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GMO Regulatory Aspects of Novel Investigational Vaccine Candidates

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Abstract

Recent scientific and technical developments create novel opportunities for vaccine development. Regulatory compliance has to be ensured from preclinical research to market authorization, whereby different legal frameworks that go beyond quality, efficacy or patient safety aspects need to be taken into account. As academia and start-ups are often focused on gathering scientific evidence, the regulatory maze is often regarded by applicants as challenging in the overall pathway to clinical translation. This is particularly true for applications concerning vaccine candidates containing or consisting of genetically modified organisms (GMOs). Active communication between applicants and competent authorities or advisory bodies early in the development stages facilitates a correct implementation of the regulatory frameworks and is of utmost importance to identify challenges or hurdles in order to avoid unnecessary delay in scientific review. Based on the state-of-play in Belgium, this chapter discusses examples of regulatory journeys of applications with genetically modified viral vectors and novel vaccine candidates that have been reviewed by GMO national competent authorities in Belgium and in Europe. They highlight the need of having a comprehensive view of global perspectives early in the development to facilitate the translation of research to clinical development or even market authorization.

Keywords: novel vaccine candidates, GMO, European directives, regulatory challenges, environmental risk assessment

1. Introduction

Recent progress in disease comprehension combined with new technology performances creates novel opportunities for vaccine development in various health sectors. The last decade has seen a significant increase in the development of prophylactic medicines aiming at preventing infectious diseases or immunotherapeutic products to fight non-infectious diseases such as cancers. Both biopharmaceuticals are regarded as vaccines because they elicit an immune response, either against a pathogenic microorganism or against the host's own tumour cells. Among these investigational medicinal products (IMP) for human use currently studied in clinical trials (CT), various candidate vaccines contain or consist of genetically modified organisms (GMOs). For the purpose of this chapter, this subset of IMPs will be further referred to as GMO vaccine candidates.

As for any medicinal product, the clinical translation of research data is subject to a stringent regulatory framework, with procedures to ensure the quality, safety and efficacy of the product in humans. To conduct a new CT in one country of the

European Union (EU), a clinical trial authorization must be obtained from the national competent authority, and the CT must be approved by an ethics committee. In the case of a CT involving a GMO vaccine candidate or any IMP containing or consisting of a GMO, an authorization should also be compliant with the provisions of the legislation regarding the use of GMOs.

With the increasing number of authorization requests for CT with a GMO vaccine candidate and the new techniques that emerge for the construction of GMOs, the applicants, the national competent authorities and the different advisory bodies are facing some hurdles that may hamper the initiatives undertaken in the clinical translation of vaccine development in Europe. A first challenge originates from the several legislations with which the conduct of a CT with a GMO should comply in the country where the CT is planned. Different legislations are often under the control of different institutional bodies that may not necessarily interrelate and that may not be easily identified by applicants (see the example of Belgium in **Figure 1**). To a lesser extent, the obligation to follow distinct procedural regulations and the subsequent administrative burden may be a deterrent for them. Similarly, the applicant who plans to undertake a CT with a GMO vaccine candidate in multiple member states of the EU can be confronted with an equal number of country-specific procedures. Indeed, contrary to the standard CTA and ethics committee approval procedures for a CT, national GMO regulatory frameworks are not fully harmonized across the EU, and procedures for application may differ from one country to another. Finally, along with the emergence of new techniques intended for genetic modification, both applicants and authorities or advisory bodies are confronted with an increasing number of questions with respect to the interpretation of the definition of a GMO as laid down in the European GMO legislation.

These challenges have the merit of prompting the debate between the different actors and to initiate exchanges at national and European level with the aim to foster a continued dynamic in innovative research, while ensuring the safety of human health and environment. By means of several examples, this book chapter illustrates several aspects of the implementation of the GMO legislation that are of

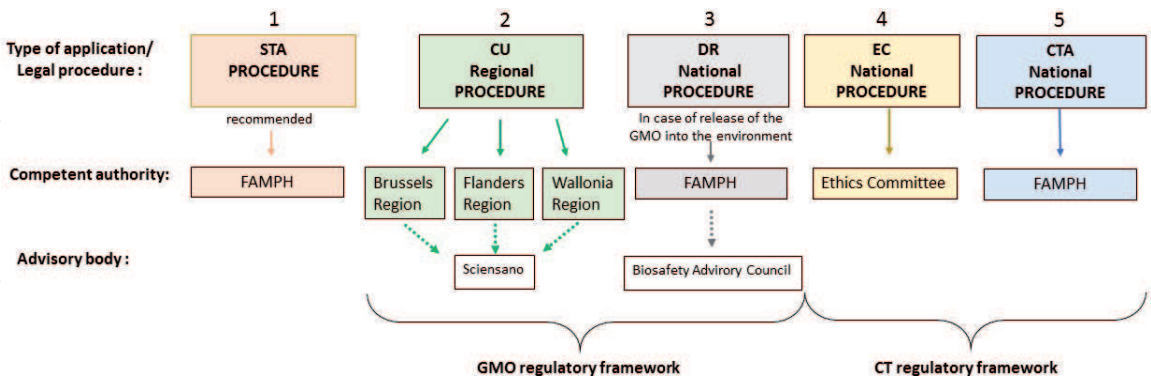


Figure 1. Overview of Belgian regulatory framework for clinical trials involving an investigational medicinal product containing or consisting of GMOs. STA, scientific and technical advice; FAMHP, Federal Agency of Medicines and Health Products; CU, contained use; DR, deliberate release; EC, Ethics Committee; CTA, clinical trial application. (1) The FAMHP offers to the applicant the possibility to request a STA prior to other mandatory procedures. The STA provides clarity on the GMO status of the IMP involved and the mandatory procedures to follow. (2) The CU procedure is applied to activities with the GMO vaccine candidate taking place in a 'contained' facility. The regional authorities and Sciensano as the advisory body are involved in the CU procedure. The CU procedure and approval are independent of those also associated to a clinical trial. (3) The DR procedure is required when there is a probability of possible release of the GMO into the environment during the clinical trial. An application is submitted to the competent authority, the FAMHP. The application is evaluated by the advisory body (Biosafety Advisory Council) which transmits its advice to the FAMHP. An application under DR framework does not exempt an application under the CU procedure. (4 and 5) Following the national law of 7 May 2004 related to experiments on human, a clinical trial cannot start without a positive advice of the (leading) ethics committee and competent authority.

relevance to CTs with a GMO vaccine candidate. The current state of discussions, an analysis of some of the hurdles that may hamper a smooth clinical translation as well as different options that are available to applicants are reviewed with respect to the Belgian and European regulatory frameworks.

2. Regulatory requirements for GMOs

2.1 The European regulatory framework

The European legislation on GMOs consists of two main Directives covering the use of genetically modified microorganisms (GMMs) in a contained facility (Directive 2009/41/EC) [1] and the deliberate release of GMOs into the environment (Directive 2001/18/EC) [2]. These Directives are mainly aimed at protecting the general population and the environment from potential risks arising from the use of GMMs and GMOs.

According to these Directives GMOs and GMMs are defined as organisms, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. The definition of a GMO is both technology and process oriented: an organism will fall under the scope of the GMO regulations if it has been developed with the use of certain techniques. Therefore the EU Directives include annexes supplying information regarding the techniques that result in genetic modification, those that are not considered to result in genetic modification or those that result in genetic modification but yield organisms that are excluded from the scope of the directives (**Table 1**).

The GMO aspects of clinical trials with medicinal products containing or consisting of GMOs, including GMO vaccine candidates, are governed by national procedures implementing the GMO Directives. However, not all member states have the same approach in implementing provisions relating to deliberate release (DR) into the environment (Directive 2001/18/EC) and/or contained use (CU) (Directive 2009/41/EC) in the specific case of clinical trials. A first report on the approaches adopted by several member states in this matter has been commissioned by the European Commission (EC) and dates back to 2007 [3]. In 2018, recognizing the developments in novel medicinal products and the need of applicants of investigational products to have an up-to-date overview of regulatory requirements, a repository of national requirements was created [4]. The approaches adopted by Bulgaria, Germany, Hungary, Ireland, Slovakia, Slovenia, Spain, Sweden and the Netherlands on the one hand, and those prevailing in Denmark on the other, illustrate two extremes. In the first mentioned member states, only the “Deliberate Release’ framework is used to assess and manage the risks for human health and the environment, while in Denmark the biological confinement of medicinal products containing or consisting of GMOs and their use in controlled hospital environments trigger the application of the ‘Contained Use’ regulatory framework only.

One of the important differences between Directive 2009/41/EC and Directive 2001/18/EC is that the latter requests the applicant to submit an environmental risk assessment (ERA). An ERA implies an assessment of the environmental impact of the GMO with regard to the potential risks for human health and the environment. Purely medical aspects concerning the efficacy of the IMP and its safety for the treated patient, as well as aspects related to social, economic or ethical considerations, are outside the scope of the ERA report. The ERA methodology for GMOs developed over the past decades is largely harmonized in many legislative systems and comprises the following steps: (1) hazard identification, (2) hazard characterization, (3) assessment of likelihood, (4) risk estimation and (5) evaluation of

Directive 2009/41/EC	Directive 2001/18/EC
<p>Article 2</p> <p>(a) ‘micro-organism’ means any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, animal and plant cells in culture;</p> <p>(b) ‘genetically modified micro-organism’ (GMM) shall mean a micro-organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.</p> <p>Within the terms of this definition:</p> <p>(i) genetic modification occurs at least through the use of the techniques listed in Annex I, Part A;</p> <p>(ii) the techniques listed in Annex I, Part B, are not considered to result in genetic modification;</p> <p>Article 3</p> <p>[...] this Directive shall not apply:</p> <p>- where genetic modification is obtained through the use of the techniques/methods listed in Annex II, Part A</p>	<p>Article 2</p> <p>(1) ‘organism’ means any biological entity capable of replication or of transferring genetic material;</p> <p>(2) ‘genetically modified organism (GMO)’ means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination;</p> <p>Within the terms of this definition:</p> <p>(a) genetic modification occurs at least through the use of the techniques listed in Annex I A, Part 1;</p> <p>(b) the techniques listed in Annex I A, Part 2, are not considered to result in genetic modification.</p> <p>Article 3.1</p> <p>This Directive shall not apply to organisms obtained through the techniques of genetic modification listed in Annex I B.</p>
<p>Annex I</p> <p>Part A</p> <p>Techniques of genetic modification referred to in Article 2(b)(i) are, <i>inter alia</i>:</p> <p>(1) Recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation.</p> <p>(2) Techniques involving the direct introduction into a micro-organism of heritable material prepared outside the micro-organism including micro-injection, macro-injection and micro-encapsulation.</p> <p>(3) Cell fusion or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.</p>	<p>Annex I A</p> <p>Techniques referred to in Article 2(2)</p> <p>Part 1</p> <p>Techniques of genetic modification referred to in Article 2(2)(a) are <i>inter alia</i>:</p> <p>(1) Recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation;</p> <p>(2) Techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation;</p> <p>(3) Cell fusion (including protoplast fusion) or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.</p>
<p>Annex I</p> <p>Part B</p> <p>Techniques referred to in Article 2(b)(ii) which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant-nucleic acid molecules or GMMs made by techniques/ methods other than techniques/methods excluded by Annex II, Part A:</p> <p>(1) <i>in vitro</i> fertilisation;</p> <p>(2) natural processes such as: conjugation, transduction, transformation;</p> <p>(3) polyploidy induction.</p>	<p>Annex I A</p> <p>Techniques referred to in Article 2(2)</p> <p>Part 2</p> <p>Techniques referred to in Article 2(2)(b) which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms made by techniques/methods other than those excluded by Annex IB:</p> <p>(1) <i>in vitro</i> fertilisation,</p> <p>(2) natural processes such as: conjugation, transduction, transformation,</p> <p>(3) polyploidy induction.</p>

Directive 2009/41/EC	Directive 2001/18/EC
Annex II Part A Techniques or methods of genetic modification yielding micro-organisms to be excluded from the Directive on the condition that they do not involve the use of recombinant-nucleic acid molecules or GMMs other than those produced by one or more of the techniques/methods listed below: (1) Mutagenesis. (2) Cell fusion (including protoplast fusion) of prokaryotic species that exchange genetic material by known physiological processes. (3) Cell fusion (including protoplast fusion) of cells of any eukaryotic species, including production of hybridomas and plant cell fusions. (4) Self-cloning consisting in the removal of nucleic acid sequences from a cell of an organism which may or may not be followed by reinsertion of all or part of that nucleic acid (or a synthetic equivalent) with or without prior enzymic or mechanical steps, into cells of the same species or into cells of phylogenetically closely related species which can exchange genetic material by natural physiological processes where the resulting micro-organism is unlikely to cause disease to humans, animals or plants. Self-cloning may include the use of recombinant vectors with an extended history of safe use in the particular micro-organisms.	Annex I B Techniques referred to in Article 3 Techniques/methods of genetic modification yielding organisms to be excluded from the Directive, on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms other than those produced by one or more of the techniques/methods listed below are: (1) mutagenesis, (2) cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods.
<i>Modified from [31].</i>	

Table 1.
The definition of a GMO according to the EU directives [1, 2].

risk management options followed by (6) a conclusion on the acceptability (or not) of the overall impact of the use of the GMO on human health and the environment, taking into account the management strategies applied. Another feature of Directive 2001/18/EC is the mandatory public consultation.

In the context of a marketing authorization application (MAA), it is important to note that Regulation (EC) No 726/2004 [5] laying down procedures for authorization and supervision of medicinal products requests an ERA similar to the ERA applied under Directive 2001/18/EC for medicinal products containing or consisting of GMOs. In practice, the scientific evaluation of the GMO, like any other MAA, is performed through a centralized authorization procedure across the EU. During this process, the European Medicines Agency holds consultations with the competent authorities (CA) of each member states established under Directive 2001/18/EC with respect to the evaluation of the environmental risk aspects. Therefore, even though a contained use-only procedure may have been accepted for a CT involving a GMO, an ERA will need to be submitted according to the provisions of Regulation (EC) 726/2004 should the IMP reach MAA.

2.2 State of the art in Belgium

In Europe, Belgium is one of the most active countries in terms of CTs undertaken with GMO vaccine candidates [6]. This is also observed by the total number of requests submitted to the Belgian authorities for new CTs involving an IMP containing or consisting of a GMO from 2009 to 2018 (Figure 2). Until 2018, the number of requests registered annually remained relatively stable, after which a marked increase was observed. These applications involve CT with IMP containing or consisting of a

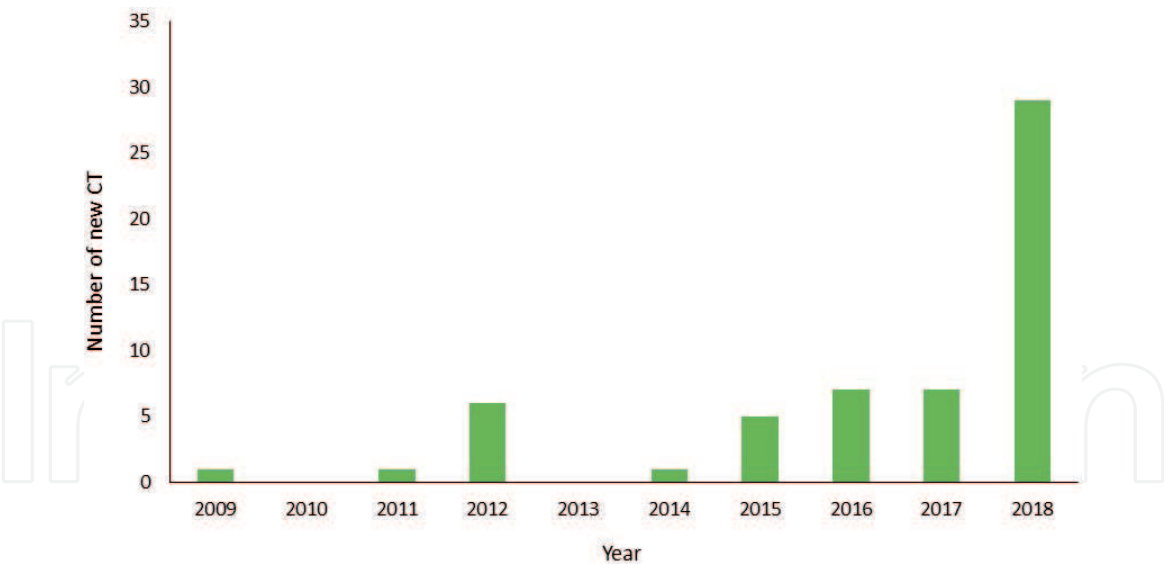


Figure 2.
Number of new clinical trials involving an investigational medicinal product containing or consisting of GMOs since 2009 to 2018 in Belgium. A new clinical trial with a GMO can take place in different clinical centres at the same time. The investigational medicinal product can be directed against infectious, cardiovascular, autoimmune or hereditary diseases but also gastrointestinal disorders, inflammation or cancer.

GMO developed against infectious diseases or cancer, as well as CT with GMOs aiming to treat cardiovascular, autoimmune or hereditary diseases, gastrointestinal disorder or inflammation. Among all these IMPs, around 70% consist of GMO vaccine candidates for prophylactic or therapeutic purposes (data not showed).

GMO vaccine candidates are mainly composed of viral vectors containing one or more specific genetic sequences whose expression in the human body will enhance the immune response against an infectious agent or tumour cells (**Figure 3**). Recently, an increasing number of clinical studies have been realized using autologous or allogenic immune cells that have been genetically modified *ex vivo* in order to express specific receptors able to recognize tumour cells when infused back into the patient body [7]. A minor part of the GMO vaccine candidates consists of the targeted infectious microorganism which is genetically modified *in vitro* to become attenuated yet still capable to trigger an immune response.

In Belgium a clinical trial with an IMP containing or consisting of a GMO can fall into the framework of the CU only or the CU and DR legislations. The GMO procedural pathway is chosen and applied on a case-by-case basis in order to guarantee proportionate and scientifically robust evaluations. To aid in the determination of the legal procedure(s), the applicant is invited to evaluate if at any stage of the CT, the general population and the environment can be exposed to the IMP.

In case physical barriers, or a combination of physical barriers together with chemical and/or biological barriers, are used to limit the contact with the general population and the environment, the CT and related activities have to comply with the Belgian legislation on CU of GMOs. Generally, activities such as the preparation, administration or storage of the IMP should follow the CU procedure only.

In general, a CU procedure suffices when there is no possible release of the GMO in the environment (e.g. the GMO is administered in clinical centres only, and there is no spreading of the GMO when subjects leave the centre) or if proper management procedures and/or working practices are implemented to prevent such a release. On the contrary, when there is a probability of release into the environment (e.g. the subject leaves the clinical centre, and close contacts of the subject may become exposed to the GMO) which cannot be avoided by proper management procedures or working practices, a notification according to the DR procedure

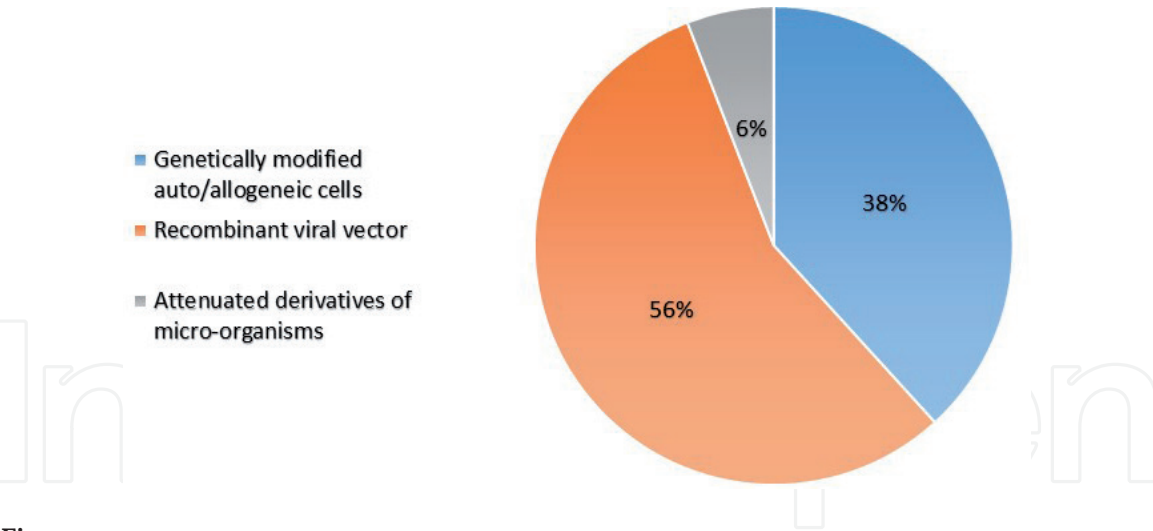


Figure 3.
Types of GMOs used as investigational medicinal products in clinical trials carried out in Belgian clinical centres and corresponding percentage of the total requests from 2009 to 2018. Globally, three main types of GMOs are used: autologous or allogeneic human cells that have been genetically modified ex vivo and reintroduced in a human body (blue), viral vectors genetically modified to carry the gene sequence of interest (orange) and attenuated derivatives of microorganisms that can operate as vaccines (grey).

will additionally be required, and an ERA should be performed. Considerations that are taken into account to determine if a DR notification is needed are the probability of shedding of the GMO, hazards associated to the shedding should it occur, probability of spreading, or whether the GMO is also taken (administered) at home. Procedures for clinical trials within the DR framework are perceived as more cumbersome than those under CU, both for the applicants and for the governmental institutions that are reviewing the applications.

3. New technologies for vaccine development facing regulatory frameworks

For many human infectious diseases no satisfactory vaccine is currently available. Hence, public health needs are continuous incentives for further research and development. Scientific advances not only contributed to the development of novel vaccines that trigger the immune system for prophylactic purposes against infectious diseases but also offered opportunities in gene transfer for (cancer) immunotherapy and the treatment of tumours. Numerous examples have reached clinical development and in some cases even commercialization, even though the interpretation and/or implementation of the regulatory maze is often regarded as challenging by applicants. This section discusses recent developments illustrating the unique features and challenges for GMO vaccines with respect to the current GMO regulation.

3.1 Dengvaxia

Dengvaxia is a GMO vaccine that recently obtained marketing authorization in the EU. This live attenuated vaccine is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals living in endemic areas. The vaccine was developed using the attenuated yellow fever vaccine strain as a vector, which has been genetically modified to express the prM and E genes from the four different dengue virus serotypes. The administered vaccine thus contains four different virus constructs, each of which contains the prM and E genes from a different dengue virus serotype. Dengue is by far the most common

mosquito-borne viral disease. It is transmitted by *Aedes* mosquitoes and infects people worldwide (mainly in tropical areas). Tens of millions of cases occur each year resulting in approximately 20,000–25,000 deaths, mainly in children [8]. Because four serologically distinct dengue viruses coexist in dengue-endemic areas, several dengue infections are possible during the patient's lifetime.

The ERA, conducted according to the principles laid down in Directive 2001/18/EC, included among others a consideration of the severity and likelihood of recombination or mutational events that would change the attenuated phenotype of the viral vector to one of virulence. The capacity of the GMO to replicate, disseminate and be transmitted by the *Aedes* mosquitoes was also evaluated. In addition, both shedding data of subjects receiving the recombinant viruses and the probability of mosquitoes or ticks transmitting the recombinant virus after oral feeding were considered in order to assess the likelihood of dissemination in the human population.

Another aspect that was considered in the context of Directive 2001/18/EC is the detection, traceability and labelling of GMOs. These legal aspects have been further regulated into sectoral legislation for genetically modified food and feed as part of their EU authorization procedure (Regulation EC 1829/2003) [9], and several recommendations have been issued on how analysis methods should be evaluated and validated by the EU Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF). Though Directive 2001/18/EC also covers IMP containing or consisting of GMOs, no sectoral legislation has been developed for IMP. Instead, as Regulation 726/2004, Art 6 (2), refers to Annex IV of Directive 2001/18/EC, traceability must be ensured at all stages of the placing on the market of GMOs (**Table 2**). However, compared to traceability requirements of genetically modified food and feed, it should be noted that much less experience has been gained so far with the validation of methods for the traceability of medicinal GMOs and no such laboratory network has been established to enforce traceability requirements at the European level. During the evaluation of the marketing authorization application of Dengvaxia, it was noticed that traceability methods proposed by the applicant referred to control and monitoring approaches for potentially contaminated effluents at manufacturing sites. However such methods are usually not adapted nor validated for detecting transfer of the donated genetic material to other organisms because the matrix in which the GMOs are supposed to be detected usually differ from those such as effluents of manufacturing sites.

3.2 Plasmid-based live attenuated virus

One of the new avenues to develop novel types of vaccines is the plasmid-based live attenuated virus technology [10]. Upon identification of the protective antigen, this next-generation vaccine platform technology potentially provides for a rapid, versatile and cost-effective vaccine response platform to infectious diseases. The technology circumvents the manufacturing of free live attenuated viral particles as such, which is subject to high-quality control requirements. Instead the genome of an attenuated virus is inserted into human cells by means of a plasmid vector. Human cells that are harbouring the plasmid vector enable the *in vivo* replication of live-attenuated virus (LAV), thereby potentially eliciting immune response and hence immunogenicity. Proof of concept has been delivered for a yellow fever virus strain, and ongoing research will now explore the use of recombinant LAVs as novel vaccines.

The approach of plasmid-based LAV vaccines exemplifies how the pace of innovation and converging technologies may blur current distinctions between a

Regulation (EC) 726/2004	Directive 2001/18/EC
<p>Article 6 (2)</p> <p>In the case of a medicinal product for human use containing or consisting of genetically modified organisms within the meaning of Article 2 of Directive 2001/18/EC, the application shall be accompanied by:</p> <p>(a) a copy of the competent authorities' written consent to the deliberate release into the environment of the genetically modified organisms [...];</p> <p>(b) the complete technical dossier supplying the information required by Annexes III and IV to Directive 2001/18/EC;</p> <p>(c) the environmental risk assessment in accordance with the principles set out in Annex II to Directive 2001/18/EC; and</p> <p>(d) the results of any investigations performed for the purposes of research or development.</p>	<p>Annex IV</p> <p>This Annex describes in general terms the additional information to be provided in the case of notification for placing on the market and information for labelling requirements regarding GMOs:</p> <p>Part A (7)</p> <p>Information on the genetic modification for the purposes of placing on one or several registers modifications in organisms, which can be used for the detection and identification of particular GMO products to facilitate post-marketing control and inspection. This information should include where appropriate the lodging of samples of the GMO or its genetic material, with the competent authority and details of nucleotide sequences or other type of information which is necessary to identify the GMO product and its progeny, for example the methodology for detecting and identifying the GMO product, including experimental data demonstrating the specificity of the methodology. Information that cannot be placed, for confidentiality reasons, in the publicly accessible part of the register should be identified.</p>

Table 2.
Legal requirements regarding traceability of an IMP containing or consisting of a GMO in the case of notification for placing on the market.

GMO and a non-GMO, and hence the legal status with regard the legislation on GMOs. Such rapidly evolving fields may also challenge a harmonized understanding of legal definitions across different countries. With respect to GMO regulation, it is not yet clear whether the status of plasmid-based live attenuated viruses will be based on a common interpretation among GMO national competent authorities.

A first element that will contribute to the interpretation can be sought in the GMO status of vaccines that are based on plasmid DNA derived from bacterial cells for use in humans or in animals, the so-called DNA vaccines [11]. Most of the EU member states do not regulate DNA vaccines as a GMO. The reasoning behind this is that a DNA vaccine is not considered an organism. Likewise, human cells transfected with plasmids should not be classified as GMOs, provided that the plasmid is not replicative and is unlikely to integrate into the cell genome. Taking into consideration Article 2 of Directives 2001/18 and 2009/41/EC, and corresponding annexes (**Table 1**), nucleic acid material (DNA or RNA) such as plasmids present under episomal form in a human cell is not considered as heritable material (of the human cell) unless the nucleic acid material is capable of continued propagation, for example, by integration of the nucleic acid material into the genome of the human cell, or when the plasmid contains an origin of eukaryotic replication. It should be noted that the probability of integration cannot be totally excluded for plasmids not known to contain integrative elements or homologous sequences. However, should an integration event occur, the risk for the human population or the environment, associated to the use of transfected human cells, would be negligible. Indeed, human cells can only propagate inside the human body or under controlled *in vitro* conditions. In terms of potential risks for the human population and the environment, it follows that the risk associated to the use of a transfected human cell is negligible provided that the plasmid is not replicative or not integrative.

A second element that prompts reflection on the GMO status, which is of particular relevance for the plasmid-based LAV technology, is associated to plasmids harbouring the full sequence of a virus. In that case the plasmid-transfected human cells may lead to the generation of replication-competent virus particles in the host human cells that eventually can be released into the environment. A plasmid harbouring a virus strain that has been genetically modified would be subjected to the GMO framework. However, the GMO status of plasmids harbouring the full sequence of a naturally occurring virus or attenuated virus has not been determined yet.

3.3 CAR T cells

Therapeutic vaccines for cancer immunotherapy with chimeric antigen receptor (CAR) T cells are another medical development exploiting modern biotechnology tools. Cancer immunotherapy uses the patient's own T cells that have been engineered to express a receptor targeting an antigen on the surface of tumour cells [12–14]. CAR T cell-based immunotherapy has shown remarkable efficacy against human malignancies, thereby providing a promising alternative to allogeneic haematopoietic stem cell transplantation which is known to be associated with severe side effects. It is anticipated that the number of developments using CAR T cells will continue to expand as current research now explores, for example, the potency of (CAR) T cells in solid tumours or the use of allogeneic 'off-the-shelf' T cells. The rapid pace of developments in part has been facilitated by the implementation of gene-editing tools to genetically modify human T cells.

Genome-editing techniques involving the use of site-directed nucleases (SDN), like the ribonucleoprotein-mediated gene editing of cells, make it possible to induce modifications in a predefined region of the genome without the need to introduce foreign (exogenous) DNA [15]. For some applications, the resulting organisms cannot be distinguished from those generated through classic mutagenesis or spontaneous mutations. While addressing how these techniques relate to the European GMO legislation and taking into account that organisms developed through classical mutagenesis are excluded from the EU regulatory framework for GMOs, a number of authorities or advisory bodies of EU members have expressed the opinion that applications of SDN, resulting in small point mutation or indels, could be exempted from the EU GMO regulation on the condition that the nuclease is not stably expressed from a recombinant nucleic acid molecule [16]. Therefore the advent of gene-editing techniques was challenging the boundaries of the GM regulation in the EU, at least until a legal opinion of the Court of Justice of the EU (ECJ) was issued. The ruling declared that organisms produced by mutagenesis techniques, including directed mutagenesis and applications of gene editing, should be regulated under EU GMO law, unless the mutagenesis technique has conventionally been used in a number of applications and has a long safety record [17]. Much opposition has been raised against this ruling, in particular with respect to the inclusion of gene-edited plants within the remit of GMO legislation [18, 19].

The ruling applies to medicinal GMOs as well and will determine the legal status of CAR T cells obtained with gene-editing techniques with respect to GMO legislation. Apart from notifications in the context of CTAs, such an IMP would also require an ERA when reaching MAA (Regulation (EC) 726/2004). However, though human cells may survive in whole blood or synthetic media with a composition similar to human blood, these cells can only propagate inside the human body or under controlled *in vitro* conditions. Human cells will not survive in non-optimized conditions, and it is highly unlikely that the genetic modification of these cells, by means of gene-editing tools, will alter the inherent fitness of human cells for survival in the environment upon their release, much less to cause

any adverse effects to human health or animal health. It follows that for MAA involving the use of gene-edited human (autologous) cells, the risks for human health and/or the environment associated to the handling/use of the medical product is negligible.

3.4 Novel oral poliovirus type 2 vaccine candidates

The continuous improvements in DNA synthesis technology also hold promise in the design of new vaccines. Developers may go further than recombinant DNA technology and make a step towards increased rational design, away from existing nucleotide sequences. For example, the genetic code of the virus can be redesigned so that 100% identity is preserved at the protein level with significant differences at the nucleotide level. This codon deoptimization has been described as an approach to generate attenuated viruses that can be used as vaccines [20, 21]. The synthesis of poliovirus (PV) as early as 2002 is considered as one of the first milestones in synthetic genomics [22]. Most recently, a clinical trial has been set-up involving two GMOs consisting of novel live attenuated polio vaccine candidates that have been developed through advanced DNA synthesis technology and codon deoptimization [23]. Though few preliminary questions were related to the GMO status of the novel live attenuated polio vaccines, it was merely the context of the global Polio Eradication Initiative and the associated efforts to minimize poliovirus facility-associated risks, as defined in the WHO's Global Action Plan III (GAP III) [24] that added to the complexity of the legal procedure.

Poliovirus has a particular status from a global world health perspective since the launch of the global Polio Eradication Initiative in 1988. Immunization with trivalent live, attenuated oral poliovirus vaccine (OPV), composed of three strains OPV1, 2 and 3, has led to a drastic decline in the number of polio cases worldwide. However OPV is genetically unstable and can regain neurovirulence, leading to outbreaks of circulating vaccine-derived poliovirus (cVDPV). In a context of PV type 2 eradication worldwide and because the risk associated to the use of OPV2 was outweighing the benefits, it was decided to withdraw the type 2 component from OPV vaccination campaigns and to introduce the inactivated poliovirus vaccine, which is more expensive and relatively more cumbersome to administer. Nevertheless, due to its induced superior mucosal immunity, monovalent OPV2 is still used in responses to outbreaks of cVDPV2, thereby challenging the feasibility of eradication of PV2 [25]. It is within this context that a global consortium of investigators, governmental, non-governmental, academic and global health organizations worked on the development of two novel OPV2 vaccine candidates (nOPV2) with better genetic stability and reduced risk to regain a neurovirulent phenotype.

The first-in-human (FIH) phase 1 study was conducted in Belgium. Although the clinical development of such novel vaccines was considered highly desirable from a world health perspective, the launch of a FIH study was considered under severe scrutiny in order to avoid any risk of introducing VDPV in a country declared polio-free for several years. As shedding of the nOPV2 was anticipated for a mean time of 2 weeks, the consortium decided to conduct the FIH phase 1 study with voluntary participants under full containment during 28 days, with strengthened containment measures. Rather unexpectedly, the study showed that ~50% of the subjects still were shedding after having left the full containment period of 28 days. Post-discharge biorisk management measures were applied to prevent the release of the candidate vaccines in the environment and to avoid contact with immune-compromised individuals.

The WHO's Polio Eradication Department encouraged further progress in the clinical study of the novel OPV vaccines and the consortium applied for a phase

II CT with the nOPV2 vaccine candidates. On the basis of shedding and genetic stability data obtained with the FIH study, and the larger size of cohorts to be involved during the phase II study, the consortium applied for an authorization for deliberate release into the environment of the GMOs. In accordance with the Royal Decree transposing Directive 2001/18/EC into Belgian law, an ERA was submitted [26], a public consultation was organized, and a notification according to the provisions of Council Decision 2002/813/EC [27], the so-called summary notification information format (SNIF), was circulated. This enables an exchange of information between the EU member state and the Commission on the basis of relevant information.

It has been the first example to our knowledge of EU member states commenting on the SNIF. One of the concerns raised by neighbouring countries was the transboundary release of the nOPV2, should the healthy volunteers not stay in Belgium during the period of virus shedding. Those concerns are not only related to GMO regulatory provisions but also to GAP III requirements, which describe a biorisk management system addressing areas associated with the design, operation and management for facilities handling poliovirus facilities [28]. It can be concluded that the regulatory pathway to the setup of the two first CT involving nOPV2 revealed an additional complexity involving increased exchanges both at national and international levels.

4. Engagement with regulatory agencies or advisory bodies

4.1 Importance of networking

Medicines become more and more the result of different and converging technologies. For many human infectious diseases, no satisfactory vaccine is currently available, and the development of vaccines containing or consisting of GMOs is one of the innovative technologies implemented to meet some of the public health needs. Regulators involved in medicinal products for humans, as well as in GMOs, need to anticipate these developments, not only by enforcing safety regulations but also by ensuring the scientific review adheres to the principles of proportionality and case-by-case approach. From the applicants' side, it is recognized that the regulatory pathway for novel technologies is complicated and not always straightforward. Early dialogue between applicants, risk assessors and the regulatory authorities is therefore paramount in addressing challenges with clinical translation of novel GMO vaccines.

The steps that were undertaken towards the approval of early phases of a clinical trial investigating nOPV2 vaccine candidates in a post-OPV2-withdrawal era exemplify the importance of liaising among several regulatory agencies and public health institutions covering (international) public health objectives at national, European and global level. At the time the consortium that worked on the development of the nOPV2 vaccine candidates applied for its second clinical trial, it was still not clear whether the nOPV2 vaccine candidates were to be considered under the scope of GAP III. Because the consortium engaged as early as possible with advisory and/or regulatory institutions, the Belgian authorities were prompted to ask the WHO's Containment Advisory Group to clarify the GAP III status of the nOPV2 vaccine candidates and, if these were to fall under the scope of GAP III, how to interpret or implement the GAP III guidelines in a phase II clinical study. The WHO's Containment Advisory Group concluded that, according to the specific terms of usage proposed in the context of the protocol of the CT, the nOPV2 could be used outside the containment requirements of GAP III. It also requested the

addition to the trial protocols of environmental monitoring for polioviruses around the trial sites, as well as monitoring of close and family contacts of trial subjects who continued to shed virus after the end of the trial period [29].

This case exemplifies how different aspects of public health interrelate and contribute to the complexity of the regulatory maze to which applicants may be confronted when submitting clinical trial applications. Both the consortium and Belgian authorities liaised with several regulatory agencies and public health institutions covering different (international) public health objectives in order to ensure that risk management measures were proportional to the risk/safety assessment taking into account the intended use, the receiving environment and the likelihood of exposure.

4.2 Interplay GMO-pharma

A concern of developers that is acknowledged by the European Commission is the lack of harmonization of regulatory procedures of clinical trials with regard to the GMO legislative framework. The approval of clinical trials is within the remit of the member states and the interplay between the CT regulation and the GMO regulation might differ between the member states. This non-harmonized approach among member states, detailed under Section 2 of this chapter, is perceived as ineffective for the conduct of multinational clinical trials and as an impediment to the effective translation of research findings into clinical applications.

Very recently the first steps towards a common procedure for a subset of innovative therapies for human use involving the use of GMOs has been agreed upon among member states [30]. It concerns an application form specifically developed for clinical trials involving the use of human cells transduced with retroviral or lentiviral vector systems. Taking into account that the evaluation of clinical trial with respect to the GMO legislation will remain within the remit of national authorities, this initiative can be seen as a significant step towards enhanced communication among regulators. It is also of high value in light of the upcoming therapeutic strategies based on the genetic modification of T cells that target defined antigens presented by tumour cells and aids the patient's own immune system to combat malignant diseases, the so-called CAR- and TCR-modified T cells.

The European Commission continues to foster exchanges among member states with the aim of developing common application forms, increasing cooperation in the risk assessment of applications and identifying issues and questions with respect to the scope of the GMO regulatory framework.

4.3 Scientific and technical advice

As already mentioned, at the time of planning a CT with a GMO in a European member state, the applicant may be confronted to a complex regulatory procedure, exceeding what is required for a standard study with an IMP. Alongside the standard Clinical Trial Application (CTA) and obtaining the advice from the ethics committee, questions regarding the GMO status of the IMP and the proper mandatory procedural steps may arise. In addition, should the IMP be identified as a GMO, the developer will need to identify a distinct competent authority in charge of reviewing the application according to the adequate procedural steps.

As outlined earlier, the determination of the GMO procedural pathway for a CT in Belgium (meaning whether the CU only or both CU and DR procedures must be followed) is subject to a case-by-case examination taking into account the possible release of the GMO into the environment and the possible associated risks. To help the applicant, the competent authority for CTAs offers the

possibility to request a scientific and technical advice (STA) prior to the CTA. The main objective of the STA is to facilitate the development of vaccines and therapeutic products by centralizing and analysing the applicant's concerns at the time of starting the CT.

Within the STA, the applicant is invited to request clarifications on the GMO status of the IMP, and on the GMO procedures to be followed, should it decide to proceed with the application. The competent authority for CTAs coordinates the contacts with experts, centralizes their responses and delivers a formal advice to the developer. As such, the STA is a means for developers to engage with the competent authorities and advisory bodies early in the process in order to (i) provide information that would facilitate further process and (ii) avoid possible misunderstandings with regard the GMO status and procedures, which consequently may save time for the developer.

5. Discussion and conclusions

With novel technologies poised to result in the development of novel IMPs and the overall drive for sustained innovation, a number of regulatory hurdles can be identified, which developers face during the development of GMO vaccines. First, EU GMO Directives have been transposed into national legislations that include different regulatory specificities between member states. Second, new technologies may lead to the generation of organisms that are prone to different interpretations with regard to their (GMO) regulatory status, thereby hampering further harmonization of legislations. In addition, aspects such as the relevance of an ERA or detection and traceability requirements become in some cases disproportionate with respect to the actual risks that novel IMP represent for the general population and the environment. Overall, these aspects will increase the need of regulatory agencies and advisory bodies to anticipate the deployment of novel IMP, through continuous engagement with all stakeholders.

The ECJ ruling has initiated the debate concerning the need to rewrite the GMO Directive 2001/18/EC, to have it more fit-for-purpose for the rapid pace of emerging technologies. This is particularly true for gene-editing technologies. Although it is primarily the impact on agri-food applications that has sparked these debates, the ECJ ruling will also have an effect on research and development activities and the development of a category of IMP. It is anticipated that the current debate on the appropriateness of the existing GMO regulatory framework may affect the future regulatory status of GMO medicines. This may have consequences through all stages of development, from R&D, through clinical translation and marketing authorization application.

Aiming to overcome existing hurdles in the regulatory pathway, a number of initiatives have been taken in Belgium and among member states. Key challenges are being addressed, for example, with the STA at Belgian level, and tangible solutions have been formulated, such as the common form for CT with human cells genetically modified *ex vivo* by retro- or lentiviral vectors. It is expected that the need for harmonization and reviewing regulatory frameworks will be the basis of further engagement and exchange between all stakeholders.

New technologies in the field of biotechnology spark promising avenues for the development of novel biopharmaceuticals involving GMOs. Enhanced networking among all stakeholders should be further promoted in order to subject regulatory frameworks to critical review with the aim of keeping them up-to-date with upcoming developments and to support innovation while ensuring quality and safety for patients, the general population and the environment.

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Conflict of interest

The authors declare that they have no conflict of interest.

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