Molecular therapeutics is a new and developing science which involves genetics, recombinant DNA technology, biochemistry, protein production and purification, microbiology, molecular biology, immunology, pathobiology and biotechnology. It addresses the treatment of human beings with ‘new drugs’ and poses a range of ethical issues, particularly with respect to clinical trials, animal models, financial considerations and availability of treatment. Some therapies that have been developed in animal models, such as germ-line gene therapy and cloning, have been banned for use in humans and others are only available under strict licence.

The technology covers a wide range of disease therapies from protein supplementation to gene therapy and is applicable to microbial, inherited and acquired diseases. Some of the therapies to be discussed are currently being tested in cell culture. We need to address the validity of such studies in the light of the fact that cultured cells are not normal in as much as normal cells do not grow indefinitely in culture. Other therapies are being evaluated in animal models. We will need to discuss the ethics of the production of such models and the validity of results we may obtain. Other therapies to be described are still at the clinical trials stage and are not yet available for patient treatment. Such therapies are being evaluated by treating terminally ill patients who are unlikely to gain any benefit from the trials. Again, we must address the ethical and moral issues surrounding such practices.
1.1 Microbial diseases

Diseases caused by bacteria, viruses and protozoans that affect mankind are common, particularly in the developing world. Indeed, during a year many of us will have suffered a virally induced infection, and worldwide the WHO estimates that 8000 people die of AIDS-related conditions every day, which equates to 3 million deaths per year. Every year there are 8.8 million new cases of tuberculosis (TB) reported, with 5500 deaths a day or a million deaths worldwide each year. Worldwide, there are about 300 million cases of acute cases of malaria reported each year.\(^1\) However, many microbial diseases can be cured by the administration of antibiotics or prevented by vaccination. The WHO statistics for 2002 showed that 32% of people died from communicable diseases, 59% from non-communicable disease and the remaining 9% from injuries.\(^2\) However, there are an increasing number of bacteria that are antibiotic-resistant, for example methicillin-resistant *Staphylococcus aureus* (MRSA) which can affect hospitalised immunocompromised patients and drug-resistant strains of *Mycobacterium tuberculosis* which are an increasing problem in patients infected with HIV. Drug-resistant strains of bacteria are thought to be caused by the overuse of antibiotics not only in human populations but also in farming where antibiotics are used to encourage fast growth of animals.

Viruses also pose a great threat to the well-being of humankind. For example, there are few effective treatments for human immunodeficiency virus (HIV), the causative agent of AIDS, human papilloma virus (HPV), which is implicated in cancer of the cervix, or hepatitis A, B and C which may cause long-term liver damage. It is also important to realise that we know much about the genome of these organisms but cannot easily formulate cures. All viral infections are difficult to treat effectively, some are potentially life-threatening and new viruses are emerging, for example Ebola virus.

Parasitic diseases are a particular problem in developing countries where the parasite burden leaves whole populations immunocompromised and open to opportunistic infection. Malaria for example affects hundreds of millions of people worldwide, but current treatment strategies are failing, new treatments are too expensive for use and no effective vaccines exist. There is therefore a need to develop new drugs, immunotherapy, gene therapy and vaccines to alleviate the problems. These therapies must be cheap enough to be used worldwide and stable enough to allow shipment and storage at room temperature.

In order to treat diseases effectively we need to understand the causative agent and the mode of infection. This may allow us to formulate preven-
tion strategies that are simple and cost-effective. Simplistically, if we know the vector of a disease breeds in stagnant water then we may treat stagnant water with pesticide and kill the vector. Such an approach was successful in the prevention of malaria. In this case the stagnant pools were treated either with oil to decrease surface tension and essentially suffocate the larvae of the vector or with a pesticide to kill the larvae directly. Such schemes are effective until the vector develops resistance or until the political situation in the country becomes unstable, resulting in failure to treat the breeding grounds so that subsequently the problem re-emerges.

An understanding of the genetic and biochemical make-up of the organism allows us to formulate a range of strategies based on our knowledge. For example, analysis of the biochemistry of HIV showed that a protease was vital for cleaving a specific protein needed by the organism for survival. This protease was distinctly different from human proteases and therefore specific inhibitors could be designed to affect the viral enzyme.3,4

The host also plays a role in infection and its control. The host immune response will affect whether an infectious agent will be removed from the host or retained and allowed to multiply. The host genotype also plays a role. Again HIV is a good example where individuals with the rare genotype ccr5−/− lack a cell surface receptor vital for infectivity. Another example involves smallpox, which is known to carry the blood group A antigen. Individuals who are blood group O have natural antibodies to this structure, which will bind to the virus and elicit destruction via the immune response. Blood group A individuals lack this first line of defence since they cannot make antibodies to a structure which is seen as ‘self’.

1.2 Cancer and heart disease

In developed countries cancer and heart disease are the major causes of death. They are both multifactorial diseases and therefore involve genetic predisposition and environmental factors. Although we can now identify genetic risk factors and environmental stimuli it is difficult to assess risk for an individual patient. Indeed, everyone seems to know someone who has smoked cigarettes all their life and lived into their 80s. Nevertheless, we all appreciate that smoking is a trigger in susceptible individuals and is the major cause of lung cancer. Presumably, if we could identify those
with genetic predisposition we could more effectively target our anti-smoking campaigns.

1.2.1 Cancer

In the 1970s Richard Nixon, the president of the USA, stated that he would cure cancer in his term of office: huge sums of money were invested but no positive results were obtained. Indeed, cancer is probably as important today as a killer as it was 100 years ago. One problem faced by scientists involves understanding the mechanisms of cancer. However, although many cancers may now be defined with respect to mutations in oncogenes and tumour suppressor genes, most cancers involve numerous genetic mutations and determining which mutated protein, if any, will provide a suitable therapeutic target is difficult. Additionally, even though a mutation is detected an environmental stimulus is often a key factor without which the disease does not progress.

Currently the most effective treatment for cancer is surgery. This is only effective if the tumour has not metastasised and spread to other parts of the body. There are fears, however, that surgery itself may liberate cancerous cells into the host system, causing further problems. Chemotherapy and radiotherapy are useful but toxic and many of the agents used damage DNA in healthy cells, leading to fears that the treatment may well cause mutations which will cause different forms of cancer to develop later. Currently researchers are investigating new therapies that either stop cancers from growing or spreading or allow the immune system to recognise the cancers more effectively and destroy them. The following therapies are undergoing trials:

- turning off oncogenes
- adding tumour suppressor genes
- manipulating cell cycle
- killing cancer cell with cytotoxic drugs
- manipulating transcription
- using antibodies bound to toxins to kill cells
- using personal vaccines
- using recombinant cytokines
- delivering tumour necrosis factor genes.
1.2.2 Heart disease

Heart disease is responsible for 50% of ‘natural’ deaths in the USA. It is currently treated with drugs, diet, lifestyle management, surgery and transplantation. Heart disease is another multifactorial disease that requires both genetic predisposition factors and environmental triggers. Many of the triggers have been identified, and these include smoking, stress, high fat diet and salt intake. Campaigns addressing the reduction of risk factors in the population are largely ineffective. Current research is focused on the treatment of thromboses, with recombinant drugs such as tissue plasminogen activator factor, and the manipulation of animal organs for transplant to alleviate the current shortage of organs. Gene therapy has been attempted to treat familial hypercholesterolaemia.

Figure 1.1 Therapies undergoing trials for heart disease

1.3 Genetic diseases

Although genetic diseases are relatively uncommon they are frequently incurable and often untreatable and therefore threaten the lives of sufferers. However, some diseases occur at high frequency within affected populations, for example thalassaemia in individuals of Mediterranean origin (1 in 20 carriers). Many problems associated with inherited disease may be overcome by using screening strategies: for example population screening and prenatal diagnosis where appropriate. However, abortion of
affected fetuses is an ethical issue and many families do not realise they have an inherited disorder until they produce an affected child.

New therapies are currently being assessed and these range from drugs, recombinant proteins and gene therapy to immunotherapy. In designing treatments for inherited diseases we need to understand the basis of recessive and dominant diseases.

### 1.3.1 Dominant diseases

Dominant diseases require only one mutated gene for an affected individual to show symptoms and the bad gene always ‘over-rides’ the good gene. Examples of dominant disease include Huntington’s disease and Marfan’s syndrome. This type of disease can only be treated by removal of the bad gene or its product, therefore gene therapy, which currently involves the addition of genes, and recombinant protein therapies are not useful. It may be possible to introduce proteins to sequester the product of the action of the dominant gene. For example, in certain forms of hypercholesterolaemia, the defect lies in the failure to form low-density lipoprotein receptors. This results in excess free cholesterol which is effectively toxic. A chemical sequestration therapy is available which is taken orally to remove excess cholesterol from the patient and restore health.

In some cases transplantation may provide a treatment since in this case a new organ with normal genes replaces the affected organ and its defective genes. Additionally, since gene augmentation therapy will not provide a cure unless the affected gene is removed or replaced, research is focused on deactivating genes.

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<thead>
<tr>
<th>Table 1.1</th>
<th>Potential strategies for dominant diseases</th>
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<tbody>
<tr>
<td>• turn off genes by mutagenesis – not yet feasible in humans</td>
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<tr>
<td>• gene correction by homologous recombination; some success in mice</td>
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<tr>
<td>• germline therapy</td>
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<tr>
<td>• turn off genes using oligonucleotides; need constant supply</td>
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<tr>
<td>• remove poison gene product</td>
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<td>• manipulate gene regulation</td>
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### 1.3.2 Recessive diseases

Recessive diseases, such as cystic fibrosis and sickle cell disease, are theoretically easier to treat since in the presence of one good gene the symptoms
do not appear. These diseases are good candidates for gene therapy and transplantation since there is no requirement to remove the defective gene. Recombinant protein therapy may be useful in cases where delivery of the protein into the blood stream, intestinal tract or lungs will effect a cure. For example, Haemophilia A is caused by a fault in the production of the clotting factor, Factor VIII. This can be provided as a recombinant protein into the blood stream and effect a cure. However, the CFTR gene that is affected in cystic fibrosis produces a protein that must lodge within the cell membrane. Recombinant protein therapy could deliver the protein to the cell, but the protein would not be taken up into the membrane to perform a function and cure the disease.

1.4 Role of molecular biology in therapeutics

Advances in molecular biology have facilitated diagnosis of disease at the molecular level: such an understanding of the disease process may suggest a treatment strategy. Recombinant DNA technology may be used to produce therapeutic proteins, transgenic animals, cloned cells or gene constructs for gene therapy. An understanding of the molecular biology and genetics of a disease are vital to allow us to decide which ‘mutant’ genes to target. In infectious disease, an understanding of microbial genetics and molecular biology allows us to formulate vaccines and molecular
therapies. Once the mechanism of infection has been clarified, novel drugs can be developed.

However, for many diseases we do not need molecular therapeutics. In these cases we use conventional methods to treat the patient. We do not achieve a cure but we improve the life of the patient. If a disease causes a reaction to a particular foodstuff we would advise avoidance rather than gene therapy. In the case of phenylketonuria we can treat the patient by suggesting a phenylalanine-free diet, which prevents the damage caused by the accumulation of the breakdown products of phenylalanine. The diet in these cases must be maintained until puberty when the organs are fully developed. At this stage most affected individuals are able to eat normally again. However, we must be aware of potential problems to fetuses carried by affected females. The phenylalanine and by-products will cross the placenta and affect the developing fetus; therefore females with the disease must maintain their phenylalanine-free diet throughout pregnancy. Lactose intolerance is treated by suggesting a lactose-free diet, although some individuals take lactase powder to digest the milk safely.

For other diseases the most appropriate therapy may be a drug, for example anti-inflammatory drugs for arthritis, beta-blockers for heart disease, antibiotics for new born babies with sickle cell disease or cystic fibrosis. For other diseases early exercise plans help, for example in the case of cystic fibrosis physiotherapy helps remove the thick mucus, and sufferers of Duchenne muscular dystrophy are kept mobile by physiotherapy. Some diseases respond to blood or bone marrow transfusion, for example, sickle cell disease, thalassaemias and leukaemia patients after chemotherapy. Other patients are treated by organ transplant; for example, cystic fibrosis may be treated by heart–lung transplant.

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\text{Table 1.2 Uses of molecular biology in therapeutics}
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- diagnosis of the disease at the molecular level
- production of therapeutic proteins
- identification of the proteins involved in a disease process
- production of gene constructs for gene therapy
- understanding of a disease; inform which mutant genes to target
- formulation of vaccines and molecular therapies
- animal models may yield clues to therapeutic strategies
- cloning may allow us to rapidly increase animal models
- therapeutic cloning may allow production of stem cells for organ regeneration
- manipulation of rejection targets in transgenic animals to produce xenotransplants
- recombinant products may be used \textit{ex vivo} to stimulate cell
Points to consider

Ask your friends about the diseases covered. Are they well informed on the novel technologies?
Do you think such therapies will ever be used in developing countries?\textsuperscript{5,6}
What problems do we face when treating genetic disease?

Notes

1 www.who.int/mdg/goals/goal6/en/
6 www.ccne-ethique.fr/english/avis/a_046p02.htm