Vector-based genetically modified vaccines: Exploiting Jenner's legacy

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ABSTRACT

The global vaccine market is diverse while facing a plethora of novel developments. Genetic modification (GM) techniques facilitate the design of 'smarter' vaccines. For many of the major infectious diseases of humans, like AIDS and malaria, but also for most human neoplastic disorders, still no vaccines are available. It may be speculated that novel GM technologies will significantly contribute to their development. While a promising number of studies is conducted on GM vaccines and GM vaccine technologies, the contribution of GM technology to newly introduced vaccines on the market is disappointingly limited.

In this study, the field of vector-based GM vaccines is explored. Data on currently available, actually applied, and newly developed vectors is retrieved from various sources, synthesised and analysed, in order to provide an overview on the use of vector-based technology in the field of GM vaccine development. While still there are only two vector-based vaccines on the human vaccine market, there is ample activity in the fields of patenting, preclinical research, and different stages of clinical research. Results of this study revealed that vector-based vaccines comprise a significant part of all GM vaccines in the pipeline. This study further highlights that poxviruses and adenoviruses are among the most prominent vectors in GM vaccine development.

After the approval of the first vectored human vaccine, based on a flavivirus vector, vaccine vector technology, especially based on poxviruses and adenoviruses, holds great promise for future vaccine development. It may lead to cheaper methods for the production of safe vaccines against diseases for which no or less perfect vaccines exist today, thus catering for an unmet medical need. After the introduction of Jenner's vaccinia virus as the first vaccine more than two centuries ago, which eventually led to the recent eradication of smallpox, this and other viruses may now be the basis for constructing vectors that may help us control other major scourges of mankind.

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1. Introduction

Ever since the discovery by Edward Jenner, more than two centuries ago, that vaccinia virus could be used to protect people from variola, vaccines have been of utmost importance in fighting infectious diseases [1], as they are the most cost effective tools for the prevention of infectious diseases. To date several types of vaccines are available, including live-attenuated, inactivated, subunit or split, toxoid, conjugate, DNA, and recombinant vectored vaccines [2]. While conventional vaccines, like live-attenuated or inactivated wild-type, have successfully protected vaccinees from various infectious diseases over the years, they are not available for most infectious diseases and for those who cannot afford them.

Conventional vaccine production methods, which predominantly use viruses and bacteria or their products, produced with classical production methods, are labour intensive, expensive, and time consuming, while some of the desired antigens cannot be produced in this way [3]. Furthermore, highly virulent pathogens can only be produced under expensive special safety conditions, while attenuated agents may have a tendency of reverting to their pathogenic form and can usually only be used in fully competent individuals [4].

To overcome the challenges of traditional vaccine production, the development and use of novel generations of vaccines, like those based on GM technologies, are being considered more and more frequently. The advent of these novel technologies may also be expected to create opportunities for the development of vaccines targeting new indications and/or application fields. Since there are many major indications for which no or only unsatisfactory vaccines are available, like AIDS, malaria, and tuberculosis, the exploitation of novel technologies, like the use of vector-based vac-

cine candidates or vector-based production of protective antigens, may eventually allow us to fill the gap of this unmet medical need. To date several vaccines for humans, based on GM technologies have been licensed (for review see e.g. [5–8]) and a lot of candidates are in the pipeline.

An interesting approach for vaccine development based on GM technology is the use of vectors, which carry selected genes encoding antigens that induce protective immunity. They can either be used as vaccines proper, or for the production of antigens that are incorporated in vaccines. The present paper only deals with vectors that are actually used as vaccines and not just for the production of immunogens. Vectors can be classified in three different categories: viral, bacterial, and plasmid [9]. Vectors can either be fully replicative or only cause abortive infection, still allowing the expression of the desired immunogens. They can be administered either parenterally or via mucosal membranes [10]. A major advantage of vector-based GM technology, is that the immunogens of interest are *de novo* synthesized, thus not only allowing for the induction of antibody and T helper cell mediated immunity, but also for the induction of protective cytotoxic T cell responses, mimicking a natural immune response against the immunogen. This balanced immune response opens pathways that were previously inaccessible with traditional vaccine technology using 'non-live' immunogens. Especially the induction of CD8+ CTL responses may be of particular interest for vaccines against certain virus infections and cancers [11]. Our previous study provides additional insights regarding the strengths, weaknesses, opportunities, and threats of such technology [12].

In the present study, the potential of vector-based vaccines is evaluated. Data obtained from literature, granted patents, and different stage clinical trials are synthesised and analysed in the light of data from currently registered vaccines providing an overview of the potential of currently used and newly generated vectors in the field of vaccine development. The data suggest that vector-based vaccines may offer a cost-effective alternative for the production of safe vaccines against diseases for which no or less perfect vaccines exist today, thus catering for a huge unmet medical need.

2. Methodology

The methods applied in this study have been split in four different stages: evaluation of literature, patents, clinical trials, and registered GM and non-GM vaccines. Each stage was individually examined in detail and the complete data set was compiled. These stages were decided upon in order to provide a complete overview of the genetically modified (GM) vector-based vaccine pipeline and market.

2.1. Literature research

To map the early research stage of emerging vectors, a literature search was performed on available candidate vector vaccine studies. Data was collected on various types of GM vectors and their properties, as mentioned in both research publications and reviews. The search was conducted using a combination of Embase, Medline, Web-of-science, Pubmed, Cochrane, and Google Scholar. Medical Subject Headings (MeSH) and Boolean Operators were utilised in order to develop a basis for the syntax. The search was restricted to publications/translations in English. This syntax and the search results were analysed by an independent biomedical information specialist from Erasmus Medical Centre medical library. Additional information on the search terms for different search engines can be found in the supporting information (S1).

A total of 1756 hits were obtained [13]. 511 duplicates were removed, resulting in 1245 publications. Restrictions for further

analysis included articles not describing vaccines or vaccine technologies, and articles not describing novel vaccine technologies. Publications were restricted to those published in the period 2009–2014. The total set contained 87 review articles on GM vaccines.

In order to retrieve more papers on vector-based GM vaccine candidates, an additional search was performed on Pubmed including relevant search terms "*vaccine*", "*vector*" and "*GM*". Reviews were retrieved adding the search term "*review*" to the previously mentioned terms or by searching for reviews only. Papers dating from the period 1998 to 2014 were collected and 18 new results were added to the previous 87 (Table 1).

A total of 38 publications, specifically on the topic of vector-based vaccines, were selected from this pool and analysed in detail. The clinical studies and reviews evaluated are shown in Table 2, and the results of this literature study can be found in Table 6.

2.2. Search for patents

Patents have multiple technology classifications based on their claims, and since they are classified in technological classes, patents related to GM vaccines were collected into a database. Patent data concerning GM vaccines was retrieved from Espacenet, which provides access to over 90 million patent documents worldwide [14]. Search terms used were "*Medicinal preparations containing antigens or antibodies*", "*Medicinal preparations containing genetic material which is inserted into cells of the living body to treat genetic diseases; Gene therapy*" and "*Mutation or genetic engineering; DNA or RNA concerning genetic engineering, vectors, e.g. plasmids, or their isolation, preparation or purification; Use of hosts therefore*", in combination with search words vaccin* (Boolean operator), and genetic* OR modif*, respectively. The results were deduplicated based on the priority numbers. The syntax and search results were analysed by a patent specialist from the Netherlands Enterprise Agency (RVO) [15], a governmental institution in the department of Economic Affairs. A total number of 40.308 unique patents were found and an original database was created, including all classes and subclasses.

As patent information in the patent database is condensed into Cooperative Patent Classification (CPC) codes, the previous search was repeated, combining the previous search with CPC codes for vectors and search term vaccine*. A total of 96 unique CPC codes were used, resulting in 32.738 vector-based vaccine patent documents. As CPC codes describe the classification in each technical area on various levels, the definitions of the CPC codes used were retrieved from Espacenet, and a comprehensive table was created including the CPC codes, their definitions, and the number patents containing this specific code. All search terms can be found in Table 3. The results are illustrated in Fig. 1 and a complete overview of these CPC codes and their description can be found in the supporting information (S2). It should be noted that the

Table 1
Results of literature search.

Database	Hits	Hits after deduplication
Embase.com	945	940
Medline (OvidSP)	364	97
Web-of-science	323	123
PubMed publisher	8	4
Cochrane DARE	7	2
Google scholar	100	79
Total initial search	1756	1245
Total set after applying restrictions		87
Additional vector search results		18
Final set used for detailed analysis		38

Table 2
List of clinical studies and reviews evaluated.

Altenburg et al. [48]	Nébié et al. [44]
Arroyo et al. [34]	Nieto and Salvetti [30]
Babu Appaiahgari and Vrati [33]	Ondondo [52]
Banchereau and Steinman [36]	Pandey et al. [27]
Bermúdez-Humarán et al. [58]	Paris et al. [43]
Bråve et al. [28]	Ploquin et al. [31]
Chin'ombe et al. [60]	Rimmelzwaan and Sutter [55]
Choi and Chang [35]	Robertson [51]
Cottingham et al. [56]	Rollier et al. [11]
Croyle et al. [41]	Saxena et al. [39]
Dicks et al. [40]	Smith et al. (2011)
Dung et al. (2012)	Tatsis and Ertl [37]
Ewer et al. [45]	Tripp and Tompkins [47]
Gómez et al. [49]	Ulmer et al. [3]
Hessel et al. [54]	Ura et al. [32]
Kreijtz et al. [26]	Verheust et al. [53]
Lundstrom [46]	Weaver and Barry [42]
Mooney and Tompkins [38]	Williams et al. [29]
Myhr et al. [50]	Youngjoo et al. (2013)

method used for this search was iterative, the original data was used to reproduce search terms for the vector search. Because of this iterative method, a complete dataset was collected.

2.3. Search for clinical trials

Clinical trials data (phase 1, 2, and 3) was gathered from the World Health Organisation International Clinical Trials Registry Platform, which currently lists 191,038 studies in 190 countries (data retrieved: May 29th, 2015 [16]). Search terms applied can be found in Table 4. The results were deduplicated based on the Trial ID number. A total of 1146 unique clinical trials were used to create an original database.

To provide a detailed outlook on the use of viral vectors in GM vaccine trials, the clinical trials database was analysed in a vector specific way. The progress of vector-based vaccines as a share of all GM vaccines was examined, as well as the spread of specific vectors and their prevalence for specific indications. Data entries

on vector trials were sorted by their indication and frequency, and the 10 most prevalent indications were selected to form a new data subset. This subset was analysed on the specific types of vectors used per indication, and a comparison was made for these indications with the complete dataset of all GM vaccines. The results are shown in Table 7.

2.4. Search for registered GM and non-GM vaccines

Data concerning registered vaccines was obtained from governmental databases of the following regions; USA, EU, Brazil, India, China, South-Africa, Australia and Japan (Table 5). BRICS countries were selected (only four out of five BRICS countries were included, Russia being omitted due to the general inaccessibility of Russian registers), because of their rapidly growing economies and potential for the industry. Currently, a total number of 821 registered human vaccines are on the market. After deduplication, 797 registered vaccines remained, of which 124 related to GM vaccines. Boolean search terms used to classify vaccines were: "*Genet**", "*Modif**", "*Engin**", "*DNA/RNA*", "*Recombin**", "*Vector*", "*Chimeric*", "*VLP/Virus-like*" and "*Virosome*". In order to analyse the availability of vector-based vaccines on the market, an analysis was performed on this database on vaccines classified as vector-based.

2.5. Data convergence

2.5.1. Patents and clinical trials

In order to provide an overview on the prevalence of vectors that have been patented and/or registered for clinical trials, two more data analyses were performed. Initially, the comprehensive patent database, that was created as described above, was analysed for data on the specific vector types. This data was then combined with data on vector types from the clinical trial database.

Relevant patent entries were selected from our database based on the presence of CPC codes related to vectors in the patent application and a sub database was created, including 73 different vectors or vector combinations extracted from 10287 unique patents. As many of these vectors only appeared a few times, the top 21 of
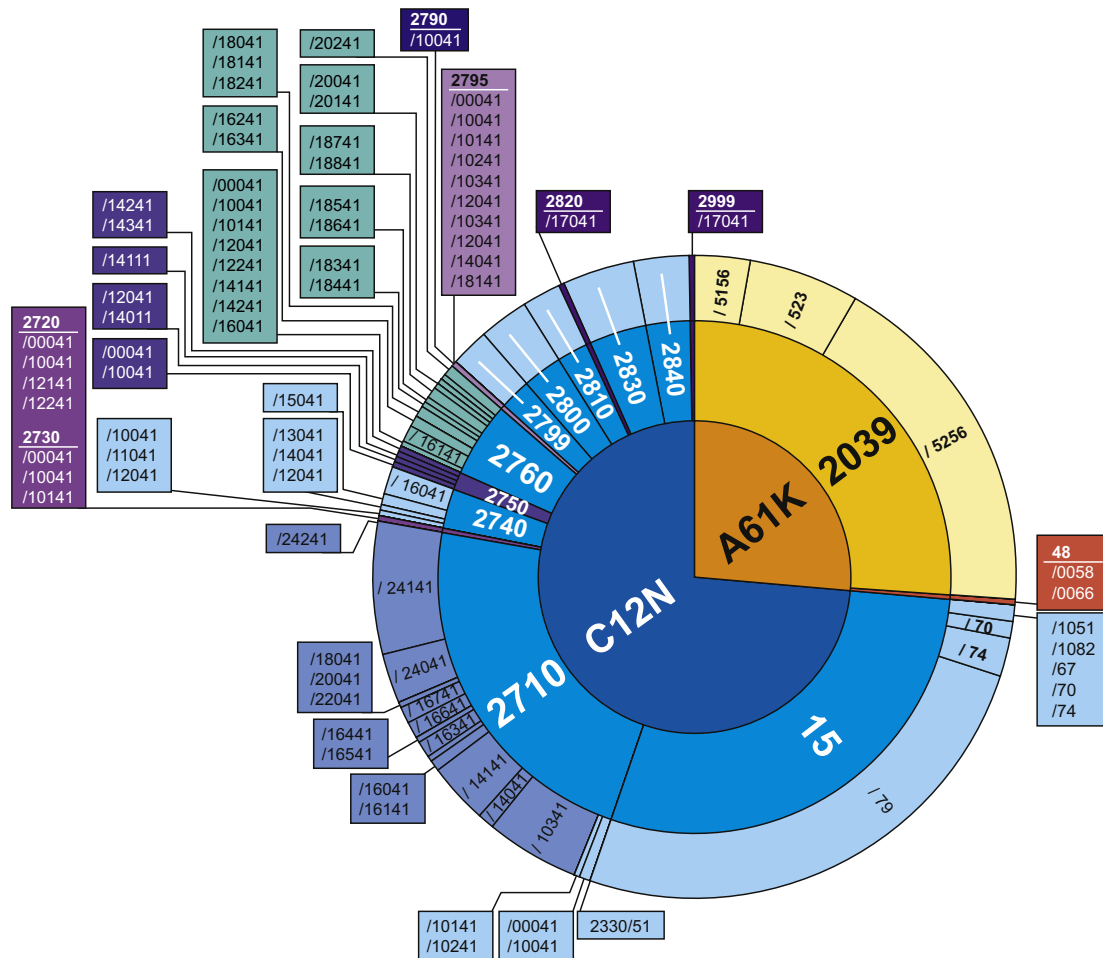
Table 3
Patent search.
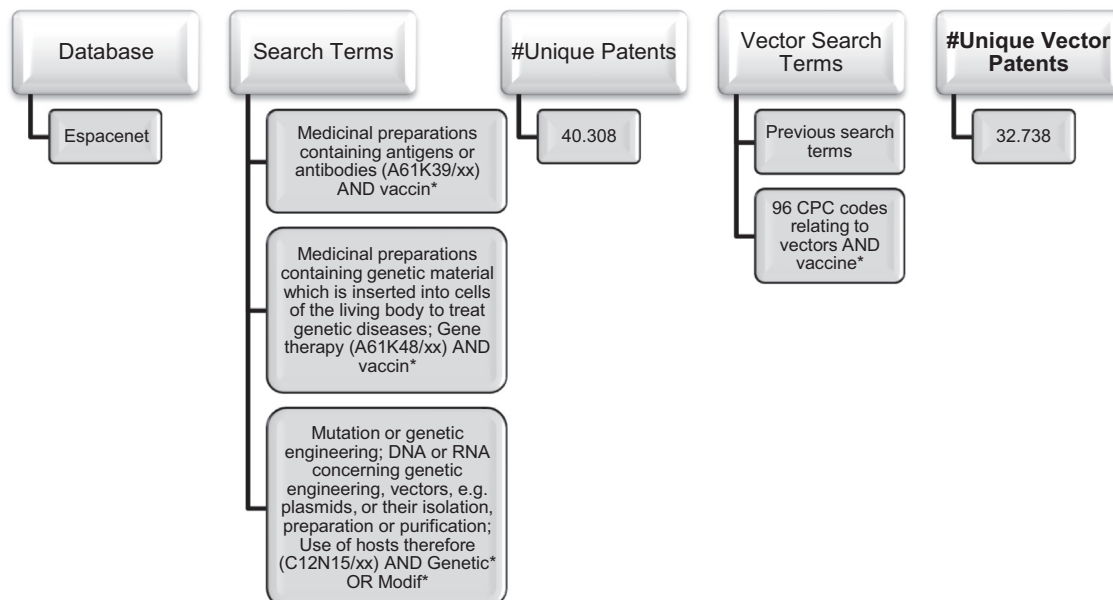
Fig. 1. Predominant CPC codes for GM vector-based vaccine patents. Illustration of the CPC codes present in the patent database for GM vaccines. The figure should be read from the inside out, starting with the middle circle, each additional layer adds a new subsection to the code of the previous layer. The outer shell consists of the numbers behind the "/", completing the CPC code.

most prevalent entries was used for further analysis. This resulted in 21 different vectors mentioned in 9088 unique patents.

For the clinical trials analysis, a similar procedure was applied. Instead of searching for CPC codes, data on the technology class of the vaccine was extracted from the previously generated clinical trial database by searching the variable "*Expression System*". Relevant data on vectors was extracted, resulting in 17 vectors in 117 phase 1 trials, 14 vectors in 66 phase 2 trials and 2 vectors in 2 phase 3 trials. The results of these analyses are shown in Fig. 3.

2.5.2. Evolution of GM vaccines: convergence of all three data bases

In order to visualise the progress of GM vaccines over the years, a timeline was created using data from all available databases (patents, clinical trials, and registered) on the prevalence per indication per year. 16 Indications were selected based on their presence in all databases, and six indications were selected that were present in patents and clinical trials, albeit absent in registered. This resulted in a timeline of vaccine presence per year for 22 indications spanning from 1976 to 2013. Data on registered vaccines from India have been omitted from this analysis, as no information on the dates of application was given in Indian registers. Patents and clinical trials databases comprise of only GM vaccines. The registered database has been used in its entirety, both GM and non-GM vaccines. This visualisation is shown in Fig. 4.

3. Results

3.1. Analysing the market

Table 6 provides information on the most frequently used vectors, as mentioned in literature. In total 21 viral, 3 bacterial and 1 plasmid DNA vectors are presented in this table, covering the most essential vectors of each type for vaccine production or delivery. This table shows data on several upcoming vectors, which are being researched, e.g. new subtypes of poxviruses, adenoviruses, and novel bacterial vectors. Furthermore, the table comprises indications mentioned for these vectors, their advantages and challenges. The viral vector part covers general viral vector species, and several important main viral families followed by their relevant species. At this point, viral vectors have been researched in more detail than bacterial vectors. Poxviruses and adenoviruses are most frequently mentioned in literature. Moreover, details on the application of these two viral families and several of their species and subspecies, as vectors for vaccine development, are more common.

Fig. 1 shows the most prevalent CPC codes in vector-based vaccine research. The most prevalent patent precursors are "*Vectors or expression systems specially adapted for eukaryotic hosts - note: This group covers the use of eukaryotes as hosts*" (C12N15/79) with 8216 patent entries, "*Virus: expressing foreign proteins*" (A61K2039/5256)

Table 4
Clinical Trials search.

Database	Search terms	Variables
WHO International Clinical Trials Registry Platform	"Attenuated NOT Live-attenuated"	"Development phase"
	"Chimeric"	(1, 2, or 3)
	"DNA"	
	"Engineered"	"Expression system"
	"Genetic"	
	"Genetically Engineered"	"Indication"
	"Genetically Modified"	
	"Live"	"Production system"
	"Live-attenuated"	
	"Modified"	"Specific target"
	"Recombinant Protein"	
	"Recombinant"	"Technology Class"
	"RNA"	
	"Vector"	
	"Virosome"	"Type of Organism"
	"VLP"	
Total number of vaccines after deduplication		1146
Of which GM vaccines		762
Of which vector-based GM vaccines		226

Table 5
Registered database search.

Region	Database	Results	Reference
US	US Food and Drug Administration (FDA)	100	[17]
EU	European Medicines Agency (EMA)	41	[18]
Brazil	Oswaldo Cruz Foundation (Fiocruz)	9	[19]
India	Central Drugs standard control organisation (CDSCO), Medguide India	218	[20,21]
China	China Food and Drug Administration (CFDA)	317	[22]
South Africa	South African Vaccination and Immunisation Centre (SAVIC)	37	[23]
Australia	Government Department of Health, Register of Therapeutic Goods	75	[24]
Japan	Pharmaceuticals and Medical Devices Agency (PMDA)	24	[25]
Total results		821	
Total results after deduplication		797	
Of which GM vaccines		124	

with 5804 entries and "*Bacterial cells; Fungal cells; Protozoal cells: expressing foreign proteins*" (A61K2039/523) with 1813 entries, respectively.

Notable prevalent viral vectors are "Orthopoxvirus, vaccinia/-variola" (C12N2710/24141, 2200 entries), "Mastadenovirus" (C12N2710/10341, 1549 entries), "Nucleopolyhedrovirus" (C12N2710/14141, 1050 entries) and "Poxviridae" (C12N2710/24041, 744 entries).

As demonstrated in Table 7, analysing clinical trials in detail shows that out of 762 GM vaccine trials, 198 are vector-based. This corresponds to a percentage of 26%. Indications with a high percentage for vector-based GM vaccine trials are variola (89%), epstein-barr (67%), HIV (56%), tuberculosis (TB) (42%), cancer (38%), and malaria (38%). Indications that have very little vector-based trials are influenza (3%), human papillomavirus (HPV) (1%), and hepatitis B (1%). The most prominent vectors are the vaccinia virus (modified vaccinia Ankara (MVA) & New York strain (NYVAC), 36.3%) and adenoviruses (17.7%).

The cumulative frequency of the aforementioned vectors per year is shown in Fig. 2. Results from this graph and table illustrate a significant increase in MVA and adenovirus application over the years, while growth of vaccines based on vaccinia virus, ALVAC, and fowlpox virus has stagnated.

The results from our registered vaccines search show that the first vector-based vaccine registered for use on the market is IMO-JEV (2010), a Japanese Encephalitis vaccine, based on a yellow fever virus (family *Flaviviridae*) vector [61]. The second is the tetravalent dengue vaccine, Dengvaxia (2015) comprising a Yellow Fever virus (YFV) encoding two JE viral proteins [62].

3.1.1. Data convergence

The analysis of the prevalence of specific vectors in patents and clinical trials is presented in Fig. 3. A total of 9088 vector-based vaccine patents were evaluated for the patent database. The orthopoxvirus (vaccinia/variola) is most prevalent with 2200 occurrences, followed by the mastadenovirus with 1549 entries. Other frequent vectors were the nucleopolyhedrovirus (1050 entries), poxviridae (744), and HIV (407). For clinical trials, a different, less diverse set of vectors was obtained. In Phase 1, MVA is most prevalent, 37 out of 117 trials. Other frequently present vectors are adenovirus (25), vaccinia virus (21), and ALVAC (9). For Phase 2, MVA is again prevalent with 14 out of 66 trials. Other vectors include adenovirus (10) and fowlpox-vaccinia combination (10). In phase 3 the use of ALVAC and allogeneic cells are present once each.

The convergence of all databases is presented in Fig. 4. This figure illustrates patent applications and clinical trials for the indications cancer and HIV over the years, yet with plenty of results yet very little success (one registered vaccine for cancer, bladder carcinoma, in 2009) [63]. For indications like *Haemophilus influenzae* (Hib) infection, hepatitis A (Hep A), Japanese encephalitis (JEV), and meningococcus, several vaccines have been registered in the past 20 years. Less patents and clinical trials are present for these indications, compared to HIV and cancer. Influenza has a significant amount of both registered vaccines and clinical trials.

4. Discussion

This study provides an overview of the vector-based GM vaccine pipeline and market, indicating that poxviruses and adenoviruses are among the most prominent vectors in GM vaccine development. Our findings show that vector-based vaccines comprise a significant part of all GM vaccines (26%) in the pipeline.

To realise data completeness, four different stages of research were conducted and analysed in detail. These stages covered literature, patents, clinical trials, and the registered market of vector-based GM vaccines, generating an idea of evolution these vaccines have gone through over the years. During the start of this project it became clear that GM vaccines have no unambiguous definition.

Table 6

Main types of vectors for GM vaccine application. Summary of properties of various vectors, the indications they are associated with and their advantages and disadvantages, as retrieved from literature. In bold the important families of viral vectors are shown, below these, in regular font, the subsequent species. Abbreviations: HCV: Hepatitis C virus, HIV: Human immunodeficiency virus, hMPV: Human metapneumovirus, hPIV: Human parainfluenzavirus, HPV: Human Papillomavirus, JEV: Japanese encephalitis virus, MERS: Middle east respiratory syndrome, NDV: Newcastle disease virus, NOS: not otherwise specified, RSV: Respiratory syncytial virus, SARS: Severe acute respiratory syndrome, SFV: Semliki forest virus, SIN: Sindbis virus, SIV: Simian immunodeficiency virus, TB: Tuberculosis, VEE: Venezuelan Equine encephalitis virus.

Vectors	Possible indications (CT, Pre-CT, in vitro)	Advantages	Challenges	Ref.
Nucleic acids Plasmid DNA	– Infectious diseases (NOS) – Influenza	– Easy production and low costs – Stable (genetically, shelf life) – Production is independent of classical production technology – Induces both humoral and cellular immune response – No interference by pre-existing immunity – Safer compared to viruses	– Low immunogenicity – Requires dose increases, multiple doses or adjuvants – Risk of integration of vaccine DNA in host genome – Risk of tolerance induction	[3,9,26–29]
Viruses Adeno associated virus (AAV)	– Cedar virus infection – Hendra virus infection – HIV infection – HPV infection – Influenza – Nipah virus infection	– Infects a wide range of tissues – Induces both humoral and cellular immune response – Non-pathogenic – Unable to replicate in normal human cells – Expresses transgenes at a high and sustained level – Several serotypes available, avoids pre-existing immunity – Flexible modification of viral genes possible	– Pre-existing immunity – Low titer production – High production costs – Limited transgene capacity – Lack of CD8+ T cell responses with natural AAV serotypes – Low immunogenicity compared to other viral vectors (Ad)	[9,28,30–32]
Yellow Fever virus	– Yellow Fever virus infection – Japanese Encephalitis – Dengue – West Nile virus infection	– Only vector-based vaccine on the market so far – Easy production and low costs – Single dose effective – Absence of tropism – No pre-existing immunity in non-endemic areas (North America, Eurasia)	– Pre-existing immunity in endemic areas (South America, Africa) – Risk of YFV associated viscerotropism	[33,34]
Adenoviruses	– Anthrax – Cancer – Ebola – Hepatitis B – HIV infection – Influenza – Malaria – Measles – Plague – Rabies – SARS – TB	– Easy production and low costs – Stable (Thermally, shelf life) – Infects a wide range of hosts – Grows at high titers in cell culture – Can be mutated to render it unable to replicate in normal human cells – Can be modified to circumvent pre-existing immunity – Can induce both mucosal and systemic immunity – Strong T cell effector memory, little T cell central memory responses (Suitable for priming) – Several serotypes available	– Pre-existing Immunity – Risk integration of vaccine DNA in host genome – Rapid elimination of transduced cells in vivo – Human adenoviruses are oncogenic in animals	[9,11,26–28,30,32,35–42]
Human serotypes (Ad4, Ad26, Ad35)		– Considerably less pre-existing immunity than regular serotype Ad – Grows at high titers in cell culture	– Cross-reactivity after immunisation	[42,43]
Simian serotypes (ChAd63, ChAdOx1)	– Ebola – Hepatitis C – Malaria	– Low pre-existing immunity in humans – Highly immunogenic – Can be mutated to be unable to replicate in normal human cells	– Requires booster for high T-cell response	[40,43–45]
Alphaviruses	– Cancer – Ebola – Hendra virus infection – HIV infection – hPIV infection – HPV infection – Influenza – Malaria – Marburg virus infection – Nipah virus infection – SFV infection – SIN – TB – VEE	– High expression capacity – Can infect dendritic cells – Induces apoptosis in infected cells – Absence of pre-existing immunity in humans – RNA virus, unable to integrate in host genome	– Cytotoxicity – Difficult production – High production costs – Possibly instable – Relatively unexplored in humans – Small to moderate foreign antigen load	[9,27,28,35,38,46]

Table 6 (*continued*)

Vectors	Possible indications (CT, Pre-CT, in vitro)	Advantages	Challenges	Ref.
Semliki Forest virus	– Cancer – Chikungunya virus infection	– Induces both humoral and cellular immune response – High expression capacity – Absence of pre-existing immunity in humans – RNA virus, unable to integrate in host genome – Encapsulated particles prevent vector specific immunity due to repeated use	– Biosafety issues – Instable genome	[46]
Sindbis virus		– Absence of pre-existing immunity in humans – RNA virus, unable to integrate in host genome	– Biosafety issues – Instable genome	[46]
Venezuelan Equine Encephalitis virus	– Cancer	– Induces both humoral and cellular immune response – Can induce both mucosal and systemic immunity – Absence of pre-existing immunity in humans – RNA virus, unable to integrate in host genome	– Biosafety issues – Instable genome	[46]
Nonsegmented Negative-sense ssRNA viruses	– Influenza	– Simple well known genomes – Stable genome compared to psRNA – Grown in high titers in many cell lines – Can induce both mucosal and systemic immunity – Able to carry large and multiple inserts while maintaining a relatively small genome – Gradient gene expression	– Instable genome	[38]
Measles virus	– HIV infection – Measles/HIV combination – West Nile virus infection	– RNA virus, unable to integrate in host genome – Well known homologous vaccine – Can induce both mucosal and systemic immunity	– Pre-existing immunity – Moderate foreign antigenic load	[9,28,38]
Newcastle disease virus/avulavirus	– Avian influenza – Cancer – Ebola – Influenza – NDV infection – RSV infection – SARS – SIV infection	– Can be grown in either eggs or cell culture – Grows at high titers in Vero cells – Bivalent vaccine for influenza and NDV for poultry – Intranasal or pulmonary delivery possible – No pre-existing immunity – Administration both mucosal surfaces of respiratory and alimentary tracts – Needle free administration possible	– Risk of tolerance induction – Instable genome	[27,38]
Para Influenza Virus 5 (PIV5)	– Influenza – Vaccinia	– Non virulent – Infects a wide range of cell types – Grows high titers in Vero cells – Gradient gene expression – Flexible modification of viral genes possible – Administration both intranasally and intramuscularly – No pre-existing immunity	– No clinical safety data for use in humans available	[38,47]
Sendai virus		– High immunogenicity		[32]
Vesicular Stomatitis virus	– Ebola – Filovirus infections – Hantavirus infection – Hepatitis B – Hepatitis C – HIV infection – HPV infection – Influenza – RSV infection – TB	– Low seroprevalence in humans – Infects a wide range of tissues and hosts – Stimulates a strong interferon response – Potential to protect against subtypes of avian influenza in poultry – High expression levels of inserted genes – Low pre-existing immunity		[9,27,28,38]
Poxviruses	– HIV infection – Malaria – Rabies – TB	– Easy production and low costs – Stable (genetically, shelf life) – Broad tropism for mammalian cells – Induces both humoral and cellular immune response	– Pre-existing immunity – Biosafety issues – Competition for antigen presentation pathways	[9,27,28,30,38,48–51]

Table 6 (*continued*)

Vectors	Possible indications (CT, Pre-CT, in vitro)	Advantages	Challenges	Ref.
		– Cytoplasmic site of gene expression – Able to carry large and multiple DNA inserts	– Rapid elimination of transduced cells in vivo – Tropism	
ALVAC (Canarypox)	– Avian influenza – Fowlpox – Influenza – HIV infection	– Induces both humoral and cellular immune response – Stable (genetically, shelf life) – Unable to replicate in mammalian cells – No pre-existing immunity – Can induce strong CD8+ T cell immunity	– Low efficacy	[26,39,50,52]
NYVAC (Vaccinia)	– Cancer – HIV infection – Influenza – Japanese Encephalitis – Malaria – Rabies (animal) – Smallpox	– Stable (thermally, genetically, shelf life) – Reduced ability to replicate in human cells – High level of safety and gene expression/immune response – Can induce both mucosal and systemic immunity – Induces a delayed antiviral response – Able to carry large and multiple DNA inserts	– Pre-existing immunity	[28,39,49]
Modified Vaccinia Ankara (MVA)	– Cancer – Coronavirus infections (SARS, MERS) – Hepatitis C – HIV infection – hMPV infection – hPIV infection – Influenza – Malaria – RSV infection – Smallpox – TB	– Stable (thermally, genetically, shelf life) – Induces both humoral and cellular immune responses – Unable to replicate in mammalian cells – Can induce both mucosal and systemic immunity – Induces both CD4+ and CD8+ T cell responses – Induces strong CD8+ T cell central memory over effector memory (Suitable for booster) – Can encode one or more foreign antigens (multivalent vaccine) – Intrinsic adjuvant capacities – Rapid clearance – Fast construction of recombinant MVA (6-12wks) – Little pre-existing immunity	– Limited priming capacity – Vector specific immunity on repeated use	[11,26–28,32,38,39,48–50,53–56]
Retroviruses		– Long term gene expression	– Generation of replication-competent virus – Infects dividing cells only	[32]
Lentivirus		– Long term gene expression – Infects non-dividing and dividing cells – High immunogenicity	– Generation of replication-competent virus – Potential for tumorigenesis	[32,57]
Bacteria				
Lactic Acid Bacteria (Lactococcus, streptococcus, pediococcus, leuconostoc, lactobacillus)	– Autoimmune diseases	– Naturally present in host – Much safer than traditional attenuated vaccines in children and immunocompromised people – History in food industry, recognised as safe – Probiotics, have health promoting properties – Capacity to survive the gastrointestinal tract – Mucosal administration could reduce traditional side effects	– Limited knowledge available for use as vector vaccine compared to viral vectors	[39,58]
Listeria	– Cancer	– Induces both CD4+ and CD8+ T cell responses – Naturally present in host – Pre-existing immunity can lead to stronger immune response – Much safer than traditional attenuated vaccines in children and immunocompromised people – Induce robust T-cell immune response – Can invade a variety of cells, including antigen presenting cells – Can reside in the cytoplasm	– Limited knowledge available for use as vector vaccine compared to viral vectors	[39,59]

Table 6 (continued)

Vectors	Possible indications (CT, Pre-CT, in vitro)	Advantages	Challenges	Ref.
Salmonella	– Salmonellosis (in animals) – Typhoid fever – HIV infection	– Naturally present in host – Pre-existing immunity can lead to stronger immune response – Much safer than traditional attenuated vaccines in children and immunocompromised people – Induces robust T-cell immune response – Induces both humoral and cellular immune responses – Can induce both mucosal and systemic immunity – Able to carry large DNA inserts	– Pre-existing immunity could still be a limiting factor – Limited knowledge available for use as vector vaccine	[9,39,60]

Table 7
Use of viral vectors in GM vaccine Clinical Trials. Types of vectors that are being used for specific indications (top 10 vector vaccine indications) in GM vaccine trials, and a comparison of vector-based vaccine GM trials compared to all GM vaccine trials.

Indication	All GM vaccine trials (n)	Vector-based vaccines (n)	(%)	Type of vector					
				Adenovirus	Vaccinia (MVA & NYVAC)	Fowlpox	ALVAC	Fowlpox & vaccinia	Other
Cancer	208	78	*38*	11	11	12	6	14	24
Influenza	157	5	*3*	1	3	0	0	0	1
HIV	153	86	*56*	25	43	3	11	0	4
HPV	105	1	*1*	0	1	0	0	0	0
Hepatitis B	82	1	*1*	0	1	0	0	0	0
Malaria	29	11	*38*	2	8	0	0	0	1
Ebola	4	1	*25*	1	0	0	0	0	0
Variola	9	8	*89*	0	8	0	0	0	0
TB	12	5	*42*	0	5	0	0	0	0
Epstein-Barr	3	2	*67*	0	2	0	0	0	0
Total	**762**	**198**	26	**40**	**82**	**15**	**17**	**14**	**30**

Bold/italic values represent indications with a high percentage for vector-based GM vaccine trials.
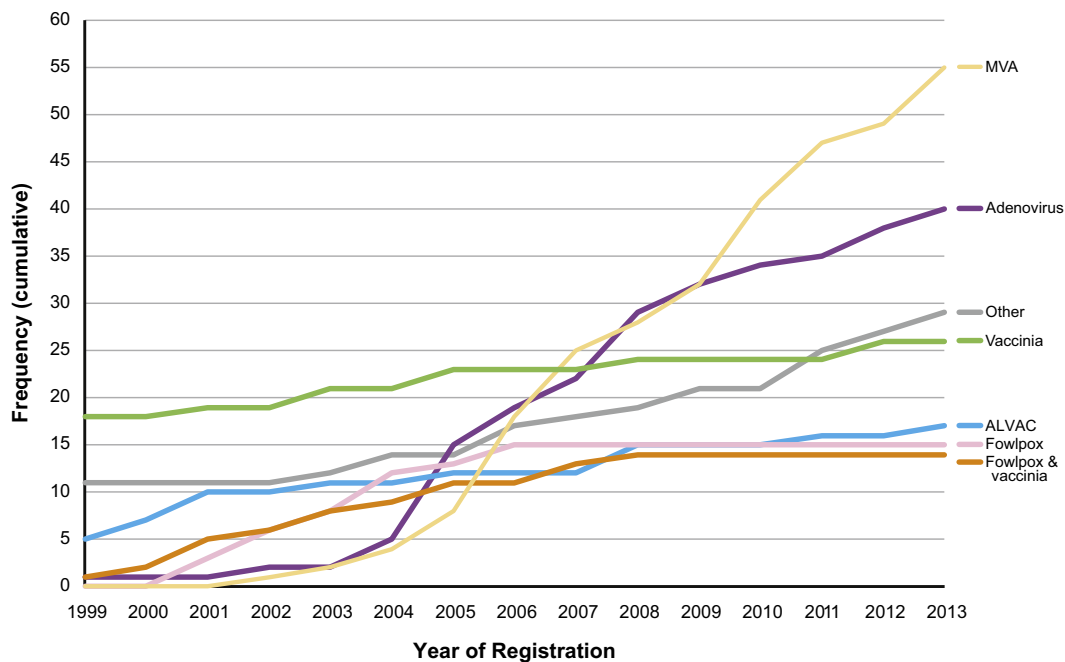

Fig. 2. Cumulative frequency of vectors in clinical trials. Supporting figure for Table 2, showing the cumulative frequency of the various vectors used from 1999 until 2013.
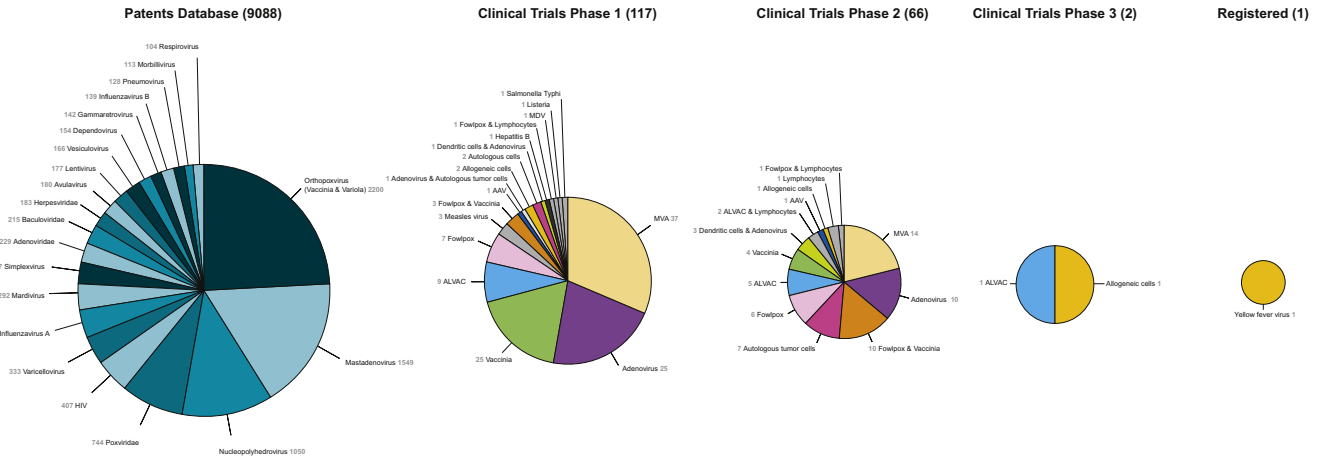
Fig. 3. Prevalence of vectors used in Patents and Clinical Trial phases for GM vaccines The illustration indicates the prevalence of various vectors used for GM vaccines for each phase of research. Each pie chart represents the contribution of each individual vector to the total amount of vectors in the specific database (Patents, CT1, CT2, CT3). Abbreviations: HIV: Human immunodeficiency virus, AAV: Adeno associated virus, ALVAC: Canarypox virus, MVA: modified vaccinia Ankara.
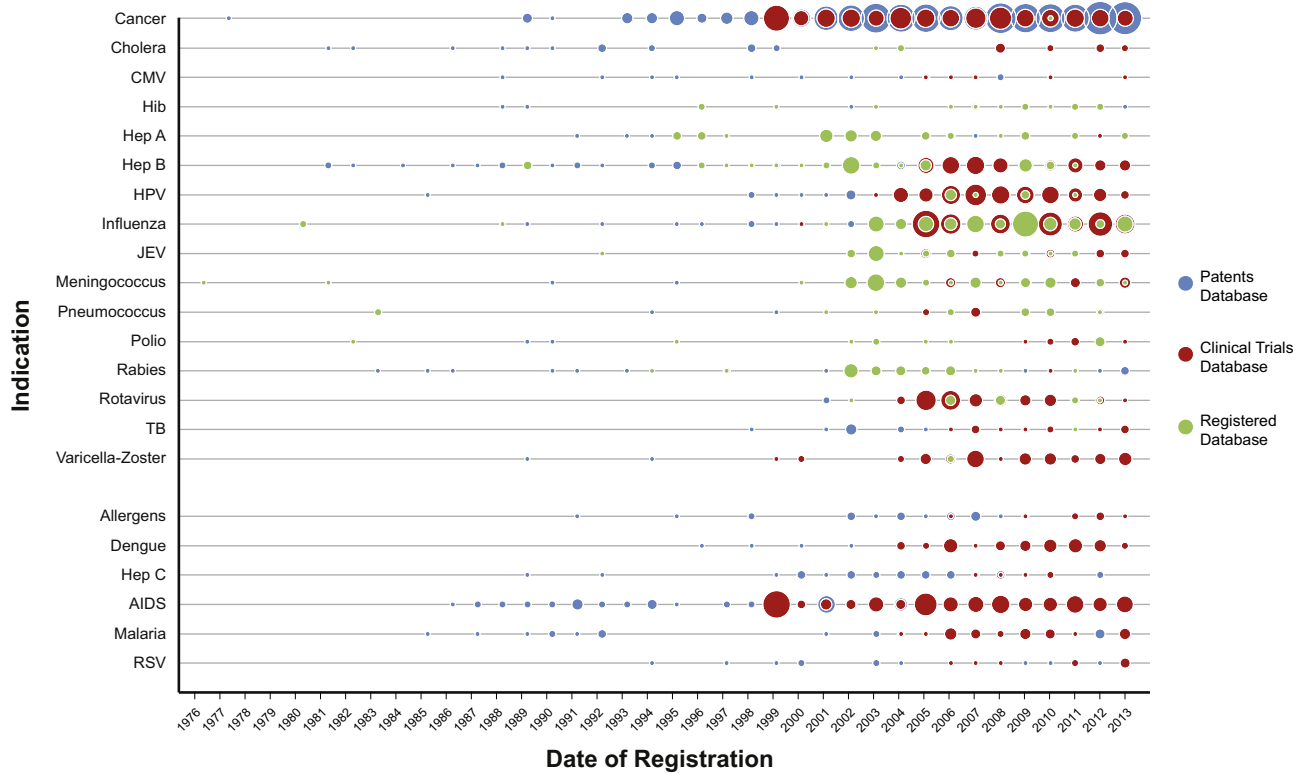

Fig. 4. Evolutionary timeline of indications and prevalence generated from various databases. The motion of indications through time in three different phases of research (Patents (blue), Clinical Trials (red), Registered (Green). Dot size indicates frequency of vaccines for this indication per year per database. The top group of indications are present in each database, the bottom six are absent from the Registered database but present for both Patents and Clinical Trials. Abbreviations: CMV: Cytomegalovirus, Hib: Haemophilus influenzae, Hep A/B/C: Hepatitis A/B/C, HPV: human papillomaviru, JEV: Japanese Encephalitis virus, TB: tuberculosis, HIV: Human immunodeficiency virus, RSV: Respiratory Syncytial virus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Various search terms were often found in literature to define GM vaccines, nevertheless these terms were all used in an inconsistent manner. The definition of GM vaccines was narrowed down by delineation of search terms found in CPC codes and literature.

Literature widely acknowledged that, compared to bacterial or DNA vectors, viral vectors have been researched in more detail (Table 6). Poxviruses and adenoviruses are referenced often and a lot of details are provided on the use of these families as viral vectors. Nevertheless, pre-existing immunity is still a major obstacle for several viral vectors (Table 6). This is of special concern for

the use of adenovirus vectors, although several strategies to circumvent this problem have been developed [40,44,45,64]. Interestingly there are many data suggesting that this seems to be les of a problem for MVA based vaccine candidates [65–67].

In comparison with viral vectors, DNA and bacterial vectors show potential in this respect, but this require more research. Furthermore there are several other limitations to overcome. The primary limitation of bacterial vectors is their lack of immunogenicity compared with viral vectors [68]. In addition, viruses are relatively easier to work with, since they have less complex genome than

bacteria [69]. Although several types of bacteria have been mentioned in literature (e.g. *Escherichia coli*, *Vibrio cholerae*, Mycobacteria, and Shigella spp.), these are not mentioned in the table due to lack of sufficient information on their advantages and disadvantages [39].

The results of the patent search show that the most prominent CPC codes in the patent database on vector-based vaccines cover general information on vectors (C12N15/79, A61K2039/5256, A61K2039/523). These CPC codes do not comprise specific vectors but are general indicators for vector-based vaccines. A large variety of different vector types are being patented for vaccine development (Fig. 1). According to the patent database, both "mastadenovirus" and "orthopoxvirus: vaccinia/variola" are the most prominent vectors. Human adenoviruses are part of mastadenovirus genus, which in turn is a large genus of the adenoviridae family. It is notable that orthopoxvirus: vaccinia/variola as well as poxviridae appear in the patent results. The species vaccinia virus and variola virus are part of the genus orthopoxvirus, which belongs to the family of Poxviridae. The explanation behind this seemingly double occurrence is that CPC codes for both the species and the family are present in Espacenet. There is one CPC code for both vaccinia and variola combined, rather than a separate code for each of these species.

For clinical trials, vector-based vaccines play quite an important part in GM vaccine trials as a whole. 26% of all GM vaccine trials use vector-based candidate vaccines, especially for currently undefeated indications, such as cancer, HIV, TB, and malaria (Table 7). Although no active clinical trials on malaria based on fowlpox vectors are provided in the clinical trials database (clinicaltrial.gov), literature shows that fowlpox vectors are being examined in combination with different vaccination regimens [70,71]. Intervention strategies for these diseases represent a large unmet medical need, still causing over a million deaths every year [72]. With limited if any cure available, new methods might provide additional value to vaccine research and development, in hope for a breakthrough. This premise is confirmed by Fig. 4. Cancer, HIV, and malaria show a large presence in both patent and clinical trial databases, while Hib, Hepatitis A, and meningococcus show little development in patent and clinical trials stages in recent years. This implies a lower need for new vaccine technologies for these indications, as current vaccines based on conventional methods are apparently sufficiently satisfactory [73–75]. Table 7 also shows that very little vector-based vaccine trials are present for more or less treatable diseases such as HPV induced neoplasias, hepatitis B, and influenza. Apparently there is less medical need for vector-based vaccines for these indications, as vaccine candidates are available based on GM-based and non-GM-based techniques [76–78]. There is, a significant amount of active non-vector-based GM trials for these three indications, demonstrating the variety of GM techniques applied in vaccine development. For influenza and hepatitis B vaccine production, the use of recombinant DNA technology is already common practice [6,79]. Therefore, the focus of research is on optimising the technology used, rather than on investing in new vector-based technology research.

The increase in MVA vector usage compared to the use of vaccinia virus indicates that MVA has started to replace regular vaccinia virus in vector-based vaccine development, as vaccinia trials have stagnated while MVA trials are increasing rapidly (Fig. 2). The reasons for this replacement is predominantly related to the safety and efficacy of MVA, as it only causes an abortive infection, while inducing an abundant expression of the target immunogen, leading to impressive protective immune responses [12]. The prominent use of adenoviruses as viral vectors is probably due to the considerable knowledge on this virus family, the ease of manipulation of the virus for use in vector-based vaccines, and the broad tissue tropism associated with this virus [40,80].

The consensus that both poxviruses and adenoviruses are important for vector-based GM vaccine research is also strengthened by data shown in Fig. 3, indicating high prevalence of these vectors in both patents and clinical trials. For patents and the first two phases of clinical trials, both orthopoxviruses vaccinia virus (mentioned in combination with variola virus), and adenoviruses are well represented (though in patents adenoviruses are not mentioned directly, these are part of the Mastadenovirus genus).

While analysing this data set, it is important to keep in mind that the data bases used are snapshots of each phase of research and development pipeline. Patents are made public 18 months after submission, but when patents are retracted before this 18 months period, they disappear from Espacenet. Clinical trials only show active and on-going trials (hence the database starts in 1999). Discontinued or terminated trials are removed from the database, consequently, making direct correlations between databases unjustifiable. Therefore, the analyses conducted in this study are not directly between databases but each database is seen as an individual snapshot.

Even though numerous vectors are being studied in different phases of pre-clinical and clinical research, the presence of their majority in phase 1 indicates that the evolution of vector-based vaccines has only just begun. The large amount of vector types being patented, or having reached phase 1 clinical trials, show a lot of promise, as new techniques might lead to a new generation of safer, more efficient, and cost-effective vaccines.

Comparing the data presented with the literature study we conducted initially, it seems that a lot of new vectors are being patented while little published information is available. This could indicate that some vectors are being patented beforehand, not necessarily in order to start a new study, but in case a method or technique is developed to make them suitable for vaccine production. Without patents, anyone could start developing these vectors without legal consequences, leading to companies competing to sell the vaccine for the lowest price possible. When revenues from vaccine sales eventually do not lead to return on investment for past and future research and development there would be no incentive for the development of new generations of vaccines. Therefore the use of a patent search was considered a valid and valuable approach to gather part of our dataset.

In conclusion, our data suggest that although currently there are two licensed, vector-based human vaccine on the market and that this field is still in its early days, vector-based vaccines may offer a cost-effective alternative for the production of safe and effective vaccines against diseases for which no or less perfect vaccines exist today. The most promising vectors for vaccine development at this moment appear to be poxvirus and adenovirus vectors. This may be concluded for their abundant use in the development of vaccines against diseases like HIV-AIDS, malaria, tuberculosis and different forms of neoplastic disease. It may be expected that the current efforts spent on developing vector-based vaccines, may lead to promising vaccine candidates for these indications and therefore hold promise for current and future unmet medical needs. Therefore, after the recent eradication of smallpox using Jenner's vaccinia virus as the first vaccine, this and other viruses may now be the basis for constructing vectors that may help us control other major scourges of mankind.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.06.059.

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