autism. These are metropolitan legends to be strongly countered since they make the vaccines feel dangerous and thus create a serious risk for both children and global health.

5c. Combating rogue interests of Big Pharma.

Numerous hypotheses, theories and conjectures allege the first cause of human events to plots. These *conspiracy theories* are often elaborated on events making a strong impression on public opinion also because of their extensive dissemination by the mass media (Conspiracy theory, 2017). The suspicion that mass vaccination practices are the result of international conspiracies frequently runs on the net and even slithers in the requests of some political movements. A particular form of conspiracy theory is the one whereby big vaccine producing companies (*the Big Pharma*) bribe physicians, healthcare workers and governments to spread vaccination by hiding the collateral

Table I. Costs of vaccines and care: Hepatitis B and C

Vaccine		Therapy [0]
Anti HBV:	32,62 €[+]	Hepatitis B: 3,000-3.500 €/year
Anti HBV	16,31 €[§]	Hepatitis C: 15.000-20.000 €/cicle

[+] Territorial pharmacies;
[§] Hospital pharmacies;
[0] Considering drug cost only

Table II. Hepatitis virus infection sequelae costs

Cirrhosis F0-F3 annual cost	522 €
Cirrhosis F4 annual cost	1.512 €
Decompensated Cirrhosis	6.350 €
Chronic Hepatites C annual cost	12.744 €
Transpiant cost (one-off)	90.986 €
Cost of the Post-Transplant year	17.612 €

*Eradicating treatments excluded

Against Hepatitis C there is no vaccine but only drugs. The data are shown for comparison with Hepatitis B. They illustrate the savings that could result from the introduction of an effective vaccine.
In Annex 1 to the Italian 2017-2019 National Plan for Vaccine Prevention it is reported how much it costs to the National Health Service every case of illness caused by avoided vaccination. The Annnex also discloses the large annual savings (around 10 millions of euro) due to vaccine-prevented illnesses (*Piano Prevenzione Vaccinale* 2017-2019, 2017).

dangers *(see Box 9)*. Television and newspaper information in favor of vaccination would be motivated by the more or less hidden funding of *Big Pharma*.

Scandal and corruption related to the trade of vaccines, their administration by health workers, and their preservation certainly exist and have been highlighted by the media and the judicial authorities, but not more than in any other business field (Grignolio, 2016).

To favor the poorest populations, prices of the vaccines are modulated according to the income of the nations they are being sold to, exploiting the revenues originating from the business in the richer nations.

Nevertheless it is no coincidence that the policies applied to the cost of vaccines and the producers' oligopoly are often subject to government investigations and continued controversies by the anti-vaccination movements. Financial surveys indicate that nowadays the vaccine business is expanding and that there is a profit related to their marketing; still vaccine spending is only 2-3% of worldwide drug expenditure.

Furthermore, as illustrated in the Annex 1 to the Italian 2017-2019 National Plan for Vaccine Prevention, the expenditure of the National Health Service for medicines and treatments necessary for disease therapy may be far superior relative to the cost of the vaccination campaigns leading to prevention of that disease (Piano Nazionale Prevenzione Vaccinale, 2017). The cost of drugs for the treatment of diseases hitting people who avoided vaccination should add to the overall cost of the disease, with its socioeconomic implications and personal suffering.

5d. *VI.* First of all, respect for Nature.

In the eighteenth-century, variolation and anti-smallpox vaccination appeared as an intolerable technological interference with God's will and the order of nature. Even today a conception of nature seen as a good order leads to consider vaccines as unacceptable interference (Grignolio, 2016). If an infectious disease is no longer seen as an occasion of repentance and redemption, it may be considered as a natural opportunity to suspend daily routine, a stimulus for individual maturation, a different body condition, and a natural way to strengthen immune defense. In any case infectious diseases can be avoided simply by living more naturally, with better nutrition and adopting better lifestyles. These interpretations are generally believed by the same people that follow alternative therapies; in fact, 50% of parents rejecting vaccinations believe in homeopathy practices.

5e. *V.* The defense of individual freedom against the paternalistic and despotic attitude of institutions.

The conflict between individual freedom and the protection of the common good, was emblematically debated in Antigone, a tragedy by Sophocles written in 442 BC. This contrast remains one of the nodal points of western culture. If the social contract is not shared, to what extent the liberty to reject the law should be tolerated?

Faced with the refusal of vaccination, the various national and regional authorities have assumed a different approach from time to time: from explicit clash (Mello *et al*, 2015) to the possibility to circumvent the conflict by limiting the rejection to minorities that take advantage of the *herd immunity* (Salmon and Omer, 2006; Grignolio 2016). The utilitarian tradition suggests that the individual freedom to refuse vaccination should be respected as long as:

- This choice does not seriously jeopardize the well-being of the community at large;
- The decision not to vaccinate (or not to vaccinate a child) is based on some strong belief and it is not just the result of behavioral indolence;
- The most recent and authoritative findings of scientific research are constantly taken into account (Salmon and Omer, 2006).

In the controversy against vaccines, these five sets of anti-vaccine motivations are commonly intertwined with arguments that blend from one to the other. As typical of the movements against power, the minority syndrome leads to the arguing and spreading of the reasons for fear and objection with a militant enthusiasm that contrasts with the well-documented but lay-back responses of the institutions and of the experts (Vaccines Safety Net, 2017).

Box 10. **The most common narrative frames of *posts* against vaccines on the COMILVA site, listed in order of frequency:**

- The negative effects of vaccines on health;
- Disinformation of lay people on the negative effects of vaccines;
- The plots associated with vaccination;
- The inefficiency of vaccines;
- Fight for freedom of choice on vaccination;
- Compensation for damages caused by vaccines;
- The difficulty of facing psychological pressure exercised by doctors, relatives and friends on the decision not to vaccinate
- The controversy with people in favor of vaccination

5f. The fight against vaccines at the time of Internet.

The anti-vaccination groups, who were relegated to niches because of the difficulty of contact with the general public carried out by distributing their publications, have discovered a very effective vehicle to spread their ideas via Internet. In the world-wide web, blogs, networks, the discussion on vaccines is particularly common in the United States, England, Canada and Australia, and as yet less common in Europe. However, while in the first group of countries *posts* and sites are predominantly in favor of vaccines, in the European countries the opposite is observed (Bello-Orgaz *et al*, 2017) and the debate polarizes almost exclusively on the extremist positions (Grignolio, 2016). In Italy, anti-vaccination sites are more numerous than pro-vaccination sites (67 vs. 27%), while only a small percentage holds both positions (Poscia *et al*, 2012).

On Facebook, the National Coordination Group of the Italian Movement for Vaccination Freedom (COMILVA, 2017), one of the most active *anti-vax* groups, has over 18,000 members and receives on average more than one hundred posts per day, containing information, news, comments, announcements of events, testimonials and petitions against vaccines (*see Box 10*) (Bellone, 2014).

5g. Tradition, politics and religion against vaccines.

We have to admit that opposition to vaccines is a real, widespread and complex problem that will persist and may even become more acute. It permeates different sections of the world population and is often common even among the higher income social groups because of the stronger desire to defend decision-making independence. In many affluent societies of the western world personalities with significant social influence, various political movements, and even some physicians express opinions against vaccination (Nature editorial, 2017b). On the other hand, current experience and the history of the eighteenth-century smallpox epidemics show that when the danger is actually perceived as a global emergency objection to vaccination disappears and is replaced by a rush to be vaccinated just when vaccines are more difficult to find.

In Africa, the anti-vaccination movements have some analogies to those of the eighteenth-century against smallpox vaccination, but mixed with a strongly anti-western sentiment. Rebellious movements, often violent, see vaccination as an intrusion of medical practice and conception of life outside of traditional African cultures, a new form of cultural violence, echoes of colonial oppression. There are well known cases of healthcare workers who have been in great difficulty because of violent reaction to vaccines. In 2004, eight health workers involved in a vaccination program were killed in Guinea (Pearson-Patel, 2015). In Nigeria, the most populous African nation, the initial resistance of religious leaders was later followed by civil authorities.

More dramatic were the attacks on the vaccination centers and the killing by the Talibans in Afghanistan and Pakistan of many healthcare workers involved in UNICEF- and WHO-sponsored anti-polio vaccination campaigns. In addition to violent acts, the Talibans spread to the population the belief that anti-polio vaccination is nothing more than a US conspiracy to make males impotent and women infertile. As a consequence of these obstacles in continuing the vaccination programs, polio has emerged endemically with a cruel punctuality among the more than 100,000 unvaccinated children living in border areas between Afghanistan and Pakistan, spreading back to Syria (Vaccines controversies, 2017). Despite the dreadful difficulties, UNICEF and WHO are enduring with their vaccination programs, attempting to associate vaccination programs to a campaign of persuasion of the population (The Guardian, 2016).

The widespread difficulty in accepting the undeniable epidemiological evidence of the advantageous risk/benefit relationship associated with vaccination should be carefully considered in order to implement effective information policies more effective in permeating all social classes, scraping off or bypassing emotionally rooted convictions. When it comes to important decisions concerning the new realities of science and technology, democratic societies appear to have some difficulties in understanding what is the choice that contributes better to social well-being (Kahan, 2013).

While on the one hand the rejection of vaccines must be faced as a fairly widespread social reality, on the other hand, epidemiological data constantly highlights the dramatic cost of suffering, illness and death caused by these movements: *Scientists, medics and commentators who have fought disinformation vaccine in the past must take a deep breath and return to the fray* (Nature editorial, 2017b).

6. CONCLUDING REMARKS: The journey of vaccines between epidemiological data, political issues and the Internet.

The World Health Organization (WHO) tells us that every year in the world, between two and three million children are saved from death by vaccines. Unfortunately, however, there are still more than 21 million children (about 1 out of 5!) who do not receive the most elementary vaccinations.

The DPT vaccine protects children against three deadly diseases, diphtheria, tetanus, and whooping cough (pertussis). This effective, no risk and low cost vaccine could be considered as the minimum level of global health implementation. During 2016, about 86% of infants worldwide received the DTP vaccine. A similar coverage rate was achieved with the measles vaccine. Slightly lower (81%) is the coverage with the vaccine against hepatitis B virus (HPV). Significantly lower still is the vaccine coverage against *Haemophilus influenzae* (52%), a bacterium causing meningitis and pneumonia, against pneumococcus (25%), and Rotavirus (less than 18%), the most common cause of severe diarrhea.

Italy has always been at the forefront, with a wise tradition of promoting vaccination policies as a measure of public health. The Italian 2017-2019 Vaccine Prevention Plan is based on sound scientific evidence (Piano Nazionale Prevenzione Vaccinale, 2017). Nevertheless, slowly but inexorably vaccination coverage has worsened, falling below the safety threshold for several diseases as warned by WHO.

Maintaining high vaccine coverage markedly reduces the chances of transmitting microbes, protecting people who cannot be vaccinated, because they suffer from immunodeficiency, cancer, and chronic illnesses. Vaccinations are therefore important not only for the individual who is actually vaccinated, but indirectly for the entire community. High coverage against a particular disease over a long period of time prevents diffusion of the microbe, eventually leading to eradication of the disease. Such is the case for smallpox, a deadly scourge which in pre-vaccine era claimed 700,000 lives a year in Europe. Our children do not need to be vaccinated against smallpox since this virus has now been eliminated worldwide. This major achievement shows how vaccination is also an act of social solidarity and global responsibility.

Reduced risk perception often makes parents reluctant to vaccinate their children. Wherever there is, however, a reduction in the vaccine coverage – i.e. for a war - almost forgotten diseases often hit again, with the risk of spreading. Proof is the recent resurge of polio in Syria, Afghanistan, Pakistan and Nigeria where, for situations of social fragility, it is not possible to vaccinate all children. If vaccination policies are not strengthened, polio reemergence may happen even in Europe.

In other cases, the scarce propensity to vaccination stems from insufficient awareness of the potential severity of some infectious diseases - such as measles - and their consequences (*Roberts, 2015*). Over and above, vaccine refusal is due to the spreading the false belief that it is best for the immune system to catch an infectious disease rather than to vaccinate: in reality the opposite is true. Clinical outcomes of an infectious disease are diverse and uncertain since many adverse complications may occur. By contrast, vaccines are probably the best workout for the immune system.

In recent years, England has had to deal with a measles outbreak that took a heavy toll given that about one million young people aged between 10 and 16 are not vaccinated. Parents of these adolescents and young adults failed to have their children immunized as a result of the completely false study by Dr. Andrew Wakefield (UK) who claimed a relationship between vaccination and autism. Despite the repeated unequivocal evidence that **autism is not caused by vaccination** as declared officially by UNICEF, WHO and scientists, fake news without a scientific basis continue to spread vaccine hesitancy in western Countries (*see Box 9*). A new, serious challenge is to counteract anxieties about vaccination spurred by lies and fake news that unfortunately spread around through the media and especially Internet.

Reliable scientific data on vaccine safety show that possible side effects are rare, usually mild and temporary while the benefits of vaccination largely outweigh the possible risks. Responsible peoples should promote the diffusion of vaccines , chiefly in places and social strata that are at high risk. It is a serious mistake to think that there is no reason to vaccinate against preventable diseases because they are virtually eradicated. Many infectious agents are still in circulation in some areas of the planet, and globalization - with travel, migratory flows and pockets of poverty - makes vaccination the key to protecting everyone's health.

The two keywords of a responsible vaccination policy are *research* and *sharing***.** To develop new, more and more effective vaccines, it is crucial to elucidate how the immune system works, from immune memory to the more recent discovery of innate immunity. We need new adjuvants that activate the most suitable defenses, to orient the immune response in the most effective direction. We need vaccines that stop microbes before they penetrate our body, blocking them when they come into contact with mucosal membranes. An entirely new field, currently in its infancy, is represented by vaccines for degenerative diseases: tumors, arteriosclerosis, and neurodegenerative diseases. Therapeutic vaccines are an additional challenge. We have the first proof of principle that this approach can work with cancer. Certainly the road is still long, but if these researches are successful, they will be pave the way to vaccine approaches against some degenerative diseases, as well as reopen the way for the use of vaccines to cure infectious diseases.

Italy boasts an extraordinary tradition for invention, development and industrial production of vaccines. Billions of people around the world have been vaccinated against polio with the vaccine produced in Italy: Albert Bruce Sabin, a Polish physician and virologist, granted the vaccine that he had formulated - and decided not to patent – to Sclavo, an Italian vaccine company. This effective tradition continues. Conjugated vaccines against type A and B meningococcal disease are in fact the result of Italian research, which today drives many of the European efforts in the field of setting up and transferring industrial vaccines.

The other current and pressing challenge is *sharing*. Today we have extraordinary tools to prevent and contain global scourges: basic vaccines for children, vaccines against Human Papilloma Virus for female health, and against hepatitis B to prevent certain forms of liver cancer. Such effective weapons, however, are often not accessible in the poorest Countries because of their cost. Sharing and extending vaccinations as well as other health protection tools being actively developed are crucial to reduce inefficient and dangerous health inequalities in different areas of the world.

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