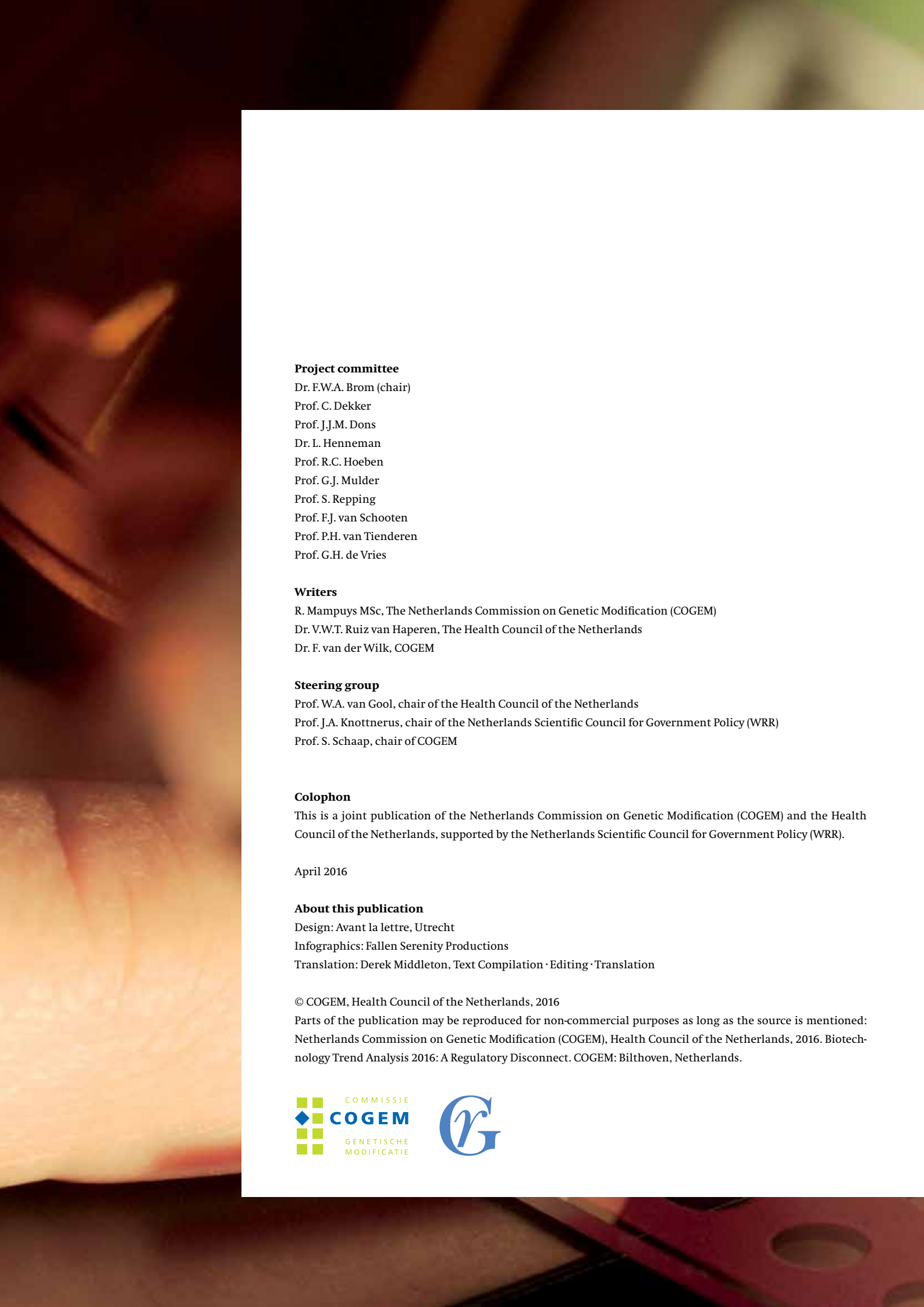
BIOTECHNOLOGY TREND ANALYSIS 2016

A REGULATORY DISCONNECT



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SUMMARY

In the past few years the advances made in biotechnology have been rapid. New techniques and applications are appearing in quick succession and growing numbers of biotechnology products are coming onto the market. Moreover, biotechnology is becoming strongly integrated into other research areas and industries, and has become a mainstream technology within the life sciences. These developments offer new opportunities and possibilities for further innovation, economic growth, food production and healthcare, but they also have major consequences for policy, legislation and how we view biotechnology itself. They raise ethical questions and pose social dilemmas concerning the acceptability and regulation of new techniques, property rights and how to deal with differences between national approaches.

It is impossible to discuss all the developments in biotechnology in this Trend Analysis. It was therefore necessary to limit the scope of this analysis to eight trends that are illustrative of the field of biotechnology as a whole and that raise important dilemmas and social questions requiring political and policy responses.

The first two trends – next generation sequencing and CRISPR-Cas – are driving new developments within biotechnology. They give a strong impetus to the other trends discussed in this report: the development of specific medicines, medical aids and therapies for individuals or specific diseases (personalised medicine, gene therapy, 3D bioprinting), new techniques in agriculture (RNAi), interventions in ecosystems (genetically modified insects), and even the prospect of designing organisms and turning nature to our advantage (synthetic biology).

1. NEXT GENERATION SEQUENCING (NGS): THE NEW STANDARD

Sequencing is determining the order of base pairs in the genetic material (DNA or RNA) in microorganisms, plants, animals and humans. The speed and scale at which DNA can be sequenced has increased enormously in recent years, while the cost has plummeted. In the health sector, sequencing is already being used in genetic diagnostics and increasingly for screening as well, which makes it particularly important that both professionals and the public are better informed about the facts of genetics and its advantages and disadvantages. The debate about NGS focuses mainly on the interpretation, communication, ownership, use and storage of the large amounts of data produced by NGS techniques.

The wider opportunities for genome sequencing raise questions about the boundaries between generating and sharing data in the interests of furthering scientific knowledge and medical possibilities on the one hand, and privacy, intellectual property rights and costs on the other hand. Traditional informed consent is no longer adequate because the nature and impact of the data cannot be foreseen (including incidental findings). Moreover, the information obtained is not limited to the individual concerned and may raise questions about things like the rights of family members, both adults and children, to know or not to know their genetic risk factors. These issues may give due cause to review the adequacy of current legislation, such as the Special Medical Procedures Act (*Wet op Bijzondere Medische Verrichtingen*), privacy law and patent law.

2. CRISPR-CAS:
ALTERING GENETIC TRAITS

CRISPR-Cas9 is a revolutionary, but simple and inexpensive new technique for making highly targeted changes to genes in microorganisms, plants, animals and humans. It opens the door to new types of research, products and treatments. However, the possibility of making targeted changes to the genome also blurs the distinction between products of genetic modification, products of classical mutagenesis and 'natural' products or organisms. This undermines the legal basis for the EU legislation on genetically modified organisms (GMOs), which is based on this distinction.
CRISPR-Cas9 is one of a growing list of 'new techniques' whose products do not clearly fall within the scope of the EU GMO legislation, and if they do it is not clear whether or not they should be exempted. A conclusive decision from the European Commission on these techniques has been expected for some years now. Given the speed at which CRISPR-Cas9 has been embraced by the scientific community, the broad scope of its application – which covers not only the agro sector but also the medical sector – and the far-reaching implications and economic interests involved, a decision is now urgently needed. Moreover, the new possibilities offered by CRISPR-Cas raise ethical questions about the acceptability of certain applications, including germline modification, the genetic modification of monkeys so that they can be used as disease models for humans, and interventions in ecosystems that virtually exterminate a species. These ethical questions also urgently require a political decision and policy response.

3. PERSONALISED MEDICINE:
PREVENTION, DIAGNOSTICS AND PERSONALISED TREATMENT

Personalised medicine involves the use of treatments or preventive treatments tailored to the genetic profile, lifestyle and environment of an individual patient or specific group of patients. The advent of next generation sequencing has expanded the possibilities for personalised medicine and stimulated further debate. In particular, there are high expectations for cancer treatments based on the genetic characteristics of tumour cells.
Although personalised medicine offers clear benefits, such as bespoke treatment with better results and reduced side-effects, it presents a challenge to the current healthcare system in the Netherlands and elsewhere. It raises questions such as How can we make the best use of the possibilities for personalised medicine without having to make concessions on the need for thorough investigation of the effectiveness and safety of treatments (smaller patient groups) or compromise patient privacy? and How can healthcare be kept affordable if personalised medicines, which are expensive because of their limited applicability, are increasingly used? Various organisations are investigating whether changes to authorisation procedures and protocols for conventional clinical trials can provide a way forward for a more individual approach to medical treatments.

4. GENE THERAPY:
PROMISE FULFILLED

Gene therapy involves introducing genetic material (DNA or RNA) into the body cells of an individual and bringing it to expression to treat a disease. In recent years the number of clinical trials has increased, clinical trials of treatments for cancer, blood and immune system conditions, and other diseases have delivered promising results, and the first gene therapy products have appeared on the market. The nature of gene therapy and the risks attached to it are highly diverse. Some new methods and techniques, including the use of replicating viruses to destroy tumour cells, involve risks that require additional measures, whereas other

types of gene therapy present little or no risk. Under the current legislation, all gene therapy treatments have to go through the same safety assessment and licensing procedures. Shortening the licensing procedure for gene therapy treatments that present no risks to third parties and the environment could provide an important boost to innovation and the exploitation of the potential benefits of gene therapy in the Netherlands. However, like personalised medicine, a problem with gene therapy is the small numbers of patients per treatment and the affordability of healthcare.

5. NEW TECHNIQUES: SILENCING GENES WITH RNA

Discussions on the legal status of various new plant breeding techniques have been going on in the EU for years without any decisions being reached. Meanwhile, new techniques are continuing to emerge. One of these developments is the use of RNAi technology. RNA interference (RNAi) is a process in the cell that regulates gene expression or destroys viruses. It can be used to block genes without changing the cell's DNA. RNAi has become an important research tool for studying the functioning of genes and already has some practical applications, both in the development of medicines and in agriculture.
The first genetically modified (GM) insect-resistant crop based on RNAi technology is already on the market. RNAi technology can also be used on conventional crops in the form of RNA sprays to combat pests or influence plant characteristics. For example, resistance to herbicides in weeds can be suppressed by adding RNAi molecules to the herbicide. The use of RNAi in the form of sprays or adding it to herbicides is not specifically covered by the current GMO legislation, but there is a possibility that residual genetic material (such as RNA) associated with genetic modification could remain on food or other products. Even if the presence of such material presents no risk to humans and the environment, it could lead to public debate about the implications for consumers' freedom of choice.

6. GENETICALLY MODIFIED INSECTS: INTERVENING IN ECOSYSTEMS

The corporate and scientific communities are investigating whether GM insects can be used to suppress infectious diseases and agricultural pests and field trials have already been held in various countries around the world. These developments present opportunities to improve public health and reduce the use of insecticides, but are meeting resistance from objectors on the grounds of safety, possible impacts on ecosystems and biodiversity, and economic damage.
In recent years a number of infectious diseases have spread rapidly to other countries and regions. Many of these, including Zika, chikungunya and dengue virus, are transmitted by insects. Field trials with GM mosquitoes to tackle dengue fever have already been held outside the Netherlands and it is expected that similar field experiments will in future be carried out on the islands of St Eustatius and Saba in the Caribbean Netherlands. The situation regarding the regulations and permits for such experiments in the Caribbean Netherlands is not clear, however. The Dutch Genetically Modified Organisms Decree (GMO Decree) does not apply to the three special municipalities that make up the Caribbean Netherlands[a] and the Housing, Spatial Planning and Environment Act that applies to these municipalities (*Wet VROM BES*) contains no provisions relating to GMOs. Before GM insects can be used to suppress infectious diseases, the Dutch government has to ensure that a sound risk assessment is made, proper measures are taken to manage any risks and provisions are made to consult stakeholders and citizens.

a The Dutch islands of Bonaire, St Eustatius and Saba in the Caribbean Sea.

7. 3D BIOPRINTING:
BODY PARTS TO ORDER

3D bioprinting is a multidisciplinary field that draws on engineering, biology, chemistry and mathematics to generate one or more types of living tissue, structures or biomedical implants using 3D printing technology. In the past, 3D printing has been used in the medical sector mainly to produce personalised implants and models, but the trend now is towards implants covered with living cells and biocompatible implants that contain living cells, such as printing soft tissue (skin, cartilage) and bone tissue for reconstructive surgery. 3D bioprinting has so far mainly been used in research and preclinical tests, for example for making disease models and testing medicines and cosmetics. European companies in particular are interested in these applications because the use of animals to test cosmetics products or their ingredients is no longer permitted.
3D bioprinting is still in the early stages of development and faces considerable technical challenges. A special 3D bioprinting facility has been built in the Netherlands and a specialised course of study established. In the future, 3D bioprinting will raise questions concerning legislation, ownership and liability. Should the products of this technology be considered medicines or implants? How and under what regulations will the safety assessment be carried out? Who will be legally liable? Who will own products made with the use of donor cells or body cells? The technology also raises ethical and social issues to do with access to the benefits it offers and its impact on how we view the integrity of the human body.

8. SYNTHETIC BIOLOGY:
FROM CLOTHES TO VACCINES

Synthetic biology is a collective term for the deliberate (re)design and construction of new biological molecules, cell components and systems with the aim of inserting non-natural systems into natural systems for useful purposes. The applications of synthetic biology include the production of raw materials and fine chemicals, the development of drug delivery systems and the synthesis of vaccines. It is a multidisciplinary field that involves changing the genetic code in existing cells or organisms (the top-down approach) as well as designing and building new cells (the bottom-up approach).
The term ‘synthetic biology’ covers a large number of widely differing applications and techniques, which makes it impossible to provide a comprehensive definition. The existing applications can be considered to be forms of genetic modification, but this will change in future. Given the broad range of applications and the speed at which the field is developing, drawing up regulations and policy specifically for synthetic biology is not only undesirable, but also impossible. It is important to be able to respond in a flexible manner to new developments and this requires a different form of governance in which the need for regulation and the suitability of existing legislative frameworks can be assessed for each application as it arises. National and international cooperation and knowledge sharing between stakeholders are required if the full potentials of this technology are to be realised, while at the same time responding constructively to issues of biosafety, biosecurity, intellectual property rights, sustainability and social acceptance.

Political and policy issues

The trends show that biotechnology is opening up new opportunities and is of great importance for the Dutch economy. However, these technological possibilities also throw up dilemmas and raise important questions. A number of these issues are found in each of the trends and are becoming more urgent as biotechnology develops and becomes increasingly integrated into a range of applications:

Ethical issues: New biotechnological possibilities such as personalised medicine, gene therapy and next generation sequencing in diagnostics and screening raise ethical questions about things like privacy, ownership, the right to self-determination and autonomy, as well as questions about social costs versus individual benefits and the borderline between healing or treating diseases and 'enhancement' – making changes without any direct medical reason for doing so. The most striking topic is germline (genome) modification. The clinical application of this technique is currently prohibited almost everywhere in the world, but now that it seems to be technically possible to use this technique to prevent genetic disorders being passed on, the ban on its use will probably be reconsidered. This in turn makes it necessary to debate the desirability, ethical acceptability and potential applications of the technique. Determining the limits of ethical acceptability and assessing the values and interests of the various stakeholders is primarily a political task.

Guaranteeing public sector expertise: The challenge facing government is to capitalise on the opportunities presented by the new technologies while at the same time guaranteeing safety and remaining alert to the ethical and social aspects. However, the ability of the public sector to build up an independent body of knowledge is increasing coming under pressure due to the expansion of public-private cooperation. It is important for the public sector to maintain its own pool of scientific expertise and data from sources other than just the private sector. Only then can the government be an equal negotiating party and play a constructive role in developing protocols for things like safety and test methods.

Intellectual property rights: The accelerating rate at which large volumes of data on biological material, DNA and cellular mechanisms are becoming available makes the question of intellectual property all the more urgent. The increasing use of patent law in the plant breeding industry at the expense of plant variety rights reflects the introduction of biotechnology into this sector. The collection and analysis of genetic data and material from patients is generating better understanding of the emergence and course of diseases and is leading to new, more targeted diagnostic tools and treatments. These in turn give rise to questions about ensuring the privacy of patients, and where relevant of family members, and about who owns the property rights to the information or the biological material. These issues may make people unwilling to contribute to biobanks.

Legislation: The EU's GMO legislation is based on the distinction between genetic modification and non-genetic modification, the latter being referred to as conventional techniques or applications.
Genetic modification and its products are subject to an extensive body of laws, regulations and safety assessments, whereas the products of conventional and other biotechnological or exempted techniques are not. The high costs of the safety assessment means that the consequences of an application falling within the scope of the GMO legislation are considerable. As these high costs can only be borne by large companies, avoiding the GMO regulations has become a driver for innovation.
However, the scientific distinction between genetic modification and other biotechnological techniques is becoming increasingly blurred and the different products can often hardly be distinguished from 'natural' products, if at all. This is undermining the legal basis of the EU GMO regulations. The approach that has always been taken of assessing whether the products of each individual technique fall under the regulations is no longer adequate. The EU legislation needs to be revised to establish certainty for citizens, consumers and businesses. It seems inevitable that the regulatory regime will take a more integrated approach and be more concerned with the consequences of introducing a product than on the techniques used to create it. Moreover, the current GMO regulations focus only on the safety aspect, which does not help to resolve the debate about genetic modification because it ignores the need to weigh up the benefits against the disadvantages. An assessment that balances the risks of biotechnological applications against their usefulness or their advantages would

better serve the wishes of the patient and consumer, as well as the desire for an integrated assessment framework for determining the safety of applications and technologies.

The international context: The possible consequences of the developments in biotechnology for Dutch legislation, policy and society must be seen in their international contexts. While many things can be regulated at the national level, the international context must always be taken into account. Companies may decide to relocate their research and development operations to countries where legislation on GMOs is more favourable to them. Differences in the legal definition of a GMO between countries play an important role in negotiations on trade agreements, such as the Transatlantic Trade and Investment Partnership (TTIP) and the importation and labelling of GMOs. And patients may decide to go abroad to have certain treatments. The international context is unavoidable, but at the same time is not necessarily the deciding factor for the Netherlands. At the national level, consideration should be given to the political direction to be taken and the nature of the policy response, because failure to take decisions means that the international context will make them instead.
Clear political decisions will therefore have to be taken on how to deal with the ethical aspects, public sector expertise in the Netherlands, intellectual property rights and the fundamental principles underlying the legislation.

CONTENTS

GLOSSARY

3D bioprinting
3D bioprinting is a multidisciplinary field that combines engineering, biology, chemistry and mathematics to generate one or more types of living structures or biomedical implants using computer-controlled 3D printing technology.

Big data
The rapid collection of large amounts of data with a high degree of complexity and/or diversity.

Biotechnology
Biotechnology is the application of science and technology to living organisms, as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services – accompanied by a list of techniques (source: OECD)

Chromosomes
DNA structures; carriers of the genetic material.

Complex disorders
Disorders with a complex aetiology or multifactorial disorder; conditions whose cause lies in a combination of genetic and environmental factors (nutrition, exposure, etc.).

CRISPR-Cas9
CRISPR-Cas9 is a system for targeted genome modification (*see below*) by various means, including introducing point mutations, removing genes or parts of genes, and inserting new genes or DNA fragments at specific locations in the genetic material.

Enabling technology
An enabling technology is a technology that facilitates or supports another technology.

Enhancement
'Improving' human beings without a strict medical need.

Epigenetics
The study of heritable changes in gene function that cannot be explained by changes in the DNA sequence.

Exome
All coding sequences (exones, genes and parts of genes, etc.) on the genome. In humans this amounts to about 1% of the genome.

Gene therapy
Gene therapy (somatic genome modification) involves introducing genetic material (DNA or RNA) into the cells of an individual and bringing it to expression to treat a disease (as opposed to altering the existing DNA by genome modification).

Genome modification
The targeted introduction of changes to the DNA of animals, plants or microorganisms.

Genotype
The genetic make-up of an organism; its DNA code.

Germline (genome) modification
The modification of genes in gametes (reproductive cells) or fertilised egg cells by genome modification, after which all the cells of the embryo carry the modification, which can then be passed on to the following generations.

GMO
Genetically modified organism

Habitat
The living environment of a plant, animal or microorganism.

Incidental findings
Variations or conditions discovered unintentionally during a diagnosis or screening that are unrelated to the reason for carrying out the test.

Informed consent
Carefully considered assent. A statement of voluntary permission to carry out a treatment, such as an extensive diagnostic intervention, operation or participation in a scientific study, having been informed of the risks involved.

Marker-assisted breeding or marker-assisted selection
A plant breeding technique which makes use of sequences of DNA (marker sequences) that are known to be located near to the gene or section of genetic code associated with the desired trait. This can be used in the laboratory to select young plants that possess the marker sequence and therefore also the desired trait before the trait is expressed.

Microbiome
A complex community of microorganisms (bacteria, fungi, algae, viruses) on and in each organism and in each habitat.

Mitochondria
Organelles (structures in the cell) with their own DNA which take care of energy conversion in the cell.

Non-invasive prenatal testing (NIPT)
A test method in which foetal (placental) DNA in the mother's blood is examined for genetic abnormalities using sequencing techniques. If any abnormalities are discovered, an amniocentesis or chorionic villus sampling is still needed to confirm the finding.

Personalised or precision medicine
Personalised or precision medicine is an approach to the treatment and prevention of diseases that takes account of individual variations in genes, environmental factors and lifestyle.

Pharmacogenetics
Pharmacogenetics investigates the genetically determined speed at which drugs are broken down and the occurrence of side-effects in the body in relation to their therapeutic effect in order to better adjust dosages to the individual patient.

Phenotype
The observable characteristics or traits of an organism; the result of the genotype in combination with environmental factors.

RNAi
RNA interference (RNAi) is a natural process in the cell that regulates gene expression or

destroys viruses. The messenger RNA (mRNA) molecule copied from the genes is broken, thus preventing the synthesis of the proteins it codes for.

Sequencing
Sequencing is the process of determining the order of the bases (A, C, T (or U) and G – adenine, cytosine, thymine (or uracil) and guanine) – on a fragment of DNA or RNA, which carries an organism's geneticinformation.

Synthetic biology
The (re)design and construction of new biological molecules, cell components or cell systems in the laboratory with the aim of incorporating non-natural systems into natural systems for useful purposes. Synthetic biology can be divided into five sub-fields: synthetic genomics, minimal genome cells, synthetic cells, (molecular) xenobiology and metabolic reprogramming.

Theranostics
A combination of diagnostics and therapy in a single concept or product.

X-omics
X-omics refers to various fields of research in cell biology ending in the suffix -omics: genomics maps the DNA code; transcriptomics studies the expression of genes in RNA; proteomics is the study of all proteins; and metabolomics is the study of all metabolic products.

1 INTRODUCTION

Biotechnology Trend Analysis 2016

The Netherlands Commission on Genetic Modification (COGEM) and the Health Council of the Netherlands were asked by the State Secretary for Infrastructure and the Environment to prepare a Trend Analysis of developments in biotechnology to inform the political and policymaking process. The Scientific Council for Government Policy (WRR) was asked to assist with the preparation of this Trend Analysis (*see Appendix 1*).

The Biotechnology Trend Analysis is published at irregular intervals at the request of the Dutch House of Representatives. Its purpose is to describe the latest developments in biotechnology and the possible consequences for policy and the regulatory framework. In view of this brief, the Trend Analysis does not contain any advice on how to respond to the identified trends, but is limited to identifying and describing important problem areas.

This is the fourth Biotechnology Trend Analysis. The first was published in 2004, the second in 2007 and the third in 2009. In the third publication the authors noted that two years between publications was too short a period because the development pathway from a scientific finding to a product takes longer. During the parliamentary debate on the Trend Analysis 2009 it was therefore decided to reduce the frequency of publication, with a tentative date for the next publication set at 2015 or later.[1]

It is impossible to discuss all the developments in biotechnology in this Trend Analysis as it has developed into a highly diverse field with branches in numerous sectors. There are countless developments within the field that could provide opportunities to tackle all sorts of social problems and which consequently deserve political attention. We are therefore compelled to limit the scope of this analysis to eight trends that are illustrative of the field of biotechnology as a whole, and that raise important dilemmas and social questions requiring political and policy responses (*see Appendix 2*).

These trends and the issues they raise are not all 'new' (*see Appendix 3*), but biotechnology in general, and genetic modification as one of its core elements in particular, appear to have entered a new phase. The approach taken during the past 40 years, in which genetic modification and biotechnology were clearly distinct entities for which specific policies and regulations were needed, has been overtaken by technological advances and can no longer be justified. In recent years, scientific and technological developments within the field of biotechnology have moved up a gear. To be able to capitalise on these opportunities, the political community must quickly address the questions and dilemmas they throw up and devise appropriate policy responses. The authors hope that this Trend Analysis will make a valuable contribution to the process.

Definition of biotechnology

This Trend Analysis explores the trends and developments in modern biotechnology. Biotechnology encompasses a broad range of activities, applications and products, which makes it difficult to provide a comprehensive definition. Various definitions of biotechnology are in use. The most common definition is that by the Organisation for Economic Cooperation and Development (OECD),[2] which consists of two parts: a list of techniques (list-based definition, *see OECD text box*) and a descriptive single definition:

The application of science and technology to living organisms, as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services.

This description also covers traditional activities such as brewing and cheesemaking, and so it should always be accompanied by the list-based definition, which includes seven categories of techniques (*see text box on the OECD definition of biotechnology*). In this Trend Analysis we use the OECD definition, noting that the emergence of molecular biology in the 1970s can be considered to be the birth of modern biotechnology.

OECD definition of biotechnology

The OECD developed both a single definition and a list-based definition of biotechnology. The single definition is deliberately broad: 'The application of science and technology to living organisms, as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services.' This definition covers all modern biotechnology but also many traditional or borderline activities. For this reason, the single definition should always be accompanied by the list-based definition.

The (indicative, not exhaustive) list-based definition, which serves as an interpretative guideline to the single definition includes seven categories:

1. iDNA/RNA: Genomics, pharmacogenomics, gene probes, genetic engineering, DNA/RNA sequencing/synthesis/amplification, gene expression profiling, and use of antisense technology;
2. Proteins and other molecules: Sequencing/synthesis/engineering of proteins and peptides (including large molecule hormones); improved delivery methods for large molecule drugs; proteomics, protein isolation and purification, signalling, identification of cell receptors;
3. Cell and tissue culture and engineering: Cell/tissue culture, tissue engineering (including tissue scaffolds and biomedical engineering), cellular fusion, vaccine/immune stimulants, embryo manipulation;
4. Process biotechnology techniques: Fermentation using bioreactors, bioprocessing, bioleaching, biopulping, biobleaching, biodesulphurisation, bioremediation, biofiltration and phytoremediation;
5. Gene and RNA vectors: Gene therapy, viral vectors;
6. Bioinformatics: Construction of databases on genomes, protein sequences; modelling complex biological processes, including systems biology; and
7. Nanobiotechnology: Applies the tools and processes of nano/microfabrication to build devices for studying biosystems and applications in drug delivery, diagnostics, etc.

2 BIOTECHNOLOGY AS AN INSTRUMENT

2.1 BIOTECHNOLOGY IS INTEGRATIVE

Biotechnology is becoming increasingly integrated into other research areas and applications. It has become an essential enabling technology[b] within the life sciences as a whole, making it much less visible as a separate sector. This can be seen, for example, in the analysis of the economic position of the Dutch biotechnology sector commissioned by COGEM in support of this Trend Analysis (*see the text box 'Economic importance of biotechnology'*).[3] A comparison with a similar analysis[4] conducted for the Trend Analysis 2007 shows that by 2015 it had become much more difficult to obtain statistics relating specifically to biotechnology. Organisations like the OECD no longer maintain such statistics because it is impossible to distinguish between the various commercial activities, and neither are such distinctions used for research grants.[5]

An example of the ongoing integration of biotechnology with other scientific fields is 3D bioprinting. 3D bioprinting employs knowledge and techniques from engineering, biology, chemistry and mathematics to make tissues or other structures from one or more types of cells and other biomaterials (e.g. cartilage) using 3D printing technology (*see §3.7 3D bioprinting*). Genomics is one of the driving forces behind the integration of biotechnology into other technologies. It provides insights into the functions of genes, the processes in cells and the interactions with other organisms, such as pathogens, throwing light on the origins of plant, animal and human diseases and opening up possibilities for developing therapies, producing biochemicals and adapting crops to the needs of producers and consumers.

As an enabling technology, biotechnology contributes to solving diverse challenges facing Dutch society and the wider global community in areas such as public health, food security and quality, environment, climate and sustainable economic development. At the same time, the integration of biotechnology with other technologies poses new challenges and raises political and policy issues. This chapter investigates these challenges and issues by looking at three traditional application areas of biotechnology: health, agriculture and industry (the biobased economy).

Economic importance of biotechnology

The biotechnology sector is highly important for the Dutch economy.[3] There are about 600 biotechnology companies in the Netherlands and in 2013 they provided around 35,000 jobs. The production value of the Dutch biotechnology sector is estimated at € 13.8 billion, with an added value of € 4.6 billion. The Dutch biotechnology sector contains a large number of start-ups with fewer than ten employees. Many of them have long lead times and few succeed in growing. However, in 2015 several Dutch start-ups were taken over by American pharmaceutical companies, which may indicate a shift in the fortunes of the sector. A threat to the Dutch economy is that mergers, takeovers and the emergence of rapidly growing economies outside Europe are enticing companies to relocate part of their R&D activities elsewhere.[6]

b An enabling technology is a technology that facilitates or supports another technology.

2.2 BIOTECHNOLOGY AND SOCIAL CHALLENGES

2.2.1 Public health

Biotechnology plays an increasing role in the healthcare sector across the range of services from diagnosis to therapy. Genomics research has become essential for understanding the origins and courses of diseases, but despite broad public support for medical biotechnology (*see text box 'Eurobarometer (2010): broad support for medical biotechnology in the EU'*), it raises questions about how to treat this information (*see §3.1 Next generation sequencing*). A range of biotechnological applications is also used in the development of prevention and treatment methods, including medicines. Some diseases are caused almost entirely by genetic factors, whereas others, such as asthma, obesity and psychiatric conditions, are often the result of a combination of environmental and genetic factors. Genetic research and sequencing and association studies are used to identify genetic factors and learn more about the origins of diseases as well as to develop new therapies. The focus of research on infectious diseases is the genetic make-up of the pathogen. It is still difficult to understand and diagnose diseases and to develop methods for prevention and cure, and it often takes many years before new therapies become available. Biomedical applications only become relevant for the general public when they become available to individual patients.

Eurobarometer (2010): broad support for medical biotechnology in the EU

The European Commission regularly commissions studies of the public perception of biotechnology and the life sciences in the EU. The last study was published in 2010.[7] It indicates that a majority of European citizens are optimistic about biotechnology and believe it will make a positive contribution to our way of life over the coming twenty years. Europeans are mainly positive about medical applications such as stem cell research (on embryos), gene therapy and xenotransplantation. Interestingly, research into non-therapeutic applications, such as human enhancement, was approved of by the majority of respondents. A large majority of the Dutch population takes a positive view of genetic testing and other medical applications.[8,9] There is also support for biobanks in the Netherlands and across Europe. On the other hand, in 2010 most people were not yet aware of the storage and use of human tissue.[10] Although most people agreed with participation in biobanks, they did want more information and control over what their material could be used for. Use for research into cosmetics was generally rejected and commercial use was also a sensitive issue. Support for medical technology is not unconditional, but dependent on adequate supervision and regulation by government. The study also revealed a desire for legislation that serves the public interest and requires the benefits, safety aspects and sustainability to be properly weighed up.[11] Moreover, Europeans want a voice in the decision-making when social norms and values are at stake.

This section explains the possibilities that biotechnology opens up for solving several important public health problems.

Genetic disorders

Genetic factors play an important role in the development of various diseases and disorders. There are estimated to be about 7,000 genetic disorders caused by a mutation in a single gene, which can be transmitted in different ways (e.g. via normal chromosomes, sex chromosomes or via mitochondria[c]).[12] Although in general these are rare or very rare conditions, they affect millions of people worldwide. The seriousness of the symptoms vary, as do the possibilities for prevention and the treatment of patients and, where relevant, family members. For some diseases it is possible to maintain a good quality of life, while other conditions lead to serious

c The cell's energy factories.

symptoms and premature death. Worldwide, there are just over 400 medicines available to treat genetic diseases, but most of these diseases are still untreatable. For a long time the limited number of patients made it particularly difficult to trace the causes of these diseases, but the genes responsible for around half of all genetic disorders are now known.[12] However, the small number of patients per disease is also the reason why very few medicines are developed to treat these diseases.

The ability to rapidly determine the base pair sequence of the human genome (*see § 3.1 Next generation sequencing*) marks a breakthrough in discovering disease mechanisms much more quickly and developing diagnostic methods, and offers new possibilities for developing therapies (*see text box '100,000 genomes project'*).

100,000 genomes project
The 100,000 genomes project began in 2012 in England and over the next few years it will map the genetic code of about 70,000 people.[13] The target group consists of cancer patients and patients with rare conditions and their families. At the moment, the project is the biggest national genomics project in the world. It aims to stimulate medical research and enable the development of new therapies. The project has already led to the diagnosis of patients with rare or unknown conditions.[14]

Therapies may consist of introducing missing endogenous proteins or other substances (as for Pompe disease[15]), inhibiting certain genes that are too active (overexpression)[16] or repairing gene functions by gene therapy. Gene therapy involves introducing 'healthy' genetic material into a small number of the patient's body cells. Clinical trials of gene therapy techniques have yielded hopeful results, in particular for the severe immune disorders X-SCID and ADA-SCID and the metabolic disease X-ALD.[17] The first gene therapy to come onto the European market was alipogene tiparvovec (Glybera®) in 2014.[18]

New possibilities for curing or even preventing genetic disorders are also coming forward. In the United Kingdom, cell nucleus transplantation to prevent mitochondrial diseases was approved for use starting in 2015 (*see text box 'Cell nucleus transplantation to prevent mitochondrial diseases'*). In addition, over the last few years a number of gene editing techniques developed in quick succession have the potential for use in plants and animals, but have also reopened the debate about germline modification (*see §3.2 CRISPR-Cas*).

Cell nucleus transplantation to prevent mitochondrial diseases
Mitochondria are the powerhouses of the cell and have their own genetic material. Mitochondrial DNA makes up a tiny part (0.1%) of our total DNA, but abnormalities in this DNA can have serious consequences.[19] Mitochondrial diseases are very serious hereditary metabolic diseases. Mitochondria are inherited only via the mother (via the egg cell). If the mother is the carrier of a disorder, a healthy embryo can be obtained via in vitro fertilisation (IVF) by removing the cell nucleus from one of the mother's egg cells and implanting it into an egg cell from a healthy donor (from which the nucleus has been removed) and then fertilising this egg with sperm cells from the father. Each cell of the resulting embryo will then contain 'healthy' mitochondrial DNA from the donor. Cell nucleus transplantation has been permitted in the United Kingdom since 2015.[20]

Complex disorders
Diseases may be caused directly by genetic mistakes, but in most cases the cause is a combination of genetic and environmental factors. The latter diseases are referred to as disorders

with a complex aetiology, or multifactorial disorders. Cardiovascular diseases, cancer, obesity and psychological disorders (with a few exceptions) are all examples of this type of condition. The 200 or so different forms of cancer are the biggest cause of death in the Netherlands. Major improvements have been made in the diagnosis and treatment of cancers over the years.[22] When diagnosing cancers, such as breast cancer, increasing use is made of analyses of the genomes of tumour cells, which makes it possible to investigate which cytostatic drugs the tumours are likely to respond to. This is a valuable aid in choosing the most effective treatment and avoiding subjecting patients to an incorrect treatment (*see §3.3 Personalised medicine*). A special group of cancers are the inherited cancers, such as breast cancer, in which the BRCA 1 and 2 genes play an important role, and inherited bowel cancers, such as Lynch syndrome and familial adenomatous polyposis. It is useful for family members and patients to know whether they have a inherited disposition to one of these forms of cancer, because preventive measures can be largely successful in preventing the onset or fatal outcome of these cancers.

Cancer treatments make increasing use of biotechnological medicines and therapies, such as immunotherapy[23,24,25] and gene therapy.[26,27] The search for new methods of treatment continues unabated, but the time from development to marketing authorisation of new medicines is long and the pressure on the authorisation process is high (*see text box 'New cancer therapies'*).

New cancer therapies

Progress is being made with diagnostic and treatment methods for various sorts of cancer, but commercial and other interests are intense and expectations high. This makes it difficult to manage expectations and complicates the process of coming to a balanced marketing authorisation assessment. Drug authorisations are sometimes rushed through, but doubts can arise at a later stage about their efficacy, effectiveness or efficiency.[28,29] The 'compassionate use' treatment option allows doctors to prescribe unregistered experimental drugs that are still in the development phase to terminal or chronically ill patients. Such drugs are not covered by health insurance. Pharmaceutical companies sometimes provides these drugs free of charge in return for data on their effectiveness. Other companies, including some in the Netherlands, aim to make experimental drugs available to patients more quickly,[30,31] but the costs are charged to the patient. This approach is not without controversy and the companies involved are accused of tempting vulnerable and susceptible patients with expensive drugs that may have no effect, or may even be dangerous.[32] Moreover, the popularity of compassionate use may have adverse effects on the willingness of test subjects to take part in clinical trials, partly because these patients will not want to run the 'risk' of being allocated to the control group. Besides drug availability and effectiveness, the extremely high costs of therapeutics is playing an increasing role in the debate, particularly if they only offer a few weeks or months more life.

Cardiovascular diseases are the second biggest cause of death in the Netherlands. Although the number of smokers has declined, the number of people who are overweight or seriously overweight is increasing and women's lifestyles are worsening in relation to those of men (smoking, alcohol consumption). About 40% of the Dutch population are overweight or obese.[33] These risk factors make cardiovascular diseases a major health concern. Efforts are being put into reducing these risk factors as well as preventing and curing cardiovascular diseases, but the use of preventive medicines is not without problems (*see text box 'Anti-cholesterol drugs: blessing or curse?'*).

Anti-cholesterol drugs: blessing or curse?
The US Food and Drug Administration (FDA) recently assessed two new drugs that lower blood cholesterol.[34] Both are intended for use by people with raised cholesterol levels in their blood, which is a risk factor for cardiovascular and other diseases. The drugs are highly effective and are claimed to have virtually no side-effects ('the triumph of the modern genetic revolution'). The drugs have to be administered twice a week or once a month. The potential group of users (people with high blood cholesterol) is enormous (estimated to be 8 to 11 million people in the USA alone), as are the costs ($7,000 to $12,000 per year). This poses a problem for health insurers as well as users. The question is whether use of the drugs should be restricted, for example to people who have already had a heart attack, or whether they should be available to everyone with high blood cholesterol irrespective of the cause. This latter group do not yet have any complaints and are therefore not patients, but would be taking medication. Moreover, it raises the question of whether this group should be encouraged to take these drugs rather than taking preventive measures, such as eating healthily and exercising more. The increasing knowledge of the human genome and certain genetic and other risk factors for diseases will probably lead to an increase in the use of preventive medicines such as cholesterol-lowering drugs.

In recent years molecular techniques have been used to develop and refine medicines. The use of stem cell treatment to repair damage following a heart attack is being investigated. In cardiogenetics, the use of sequencing in recent years has led to good progress with research into the causes and treatment of early heart death and dozens of genes associated with early heart death have been identified. Patients with family members who have died young can now be tested via this gene panel and if they are found to have any affected genes, they can receive medication or can be fitted with an internal automatic defibrillator.

Genetic factors also play a role in the development of obesity.[35] In rare cases a mutation in a gene may be inherited, which causes the holder to continually feel hungry and overeat. However, the relation between obesity and genetic disorders is more complex and several or many genes are involved. In a genome-wide association study, data from more than 300,000 people were analysed and compared and 97 regions in the human genome were identified that influence the occurrence of obesity, but which can explain just 2.7% of the differences in body mass index (BMI).[36] Identifying effective means of preventing and treating obesity based at least in part on genetic information is therefore a long way off.

Psychiatric disorders are also influenced by a combination of genetic and environmental factors, and recent research into genetic factors has delivered many new insights. A wide range of disorders, such as autism, schizophrenia, bipolar disorders, depression and ADHD appear to share a number of genetic risk factors.[37,38] The first step in the development of new medicines or diagnostic methods is identifying genes that play a role in the origin of these disorders. A complicating factor, though, is the large number of genes and variations within genes that are involved. The hope is that many of these variations influence the same molecular routes, cells or cell functions in the brain. Research into the genetic factors involved in psychiatric disorders takes many forms, including genetic modification in animal models and optogenetics (*see text box 'Optogenetics'*).

Optogenetics
Researchers use animal models into which they induce mutations in specific genes to study physiological and behavioural changes. Optogenetics involves genetically modifying an animal with genes coding for light-sensitive proteins. Light can then be directed

onto specific neurons in the brain to either activate or deactivate them and the resulting changes in behaviour can be used to investigate the function and role of the neurons in certain disorders. Researchers are using optogenetics to try to find target molecules for medicines by identifying brain cells involved in the disorders. Possible options include therapeutic interventions for psychiatric and neurological disorders, such as schizophrenia and Parkinson's disease.[39] Optogenetics illustrates the integration of biotechnology with other technologies.

Over the past 50 years no breakthroughs have been made in the development of medicines to treat psychiatric disorders.[40,41] Many of the most widely used medicines, such as antipsychotics and antidepressants came onto the market in the 1960s and 1970s, but these drugs are often ineffective and can have serious side-effects. Many pharmaceutical companies have stopped their research into new psychiatric medicines because of the lack of success and there is no evidence that any new medicines are in the pipeline. However, pharmacogenetics does make it possible to make patients respond faster and more easily to existing and new drugs. Pharmacogenetics investigates the genetically determined speed at which drugs are broken down in the body in relation to their therapeutic effect in order to better adjust dosages to the individual patient.

Infectious diseases

Infectious diseases are one of the biggest threats to public health worldwide. Half the world's population runs the risk of becoming infected with malaria, HIV still claims countless victims, mainly in Africa and Asia, and each year between 250,000 and 500,000 people die from influenza.[42] Globalisation and urbanisation have facilitated the rapid spread of a number of tropical and subtropical viral diseases, including dengue fever, West Nile virus and recently the Zika virus, and new viral diseases such as SARS and MERS[d] have appeared. In addition, resistance to antibiotics is increasing around the world, raising the real prospect of bacterial infections once again becoming a serious threat to public health. In the Netherlands, for example, preventing death from septicaemia (blood poisoning) and endocarditis (inflammation of the heart valves) are among the biggest challenges.

Genome sequences of viruses, bacteria and parasites can be used to develop better and faster diagnostic tests and drugs. Sequencing also plays an important role in the battle against resistance to antibiotics. Greater knowledge of bacterial genetics will provide an inroad to the development of new antibiotics and the prevention of resistance. Sequencing of intestinal flora can help to speed up the identification of resistance.[43] The ministerial conference on antimicrobial resistance, held on 10 February 2016 at the initiative of the Dutch Minister of Health, Welfare and Sport as part of the EU presidency, underlines the urgent need for solutions to this pressing problem.

Vaccination is one of the most important and effective methods of controlling infectious diseases. It has led to the eradication of smallpox around the world; the same can just about be said for polio. However, vaccines are not yet available for most of the global infectious diseases. Biotechnology makes it possible to develop and produce vaccines more quickly (for example, against Ebola, *see text box 'Experimental Ebola vaccine'*). In 2013 researchers presented a method for generating vaccines against flu strains that makes use of sequencing data,[44] reducing the time taken to produce a vaccine from about six months to one week (*see §3.8 Synthetic biology*). Moreover, new techniques are making it possible to develop vaccines against diseases for which traditional methods have failed (malaria, HIV).[45,46,47,48,49,50] Research indicates that various GM vaccines for human and veterinary application are in the pipeline.[46] Al-

d SARS: severe acute respiratory syndrome; MERS: Middle East respiratory syndrome

though experts say these vaccines will be safer and more effective than traditional vaccines, more extensive use of GM vaccines could fuel public concern about vaccination.

Experimental Ebola vaccine
The Ebola outbreak in West Africa at the end of 2014 led to the expedited use of several experimental drugs and vaccines (including TKM-Ebola, ZMapp™ and Ebola_Tx). One of these, ZMapp™, consists of a combination of antibodies and is produced in genetically modified tobacco plants. The vaccine was successfully tested on monkeys, but had not yet been used on humans.[51] During the Ebola outbreak, permission was given to expedite the use of this vaccine on people.[52] The first American Ebola patient was given the vaccine and recovered, but because the vaccine had not been used in the usual setting of a clinical trial, no firm conclusion could be drawn about its effectiveness. Progress has also been made in the Netherlands with the development of a vaccine against Ebola. A Dutch company began human tests with a vaccine in 2015.[53,54]

To tackle infectious diseases transmitted by insects, researchers are investigating whether the vector can be controlled or eradicated by releasing GM insects (*see §3.6 'GM insects'*). This approach is based on the principle of competition with the wild population and the suppression of the spread of 'infected' natural disease vectors by reducing their reproductive success. Reactions to the field experiments indicate that releasing living GM insects into the environment raises questions that go beyond the issue of environmental risks. Before these types of applications can be licensed and authorised for putting on the market, full discussion is needed on not only their effectiveness but also how appropriate or desirable they are.[55]

2.2.2 Agricultural production

Arable and livestock farming face the challenge of providing the growing world population with sufficient healthy food in a sustainable and ecologically sound way.[56,57] The United Nations forecasts that the global population will grow to 8.4–8.6 billion in 2030 and to between 9.5 and 13.3 billion in 2100.[58] Not only will this growth create higher demand for food, but also the associated urbanisation will reduce the amount of available agricultural land and increase claims on freshwater resources. In addition, rising prosperity and growth in disposable incomes, especially in Asia, will push up demand for inefficient animal products.[59] At the same time, global climate change will lead to drought in important agricultural areas and more frequent extreme weather conditions will threaten harvests and add to the uncertainty of food production.[60] Africa is expected to be particularly hard hit. Population growth is strongest here, malnutrition is already a problem in some areas, and water resources will become depleted in the north and south of the continent. Countering these threats will require action in several areas.[61] In addition to changes in consumption patterns, better distribution of food and a reduction in post-harvest losses, the quantity and quality of food production will have to be improved in a sustainable manner. Biotechnology can be an important tool for developing better crop varieties.

Biotechnology and crops
Crop yields per hectare vary considerably across the globe and in many agricultural areas yields can be increased by adopting improved cropping systems. However, water table drawdown and salt water intrusion (e.g. due to irrigation) are major obstacles. Raising crop yields will depend in future on the availability of new crop varieties. Crops are needed that produce higher yields, are resistant to pests and diseases, and are more stress tolerant (drought and saline conditions). Biotechnology has become indispensable in plant breeding: the use of molecular markers (marker-assisted breeding) has speeded up the

process of breeding new varieties, genomics research identifies genes associated with favourable characteristics, and much use is made of biotechnological techniques from cell biology and related disciplines. These techniques are not only relevant for the cultivation of bulk or field food crops; the highly important horticultural sector in the Netherlands (breeding and cultivation of vegetables and ornamental plants) also profits from the advances made in our understanding of genetics and physiology and the growing number of related model systems. Businesses can further increase the quality and diversity of their products by applying this knowledge and making use of widely applicable biotechnological techniques.

Public less sympathetic to genetic modification than to other technologies

The public perception of biotechnology is to a large extent determined by the public debate on genetic modification in agriculture. At various times in the past the opinions of the European (and Dutch) population on biotechnology, genetic modification and other technologies have been surveyed. The results of these surveys have consistently shown public resistance to the use of genetic modification in agriculture and food, whereas medical applications have met with little or no resistance. The 2010 Eurobarometer survey reported a decline in support for GM food, with the exception of the countries where GM crops were grown. Many Europeans were concerned about the safety of GM food, which they overwhelmingly felt to be unnatural. To find out whether or not public opinion in the Netherlands about genetic modification has changed since the 2010 Eurobarometer study, COGEM commissioned a survey of the Dutch public opinion in 2014.[62] This showed that people were still less sympathetic to genetic modification than to other technologies. The majority of the respondents somewhat agreed or totally agreed with the statement that science and technology improve our lives, but also think that we should not tamper with nature. When people are asked to list technological developments in order of importance or desirability, genetic modification of food comes at the bottom of the list. They are most enthusiastic about growing organs from human stem cells and genetic tests for genetic disorders, followed by online shopping and self-driving cars. More than two-thirds of the respondents considered medical applications to be improvements, but just a quarter thought genetically modified food products were an improvement.

Genetic modification of plants

For years the cultivation of genetically modified crops has been restricted to maize, soy, cotton and oilseed rape with herbicide tolerance and insect resistance traits (*see text box 'Growth in the area of GM crops levels off'*). This is partly because there were few interesting genes or traits available to introduce into crops. This situation appears to be changing as a result of genomics research, the increased knowledge of plant physiology and the role of specific genes in it, as well as the availability of 'new' genes from other plant species. The companies involved are focusing primarily on stress tolerances, partly because these traits involve a number of different genes and are therefore difficult to cross using traditional methods.[63] The crops being worked on are largely limited to the 'bulk crops' grown around the world, because the development costs and high authorisation costs of GM crops can only be recouped for crops that are cultivated on a large scale. Another reason is the public resistance to GM food in many countries (*see text box 'Public less sympathetic to genetic modification than to other technologies'*).

New techniques such as CRISPR-Cas9 offer new possibilities for altering plant traits. However, for many techniques it is still not clear whether or not they fall within the scope of the GMO regulations, which makes companies reluctant to invest in these applications (*see §3.2 CRISPR-Cas and §3.5 New techniques in agriculture*).

Growth in the area of GM crops levels off

Since 1995 the total area under GM crops in the world grew to 1.8 billion hectares in 2014. In the past two years the growth in the area of GM crops has levelled off and in 2015 for the first time it fell slightly.[64] This is because just a few GM crop varieties with a limited number of traits are grown, the number of countries where GM crops are grown is not increasing and the areas of land where these crops are grown have reached their maximum. The area will only start to increase again if other countries permit the cultivation of GM crops or new GM crops appear on the market. An analysis of field trials worldwide shows that the emphasis continues to be on GM maize and GM soy with herbicide tolerance and insect resistance.[65] In addition, field trials have been carried out with GM crops, mainly in the US and Canada, in which the biological characteristics (e.g. growth rate, yield) were altered or with GM crops with resistance to abiotic stress factors (such as drought resistance or efficient nitrogen use). The number of field trials worldwide remained about the same during the period covered by the study, but there are signs of a shift in the location of field trials with GM crops to Africa.

Biotechnology in animals

The growing demand for animal protein pushes up demand for livestock feed and contributes to overfishing, while intensive livestock farming and aquaculture are facing problems such as disease pressure and manure surpluses. Besides using biotechnology to speed up conventional breeding, scientists have been working on the genetic modification of animals at various places in the world since the 1980s. These GM animals produce more milk or wool, are resistant to diseases like bird flu,[66] grow more quickly or produce less waste (manure). Although GM cows and pigs that produce better milk and meat are being developed at various places in China,[67,68,69] the development of GM animals for food production has more or less ground to a halt in recent years. The Canadian research programme on GM pigs that produce less phosphate (Enviropig) was stopped in 2012 due to a lack of funding, while disappointing results and funding problems have raised doubts about the future of a long-running research programme on GM cows in New Zealand.[70]

Besides, obtaining marketing authorisation for GM animals is a tortuous process. Although the fast-growing GM salmon produced by the American-Canadian company Aquabounty was authorised for the American market at the end of 2015, the original application was made in 1999. At the same time, public concern about GM animals flares up at regular intervals, for example when it was discovered that a GM sheep from a research institute in France may have entered the food chain.[71]

Besides increased production and specific food traits, animals are also genetically modified to produce xenobiotic substances such as medicinal drugs.[72,73] For example, at the end of 2015 authorisation was given in the US for GM chickens that produce a human enzyme in their eggs.[74] A growing trend is the genetic modification of insects to control pests. This is discussed further in §3.6 'Genetically modified insects: Intervening in the ecological system'.

Nevertheless, the most important reason to genetically modify animals is for scientific research. The animal that is used most in this type of research is the genetically modified mouse, as a disease model. In 2013 more than 17% of the laboratory animals in the Netherlands were genetically modified.[75]

Cloning animals

Another biotechnology application in animals is cloning. A Korean company is working on cloning dogs for professional use (e.g. for customs and the police) and as pets. The company says it has already made 600 successful clones. The cost of a cloned dog is currently about

$100,000.[76] In North and South America cloning valuable parent animals appears to have become a standard option in the package of livestock reproductive techniques.

Offspring of clones off the radar

In 2008 the American FDA concluded that cloned animals (cows, pigs and goats) present no specific safety or health problems.[77] In the US cloned animals and products from these animals do not have to be labelled as such because the currently used analytical methods cannot show that they are any different from products of non-cloned animals. Since 2008 the products of cloned animals and their progeny have therefore been approved for use. Two of the three companies in the US have voluntarily set up a tracking system in which each clone is given a unique identification number. The voluntary tracking system does not apply to the offspring of clones. Cloning for commercial purposes is permitted in Argentina, where no register is held of cloned animals. In Argentina animal cloning is used to produce cloned cattle, including beef cattle, and goats, and transgenic cows are cloned for the production of pharmaceutically important proteins. In Brazil the cloning of beef cattle is also gaining ground.

Despite the limited regulatory possibilities (*see text box 'Offspring of clones off the radar'*), the European Parliament recently amended the rules to prohibit the importation of cloned animals and their products and their use in food chains.[78] In response, the European Commission reported that it could not adopt the proposed amendments because they could not be justified on the basis of food safety or animal welfare arguments.[79]

2.2.3 The biobased economy

The transition to an economy based on renewable resources and renewable energy is seen as a solution to problems such as resource depletion and climate change driven by greenhouse gas emissions. The idea of a circular, biobased economy originated in 2000. Since 2004 it has been promoted by the European Commission and supported by EU research programmes.[80] Before the transition to a biobased economy can be made, a number of technological challenges must be overcome.[81] The key challenge lies in the availability and use of biomass as a raw material. Most of the biomass currently used as raw material is maize and sugarcane (for bioethanol), soy (for biodiesel), wood pellets (for energy: co-combustion in power plants), waste oils and fats (e.g. frying oil) and sugars from plant material (industrial production of biochemicals). Techniques for using sugar beet as a raw material for bioethanol and biochemicals are being developed in the Netherlands[82,83] and a trial installation for the production of bioethanol has been opened in Lelystad.

However, the present uses of biomass also have their disadvantages. The use of food crops as raw materials for biofuels can compromise food production. Forests are felled to produce wood pellets and the availability of this biomass could become problematic in future. The EU is already the biggest market for wood pellets from the US.[84] To make to transition to a real biobased economy, therefore, other routes will have to be pursued, such as the conversion of waste streams from agriculture into biofuels and the production of biochemicals and biofuels by algae and microorganisms. Innovations for the biobased economy will not be possible without the use of biotechnology and genetic modification.[85,86]

Biochemicals

Biotechnology is already widely used in industrial biotechnology for the production of vitamins, additives, fine chemicals and other raw materials (*see §3.8 Synthetic biology*). Algae are thought to be one of the most promising production platforms for biochemicals (and biofuels). However, the cost of producing biofuels from algae are still too high and production is only profitable as a residual product of the biorefining of other substances (such as proteins,

biochemical and food additives) from algae.[87] To raise the production efficiency of algae and make it possible to produce biochemicals and other substances, the algae will have to be genetically modified.[88] Numerous research groups and companies around the world are working on this, as well as on the conversion of residual streams from the agricultural sector (such as straw). Yeasts have been developed that can break down the lignocelluloses in straw and woody parts of plants into sugars,[89] which is the first, crucial step in the production process.

The need for and growth of genetic modification in industrial biotechnology and the biobased economy has proceeded without fuss and has largely gone unnoticed. This seems set to change now that semi-manufactures produced by GMOs are turning up in consumer products (*see §3.8 Synthetic biology*).[90] The presence in detergents of oils produced by algae as an environmentally friendly alternative to palm oil has led to concern and objections by activists.[91]

Biofuels

Various pilot projects are underway on producing biofuels with genetically modified algae and yeast. These applications are all considered to be part of synthetic biology (*see §3.8 Synthetic biology*). The projects vary from the production of biodiesel from algae to the conversion of sugars into bioethanol and biodiesel by algae and yeasts to the production of hydrogen.[92] The question is how economically viable these projects can be. Biofuels are 'high volume, low value' products, especially at the current low price of oil.

The main uses of biomass for the generation of energy at present are the co-combustion of wood or wood waste in conventional power stations and the conversion of plant materials (sugar cane, maize) to produce biofuels. These applications are controversial, even though, according to an economic impact analysis of the American 'bioproduction sector', they save about 1,136 million litres (300 million gallons) of fuel.[93] Critics point out that the use of food crops is having an adverse impact on food production, the cultivation of bioenergy crops takes up agricultural land and that the conversion of crops cannot provide the volumes required to be an alternative for fossil fuels.[94,95] it is also doubted whether they can make a real contribution to sustainability and reducing carbon emissions. Regarding the use of residual streams from agriculture, it is noted that these products are often already used elsewhere and that the costs of these raw materials are underestimated. Other scientists say that biofuels can indeed make a real contribution to reducing CO2 emissions and that the production of biofuels does not have to compete with food production or impact on forests and natural areas. They point out that in large parts of the world agricultural production can be raised to a much higher level.[96] Given the rising demand for energy and food in future, it remains to be seen whether this is indeed possible and offers a way forward.

2.2.4 New fields and applications

Biotechnology is expanding into an increasingly broad array of applications, not only in the food and medical sectors, but also, for example, in cosmetics, clothing and data storage.

Cosmetics companies are making increasing use of biotechnology when developing new products. An Icelandic cosmetics company has developed a skin care line in which the active ingredient is human epidermal growth factor (EGF),[97] a protein produced in GM barley plants in greenhouses in Iceland. They claim that by increasing the elasticity of the skin, the EGF has a rejuvenating effect. These skincare products are sold worldwide. According to the company, 30% of Icelandic women above 30 use their face creams. Other cosmetics companies are also investing heavily in biotechnology for the development of new products and in some cases the boundary between pharmaceutical and cosmetic applications is hard to discern. In recent years various cosmetics companies and raw materials suppliers have bought small biotechnology companies or have entered into partnerships with them.[98]

Many clothes nowadays consist entirely or partly of synthetic fibres. Chemical concerns are developing new types of fibre with specific properties, for example for use in sports and outdoor clothing. One of these concerns manufactures a biopolymer by using genetically modified E. coli bacteria to convert sugar from maize into 1,3-propanediol. The resulting fibres are described as being soft, stain-resistant and hard-wearing, and are used in products like carpets, furniture and sports clothing. A number of well-known clothing brands have been using these fibres in their collections for some time.[99] Moreover, the manufacturer claims that the production process for these fibres is more sustainable than for nylon and other artificial fibres.

Computers and digital networks are becoming faster as microchips become increasingly efficient. However, the reduction in size and increase in speed of standard silicon microchips are expected to reach the physical limits of the materials. Chip manufacturers are therefore looking for other materials for data storage and processing. One of these materials is DNA, which is thought to be able to increase the speed of computers. The computing power of DNA was first described in 1994 by the computer scientist Leonard Adleman for solving mathematical problems.[100] It can also be used to store large amounts of data for long periods.[101,102] Partnerships between the major computer companies and biotechnology businesses are working on developing such applications and claim that DNA computers will have a key role to play in encrypting and storing sensitive data.[103,104]

New applications are also being devised for use in sectors where biotechnology is already well established, such as food production. Various recent initiatives and new start-ups are working on cellular agriculture, in which meat, milk, eggs, cheese, shellfish and crustaceans are produced without the use of animals. It is uncertain whether these initiatives will be successful and lead to products actually coming onto the market, as they depend on both technological and social factors, but they do illustrate that biotechnology has the potential to deliver creative possibilities for making the food chain more sustainable.

2.3 RAPID DEVELOPMENTS AND INTEGRATION DEMAND CHANGES TO BIOTECHNOLOGY POLICY

The trends described in this chapter show that biotechnology is becoming increasingly integrated into a range of sectors and has become one of the key technologies for finding solutions to the challenges facing society in the fields of health, food security, environment and innovation. But as it increases in importance, its integration into these sectors is making biotechnology in general, and genetic modification in particular, less visible to people. This may be one of the reasons why public knowledge of and interest in the most controversial part of biotechnology, genetic modification, is waning (*see text box 'Public survey 2014: majority of people have little interest in genetic modification'*). But as the visibility of and public interest in the topic declines, the growing technological capabilities are raising important ethical and social questions. These include categorical questions about risks and benefits as well as broader questions of ownership, accessibility, cost, privacy and ethical boundaries.

Public survey 2014: majority of people have little interest in genetic modification
A study commissioned by COGEM surveyed public opinion about genetic modification.[62] The results indicate that most people have no knowledge or firm opinions about the subject. A large group of respondents had no direct or clear associations with the terms 'genetic modification' or 'genetically modified organisms'. The most common associations were 'no idea', 'don't know' and 'unknown' or 'what's that?' This was repeated

in the remainder of the survey. About a third of the respondents gave neutral replies to almost all the questions. A small number (about 8%) had positive associations with genetic modification, such as ‘good idea’ and ‘progress’. Specific negative associations (such as ‘unnatural, ‘wrong’ and ‘dubious’) were given in about 20% of the cases. The study also revealed that people are not actively looking for information about genetic modification: one in ten people regularly or often search for information about GMOs on the internet, but about 60% never do this, and few people attend special information meetings about genetic modification.

The next chapter examines eight biotechnological trends, discusses the dilemmas and social and ethical questions they raise, and identifies where current policies prevent the opportunities provided by the technology from being fully exploited.

3 TRENDS IN BIOTECHNOLOGY

In this chapter eight trends in biotechnology are discussed. The fields covered by these trends vary in breadth, but what they all have in common, in our opinion, is that they raise issues which require political and policy responses. The first two trends are driving forces behind developments within biotechnology. Next generation sequencing makes it possible to determine the functions of genes and identify the influence of mutations in genes, and therefore to develop diagnostic methods and treatments. The new genome editing technologies for making alterations to the genome are taking the scientific world by storm, and in doing so are giving a powerful impetus to the other trends discussed in this report: the development of specific medicines, medical aids and therapies for individuals or specific diseases, new agricultural breeding and crop management techniques, interventions in ecosystems, and even the prospect of designing organisms and turning nature to our advantage. But these trends also raise social questions and ethical dilemmas. Just because we can do these things, does that mean we should? Does our legislation properly address the new possibilities? How are other countries responding? Who actually owns information and technologies?

3.1 NEXT GENERATION SEQUENCING: THE NEW STANDARD

In the Trend Analysis 2007 the search for mutations in BRCA genes[e] associated with cancer was still a time-consuming process, although the emerging high-throughput technologies were heralded as having the capacity to considerably speed up the development of diagnostic tests. The Trend Analysis 2009 highlighted the increasing importance of X-omics[f] and the coming of the $1,000 genome. Now, in 2016, NGS/genome sequencing has become an indispensable technology in medical genetic diagnostics and is also starting to be used in agricultural applications.

3.1.1 What is NGS?

Sequencing is determining the order of DNA bases (letters),[g] which carry the genetic information of organisms. For a long time the most commonly used method was Sanger's technique, which dates from 1977 and is called 'first generation sequencing'.[105,106] This technique was used to sequence a major part of the first human genome, which was completed in 2000 after 15 years of work and cost $2.7 billion. Major technological advances have been made over the past 15 years and it is now possible to sequence long strands of DNA within just a few weeks at much lower, and falling, costs (in 2015 between 1,000 and 3,000 euros[h]). The techniques used are called 'next generation sequencing' (NGS).[107,08,109,110] One company has even reduced the price to below the psychological barrier of $1,000, quoting $999 (*see*

e BRCA (breast cancer) genes are genes associated with a inherited form of breast and ovarian cancer.

f X-omics refers to various fields of research in cell biology ending in the suffix -omics: genomics maps the genes, proteomics is the study of all proteins, metabolomics is the study of all metabolic products, etc.

g The genetic code is determined by the configuration of four DNA bases, A, T, G and C, which stand for adenine, thymine, guanine and cytosine.

h These are the prices charged in hospitals. Commercial parties offer sequencing at lower prices, but the quality is not always as reliable.

Figure 1). The debate about NGS focuses mainly on the interpretation and use of the data produced by these techniques.
NGS has become a fact of life in the Dutch healthcare system, where it is increasingly used in the clinical genetic centres and university hospital laboratories for diagnosis and for scientific research (*see Figure 2*). In addition, the first steps have been taken in its use for screening, for example in non-invasive prenatal testing (NIPT).[111] Rapid progress is also being made with the development of prenatal carrier screening for couples wishing to have children. Several University Medical Centres (UMCs) offer these tests for several diseases at the same time and they are increasingly performed using sequencing.
NGS is also increasingly used in other sectors, such as plant breeding and veterinary medicine, where it is used among other things for faster and more efficient development of crop varieties and faster and better diagnosis of infectious diseases.

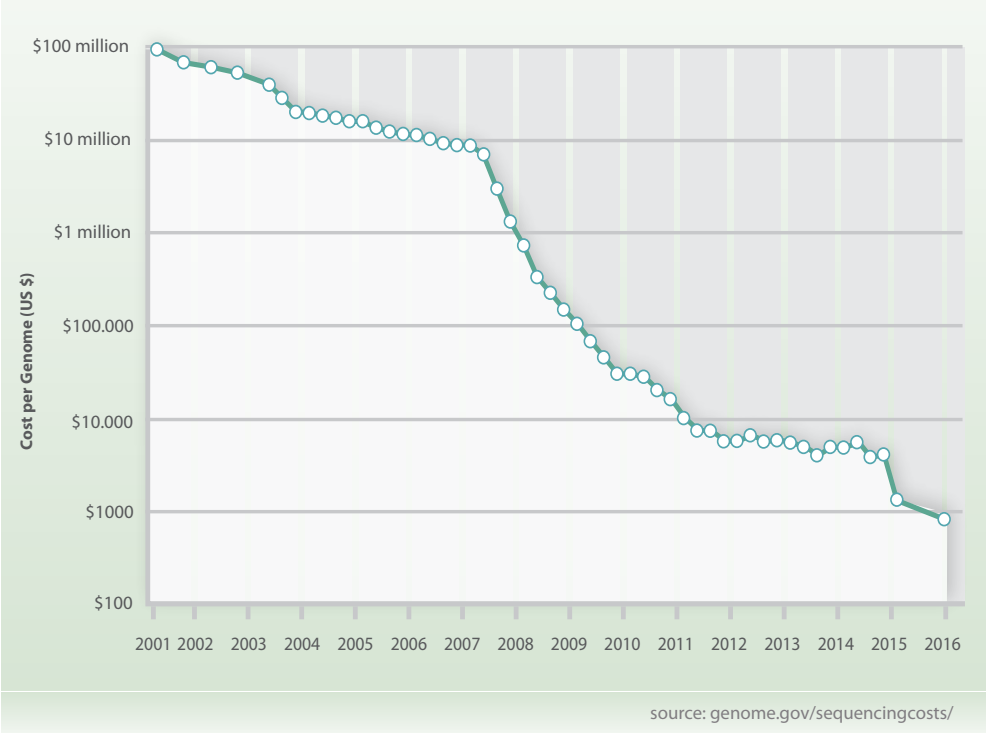

Figure 1: Reduction in the cost of sequencing a human genome over time

3.1.2 Applications

An important application that is under rapid development is the use of NGS in the characterisation of microorganisms. Large projects like the Human Microbiome Project[112] in the US and MetaHIT[113] in Europe aimed to study the role of microorganisms in disease and health, which turned out to be greater than expected (*see text box 'The importance of the microbiome'*). The number of microorganisms the human body carries is greater than the number of its own cells. These microorganisms play an important role in our digestive and immune systems. The advantage of using NGS techniques is that they avoid the need to culture microorganisms, which saves a lot of time and makes it possible to study microorganisms that cannot be cultured in the laboratory. In surveillance of infectious diseases, NGS help to speed up the process of tracking down resistance to antibiotics.
Microorganisms are also a major factor in the environment, influencing things like soil condition, water quality and the health of plants and crops. To study the composition and functioning of this microbiome in the world's biosphere there has been a call in the United States

to establish an American[114] or International Microbiome Initiative[115] involving researchers from a range of disciplines. So far NGS has helped to identify a far greater diversity of micro-organisms than had previously been thought existed.[116]

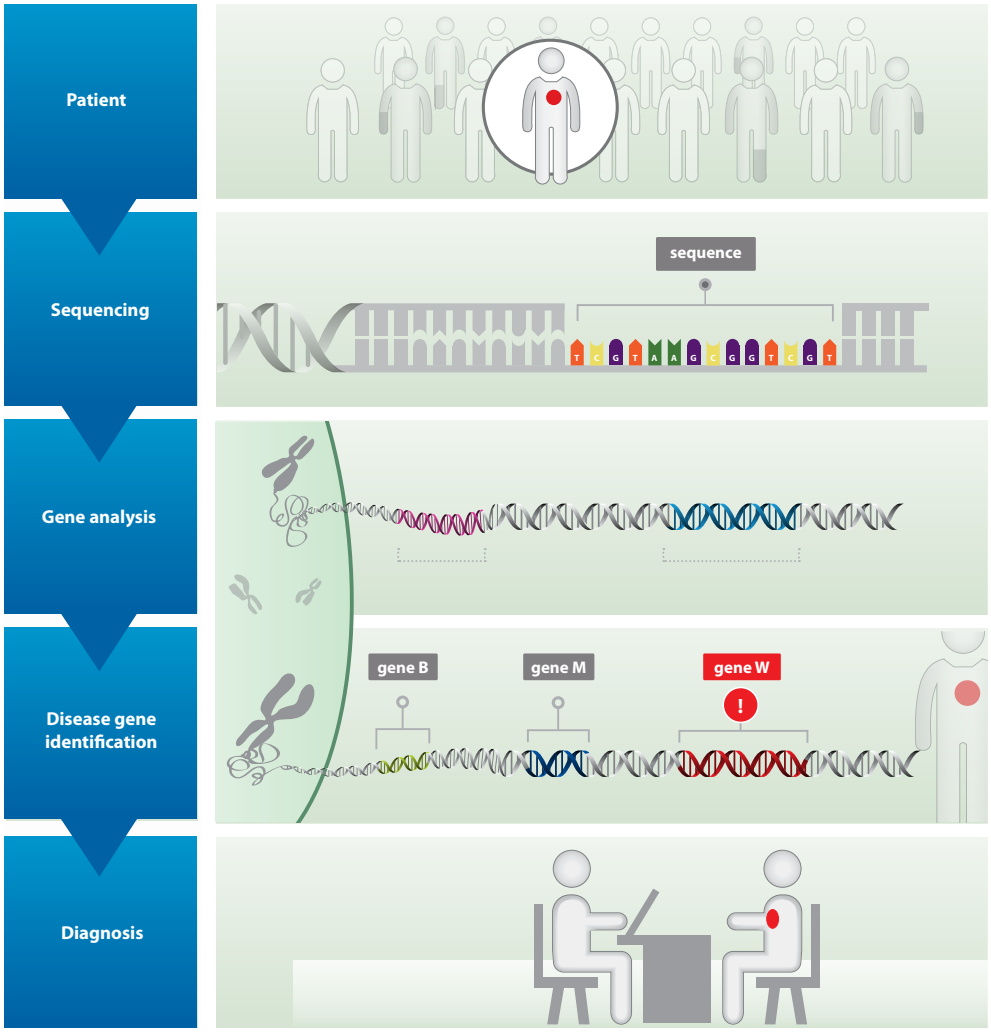

Figure 2: Sequencing in patient care

The importance of the microbiome

Each organism and habitat[i] is home to a complex community of microorganisms (bacteria, fungi, algae, viruses). For example, the human gut contains about a kilogram of microorganisms. These communities are referred to as the microbiome. Little is known about which microorganisms are present in the microbiome. Many microorganisms cannot be cultured or are only present in very small numbers. The mutual interactions between these microorganisms and between them and their environment, such as the host organisms, are unknown. Nevertheless, it is beyond any doubt that microbial systems are very important. Microorganisms are crucial in the decomposition of organic material and in the nitrogen and carbon cycles, but also in the protection of plants, animals and humans against disease. The composition of the human microbiome appears to play a role in asthma and diabetes. The intestinal flora influence the human immune system.

i The living environment of a plant, animal or microorganism.

The new large-scale sequencing methods make it possible to sequence and determine a microbiome in its entirety without first having to isolate and culture all the different microorganisms. The great importance of microbiomes has prompted a consortium of researchers to propose a project to characterise the various microbiomes inside and outside the human body and to shed light on the function of the various genes and the interactions between microorganisms and their environment.[117] Doing this will require the development of new technologies and methods.

Previously, genetic diagnosis was done by sequencing genes thought to contain a mutation one by one. Now, with NGS, it is possible to sequence many genes at the same time using techniques such as gene sequencing panels, whole exome sequencing[j] and whole genome sequencing. Single genes can also be sequenced more quickly, which can save time when making a diagnosis or ruling it out. Whole genome sequencing sequencing (WGS) seems to have taken off in the Netherlands as a 'one test fits all' technique in which a genome-wide sequencing is out carried straight away for every condition. Once a whole genome sequence has been obtained, the analysis may be restricted to one or more specific genes rather than the whole sequence, but the whole sequence is nevertheless available should further analysis be needed. It is expected that in the near future sequencing will be so cheap that it will be more efficient to make a sequence every time one is needed to avoid the need to store the information. Clinical genetic centres in the Netherlands are cooperating in a national gene sequencing centre.[118]

Another advantage of NGS is that the greater understanding of the significance of genetic variety it brings provides a genetic evidence base for an increasing number of diagnoses. This helps to reassure patients because they can be given a clearer idea of what is causing their complaint or disorder, even if there is no treatment available. It gives parent something to hold onto regarding the development of a prognosis for their child.

NGS is used in oncology to sequence the DNA of tumour cells in order to decide which therapy will have the most chance of success and thus avoid unnecessary treatment with drugs that have no effect on the tumour, speeding up the treatment pathway and averting side-effects. The genetic characterisation of tumours has led to the development of drugs that target a specific defect or characteristic of the tumour, which is an example of personalised medicine (*see §3.3*).

In time it is expected that NGS may be able to obviate the need for other tests or replace them. An example is NIPT, which avoids the need for many invasive prenatal tests (amniocentesis and chorionic villus sampling). Similarly, in the longer term, it is expected that NGS can be used in population screening tests, such as the neonatal heel prick test.[119] However, optimism should be tempered (not all metabolic diseases can be identified by DNA sequencing because not all the disorders tested for in the heel prick screening have a genetic origin, such as congenital hypothyroidism) and full consideration should be given to the ethical and financial consequences. Moreover, it is important to realise that many genetic variations are not yet known, or it is not known whether or not they can cause diseases and, if so, to what extent.

3.1.3 Issues and challenges

As for any new technology there are certain aspects that require further reflection from scientists, doctors, policymakers, patients and the public.

j The exome is the complete set of coding sequences (exones, genes and parts of genes, etc.) on the genome; in humans this is about 1% of the genome.

Further research needed

It is import to beware of placing too much faith in and emphasis on genetic information. Although knowledge is increasing at a rapid rate, little is known about the significance of genetic variants for disease and health and about the soundness of the correlations found. Besides, illness and health are not only determined by genetic factors (with the exception of monogenetic disorders[k]); environmental factors are just as important. This is particularly important regarding advice on the reproductive choices of patients with these types of genetic variants. Furthermore, the quality and accuracy of sequencing, especially the interpretation of the sequence analysis, can and must be much better. Biobanks, such as BBMRI and Parelsnoer, make an important contribution to improving our knowledge on these issues.[120,121] A further issue is who should be the owner of human tissue and the information and knowledge obtained from it (*see text box 'The immortal cells of Henrietta Lacks*).

The immortal cells of Henrietta Lacks

Biobanks and information on genome sequences are indispensable for identifying genes that are linked to diseases and for developing new diagnostic tools and treatment methods, but they depend on patients making samples of blood and body tissue available. This raises questions of who owns the material and the data, who has access to this information, the purposes for which the material may be used, and whether or not the privacy of the patient and his or her family and descendants is guaranteed. The example of the HeLa cells is illustrative of these issues.[122] The 'HeLa cells' are a tissue culture cell line dating from 1951 derived from a tumour in Henrietta Lacks. HeLa cells are the most used cell line in biomedical research, but the Lacks family have never received any benefit from this use (and for a long time even had no knowledge of it). The rights of the descendants of Henrietta Lacks were only recently recognised, after the full genome sequence of these cells, among other information, had been published. Researchers did not properly realise that publication of this information would affect her descendants. The American National Institute of Health (NIH) eventually came to an agreement with the family, under which access to the data is restricted and requests for access to the data are decided on by a commission in which the family is represented. Use of the information is limited to biomedical research.

Big data[l]

Another issue is the enormous amount of data generated by this technique. It raises technical questions not only about algorithm development and statistical computing and storage capacity, but also ethical and legal questions about who owns the data (the patient, the family, the doctor, the sequencing company?), who should have access to them (patient, family members, doctor?) and when and for how long these data should be stored, and where (on a USB stick held by the patient, in the cloud, in the electronic patient record, in the hospital?).

Incidental findings and consent

Whole exome and whole genome sequencing, in particular, can give rise to incidental findings: variations or abnormalities that are discovered unintentionally and unrelated to the condition for which the tests were performed. When gene panels (a set of genes known to be associated with a specific group of disorders, such as cardiovascular disorders) are used, the risk of incidental findings is small. It should be noted, though, that many incidental findings are not clinically relevant. However, the problem with genetic variations is that their implications are not always known. Because it is impossible to anticipate the whole range of

k A monogenetic disorder is caused by one or more changes (mutations) in a single gene.

l There is no generally accepted definition of big data. Roughly speaking, it is taken to mean many rapidly generated and complex data.

potential incidental findings and their impacts on the patient and the patient's family and friends, the traditional informed consent[m] is no longer adequate and there is a need for new forms of layered consent. In addition, there is the question of what should be reported back to the patient and what should not.[123] This technique also blurs the boundaries between diagnostics, screening and scientific research, which has consequences for re-contacting patients (for example, about incidental findings), financing the tests and ethical questions associated with the tests.

Legislation
In the publicly financed healthcare system, the legislative basis for the provision and financing of screening, diagnosis and care – services which are more influenced by the possibilities presented by sequencing than the care provided under the Care Insurance Act [*Zorgverzekeringswet*] – is provided by the Population Screening Act [*Wet op het Bevolkingsonderzoek*] and the Special Medical Procedures Act [*Wet op Bijzondere Medische Verrichtingen*]. For a while now questions have been regularly raised in the medical community about the ability of these laws to manage or prevent undesirable developments without unduly blocking innovation in genetic screening and diagnostics, the boundaries between which are becoming increasingly blurred.

Public knowledge of genetics
An important underlying problem in society is that at the moment the public and many medical professionals (general practitioners, nurse practitioners, midwives, medical specialists and nurses) do not know enough about genetics to fully appreciate the implications of NGS. This is important because genetic findings not only have important consequences for the person in question, but sometimes also for family members, who in most cases would not have asked for the information.

APPLICATIONS, BENEFITS AND ISSUES IN OTHER SECTORS

Applications and benefits
NGS is also finding its way into other sectors. For example, in agriculture it is used to speed up the plant breeding process. Long sequences and, more particularly, single molecule sequencing have major advantages when complicated (polyploid) genomes and metagenomes are involved. It also makes it much easier to make gene maps – an added benefit besides the lower costs. Genome sequences have already been determined for many plant species. Determining and comparing the genomes of different varieties of the same plant species has led to the identification of genes that code for favourable traits, such as a change in flowering period, the quality of cereal grains and tomato ripening.[124,125] NGS is also used to study the interactions between plants and microorganisms[126] and to characterise and study the soil flora to aid with improving soil quality. It is also being adopted in veterinary medicine, for example for the diagnosis of infectious diseases,[127,15] and is used in scientific research into the genetic make-up of a range of organisms.

These rapid developments are making model systems less relevant, because every organism can be sequenced, often quickly. Whereas in the past a company working on a small crop could not afford to invest heavily in genomics, the costs are now no longer an obstacle. Moreover, the numbers of annotated genomes (genomes for which information about the function and position in the genome of sequences has been added to the genome database) is growing, making it possible to transfer information to other species. However, to do this companies will have to acquire the necessary genomic expertise.

m Informed consent: a statement of voluntary permission to carry out a treatment, for example extensive diagnostics, an operation or participation in a scientific study, having been informed of the possible risks of the treatment.

Issues

Some of the applications mentioned here face the same challenges as the medical sector, such as computing and storage capacity for the data and interpreting the information. There are also parallels between the discussion about privacy in the medical sector and intellectual property rights in the agricultural sector. The core question is who owns these data.

3.1.4 Stakeholder implications

The development of NGS and its applications has implications for various stakeholders and should therefore be considered within the national political and social context.

- **Patients:** For patients and their families, NGS will open up more possibilities for diagnosis, and it is expected that this will in turn lead to more possibilities for personalised prevention and treatment (*see §3.3 Personalised medicine*). But not everyone will want to know everything; people have a right not to know and a right to an open future. Who decides what to test for? In the US a fierce debate is raging about the recommendation by the American College of Medical Genetics to make it standard practice in WGS diagnoses to test for known disease-causing mutations in 57 genes associated with serious disorders, regardless of the medical reason for the diagnostic test[n,128] and to inform the patient of any mutations discovered by the test. The proposal has now been amended to include an opt out clause for the patient. A particularly difficult issue is how far parents should be allowed to make decisions for their children (and unborn children) and have access to their genetic information. A further important issue is the need to provide and present information as clearly as possible so that patients can properly comprehend the implications and options open to them. By the same token, it is important to consider the desirability or not of storing genome information in electronic patient records.
- **Consumers:** The introduction of market principles into the healthcare system encourages patients to act like consumers to obtain the best possible care for the best price. Companies are responding to this by offering healthy consumers extensive genetic tests, claiming that they can identify the risks of a whole range of disorders. (At the moment these tests work with markers, but increasingly use sequencing, which gives the companies information about the whole genomes of their clients.) The problem is, the real meaning of a probability of having a disorder is hard to grasp. For example, a ten times higher risk of having a disorder that is relatively rare still means that the absolute risk is small, but the news can still be distressing. Having an annual general health check to identify or prevent emerging health problems is of dubious value, but including a genetic analysis, particularly for multifactorial disorders, as part of such a health check is definitely controversial. It entails a risk of overdiagnosis (and overtreatment). It is also important that consumers are given clear information on sequencing techniques, especially on what they can and cannot do. Another question for stakeholders is the financing of the test and any resulting treatment and care. Just because a certain treatment is possible, does it mean it should always be provided? These considerations also apply to personalised medicine (*see §3.3 Personalised medicine*).

  The benefits to consumers of the use of NGS in plant breeding are that as knowledge of the characteristics and growth of crops rapidly increases, breeding is becoming quicker and more efficient, resulting in crops with better growth, better taste and other desirable attributes. On the other hand, the relation between genetics and food is a sensitive one. Applications in veterinary medicine, for example for faster and better diagnosis of infectious diseases, can lead to less use of antibiotics and 'cleaner' meat.
- **Medical professionals:** NGS generates large quantities of data and information, which must be interpreted and placed in the right context. Most of the time this will be done by medical professionals, assisted where necessary by laboratory specialists. At the moment,

n Except prenatal and neonatal screening.

most questions about genetics are dealt with in clinical genetic centres, although this is expected to expand to other specialisms and even general practice. It is therefore important that these centres have sufficient in-house knowledge and expertise about sequencing, what the results mean and any uncertainties surrounding this information. At the moment, though, this may not always be the case.

Another aspect of the growth in NGS for medical professionals is a growing need for 'e-lab' technicians as opposed to the traditional 'wet lab' scientists. An 'e-lab technician' is conversant with bioinformatics and can process sequencing data, whereas the 'wet lab technician' prepares the samples and carries out the sequencing. The higher laboratory technology courses will have to take these requirements on board.

- **Scientific researchers:** Medical professionals and researchers will have to share data in order to increase their understanding of genetic variation and what it means for illness and health. On the other hand, patients and their families have a right to privacy, to not know and to an open future, while researchers, who are under pressure to publish and extract value from their research, have a tendency to jealously guard their data. The question is how data can be shared in a safe and effective way. A current development is the growing number of foreign providers of cheap sequencing services (both commercial and semi-public, such as the Beijing Genomics Institute). Making use of these services raises questions of the quality of the sequencing, privacy issues ('genome in the cloud') and the ownership of the data.

  In a recent monitoring report the Health Council of the Netherlands advised holding a broad public debate among the stakeholders on the desirability and consequences of various aspects of these techniques, and placed particular importance on increasing the understanding of genetics among professionals and the public.[129]

  In other sectors, sharing information about genomes, genetic variation and their implications for the phenotype of the crop or animal is also important. Here, the issue is not one of privacy, but of intellectual property rights.
- **Companies:** Companies in the healthcare and other sectors are confronted with the rapid evolution of sequencing technology and the data it produces. They have to make decisions about whether to invest in sequencing themselves or to outsource it, and whether or not to provide services to third parties or buy in the necessary expertise.

3.1.5 Conclusions

Next generation sequencing offers many opportunities, from more effective plant breeding to faster genetic diagnostics for patients, and from a better understanding of species diversity to a better grasp of the role played by bacteria in soil quality.

Issues and dilemmas that arise across the whole field of application of NGS:

- What is the optimum balance between generating, analysing and sharing data in the interests of furthering scientific knowledge and medical possibilities on the one hand, and privacy, intellectual property rights and costs on the other hand?
- What is the optimum balance between the benefits of using NGS for the individual and the rights of family members not to know their genetic risk factors?
- These two issues could be a reason to review the adequacy of current legislation, such as the Special Medical Procedures Act [*Wet op Bijzondere Medische Verrichtingen*], privacy legislation and patent law.
- Another wide-ranging issue is how to better educate the public and professionals about the facts and fables of NGS across all its application areas in a balanced and sensitive manner. A range of organisations should be willing to participate in this, such as government departments, educational and research institutes and businesses. The Health Council of the Netherlands has already recommended taking an integrated approach to tackling these issues in a so-called 'genome clinic', a partnership between various professions (such as molecular geneticists, epidemiologists, bioinformaticians, health economists, ethicists,

clinical geneticists, diverse medical specialists and patient representatives), and centres for NGS and genetic diagnostics.[129]

3.2 CRISPR-CAS: ALTERING GENETIC TRAITS

In recent years the possibilities for making specific changes in the genomes of animals, plants and even humans have increased enormously. The latest development is the CRISPR-Cas9 system. This offers previously unheard of possibilities for altering genes and the genome and opens the door to new types of experiments, products and therapies. The journal *Science* elected CRISPR as its 'Breakthrough of the Year'.[130] However, the technology does raise questions about the differences between the possible and the desirable.

3.2.1 What is CRISPR-Cas?

Over the past three years the CRISPR-Cas9 technology has taken the world by storm.[131] CRISPR-Cas9 is used to make specific changes in the genetic material of animals, plants or microorganisms (genome editing). The system consists of a complex of RNA molecules, which recognise the complementary sequence in the DNA of an organism, and proteins (Cas9), which then cut the DNA at the desired location. This system makes it possible to regulate the expression of genes, make targeted point mutations, remove genes or parts of genes, and insert new genes or DNA fragments at specific locations in the genetic material (*see Figure 3*). Compared with CRISPR, earlier forms of genome editing techniques, such as oligo-directed mutagenesis, TALEN and zinc fingers, are complicated, time-consuming and of limited applicability.[132,133,134]

The simplicity, speed and low cost of the CRISPR-Cas9 technique makes genome editing a standard technique in every research laboratory.[135] It is therefore no surprise that applications of CRISPR-Cas9 are springing up in all research areas in biology and the life sciences at an unprecedented rate. Researchers hope that CRISPR-Cas will help them to better understand the role played by genes in diseases such as cancer, to repair human genes associated with genetic disorders and control infectious diseases, and to speed up the plant breeding process. The rise and potentials of CRISPR-Cas9 are closely associated with next generation sequencing, which provides the necessary sequence information and insights into the role played by genes.

3.2.2 Applications

New applications and improvements to the CRISPR-Cas system are published virtually every day.[136] CRISPR-Cas9 has been successfully used in plants, animals, microorganisms and viruses. It has been used to repair genes in human stem cells, which can then be returned to the host to control diseases.[137] CRISPR systems have also been developed to control virus infections in humans and plants.[138] Researchers are combining the CRISPR system with light-sensitive proteins in an attempt to induce modifications (under the influence of a light beam) in only certain tissues during a certain period of time.[139] This combination of CRISPR and optogenetics can be used, for example, to control tumours and make changes in the brain. CRISPR-Cas9 has great potential for plant breeding, because many agricultural crops have genome duplications and therefore possess several copies of the same gene, and CRISPR-Cas can be used to alter all the copies of the target gene in the cell. It has already been demonstrated that CRISPR-Cas9 works in a range of plants species, including wheat, rice, tomato, soy, tobacco and poplars. The technique has also been called the new driver for research into xenotransplantation, the transplantation of humanised organs from animals into humans.[140,141] The power of the CRISPR system is illustrated by an experiment in which a record number of 62 sequences of retroviruses (PERVs) were removed from the genome of a pig embryo.[142]

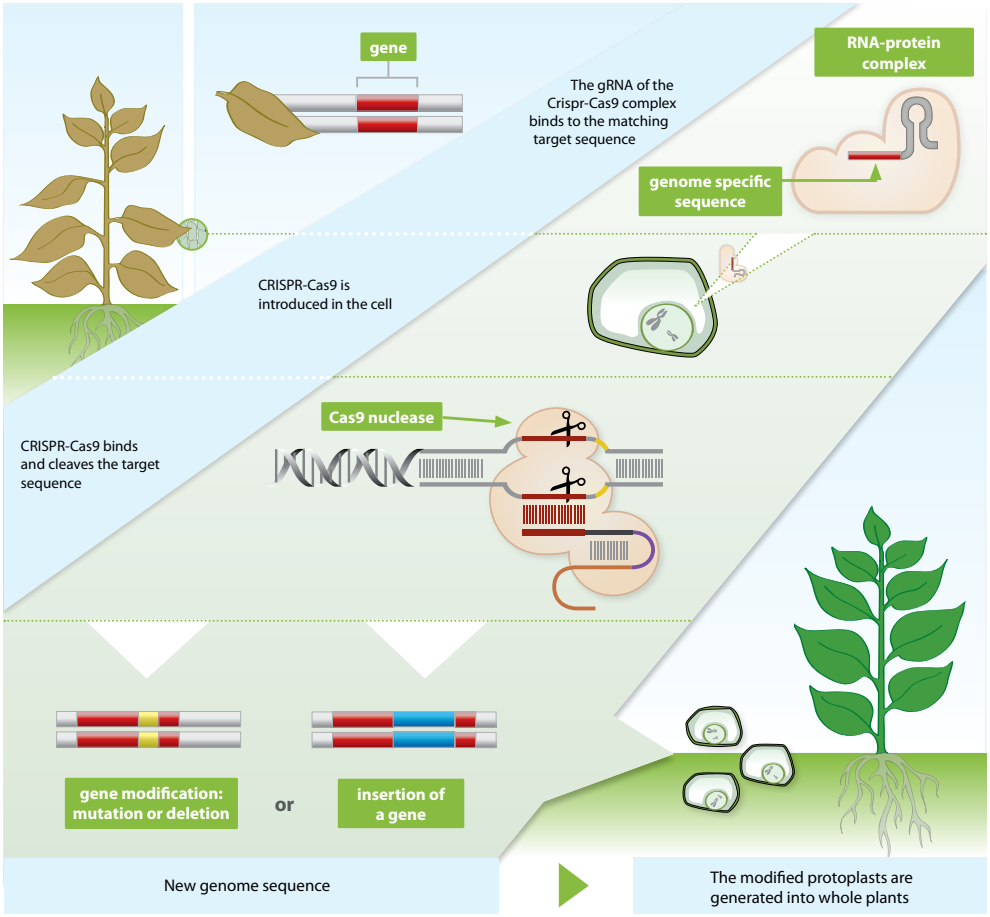

Figure 3: The CRISPR-Cas9 system

3.2.3 Issues and challenges

Besides the great potential and opportunities that CRISPR offers, the technology raises a number of issues and ethical questions.

Technical obstacles that still have to be overcome: off-target modifications

The success of CRISPR-Cas is down to its effectiveness and ease of use. However, removing sequences and turning genes off is easier than introducing mutations or inserting and exchanging sequences. And before CRISPR-Cas9 can be used for medical purposes, more needs to be known about the occurrence of off-target modifications and how to avoid them.[143] Off-target modifications are caused if the CRISPR-Cas9 system unintentionally recognises or binds to a location on the DNA and induces an unwanted change. It is not yet clear how frequent such off-target modifications are. Research into the use of CRISPR-Cas in stem cells has shown that off-target effects are rare,[144,145,146,147] although a large number of off-target modification have been reported in studies on human embryos.[148] Research aiming to improve the CRISPR system and prevent off-target modifications is well underway. Results are now appearing in rapid succession and may soon make this problem irrelevant. A new variant of the CRISPR-Cas system has recently been discovered which makes use of the Cpf1 enzyme instead of Cas9.[149] The claimed advantages of CRISPR-Cpf1 are that it has a higher specificity, which considerably reduces the likelihood of off-target modifications, and that it is simpler to insert and exchange sequences. At the end of 2015, the same researchers announced that they had 'dramatically improved' the CRISPR system

by making changes in the Cas9 protein.[150] At the start of 2016 another group in the US claimed even claimed to have improved the system to such an extent that off-target effects could no longer be detected.[151]

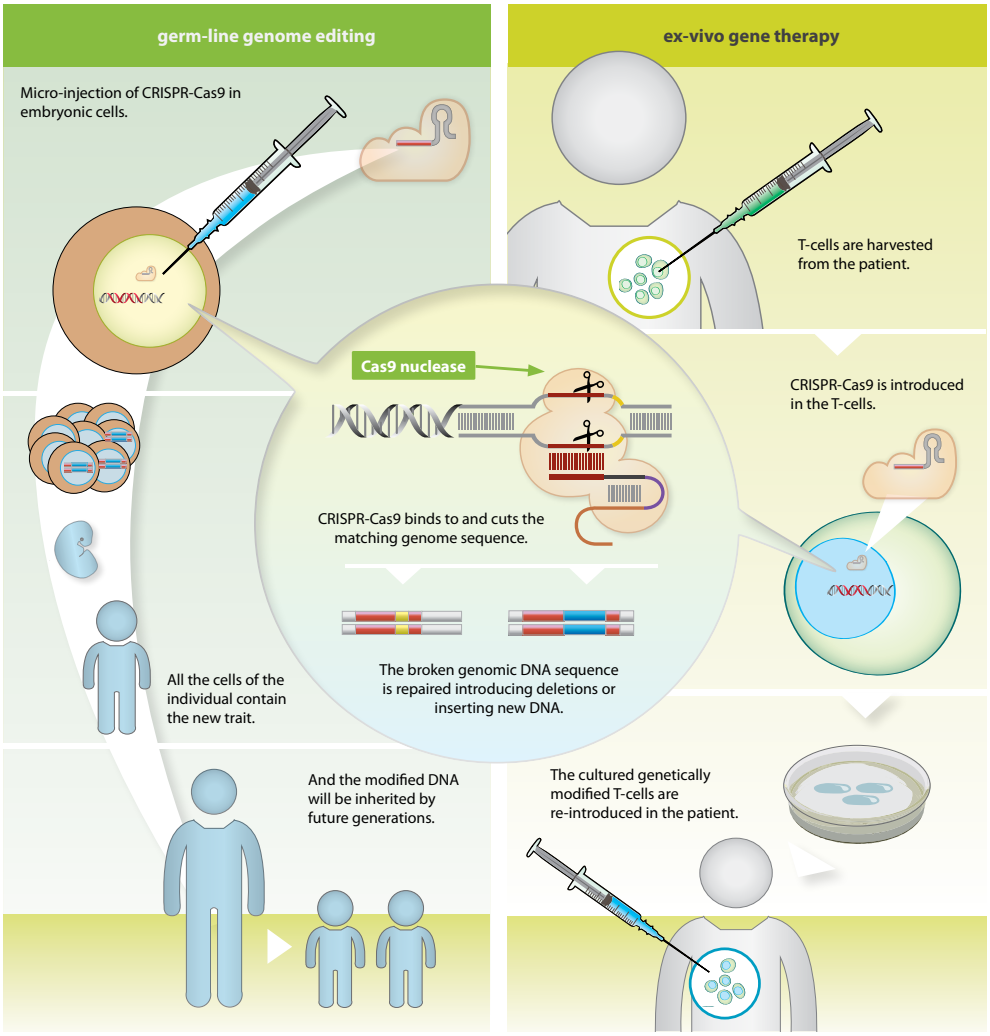

Figure 4: Use of CRISPR-Cas in humans, germline modification and gene therapy

Germline modification

Regarding the medical use of CRISPR, an important distinction must be made between two types of application. The first is its use in body tissue (somatic cells), in which changes made to the DNA are not passed on to future generations. This use of the CRISPR technology is considered to be a refinement of gene therapy (*see §3.4 Gene therapy and Figure 4*). The second is use of CRISPR in reproductive cells (egg cells and sperm cells) or in pre-implantation embryos at an early stage of development, in which changes made to the DNA are transmitted to future generations. This form of the technology is called germline modification, or human genome editing.[o] Modification of the genome in human germline cells is prohibited in the

o Germline modification is the modification of gametes (reproductive cells) or fertilised egg cells, after which all the cells of the embryo carry the modification, which can then be passed on to the following generations. The Dutch Embryo Act prohibits modification of the genome in human germ-line cells. This prohibition does not include cell nucleus transplantation to prevent mitochondrial diseases. To clarify that CRISPR-Cas concerns the modification of genomic DNA, its use is often described as germline genomic modification or germline gene therapy. In this Trend Analysis we use the more usual term 'germline modification'.

EU.[152] However, the advances made in the possibilities of the technology may lead to questions about the desirability of such an absolute ban.

In early 2014 Chinese researchers reported that they had succeeded in genetically modifying monkeys using CRISPR-Cas9 targeted mutations in embryos.[153] Various research groups have announced that they also want to make GM monkeys for use as model systems for research into human diseases, etc. Quite apart from the question of whether more widespread use of GM monkeys as model systems for diseases is desirable or not, it brings the possibility of genetic modification of humans a step closer. From a scientific point of view, the step from monkeys to humans is a small one. Early in 2015 and 2016 two different Chinese research groups succeeded in modifying the genetic material in non-viable human embryos[148,154] and it is thought that other Chinese research groups are also experimenting with human embryos.[155] A researcher in the United Kingdom has obtained a licence to modify human embryos for research into the causes of miscarriages.[156,157] The conditions are that the embryos must not be used to initiate a pregnancy and must be destroyed on completion of the experiment. Permission has been given for similar experiments in Sweden.[158]

Until recently, the objections to germline modification were mostly practical in nature, rather than ethical. It was not certain that germline gene therapy was actually possible and the risks of randomly inserting the introduced gene into the genome of the progeny (until now a random process) are great. CRISPR-Cas9 appears to negate many of the practical objections to genetic modification of humans, making the repair and prevention of genetic disorders in future generations a real possibility. Monogenetic disorders (caused by a defect in a single gene) will be the first to become treatable. Disorders or traits that involve multiple genes are technically much harder to tackle.

Before CRISPR-Cas can be used for germline modification in humans, further research is needed to determine when the risks will be small enough to be able to use the technique and how long-term risks and risks to future generations can be estimated and taken into consideration. Researchers will have to eliminate uncertainties, including the possibility of off-target modifications, whether or not all cells do indeed contain the modification (the 'mosaic effect') and questions about efficiency and specificity. This inevitably raises the question of how far effects are reversible or repairable.

As studies of animal models such as mice and monkeys will never be able to provide sufficient evidence, the Hinxton Group and a meeting in Washington of the Chinese, British and American Academy of Sciences, among others, argue that research on CRISPR-Cas in human embryos will have to be permitted under a set of conditions.[159,160] Some countries permit research involving the use of human embryos, making a distinction between the use of 'excess embryos', for example from in vitro fertilisation (IVF) clinics, and embryos created specifically for research purposes. Because the availability and suitability of human embryos is limited, to assess the safety of the techniques like CRISPR-Cas it is often necessary in practice to create embryos specifically for research purposes.[159] This is currently not permitted in the Netherlands, but it is in several European countries.[161,162] As far as we know, at this time it is not permitted anywhere in the world to place genetically modified embryos in the womb.[163]

Removing some of the technical objections shifts the focus of the discussion towards the ethical aspects of genetic modification in humans. Many researchers have already expressed their concern about this development and have called for a moratorium on germline modification and for international discussions about how to proceed.[164,165,166] In 2015, statements were issued by various organisations, including the International Society for Stem Cell Research (ISSCR), the Hinxton Group and the International Bioethics Committee of UNESCO (IBC), and meetings were held in Amsterdam, Washington and other cities to discuss the possibilities for and desirability of germline modification.[159,160,167,168,169,170]

The question of whether germline modification is ethically acceptable or not involves a broad spectrum of considerations. To start with, to what extent are alternatives to germline modification available? For some patient groups there are alternatives for preventing the disease in question, such as pre-implantation genetic diagnostics (PGD) in which embryos are created through IVF and only those free of the genetic mutation are selected for implantation. Apart from the fact that this raises ethical questions regarding the use of embryos, there may also be practical problems, such as the production of sufficient egg cells in the first place to permit the use of PGD. Besides alternatives that can be used to prevent illness in the first place, an alternative for some patients and diseases can be somatic gene therapy. However, in most cases, somatic or other gene therapy treatments are not yet possible.

When there are no alternatives, the question becomes whether or not germline modification should be permitted. To answer this question, consideration should first be given to how effective and safe the proposed modification is. How much DNA has to be modified, how big is the probability that this will adversely affect other functions, and are off-target effects a possibility? Besides the question of safety, an important consideration is the burden of the genetic disorder itself. Not all genetic disorders lead to insuperable disabilities in those affected, and the severity of the condition and the patient's ability to cope also differ. As this concerns people who 'do not yet exist' and therefore cannot speak for themselves, a key issues is who has the right to decide which genetic disorders are 'severe enough' to justify the use of germline modification. Who decides what is a 'sufficiently healthy' future? Should that be the future parents; do the medical practitioners have a responsibility; or should the government lay down the rules?

Underlying these questions is the concern that what may begin as treating a few serious genetic disorders could eventually turn into the promotion of all sorts of specific desirable characteristics. The debate about germline modification, therefore, seems to be inseparable from the debate about human enhancement. In fact, this debate already plays a role in embryo selection.

Finally, the ethical debate also raises broader social questions. There are major concerns about the desirability of these developments from a social justice perspective.[171,172] These scientific possibilities are emerging within existing power relations in society, both in the Netherlands and internationally, which will influence the direction developments take. The concern is that germline modification will aggravate the existing problems and differences between people at the local, regional and international levels, that expensive technological treatments will only be accessible to a small group of people, and that an elite few will use human enhancement to give their descendants an even greater competitive advantage. But the new developments are also welcomed and give patients and others new hope for the future *(see text box 'Public opinion on germline modification')*.

Public opinion on germline modification

An opinion poll of 1,000 viewers of the TV science programme *De Kennis van NU* [Knowledge Today] gave a positive view of germline modification.[173] This survey of public opinion, albeit non-representative, indicated that an overwhelming majority (85%) of participants would have their DNA altered to avoid a hereditary disease. The respondents were a bit more hesitant about altering the DNA of their children: 65% would have the DNA of their unborn child altered to prevent them having a genetic disorder. They were much less enthusiastic about altering DNA to obtain resistance to disease (30%) or increase intelligence (15%).

The question then is whether the potential risks of germline modification and the ethical objections mentioned above outweigh the possibilities of preventing genetic disorders and

giving carriers of these disorders the opportunity to have children of their own. It does not seem to be possible to give a simple yes or no answer to the question of whether germline modification is justified, or even desirable, from an ethical point of view. The ethical discussion is also about the limits of what is acceptable and how these can be determined, where the line can or should be drawn between healing and enhancement, if the amount of someone's DNA or number of their genes that are altered makes a moral difference, and whether or not this new technology will heighten existing social tensions and injustices and widen the gulf between generations.

The Dutch authorities will have to review the extent to which current legislation is up to the task of meeting the challenges posed by these developments. They will also have to think about how to deal with international differences in the regulatory framework. Human germline modification is banned in the EU. In some other countries the legislation is more ambiguous, or is even entirely lacking.[174] If germline modification becomes technically possible, continuation of the ban in the Netherlands could result in medical tourism to countries where it is permitted or where it can be carried out without any regulatory control. But even if germline modification were to be permitted in the Netherlands for certain medical purposes, medical tourism for enhancement cannot be ruled out.

Gene drives: intervening in ecosystems

An application of CRISPR that illustrates both the power and the potential risks of the technique is the CRISPR gene drive (also called mutational chain reaction). Introducing or changing a genetic character in a population may be desirable in some situations, such as genetically modifying mosquitoes so that they can no longer transmit malaria (*see text box 'Mosquitoes that cannot spread malaria'*), but the question is whether such a genetic alteration can be introduced into a population so that most if not all the individuals in the population contain the modification. As a rule only some of the descendants of a modified organism contain the inserted or modified gene. The speed and scale of the introduction of a trait are a function of the number of genetically modified individuals released in relation to the size of the wild population, which is a constraint on the introduction of the desired trait. In view of this problem, research has been ongoing for some time into 'gene drive' mechanisms, which can ensure that a modification is transmitted.[175] Various systems have been developed with limited success.[176] In a recent development a mechanism using CRISPR-Cas9 has been proposed.[177] If a CRISPR-Cas complex is inserted into the target gene, the desired modification will be introduced into each copy of the gene in the genome and the CRISPR complex will be inserted into each chromosome. All progeny will therefore contain the CRISPR complex and pass it on to all individuals in the next generation. In theory, this will ensure that the genetic modification will be transmitted throughout the whole population and in time the 'wild-type' gene will disappear (*see Figure 5*). This means that if a gene drive is used, in theory it would only be necessary to release a limited number of genetically modified individuals in order to modify the whole population.

Mosquitoes that cannot spread malaria

Mosquitoes transmit numerous viruses and parasites to humans, including malaria. Malaria is caused by parasitic protozoa of the *Plasmodium* genus. The life cycles of these single-celled parasites include stages in infected mosquitoes and humans. Malaria is one of the most serious infectious diseases in the world and is endemic in large parts of Africa and Asia. In 2015 an estimated 438,000 people died from malaria, mostly children. The fight against malaria focuses on reducing the risk of infection by spraying with insecticides, the use of mosquito nets and providing medicines. Scientists have succeeded in finding genes that prevent mosquitoes from transmitting the parasite to humans, but no method had been found for spreading these genes through the population. However, by

using CRISPR-Cas9 a gene drive system has been developed for the mosquito *Anopheles stephensi* that can transmit the gene throughout more than 98% of the population via their progeny.[178] *A. stephensi* is the most important malaria vector in India. The researchers do not yet have any plans to test the system in field trials, and are waiting to see how the public debate about the acceptability of these types of experiments develops.

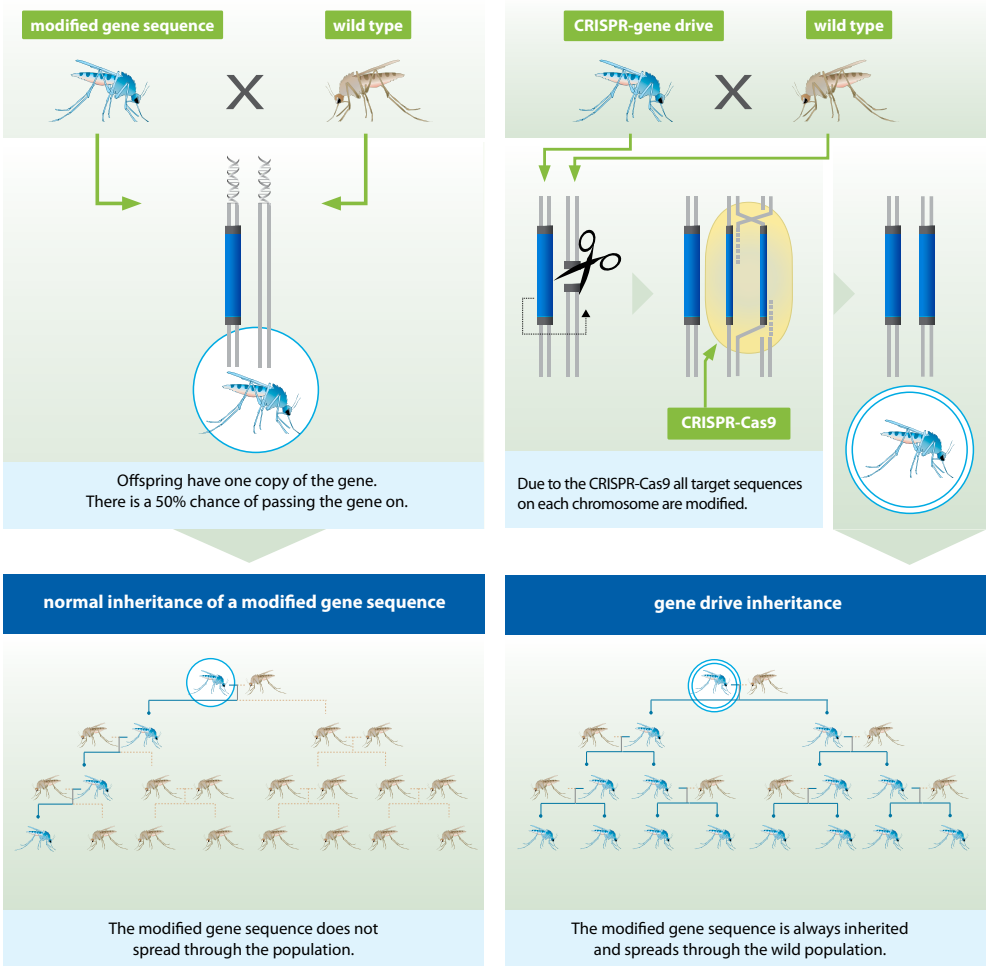

Figure 5: Molecular mechanism of gene drives in insects

CRISPR gene drive systems may offer major advantages for transmitting a desired character through a wild population, but there are also risks involved.[179] A gene or altered trait could possibly be transmitted unintentionally to another species or population by interbreeding. The trait will then spread through the population and become established, seemingly without any way of stopping it. It may even be possible to use this technique to eradicate an organism (*see text box 'Eradicating pathogens and exotics'*).[180] Researchers are therefore quick to warn of the risks associated with this technique.[181] For example, should a modified insect escape from the lab the consequences could be very serious[182]; some warn of the risk of bioterrorism and think guidelines should be drawn up on what may and may not be published in scientific journals on the subject. Other researchers question how real the supposed risks of gene drive systems actually are,[183] pointing out that functioning gene drive systems have as yet only been demonstrated for yeast, fruit flies and mosquitoes under laboratory conditions.[184] These are organisms that with rapid reproduction and short generation times. It is questionable

whether gene drive systems can be effective in organisms that do not meet these conditions, such as mammals. CRISPR gene drive systems will also be less effective in plants, because the underlying system of recombination works less effectively in plants. Also, it is doubtful that a gene drive system will actually be able to spread throughout a natural population, because this will depend, among other things, on the fitness of the GMOs compared with the wild-type organisms, the spatial genetic structure of the natural population and the stability of the genes in question. It is known that 'resistance mechanisms' can emerge within just a few generations and inhibit the further spread of the altered gene.[176] If the changes introduced by a gene drive system have an adverse effect on the fitness and reproduction of the organism, the trait will spread more slowly, or not at all, because the number of descendants of the wild-type individuals will be larger. Highly adverse effects on fitness will eventually lead to the gene drive disappearing altogether from the population,[185] which can only be compensated for by releasing large numbers of modified individuals. If the change has a beneficial effect on fitness and reproduction, the gene drive will spread through the population more rapidly, like all traits that increase fitness. The efficiency of gene drive systems also depends on the CRISPR RNA molecule and the sequence to be modified. In mosquitoes an efficiency of between 24% and 90% has been observed.[186]

Developments in this area are moving fast and at the moment it is hard to tell what the real potential of gene drives is. For example, researchers have been able to increase the efficiency of gene editing in plants by combining CRISPR-Cas9 with a GM virus system.[187] Theoretically, it is also possible to insert a gene drive system into a GM virus that can spread through a population. This would circumvent barriers to the transmission of the gene drive caused by low reproductive rates or long generation times.

Eradicating pathogens and exotics

In theory a gene drive system can be used to eradicate or virtually extinguish populations of pathogens or invasive exotics. Researchers have announced that they have developed a CRISPR-Cas gene drive system for the A. gambiae mosquito that makes the females sterile.[188] In theory, this should reduce the population of these mosquitoes to such a low level that they would no longer be able to transmit malaria to humans. Invasive exotics can cause extensive ecological and economic damage, examples being the musk rat in the Netherlands, the cane toad in Australia and the black and brown rat on Pacific islands. Such exotic species are now controlled by releasing natural enemies or by hunting them. In the past researchers have proposed developing gene drive systems to control invasive species, but the techniques to do this were not yet available and the idea remained a theoretical exercise. CRISPR may have changed all this. However, using a gene drive system to control an invasive exotic species involves huge risks, because the gene drive could spread to other areas. The rabbits in Australia are considered to be a pest, but the decline in the rabbit population in Spain is a source of concern.

The US Academy of Science, Engineering, and Medicine has taken the lead in the debate about gene drives by setting up a working group[189] and has organised several meetings with the aim of finding a balance between the benefits and risks of this technique.

Legislation

The legislation in Europe and the Netherlands does not take account of new technological developments, such as CRISPR-Cas9.[132,190] These scientific developments undermine the application and consistency of the EU legislation on GMOs.

Directive 2001/18 states that products and organisms fall within the scope of the GMO legislation if the genetic material of an organism is altered in a way that does not occur nat-

urally by mating and/or natural recombination (this is called process-based legislation).[191] The Directive also describes a number of techniques for genetic modification. Several other techniques and their products, including mutagenesis using chemicals and radiation, are exempted because they were already in use some time before the GMO legislation came into force. Whether or not an organism is covered by the GMO legislation has major consequences (*see text box 'Legislation determines the success or otherwise of biotechnological innovations'*). If it falls within the scope of the GMO legislation, an extensive safety file has to be compiled before it can be placed on the market. The costs of doing this are considerable, running into many millions for GM crops, and can only be afforded by large companies.[192,193]

Legislation determines the success or otherwise of biotechnological innovations
Whether or not an application falls within the legal definition of genetic modification has major consequences. If a commercial application is deemed to be a genetic modification, a risk assessment and safety study must be made, both of which require considerable expenditure of time and money. Compiling a file for the authorisation of a GMO crop in the EU is estimated to take about 5.5 years and cost 32 million euros.[193] These costs do not have to be incurred for crops that do not fall under the GMO legislation. To get permission to import a herbicide-tolerant GM crop an approval procedure must be gone through and the products have to be labelled, whereas a herbicide-tolerant crop obtained via 'conventional' mutagenesis can be placed on the market without any additional approval procedure. This makes the GMO legislation a driver for technological developments in the agro sector in particular. In addition, everything is done to circumvent the GMO legislation, often by taking advantage of new technological possibilities. It is cheaper to use conventional techniques to make random mutations in the genome of bacteria and then use new sequencing techniques to identify the desired mutated bacteria from the many hundreds of candidates rather than to directly make the desired mutation by targeting the relevant part of the genome.

The text of the EU Directive would seem to place some CRISPR applications within the scope of the GMO legislation. However, if CRISPR is used only to make mutations or deletions in a genome, the end result is the same as the outcome of conventional mutagenesis or natural mutations. But conventional mutagenesis makes numerous random deletions and rearrangements in the genome, whereas CRISPR-Cas only makes the specific mutations that are wanted, which makes the technique safer.[194]

It has recently been demonstrated that it is possible to make changes in the genome of lettuce, rice and other plants without inserting the CRISPR genes and sequences into the plant genome, or even to make use of DNA at all.[195] It is not clear whether or not such applications of CRISPR fall under the GMO legislation. The Swedish Board of Agriculture recently announced that they had decided that certain field trials with CRISPR-Cas9 mutated *Arabidopsis* plants did not require a permit under the GMO legislation because the plants did not contain any foreign DNA.[196]

This problem is not peculiar to crops. Why, for instance, must a cell genetically modified with CRISPR that is put back into a human fall under the GMO legislation? Moreover, CRISPR-Cas can also be used as a drug to influence the expression of a gene by binding to the sequence, but not modifying it. This is comparable to medicines that have an effect on gene expression. Given that no changes are made to the genome, it is illogical to invoke the GMO legislation in such cases.

Intellectual property rights
The issue of intellectual property rights for CRISPR-Cas has not yet been resolved.[197,198] The first patent for a CRISPR-Cas9 application was awarded in 2014.[199] Various other institutes and scientists have also submitted patent applications for the technology or applications of the

technology, and there are probably many more patent applications that have not been published yet.[197] At the moment there is a risk of a 'patent war' between the University of Berkeley on one side and the Broad Institute and MIT on the other.[200] Both groups have set up competing companies. One of those companies has entered into an alliance with a multinational which includes the cross-licensing of each other's CRISPR-Cas patents.[201] It is still uncertain how the patent landscape will develop in future. For one thing, patent applications for similar technologies or working mechanisms made at an earlier date may also have an influence on the situation regarding intellectual property rights for CRISPR-Cas. The development and patenting, or not, of similar technologies and improvements to the CRISPR-Cas9 system, such as CRISPR-Cpf1, may also have a big influence on the patent landscape.
Uncertainty about intellectual property rights or control over the technology by one or more people or organisations may inhibit implementation of the technology and hold up the appearance of commercial applications for longer than expected. It may also generate public resistance to the idea that a potentially crucial technology could be the property of a small group of scientists, institutes or companies.

Innovation
The CRISPR-Cas technique has been embraced by workers in the field as a quick and simple genome editing method for prokaryotic (e.g. bacteria) and eukaryotic (plants, animals) organisms,[p] and for both medical and agricultural research. The technique also opens up considerable opportunities for industrial production ('white biotechnology') because of the possibility it offers for improving production organisms. CRISPR-Cas is also considered to be an essential technique for the upcoming field of synthetic biology.[202,203,204] CRISPR will therefore play an important role in innovation across the whole field of biotechnology, which in turn has considerable economic implications. If the use of CRISPR-Cas is viewed in a different light in Europe than outside Europe (in other words, if it falls under the GMO legislation), commercial and research activity could be lost from the EU. This would affect the whole biotechnology sector, including the medical sector.

Trade
In the United States and elsewhere crops in which targeted mutations have been made are not considered to be GMOs and are not subject to any GMO regulations.[205] An example is a mushroom variety made with CRISPR-Cas9 that does not turn brown.[206] If the EU decides that these are in fact GMOs and therefore have to be labelled as such, importing these products into the EU will become problematic. Given that in the US these crops are not classified as GMOs or registered as such, and neither the crops nor products made from them can be distinguished from conventional crops and their products, they might not be labelled as GMOs when they are imported into the EU. This would undermine consumer choice in the EU and strain the credibility of government policy. It remains to be seen how these different views about the status of these types of organisms and products will be dealt with in the negotiations on the Transatlantic Trade & Investment Partnership (TTIP).

3.2.4 Stakeholder implications

CRISPR-Cas applications have implications for a range of stakeholders.

- **Patients:** For patients suffering from genetic disorders, they open up new possibilities for treatment, but also raise questions about their own treatment and possibly that of their children and subsequent generations.

p Prokaryote organisms (such as bacteria) do not have a cell nucleus or definite organelles. Their DNA floats freely inside the cell. Eukaryote organisms (plants and animals, including humans) have cells with a clear nucleus, which contains the DNA. They also have several types of organelles which carry out various tasks, including energy supply (mitochondria) and the production of proteins (ribosomes, endoplasmic reticulum).

- **Risk assessors:** How should the opportunities provided by gene drives to control pests and diseases transmitted by insects be weighed against the risks to ecosystems? Should this be in the form of a benefit-risk appraisal, or should it fall under the principle of a negligible risk established in EU law?
- **Industry:** CRISPR provides huge opportunities for industry. However, as long as the EU and the Dutch government make no decision about the status of this technique and its applications, investing in the development of these possibilities will remain a risky business. If it is decided that CRISPR falls within the scope of the GMO legislation, an uneven playing field will be created with industry based outside the EU. Companies will then consider the option of relocating their research & development operations outside the EU. In such a situation, trading companies and importers and processers would have to take measures to protect themselves against liability claims and damage to their reputation, because detecting products considered not to be GMOs outside the EU is virtually impossible.
- **EU and the Dutch government:** The GMO legislation will have to be amended or a decision will have to be made on whether or not certain applications and products fall under the existing GMO legislation. Any such decision will have important consequences. Besides possible trade difficulties with the US and other countries, the credibility of the government will come into question. Many of the products cannot be distinguished from natural organisms or products, which means when they are imported they will not be recognisable as falling under the EU GMO legislation. In the TTIP negotiations, the government will also have to decide how to deal with the differences between the EU's position and that of the US and other countries on what a GMO is.
  Human germline modification is currently prohibited in the EU.[152] The new technical possibilities and the public debate that has arisen in response present the government with the question of whether or not germline modification should be permitted in certain cases, and if so, for which applications and under what conditions. Should a distinction be made between treating genetic disorders and human enhancement, and if so, where should the line be drawn?

3.2.5 Conclusions

CRISPR-Cas is a new technique for genome editing that is being adopted in the field of biotechnology at an unprecedented rate. As a consequence, genome editing is becoming a standard technique for use in every research laboratory within the life sciences. The technology is still new and in the future as yet unforeseen applications will be developed. The CRISPR technology has huge potential for controlling diseases, industrial production (biobased economy), speeding up selective breeding, and for better understanding the genetic basis of mechanisms in plants, animals and humans.

Three fundamental issues need to be addressed in relation to this trend:

- The formal legal basis of the EU GMO legislation has been overtaken by technological developments. Amendment or revision of the legislation is urgently needed. In turn, this posits the key question of what genetic modification is and when and why organisms or products fall under the EU GMO legislation.
- Ethical questions about the acceptability of certain applications of this technique, such as germline genetic modification, the use of GM monkeys as disease models, and intervening in ecosystems by eradicating (for all intents and purposes) an organism.
- Who owns the intellectual property rights to this technique, and should a potentially crucial technology be in the hands of a small group of scientists, institutes or companies?

3.3 PERSONALISED MEDICINE: PREVENTION, DIAGNOSTICS AND PERSONALISED TREATMENT

The term 'personalised medicine' did not appear in the Trend Analysis 2007 of biotechnology, but it was hinted at in the anticipated growth in possibilities for genetic diagnostics and the wider significance of ethnicity for techniques such as genetic diagnostics and population studies. Two years later, personalised medicine was included as a trend in itself. The reason for including it in the present Trend Analysis is that the rapid progress with sequencing our DNA has opened up whole new possibilities for personalised medicine, generating a heated debate in the process.

3.3.1 What is personalised medicine?

Personalised medicine is a new concept. Hippocrates said that it is more important to know what sort of person has a disease than to know what sort of disease a person has; medicine is by definition about healing individual patients. Over time, doctors have acquired a growing diagnostic toolbox to allow them to treat their patients, such as blood analyses and imaging techniques. However, new techniques that can quickly provide a picture of the patient's complete genetic make-up and their diseased tissue (such as tumours) have taken this to a new level and given the concept of personalised medicine a whole new dimension. In the Trend Analysis 2009 personalised medicine was described as *the trend in medical practice in which treatments are developed for smaller groups of patients and individual molecular biological characteristics are sought that can provide indications of the likely success of a treatment*.[207] Another frequently used term is 'precision medicine'. In the Precision Medicine Initiative launched in January 2015 by President Obama this is described as follows: *Precision medicine is an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person*.[208,q] In essence, it means providing highly specific treatments based on the genetic and other characteristics, lifestyle and social context of the individual patient (*see Figure 6*). In 2014 the term 'individualised medicine' was proposed for this.[209]

3.3.2 Applications

In recent years personalised, or precision medicine, has been given a considerable boost by the rapid developments in sequencing and its widespread application in mapping DNA, gene expression and regulation (including epigenetics[210]), metabolism and intestinal flora. It is expected that the new knowledge about disease and health this will provide will enable the development of new preventive interventions, treatments and medicines. An example of personalised medicine is cancer treatment based on the genetic make-up of the tumour tissue (*see §3.1 Next generation sequencing*). To support research into personalised medicine, NWO honoured seven applications for innovative scientific equipment under its NWO Investment Grant Large programme starting in 2016. Three of these are important for personalised medicine. Five UMCs are working with a number of industrial partners to develop a META Scan: an imaging instrument based on MRI which can accurately follow the effects of personalised medicine treatments by measuring changes in metabolism. Other researchers are aiming to use an innovative database to obtain a greater understanding of the quality of life of cancer survivors. And lastly, the Netherlands Twins Register has been awarded a grant to continue and expand its research into the interaction between the genome and the environment. Theranostics, the combination of diagnostics and therapy in a single concept or product, which was also mentioned in the previous trend report, has in recent years moved in the direction of nanomedicine, particularly in oncology. Drugs are attached to nanoparticles that

q Personalised medicine is an emerging approach to the treatment and prevention of diseases that takes account of individual variations in genes, environmental factors and personal lifestyle.

can be made visible with imaging equipment (e.g. gold particles) to verify whether or not the drug is delivered accurately to its intended destination.[212] It is also expected that pharmacogenetics will deliver new insights that will enable more accurate delivery of existing medicines and other therapies and preventive treatments. On 1 January 2016 the U-PGx research project was launched (Ubiquitous Pharmacogenomcs: Making actionable pharmacogenomic data and effective treatment optimisation accessible to every European citizen). Financed under the EU Horizon 2020 programme and coordinated by Leiden University Medical Centre (LUMC), this project aims to set up and roll out the infrastructure needed to map 80 genes in patients that play a role in the conversion of drugs, and then make the information available to doctors and pharmacists, along with a set of guidelines.[213]

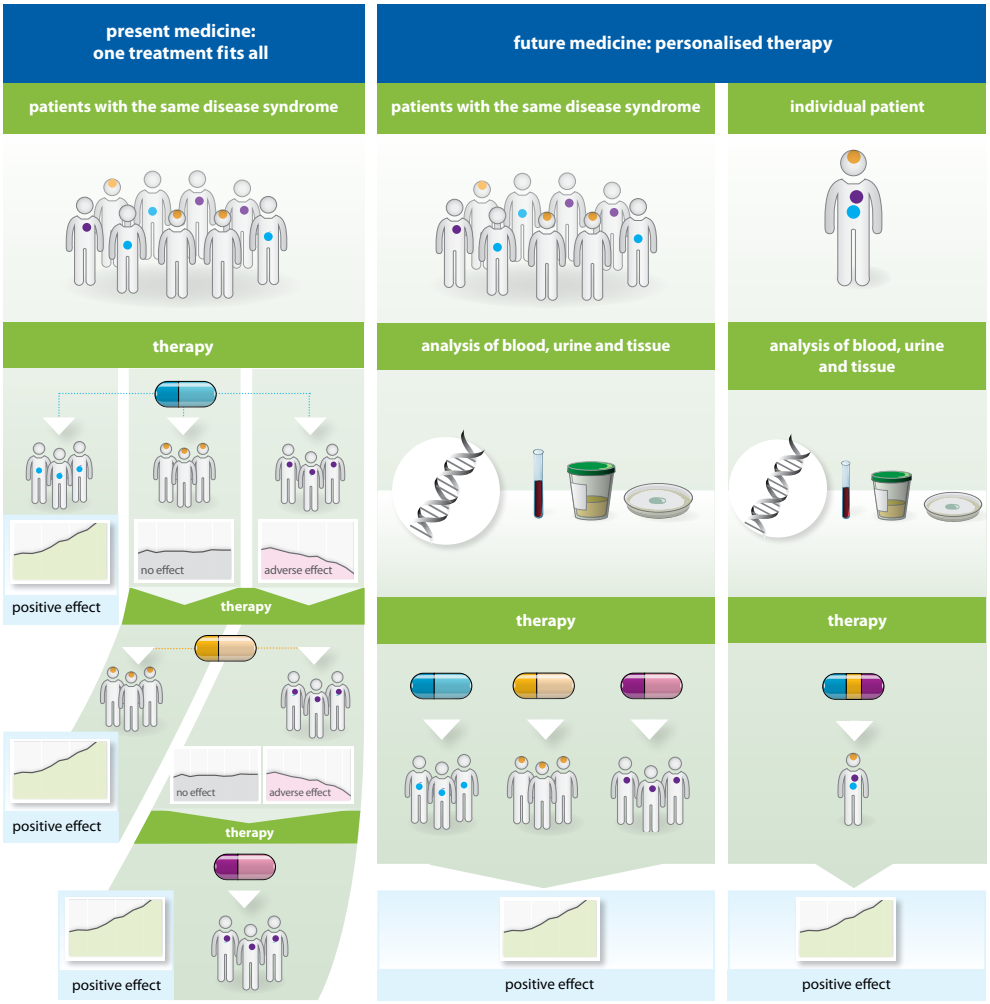

Figure 6: The various forms of personalised medicine

3.3.3 Issues and challenges

The big advantage of personalised medicine is that treatments are specific to each patient so that unwanted side-effects can be prevented, the right dose can be identified more quickly, and drugs that are not effective in treating the patient's condition can be avoided. To do this use is made of information from sequencing and pharmacogenetics. The results are satisfying to the patient and this general approach can reduce costs.

However, in the stricter application of this principle – the development and use of essentially individual medicines, as is increasingly being used in oncology[214,215] – leads to more expen-

sive medicines. Because the medicines are used to treat a limited number of patients only, pharmaceutical companies charge high prices to recover the investment costs of developing them.[216,217] It is not easy to judge the extent to which these high prices are justified, but they have already forced hospitals to make choices as they have fixed medicine budgets which are insufficient to cover the costs of all expensive medicines.[218] Another case that clearly demonstrates that financing and access to drugs can lead to problems for small groups of patients is that of 'orphan medicinal products' for rare conditions. In 2012 the National Health Care Institute (Zorginstituut Nederland, ZiN) advised the minister to stop financing the drugs used to treat Fabry disease and Pompe disease from the basic (statutory) health insurance package because there was insufficient evidence that these drugs are effective for the patient groups as a whole. This was met with a wave of protest, because patients who do benefit from the drugs would be affected as well. A different arrangement has now been made.[219] The concern is that developing new drugs for smaller groups of more similar patients will eventually lead to all conditions being split up into subgroups of rare disorders, with the problem described above as the inevitable result. Concerns about the affordability of more specific drugs are not limited to the United States and Europe, but are universal.

Orphan medicinal products

Drugs developed to treat rare diseases ('orphan medicinal products' for diseases that affect less than 1 in 2,000 people) are subject to special rules, because it is more difficult to recoup the investments made. The drug companies producing these medicines have exclusive rights for a period of ten years and the duration of the patent is also extended. The authorisation procedure is also shortened and simplified in recognition of the difficulty or impossibility of testing the efficacy of the drug on a large number of patients in a short time. Numerically, the scheme for orphan medicinal products has been a success, because the number of drugs approved in the EU has risen sharply and more than 1,300 applications for orphan medicinal product status have been approved by the EMA. How many of those will reach the market remains to be seen.[220] The downside is that prices for orphan medicinal products are often extremely high. Moreover, there is a growing tendency for drug companies to try to obtain orphan medicinal product status for the drugs they develop. They can obtain several orphan medicinal product approvals for the same drug by applying for approval for their use on different groups or subgroups of patients. But if all these patients are added up the number would exceed the limit for orphan medicinal products.[216,217] To a certain extent this practice is made possible by advances in scientific knowledge that permit better understanding of the differences between similar or related diseases and disorders.

Another frequently mentioned expected benefit of personalised medicine is the prospect it holds for preventing illness in the first place. The idea is that increasing scientific knowledge of genetic predisposition to disorders with a complex aetiology (also called multifactorial disorders) will allow preventive measures to be taken in people with a high risk. These measures may include adjustments to lifestyle and medicines. However, it must be noted that the connections found between genetic make-up and environmental and lifestyle factors are based on epidemiological research, which involves considerable statistical analysis. One should be cautious about being too optimistic about the reliability of such connections and therefore the possibilities for prevention. Also, the effectiveness of any such interventions still has to be demonstrated. A recent meta-analysis has shown that informing healthy people of their genetic risk factors does not lead to changes in behaviour and lifestyle.[221] It is therefore important to be wary of too much optimism about the possibilities for preventing disorders.

3.3.4 Stakeholder implications

The development of the concept of personalised medicine and its applications has implications for various stakeholders and should therefore be considered within the national political and social context.

- **Patients:** Personalised medicine has considerable advantages for the individual patient: optimal diagnostics and treatment with a minimum of side-effects and the best possible outcome, taking account of the patient's specific genetic, physical and mental make-up and social context. The concept can be seen as the ultimate goal of medicine. On the other hand, personalised medicine may threaten the privacy of the individual if it involves sequencing in all stages of life, continuous monitoring of vital functions and blood levels with nanosensors, and mobile apps that continually register the individual's activity pattern. Furthermore, the potential costs of all this raises the question of the affordability of care for everyone. In this time of collective decision-making, patient empowerment is essential in the further development of personalised medicine. The further growth of personalised medicine will necessarily involve the further specialisation and centralisation of healthcare. Even now, expertise on rare conditions is increasingly concentrated in one place in the country, or even within Europe, for example in European Reference Networks.[222,223,224] The position and input of patients in these structures will become increasingly important.
- **Consumers:** The advantages for consumers are less clear. On the one hand, there are the benefits described above when the consumer becomes a patient. This will deliver certain cost savings for healthcare, which could have advantages for the consumer in the form of lower health insurance premiums. On the other hand, personal medicines may become so expensive that premiums will have to rise considerably or the principle of universal access to pharmaceutical care is put at risk. Expectations regarding prevention are also high. As described in Trend 3.1 about NGS, companies are responding to this by offering healthy consumers extensive genetic tests, claiming that they can identify the risks of a whole range of disorders. Besides the risk of unnecessary worry, there is also the issue of what the possibilities for prevention really are. In principle, preventing disease means maintaining quality of life, but this raises questions about what prevention should involve (adapting lifestyles, moving to a better environment, use of medicines, etc.?), how preventive 'care' should be financed (from the individual's own pocket or the health service?), the consequences for solidarity regarding health insurance premiums and how compulsory preventive measures should be (e.g. stopping smoking, high blood pressure medication) and the privacy implications of preventive medicine, as discussed above.
- **Medical professionals:** For medical professionals, personalised medicine in the sense of the integrated use of sequencing and biomonitoring presents a challenge. To successfully turn research results into practical applications they will quickly have to understand what these data mean and how they can be used. Clinical studies will have to be designed differently, because patient groups will become increasingly diverse. Much more than at present, doctors will have to apply their general medical knowledge to the specific characteristics and situation of the individual patient. Pharmacists may have an increasing part to play in advising which medication to prescribe and in making and administering of medicines, which in turn will have consequences for their training and degree of specialisation. In the more distant future of personalised medicine, prevention as described above will take on an increasingly prominent role. However, most medical professionals (GPs and specialists) are not yet oriented towards primary prevention. Those that are (community health doctors, paediatricians) are primarily concerned with public health of large groups of people and are not yet properly conversant with the relevance of genomic information to prevention. The traditional division between public and individual healthcare may have to be reviewed.
- **Scientific researchers:** Scientists are acquiring knowledge about the significance of genomic and phenotypic variation and various mechanisms of gene regulation, such as epigenetics and the genetic make-up of tumour tissue. Researchers are also working to improve the

accuracy of various techniques. Clinical trials will have to be set up differently: the groups of patients for which the effectiveness of new medicines are tested will become smaller, with consequences for the design of preclinical trials and the interpretation of results and statistics. Some scientists feel that the potentially radical implications for individual consumers and patients call for a debate about whether everything that can be done should be done.

- **Insurers:** Personalised medicine may deliver major healthcare savings because prevention, diagnosis and treatments will become more specific. On the other hand, the use of individualised medicines may push up costs. The financing and reimbursement of the costs of orphan medicinal products for rare disorders is already a problem, because they are so expensive. An increase in the number of medicines for small groups of patients may make it impossible to sustain the current system for pricing and reimbursement.
- **Pharmaceutical companies:** The pharmaceutical industry has embraced genomics and is developing new therapies based on the latest knowledge and products, often acquired from university research groups and spin-off companies. Gene therapy and other targeted interventions are gaining ground, but make heavy demands on trial design before they can be authorised for placing on the market. Another big problem is that these therapies are currently very expensive. Drug companies will have to enter into discussions with the government about new ways to finance the healthcare system. The current system of reimbursement based on large groups of patients who can use the same medicine will not be workable in the context of personalised medicines. The whole way in which new medicines are developed (led by the pharmaceutical industry) may even have to be overhauled completely. Tentative consideration is being given to the additional possibilities of extemporaneous (magistral) preparation of precision medicines by UMCs and the development and production of medicines by publicly financed not-for-profit institutes. Thought is also being given to new ways of assessing drugs for market registration and reimbursement in which the price is included in the assessment.[225,226,227,228]
- **Government:** The Dutch authorities are giving much attention to the phenomenon of personalised medicine. The National Institute for Public Health and the Environment (RIVM) recently published a report titled 'Personalised medicine products, evaluation of the regulatory framework', which points to the lack of consistency in the legislation, particularly between the treatment of medicines and in vitro diagnostics, which fall under the regulations governing medical devices.[229] Further, in 2015/2016 RIVM will be reviewing the application of personalised medicine in the Netherlands and is carrying out research into the possible uses of personalised medicine/pharmacogenetics in primary health care.[230] Under the 'Good Use of Medicines' [Goed Gebruik van Geneesmiddelen] programme, The Netherlands Organisation for Health Research and Development (ZonMw) has established a sub-programme on personalised medicine which looks at research into applications of NGS techniques for diagnosis versus traditional diagnosis of rare disorders and cancer.[231] This programme includes policy research. In addition, ZonMw is working on two reports on advanced-therapy medicinal products (ATMPs), identifying constraints and how to resolve these, and on the results of the gene therapy efficiency study. The purpose of these government activities is to collect and share knowledge with the aim of taking full advantage of the benefits of personalised medicine, to implement findings and remove constraints.

3.3.5 Conclusions

The rapid development of sequencing and biomonitoring have opened up new possibilities for personalised medicine. There are three main issues and dilemmas in this field:

- How can the best use be made of the possibilities of personalised medicine without adversely affecting people's individual choices regarding lifestyle and their privacy (*see also §3.1 Next generation sequencing*)? Can medical professionals remain free to select a treatment based on their expertise, or will they be expected to always offer the same treatment to patients with a specific profile?

- How can fads be quashed and hopes fulfilled? In other words, what is a realistic and ethical approach to the possibilities and constraints of personalised medicine?
- How can the healthcare system remain affordable as the number of personalised medicines increases?

Personalised medicine will put pressure on the current healthcare system in the Netherlands and elsewhere. In this context, the health minister has given priority to the theme of 'innovative medicines delivered quickly to the patient at an acceptable cost' for the Dutch presidency of the EU in the first half of 2016.

3.4 GENE THERAPY: PROMISE FULFILLED

Gene therapy was also discussed in the previous Trend Analysis, when it was concluded that it was making a comeback. This trend has continued over the past few years. The media regularly contain stories of spectacular successes in patients with cancer or other diseases. But besides these promising developments, there are also a number of issues that need to be addressed: Dutch legislation can sometimes unnecessarily restrict clinical research involving humans; the use of viruses involves risks; 'medical tourism' will increase in future; and the costs of gene therapies are high.

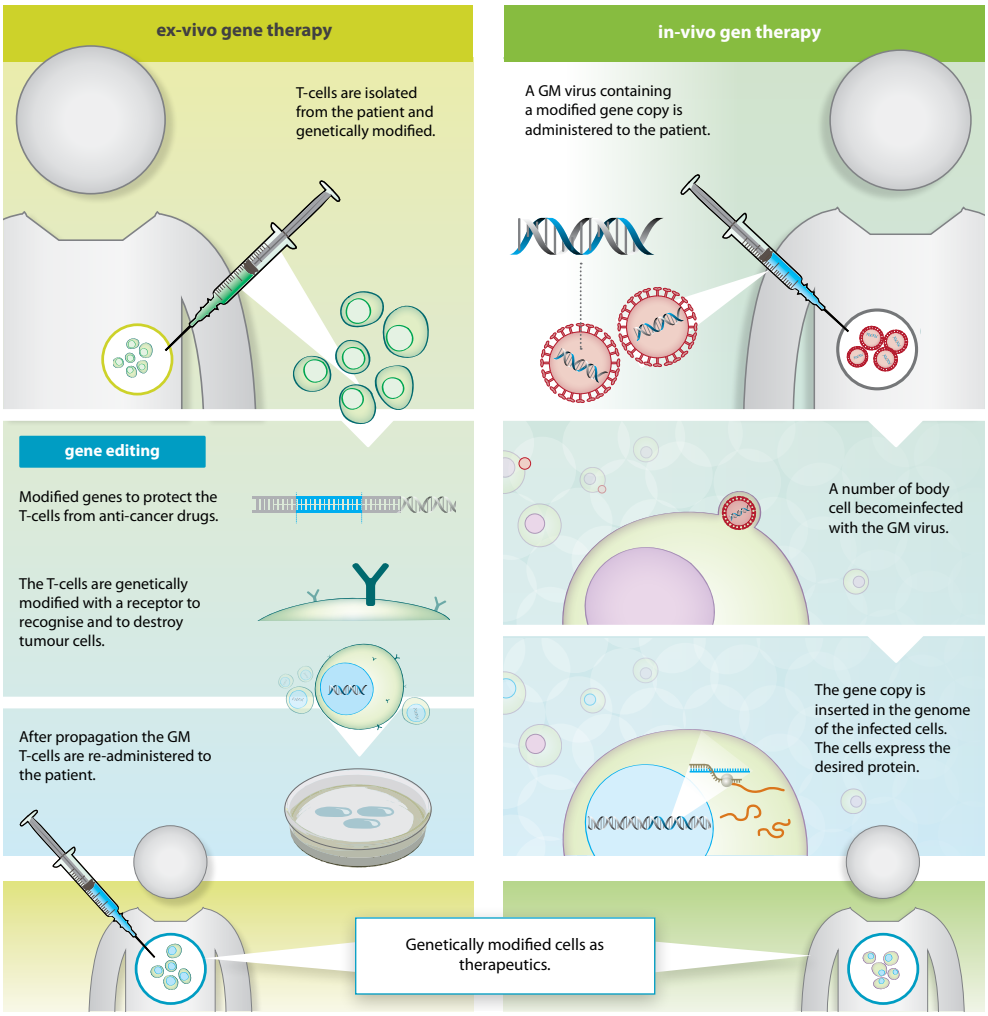

Figure 7: In vivo and ex vivo gene therapy

3.4.1 What is gene therapy?

Gene therapy involves introducing genetic material (DNA or RNA) into the cells of an individual and bringing it to expression to treat a disease. The introduced material may be a 'healthy' copy of a defective gene to repair a genetic disorder or a 'foreign' gene (from another species) to bring about the desired therapeutic effects. The genetic material or transgene is transported to the right place in a cell by a 'vector'. This is often a disabled virus, but other systems are also used, such as minuscule lipid droplets and nanoparticles. DNA can also be tattooed into the skin. Another method is to isolate cells from the body and return them to the body after genetic modification (*see Figure 7*).

When considering the genetic modification of human cells, a distinction must be made between the modification of normal body cells (somatic gene therapy) and the modification of gametes (germline modification). Germline genome modification in humans is not permitted in the EU and is discussed in §3.2 CRISPR-Cas.

Since the first successful experiment in humans in the early 1990s, gene therapy has been surrounded by high expectations and deep disappointments in equal measure. At the end of the 1990s interest in gene therapy fell considerably. This was accompanied by several setbacks, including the death of a patient and cases of leukaemia in children who had been treated for the immune disorder X-SCID, which sparked much debate about the safety of gene therapy. But in recent years there has been a strong revival of interest and gene therapies are now being developed for a whole spectrum of disorders and diseases, many of them forms of cancer. The number of clinical studies is increasing and promising results have been obtained. Companies are again investing in gene therapies and some have already received market approval. Gendicine® was registered in China in 2003 and the oncolytic virus Oncorine® for treating head and neck cancer was registered in China in 2005.[232] Glybera®, produced by the Dutch company UniQure for treating the rare hereditary disease lipoprotein lipase deficiency (LPLD), was approved in Europe in 2012 and T-Vec, an oncolytic virus for cancer therapy, was approved in the US in 2015.[233,234,235] Mydicar®, a gene therapy for heart failure, has been given 'breakthrough therapy' status by the FDA in the US.[236]

Haemophilia B

Haemophilia B patients suffer from spontaneous bleeding caused by a lack of the clotting factor IX. Treatment consists of injections of factor IX every two to three days. Giving patients a one-time injection of a GM virus containing the gene for factor IX allowed the number of injections of purified factor IX to be reduced considerably over a long period of time (16 to 48 months).[239] Some patients did not even need any more injections of purified factor IX at all. Treatment with purified factor IX is costly and for severe cases can amount to as much as 135,000 per year.[237]

3.4.2 Applications

Promising results have been achieved in various clinical experiments, including for the treatment of the immune disorder X-SCID, B-cell leukaemia and haemophilia (*see text box 'Haemophilia B'*).[238,239,240] Encouraging results have also been obtained for the treatment of cancer patients by genetically modifying their own immune cells so that they recognise tumour cells and attack them.[241,240,242] T-cells are isolated from the body, genetically modified by inserting a receptor gene and then reintroduced into the patient. Spectacular results have been reported from small-scale clinical trials with T-cell therapy for leukaemia and lymph node cancer (*see text box 'Layla'*). Among patients with untreatable acute lymphatic leukaemia (ALL) and lymphoma, remission rates of 94% and more than 50% respectively have been reported.[243,244] These results were presented at a conference and have not yet been confirmed.

Further research is required. However, these forms of treatment are not without risk; of the 35 ALL patients, 20 displayed serious side-effects and two died.

Layla

One of the most striking success stories of gene therapy treatment is the treatment of a one-year-old baby called Layla in an advanced stage of acute lymphatic leukaemia who had exhausted all other available treatments. This form of leukaemia leads to an overproduction and accumulation of immature immune cells (B and T lymphocytes) in the bone marrow. The production of normal blood cells is disrupted, which causes anaemia and an increased chance of bleeding and infection. In very young children the normal treatment of chemotherapy followed by a bone marrow transplant is often unsuccessful. The highly experimental treatment, which had never before been carried out on a human, consisted of introducing genetically modified T-cells. These GM cells have a receptor (CAR19) which enables them to recognise cancer cells and destroy them. Because the patient herself was no longer producing T-cells, foreign donor T-cells were used that had been genetically modified so that they would not be recognised as foreign to her body and destroyed. Moreover, a further modification was made to ensure they would not be recognised and destroyed by a medicine (antibodies) given to leukaemia patients. Modifying the T-cells was made possible by the availability of genome modification techniques. Within a few months of giving the treatment, no cancer cells could be found in the baby's body. It will only be possible to determine whether or not she has been definitely cured after a few years. The ability of the treatment to be successful in other patients will also have to be demonstrated.

3.4.3 Issues and challenges

Expectations too high

It is undeniable that major successes have been achieved and many developments give cause for optimism. Successful experiments regularly receive extensive media attention. The impression is sometimes created that it is just a question of time before a treatment for a serious disease will become available. However, initially hopeful results can later turn out to be disappointing, as in the case of a gene therapy for an eye disease. In the first instance, good results were reported for the treatment of Leber congenital amaurosis, a hereditary disease that impairs the functioning of the light-sensitive cells in the eye. Sight was initially restored following an injection with a GM virus, but after five to six years the effect had worn off and the breakdown of the photoreceptors in the eye was the same as in patients who had not received the treatment.[245] Gene therapy does appear to lead to long-term improvement of the hereditary eye disease choroideremia.[246]

Risk analysis

From an analysis of the preclinical studies from around the world, it seems probable that more gene therapies will come onto the European market[247] and gene therapy will increasingly become a standard part of medical practice. Medicine will continue to develop through the implementation of the latest techniques, such as the use of exosomes (cell-derived vesicles that can be used as vectors), genome editing and vector barcoding, which is a new method for improving patient safety.[248] This 'barcode' is a small piece of DNA with a 'random' nucleotide sequence, which is inserted into the genome of each GM virus particle. When the target cells in the patient are infected with this virus the barcode is transmitted to the cells, making each genetically modified cell in the patient unique. Any deviant cell behaviour, such as uncontrolled growth (tumour), can then be detected more easily and much earlier so that treatment can begin. The impact of new techniques on the environmental risk assessment system therefore needs to be looked into.[249]

The use of GM viruses that multiply in tumour cells and thus kill those cells is a promising development within gene therapy. Viruses are modified so that they can infect cancer cells and can only multiply inside cancer cells. As this involves the use of viruses that can multiply, and which in theory can be dispersed outside the patient, this technique must be carefully investigated in the risk assessment.

Licensing
A GMO consent must be obtained for gene therapy experiments and for their use as a medicine. The legal definition of gene therapy in the Netherlands not only includes treatments in which DNA is inserted into the cells of a patient, but also clinical experiments with GM viruses, GM vaccines and cells (T-cells, stem cells, etc.) that have been genetically modified outside the body and then introduced into the patient.

The Dutch GMO authorisation procedure for clinical gene therapy studies seems to take longer than in many other countries, and companies say that this is a reason to move clinical studies to other European countries. One of the reasons why the Dutch licensing procedure takes longer is that in the Netherlands all gene therapies are classified as 'deliberate release into the environment' and the procedure for this category includes six weeks for stakeholder consultation. In some other EU member states gene therapy or certain types of experiments are classified as 'contained use', for which the authorisation procedures are shorter. Also, some countries do not carry out a stakeholder consultation. In view of this, one can question what the six week consultation period contributes to ensuring the safety and protection of the interests of third parties.

The examples given above indicate that some types of gene therapy may indeed involve environmental risks and that a comprehensive risk assessment is therefore needed, for example for GM viruses. However, there are also other systems, such as GM immune cells, which appear to involve little or no risk to anyone other than the patient because there is no risk of these cells being dispersed outside the patient. A flexible authorisation procedure that can be adapted to the different types of gene therapy would therefore be more suitable. The environment ministry has already taken a step in this direction by introducing a simplified authorisation procedure (under certain strict conditions) for gene therapies in which DNA is injected and contains no parts of viruses. It is not clear why there is no simplified procedure for more types of gene therapy studies. Thought should also be given to making some types of gene therapy subject to the quicker authorisation procedure for contained use.

Animal viruses in cancer research
Preclinical and clinical studies into the use of viruses for treating cancer use not only GM viruses but also wild-type viruses.[247] Besides human viruses, these include a few animal viruses that can cause economic damage if they infect farm animals. Any studies involving viruses that cause notifiable animal diseases must meet specific regulatory requirements. However, viruses being investigated for potential use in cancer therapies include lesser known and non-notifiable animal viruses, such as Maraba virus and the Seneca Valley virus (SVV),[250] which can be transmitted unintentionally to livestock.[251] Assessments of the clinical application of wild-type viruses such as SVV appear to focus on ethical and medical issues and not on the possible animal welfare and economic impacts.

Costs of gene therapies
Some gene therapy treatments are marketed at very high prices, which raises the question of how these prices are arrived at. The development costs of a treatment can be high, but the production costs are relatively low. Gene therapy treatments developed for rare or chronic diseases are priced in line with the often very high costs of the available conventional treatments – but can this always be justified? The high cost of new medicines is not specific to gene therapies and has been a problem for some time throughout the world.[252,253,216]

Treatment abroad

Occasionally Dutch patients travel abroad to receive gene therapy treatments,[254] either experimental treatments as part of or outside clinical or other studies, or gene therapies registered in the other country. These treatments include the use of GM viruses and wild-type viruses. It is expected that the number of patients going for treatment abroad will increase, partly because within the EU there will be increasing specialisation in the treatments offered, with just one or a few hospitals offering treatments for relatively rare disorders. Patients undergoing gene therapy treatments abroad are not registered, which means that public health risks may arise if patients are treated with GMOs that can be transmitted, such as viruses. Moreover, for treatments abroad it is often not clear whether there is any system of checking for possible shedding and dispersal of the viruses used outside the patient, and if so, how. And should there be incidents involving returning patients, it will be difficult to take appropriate measures if there is no information on the treatment and the precise nature of the gene therapy.

3.4.4 Stakeholder implications

The developments and issues outlined above have several consequences for the various stakeholders in the field:

- **Patients and consumers:** Patients hear from the media about possible new treatments and want these to become available as soon as possible. However, before new gene therapies can be authorised or tested, they have to be assessed for their safety for patients and third parties, effectiveness, etc. This evaluation of the possible benefits to the patient and the possible risks to society may result in treatments not being made available to patients, or becoming available too late.
- **Medical professionals:** The use of some gene therapies, such as replicating viruses, involve not only a risk to the patient (as in all treatments) but also a risk of environmental or third party exposure. For this reason the authorisation procedure includes an environmental risk assessment. For off-label use – use of a medicine outside the terms of the licence – a new risk assessment has to be made and a new licence applied for, which may conflict with medical interests. A point to consider is raising awareness among doctors and treatment providers of the possible risks of off-label use and the GMO legislation.[255,256]
- **Pharmaceutical companies:** Companies face the same issues as for personalised medicine. They will be reminded by government and others that they have a moral obligation not to market gene therapies at excessively high or unrealistic prices. If they do, the government will eventually be forced to intervene. They will have to talk to government and other parties about setting realistic prices and establishing new ways of financing healthcare.
- **Government:** The challenge facing the government is to amend the legislation to ensure public safety, while removing the unnecessary restrictions on some types of clinical gene therapy research. More gene therapies will soon be coming onto the market and the cost of these therapies will drive up the cost of the healthcare system. A dialogue with the pharmaceutical and gene therapy industry about realistic prices will be needed.

  An increasing trend will be for people to have treatments in other countries. If returning patients spread viruses or other GMOs, this could lead to possible environmental or public health problems. Such problems could be prevented by establishing a uniform international system for registering patients undergoing gene therapy treatments abroad, but this would run up against privacy and medical confidentiality requirements. Solving these issues will require discussions with health insurers, patient organisations, treatment providers and institutions in other countries.

3.4.5 Conclusions

- Gene therapy is on the rise. The number of clinical studies is increasing, promising results have been obtained for various disorders, companies are investing in gene therapies and the first products have already come onto the market.
- New methods and technologies, including the use of replicating viruses to destroy tumour cells, must be subject to a thorough risk assessment, whereas other types of gene therapy involve no risk of the environment or the population being exposed to a GMO.
- To make full use of the potential of gene therapy to control diseases and support innovation in the Netherlands, the authorisation procedures should be reviewed and, where necessary and possible, amended.
- In the future the number of gene therapies coming onto the market will increase and the costs of these treatments will be high. A clear system for reimbursing healthcare costs is urgently needed.

3.5 NEW TECHNIQUES: SILENCING GENES WITH RNA

The subject of new biotechnological techniques in agriculture also came up in the last Trend Analysis. Partly in response to a COGEM topic report published in 2006, the question of whether or not these techniques fall under the GMO legislation has been debated within the EU for many years, but the European Commission has not yet come to a decision. Some EU member states have already taken unilateral decisions on specific applications. Meanwhile, new techniques and applications are emerging and being adopted outside Europe, but their legal status in Europe remains unclear. This trend has been included yet again because of the emergence of new techniques, the absence of a decision in the EU, and the social and politically controversial nature of the subject. [r]

3.5.1 What are the new biotechnological techniques?

To most people, agricultural biotechnology is almost synonymous with GM crops. However, agricultural biotechnology is much broader and has become an essential part of conventional plant breeding. For example, marker-assisted breeding is now standard practice in the development of new plant varieties. The ability to map and compare the genomes of plant species and varieties on a large scale and use this information to identify genes associated with specific characteristics gives a further impetus to the increasing importance of biotechnology in the agricultural sector.

In addition, new biotechnological techniques have been developed that occupy the grey area between genetic modification and conventional techniques, and others are on the way.[132] Previous trend analyses and various reports have explored in some detail these new techniques and their consequences for the legislation.[190,194,207] Politicians and policymakers have also given considerable attention to these techniques over the years.[257] Various scientific advisory bodies have argued for a revision of the legislation or for exempting them from the regulations.[258,259] An EU working group has examined this issue, a legal analysis is being carried out and the European Food Safety Authority (EFSA) has been asked to evaluate the environmental risks of several of these techniques.[260] Despite these efforts, after nine years still no decision has been made. In the meantime, the United Kingdom and Germany have taken matters into their own hands and decided that field trials with crops produced with the use of site-directed mutagenesis are not subject to the GMO legislation, and Sweden has decided

r This chapter covers only agricultural applications. However, techniques are also being developed in other sectors that fall within the grey area of what may or may not be genetic modification. For example, RNA vaccines are being developed in which mRNA is injected into the skin and the skin cells of the person receiving the vaccine then make the antigen. Whether or not this falls under the GMO legislation is not yet clear.

that certain crops in which mutations have been made using CRISPR are not subject to the GMO legislation. Differences between the EU member states in how they assess these techniques and their products may have serious consequences for the internal market.

New biotechnological techniques under discussion

Technique	Aim of the technique	Remarks
Reverse breeding	Creating parental lines from hybrid (non-GM) lines.	Use is made of genetic modification, but the plants produced are not genetically modified.
Agro-infiltration	Insertion of transgene sequences without genetically modifying the plant.	The plant is not genetically modified. The technique is used during the breeding process to test plants for resistance, etc.
Grafting onto GM rootstock	Strengthening the rootstock through genetic modification.	The fruit from the scion are not genetically modified.
Site-directed mutagenesis with oligonucleotides	Making site-specific mutations in the genome. Use is made of short pieces of DNA which are almost the same as the sequence in the genome.	Classical mutagenesis using chemical mutagens or radiation is exempt from the GMO legislation.
Zinc finger technology	Technology for making site-specific mutations in the genome.	Both medical and agricultural applications.
RNA-dependent DNA methylation (RdDM) or RNAi technology	Technology for switching genes on and off.	The DNA sequence is not altered. Both medical and agricultural applications.
TALEN	Technology for making site-specific mutations in the genome.	Both medical and agricultural applications.
CRISPR-Cas	See §3.2	
Cisgenesis and intragenesis	Genetic modification in which genes from a crossable relative are introduced.	The trait can also be introduced via classical breeding. The advantage lies in the much faster breeding process.

Technological and scientific progress are continuing unabated and new techniques and applications continue to be developed. One of these developments is the use of RNA interference (RNAi) technology. In every cell genes are continually being read and DNA transcribed to mRNA (messenger RNA), which acts as a blueprint for making proteins. RNAi is a natural process in the cell to regulate gene expression or destroy viruses.[261] The RNAi system works by breaking down the mRNA transcribed from a gene so that no protein can be manufactured. The destruction of the mRNA is induced by a double-stranded RNA molecule with the same sequence as the RNA molecule to be broken down (*see Figure 8*). RNAi effectively blocks the gene. Such gene silencing can be heritable.
If the sequence of a gene or the genome of an organisms is not altered, the use of double-stranded RNA as an RNAi technique does not fall within the definition of genetic modification. RNAi has blossomed into an important research method for studying the functioning of genes, but also has practical applications, including acting as an insecticide and controlling disease in humans, such as cancer and infections.[262,263]

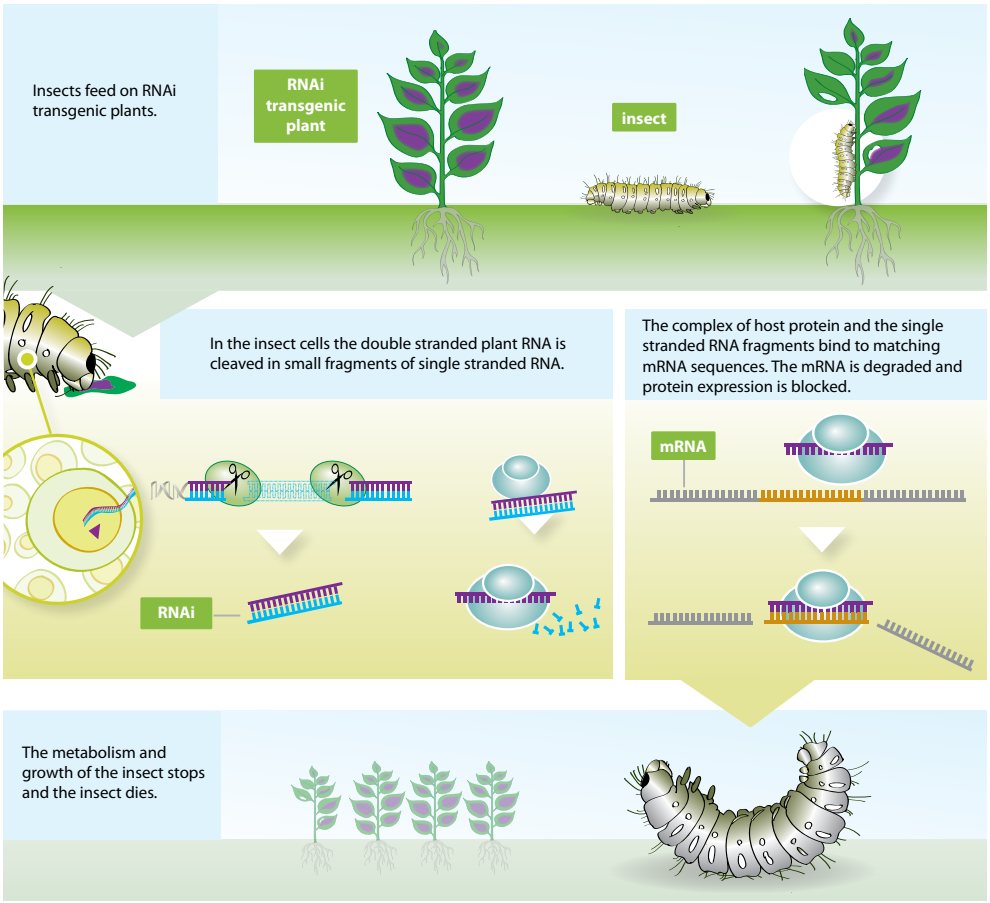

Figure 8: Insect resistance in GM plants using RNAi

3.5.2 Applications

Genetically modified RNAi crops

GM crops[s] make use of RNAi technology to adapt the plant's own metabolism by switching off or regulating genes or to induce resistance to pests.[264,265,266,267] Changing a plant's metabolism is a technique that has been in use for some time. Examples include the amylose-free potato and the GM Arctic® apple. The amylose-free potato contains a copy of a potato gene inserted into the DNA back to front. This causes both the inserted gene and the original gene to be blocked, making it impossible for the cell to make the enzyme (granule bound starch synthase) that synthesises amylose. The GM Arctic® apple recently approved for sale in the US and Canada[268,269,270] does not turn brown after it has been cut and exposed to the air. This trait has been achieved by silencing the enzyme polyphenol oxidase (PPO) using a similar technique to that used in the potato.

Whereas sequences from the plant itself are used to adapt its metabolism, pest control traits are created using sequences from the pest organism. The first insect-resistant GM crop based on RNAi technology is already on the market and an application has been submitted for authorisation to import and process the crop in the EU. The crop is a GM maize made resistant to the western corn rootworm by inserting part of a gene (Sfn7) from an insect into its DNA.

s RNAi applications are not only being developed for agricultural purposes, but also in the medical sector, for example. Clinical studies are being conducted with experimental 'RNAi medicines' to treat various conditions, including liver diseases, viral infections and cancer.

This gene synthesises a double-stranded RNA molecule. When the insect eats the plant it ingests this RNA molecule, which eventually blocks the formation of the essential Sfn7 enzyme in the insect's cells, leading to its death. Work is also progressing to develop other resistances, such as resistance to viruses.

RNAi and crop cultivation

The cultivation of herbicide-tolerant GM crops in the US and elsewhere has led to a major problem of herbicide resistance in weeds.[271,272] The appearance of such weeds has been encouraged by an almost complete lack of resistance management. In many areas over the years farmers have applied increasingly higher doses of the same herbicide (glyphosate – RoundUp) to control weeds and in doing so have unintentionally created an ideal situation for the emergence of resistance to the chemical. In response to this resistance to glyphosate, GM crops are now being developed with resistance to other, often more toxic herbicides, such as atrazine, 2,4-D or dicamba. Another approach is to make use of RNAi technology to make the weeds susceptible to the herbicide again, by adding dsRNA to counteract the 'resistance gene' to the glyphosate mixture.[273] The developer of this technology claims that the technical obstruction preventing plant cells taking up RNA has been overcome and that the effectiveness of their approach has been demonstrated in field trials.[274] The advantages to the industry of this RNAi technology are, first, that the existing glyphosate-tolerant GM crops can still be grown, and second, that the developer now has a unique herbicide formula. Since the patent period ended in 2000, glyphosate has been produced by numerous companies, but this new formula is the only one that works against glyphosate-resistant weeds.

RNAi technology can also be used to control pests and diseases in conventional crops. For example, RNA sprays have been developed to control Colorado beetle in potato and bacterial diseases and pests of citrus fruits, and to protect bees against viruses and the Varroa mite.[275] Sprays are also being developed that can influence the traits of plants growing in the field when the growing conditions demand it.

3.5.3 Issues and challenges

Applications and products are also coming onto the European market

Most new RNAi applications are developed in the US. Herbicide-tolerant GM crops are not grown in Europe. However, the appearance of resistance in weeds is not exclusively associated with the cultivation of GM crops; resistance to plant protection products and herbicides is an almost inevitable consequence of the use of these compounds, because natural selection favours mutations and insensitive organisms.[276] Herbicide-resistant weeds are also found in Europe and the Netherlands. Continual use of a single compound encourages the development of resistance. As the number of herbicides available for use in Europe declines, resistance among weeds may become an increasing problem. A herbicide formula that can break this resistance would be of significant value for Europe as a whole. RNAi sprays to control insects and other pests are definitely an interesting proposition in European agriculture. It can therefore be expected that if these applications prove to be effective they will eventually appear on the European market.

Legislation

Genetically modified crops containing an RNAi construct fall within the scope of the GMO legislation. However, the other RNAi applications are in the form of RNA sprays and do not involve modifying the genome of any organisms. As such, therefore, these application would not seem to fall under the GMO legislation,[191] but under the regulations for plant protection products.

Risk analysis

The application of RNAi technology raises new questions regarding the risk assessment required by the GMO legislation.[277,278] It can be assumed that the risks to human health and the

environment posed by GM crops in which genes are switched off by the insertion of the plant's own genes will generally be low. This is because no traits or genes are added to the plant's genome. However, the situation is different when insect resistance is added to 'RNAi GM crops' and the risk assessment also looks at possible effects on organisms other than the pest insect. For plants containing an inserted gene that codes for a protein, the first step is to feed the pure protein to various insects and organisms under laboratory conditions. However, for RNAi this is difficult if not impossible to do. Besides questions about the specificity of the RNAi and how to test it, there are questions about the persistence of the RNAi in the environment.[279]

New technological developments require new knowledge

New technological developments, such as the use of RNAi technology, raise questions about the safety of the products and the methods that can be used to assess their safety. Much of the research into the safety of these types of new applications is carried out or sponsored by the companies that develop them. Companies need this information to be able to put their products on the market. For scientists, research into the safety of applications is often not very interesting and is also difficult to carry out, partly because the products or materials to be tested are not always available to them. This situation hampers the independent acquisition of a robust knowledge base in the public domain (*see text box 'Public knowledge in the Netherlands'*).

Public knowledge in the Netherlands

Public knowledge provision in the Netherlands is under pressure. Spending on public sector R&D in the Netherlands has declined in recent years,[280] while private sector investments are increasing. The EU is an increasingly important source of public sector research funding, which appears to mark a shift from national funding and the setting of national research agendas towards a European research and innovation agenda. In addition, public research funding is often conditional on a certain degree of 'knowledge valorisation' (extracting value from or commercialising the results) in partnership with private parties and so the knowledge and materials gained are often owned by private enterprises. As a result, many scientists become associated with companies, compromising independent research to support public policy in areas such as safety, public order, healthcare, housing, social security, education and science.

3.5.4 Stakeholder implications

The developments and issues outlined above lead to several consequences for the various stakeholders in the field:

- **Consumers:** In the future, some food and other products may possibly contain traces of additional material (such as RNA) that some people associate with genetic modification. Although the presence of such material presents no risk to humans and the environment, it could become a public issue. People who reject genetic modification may see this as an infringement of their freedom of choice, especially because there is no legal requirement to label these products as containing genetically modified material.
- **Scientists and risk assessors:** Insufficient resources and access to products and knowledge hampers independent research, for example into the safety and effectiveness of new technologies, which may lead to the developers of these technologies having an information monopoly.
- **Industry:** As long as the government does not make a decision on the legal status of these technologies, it will not be attractive for companies to invest in them. This inhibits innovation by companies and worsens their competitive position in relation to companies outside the EU.
- **Government:** New technologies are being developed that have no clear status with respect to the GMO legislation. The government urgently needs to make a decision on the status

of these technologies. There are also technologies that some people intuitively feel involve genetic modification, but which do not fall within the scope of the GMO legislation. The government will have to respond to these concerns.
The developers of the RNAi technology almost have a monopoly on knowledge about the technology. When assessing these technologies, therefore, the government runs the risk of not being able to draw on sufficient independent scientific expertise and information. It is important that the government maintains its own pool of scientific expertise and data from sources other than just the private sector. Only then can the government be an equal negotiating party and play a constructive role in developing safety requirements, test methods and criteria, etc. This will depend on the stimulation of independent research into these applications.

3.5.5 Conclusions

- Biotechnology is becoming ever more pervasive in agriculture. In the past, biotechnological applications were limited mainly to plant breeding, but in future biotechnology will also play a part in the cultivation of crops.
- The challenge facing government is to capitalise on the opportunities presented by these new technologies, while at the same time guaranteeing their safety. Over-regulation can pose a threat to innovation, but regulation is also necessary to guarantee safety and can also encourage innovation by companies by removing uncertainties. The government will have to enter into a dialogue with companies and other stakeholders to establish criteria and safety standards for new technologies. To support this process it is important that the government maintains its own pool of scientific expertise and data from sources other than just the private sector. Only then can the government be an equal negotiating party and play a constructive role in developing safety requirements, test methods and criteria, etc. This will depend on the stimulation of independent research into these applications.

3.6 GENETICALLY MODIFIED INSECTS: INTERVENING IN ECOSYSTEMS

The genetic modification of animals is an important tool in scientific research. Other applications have largely disappeared in recent years, with the exception of the modification of insects. Research is underway to establish whether or not GM insects can be used to suppress infectious diseases and agricultural pests. Field trials have already been held in various countries around the world. These developments present opportunities to improve public health and reduce the use of insecticides, but are meeting resistance from objectors. Given the degree of public discussion this trend can generate, it has been decided to include it in this year's Trend Analysis.

3.6.1 What are GM insects?

In recent years a number of infectious diseases have spread rapidly to other countries and regions. They are referred to as 'emerging diseases' and many of them are transmitted by insects. Well-known examples are dengue fever, chikungunya and Zika (*see text box 'Dengue fever: an emerging disease'*).

Dengue fever: an emerging disease
Dengue fever, a viral infection spread by mosquitoes, is illustrative of emerging diseases. The disease was originally only found in Africa and South-East Asia. The global spread of the disease began during the Second World War and accelerated in the 1980s. From a lo-

cal disease restricted to limited geographical areas, dengue fever has spread to every continent except Antarctica and Europe. Each year 50 to 100 million people become infected and 22,000 die from the disease, mostly children. The rise of dengue fever is associated with the spread of the mosquitoes Aedes aegypti and A. albopictus. At the end of 2015, after twenty years of research, it was announced that the first dengue vaccine will become available on the market in Mexico.[281]

Besides vaccines (if available), the use of insecticides is an important way of controlling these types of diseases. The disadvantages of chemical control, though, are the development of resistance to the compounds used, possible environmental and health risks, and the need for repeated applications because breeding places are difficult to access. Moreover, chemical compounds are not always available and affordable in the poorer regions of the world.[282]

Genetically modified insects are an alternative to the use of insecticides. Research focuses on developing GM insects that are incapable of transmitting the pathogen (parasite or virus) and on ways to greatly reduce the insect population and so reduce the likelihood of infection. The advantages include a much lower risk of the development of resistance, no use of substances harmful to humans or the environment, and species-specific control.

Biotechnology has breathed new life into the old technology of reducing population size by releasing sterile males55 (*see text box 'Sterile Insect Technique' (SIT)*). In the classical sterile males technique, insects are irradiated with ionising radiation to make them sterile and then large numbers of these sterile male insects are released. The population size is reduced because females that mate with sterile males will not produce any offspring. The disadvantages of this technique are that the radiation makes the male insects less fit than the males in the natural population, which in turn means that the treatment needs to be repeated to eradicate or control a population, and that it is sometimes difficult to separate the cultured male and female insects.

Sterile Insect technique (SIT)
The technique was successfully used for the first time in the 1950s to exterminate the screw-worm fly (*Cochliomyia hominivorax*) from parts of North America. In the 1950s the larvae of this fly caused extensive damage (US$ 200 million per year) to the American meat and dairy industry. In 1954 the first successful trials with SIT were carried out on Curaçao, followed by the US, Mexico and other regions in Central America. Between 1944 and 1994 more than 30 SIT releases of organisms were made worldwide to combat pests and diseases. The technique has also been used in Mexico and Japan to control fruit flies and in Africa to control the tsetse fly. The technique is still used in various countries, including the Netherlands, where it is used to control the onion fly. Besides these success stories, mistakes have also been made with SIT. The mass irradiation of insects has not always been properly carried out, resulting in the release of large numbers of non-sterilised laboratory-reared insects into the environment.[284]

3.6.2 Applications

A British company has developed an alternative technique that uses genetic modification[285] to insert a gene that is lethal to the insect. In the laboratory the gene is suppressed by adding an antibiotic to the insects' food so that they can be bred. After the insects have been released into the wild they breed with wild individuals of the same species. The offspring die while they are still larvae because the gene is no longer suppressed by the antibiotic. The

released insects also die after some time. The advantage of using these GM insects over the traditional sterile males technique is that there is no loss of fitness through irradiation and that selecting males is simple to do. The company initially concentrated on controlling mosquito populations to reduce the incidence of dengue fever and malaria. Field experiments in which millions of GM mosquitoes were released have been carried out in various places, including in the Caiman Islands, Panama and Brazil.[286,287,288] A big experiment in Florida is also planned.[289,290] Interest has also been expressed for field experiments in the Caribbean Netherlands (St Eustatius and Saba).

It is generally not possible to completely eradicate an insect population using this technique. It has only been achieved using the sterile males technique on relatively small islands which cannot be reached by insects from elsewhere, and even then by releasing an excessive number of insects to flood the population. The bigger the area and the target insect population and the bigger the chance of an influx of new insects, the bigger the number of insects that have to be released. The company says that in its field trials the size of the mosquito populations were reduced by 90% to 99%.

Other researchers are investigating the use of gene drive systems (*see §3.2 CRISPR-Cas*). The advantage of this approach is that only a limited number of GM insects have to be released, because the inserted gene or introduced trait spreads rapidly throughout the population. A research team at Imperial College London has developed a gene drive system that disrupts the genes involved in the production of eggs in female mosquitoes.[188] These types of system are still in their infancy and have not yet been tested in field trial conditions. If a gene drive system works in the field, it can substantially reduce the population of disease-transmitting insects. It is unlikely that a gene drive system would be able to completely eradicate an insect population.

Besides reducing the insect population, work is also progressing on replacing wild-type populations with insects no longer capable of transmitting pathogens. Promising results have recently been obtained for malaria. Malaria is one of the biggest infectious disease problems in the world and, like dengue fever, it is transmitted by infected mosquitoes. The mosquito not only transmits the parasite, but is the second host after humans and therefore essential for the life cycle of the parasite. If the development of the parasite in the mosquito can be disrupted, the whole life cycle can be broken and the parasite will no longer be able to infect humans. Mosquitoes can be made more or less 'resistant' by creating GM mosquitoes with an antibody against the parasite.[291] Until recently, however, it has not been possible to disperse such a gene or trait through the wild population, but researchers have now succeeded in combining the antibody gene with a CRISPR-Cas gene drive system in GM mosquitoes. Virtually all (>99%) of the offspring of these mosquitoes had this 'resistance gene'.[178] The researchers say they will be able to carry out a field trial within a year, but will wait before doing so because they do not want to prejudice the public debate about gene drives.[292]

An alternative method: the Wolbachia bacterium

Another method of controlling infectious diseases is by infecting mosquitoes with the *Wolbachia* bacterium, which can be thought of as a natural gene drive system. In nature, *Wolbachia* species are found in numerous insect species. The bacterium lives in the insect cells and can infect many different tissues, but is concentrated in the female sex organs. Some *Wolbachia* species are symbiotic, others trigger asexual reproduction in their hosts or the production of only female progeny, and a third group are parasitic and adversely affect the lifespan of their host. *Wolbachia* bacteria do not naturally occur in the mosquito which transmits dengue fever, A. aegypi, but it is possible to introduce it into this species.[293] Australian researchers have found a Wolbachia strain that blocks the replication of the dengue virus in this mosquito.[294] This strain of bacteria has no influence on the lifespan of the mosquito and is able to spread through a mosquito population because only (infected) female offspring are

produced.[295] In field experiments in Australia the dispersal of limited numbers of infected mosquitoes led eventually to 80% of the mosquito population being infected.[296] Field experiments with this Wolbachia strain have been carried out in Vietnam, Indonesia and Brazil.[297] Other researchers are trying to combat the Asian tiger mosquito (*A. albopictus*) by making use of the fact that if two mosquitoes carrying different *Wolbachia* bacteria mate, the eggs cannot develop and so no offspring will be produced.[298] This is variant of the sterile males technique. Releasing male mosquitoes carrying a different *Wolbachia* strain than in the natural population will strongly inhibit population growth.

Agriculture and GM insects

The British company that developed the sterile GM mosquitoes wants to use the same technique to control agricultural insect pests.[299] In the US, three-year field trials have been carried out on controlling the pink bollworm larva on cotton.[300] According to the company, about 20 million GM moths were released during these field trials. Field trials have also been carried out with GM cabbage moths (a pest of all cruciferous crops) under semi-contained conditions in the US and open field trials will be held in 2016.[301] Greenhouse trials on controlling the Mediterranean fruit fly will be held in Australia and a field trial in Brazil. An application has been made for a semi-contained field trial in Spain on controlling the olive fruit fly using GM flies. Permission has not yet been given in connection with questions about containment of the insects and how to deal with the presence of living and dead GM insects on the olives.

3.6.3 Issues and challenges

Public debate: utility and risk

The use of GM insects provides opportunities to curb human and animal infectious diseases and agricultural insect pests and to reduce the use of insecticides. The UK House of Lords is even of the opinion that there is a moral imperative to conduct field experiments as soon as possible to ascertain whether or not GM insects can be used to eliminate or control serious diseases and agricultural pests.[302] The members of the House of Lords also feel that the EU legislation on GM insects is 'woefully inadequate' and presents unnecessary obstacles to carrying out field trials, and should be amended as soon as possible. However, there are objections to releasing GM insects in the wild. Objections to field trials on controlling infectious diseases include issues like safety and potential effects on ecosystems and biodiversity. The loss or decline of a population can in theory have consequences for the whole ecosystem. Trust, freedom of choice and social and economic considerations also play a part in the debate. The lack of a transparent public consultation on the first field trials with GM mosquitoes has also come under criticism. It can be argued that controlling pests is a government responsibility, but genetic modification has always been an exception in which individual freedom of choice can be used as an important bargaining chip, although there is little scope for this in the case of the introduction of GM mosquitoes. What is an appropriate response to groups of people (however small) who have ethical or religious objections to the introduction of the GM mosquitoes? To what extent can developing countries be put in a position of dependence on a Western company, and who can be held to account should the release of the GM mosquitoes lead to problems? Objections to agricultural applications by the organic farming sector are that the GM insects may spread to fields owned by organic farmers and may be present on their products.[304]

Applications and products are also coming onto the European market

GM insects are not likely to be used in the European part of the Netherlands for some time. However, should GM insects be used on crops grown outside the Netherlands, the presence of material from dead GM insects on imported products cannot be ruled out. Although this is unlikely to present any risk to humans or the environment, some people may still see this as an infringement of their freedom of choice, particularly as there is no obligation to mention this on the product label.

Field experiments in the Kingdom of the Netherlands
Caribbean islands where infectious diseases such as dengue fever are endemic are suitable places to hold field trials. As they are isolated areas there is little chance of the arrival of new mosquito populations and so the results of the tests will be reliable. It is likely that field experiments on the control of mosquitoes will be held in the Caribbean Netherlands (the islands of Bonaire, Sint Eustatius and Saba). Dutch GMO legislation does not apply to these territories and the question is what regulatory framework does apply in this case. Other issues to be resolved include which authority is competent to issue a consent for the field experiments, whether or not this authority has sufficient expertise to carry out a risk assessment, what arrangements are in place to ensure the necessary scientific advice is obtained during the licensing process, and what enforcement arrangements are in place.

3.6.4 Stakeholder implications

The use of GM insects affects various stakeholders:

- **Public and consumers:** Consumers may be confronted with imported food products containing material from dead GM insects. Tourists may also come into contact with GM insects. During the football World Cup in Brazil in 2014 there were plans for a field trial with GM insects to protect visitors from contracting dengue fever.[305] With the Olympic Games coming up in the summer of 2016 and the outbreak of the Zika virus, trials were held with sterile males, Wolbachia and GM insects.[306,307,308,309]
- **Businesses:** The development and particularly the use of GM insects can be expected to meet with public resistance and businesses should be aware of the objections people have. This requires good communication and consultation with all stakeholders.
- **Risk assessment bodies:** Risk assessors must ensure they have sufficient expertise in house to assess new applications of GM insects.
- **Government:** The regulations must contain adequate provisions for the assessment of field experiments and similar tests involving GM insects, and apply not only to the territory of the Netherlands, but also to the other countries and special municipalities in the Kingdom of the Netherlands.

3.6.5 Conclusions

- At the moment, many experiments are being conducted with GM insects to control infectious diseases like Zika, dengue fever and malaria. It is expected that experiments will also be carried out on the Dutch Caribbean islands of Sint Eustatius and Saba. The situation regarding the regulations and permits for such experiments in the Caribbean Netherlands are not clear. The Dutch Genetically Modified Organisms Decree (GMO Decree) does not apply there and the Law on Public Housing, Urban Planning and Environmental Management BES (the law that applies on the three islands of the Caribbean Netherlands) does not contain any provisions relating to genetic modification.
- In the past, biotechnological applications in agriculture were limited mainly to plant breeding, but in future biotechnology will also play a part in the cultivation of crops, for example for controlling pests with GM insects.

3.7 3D BIOPRINTING: BODY PARTS TO ORDER

3D printing is all over the news. The applications of 3D printing are diverse, both in the consumer sector and in professional markets such as architecture, marketing and the medical sector. 3D printing technology is improving at a rapid rate and can be used to print objects in all sorts of materials, including paper, plastic, metal and food, and more recently organic material such as living cells and tissue – 3D bioprinting. Developments are in the 'peak of in-

flated expectations' stage in the Gartner Hype Cycle[t] and speculation is rife about the almost unlimited possibilities of printing tissues and organs. At the moment, the biggest obstacles to implanting tissues and organs printed from cells derived from the patient are technical in nature. However, the technology is advancing at a rapid rate and when the feasibility of such applications is within reach, other issues will have to be dealt with, such as safety and the rights to the ownership of 3D printed tissues, the differences in the international legislation on the use of 3D printed products, and liability for adverse effects.

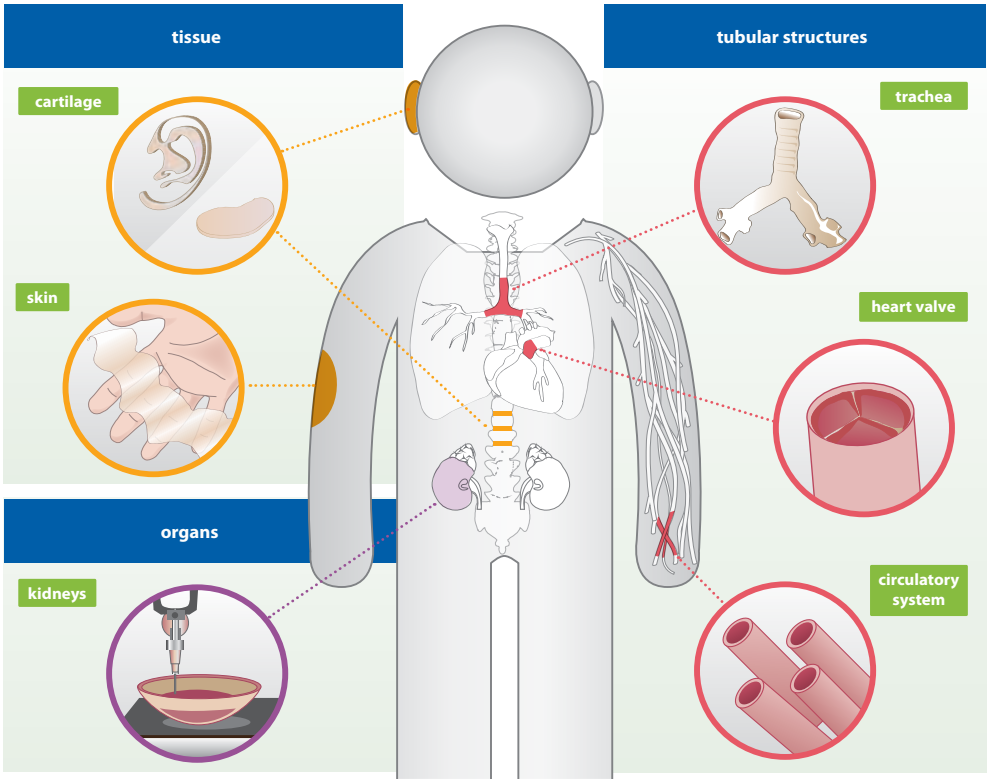

Figure 9: 3D bioprinting: Current research focuses on printing uniform structures such as skin and cartilage and more complex structures such as blood vessels. Eventually the aim is to be able to print fully functioning organs consisting of different types of tissue.

3.7.1 What is 3D bioprinting?

3D bioprinting is a multidisciplinary field that draws on engineering, biology, chemistry and mathematics to generate one or more types of living structures or biomedical implants using computer-controlled 3D printing technology.[310,311,312] The first patents for this technique were awarded in the US in 2006.[313] 3D printing was first used in the medical sector mainly to produce personalised prostheses, implants and scale models, but in recent years the trend has been towards biocompatible implants that contain living cells. Companies aim eventually to produce 3D printed tissues and organs consisting entirely of living cells. Besides printing relatively simple structures such as skin and cartilage, researchers are also aiming to print more complex forms such as heart valves and even whole organs (*see Figure 9*).

To print body tissue, living cells are first isolated from a donor or patient. The cells are then replicated and may even be altered, for example by generating specific types of cells from stem cells. Before living cells can be 'printed' they must be mixed with a liquid material that

t http://www.gartner.com/technology/research/methodologies/hype-cycle.jsp

provides them with oxygen and nutrients. The mixture, called 'bio-ink', can then be put into a printer cartridge and used to print the desired three-dimensional form (obtained, for example, from medical scans of the patient). At the moment, the cells are usually printed onto a biocompatible mould or model. These models are also printed using 3D printing technology.

3.7.2 Applications of 3D bioprinting

The direct applications of 3D printing for patients are currently limited mainly to the production of individual models,[314] prostheses[315] and implants made of foreign materials, such as plastics and titanium. Using 3D technology these can be made to exactly the right dimensions, based on patient scans. The number of medical implants produced this way is increasing rapidly. In 2012 a patient was given a 3D-printed lower jaw.[316] Similar implants have been printed for skulls (*see text box '3D printed skull'*), neck and vertebra, pelvis, hip, sternum and ribs, and successfully placed in the body.[317,318,319,320,321,322,323]

3D printed skull

In 2013 a patient was given a 3D printed plastic implant to replace 75% of his cranium.[324] Specialists analysed the form of his head and printed the 3D implant layer by layer. The cranium parts were made from a biomedical polymer with very similar properties to bone. Because the 3D printer can recreate very precise surface details, the implant fitted the patient's head perfectly and cell growth over the surface was encouraged. This technique was also successfully carried out in the Netherlands, in 2014, when the cranium of a 22-year-old patient was almost entirely replaced during a 23-hour long operation.[325]

The step from 3D printing to bioprinting involves a transitional stage in which biocompatible foreign (non-somatic) material is printed as a platform for living cells. These can be used as a permanent or temporary replacement or implant/prosthesis. 3D printing is also an important tool in tissue engineering for printing a scaffold of biomaterial to be coated with living cells.[326] Examples of these biomaterials are alginate and synthetic hydrogels.[327,328] Alginate is a natural polysaccharide based on algae. Hydrogels are soft, biocompatible materials consisting of water molecules encapsulated in a polymer network that determines the structure and form of the printed living tissue. The first 3D printed products in this category are already available on the market. An example is a product used as a temporary replacement for the cerebral membrane (dura mater) after brain operations. The material dissolves after a time, giving the body time to repair its own dura mater.[329] This product was allowed onto the European market in 2011.[330] Various other products are under development, such as a replacement for cartilage consisting of biocompatible hydrogels and plastic substrates containing living cells for the restoration of ears and noses.[331,332,333] In 2013 a patient received a 3D printed tracheal splint.[334] More complex structures, such as heart valves, are also in the pipeline.[327] At the beginning of 2016 researchers in the US reported that they had printed life-size bone, muscle tissue and ears from living cells in combination with biocompatible polymers and hydrogels. The structures were implanted in mice where they became functional tissue, including a system of blood vessels.[335] During the annual meeting of the Endocrine Society in the US researchers presented their first attempts at a 3D-printed ovary consisting of biocompatible material in which hormone producing cells and immature egg cells were placed.[336]

3D printed structures consisting entirely of living material are not yet permitted for use in humans, but are used in research and preclinical tests, for example for making disease models and testing medicines. An American company aims to produce commercially available tissues, such as liver, kidneys and bone, for preclinical tests and research into bodily functions.[337] Testing drugs on human tissues is mentioned as a valuable step towards bridging the gulf between preclinical testing and clinical trials because findings from successful animal tests often prove unreproducible in clinical trials due to the differences between animals

and humans.[338,339] The company's first commercial product is a 3D printed liver tissue (*see text box 'Printed liver for pharmaceutical testing'*).

Printed liver for pharmaceutical tests

The exVive3D™ Liver is a tissue model for the liver created using liver cells (hepatocytes) from three different human donors. It is a human model suitable for determining toxic or other effects (acute and chronic) on liver function and metabolite formation.[340] The liver tissue is functional and stable for at least 40 days. The product supplied by the company is a service contract, under which pharmacists can have a potential medicine tested on the liver model to see what effects it has. The company is working on the development of a similar model for kidney tissue.[341]

Besides the pharmaceutical industry, 3D printed tissues also has potential uses for cosmetics companies. 3D printed skin tissue is the next step towards a realistic skin model to test products on.[342] European cosmetics manufacturers in particular are interested because animal testing of cosmetics has been prohibited in Europe since 2004.[343]

Besides a flush of bioprinting start-ups, much research is being done into the potential of bioprinting. Scientists are focusing on the production of 3D-printed skin tissues to be used as grafts for burn sufferers.[342|4,345] Researchers in the UK are working on printing soft tissues and bone tissue for reconstructive surgery.[346] In the US, a biotechnology company is working with a university on the development of a specialised 3D printer for bones.[347] Also in the US, researchers are combining bioprinting with nanotechnology to print tissues that can facilitate the regeneration of nerve cells.[348] In 2015, American researchers succeeded in restoring the motor function of a rat using a nerve printed to order in which nerve cells could grow.[349] In autumn 2015, Scottish researchers were the first to print with highly delicate stem cells,[350] which remained intact after the printing process and were able to differentiate into specific cell types. To print functional organs it is essential that blood vessels are formed. Researchers at a German company are combining different printing techniques to print branched and porous blood vessels in high resolution.[351,352] The required branching is calculated from mathematical simulations to obtain a uniform flow of blood through the tissue. At the end of 2015, researchers in the US succeeded in printing tissue in which small blood vessels (capillaries) were formed from introduced blood vessels.[353] At the beginning of 2016, researchers presented another method for printing multiple layers of tissue that are sufficiently permeated with blood to remain functional for up to six weeks.[354]

The first reports of fully printed organs have also made the news. At the end of 2015, Russian scientists claimed to be the first to have printed a thyroid gland and implanted it into a mouse.[355,256,357] Bioprinting can also be used to create 'organoids' (*see text box 'Miniature organs from the printer'*). Organoids are miniature organs used for testing medicines or organ function.

Miniature organs from the printer

Organoids are structures made of specialised cells that represent organ functions such as the heart, lungs, liver, kidneys, etc. Organoids are round and hollow inside and are made up of all the types of cells found in the complete organ.[358] These miniature organs can be attached to each other with a circulating blood substitute to test for the effects of chemical and biological substances. This application also offers opportunities for testing potential treatments for patients. The first organoids for intestinal function were developed by a team at the Hubrecht laboratory in Utrecht.[359] They are now working on other organoids that represent the brain, heart and liver.[360] Initially, the 3D structure of these organoids was created by growing specified cells around a platform, but now 3D printing has been used to make organoids that can reproduce the functions of heart and liver cells.[361]

3D bioprinting is being used primarily to develop human medical applications. However, other applications are also being explored, such as printing meat and leather.[362] In 2013 a team of Dutch researchers at Maastricht reported the first 3D-printed hamburger.[363] Manufacturing in vitro or cultured meat for the consumer market faces a number of challenges, both technical (taste and structure) and financial. The Dutch researchers started a company in early 2016 and expect to be able to market affordable cultured meat within five years.[364]

3D bioprinting in the Netherlands

Much work is being done on 3D bioprinting in the Netherlands. In 2014 a special bioprinting facility was established in Utrecht which also offers a specialised biomedical engineering training course called BIOFAB (Biofabrication Training for Future Manufacturing).[365,366,367] Most developments in 3D bioprinting described so far are for in vitro printing of full or partial biological structures, but the first proof-of-concept studies for in vivo bioprinting have already been reported.[368,369] In vivo bioprinting is when cells or tissues are printed directly onto the body. At the end of 2015, researchers at LUMC and Erasmus MC won the Open Mind award for their plans to develop a 3D printer to treat foetuses with spina bifida[u] while they are still in the womb.[370] Children are now operated on immediately after birth to close the back, but by then the nerves are already damaged. In the proposed new method, a thin needle linked to a 3D printer is inserted into the amniotic sac and a cap is printed over the defect in the baby's back to protect it until after the birth.

3.7.3 Issues and challenges

Technical challenges

The technical challenges facing 3D printing include the high temperatures during printing and the use of organic and other solvents, which damage the viability, function and stability of living cells. A further challenge is the complexity and diversity of organs and tissues. Almost all organs and tissues consist of several types of cells and must be provided with oxygen and nutrients via blood vessels. Various printing techniques are used, such as photolithography, magnetic bioprinting, stereolithography and direct cell extrusion, each with its own advantages and disadvantages. A number of start-ups, established companies and research groups are developing 3D printers specifically for printing with living cells and biocompatible materials ('bio-ink').[371,372,335] These materials must be suitable for printing while retaining the function and stability of the living cells, and must not be rejected by the recipient's body.

Legislation

The use of 3D printing raises questions about legislation, ownership and liability. For example, are products containing combinations of endogenous and exogenous materials medicines or implants? The legislation and regulations covering these things differ considerably. Printed tissues are based on cells from a donor, whereas in future clinical applications will probably be based on material derived from the patient. It may be possible to patent printed human tissue, as long as it is not structurally identical to the original tissue from which it was derived.[373] However, patenting bioprinting processes and printers would seem to be a more obvious option than patenting the tissue itself. An additional question is if and to what degree the legislation contains provisions for assessing the safety and effectiveness of new products. 'Print to patient' applications are still some way off, but printed tissues are already being used to test drugs, hazardous materials and cosmetics. How adequate are these tests and what part can they play in the risk assessment of assessment agencies? Companies in the US are working with the Food and Drug Administration (FDA) to identify any legislative inadequacies in good time and where possible iron out the wrinkles.[374] As for some other biotech-

u Spina bifida, or 'split spine', is a birth defect due to a developmental disorder of the spinal cord and spinal column. The disorder occurs in 4.5 per 10,000 births.

nological applications, international differences in legislation can lead to trade difficulties and an uneven playing field. Besides the adequacy of current regulations for assessing new products, there are questions about liability for any adverse effects that might arise after these tissues are used in patients.[375,375] If the printed tissues and organs are used in people, it must first be established whether they will remain stable and functional and not present any health risks (e.g. cancer caused by uncontrolled growth of the printed cells).

Ethics

3D printing of living cells raises ethical questions about the direction new legislation should take.[377] These are not restricted to technical issues, such as standardisation, quality assurance and intellectual property rights, but also include questions about social justice and human enhancement.[378] And, as for many expensive and possibly life-saving technologies, the questions of who is going to pay for the technology and who will have access to it are crucial. The possibilities for human enhancement also raise ethical questions. If bioprinting makes it easy to replace organs, will this change how we view the human body? Will this technology be able to make improved organs or tissues that contain non-human material?[379] Will the boundary between medicine and human-improving technologies be a fundamental consideration? Many of these questions are actually not new and are also relevant to tissue engineering.

3.7.4 Stakeholder implications

3D printing and bioprinting are expected to have a big influence on the medical sector and industrial production. Several issues of potential concern to stakeholders are mentioned below:

- **Patients:** Patients requiring prostheses and implants will have more treatment options, but they will also face uncertainties regarding the effectiveness and risks associated with this new technology.
- **Industry:** Pharmaceutical and cosmetics companies will have to transition from the use of laboratory animals to 3D-printed tissues and miniature organs. The adequacy of these tissues as an alternative to testing on animals will need to be investigated. Companies developing 3D printing products and printers will have to investigate (with governments) which national and international legislation is applicable and how the safety and effectiveness of the new products can be assessed.
- **Risk assessors:** Are the current regulations and assessment agencies up to the task of assessing test results for medicines or other substances on 3D-printed tissues and the use of these tissues in living organisms? The need to integrate knowledge from various disciplines will require a broadening of expertise and collaboration with industry and researchers.
- **Government:** The government must establish whether the current legislation is adequate for regulating the safety of bioprinting products, and if so, which regulations are relevant.

3.7.5 Conclusions

- 3D bioprinting is used in patients in the form of temporary and permanent prostheses and implants. Research is being conducted into the use of combinations of biocompatible materials and living cells, with the ultimate aim of printing complete tissues, structures and organs using the body's own cells.
- The safety assessment of living tissue and combinations of living tissue and compatible materials should be regulated as soon as possible, because it will probably be used within the foreseeable future. The same goes for liability and insurance cover.
- Other issues that need to be addressed are the ownership of 3D printed tissues that contain material from the patient or donor, inconsistencies within international law, and medical tourism.
- The technology also raises ethical and social issues around access to and the desirability of this technology and its impact on how we view the integrity of the human body.

3.8 SYNTHETIC BIOLOGY: FROM CLOTHES TO VACCINES

Synthetic biology was first mentioned as a new scientific field in the Trend Analysis 2007. In the years that followed it became a hype and many researchers and companies adopted the term to describe their activities. COGEM, the Rathenau Institute, the Royal Netherlands Academy of Arts and Sciences (KNAW), the Advisory Council on Health Research (RGO) and the Health Council of the Netherlands, among others, published reports on the development and consequences of synthetic biology for the market, society and the legislation.[380,381,382,383] In view of this, synthetic biology was not included in the Trend Analysis 2009 as a separate trend. Almost ten years after the emergence of synthetic biology, the hype seems to have died down, but developments continue apace. Although the field is mainly limited to fundamental research, the first products (medicines, industrial raw materials and fine chemicals) are coming onto the market. Other than genetic modification, which has been used for some time in industrial production, synthetic biology can also be used to introduce non-natural metabolic pathways into microorganisms. These applications make previously identified difficulties in the risk assessment of immediate concern and raise new political and policy issues regarding visibility and labelling.

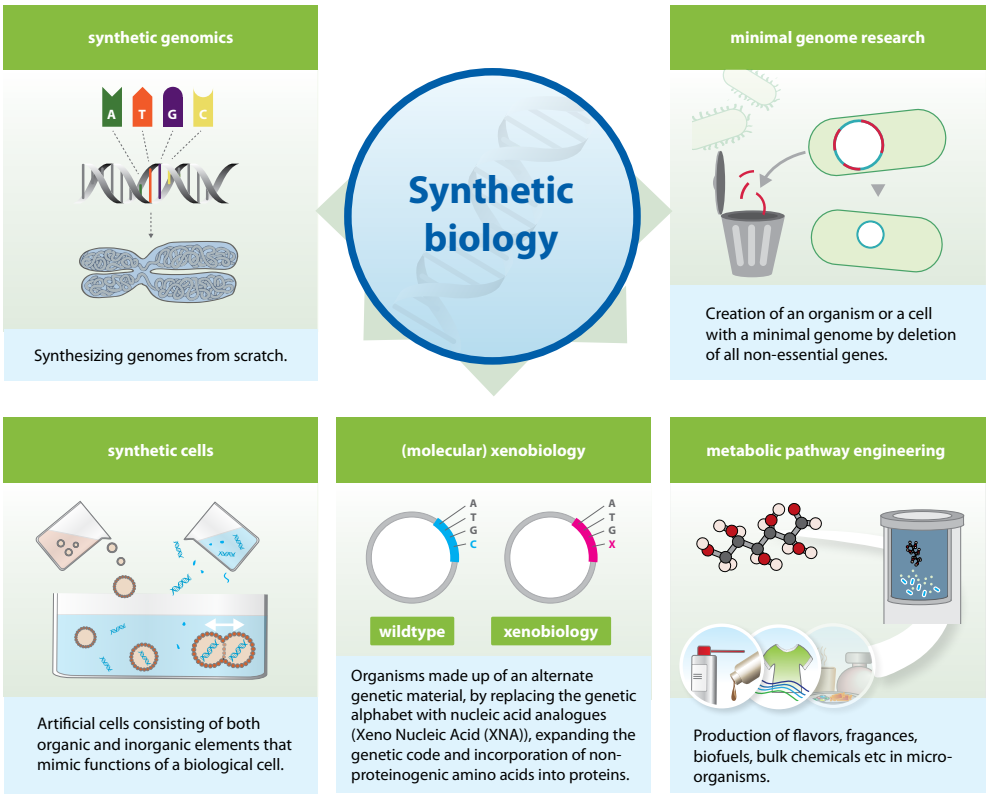

Figure 10: Sub-fields: synthetic biology can be divided into five sub-fields: synthetic genomics, minimal genome cells, synthetic cells (protocells/artificial cells), (molecular) xenobiology and metabolic reprogramming (metabolic pathway engineering).

3.8.1 What is synthetic biology?

Synthetic biology is a broad field of research that opens the way to new biotechnological applications and research. The applications of synthetic biology include the production of raw materials and fine chemicals, the development of drug delivery systems and the synthesis of vaccines. It also expands the possibilities for fundamental research into mi-

croorganisms, plants and insects. Synthetic biology brings together a range of disciplines, including molecular biology, information technology, nanobiotechnology and systems biology. It seeks to change the genetic code in existing cells or organisms (the top-down approach) as well as to design and build new cells (the bottom-up approach). The European synthetic biology network ERAsynbio defines synthetic biology as 'the deliberate (re)design and construction of novel biological and biologically based systems to perform new functions for useful purposes, that draws on principles elucidated from biology and engineering'.[v] Synthetic biology can be divided into five sub-fields: synthetic genomics, minimal genome cells, synthetic cells, (molecular) xenobiology and metabolic reprogramming (*see Figure 10*).

Synthetic genomics is the bottom-up, artificial synthesising of DNA, from genes to chromosomes, and even complete genomes. This sub-field is an enabling technology that opens up possibilities for various developments within other sub-fields of synthetic biology. Besides the rapid progress being made in DNA sequencing, the possibilities for DNA synthesis are also growing.[384,385] Although increasingly large pieces of DNA can be synthesised without error, it is not yet possible to construct the complete DNA of an organism in one go. For the time being, several smaller pieces have to be synthesised and then joined together. In 2010 researchers presented a first bacterial (prokaryotic) cell with a fully 'synthetic' genome.[386] Eukaryotic organisms (plants and animals) have a much bigger and more complex genome and present a challenge. The first synthetic eukaryotic chromosome, based on the unicellular yeast Saccaromyces cerevisiae, was made in 2014. The researchers aim to fabricate the complete genome of a yeast cell using synthetic DNA.

Minimal genome research seeks to develop a model organism that possesses only the most essential genes for survival.[389,390] Research in this field is concerned mainly with the development of an ideal production organism. In the top-down approach, genes are removed from an existing organism to determine the minimum required for cellular life. Various techniques have been developed for identifying and removing the 'non-essential' regions in the genetic material.[391] Over the past few years researchers have been able to remove about 20% of the genome of the bacterium *Escherichia coli* without damaging the essential functions of this organism.[392,393,394] Experiments in reducing the genome have also been successfully carried out with other bacteria.[395] In March 2016, researchers at the J. Craig Venter Institute in the US revealed that they had made an organism based on the bacterium *Mycoplasma mycoides* with the smallest genome so far (473 genes).[396] Minimal genome research can also take a bottom-up approach in which a synthetic minimal genome is designed, synthesised and assembled, and then inserted into a cell and activated.[397] This approach overlaps with the following sub-field.

Synthetic cells (protocells/artificial cells) are emblematic of the bottom-up approach in synthetic biology. A synthetic cell is a simplified artificial chemical model of a living cell, consisting of organic and/or inorganic elements, that mimics the functions of some, but not necessarily all, natural cell components and molecules.[398] A functioning synthetic cell must, at the minimum, be able to maintain itself, reproduce and adapt/evolve.[399] Three elements are necessary: a 'container' or membrane in which reactions can take place, a metabolism to convert and store energy, and molecules that can convey information and react to a changing environment. Research into developing fully or partially synthetic cells is mainly fundamental research, but applications have already been mentioned, such as cleaning up pollution, producing biofuels and developing medical and pharmaceutical systems for drug production and delivery.[400] Over the past few years results have appeared of the first attempts to replicate both an information carrier (DNA) and a cell membrane over several generations as the first step towards a replicating and evolving synthetic cell.[401] The first steps

v www.erasynbio.eu/about/synthetic-biology

have also been taken in the diversification ('evolution') of synthetic molecules.[402] No fully synthetic cells have yet been made; the sub-field acts as a driver for fundamental research into the functioning of living cells. A growing number of researchers in the Netherlands and Europe are active in this field.

(Molecular) xenobiology – which should not be confused with xenotransplantation[w] – is the alteration of existing genetic code by changing the chemical composition of nucleic acids or incorporating non-natural amino acids into proteins.[403,404] Research seeks primarily to answer fundamental questions, but in the future applications are expected in the medical sector, such as the production of proteins with unique pharmacological properties. In 2011, researchers succeeded in replicating an altered form of DNA (XNA) in vitro and in 2014 they also succeeded in vivo.[405] Xenobiology is also mentioned as a biosafety measure, because the artificial system is not supposed to be able to survive outside the laboratory,[406,407,408] although this has been questioned.[202,409,410]

Metabolic reprogramming (metabolic pathway engineering) aims to produce specific molecules in a genetically altered organism that turn it into a mini factory.[411] This sub-field is distinct from regular genetic modification because non-natural metabolic pathways can also be introduced into the cell.[412] Researchers are also working on mapping and altering existing genetic circuits, for example in plants.[413] High-throughput systems which can rapidly produce and test enormous numbers of genetic variants are used to identify new organisms with interesting traits.[414,415] Metabolic reprogramming is the field of synthetic biology that is furthest down the road to practical applications. At first metabolic reprogramming was used mainly to produce materials like biofuels and bioplastics, but in recent years the industry has shifted its focus to the consumer market, producing fine chemicals such as fragrances and flavouring agents.[416] The products of greatest interest are those that are produced only in small quantities in their natural form or are difficult to process. Synthetic biology can help to make manufacturing processes more sustainable by cutting energy use and reducing the pressure on natural resources. However, biomass is still needed for various processes that make use of synthetic biology, which can even lead to greater pressure on natural resources. In recent years, metabolic reprogramming has been used to produce various products in different industries (*see Figure 11*). Dutch companies such as DSM are among the world leaders in this sub-field of synthetic biology.

3.8.2 Applications

Between 2009 and 2013 the number of businesses dedicated to synthetic biology worldwide tripled from 61 to 192. The majority of these businesses (133) can be found in the United States, followed by Europe (43) and Asia/Oceania (13).[417] In the same period, the number of universities and research institutes active in the area of synthetic biology increased from 127 to 204. Businesses and research institutes are interested mainly in medical drugs and vaccines (*see text box 'Vaccine to order'*), fine chemicals, fuels and plastics. Fine chemicals are used in industrial processes (precursors, semi-finished products and enzymes) and in consumer products (cosmetics, fragrances and flavouring agents, and cleaning products).[418] A few examples are discussed below:

w Xenotransplantation is the transplantation of organs or tissues between different species (mostly from an animal to a human). Research is being conducted in this area, but no successful cases are known due to complications arising from the immune system attacking the donor material.

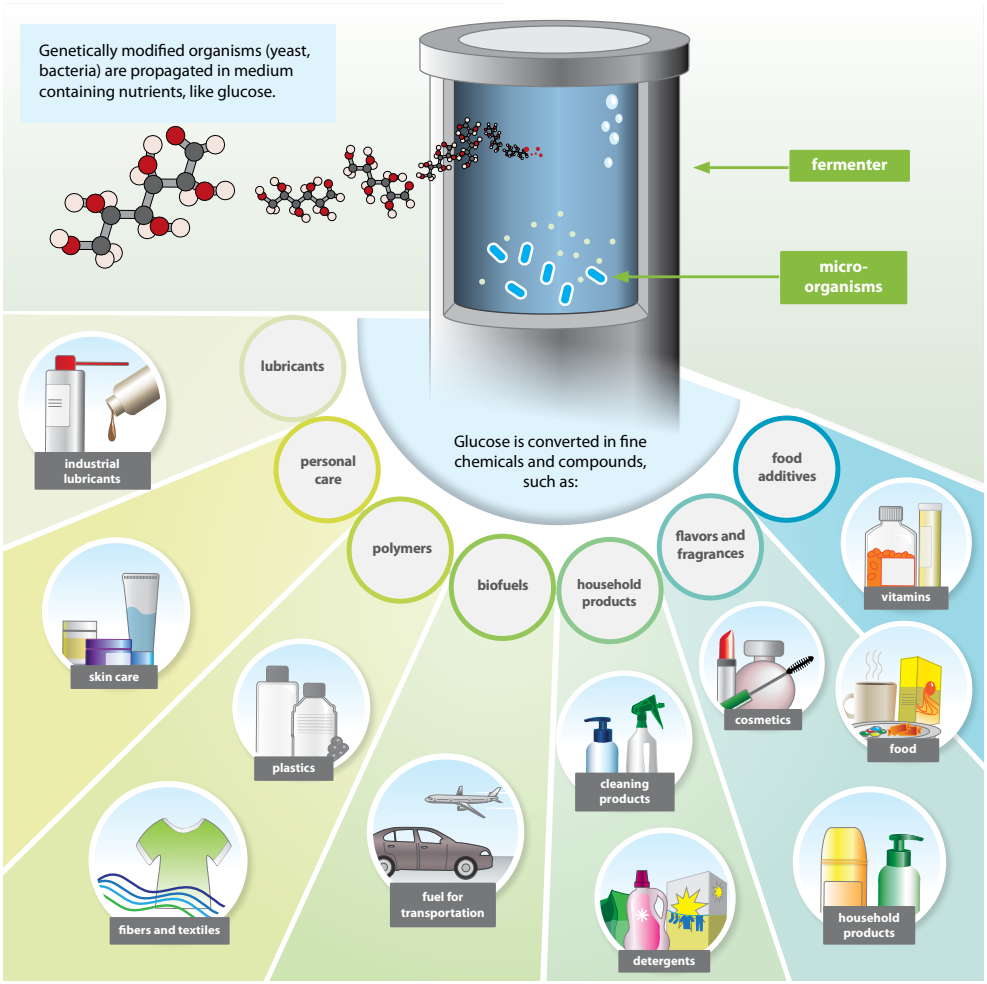

Figure 11: Metabolic reprogramming: inserting genetic code for new metabolic routes into microorganisms enables them to convert nutrients into a wide variety of products, such as food additives, fragrances and flavouring agents, household products, fuels, polymers, personal care products and lubricants

Vaccine to order

Many vaccines are based on inactivated or attenuated pathogens, which stimulates an immune response in the body to protect it against future infections. Besides these traditional vaccines, increasing use is made of proteins (from the pathogen) produced in cell systems and vectors which express the proteins. Despite the advances made in recent years, the development and production of vaccines is still a time-consuming process. Many problems could be avoided by using DNA and RNA vaccines, which can also produces more quickly.[419,420,421] Various pharmaceutical companies are working with start-ups on the production of synthetic vaccines.[422] In 2013, researchers presented a method for generating vaccines against flu strains based on sequencing data.[44] This method is expected to reduce the time taken to produce a vaccine from about six months to one week.[423] The same method based on sequencing data has recently been used in the production of antivenom to treat snake bites,[424] which claim the lives of an estimated 100,000 people each year. Antivenoms are traditionally made by extracting antibodies from animals injected with a small amount of the snake venom. In 2015 experts warned that shifts in the antivenom producing sector will lead to shortages.[425] Synthetic production could provide an alternative.

Textile – A chemical concern and sugar manufacturer have produced a new type of fibre by using genetically modified yeast cells to convert maize sugars into a raw material for the manufacture of synthetic textile. These fibres are used in various products, including carpets and sports clothing.[426]

Medicines – One of the first synthetic biology companies became known for developing synthetic artemisinin, a precursor to antimalarial drugs. This drug is produced in small quantities by the sweet wormwood plant (*Artemisia annua*). A pharmaceutical company took over the development process and in 2014 produced the first batch of artemisinin, which was used to produce an antimalarial medicine and 1.7 million doses were shipped to six African countries.[427] The company has won various prizes for innovation.[428] Nevertheless, the global market for artemisinin proved to be unstable and various factors led to the production facility becoming inoperative in 2015.[429]

Food – Various companies are working on the production of substitutes for natural fragrances and flavouring agents in yeast cells or algae. A Swiss company is using synthetic biology in the production of various substances including stevia (sweetener), saffron and vanilla.[430,431] A company in the Netherlands is working on the production of valencene, an aroma and flavour component of citrus fruit.[432] In addition, various foreign synthetic biology start-ups are aiming to produce meat, milk and eggs without the use of animals.[433,434,435]

Cosmetics – Several companies are working on the production of industrial precursors and semi-finished products. An example is farnesene, the building block for a variety of chemical products, detergents, cosmetics, perfumes and industrial lubricants.[436] One of these companies is responsible for 18% of the global squalene production, a skin care product that is currently obtained mainly from shark liver oil and to a lesser extent from olive oil.[437,438] Each year six million sharks are killed for the production of squalene. Other companies also use synthetic biology in the production of cosmetics, for example based on GM algae.[439,440,441]

Industrial raw materials & biofuels – Synthetic biology is being used for the production of isoprene (BioIsopreneTM) in bacteria. BioIsoprene can be used, among other things, in the manufacture of car tyres.[442] Various other raw materials can also be produced using synthetic biology techniques.[443] Other companies are focusing on the production of biofuels. In 2010 more than 80,000 litres of biodiesel were produced by GM algae for the US navy.[444] The same company is also working on biofuels for cars and aircraft.

Synthetic biology in the Netherlands

In the Netherlands there has been much interest in synthetic biology from universities and industry for some years. Scientists at Delft University of Technology are using bionanotechnology to work on synthetic cells.[445] In 2008, the Center for Synthetic Biology was established in Groningen,[446] where work is also progressing on synthetic cells.[447] The popularity of synthetic biology is reflected in the establishment of various 'community labs' and the international IGEM synthetic biology competition, in which more than 250 teams from the whole world 'build' organisms for practical applications of benefit to society (*see text box 'DIY bio: science for the masses?'*). In 2012, the Groningen team won this international competition and in 2015 the honour went to the Delft team.[448,449]

DIY bio: science for the masses?

The growth of synthetic biology and other developments have given rise to the impression that creating organisms with new traits is just a question of combining the right set of building blocks. Although this is easier said than done, new instruments and techniques have certainly simplified genetic modification. This has brought biotechnology

out of the scientific domain and made it more accessible to other interested parties. At the popular IGEM[x] competition – from 2005 to 2015 the number of participants grew from 13 to 268 teams – international student teams compete to produce the most innovative design for an organism.
The accessibility of genetic modification can also be seen in the emergence of Do It Yourself Biology (DIYBio) and Community Labs. DIYBio can be described as an international network movement of a select group of amateur researchers, artists, students and professional and semi-professional scientists who carry out biological experiments with simple and affordable materials.[450] Although there are very few home laboratories, several community labs have been established in the US and the UK where enthusiasts can come to brainstorm or carry out experiments.[451] A number of these experiments are financed from crowdfunding initiatives.[452,453,454,455,456,457] DIYBio activities in the Netherlands are concentrated around three DIYBio initiatives in Amsterdam, Eindhoven and Groningen. In 2015 the Amsterdam lab obtained a permit to conduct experiments with GMOs.[458]

Besides research, various synthetic biology commercial applications have come out of the Netherlands. The Woodrow Wilson International Center for Scholars in the US maintains a list of products made using synthetic biology.[y] The list contain 51 products (worldwide) already on the market or nearing marketing authorisation, 44 of which are from the US. The Netherlands is represented as a manufacturing country on this list by five products: 2 flavouring agents, 2 antibiotics and 1 soap.[459] In addition, products can be found in the Netherlands that are produced in other countries, such as cleaning products and textiles.[460,461]

3.8.3 Issues and challenges

The field of synthetic biology is continuing to develop and various products are starting to emerge on the market. But synthetic biology also faces challenges in the technical, legislative and social spheres.

Technical challenges

Synthetic biology still faces numerous technical challenges. The key challenge facing synthetic genomes has for some time been to faultlessly synthesise longer strands of DNA. Minimal genome research is still a process of trial and error in which genes that in one organism do not appear to have any (essential) function turn out to be essential for the functioning of another organism.[462] Major challenges still have to be overcome before it will be possible to develop autonomously functioning synthetic cells that resemble natural cells. A truly synthetic cell has to possess a number of self-organising subsystems if it is to be able to maintain itself, grow, replicate and evolve.[463] Each of these steps is in itself a challenge and combining them even more so. Within xenobiology, efforts are being made to find changes in DNA that are sufficiently distinct from the natural variant while remaining functional enough. The technical challenges facing metabolic reprogramming arise from the complexity of the interactions within metabolic networks and making cells suitable for production on an industrial scale.

Legislative challenges

Most synthetic biology experiments and applications fall under the GMO legislation. In 2013, COGEM observed that the current risk assessment method was adequate for the research being conducted in the field of synthetic biology. This opinion was shared by advisory bodies from various other member states and by European and international colleagues.[409,464,465,466,467,468]

x International Genetically Engineered Machine

y The survey by the Woodrow Wilson Institute is an open source database which can be searched by product category, company or market status. It is not an exhaustive list.

Should current trends continue, the risk assessment may in future be made more difficult by an increase in the complexity of the interactions, a blurring of the distinction between donor and host, and the absence of a natural reference. These development may in future lead to a situation in which there is no known and characterised host organism for use as a reference in the risk assessment, or in which the introduced traits and interactions with the host organism are unpredictable. High-throughput techniques can be used to create large numbers of different mutants, and increase in scale and speed that can lead to practical and organisational problems in relation to the current case-by-case approach and statutory time limits. Most synthetic biology applications take place in laboratories and in closed systems such as bioreactors. As for other applications of genetic modification, applications involving deliberate release into the environment must be subject to a detailed environmental risk assessment.

Synthetic biology has been around for some time, has much in common with genetic modification and also integrates with other technologies, such as nanotechnology. Most applications fall under the existing GMO legislation or, in combination with other technologies, will raise general issues which transcend the field of synthetic biology. An OECD report from 2014 stresses the importance of flexible policy, international cooperation and knowledge sharing between stakeholders to realise the full potentials of this technology, while at the same time responding constructively to issues of biosafety, biosecurity, intellectual property rights, sustainability and social acceptance.[465] That these issues can sometimes arise in unexpected places is shown by recent reports of the possible production of opiates using synthetic biology (*see text box 'Home-made morphine produced by yeast cells'*).

Home-made morphine produced by yeast cells

Three research groups in the US and Canada inserted genetic components from poppy, beetroot and soil bacteria into a yeast cell. The resulting cell could perform parts of, but not the entire, process of the converting glucose into morphine.[469,470,471,472,473] A fourth research group developed a yeast cell that can convert the missing intermediary component (S)-reticuline into (R)-reticuline.[474] Combining the characters of these two yeast cells would create a yeast cell that can convert glucose into morphine. Given the ease with which yeast cells can be replicated (a beer home brew kit is good enough), the publication of this research in 2015 generated considerable debate.[475,476] Morphine is still produced from poppies (*Papaver somniferum*), but producing morphine in modified yeast cells may be a cheaper, faster and more efficient manufacturing process. Some news reports argue that producing opiates in a self-replicating source that is easy to maintain, hide and distribute will bring about a structural shift from a defined and restricted opium market to one of decentralised local production, which will make it much easier to access the market.

In recent years synthetic biology has regularly been in the news with items about the production of useful products in modified yeast, bacteria and plants, such as antimalarial agents, fragrances and flavouring agents and fuels. However, the production of a prohibited substance shows that synthetic biology can also be used to facilitate the production of illegal or problematic substances. This development raises policy questions about the dual use of this technology.

Social challenges

Over the past few years, organisations like the Rathenau Institute and COGEM have published various reports and organised events to identify the technical, scientific and social implications of this technology,[202,380,381,477,478] Although there has been no sign of a major public debate about synthetic biology, various international NGOs have drawn attention to their concerns about the risks and social impact of this technology.[479]

At first synthetic biology was used mainly to produce things like biofuels and medicinal drugs. However, in recent years there has been a shift away from industrial raw materials

towards consumer products such as fragrances and flavouring agents, cleaning products and cosmetics.[439,480] This shift has generated some debate over the past few years. At the same time, studies show that consumers say utility and affordability are their two main considerations when buying products (*see text box 'Utility key factor in consumer decision-making'*).

Utility key factor in consumer decision-making

Dutch respondents were asked for their opinions on three applications of genetic modification: enzymes in washing powder, disease-resistant potatoes and the production of medicinal insulin.62 They mentioned affordability and effectiveness as the decisive factor in their choice of detergent. More than half the respondents agreed or somewhat agreed with the statement that affordability is more important than the ingredients. One of the main considerations for the majority of respondents was washing at low temperatures. Interestingly, a majority of the respondents agreed or somewhat agreed with the statement that farmers should decide for themselves whether or not they want to grow GM potatoes and that these potatoes should be sold in the shops. A small proportion of the respondents answering these questions were explicitly opposed to genetic modification: 2% said they check for the use of genetically modified ingredients in detergents (versus 57% who never do); 6% thought that farmers should not be allowed to cultivate GM potatoes (versus 29% who thought farmers should be allowed to choose); and 3% check for the use of genetically modified ingredients in medicines (versus 37% who never do).

Although the synthetic production of raw materials, semi-finished products and fine chemicals seemed initially to be a good alternative to existing practices (e.g. for reasons of animal welfare, because the natural resources are difficult to obtain, or because synthetic production is cheaper or quicker), opinions are mixed. For example, NGOs argue that producing squalene[z] in microorganisms cannot be called sustainable, because large amounts of sugar cane are required as a raw material input.[481] Another criticism is that large-scale synthetic production can undermine the competitive position of the local production of natural substances (such as vanilla).[482,483] In its topic reports, COGEM has pointed out that the production of natural substances in GMOs or synthetic organisms can be a source of disquiet or concern for some people.[484,485] This was the argument used in protests against a company that makes cleaning products when it announced it would be using oil from GM algae instead of palm oil (*see text box 'Green company attacked for using synthetic biology'*).

Green company attacked for using synthetic biology

In 2014, Ecover, a company that promotes itself as green and ecological, attracted attention when it announced it was switching from the use of palm oil[aa] to oil produced in GM algae for the production of various soap products. Various NGOs launched petitions and argued that the oil from these 'synbio' algae could not be called natural[ab,ac], and the products were wrongly being marketed as 'natural' and 'sustainable'.[486] Various health food stores and chain stores are reported to have removed the products from sale. However, other manufacturers who switched to oil produced by GM algae have not drawn the same response.[441] The debate about genetic modification indicates that the visibility of products made using synthetic biology is something that not only industry needs to be aware of, but also politicians and policymakers.

z Squalene was originally obtained from sharks' livers.

aa Large-scale production of palm oil is criticised by environmental organisations because of the related destruction of tropical rainforest.

ab www.syntheticisnotnatural.com

ac http://www.etcgroup.org/content/open-letter-ecover-method

3.8.4 Stakeholder implications

Applications of synthetic biology can be found in many different fields, which means that various stakeholders are affected or will be in future.

- **Citizens:** As consumers and patients, the public will come into contact with products made using synthetic biology. This can have a financial effect if the ingredients can be produced more cheaply. On the other hand, it may influence their purchasing behaviour if these products do not match consumers' ideas on healthy, sustainable or natural food and this is directly associated with the visibility of synthetic biology in the production of the end product and on the label.
- **Industry:** The availability of raw materials and fine chemicals produced using synthetic biology will present businesses with choices regarding products and production methods. Synthetic biology opens up possibilities for innovation, but at the same time it will create differences in legislation and regulations between countries. Businesses will have to make choices about how they respond to the way consumers view the sustainability and naturalness of products made using synthetic biology, which may be conflicting.
- **Risk assessors:** The developments in synthetic biology will possibly go beyond the scope of the current risk assessment. Integration of various disciplines and broadening of the expertise and cooperation between scientists and risk assessors can contribute to the streamlining, and if necessary adaptation, of the risk assessment.
- **Government:** Politicians and policymakers are confronted by advances in synthetic biology and their integration into other fields of knowledge. These developments may lead to products of synthetic biology not being recognisable as such to the consumer and raise the question of whether the existing legislation is sufficient and at what point it may no longer be adequate. From an international perspective, government is faced with the question of how to respond to the importation of products made using synthetic biology that cannot be distinguished from the conventional version. The visibility of this production method to consumers is another governance issue in relation to freedom of choice and transparency.

3.8.5 Conclusions

- The growing possibilities for producing every possible substance in microorganisms throws up technical and policy challenges as well as social concerns. Examples are the possibility of producing prohibited substances in synthetic organisms and the visibility to the consumer of products made with synthetic biology.
- The potential risks to human health and the environment have been on the radar of advisory bodies and licensing authorities for some time. The distinction between genetic modification and synthetic biology is not always clear-cut. Most applications fall under the GMO legislation and can be adequately assessed using the existing environmental risk methodology.
- However, in some situations an increase in the complexity of the interactions, and the absence of a natural reference for the risk assessment will make this more difficult.
- Given the amorphous nature of synthetic biology, the broad range of applications and the speed at which the field is developing, drawing up regulations and policy specifically for synthetic biology is an impossible task. It is important to be able to respond in a flexible manner to new developments as they arise. This demands a form of governance in which assessments are made of the need for regulation for each application as it arises and, if it is needed, what existing legislative frameworks are most suitable.
- National and international cooperation and knowledge sharing between stakeholders is required to realise the full potentials of this technology and at the same time respond constructively to issues of biosafety, biosecurity, intellectual property rights, sustainability and social acceptance.

4 POLITICAL AND POLICY ISSUES

From the trends described in Chapter 3 we can distil several recurring issues relevant to policy. In this final chapter, we identify these issues and go into them in more depth.

4.1 DEFINING THE ETHICAL ISSUES IS A POLITICAL TASK

The developments in biotechnology open up opportunities and possibilities, but besides these positive aspects they also raise issues that must be dealt with. The trends where most medical ethics issues arise are in next generation sequencing (NGS), CRISPR-Cas, personalised medicine and gene therapy. These issues include questions of privacy (*see text box 'New initiatives by technology companies raise questions'*), ownership, the right to self-determination, autonomy and questions of social costs versus individual benefits, and the dividing line between healing and treating diseases and enhancement.

New initiatives by technology companies raise questions

In recent years various new players have entered the lucrative health market. For some years companies have been offering genetic tests or self-tests via internet, but the quality of these tests is not always assured and the protection afforded to individual privacy is often couched in vague terms. The big technology companies are also not sitting still. Apple has launched a mobile platform where iPhone owners can take part in 'observational studies' into Parkinson's disease, breast cancer and other conditions.[487] Google is developing similar initiatives and is compiling DNA databanks.[488] The advantage of a mobile platform is that it allows huge numbers of people to take part in the studies; the disadvantages are the reliability of the information, non-representative populations, etc. Besides the possible 'technical' disadvantages, one can question whether companies like Google and Apple, who already accumulate large amounts of data about their users, ought to be allowed to acquire medical and genetic information as well.

The most pressing issue is germline modification in humans. Whereas before it was only theoretically possible to alter the genetic code in a fertilised egg cell, the technology is now not the biggest problem. Given the possibilities that germline modification provides, such as eliminating genetic disorders, the current ban on germline modification is expected to come under scrutiny. In view of this, a debate on the desirability and ethical acceptability of the technique is urgently needed. The question of whether germline modification is desirable or acceptable cannot be answered with a simple yes or no, but is more likely with either 'yes, as long a certain conditions are met' (e.g. only in clearly defined cases), or 'no, unless there are exceptional circumstances' (which will also have to be precisely defined).

The recent public debate on making NIPT (non-invasive prenatal test) available to pregnant women with no increased risk – and the fear among some people of prenatal screening become a routine procedure – is illustrative of the types of ethical issues that may be thrown up by developments in sequencing. Whole exome and whole genome sequencing, as now used in clinical genetics for making molecular diagnoses, will give us a good picture of our genetic make-up – and that of our family members – and this information can be used to

improve individual care. The question, though, is what this means for the 'right not to know'. Also, clinically relevant and non-relevant incidental findings may also be revealed, which may challenge the principle of informed consent.

Regarding personalised medicine, the question is how far it can meet expectations and whether it will actually deliver substantially better healthcare. Also, how much pressure will it put on patients to change their lifestyle or undergo treatment? And what about the pressure on professionals (e.g. from the health insurers) to offer certain treatments, or indeed withhold certain treatments? Solidarity within the insurance system may also come under pressure. Will people want to pay premiums to allow someone with a genetic disorder to take out a bigger mortgage, or to allow a smoker to receive treatment for lung cancer?

Determining the framework for weighing up the values and interests of stakeholders and evaluating the consequences for the public is a political task. Politicians must take up this challenge and then turn their decisions into policy.

4.2 STAKEHOLDER PARTICIPATION IS ESSENTIAL BUT CONSENSUS IS NOT A REQUIREMENT

As biotechnology is having an impact in various sectors – as can be seen in the trends described in Chapter 3 – and has consequences for stakeholders, it is inevitable that these stakeholders will play an active role in discussions on policy and decision-making procedures. Some groups of stakeholders, such as patients' associations, are already actively engaged in the development of medical biotechnology applications. The decentralisation of medical and social care has also strongly encouraging patient/client participation. 'Caring for' is increasingly becoming 'caring with'. This means that patients and relatives have to be properly informed about the possibilities and limitations of biotechnological applications so that they can make informed decisions. As this information is so complex, it will have to be given in careful doses, with consequences for the procedures for informed consent and how much the public should be expected to know.

It is evident that there is an urgent need to involve stakeholders at an early stage of policy development and decision-making procedures. However, there are different sorts and levels of stakeholder participation, each with its own role, function, possibilities and limitations. Involving stakeholders is often unjustifiably seen or presented as an instrument for consensus building. However, consensus is usually impossible to achieve on controversial topics and proposals in which individual values and interests differ widely, neither is it necessarily what participants want. Besides, it is not necessary for making decisions. Nevertheless, in these situations stakeholder participation can be an important tool for obtaining different sorts of knowledge (including technical, economic and first-hand knowledge) and collecting different perspectives and visions as inputs to the political debate and policymaking. Before involving stakeholders it is important to think about the goal and timing of the participation exercise. Different sectors may be affected in different ways, with consequences for the nature and outcome of the discussions. For example, innovations in healthcare can affect stakeholders directly (e.g. as patients), field trials with GM insects to control pests and infections (*see §3.6 'Genetically modified insects'*) can have an impact on the environment, and GM crops or foods produced using synthetic biology (*see §3.8 Synthetic biology*) can affect consumer choice.

Stakeholder participation in policymaking and decision-making on biotechnological developments is essential. At the same time, it is particularly important that the nature and purpose of this involvement is made explicit, both in advance and after the event.

4.3 PUBLIC SECTOR MUST GUARD ITS KNOWLEDGE INDEPENDENCE

Biotechnology is becoming increasingly integrated into broader application areas – making it less visible – and much of the rapid development in fields like sequencing and plant breeding is happening in the private sector. However, decisions on the type and scope of applications to be permitted on the market and making policy on this is a political, public sector task – and this depends crucially on having objective, independent and verifiable knowledge. The growing cooperation between public and private sectors – stimulated by government – and the increasing outsourcing of research to support policymaking is making it more difficult to find experts based in the Netherlands who are capable of assessing new developments and also have no relations with private sector players. Politicians and policymakers must ensure that the public sector continues to have access to good quality independent knowledge. Several issues need to be addressed, including the question of whether politics and policy have a clear picture of what information and expertise is lacking, whether or not this is available from independent sources, and if it can be made available in a transparent manner. Furthermore, it is important that the government departments themselves possess the content knowledge required to determine what knowledge is missing and to ask research centres and knowledge institutions the right questions.

This requires an analysis of the current state of knowledge about biotechnology in general and the described trends in particular, specifying the public sector knowledge available in the Netherlands and the knowledge that the Dutch government and an independent academy must have.

4.4 INTELLECTUAL PROPERTY: BALANCE NEEDED BETWEEN STIMULATING INNOVATION AND PREVENTING MONOPOLISATION

The rapid developments, rising number of patents and the scaling up of biotechnology are reasons to be concerned about the risk of monopolisation. In the plant breeding sector the issue is one of plant variety rights versus patent law (*see text box 'Patent law versus plant variety rights'*). Under plant variety rights, plants may be used to breed new varieties without breeders having to pay a fee to the owner of the variety used for breeding. This is not possible under patent law. Plant variety rights is seen as the motor behind innovation in plant breeding in the Netherlands, but it is in danger of being displaced by patent right. Moreover, it is feared that patenting plant or crop varieties will facilitate monopolisation.

Patent law versus plant variety rights

In recent years, patenting crops with specific traits (as opposed to using plant variety rights) has been in the political and public limelight.[489] Plant variety rights (also known as plant breeders' right) is an intellectual property right that gives a breeder the exclusive right to exploit propagating material from a new plant variety for a period of 25 years (depending on the type of crop). Others may therefore not produce and sell seeds or other propagating material to farmers and growers. Other breeders, though, are free to use a new plant variety in their own breeding programmes to create new varieties, without having to pay a fee to the breeder of the original variety ('breeders' exemption').

In recent years patent law has been on the increase in the plant breeding industry; more than 300 patent applications have been submitted and 71 granted. Recently the Enlarged

Board of Appeal of the European Patent Office (EPO) ruled that while essential biological processes may not be patented, the products arising from them, such as seeds and plants, may be.[490,491] This decision has far-reaching consequences as it means that patents can be obtained for the products of traditional plant breeding, which will further squeeze out plant variety rights. To restore the balance between patent rights and plant variety rights, the Dutch government is committed to including a (form of) breeders' exemption in European patent law.[492] This proposal has met with strong criticism from the Dutch biotechnology industry association HollandBio, which sees it as a threat to the Dutch biotechnology sector.[493]

The concerns in the pharmaceuticals sector relating to monopolisation are mainly to do with the risk of extremely high prices, as illustrated by the success of the orphan medicinal products regulations. These have led to an increase in the number of medicines with the status of orphan medicinal product, although a number of these medicines are not orphan medicinal products in the strict sense of the term, but actually rather cleverly registered for several small indications. Developments in the field of personalised medicine are also provoking fears of expensive drugs; if pharmaceutical companies make medicines for small groups of patients, they will be able to ask higher prices for them. This practice is defended with the argument that the high development costs have to be recouped, despite the sometimes relatively low production costs.

The developments described in this Trend Analysis show that the issues around pricing and monopolisation in the pharmaceutical sector are urgent and will only intensify in future. It is crucial that a balance is found between preventing monopolisation and protecting intellectual property rights so that it will continue to be possible to stimulate innovation and maintain the economic value of biotechnology. Edith Schippers, the Minister of Health, Welfare and Sport, and Sharon Dijksma, Minister for the Environment, have taken initial steps to this end.[494,495,496]

A study by the Netherlands Patent Office and a supplementary study by COGEM show that between 2003 and 2011 the Netherlands was among the top ten countries for biotechnological patent applications (*see text box 'Patents in the Netherlands'*).[497]

Patents in the Netherlands

Since 2006[498] the Netherlands has lost ground against both Asian and other EU countries in the field of red (medical) and white (industrial) biotechnology, but has strengthened its position in the green (agriculture) biotechnology patent applications. From 2003 to 2011 the Netherlands was 4th on the list for green and 9th on the list for white and red biotechnology patent applications. In absolute terms, by far the most patent applications in the Netherlands are in the field of red biotechnology, followed by white biotechnology. The number of patent applications in green biotechnology is significantly smaller than in the other two sectors. An analysis of patent applications shows that the standard division of biotechnology into green, white and red is becoming less useful. Many of the patent applications cover several fields within these sectors (such as industrial production of food for patients with a stomach disease). Also, many patent applications are for topics that do not fit comfortably into one of the green, red or white categories, such as cosmetics and biosensors to detect pollution or explosives.

4.5 LEGISLATION HAS BEEN OVERTAKEN BY SCIENCE AND NEEDS REVISION

The legislation is no longer in step with the technological advances that are being made (*see text box 'The GMO legislation in the EU'*). While developments in biotechnological techniques such as RNAi and CRISPR-Cas (*see §3.2 and §3.5*) are rapidly gaining momentum, political decisions on the status of these techniques and changes to the legislation have not been forthcoming.

The GMO legislation in the EU

Genetic modification is strictly regulated in the EU. The requirements and obligations are set down in various EU directives and regulations on a range of topics, such as working with GMOs in laboratories, field trials and commercial activities, labelling and the traceability of GM foods, and food safety. In the Netherlands these rules are implemented via the GMO Decree.[499] The GMO authorisation procedure does not include a benefit-risk appraisal. The principle behind EU policy on GMOs, and therefore also Dutch policy, is that all appropriate measures should be taken to prevent any adverse consequences for human health and the environment.[500] These measures are drawn up on the basis of an environmental risk assessment and imposed as conditions in a consent for the relevant activities involving GMOs. A consent and risk assessment are required for all experiments, activities and commercial activities involving GMOs. A consent is granted if the activities do not put human, animal and environmental safety at risk.[501] Consents can therefore in principle only be refused in the interests of protecting human and environmental health.[502,ad]

For many years, politicians and policymakers in the EU have been critically examining various new techniques. Despite these efforts, after nine years the European Commission has still not come to any decision. In the meantime, the United Kingdom and Germany have taken matters into their own hands and decided that field trials with crops developed using site-directed mutagenesis are not subject to the GMO legislation. Sweden has decided that the GMO legislation does not apply to crops in which mutations have been made using CRISPR. Differences in how individual member states assess these techniques and their products may have serious consequences for the internal market.

Decisions on the status of these 'new techniques' are being held up because the scientific basis of the regulations has been overtaken by events. The regulation of biotechnological applications in the EU has so far been based on the ability to clearly distinguish between organisms that have been genetically modified and those that have not. The emergence of genome modification and other techniques (*see §3.2*) has shattered that distinction, undermining the foundations of the EU GMO legislation. Products made using these techniques can hardly, if at all, be distinguished from 'natural' products or products made using other techniques, neither can they be identified when imported. Without clear criteria or reference frameworks for decisions on the status of new techniques, inconsistences between assessments of techniques and their products can easily creep in.

The continued absence of a decision on the status of new techniques or a revision of the legislation is making Europe less attractive to biotechnological companies because of the uncertainty about current and future legislation. This situation also creates an uneven playing field with companies in other parts of the world where these techniques and products are not regulated. The fact that products are not identifiable as such heightens the problem. Dif-

ad An exception to this is the 'national cultivation authorisation'. Directive 2015/412 gives member states the option to restrict or prohibit the cultivation of GM crops in their territory based on concerns which do not solely relate to safety issues.

ferences in opinion about what a GMO is can lead to trade conflicts and have to be addressed in negotiations on trade agreements, such as TTIP.

These developments make revision of the EU GMO legislation very urgent. The current approach in which the need to obtain consent and submit a risk assessment depends on the method of production is no longer workable or consistent with the possible risks associated with products. Furthermore, biotechnology has spread to numerous different fields and the potential risks of the applications must be assessed in their specific contexts. Among the issues to be investigated is whether to develop an integrated safety policy or make biotechnological applications subject to existing or new sector-specific legislation, or perhaps take the Canadian product-based approach (*see text box 'GMO legislation in the EU and elsewhere'*). A revision of the legislation could also take on board the public's preference for weighing up the risks and benefits of individual applications.

GMO legislation in the EU and elsewhere

The approach taken in the EU is to introduce specific legislation on GMOs to ensure human and environmental safety. The principles underlying the EU legislation are set out in Directive 2001/18. This defines a GMO as: 'an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination'. In addition, Annexes 1A and 1B list a number of techniques through which genetic modification occurs or does not occur, as well as techniques which are excluded from the Directive, such as mutagenesis. As this legislation is based on the method used to make the organism or product, it is known as *process-based* legislation.

The US and Canada have chosen a different approach, called product-based legislation, which is based on the characteristics of the organism. Nevertheless, there are still considerable differences between the American and Canadian systems. In Canada, regulation is based on the presence of a new trait, irrespective of the way this was introduced. This means, for example, that a safety assessment has to be carried out for a new type of herbicide tolerance, irrespective of whether the plant was made via genetic modification or via classical mutagenesis. The US has decided to make all products subject to existing legislation. This means, for example, that if a gene from a plant pathogen is introduced into a plant, this plant must in principle be assessed by the USDA.

A particular issue regarding the Dutch legislation is the status of the Caribbean Netherlands (Bonaire, St Eustatius and Saba). The Dutch GMO Decree does not apply to the three special municipalities that make up the Caribbean Netherlands and the Housing, Spatial Planning and Environment Act that applies to these municipalities (Wet VROM BES) contains no provisions relating to GMOs. This has consequences, for example for the authorisation of field trials with GMOs (such as GM insects).

Biotechnological developments in the medical sector (NGS, CRISPR-Cas, personalised medicine, gene therapy and 3D bioprinting) have an influence on the regulation of medical research, marketing authorisation of medicines and medical devices, the reimbursement of the costs of these medicines and devices, and population screening.

Given the developments in the medical sector, the existing legislation should be screened to identify any unnecessary obstacles it poses to innovation and the treatment of patients, and whether or not it can adequately guarantee the safety of new products and treatments. 3D bioprinting, in particular, raises issues that highlight uncertainties regarding the legislation and safety.

4.6 ABSENCE OF POLITICAL DECISION IN THE NETHERLANDS MAKES INTERNATIONAL CONTEXT DECISIVE

This Trend Analysis is written from a Dutch perspective, drawing on the international scientific literature and news sources. It shows that the Netherlands does not stand in isolation. Many things can be regulated at the national level, but the international context must always be taken into account. The ready availability of scientific knowledge and international travel provide additional healthcare possibilities for the individual patient and consumer (medical tourism). However, medical tourism can be risky for society (gene therapy) and provoke debate about developments in the Netherlands considered to be undesirable. Nevertheless, medical tourism is here to stay, businesses will continue to consider where they can most profitably develop and market new products, and food containing genetically modified components will inevitably become indistinguishable from foods that do not, with consequences for imports and exports (*see §3.5 RNAi)*. This makes international differences in the legal definition of a GMO a crucial point in negotiations on trade agreements such as the TTIP.

The international context is unavoidable, but at the same time is not necessarily the deciding factor for the Netherlands. At the national level, consideration should be given to the political direction to be taken and the nature of the policy response, because failure to make such decisions means that the international context will make them instead.

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APPENDICES

APPENDIX 1: MEMBERS OF THE PROJECT COMMITTEE, WRITING GROUP AND STEERING GROUP

The Biotechnology Trend Analysis 2016 was prepared by a project committee consisting of the following members:

Chair
Dr. F.W.A. Brom, Scientific Council for Government Policy (WRR)[ae]

Members
Prof. C. Dekker, TU Delft
Emeritus Prof. J.J.M. Dons, Wageningen UR
Dr. L. Henneman, VU University Medical Center
Prof. R.C. Hoeben, Leiden University Medical Center
Emeritus Prof. G.J. Mulder, Leiden University
Prof. S. Repping, Academic Medical Center
Prof. F.J. van Schooten, Maastricht University
Prof. P.H. van Tienderen, University of Amsterdam
Emeritus Prof. G.H. de Vries, University of Amsterdam

Observer: Dr. D.W.G. Jung Ministry of Infrastructure and the Environment

The project committee was supported by a **writing group** of staff members of the Health Council of the Netherlands and COGEM:

R. Mampuys MSc, COGEM
Dr. V.W.T. Ruiz van Haperen, Health Council of the Netherlands
Dr. F. van der Wilk, COGEM

The three organisations involved established a joint **steering group** consisting of the chairs of these organisations and headed by the COGEM chair. The steering group adopted the action plan, established the project committee, supervised the process of preparing the Trend Analysis, and monitored progress.

Steering group
Prof. W.A. van Gool, chair of the Health Council of the Netherlands
Prof. J.A. Knottnerus, chair of the Scientific Council for Government Policy (WRR)
Prof. S. Schaap, chair of COGEM

ae Chair in a personal capacity. When the study began Dr Brom was not yet working at WRR.

APPENDIX 2: METHODOLOGY

Biotechnology is a very broad field encompassing numerous developments. The trends presented in this Trend Analysis were identified and selected through the following procedure.

1) Identification of trends Trends for possible inclusion in the Trend Analysis were identified from:

- the expertise of members of the project committee preparing the Trend Analysis;
- interviews with members of the Health Council of the Netherlands and COGEM;
- recent publications by the Health Council of the Netherlands, COGEM and WRR;
- a literature review of developments in biotechnology maintained by the COGEM secretariat;
- several research reports prepared for COGEM as possible inputs to the Trend Analysis;
- input from stakeholders: in June 2015 a large number of stakeholders in the field of biotechnology were approached to submit suggested topics and trends in writing. Responses were received from:
  Central Committee on Research Involving Human Subjects (CCMO)
  Dutch Forum for Biotechnology and Genetics
  HollandBIO
  Royal Netherlands Academy of Arts and Sciences (KNAW)
  Leiden University Medical Center (LUMC)
  Netherlands Society of Gene and Cell Therapy (NVGCT)
  Dr J.M. Fentener van Vlissingen, Laboratory Animal Science Center, Erasmus MC
  Nefarma
  Plantum NL
  VSOP
  Cells4Therapy

The stakeholders' inputs are included in the Trend Analysis in a separate Appendix, with the exception of the submission by the Royal Netherlands Academy of Arts and Sciences (KNAW). The KNAW could not agree to the publication of its input because when its submission was prepared the Academy did not take into account its eventual publication and so the necessary relevant pre-publication procedures were not followed.

2) Selection of trends The project committee used the following criteria to select the trends for inclusion in this Trend Analysis:

- **Impact of a development** – The size and intensity (either positive or negative) of the possible effects on individuals or society. This may be described in terms of the numbers involved, the financial scale (pros and cons) of the consequences, or the seriousness and gravity of the effect. This criterion can be summed up by the following equation: Impact = intensity x size.
- **Social relevance** – Political and policy action are possible and necessary. The Government and the House of Representatives are the primary target audience of the Trend Analysis.
- **Time period** – The trend must be a topical issue within five years, or require political or policy action/decision.
- **Plausibility/Realism** – The topic or trend must be able to be empirically or otherwise substantiated.

3) Review of the Trend Analysis The draft Trend Analysis was submitted for review to the Health Council of the Netherlands (Standing Committees on Public Health and on Health Care), COGEM and the Scientific Council for Public Policy (WRR). The comments made were taken in account when drawing up the final version of the Trend Analysis.

APPENDIX 3: REFLECTION ON THE BIOTECHNOLOGY TREND ANALYSIS 2007 'OPTIONS AND OPPORTUNITIES' AND THE TREND ANALYSIS 2009 'GLOBAL MOMENTUM'

Previous trend analyses on biotechnology were published in 2004, 2007 and 2009. The first was compiled by the Committee on Animal Biotechnology (CBD), the Central Committee on Research Involving Human Subjects (CCMO) and COGEM. The remits for each of these trend analyses were different. In 2004 the main issue for investigation was the moral dilemmas arising from biotechnological developments. In 2007 the focus was on the opportunities provided by biotechnology. In 2009, at the request of the Ministry of Housing, Spatial Planning and the Environment, the Centre for Society and Genomics (CSG) was involved in the preparation of the Trend Analysis to introduce a more in-depth analysis of the social aspects.

As can be seen from the table below, several trends that were previously identified have been revisited in this Trend Analysis. The issues surrounding these trends have intensified because no decisions have been made during the intervening period, whereas the technologies have continued to advance – particularly abroad – and the need for decisions has become urgent.

Trend Analysis 2007

Trend	How has the trend developed?
Environment: Biotechnology offers opportunities for the environment	This trend is continuing undiminished. Given the political attention this topic enjoys and the policy action being taken, the project committee sees no need to raise this trend again.
Economy: The potential of the Dutch biotechnology sector is insufficiently exploited	An economic analysis of the period 2010–2014 revealed that the position of the Dutch biotechnology sector is largely the same as it was in 2007. A number of developments that took place in 2015 suggest that the economic position of the Dutch biotechnology sector is improving.
Vaccines: Vaccines and the rise of infectious diseases	The expected spread of infectious diseases (emerging and re-emerging diseases) in humans and animals (including zoonoses), often tropical and subtropical in origin, has continued. The possibilities for treating these diseases are often limited. Resistance to antibiotics among pathogenic bacteria continues to grow. The conclusion in 2007 that vaccines are needed to halt the further spread of these diseases and protect humans and animals remains undiminished.
Legislation: Technological developments in plant biology call for a reconsideration of the legislative and regulative frames	The points raised in 2007 are still relevant and have been heightened by CRISPR-Cas9. The problems have also expanded to cover not only the agro sector but also the medical sector. This problem is found in several trends in the Trend Analysis 2016.
Food: In the years ahead the number of genetically modified food products on the store shelves will increase	The number of GM foods in the EU and NL has not increased. In fact, the numbers on the shelves has decreased rather than increased, despite a considerable difference in price between genetically modified and conventional commodities. This is because: • genetic modification is restricted to the bulk products of maize, soy, oilseed rape and cotton and these products are present in food products largely is processed forms; • Europe is self-sufficient in maize; • large amounts of GM soy are imported as animal feed; • companies are afraid to lose income because of the public's aversion to GMOs; • raw materials make up a relatively small proportion of the total costs of a consumer product; • GM products are therefore limited to the cheaper margarines, salad dressings and mayonnaise.
Animals: Products of genetically modified animals end up in pharmacies	At the moment it is not known whether or not there has been further growth in drugs produced by GM animals. A limited number of products have been allowed onto the European market. Other production methods (cell systems) seem to be becoming predominant.

Diagnostics: Strong increase of the opportunities for genetic diagnostics while treatment possibilities are lagging	The observed gap between the possibilities for diagnosis and the available treatment methods is still an issue. Also, the availability of 'self-tests' via internet is now a fact.
Ethnicity: Ethnicity as a factor in scientific research, genetic diagnostics and genetic screening	This topic has been taken up by the health minister and the profession.

Trend Analysis 2009

Trend	How has the trend developed?
1 Searching for the significance of the human genome	
X-omics and the $1000 genome	This trend comes up again in the Trend Analysis 2016. NGS is a fact in Dutch clinical genetics labs – thus far gene panels and whole genome sequencing for diagnostics, whole genome sequencing increasingly within reach, first steps to NGS in screening (NIPT) in the Netherlands. Steps towards the use of WGS in screening are also being made internationally. The dilemmas and recommendations are still relevant and even more urgent. • Privacy – consent • Right to know or not know • Advances in knowledge • Provision of information
Biobanks	The development of biobanks continues undiminished. Since the previous Trend Analysis, BBMRI has been set up and has grown into a European network of biobanks. The Netherlands is also a member via BBMRI-NL (www.bbmri.nl). There is a strong need to share data for genome analyses and update sequences in databanks for interpreting NGS results.[af] The dilemmas and recommendations are still relevant and even more urgent.
2 Possible applications in healthcare	
Personalised medicine	This trend comes up again in the Trend Analysis 2016. This development is continuing and becoming increasingly integrated into medicine. The issues raised in relation to marketing authorisation and the reimbursement of costs still apply without reservation.
Stem cells	The hype and public interest surrounding induced pluripotent stem cells (iPS) has subsided. Stem cells appear not to be living up to the initial overblown expectations, but research into stem cells has paved the way for many other developments, including bioprinting.
Gene therapy	This trend comes up again in the Trend Analysis 2016. Gene therapy is starting to bear fruit, but outdated legislation is hampering the exploitation of the opportunities gene therapy offers to patients and industry.
3 Industrial biotechnology: towards a biobased economy	
	The transition to a biobased economy is proceeding slowly but surely. Many of the issues are still relevant. The project committee feels the developments are not substantial enough to be considered a priority trend for inclusion in the Trend Analysis 2016.
4 Plant biotechnology: the divide between the EU and the rest of the world	
Rising prices	The price difference between GMO and non-GMO has increased and the predicted decline in the importance of the EU as a market has come about. This can be seen, among other things, in the fact that South American countries are no longer aligning their cultivation authorisations for GM crops to European import licences.
Inadequate legislation	This trend comes up again in the Trend Analysis 2016. EU legislation lags far behind the state of the technology. This situation has not changed at all during the intervening years. The European Commission is expected to publish a viewpoint on 'new techniques' at the end of this year.

af 27 428 Beleidsnota Biotechnologie [White Paper on Biotechnology] nr. 114. (2008). Letter from the State Secretary for Health, Welfare and Sport.

Monopolisation	The number of plant breeding companies is declining further. For the time being this has not led to a reduction in the supply of seeds and propagating material.
Patent law	The problem of protecting intellectual property rights by patents versus plant variety rights is a political issue in the Netherlands.
5 Biotechnology in animals: the advance of cloning	
	The Netherlands has decided to ban cloning, but products and progeny of clones may be imported. Although the cloning of sport horses only takes place outside the Netherlands, offspring from these clones can be found in the Netherlands. Various research groups (outside the Netherlands) are still working on cloning extinct animals (e.g. the passenger pigeon and the mammoth).