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Review

Clinical trials with GMO-containing vaccines in Europe: Status and regulatory framework

Florence Kauffmann [a], Pierre Van Damme [b], Geert Leroux-Roels [c], Corinne Vandermeulen [d], Nele Berthels [e], Claire Beuneu [e], Stéphanie Mali [e,*]

[a] GlaxoSmithKline Biologicals S.A., Rue de l'Institut 89, 1330 Rixensart, Belgium
[b] Centre for the Evaluation of Vaccination, Vaccines & Infectious Disease Institute, University of Antwerp, Belgium
[c] Centre for Vaccinology (CEVAC), Ghent University and Ghent University Hospital, Belgium
[d] Leuven University Vaccinology Centre, KU Leuven, Belgium
[e] Federal Agency for Medicines and Health Products (FAMHP), Belgium

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ABSTRACT

Recombinant technology has revolutionised the way novel vaccines are developed and manufactured. The possibility to genetically modify micro-organisms to bring immunogenic material (antigens/epitopes) to the human (or animal) immune system to provoke an immune response, provides new hope to producing prophylactic vaccines against HIV, malaria and tuberculosis and emerging diseases. Regulatory requirements associated with the development of genetically-modified organism (GMO)-containing vaccines in Europe add an additional burden to the clinical trial application procedure and to the preparation and initiation of a clinical trial of such vaccines. Moreover, the GMO regulatory framework is complex and only partially harmonised across Europe, which may hamper multi-country clinical trials with GMO-containing vaccines. This paper provides an overview of clinical trial applications with GMO-containing vaccines in Europe and reviews the regulatory framework in countries where GMO-containing vaccine clinical trial authorisation (CTA) applications were submitted between 2004 and 2017.

Contents

* Corresponding author.
E-mail address: Stephanie.Mali@fagg-afmps.be (S. Mali).

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1. Introduction

Increasing knowledge of microbiology and technological progress have unceasingly expanded the number and quality of vaccines available today. Innovative solutions such as conjugate, recombinant, vectored, nucleic acid-based or somatic cell therapy vaccines contribute to the diverse palette of modern-day vaccines designed to bring new immunogenic material to a host's immune system [1,2].

Genetically engineered (GE) vaccines can be generated through the use of a GMO, for example by *in vitro* expression of recombinant genes encoding antigen proteins, but not necessarily contain a GMO. As per the European directive 2001/18/EC, a GMO is 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 [3]. GMO-containing vaccines thus comprise recombinant micro-organisms which deliver the vaccine antigen to the human body. Although viruses are biologically speaking no organisms, they are considered as GMO when genetically modified. Clinical trials using gene therapy technology (which also includes non-vaccines) have significantly increased between the 1990 and 2000 [4]. The number of registered GE vaccines has seen a surge since 2000 and keeps increasing [5]. However, only 5% of human GE vaccines in clinical development seem to contain GMOs [6]. To our knowledge, the status of clinical trials with GMO-containing vaccines in Europe has not been documented recently [7].

Although numerous GMO-containing vaccines for human use are in development, only a few have reached the market so far: (1) IMOJEV, a vaccine against Japanese encephalitis, is a genetically modified yellow fever virus (YFV) wherein the envelope proteins of the yellow fever attenuated 17D strain vaccine are replaced by those of the Japanese encephalitis virus, (2) Dengvaxia, a vaccine against dengue, consists of genetically modified yellow fever viruses wherein structural genes of the yellow fever attenuated 17D strain vaccine are replaced with those from the four dengue serotypes and (3) Fluenz Tetra, a live-attenuated seasonal influenza vaccine containing four cold-adapted, temperature sensitive, reassortant influenza viruses, representing the circulating influenza A and B strains [8–10].

The development of vaccines abides by strict regulatory requirements to ensure that safe, efficacious and good quality vaccines reach the market. Considering that most vaccines are administered to healthy individuals and young children, little compromise can be made on the benefit/risk assessment. However, increasingly stringent regulatory requirements are considered as one of the factors responsible for the decreasing numbers of pharmaceutical companies that are still developing and producing vaccines for human use since the 1950s [11–13].

The EU GMO legislation ensures the protection of human health and the environment from the introduction of potentially hazardous genetically modified organisms. This legislation was initially developed to regulate the introduction of genetically modified plants and food products within the EU. However, as the GMO definition includes micro-organisms as well, this legislation is also applicable for the introduction of GMO-based medicines. As a result, conducting a clinical trial in Europe with a medicinal product consisting of/or containing GMOs requires three levels of review, whereas only two levels of review for non-GMO medicinal products are needed. In addition to the standard review of a CTA application and the Ethics Committee review, a GMO-specific procedure encompassing a risk assessment relating to the environmental and biosafety aspects of the use/release of the GMO is required [14,3].

Previously, an overview of gene therapy clinical trials using plasmid DNA and viral vectors without distinction between vaccines and non-vaccines purpose has been published [4]. A vaccine-related regulatory overview has also been reported in view of general toxicology requirements for conventional vaccines [15]. The clinical trials using viral vectored and DNA-based vaccines worldwide and the regulatory framework that relates to these vaccines in the USA, Europe and Japan was reviewed [16], but this review excluded therapeutic vaccines and was not specifically focused on vaccines consisting of/or containing GMOs.

To ensure the future development of innovative vaccines such as through the genetic modification of micro-organisms to target emerging diseases, a better understanding of the status of GMO-containing vaccines and their regulatory framework is needed. In this paper, we review the clinical development and regulatory framework in Europe of GMO-containing vaccines for prevention or treatment of infectious and non-infectious human diseases.

2. Methods

2.1. Analysis of clinical trials with GMO-containing vaccines

Using the EudraCT database of human clinical trials from its initiation in 2004 until 2017, a first selection was performed using the criteria requested in Annex I of the CTA form. Only clinical trial applications which answered "YES" to both questions D3.11.6 "Immunological medicinal product (such as vaccine, allergen, immune serum)?" and D3.11.10 "Medicinal product containing genetically modified organisms?" were considered further.

From this selection encompassing 307 studies, a further refinement was performed to exclude non-vaccines studies, such as: (1) somatic cell therapy investigational medicinal products, which consists of *ex-vivo* genetically-modified human cells to act as effector cells (e.g. CAR-T cell therapy), (2) tissue-engineered products and (3) gene therapy medicinal products, which consist of genetically modified viral, bacterial or parasitic vectors, for the "direct" *in vivo* targeting of the infectious/non-infectious disease (e.g. a thymidine kinase-deleted vaccinia virus encoding GM-CSF or other effector molecule) that do not elicit an immune response against the expressed molecule. The obtained selection contained vectored (viral, bacterial, parasitic) vaccines, cell-based vaccines and nucleic-acid based vaccines. A resulting 201 GMO-containing vaccine clinical trial applications (both prophylactic and therapeutic) were further used to determine the number of clinical trials per country.

Then, the clinical trials submitted in multiple countries (*i.e.* multi-country trials) were considered as just one clinical trial, which led to a final number of 147 studies. These 147 studies were classified by year, clinical trial phase, targeted disease and vector type.

2.2. Overview of the EU regulatory framework and guidelines regarding GMO-containing vaccines

An overview of the relevant European regulations, directives and guidelines regarding the conduct of clinical trials with

(GMO-containing) vaccines is given. The decision-tree that resulted from the analysis categorises the different types of vaccines, makes a distinction between GMO- versus non-GMO-containing vaccine regulations and between vaccines against infectious diseases versus non-infectious diseases. Information regarding Ethics Committees procedures was not considered.

Excluded from this review are: European regulations and directives regarding pharmacovigilance (Directive 2010/84/EU), quality/good manufacturing practices of investigational medicinal products (Directive 2003/94/EC) and medicinal products for paediatric use (Regulation (EC) No 1901/2006, Regulation (EC) No 1902/2006), European disease-specific (e.g. cancer, influenza, smallpox, polio, RSV) and technological (e.g. adjuvants) guidelines.

3. Results

3.1. GMO-containing vaccine clinical trial in Europe: Applications and phases.

Out of all European CTA applications with GMO-containing vaccines in Europe between 2004 and 2017, 21 countries were identified (Table 1). The countries with the highest number of clinical

Table 1
Absolute number and percentage of GMO-containing vaccine clinical trial applications per country in Europe (2004–2017). Top five countries with highest numbers/percentages are highlighted in grey. NA: not applicable.

Number° of GMO-containing vaccine clinical trials per country in Europe: 2004-2017	Absolute n°	% (out of 147 trials)
Austria	4	2.7
Belgium	13	8.8
Bulgaria	2	1.4
Czech republic	2	1.4
Denmark	3	2.0
Estonia	1	0.7
Finland	5	3.4
France	14	9.5
Germany	18	12.2
Hungary	7	4.8
Iceland	1	0.7
Ireland	1	0.7
Italy	4	2.7
Lithuania	1	0.7
Netherlands	8	5.4
Norway	1	0.7
Poland	3	2.0
Romania	1	0.7
Slovakia	1	0.7
Spain	16	10.9
Sweden	4	2.7
UK	91	61.9
TOTAL including all multi-country trials	201	NA
TOTAL representing each multi-country trial as one trial	147	100

Table 2
Type of GMO-containing vaccines used during clinical trials in Europe (2004–2017). "Mixed" GMO-containing vaccines refers to studies combining plasmid DNA and viral vector vaccines (9) and studies combining a parasitic vector with a viral vector vaccine (1). ID: infectious disease targeted, NID: non-infectious disease targeted.

Types of GMO vaccine CT in Europe: 2004–2017	Total n°	ID	NID	Prophylaxis	Therapy
Viral vector (VV)	121	109	12	95	26
Bacterial vector (BV)	11	9	2	6	5
Parasitic vector (PV)	2	2	0	2	0
Plasmid DNA (pDNA)	2	2	0	0	2
Cell-based vaccine (CBV)	1	1	0	0	1
Mixed	10	9	1	5	5
TOTAL	147	132	15	108	39

trial applications were the UK (61.9%), Germany (12.2%), Spain (10.9%), France (9.5%) and Belgium (8.8%) (Table 1). From the 91 studies in the UK, the clear majority (*i.e.* 52) of GMO-containing vaccine clinical trial applications were submitted by Oxford University and to a lower extent (*i.e.* 27) by several commercial sponsors. In contrast, more than 60% of the GMO-containing vaccine clinical trial applications in Germany, Spain, France and Belgium originated from commercial sponsors (data not shown).

More than half (85 studies, 57.8%) of the GMO-containing vaccine clinical trial applications in Europe between 2004 and 2017 were Phase 1 (Suppl. Fig. 1). Phase 1/2 and Phase 2 studies accounted for 19.0% (28 studies) and 16.3% (24 studies), respectively (Suppl. Fig. 1). Phase 2/3 and Phase 3 represented only 1.4% (2 studies) and 5.4% (8 studies) of the studies, respectively (Suppl. Fig. 1). The Phase 3 studies were targeting cancer, dengue, ebola and influenza (data not shown). The total number of studies per year was fairly stable between 2004 and 2017, with an average of 10.5 ± 4.7 studies per year (data not shown) (Suppl. Fig. 2). The number of studies with a commercial sponsor compared to a non-commercial sponsor was 60 and 87, respectively (data not shown).

3.2. GMO-containing vaccines in Europe: Diseases targeted and GMO types

The 147 European clinical trial applications for the use of GMO-containing vaccines between 2004 and 2017 targeted a variety of diseases, non-infectious (cancer) as well as infectious diseases (viral, bacterial or parasitic) (Suppl. Fig. 2). The most frequently targeted diseases by GMO-containing vaccines were: (1) HIV (26 studies, 17.7%), (2) Malaria (23 studies, 15.6%), (3) Influenza virus (18 studies, 12.2%), (4) Cancer (15 studies, 10.2%) and (5) Tuberculosis (14 studies, 9.5%) (Suppl. Fig. 2). These five diseases account for about 65% of the total number of GMO-containing vaccine studies in European clinical trial applications between 2004 and 2017 (Suppl. Fig. 2).

The majority of GMO vaccine clinical trials used viral vectors (121 studies, 82.3%), followed by bacterial vectors (11 studies, 7.5%) and mixed types (10 studies, 6.9%) (Table 2). Most GMO-containing vaccines were directed against infectious diseases (132 studies, 89.7%) compared to non-infectious diseases (15 studies, 10.9%), and meant to be prophylactic (108 studies, 73.5%) compared to therapeutic (39 studies, 26.5%) (Table 2). Only two studies were conducted with parasitic vectors (against malaria, data not shown), two with plasmid DNA (against HIV and Cytomegalovirus, data not shown) and one with cell-based vaccines (against hepatitis C virus, data not shown) (Table 2). Interestingly, the two plasmid DNA vaccines were described as a "medicinal product containing genetically modified organisms" in the clinical trial application submitted in France, although plasmid DNA is not generally considered as a GMO (see also Discussion below) [17].

From the 121 clinical studies using viral vectors, the most utilised vectors were: (1) a combination of an Adenoviral vector with a Modified Vaccinia Ankara (MVA) vector in a heterologous prime-boost regimen (40 studies), (2) MVA vector system only (36 studies) and Adenoviral vector only (13 studies) (Fig. 1).

3.3. Regulatory framework for GMO-containing vaccines in the EU

The European Union has established a legal framework to ensure that the development of GMOs takes place in safe conditions. A clinical trial involving a GMO-containing medicine thus needs to comply with the regulatory provisions of both the clinical trial and the GMO legal framework (Fig. 2).

The EU 'Clinical Trial Directive' 2001/20/EC and the future EU 'Clinical Trial Regulation' 536/2014 (entry into application currently estimated to occur in 2019) aim to simplify and harmonise the administrative provisions governing clinical trials by establishing a clear, transparent procedure and creating conditions conducive to effective coordination of such clinical trials in the European Community by the authorities concerned. A formal approval of the CTA application and a positive opinion from the Ethics Committee are at least mandatory. The EU Clinical Trial Directive as well as the EU Clinical Trial Regulation do not cover biosafety and environmental aspects. Therefore, when a GMO-containing medicine is involved, the clinical trial must then also comply with legislative provisions on biosafety, implementing the 'Contained Use Directive' 2009/41/EC and the 'Deliberate Release Directive' 2001/18/EC and with the Directive 2000/54/EC on the protection of workers to the risks related to the exposure to biological agents [14,3,18]. These directives provide a harmonized legal framework yet allowing for country-specific requirements set in Member State legislation.

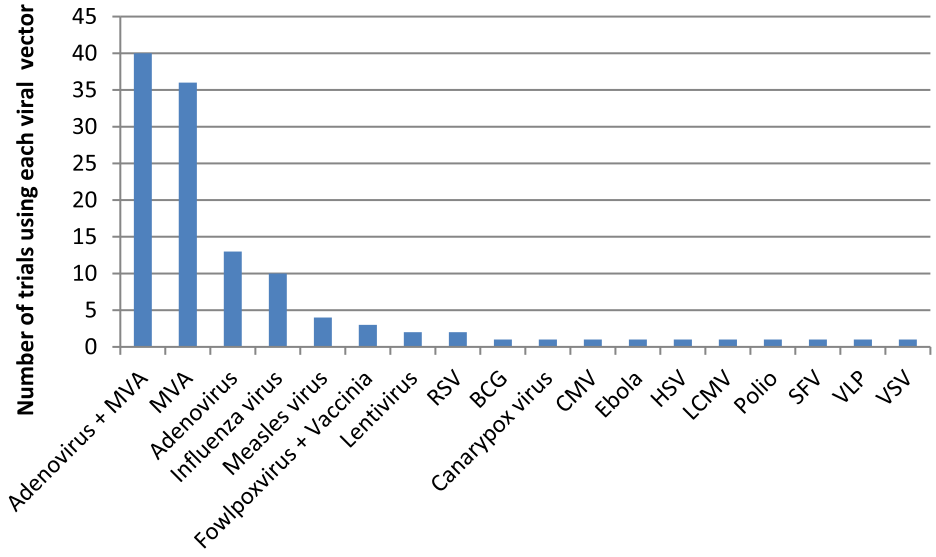

Fig. 1. Overview of number of GMO-containing vaccine clinical trials using each viral vector type. MVA: Modified Vaccinia Ankara virus, RSV: Respiratory Syncytial virus, BCG: bacillus Calmette-Guérin, CMV: Cytomegalovirus, HSV: Herpes Simplex virus, LCMV: Lymphocytic Choriomeningitis virus, SFV: Semliki Forest virus, VLP: Vaccine-like particle, VSV: Vesicular Stomatitis virus. n = 121.

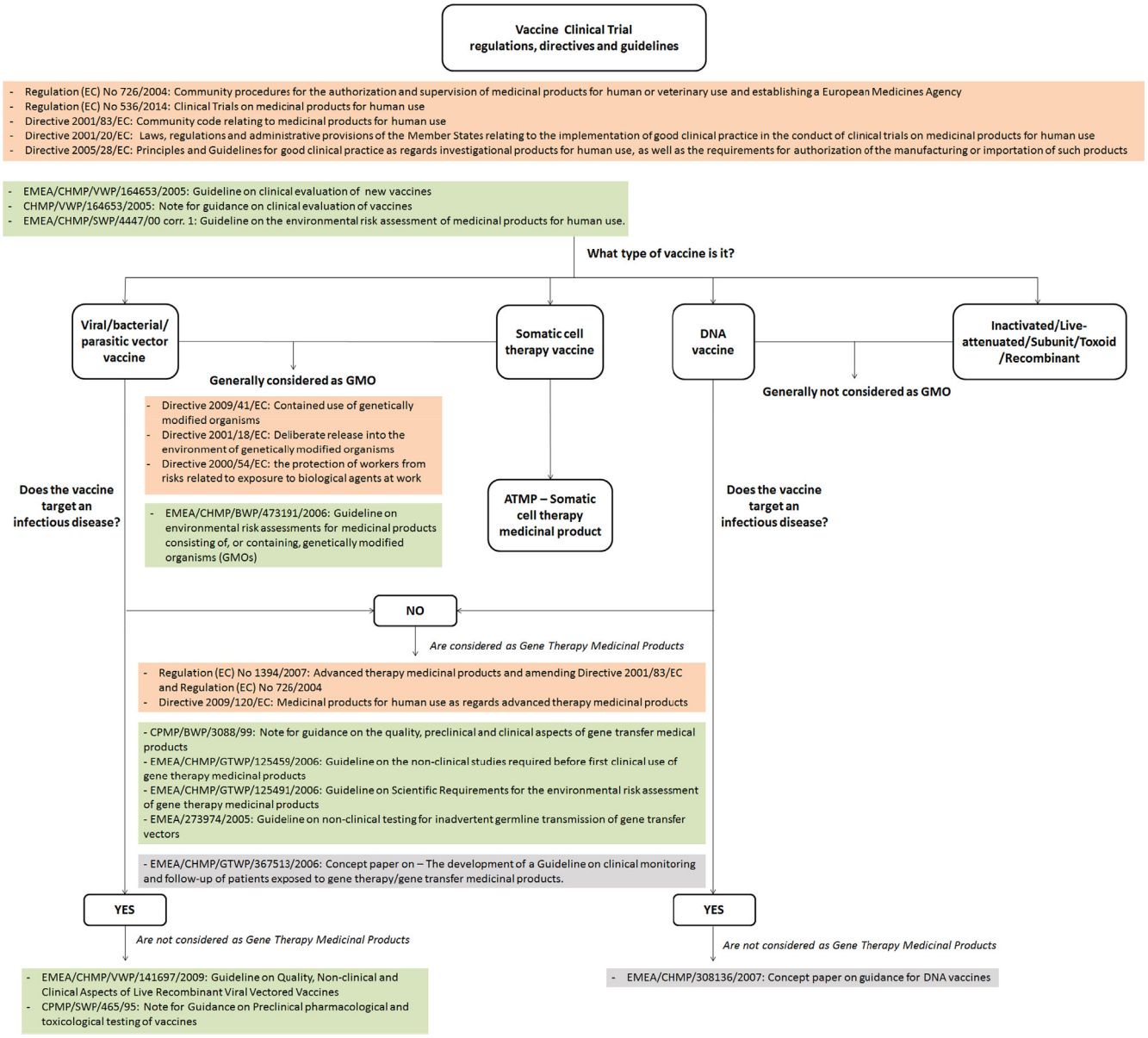

Fig. 2. Overview of European regulations, directives and guidelines for the use of GMO-containing vaccines in a clinical trial setting. ATMP: Advanced Therapy Medicinal Products. Regulations and directives (orange boxes), guidelines (green boxes) and concept papers (grey boxes).

The GMO-specific regulatory requirements in Europe, which go beyond biotechnologies of micro-organisms also includes food, plants, livestock, etc., are captured under three main directives. The first two relate to the way in which the GMO-associated activity is performed: (1) the "contained use" (CU) directive 2009/41/EC applies to any activity in which (micro)-organisms are genetically modified or in which such GMOs are cultured, stored, transported, destroyed, disposed of or used in any other way, and for which specific containment measures are used to limit their contact with, and to provide a high level of safety for the general population and the environment [14]; (2) the "deliberate release" (DR) directive 2001/18/EC applies to any intentional introduction into the environment of a GMO or a combination of GMOs for which no specific containment measures are used to limit their contact with and to provide a high level of safety for the general population and the environment [3]. The third directive 2000/54/EC refers to the protection of workers to the risks related to the exposure to biological agents at work [18].

Biological agents can be classified into four risk groups according to their level of risk of infection: (1) a group 1 biological agent is unlikely to cause human disease; (2) a group 2 biological agent can cause human disease and might be a hazard to workers; it is unlikely to spread to the community; there is usually effective prophylaxis or treatment available; (3) a group 3 biological agent can cause severe human disease and presents a serious hazard to workers; it may present a risk of spreading to the community, but effective prophylaxis or treatment are usually available; (4) a group 4 biological agent causes severe human disease and is a serious hazard to workers; it may present a high risk of spreading to the community; no effective prophylaxis or treatment is usually available [18]. Associated to these risk groups are the containment levels that are appropriate to protect human health and the environment from these biological agents. These are categorised as activities of no or negligible risk (level 1), low risk (level 2), moderate risk (level 3) and high risk (level 4) [14].

Table 3
Overview of country-specific GMO procedures for 20 EU member states, including the authorities involved, the contained use (CU), deliberate release (DR) and submission procedures and public consultation.

EU Member State	Authorities involved	CU or DR	Submission procedure	Public consultation
Austria	CT: BASG - Bundesamt für Sicherheit im Gesundheitswesen GMO CU: Bundesministerium für Gesundheit und Frauen	CU (1)	CTA and GMO submission can be done in parallel	No
Belgium	CT: Federal Agency Medicines Health Products (FAMHP) GMO DR: BAC: Biosafety Advisory Council/FAMHP GMO CU: Regional competent authority/SBB	CU or DR (2)	CTA and GMO submission can be done in parallel	Yes, if DR
Bulgaria	CT: Bulgarian Drug Agency (BDA) GMO: Ministry of Environment and Water (MoEW)	CU or DR (3)	GMO authorization required prior to CTA submission	No
Czech republic	CT: State Institute for Drug Control GMO: Ministry of the Environment	CU or DR (4)	CTA and GMO submission can be done in parallel	Yes, if DR
Denmark	CT: Danish Medicines Agency/National Committee on Health Research Ethics (NEC) GMO: Environmental Protection Agency (EPA)/Working Environment Authority (WEA)	CU	CTA and GMO submission can be done in parallel	No
Estonia	CT and GMO: State Agency of Medicines (SAM)/Gene Technology Commission	DR	Single CTA and GMO submission	No
Finland	CT: Finnish Medicines Agency (FIMEA)/National Committee on Medical Research Ethics (TUKIJA GMO: Board for Gene Technology	CU or DR (5)	CTA and GMO submission can be done in parallel	Yes
France	CT: Agence Nationale de Sécurité du Médicament et des produits de Santé (ANSM) GMO CU: Ministère de l'enseignement supérieur, de la recherche et de l'innovation (MESRI) GMO DR: Ministère Transition écologique et solidaire	CU or DR (6)	CTA and GMO submission can be done in parallel	Yes
Germany	CT: Paul-Ehrlich-Institut (PEI) GMO: Federal Office of Consumer Protection and Food Safety (BVL)	DR	Single CTA and GMO submission (PEI)	Yes
Hungary	CT: National Institute of Pharmacy and Nutrition (OGYÉI) GMO: National Institute of Pharmacy and Nutrition Ministry of Agriculture /GMO committee as advisory	DR	CTA and GMO submission can be done in parallel	Yes
Ireland	CT: Health Products Regulatory Authority GMO: Environmental Protection Agency (EPA)	DR	CTA and GMO submission can be done in parallel	Yes
Italy	CT: Italian Medicines Agency (AIFA) GMO CU: Ministry of Health GMO DR: Ministry for Environment, Land and Sea Protection	CU or DR (7)	CTA and GMO submission can be done in parallel	Yes
Lithuania	CT and GMO: State Medicines Control Agency/Ministry of Health	DR	Single CTA and GMO submission	No
Netherlands	CT: Medicines Evaluation Board: MEB/Central committee on research involving human subjects (CCMO) GMO DR: Ministry of Infrastructure, Environment and Water Management (IenW)/ Gene therapy office (as central point of contact)	DR	CTA and GMO submission can be done in parallel	Yes
Norway	CT: The Norwegian Medicines Agency GMO CU: The Norwegian Directorate for Health GMO DR: The Norwegian Environment Agency	CU or DR (8)	CTA and GMO submission can be done in parallel	Yes
Poland	CT: The Office for Registration of Medicinal Products, Medical Devices and Biocidal Products GMO: Ministry of the Environment	CU	GMO authorization required prior to CTA submission	Yes
Romania	CT: National Agency for Medicines and Medical Devices/Ministry of Health GMO: National Environmental Protection Agency (NEPA), The Biosafety Commission (BC) as advisory	DR	GMO authorization required prior to CTA submission	Yes
Slovakia	CT: The State Institute for Drug Control GMO: Ministry of Environment	DR	GMO authorization required prior to CTA submission	Yes
Spain	CT: Agencia Española de Medicamentos y Productos Sanitarios GMO: Consejo Interministerial de OMG (CIOMG)/Comisión Nacional de Bioseguridad (CNB)	DR	CTA and GMO submission can be done in parallel	Yes
Sweden	CT and GMO: Medical Products Agency (MPA)	DR	Single CTA and GMO submission	Yes
UK	CT: Medicines and Healthcare products Regulatory Agency GMO CU: Health and Safety Executive (HSE) GMO DR: Department for Environment Food & Rural Affair	CU or DR (9)	CTA and GMO submission can be done in parallel	Yes, if DR

(1) If IMP do not fall under the definition of somatic gene therapy, a separate GMO authorization is not required (e.g. GMO-vaccines).
(2) See Section 3.3.5.
(3) Clinical trials of human medicinal products are treated as experimental DR into the environment. In addition, the premises of the hospitals and other facilities where the clinical trials will take place should comply with the safety requirements for CU of the specific class but no special notification is needed. In some cases, when the whole clinical trial takes place in a hospital, it might be considered entirely under the CU framework.
(4) While the manufacturing and application of the medicinal product to the patient is in principle regulated under CU, the DR framework applies if there is a possibility of shedding of the GMM into the environment after the application.
(5) A decision is taken case-by-case depending on factors such as the replicative capacity of the GMOs and whether specific containment measures are used to limit their contact with the general population and the environment and to provide a high level of safety for the general population and the environment.
(6) See Section 3.3.4.
(7) If the assessment of a genetically modified microorganism (GMM) contained use shows that the medicinal product contains a GMO that can replicate, transmit and disseminate into the environment, an DR authorization should also be obtained from the Ministry for Environment, Land and Sea Protection.
(8) A decision is taken on a case-by case basis. For example, ex-vivo stable modification of patients cells is considered under contained use and, due to the absence of risk of spreading after transfusion of the GM-cells back to the patients, patients may leave the clinic after treatment. On the other hand, persisting, circulating GMO medicinal products may require a DR approval. This will partly depend on the condition to be treated. Thus, long time hospitalization (as for cancers) may be assessed under CU, since the GMO will be eliminated by/from the body within the time the patient is in hospital. In contrast, when the treatment only involves a short visit (as for receiving a GMO vaccine), the DR framework applies, due to the risk of spreading the GMO when leaving the site after vaccination.
(9) See Section 3.3.1.

Each European country has implemented these European GMO directives into their respective national legislation and appoints a competent authority in charge of reviewing the GMO dossier submitted by the vaccine developers. The competent authorities can be governmental, regional or separate entities and can work together or independently with the authority reviewing the CTA application. They determine whether the "contained use" and/or the "deliberate release" procedure must be applied. Depending on the country, the review of the GMO dossier can be conducted before, simultaneously with, or after the CTA and Ethics Committee (EC) review. An overview of the different country-specific procedures for GMOs is provided below for 20 countries listed in Table 3 (with the exception of Iceland, for which no information could be retrieved).

In addition, a more in-depth review is described below for the five countries identified as main contributors to GMO-containing vaccine CT application in Europe between 2004 and 2017. This selection is based on the Results Section 3.1.

3.3.1. United Kingdom

Clinical trials with GMOs in the UK can be regulated under either the contained use or the deliberate release framework. This decision is taken case-by-case taking into account the biological characteristics and environmental risk assessment of the GMO. When there is uncertainty on procedure to follow, applicant can seek guidance from regulatory authorities (including the Scientific Advisory Committee on Genetic Modification (SACGM) compendium of guidance on using GMO in clinical setting) [19,20,21].

When the clinical trial is regulated as deliberate release, the deliberate release application dossier is submitted to DEFRA (Department for Environment Food and Rural Affairs) and a public consultation is organized. The ACRE (Advisory Committee on Release to the Environment) advises Defra. Deliberate release licences are granted by Defra within a 90-day period [22] (Suppl. Fig. 3).

When the clinical trial is regulated as contained use, the participating site must submit a notification to Health and Safety Executive (HSE) as per GMO contained use regulation. If the micro-organisms are classified as risk group 1, a premise notification (CU1 form) is sent HSE for the contained use for the first time, while for future contained use class 1 activities no further notification is required and the trial can start immediately [23]. For micro-organisms of class 2 or higher, a contained use notification (CU2 form) is required. Each site must have a genetic modification safety committee (GMSC) and a biological safety officer (BSO), which provide site-specific advice on the use of GMOs. Additionally, a risk-assessment (which includes the comments from the GMSC) is required (Suppl. Fig. 3).

3.3.2. Germany

Clinical trials with GMO in Germany are regulated under the deliberate release framework [24]. This procedure requires the submission of an environmental risk assessment (ERA), a technical description of the GMO, the summary notification information sheet (SNIF), and "additional information" (requesting information on vaccine handling, storage and transport, sampling, waste management and emergency response) to the Paul-Ehrlich Institute (PEI) [25]. A single submission procedure applies to seek CTA and GMO authorization. The dossier will be forwarded to the BVL (Federal Office of consumer protection and food safety) which is the competent federal authority for the deliberate release of GMO. The ZKBS (Central Committee on Biological Safety) of the BVL subsequently provides a recommendation to the PEI regarding the risk group classification of the GMOs. The authorisation is provided by the PEI after a maximum of 90 days after submission of the GMO dossier (Suppl. Fig. 3). Subsequently, the biosafety committees of each study site need to approve the dossier at a local level.

3.3.3. Spain

In Spain, clinical trials with GMOs fall under the deliberate release legislation as per Spanish legislation [26]. The biosafety dossier is submitted to the Ministry of Agriculture, Food and Environment (MAPAMA) and should comprise the following documents: a request form, a technical dossier, the SNIF (in English and Spanish) and ERA. Additionally, the protocol or any other document can be requested [27]. The dossier will be forwarded to the advisory body, the National Biosecurity Commission (CNB). An authorisation from the Inter-Ministry GMO council CIOMG (Consejo Interministerial de OMG) is required before starting the clinical trial and should be obtained within 90 days after submission of the GMO dossier (Suppl. Fig. 3) [28].

3.3.4. France

Although clinical trials with GMOs in France can be regulated under either the contained use or the deliberate frameworks, they generally fall under the contained use legislation (implemented from the European directive 2009/41/EC) [29]. A biosafety dossier is submitted to the Ministry of Higher Education and Research (MESR), which forwards the dossier to the advisory body, the High Council of Biotechnology (HCB). The HCB advises the MESR on the classification of the micro-organisms and conditions of contained use. For micro-organisms classified as risk group 1, a notification to the MESR is required, for risk groups 2, 3 and 4 an authorisation from the MESR is needed. When a risk of deliberate release is identified, the applicant should submit a second dossier to the Ministry of Environment MTES (Ministère de la Transition Écologique et Solidaire) for the deliberate use assessment, which follows the standard 90-day period, as per EU Directive 2001/18/EC. The CTA application can be submitted to the competent authority (the French Agency for the Safety of Health Products, ANSM) in parallel with the GMO application (Suppl. Fig. 3) [30,31].

3.3.5. Belgium

In Belgium, clinical trials with GMOs can fall under the contained use procedure and/or the deliberate release framework [32,33]. Before submission, sponsors can determine what procedure to follow by requesting a scientific-technical advice (STA) to the national competent authority, the Federal Agency of Medicines and Health Products (FAMHP) [34]. To determine the applicable GMO procedure(s), the applicant should evaluate if, at any stage of the CT, the general population and the environment can be exposed to the IMP containing or consisting of a GMO.

In the case where 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 must comply with Belgian legislation on the CU. Generally, activities such as the preparation, administration or storage of the IMP should follow the CU procedure. The contained use procedure is implemented at regional level (Brussels, Flanders, Wallonia). Each region has its independent procedure (which differs whether the risk classification of the micro-organism and whether the study is first or subsequent use), timelines and documents written in the regional language [35]. A notification/authorisation for the contained use needs to be sent/obtained for each new study.

When there is a probability of release into the environment as a result of the shedding and spreading of the GMO into the environment, a notification under deliberate release will additionally be required. This is the case when the subject leaves the clinical centre but still sheds and spreads the GMO, thereby potentially exposing his or her close contacts and the environment to the GMO.

Considerations that are taken into account include the probability of shedding, hazards associated to the shedding should it occur, probability of spreading, probability of recombination with wild type viruses (in case the IMP contains or consists of a viral vector) or whether the GMO is also administered at home. The deliberate release procedure is implemented at the federal level. It follows the Royal Decree of 21/02/2005 and therefore requires the standard documents such as the ERA, a technical description of the GMO and the SNIF, which is made publicly available [36]. Additionally, information to the public, a declaration of civil responsibility (liability declaration) and a declaration to provide a control sample and relevant protocol for detection are requested. The GMO dossier (together with the CTA) is submitted to the national competent authority, the FAMHP, which transfers the GMO dossier to the relevant advisory body, the Biosafety Advisory Council (BAC). The deliberate release approval requires a 90 days review period, which runs in parallel with the CTA procedure (Suppl. Fig. 3) [37].

4. Discussion

Our analysis of the GMO-containing vaccine development landscape, based on the EudraCT database, indicated that clinical trial applications with GMO-containing vaccines were submitted in 21 EU countries between 2004 and 2017. The observation that the UK was the largest contributor to GMO vaccine clinical trials can be explained by the extensive GMO vaccine experience in the UK. This might be partially due to the creation of the Oxford Vaccine Group at Oxford University in 1994 and the Edward Jenner Institute for Vaccine Research [38], the high number of GMO centres (1009, according to the public register) with a premise notification [39] and/or the relatively fast regulatory authorisation process by the competent authorities [23,40]. On second and third position were Germany and Spain, which are both countries that generally follow the deliberate release procedure. Through the use of standardised SNIF and ERA templates, the deliberate release content and format requirements are harmonised across the EU countries and could possibly contribute to making the GMO authorisation process more efficient in these countries [3]. A previous review made a similar observation, in which gene therapy clinical trials (which include non-vaccine studies) worldwide were mostly performed in the USA, the UK, Germany, France and Switzerland [4]. An interesting observation was that higher proportion of non-commercial sponsors (universities, university hospitals, research institutes, etc.) versus commercial sponsors in the UK was higher than in the four other countries.

The numbers of clinical trial applications for GMO-containing vaccines were fairly stable between 2004 and 2017 and were mostly in Phase I. The Phase III studies were targeting cancer, dengue, ebola and influenza and these vaccines can possibly be added to the relatively small list of commercialised GMO-containing vaccines soon. Although the inclusion criteria for the selection of clinical trials were different, the data obtained in this review align with the proportion of Phase I, II and III clinical trials for gene therapy and prophylactic gene-based vaccines worldwide described previously [4,16]. The five most frequently targeted diseases by GMO-containing vaccines included a variety of pathogens: viruses (HIV, Influenza), bacteria (Mycobacterium tuberculosis), parasites (Plasmodium falciparum causing malaria) as well as non-infectious diseases (cancer). The fact that 22 different diseases (cancer and 21 infectious diseases) were targeted, highlights the diversity of vaccines solutions available with GMO-containing vaccines and the possible impact these may have on public health in preventing or curing infectious and non-infectious diseases in the future.

Most GMO-containing vaccine clinical trials in Europe were performed with viral vectors, mostly with MVA, adenoviral vector or a combination of both. Viral vectored vaccines have been used for many decades due to their interesting properties and their ability to overcome some of the obstacles encountered with plasmid DNA vaccines [41]. They are the only GMO-containing vaccine type that has led to commercialised products so far [42,8,9,10]. Interestingly, two clinical trial applications (from France) using plasmid DNA vectors have indicated these as a "medicinal product containing genetically modified organisms" in the CTA application form. Although a plasmid is not an organism, it may contain other elements that warrant assessment under the GMO framework. For example, a plasmid that harbours a virus strain that has been genetically modified would be subject to the GMO framework. The classification of such vaccines as GMOs is performed at an individual country-level and on a case-by-case scenario. The definition of GMO is thus subject to interpretation, especially when it comes to what is meant with an "organism" (*i.e.* any biological entity capable of replication or of transferring genetic material, as per Directive 2001/18/EC) [3].

Clinical trials in Europe are strongly regulated, also in accordance with international agreements, and need to comply with an extensive amount of European and country-specific legislations. The classification of modern-day vaccines, such as viral-vectored or DNA vaccines for gene therapy or against infectious diseases, within the scope of their relevant legislation, can be perceived as a complex exercise. Interestingly, vaccines consisting of gene therapy medicinal products and somatic cell therapy medicinal products fall under the definition of "Advanced Therapy Medicinal Product (ATMP)" and have their own specific legislations [43,44]. Others, such as vaccines against infectious diseases, which use the same vaccine technology as gene therapy vaccines (*i.e.* DNA vaccines or viral/bacterial vectors), however, do not fall within this scope and require separate directives and/or guidelines [44].

Vaccines consisting of/or containing GMOs for use in clinical trials in the EU need to comply with the GMO-specific "deliberate release", "contained use" and "workers protection to biological agents" directives [14,3,18]. Most countries request either procedure based on a case-by-case evaluation, e.g. Belgium (see Table 3 and Results Section 3.3.5). Some countries, however, like the UK, generally consider clinical trials with GMOs as 'contained use', whereas other countries, like Germany and Spain, generally require the 'deliberate release' procedure. Although it is a more extensive process involving an elaborated environmental risk assessment and a detailed technical/scientific description of the GMO, the content and format requirements of the deliberate release procedure are harmonised across EU countries through the standardised use of the SNIF and ERA templates [3]. In contrast, the content requirements of the contained use dossier which focuses on a description of the GMO facilities, the GMO handling, waste management and protection of workers, is broadly harmonised in the EU but the format differs from country to country. In the five countries that have been examined more in detail in this review, the timelines for approval of a clinical trial could vary from 90 days for countries that follow the deliberate release procedure and from 0 to 15 days (risk group 1 micro-organisms), and 45 days (risk group 2 organisms), for countries that follow the contained use framework. The sequence of consecutive procedural steps (e.g. if the CTA and GMO application occur simultaneously or consecutively) and the identity of competent authorities and advisory bodies (whether being part of the ministry of health/environment or an external body, active at a national or regional level) for both the CU and DR procedure are not streamlined between European countries [45]. Added to the varying and often substantial costs for the assessment of the DR/CU dossiers, a low level of harmonisation of the GMO regulatory procedures across EU countries could partially explain the differences observed in the number of GMO-containing studies between EU member states and could add to

the complexity of starting multi-country vaccine clinical trials with GMOs. Additionally, the new Clinical Trial regulation (EU) No 536/2014 will not address the environmental risk assessment associated with GMO-containing clinical trials [46]. This complexity may place a barrier for conducting clinical trials with GMO-containing vaccines in European countries and provide an incentive for vaccine developers to perform such clinical trials in countries with easier approval procedures. A recently published industry position paper describes the issues and possible solutions to streamline the GMO application and review processes by the relevant authorities in the EU. It also suggests solutions to shorten the time before clinical trials of ATMPs that consist of/or contain GMOs can be initiated [45]. The national competent authorities and the European Commission services have published a Good Practice Document and a common application form for some ATMPs consisting of/or containing GMOs to reach harmonisation based on the existing legislation. These have been endorsed by several European members states including Belgium and future achievements will be published on the EC website [47].

In the event of emerging disease outbreaks (e.g. ebola), it would be essential to understand how GMO legislations should be considered and/or to clarify the possibilities to accelerate these procedures. An emergency regulatory procedure could be highly beneficial for GMO-containing vaccines. For example, the approval of GMO platform technologies may fasten the licensing process and availability of new disease strategies in case of emergencies.

A limitation of this review was the unknown rationale behind choosing the CU or DR procedure by individual EU member states. Obtaining this information would help investigators and sponsors understand the drivers behind these choices and enable to better addressing concerns raised by the competent authorities. Unfortunately, the information regarding the CU/DR procedure followed for each GMO-containing vaccine clinical trial analysed here is not captured in the EudraCT database. Although the deliberate release directive 2001/18/EC requires a public consultation, and makes the SNIF dossier publicly available on the EU GMO register [48], the contained use directive 2009/41/EC allows for a public consultation if the Member state considers it appropriate [14,3,18]. Therefore, the information about the CU/DR procedure followed for each GMO-containing vaccine clinical trial was not included in our analysis.

Another interesting finding would have been to obtain the "real" GMO procedure approval timelines, as experienced by the industry, for each of these GMO-containing vaccines clinical trials. These would include stop-clock times in which the sponsor is requested to provide changes, clarifications, and/or additional documentation and would allow all stakeholders involved a better planning of such clinical trials.

5. Conclusion

This study provides an updated view of the clinical development of GMO-containing vaccines in Europe: the countries involved, the stage of development, the numerical evolution between 2004 and 2017, the diseases targeted and the vectors applied. The overview of the regulatory environment at the EU and individual country level highlights the complexity and the urgent need for further harmonisation in the implementation of GMO-related legislations. To stimulate the development of GMO-containing human vaccines and to favour an even distribution of GMO-containing human vaccine studies across European countries, it is important to minimise the regulatory burden associated with GMO-containing vaccine human studies and to better harmonize the procedures across the EU.

ICMJE criteria

All authors attest they meet the ICMJE criteria for authorship.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.08.018.

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Further reading

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