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**HUMAN GENETICS: AN INVENTORY  
OF NEW AND POTENTIAL  
DEVELOPMENTS IN HUMAN  
GENETICS AND THEIR POSSIBLE  
USES**

**Final Study**

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## BIOINFORMATICS

### 1. DESCRIPTION OF THE TECHNOLOGY

Bioinformatics is the combination of genomics and advanced computation. The vast amounts of information produced in recent years by the Human Genome Project (HGP) and other sequencing initiatives have generated the need for an informatics supported means of collecting and disseminating data. Furthermore, much of the promise of the current genetics and genomics research rests on moving from the study of single genes to understanding the interaction of multiple ones. The study of multifactorial diseases is a complex process that requires high-throughput technologies capable of generating and analysing large amounts of information. The sequencing, storage and retrieval of genetic data, together with new silicon-based genetic technologies have offered new possibilities for the understanding of the contribution of genes and proteins to health.

For instance, bioinformatics is portrayed as having significant implications for all phases of drug discovery. These technologies are supposed to contribute to drug discovery by finding targets for therapeutic intervention (e.g. receptors, enzymes); comparing gene expressions for those individuals with and without disease; determining the function of genes; identifying potential therapeutic compounds and guiding the organisation of chemical libraries used for drug identification. In these ways, bioinformatics is hoped to lower the attrition rate in drug discovery and help rationalise the drug development process.

Whatever the possibilities opened up by bioinformatics, research in this area is situated at the intersection of academic, government, and commercial interests, often combining multiple funding sources in particular initiatives. The large amount of financial and technical resources required to undertake much of bioinformatics related activity has required the creation of new research alliances and specialisations that span conventional boundaries between the public and private. This situation raises pressing questions about the how diverse and divergent strategies do and should come to bear on the production new therapies.

### 2. CURRENT STATE OF DEVELOPMENT

Bioinformatics refers to a number of highly inter-related technologies that are developing rapidly:

*Genetic sequencing:* Various techniques have been offered for locating specific genes and identifying the proteins with which they are associated. The different strategies for sequencing used in the HGP have led to a series of disputes about the accuracy of sequences between the US company Celera that has utilized whole genome shotgun methods and HGP funded researchers who have mainly used clone-by-clone methods.

*Combinatorial chemistry and high throughput screening:* Combinatorial chemistry has enabled the automation of the discovery process for finding new drug candidates through the creation of large libraries of chemicals. High throughput screening provides a rapid means of finding or screening compounds against various biological

targets. The recently developed so-called 'lab on a chip' technology enables researchers to carry out combinatorial and screening tasks simultaneously.

*Time-of-Flight Mass Spectrometry:* This technique allows for the rapid identification of DNA and relies on high-speed computational electronics and processors. In an effort to further reduce costs, firms are now combining mass spectrometry with lab-on-a-chip technology to provide high-throughput sample analysis for evaluating and characterizing drug targets and drug candidates. This has applications in proteomics, drug discovery, pharmacogenomics, and SNP technology.

*Genechips:* These are silicon devices that monitor the activities of thousands of genes and thereby generate indicators for complex multi-factorial conditions. Recent years have witnessed an intense interest in genechips and a widening of the range of gene targets that can be detected. The large amount of data produced by this technology in turn requires even more sophisticated information handling devices and programmes.

*Databases:* Information on both nucleic acid sequences and proteins have been stored in databases that allow for the retrieval, exchange and analysis of data.

Many of these bioinformatics hardware technologies and software programmes have developed over time in response to local circumstances. This historical development combined with the variety of data sources and formats currently in operation have created a situation in which biological data from multiple sources is often difficult to handle and compare (the question of interoperability). For instance, much debate is currently taking place regarding the most appropriate data language for browsing, publishing or analysing genetic information across the World Wide Web (e.g., HTML vs. XML). A number of bioinformatic companies specialise in providing tools designed to help access, manipulate and analyse data held by different organisations.

### **2.1 Dependent and related technology areas:**

Bioinformatics is a set of generic technologies that underpin a wide range of developments in automation, computational chemistry, genetics, genomics, proteomics and structural biology. As such its development both depends on and will influence a wide range of other technologies mentioned in this report including pharmacogenetics, gene testing, genetic health registers, pharmaceuticals, informatics and communication technologies (e.g., the next generation world-wide web technology called GRID).

## **3. KEY ACTORS**

### **3.1 Public, commercial and extent of interaction:**

A diverse range of bioinformatic-related organisations from university, charity, government and commercial settings exist. Such organisations operate as data suppliers, customers of programmes and services, or funders of research. Table 1 lists a number of the major organisations that contribute to the developments of bioinformatics.

**Table 1: Examples of major bioinformatics-related organisations**

<b>Major Public or Charity Funded</b>	<b>Commercially funded</b>
<ul style="list-style-type: none"> <li>· European Bioinformatics Institute (UK) under the European Molecular Biology Laboratory</li> <li>· Stanford Human Genome Center (US)</li> <li>· The Sanger Centre (UK)</li> <li>· Human Genome Mapping Project Resource Centre (UK)</li> <li>· Washington University, St. Louis (US)</li> <li>· Sylvius Laboratories, Leiden University Medical Center (The Netherlands)</li> <li>· Weizmann Institute of Science (Israel)</li> <li>· Whitehead Institute (USA)</li> <li>· Genbank (USA)</li> <li>· DDBJ (Japan)</li> <li>· SWISS-PROT (Switzerland)</li> </ul>	<ul style="list-style-type: none"> <li>Incyte Pharmaceuticals(US)</li> <li>LION (US)</li> <li>Celera Genomics (US)</li> <li>Genset (France)</li> <li>Oxford GlycoScience (UK)</li> <li>Large Scale Biology (US)</li> <li>GlaxoSmithKline (UK-US)</li> <li>Bristol-Myers Squibb (US)</li> <li>Rosetta Inpharmatics (US)</li> <li>Genomatix Software (Germany)</li> </ul>

Given the importance associated with bioinformatics as an underpinning technology, there are a number of companies working in this area. These range from large pharmaceutical firms to small dedicated informatics ones. The UK-based *The Bioinformatics Resource* lists over 65 bioinformatics-related companies in Europe and over 250 in the rest of the world (mainly in the US). Many of these are small or medium sized dedicated firms that write programmes or generate data.

As mentioned above, the scale and resources involved in bioinformatics often requires the creation of strategic alliances that cross public and commercial divides. Some of the most notable bioinformatics-related alliances include:

- *SNP Consortium* – A group of major drugs companies, bio-informatics firms, academics and the Wellcome Trust (UK) charity that are producing a SNP map of the human genome. See <http://snp.cshl.org/>.
- *Human Proteome Organisation* – Similar to the Human Genome Organisation in the HGP, HUPO has been set up to co-ordinate the large-scale identification of proteins.
- *COBRA* – A large non-profit consortium between software vendors and the EBI formed in 1997 to establish interoperable standards for accessing data.
- *BIO* – A group of largely US-based biotechnology and informatics companies, research charities and large pharmaceutical firms established in 2001 to create a standardised language for the exchange of data.

#### **4. PROSPECTS AND UNCERTAINTIES**

##### **4.1 Medium prospects (5-10 years):**

Within 5-10 years, it is likely that commercial organisations will achieve interoperability across their major R&D internal systems. Some of the most hoped for clinical applications associated with bioinformatics are unlikely to be achieved in the medium term though. Many have suggested that developments in bioinformatics will one day lead to the marketing of over-the-counter or doctor administered chip kits that can test for diseases and drug response. Even assuming that current difficulties associated with chip technology are satisfactorily resolved, and that the price of chips may go down significantly in the next few years, the process of interpreting genetic data is likely to be quite expensive and problematic. Many of the potential developments in bioinformatics might be arrested due to the lack of qualified personnel.

#### **4.2 Long term prospects (10-20+ years):**

The field of bioinformatics is developing rapidly and it is difficult to speculate on the long-term prospects of a technology that is situated at the intersection of two highly dynamic fields. The extent of introduction of bioinformatics tools into clinical settings is likely to be dependent on a range of wider social and ethical issues associated with the acceptability of genetic technology. The long term prospects of the field will also depend on the extension of bioinformatics-related education out of the confines of R&D activities.

#### **4.3 Known current uncertainties:**

Many of the existing concerns about the development of bioinformatics relate to limitations due to storage capacity, data complexity, network exchanges, and other such considerations. Available gene chips, for instance, are highly variable across model types and locations due to considerations such as the effects of temperature. Until further standardisation takes place, such technology is going to be limited in its application. The technical and organisational issues regarding interoperability pose basic uncertainties regarding the viability of this technology. Much of the current uncertainty about developments in the field relate to the shortage of qualified bioinformatics personnel. Making computational analysis and assessment part of the scientific process, requires a wide range of skills and knowledge across an increasing number of disciplines. Currently there is growing recognition of the need to further incorporate statistical knowledge with that already existing in bioinformatics. All of these considerations will have an important impact on the possibility of being able to understand and intervene in the interaction of multiple genes and proteins in the creation and so management of disease.

### **5. ELSI ISSUES**

As bioinformatics underpins many other technologies detailed in other sections of this report, it has the potential for exacerbating many of the ethical, legal, and social dilemmas that arise regarding particular facets of genetics. Key areas among these include:

- **Genetic Testing:** The further development of bioinformatics is likely to enhance the possibility and speed of the testing for multi-factorial genetic traits.
- **Biological Samples:** The full utilisation of bioinformatics depends on the analysis of large sets of biological samples. This issue raises important questions about informed consent, confidentiality, and the protection of information from third parties (e.g., police, legal, insurance systems). Bioinformatic technologies heighten such concerns by facilitating the exchange of large amounts of integrated data.

### **6. REGULATORY AND PUBLIC POLICY RESPONSE**

Given both the growing interdependence and continuing tensions between commercial and publicly funded organizations in bioinformatics research, there are a number of areas of policy tension. Some of the major ones include:

*Openness of research:* As with many areas surrounding genetics, the control over knowledge and intellectual property rights are important issues. The philosophy of

free access has directed much of the thinking behind publicly funded (and even some privately funded) work the HGP and genomics more generally. But such research is also seen as an investment that is supposed to produce a pay-off. There are various tensions then about how policy-makers should respond. The degree of openness of research will depend on a wide range of factors including the source of funding and accompanying terms and conditions for funding, the type of research and its applicability, the extent of exchanges between researchers as well as intellectual property and commercialisation policies.

*Proprietary/non-proprietary databases:* The range of actors active in bioinformatics means that the data sources compiled have been designed for differing purposes. In the past public domain databases have served as an important source of data and programmes. The growing proliferation of providers and financial costs of care taking and enhancing databases raise concerns over long term viability of non-proprietary databases. There are further questions about the desirability of some non-proprietary sources. So, despite the availability of free access to the map of the human gene, Celera's database has been under increasing demand by public and commercial actors due to perceptions of its user-friendliness and high quality. The costs of accessing it have, however, meant that, in the UK at least, those funded by the Wellcome Trust are required to use the public sector database available at the Sanger Centre in Cambridge.

*Skills shortage:* Bioinformatics is a demanding area requiring intense study across a number of disciplines. Some Member States (e.g., France, Germany, the UK) have established training programmes to alleviate existing skills shortage. Despite these initiatives, academic and other public sector based institutions are likely to struggle to retain staff due to more lucrative terms of employment in the private sector.

*Cost distributions:* While bioinformatics might be able to reduce the cost of drug discovery, it is more questionable whether it will lead to a reduction in overall health care costs. The incorporation of bioinformatics into clinical settings is likely to introduce significant costs related to the diagnosis of diseases and the interpretation of data.

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## GENETIC TESTING.

### 1. DESCRIPTION OF THE TECHNOLOGY

Genetic testing is usually accompanied by the process called ‘genetic counselling.’ This is the provision of information about the procedures, results and implications of a genetic test to patients. A core theme of genetic counselling is that it should be ‘non-directive’; i.e. the counsellor should not recommend any particular course of action to the patient (e.g. whether to be tested or not) but just provide information without directing the patient.

#### 1.1 Prenatal testing:

A variety of technologies are used in pregnancy to test the foetus for chromosomal abnormalities (e.g. Down Syndrome) and genetic diseases (e.g. Muscular dystrophy) (see Table 1). The invasive techniques involve some risk of inducing miscarriage, although developments like the use of ultrasound to guide the sampling have reduced the chances of this happening.

Some of these tests are routine; for example in the UK it is usual practice to offer pregnant women over 35 years the option of testing for Down syndrome (since it has a higher chance of occurring with an older mother). Other tests, such as those for genetic diseases, are only likely to be offered if there is a family history of the condition concerned.

With many of the conditions being tested for, there is no therapeutic option available should the test prove positive, and the only medical intervention open is a termination of the pregnancy. But in some cases, early diagnosis of a condition may allow clinical treatment to start at an early stage, and thus improve the child’s health.

#### 1.2 Postnatal testing:

Many genetic conditions can now be tested for using a simple blood test or even a saliva sample (see table 2). Although the actual mechanics of testing are similar for children and adults, over the years a different set of issues have grown up around each area.

*Testing children:* Testing children for diseases that onset during childhood is fairly straightforward. For example many countries test newborns for PKU, an inherited condition which can lead to mental retardation. It is easily treated through changes in diet, and although the disease is rare, the testing programme is itself generally seen a useful addition to public health.

Issues become more complex when testing is offered for conditions which will not become evident until the child becomes an adult (for example, testing a girl for the genes that lead to familial breast cancer). It is important that patients’ autonomy be respected, although this is harder in situations where legally, parents have a great deal of say over how their children get treated. It is important to note that in late onset conditions (e.g. Huntington’s disease), although adults might think it would be good to be tested to find out whether they will develop the condition, the numbers of people actually taking up this test has been far lower than expected. This suggests that adults who decide for their children to be tested ‘because they would want to know, when the grow up’ are perhaps out of step with how people at risk see testing.

**Table 1: Technologies for prenatal testing**

Technology	Time period	Details	Test for:	Invasive ?	Risk of Miscarriage
Ultrasound	18-20 weeks	based on reflection of sound waves	physical abnormalities of organs such as nervous system, kidneys, heart, gut and limbs	No	N/A
Amniocentesis	16-20 weeks	the removal of a small amount of amniotic fluid which contains foetal cells	spina bifida; chromosomal disorders (e.g. Down syndrome); metabolic disorders (e.g. PKU); DNA studies (many genetic diseases)	yes	0.5%
Chorionic Villus Sampling (CVS)	8-12 weeks	removal of cells from the edge of the placenta; yields a greater amount of DNA than the sampling of amniotic fluid	genetic conditions (e.g. muscular dystrophy, cystic fibrosis, Huntington's disease)	yes	1%
Foetal Blood Sampling	18+ weeks	removal of foetal blood from umbilical vein; useful when other studies not available, produces rapid results, though technically difficult	genetic conditions	yes	1-2%
Maternal blood sampling	18-20 weeks	tests mother's blood for traces of foetal cells; measures levels of hormones associated with different conditions; used to decide whether other techniques should be used	spina bifida; Down syndrome	no	N/A
'Oscar' screening	12 weeks	Experimental combination of ultrasound, maternal serum screening; allows earlier screening for Down syndrome	Chromosomal abnormalities	no	N/A

**Table 2: Examples of genetic diseases tested for.**

Alpha-1-antitrypsin deficiency (emphysema and liver disease) Amyotrophic lateral sclerosis (Lou Gehrig's Disease; progressive motor function loss leading to paralysis and death) Alzheimer's disease* (APOE; late-onset variety of senile dementia) Gaucher disease (enlarged liver and spleen, bone degeneration) Inherited breast and ovarian cancer* (BRCA 1 and 2; early-onset tumors of breasts and ovaries) Cystic fibrosis (disease of lung and pancreas resulting in thick mucous accumulations and chronic infections) Duchenne muscular dystrophy/Becker muscular dystrophy (severe to mild muscle wasting, deterioration, weakness)	Factor V-Leiden (blood-clotting disorder) Fragile X syndrome (leading cause of inherited mental retardation) Hemophilia A and B (bleeding disorders) Huntington's disease (usually midlife onset; progressive, lethal, degenerative neurological disease) Myotonic dystrophy (progressive muscle weakness; most common form of adult muscular dystrophy) Neurofibromatosis type 1 (multiple benign nervous system tumors that can be disfiguring; cancers) Phenylketonuria (progressive mental retardation due to missing enzyme; correctable by diet)	Adult Polycystic Kidney Disease (Kidney failure and liver disease) Sickle cell disease (blood cell disorder; chronic pain and infections) Spinocerebellar ataxia, type 1 (involuntary muscle movements, reflex disorders, explosive speech) Spinal muscular atrophy (severe, usually lethal progressive muscle-wasting disorder in children) Thalassemias (anemias - reduced red blood cell levels) Tay-Sachs Disease (fatal neurological disease of early childhood; seizures, paralysis)
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*Testing adults:* Counselling of adults should ensure that they understand the implications of being tested: there are psychological aspects (such as fatalism towards genetic conditions which can be treated) as well as the social impact of being diagnosed with a genetic condition (e.g. employment prospects). Genetic information by definition tells us about our family, and the result of a genetic test may reveal unforeseen facts about family history (e.g. non-paternity).

*Carrier testing:* This is an issue in families with a history of a specific condition, where people may want to find out if they carry the gene for that disease. This means that although they will not develop the disease themselves, they can pass the gene on to a child who may inherit a second copy from another parent and thus develop the condition. This gives people information for reproductive decisions (whether to have children or not, or to test one's partner to see if they are also a carrier).

### **1.3 Types of conditions:**

*Chromosomal abnormalities:* These conditions are caused by errors at the level of the chromosome (the large blocks of genetic material in the nucleus of the cell: humans have 46 of them in 23 pairs). Sometimes there are more chromosomes than normal (e.g. Down's syndrome which is caused by having 3 copies of chromosome 21), in other conditions there are smaller changes.

*Mendelian Disorders:* These diseases are inherited in a predictable way, according to Mendel's laws of inheritance. Recessive conditions such as Cystic fibrosis or muscular dystrophy only become apparent if someone inherited two copies of the faulty gene. Dominant conditions like Huntington's disease can affect you if you only inherit one copy of the gene concerned. Although these diseases are well studied, they are relatively rare and do not affect the majority of the population.

*Complex multifactoral Diseases:* These conditions are caused by a mixture of genetic and environmental factors. Many of the biggest diseases are included in this category, such as heart disease, diabetes, cancer and schizophrenia. Over the past few years scientists have begun to unravel the complex relationship between certain genes, the environment and diseases (e.g. the p53 oncogene and cancer causation). Although genetic testing for these conditions provides some information and may suggest a predisposition to develop a condition, it would be wrong to say that such a test determined whether one will develop the disease or not. For many of these disease, non-genetic factors are just as important.

## **2. CURRENT STATE OF DEVELOPMENT**

The genetic conditions tested for at any single clinic varies, but there are a large number of possible conditions which can be tested for (see Table 2 for examples); Online Mendelian Inheritance in Man (OMIM), lists over 12,000 entries for genetic diseases, with 9,000 of these being to established gene positions. Many of these are for rare single gene disorders affecting very few people, but developments in genetic technologies mean that increased information is being gathered on more common conditions.

## **2.1 Dependent and related technology areas:**

*Gene chips / DNA arrays:* these allow one to test a single DNA sample for hundreds of different genetic conditions. This will bring down the cost of genetic testing and make testing for complex disorders, which may involve a number of different genes, far easier.

## **3. KEY ACTORS**

### **3.1 Public, commercial and extent of interaction:**

Although most national health systems provide some forms of genetic testing, commercial interest in this area is high. Industry is involved in developing genetic testing kits and marketing them to health services. Companies have also been begun selling genetic testing services directly to the public which has raised concerns over the degree of counselling provided in such 'over the counter' situations.

Industry's main involvement in genetic testing is through the patents for genes that a number of companies hold. This means that companies have a degree of control over the services offered by national health services. For example, in the UK, breast cancer genetic screening is offered for the BRCA1 gene (which causes a rare form of familial breast cancer). Recently, the patent holders for the gene (Myriad genetics, a US company) have licensed commercial testing to a UK firm (Rosgen Ltd). There was the possibility that Rosgen (as license holders) could have sued the NHS for using the BRCA1 test; the company and the NHS are currently in negotiation to reach a settlement whereby the NHS can continue to provide testing for this gene.

### **3.2 Others (gov R&D, advocacy organisations):**

Charities representing people with genetic diseases are often involved in genetic research by the donation of material and the raising of research funds. They are also involved in the public and policy discussions that surround genetic testing and are represented by umbrella groups such as the European Alliance of Parent and Patient Organisations for Genetic Services and Innovation in Medicine (EAGS). The EAGS has been involved in lobbying both the European Parliament and Commission on the Directive on the Legal Protection of biotechnological inventions and the Convention on Human Rights and Biomedicine.

## **4. PROSPECTS AND UNCERTAINTIES**

### **4.1 Medium prospects (5-10 years):**

Over the next five years, the number of genes linked to common disorders will increase, and scientists' understanding of how genes and environmental factors interact to cause disease will begin to improve. The use of genetic testing for common conditions will develop in the specialist clinic, with testing for monogenic disorders developing as well. Whether the market for commercial testing develops will depend upon the availability and range of tests offered by national health systems.

#### **4.2 Long term prospects (10-20+ years):**

The visions painted by advocates of genetic testing suggest that further into the future, genetic testing will become commonplace as a normal part of the clinical encounter. Individuals' medical records (perhaps in the form of an electronic health card) will contain all relevant genetic information with regard to both specific genetic conditions and complex diseases which have a significant genetic component. People will be able to structure their lifestyle around predispositions to diseases (for example low fat diets for those predisposed to heart disease).

#### **4.3 Known current uncertainties:**

The current provision and understanding of genetic testing is largely based on very genetic conditions (usually Mendelian, single gene disorders) which are inherited in clearly predictable ways. Even these 'simple' disorders are complicated by genetic variation (e.g. Cystic fibrosis being caused by any one of over 1,000 possible mutations in the same gene). It is not clear that the genetic component of complex, multifactorial conditions such as heart disease, sporadic cancers and schizophrenia can be identified to the degree required to allow genetic testing. Even if such tests can be shown to be accurate, it is not clear how to incorporate them into clinical practice: should testing continue to be provided by specialist centres, or should it be incorporated into general practice. Do general practitioners know enough about genetics to provide a suitable context for testing?

### **5. ELSI ISSUES**

- **Informed Consent:** patients need to be given accurate information about nature of genetic test and possible implications before making a choice.
- **Non-directiveness:** usually held that genetic counselling should not recommend particular choices to patients, but some suggest that simply offering a test implies that testing (and abortion if relevant) are the 'right' choices.
- **Testing children:** while testing for diseases which have an onset during childhood makes sense, evidence is not clear about the benefit of testing children for diseases which will not take hold until adulthood. Option is always open to children to choose testing when they grow up.
- **Commercial involvement in testing provision:** are genetic tests marketed directly to the public offered with genetic counselling? What information is supplied to customers?
- **Commercial restrictions on testing provision:** as pointed out during the debates over allowing the patenting of genes, companies with rights over genes can prevent testing for that sequence, even if it is being done on a not for profit basis.
- **Confidentiality:** are the results of genetic tests kept confidential? Are third parties (insurance companies, employers) allowed access to results? There is general resistance among policymakers to allowing insurers to have access to the results of genetic tests. Many states in the US have adopted 'genetic privacy' acts which legally prevent insurers from asking for the results of genetic tests.
- **Eugenics:** Some people claim that that use of prenatal testing is eugenic since it is preventing certain kinds of people from being born, yet the context is very different from the state-sponsored eugenics of the early/mid 20<sup>th</sup> Century.

- Discrimination: Some people with disabilities suggest that prenatal diagnosis and abortion discriminate against them since these technologies imply that people with disabilities shouldn't be born.
- Justice: if genetic screening is only available to those who can afford to pay for it then over time genetic diseases will tend to occur among those sections of the population who have less money.

## **6. REGULATORY AND PUBLIC POLICY RESPONSE**

### **6.1 Bioethics convention:**

Several articles of the convention are relevant to the regulation and provision of genetic tests. Article 11 prohibits any discrimination against a person on the grounds of their genetic heritage, and thus prohibits the use of genetic tests by insurers or employers. Article 12 refers to the need for genetic testing for disease to be performed only for health purposes or related scientific research, and subject to 'appropriate genetic counselling.' This may reflect on the provision of commercial testing, requiring companies to provide a counselling service.

### **6.2 Medical Devices Directive:**

Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices regulates genetic tests in terms of quality control. Its main requirement is to ensure that tests are manufactured to certain standards, but does not comment on the use of such tests.

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## GENETIC DATABASES/FUNCTIONAL GENOMICS/ASSOCIATION GENETICS

### 1. DESCRIPTION OF THE TECHNOLOGY

The completion of the first phase of the Human Genome Project in February 2001 marked the start, not the end, of a major programme of research, which is likely to occupy the biological sciences for decades to come. The next stage of investigation will be to understand exactly what the information coded in the human genome means and how this new knowledge might be used to improve health and healthcare. At present, the biology of the great majority of the estimated 30,000-45,000 genes that have already been sequenced is unknown and until the function of a particular gene and its role in pathology has been established the raw sequence data is of little clinical value. Work is required to link gene sequences (genotypes) to particular biological functions or diseases (phenotypes). This area of research has become known as functional genomics and is one of the most rapidly expanding areas of molecular biology.

Traditionally, researchers have worked 'backwards', starting with the identification of an inherited pattern of a disease and then trying to find the genetic changes responsible for that condition. Family studies of this sort have been routinely undertaken by clinical geneticists, and include research on rare single gene disorders, familial cancers and familial forms of other common diseases. In these cases, where the inheritance of the disease is well characterised, relatively small numbers of subjects/DNA samples are required for the analysis and little additional clinical information is needed beyond a positive diagnosis for the condition.

However, for most common diseases it is difficult to demonstrate a simple pattern of inheritance and the biological changes responsible for the pathology are often not well characterised. To identify disease susceptibility genes for these disorders, biological samples from large, extensively and consistently characterised populations are required. Where a pattern of inheritance can be established, genetic linkage studies are carried out comparing the pattern of inheritance of short DNA marker sequences to the pattern of inheritance of the disease within the population studied. Where no pattern of inheritance is obvious, a large collection of human biological samples is needed to carry out genetic association studies. In this approach a statistical correlation is made between specific DNA sequences and particular diseases, in the hope of identifying particular single base pair changes or single nucleotide polymorphisms (SNPs) that closely correlate with the disease being investigated. In effect the SNPs act as landmarks amongst the genome. In the case of many common diseases, which might only have a small genetic component, it is perhaps more useful to think of genes as being risk factors. If a person has a particular genotype they may be at greater risk of getting a disease than people not carrying that specific genetic change. It should also be noted that the same broad approach can be used to study the genetic basis of drug response (see section on pharmacogenetics).

As increasing knowledge is gained of human genetic variations and genes associated with particular diseases, it will become increasingly possible to analyse the role which genetic risk factors and specific environmental hazards play in the cause of common conditions such as cancer or heart disease. The study of the interaction between genes and environment – genetic epidemiology, will also require the use of large population

based sample collections and access to detailed patient information. Large genetic epidemiological databases are a potentially valuable research resource that can be used to study: the natural history of diseases including onset and severity in multiple populations, the interactions of susceptibility genes with each other and environmental factors, the impact of various health interventions on the onset and course of the disease, and the effectiveness of preventative therapies for disease.

## **2. CURRENT STATE OF DEVELOPMENT**

A small number of both publicly funded and private sector genetic databases are under construction in Europe. The most well known example of this type of research resource is the Icelandic Health Sector Database, which is being financed by and exclusively licensed to deCODE Genetics, an Icelandic biotechnology firm. Iceland provides a good location for genetic studies as it has high quality genealogical records and a geographically isolated and relatively homogenous population, allowing the construction of extended family trees and making the genetic component of a disease much easier to spot. To undertake the analysis of common diseases, deCODE has established two core technologies: a computerised genealogical database – containing records on over 600,000 Icelanders (living and dead) and high-throughput genotyping in order to scan DNA samples using large numbers of genetic markers. The Health Sector Database will consist of electronic records on almost all of the current Icelandic population of 250,000 and will contain data taken from their medical records. This will be linked to the genealogical data and the results of genotyping tests, and will enable both genetic linkage and association studies of the entire population. Although the database has not been fully constructed deCODE has already been successfully using this strategy to carry out genetic research and claims to have found disease susceptibility genes associated with stroke, diabetes, osteoporosis, and Alzheimer's. This information can then be used to develop new diagnostic tests and therapies.

The controversial project has provoked fierce debate in Iceland and around the world. In particular concern has focused on the security of the database, the possible misuse of the data, the need for oversight of research, and the fact that a private company has a monopoly over the use of the database. However, the most contentious issue has been the fact that information on all Icelandic citizens will be included in the database unless they explicitly opt out.

Several other large genetic databases are also being established by public sector organisation. In Britain the Medical Research Council and the Wellcome Trust are developing a study that would involve collecting genetic, health and lifestyle data over a number of years from 500,000 volunteers in an attempt to examine the relative contributions of genetic and environmental factors in diseases of mid-life. A similar database is also being created in Estonia and will cover more than 70% of the country's population of 1.4 million.

In addition to the construction of very large population databases a number of biotechnology companies and academics are also collecting DNA samples and clinical information from smaller groups from families and patients suffering from particular diseases. For example, the UK based company Oxagen is looking for genes involved in coronary artery disease and is collecting samples from over 2,000 affected families in four different European countries.

As researchers try to understand the relationship between genes and disease, increasing use will be made of strategies and resources, which bring together genetic, clinical and personal data. This will inevitably mean closer ties between basic and clinical research and a blurring of the boundary between the public and private sector. Furthermore, the information used and produced by studies of this kind will become increasingly valuable, creating a growing commercial market for genetic data about populations and individuals. Important social, ethical and legal issues are raised by the emergence of this market and how it should be governed.

### **2.1. Dependent and related technologies:**

*High throughput genotyping:* the ability to rapidly sequence or screen very large numbers of DNA samples for particular markers (e.g. SNPs). The speed of genotyping is increasing very rapidly at present, whilst the cost is falling sharply.

*SNP mapping:* a grouping of major drugs companies, academics and the charity *The Wellcome Trust* (the SNP Consortium) is in the process of producing a publicly available high density SNP map of the human genome. This will greatly facilitate association genetic studies and the identification of disease markers.

*Diagnostics:* by linking a particular genetic variant with a given disease it may be possible to develop pre-symptomatic genetic tests.

*DNA arrays/Gene Chips:* will be required to allow doctors to quickly identify which genes and gene variants a person carries. Although currently widespread in genomic research, their clinical use is still rare.

*Pharmacogenetics:* the identification of DNA markers that can predict an individual's response to a medicine.

## **3. KEY ACTORS**

### **3.1. Public, commercial and extent of interaction:**

The biotechnology and pharmaceutical industry will be at the forefront of developing genetic databases, given the very large capital investment required. Table 1 describes some of the leading European dedicated biotechnology firms working in this area. In addition, many leading pharmaceutical companies are already collecting large numbers of DNA samples from participants in their clinical trials of new medicines in order to understand the genetic basis of drug response. Very close academic-industry links are a general feature of research in the area of human genetics and whilst this enables effective technology transfer, it also gives rise to concerns about academic conflicts of interest and the extent to which biological samples donated for public research are being used for profit. However, it must be stressed that these private sector activities depend heavily on public funded research and widespread public participation. It is therefore difficult to disentangle public and private research, as scientists from both sectors are often involved in supporting the same project.

**Table 1: Leading European firms involved in association genetics and creating genetic databases**

<b>Company</b>	<b>Location</b>	<b>Type of sample collection</b>
Decode Genetics	Iceland	Large database covering entire population. Samples taken from particular patient groups
Gemini Genomics	UK	DNA from twin studies and creation of population database in Canada
Genset	France	Samples collected from affected families by collaborating clinicians in a number of countries
Oxagen	UK	Samples collected from affected families by collaborating clinicians in a number of countries

### **3.2. Others (government R&D, advocacy organisations):**

An increasing number of medical research charities becoming involved in funding the development of DNA ample collections and genetic databases. They are also helping researchers get access to the patient groups they represent.

## **4. PROSPECTS AND UNCERTAINTIES:**

### **4.1 Medium term prospects (5-10 years):**

It is likely that many genes and genetic markers associated with common conditions, such as cancer, heart disease and neuro-degenerative disorders, will be identified in the next decade by both public and private researchers. In a number of cases these will be used to develop diagnostic tests to identify people at high risks of becoming ill e.g. the increased risk of women getting breast cancer from carrying the BRCA I or II mutations. The ability to identify genes directly involved in disease will also provide a new set of drug targets for which the pharmaceutical industry will seek to develop new medicines.

### **4.2 Long term prospects ( 10-20+ years):**

Once a diagnostic test based on statistical association can be validated and linked to improved medical outcomes, then it is likely to be rapidly integrated into routine clinical practice. In the long term the availability of a large number of such tests is likely to lead to significant improvements in people's health and healthcare, with increasing emphasis placed on health promotion and prophylactic treatment. A greater understanding human genetic polymorphisms (i.e. the genetic variation within and between populations) will also lead to a greater understanding of the genetic basis of the response to drugs and other forms of therapy. New classes of pharmacogenomics drugs might also be developed, targeted at people with specific genotypes.

### **4.3 Known current uncertainties:**

One of the major problems with using statistical correlations as the basis for predicting people's likelihood of getting a particular diseases is the extent to which these associations can be shown to be of value in the clinic. The rate of introduction of these tests will therefore be limited by the speed and ability of these statistical associations to be validated in clinical practice by prospective and other types of epidemiological study. There is also likely to be a low take up of such tests if their results cannot be linked directly to improved treatment and care, as is currently the case with tests for Huntington's disease. In addition, the construction and use of large human genetic databases raises many questions regarding new forms of property rights, the

relationship between academia and commerce, as well as critical issues of human rights. Furthermore, the use of biological sample collections cannot easily be separated from the use of medical records and data about individuals and family relationships. The adequacy of the current legal framework in many countries to adequately deal with the complex issues of the ownership of biological materials/ genetic data, and patient confidentiality and consent, is open to question.

## **5. ELSI ISSUES:**

- Consent of research subjects and the use of data/materials collected. At the time of sample collection is it possible to foresee all the potential research applications that the database may be used for? Is it possible to give informed consent given the complexity of genetic research? Do different forms of consent need to be obtained for different uses?
- Confidentiality and security of medical information, privacy and data protection. Is it possible to integrate large databases of anonymous genetic, medical and family information in such a way that confidentiality can be maintained?
- The value of pre-symptomatic testing where there is no treatment available;
- Third party access to materials and medical information. Should medical information be routinely used in research without obtaining consent? On what basis should access be granted to third parties?
- Ownership (patenting) of human tissues/genes. Important questions remain unanswered about the social acceptability of the private ownership of gene patents, and the impact this might have on scientific research, innovation and the costs of new medical technologies;
- The commercial exploitation of biological sample collections. When tissue samples are donated freely by participants in research studies there may be objections to the subsequent use of these resources for profit;
- The potential for discrimination as a result of the misuse of the personal information contained in genetic databases;
- The social acceptability of the research being undertaken with personal samples and data, especially in controversial areas such as mental health, behaviour and race;
- Oversight and governance mechanisms. How will the creation and operation of genetic databases be controlled to ensure that companies and researchers behave in an ethically and socially acceptable manner?
- The use of genetic databases established from medical research by the police and criminal justice system;

## **6. REGULATORY AND PUBLIC POLICY RESPONSE:**

### **6.1 EU:**

*The European Union Directive 95/46/EC:* on the protection of individuals with regard to the processing of personal data and on the free movement of such data. All genetic databases in the EU will have to operate within these guidelines.

*Opinion of the European Group on Ethics in Science and New Technologies to the EC: No. 11: Ethical Aspects of Human Tissue Banking ( 1998).* Recommendations included the importance of preventing possible discrimination, the importance of confidentiality, consent and the need to provide maximum health benefits.

## **6.2 International:**

*HUGO Ethics Committee: Statement on DNA Sampling – Control and Access (1998):* Recommended that there should be no disclosure to third parties of an individual's participation in a research project and that security measures should ensure that desired levels of confidentiality are respected. Considered the international standardisation of the ethical requirements for the control and access of DNA samples and information to be essential.

*UNESCO: Universal Declaration on the Human Genome and Human Rights (1999):* Recommendations concentrated on the importance of consent, confidentiality and international co-operation. Describing the Human Genome as “the heritage of humanity”, UNESCO also specified that the human genome in its natural state should not give rise to financial gain.

*World Health Organisation: Proposed International Guidelines on Ethical Issues in Medical Genetics and Genetic Services (1995):* Adopted the position that existing stored specimens or samples, such as those in university or hospital departments, should not be subject to new rules for consent that may be established in the future. Also propose that blood relatives should be entitled to access a sample for the purpose of learning their genetic status, but not the donor's genetic status.

## **6.3 National:**

*Icelandic Legislation relating to the Health Sector Database:* Law passed establishing the licensing arrangements for the operation of the database, data protection measures, a regulatory body, an ethical oversight committee and sanctions if the terms of the license are breached.

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## PHARMACOGENETICS AND PHARMACOGENOMICS

### 1. DESCRIPTION OF THE TECHNOLOGY

Pharmacogenetics (and its more modern equivalent pharmacogenomics) is the study of the genetic basis for drug metabolism. For over 60 years scientists have known that for some pharmaceutical products, there are variations in drug reaction based on underlying genetic factors. There may be certain people for whom a drug does not work; doctors will then move them onto another drug. For example up to 7% of whites in the USA cannot take codeine as a pain killer, since their bodies do not produce the enzyme required to break it down into morphine in the brain and give it its analgesic effect. In addition, a small number of patients will have serious Adverse Drug Reactions (ADRs) with certain drugs which may lead to hospitalisation or even death: a recent survey suggest that ADRs are between the 4th and 6th biggest killers in the US.

Research into pharmacogenetics aims to spot those genes associated with ADRs and non-reaction and allow doctors to prescribe alternative drugs to patients. There are also more subtle aims, such as altering drug dosage to fit particular people's metabolisms. Traditionally pharmacogenetics has been a hit and miss affair, with genetic drug reactions only being spotted once they had occurred, but a more modern approach (pharmacogenomics) uses the results of the human genome project and its attendant technologies to provide a systematic search for those genes related to reactions to different drugs. Most recent interest in pharmacogenomics centres around Single Nucleotide Polymorphisms or SNPs, which are very small differences between individuals' genetic make-ups. There are roughly 500,000 SNPs in coding regions of the genome, and the hope is that certain SNPs will be linked to reactions to particular drugs. Since there are a large number of SNPs, the hope is that they will provide a 'map' of the genetic basis to drug reaction.

Pharmaceutical companies are interested in this technology for a number of reasons. They hope that pharmacogenomics will reduce the cost of developing drugs since they will be able to screen the subjects of a clinical trial beforehand, removing those people who are likely to have a negative reaction to the drug from the trial. This will get products through trials more quickly, and reduce the chances of trials for a particular drug having to stop because of ADRs. Although a drug tested like this would be prescribed to a smaller number of people than before (since some potential users will be ruled out on pharmacogenetic grounds), companies hope to make money from the increased efficacy of their products (thus better sales) and the sale of gene tests for reactions to their drugs. Another boost for the companies is the so called 'Lazarus' effect, where drugs that failed the safety aspects of clinical trials are resurrected for specific populations, recouping the money originally invested in them.

### 2. CURRENT STATE OF DEVELOPMENT

There are a number of different genes which are known to affect people's reactions to drugs (see Table 1). The most well known are the genes which encode for a family of enzymes associated with the liver, the cytochrome P-450 enzymes. These are directly involved in drug metabolism and one enzyme in particular, CYP2D6 (or debrisoquine hydroxylase) is extremely variable in its genetics (polymorphic). One version of the gene for CYP2D6 inactivates this enzyme and means that the people who carry it are 'poor metabolisers'; i.e. their bodies break down drugs at a much slower rate than

most people. They are therefore at a higher risk of adverse drug reaction. Another version of the gene for CYP2D6 amplifies drug metabolism, leading to 'rapid metabolisers' whose bodies break down drugs so quickly that they may have to be prescribed many times the normal dose of a drug to gain any therapeutic effect. Although CYP2D6 polymorphisms do not effect all drugs, they are relevant in the case of many products (such as Prozac), used to treat psychiatric and neurological conditions as well as some drugs for cardiovascular disease. Since between 5-10% of the population can be described as slow metabolisers, and 1-10% as rapid metaboliser, CYP2D6 could be seen as a serious public health issue. Some commentators suggest that we now know enough to have population-wide screening for well-studied polymorphisms (such as CYP2D6) to reduce the chances of ADRs and misprescription of drugs.

Already in some centres, genetic testing is routine when certain drugs are going to be prescribed: for example testing for TPMT in cases of childhood leukaemia. Although wholesale searches of the genome for pharmacogenetic genes has only just begun, the testing of people for fast/slow metaboliser status would be possible and some claim that this would significantly reduce the occurrence of ADRs.

As well genes for drug metabolism, there are some genes involved in the actual progression of disease (receptors/drug targets). For example, in Atherosclerosis, the gene that predicts good response to prevastatin also predicts a poorer prognosis. Thus if you genotype patients to determine whether they should be treated with prevastatin or not, you are also giving them a more conventional disease genetic test, with the ethical implications that involves.

**Table 1: Current examples of pharmacogenetic reactions**

Disease	Gene	Drug	Reaction
Atherosclerosis	cholesterol ester transfer protein (CETP)	prevastatin	Mutation TaqIB, associated with greatest risk of heart attack also associated with most positive response to treatment.
Ovarian cancer	p53	common anti-cancer drugs	p53 makes tumours chemoresistant: more aggressive treatment required.
Asthma	ALOX5 promoter locus	ABT-761	Carriers of certain variations in gene showed improvement.
Childhood leukaemia	gene for TPMT	Antitumour agents (6-mercaptopurine/6-thioguanine)	Children with TPMT deficiency require much lower doses to avoid toxicity. Those with 'over active' TPMT require higher doses for effect.
Schizophrenia	Serotonin neurotransmitter receptor 2A	clozapine	Certain alleles (carried by ~50% of pop.) lead to improved response to drug.

### 2.1 Dependent and related technology areas:

*DNA arrays/Gene Chips:* these are required if the testing of patients is to become regular and commonplace. These would allow doctors to quickly ascertain which genes a person carries and which drugs they should not be prescribed. Although currently widespread in genomic research, their use in clinical situations is still rare.

*DNA databanks:* To generate their SNP-maps of drug reaction, companies need samples of DNA linked to clinical information concerning drug reactions. Many

companies are already taking blood samples from participants in their clinical trials to store in case future pharmacogenetic work needs to be done and others have links to smaller biotech firms which have DNA databanks. Best practice in this area involves specific consent for the DNA sampling, as well as the general consent for the clinical trial.

### 3. KEY ACTORS

#### 3.1 Public, commercial and extent of interaction:

*Pharmaceutical industry:* sees pharmacogenomics as the way forward in terms of clinical trials and drug development (see table 2).

**Table 2: Examples of Industry Involvement**

<b>Dedicated Pharmacogenomics firms operating in Europe</b>	<b>Major European Pharmaceutical firms adopting pharmacogenomics</b>
DeCODE Genetics	Amersham Pharmacia
Gaifar	AstaZeneca
Genset	Bayer
ExonHit	GSK
Oxford Glycosystems	Hoffman-La Roche
Gemini Genomics	Novartis
Oxagen	

*SNP consortium:* a grouping of major drugs companies, academics and the charity *The Wellcome Trust*. Has sequenced a SNP map of the human genome. All information is released in to the public domain; project due to finish at end of 2001.

*Public Academic researchers:* have been involved in discovery of individual pharmacogenetic links and may be involved in industry research.

*Public health providers:* limited involvement in pharmacogenomics to date. Some screening for certain pharmacogenetic response in certain countries, but no widespread policy as yet.

### 4. PROSPECTS AND UNCERTAINTIES

#### 4.1 Medium term prospects (5-10 years):

Supporters of pharmacogenomics suggest that within 5 years testing may be used in general practice as well as being common-place in specialists centres ( e.g. cancer treatment clinics). Clinical trials may also have changed, with their participants being genotyped beforehand to select those who are least likely to react negatively with the drug being tested. More extreme predictions suggest that populations as a whole will have a ‘one off’ genetic test which will provide a personalised SNP-map. This will be stored in their medical records and will inform doctors of prescription choices in the future. By the end of this period, we might expect a number of drugs designed to fit particular genetic targets to be coming through clinical trials and onto the market.

#### 4.2 Long term potential (10-20+ years):

If the predictions about pharmacogenomics are right, then this technology has the potential to reshape the way in which pharmaceutical products are designed, tested and prescribed. It may significantly reduce healthcare costs by ensuring accurate prescription and reducing the number of adverse drug reactions which have to be treated. At the same time, pharmacogenomics will involve wholesale expansion of genetic testing, with a great deal more genetic information being available. Some of

this data will be relatively innocuous (P450 status for example), but other pharmacogenomic information will relate to disease genes and possible prediction.

#### **4.3 Known current uncertainties:**

Although a number of individual drugs and conditions are known to have pharmacogenomic aspects, it is not clear that such links will be relevant to anything but a limited number of drugs. This would restrict industry interest in pharmacogenetics. Uncertainty also surrounds the use of SNPs, since it is not clear how many need to be searched through to provide evidence of a pharmacogenomic link. It may be the case that many more SNPs are required than currently thought; this would slow down the production of accurate pharmacogenomic SNP-maps. Finally, there is doubt over whether pharmacogenomics will speed up clinical trials and reduce their cost since some commentators claim that the numbers of people enrolled in such trials would need to be increased significantly if genetic information relevant to the population at large is to be gathered.

### **5. ELSI ISSUES**

- Informed consent in clinical trials: pharmacogenetics adds another element of complexity to clinical research. Participants must feel free to enter into trials for drugs without being obliged to donate DNA for additional pharmacogenetic research.
- Overlap between pharmacogenetic testing and disease gene tests: genetic counselling, informed consent, right not to know, family aspects.
- Potential expense of pharmacogenetic drugs, and subsequent reaction of health services and insurers.
- Exclusion of certain groups from healthcare if they react to a large number of drugs and alternatives are not economic to produce.
- Role of ethnicity in pharmacogenetics since some drug reactions are higher in specific ethnic groups.
- Possible stigmatisation
- Dangers of off-label prescription increased if certain groups guaranteed to suffer ADRs with a specific drug.

### **6. REGULATORY AND PUBLIC POLICY RESPONSE**

#### **6.1 EU Regulations:**

As yet, there are no regulations which specifically refer to pharmacogenetics, but there are those which relate to some aspects of it. For example, in terms of European Union Directives, Directive 2001/20/EC on clinical trials requires that participants give full informed consent and that such research is approved by an ethics committee. Inclusion of genetic testing in such trials raises issues to do with added informed consent and extending information provided to ethics committees. The EU's drug assessment agency, European Agency for the Evaluation of Medicinal Products (EMA) has discussed pharmacogenomics with industry. It has not yet decided to allow incorporation of pharmacogenomic information into clinical trials, but accepts in principle the value of such results.

## 6.2 The Bioethics Convention:

The Bioethics convention has a number of articles which might relate to pharmacogenetics. Article 3 requires signatories to ensure 'equitable access to health care', which may affect the role of pharmacogenetics in stratifying patient populations. This does seem to imply that no groups should be denied any drugs at all on the strength of their genotype. Article 10, on private life and right to information suggests both that confidentiality concerning pharmacogenetic information needs to be respected, and that people have to right to know any information about their health. This means that the current policy in clinical trials of DNA typing participants, but *not* feeding the results of the genetic test back to the participants, is in contravention of this article. In addition, articles 11 (Non-discrimination on genetic grounds) and 12 (Predictive genetic testing) both obviously relate to aspects of pharmacogenetic testing.

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## NEW REPRODUCTIVE TECHNOLOGIES RELATED TO GENOMICS

### 1. DESCRIPTION OF THE TECHNOLOGY

These are a range of different techniques aimed to help infertile people have children. Because of the nature of this report, this section will focus on those technologies which relate to the new genetics, rather than the broad range of techniques (such as various forms of surgery) which deal with infertility per se.

#### 1.1 InVitro Fertilisation (IVF):

InVitro Fertilisation (IVF) is the original 'test-tube baby' technique pioneered by Steptoe and Edwards, resulting in the birth of Louise Brown in 1978. It involves the removal of eggs from the mother, their fertilisation by the father's sperm in a test-tube, and their transfer to the mother a few days later. It is appropriate in cases of female infertility where, for example, the fallopian tubes are damaged, or a woman suffers from endometriosis, or cases where the male partner has a low sperm count. There are various developments of the technique which have occurred over the past few years (Table 1), but the basic approach has remained constant, with a success rate of around 17%.

To date, over 300,000 children have been born world-wide using IVF and related techniques. There is no conclusive evidence of physical harm or abnormality in IVF children, in fact, they may have a lower rate of conditions such as Down syndrome. One concern in the early years was that these children would be emotionally harmed because of the way in which they were conceived. The most recent research on this suggests that children resulting from IVF are emotionally unaffected by the way in which they were conceived, and that their families display high levels of stability, emotional warmth and appropriate discipline and control. One of the elements of IVF which has raised issues in the past is that because of the failure the rate of this techniques, more than one embryo is usually implanted in the mother, on the assumption that one or two will fail to develop. This has led on occasion to multiple births.

#### 1.2 Prenatal Genetic Diagnosis (PGD):

Prenatal Genetic Diagnosis (PGD) is related to both IVF and prenatal genetic testing, and involves testing embryos in IVF for specific genetic diseases prior to their reinsertion into the mother. Those embryos carrying the genes being looked for are discarded and only those found to be clear are put back. Developed in the late 1980s, this technique is usually applied in families where there is a history of genetic disease. The range of conditions that are tested for ranges from monogenic disorders which affect children, such as Cystic Fibrosis and Tay-Sachs disease through chromosomal abnormalities, including trisomies (e.g. Down Syndrome) and deletions (Turner's syndrome) to late onset disorders such as Huntington's disease and early onset Alzheimer's disease.

## 2. CURRENT STATE OF DEVELOPMENT

Technology	Details	Discussion
<b>IVF</b>		
Sperm sorting for sex selection	'Microsort' technique sifts heavier 'female' sperm (X chromosome carries more DNA) from lighter male sperm. At trial stage but developers claim substantial success.	Developed to avoid implanting male embryos which might develop X-chromosome linked genetic disorder, such as haemophilia. Females will carry these kinds of disorders but will not suffer from them.
ICSI: Intracytoplasmic Sperm Injection	Used when a man's sperm are barely motile (i.e. of very low fertility) or are very low in number, this procedure injects a single sperm into an egg, allowing it to fertilise.	Originally it was to have a better success rate but recent studies have suggested that a) ICSI is no more efficient than 'normal' IVF and b) that the children produced by ICSI do not have higher rates of congenital abnormalities, as was first thought.
Ooplasmic transplantation	This technique combines DNA from 3 people (2 women and a man) to increase the chances of successful IVF in older women. It introduces mitochondria (small structures from inside the cell which contain a small amount of DNA) from younger women into the egg of the mother.	Technically the resulting children (30 have been born worldwide) have 3 parents since they carry DNA from 3 people. Concerns have been raised in the scientific press since the action of mitochondrial DNA on the body is not clear. This technique is not permitted in many countries which regard it as unsafe (e.g. UK).
Egg fertilised by Body cells	Tested in mice, this technique allows doctors to use body cells to fertilise an egg. Chemicals are used to make egg eject excess chromosomes (since body cells carry 2 sets of 23, while sperm/egg cells only one set).	If it works in humans, would allow infertile man to father child with his own genetic material, instead of relying on donor sperm.
Frozen Eggs	Although frozen sperm has been used for years, the freezing and use of ova has been much less common. Developed to allow women who have treatments such as cancer chemotherapy to have children at a later stage.	Worldwide, only 30 babies have been born using this technique, although it has been around for 15 years. The method has a low success rate. Some commentators have suggested that it might be used as a 'lifestyle' choice, allowing women to have a career, but also to ensure having children at a later date.
<b>PGD</b>		
Aneuploidy Screening	Screening for aneuploidy, a condition where an embryo has more than the normal number of chromosomes, allows doctors to implant only those embryos with the best chance to developing.	40 to 70% of IVF embryos suffer from aneuploidy and are thus very unlikely to develop in the womb. In women with a history of miscarriage, this technique increases their chances of successful IVF. This will screen out conditions such as Down's syndrome which are caused by extra chromosome
Familial cancer screening	The recent birth of a baby boy resulting from IVF and PGD screening for Li-Fraumeni syndrome, a form of hereditary cancer. Embryos were screened for mutations of the p53 gene.	Those born with the mutation have 50% chance of cancer by age 40, and 90% by age 60.
Tissue type selection	PGD can be used to ensure that implanted embryos are of a particular tissue-type (relevant for transplants). This means that a child can be born which can then act as a tissue donor for someone else.	The most high profile example of this was the 2000 case of Adam Nash who was the result of IVF and PGD. His sister Molly suffers from Fanconi's anaemia and required a bone marrow transplant. Their parents had PGD to select embryos that a) did not carry this condition and b) that were tissue-type compatible, so that they might provide bone marrow in the future.

## **2.1 Dependent and related technology areas:**

IVF depends on technologies outside of genomics, but PGD obviously depends on advances in genetic testing such as DNA arrays and SNP screening.

## **3. KEY ACTORS**

### **3.1 Public, commercial and extent of interaction:**

In most countries there is a mixed public/private provision of NRT treatments with private funding outweighing public considerably. For example, in the UK, only 10% of IVF carried out is done on the National Health Service, with many health insurer refusing to cover these technologies.

## **4. PROSPECTS AND UNCERTAINTIES**

### **4.1 Medium prospects (5-10 years):**

IVF: We can expect improved success rates for IVF resulting from: better (i.e. genetically engineered) drugs to stimulate ovaries; maturation of ova outside the human body (reducing cost of treatment); improved embryo and egg freezing procedures; and screening of embryos' metabolisms to access those which will implant better.

PGD: Increased range of genetic conditions screened for.

### **4.2 Long term prospects (10-20+ years):**

A major possible development is the artificial womb which will allow for foetal development outside the human body (ectogenesis). Experiments have been carried out on sheep and goats, but it is still far from certain that this technology will ever be safe for human use. Many fertility experts are dubious about its viability.

## **5. ELSI ISSUES**

- Long term health effects of IVF: current opinion is that children born through IVF do not have higher rate of congenital disorders, but still question mark over the fertility of these people in the case of ICSI.
- Eugenics: like prenatal genetic testing, PGD raises the objection that it is eugenic, in that it selects the kinds of people to be born, and thus implies that people with diseases/disabilities are to be undervalued.
- Resources: should New Reproductive Technologies be funded by public money when there are other conditions which may be seen as more serious which need funding? This focuses on whether we see infertility as a medical condition to be treated; do people have right to have children, and if so, does this right require public funding?
- Status of the embryo: at the heart of the ethical issues raised by NRT's is whether the embryo is given moral status, and if so what that requires of us. Almost all IVF treatment will result in spare, unwanted embryos, and PGD and fertility research inevitably result in the destruction of embryos.

## **6. REGULATORY AND PUBLIC POLICY RESPONSE**

### **6.1 National Regulations:**

Across Europe there is a wide range of regulatory responses, ranging from no regulation at all (e.g. Belgium), through moderate positions which permit access to technologies by unmarried couples and single women (e.g. UK) to restrictive positions which only allow married couples access to these technologies (e.g. France).

### **6.2 Bioethics Convention:**

The Bioethics convention refers explicitly to embryos in Article 18, which requires national laws to provide 'adequate protection' of the embryo. It also prohibits the creation of embryos for research purposes, which conflicts with UK law, for example, which allows this (although only about 100 research embryos have been created in ten years). Article 14 of the convention prohibits sex-selection, unless it is to prevent the transmission of a sex related hereditary disease. This obviously relates to sperm sorting for sex selection and PGD.

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## GENE THERAPY (SOMATIC AND GERMLINE)

### 1. DESCRIPTION OF THE TECHNOLOGY

Gene therapy is best thought of as a family of technologies resulting from the application of gene transfer techniques to clinical problems. Two broad types of gene therapy can be distinguished, somatic and germ line. Somatic cells form the non-reproductive tissues of the body, for example liver, muscle or blood cells. Somatic gene therapy can be defined simply as the delivery of functional genes to somatic tissue for the treatment of disease. Here a therapeutic gene is administered to the patient in order to make changes to diseased somatic cells in the body, but not to the germ cells (sperm and eggs), which are involved in reproduction. The therapy therefore only affects the person to whom it is given. In contrast, germ line gene therapy is aimed at the genetic alteration of future generations. Here the therapeutic gene would be given to the sperm or eggs and could be inherited by the children of the treated individual. It would become a stable part of their genetic makeup and be passed to future generations. For ethical reasons, germ line gene therapy is not currently being developed in humans.

The efficient transfer of a gene to human tissue and its subsequent expression within the body lies at the heart of gene therapy technology. The systems used for somatic gene therapy have three central components; the therapeutic gene, the adjacent DNA sequences which will regulate the gene's expression, and the delivery system or vector by which the gene is transferred into the patient and targeted to the desired cell type. Gene transfer technology can be applied to the patient in two distinct ways. *Ex vivo* gene therapy is where the modification of a patient's cells occurs outside the body before returning the cells back to the patient, for example by blood transfusion or bone marrow transplantation. *In vivo* gene therapy is where the genetic alteration of the cells occurs by direct administration of the therapy to the patient, mainly by injection or using an aerosol spray to the lung.

The efficiency and safety of a number of different therapeutic strategies and gene delivery systems are currently being studied in human clinical trials. The most widely used approaches involve the use of disabled viruses as vectors. Whilst these are more efficient than other non-viral approaches, they are associated with greater safety risks (see below).

Gene therapy was initially developed for the treatment of rare single-gene disorders, such as growth hormone deficiency, cystic fibrosis and Gaucher's disease. However, the great majority of clinical trials undertaken to date have been aimed at the treatment of cancer and cardiovascular diseases. In principle, gene therapy could be used to treat a very wide range of diseases and conditions. For example, it is already being investigated as a post-surgical intervention following widening of the cardiac arteries (restenosis), as a treatment for burns, and as a means of preventing degenerative diseases such as arthritis.

In principle, gene transfer technology could also be used to either enhance particular traits in an individual (e.g. height, skin colour, etc.) or change the characteristics of future generations using germ line interventions. At present little work is being actively carried out on gene therapy based enhancements. However, therapies are being developed to increase the growth of children suffering from very short stature and international sporting bodies fear that gene therapy might be used to illegally deliver the gene

encoding EPO, a banned drug that can enhance athletic performance. Although a broad international consensus has been in place for the last 20 years agreeing that it would be unethical to develop germ line gene therapy, there are increasing calls within the American scientific community to allow this technique to be developed on a very limited basis for the treatment of families suffering from rare incurable genetic disorders. If this were sanctioned it would represent the first attempt to genetically alter future generations.

## **2. CURRENT STATE OF DEVELOPMENT**

Despite the fact that over 500 completed, ongoing or pending clinical trials of somatic gene therapy have been approved since the first one took place in 1990, no gene therapy has yet received marketing approval anywhere in the world. Furthermore, major technical problems remain to be overcome, most notably the lack of high efficiency gene transfer and the risk of adverse immune response provoked by using viruses as gene transfer vectors. As a consequence nearly 70% of trials have remained in Phase I, although a significant number have progressed to Phase II and a few therapies are now in pivotal trials, most notably for cancers of the ovary, and head and neck.

Although progress has been slow, significant milestones have been reached. It has been shown that genes can now be transferred to almost any tissue in the human body and in 2000 the technique was used to permanently cure two French children suffering from a rare fatal enzyme deficiency (ADA-SCID).

Very heavy investment in gene therapy has been made by industry during the last decade. Over 60 new firms have been formed in the US and Europe explicitly to develop the technology, and biotechnology and pharmaceutical companies have committed over ECU 3 billion in research and development in this period. Since the end of 1999 there has been a significant decline in investment in gene therapy following the failure of various clinical trials and the controversy following the death of the first person as a direct result of a gene therapy. Jesse Gelsinger died after a genetically altered virus was injected into his liver during a gene therapy Phase I clinical trial at the University of Pennsylvania Institute for Human Gene Therapy. Several of the researchers involved were heavily criticised at the time for failing to follow regulatory guidelines. As a result, new moves have been made to further protect research subjects and tighten controls over the conduct of clinical research. The scandal surrounding Gelsinger's death has been a major set back for the field as a whole.

In relation to germ line gene therapy, it must be stressed that this technique is not currently being developed in humans. However, technologies that might enable this type of treatment to be introduced in the future are already being routinely used for the creation of transgenic animals. Before germ line interventions could be undertaken, major safety problems would have to be overcome associated with the transfer of foreign genes into the human genome. This might result in genetic defects and the creation of tumours. Developing the technology would therefore require the instrumental use and waste of human embryos, and as no one could be certain whether principle safety could be guaranteed, there would be unforeseeable risks for the first generation of patients and their progeny.

## **2.1 Dependent and related technologies:**

*Gene transfer technology:* Great progress has been made in the safe and controlled transfer of genes into human tissues. However, significant improvements would need to be made before gene therapy could be widely used. Artificial Chromosomes might offer an alternative method to viruses for transferring DNA into cells.

*Cell and tissue engineering:* The ability to isolate, manipulate and transplant particular cell populations would greatly assist the development of many types of gene therapy.

*Genomics:* the identification of thousands of new genes involved in causing common diseases opens up the possibility of creating a new generation of gene-based therapeutics.

*Fertility Research:* As society has come to accept the expansion of reproductive options for infertile couples new reproductive technologies continue to be developed. Following recent moves towards human reproductive cloning it seems likely that at some point in the medium terms someone will attempt germ line gene therapy.

## **3. KEY ACTORS**

### **3.1 Public, commercial and extent of interaction:**

Very significant investment in gene therapy has been made by both the public and private sectors. Since the mid 1990s a number of European governments have adopted policies explicitly aimed at promoting the technology and have spent tens of millions of Euros funding basic research. This investment has helped stimulate the creation of new firms and in May 2000 there were 27 biotechnology firms in Europe dedicated to the development of gene therapy, compared to just 10 in 1996. Though now highly competitive when judged internationally, the European industry is still less mature than it's US counterpart when measured by the number of staff employed, the number of firms organising clinical trials, the number of public companies and the number of corporate partnerships with the pharmaceutical industry. This is largely due to the head start achieved by American firms, many of whom were founded in the early 1990s. The sector is marked by very close industry-academic linkages, with over 60% of leading European researchers having some form of industrial collaboration

### **3.2. Others (government R&D, advocacy organisations):**

In addition to public funding measures promoting gene therapy, a number of leading medical researcher charities have been very actively involved in supporting the technology. The best-known example is the French Muscular Dystrophy Association (AFM), which has created the largest public gene therapy laboratory in Europe (Genethon III).

## **4. PROSPECTS AND UNCERTAINTIES**

### **4.1 Medium term prospects (5-10 years):**

Given that a number of gene therapies are now in pivotal Phase III trials, it seems very likely that the first products will enter the market within the next decade. However, progress is likely to remain slow until major improvements are made in gene delivery technology. With respect to germ line therapy, it seems unlikely that any serious attempt will be made at modifying future generations whilst so many problems remain unresolved in somatic therapy.

#### **4.2 Long-term prospects (10-20+ years):**

Despite the current lack of progress somatic gene therapy still promises to be one of the most important developments in medicine in the next 20 years. Gene transfer has already been successfully applied to almost every tissue and progress is being made in developing treatments for a number of important human diseases. Furthermore, as information accumulates about the human genome and more important genes associated with common diseases are identified, gene therapy will become increasingly feasible. The technology also offers a number of clear potential advances over the use of conventional small molecule drug therapies.

In contrast, the long term prospects for germ line therapy are highly uncertain given that major technical obstacles and safety issues raised by trying to genetically modify future generations remain unexplored. It does, however, seem likely that some sections of the biomedical research community will want to pursue this option in coming decades.

#### **4.3 Known current uncertainties:**

There are a number of important uncertainties that still surround somatic gene therapy. The death of Jesse Gelsinger has reminded the scientific community of the very real risks involved in using genetically modified viruses as therapeutic agents. Until these risks are reduced, gene therapy is likely to be mainly developed for life threatening diseases where the risk/ benefit ratio is more favourable. In addition, whilst the idea of transferring genes already found in the body is an attractive basis for therapy, it remains to be seen if it can be successfully used to treat many common diseases which are likely to have complex multi-factorial causes.

In addition to the many technical uncertainties that are likely to hamper the development of germ line gene therapy, there are important ethical questions which remain unanswered (see below). In particular, the idea of human genetic engineering being used as the basis of a new eugenics is widely held. As a consequence, the development of germ line engineering is likely to meet stiff opposition from within the scientific community and the public at large.

### **5. ELSI ISSUES**

- Safety - Are the long-term effects of somatic gene therapy sufficiently understood? Whereas many conventional treatments that prove harmful to a patient can be stopped, some forms of gene therapy permanently change patient's biology. Should these types of gene therapy be provided if simpler and safer treatments exist?
- Availability – Will the likely high cost of gene therapy exacerbate problems of access and social discrimination?
- Social dangers – Will the pursuit of genetic treatments lead to society ignoring the social and cultural factors of disease and disability? Will somatic gene therapy open the door to enhancement techniques that might alter traits not associated with disease and therefore increase social discrimination?
- “Slippery slope” argument – Opponents of somatic gene therapy argue that allowing modifications of somatic cells for therapeutic purposes might inevitably lead to germ-line gene therapy.
- The right for autonomy – Would germ-line gene therapy violate the rights of future generations to inherit a genetic makeup that had not been modified? Do we have an obligation to future generations to allow them to make their own choices?

- Eugenics – would moves towards the genetic enhancement of human traits constitute a form of eugenics? Would market forces and cultural pressure to enhance certain qualities inevitably lead to discrimination?

## 6. REGULATORY AND PUBLIC POLICY RESPONSE

*Group of Advisors on the Ethical Implications of Biotechnology to the EC: Opinion No. 4: Ethical Implications of Gene Therapy: (1994):* The Group recommended that somatic gene therapy should be encouraged at different necessary levels (basic research, clinical trials, biotechnology), by supporting research actions. Special regulations should provide for evaluation at European level the risks and results of gene therapy technology. It also believed that germ line human gene therapy was not at present ethically acceptable.

*UNESCO: Report on Gene Therapy: 1994:* Recommended that somatic gene therapy is permissible, regulated as an experimental therapy and that the use of germ line interventions for enhancement purposes should be categorically prohibited.

*The Parliamentary Assembly of the Council of Europe:* recommends that an individual has the right to be born with their genetic substance artificially unaltered with the exception being if interventions are “ recognized as being fully compatible with respect to human rights”.

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## STEM CELLS / THERAPEUTIC CLONING

### 1. DESCRIPTION OF THE TECHNOLOGY

Stem cells are thought to be the precursors or parent cells of all other cells in the body. A stem cell has the ability to divide for long periods, often throughout the life of the organism, and under the right conditions, or given the right signals, can give rise (differentiate) to form the many different specialized cells that make up the body. As an animal or human develops most cells become specialized, or committed, to one of two hundred different cell types that exist in an adult, such as nerve, skin or bone cells. In contrast, a number of stem cell populations found in the adult retain varying abilities to differentiate into a range of specialised tissues. Stem cells have been classified depending on their source and much work is ongoing to determine the similarities and differences between those derived from the embryo, the foetus and the adult. Current science suggests these cells differ in important ways.

Embryonic stem (ES) cells have been derived from both human and mice blastocysts, the term for the early 4- to 5-day embryo. What is remarkable about these ES cells is that they have been found to be pluripotent, that is they can give rise to a very wide range of tissues and cell types in the body. Mouse ES cells have already been genetically manipulated in culture and then implanted into early stage embryos, resulting in animal with many tissues derived from the ES line. These animals have subsequently been bred to produce offspring wholly derived genetically from the original ES cells. This procedure has allowed targeted genetic modification of the mouse genome that can be passed on to future generations. It is this ability to be the source of a wide range of cell types, combined with the fact that they can replicate indefinitely in culture, which underpins the potential role of ES cells in tissue transplantation and cell-based therapies. In principle, ES cells could be used to repair or replace cells damaged in many degenerative disorders, such as Parkinson's disease and muscular dystrophy, which occur due to the loss of specialized cells.

The origin of stem cells in adult tissues is unclear, although some scientists have suggested they are 'put aside' in some way during foetal development and are restrained from fully differentiating. Until recently, it was widely believed that adult stem cells were rare and diminished further with age. Stem cells derived from adult tissue do not appear to have the same capacity to differentiate as embryonic stem cells or embryonic germ cells. Though also possessing the ability of long-term self-renewal, the primary function of an adult stem cell is the replacement of the specialised cells in the particular tissue in which it originated. For example, neural stem cells give rise to specialized cell types required in nervous tissue. These adult stem cells have been described as multipotent, only being able to give rise to a limited number of other cell types. For these reasons the potential of adult cells to be a source of tissue for transplantation has been considered limited in comparison to embryonic stem cells. However, recent studies have started to challenge this assumption (see below).

For over 40 years doctors have been using the unique properties of adult stem cells for therapeutic purposes in bone marrow transplantation. This life saving procedure for the treatment of certain cancers and diseases of the immune system is now known to be based on the transplantation of haematopoietic stem cells, the immortal precursors of the blood and immune system. It is hoped that if other types of stem cells can be successfully

isolated from adult tissues, it may be possible to manipulate them to differentiate into a wide variety of replacement adult cell types. Such cells would have broad applications in tissue transplantation and cell-based therapies for the treatment of degenerative disorders such as multiple sclerosis and Alzheimer's disease, and even for the treatment of certain cancers.

In principle, stem cells for use in therapy can be collected from a number of different sources. Embryonic stem cells can arise from embryos 'left over' from infertility treatments, embryos created specifically for research purposes, and somatic cell nuclear transfer. The use of nuclear transfer for medical purposes has become known as therapeutic cloning. This would involve replacing the genetic material of a human oocyte (egg) with the DNA from an adult cell taken from the patient, a technique pioneered in mice and sheep. The resulting embryo would then be grown in culture only until a stage from which embryonic stem cells could be derived. At no time would these cells be placed back into a woman, as would be the case if cloning of the adult were intended (reproductive cloning). Potentially, these embryonic stem cells could then be directed to differentiate into the particular cell type required, for example replacement pancreatic islet cells for patients suffering from diabetes, and as the cells would carry the patients own DNA there would be fewer problems with rejection of the transplant. However, the use of early human embryos grown in culture as a source of cells for therapy is highly controversial and raises many ethical issues.

Adult stem cells can already be recovered in very small quantities from a number of tissues or from the organs of recently deceased individuals. While evidence is accumulating that suggests adult stem cells are more common than originally thought, they are often very difficult to isolate. Ultimately as more becomes known about adult stem cells it may be possible to bypass the use of ES cells and simply reprogramme adult stem cells, or ultimately even specialised adult cells, into virtually any tissue.

## **2. CURRENT STATE OF DEVELOPMENT**

### **2.1 Embryonic stem cell therapy:**

Since the first isolation of human ES cells in 1998, molecular cell biology has progressed sufficiently to allow scientists to grow human ES cells for up to a year in the laboratory. ES cells isolated and cultured from human blastocysts have been shown to be pluripotent, contributing to tissues in all parts of the early embryo. One of the next research goals is to derive pure cultures of a specific specialised cell type from a human ES cell line, an important progression if ES cells are ever going to be used in transplantation therapies. In terms of so-called therapeutic cloning, involving the use of nuclear transfer to create embryonic stem cells specifically for a given patient, this prospect has been boosted by research on animal cloning. The ability to reconstruct embryos from cultured adult somatic cells was first shown in the cloning of Dolly the sheep in 1997. These experiments have since been repeated in pigs, cattle and mice, and have demonstrated in principle that adult cells can be used to create a genetically identical embryo from which cells for therapy might be derived.

## **2.2 Adult stem cell therapy:**

The location and prevalence of adult stem cells is still a highly contentious issue, with the usefulness of technologies based on adult cells thought to be limited by the apparent lack of detectable stem cells in many tissues. However, the isolation of stem cells in organs such as the brain and muscle, previously thought to lack stem cells and regenerative potential has lessened these concerns. Furthermore, certain adult stem cells have been found to be capable of developing into cells that are characteristic of quite different tissues. For example, it has been shown that it may be possible for bone marrow derived stem cells to differentiate into skeletal muscle and certain types of brain cells. These findings have therefore challenged the idea that adult stem cells are “committed” to differentiate only into a limited range of other cells and their potential may not be as limited as originally thought. Further support for the blurring of the boundary between embryonic and adult stem cells has been provided by two companies (PPL Therapeutics and Anthrogenesis) who claim they have identified pluripotent cells without the use of embryos or foetal tissue<sup>7</sup>. However, a great deal of research is still required to develop cultured cell lines that can generate replacement cells and tissues for use in therapy.

## **2.3 Dependent and related technologies:**

*Molecular Cell Biology:* Over the last five years significant progress has been made in isolating and culturing ES and adult stem cells as a consequence of advances in understanding the cellular signals that control their development. Research looking at the mechanisms that determine stem cell differentiation should further help moves to produce different tissues from either adult or embryonic stem cells.

*Tissue and Cell Engineering:* Combining the use of stem cells with other innovations, such as the development of collagen infrastructures for the replacement of organs, may allow the creation of replacement organs using a patient’s own cells.

*Somatic Cell Nuclear Transfer:* Advances in somatic cell nuclear transfer may allow the development of therapeutic cloning, however, major technical problems would need to be overcome in order to make this process safe and efficient.

## **3. KEY ACTORS**

### **3.1 Public, commercial and extent of interaction:**

Major public investment into basic research in developmental biology and human and animal reproduction has laid the foundation for the growth of stem cell research. However, whilst there has been some public investment in the development of cell and tissue engineering technologies, the majority of work on their practical application has been conducted by industry. In relation to human ES cells, a number of UK academic laboratories have announced their intention to work in this area following a recent change in the law. Despite this, most research on this topic is currently confined to the private sector due to the public controversies surrounding it (see Table 1.). In the US, Geron Corporation has an exclusive licence to a patent covering the isolation of human embryonic stem cells. The dominance of the private sector is likely to be reinforced by moves in the US to cut all Federal funding of embryonic stem cell research. Similarly work on the use of adult stem cells has also been led by the biotechnology industry with a number of companies carrying out research into the isolation, production and transplantation of stem cells from a range of tissues.

**Table 1. Leading biotechnology firms working on stem cell therapies**

<b>Company</b>	<b>Location</b>	<b>Employees</b>	<b>Speciality</b>
Geron	California, US	100	Embryonic, foetal stem cells
Nexell Therapeutics	California, US	120	Haematopoietic stem cells
ReNeuron	London, UK	17	Neural stem cells
Stem Cells	California, US	16	Adult neural stem cells
Stem Cell Sciences	Melbourne, Aus	-	Embryonic stem cells

**Science 287, pg1420.**

### **3.2. Others:**

A number of other groups are actively involved in the debate on the development of stem cell research, including religious organisations, many of whom are opposed to the use of human embryos for research, and patient groups, who support it as a potential source of new cures.

## **4. PROSPECTS AND UNCERTAINTIES**

### **4.1 Medium term prospects ( 5-10 years):**

The rapid development of these technologies in recent years may have created the misconception that tissues derived from stem cells and nuclear transfer will be available soon. Despite the high profile that this area of research has received, animal studies examining the benefits of stem cell transplantation are few and still in their infancy. However, both policy makers and scientific researchers suggest that the first human clinical trials of therapies based on both adult and embryonic stem cells will take place within the next 5-10 years.

### **4.2 Long term prospects (10-20+ years):**

One of the ultimate aims of stem cell research is to learn how to reprogramme adult stem cells, so that a patient's own tissue can be used as a potential therapy. This might be a similar procedure to bone marrow transplantation and would also open up the possibility of people donating stem cells to others in a similar fashion to organ donation today. It may also be possible to produce 'universal' donor cells grown on an industrial scale in culture, which would be engineered so they could be transplanted into any patient without the fear of rejection. Furthermore, in the long term it is hoped that stem cell technology will enable the production of complex solid organs, such as lung and kidney, using either extracellular matrices or existing organs as scaffolding.

### **4.3 Known current uncertainties:**

A significant number of technical and social uncertainties currently surround the field. In particular, the extent to which adult and embryonic stem cells can be isolated, manipulated and successfully transplanted is unknown at present. Fundamental advances in developmental biology, cell isolation and purification, and tissue engineering will have to be made before any of the therapies can become a clinical reality. There are also a number of important safety issues. In particular, the transmission of infectious agents and the formation of tumours after uncontrolled cell proliferation or the incomplete reprogramming of the transplanted cells, are two potential risks. Other uncertainties surround the limited availability of human oocytes and the significance of the age of the donor cells in therapeutic cloning. In social and ethical terms it is unclear if the use of human embryos to create therapies will ever be acceptable to a significant part of the

population in many European countries. This may be a significant factor driving the creation of alternative technologies based on the use of adult stem cells. It will take many years before all the factors that determine the successful, safe and ethical use of stem cells are understood and progress is likely to be limited by a wide range of factors.

## **5. ELSI ISSUES**

The main focus of debate on stem cell technologies has been on the ethics of human embryo stem cell research and 'therapeutic' cloning, and issues surrounding commercial development.

- Is stem cell technology ethically objectionable *per se* or is the key issue the origin of the cells and the way in which they were derived?
- The experimental creation of pre-implantation embryos is highly controversial to groups opposed to embryo research. To subvert the development of a potential human being is seen as morally objectionable;
- The creation and manipulation of living human embryos for the sole purpose of generating therapeutic tissue seems incompatible with respect for human life. Can the "special status" of the human embryo be over-ridden in the interests of therapeutic research?
- Does using "spare" embryos no longer needed for infertility treatment raise fundamentally different ethical issues than creating them solely for research?
- Fear that allowing therapeutic cloning will be the start of "slippery slope" to reproductive cloning / genetically engineered human beings;
- Consent – the extent to which couples undergoing fertility treatment are made aware of all uses of embryos created by their gametes;
- Patenting – there are objections to the patenting of therapies based on naturally occurring human cell populations e.g. the recent controversy around the patenting of placental cord blood therapies.

## **6. REGULATORY AND PUBLIC POLICY RESPONSE**

### **6.1 European Regulation**

The main focus of policy and regulatory activity has been on the ethics of embryonic stem cell research.

*Fifth Programme of the European Community for research, technological development and demonstration activities (1998-2002):* Stipulates that " ... no research activity, understood in the sense of the term "cloning", will be conducted with the aim of replacing a germ or embryo cell nucleus with that of the cell of any individual.

*Opinion of the European Group on Ethics in Science and New Technologies to the European Commission: No.15: Ethical Aspects of Human Stem Cell Research and Use:* The Group deems it essential to underline the sensitivity attached to the use of embryonic stem cells and that ES cell research, in countries where it is permitted, is placed under strict public control by a centralised authority.

### **6.2 National:**

Only then the *UK government* has regulated to allow this research: In 2001 the UK Parliament voted to allow research on therapeutic cloning to be undertaken on human embryos <14 days within the framework of existing statutory legislation.

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## HUMAN REPRODUCTIVE CLONING

### 1. DESCRIPTION OF THE TECHNOLOGY

The use of the term 'Reproductive Cloning' is fraught with definitional confusion and inconsistency and it is important at the outset of this report to offer some clarification of a highly contested term. For instance, in the original article by Wilmut and colleagues (1997) announcing the birth of a cloned ewe from somatic cell nuclear transfer (see below), the word clone or cloning was never used. This is, in part attributable to the desire to avoid potentially negative forms of language and indeed accounts for the preference amongst many to substitute 'cell expansion techniques' for cloning. Another recent adaptation to terminology has seen the emergence of the distinction between 'reproductive' and 'therapeutic' cloning. Notwithstanding these notes of caution, we can refer to a number of processes as 'cloning'.

#### 1.1 Embryo Splitting (Blastomere Separation):

Embryo splitting is a method whereby the cells of a very early embryo (2, 4 or 8 cell stage) are separated out to continue developing individually. Since at this stage each blastomere is 'totipotent', the undifferentiated cell will continue to divide, then differentiate into functionally specific cell types and eventually develop into a complete organism. Each of the resulting organisms will be genetically identical. This process of embryo splitting is, in many respects, a technically induced means by which identical twins (AKA monozygotic twins) occur naturally.

In the context of human reproductive fertility, this form of cloning was first used experimentally in 1992 when researchers split the cells of 17 chromosomally abnormal human embryos to determine if development would continue. Whilst there was no intention to re-implant the developing embryos to develop in vivo, it nevertheless demonstrated the application of the technique in human reproduction. Numerous infertility clinics are capable of producing clones in this way. There are however, important differences between this and somatic cell nuclear transfer. First, somatic nuclear transfer is used to produce a genetic replication of an already known adult being. On the other hand, embryo splitting requires the complementary gametes of two parents before being split into separate clones. Second, the method of embryo splitting, like the method of nuclear transfer discussed directly below is somewhat inefficient in that it is restricted by the number of developing embryonic cells available.

#### 1.2 Nuclear Transfer:

Until the events surrounding Dolly in 1997, nuclear transfer in mammals had only been accomplished by inserting an undifferentiated embryonic cell into either a fertilised zygote or egg from which the nucleus had been removed. This has been possible in sheep since the mid 1980s.

In the year preceding the announcement of Dolly, the Roslin Institute published findings from a nuclear transfer procedure resulting in the birth of two genetically identical sheep, Megan and Morag. The key difference between the 1996 and 1996 events is the source of nuclear donation. In the 1996 case, the nuclear source was an embryonic sheep cell. Dolly on the other hand was derived from an already adult somatic and differentiated cell.

### **1.3 Somatic Cell Nuclear Transfer (SCNT):**

The announcement by the Roslin Institute in 1997 represents the first instance of using a somatic nuclear source to produce a cloned adult mammal. Dolly was derived from cells taken from the udder of a 6-year-old ewe. 277 such cells were then cultured in vitro and fused with unfertilised eggs from which the nucleus had been removed. This process resulted in 29 viable reconstructed eggs, each with a nucleus from the adult animal, which was then implanted into surrogate ewes. Only one of these would result in a viable adult mammal, Dolly.

The technical shift from embryonic to somatic nuclear transfer holds the possibility of much greater flexibility in the production of cloned and transgenic mammals. By using treated and cultured somatic cells, researchers need not be overly restricted by having to rely on a limited number of available embryonic cells.

### **1.4 Reproductive Vs Therapeutic Cloning:**

Policy and decision-making around cloning, particularly in the UK, has sought to distinguish between different end uses for SCNT resulting in the widespread adoption of the distinction between 'reproductive' and 'therapeutic' cloning. The function of the distinction has been to permit the use of the technique to generate potentially beneficial therapeutic applications whilst prohibiting its use in human reproduction.

*Reproductive SCNT:* use of somatic cell nuclear transfer to generate genetically identical individuals.

*Therapeutic SCNT:* use of somatic cell nuclear transfer to create genetically identical embryos that would not be allowed to continue developing. Such embryos could be used as a source from which cells and tissues of a desired genetic type might be derived.

This report confines itself to addressing the potential use of SCNT in human reproductive cloning. However, it must be acknowledge the above distinction has been the subject of continuing policy discussion especially since technical refinement of therapeutic SCNT is likely to contribute to making reproductive SCNT more feasible.

To date, there is no known evidence of SCNT having been used to create a human person beyond an early embryological stage in vitro.

### **1.5 Unintentional Cloning:**

There is some, largely anecdotal, evidence of assisted conception techniques having accidentally resulted in humans inheriting the chromosomal DNA of only one parent rather than two.

Dr Steen Willadsen has suggested that a procedure for treating male infertility, involving the removal of immature sperm cells which are then microinjected into the egg (oocyte) may result in the transfer of exclusively male DNA. Immature sperm cells are diploid (containing both sets of male chromosomes) rather than haploid (one set of chromosomes which during fertilisation bind with the female complementary set of chromosomes). If, following microinjection, the egg subsequently sheds its own nucleus, the resulting embryo will inherit the male DNA exclusively.

## **1.6 Exceptions:**

An important exception to the concept of nuclear cloning concerns mitochondrial DNA that remains with the egg after the nucleus has been substituted. The mitochondria, roughly 16,000 bases in length, resides outside of the nucleus close to the surface of the cell and is not replaced during nuclear transfer techniques. This sets important limits to the notion of what we mean by the word 'clone' since many examples of cloning are drawn from applications where the derived organism is not strictly speaking an exact genetic copy of its nuclear source.

## **2. CURRENT STATE OF DEVELOPMENT**

### **2.1 Immaturity of SCNT:**

The procedure used by the Roslin Institute to produce a viable offspring by SCNT in 1997 represented a single success amongst 276 failed attempts in a species with which the research team had detailed experimental experience. Extrapolating from such poor results, one commentator has suggested that the production of a single cloned human neonate would currently require roughly one thousand willing surrogate mothers and result in nearly as many miscarriages, still births and fatal defects.

Whilst cloning efficiency remains relatively low (<2%) with high perinatal mortality, refinement of the technology has proceeded apace and has extended the application of SCNT to numerous other species.

However, ongoing work has demonstrated the serious practical and ethically prohibitive difficulties that would face any current attempt to apply SCNT to human reproduction. They include:

- The current paucity of knowledge regarding cell cycle regulation required to return donor somatic nuclei to an undifferentiated totipotent state
- Lack of current understanding regarding the role of telomeres and aging
- Implications of mitochondrial DNA residue from egg donors
- X-Chromosomal inactivation
- The possibility that stem cell nuclei are being mistaken for adult somatic nuclei accounting for those rare successes where viable organisms are produced

### **2.2 Current Attempts to Apply SCNT in Human Reproduction:**

There are currently several known examples of teams actively pursuing the application of SCNT to human reproduction. Each case has provided strong impetus towards statutory prohibition of human reproductive cloning (discussed below).

*Raelian Movement (Canada)* claim to be conducting active research after receiving private finance from, amongst other sources, a couple seeking to clone their deceased child. Whilst monitoring of the work has been difficult, it has been taken seriously by the US Congressional Hearing in March 2001, especially since the Raelian's claim to be conducting the work in the US but beyond current regulatory reach.

*Severino Antinori (Italy)* formally announced in January of 2001 his intention to proceed with human reproductive cloning. Antinori leads a consortia with Dr Panos Zavos who stated in January their expectation to have successfully applied SCNT to humans within two years outside of the US.

Notwithstanding the intentions of these groups, there is a wide-ranging consensus that in the near term (<5 years) such attempts are likely to meet with insurmountable practical difficulty.

### **2.3 Dependent and Related Technology Areas:**

Clearly, SCNT is in its infancy on numerous fronts and is unlikely to be feasibly applied to humans in the near future, even if there were a legal basis on which to conduct such a procedure. Related technology areas place an emphasis on:

- Cell cycle knowledge
- Processes of cell differentiation
- Improved understanding of the mechanisms genetic reconstruction of embryo

## **3. KEY ACTORS**

### **3.1 Public / Commercial and Degree of Interaction:**

There is extensive public and commercial interaction in the development of SCNT in nonhumans. Indirectly, advances in other species will contribute to a knowledge base which will have applications in human reproduction. Excepting rare examples of actors promoting SCNT, regulatory institutions are by far the most relevant actors with any influence over the application of cloning in humans.

## **4. PROSPECTS AND UNCERTAINTIES**

### **4.1 Medium Term Prospects (5-10 years):**

Any near term attempt to apply SCNT to human reproduction is likely to result in ethically prohibitive trauma for parents and resulting neonates. Given those technical constraints already identified, it is enormously unlikely that a viable human offspring can be currently produced.

Many domestic regulatory systems are currently in the process of tabling prohibitive legislation is expected to be in place within a medium term timeframe. Nevertheless, such regulation is unlikely to apply uniformly from country to country and some 'research tourism' is to be expected.

### **4.2 Long Term Potential (10-20+ years):**

It is conceivable that, in the long term, SCNT will reach a stage where current inefficiencies are significantly improved. However, this is unlikely to reach the point where routine successful application of the technique in humans can be expected.

### **4.3 Known Current Uncertainties:**

Some of the known uncertainties around human SCNT have been reviewed above but broader considerations also need to be addressed, especially.

- Technical uncertainties (see above)
- Extent of unregulated research activity
- Changing cultural factors around acceptability of human reproductive science

## **5. Ethical, Legal and Social Implications**

- **Safety:** The current inefficiencies of SCNT would require a wholly prohibitive level of invasiveness and distress, leading to potentially serious disabilities amongst live births.
- **The contravention of the right to unique identity:** Even if safety issues were substantially addressed in the longer term, cloning has been uniformly taken as burdening a person with the knowledge of having been created to substitute for, replace, or mimic another. A central ethical tenet here is the widely accepted moral principle that persons must not be regarded as an instrumental means to an end but, instead, ends in their own right.
- **Destabilisation of kinship:** SCNT represents quite unprecedented disruptions to even the most flexible of contemporary kinship arrangements. For example, the fact that a single parent is the somatic source may introduce an acute unbalance into kinship arrangements where adult partners seek to share the responsibilities of rearing.
- **Possible justifications:** Persuasive counter arguments for allowing SCNT in humans might emerge from the equally important obligation to relieve suffering. For example, in some cases SCNT might represent the only measure to assist conception. In these circumstances, policy makers may be challenged to acknowledge that there are strong reasons to assume that a resulting offspring would be valued for his or her own sake.
- **Genetic Determinism / Reductionism:** Much of the opposition to SCNT in humans arises from a sense of equivalence a person and their genetics. Numerous commentators have pointed to the potential harms and distortions of what might be called genetic determinism or reductionism. Individuals have an entirely unique and unreproducible relationship to their environment, times and events, irrespective of genetic factors. Therefore, the assumption that SCNT will lead to exact copies of individuals is, in many ways, based on an unmerited notion of the role played by genetics in identity and distinctiveness. Quite justifiable opposition to cloning also needs to be measured against the potentially equal harm of genetically reductionist assumptions.
- **Consent:** Additional problems arise from the impossibility of securing consent for such a radical procedure from the person who would bear the psychological burden of having been brought into being by SCNT.
- **Institutional Trust:** One of the main points made by the Wellcome Trust Report (1998) on public perspectives regarding cloning is that people regard themselves as being prevented from knowing about research developments. Institutional science and policy formation is regarded as being conducted without attention to potentially deleterious applications. Policy-formation around SCNT needs to take account of measures that can alleviate a lack of trust in institutions by fostering transparency and inclusion.

## **6. REGULATORY AND PUBLIC POLICY RESPONSE**

Whilst regulatory capacities have rallied to the need for prohibitive regulation on human SCNT for reproduction, few have in fact produced legally binding and enforceable legislation. This is, in part, because of continuing discussion over how and whether to permit SCNT for therapeutic research purposes. There is considerable national variation on governance in this matter and what follows is more of an illustrative sketch than a comprehensive review.

### 6.1 Supra/International:

A number of supranational agreements have sought to enforce a prohibition of human reproductive cloning, including:

- UNESCO Declaration on the Human Genome and Human Rights, unanimously adopted on 11 November 1997, of which Article 11 states that “Practices which are contrary to human dignity, such as reproductive cloning of human beings, shall not be permitted”
- Council of Europe (1997) Additional Protocol to the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine, on the Prohibition of Cloning Human Beings. Strasbourg: Council of Europe 1997
- European Commission Directive on the Legal Protection of Biotechnological Inventions (COM(97)446 final) prohibiting the issue of any patent on work leading to the intentional cloning of human beings.
- World Health Organisation (WHO) 51<sup>st</sup> World Health Assembly, implementation of resolution WHA50.37 – A51/6 Add.1 – 8 April 1998

### 6.2 National (illustrative and in alphabetical order):

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<b>Australia</b>	Agreement has been reached by leaders of the six states and two territories to uniformly prohibit human reproductive cloning. Legislation is already in place in three states. The completion of a consultative report is expected before the end of 2001 with prohibitive legislation in place in all jurisdictions by June 2002.
<b>Canada</b>	Draft legislation has been presented to the Canadian Standing Committee on Health which would prohibit human cloning whilst regulating assisted reproductive technologies and embryo research. There is currently no such comprehensive federal or provincial legislation.
<b>France</b>	Draft legislation prohibiting human cloning but allowing limited research on surplus and donated embryos was adopted in June 2001 and is expected to become law in early 2002. Human reproductive cloning would be punishable with up to 20 years imprisonment.
<b>Germany</b>	Cloning and embryological science operates under relatively strict controls in Germany. Stem cells cannot be generated in Germany though importation from elsewhere is permitted. With regard to statutory regulation, the Chancellor has instituted a new National Bioethics Institute to advise government on appropriate legislation.
<b>UK</b>	Any procedure involving human embryos in the UK requires a licence from the Human Fertilisation and Embryology Authority which has formally stated that it will not consider issuing a license for human cloning. Contravention of these terms is a criminal offence though the UK Parliament is still awaiting the tabling of Parliamentary legislation criminalizing cloning. However, the terms of reference of the HFEA have had to be broadened in order to take account of possible breaches.
<b>US</b>	There is currently no regulatory legislation in place on human NCST in the US, although federally funded research is prohibited. Proposed bills to legislate on cloning are currently under consideration but it is yet unclear which is likely to enter law. A recent statement by the Health and Human Services (HHS) department indicates that the Bush administration is expected to favour the more prohibitive of the legislative options that would see the federal criminalisation of virtually all human somatic cell nuclear transfer, including therapeutic cloning. The US House of Representatives Judiciary Committee recently endorsed this position. It is however still possible that more moderate legislation will enter law.

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## 7. FURTHER READING

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## PHARMING: ANIMAL TRANSGENICS AND CLONING

### 1. DESCRIPTION OF THE TECHNOLOGY

In recent years, far reaching changes in the health and life sciences have generated an unprecedented degree of innovation aimed at expanding the potential utility value of animals in both medicine and agriculture. The techniques that have fuelled this expansion are wide ranging but include gene 'splicing', transgenics, cloning, gene deletion and various combinations of approaches.

The scale and variety of these activities prevents a detailed audit of animal transgenics and cloning, although we attempt to present here a reasonably comprehensive assessment of current and future areas of development.

#### 1.1 Selective Breeding:

For millennia, techniques have been employed to alter the genetic characteristics of animals for many reasons including the creation of a vast spectrum of canine varieties bred for appearance or temperament, and the agricultural production of high yield ruminants.

Genetic engineering differs from traditional methods of selective breeding in a number of respects. Genes from one organism can be extracted and recombined into that of another (using recombinant DNA technology or rDNA) without either organism having to be of the same species. Removing the requirement for species reproductive compatibility, new genetic combinations can be produced in a much more highly accelerated way than before.

#### 1.2 Transgenics:

Transgenic animals can be defined as organisms 'containing integrated sequences of cloned DNA (transgenes), transferred using techniques of genetic engineering (to include those of gene transfer and gene substitution)'. The purpose of producing such an organism is usually to either introduce or delete specific characteristics of the phenotype. The first transgenic animals produced in 1980 were mice, followed by larger ruminants in the mid 1980s.

#### 1.3 Pronuclear Microinjection:

Pronuclear Microinjection is the most common method employed to produce transgenic animals and involves the injection of exogenous DNA into a fertilised egg. Though often unpredictable, the intention is to incorporate the newly introduced DNA into the subsequent cell division of the developing embryo.

However, in most instances the cells of the organism will carry only one insertion of the imported genetic material and not in both male and female complementary gametes. Subsequent breeding is usually required to produce GM animals expressing the desired phenotypic properties.

Generally, the method is highly inefficient (<10% for rodents) because of the randomness of the insertion process, often resulting in unwanted gene regulatory disruption. However, cloning techniques have recently improved the possibilities for targeted gene insertion. This follows publication of research to place a therapeutic transgene at a specific site the

ovine 1(I) procollagen (COL1A1) gene locus and the subsequent production of live sheep by nuclear transfer. Targeted gene insertion was only previously possible in mice.

#### **1.4 Retroviral Transfer:**

The use of retroviral vectors to deliver DNA to an organism either at an early or advanced stage of development is somewhat less efficient than pronuclear injection but has been used widely in avian species.

#### **1.5 Foetal and somatic cell nuclear transfer:**

Another approach includes the genetic manipulation of stem cells derived from embryos or, more recently, the derivation of nuclei from adult somatic cells (SCNT). In the former method, ES cells are introduced back into early embryos subsequently giving rise to 'mosaics' (animals where some cells are genetically altered). Again, breeding of the founder stock can eventually result in a consolidation of the desired phenotypic attributes.

More recent research has sought to exploit the benefits of reprogramming adult somatic and differentiated embryonic nuclei so that they regain their totipotency resulting in an increasing number of cloned transgenic offspring.

#### **1.6 Gene Deletion ('knock-out'):**

Numerous gene-targeting procedures are designs to stop or inhibit the production of a protein by gene deletion. In these cases the gene function is 'knocked-out'.

## **2. CURRENT STATE OF DEVELOPMENT**

### **2.1 Trends in the production and use of transgenic and cloned animals:**

A accurate assessment of changes in the production of transgenic and cloned animals for a variety of purposes can be gained by observing recent statistical trends in animal licensing procedures. Whilst there are no international figures on which to base such an estimate, domestic figures from countries where this information is available can be taken as indicative of general current direction.

In the UK, for instance, it is possible to observe 3 main trends since 1990:

- Overall decline in the number of animal procedures, due to a fall in the use of genetically normal animals (from just over 3 million in 1990 to slightly under 1.9 million in 1999).
- Overall rise in the use of animals with harmful mutations (from ~143,000 to ~251,000).
- Tenfold rise in procedures using transgenic/GM animals from just under 50,000 to over 500,000; around one in five of all animal procedures in 1999 involved GM animals.

Mice account for the vast majority of animals produced with genetic modifications (98%) although the use of other species is set to increase. This is a consequence of several factors, especially the movement to production in larger animals of products developed in murine varieties. This is especially likely to be the case in applications like protein production and xenotransplantation.

### **2.2 Toxicological Testing:**

At present, relatively few genetically altered animals are used in routine toxicological testing though this is expected to change as product developments approach the market place. New products, including proprietary mouse mutations are soon to become

commercially available for assessing the relationships between genetic mutations and different carcinogenic compounds.

### **2.3 Animal Models of Human Disease:**

Since the early 1990s, numerous transgenic and knockout rodent varieties have become commercially available with which to model human disease processes and their response to potentially therapeutic compounds. On a smaller scale model species have now been extended to larger mammals.

Disease models include neuropsychiatric, cardiovascular, pulmonary, oncological, inflammatory, autoimmune diseases, in addition to metabolic, reproductive and developmental conditions. A number of databases of mouse transgenic mutations exist including TBASE and Induced Mutant Resource (IMR). Over 60 'oncogenes' and 20 tumour suppressor genes have been produced in mouse mutants.

While many mammals share similar biogenetic and chemical pathways, physiological and metabolic processes can differ radically, especially in those conditions where environmental and behavioural factors need to be taken into account. Future areas of development are increasingly likely to concentrate on producing models for the analysis of polygenic disorders (hypertension, schizophrenia, etc.) which will inevitably prove highly problematic in producing extrapolative findings to human behaviour, environment and genetic constitution.

### **2.4 Use of Transgenic Animals for Recombinant Protein Production:**

The genetic modification of animals has long been recognised as a more effective platform for the production of novel recombinant proteins. The method has a number of advantages over traditional cell culture production inclining the ability to manufacture much larger amounts of protein using heightened expression levels with higher production volumes. Gene expression and heterologous protein production can be achieved in numerous tissue and fluid secretion sites in the body of a source animal (including blood, urine, semen and milk). To date, the expression of proteins in the milk of grazing ruminants has proved to be the most active area of research and product development. Mammary gland production of proteins promises significant reductions in the cost per unit of protein with the source animal requiring fewer inputs and less sophisticated continual monitoring than traditional recombinant cell culture systems.

The technique has additional advantages in that many proteins (blood-clotting factors and antibodies) can only be produced in live animal cell bodies. Other proteins (i.e. human albumin used in a wide variety of applications) cannot be produced in a sufficient volume using other available techniques. By contrast to the derivation of proteins from humans, the use of transgenic source animals permits greater levels of control of the biosecurity and the SPF (Specific Pathogen Free) character of production.

As many as 50 varieties of genetically modified animals have been produced which express human proteins by three particularly strong industry leaders (Genzyme Transgenics Corp.[USA], PPL Therapeutics [UK] and Pharming B.V. [NL] ). Preclinical and clinical trials of various product lines (see table 1 below) include anti-thrombin III, monoclonal antibodies, factor VIII, alpha-1-proteinase inhibitor, alpha-glucosidase and other therapeutic applications. The following table is illustrative of products currently in development.

**Table 1: Products currently in development**

<i>PreClinical</i>	Developer: Genzyme Transgenics Indication: cancer Marketer(s): Bristol-Myers Squibb Product: BR96 monoclonal from transgenic goat milk Current Status: Preclinical BR96 monoclonal from transgenic goat milk	
<i>Phase I</i>	Developer: Avant Immunotherapeutics Indication: atherosclerosis prevention - cholesterol lowering Product: injectable vaccine to increase HDL cholesterol Tradename: CETP vaccine Disease: Cardiovascular Technology: vaccine	Developer: Pharming Group Indication: heparin-neutralization Product: lactoferrin Tradename: Disease: Cardiovascular Technology: Transgenics, Coagulation control
	Developer: Pharming Group Indication: angioedema Marketer(s): Hyland Immuno Product: human C1 Esterase inhibitor Tradename: Disease: Excessive edema Technology: Transgenics, Natural products	Developer: Pharming Group Indication: GI Infections Product: lactoferrin Tradename: Disease: Infections Technology: Transgenics, Natural product
<i>Phase II</i>	Developer: Progenics Indication: AIDS Product: humanized antibody to CCR5 Tradename: PRO 542 / Pro 367 and PRO140 Technology: Monoclonals - Humanized Abs Transgenic production in goat's milk with Genzyme Transgenics.	Transgenic production  Developer: Abgenix Indication: renal cancers Marketer(s): Immunex Product: humanized Mab against epidermal growth factor Tradename: ABX-EGF Disease: Cancer Technology: Monoclonals - Humanized Abs
	Developer: PPL Therapeutics Indication: cystic fibrosis Product: alpha-1 antitrypsin Tradename: AAT	
<i>Phase III</i>	Developer: Genzyme Indication: Pompe's Disease Marketer(s): JV with Pharming Group NV Product: human alpha glucosidase Disease: Metabolic Disorders Technology: Transgenics	Developer: Genzyme Transgenics Indication: reduce bleeding & transfusions in CABG Marketer(s): Aventis, Sumitomo Metal Product: goat milk-produced human recombinant antithrombin III Tradename: AT-III / aaATIII Disease: Blood & Hematopoietic Factors, Cardiovascular Technology: Transgenics transgenically produced recombinant human antithrombin III (rhATIII)

**2.5 Xenotransplantation:**

The use of cloned and transgenic animals as a source of tissues, cells, organs and secretions has become a key area of research both within Europe and the US since the early and mid 1990s. Recent changes in the industry, particularly the withdrawal of

Novartis from the field, represent a significant shift in perspective on the future viability of xenografts. The lead-time for developments is likely to be much more long term than once envisioned and product concepts will be strongly affected other competing alternatives (mechanical devices, stem cells, etc). The field is addressed in greater depth in an accompanying Annex to this report.

## **2.6 Agricultural Production:**

New genomic techniques in the production of farm animals concentrates on:

- Disease-resistant strains
- Development of trypanosomiasis (foot and mouth)-resistant ruminants.
- Desirable alterations to growth rate / feed conversion efficiency
- Reduction of fat to muscle ratio
- Enhancement of anti-microbial properties in milk

The field is currently somewhat under-researched and has not produced sufficient risk management data to merit widespread licensing and application. Lack of understanding of muscle growth regulation and wider environmental implication has been noted by numerous authorities.

Of particular recent concern, is the highly successful growth modification of fish including trout, salmon, catfish, tilapia, chinook and carp. In many cases, modification has produced a x3 increase in mature body weight and evidence of a colder water tolerance. Studies have also demonstrated the heightened feeding motivation of GM salmon (~250%) and led to expressions of concerns on environmental impact.

## **2.7 Dependent and Related Technology Areas:**

There are a number here, principally:

- Bioinformatics – gene function and integrated trans-organism databases
- Biosecurity – pathogenic containment (particularly endogenous and retroviral)
- Animal husbandry – methodological adaptation to new requirements
- Cell-cycle and regulation research
- Gene restriction methods and targeted genetic insertion.

## **3. KEY ACTORS**

### **3.1 Public / Commercial Interaction:**

There is extensive public and private coupling undertaken in pharming-related areas of innovation though this will vary considerably across member states of the EU according to local regulations governing university research involvement in commercial development. A particularly prominent illustration of strong relationships between public and commercial research is the Roslin Institute and the integration of somatic cloning techniques into industry.

## **4. PROSPECTS AND UNCERTAINTIES**

### **4.1 Medium Term Prospects (5-10 years):**

- Significant increase in the number of GM animals being used in toxicological testing
- Possible reductions in the overall numbers of animals being used in testing because of the production of statistically more appropriate animals

- Significant increase in the number of GM animals being used in gene function studies as a consequence of HGP – this could potentially reverse the overall general decline in the use of research animals
- Arrival on the market of medicinal proteins from transgenic animals
- Use of cloning and the banking of cells for the protection of endangered species
- Gradual reductions in the failure rate of cloning and transgenic animal production

#### **4.2 Long Term Potential (10-20+ years):**

Increasing range of ‘medicinal’ foodstuffs (for example, milk lacking human allergenic proteins [lactoglobulin]).

#### **4.3 Known Uncertainties:**

The political legitimacy and public trust in pharming, particularly in food production rather than other areas like toxicological testing.

Whether overall number of animals used in medical research will increase or decrease.

### **5. ETHICAL, LEGAL AND SOCIAL ISSUES**

#### **5.1 Environmental:**

- Serious concerns pertain to the ecological implications of pharming and the application of transgenics in food production.
- Some applications present inherent environmental containment limitations such as the production of GM fish varieties. Canada has been a strong advocate of a moratorium on GM fish because of the possibility of wider fish stock decimation by novel voracious strains. Whilst sterilisation is not widely seen as an adequate solution to the problem, the use of land-lock sites has some potential in providing environmental protection.
- Environmental consideration also needs to involve innovation where the intention is to create products that can tolerate longer travel times without spoiling. This is likely to run counter to policies aimed at reducing dependence on road and air haulage.

#### **5.2 Public Health:**

- The public health implications of transgenics, cloning and pharming extend into application areas like xenotransplantation, where there are risks of transpecies infections which have the potential to affect a recipient’s wider contacts. Most regulatory authorities have advised that not enough is currently known about the scale and seriousness of these risks to endorse widespread clinical trials.
- Other risks relate to potential allergenic reactions in humans to animals produced for meat or toxicity from biologically active proteins (in milk for example).

#### **5.3 Animal Welfare:**

- Widespread commentary has emphasised the need to reappraise animal welfare assessment procedures in the light of new genetic methods for producing animals. Muscle growth, physiology, nervous systems and normal development are known to be adversely affected by pronuclear recombination and cloning. Genetically altered traits need to be consistent with established welfare standards.
- Inefficiency in the production of GM animals means that a much greater number of invasive procedures need to be currently conducted on animals to produce even small population of research valid animals.

- Whilst invaluable to research, animals that have been produced to exhibit human disease disorders may indicate prohibitively severe levels of suffering.
- Biosecurity measures, used to produce specific pathogen free (SPF) animals (i.e. for tissue and cell sourcing) may prevent animals from normal behavioural traits such as rooting etc.

## 6 REGULATORY AND PUBLIC POLICY RESPONSE

The recent development of European regulatory capacity has relied heavily on existing edicts relating to recombinant technology and production, especially from the European Medicines Evaluation Agency (EMA) and the Committee for Proprietary Medicinal Products (CPMP). The CPMP document on the use of transgenic animals is recognised as bearing substantive similarities to FDA guidance in the US. More recently, the International Conference on Harmonization has played an increasing role in pressing for new regulations that take account of the production of genetically tailored test animals.

**Table 2: European regulation**

<b>Medicines Directive (65/ 65/ EEC)</b>	Applies to aspects of xenotransplantation including cell therapies and gene therapies involving viable animal tissues
<b>Medical Devices Directive (93/ 42/ EEC)</b>	Applies to xenotransplantation procedures involving the use of a medical device
<b>Genetically Modified Organisms (Deliberate Release) Directive (90/220/EEC) (to be repealed by the coming into force of Directive 2001/18/EC in 2001)</b>	Applies to the release and marketing of all GMOs – no applications relating to GM animals to date in the EU
<b>Directive (86/ 609/ EEC) on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (currently being updated)</b>	Applies to procedures involving the experimental scientific use of animals
<b>Council of Europe European Convention ETS 123 (1986).</b>	Protection of vertebrate animals used for experimental or other scientific purposes.
<b>Council Regulation concerning novel foods and novel food ingredients (EC 258/ 97)</b>	Applies to the use of GM animal material in the human food chain. No applications in the EU to date.
<b>Council Directive on the protection of animals kept for farming purposes (98/ 58/ EC)</b>	Affords protection of all species used in the production of food, wool, skin or fur or for any other farming purpose including fish, reptiles or amphibians. Based on the European Convention for the Protection of Animals.

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## XENOTRANSPLANTATION

### 1. DESCRIPTION OF THE TECHNOLOGY

Whilst the issues are highly complex, there is a general consensus that developments in specialist fields like surgery and immunology together with organisational initiatives (transplant co-ordination) have produced effective medical procedures for a wide range of patient groups who would otherwise not have been treatable.

Yet the demand for human tissues and organs has become more acute as a consequence of several recent trends. Improvements in the efficacy of human-to-human (allotransplantation) have expanded the number of patients on waiting lists. Better health and safety standards have reduced the number of people dying inappropriately young and thus had a negative effect on the allotransplantation donor pool. In some contexts, abuses of clinical expert autonomy have had an adverse effect on professional status and on willingness to donate tissues.

The widening gap between the demand for and availability of replacement tissues has prompted renewed interest in a wide range of approaches including the possible use of nonhuman derived tissues and organs (xenotransplantation). In addition, developments in the fields of transgenics and cloning has raised expectations that it may well become possible to overcome natural immunological barriers between species that would otherwise rule out the use of other species as a tissue source. It is important to recognise that whilst xenotransplantation may possibly have a future role to play amongst a number of strategies it is not likely to solve the many problems currently facing transplantation. Future innovation in transplantation will instead be drawn from a complex combination of the following:

- Improved preventative public health measures
- Mechanical and biomechanical devices
- Improved organisational procurement and distribution of organs
- Policy changes in the consent procedures for donation
- Stem cell applications
- Xenotransplantation applications

The remainder of this report will focus on the last of these options.

Research into xenotransplantation has concentrated on numerous applications including some areas not currently treatable by allotransplantation:

- Whole Organs (kidney, heart, lung and liver)
- Ex vivo whole organ perfusion (circulation of blood through non-human organs, particularly the liver, as a 'bridging device' to allogenic transplantation)
- Cellular implants (use of dopamine producing implanted porcine foetal cells for Parkinson's Disease, regenerative nerve cells for use in the brain or spinal cord, chronic intractable pain syndromes, insulin producing islet cell implants)
- Use of nonhuman cells in human skin cell culturation (skin cultured on nonhuman cell cultures)

Definitions of xenotransplantation vary to some extent though most compare quite closely to that of the US Food and Drug Administration. The FDA definition of xenotransplantation has been altered recently to take account of the skin cell culturation method mentioned above and is in the process of being adopted by other regulatory authorities including the United Kingdom Xenotransplantation Interim Regulatory Authority (UKXIRA):

any procedure that involves the transplantation, implantation, or infusion into a human recipient of either (a) live cells, tissues, or organs from a nonhuman animal source or (b) human body fluids, cells, tissues or organs that have had ex vivo contact with live nonhuman animal cells, tissues, or organs. Furthermore, xenotransplantation products have been defined to include live cells, tissues or organs used in xenotransplantation.

The definition excludes the transplantation of non-live tissues such as porcine heart valves or porcine derived insulin for the treatment of diabetes because they do not pose the same immunological and transpecies disease risks. These exclusions have been in routine medical use for some years, as has the recently included human skin products cultured on mouse cells.

## **2. CURRENT STATE OF DEVELOPMENT**

### **2.1 Production of Transgenic, Cloned and Genetically Modified Animals:**

Enthusiasm for xenotransplantation has intensified with the development of techniques that allow the donor animal's immune system to be genetically modified to mimic more closely that of the recipient species. Biotechnological approaches have broadened the range of species potentially useable in xenotransplantation though much of the research has focused on the modification of the pig as the donor species of choice. Some of the reasons for this include the pig's comparable size to humans, its established use in the food industry, its rapid rate of reproduction (relative to other potential donor species such as nonhuman primates).

The key areas of current innovative research focus on:

- Reducing human immune response to nonhuman tissues and cells through transgenic and clonal adaptation of the donor, and appropriate immuno-suppression of recipient.
- Reducing the risks of transpecies disease (viral pathogens, bacterial and fungal agents etc.).

### **2.2 Reducing Human Immune Rejection Responses:**

*Hyperacute rejection (HAR):* Hyperacute rejection occurs when antibodies on the donor organ trigger a

response in the recipient, leading to rapid destruction of the xenograft. The HAR is triggered by the most severe rejection action in the human body (complement cascade) and represents the most intense area of research to date.

There is general consensus amongst researchers in the field that HAR arises because of a carbohydrate on the surface of pig cells called alpha 1,3-galactose (alpha gal). Whilst humans lack alpha-gal, we possess antibodies against it because of prior exposure to bacteria bearing the same sugar. Various strategies have been adopted to manage the HAR response. But relatively modest animal study successes have so far not merited approval of large-scale clinical trials.

In the early 1990s, *Imutran* (Novartis) produced transgenic pigs whose immune systems had been engineered to include two human complement inactivating proteins (C5-C9 and Decay Accelerating Factor). More recently, *Nextran* in the US, has used a similar approach to produce porcine donors genetically engineered with the human complement regulatory proteins CD59 and decay-accelerating factor (DAF).

Other companies, Ophidian Pharmaceuticals, Inc. for example, have approached the problem pharmacologically by seeking to block the binding of naturally occurring antibodies reactive to the alpha-gal epitope. BioTransplant Inc. has also used pharmacological absorption techniques to 'clean' natural antibodies from the recipient's blood prior to the transplant of porcine organs into monkeys.

More recent approaches have been directed at using cloning techniques to produce pigs whose alpha gal gene has been deleted. Cloning is more versatile than the older technology of DNA microinjection into pig eggs used to introduce new genes (the strategy adopted by companies like *Imutran* and *Nextran* above). Instead, cloning means that it is possible to delete parts of the genome in advance of nuclear cloning. In 2000, PPL Therapeutics Inc. announced the production of 5 non-transgenic cloned pigs demonstrating for the first time the application of nuclear transfer in xenotransplantation's donor species of choice. The development was followed more recently by a repeat of the procedure but with the addition of a transgenic gene to the clones.

A number of teams are now working to combine these techniques in the production of cloned alpha gal 'knock out' porcine donors. Edinburgh's Roslin Institute recently demonstrated alpha gal deletion in sheep creating the possibility to determine the importance of the gene to graft rejection (Denning et al, 2001).

The success of the approach still faces many uncertainties. Even if an alpha gal deletion inhibits HAR, other acute and chronic rejection processes need to be addressed.

*Acute and Chronic Rejection:* Acute rejection of xenografts (tissue from different species) is generally believed to be mediated by T-cells rather than the human immunological complement system.

Illustrating work in this field, *Nextran* is exploring the development of pharmacological immunosuppressive regimes that will have to operate differently to allotransplantation immunosuppressives. This highlights the need for new understanding of rejection acute and chronic processes for xenotransplantation.

Other approaches being considered involve preparing the recipient's immune system to accept grafted tissues by transplanting donor bone marrow in advance of xenotransplantation. The concept, pursued by companies such as BioTransplant, assumes that T cells derived from the transplanted bone marrow will migrate to the thymus, where potentially harmful T cells will be deleted. The recipient's immune system is then expected to accept both donor and recipient tissues.

Nevertheless, since research is still focused on hyperacute rejection, uncertainty abounds as to the nature of potential acute and chronic problems.

### 2.3 Cell-based applications:

Cell-based applications do not present the same degree of immunological difficulty faced by solid organs because they do not involve the use of endothelial cells which line blood vessels in solid organs and are far more immunogenic than other cell types. Transplanting cells rather than the structural framework of blood vessels does not trigger the acute rejection associated with solid organs. Nevertheless, this still leaves some T cell mediated responses which are managed in a number of ways. The use of standard immunosuppression (cyclosporin) reduces potential difficulties in addition to masking pig-graft antigens.

Neural cell applications, for the treatment of stroke or Parkinson's disease, also benefit from the protection afforded by the blood / brain barrier which separates the central nervous system from antibodies circulating in the blood. Illustrating this approach, Diacrin Inc. is now in Phase II/III clinical trials for NeuroCell-PD, a cellular approach using porcine foetal neural cells to treat Parkinson's disease. Although Diacrin recently suspended Phase I trials for similar stroke treatments after several adverse events. Other applications being pursued include cell xenotransplantations for focal epilepsy and intractable pain, spinal injury treatments and NeuroCell-HD for Huntington's disease.

### 2.4 Products Currently in Development:

**PreClinical** Extensive preclinical research to produce immunologically and virologically safe xenografts – extensive use of transgenics and some mammalian cloning

#### Clinical Trials

##### Phase I

Temporary liver support system developed by Excorp Medical. Product: use of animal cells for liver failure

Neural Xenograft developed by Diacrin Inc. Product: fetal pig brain cells for treatment of stroke patients

NeuroCell-HD developed by Diacrin / Genzyme (Neurocell). Product: fetal porcine neural cell transplant.

##### Phase II

NeuroCell ND developed by Diacrin Inc / Genzyme (Neurocell JV). Product: Porcine Brain Cell Xenotherapy for the treatment of Parkinson's disease

ELAD developed by VitaGen. Product: extracorporeal porcine liver assist device

##### Phase III

HepatAssist Liver Support System developed by Circe Biomedical Inc. Product: HepatAssist liver supportsystem using a hollow fibre ex-vivo bioreactor.

NeuroCell-PD developed by Diacrin Inc / Genzyme (Neurocell JV). Product: foetal porcine neural cell transplant for advanced Parkinson's disease

#### In Clinical Use

Epichel developed by Genzyme Corp and marketed since 1987. Product: a human autologous skin cell keratinocyte product that has been expanded on irradiated feeder layers of murine fibroblast 3T3 cells from NIH 3T3. Only with recent changes to the FDA xenotransplantation definition does Epichel now count as a xenotransplant application. Over 100 patients per year have been treated with Epichel though genzyme does not have a surveillance registry considered necessary by most xeno-regulatory authorities.

### 2.5 Production of Pathogen Free Donor Animals:

Since the early/mid 1990s serious concerns have been raised with regard to the potential transfer of infectious agents between the donor and host species (bacteria, fungi,

parasites, viruses and Porcine Endogenous Retroviruses [PERV] embedded in the pig genome). Because of the novelty of infection to human immunity, an infected recipient would also represent a biological hazard to close contacts and the wider population.

Ironically, many of the strategies for overcoming immunological difficulties result in greatly increased risks of transpecies disease transfer because patients will be iatrogenically immunocompromised thus increasing disease susceptibility. Many strategies for improving graft acceptability involve depletion of human anti-Gal antibodies and suppression of Gal-reactive B cells. This in turn may remove a barrier to infection by PERVs and other infectious agents.

A recent retrospective study could find no PERV infection in 160 patients who had been treated with porcine transplant and perfusion technologies. However, a number of patients showed an antibody response to PERV and the persuasiveness of the study was somewhat compromised because of the limited DNA assays used to detect PERV and for having used blood tests rather than tissue biopsies.

Research innovation directed at overcoming these risks has intensified of late by approaching the problem from a number of angles:

Removal of PERVs from the porcine genome. Most pigs possess as many as 40 endogenous retroviruses. A number of research initiatives are now considering the use of genetic engineering to remove genomically embedded viruses. Immerge BioTherapeutics, Boston MA, is working with miniature swine not found to possess PERVs.

Biosecurity containment – in addition to those measures being taken to remove PERVs, all research institutions developing donor animals are having to adopt new techniques for the production of Specific Pathogen Free (SPF) animals. These measures include new sterile housing facilities, aeration of housing under positive pressure, delivery of piglets by caesarean or hysterectomy, etc.

## **2.6 Dependent and related technology areas:**

*Cloning:* the production of cloned alpha gal deleted pigs is seen as an important near-term milestone in a better understanding of potential therapeutic efficacy.

*Stem cell:* animal derived applications are likely to depend upon improved understanding of cell function and differentiation

*Gene targeting:* refining gene targeting techniques for the removal of potentially harmful immunological and endogenous virological properties is seen as essential for potential development.

*Immunology:* it is thought extremely likely that unforeseen acute and chronic immunological difficulties face xenotransplantation, even if hyperacute rejection is resolved.

*Virology:* knowledge of potentially harmful virological pathogens is widely judged to be in its infancy.

*Biomechanics:* animal derived tissues are likely to be used in hybrid biomechanical devices offering nonhuman cells a measure of protection from human immunity and offering the patient protection from potentially harmful pathogens.

*IT & Bioinformatics:* any move towards clinical trials is likely to depend on the establishment of interoperable surveillance databases.

### 3. KEY ACTORS

#### 3.1 Pharmaceutical industry:

In whole organ research, the situation has now altered significantly with the withdrawal of big pharma (Novartis) from direct involvement. The composition of the industry, even in cell-based application, now tends to be comprised of SME biotech organisations. The long lead-time to clinical viability is seen as a reason for larger pharma disinterest in xenotransplantation. Though this is likely to change in the long term if the pharmaceutical industry observes significant improvements in biosecurity and pre-clinical immuno-tolerance.

#### 3.2 Public, commercial and extent of interaction:

There is a relatively high degree of interaction between academic clinical research and commercial science in this sector, especially because the concept of the technology requires the enrolment of clinical specialists such as transplant surgeons, renal specialists and neurologists.

Basic science is likely to continue feeding reasonably novel approaches into the xenotransplantation concept, although recent trends have demonstrated a scaling down of basis R&D activity directed specifically at XT.

### 4. PROSPECTS AND UNCERTAINTIES

#### 4.1 Medium prospects (5-10 years):

It is highly likely that alpha gal knockout porcine founder stock will be developed within this time frame. Subsequent preclinical studies will then have the opportunity to empirically verify the hypothesis that alpha gal represents one of the more significant immunological factors in graft rejection.

It is also somewhat likely that PERV free pigs will be produced, but this will not necessarily lay to rest anxieties surrounding transpecies disease.

<b>Heart</b>	Recent progress in mechanical assist and replacement devices are seen as potentially more feasible – though long term prospects remain unclear
<b>Kidney</b>	Of all the whole organ applications, kidney xenografts appear the most feasible – although contingent upon major advances in immunology and biosecurity (<15 years)
<b>Liver</b>	Physiological complexity is likely to rule out permanent xenografting. Ex vivo assist devices are likely to have a role in end stage bridging to allograft (<5 years).
<b>Pancreatic Islet Cells</b>	Potential feasibility – although widespread application is unlikely in the medium term (<10 years).
<b>Dopamine producing neural implants</b>	Potential feasibility (<15 years) – although major concerns over current biosecurity and poor graft survival. May well be substituted by stem cell applications in long term (>15 years).
<b>Other nerve and neural applications</b>	Potential feasibility (>15) May well be substituted by stem cell applications in the long term (>15 years)

#### **4.2 Known current uncertainties:**

Whether new immunological factors will become evident

Whether current knowledge of retroviruses is sufficient – other unknown pathogens

Whether mechanical devices (for heart esp) are likely to progress further / whether human stem cell applications will remove R&D incentives around xenografting.

### **5. ELSI ISSUES**

#### **5.1 Human:**

- Public Health – there are likely to be persistent problems in resolving the conflict between the willingness of the patient to undergo risky treatment and, on the other hand, the impossibility of securing consent from their contacts and the population at large.
- Human recipient surveillance measures – the requirement for potentially invasive and life long surveillance measures represent almost unprecedented levels of biosecurity. These can only be taken on a voluntary basis and authorities may consider this to be practically untenable.
- Psycho-social - Appropriate attention to the psycho-cultural implications of being implanted with nonhuman cells and tissues needs to be considered, especially where this is seen to represent a valid ‘lay’ apprehension of material risk.

#### **5.2 Animal:**

- Pigs - There are continued welfare considerations for donor source species, especially the need to incorporate natural features of the pigs’ behavioural environment (rooting) into the sterile conditions of pathogen free containment facilities
- Primates – preclinical trials of xenotransplantation require a higher number of primate models than almost any other preclinical area of medical innovation. This is because of a number of highly distinctive features to the xenotransplantation concept including: A. The need to test for complex immune-rejection processes and the requirement to do this research in a species as closely related to humans as possible. B. The requirement to demonstrate potential efficacy through post-xenotransplantation survival times. This means that primates have to endure long periods of survival in relatively traumatising experimental conditions. C. Disappointing preclinical trials of solid organ xenotransplantations have considerably increased the number of primates being used. D. The high demand for primates has increased the use of wild caught Primates (mainly baboons and cynomolgus monkeys) in trials because primates are relatively difficult to breed in captivity.

### **6. REGULATORY AND PUBLIC POLICY RESPONSES**

There is a reasonably high degree of international regulatory activity being facilitated through the participation of domestic regulators in numerous transnational initiatives. Such activity is largely geared towards fostering information sharing rather than arriving at international binding rules and principles. Examples of this kind of learning include the recent efforts to update the new FDA definition of xenotransplantation to include cells cultured on nonhuman tissues. Xenotransplantation is currently receiving consideration by:

- Organisation for Economic Cooperation and Development
- World Health Organisation

- European Commission – until recently the Commission recommended a voluntary freeze on
- Council of Europe Working Party on Xenotransplantation – currently seeking to develop a European policy on xenotransplantation

Changes in US governance arrangements have been particularly important in respect to Xenotransplantation because of continued and intensive research work taking place there. In a recent move, oversight of xenotransplantation clinical studies has shifted from a devolved local model (Local Review Bodies, Institutional Review Boards, Institutional Animal Care and Use Committees, Institutional Biosafety Committees) to a centralised model managed by the FDA with review by the Secretary's Advisory Committee on Xenotransplantation.

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