Global Challenges Report

Patent Information, Freedom to Operate and "Global Access": A Case Study of Dengue Vaccines Under Development

ANATOLE KRATTIGER
Director, Global Challenges Division, World Intellectual Property Organization, Geneva, Switzerland

RICHARD T. MAHONEY
Coordinator, Policy & Access, Dengue Vaccine Initiative, International Vaccine Institute, Seoul, Republic of Korea

AMRITA CHILUWAL
Fellow, International Technology Transfer Institute, Franklin Pierce Center for Intellectual Property, University of New Hampshire School of Law, Concord, United States of America

STANLEY P. KOWALSKI
Professor, Franklin Pierce Center for Intellectual Property, University of New Hampshire School of Law, Concord, United States of America

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Abstract

This *Global Challenges Report*[1] presents a "global access" freedom to operate (FTO) analysis[2] of six vaccines under development against dengue hemorrhagic fever (hereinafter referred to as "dengue"), a Neglected Tropical Disease (NTD)[3] endemic to tropical regions. Developing a vaccine against dengue is challenging because there are four closely related viruses that can cause the disease. Several vaccines are in various stages of development, including by developing country institutions from both the public and private sector. Clinical trials are underway on five candidate vaccines with encouraging results.

After an extended executive summary (Section 1) and an introduction and description of the methodology (Section 2), the report reviews the scientific basis of the various vaccines under development (Section 3). A successful vaccine should immunize against all four types, and substantial progress towards the development of such a vaccine has been made in the last decade. The range of vaccines under development include live attenuated vaccines, live chimeric virus vaccines and live recombinant, DNA and subunit vaccines. Each type comes with its own unique challenges and benefits, though live attenuated vaccines have been the most successful to date[4].

Section 4 presents the patent situation relating to six of the dengue vaccines under development. Some 10,800 patents and patent applications were found to have "dengue" in the abstract, title, text or claims, corresponding to 4,500 patent families. Of these, 700 families were found to be outside the scope. Of the remaining 3,800 patent families, 55 patents or patent families were deemed pertinent to the six vaccines discussed in this report. The number of patent families related to a given vaccine ranged from five to 22. Most of these were filed in developed countries with only a small number also filed in select developing countries. Each of the patent groups occupied a well-defined space in the patent landscape, with little overlap in the specific technological field. This finding has important implications for IP management strategies in that few, if any, cross-licensing deals may be required to bring any given vaccine to market. This aspect is further discussed in Section 5 on the licensing status of the vaccines under development.

The results of this "global access" FTO suggest that there are few major constraints related to patents that could complicate developing-country access to the vaccines under development. It should be noted that the analysis is limited to patent data and licensing information. Market considerations such as economies of scale, pricing and regulatory approval, or efficacy of the vaccine itself, are beyond its scope. Notwithstanding the relatively few patents applied for or issued in developing countries, an effective transfer of productive capacity of any of the vaccines to developing countries would require consideration of additional elements beyond patent data. Those include regulatory requirements, issues relating to know-how, and possible access to materials, such as cell lines. The report, nevertheless, identifies the state of product development, identifies key players, and the patent and licensing status, which together facilitate the development of effective strategies, including collaborations, as appropriate. These should enable early product deployment in areas where dengue most affects people's lives and thus lead to accelerated access to dengue vaccines by those most in need.

This report provides an informal guide for those wishing to better understand the important interplay of intellectual property (IP) with product development, manufacture and delivery (viz. access). It can be seen as an example of using patent information to address major global challenges, including access to medicines, and thus contribute to informed policy discussions, strategic research planning and technology transfer, and in that way, benefit humanity.

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[1] This report is based on a confidential "global access" FTO review, prepared by *bio*Developments-International Institute, and commissioned by the Dengue Vaccine Initiative (DVI), a consortium of the International Vaccine Institute (IVI), the World Health Organization (WHO), the International Vaccine Access Center of the Johns Hopkins University Bloomberg School of Public Health and the Sabin Vaccine Institute. Funding for the confidential FTO review was provided by The Bill & Melinda Gates Foundation through DVI. The report has been updated, expanded and edited for public release by the World Intellectual Property Organization (WIPO) in collaboration with both the Franklin Pierce Center for IP, University of New Hampshire School of Law, and DVI. As with any FTO review, this report provides a snapshot of the situation at present and does not constitute a legal opinion on patent infringement in relation to dengue vaccine development. The situation will evolve over time as patents are filed, issued, modified and withdrawn or licensed. The vaccine development and licensing information is current as of June 2011 and the patent information as of December 2010. The website links were last accessed in June 2012

[2] An FTO opinion is a legal opinion by patent counsel, assessing whether making, using, or selling a specific product in a specific market is likely to infringe existing patents or other types of IP rights. The resulting information contributes to risk assessment and management strategies that may involve various options. The latter include in-licensing, cross-licensing, substituting technologies, "waiting-and-seeing", investing in work-around technologies, abandoning a project, or acquiring the company with relevant IP assets. A "global access" FTO review differs from an FTO opinion in that it is a broad patent analysis without specific legal opinions as to infringement, with a specific focus on developing country "access".

[3] See WHO resources (http://tiny.cc/4fajhw and http://tiny.cc/llebiw) for information on dengue and http://tiny.cc/aiajhw for WHO's list of NTDs.

[4] See the Dengue Vaccine Initiative's resources on http://tiny.cc/5bbjhw.

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Section 1:
Executive Summary

1.1 DENGUE FEVER

Dengue hemorrhagic fever (hereinafter referred to as "dengue") is an acute febrile disease that primarily afflicts children and young adults. Endemic in tropical regions, an estimated 500,000 individuals are seriously affected every year by this disease, which can be fatal. For the global health community, ensuring broad access to any dengue vaccines that are developed is therefore a priority. Development of and access to vaccines require a range of activities, including research and development (R&D), navigation of the relevant regulatory processes, manufacturing, marketing and trade, and intellectual property (IP) management.

1.2 THE DENGUE VACCINE GLOBAL ACCESS FREEDOM TO OPERATE (FTO) REVIEW

A vaccine or drug is typically manufactured by the developer of the product and/or licensed to a third party for production. In any case, the vaccine developer needs to have assembled all of the relevant IP rights (IPRs) information in order to enjoy FTO. FTO means that, for a given product or service, at a given point in time, with respect to a given market, no intellectual property or tangible property rights from any third party are infringed.

The goals of this "global access" FTO review[5] (see note at the end of this Section) are to:

1. understand how IPRs may affect access to dengue fever vaccines in developing countries;
2. assess the ways in which some vaccine developers may be affected by IPRs and the extent of freedom they have to license their products to developing countries; and
3. evaluate the freedom of vaccine developers in developing countries to market their vaccines outside their home countries.

The results presented here are based on a product deconstruction analysis as well as patent searches that were conducted using both open and subscription-based services. A production deconstruction analysis consists of understanding, analyzing and dissecting the technology into its components, and formulating a series of FTO analytical questions. A "global access" FTO review differs from a legal opinion in that a thorough legal status search of pertinent patents was not conducted. Much of the relevant information was obtained from vaccine developers through interviews. Further, possible patents applicable to future large scale production technologies are not included; methods are still in early stages of development and will be specifically tailored to the downstream requirements of the discrete vaccine technology. Five of the six vaccines considered by this analysis are still under development, the exception being the discontinued Mahidol University/Sanofi Pasteur vaccine.

1.3 PATENT SEARCH RESULTS

Some 10,800 patents and patent applications were found to have "dengue" in the abstract, title, text or claims, corresponding to 4,500 patent families. A patent family is a group of patents/patent applications that are issued or published in various countries to protect a single invention by the same inventor(s). Of these, 700 families[6] were outside the scope of dengue vaccines, diagnostics or therapeutics. Of the remaining 3,800 patent families, merely 55 patents or patent families were deemed pertinent to the six vaccines discussed in this report. For any given vaccine, the total number of related patents ranged from five to 22. Most of these patents were only filed in developed countries, though a small number of patents were also filed in select developing countries, as shown in Table 1.

Among the search databases used were WIPO PATENTSCOPE®, USPTO, esp@cenet® and Patent Storm (free of charge), and Thomson Innovation and MicroPatent® (premium pay-per-view or subscription-based services).

Annex A also lists the family data for the 55 relevant patent documents identified in this report with corresponding International Patent Documentation Center (INPADOC)[7] family members, as per jurisdictional codes in alphabetical order.

Patents and patent applications that were identified were classified as:

1. relevant;
2. might be relevant, pending further discussion;
3. not immediately relevant, but warranting consideration within the context of future developments in dengue vaccines; and
4. definitely not relevant.

Each of the patents/patent families occupies a well-defined space in the patent landscape, with little overlap in the specific technological field. This can be seen from the map in Figure 1, generated using Aureka® Themescape™ software. It illustrates the distance between patents as well as major concentrations of patents. The number of letters is indicative of the number of patents, but, due to proximity, not all patents are visible.

Following interviews with a variety of R&D organizations, we eliminated most of the patents that fell into the second category. Careful analysis resulted in 55 patent families (of which many are composed mainly of patent applications) being deemed relevant to the six vaccines under development. Depending on the specific vaccine, between five and 22 patent families are of core relevance. Overall, few patents have been filed in developing countries.

Table 2 lists the applicants or assignees of potentially applicable patents. Details regarding these applicants and assignees are provided in Section 3, while data on the countries in which patents have been filed are provided in the tables of Section 4. Section 5 discusses areas in which there is disagreement over the applicability of patents to a given vaccine. Many of the above listed documents are patent applications that have not yet matured into issued patents. Thus, the number of applicable patents may change over time. The specific claim formulation in different jurisdictions will also likely vary due to differences in patent law, resulting in a given patent to be applicable in one jurisdiction, but not necessarily in another. In addition, although we reviewed WIPO filing data and interviewed many research groups and applicants, some applicants may decide to enter the national phase under the PCT filing at a later time; such data have not been included to date. Finally, new patents will be filed and published, so that the number of patents listed in the table below will constantly evolve.

Table 1:

VACCINES UNDER ADVANCED DEVELOPMENT AND PERTINENT PATENT INFORMATION

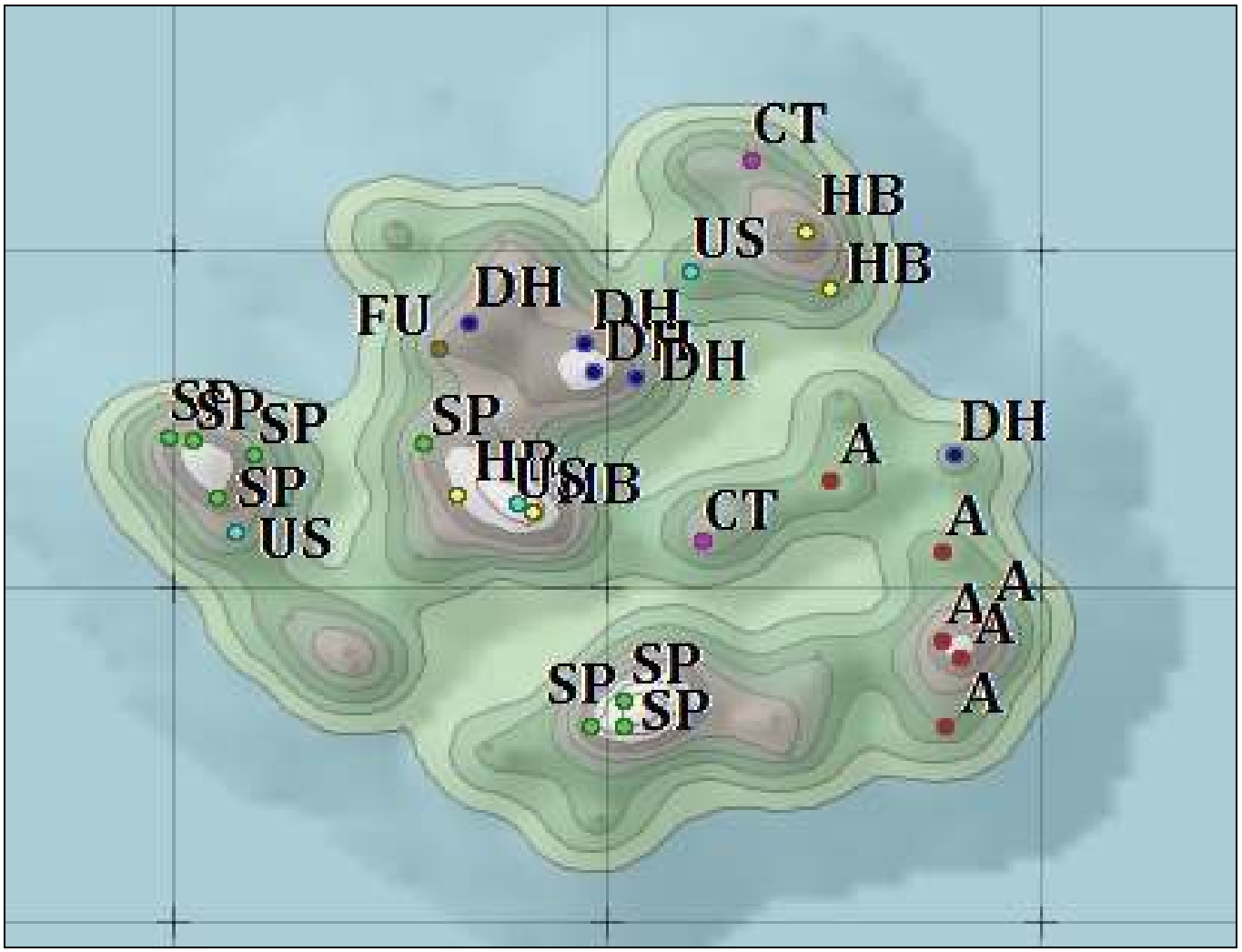
Originator or Developer	Partner or Producer	Technological Approach	Development Status (as of June 2011)	Possible Low and Middle Income Country Coverage*)
Acambis Plc. (now owned by Sanofi SA)	Sanofi Pasteur (the vaccine division of Sanofi), France	Yellow fever – Dengue chimera	Phase II, adults	AR, BR, CL, CN, HK, IN, KR, MX, OA, RU, ZA
Hawaii Biotech, Inc.	Hawaii Biotech, Inc. (now owned by Merck & Co.), United States	Envelope protein subunit	Phase I	AR, BR, CN, CU, HK, IN, KR, MX, PH, RU, ZA
Mahidol University, Thailand	Sanofi Pasteur, France	Cell culture passage	Recently discontinued	AR, BR, CN, IN, KR, MX, ZA
US Center for Disease Control and Prevention	InViragen, Inc., United States	Dengue – Dengue chimera	Phase I	AR, CN, IN, KR, MX, ZA
US National Institutes of Health	Butantan, Brazil Biological E, India Panacea, India VABiotech, Vietnam	Dengue – Dengue chimera with gene deletions	Phase I-II, adults	AR, BR, CN, IN
US Walter Reed Army Institute of Research	GlaxoSmithKline, United Kingdom	Cell culture passage**)	Phase II, adults and children	AR, BR, CN, ID, IN, KR, MX, MY, VN

*) Country Codes: AR: Argentina; BR: Brazil; CL: Chile, CN: China; CU: Cuba; HK: Hong Kong, China; ID: Indonesia; IN: India; KR: Korea (whereas KR is a high-income country, we listed it in this table since IVI/DVI are located in the Republic of Korea); MY: Malaysia; MX: Mexico; OA: African Intellectual Property Organization; PH: Philippines; RU: Russia; VN: Vietnam; ZA: South Africa. Tables 6 to 11 (Section 4) provide patent filings in high-income countries and economies in transition.

**) GSK has recently begun working on a "purified inactivated vaccine" developed by WRAIR. GSK intends to give up the development of the "live attenuated vaccine" but WRAIR is interested in its continued development.

Figure 1:

PATENT LANDSCAPE MAP WITH THE KEY PATENTS OF THE SIX VACCINES



A: Acambis/Sanofi;
FU: Fundação Oswaldo Cruz (Fiocruz);
HB: Hawaii Biotech/Merck;
US: US Army (essentially Walter Reed Army Institute of Research)/GSK.
CT: CT Ingeniera Biotecha;
DH: US Dept. of Health, National Institutes of Health;
SP: Sanofi Pasteur;

Note that the Mahidol University/Sanofi Pasteur patents are not shown because the vaccine development has been discontinued. The analysis is using Derwent or Micropatent data, titles and abstracts (see also Annex B).

The figure is for illustrative purposes only and does not, in itself, provide proof that a given patent does not relate to a certain technology or product depicted in a different "island". Different ways of writing patent specifications (e.g. chimera vs. gene replacement) and claim language may lead to patents being represented on different "islands".

Table 2:

NUMBER OF PATENTS OR PATENT FAMILIES RELATED TO EACH OF THE SIX VACCINES

Assignee or Applicant[*)]	Number[**)] of Patents/Patent Families
"Acambis" vaccine	
Acambis	9
Intercel	1
Mayo Foundation	1
Oswaldo Cruz Foundation (Fiocruz)	2
Sanofi Pasteur	6
University of Texas	1
US Department of Health	2
Hawaii Biotech, Inc. (now Merck & Co.)	
Bavarian Nordic	1
Centro de Ingenieria Genetica y Biotecnologia, Cuba	3
Galenica Pharmaceuticals	1
International Centre for Genetic Engineering & Biotechnology	1
Maxygen	1
SmithKline Beecham	1
US Army	1
US Department of Health and Human Services	3
"Mahidol" vaccine	
Acambis	1
Aventis Pasteur	1
Mahidol University	1
Sanofi Pasteur	2
"US CDC - InViragen" vaccine	
Sanofi Pasteur	3
US Department of Health and Human Services	3
"US NIH" vaccine	
Sanofi Pasteur	1
US Department of Health and Human Services	4
"US WRAIR - GSK" vaccine	
Sanofi Pasteur	1
US Army	6
US Department of Health and Human Services	1

[*)] Note that licensing information on Mahidol University/Sanofi Pasteur's vaccine is not given since the development of the vaccine has recently been discontinued.

[**)] The total number of patent families and patent applications is greater than 55 because two patents (Barban *et al.* and Lai *et al*.) apply to more than one vaccine. Also, several patents or patent applications have more than one assignee or applicant.

1.4 LICENSING

A number of IP licensing agreements, covering both in-licensing of inputs and out-licensing of products, were identified. These are discussed in Section 4. They are summarized below in Table 3. Table 12 in Section 5 provides additional details.

1.5 CONCLUSIONS OF THE FTO REVIEW AND POSSIBLE IMPLICATIONS FOR THE DENGUE VACCINE INITIATIVE (DVI)

Overall, the results of this "global access" FTO review suggest that there are relatively few major IP constraints that would complicate global deployment of any of the six types of vaccines in various stages of development. This appears to be the case for several reasons:

Many of the inputs are already in the public domain. Basic technologies for developing and manufacturing vaccines are well-established, and many have been in use for well over a century. However, with the advance of biotechnology in vaccine research, development and manufacturing, IP issues may arise, depending on the type of methodologies and materials employed. Issues stemming from the use of recombinant DNA technology are the principal ones identified in this report because these technologies enabled tremendous advances in vaccinology.

Table 3:

IP LICENSING STATUS OF THE SIX VACCINES

Technology Developer	In-licensed Enabling Technology	Out-licensed Vaccine Technology
Acambis/Sanofi Pasteur	Non-exclusive license from NIH, possibly from the Mayo Clinic, and from St. Louis University.	Acambis has exclusively licensed the ChimericVax™ DEN2 platform technology to Sanofi Pasteur for subsequent development.
Hawaii Biotech, Inc. (now Merck & Co.)	Hawaii Biotech has licensed the proprietary expression vector from GlaxoSmithKline for production of flavivirus vaccines for use with all flavivirus vaccines worldwide.	Hawaii Biotech has not licensed its vaccine patents. The rights to the Hawaii Biotech, Inc. vaccine were procured by Merck & Co. in 2010.
US CDC – InViragen	CDC granted an exclusive license to InViragen for its DEN-2 PDK-53 chimeras. InViragen has obtained an exclusive license from the CDC for a patent family. InViragen has obtained a non-exclusive license for another patent family.	InViragen has signed a manufacturing agreement with Shantha Biotechnics, Hyderabad, India.
US NIH – Developing Country Manufacturers	Not known. Presumably few or none.	Several industrial sponsors in Asia and Brazil have been awarded non-exclusive licenses for the rDenΔ30 formulations, including Butantan Foundation (Brazil), Biological E (India) and Panacea Biotech (India).
US WRAIR – GSK	Not known. Presumably few or none.	The vaccine development partnership between WRAIR and GSK is based on a Cooperative R&D Agreement (CRADA) which captures all relevant patents.

CDC: US Center for Disease Control and Prevention; NIH: US National Institutes of Health; WRAIR: Walter Reed Army Institute of Research.

The degree of overlap in terms of patents among the six major vaccines is quite low, with each candidate vaccine occupying a distinct, defined area, as illustrated by Figure 1 above. The proximity of the InViragen, Acambis and NIH patent documents in the Themescape Map is not an unexpected result, as these technologies are all variants of the Chimeric Live Attenuated Dengue Vaccines. Nevertheless, each group of patents is distinct. This leads to the practical conclusion that from an IP management perspective, the commercialization of any given vaccine can take place independently of the others.

The manufacturing and distribution of any of the six types of vaccines could therefore likely take place in most developing countries without the need for a license from any of the vaccine developers. This is due to the fact that, to date, few patents have been issued or filed in developing countries. Exceptions to this have been listed in Table 1 above, which lists the countries in which patents have been issued or applications filed.

Table 3 only provides information about patents, and not other IP, such as trademarks, trade secrets, regulatory data or know-how. Further, the table does not include possible constraints related to access to essential materials, such as cell lines, that may be required for the efficient production of the vaccine. In other words, taking the Acambis/Sanofi Pasteur vaccine as an example, any entity wishing to manufacture or commercialize the vaccine outside China, the Republic of Korea or the Philippines may be able to do so without a license. Notwithstanding this conclusion, which is purely based on patent rights, a manufacturer might accelerate the production process and vaccine registration with a license to regulatory data and know-how.

In sum, the primary issues identified in this “global access” FTO review include:

1. For any given technological approach to the six dengue vaccines under development, there appears to be minimal overlap, in terms of proprietary rights, with other approaches. This suggests that the patents on one vaccine technology will not materially interfere with any other vaccine technology. This is reflected in the Aureka® Themescape™ MapManager data. For DVI, the result is that any discussions on licensing with one vaccine developer can take place independently of those with other vaccine developers.

2. Each of the entities developing the six vaccines appears to enjoy FTO. Our findings suggest that, with respect to major IP, the developers either used their own IP or had already in-licensed from third parties. Notwithstanding this, the Lai-related technologies may merit further analysis. Specifically, the Lai *et al.* patent family, of which US6676936 is a representative document, covers fundamental technologies that are potentially relevant to the current advanced phase dengue vaccine approaches employed by NIH, WRAIR, Hawaii Biotech and Acambis/Sanofi Pasteur, while US5494671 and its family members are likely to be relevant to the technologies developed by Hawaii Biotech. All late stage vaccine developers should thus consider revisiting the entire list of Lai *et al.* patents in order to determine whether the patents might present downstream constraints on their vaccines. Possible IP management approaches might include licenses or non-assertion covenants[8].

   The Sanofi Pasteur patent application, (US20080014219), which covers both a method of application and a kit thereof could potentially apply to all six vaccines under consideration here[9]. The application covers dengue vaccine preparations comprising two or four dengue serotypes, as well as any kit that has any combination of any two dengue serotypes. The claims also cover any scheme that administers two serotypes (whether separate or mixed together as one unit) followed at some future date (30 days to approximately one year) by the administration of two more serotypes (whether separate or mixed together as one unit). A number of strategies could be pursued to overcome some of the potential constraints presented by this application. These could include: patent challenges based on prior art or non-obviousness arguments; the production of a kit with three serotypes; a non-assertion covenant from Sanofi Pasteur applicable to low and middle income countries; or licensing.

3. The only significant potential overlap identified during this “global access” FTO review was found to lie between the vaccine technologies under development by Fiocruz and those by Acambis/Sanofi Pasteur. The primary concern is that the Fiocruz vaccine technologies (the subject of patent family as represented by WO2007051267) would overlap with the Acambis technology (ChimeriVax™-DEN patent portfolio) and thus would potentially be subject to a prior art challenge or obstructed by FTO restrictions. That is, Acambis/Sanofi Pasteur may face certain challenges because vaccine technologies under development may embody elements that could impinge on the proprietary rights of Sanofi Pasteur. As of this writing, the issue is unresolved and will likely require additional legal analysis in order to delineate both the prior art and the FTO issues.

4. For the six major vaccine technologies examined in this study, only a few patent applications have been

filed in developing countries. This has been confirmed through interviews with the institutions developing the vaccines. The countries in which one or more patents have been issued or filed include Brazil, China, India, Indonesia, Republic of Korea, Mexico, Philippines, South Africa and Vietnam. Over time, however, one would expect a trend towards greater patent application filings in developing countries, and past activity should not be construed as a reliable predictor of future activities. Since a number of patent applications may still enter the national phase under PCT filing in developing countries, it may be advisable to regularly update the "global access" FTO review or, at a minimum, monitor the 55 patents discussed in this report.

In view of its above-mentioned goals and results, the study can be seen as an example of using patent information to address major global challenges, including access to medicines. Furthermore, its findings can contribute, among others, to inform policy discussions, strategic research planning, technology transfer, and in that way, benefit humanity.

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[5] Since DVI is not a developer or manufacturer of vaccines, a detailed and costly legal FTO opinion was not required.

[6] This report predominantly cites 'WO', or Patent Cooperation Treaty (PCT), patent applications because when patent families are generated, the algorithm used by Derwent® typically lists WO patents. Only representative parent patents are cited, rather than entire patent families. Due to differences in international and United States (US) patent law, claims in PCT and US applications are often not identical. To discern the precise extent of the patent grant for any given country, an analysis of the specific patent as issued in that country would be necessary. Further, patent families are not identical in their claim structures; rather, they are related via lineage to a common filing. This limitation does not materially affect the conclusions of this report, although it would have a direct bearing on the number of patents to be licensed within a patent family, which would typically originate from the same licensor.

[7] The **In**ternational **Pa**tent **Do**cumentation **C**enter (INPADOC), is an international patent collection. The database is produced and maintained by the European Patent Office (EPO). It contains patent families and legal status information, and is updated weekly (http://tiny.cc/2nbjhw).

[8] Krattiger A., *The Use of Nonassertion Covenants: A Tool to Facilitate Humanitarian Licensing, Manage Liability, and Foster Global Access*, Intellectual Property Management in Health and Agricultural Innovation: A Handbook of Best Practices (eds. Krattiger A., Mahoney R.T., Nelsen L., *et al*.). MIHR: Oxford, United Kingdom, PIPRA: Davis, United States of America, Fiocruz: Rio de Janeiro, Brazil, and bioDevelopments-International Institute: Ithaca, NY. www.ipHandbook.org (2007).

[9] This would, however, not apply to DNA vaccines, such as those currently under development by the US Naval Medical Research Center (NMRC) mentioned in Section 3.6.3 of this report.

Section 2:
Background of the Study and Methodologies

2.1 INTRODUCTION

Dengue is an acute febrile disease that afflicts children and young adults. It is endemic in tropical regions, where it affects an estimated 500,000 people every year. Symptoms include fever, headache, muscle and joint pain, nausea, rash, and vomiting; they can progress to bloody stools, bloody urine, dehydration, shock, and death. The development and dissemination of vaccines for dengue is therefore a priority for the global health community.

For a vaccine to be created and made available, a range of activities are necessary, including research and development (R&D), navigation of the relevant regulatory systems, IP management, manufacturing, marketing, and trade. Within the context of IP management, there are three principal elements:

1. In-licensing of technologies related to the vaccine and its manufacture;
2. Protecting new inventions, including the protection of trademarks and know-how, as appropriate; and
3. Out-licensing of the vaccine and production process to manufacturers.

Typically, a vaccine or drug is either manufactured by the developer of the product and/or licensed to a third party for production. In any case, the manufacturer needs to have "assembled" all of the IP required in order to have FTO[10]. For instance, third party manufacturers will need to obtain licenses that cover the developer's IPRs as well as the IPRs of others; ideally, the developer would be in a position to offer a master license that includes all relevant IPRs. Ensuring FTO is an ongoing process, since the patent landscape changes as new patents are issued and old ones expire. FTOs are typically initiated before significant resources have been spent on manufacturing or commercialization, and they are regularly updated. This approach provides for the early identification of key patent holders and it facilitates early licensing discussions or the implementation of alternative strategies.

2.2 OBJECTIVE OF THIS STUDY: A "GLOBAL ACCESS" FTO

This study was carried out for the Pediatric Dengue Vaccine Initiative (PDVI), now called the Dengue Vaccine Initiative (DVI), which is concerned with global access to dengue vaccines. DVI is seeking a clearer understanding of the probable development of both the market and the IP landscape, both of which will influence licensing between vaccine developers and manufacturers, especially in developing countries. As DVI is neither a developer nor manufacturer of vaccines, a detailed and costly legal FTO opinion is not necessary. A "global access" FTO, however, provides DVI with a valuable strategic input allowing it to define appropriate partnership strategies and strengthening its position when negotiating with the vaccine developers.

The objective, of this "global access" FTO, therefore, is:

- to understand how IPRs may affect access to dengue vaccines in developing countries;
- to assess how some of the companies that are developing vaccines will be affected by IPRs and what freedom they have to license the product in the event they need a manufacturing partner; and
- to evaluate the freedom of vaccine developers in developing countries to market their vaccines outside their home countries.

For reasons related to patent searches, the vaccines under development were classified into six different technologies or approaches (see Sections 3.1 to 3.6 for a brief description of each):

1. Traditional live attenuated vaccines;
2. Inactive virus (classic approach);
3. Chimeric live attenuated dengue vaccines;
4. Reverse genetically-engineered, live attenuated vaccines;
5. Recombinant dengue virus protein vaccines; and
6. DNA vaccines.

2.3 METHODOLOGIES

Scientific and technical literature review

The authors reviewed the relevant scientific literature, together with pertinent reports supplied by DVI. This enabled them to identify the principal scientific and technical strategies being pursued by each vaccine developer and the key components of the various vaccines. It should be noted that the specific laboratory protocols were not reviewed and the planned large-scale production methods were not analyzed. This would have been premature and would have exceeded the scope of this review.

Deconstruction of the vaccines under development

Following the scientific and technical review, each of the main candidate vaccines was deconstructed, leading to the creation of a list of the vaccine component parts.

It was assumed that the various entities developing the vaccines under consideration have the appropriate commercial licenses for the enabling technologies, such as polymerase chain reaction (PCR), restriction enzymes, reverse genetics, genetic constructs, promoters, plasmids, various molecular technologies and transformation technologies.

Patent searches

Based on the deconstruction of each of the candidate vaccines, searches for relevant patents were conducted using a combination of readily available patent databases, Among the search databases used, WIPO PATENTSCOPE®, USPTO, esp@cenet® and Patent Storm were free of charge, and Thomson Innovation and MicroPatent® were premium pay-per-view or subscription-based services. A subscription to Aureka® was also made for this review, which was instrumental in developing the three-dimensional results in Section 4. An iterative, redundant patent search was also conducted to ensure that no major patents or patent applications would be missed.

Patent mapping with Aureka® ThemeScape™ MapManager

Mapping programs such as Themescape parses documents and statistically analyzes the key terms, or topics, that those documents have in common. This tool draws on US (United States), DE (Germany), EP (European Patent), GB (United Kingdom), and WO (PCT applications - WIPO) data.

Aureka® Themescape™[11] is a text mining tool that analyzes text in large sets of documents and creates an overview of the subject matter. The analysis is faster and identifies more subject categories than could reasonably be accomplished by a human reader. In addition, results are condensed into a visual representation of the topics that can be further investigated.

Based on the topics in patent documents, Aureka® ThemeScape™ creates interactive, self-organizing content maps that visually provide an overview of patent portfolios while also representing the conceptual relationships among the patent documents. The program identifies the relevant key themes (coordinately expressed topics) and then visually portrays them and their relationship to each other on a contour map. The Aureka® ThemeScape™ map function thus transforms a set of patent documents into a topographical landscape, based on its assessment of a range of categories, themes, and concepts.

By showing where patents exist in relation to other patents, this geographic, big picture view facilitates identification of areas of potential overlap and enables the reader to compare the concentration of efforts within the given technology space. See Annex B for additional, detailed information regarding the Aureka® Themescape™.

Aureka® patent maps for patents related to the six types of advanced stage dengue vaccine technologies were generated based on the 55 patents/patent families deemed most likely to be relevant.

Interviews

Once the key patents had been identified and mapped, the authors interviewed a range of individuals from institutions (both public and private) that were either working on the development of the vaccines or that had out-licensed relevant technologies to be developed. Institutions included Acambis, Biological E, Bio-Manguinhos, the US Center for Disease Control and Prevention, Hawaii Biotech, Inc./Merck & Co, InViragen, National Institutes of Health, Panacea, the Dengue Vaccine Initiative, Sanofi (formerly Sanofi Aventis), and the Walter Reed Army Institute of Research. The first and last authors of this report also visited Butantan, Fiocruz, and the International Vaccine Institute. These interviews allowed the authors to more accurately analyze the relevance of the identified patents and to clarify specific issues. As part of the interviews, the licensing status of each patent was discussed to the fullest possible extent.

2.4 IMPORTANT REMARKS & LIMITATIONS ON THE MEANING OF THE RESULTS

The results of the patent searches are provided in two forms. *First*, in Section 3, the primary patents identified for each of the *technologies* and/or inventors and/or research groups are presented. *Second*, in Section 4, the relevant patents for each of the *products* under development are discussed.

In Section 3, for purposes of clarity, generally only one representative patent was listed, based on a given priority date (as opposed to listing entire patent families, such as Derwent® World Patent Index or INPADOC patent families). Due to the complexities of patent prosecution that often follows a priority document, redundancy with overlapping US patents, applications, and PCT applications was allowed in order to avoid excess, undue and/or overzealous stringency in terms of data inclusion. This approach permits a workable balance, and allows for crisper conclusions but may lead to the exclusion of a small number of patents. Nevertheless, this approach is practical and reflective of the reality that if a license is required for one patent of a given family, negotiations with the same entity are likely to be conducted. Hence, patents that may have been omitted from this study are likely to be included should licensing negotiations be initiated at a later date.

The results presented in this study only include partial vaccine production methodology patents, as most large scale production methods are still in the early stages of development, and/or will be specifically tailored to the downstream requirements of the discrete dengue vaccine technological approaches. For instance, recombinant protein will differ from chimeric viral vaccines in terms of production specifics and scale-up requirements. Hence an update of this “global access” FTO will have to be conducted at some appropriate stage in the future.

In several instances, patents and/or patent applications have been identified that could have broad applicability to all of the dengue vaccine technologies currently in the late production stage and/or clinical trial pipeline.

Identified patents and patent applications were initially classified as given in Table 4.

Following interviews with the various R&D groups, we were able to eliminate most patents in group 2.

Many of the patent documents listed in this report are published patent applications. During the patent prosecution process, claims are subject to change at the PCT or national level. Hence, many claims as written in the patent applications may not be included in issued patents. The re-sult of this would be that fewer patents might be applicable than identified at this stage.

Issued patents and published applications in various countries around the world are also listed. Such data was identified either by searching national or PCT databases. There is a delay of approximately 30 months between PCT and national filing. Therefore, we expect more patents to have entered, or to be entering, the national phase in various countries. Our interviews with the inventor institutions allowed us to reduce such uncertainties.

Although assignees are listed in patents and patent applications, this does not necessarily mean that these are currently the entities holding rights to issue licenses. Our interviews allowed us to ascertain licensor information, which is provided in the relevant sections. Some patents may not need to be licensed because they will expire or have expired before commercialization (e.g., JP1120285, with the application date of November 5, 1987).

Where EP is listed, it is important to note that this may include any of the countries that are members of the European Patent Organization (EPO).

Finally, we predominantly cite WO (i.e. patent applications under PCT) because these numbers and website links concurrently provide national phase information of relevance. Due to different patent statutes and patent laws, claims in the US and PCT application are generally not identical. Hence, in order to precisely know the extent of the patent grant for a given country, it would be necessary to analyze the patent as issued in a given jurisdiction of interest. Patent families are not identical in terms of claim structure; they are related via lineage to a common filing. We do not believe that this limitation materially or substantially affects the conclusions of this report.

Table 4:

CLASSIFICATION OF PATENTS ACCORDING TO THEIR RELEVANCE TO A GIVEN PRODUCT

1. Patents and/or patent applications that appear to be relevant, even "core" to the analysis, and must therefore be carefully considered;

2. Patents and/or patent applications that might be relevant, pending further discussions with specific dengue vaccine development groups and leaders;

3. Patents and/or patent applications that do not appear to be immediately relevant, but should be considered within the context of future developments in dengue vaccines; and

4. Patents that are definitely not relevant.

[10] FTO means that, for a given product or service, at a given point in time, with respect to a given market, no intellectual property or tangible property rights from any third party are infringed. Tangible property limitations may also arise through material transfer agreements on materials used in the development of a product. "Assemble" in this context means to have in-licensed, cross-licensed, or otherwise obtained rights to a particular IP or set of IP protected properties. See Krattiger A., *Freedom to Operate, Public Sector Research and Product-Development Partnerships: Strategies and Risk-Management Options* (2007). And Kowalski S.P., *Freedom to Operate: The Preparations* (2007). And Fenton G., Chi-Ham C., and Boettiger S., *Freedom to Operate: The Law Firm's Approach and Role.* All three in Intellectual Property Management in Health and Agricultural Innovation: A Handbook of Best Practices (eds. Krattiger A., Mahoney R.T., Nelsen L., *et al.*). MIHR: Oxford, United Kingdom, PIPRA: Davis, United States of America, Fiocruz: Rio de Janeiro, Brazil, and *bio*Developments-International Institute: Ithaca, NY. www.ipHandbook.org (2007).

[11] http://www.micropat.com/static/aureka.htm

Section 3:
Principal Dengue Vaccine Approaches

3.1 TRADITIONAL LIVE ATTENUATED VACCINES

3.1.1 Technical Background

Traditional live attenuated vaccines are generated by means of serial passage through cell cultures, with periodic/systematic screening for naturally occurring mutations. A viral vaccine produced via this route offers several distinct advantages, such as durable humoral and cellular immune responses that have a broad antigenic response to both structural and non-structural viral proteins (antigens). However, there are at least some theoretical concerns (see below).

This classic virology-approach has been the workhorse of vaccine development for over a century. For example, more than 60 zears ago, the polio vaccine, for example, was developed via passage through monkeys, and Albert B. Sabin produced a workable dengue vaccine via passage in mouse brain.

In the case of dengue, a more reliable method for production of a live attenuated vaccine has been via serial passage through cell lines (e.g., primary dog kidney (PDK) cells certified as vaccine substrate). This useful method was discovered by Scott Halstead while at the University of Hawaii. With each viral passage, there is a probability of an attenuating point mutation arising in the viral genomes (virions) which are cultured in foreign host cells (e.g., PDK cells).

Used to screen and evaluate the biological properties of the virus after a series of (typically 10) passages, markers of attenuation include:

- temperature-restricted replication;
- small plaque size;
- cytopathic effect;
- mouse neurovirulence; and
- growth in human monocytes.

Vaccine candidates are typically identified at passages ranging from 10 to 50.

A concern with traditional live attenuated viruses includes the possibility of such a virus reverting to a virulent, wild-type phenotype, which is a theoretical yet a potentially very serious event. This could occur, for example, if there were a recombination between viruses that constitute the tetravalent mix. An additional concern with these tetravalent vaccines is viral or immune interference between serotypes.

Key publications: Innis B.L. and Eckels K.H., *Progress in Development of a Live-Attenuated, Tetravalent Dengue Virus Vaccine by the United States Army Medical Research and Materiel Command,* American Journal of Tropical Medicine and Hygiene 69:1-4 (2003).

Bhamarapravati N., Sabchareon A., Yoksan S., Forrat R. and Lang J., *Progress in Live Attenuated, Tetravalent Dengue Vaccine Trials in Thailand*, Journal of Clinical Virology 28:S25-S25 (2003).

3.1.2 Relevance for and Status of Traditional Live Attenuated Vaccines

A tetravalent vaccine candidate has been developed by Mahidol University's vaccine research group, and licensed to Aventis Pasteur. This vaccine was developed by conventional serial passages through PDK cells. The primary sequence of the attenuated strain is known, but the molecular basis for attenuation is not known. This vaccine candidate encountered problems at the clinical trials phase.

A tetravalent vaccine candidate has also been developed at the Walter Reed Army Institute of Research (WRAIR) and licensed to GlaxoSmithKline (GSK). This vaccine was developed by conventional passage techniques, via multiple passages through PDK cells with a final passage through fetal rhesus lung (FRhl) cells. The primary sequence of the attenuated strain is known, but the molecular basis for attenuation is not known. The vaccine is in phase II clinical trials.

Key Publications: Chanthavanich P., Luxemburger C., Sirivichayakul C., Lapphra K., Pengsaa K., Yoksan S., Sabchareon A. and Lang J., *Immune Response and Occurrence of Dengue Infection in Thai Children Three to Eight Years After Vaccination With Live Attenuated Tetravalent Dengue Vaccine*, American Journal of Tropical Medicine and Hygiene 75:26-28 (2006).

Edelman R., Wasserman S.S., Bodison S.A., Putnak R.J., Eckels K.H., Tang D., Kanesa-Thasan N., Vaughn D.W., Innis B.L. and Sun W., *Phase I Trial of 16 Formulations of a Tetravalent Live-Attenuated Dengue Vaccine*, American Journal of Tropical Medicine and Hygiene 69:48-60 (2003).

3.1.3 Key Players, Publications and Patents

- **WRAIR/GSK Biologicals**

Key Scientist(s): K.H. Eckels (WRAIR), J.R. Putnak (WRAIR), B.L. Innis (WRAIR)

R&D Status: WRAIR/GSK Biologicals, tetravalent vaccine is in phase II (it has been evaluated in rhesus monkeys).

Key Publication(s): Eckels K.H., Dubois D.R., Putnak R., Vaughn D.W., Innis B.L., Henchal E.A. and Hoke C.H., *Modification of Dengue Virus Strains by Passage in Primary Dog Kidney Cells: Preparation of Candidate Vaccines and Immunization of Monkeys*, American Journal of Tropical Medicine and Hygiene 69:12-16 (2003).

Core Patent(s)/Patent Applications:

US7217418 Multivalent dengue virus vaccine

Inventors: Eckels, Kenneth H. (Rockville, MD), Putnak, Joseph B. (Silver Spring, MD), Dubois, Doria R. (Wheaton, MD), Innis, Bruce L. (Haverford, PA), Hoke, Charles H. (Columbia, MD), Sun, Wellington (Rockville, MD), Kanesa-Thasan, Niranjan (Rockville, MD)

Assignee: United States of America as represented by the Secretary of the Army (Washington, DC)

Filed: July 24, 2003

US7217418 Multivalent dengue virus vaccine

Inventors: Eckels, Kenneth H. (Rockville, MD), Putnak, Joseph R. (Silver Spring, MD), Dubois, Doria R. (Wheaton, MD), Innis, Bruce L. (Haverford, PA), Hoke, Charles H. (Columbia, MD), Wellington, Sun (Rockville, MD), Kanessa-thasan, Niranjan (Rockville, MD)

Assignee: The United States of America as represented by the Secretary of the Army (Washington, DC)

Filed: March 24, 2000

WO2000058444 Adaptation of virus to vertebrate cells

Inventors: Eckels, Kenneth H. (Rockville, MD), Putnak, Joseph R. (Silver Spring, MD), Innis, Bruce L. (Haverford, PA)

Assignee: The United States of America as represented by the Secretary of the Army (Washington, DC)

Filed: March 24, 2000

US7217418 Multivalent dengue virus vaccine

Inventors: Eckels, Kenneth H. (Rockville, MD), Putnak, Joseph R. (Silver Spring, MD), Dubois, Doria R. (Wheaton, MD), Innis, Bruce L. (Haverford, PA), Hoke, Charles H. (Columbia, MD), Sun, Wellington (Rockville, MD), Kanesa-Thasan, Niranjan (Rockville, MD)

Assignee: US Army Medical Research and Materiel Command

Filed: July 24, 2003

- **Mahidol University/Sanofi Pasteur**

Key Scientist(s): N. Bhamarapravati (Mahidol, Thailand) J. Lang (Aventis Pasteur, France)

R&D Status: Mahidol University/Sanofi Pasteur, no current testing (this vaccine candidate does not induce a balanced immune response and has caused systemic symptoms in vaccine recipients).

Key Publication(s): Bhamarapravati N. and Sutee Y., *Live Attenuated Tetravalent Dengue Vaccine,* Vaccine 18:44-47 (2000).

Core Patent(s)/Patent Applications:

EP1159968 Attenuated strains of dengue virus and their use in a vaccine composition

Publication date: 2001-12-05

Inventor: Bhamarapravat Nath (Thailand), Yoksan Sutee (Thailand)

Applicant: Mahidol University (Thailand)

WO200191790 Vaccine composition

Publication date: 2001-12-06

Inventor: Lang Jean (France), Saluzzo Jean François (France)

Applicant: Aventis Pasteur (France), Lang Jean (France), Saluzzo Jean François (France)

3.2 INACTIVATED VIRUS (CLASSIC APPROACH)

3.2.1 Technical Background

Whole virus inactivated vaccines consist of live virus that is inactivated and then used as the vaccine. Historically, there has been a persistent problem of relatively low level of viral replication in certified mammalian cultures. How-

ever, Putnak and colleagues have largely overcome these obstacles. Now propagated in certified Vero cell cultures, virus is purified on sucrose gradients, inactivated with 0.05% formalin at 22°C, concentrated via ultracentrifugation, and purified on sucrose gradients. These preparations exhibit high titers capable of conferring immunity in mice and monkeys.

Key Publications: Putnak J.R., Coller B.A., Voss G., Vaughn D.W., Clements D., Peters I., Bignami G., Houng H.S., Chen R.C.M., Barvir D.A., Seriwatana J., Cayphas S., Garcon N., Gheysen D., Kanesa-thasan N., McDonell M., Humphreys T., Eckels K.H., Prieels J.P. and Innis B.L., *An Evaluation of Dengue Type-2 Inactivated, Recombinant Subunit, and Live-Attenuated Vaccine Candidates in the Rhesus Macaque Model,* Vaccine 23:4442-4453 (2005).

3.2.2 Relevance for and Status of Inactivated Virus Vaccines (classic approach)

Whole virus inactivated vaccines have been produced at the Walter Reed Army Institute of Research (WRAIR) by Putnak and colleagues.

Key Publications: Putnak R., Cassidy K., Conforti N., Lee R., Sollazzo D., Truong T., Ing. E., Dubois D., Sparkuhl J., Gastle W. and Hoke C., *Immunogenic and Protective Response in Mice Immunized With a Purified, Inactivated, Dengue-2 Virus-Vaccine Prototype Made in Fetal Rhesus Lung-Cells,* American Journal of Tropical Medicine and Hygiene 55:504-510 (1996).

3.2.3 Key Players, Publications and Patents

- **Purified, Inactivated, Dengue Virus-Vaccine (US Army)**

Key Scientist(s): R. Putnak (Walter Reed Army Medical Center, Washington) K.H. Eckels (Walter Reed Army Medical Center Washington)

R&D Status: Preclinical (the DENV-2 vaccine, with alum and other adjuvants, induces a strong immune response in primate models)

Key Publication(s): Putnak R., Barvir D.A., Burrous J.M., Dubois D.R., Dandrea V.M., Hoke C.H., Sadoff J.C. and Eckels K.H., *Development of a Purified, Inactivated, Dengue-2 Virus-Vaccine Prototype in Vero Cells - Immunogenicity and Protection in Mice and Rhesus-Monkeys,* Journal of Infectious Diseases 174:1176-1184 (1996).

Core Patent(s)/Patent Applications:

US6254873 Inactivated dengue virus vaccine

Inventors: Putnak, J. Robert (Silver Spring, MD), Eckels, Kenneth (Rockville, MD), Dubois, Doris R. (Wheaton, MD)

Assignee: The United States of America as represented by the Secretary of the Army (Washington, DC)

Filed: April 17, 1995

US6190859 Method and kit for detection of dengue virus

Inventors: Putnak, J. Robert (Silver Spring, MD), Eckels, Kenneth (Rockville, MD), Dubois, Doria R. (Wheaton, MD), Cassidy, Kevin (Toronto, CA)

Assignee: The United States of America as represented by the Secretary of the Army (Washington, DC)

Filed: April 17, 1995

3.3 CHIMERIC LIVE ATTENUATED DENGUE VACCINES

3.3.1 Technical Background

Chimeric live attenuated vaccines are genetically engineered constructs, typically engineered by replacing the prM and E segments of the cDNA clone of another flavivirus (backbone) with the corresponding genomic segment of the desired virus selected for vaccine development. The backbone (platform) sequence is either selected from a well-characterized attenuated vaccine (e.g., CDC uses the DENV-2 PDK-53 and Acambis uses YFV 17D), or an attenuated wild-type derived via genetic engineering mutations (e.g., NIH uses the genetically engineered attenuated rDen1Δ30 virus). The chimeric virus constitutes the vaccine: it combines a required attenuation phenotype with expression of the target antigens, thus the best of both worlds.

Chimeric live attenuated vaccines have been developed by one of two broad routes. Chimeras are built in such a way that they are either:

1. heterologous (comprised of both dengue and non-dengue genomic viral segments); or
2. homologous (comprised of dengue genomic viral segments derived from different strains/serotypes).

A distinct advantage of genetically engineered chimeric live attenuated vaccines over traditional live attenuated vaccines is the precision of their construction. Tetravalent chimeric vaccine formulations are assembled with standardized attenuated backbone sequences. This eliminates the theoretical possibility associated with traditional vaccines of reversion via intra-vaccine/viral genetic recombi-

nation. In addition, chimeric vaccines may reduce the potential for viral or immune interference among serotypes.

Key Publications of Chimeric Vaccine: Heterologous: Trent D., Guirakhoo F., Arroyo J., Pugachev K., Bedford P., McCarthy K., Kitchner S. and Monath T., *ChimeriVax YF17D/Dengue Tetravalent Vaccine.* Journal of Clinical Virology 28:26-27 (2003).

Guirakhoo F., Kitchener S., Morrison D., Forrat R., McCarthy K., Nichols R., Yoksan S., Duan X.C., Ermak T.H., Kanesa-Thasan N., Bedford P., Lang J., Quentin-Millet M.J. and Monath T.P., *Live Attenuated Chimeric Yellow Fever Dengue Type 2 (ChimeriVax (TM)-DEN2) Vaccine: Phase I Clinical Trial for Safety and Immunogenicity,* Human Vaccines 2:60-67 (2006).

Key Publications of Chimeric Homologous: Huang C.Y.H., Butrapet S., Tsuchiya K.R., Bhamarapravati N., Gubler D.J. and Kinney R.M., *Dengue-2 PDK-53 Virus as a Chimeric Carrier for Tetravalent Dengue Vaccine Development,* Journal of Virology 77:11436-11447 (2003).

Durbin A.P., McArthur J.H., Marron J.A., Blaney J.E., Thumar B., Wanionek K., Murphy B.R., Whitehead S.S., *rDEN2/4 Delta 30(ME), A Live Attenuated Chimeric Dengue Serotype 2 Vaccine, Is Safe and Highly Immunogenic in Healthy Dengue-Naive Adults,* Human Vaccines 2:255-260 (2006).

3.3.2 Relevance for and Status of Chimeric Live Attenuated Dengue Vaccines

Monath and Chambers *et al.* have developed at Acambis a chimeric construct utilizing a yellow fever viral infectious clone (YFV17D) as a vector platform: ChimeriVax™-DEN3. To develop a tetravalent formulation, the prM and E genes from the four dengue serotypes were used to construct four chimeric viral vaccines, with DENV-1-4 cloned into the platform construct. These vaccines are replication competent, genetically stable, and do not become more neurovirulent upon 20 passages in Vero cells. Acambis has exclusively licensed the ChimeriVax™-DEN2 platform technology to Sanofi Pasteur for subsequent development. Acambis obtained two, nonexclusive licenses from NIH to practice the technologies covered by the Lai *et al.* patent family, of which US6676936 is a representative document.

The Washington and St. Louis Universities in St. Louis, Missouri, and Acambis in Cambridge, Massachusetts, have licensed these technologies to Aventis Pasteur, Lyon (YFV [17D] backbone: ChimeriVax™D2). The original owner of these licensed technologies was St. Louis University, which granted an exclusive license to Acambis, which was then exclusively sub-licensed to Sanofi Pasteur along with the entire package of Acambis patents.

At the US Center for Disease Control and Prevention (CDC), Huang, Kinney, and colleagues have developed a dengue-dengue homologous chimera. This chimera was produced via cloning the prM/E genes of DENV-1, 3, and 4 into the DENV-2 16681 PDK-53 virus backbone (originally from Mahidol University, Thailand). Subsequent development of the DEN-1, 3 and 4 chimeras has been pursued by a collaborative effort involving the CDC and Mahidol University. It appears that this research has substantially moved to InViragen (Fort Collins, Colorado). In 2006, the CDC granted InViragen an exclusive license to its DEN-2 PDK-53 chimeras. In addition, InViragen signed a manufacturing agreement with Shantha Biotechnics in Hyderabad, India.

At the US Department of Health/NIH, Whitehead and colleagues have taken a different approach. Using reverse genetic engineering methodologies, they have developed an attenuated virus (e.g., rDen4Δ30). Attenuation was achieved by the introduction of non-lethal deletions into the 3' untranslated region (UTR). Although these products have demonstrated immunogenic potential *per se*, they are not entirely suitable as vaccine candidates. Whereas rDen1Δ30 and rDen4Δ30 appear to be suitable components for the candidate tetravalent vaccine, rDen2Δ30 and rDen3Δ30 have been further genetically engineered into chimeric constructs (rDen2/4Δ30, rDen3/4Δ30) in order to confer suitable attenuation for vaccine development. Hence, the rDen4Δ30 strain has been utilized as the backbone for the assembly of recombinant attenuated dengue-dengue chimeric vaccine candidates containing the 30-nucleotide deletion and the prM/E genes of DEN1, 2, and 3. These vaccine candidates were determined to be attenuated and immunogenic in monkeys. Several industrial sponsors in Asia and Brazil have been awarded nonexclusive licenses for the rDen4Δ30 formulations.

The US Food and Drug Administration (FDA) has also developed a chimeric virus. In this case, the DEN 2-4 prM and E genes were inserted into a DEN-1 backbone attenuated by replacing three nucleotides in the terminal 3' stem structure (DEN2mutF).

In collaboration with the California Institute of Technology, Emory University has developed a chimeric dengue vaccine candidate (YFV-DENV-2 [PR-159] chimera) which protects mice intracranially challenged with DENV-3.

Another organization that is working on heterologous chimeric dengue vaccine candidates is Fiocruz, Brazil (YF-17D flaviviral chimeras).

Key Publications of Chimeric Vaccine: Heterologous: Guirakhoo F., Pugachev K., Zhang Z., Myers G., Levenbook I., Draper K., Lang J., Ocran S., Mitchell F., Parsons M., Brown N., Brandler S., Fournier C., Barrere B., Rizvi F., Travassos A., Nichols R., Trent D. and Monath T.,

Safety and Efficacy of Chimeric Yellow Fever-Dengue Virus Tetravalent Vaccine Formulations in Nonhuman Primates, Journal of Virology 78:4761-4775 (2004).

Guirakhoo F., Pugachev K., Arroyo J., Miller C., Zhang Z.X., Weltzin R., Georgakopoulos K., Catalan J., Ocran S., Draper K. and Monath T.P., *Viremia and Immunogenicity in Nonhuman Primates of a Tetravalent Yellow Fever-Dengue Chimeric Vaccine: Genetic Reconstructions, Dose Adjustment, and Antibody Responses Against Wild-Type Dengue Virus Isolates*, Virology 298:146-159 (2003).

Deauvieau F., Sanchez V., Balas C., Kennel A., De Montfort A., Lang J. and Guy B., *Innate Immune Responses in Human Dendritic Cells Upon Infection by Chimeric Yellow-Fever Dengue Vaccine Serotypes 1-4,* American Journal of Tropical Medicine and Hygiene 76:144-154 (2007).

Jaiswal S., Khanna N. and Swaminathan S., *Replication-Defective Adenoviral Vaccine Vector for the Induction of Immune Responses to Dengue Virus Type 3,* Journal of Virology 77:12907-12913 (2003).

Markoff L., Pang X., Houng H.S., Falgout B., Olsen R., Jones E. and Polo S., *Derivation and Characterization of a Dengue Type 1 Host Range-Restricted Mutant Virus That is Attenuated and Highly Immunogenic in Monkeys*, Journal of Virology 76:3318-3328 (2003).

Van Der Most R.G., Murali Krishna K., Ahmed R. and Strauss J.H., *Chimeric Yellow Fever/Dengue Virus as a Candidate Dengue Vaccine: Quantitation of the Dengue Virus-Specific CD8 T-Cell Response,* Journal of Virology 74:8094-8101 (2000).

Holman D.H., Wang D., Raviprakash K., Raja N.U., Luo M., Zhang J., Porter K.R. and Dong J.Y., *Two Complex, Adenovirus-Based Vaccines That Together Induce Immune Responses to all Four Dengue Virus Serotypes,* Clinical and Vaccine Immunology 14:182-189 (2007).

Key Publications of Chimeric Homologous*:* Kinney R.M., Butrapet S., Chang G.J.J., Tsuchiya K.R., Roehrig J.T., Bhamarapravati N. and Gubler D.J., *Construction of Infectious cDNA Clones for Dengue-2 Virus: Strain 16681 and its Attenuated Vaccine Derivative, Strain PDK-53,* Virology 230:300-308 (1997).

Huang C.Y.H., Butrapet S., Pierro D.J., Chang G.J.J., Hunt A.R., Bhamarapravati N., Gubler D.J. and Kinney R.M., *Chimeric Dengue Type 2 (Vaccine Strain PDK-53)/Dengue Type 1 Virus as a Potential Candidate Dengue Type 1 Virus Vaccine,* Journal of Virology 74:3020-3028 (2000).

Blaney J.E., Sathe N.S., Hanson C.T., Firestone C.Y., Murphy B.R. and Whitehead S.S., *Vaccine Candidates for Dengue Virus Type 1 (DEN1) Generated by Replacement of the Structural Genes of rDEN4 and rDEN4 Delta 30 With Those of DEN1,* Virology Journal 4:23 (2007).

3.3.3 Key Players, Publications and Patents[12]

- **ChimeriVax™-DEN2**

Key Scientist(s): F. Guirakhoo (Acambis Inc., Cambridge, MA 02139) T. Monath J. Lang (Sanofi Pasteur, Marcy Letoile, France)

R&D Status: Acambis/Sanofi Pasteur: Tetravalent, Phase I

Key Publication(s): Guirakhoo F., Kitchener S., Morrison D., Forrat R., McCarthy K., Nichols R., Yoksan S., Duan X.C., Ermak T.H., Kanesa-Thasan N., Bedford P., Lang J., Quentin-Millet M.J. and Monath T.P., *Live Attenuated Chimeric Yellow Fever Dengue Type 2 (ChimeriVax (TM)-DEN2) Vaccine: Phase I Clinical Trial for Safety and Immunogenicity,* Human Vaccines 2:60-67 (2006).

Core Patent(s)/Patent Applications:

US6962708 Chimeric flavivirus vaccines

Inventors: Chambers, Thomas J. (St. Louis, MO), Monath, Thomas P. (Harvard, MA), Guirakhoo, Farshad (Melrose, MA), Arroyo, Juan (S. Weymouth, MA)

Assignee: Acambis, Inc. (Cambridge, MA), St. Louis University (St. Louis, MO)

Filed: July 23, 1998

US6962708 Chimeric flavivirus vaccines

Inventors: Chambers, Thomas J. (St. Louis, MO), Monath, Thomas P. (Harvard, MA), Guirakhoo, Farshad (Melrose, MA)

Assignee: Acambis, Inc. (Cambridge, MA) and St. Louis University (St. Louis, MO)

Filed: December 1, 1999

US20040259224 Tetravalent dengue vaccines

Inventors: Guirakhoo, Farshad (Melrose, MA)

Filed: June 2, 2003

EP1924280 Vaccination against dengue virus infection

Inventor: Monath T.P (US), Guirakhoo F. (US), Kanesa-Thasan N. (US), Ermak T.H. (US), Lang J. (FR), Forrat R. (FR)

Applicant: Acambis Inc. (US), Sanofi Pasteur (FR)

- **Dengue-2 PDK-53 Chimeric Virus Vaccine**

Key Scientist(s): C.Y.H. Huang, InViragen, Fort Collins, CO, R.M. Kinney, InViragen, Fort Collins, CO

R&D Status: Preclinical (phase I in humans is anticipated)

Key Publication(s): Huang C.Y.H., Butrapet S., Tsuchiya K.R., Bhamarapravati N., Gubler D.J. and Kinney R.M., *Dengue-2 PDK-53 Virus as a Chimeric Carrier for Tetravalent Dengue Vaccine Development,* Journal of Virology 77:11436-11447 (2003).

Core Patent(s)/Patent Applications:

US7641909 Avirulent, immunogenic flavivirus chimeras

Inventors: Kinney, Richard M. (Fort Collins, CO), Kinney, Claire Y.H., (Fort Collins, CO), Gubler, Duane J. (Fort Collins, CO), Butrapet, Siritorn (Bangkok, TH), Bhamarapravati, Natth, (Bangkok, TH)

Filed: February 16, 2001

US7641909 Avirulent, immunogenic flavivirus chimeras

Inventors: Kinney, Richard M. (Fort Collins, CO), Kinney, Claire Y. H. (Fort Collins, CO), Gubler, Duane J. (Fort Collins, CO), Butrapet, Siritorn (Bangkok, TH), Bhamarapravati, Natth (Bangkok, TH)

Assignee: The United States of America as represented by the Department of Health and Human Services (Washington, DC)

Filed: February 16, 2001

- **rDEN2/4 Delta 30(ME)**

Key Scientist(s): S.S. Whitehead, Johns Hopkins, Baltimore, MD 21205, NIAID, NIH Bethesda, MD 20892

R&D Status: The Monovalent (DENV 1-4) is in phase I/II. Phase I & II clinical trials have been scheduled by the National Institute of Allergy and Infectious Diseases (NIAID) (phase I by the end of 2008). Additional phase II and III trials are in the planning stages (Butantan). These trials appear to be for the tetravalent reverse genetically engineered (rDen1Δ30, rDen4Δ30) and chimeric constructs (rDen2/4Δ30 and rDen3/4Δ30) vaccine candidate(s).

Key Publication(s): Durbin A.P., McArthur J.H., Marron J.A., Blaney J.E., Thumar B., Wanionek K., Murphy B.R. and Whitehead S.S., *rDEN2/4 Delta 30(ME), A Live Attenuated Chimeric Dengue Serotype 2 Vaccine, is Safe and Highly Immunogenic in Healthy Dengue-Naive Adults,* Human Vaccines 2:255-260 (2006).

Core Patent(s)/Patent Applications:

US20090263424 Development of mutations useful for attenuating dengue viruses and chimeric dengue viruses

Inventors: Whitehead, Stephen S. (Montgomery Village, MD), Murphy, Brian R. (Bethesda, MD), Hanley, Kathryn A. (Bethesda, MD), Blaney, Joseph E. (Frederick, MD)

Assignee: The United States of America, as represented by the Secretary, Department of Health and Human Services (Washington, DC)

Filed: November 21, 2003

- **Yellow Fever (YF17D) Flavivirus Chimera**

Key Scientist(s): R. Galler (Fiocruz, Brazil)

R&D Status: Research, not clinical yet. Their approach is different to others in that the research by Fiocruz (and patent applications) cover two methods of inserting (as opposed to replacing) foreign sequences into the 17D genome: one allows insertions of epitopes (8-36 amino acids) into the surface of the envelope E protein, the other allows insertion of larger segments (up to 300 amino acids) between E and NS1 (intergenic region). These represent conceptually totally different approaches as compared to ChimeriVax.

Key Publication(s): Bonaldo M.C., Garratt R.C., Freire A.S. and Galler R., *Expression of Foreign Protein Epitopes at the Surface of Recombinant Yellow Fever 17D Viruses Based on Three Dimensional Modeling of its Envelope Protein,* 10th IUBMB Conference and 36th Annual Meeting of the SBBQ, May 21 to 25, 2007, Abstract (2007).

Core Patent(s)/Patent Applications:

WO2007051267 Method for the Production of Recombinant Virus, DNA Constructs, Recombinant Virus and Vaccine Compositions

Inventors: Bonaldo MC (BR), Galler R (BR)

Applicant: Fiocruz Fundação Oswaldo Cruz (BR)

Publication date: 2007-05-10

The Fiocruz construct differs from the ChimeriVax platform of Acambis by following a different approach to inserting

foreign sequences into the YF genome. ChimerVax is a platform that covers the replacement of structural genes of YF 17D virus with those equivalent from other flaviviruses, whereas the Fiocruz technology covers the insertion of certain sequences. To what degree there might be overlap in claims has not been analyzed.

- **GenPhar**

GenPhar has a long history of working closely with the US Government to provide needed vaccines for biodefense applications. GenPhar has established working relationships with the National Institutes of Health, the US Army Medical Research Institute of Infectious Diseases, and the US Naval Medical Research Center[13]. The GenPhar technology involves a hybrid adenoviral platform.

Key Scientist(s): Danher Wang and Jianyun D; US Navy

R&D Status: Research, development of vaccine platform involving hybrid adenovirus.

Key Publication(s): Raja N.U., Holman D.H., Wang D., Raviprakash K., Juompan L.Y., Deitz S.B., Luo M., Zhang J., Porter K.R. and Dong J.Y., *Induction of Bivalent Immune Responses by Expression of Dengue Virus Type 1 and Type 2 Antigens From a Single Complex Adenoviral Vector,* American Journal of Tropical Medicine and Hygiene 76(4):743-751 (2007).

Two tentatively identified patents that are assigned to GenPhar:

US6544780 Adenovirus vector with multiple expression cassettes

Inventors: Wang; Danher (Mt. Pleasant, SC)

Assignee: GenPhar, Inc. (Mt. Pleasant, SC)

Filed: June 2, 2000

US6964762 Composition and method for stimulating immune response to pathogen using complex adenoviral vector

Inventors: Wang; Danher (Mt. Pleasant, SC), Dong; Jianyun (Mt. Pleasant, SC)

Assignee: Genphar, Inc. (Mt. Pleasant, SC)

Filed: December 19, 2002

3.4 REVERSE GENETICALLY-ENGINEERED, LIVE ATTENUATED VACCINES

3.4.1 Technical Background

A method that facilitates the production of live virus from cloned cDNA; reverse genetic engineering involves rationally modifying the viral genome to confer attenuation via the introduction of specific virulence-attenuating mutations. In brief, the technological steps of reverse genetics are:

1. Synthesis of full length cDNA of the flaviviral genomic RNA;
2. Modification of the cDNA via molecular engineering;
3. Re-derivation of RNA from transfected cells; and
4. Derivation of infectious particles when the resulting RNA is transfected into permissive Vero cells.

An important advantage of this approach over traditional passage attenuation methodologies is precision. Instead of random mutations, which might arise in the critical E-protein (antigenic determinant regions), reverse genetics technology provides the tools whereby mutations can be rationally designed and developed, conferring attenuation while retaining immunogenicity.

Key Publications: Durbin A.P., Karron R.A., Sun W., Vaughn D.W., Reynolds M.J., Perreault J.R., Thumar B., Men R., Lai C.J., Elkins W.R., Chanock R.M., Murphy B.R. and Whitehead S.S., *Attenuation and Immunogenicity in Humans of a Live Dengue Virus Type-4 Vaccine Candidate With a 30 Nucleotide Deletion in its 3 '-Untranslated Region,* American Journal of Tropical Medicine and Hygiene 65:405-413 (2001).

3.4.2 Relevance for and Status of Reverse Genetically-Engineered, Live Attenuated Vaccines

Utilized by several research groups, this approach has been applied to three strategies to generate attenuated viruses vaccine candidates:

1. The molecular attenuation of the dengue virus by introducing selected mutations/deletions;
2. The insertion of dengue structural genes (prM/E) into the genomic backbone of a classically attenuated dengue strain (see chimeric vaccines); and
3. The insertion of prM/E into the genomic backbone of the yellow fever (YF) 17D vaccine strain as a vector (see chimeric vaccines).

A group led by Dr. Stephen Whitehead at the US NIAID has produced a recombinant dengue virus that harbors a 30 nucleotide deletion in the 3' non-coding region for each of the four serotypes (the rDen1Δ30, rDen2Δ30, rDen3Δ30, and rDen4Δ30 viruses). Using modern genetic

engineering technology (reverse genetics), presumed pathogenic viral genomic sequences were removed, and these were then developed into clones of the engineered virus capable of replication in Vero cell-lines. These have been used directly as vaccine candidates, or as components of chimeric constructs (see chimeric vaccines). Although they have demonstrated immunogenic potential *per se*, they are not entirely suitable as vaccine candidates. Whereas rDen1Δ30 and rDen4Δ30 appear to be suitable components for the candidate tetravalent vaccine, rDen2Δ30 and rDen3Δ30 have been further genetically engineered into chimeric constructs (rDen2/4Δ30, rDen3/4Δ30) in order to confer suitable attenuation for vaccine development.

Researchers at the US FDA (Markoff and colleagues) have introduced several mutations into the stem and loop structure of the terminus of the 3' untranslated region of the viral genome to produce a molecularly engineered attenuated virus (DEN2mutF). Although replication is defective in mosquito cell lines, this mutant replicates well in monkey cells and is both attenuated and immunogenic in challenged monkeys.

Key Publications: Blaney J.E., Durbin A.P., Murphy B.R. and Whitehead S.S., *Development of a Live Attenuated Dengue Virus Vaccine Using Reverse Genetics,* Viral Immunology 19:10-33 (2006).

McArthur J.H., Marron J.A., Thumar B., Wanionek K.A., Lovchik J.M., Blaney J.E., Murphy B.R., Whitehead S.S. and Durbin A.P., *The Live Attenuated Dengue Serotype 2 Vaccine rDEN4 Delta 30 is Safe and Immunogenic in Healthy Volunteers,* American Journal of Tropical Medicine and Hygiene 75:281-283 (2006).

3.4.3 Key Players, Publications and Patents

- **Reverse Genetics Technology**

Key Scientist(s): C.J. Lai

R&D Status: N/a

Key Publication(s): Lai C.J., Zhao B.T, Hori H., and Bray M., *Infectious RNA Transcribed From Stably Cloned Full-length cDNA of Dengue Type 4 Virus,* Proceedings of the National Academy of Sciences, U.S.A., June 15, 1991; 88(12): 5139–5143 (1991).

Core Patent(s)/Patent Applications:

US6676936 Chimeric and/or growth-restricted flaviviruses

Inventors: Lai, Ching-Juh (Bethesda, MD), Bray, Michael (Bethesda, MD), Pletnev, Alexander G. (Rockville, MD), Men, Ruhe (Kensington, MD), Zhang, Yi-Ming (Bethesda, MD), Eckels, Kenneth H. (Bethesda, MD), Chanock, Robert M. (Bethesda, MD)

Assignee: The United States of America as represented by the Department of Health and Human Services (Washington, DC)

Filed: May 27, 1994

US6676936 Chimeric and/or growth-restricted flaviviruses

Inventors: Lai, Ching-Juh (Bethesda, MD), Bray, Michael (Bethesda, MD), Pletnev, Alexander G. (Rockville, MD), Men, Ruhe (Rockville, MD), Zhang, Yi-Ming (Falls Church, VA), Eckels, Kenneth H. (Rockville, MD), Chanock, Robert M. (Bethesda, MD)

Assignee: The United States of America as represented by the Department of Health and Human Services (Washington, DC)

Filed: August 18, 2000

US6676936 Chimeric and/or growth-restricted flavivirus

Inventors: Lai Ching-Juh (US), Bray Michael (US), Pletnev Alexander G (US), Men Ruhe (US), Pethel Michele (US)

Assignee: US Health (US)

Publication: 1993-04-01

Key scientist(s): P. Palese, The Mount Sinai School of Medicine of the City University of New York (New York, NY)

R&D Status: N/a

Key Publication(s): Palese P., *Making Better Influenza Virus Vaccines*? published in Emerging Infectious Diseases 2006:61-65 (2006).

Core Patent(s)/Patent Applications:

US5166057 Recombinant negative strand RNA virus expression systems

Inventors: Palese, Peter (Leonia, NJ), Parvin, Jeffrey D. (Belmont, MA), Krystal, Mark (Leonia, NJ)

Assignee: The Mount Sinai School of Medicine of the City University of New York (New York, NY)

Filed: May 22, 1990

US5578473 Recombinant negative strand RNA virus

Inventors: Palese, Peter (Leonia, NJ), Parvin, Jeffrey D. (Belmont, MA), Krystal, Mark (Leonia, NJ)

Assignee: Aviron, Inc. (Mountain View, CA)

Filed: March 10, 1994

US5820871 Recombinant negative strand RNA virus expression systems and vaccines

Inventors: Palese, Peter (Leonia, NJ), Garcia-Sastre, Adolfo (New York, NY)

Assignee: The Mount Sinai School of Medicine of the City University of New York (New York, NY)

Filed: June 6, 1995

US5854037 Recombinant negative strand RNA virus expression systems and vaccines

Inventors: Palese, Peter (Leonia, NJ), Garcia-Sastre, Adolfo (New York, NY)

Assignee: The Mount Sinai School of Medicine of the City University of New York (New York, NY)

Filed: June 1, 1994

US6544785 Helper-free rescue of recombinant negative strand RNA viruses

Inventors: Palese, Peter (Leonia, NJ), Garcia-Sastre, Adolfo (New York, NY), Brownlee, Georg G. (Oxford, GB)

Assignee: Mount Sinai School of Medicine of New York University (New York, NY)

Filed: July 14, 2000

US6649372 Helper-free rescue of recombinant negative strand RNA virus

Inventors: Palese, Peter (Leonia, NJ), Garcia-Sastre, Adolfo (New York, NY), Brownlee, Georg G. (Oxford, GB), Fodor, Ervin (Oxford, GB)

Assignee: Mount Sinai School of Medicine of New York University (New York, NY)

Filed: November 28, 2000

- **rDEN4 Delta 30**

Key scientist(s): SS Whitehead (NIH, NIAID, Bethesda, MD)

R&D Status: Phase I & II clinical trials have been scheduled by NIAID (phase I by the end of 2008). Additional phase II and III trials are in the planning stages (Butantan). These trials appear to be for the tetravalent reverse genetically engineered (rDen1Δ30, rDen4Δ30) and chimeric constructs (rDen2/4Δ30 and rDen3/4Δ30) vaccine candidate(s).

Key Publication(s): Durbin A.P., Whitehead S.S., McArthur J., Perreault J.R., Blaney J.E., Thumar B., Murphy B.R. and Karron R.A., *rDEN4 Delta 30, a Live Attenuated Dengue Virus Type 4 Vaccine Candidate, is Safe, Immunogenic, and Highly Infectious in Healthy Adult Volunteers,* Journal of Infectious Diseases 191:710-718 (2005).

Core Patent(s)/Patent Applications:

US6685948 Dengue viruses that are replication-defective in mosquitoes for use as vaccines

Inventors: Zeng, Lingling (Brooklyn, NY), Markoff, Lewis (Bethesda, MD)

Assignee: The United States of America as represented by the Department of Health and Human Services (Washington, DC)

Filed: March 2, 2001

US20090258036 Dengue tetravalent vaccine containing a common 30 nucleotide deletion in the 3'-UTR of dengue types 1, 2, 3, and 4, or antigenic chimeric dengue viruses 1, 2, 3, and 4

Inventor: Whitehead SS (US), Murphy BR (US), Markoff L (US), Falgout B (US), Blaney J (US), Hanley K (US)

Applicant: US Government (US)

Publication date: 2003-11-13

3.5 RECOMBINANT DENGUE VIRUS PROTEIN VACCINES

3.5.1 Technical Background

In recombinant dengue virus protein vaccines, subunit proteins (primarily E proteins cloned into several expression systems) serve as antigens. In other words, the protein

itself constitutes the vaccine. In general, the humoral immune response for these vaccines tends to predominate over cellular response: most protein-based vaccines formulated with adjuvant primarily stimulate CD4 cells, which elicit B cell differentiation and antibody synthesis and not the CD8 cytotoxic T-cell response. Hence, recombinant protein vaccines share many, if not all, of the limitations of inactivated DENV vaccines.

Key Publications: Coller B.A., Lieberman M., Putnak J.R., Clements D., Ogata S., Thorne M., Martyak T., Chang D., Lehrer Ang T. and Weeks-Levy C., *Safe, Effective, Recombinant Subunit Vaccine for Protection Against Dengue Virus Induced Disease,* American Journal of Tropical Medicine and Hygiene 75:281-281 (2006).

Lazo L., Hermida L., Zulueta A., Sanchez J., Lopez C., Silva R., Guillen G. and Guzman M.G., *A Recombinant Capsid Protein From Dengue-2 Induces Protection in Mice Against Homologous Virus*, Vaccine 25:1064-1070 (2007).

3.5.2 Relevance for and Status of Recombinant Dengue Virus Protein Vaccines

Researchers at Hawaii Biotech, Inc. (now owned by Merck & Co.) have developed a recombinant, subunit, tetravalent vaccine for the dengue virus. Produced in *Drosophila* cells (Drosophila S2 expression system which maintains glycosylation and tertiary conformation), the vaccine is composed of fusion proteins. Four truncated dengue serotype subunit proteins (the NH2-terminal 80% of the E-protein) are joined to the complete NS1 protein of DEN3. The expressed proteins maintain the native tertiary structure critical for generating an immune response. Furthermore, the recombinant proteins do not seem to interfere with each other when given in combination (in contrast to live-attenuated vaccine candidates). The Drosophila S2 cells, obtained from the American Type Culture Collection (ATCC), are in the public domain. The vaccine is formulated in proprietary adjuvant (GPI-0100), among others. The specific adjuvant for the commercial product has not been determined as yet. Hawaii Biotech maintains the patent family, of which US6080725 is a representative document, on this adjuvant. These patents were acquired via the Advantogen merger. However, they subsequently switched to alum as the adjuvant.

Led by Mune and Guzman, a group working at the Center for Genetic Engineering and Biotechnology, Havana, Cuba, has developed a recombinant truncated E protein of the Den 4 virus expressed in *Pichia pastoris*. Although immunogenic, this antigen provided only partial protection in challenged monkeys. In addition, structural subunits of the DEN E protein have been fused to the meningococcal P64k carrier protein to produce a bacterial (*E. coli*) expression system.

In the late 1980s and early 1990s, Putnak, Eckels, and colleagues at the WRAIR conducted experiments to produce dengue virus structural and non-structural proteins (NS1) in a recombinant baculovirus (Baculovirus/Sf9) system. DEN-1 virus envelope glyocoproteins expressed in this system elicited an immune response in challenged mice.

Although not viruses *per se*, virus-like particles (VLPs) are like live viruses insofar as they stimulate and prime both B-cell differentiation and antibody synthesis, as well as the cytotoxic T-cell response. VLPs consist of viral structural proteins (prM/M and E) that are self-assembled into polymeric particles in recombinant cell cultures. Primarily worked on by Konishi and colleagues, VLPs have mostly been developed for potential vaccine strategies against West Nile virus, Japanese encephalitis, and tick-borne encephalitis, with the dengue genome functioning as the carrier backbone in (or platform of) the hybrid constructs.

Relevant Publications: Simmons M., Murphy G.S. and Hayes C.G., *Short Report: Antibody Responses of Mice Immunized With a Tetravalent Dengue Recombinant Protein Subunit Vaccine,* American Journal of Tropical Medicine and Hygiene 65:159-161 (2001).

Zhang Z.S., Yan Y.S., Weng Y.W., Huang H.L., Li S.Q., He S. and Zhang J.M., *High-Level Expression of Recombinant Dengue Virus Type 2 Envelope Domain III Protein and Induction of Neutralizing Antibodies in BALB/C Mice*, Journal of Virological Methods 143:125-131 (2007).

Wei H.Y., Jiang L.F., Xue Y.H., Fang D.Y. and Guo H.Y., *Secreted Expression of Dengue Virus Type 2 Full-Length Envelope Glycoprotein in Pichia pastoris,* Journal of Virological Methods 109:17-23 (2003).

Bisht H., Chugh D.A., Raje M., Swaminathan S. and Khanna N., *Recombinant Dengue Virus Type 2 Envelope/Hepatitis B Surface Antigen Hybrid Protein Expressed in Pichia pastoris Can Function as a Bivalent Immunogen,* Journal of Biotechnology 99:97-110 (2003).

Chan L.C.L., Young P.R., Bletchly C. and Reid S., *Production of the Baculovirus-Expressed Dengue Virus Glycoprotein NS1 can be Improved Dramatically With Optimised Regimes for Fed-Batch Cultures and the Addition of the Insect Moulting Hormone, 20-Hydroxyecdysone,* Journal of Virological Methods 105:87-98 (2003).

Staropoli I., Frenkiel M.P., Megret F. and Deubel V., *Affinity-Purified Dengue-2 Virus Envelope Glycoprotein Induces Neutralizing Antibodies and Protective Immunity in Mice,* Vaccine 15:1946-1954 (1997).

White L.J., Parsons M.M., Whitmore A.C., Williams B.M., de Silva A. and Johnston R.E., *An Immunogenic and Pro-*

tective Alphavirus Replicon Particle-Based Dengue Vaccine Overcomes Maternal Antibody Interference in Weanling Mice, Journal of Virology 81:10329-10339 (2007).

3.5.3 Key Players, Publications and Patents[14]

- **Recombinant Subunit Vaccine for Protection Against Dengue Virus**

Key Scientist(s): Beth Ann Coller, Hawaii Biotech, Aiea, HI

R&D Status: Clinical trials are scheduled to start in 2009.

Key Publication(s): Coller B.A., Lieberman M., Putnak J.R., Clements D., Ogata S., Thorne M., Martyak T., Chang D., Lehrer Ang T. and Weeks-Levy C., *Safe, Effective, Recombinant Subunit Vaccine for Protection Against Dengue Virus Induced Disease,* American Journal of Tropical Medicine and Hygiene 75:281-281 (2006).

Core Patent(s)/Patent Applications:

US6749857 Recombinant dimeric envelope vaccine against flaviviral infection

Inventors: Peters ID (Bozeman, MT), Coller BAG (Woluwe Saint Lambert, BE), McDonell M (Bogart, GA), Ivy JM (College Station, TX), Harada K (Honolulu, HI)

Assignee: Hawaii Biotechnology Group, Inc. (Aiea, HI)

Filed: August 18, 1999

US6432411 Recombinant envelope vaccine against flavivirus infection

Inventors: Ivy J (College Station, TX), Bignami G (Honolulu, HI), McDonell M (Bogart, GA), Clements DE (Honolulu, HI), Coller BAG (Woluwe Saint Lambert, BE)

Assignee: Hawaii Biotechnology Group (Aiea, HI)

Filed: July 13, 1999

US6165477 Subunit immonogenic composition against dengue infection

Inventors: Ivy J (Kailua, HI), Nakano E (Hon., HI), Clements D (Honolulu, HI)

Assignee: Hawaii Biotechnology Group, Inc. (Aiea, HI)

Filed: August 20, 1997

- **Recombinant Dengue Capsid/Envelope Protein Vaccines**

Key Scientist(s): M.G. Guzman, Autopista Novia Med, Havana, Cuba, Genet Engn & Biotechnol Ctr, Havana, Cuba

R&D Status: Likely still preclinical

Key Publication(s): Guzman M.G., Rodriguez R., Rodriguez R., Hermida L., Alvarez M., Lazo L., Mune M., Rosario D., Valdes K., Vazquez S., Martinez R., Serrano T., Paez J., Espinosa R., Pumariega T. and Guillen G., *Induction of Neutralizing Antibodies and Partial Protection From Viral Challenge in Macaca Fascicularis Immunized with Recombinant Dengue 4 Virus Envelope Glycoprotein Expressed in Pichia pastoris*, American Journal of Tropical Medicine and Hygiene 69:129-134 (2003).

Core Patent(s)/Patent Applications:

US20080311157 Dengue virus capsid protein which induces a protective response and pharmaceutical composition

Publication date: 2007-03-22

Inventor: Lazo VL (CU), Hermida CL (CU), Lopez AC (CU), Sierra VB (CU), Vazquez RS (CU), Valdez PI (CU), Guillen NGE (CU), Guzman TMG (CU), Zulueta MA (CU)

Applicant: Centro de Ingenieria Genetica y Biotecnologia (Cuba)

US7566457 Chimeric proteins that induce effects directed against viruses

Inventors: Cruz LH (CU), Diaz RR (CU), Vazquez LL (CU, Morales AZ (CU), Abarrategui CL (CU), Prado IV (CU), Silva Rodriguez RC. (CU), Santiago GC (CU), Guillen Nieto G E (CU), Guzman TMG (CU), Sierra Vazquez BC (CU), Espinosa Perez R (CU)

Assignee: Centro de Ingenieria Genetica y Biotecnologia (Cuba)

Filed: February 13, 2007

Recombinant Baculovirus (Baculovirus/Sf9) System

Key Scientist(s): JR Putnak, EP Kelly and AD King, WRAIR, Washington, D.C.

R&D Status: Not known

Key Publication(s): None identified.

Core Patent(s)/Patent Applications:

US7265215 Recombinant vaccine against dengue virus

Inventors: Kelly EP (Takoma Park, MD), King AD (Washington, DC)

Assignee: The United States of America as represented by the Secretary of the Army (Washington, DC)

Filed: December 21, 1999

US7265215 Recombinant dengue virus DNA fragment

Inventors: Kelly EP (Takoma Park, MD), King AD (Washington, DC)

Assignee: The United States of America as represented by the Secretary of the Army (Washington, DC)

Filed: July 20, 1995

US6117640 Recombinant vaccine made in *E. coli* against dengue virus

Inventors: Srivastava AK (Silver Spring, MD), Putnak JR (Silver Spring, MD), Hoke CH (Columbia, MD), Warren RL (Brookville, MD)

Assignee: The United States of America as represented by the Secretary of the Army (Washington, DC)

Filed: May 2, 1995

3.6 DNA VACCINES

3.6.1 Technical Background

With DNA vaccines, the substance injected is literally "naked DNA." This "naked DNA" is in the form of a recombinant plasmid that migrates into the host cells. Cellular protein machinery then synthesizes antigens encoded by the vaccine plasmid: in other words, a DNA vaccine expresses its antigen-coding sequences intra-cellularly. DNA vaccines, therefore, are dynamic agents, more akin to live viral vaccines than to non-replicating vaccines such as purified inactivated viruses or protein antigens.

Since its discovery and introduction in the early 1990s, DNA vaccine technology has been studied extensively in various infectious diseases. It has been shown to be effective in several virus systems (HIV, hepatitis B, rabies, influenza). DNA vaccines stimulate both long-term cell-mediated and humoral immune responses, in many cases effectively mimicking the effects of live (replicating) vaccines. However, since DNA vaccines drive the intracellular synthesis of antigens, major histocompatibility complex class 1 (MHC-1) antigen presentation is favored, leading to a greater cellular immune response.

DNA engineering of plasmids permits significant precision in design, making the co-delivery of targeting signals and immune modulators possible, along with the specific antigens that can be engineered for greater efficacy (for example, innovatively engineered fusion proteins).

Advantages of DNA vaccines include their non-replicating nature and long-term stability in storage, which would greatly facilitate distribution to remote endemic regions where cold storage facilities might be less than optimal. Also, DNA vaccines entail relatively low production costs. Furthermore, unlike live, attenuated viral vaccines, DNA vaccines cannot revert to a virulent strain.

Potential disadvantages of DNA vaccines include several theoretical concerns. Immunized vaccine DNA might integrate into the chromosomal DNA of the host cell, possibly leading to the activation of oncogenes or the inactivation of tumor suppressor genes, which would then lead to the development of malignancies. In addition, DNA vaccines might induce long-term immunological tolerance and also the production of anti-DNA antibodies that could contribute to the development of autoimmune disorders. However, these concerns have not been empirically demonstrated.

Key Publications: Donnelly J.J., Wahren B. and Liu M.A., *DNA Vaccines: Progress and Challenges,* The Journal of Immunology 175:633-639 (2005).

3.6.2 Relevance for and Status of DNA Dengue Vaccines

Although several groups around the world have conducted at least preliminary, laboratory research on DNA vaccines for dengue, only two identified research groups appear to be involved in the development of a dengue DNA vaccine that has the potential to progress to clinical trials.

First, the group led by Raviprakash at the US Naval Medical Research Center, has used DNA shuffling and screening technologies to produce chimeric DNA constructs expressing antigens from all four dengue serotypes. Several of these have been evaluated in *rhesus macaque*. Vaccinated monkeys developed antibodies that neutralized all four dengue serotypes *in vitro*. When challenged with live dengue-1 or dengue-2 virus, partial protection against dengue-1 was observed.

Second, the group of Konishi, Kosugi and Imoto, from Kobe University School of Medicine, Japan, has developed a dengue tetravalent DNA vaccine consisting of plasmids expressing pre-membrane and envelope genes of each of four serotypes of dengue viruses. Mice, immunized twice with the tetravalent vaccine, developed neutralizing antibodies against all serotypes, with no interference among the four components included in this combination vaccine.

Hence, as these studies have demonstrated, it is theoretically possible to generate a DNA vaccine wherein the plasmid construct would code for a chimeric protein that expresses antigenic sites for each of the four dengue serotypes, making it essentially a tretravalent vaccine. The stability of the DNA vaccines could greatly facilitate their distribution to low-income countries where cold storage capabilities might be less than optimal. Such countries are also predominant in the geographic regions where dengue is endemic.

All DNA vaccines still appear to be in the preclinical stages of development. Studies have been conducted in mice and monkeys.

Key Publications: Raviprakash K., Apt B., Brinkman A., Skinner C., Yang S., Dawes G., Ewing D., Wu S.J., Bass S., Punnonen J. and Porter K., *A Chimeric Tetravalent Dengue DNA Vaccine Elicits Neutralizing Antibody to all Four Virus Serotypes in Rhesus Macaques, Virology* 353:166-173 (2006).

Konishi E., Kosugi S. and Imoto J., *Dengue Tetravalent DNA Vaccine Inducing Neutralizing Antibody and Anamnestic Responses to Four Serotypes in Mice,* Vaccine 24:2200-2207 (2006).

3.6.3 Key Players, Publications and Patents

- **United States (US) Naval Medical Research Center, United States of America**

Key Scientist(s): K. Raviprakash, the US Naval Medical Research Center

R&D Status: The US Naval Medical Research Center (NMRC) is a major player in dengue vaccine research, and appears to be the leader in the field for dengue DNA vaccines. These vaccines appear to be in preclinical, primate-model, trials. In addition to being a major player in dengue vaccine research, the US NMRC is also a leader in global dengue surveillance.

Key Publication(s): Blair P.J., Kochel T.J., Raviprakash K., Guevara C., Salazar M., Wu S.J., Olson J.G. and Porter K.R., *Evaluation of Immunity and Protective Efficacy of a Dengue-3 Premembrane and Envelope DNA Vaccine in Aotus Nancymae Monkeys,* Vaccine 24:1427-1433 (2006).

Core Patent(s)/Patent Applications:

US6455509 Dengue nucleic acid vaccines that induce neutralizing antibodies

Inventors: Kochel, Tadeusz J. (Frederick, MD), Porter, Kevin R. (Gaithersburg, MD), Raviprakash, Kanakatte (Silver Spring, MD), Hoffman, Stephen L. (Gaithersburg, MD), Hayes, Curtis G. (Frederick, MD)

Assignee: The United States of America as represented by the Secretary of the Navy (Washington, DC)

Filed: June 4, 1997

- **Kobe University, School of Medicine, Japan**

Key Scientist(s): E Konishi

R&D Status: Preclinical, in mouse models.

Key Publication(s): Konishi E., Kosugi S. and Imoto J.I., *Dengue Tetravalent DNA Vaccine Inducing Neutralizing Antibody and Anamestic Responses to Four Serotypes in Mice,* Vaccine 24:2200-2207 (2006).

Core Patent(s)/Patent Applications

JP2004307477 Method for enhancing neutralization antibody-inducing ability of transgenic vaccine and method for administering vaccine

Publication date: 2004-11-04

Inventor: Konishi Eiji

Applicant: Kobe University, Japan

JP2005015355 Method for increasing amount of antigen produced from DNA vaccine, method for administering DNA vaccine and method for detecting antigen produced by DNA vaccine

Publication date: 2005-01-20

Inventor: Konishi Eiji

Applicant: Kobe University, Japan

- **United States Department of Health and Human Services, United States of America**

Key Scientist(s): G.J.J. Chang, US Department of Health and Human Services (DHHS), United States of America

R&D Status: Not known. Likely not applicable as this line of vaccine development does not appear to be the major focus of the efforts of the US DHHS dengue vaccine research group.

Key Publication(s): Chang G.J.J., Hunt A.R., Holmes D.A., Springfield T., Chiueh T.S., Roehrig J.T. and Gubler D.J., *Enhancing the Biosynthesis and Secretion of Premem-*

brane and Envelope Proteins by the Chimeric Plasmid of Dengue Virus Type 2 and Japanese Encephalitis Virus, Virology 306:170-180 (2003).

Core Patent(s)/Patent Applications:

US7632510 Nucleic acid vaccines for prevention of flavivirus infection

Publication date: 2002-10-17

Inventor: Chang Gwong-Jen J (US)

Applicant: Government of the US, US Department of Health, Chang Gwong-Jen J (US)

Licensing status: Not known.

- **Oswaldo Cruz Foundation, Department of Biochemistry and Molecular Biology, Brazil**

Key scientist(s): S.M. Costa, Fiocruz, Brazil

R&D Status: DNA vaccines incorporating the NS1 gene have been shown to elicit an immune response in mice.

Key Publication(s): Costa S.M., Azevedo A.S., Paes M.V., Sarges F.S., Freiere M.S. and Alves A.M.B., *DNA Vaccines Against Dengue Virus Based on the NS1 Gene: The Influence of Different Signal Sequences on the Protein Expression and its Correlation to the Immune Response Elicited in Mice,* Virology 358:413-423 (2007).

Core Patent(s)/Patent Applications: No documents found.

- **Queensland University and Australian Army Malarial Institute, Australia**

Key Scientist(s): M.P. Reid and J.G. Aaskov, Queensland University, Australia

R&D Status: Not known

Key Publication(s): Liew S.C., Reid M.P. and Aaskov J.G., *Development of a Tetravalent Dengue DNA Vaccine,* Journal of Clinical Virology 28:S31-S33 (2003).

Core Patent(s)/Patent Applications: No documents found.

- **National Defense Medical Center, Taiwan (Province of China)**

Key Scientist(s): HK Sytwu, National Defense Medical Center, Taiwan (Province of China)

R&D Status: Not known

Key Publication(s): Wu S.F., Liao C.L., Lin Y.L., Yeh C.T., Chen L.K., Huang Y.F., Chou H.Y., Huang J.L., Shaio M.F. and Sytwu H.K., *Evaluation of Protective Efficacy and Immune Mechanisms of Using a Non-structural Protein NS1 in DNA Vaccine Against Dengue-2 Virus in Mice,* Vaccine 21:3919-3929 (2003).

Core Patent(s)/Patent Applications: No documents found.

- **Medical Biotechnology Unit (BIOTEC), Thailand**

Key Scientist(s): C. Puttikhunt, Medical Biotechnology Unit (BIOTEC), Bangkok, Thailand

R&D Status: Not known

Key Publication(s): Puttikhunt C., Kasinrerk W., Srisa-ad S., Duangchinda T., Silakate W., Moonsom S., Sittisombut N. and Malasit P., *Production of Anti-dengue NS1 Monoclonal Antibodies by DNA Immunization,* Journal of Virological Methods 109:55-61 (2003).

Core Patent(s)/Patent Applications: No documents found.

- **Walter Reed Army Institute of Research, United States of America**

Key Scientist(s): R. Putnak, B. Innis, D. Vaughn, Walter Reed Army Institute of Research (WRAIR), United States of America

R&D Status: Not known. Likely not applicable as this line of vaccine development does not appear to be the major focus of the efforts of the WRAIR dengue vaccine research group.

Key Publication(s): Putnak R., Fuller J., Vanderzanden L., Innis B. and Vaughn D., *Vaccination of Rhesus Macaques Against Dengue-2 Virus with a Plasmid DNA Vaccine Encoding the Viral Pre-membrane and Envelope Genes*, American Journal of Tropical Medicine and Hygiene 68:469-476 (2003).

Core Patent(s)/Patent Applications: No documents found.

[12] In addition to the patents listed in this section, the following are also central to this technology: US 6184024, US6589531, US5744140, US6869793.

[13] http://tiny.cc/5pbjhw

[14] In addition to the patents listed in this section, the following are also relevant to this technology but not considered relevant to the specific product under development: WO03048184, US200601596990196376, and US2006 0073164.

Section 4:
Patent Activity Analysis

4.1 INTRODUCTION: OVERALL DENGUE VACCINE PATENTS

Our search of dengue patents yielded a list of nearly 3,800 patent families with dengue in the abstract, title, or text of the patent applications or issued patents in the United States of America. Figure 2 shows the resulting landscape, with patents related to vaccines highlighted in white dots.

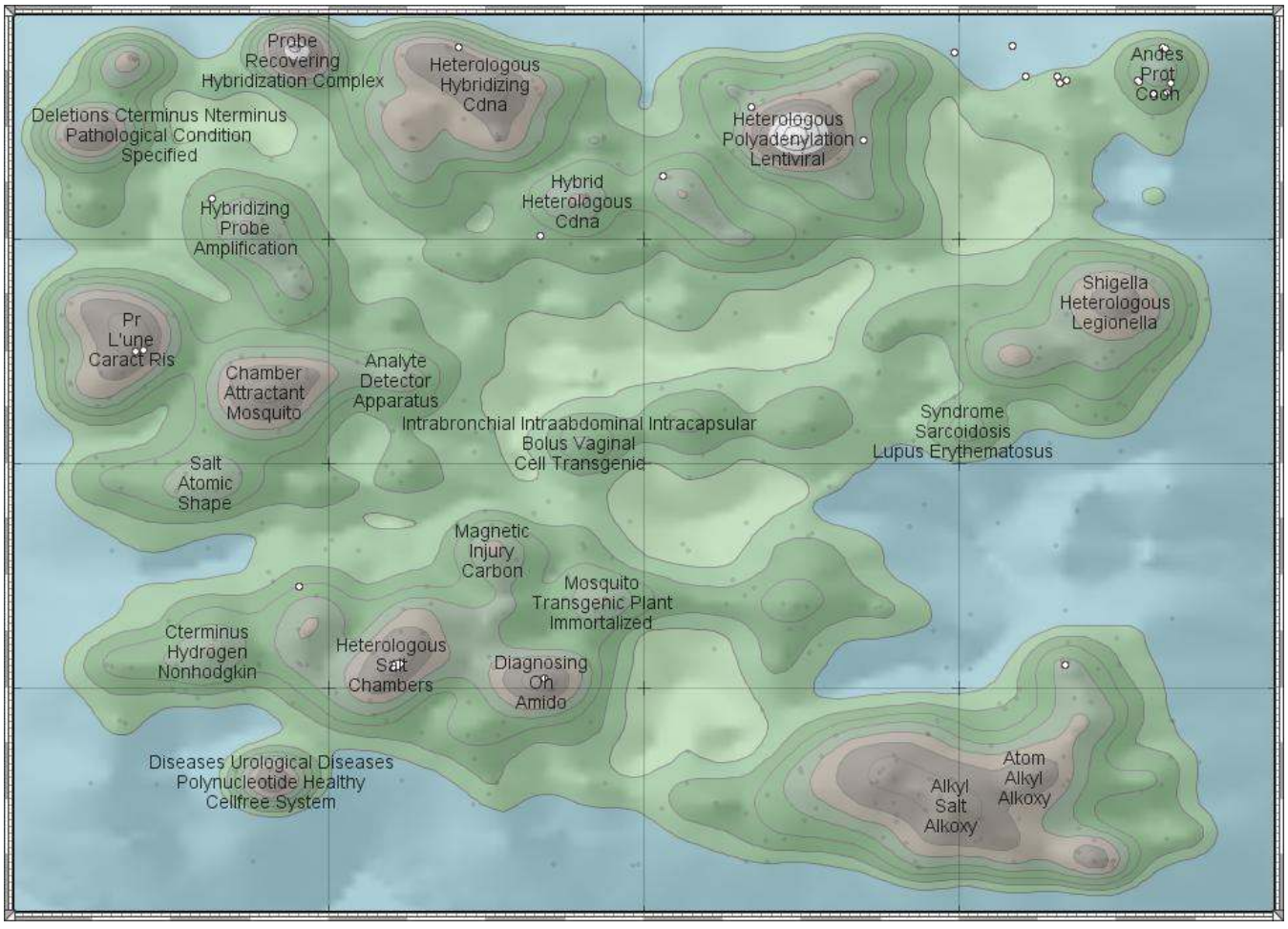
The concentrations of the patents related to the six products are overwhelmingly in the north/northeastern area. An analysis of the outliers (western and southern area) shows that these are either related to diagnostics or adjuvants, or as in the case of the southwestern area, to live DNA vaccines.

Annex C provides figures of the full dengue landscape of nearly 3,800 patents with those patents highlighted which we deemed related to the Acambis, InViragen, and Hawaii Biotech products. They show that all relevant patents are indeed in the northeastern corner. The Aureka® Themescape™ figures presented here (Figure 2) represent magnifications of this region.

Of the nearly 3,800 patent families considered, only about 225 fall within the broad area of the six products under development. Based on a detailed analysis of each of these 225 patents, 107 patents or patent families could be eliminated as they were not deemed relevant to any of the six products under development.

Figure 2:

PATENT LANDSCAPE RESULTING FROM A SEARCH FOR DENGUE-RELATED PATENTS WITH THOSE FOR DENGUE VACCINE HIGHLIGHTED (WHITE DOTS)



The remaining 118 patents/patent families were further analyzed and subsequent telephone interviews with dengue vaccine specialists and/or developers allowed for the elimination of a remaining group of 63 patents/patent applications. These 63 are listed in Table 5 below and Annex D provides the same list of patents with relevant annotations as to why they were eliminated.

There remained therefore 55 patents or patent families (consisting of both issued patents and patent applications) and PCT applications that were deemed relevant to the six advanced stage dengue vaccine technologies considered as part of this study. The summary is provided in Table 1, presented in Section 1, Executive Summary.

As noted earlier, the entire patent families are not listed; for many of the patents or patent applications listed here, one or all related patents of the same family are also deemed not relevant.

4.2 ACAMBIS/SANOFI PASTEUR: YELLOW FEVER-DENGUE CHIMERA VACCINE

Figure 3 shows the patents/patent applications by "Acambis" (Assignee) and/or "Monath" (Inventor) highlighted in white dots. Note that major activity is in the northern, and predominantly northwestern, ridges. This area has a partial overlap with the Whitehead and NIH activities.

Table 6 provides the patent details. Further, the total number of patent documents listed in the following tables is 63, more than the above-mentioned total of 55. This is due to the fact that two patents, namely US20080014219 (the Sanofi Pasteur patent) and US6676936 (one of the Lai *et al.* patents) potentially apply to more than one vaccine. The concentrations of the patents related to the six products under development are overwhelmingly in the northern and northeastern area of the landscape.

Table 5:

PATENTS/PATENT FAMILIES RELATED TO BUT NOT DEEMED RELEVANT TO THE CURRENT LIST OF PRODUCTS STUDIED AS PART OF THIS "GLOBAL ACCESS" FTO

EP1018556	US2007292453	US6685948
EP1159968	US20080063657	US6784161
JP23135085	US5690938	US6824793
JP24089185	US5723130	US6844001
JP24307477	US6017535	US6861410
JP25015355	US6083505	US6984385
JP6070760	US6086899	US7034141
JP6070760B4	US6117640	US7037499
JP6070760U2	US6149922	US7038029
US20020086403	US6190859	US7045576
US20040049016	US6258788	US7060280
US20040101862	US6355247	US7189403
US2004049016	US6372227	US7227011
US2004265324	US6416947	WO03048184
US2004265338	US6455509	WO07035530
US20050100886	US6458370	WO1992003545
US2005118698	US6558670	WO1992015672
US2005226849	US6589533	WO2000032625
US2006159699	US6630455	WO2005067968
US2006233830	US6660273	WO9203545
US2006280757	US6673591	WO9963095

Figure 3:

ACAMBIS/MONATH PATENTS

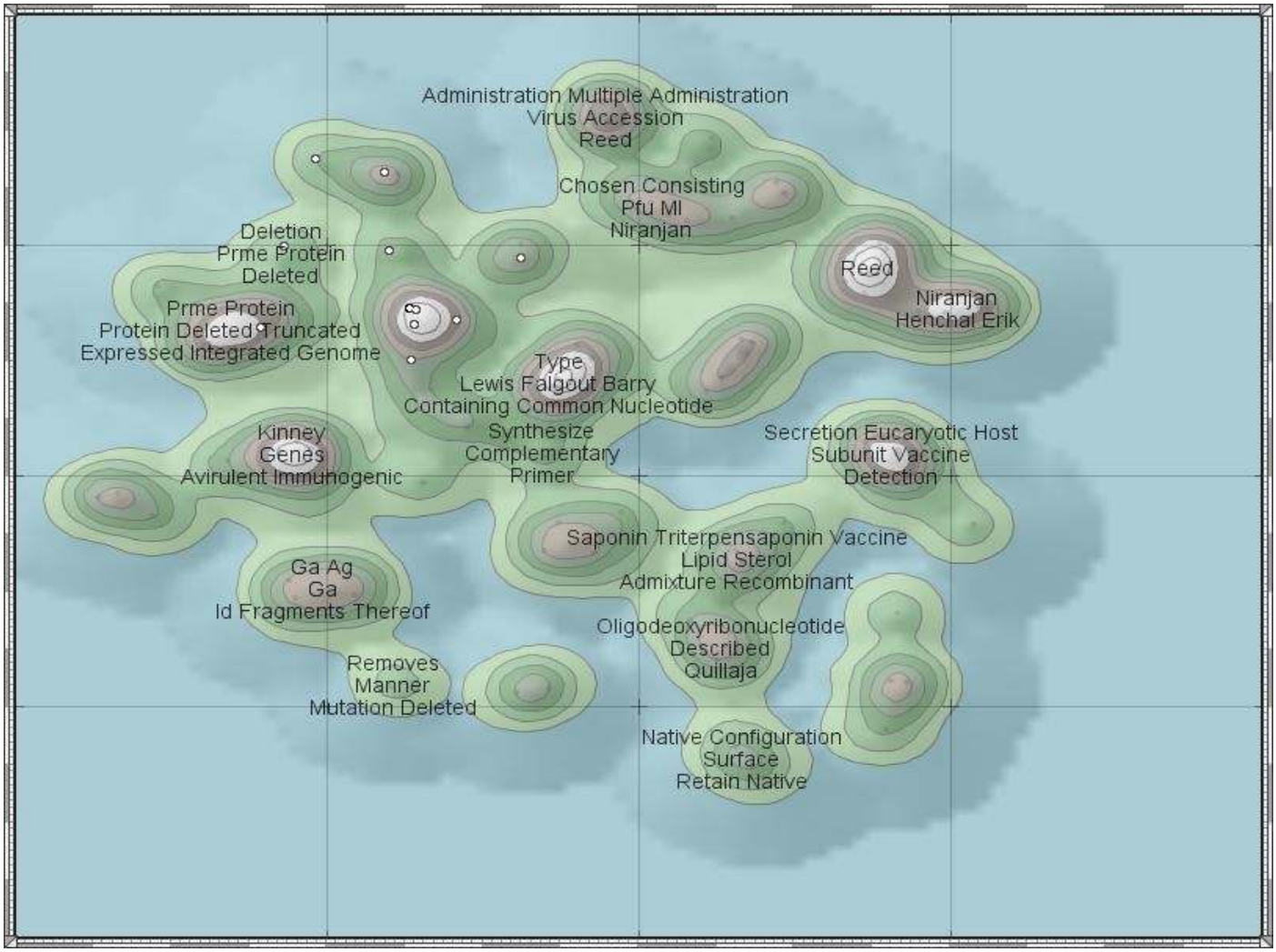


Table 6:

PATENT FAMILIES[15] RELATED TO THE ACAMBIS/SANOFI PASTEUR: YELLOW FEVER-DENGUE CHIMERA VACCINE

Publication Number	Title	Assignee *)	Inventor	Priority Date - Earliest	Application Date	National Phases (Published) **)
EP1373478	Attenuated live vaccine	Intercell AG	Heinz and Mandl	2001-02-21	2002-02-11	AT, AU, CA, CN, DE, EP, JP, US, WO
EP1441761	Methods of preventing and treating flavivirus infection in animals	Acambis Inc.	Monath *et al.*	2001-10-19	2002-10-21	AT, AU, BR, CA, DE, DK, EP, ES, IL, JP, KR, MX, NZ, PT, RU, US, WO, ZA
EP1924280	Vaccination against dengue virus infection	Sanofi Pasteur	Monath *et al.*	2005-08-10	2006-08-09	AR, AU, CA, CN, EP, FR, JP, NO, US, WO, ZA
EP2143440	Stabilizing agent and vaccine composition comprising one or several attenuated living flavivirus	Sanofi Pasteur	Françon *et al.*	2008-07-09	2008-07-09	AU, CA, CN, EP, IN, JP, KR, PL, US, WO
US20040259224	Tetravalent dengue vaccines	Acambis Inc.	Guirakhoo	2002-05-31	2003-06-02	AU, US, WO
US20080014219	Method of immunization against the 4 dengue serotypes	Sanofi Pasteur	Barban *et al.*	2006-07-12	2007-07-12	AR, AU, CA, CN, EP, FR, IN, JP, KR, MX, TW, US, WO, ZA
US20080085288	Method of immunization against the 4 dengue serotypes	Sanofi Pasteur	Barban *et al.*	2006-10-04	2007-10-02	EP, US, WO
US20080131460	Method of immunization against the 4 serotypes of dengue fever	Sanofi Pasteur	Barban *et al.*	2006-12-01	2007-11-21	AT, AU, CA, CN, CZ, DE, DK, EP, ES, HK, HU, IL, JP, NO, NZ, PL, PT, RU, US, WO
US20090191240	Flavivirus vaccines	Sanofi Pasteur / Acambis	Monath *et al.*	2002-01-15	2008-12-01	AU, BR, CA, CN, EP, IN, JP, KR, MX, NZ, SG, US, WO
US5023171	Method for gene splicing by overlap extension using the polymerase chain reaction	Mayo Foundation	Ho and Horton	1989-08-10	1989-08-10	US
US6171854	Yellow fever infectious cDNA and plasmids	Oswaldo Cruz Foundation (Fiocruz)	Galler *et al.*	1997-04-11	1998-04-10	AR, AU, CA, CL, CN, EP, JP, MX, TW, WO
US6676936	Chimeric and/or growth-restricted flaviviruses	US Department of Health	Lai *et al.*	1988-07-14	2000-08-18	CA, DE, EP, IN, MX, OA, RU, US, ZA

Publication Number	Title	Assignee *)	Inventor	Priority Date - Earliest	Application Date	National Phases (Published) **)
US6962708	Chimeric flavivirus vaccines	Acambis Inc. / St. Louis University	Monath *et al.*	1997-02-28	1998-07-23	AT, AU, BR, CA, DE, DK, EP, ES, GR, JP, US, WO
US7569383	Chimeric flavivirus vectors	Acambis Inc.	Kleanthous *et al.*	2001-06-01	2002-05-31	AU, BR, CA, CN, EP, JP, PL, US, WO
US7632510	Methods for preventing flavivirus infection	US Department of Health	Chang, Gwong-Jen J.	1998-06-04	2006-06-14	AR, AU, BR, CA, CN, EP, FR, JP, KR, MX, TW, US, WO, ZA
WO2003060088	Viral vaccine production method	Acambis Inc.	Monath *et al.*	2002-01-15	2003-01-15	AU, US, WO
WO2006116182	Recombinant flavivirus vaccines	Acambis Inc.	Monath *et al.*	2005-04-24	2006-04-24	AU, CA, CN, EA, EP, JP, KR, NO, RU, SG, US, WO
WO2007051267	Method for the production of recombinant virus, DNA constructs, recombinant virus and vaccine compositions	Oswaldo Cruz Foundation (Fiocruz)	Galler *et al.*	2005-10-31	2006-10-31	BR, EP, WO
WO2008036146	Construction of recombinant virus vaccines by direct transposon-mediated insertion of foreign immunologic determinants into Vector Virus Proteins	Acambis Inc.	Pugachev and Rumyantsev	2006-07-14	2007-07-16	AU, CA, CN, EP, HK, IL, JP, MX, NZ, US, WO, ZA
WO2008057550	Stabilization of vaccines by lyophilization	Acambis Inc.	Vellom *et al.*	2006-11-07	2007-11-07	AU, CA, CN, EA, EP, IN, JP, KR, NO, RU, US, WO, ZA
WO2008094674	Recombinant bicistronic flavivirus vectors	Acambis Inc.	Guirakhoo *et al.*	2007-01-31	2008-01-31	CA, EP, US, WO
WO2008137163	Two-component genome flavivirus and uses thereof	University of Texas	Frolov and Shustov	2007-05-07	2008-05-07	AU, CA, EP, US, WO

*) Note that many of the Acambis patents are now assigned to Sanofi or Sanofi Pasteur.

**) For a list of abbreviations, see http://tiny.cc/1qbjhw

4.3 HAWAII BIOTECH /MERCK & CO.: ENVELOPE PROTEIN SUBUNIT VACCINE

Figure 4 shows the patents/patent applications by “Hawaii” (Assignee) and/or “Ivy” (Inventor) highlighted in white dots. Note that these technologies occupy a discrete southeastern peninsula region, with little or no apparent overlap with other technologies. Table 7 provides the patent details.

Figure 4:

HAWAII BIOTECH/IVY PATENTS

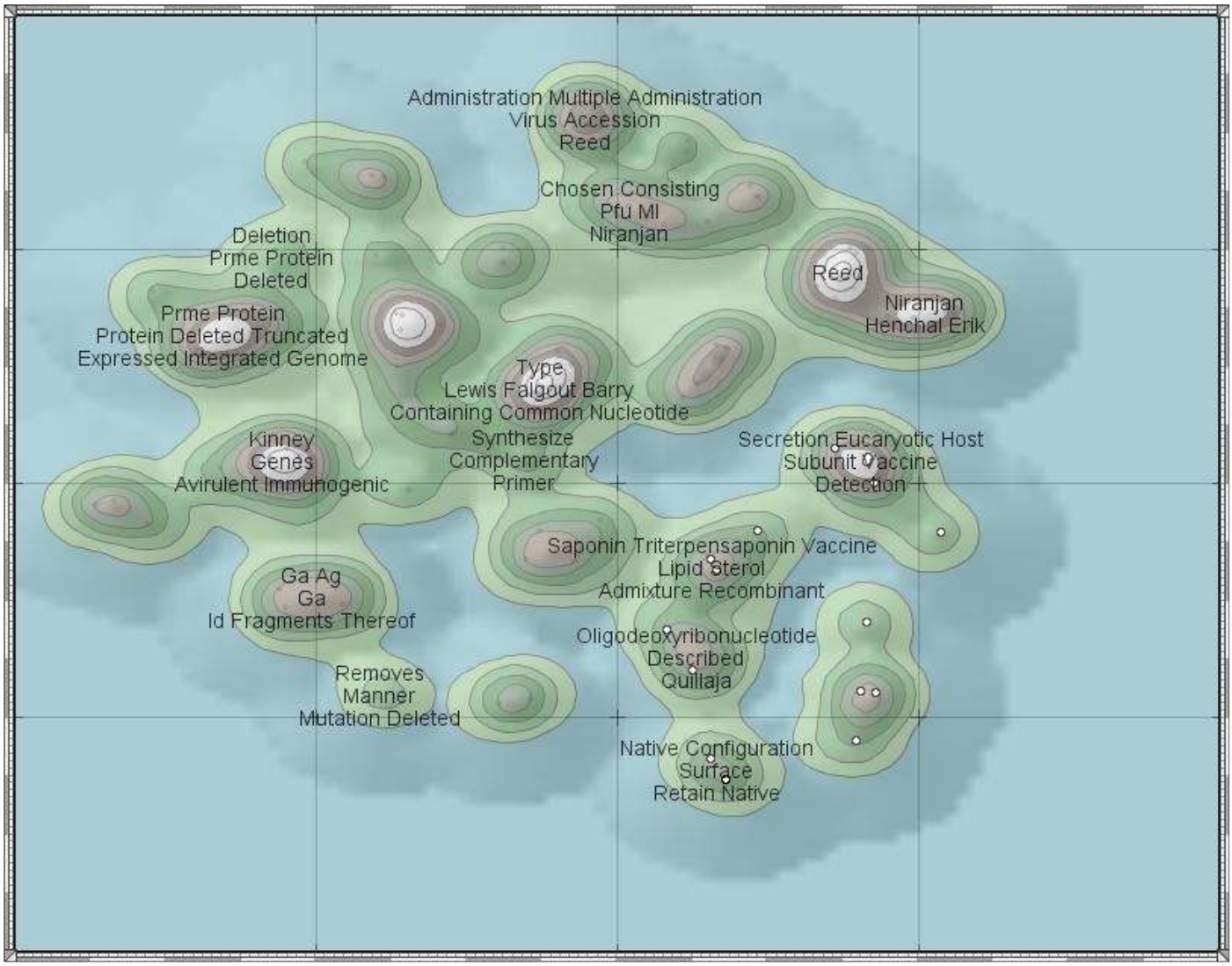


Table 7:

PATENT FAMILIES RELATED TO THE HAWAII BIOTECH INC./MERCK & CO. ENVELOPE PROTEIN SUBUNIT VACCINE

Publication Number	Title	Assignee	Inventor	Priority Date - Earliest	Application Date	National Phases (Published)
US20080248064	Localization and characterization of flavivirus envelope gylcoprotein cross-reactive epitopes and methods for their use	US Department of Health	Chang, Gwong-Jen J./Crill, Wayne D.	2004-07-27	2007-01-26	AU, CA, EP, IN, US, WO
US20080311157	Pharmaceutical compound capable of inducing immune protective response against dengue virus having the capsid protein of the dengue virus	Centro de Ingeniera Genetica y Biotecnologia	Lazo Vazquez *et al.*	2005-09-16	2008-07-23	AR, AU, CA, CN, EP, IN, JP, KR, MX, RU, US, WO, ZA
US20100047280	Flavivirus NS1 Subunit Vaccine	Bavarian Nordic	Howley *et al.*		2008-02-27	AU, CA, CN, EP, IN, JP, KR, US, WO
US5494671	C-terminally truncated dengue and Japanese encephalitis virus envelope proteins	US Department of Health	Lai *et al.*	1990-08-27	1991-08-20	AU, US, WO
US6046025	Expression of heterologous proteins in drosophila cells	SmithKline Beecham Corp.	Johansen *et al.*	1987-05-08	1995-05-03	AP, AT, AU, CA, CY, DE, DK, EP, ES, FI, HK, IE, IL, JP, KR, NO, PH, PT, US, WO, ZA
US6080725	Immunostimulating and vaccine compositions employing saponin analog adjuvants and uses thereof	Galenica Pharmaceuticals Inc.	Marciani, Dante J.	1997-05-20	1999-04-13	AT, AU, BR, CA, DE, DK, EP, ES, IL, JP, KR, MX, NZ, PT, US, WO
US6165477	Subunit immunogenic composition against dengue infection	Hawaii Biotech	Ivy *et al.*	1995-05-24	1997-08-20	AT, AU, CA, DE, DK, EP, ES, PT, US, WO
US6416763	Recombinant nonstructural protein subunit vaccine against flaviviral infection	Hawaii Biotech	McDonell *et al.*	1998-08-28	1998-08-28	AU, CA, EP US, WO
US6432411	Recombinant envelope vaccine against flavivirus infection	Hawaii Biotech	Ivy *et al.*	1999-07-13	1999-07-13	AU, BR, CN, IN, US, WO
US6676936	Chimeric and/or growth-restricted flaviviruses	US Department of Health	Lai *et al.*	1988-07-14	2000-08-18	AT, AU, CA, DE, DK, EP, ES, GR, JP, US, WO
US6749857	Recombinant dimeric envelope vaccine against flaviviral infection	Hawaii Biotech	Peters *et al.*	1997-07-31	1999-08-18	AT, AU, BR, CA, DE, EP, JP, US, WO
US7265215	DNA or RNA encoding a recombinant dengue envelope protein	US Army	Kelly and King	1995-07-20	2003-02-03	US
US7476390	Flavivirus antigens	Maxygen Inc.	Apt *et al*	2002-02-26	2003-02-26	AU, CA, CN, EP, IN, NZ, US, WO

Publication Number	Title	Assignee	Inventor	Priority Date - Earliest	Application Date	National Phases (Published)
US7566457	Chimeric proteins that induce effects directed against viruses	Centro de Ingeniera Genetica y Biotecnologia	Cruz *et al.*	2001-07-16	2007-02-13	AT, AU, BR, CA, CN, CU, DE, DK, EP, ES, IN, JP, KR, MX, PT, US, WO, ZA
WO2004052293	Recombinant vaccine against flavivirus infection	Hawaii Biotech	Lieberman	2002-12-11	2003-12-08	AU, US, WO
WO2007034507	Tetravalent dengue specific domain III based on chimeric recombinant protein	International Centre for Genetic Engineering and Biotechnology	Batra *et al.*	2005-09-20	2006-08-30	IN, WO
WO2007059715	Methods and proteins for the prophylactic and/or therapeutic treatment of the four serotypes of dengue virus and other flaviviruses	Centro de Ingeniera Genetica y Biotecnologia	Chinea Santiago *et al.*	2005-11-22	2006-11-21	AR, AU, CA, CN, EP, IN, JP, KR, MX, RU, US, WO, ZA

4.4 MAHIDOL UNIVERSITY/SANOFI PASTEUR: LIVE ATTENUATED VACCINE

Figure 5 shows the patents/patent applications by “Sanofi Pasteur” (Assignee) and/or “Guirakhoo” (Inventor) highlighted in white spots. Patents documents relevant to the Mahidol University/Sanofi Pasteur vaccine are not included. Table 8 provides the patent details.

Figure 5:

MAHIDOL UNIVERSITY/SANOFI PASTEUR PATENTS

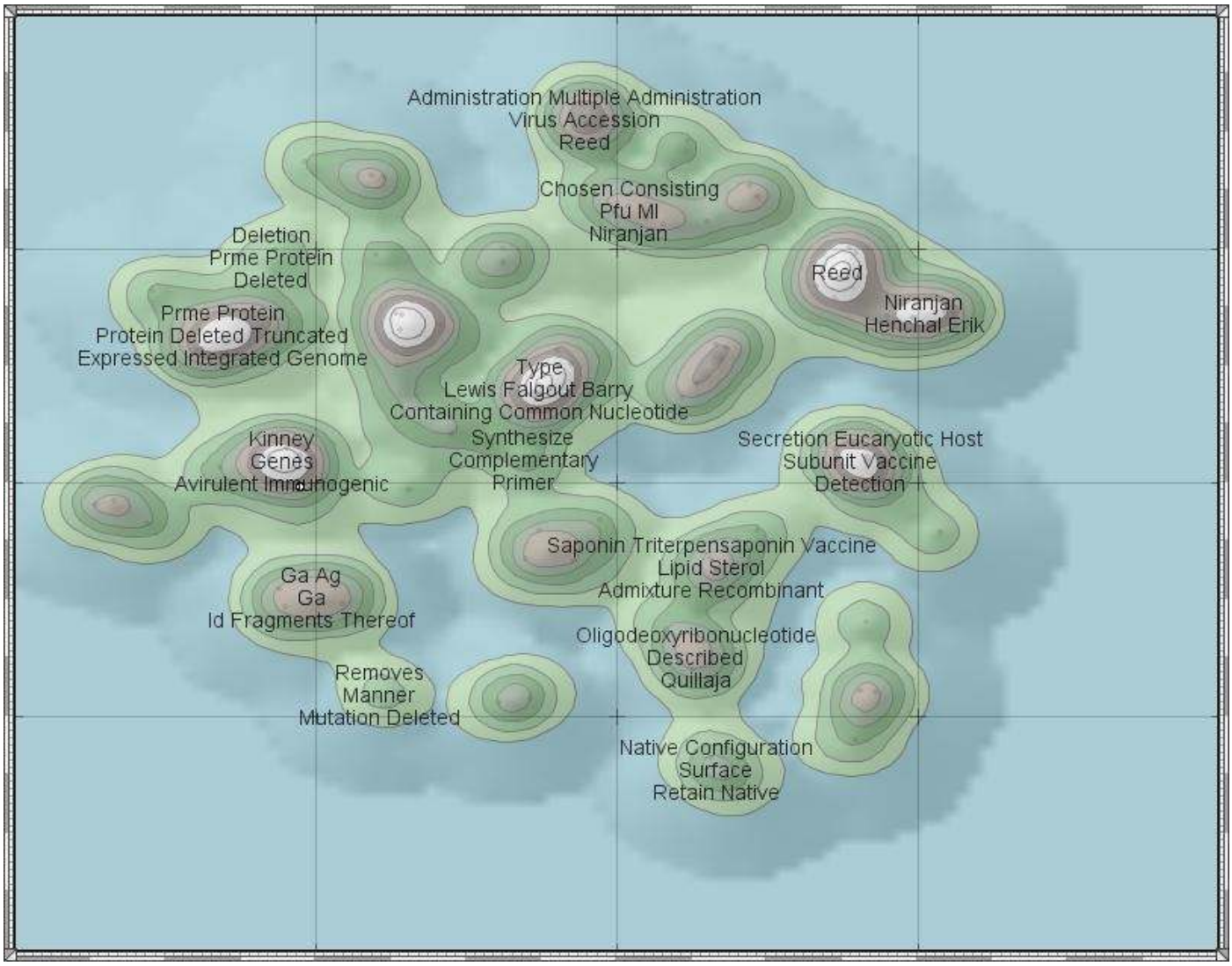


Table 8:

PATENT FAMILIES RELATED TO THE MAHIDOL UNIVERSITY/SANOFI PASTEUR: LIVE ATTENUATED VACCINE

Publication Number	Title	Assignee	Inventor	Priority Date - Earliest	Application Date	National Phases (Published)
EP1159968	Attenuated strains of dengue virus and their use in a vaccine composition	Mahidol University	Bhamaraprav at *et al.*	2000-05-30	2000-05-30	AT, DE, DK, EP, ES, PT
US20080014219	Method of immunization against the 4 dengue serotypes	Sanofi Pasteur	Barban *et al.*	2006-07-12	2007-07-12	AR, AU, CA, CN, EP, FR, IN, JP, KR, MX, TW, US, WO
WO2001091790	Vaccine composition	Aventis Pasteur	Lang *et al.*	2000-05-30	2001-05-29	AU, BR, CN, EP, IN, KR, MX, WO
WO2003101397	Tetravalent dengue vaccines	Acambis Inc.	Guirakhoo	2002-05-31	2003-06-02	AU, US, WO
WO2006134433	Dengue serotype 1 attenuated strain	Sanofi Pasteur/US Center for Disease Control and Prevention	Kinney *et al.*	2005-06-17	2006-05-18	AR, AU, CA, CN, EP, IN, JP, KR, MX, NO, TW, US, WO, ZA

4.5 US CDC-INVIRAGEN: DENGUE-DENGUE CHIMERA VACCINE

Figure 6 shows the patents/patent applications by "InViragen" (Assignee) and/or "Kinney" (Inventor) highlighted in white dots. Note that there is partial overlap with Whitehead and NIH patent documents, and that InViragen patent documents predominantly occupy a southern/southwestern hill region without apparent overlap. Figure 7 shows the patent family by "Lai *et al.*" (Inventor), of which US6676936 is a representative document. Note the proximity to InViragen/CDC patent documents in Figure 1 above (for more details, please refer to Section 5.6 on US CDC-InViragen licensing). Table 9 provides the patent details.

Figure 6:

INVIRAGEN/KINNEY PATENTS

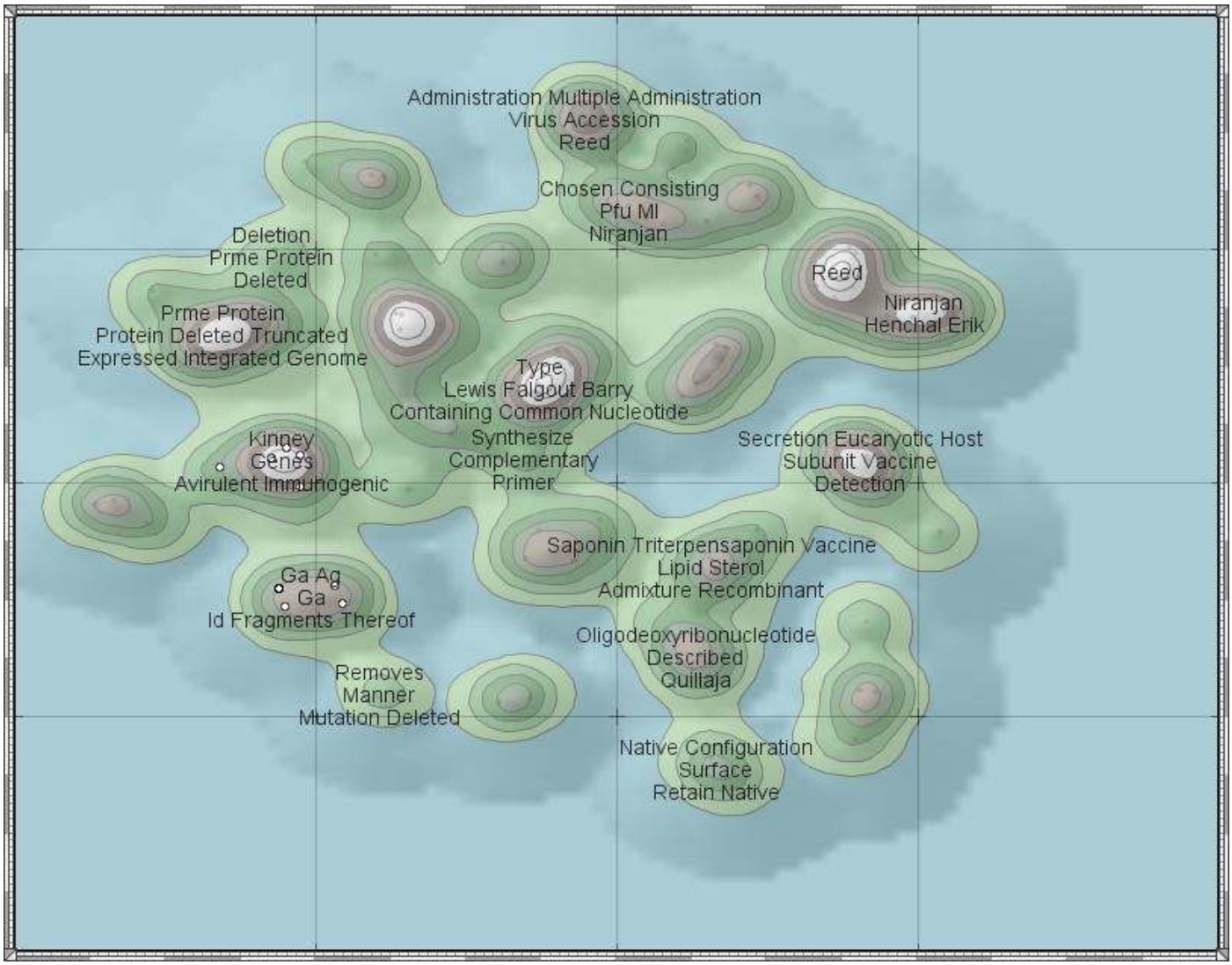


Figure 7:

LAI *ET AL.* PATENTS

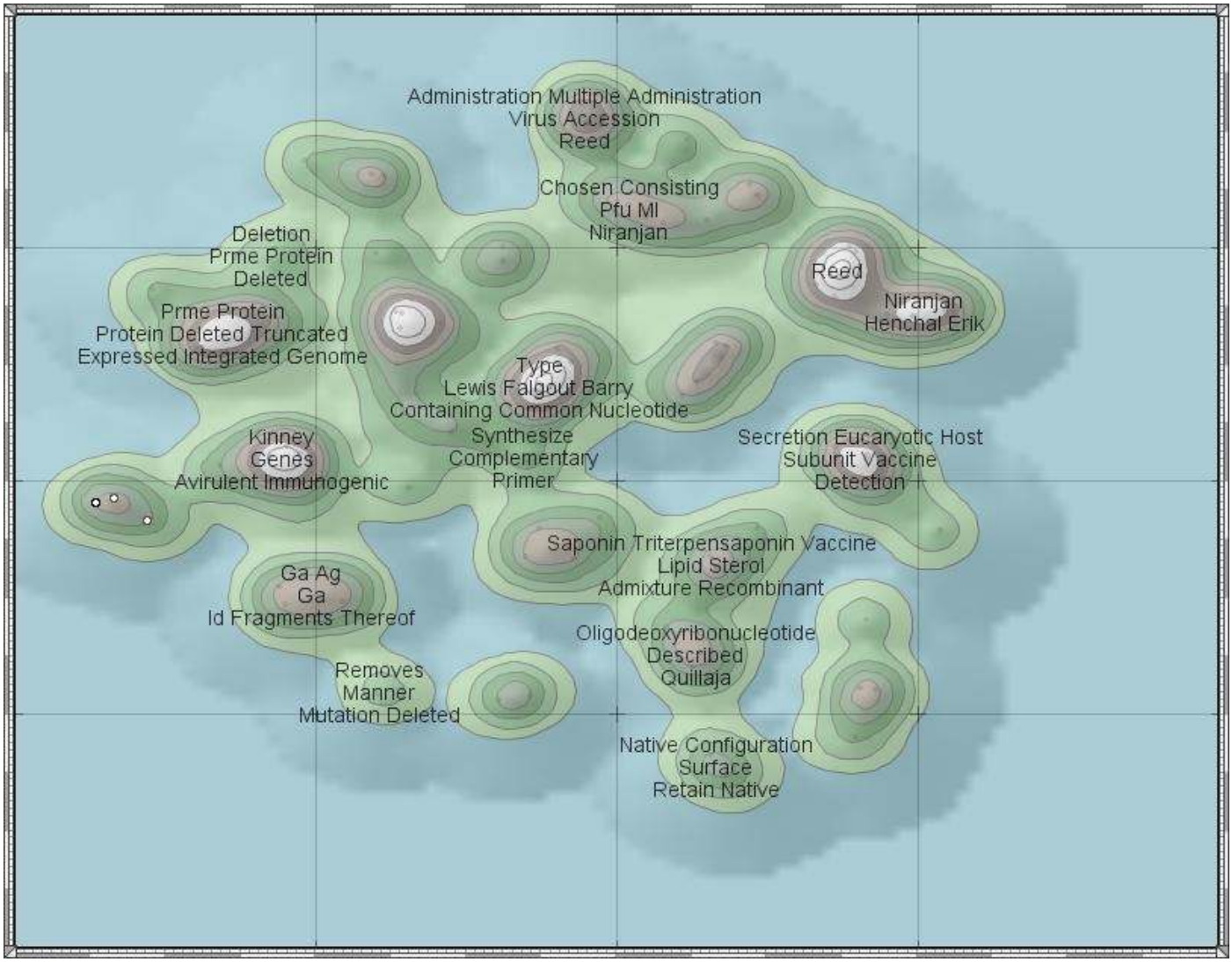


Table 9:

PATENT FAMILIES RELATED TO THE US CDC-INVIRAGEN: DENGUE-DENGUE CHIMERA VACCINE

Publication Number	Title	Assignee	Inventor	Priority Date - Earliest	Application Date	National Phases (Published)
US20080014219	Method of immunization against the 4 dengue serotypes	Sanofi Pasteur	Barban *et al.*	2006-07-12	2007-07-12	AR, AU, CA, CN, EP, FR, IN, JP, KR, MX, TW, US, WO, ZA
US6676936	Chimeric and/or growth-restricted flaviviruses	US Department of Health		1988-07-14	2000-08-18	AT, AU, CA, DE, DK, EP, ES, GR, JP, US, WO
US7641907	Dengue serotype 1 attenuated strain	Sanofi Pasteur / US Center for Disease Control and Prevention	Kinney *et al.*	2005-06-17	2006-06-09	AR, AU, CA, CN, EP, IN, JP, KR, MX, NO, TW, US, WO, ZA
US7641908	Dengue serotype 2 attenuated strain	Sanofi Pasteur / US Center for Disease Control and Prevention	Kinney *et al.*	2005-06-17	2006-06-15	AR, AT, AU, CA, CN, EP, IN, JP, KR, MX, NO, TW, US, WO, ZA
US7641909	Avirulent, immunogenic flavivirus chimeras	US Department of Health	Kinney *et al.*	2000-02-16	2006-08-18	AU, CA, EP, JP, US, WO
WO1996040933	Infectious dengue-2 virus PDK-53 as quadravalent vaccine	US Department of Health	Bhamarapravati *et al.*	1995-06-07	1996-06-06	AU, WO

4.6 US NIH-DEVELOPING COUNTRY MANUFACTURERS: DENGUE-DENGUE Δ-30 CHIMERA VACCINE

Figure 8 shows the patents/patent applications by “NIH” (Assignee) and/or “Whitehead” (Inventor) highlighted in white dots. Note that this technology group is found in the central ridge of Figure 8, with little overlap with Acambis patent documents in the west. Table 10 provides the patent details.

Figure 8:

NIH/WHITEHEAD PATENTS

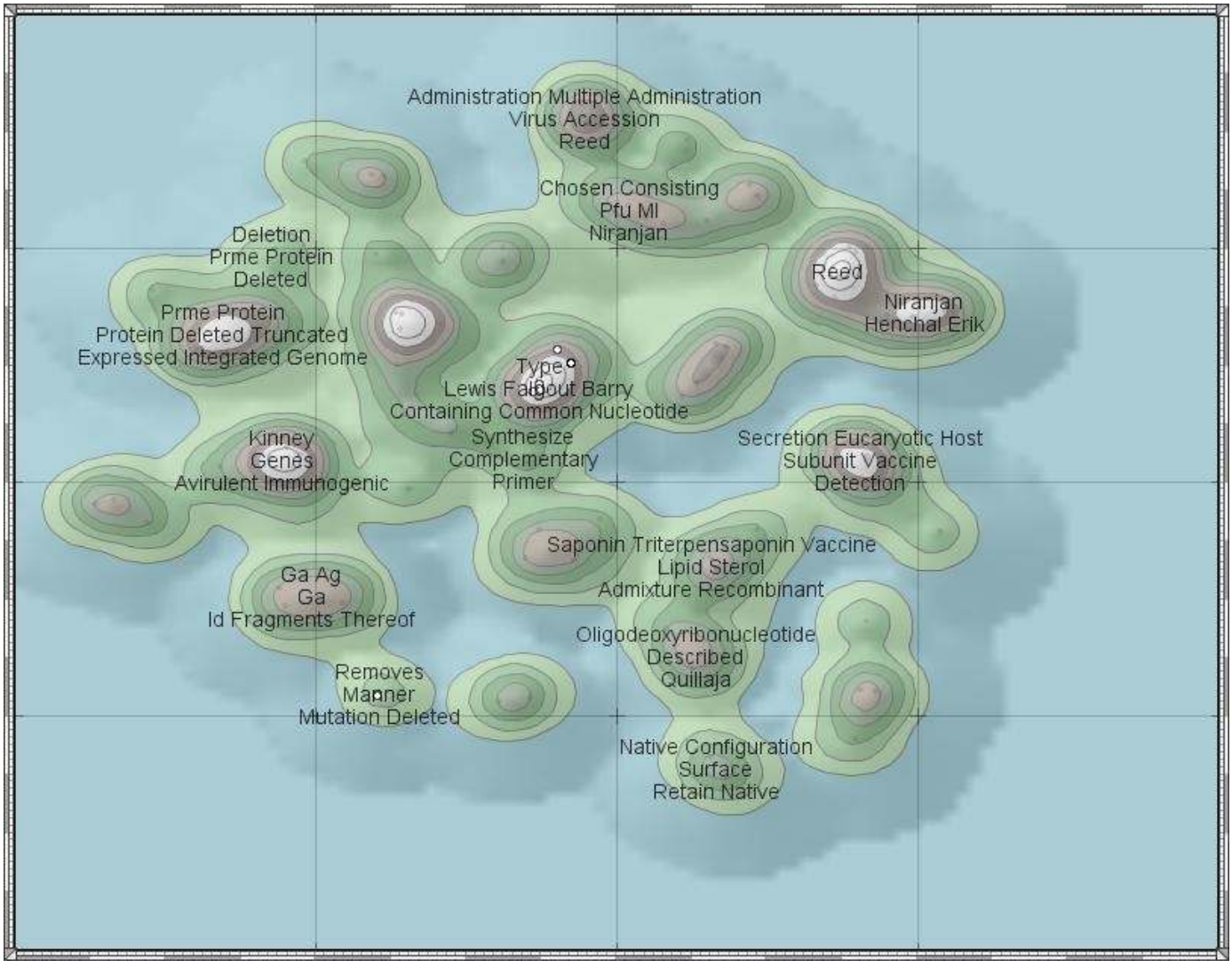


Table 10:

PATENT FAMILIES RELATED TO THE US NIH-DEVELOPING COUNTRY MANUFACTURERS: DENGUE-DENGUE Δ-30 CHIMERA VACCINE

Publication Number	Title	Assignee	Inventor	Priority Date - Earliest	Application Date	National Phases (Published)
US20090258036	Dengue tetravalent vaccine containing a common 30 nucleotide deletion in the 3'-UTR of dengue types 1, 2, 3, and 4 or antigenic chimeric dengue viruses 1, 2, 3, and 4	US Department of Health	Whitehead *et al.*	2002-05-03	2009-03-04	AU, CA, EP, IN, JP, US, WO
US20090263424	Development of mutations useful for attenuating dengue viruses and chimeric dengue viruses	US Department of Health	Whitehead *et al.*	2001-05-22	2009-03-02	AU, BR, CA, EP, IN, US, WO
US6676936	Chimeric and/or growth-restricted flaviviruses	US Department of Health	Lai *et al.*	1988-07-14	2000-08-18	AT, AU, CA, DE, DK, EP, ES, GR, JP, US, WO
WO2007141259	Live attenuated dengue 3 virus strains	Sanofi Pasteur	Barban *et al.*	2006-06-07	2007-06-05	AR, US, WO
WO2008022196	Development of dengue virus vaccine components	US Department of Health	Whitehead *et al.*	2006-08-15	2007-08-15	AU, CA, CN, EP, IN, US, WO

4.7 US WRAIR-GSK: LIVE ATTENUATED VACCINES

Figure 9 shows the patents/patent applications by "US Army" (Assignee) and/or "Putnak" (Inventor) highlighted in white dots. Note that the activity of this group appears to primarily occupy a specific peak in the northern/northeastern region of Figure 9 with minimal overlap with other vaccine technologies. Table 11 provides the patent details.

Figure 9:

US ARMY/PUTNAK PATENTS

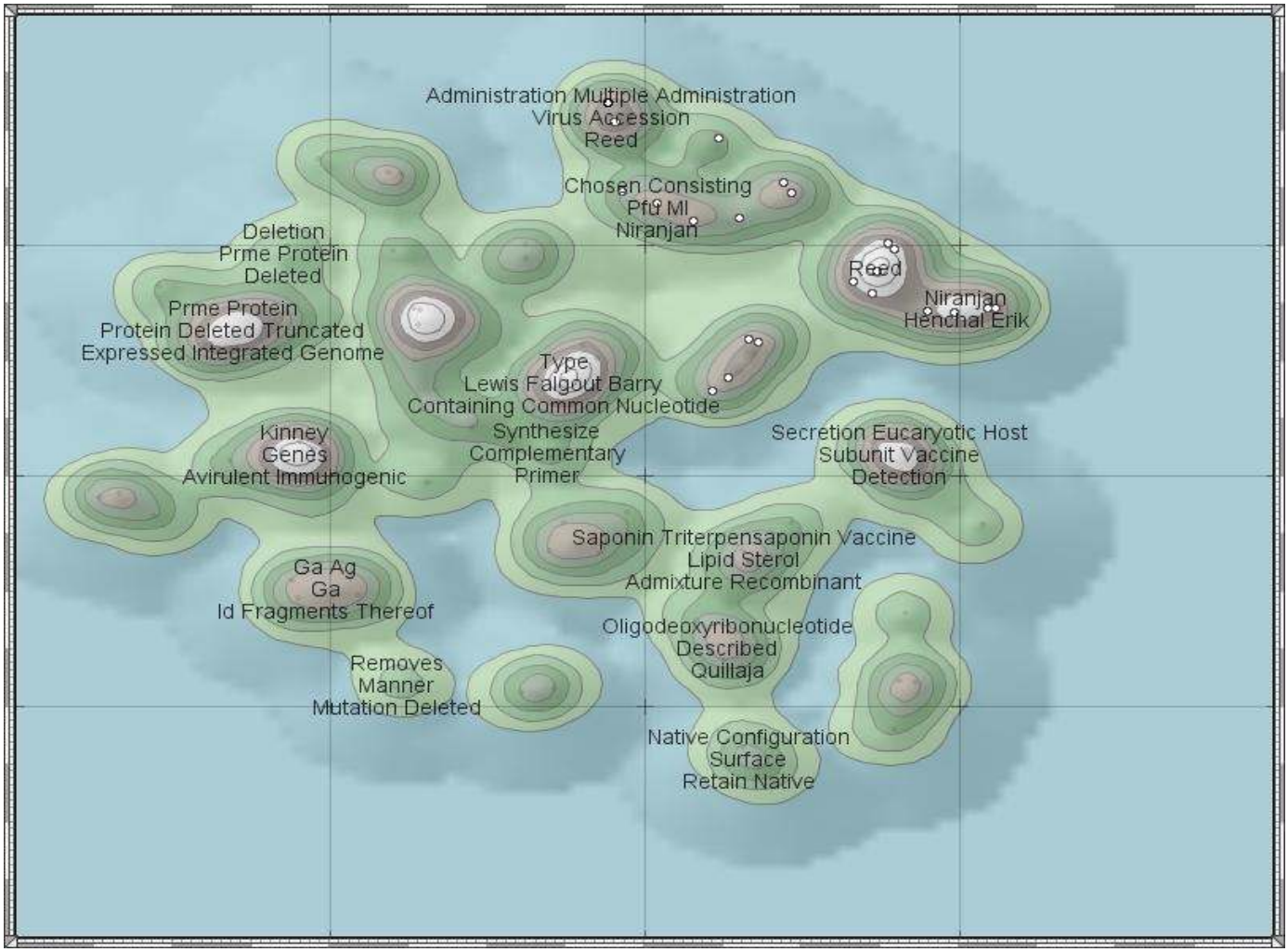


Table 11:

PATENT FAMILIES RELATED TO THE US WRAIR-GSK: LIVE ATTENUATED VACCINES

Publication Number	Title	Assignee	Inventor	Priority Date - Earliest	Application Date	National Phases (Published)
US20080014219	Method of immunization against the 4 dengue serotypes	Sanofi Pasteur	Barban *et al.*	2006-07-12	2007-07-12	AR, AU, CA, CN, EP, FR, IN, JP, KR, MX, TW, US, WO
US6676936	Chimeric and/or growth-restricted flaviviruses	US Health	Lai *et al.*	1988-07-14	2000-08-18	AT, AU, CA, DE, DK, EP, ES, GR, JP, US, WO
US7217418	Multivalent dengue virus vaccine	US Army	Eckels *et al.*	1999-03-26	2003-07-24	AT, AU, BR, CA, CN, DE, EP, ES, ID, JP, KR, MX, MY, US, VN, WO
WO2000057904	Attenuated dengue-3 virus vaccine	US Army	Eckels *et al.*	1999-03-26	2000-03-24	AU, CA, EP, JP, US WO,
WO2000057908	Attenuated dengue-1 virus vaccine	US Army	Eckels *et al.*	1999-03-26	2000-03-24	AU, CA, EP, JP, WO
WO2000057909	Attenuated dengue-2 virus vaccine	US Army	Eckels *et al.*	1999-03-26	2000-03-24	AU, CA, EP, JP, US, WO
WO2000057910	Attenuated dengue-4 virus vaccine	US Army	Eckels *et al.*	1999-03-26	2000-03-24	AU, CA, EP, JP, US, WO
WO2000058444	Adaptation of virus to vertebrate cells	US Army	Eckels *et al.*	1999-03-26	2000-03-24	AU, CA, EP JP US, WO

[15] Only one representative family member is listed per patent family. This applies to all tables in this Section.

Section 5:
Licensing Status and Discussion

5.1 OVERVIEW

The results of this "global access" FTO suggest that there are few major IP constraints that would complicate, from an IP perspective, developing-country access to the six major vaccines that are in late-stage development. This appears to be the case for several reasons:

First, many of the inputs were already in the public domain.

Second, the degree of overlap, in terms of proprietary rights, among the six major vaccines appears to be minimal, with each candidate occupying a defined area; the Aureka® Themescape™ figures in this "global access" FTO lend support to this observation.

Third, the technological approaches of third party dengue vaccine developers do not appear to overlap with those of the six major vaccine developers. For example, the Cuban (Guzman *et al.*) patents that disclose recombinant dengue protein vaccines are not applicable to the Hawaii Biotech vaccines, as they cover fusion protein constructs (Hawaii Biotech patents are for truncated dengue protein constructs). IPRs of other vaccine approaches, such as of DNA vaccines, are quite irrelevant here since there is essentially no technological overlap.

Basic technologies for developing and manufacturing vaccines are well established; many have been in use for well over a century and fall squarely into the public domain. These technologies should not pose IP constraints and would not require licensing or other IP-access strategies such as non-assertion covenants. However, with the advance of biotechnology in vaccine research, development, and manufacture, IP issues may possibly arise, depending on the type of methodologies and materials employed. A brief examination of the principal dengue vaccine technologies follows, with an emphasis on the technological inputs that might pose proprietary questions. The known licensing information is set forth in the below sections. It is important to note that the NIH, Lai *et al*. patent family, of which US6676936 is a representative document, covers fundamental technologies potentially relevant to the current advanced-phase dengue vaccine approaches (WRAIR, Hawaii Biotech, Acamis/Sanofi Pasteur, NIH). Another Lai *et al.* patent family (represented by US5494671), is possibly relevant to the technologies developed by Hawaii Biotech; however, the expiration date of these patents may precede the actual commercial distribution of a vaccine.

It is assumed that the various entities developing the vaccines under consideration have appropriate commercial licenses for the enabling technologies. Those include but are not limited to PCR, restriction enzymes, reverse genetics, genetic constructs [promoters, plasmids], various molecular technologies, transformation technologies, and access to proprietary cell lines as well as confidential know-how. The licensing status is summarized in Table 12.

5.2 ACAMBIS/SANOFI PASTEUR: YELLOW FEVER-DENGUE CHIMERA VACCINE

The Acambis ChimeriVax platform technology utilizes yellow fever virus (YF) 17D vaccine strain capsid and non-structural genes to deliver the envelope gene of other flaviviruses as live attenuated chimeric viruses.

Upstream R&D IP constraints might be associated with molecular techniques used to generate the chimeric construct, e.g., polymerase chain reaction methods and reagents, molecular cloning technologies and related technologies. When purchased through legitimate commercial channels, these are typically accompanied by a user's license that stipulates the terms and limitations of use. Reverse genetics patents might also apply.

5.3 FIOCRUZ AND ACAMBIS/SANOFI PASTEUR

There are different perspectives as to the precise situation regarding the Fiocruz chimeric dengue viral platforms. In addition, the patenting status is evolving fairly fast in this specific area. The text that follows aims at identifying the key issues that may warrant further investigation.

Table 12:

LICENSING SUMMARY OF PRINCIPAL, LATE-STAGE DENGUE VACCINE DEVELOPERS

Vaccine Developer	In-Licensed Enabling Technologies	Out-Licensed Vaccine Technologies
Acambis/Sanofi Pasteur	• Acambis obtained two, nonexclusive licenses from NIH to practice the technologies covered by the Lai *et al.* patent family, of which US6676936 is a representative document. • Acambis might have obtained a license from the Mayo Clinic to practice the technologies covered in US5023171 that describes PCR gene-splicing methodologies. • The original owner of a core technology (YFV [17D] backbone: ChimeriVax™D2) was St. Louis University, which granted an exclusive license to Acambis, which was then exclusively sublicensed to Sanofi Pasteur along with the entire package of Acambis patents.	Acambis has exclusively licensed the ChimeriVax™-DEN2 platform technology to Sanofi Pasteur for subsequent development.
CDC-InViragen	• The CDC has granted an exclusive license of its DEN-2 PDK-53 chimeras to InViragen. • InViragen has obtained an exclusive license from the CDC for the patent US7094411, which belongs to the patent family represented by US7641909; license terms include a series of low single-digit royalty payments based on sales. • InViragen has obtained a nonexclusive license for the patent US6676936 (the original assignee on this patent is listed as: the United States of America as represented by the Department of Health and Human Services).	InViragen has signed a manufacturing agreement with Shantha Biotechnics, Hyderabad, India.
Hawaii Biotech, Inc.	Hawaii Biotech has licensed the proprietary expression vector from GlaxoSmithKline (for production of flavivirus vaccines) for use with all flavivirus vaccines worldwide (US6046025, US6046025).	Hawaii Biotech has not licensed its vaccine patents. The rights to the Hawaii Biotech vaccine were procured by Merck & Co. in 2010.
NIH-Developing Country Manufacturers	Not known	Several industrial sponsors in Asia and Brazil have been awarded nonexclusive licenses for the rDenΔ30 formulations (US20090258036). The Butantan Foundation (Sao Paulo, Brazil) has taken a nonexclusive license for the rDenΔ30 candidate vaccine(s) and has also received seed virus from NIAID for vaccine development. The field of use may be limited to live attenuated vaccines against dengue in humans. Said license provides exclusivity in Brazil and in the rest of Latin America. Biological E, Hyderabad, India (nonexclusive rights for commercialization) and Panacea Biotech, New Delhi, India (nonexclusive rights for commercialization) have also been awarded licenses.
WRAIR-GSK	Not known	The vaccine development partnership between WRAIR and GSK is based on a Cooperative R&D Agreement (CRADA). This CRADA captures all relevant patents.

Licensing status: Acambis has exclusively licensed the ChimeriVax™-DEN2 platform technology to Sanofi Pasteur for subsequent development. On December 4, 2007, Maxygen, Inc. announced that it had licensed its proprietary dengue-virus-antigen technology to Sanofi Pasteur, the vaccines division of Sanofi (formerly Sanofi Aventis). Under the terms of the agreement, Maxygen will transfer a portfolio of preclinical dengue antigens for development and worldwide commercialization of a second-generation vaccine. In addition to royalties, total event payments to Maxygen, including an upfront fee, could total $24.5 million.[16]

To develop its platform, Acambis obtained two non-exclusive licenses from NIH to practice the technologies covered by the Lai *et al.* patent family, of which US6676936 is a representative document. In addition, Acambis might have obtained a license from the Mayo Clinic to practice the technologies covered in US5023171 that describes PCR gene-splicing methodologies.

Unanswered questions with regard to licensing status:

- The licensing status of any of the listed patents that are or may be relevant to the Acambis vaccine.
- Existence of any other IP licenses, such as for know-how/trade secrets.
- International filing status of patent portfolio. Some information has been provided: PCT/US98/03894 has led to two issued patents. One is relevant: US6962708, the so-called Master ChimeriVax family; US patents and applications; chimera comprising YF backbone and capsid, YF envelope and membrane proteins nonfunctional, membrane and envelope protein of second flavivirus expressed; method of administration of above chimera (where second flavivirus is JE or DEN ½/3/4) to Rx to prevent JE or dengue, respectively. Another application claims specific chimeras and therapeutic uses. Non-US patents and applications: granted in Australia, China, New Zealand, Russian Federation, Singapore, Vietnam, and the European Union (opposition period ended 03/06); Republic of Korea (09/05). Divisional European patent applications claiming signal sequences, other than YF, to produce viable chimeras. Pending patent granting in Brazil, Canada, Cuba, Czech Republic, Hungary, Israel, Japan, Mexico, Norway and Poland.

5.4 HAWAII BIOTECH/MERCK: ENVELOPE PROTEIN SUBUNIT VACCINE

Significant scientific effort has gone into the development of the current strategy to use recombinant truncated (transmembrane domain-deleted) envelope glycoproteins (80%) expressed as soluble products in the Drosophila S2 expression system. The expressed proteins have been shown by structural studies to maintain the native tertiary structure critical for eliciting a protective immune response.

Hawaii Biotech has recently hired a much-needed formulation expert. The current formulation of antigens with Alhydrogel in phosphate-buffered saline (PBS) appears to be stable. Antigen binding is approximately 80%. No effort has been made to increase the binding. Hawaii Biotech has recognized that there is considerable work to be done to optimize the formulation.

The first clinical study planned for the Hawaii Biotech dengue vaccine will be a phase I clinical trial, sponsored by the Walter Reed Army Medical Center, evaluating the GSK live attenuated dengue vaccine and the Hawaii Biotech recombinant vaccine administered sequentially. In this study, the two vaccines will be administered one before the other to see if priming with one and boosting with the other will improve the immunogenicity of either product given alone. It is expected that, in alternate studies, each vaccine will assume the role of both the “prime” and the “boost.”

A unique and efficient manufacturing method has been developed in conjunction with Xcellerex (Marlborough, MA, United States of America). The system utilizes WAVE cell factories to expand cells to the 25 L level and a 200 L disposable, plastic-lined bioreactor for final expansion and induction. The entire system is closed and disposable with the exception of the purification columns, which are reused. This system and method appear quite robust and readily scalable to the 1000 L level. Cycle time is 22 days at the 200 L level and an estimated 24 days at the 1000 L level. The media is a commercial, animal product–free media. The Xcellerex system appears to be very well suited to technology transfer. The downstream process includes purification by immune adsorption chromatography (IAC), viral inactivation by acidification (<pH 3.8, 24 hr), viral filtration through a 20 nanometers filter, and buffer exchange by diafiltration. After the final filtration, the monovalent material is stored at between -20 and -70 degrees Celsius.

Overall, the manufacturing process is well designed and the process development very advanced. The use of IAC instead of conventional chromatographic measures was found to be essential to preserving the integrity of the tertiary structure of the proteins, which are partially denatured by ion exchange chromatography.

The Hawaii Biotech vaccine might encounter IP constraints with regard to early R&D inputs, for example molecular technologies associated with cloning and expression of candidate gene sequences, as well as with subsequent production methodologies, such as IAC and optimization. Proprietary issues vis-à-vis the Xcellerex system should not be a concern but should nonetheless be examined and

clarified. In addition, contemplated prime boost approaches could present IP issues, depending on the precise approach taken. The formulation appears to be handled by in-house technologies; including the use of different adjuvants.

Licensing status: Hawaii Biotech has not licensed its own patents. To produce the vaccine, Hawaii Biotech has licensed the proprietary expression vector from Glaxo SmithKline (for production of flavivirus vaccines) for use with all flavivirus vaccines anywhere in the world. The Drosophila S2 cells, obtained from the ATCC, are in the public domain. The vaccine is formulated in proprietary adjuvant (GPI-0100), among others. The specific adjuvant for the commercial product has not yet been determined. Hawaii Biotech maintains the patent family, of which US6080725 is a representative document on this adjuvant (JPI100 Saponin). These patents were acquired via the Advantogen merger in May 2006 (they were initially owned by Galenica). Hawaii Biotech has a stated strategy of pursuing partnerships and licensing agreements for the later stages of developments. However, they subsequently switched to alum as the adjuvant for the Hawaii Biotech vaccine. The NIH Lai *et al.* patent family, of which US5494671 is a representative document, has been discussed with the NIH as to the possibility of licensing, however, the technology may never be licensed if the patent expires before the Hawaii Biotech product enters the market. The Cuban (Guzman *et al.*) patents that disclose recombinant dengue protein vaccines are not applicable because they cover fusion protein constructs (Hawaii Biotech patents are for truncated dengue protein constructs).

5.5 MAHIDOL UNIVERSITY/SANOFI PASTEUR: LIVE ATTENUATED VACCINE

This vaccine candidate was developed through conventional PDK cell passages. Techniques used to develop this type of vaccine candidate are well established, many having been in use since the early days of the last century. Cell lines used might have proprietary constraints; however, many of these are likely to be available from cell culture collections, e.g., ATCC. Sanofi Pasteur has discontinued work on this vaccine. The vaccine is no longer under development.

5.6 US CDC-INVIRAGEN: DENGUE-DENGUE CHIMERA VACCINE

The backbone (platform) sequence CDC/InViragen uses to construct its vaccine is the DENV-2 PDK-53.

The VERO-Derived Vaccine Dengue serotype 2 (LAV2=PDK strain 16681, passage 53) uses the DEN-2 16681 virus attenuated by serial passages on PDK cells. Sanitization of the strain was performed by purifying and transfecting viral RNA into Vero cells. The process comprises the following steps:

1. extracting and purifying viral RNA from plaque-purified LVA2 strain, e.g., DEN-2 16681/PDK50 viruses;
2. advantageously associating the purified RNA with cationic lipids;
3. transfecting Vero cell, in particular Vero cell LS10;
4. recovering of the neo-synthesized virus; and
5. purifying a VDV strain by plaque purification and optionally amplifying it in host cells, especially Vero cells.

The parent strain of PDK-53 was a Thai virus-coded 16681 isolated by the Mahidol University/AFRIMS teams in Bangkok and passaged 53 times in primary dog kidney cells. Subsequent development of the DEN-1, 3, and 4 chimeras was accomplished by a joint CDC and Mahidol University collaboration.

The Vero cell technology is a well-known technology that has been used for different commercial products (injectable and oral polio vaccines, rabies vaccine). In the present invention, qualified Vero cells were advantageously used. This dengue-dengue homologous chimera was produced via cloning the prM/E genes of DENV-1, 3, and 4 into the DENV-2 16681 PDK-53 virus backbone. (Adapted from: US7641908, VERO-derived dengue serotype-2 viruses).

Preliminary formulation studies have enabled InViragen to develop a unique proprietary excipient formulation that provides excellent preservation of infectivity during lyophilization and after rehydration (as noted under IP, InViragen indicated that they have already filed a patent application for the relevant formulation work). Vaccine-certified WCB-Vero cells transfected with dengue viral RNAs were sent to Shantha in Hyderabad to develop cGMP seed stocks.

With regard to potential IP constraints on the aforementioned technologies, the Vero-cell technologies (cell-culture techniques, transfection, optimization) appear to be well established, with usage in vaccine development for a long time. The excipient formulation under consideration appears to be the property of InViragen. Hence, as with other vaccine technologies, the primary questions surrounding potential IP issues relate to molecular techniques used to generate the chimeric construct, e.g., polymerase chain reaction methods and reagents, molecular cloning technologies and related technologies. When purchased through legitimate commercial channels, these are typically accompanied by a user's license that stipulates the terms and limitations of use. Reverse genetics patents might also apply

Licensing status: The CDC has granted an exclusive li-

cense to InViragen to its (the CDC's) DEN-2 PDK-53 chimeras. InViragen has signed a manufacturing agreement with Shantha Biotechnics, Hyderabad, India. InViragen has obtained an exclusive license from the CDC for the patent US7641909; license terms include a series of low single-digit royalty payments based on sales. In addition, InViragen has obtained a nonexclusive license for the patent US6676936 (the original assignee on this patent is listed as: the United States of America as represented by the Department of Health and Human Services).

Unanswered questions with regard to licensing status:

- existence of any other IP licenses (such as for know-how/trade secrets)
- international filing status of patent portfolio
- a license from CDC to InViragen and Shantha in India; nature of the relationship between InViragen and Shantha

5.7 US NIH-DEVELOPING COUNTRY MANUFACTURERS: DENGUE-DENGUE Δ-30 CHIMERA VACCINE

The Δ30 Dengue candidate vaccine takes advantage of modern genetic techniques to first excise presumed pathogenic genetic sequences of the virus, then re-establish clones of the modified virus capable of replicating at high titers in FDA-approved Vero cell lines. The backbone (platform) sequence is the genetically engineered attenuated rDen1Δ30 virus.

The technological steps of reverse genetics are:

1. Synthesis of full length cDNA of the flaviviral genomic RNA;
2. Modification of the cDNA via molecular engineering;
3. Re-derivation of RNA from transfected cells; and
4. Derivation of infectious particles when the resulting RNA is transfected into permissive Vero cells.

Three novel recombinant dengue type 3 (DEN3) virus vaccine candidates were generated from a DEN3 virus isolated from a mild outbreak of dengue fever in the Sleman district of central Java in Indonesia in 1978. Antigenic chimeric viruses were prepared by replacing the membrane precursor and envelope (ME) proteins of recombinant DEN4 (rDEN4) virus with those from DEN3 Sleman/78 in the presence (rDEN3/430(ME)) and the absence (rDEN3/4(ME)) of the 30 mutation, a 30-nucleotide deletion in the 3' untranslated region generated via reverse genetics.

The Lai *et al.* patent family, of which US6676936 is a representative document, appear to be the dominant patents for reverse-genetics technology with regard to manipulation of flaviviral genomes. The Palese patent portfolio (see below) that addresses reverse-genetics technologies is likely peripheral or irrelevant; however, it has been included in this document for the sake of completeness.

The Kinney and Huang group at InViragen have sought a license to the technology in these Lai patents, as stated in the Global Solutions for Infectious Diseases (GSID) Brief: "InViragen has secured an exclusive license from the CDC for their patent 'Avirulent, immunogenic flavivirus chimeras' (patent no. US7641909), on which Dr. Kinney and Dr. Huang are listed as co-inventors, along with Dr. Duane Gubler and others. InViragen has negotiated a series of low-cost milestone payments based on development progress, as well as low single-digit royalty payments based on any sales. In addition, InViragen has received a non-exclusive license for the 'Chimeric and/or growth-restricted flaviviruses' patent of C.J. Lai *et al.* (patent no. 6184024 belonging to the family represented by US6676936)".

Reverse-genetics technologies might be covered by a series of patents (Peter Palese *et al.* Mt. Sinai Hospital, inventors):

- US Patent 5166057 (Recombinant negative strand RNA virus expression systems):
- US Patent 5578473 (Recombinant negative strand RNA virus)
- US Patent 5820871 (Recombinant negative strand RNA virus expression systems and vaccines)
- US Patent 5854037 (Recombinant negative strand RNA virus expression systems and vaccines)
- US Patent 6544785 (Helper-free rescue of recombinant negative strand RNA viruses)
- US Patent 6649372 (Helper-free rescue of recombinant negative strand RNA virus)

MedImmune (Gaithersburg, MD) has acquired the exclusive worldwide rights to certain IP owned by Mount Sinai School of Medicine of New York University for reverse genetics in the production of influenza vaccines. MedImmune now owns or has exclusive licenses to all of the key IP for this technology.

In addition, as with other vaccine technologies, the important questions surrounding potential IP issues relate to molecular techniques used to generate the chimeric construct, e.g., polymerase chain reaction methods and reagents, molecular cloning technologies, and related technologies. When purchased through legitimate commercial channels, these are typically accompanied by a user's license that stipulates the terms and limitations of use.
Several key technological inputs should not encounter IP issues:

- Adjuvants are not used with any live attenuated vaccine (including traditional live attenuated, chimeric,

and reverse genetically engineered) thus, IP issues are immaterial.
- NIH uses Vero cell lines (Vero passage 135), certified by WHO, which are in the public domain.
- NIH uses the 1978 Indonesia dengue strains, which are in the public domain.
- Regarding downstream production, it is anticipated that production of the vaccine will employ conventional technologies that are not proprietary.

Licensing status: Several industrial sponsors in Asia and Brazil have been awarded nonexclusive licenses for the rDen∆30 formulations. The Butantan Foundation (Sao Paulo, Brazil) has taken a nonexclusive license for the rDen∆30 candidate vaccine(s) and has also received seed virus from NIAID for vaccine development. It has also been licensed to three other development partners: Biological E, Hyderabad, India (nonexclusive rights for commercialization), Panacea Biotech, New Delhi, India (nonexclusive rights for commercialization) and Vabiotech of Hanoi, Vietnam.

In the Federal Register, Vol. 69, No. 209, page 63162, it was officially announced on October 29, 2004, that the NIH was contemplating the grant of an exclusive license to practice the invention (US20090258036) "Dengue Tetravalent Vaccine Containing a Common 30 Nucleotide Deletion in the 3'-UTR of Dengue Types 1, 2, 3, AND 4, or Antigenic Chimeric Dengue Viruses 1, 2, 3, AND 5." This was anticipated to be a grant to the Butantan Foundation, Sao Paulo, Brazil. The field of use may be limited to live attenuated vaccines against dengue in humans. The licensed territory was anticipated to be Brazil.

The Butantan Foundation has had a draft licensing agreement for some months that provides exclusivity in Brazil. It also provides exclusivity in the rest of Latin America (note that the NIH does not have patent filings outside Brazil). The license includes statements about the right to "make, use, and sell" a material. This control on materials provides Butantan with a form of exclusivity protecting it from competition by the Indian licensees and vice versa, i.e., the Indian licensees do not have to worry about Butantan selling in Asia (outside China). The NIH has not filed patent applications in China but it has in India. The Indian licensees have rights for Asia and not Latin America, and Butantan would have rights for Latin America and not Asia. NIH will not grant licenses to any other party. NIH has been approached by a large pharmaceutical company asking NIH to grant the company "co-exclusive" rights with Butantan. NIH will not do this. It is likely that once a final NIH formulation is decided on, it would not be too difficult for another company or organization to create the virus strains for the vaccine and make a copy in a country, e.g., China, where NIH does not have patent protection. The copier could not distribute the vaccine in countries where NIH has obtained patents. In sum, Butantan will get the protection it wants, and it will be the sole supplier of the NIH vaccine to countries in Latin America in the foreseeable future. Thus, testing in Nicaragua and other Latin American countries and thereby entering the Latin American vaccine scene (governments, regulators, the Pan American Health Organization (PAHO), buyers, etc.) with the NIH dengue vaccine, is sensible. Finally, it might be important to note that Butantan is using Hansenula yeast cells, not saccharomyces or pichia. This could have a number of implications, too numerous to discuss here.

Unanswered questions with regard to licensing status:

- What is the licensing status of patents to Butantan etc., viz: US6676936, US6676936, US20090263424, US20090258036 and WO2008022196?
- How are patent applications handled?
- What specific clauses are included in the licenses? For example, do the licenses grant automatic forward-going IPRs related to the subject matter being licensed?

5.8 US WRAIR-GSK: LIVE ATTENUATED VACCINE

This vaccine was developed by conventional passage techniques (nonproprietary) via multiple passages through PDK cells with a final passage through fetal rhesus lung (FRhl) cells (freely available in cell collections). With each viral passage, there is a probability of an attenuating point mutation arising in the foreign host cells (e.g., PDK cells). Passage techniques could be proprietary; they were developed at the University of Hawaii by Halstead. However, no issued US patents related to this technology were identified.

Our research identified four PCT applications that addressed basic vaccine technologies:

1. WO2000057908, Attenuated Dengue-1 Virus Vaccine
2. WO2000057909, Attenuated Dengue-2 Virus Vaccine
3. WO2000057904, Attenuated Dengue-3 Virus Vaccine
4. WO2000057910, Attenuated Dengue-4 Virus Vaccine

However, only three corresponding US patents were identified:

1. US6511667, Attenuated Dengue-2 Virus Vaccine
2. US6528065, Attenuated Dengue-3 Virus Vaccine
3. US6537557, Attenuated Dengue-4 Virus Vaccine

Subsequent conversations with WRAIR indicated that the PCT application for Attenuated Dengue-1 Virus Vaccine (WO2000057908) as a US application was unsuccessful due to prior art issues.

The relationship between WRAIR and GSK is based on a CRADA (nonexclusive license in 2000). This agreement captures all patents and applications related to this project, i.e., Live Attenuated Vaccines. The NIH Lai *et al.* patent family, of which US6676936 is a representative document, is possibly also applicable to this vaccine.

Unanswered questions with regard to this vaccine:

- What is the licensing status?
- What is the international filing status of the portfolio?

5.9 A NOTE ABOUT THE LAI *ET AL.* PATENTS

It is very important to note that the NIH Lai *et al.* patent family, of which US6676936 is a representative document, covers fundamental technologies potentially relevant to the current advanced-phase dengue vaccine approaches.

In addition, another Lai *et al.* patent family, of which US5494671 is a representative document, is likely to be relevant to the technologies developed by Hawaii Biotech. Despite the existence of the Lai *et al.* patents, it was indicated that the expiration date of these patents may precede the actual commercial distribution of a vaccine. However, this poses a dilemma because even if these patents expire before the commercialization of the vaccine, the methodologies used in developing the vaccine may still be protected by another active patent.

For the Lai *et al.* patents, therefore, late-stage vaccine developers might consider reviewing the IP management approaches and determine what the potential status of their products are with regard to the various inputs. A range of options are available but discussion of these exceeds the purpose of this present report and should, in any case, be discussed internally by the various institutions.

[16] For further details visit http://tiny.cc/rgcse

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Annexes

Annex A:

FAMILY DATA FOR RELEVANT PATENT DOCUMENTS IDENTIFIED IN THIS REPORT:

REPRESENTATIVE FAMILY DOCUMENT (PUBLICATION NUMBER), WITH CORRESPONDING INPADOC FAMILY MEMBER DOCUMENTS, AS PER JURISDICTIONAL CODES IN ALPHABETICAL ORDER

Table A1:

PATENTS RELATED TO THE ACAMBIS/SANOFI PASTEUR: YELLOW FEVER-DENGUE CHIMERA VACCINE

Publication Number	INPADOC Family Members
EP1373478	AT200127220 AT323758 AT410634 AU2002229390 CA2432370 CN101658669 CN101670101 CN1492923 DE50206472 EP1373478 EP1373478 JP2004520406 US20040052818 WO2002066621
EP1441761	AT359816 AU2002365922 BR200213408 CA2464138 DE60219658 DE60219658 DK1441761 EP1441761 EP1441761 EP1441761 ES2284984 IL161501D0 JP2005516974 MX2004PA003692 NZ532385 PT1441761 RU2004115107 RU2313367 US20030129201 US6878372 WO2003063725 WO2003063725 ZA200403047
EP1924280	AR55603 AU2006280144 CA2618783 CN101287490 EP1924280 EP1924280 JP2009504654 NO200801127 US20080193477 WO2007021672 WO2007021672 ZA200802176
EP2143440	EP2143440 US20100015180 WO2010003670
US20040259224	AU2003239932 AU2003239932 US20040259224 WO2003101397 WO2003101397
US20080014219	AR61887 AU2007274100 CA2656349 CN101489585 EP2043680 FR2903605 JP2009542783 KR2009027759 MX2009000369 US20080014219 WO2008007021
US20080085288	AR63057 AU2007311792 CA2663885 CN101553251 EP2077857 FR2906724 FR2906724 JP2010505801 KR2009064593 MX2009003417 US20080085288 WO2008047023 WO2008047023
US20080131460	AR64009 AU2007327367 CA2668570 CN101541344 EP2099483 FR2909286 KR2009087890 MX2009004223 US20080131460 WO2008065315
US20090191240	AU2003267937 AU2003267937 AU2005216248 BR200306905 BR200508064 CA2473321 CA2557136 CN1871026 CN1950499 EP1471873 EP1471873 EP1755539 EP1755539 JP2005519639 JP2007525226 KR2006135844 MX2004PA006870 NZ534159 NZ549749 SG150551 US20050002968 US20080274142 US20090191240 US7459160 WO2003103571 WO2003103571 WO2005082020 WO2005082020 WO2005082020
US5023171A	US5023171
US6171854	BR199701774 CA2233932 CA2233932C DE69839169 DE69839169T2 EP877086 EP877086 EP877086 OA10791 RU1998106999 US6171854 US6589522 ZA199802760
US6676936	AT186744 AT197607 AT310824 AT443143 AU199226914 AU667836 CA2116980 CA2116980C DE69230316 DE69230316 DE69231570 DE69231570 DE69233566 DE69233566 DE69233770 DK604566 DK872553 EP1018556 EP1018556 EP1605047 EP1605047 EP604566 EP604566 EP604566 EP872553 EP872553 ES2140418 ES2153223 ES2254055 GR3032416 GR3035149 JP03531934 JP03681358 JP03681369 JP2002325593 JP2003135085 JP6511150 US6184024 US6676936 WO1993006214

Publication Number	INPADOC Family Members
US6962708	AT297757 AU199864431 AU200118139 AU740961 BR199807873 CA2282790 CN1187088C CN1253506 CZ199903064 CZ300172B6 DE69830579 DE69830579 DK977587 EP1625852 EP977587 EP977587 EP977587 ES2244050 HK1025743 HU200002139 HU200002139 IL131600D0 JP2001514622 NO199904185 NO199904185 NZ337522 PL192957 PL335481 PT977587 PT977587 RU2209082 US20040223979 US6696281 US6962708 WO1998037911 WO2001039802 WO2001039802
US7569383	AU2002322026 BR200210907 CA2448971 CN1551782 EP1401859 EP1401859 JP2005501532 PL371187 US20030044773 US7569383 WO2002102828 WO2002102828
US7632510	AU199943296 AU2007202304 AU2007202304 AU2007202304 AU778988 BR199910830 BR200208301 CA2331368 CA2331368C CA2443323 CN101002936 CN1304575 CN1500152 DE69934583 DE69934583 EP1084252 EP1084252 EP1383931 EP1383931 EP1935991 EP1935991 HK1062836 IL139844 IL139844D0 JP2002517200 JP2004532023 JP2010017185 MX2003PA008838 NZ529106 US20030022849 US20050163804 US20070166329 US20070166701 US20080241186 US20100003273 US20100040643 US7227011 US7417136 US7521177 US7632510 US7662394 WO1999063095 WO1999063095 WO2002081754 ZA200307580
WO2003060088	AU2003205169 AU2003205169 US20030180329 WO2003060088 WO2003060088
WO2006116182	AU2005295438 AU2006239954 CA2584228 CA2605924 CN101084010 CN101203240 EA200700904 EP1809325 EP1809325 EP1874346 EP1874346 JP2008520187 JP2008538698 KR2007072597 NO200701924 RU2007143529 SG156666 US20070269458 US20080175862 WO2006044857 WO2006044857 WO2006116182 ZA200704033
WO2007051267	BR200504945 EP1989316 WO2007051267 WO2007051267 WO2007051267
WO2008036146	AU2007297801 CA2659592 CN101528254 EP2043681 EP2043681 JP2009543554 WO2008036146 WO2008036146
WO2008057550	AR63606 AU2007317847 AU2007317847 CA2668834 CL20073209 CL32092007 EP2086581 MX2009004862 WO2008057550 WO2008057550
WO2008094674	CA2676689 EP2121010 US20100034849 WO2008094674
WO2008137163	AU2008248061 CA2686398 EP2150614 US20090324623 WO2008137163

Table A2:

PATENTS RELATED TO THE HAWAII BIOTECH/MERCK ENVELOPE PROTEIN SUBUNIT VACCINE

Publication Number	Expanded INPADOC Family Members
US20080248064	CA2576798 EP1784487 US20080248064 WO2006025990 WO2006025990
US20080311157	AR58049 AU2006291863 CA2622827 CN101304760 EP1944038 JP2009507864 KR2008048068 RU2008114841 US20080311157 WO2007031034 ZA200802740
US20100047280	AU2002356690 AU2002356690 CA2466413 CN101397574 CN1602316 EP1456230 JP2005511042 KR2005044726 US20060159699 US20100047280 WO2003048184 WO2003048184
US5494671A	AU199187625 AU199224972 US5494671 WO1992003161 WO1993003763
US6046025A	AP198900150D0 AT90969 AU198815510 AU199047552 AU618952 AU630649 CA1341345 CA2003794 CA2003794 CY1929 DE3881953 DE3881953 DK175461 DK175595 DK198802433 DK198802433D0 DK199101049 DK199101049D0 EP290261 EP290261 EP446272 EP446272 ES2058274 FI113475 FI199102635D0 HK1006852 IE19881373L IE63005 IL92470 IL92470D0 JP02706260 JP03085704 JP4501954 JP63304982 KR152525 NO199102099 NO199102099D0 NO314090 PT87424 PT87424 PT92477 US5550043 US5681713 US5705359 US6046025 WO1990006358 ZA198803213 ZA198909150
US6080725A	AT286399 AU199875818 AU732856 BR199809149 CA2290646 CA2290646 DE69828507 DE69828507 DK996451 EP996451 EP996451 EP996451 ES2235330 IL132886D0 JP2002504099 NZ500779 PT996451 US5977081 US6080725 WO1998052573 WO1998052573
US6165477A	AT227584 AU199660239 AU716676 CA2224724 CA2224724 DE69624815 DE69624815 DK836482 EP836482 EP836482 EP836482 ES2184869 PT836482 US6136561 US6165477 WO1996037221
US6416763	AU199957907 AU758361 CA2340788 EP1107786 US6416763 WO2000012128 WO2000012128 WO2000012128 WO2000012128
US6432411	AU200060863 BR200013154 CN1424917 US6432411 WO2001003729 WO2001003729
US6676936	AT186744 AT197607 AT310824 AT443143 AU199226914 AU667836 CA2116980 CA2116980 DE69230316 DE69230316 DE69231570 DE69231570 DE69233566 DE69233566 DE69233770 DK604566 DK872553 EP1018556 EP1018556 EP1605047 EP1605047 EP604566 EP604566 EP604566 EP872553 EP872553 ES2140418 ES2153223 ES2254055 GR3032416 GR3035149 JP03531934 JP03681358 JP03681369 JP2002325593 JP2003135085 JP6511150 US6184024 US6676936 WO1993006214
US6749857	AT321567 AU199885905 AU2002300271 AU2002300271B8 AU752191 BR199815551 CA2298538 DE69834041 DE69834041 EP1005363 EP1005363 JP2001511459 US20030175304 US6749857 WO1999006068 WO1999006068
US7265215	US20040022811 US6074865 US6514501 US7265215

Publication Number	Expanded INPADOC Family Members
US7476390	AU2003267943 AU2003267943 AU2003267943C1 CA2481479 CN1909922 EP1572941 EP1572941 NZ535690 US20040009469 US20090181044 US20090311287 US7476390 WO2003102166 WO2003102166 WO2003102166
US7566457	AT349466 BR200211178 CA2453300 CN100378123 CN1531548 CU23245 DE60217129 DE60217129 DK1418180 EP1418180 EP1418180 ES2278041 JP04417712 JP2004537306 MX2004PA000486 PT1418180 US20040234951 US20070141081 US20090274718 US7279164 US7566457 WO2003008571 WO2003008571 WO2003008571 ZA200400289
WO2004052293	AU2003300831 AU2003300831 US20040213808 US20050287170 WO2004052293 WO2004052293 WO2006115548 WO2006115548
WO2007034507	WO2007034507 WO2007034507
WO2007059715	AR58215 AU2006317350 CA2630629 CN101360758 EP1958959 JP2009524581 KR2008080137 RU2008125077 US20090312190 WO2007059715 WO2007059715 WO2007059715 ZA200804874

Table A3:

PATENTS RELATED TO THE MAHIDOL UNIVERSITY/SANOFI PASTEUR: LIVE ATTENUATED VACCINE

Publication Number	Expanded INPADOC Family Members
EP1159968	AT412738 DE60040652 DK1159968 EP1159968 EP1159968 ES2315221 PT1159968
US20080014219	AR61887 AU2007274100 CA2656349 CN101489585 EP2043680 FR2903605 JP2009542783 KR2009027759 MX2009000369 US20080014219 WO2008007021
WO2001091790	AU200167541 BR200111223 CN1188168 CN1431913 EP1159969 MX2002PA011654 WO2001091790
WO2003101397	AU2003239932 AU2003239932 US20040259224 WO2003101397 WO2003101397
WO2006134433	AR53913 AU2006257610 CA2611934 CN101238144 EP1893637 JP2008546382 KR2008027356 MX2007015873 NO200706355 US20060292172 US7641907 WO2006134433 ZA200710714

Table A4:

PATENTS RELATED TO THE US CDC-INVIRAGEN: DENGUE-DENGUE CHIMERA VACCINE

Publication Number	Expanded INPADOC Family Members
US20080014219	AR61887 AU2007274100 CA2656349 CN101489585 EP2043680 FR2903605 JP2009542783 KR2009027759 MX2009000369 US20080014219 WO2008007021
US6676936	AT186744 AT197607 AT310824 AT443143 AU199226914 AU667836 CA2116980 CA2116980 DE69230316 DE69230316 DE69231570 DE69231570 DE69233566 DE69233566 DE69233770 DK604566 DK872553 EP1018556 EP1018556 EP1605047 EP1605047 EP604566 EP604566 EP604566 EP872553 EP872553 ES2140418 ES2153223 ES2254055 GR3032416 GR3035149 JP03531934 JP03681358 JP03681369 JP2002325593 JP2003135085 JP6511150 US6184024 US6676936 WO1993006214
US7641907	AR53913 AU2006257610 CA2611934 CN101238144 EP1893637 JP2008546382 KR2008027356 MX2007015873 NO200706355 US20060292172 US7641907 WO2006134433 ZA200710714
US7641908	AR53914 AU2006257621 CA2611954 CN101238209 EP1891210 JP2008546383 KR2008018271 MX2007015872 NO200706360 US20070026016 US7641908 WO2006134443 ZA200710752
US7641909	AU2001238441 AU200138441 AU2007200319 AU2007200319 AU2007200319B8 AU2009212794 CA2398872 EP1263965 JP2003523189 US20060062803 US20090010961 US7094411 US7641909 WO2001060847 WO2001060847 WO2001060847
WO1996040933	AU199660932 WO1996040933

Table A5:

PATENTS RELATED TO THE US NIH-DEVELOPING COUNTRY MANUFACTURERS: DENGUE-DENGUE Δ-30 CHIMERA VACCINE

Publication Number	Expanded INPADOC Family Members
US20090258036	AU2003231185 CA2483653 EP1554301 EP1554301 JP2005532044 US20070009552 US20090258036 US7517531 WO2003092592 WO2003092592
US20090263424	AU2002312011 AU2008203275 BR200209943 CA2448329 EP1402075 EP1402075 US20050010043 US20070092534 US20090263424 US7226602 US7560118 WO2002095075
US6676936	AT186744 AT197607 AT310824 AT443143 AU199226914 AU667836 CA2116980 CA2116980 DE69230316 DE69230316 DE69231570 DE69231570 DE69233566 DE69233566 DE69233770 DK604566 DK872553 EP1018556 EP1018556 EP1605047 EP1605047 EP604566 EP604566 EP604566 EP872553 EP872553 ES2140418 ES2153223 ES2254055 GR3032416 GR3035149 JP03531934 JP03681358 JP03681369 JP2002325593 JP2003135085 JP6511150 US6184024 US6676936 WO1993006214
WO2007141259	AR61197 US20080107685 WO2007141259
WO2008022196	AU2007285929 AU2007285929 CA2661296 CN101657463 EP2054428 WO2008022196 WO2008022196

Table A6:

PATENTS RELATED TO THE US WRAIR-GSK: LIVE ATTENUATED VACCINE

Publication Number	Expanded INPADOC Family Members
US20080014219	AR61887 AU2007274100 CA2656349 CN101489585 EP2043680 FR2903605 JP2009542783 KR2009027759 MX2009000369 US20080014219 WO2008007021
US6676936	AT186744 AT197607 AT310824 AT443143 AU199226914 AU667836 CA2116980 CA2116980 DE69230316 DE69230316 DE69231570 DE69231570 DE69233566 DE69233566 DE69233770 DK604566 DK872553 EP1018556 EP1018556 EP1605047 EP1605047 EP604566 EP604566 EP604566 EP872553 EP872553 ES2140418 ES2153223 ES2254055 GR3032416 GR3035149 JP03531934 JP03681358 JP03681369 JP2002325593 JP2003135085 JP6511150 US6184024 US6676936 WO1993006214
US7217418	AT419006 AU200040382 AU779280 BR200010969 CA2368674 CN1191092C CN1351502 DE60041250 EP1165127 EP1165127 ES2322327 JP2002540168 MX2001PA009683 US20070087015 US6638514 US7217418 WO2000057907 WO2000057907 WO2000057907
WO2000057904	AU200041783 CA2368790 EP1165130 JP2002540166 US6528065 WO2000057904 WO2000057904 WO2000057904
WO2000057908	AU200041792 CA2365728 EP1165131 JP2002540169 WO2000057908 WO2000057908 WO2000057908
WO2000057909	AU200040402 CA2365411 EP1165128 JP2002540170 US6511667 WO2000057909 WO2000057909 WO2000057909
WO2000057910	AU200040404 CA2368673 EP1165129 JP2002540171 US6537557 WO2000057910 WO2000057910
WO2000058444	AU200040403 AU776638 CA2365415 EP1165756 JP2002539821 US6613556 WO2000058444 WO2000058444 WO2000058444

Annex B:

PATENT MAPPING WITH THE AUREKA® THEMESCAPE™ MAPMANAGER

Mapping a Technology Landscape[1] Themescape parses documents and statistically analyzes the key terms, or topics, that those records have in common. This tool draws on US, DE, EP, GB, and WO data.

Aureka® Themescape™ is a text mining tool that analyzes text in large sets of documents, creating an overview of the subject matter. The analysis is faster and identifies more subject categories than could reasonably be accomplished by a human reader. In addition, results are condensed into a visual representation of the topics that can be further investigated.

Based on the topics in patent documents, Aureka® Themescape™ creates interactive, self-organizing content maps that visually provide an overview of patent portfolios while also representing the conceptual relationships among the documents. The program identifies the relevant key themes (coordinately expressed topics) and then visually portrays them and their relationship to each other on a contour map. The Aureka® ThemeScape™ MapManager function thus transforms a set of patent documents into a topographical landscape, based on its assessment of a range of categories, themes, and concepts.

By showing where patents exist in relation to other patents, this geographic, big picture view facilitates identification of areas of potential overlap and enables the reader to compare the concentration of efforts within the given technology space.

Creation of the Themescape™ Map is a fourfold process:

1. Harvest

- Load text from document list to database
- Apply Stopwords
- Create Stems and Tokens

2. Analyze

- Calculate Term Frequency/Inverse Document Frequency (TFIDF)
- Eliminate Frequent/Infrequent Used Terms
- Creates Topic List

3. Cluster

- Apply Naive Bayesian Classifier
- Assign Document Vectors
- Apply Vector Space Modeling to Plot Documents in ‘n’ dimensions

4. Self Organizing Map (SOM) Algorithm

- Convert ‘n’ dimensions to 2-dimensions
- Simulate depth with contour lines and color shading
- Show dense clusters as mountains
- Add labels based on regional topic term TFIDF
- Show elevation decreasing with lighter shading

Technical Limits of Aureka® Themescape™ Map:

- 60,000 documents - titles and abstracts
- 30,000 documents – claims
- 10,000 documents – full text
- 20 documents minimum

The Naïve Bayesian Classifier as employed in the Aureka® Themescape™ Map generation is based on established principles of classical probabilistic theorems. Bayes’ Theorem is a result in probability theory, named after the Reverend Thomas Bayes, a British mathematician and minister, who proved a special case of it in the 18th century. In Themescape™, the Bayesian Classifier is used in combination with Vector Space Model (VSM) to derive a statistical inference. This inference updates iteratively estimates of the probability that classifications of a document are correct, based on relationships of the Topic List of each document to other documents in the set, as well as the knowledge of how likely those relationships are correct. The classifications are also derived from the Topic List.

The Bayesian Process:

- Reads the Topic List for each document.
- Uses the Bayes’ Theorem to estimate the posterior probabilities of all classifications.
- For each word in the Topic List, a classification with highest posterior probability is chosen as the prediction.
- Is called naïve because it originally makes the assumption that the classifications are independent of each other.

[1] http://www.micropat.com/static/aureka.htm

The formal algorithm is:

- Let P be the Topic List,
- Let h be a hypothesis of relationship of a document to a word in P
- Let D be the set of documents.

If we know:

- P(h), the probability of hypothesis h being correct,
- P(D), the probability of data set D being observed, and
- P(D/h), the probability of observing D, under the assumption of h being correct,

then the Bayesian Theorem provides a method for calculating P(h / D), denoting the probability of h being correct, given a specific data set D. Using the Prior Probability and Naïve Bayes Classifier Vector Space Modeling (VSM), the document is provisionally plotted in n-space. Next, the 'Likelihood' or Probability of the classification of Document X given Topic A and Topic B are calculated. In Bayesian analysis, the final classification is produced by combining both the Prior Probability and the Likely Probability to form the Posterior Probability.

Annex C:

BROAD LANDSCAPE AUREKA® PATENT MAPS

The patents we considered applicable are highlighted in white dots, showing concentration in the top right (north-eastern) corner. Those in the lower left side are live DNA vaccines. The outlier in the upper left side is a patent for a diagnostic.

Figure C1:

3,800 PATENTS/PATENT FAMILIES WITH THOSE BY "PUTNAK" (INVENTOR) HIGHLIGHTED

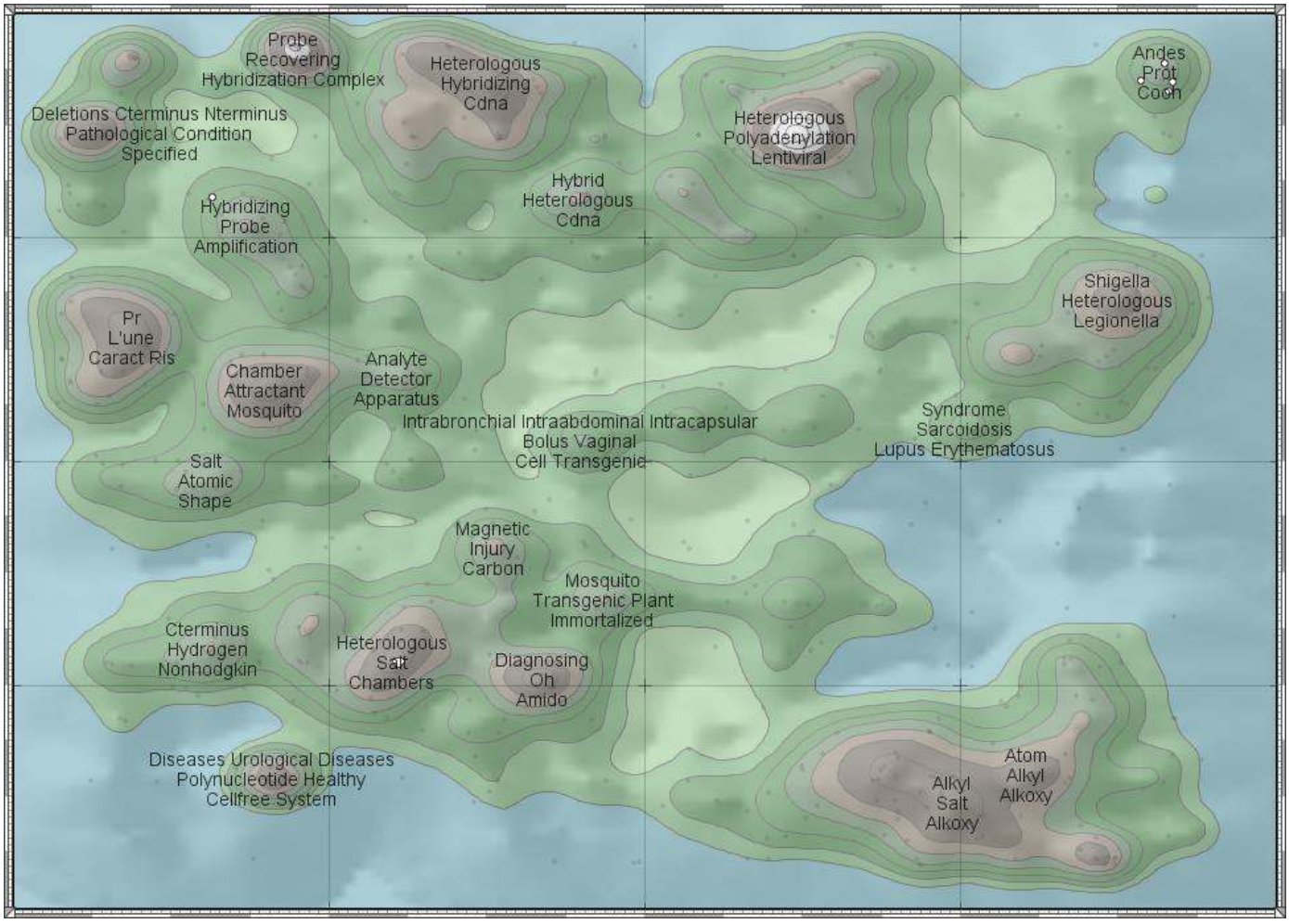


Figure C2:

3,800 PATENTS/PATENT FAMILIES WITH THOSE BY “ACAMBIS” (ASSIGNEE) OR “MONATH” (INVENTOR) HIGHLIGHTED

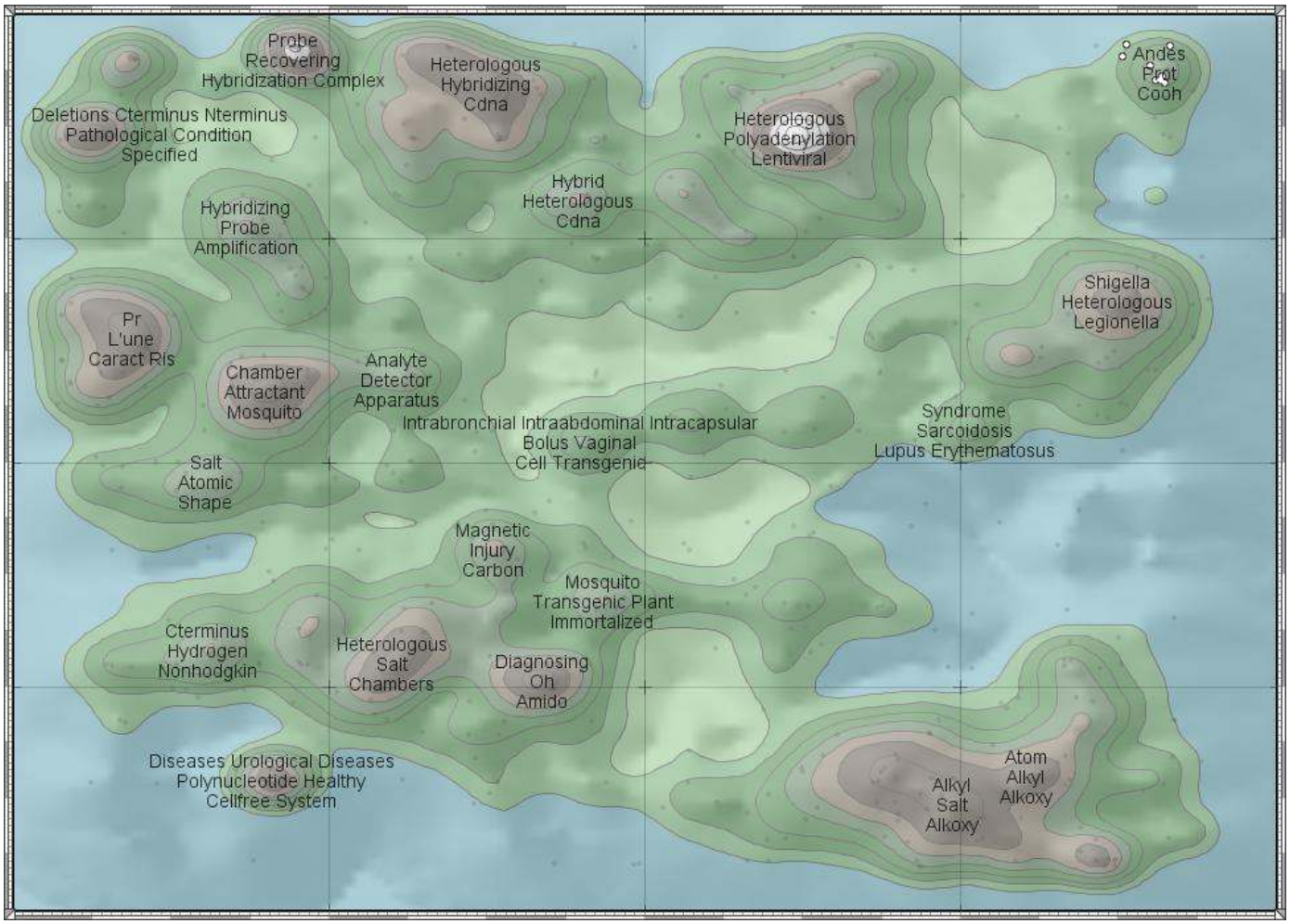
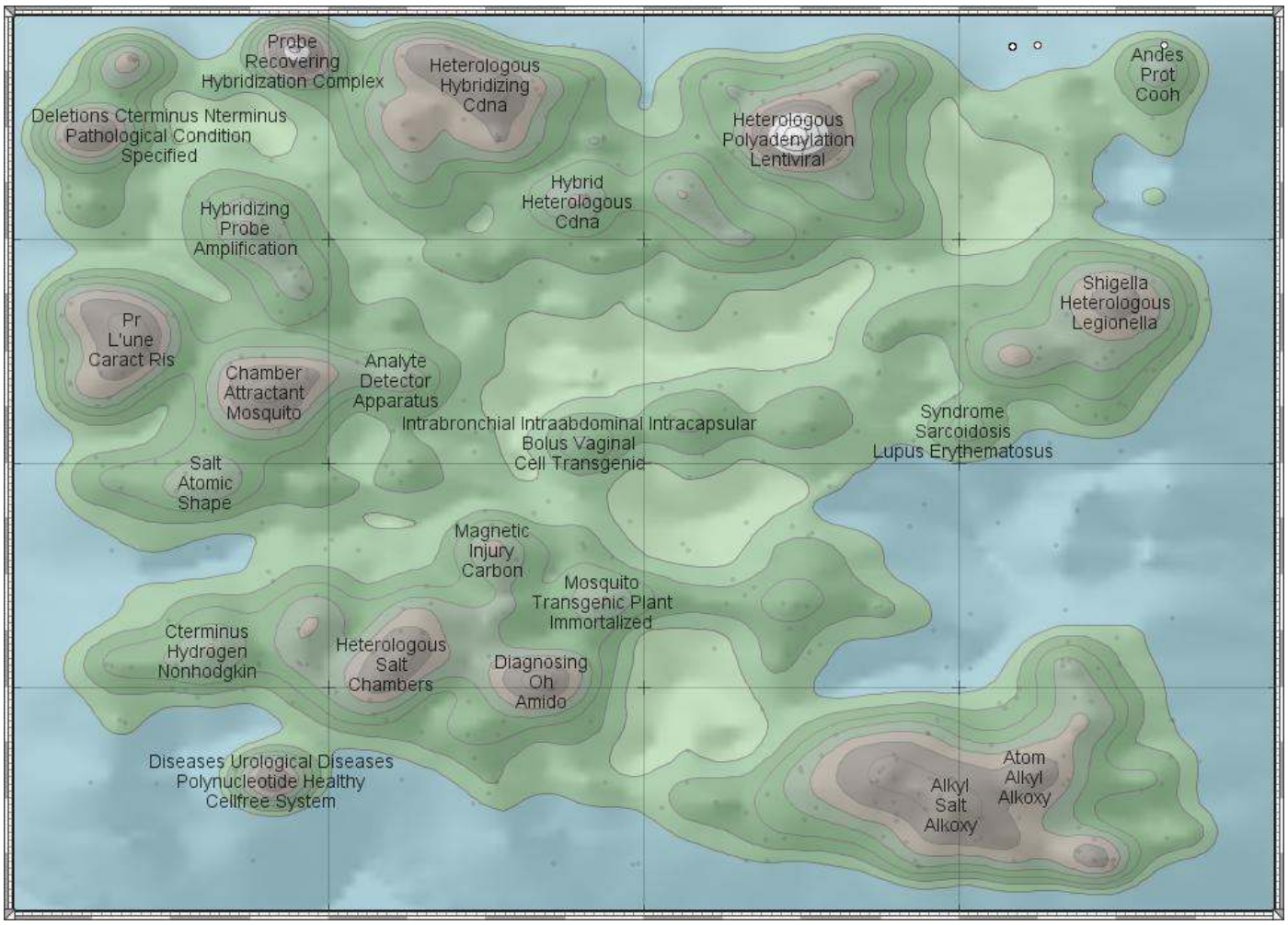


Figure C3:

3,800 PATENTS/PATENT FAMILIES WITH THOSE BY "HAWAII" (ASSIGNEE) OR "IVY" (INVENTOR) HIGHLIGHTED



Annex D:

REPRESENTATIVE OR SAMPLE LIST OF PATENTS FAIRLY CLOSELY RELATED TO BUT NOT DEEMED RELEVANT TO THE LIST OF SIX PRODUCTS STUDIED AS PART OF THIS REPORT

Patent or Application Number	Title	Comment	Technology	Assignee / Applicant	Inventor(s)	Filing date
EP1018556	Chimeric and/or growth-restricted flaviviruses	Type 4 viral RNA, at least 1 mutation with conditions. Also method related to dengue and non-dengue.	Chimeric Live Attenuated Vaccines	The United States of America, as represented by the Secretary, Department of Health and Human Services	Lai, Ching-Juh / Bray, Michael / Pletnev, Alexander G. / Men, Ruhe / Chanock, Robert M.	18-Sep-92
EP1159968	Attenuated strains of dengue virus and their use in a vaccine composition	For specific accessions only. Restricted to tetravalent and 2 dose administration. Only applicable in the unlikely event that Sanofi Pasteur continues to work with the very same accessions from Mahidol University	Traditional Live Attenuated Vaccine	Mahidol University	Bhamarapravat, Nath Vaccine Development Center / Yoksan, Sutee Vaccine Development Center	30-May-00
JP23135085	Chimeric and/or growth-restricted flavivirus	Equivalent of EP1018556	Chimeric Live Attenuated Vaccines	US Government	Lai, Ching-Juh / Bray, Michael / Pletnev, Alexander G. / Men, Ruhe	18-Sep-92
JP24307477	Method for enhancing neutralization antibody-inducing ability of transgenic vaccine and method for administering vaccine	Could be relevant to the Navy program	DNA Vaccines	Kobe University	Konishi, Eiji	9-Mar-04
JP25015355	Method for increasing amount of antigen produced from DNA vaccine, method for administering DNA vaccine and method for detecting antigen produced by DNA vaccine	Could be relevant to the Navy program	DNA Vaccines	Kobe University	Konishi, Eiji	24-Jun-03

Patent or Application Number	Title	Comment	Technology	Assignee / Applicant	Inventor(s)	Filing date
US2004049016	Pro-apoptotic fragments of the dengue virus envelope glycoproteins	Cancer treatment. Use of dengue to kill tumor	Cancer	Pasteur Institute	Despres, P. / Courageot, M.P. / Deubel, V. / Catteau, A.	6-Aug-03
US2004265324	Recombinant MVA virus expressing dengue virus antigens, and the use thereof in vaccines	Instead of yellow fever, use of MVA	Chimeric Live Attenuated Vaccines	Cardosa Mary Jane/Sutter Gerd/Erfle Volker	Cardosa, Mary Jane / Sutter, Gerd / Erfle, Volker	24-Feb-04
US2004265338	Subgenomic replicons of the flavivirus dengue	Could be applicable in 2nd generation dengue vaccines	Reverse Genetically-Engineered, Live Attenuated Vaccines	The United States of America, as represented by the Secretary, Department of Health and Human Services	Pang, Xiaowu / Dayton, Andrew I. / Zhang, Mingjie	5-Sep-03
US20050100886	Construction of West Nile virus and dengue virus chimeras for use in a live virus vaccine to prevent disease caused by West Nile virus	Dengue as backbone/InViragen Note: claim 1 proviso that NOT PDK-53, etc.			Pletnev, Alexander G. (Rockville, MD) / Putnak, Joseph R. (Silver Spring, MD) / Chanock, Robert M. (Bethesda, MD) / Murphy, Brian R. (Bethesda, MD) / Whitehead, Stephen S. (Montgomery Village, MD) / Blaney, Joseph E. JR. (Frederick, MD)	11-May-01
US2005226849	Compositions and methods of using capsid protein from flaviviruses and pestiviruses	Cancer mainly, but possibly in future as dengue vaccine	DNA Vaccines		Weiner, David B. / Yang, Joo-Sung / Muthumani, Karuppiah	14-Oct-04
US2006159699	Flavivirus NS1 subunit vaccine	US Navy related but not on DVI list	DNA Vaccines		Howley, Paul / Leyrer, Sonja / Cardosa, Mary Jane / Sum, Magdeline / Sia, Henry	20-Nov-02

Patent or Application Number	Title	Comment	Technology	Assignee / Applicant	Inventor(s)	Filing date
US2006280757	Flavivirus vaccine delivery system	In future maybe applicable to VLPs	Delivery		Khromykh, Alexander A.	7-Jun-04
US2007292453	RNA virus vaccines and methods	Emerging for DNA vaccine (RNA vaccine technology)	DNA Vaccines		Floyd, Robert A. / Dittmer, Dirk P.	14-Dec-06
US20080063657	Compositions comprising pathogen-associated molecular patterns and antigens and methods of use thereof	Composition of matter patent for a fusion protein. Might be useful as a prime boost.	Recombinant Dengue Virus Protein Vaccines		Powell, T.J. / Nakaar, V. / Song, Langzhou / Huleatt, J.W. / McDonald, WF. / Hewitt, D.D.	18-Jul-07
US5690938	Oral immunization with multiple particulate antigen delivery system	Bluetonge antigen delivery system (mucosal immune response)	Delivery	Oravax, Inc. / Natural Environment Research Council	Ermak, Thomas H. / Pappo, J/ Guirakhoo, Farshad / Nichols, Jr. Richard D. / Monath, Thomas P. / Roy, Polly	7-Jul-89
US6017535	cDNA sequence of Dengue virus serotype 1 (Singapore strain)	Serotype 1 with specific sequence. How closely are the DNA sequences of different serotype 1 viruses related?	Reverse Genetically-Engineered, Live Attenuated Vaccines	Insititute of Molecular and Cell Biology	Fu, Jianlin / Tan, Boon-Huan / Yap, Eu-Hian / Chan, Yow-Cheong / Tan, Yin-Hwee	16-Dec-94
US6083505	1H-imidazo[4,5-C]quinolin-4-amines as vaccine adjuvants	Only as adjuvant (1H-imidazo[4,5-C]quinolin-4-amines). Adjuvant	Adjuvant	3M Innovative Properties Company	Miller, Richard L. / Tomai, Mark A. / Bernstein, David I. / Harrison, Christopher J.	24-Mar-94
US6355247	Nucleic acid immunization using a virus-based infection / transfection system	Only for DNA vaccine. US Navy and Japanese group	DNA Vaccines	Chiron Corporation	Selby, Mark / Walker, Christopher	4-Nov-96
US6372227	Vaccines	Broad adjuvant	Adjuvant	SmithKline Beecham Biologicals, S.A.	Garcon, Nathalie / Momin, Patricia Marie Christine Aline Francoise	5-Sep-97

Patent or Application Number	Title	Comment	Technology	Assignee / Applicant	Inventor(s)	Filing date
US6589533	Genetically-engineered alphaviruses, flaviviruses, and bunyaviruses with modified envelope transmembrane glycoproteins and altered host-range phenotype	Hawaii raises vaccine in insect cells. Use of mosquito cells to detect		Research Development Foundation	Brown, Dennis T. (Raleigh, NC) / Hernandez, Racquel	7-Jul-99
US6630455	Methods for inducing mucosal immune responses	Mucosal immune response. With liposome carrier. Esp. HIV vaccine	Delivery	Vanderbilt University	Mitchell, William M.	13-Jan-95
US6660273	Chimeric Vaccine Against Tick-borne Encephalitis Virus	Varient of US6184024. For encephalitis		The United States of America as represented by the Department of Health and Human Services	Pletnev, Alexander / Men, Ruhe / Chanock, Robert / Lai, Ching-Juh.	9-Dec-03
US6673591	Methods for enhancing the production of viral vaccines in cell culture	Production of anything where whole virons are made. Traditional chimeric, etc.	Production	The Regents of the University of California	Lau, Allan S.	13-Dec-00
US6784161	Method for the treatment or prevention of flavivirus infections using nucleoside analogues	Diagnostic only		BioChem Pharma, Inc.	Ismaili, Hicham / Moulay, Alaoui	30-Aug-00
WO03048184	Flavivirus NS1 subunit vaccine	US2006159699 seems very close. But the priority dates (and other dates) are not the same. Could be US Navy	Recombinant Dengue Virus Protein Vaccines	Bavarian Nordic A/S / Venture Technologies Sdn Bhd	Howley, Paul / Leyrer, Sonja / Cardosa, Mary Jane / Sum, Henry / Sia, Magdeline	4-Dec-01

This report was prepared by Anatole Krattiger, Director, Global Challenges Division, World Intellectual Property Organization (WIPO), Geneva, Switzerland (anatole.krattiger@wipo.int), Richard T. Mahoney, Coordinator, Policy & Access, Dengue Vaccine Initiative, International Vaccine Institute, Seoul, Republic of Korea (rmahoney@pdvi.org), Amrita Chiluwal, Fellow, International Technology Transfer Institute, Franklin Pierce Center for Intellectual Property, University of New Hampshire School of Law, Concord, United States of America (amrita.chiluwal@law.unh.edu), and Stanley P. Kowalski, Professor, Franklin Pierce Center for Intellectual Property, University of New Hampshire School of Law, Concord, United States of America (stanley.kowalski@law.unh.edu).

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World Intellectual Property Organization
34, chemin des Colombettes
P.O. Box 18
CH-1211 Geneva 20
Switzerland

Telephone: +4122 338 91 11
e-mail: global.challenges@wipo.int