Review

Interindividual variations in the efficacy and toxicity of vaccines

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A number of currently available vaccines have shown significant differences in the magnitude of immune responses and toxicity in individuals undergoing vaccination. A number of factors may be involved in the variations in immune responses, which include age, gender, race, amount and quality of the antigen, the dose administered and to some extent the route of administration, and genetics of immune system. Hence, it becomes imperative that researchers have tools such as genomics and proteomics at their disposal to predict which set of population is more likely to be non-responsive or develop toxicity to vaccines. In this article, we briefly review the influence of pharmacogenomics biomarkers on the efficacy and toxicity of some of the most frequently reported vaccines that showed a high rate of variability in response and toxicity towards hepatitis B, measles, mumps, rubella, influenza, and AIDS/HIV.

1. Introduction

Similar to interindividual differences in drug response (Bhathena and Spear, 2008), a number of currently available vaccines have shown significant differences in the magnitude of immune responses in individuals undergoing vaccination. It has been postulated that, a number of factors may be involved in these variations in immune responses. These factors include age, gender, race, amount and quality of the antigen, the dose administered and to some extent the route of administration, and genetics of immune system. Most of these factors can be grouped into variations caused by biology and genomics of the host and the pathogen. In addition, the environmental factors such as smoking, alcohol consumption and diet can potentially alter biology and genomic factors (Poland et al., 2008a). In a recent study (Poland et al., 2007), the term “vaccinomics” was defined as the areas of immunogenetics and immunogenomics which provide a far better understanding of how an array of factors and/or molecules play critical roles in the regulation of innate and adaptive immune responses. The examples of such molecules include human leukocyte antigen (HLA), toll like receptor (TLR) and their signaling components, cytokine receptors and genes as well as transporter associated with antigen processing (TAP), which play a role in contributing to the variations in the immune response due to genetic polymorphisms (Poland et al., 2007).

The role of genomics in determining the extent of immune response is still in its infancy with only a handful of diseases investigated in this regard. Some of the most extensively researched
infectious diseases include measles, hepatitis B, hepatitis C, human papillomavirus and influenza (Ovsyannikova et al., 2004; Poland et al., 2008a). For example, HLA genes are the most highly polymorphic genes in the human genome. Moreover, HLA genes play critical roles in establishing T cell and antibody responses against infectious agents. Polymorphism in HLA genes has been shown to significantly impact processing and presentation of peptides which originate from pathogens that ultimately affects the type of T cell and B cell responses induced.

Due to the increasing amount of reports regarding the non-responsiveness or variations of responsiveness in vaccinated individuals it becomes imperative that researchers have tools such as genomics and proteomics at their disposal to predict which set of population is more likely to develop toxicity to a certain type of vaccine administered. Furthermore, the norm that, “one size fits all” which was the basis of designing vaccines so far increasingly becomes imperative that researchers have tools such as genomics and proteomics at their disposal to predict which set of population is more likely to develop toxicity to a certain type of vaccine administered. Furthermore, the norm that, “one size fits all” which was the basis of designing vaccines so far increasingly needs to be reassessed for majority of the vaccines (Jacobson and Poland, 2004; Poland et al., 2008a).

In the following sections, we have briefly reviewed the influence of pharmacogenomics on the efficacy and toxicity of some of the most frequently reported vaccines that showed a high rate of non-responders including hepatitis B, measles, mumps, rubella and influenza, AIDS/HIV (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Vaccine/disease</th>
<th>Responders or a better responders, susceptibility to virus</th>
<th>Non-responders or less responders, resistance to virus</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis</td>
<td>HLA-DRB1(DRB1<em>0101, DRB1</em>0803), HLA-DPA1(DPA1<em>0103), HLA-DPB1(DPB1</em>0402)</td>
<td>HLA-B44, HLA-B8, HLA-B13, HLA-DRBI*07</td>
<td>Davenport et al. (1995)</td>
</tr>
<tr>
<td>Measles</td>
<td>HLA-B<em>07, HLA-DRB1(DRB1</em>0303, DRB1<em>01), HLA-DQB1(DQB1</em>0201)</td>
<td>HLA-DRB1<em>0301, DRBI</em>0801, DRBI<em>1201, DRBI</em>1302</td>
<td>Hatae et al. (1992)</td>
</tr>
</tbody>
</table>
| Mumps          | HLA-DRB1(DRB1*0101, DRBI*0701),'

2. Examples of variation in vaccine efficacy and toxicity

### 2.1. Hepatitis A and B

The vaccinated individuals show significant interindividual variations in immune response to hepatitis B vaccine. For instance, the approved hepatitis B vaccine is given in vaccine doses ranging from 10 to 40 μg depending on the targeted population. In addition to dose variation, specific segments of population fail to respond to the hepatitis B vaccine, including obese individuals, smokers, and immune-compromised individuals. In general, 10–15% of the population fails to respond to the currently available three-dose vaccine, whereas around 40% of adolescent population shows antibody levels that are considered to be protective after one or two doses. There have been also cases where some individuals have even required more than six doses of hepatitis B vaccination for the generation of immune response (Alper et al., 1989).

Antibody responses to hepatitis B vaccination have been reported to be greatly influenced by genetic variability. Among the various factors, presence of specific carriers of HLA class I and II genotypes greatly influences the differences reported in responders and non-responders. For example, the presence of HLA-B46 and HLA-B15 alleles was found to be higher in non-responders in comparison to responders in hepatitis B vaccination. Individuals with HLA-DRB1*13 allele are less likely to be infected chronically with hepatitis B (Davenport et al., 1995; Ovsyannikova et al., 2004). To address some of the questions pertaining the observed differences, twin studies are widely used as a method to study the genetic basis of variability in immune response upon vaccination. Using this method it is possible to clearly differentiate the influence of genetic and environmental factors on the nature of the immune response as monozygotic twins are genetically identical whereas dizygotic twins share 50% of their genes on an average (Hohler et al., 2005). Therefore, twin studies make it possible to investigate the influence of genetic factors that show variability in immune responses (Jacobson et al., 2003; Poland et al., 2007). For instance, in a study conducted in twin pairs at the age of 5 months, 77% variability in antibody responses was observed in response to the hepatitis B virus antigen (Newport et al., 2004). In another study in Japanese population it was found that DRB1*0101, DRB1*08032, DPBI*0103 and DPBI*0402 played a major role in the production of antibody responses whereas DRBI*0405 was associated negatively with the production of antibody (Hatae et al., 1992; Mineta et al., 1996; MacDonald et al., 2000). Wang et al. (2004) reported HLA-DRBI*07 to be associated with non-response to HBsAg.

In addition to genes encoding the major histocompatibility complex (MHC), other factors such as polymorphisms in IL-10 and IL-1β may also play a role in determining the immune responses to Hepatitis vaccination (Hohler et al., 2002; Yucseoy et al., 2002). Specifically, single nucleotide polymorphisms (SNPs) were found to influence the amount of pro-inflammatory cytokines being produced. For example, in the case of hepatitis B and A vaccination, nearly 25% of total heritability can be determined by IL-10 promoter polymorphisms (Hohler et al., 2005). IL-1β gene polymorphisms at position +3953 in the exon 5 was reported to be affecting the
IL-1β expression in response to specific stimuli. However, the study also found that the allelic distribution did not deviate significantly from the Hardy-Weinberg distribution, which was found to be around 54% with the 1.1, 37% with 1.2 and 5.4% with the 2.2 genotypes. As shown in Fig. 1, the variant 2.2 of IL-1β (+3953) polymorphism showed significantly higher antibody responses in comparison to the allele 1.1. Furthermore when the lymphocyte proliferation responses to HBsAg in peripheral blood mononuclear cells (PBMCs) were determined it was found that there were significantly higher responses in heterozygous or homozygous for the IL-1β +3953 allele (Fig. 2). These studies suggest that the immune response to HBsAg was indeed influenced by the allelic variant of +3953 IL-1β (Yucesoy et al., 2002). The study led to the use of IL-1β peptide as an adjuvant in formulations of subsequent vaccine preparations (Boraschi and Tagliabue, 1999; Yucesoy et al., 2002).

2.2. Measles, mumps and rubella

For measles vaccine, during the last major US outbreak, 20–40% of those who contracted the disease had been previously vaccinated against measles, indicating that the vaccine used was ineffective in eliciting neutralizing antibodies (Jacobson and Poland, 2004). A closer examination revealed specific genetic associations correlated with poor immune responses to measles vaccine. Children vaccinated against rubella have shown immune response variations especially with regards to antibody and T cell responses, which have been linked to host genetic variations (Ovsyannikova et al., 2004).

In measles vaccinated individuals, association between HLA genes and very high levels of antibodies or hyperseropositive responses have been reported. A higher frequency of hyperseropositive responders to measles antigens was observed for individuals carrying the class I HLA-B*07 allele. In contrast, frequencies of HLA-B*44 allele were lower in hyperseropositive than in normal seropositives (Jacobson and Poland, 2004; Jacobson et al., 2003). A significant association was observed between HLA class II alleles and circulating measles virus antibodies (Ovsyannikova et al., 2004; Poland et al., 2001). This association has been reported to be specific for HLA class II DR, DQ and DP genes. For examples, HLA-DRB1*032 and HLA-DPA1*0201 alleles were found more in seronegative than in seropositive individuals (Poland et al., 2001). Furthermore, in a study conducted in 100 healthy twin pairs (45 monozygotic and 55 dizygotic), each receiving 1–2 doses of measles–mumps–rubella-II (MMR-II) vaccine, it was found that heritability of immune responses to MMR-II differed for each of the three components of the vaccine. Heritability for measles was around 90% as compared to 39 and 46% for mumps and rubella, respectively, and these data clearly indicate that genetic differences play a significant role in determining the extent of immune responses after measles vaccination as compared to mumps and rubella vaccination (Ovsyannikova et al., 2004, 2005a; Poland et al., 2008a). It was also demonstrated that the IFN-γ and IL-4 cytokine responses in response to measles vaccine is influenced by HLA genes (Ovsyannikova et al., 2005b,c; Poland et al., 2008a).

Immune responses to measles vaccine are also influenced by SNPs and cytokine gene polymorphisms. For example, polymorphisms in measles virus gene were found to influence the induction of humoral immune responses. Two of such genes having measles virus binding domains are signaling lymphocyte activation molecule (SLAM) or known as CDw150 and membrane cofactor protein (CD46). When SNPs in these genes were studied, a clear association of allele dose-relation and the magnitude of the immune response was reported (Dhiman et al., 2007a; Dorig et al., 1993; Poland et al., 2008a; Tatsuo et al., 2000). In these studies it was found that measles specific IgG responses showed an allele dose-related 4-fold decrease in the immune response. This decrease in the immune response was attributed to higher representation of SNPs in exon 7 (rs3796504) in the SLAM gene. On the contrary, a decrease in the antibody response to measles was observed due to a SNP in exon 3 (rs164288) of the SLAM gene. The authors postulated from the data that there was a possibility that such SNPs may affect the binding ability of measles virus to the receptors (Dhiman et al., 2007a). In addition, higher IgG and lymphocyte proliferation were observed if there was a SNP within the IL-12 gene and lower responses if the SNPs were within the IL-10 and IL-12R gene after vaccination with measles vaccine (Dhiman et al., 2007b; Poland et al., 2008a).

The role of TLR polymorphisms and measles specific immune responses has also been studied and it was found that immune responses were altered with a differential representation of SNPs in certain gene variants. For instance, after measles vaccination, lower antibody responses were found with a heterozygous var-
ant for rs3775291 of the TLR gene. Similarly, a SNP in the 3’UTR (untranslated region) of the TLR3 gene also showed low responses (Dhiman et al., 2008).

The association of HLA genes and mumps has also been reported. It was shown that IgA responses were increased after vaccination by mumps antigens and this was related to DR3 and DR4 antigens. Interestingly the association of immune responses with HLA was observed only in IgA responses and not in IgM and IgG responses after mumps vaccination (Hyoty et al., 1986). For rubella infection, female populations show higher antibody levels as compared to males following the infection. On the same note, after rubella vaccination, higher rubella specific immune responses were observed in females than males. This is also reflected in the fact that females show more tendencies towards developing adverse reactions after vaccination with the rubella vaccine. Lymphocyte proliferative response is however not greatly influenced by gender as well as age of the population. Interestingly, it was found that HLA class I genes do not influence the extent of the immune response after rubella vaccination, though some suggestive but not conclusive studies have been reported (Benjamin et al., 1992; Mitchell, 1999; Ovsyannikova et al., 2006).

2.3. Influenza

The exact constitution of an influenza vaccine which has been now available more than half a century is uncertain. The dose of the influenza vaccine has undergone significant changes since its approval. Currently, there are two main types of influenza vaccine that are available for the prevention of epidemic influenza yearly; the trivalent inactivated influenza vaccine and the live attenuated influenza vaccine. For the influenza vaccine, genetic polymorphisms appear to be important in explaining variations observed for immune responses made against influenza virus (Poland et al., 2008b).

There is a considerable amount of heritability in the extent of protective immune response to influenza vaccination even when using a constant vaccine dose, formulation and the same route of administration (Poland et al., 2008b). HLA associations have been reported for immune response variations to influenza vaccines (Poland et al., 2008b) specifically HLA class II molecules. In the case of trivalent influenza vaccine, DRB1*07 was reported to be contributing to nonresponsiveness in comparison to the population responding to influenza vaccine (Lambkin et al., 2004; Poland et al., 2007). Additionally, in separate studies it was found that HLA-DRB1*0701 and HLA-DQB1*0303 allele occurs in more frequency in non-responders than in responders. In contrary, HLA-DRB4*01 and HLA-DQB1*02 alleles showed no difference among both responders and non-responders. An association of decreased HLA-DQA1*0603-9/14 allele and non-responsiveness to influenza vaccine has also been reported (Gelder et al., 2002; Lambkin et al., 2004). Additionally, in the case of influenza A antigens, it was shown that HLA-Bw35 contributes to the early phase of the immune response whereas HLA-Bw16 was associated with a reduced immune response (Cunningham-Rundles et al., 1979; Mackenzie et al., 1977). In the case of live attenuated intranasal influenza A vaccine, HLA-Bw16 allele is associated with decreased immune response (Spencer et al., 1976). There have also been reported associations of heritable dispositions to death because of influenza. This study was carried out in Utah using data from the population that have died in the past 100 years due to influenza. The study reported that close as well as distant relatives of people who died of influenza had significantly higher risk of dying from influenza (Albright et al., 2008).

Moreover, developing vaccines in the elderly is a major concern as well. Nearly 90% mortality rates have been reported in elderly during an influenza epidemic. In comparison to the younger adults who show up to 65–80% protection from influenza, elderly individuals show only between 30 and 50% protection from the illness and they also reduced responsiveness to vaccine (Effros, 2003; Remarque et al., 1999; Ruh et al., 1998; Webster, 2000) and hence may require increase in vaccine doses. However the higher IgG and IgA responses do not necessarily mean better hemagglutination–inhibition response (Remarque et al., 1999). All these data are very significant as it provides us with an idea about population that might not be protected using the current vaccination strategies or against a new influenza epidemic. For instance, the April 2009 epidemic of swine-origin influenza virus A (S-OIV, H1N1) clearly indicated that the existing vaccination strategies including the seasonal trivalent, inactivated influenza vaccine and the live attenuated influenza vaccine did not provide any protection against the H1N1 virus (Chang et al., 2009; Katz et al., 2009).

2.4. AIDS/HIV

AIDS is one of the deadliest diseases that have inflicted mankind since it was first reported in early 1980s. AIDS vaccine development has for a number of years faced with one major hardship which is the diversity in the HIV type 1 (HIV-1) strains or its gene products (McBurney and Ross, 2008). The diversity particularly in the envelope (Env) glycoprotein is the major obstacle we are facing today in the AIDS vaccine design. A large number of vaccine trials have failed right from the first one using the Env only containing vaccine to the very recent Vaxgen trials. The focus of the vaccine design has over the years changed because of these failures, with initially most of the vaccines focusing on humoral antibody development to the more recent studies which are directed towards the generating T cell mediated immunity (Letvin, 2006; McBurney and Ross, 2008; Sekaly, 2008; Thorner and Barouch, 2007). However, the unsuccessful vaccine trial by Merck recently has raised concerns as this vaccine in fact led to increased risk of HIV infection (McBurney and Ross, 2008).

HIV-1 genotype has been reported to have significant impact as far as the susceptibility to the HIV infection and hence understanding the nuances of it becomes a major direction towards vaccine development (Kulkarni et al., 2008). It was reported that both the HLA class I and II loci influence the susceptibility to the infection. HIV-1 infection resistance is reported to be because of HLA-DRB1*01 (MacDonald et al., 2000). Additionally, it has been found that A2/6802 supertype which is a cluster of closely related HLA alleles is associated with decreased HIV-1 infection. It has been known for some time now that cell surface chemokine receptors CCR5 and CXCR4 plays a major role in the virus entry into the cells. Very recently Kulkarni et al. (2008) studied the role of the polymorphisms of the major co-receptor of HIV; CCR5 and CCL3L1 which is a potent CCR5 ligand and HIV suppressive chemokine in the susceptibility to the infection as well as its role in vaccine evaluation and efficacy. The authors proposed in this study that the direction of therapeutic vaccines design should be towards reducing the infectivity of the host and the role of pharmacogenomics becomes more and more important as evident from these studies. Recently in August 2007 the FDA has approved Maraviroc which is a CCR5 co-receptor antagonist and has been directed to be used in combination with antiretroviral drugs in adult patients who are infected by the multidrug resistant virus; CCR5-tropic HIV-1 virus that uses CCR5 for cell entry as compared to a CXCR4-tropic virus which uses CXCR4 for cell entry. A co-receptor tropism assay is currently available in the United States which is expensive as well as reported to be not very sensitive. Thus development of much more sensitive and cost effective assay would significantly increase the effectiveness of Maraviroc therapy (Lieberman-Blum et al., 2008). In addition, the development of a more sensitive CCR5 assay can be useful in vaccine development.
Moreover, in spite of having monoclonal antibodies (mAbs) to all the HIV strains and studies reporting the ability of these mAbs to either neutralize HIV viruses in vitro as well as complete protection in animal models (Gauduin et al., 1997; Mascola et al., 2000; McMichael, 2006; Parren et al., 2001; Rappuoli, 2007); the success regarding a AIDS vaccine still is a distant dream. This is because of the lack of complete understanding of both the host and pathogen immune system, which as we mentioned earlier has become the focus of AIDS vaccine researchers.

Finally, many other examples have also shown a link between HLA polymorphism and the immune response against a vaccine illustrating its significance. Drawing strong associations between HLA genes and immune response variation as well as with other classes of immune response genes through immunogenetic profiling will eventually result in greatly improved and much more effective vaccines. This will lead to greater vaccine coverage, fewer failures and adverse responses and ultimately reduction in vaccination costs.

3. Genomics in vaccine development

Currently the vaccine development is based on starting from a known genomic sequence of a pathogen to identify a suitable antigen. However, because of the completion of human genome project, as well as a number of bacterial and microbial pathogens genome projects, and also due to the technical advances achieved in the field of biotechnology, the identification and development of an antigen might be achievable in a few years. In contrast, in the pre-genomic era, the methods of identifying an antigen was very laborious, very expensive, and time consuming, which may run anywhere between 5 and 15 years. The current genome based vaccine development which has been termed as “reverse vaccinology” as shown in Fig. 3, by the pioneer in this field Rino Rappuoli, Global Head of Research, Novartis Vaccines and Diagnostics, promises a great future for the current deadly infectious diseases we are facing today (Rappuoli, 2000; Serruto et al., 2009).

Reverse vaccinology was first tested for the development of vaccine against serogroup B Nisseria meningitidis (MenB). The available vaccine against meningococci was not effective against the serogroup B as the capsular polysaccharide in the conjugate vaccine was poorly immunogenic as well as autoimmune. Additionally, because the MenB strains are highly variable, the current vaccine failed to provide protection against this particular strain (Rappuoli, 2000). However, with the use of reverse vaccinology which was based on the whole genome sequencing of MenB strain, a vaccine was developed which is now in clinical trials. The details of this vaccine development are beyond the scope of this review and are covered in details in several other reviews and original research articles (Giuliani et al., 2006; Pizza et al., 2000; Rappuoli, 2000; Serruto et al., 2009; Tettelin et al., 2000). In addition to the MenB vaccine, several other potential vaccine antigens have already been screened and many are under development using this methodology. Some of examples include a pilus based vaccine against S. pneumonia, pan-genome (a global repertoire of genes of a specific species) based vaccine for the S. agalactiae and the group B Streptococcus. Pan-genome vaccine development is said to be a global genome approach which offers a broader selection of strains that can be included in the vaccine formulation (Muzzi et al., 2007). Other examples that have utilized the genome based methodology are P. gingivalis, S. pyogenes, C. pneumoniae, B. anthracis. Furthermore, it has been suggested that genome based vaccine development can be possible for a number of important diseases such as malaria, hepatitis C, tuberculosis, syphilis (Rappuoli, 2000). It has been pointed out by Rappuoli, that reverse vaccinology falls short when the vaccine candidate is not a protein, for example polysaccharides (Shadan, 2004). Hence, future vaccine development needs to take into account a number of approaches combined with reverse vaccinology to be effective in producing successful vaccine candidates. This includes use of genetic engineering techniques in combination with structured vaccinology (Dormitzer et al., 2008). Recently, to address the shortcomings of a number of software programs currently available for vaccine target prediction and identification, a new vaccine design system Vaxign was developed and made publically available on web (Xiang and He, 2009). The post-genomic vaccine development brings new hopes for making vaccines against diseases that we have failed either to control the diseases despite having a vaccine available or to develop an effective vaccine target in the pre-genomic era.

4. Biomarkers and their potential in vaccine development

The genomic and proteomic profiles of vaccinated population can be compared to subpopulation that shows adverse effects to vaccination. Such comparisons can be used to identify and validate biomarkers for potential adverse effects as a result of vaccination.
For instance, vaccinia virus used for smallpox vaccination is associated with a number of adverse events. This is because the immune response generated after the smallpox vaccination is greater than what is required (Kemper et al., 2002; Reif et al., 2009). In this regard, Reif and co-workers have very recently carried out an integrated genetic and proteomic data analysis for the identification of biomarkers that is associated with adverse events after smallpox vaccination (Reif et al., 2009). In order to study the adverse events after smallpox vaccination they employed 1442 SNPs for the evaluation of genetic factors and 108 serum cytokine determinations for studying the proteomic factors. Reif et al. (2009) concluded from this study that adverse events after smallpox vaccination are associated with a set of genetic and proteomic candidates, such as ICAM-1, IL-10, IL-4 and CSF-3. The authors suggested that the model proposed in this study could be used as a diagnostic tool for predicting adverse events. However, the authors caution that there needs to be further studies carried out in this regard along with other factors that may also influence the responses (Reif et al., 2009).

The biomarkers are also useful in the development of candidate vaccines. Currently, most of the available vaccines and prospective vaccine candidates utilize determinations like T cell proliferation assays, cytokine profiling, and antibody responses specific to the antigen or peptide in question as a means of evaluating the success of a vaccine. However, with a wide variety of vaccines based on different antigens and peptides, it becomes difficult to correlate the results obtained directly to a particular clinical outcome. Hence, identification of certain common biomarkers would serve as an excellent means for successful vaccine development as far as patient compliance, cost effectiveness as well as reducing the amount of time involved in vaccine development.

5. Summary

With the increasing number of side effects associated with a number of vaccines reported over the years, it has become imperative to develop new technologies that can effectively assist in the development and evaluation of vaccines for efficacy and toxicity. The use of DNA, RNA and protein microarrays provides a number of advantages such as an increased flexibility in the number of genes and gene products that can be tested in the evaluation of vaccine and immune response over the traditionally used methods which evaluates an overall in vivo efficacy and toxicity response to a vaccine formulation (Regnstrom et al., 2008; Thomas et al., 2009). Such technology can also be used to evaluate and compare the pharmacological and immunostimulatory effect of the vaccine antigen, the adjuvant and the combination on the expression of RNA and proteins. These evaluations can be carried out initially in preclinical animal studies and later in human clinical trials. Genomics and proteomic studies in vaccine development can provide vital clues regarding the variations in pharmacological outcomes such as inflammation, stress response, apoptosis and carcinogenicity (Regnstrom et al., 2002; Regnstrom et al., 2008; Thomas et al., 2009).

The era of immunogenomics has truly arrived and may very well hold the key to future breakthroughs and advances in vaccine design and development and improvement of public health. Such information can be useful in identification of non-responders and individuals at risk of developing side effects, and in understanding the underlying mechanism of immune response to vaccination. Genomic technology also makes it possible to develop vaccines against pathogens that are currently difficult to cultivate in laboratory.

Conflict of interest statement

The authors declare that there are no conflicts of interest.


