# Genetics and Genomics in Medicine Chapter 9

# Questions & Answers

## Multiple Choice Questions

### Question 9.1

Which, if any, of the following can be classified as a type of augmentation therapy?

a) Treatment using a small molecule drug to bind a target protein and prevent it working.
b) A bone marrow transplant.
c) Corrective surgery for cleft lip and palate.
d) Insulin treatment in diabetes.

**Answer 9.1**

b) A bone marrow transplant.
d) Insulin treatment in diabetes.

### Question 9.2

With regard to treatment of inborn errors of metabolism (IEM), which of the following statements, if any, is false?

a) IEMs are all single gene disorders that have been studied for many decades, leading to the development of successful treatment in all cases.
b) IEMs can be treated by augmentation therapy, treatment to inhibit positively harmful effects, or by prevention therapy.
c) Treatment for some individual IEMs can involve both augmentation therapy plus treatment to inhibit positively harmful effects.
d) Treatment of some IEMs involves artificially forcing an increase in a minor metabolic pathway to counteract a build-up in a toxic metabolite produced by a metabolic block in a major metabolic pathway.

**Answer 9.2**

a) IEMs are all single gene disorders that have been studied for many decades, leading to the development of successful treatment in all cases.

**Explanation 9.2**

For some IEMs there remains no suitable treatment.

## Question 9.3

Concerning the efficacy of small molecule drugs, which, if any, of the following statements is true?

a) At the level of clinical trials drugs can vary widely in how effective they are.
b) Once a drug has received regulatory approval, we can be sure that it will be effective in all patients, although some people will receive more benefit from it than others.
c) Drugs used to treat psychiatric disorders are particularly effective.
d) Stains and beta blockers that were meant to reduce the risk of heart disease are good examples of drugs that are largely ineffective.

**Answer 9.3**

a) At the level of clinical trials drugs can vary widely in how effective they are.

## Question 9.4

Which of the following descriptions, if any, is false? A person's ability to absorb or metabolize a drug that is intended to treat a genetic disorder

a) is entirely due to genetic factors.
b) depends on a person's lifestyle.
c) is not modified by having a bacterial infection.
d) is independent of a person's diet.

**Answer 9.4**

a) is entirely due to genetic factors.
c) is not modified by having a bacterial infection.
d) is independent of a person's diet.

## Question 9.5

With regard to drug metabolism, which, if any, of the following statements, is true?

a) The therapeutic window is simply the range of plasma drug concentrations in which the drug has therapeutic benefit.
b) Each individual drug molecule is metabolized by a specific drug-metabolizing enzyme that is dedicated to the metabolism of that drug.
c) An ultrafast metabolizer is a person who metabolizes a drug too quickly and so is at risk of an overdose
d) A poor metabolizer is a person who cannot metabolize a drug properly and is at risk of an underdose.

**Answer 9.5**

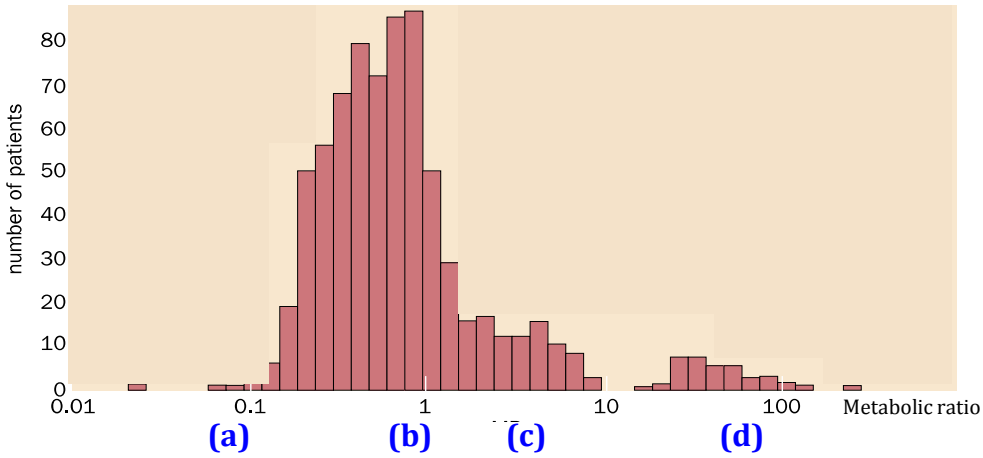
None. All are false.

**Explanation 9.5**

a) The therapeutic window is the range of plasma drug concentrations in which the drug has therapeutic benefit *without causing extra safety risks due to drug toxicity*.
b) Individual drug molecules can sometimes be metabolized by one of several drug-metabolizing enzymes, and many drug-metabolizing enzymes metabolize multiple different drugs.
c) An ultrafast metabolizer is a person who metabolizes a drug too quickly and so is at risk of an underdose.
d) A poor metabolizer is a person who cannot metabolize a drug properly and is at risk of an overdose.

## Question 9.6

The diagram below shows the urinary metabolic ratio as a measure of CYP2D6 enzyme activity in a total of about 700 individuals. After individuals were given a standard dose of a drug known to be metabolized by CYP2D6 the metabolic ratio was obtained by measuring the urinary concentration of the substrate drug and dividing it by the concentration of the metabolic product resulting from CYP2D6 acting on the drug. Classify individuals with metabolic ratios in the four ranges shown as (a) to (d) in terms of their drug-metabolizing abilities and describe the expected genotypes associated with each group.




**Answer 9.6**

a) This falls within the range of ultrafast metabolizers who will have multiple *CYP2D6* genes.
b) Extensive metabolizers with one or two normal *CYP2D6* alleles.
c) Intermediate metabolizers two mutated *CYP2D6* alleles, at least one of which makes some gene product but at a reduced level.

d) Poor metabolizers that have two null *CYP2D6* alleles (homozygous deletion, heterozygous deletion plus inactivating mutation, or inactivating mutations in both alleles)

## Question 9.7

Which of the following statements, if any, is false?

a) Monoclonal antibodies are made by identical immune cells and so will recognize and bind just one specific epitope on a target molecule.
b) Monoclonal antibodies of rodent origin are far from ideal therapeutic agents because of their short half-life in human serum and the potential for immune responses by the recipient.
c) Humanized antibodies are hybrid antibodies that have constant regions of human origin but variable regions of rodent origin.
d) An intrabody is an artificial constructs with just a single chain that is linked to variable domains and, unlike regular antibodies with four polypeptide chains, has the potential to work inside cells.

**Answer 9.7**

c) Humanized antibodies are hybrid antibodies that have constant regions of human origin but variable regions of rodent origin.

**Explanation 9.7**

The variable domains in humanized antibodies are of human origin, except for the complementarity-determining regions, which are of rodent origin.

## Question 9.8

Which of the following statements, if any, is false?

a) Gene therapy involves the direct genetic modification of the cells of a person (or animal model) to achieve a therapeutic goal.
b) Current gene therapy is directed at modifying somatic cells.
c) The only successful gene therapies are those in which cells are removed from a patient, genetically modified, and then returned to the patient.
d) Gene therapy successes have largely involved treatment of recessively inherited disorders.

**Answer 9.8**

c) The only successful gene therapies are those in which cells are removed from a patient, genetically modified, and then returned to the patient.

**Explanation 9.8**
There have been some successes with in vivo gene therapy as well as ex vivo gene therapy.

## Question 9.9

Concerning stem cells, which of the following statements is incorrect?

a) Stem cells occur frequently in our bodies.
b) A stem cell can divide asymmetrically to give a daughter stem cell plus a daughter transit amplifying cell that can undergo a series of differentiation steps to give rise to a differentiated cell.
c) If for any reason, stem cells are depleted, a stem cell can divide symmetrically to regenerate the stem cell population.
d) In an adult person, stem cells are normally multipotent or unipotent.

**Answer 9.9**

a) Stem cells occur frequently in our bodies.

## Question 9.10

Concerning transport of genes into human (or animal) cells, which, if any, of the following statements is false?

a) Transduction means using viruses to transfer DNA into human (or other animal) cells.
b) Tropism refers to the ability of certain viruses to transduce only certain types of cell, such as hepatocytes, but not neurons.
c) Tropism depends on a virus being able to recognize a specific receptor molecule on the surface of the cell.
d) Transfection means transferring DNA into the cells by any means other than using viruses.

**Answer 9.10**
None.

**Explanation 9.10**
All are correct.

## Question 9.11

Concerning gene transfer into human cells, which, if any, of the following statements is false?

a) Integrating viruses can insert genes into the chromosomes of a host cell.
b) Most integrating viruses insert their DNA into a specific location within the genome.

c) The great value of integrating viruses is that they allow foreign (and therefore, therapeutic) DNA to be stably inherited so that it passes to all descendant cells of the transduced cell.
d) Because non-integrating viruses cannot insert their DNA into the chromosomes of a cell, the transduced DNA is quickly destroyed by enzymes within the host cell.

**Answer 9.11**

b) Most integrating viruses insert their DNA into a specific location within the genome.
d) Because non-integrating viruses cannot insert their DNA into the chromosomes of a cell, the transduced DNA is quickly destroyed by enzymes within the host cell.

## Question 9.12

Concerning animal models of human disease, which, if any, of the following statements is false?

a) Primates should be the best animal models, but for mostly practical reasons, rodent models have been preferred.
b) Rats have been the preferred disease models because they offer the best balance between rapid breeding, size and the cost of maintaining colonies.
c) Rodent models are especially suited to modelling neuropsychiatric disorders.
d) All animal models have limitations regarding how far we can make inferences to help understand human disease.

**Answer 9.12**

b) Rats have been the preferred disease models because they offer the best balance between rapid breeding, size and the cost of maintaining colonies.
c) Rodent models are especially suited to modelling neuropsychiatric disorders.

**Explanation 9.12**

Mice have been the preferred disease models, but rodent models are poorly suited to modelling neuropsychiatric disorders (in part, because they are not good models of cognitive capacity).

## Question 9.13

Concerning making animal models of human disease, which, if any, of the following statements is false?

a) Pronuclear microinjection is a general way of making a transgenic animal and involves microinjection of foreign DNA into a fertilized egg cell.
b) Pronuclear microinjection is best suited to modelling recessively inherited single gene disorders.
c) Gene targeting using embryonic stem cells depends on having well-characterized embryonic stem cell lines that can readily allow transmission through the germ line.

d) Gene targeting using embryonic stem cells in mice is a popular way of modelling disease phenotypes that result from a gain-of-function.

**Answer 9.13**

b) Pronuclear microinjection is best suited to modelling recessively inherited single gene disorders.
d) Gene targeting using embryonic stem cells in mice is a popular way of modelling disease phenotypes that result from a gain-of-function.

**Explanation 9.13**

Pronuclear microinjection is not suited to modelling recessively inherited single gene disorders, but has often been used to model disease phenotypes that result from a gain-of-function. Gene targeting using embryonic stem cells in mice is not well suited to modelling disease phenotypes that result from a gain-of-function but is a popular way of modelling the loss-of-function in recessively inherited single gene disorders.

## Question 9.14

Which, if any, of the following statements is false?

a) Hematopoietic stem cells are multipotent because they can give rise to a variety of different cell types.
b) Mammalian embryonic stem cell lines are pluripotent because they can give rise to all types of cell in the body.
c) Transdifferentiation is a type of epigenetic reprogramming in which a differentiated cell is induced to become pluripotent.
d) A transit amplifying cell is a cell produced by asymmetric division of a stem cell and has the potential to give rise to differentiated cells.

**Answer 9.14**

c) Transdifferentiation is a type of epigenetic reprogramming in which a differentiated cell is induced to become pluripotent.

## Question 9.15

Which, if any, of the following statements is false?

a) Transdifferentiation means reprogramming of a differentiated cell so that it acquires the characteristics of another type of differentiated cell.
b) In dedifferentiation a differentiated cell is artificially reprogrammed so that it behaves as a pluripotent cell.
c) Human induced pluripotent stem (iPS) cell lines are usually generated by artificial dedifferentiation of readily accessible human cells, such as skin cells.

d) Human iPS cell technologies do not offer clinical applications but they are of value for studying pathways of cellular differentiation

**Answer 9.15**

d) Human iPS cell technologies do not offer clinical applications but they are of value for studying pathways of cellular differentiation

**Explanation 9.15**

Human iPS cell technologies have the potential for valuable clinical applications, most readily in creating cellular disease models and possibly in extending ex vivo gene therapy.

## Question 9.16

Which, if any, of the following statements is true?

a) Autologous cell transplantation is involved in *in vivo* gene therapies: cells from an individual are genetically modified and then returned to that individual.
b) Adenovirus vectors have the advantage that they offer very high level expression and are well suited to gene therapy for blood disorders.
c) Adenovirus vectors have a better safety profile than adeno-associated virus vectors and have a larger insert size capacity.
d) Adeno-associated virus vectors are well suited to gene therapy for blood disorders but have a low insert size capacity

**Answer 9.16**

None.

**Explanation 9.16**

All are false.

Autologous cell transplantation is involved in ex vivo gene therapy. Both adenovirus vectors and adeno-associated virus vectors are not well suited to gene therapy for blood disorders because neither of them are integrating vectors (because blood cells have short lives some kind of retroviral integrating vectors are needed for gene therapy for blood disorders – the hope is to insert the gene constructs into the chromosomal DNA of hematopoietic stem cells). Adenovirus vectors have a poor safety record because they often induce powerful inflammatory responses in the recipient of therapy.

## Question 9.17

Which, if any, of the following statements is false?

a) The great majority of clinical gene therapy trials have had limited success
b) The only successful gene therapies have been for recessive blood disorders.

c) The only successful gene therapies have been *ex vivo* gene therapies.
d) Gene therapy for inherited disorders represents a minority of clinical gene therapy trials.

**Answer 9.17**

b) The only successful gene therapies have been for recessive blood disorders.
c) The only successful gene therapies have been *ex vivo* gene therapies.

**Explanation 9.17**

There have been examples of other successful gene therapies that involve brain disorders and in vivo gene therapy for eye disorders, for example.

## Question 9.18

Which, if any, of the following statements is false?

a) RNA interference (RNAi) is a cellular defense mechanism that is triggered by the presence in cells of unnatural double-stranded RNA, as can occur after viral infections.
b) RNAi therapy is a type of RNA-targeted therapy in which specific double-stranded RNA constructs are engineered to appear in diseased cells in order to incite the cells to destroy any RNA that contains the same sequence.
c) By destroying RNAs that are related to a specifically introduced genetic construct, artificial RNAi is effectively a type of gene-specific silencing.
d) RNAi therapy is best suited to silencing genes so as to replicate a phenotype caused by loss-of-function mutations.

**Answer 9.18**

d) RNAi therapy is best suited to silencing genes so as to replicate a phenotype caused by loss-of-function mutations.

**Explanation 9.18**

RNAi is a convenient way of silencing a gene in cultured cells as a way of understanding its function but RNAi therapy is better suited to specific silencing of a positively harmful gene in diseased cells than it is to replicating loss-of-function phenotypes.

## Question 9.19

Which, if any, of the following statements is false?

a) Genome editing means making a predetermined change to the nucleotide sequence at just one locus within an intact cell.
b) The specificity of genome editing depends on an initial site-specific cleavage of double-stranded DNA following base pairing with specifically designed nucleotide sequences.

c) Genome editing has the potential to permit specific "gene correction" in which a mutant sequence in a cell is restored to the normal sequence.
d) Genome editing might also have therapeutic potential by specifically inactivating a gene in some cases.

**Answer 9.19**

b) The specificity of genome editing depends on an initial site-specific cleavage of double-stranded DNA following base pairing with specifically designed nucleotide sequences.

**Explanation 9.19**

The site-specific cleavage is also often carried out following recognition of the sequence at the target locus by a combination of zinc fingers within zinc finger nuclease *proteins*.
d) See the example of therapeutic genome editing by zinc finger nucleases to inactivate the *CCR5* gene as a cure for HIV-AIDS (see Box 9.3 on page 365.)

## Question 9.20

Which, if any, of the following descriptions is false?

a) Zinc fingers are elements of protein secondary structure in which the polypeptide chain folds back upon itself after co-ordination of a $Zn^{2+}$ ion with selected amino acids, often a pair of cysteines and a pair of histidines.
b) Zinc finger nucleases are natural proteins containing a sequence of zinc fingers that can bind to specific sequences in DNA.
c) After zinc finger nucleases bind to both DNA strands at a specific DNA sequence they attract cellular DNA cleavage enzymes, inducing them to make a double-stranded break at just that one position in the genome.
d) The CRISPR-Cas system also allows genome editing but in this case the target DNA sequences are recognized by guide RNA sequences rather than proteins.

**Answer 9.20**

b) Zinc finger nucleases are natural proteins containing a sequence of zinc fingers that can bind to specific sequences in DNA.
c) After zinc finger nucleases bind to both DNA strands at a specific DNA sequence they attract cellular DNA cleavage enzymes, inducing them to make a double-stranded break at just that one position in the genome.

**Explanation 9.20**

Zinc finger nucleases are not natural: they are proteins produced after genetic engineering to covalently join DNA sequences that can specify a series of zinc finger modules to a bacterial DNA sequence that can specify a DNA cleavage domain.

# Fill in the Blanks Questions

## Question 9.21

Fill in the blanks with single words.

In some diseases the problem is the loss of some function and a type of ____1_____ therapy is used to supplement the resulting deficiency. It may be a deficiency in some normal aspect of the ____2_____, such as deafness, a deficiency of organs or ____3____ , or a deficiency of molecules (which might be at the level of ____4_____ , ____5_____ , or downstream factors). Sometimes, however, disease is not due to a deficiency; instead, the problem is that there is some positively _____6_____ effect produced at some level (at the level of the phenotype, ____3_____ or ____4_____ ), that cannot be supplemented. Here treatment is possible by seeking to eliminate, correct or ____ 7_____ the agent causing the positively _____6_____ effect. The treatment might seek to kill dangerous _____3____ , for example, or to ____7_____ a _____6_____ _____4_____ or a _____7_____ _____6_____. In the latter case, the treatment might often be to use some kind of _____8_____ , such as a conventional ____9_____ molecule ____8_____ that usually works by binding to a cleft in the _____6_____ _____5_____ and thereby ____7____ its ____6____ effect. A third class of disease treatment seeks to use a ____8____ in order to alter a person's ______10_______ to the disease, or to alter exposure to some _____11_____ factor.

**Answer 9.21**

1. augmentation. 2. phenotype. 3. cells. 4. genes. 5. protein(s). 6. harmful. 7. inhibit. 8. drug. 9. small. 10. susceptibility. 11 environmental.

## Question 9.22

Fill in the missing blanks with single words.

In the recent past, virus vectors used in gene therapy trials were often based on a type of retrovirus called a ______1_______retrovirus. They had the advantage of allowing a _____2_____ DNA to be stably inserted into the _____3____ DNA of cells. For cells that are short-lived, such as blood cells, the hope was that a certain percentage of _____4_____ cells might be successfully transduced so that there was a self-renewing population of cells carrying the desired _____2_____ DNA. Unfortunately, vectors based on ______1_______retroviruses have a poor safety profile: there is little control over where they ______5_______ into the _____3____ DNA and sometimes when they _____5_____ they activate a neighboring _____6_____, causing _____7_____. As a result, in modern gene therapy trials it is now

commonplace to use self-_____8_____ strains of a class of retrovirus vectors known as _____9____ that are much safer to use.

**Answer 9.22**
1. gamma. 2. therapeutic. 3. chromosomal (or genomic). 4. stem. 5. integrate. 6. oncogene. 7. cancer. 8. inactivating. 9. lentiviruses.

## Question 9.23
Fill in the missing blanks with single words.

Genome _____1_____ means artificially introducing a specific change in the DNA sequence at a unique, pre-determined location within the genome of an ____2____ cell. The method relies on some form of recognition of specific sequences on both DNA strands at a locus that then allows an artificially introduced _____3____-stranded DNA break at this location. In response to the _____3____-stranded DNA break, DNA repair is carried out by the cell but after using non_____4_____ end joining DNA repair, errors can be made in the repair that can occasionally result in a desired specific DNA change. In one system genetically engineered _____5_____ _____6_____ nucleases are used in which a DNA is constructed to code for a specific sequence of _____5_____ _____6_____ and is then ligated to a DNA sequence that will specify a DNA _____7_____ domain. A plasmid containing the resulting DNA construct can encode a _____5_____ _____6_____ nuclease when transfected into a cell. Using this technology, a pair of _____5_____ _____6_____ nucleases can be designed to bind to specific sequences on the opposite DNA strands at a desired unique position in the genome and the adjoining DNA cleavage domains work to produce the required ____3_____–strand DNA break.

**Answer 9.23**
1. editing. 2 intact. 3. double. 4. homologous. 5. zinc. 6. finger. 7. cleavage.

# Essay and Listing Questions

## Question 9.24

There are three broad classes to disease treatment. Give a brief outline of the three classes.

**Answer 9.24**

1) Augmentation therapy. Designed to supplement a deficiency of some bodily function, or a failing organ/loss of cells, or a defective or missing gene, or of some downstream metabolite. Can involve providing some artificial aid to restore the bodily function (a hearing aid, for example), or a supply or healthy organs/cells (transplantation), or a supply of properly working genes (gene therapy), or gene products/downstream factors (for example, insulin).
2) Treatment to eliminate, correct, or inhibit some positively harmful effect. That can involve corrective surgery, killing of harmful cells (using antibiotics or by provoking an increased immune response), inhibiting gene expression, or by blocking the function of a harmful protein (such as by using conventional small molecule drugs or monoclonal antibodies to specifically bind to the harmful protein, thereby disrupting its function).
3) Prevention therapy. The idea is to give some treatment that reduces the risk of developing disease, such as giving statin drugs to reduce blood pressure and so lessen the susceptibility of developing various cardiovascular diseases.

## Question 9.25

Four stages are often identified in drug development: a preclinical stage plus three clinical trial stages. What is involved in these?

**Answer 9.25**

- The pre-clinical stage involves a battery of laboratory tests that can be in both cultured cells (testing the efficacy of the proposed treatment when there is a suitable cellular assay) and in animal models where efficacy and toxicity are assessed as well as the pharmacokinetic parameters (studies of the absorption, activation, catabolism, and elimination of the drug).
- Phase I clinical trials involve small-scale studies of healthy volunteers (typically, up to 100 people) and are largely a study on the safety of the treatment. Both the pharmacokinetics and also pharmacodynamics (the response of a target organ or cell to a drug) are assessed.
- Phase II clinical trials represent the first clinical trials on patients. Usually several hundred patients are treated; both safety and efficacy of the treatment are monitored.

- Phase III clinical trials represent large-scale patient trials. Typically the effect of treatment on thousands of patients in multiple centers are assessed in randomized, controlled trials. Again, the objective is to assess both the safety and efficacy of the treatment.

## Question 9.26

Many of the genes that produce the enzymes and other proteins involved in handling drugs are polymorphic. Why should that be?

**Answer 9.26**

From the body's perspective, drug metabolism is really a defense mechanism: the body's priority is to facilitate excretion of the parent drug and its metabolites, and so limit their ability to accumulate within the body and cause dose-dependent toxicity. We have a range of genes, many of them polymorphic, that make the proteins that deal with *xenobiotics*, foreign chemical substances that we ingest but that are not normally part of the human diet. The polymorphism developed initially as a form of self-protection to reduce the risk from ingested toxins (such as from certain plants and fungi). It was driven by natural selection because if a person has a wider range of proteins that can interact with potentially harmful xenobiotics, there is a reduced chance that the person is seriously affected by ingested harmful substances.

## Question 9.27

What distinguishes phase I and phase II reactions in the metabolism of small molecule drugs?

**Answer 9.27**

Small molecule drugs are based on hydrocarbon backbones and so are lipophilic, but drug metabolism allows them to be converted into hydrophilic forms that are easier to excrete from the body.

Phase I reactions are usually carried out by monooxygenases; these work by adding an oxygen atom from molecular oxygen to produce a more polar substance. Often a hydroxyl group is introduced, or a bulky alkyl group bound to a nitrogen, sulfur, or oxygen atom is replaced by a hydrogen atom. The drug derivative is typically left with a more reactive group, a molecular 'handle' that makes it easier for a secondary reaction to be carried out (see below).

Phase II reactions are conjugation reactions, catalyzed by transferases that add one of a variety of chemical groups, notably acetyl, methyl, glucuronyl, glutathionyl and sulfate groups. Phase II reactions commonly occur after phase I reactions have introduced a molecular handle for attaching the secondary chemical group. A hydroxyl group attached during phase I, for example, provides a convenient site for an acetyl group or a sugar (glucuronyl) group to be attached by a phase II enzyme, detoxifying the drug and assisting in its excretion.

## Question 9.28

Using the example of genes encoding cytochrome P450 enzymes, illustrate how genetic variation in drug-metabolizing enzymes can often stem from gene copy number variation.

**Answer 9.28**

Probably, the best example of copy number variation for genes encoding drug-metabolizing enzymes comes from the *CYP2D6* locus on chromosome 22 where the number of gene copies can range from 0 to 13. People who lack both *CYP2D6* alleles or who have a deletion of one *CYP2D6* allele and an inactivating mutation in the other allele are poor metabolizers for the drugs that this enzyme normally handles. Those who have a solitary *CYP2D6* allele that has a reduced function are intermediate metabolizers. Some individuals have many *CYP2D6* genes and are ultrafast metabolizers.

## Question 9.29

In some cases, genetic variation at multiple loci is known to affect the response to a specific drug. What is known about genetic variation that affects our responses to the anticoagulant warfarin?

**Answer 9.29**

At least three loci are involved: *CYP2C9, VKORC1* and *CYP4F2*, as listed below.

1) *CYP2C9* makes a major cytochrome p450 enzyme that is known to be involved in metabolizing multiple drugs. The CYP2C9 enzyme hydroxylates warfarin to produce 7-hydroxywarfarin.
2) *VKORC1* makes the C1 subunit of the vitamin K epoxide reductase complex (VKOR). The latter enzyme works to ensure that there is a healthy supply of vitamin K, a vitamin that is essential for activating four important blood clotting factors, Factors II, VII, IX and X).
3) *CYP4F2* makes an enzyme that works as a vitamin K oxidase that converts vitamin K quinone to hydroxyvitamin K.

## Question 9.30

Sometimes, a prescribed drug can be dangerous, and occasionally deadly, according to a patient's genotype. Give three examples of such.

**Answer 9.30**

1) Suxamethonium (succinylcholine) works as a fast-acting muscle relaxant and is used before surgery. Normally the effects of the drug wear off quite quickly when the drug is metabolized by the enzyme butylcholinesterase. Low metabolizers are at risk of apnea—

they remain paralyzed and unable to breathe after surgery because they cannot regain their muscle function quickly enough and may require extended ventilation.

2) 6-mercaptopurine and azathioprine are immunosuppressant drugs that are important in dampening down potentially harmful immune responses after organ transplantation. The enzyme thiopurine *S*-methyltransferase (TPMT) inactivates these immunosuppressant drugs by adding a methyl group. In people with two low-activity TPMT alleles, the drugs are metabolized slowly; if normal doses are given, the drugs accumulate and can result in life-threatening bone marrow toxicity.
3) Various statins and the inhalation anesthetics halothane and isoflurane are associated with usually mild myopathies but in some patients there can be severe muscle toxicity in which the muscle tissue breaks down (rhabdomyolysis) and can lead to death. Persons with inactivating mutations in the ryanodine receptor gene develop life-threatening rhabdomyolysis and an extreme rise in temperature, a form of malignant hyperthermia (OMIM 145600).

## Question 9.31

The *CFTR* gene that underlies cystic fibrosis was isolated by positional cloning in 1989. Twenty years later, Jack Riordan, one of the major contributors to this historic achievement, was quoted as saying "the disease has contributed much more to science than science has contributed to the disease". What did he mean by this, and what important developments have occurred in treating cystic fibrosis since 2009?

**Answer 9.31**

He meant that when the novel *CFTR* gene was first identified, almost nothing was known about how it worked. The CFTR protein was subsequently shown to function as a channel that regulates transmembrane conductance by allowing chloride ions to pass through the cell membrane. A great deal was also discovered about aspects of CFTR biology that would inform diverse fields such as protein trafficking and membrane transport.

In contrast to the burgeoning information on how the CFTR protein works, no successful treatment had been devised by 2009. Gene therapy for cystic fibrosis has enormous problems in terms of gene delivery and expression (because of the thick mucus layer coating the surface of lung epithelia in cystic fibrosis patients). Another problem was that six different classes of mutations could be identified to be associated with the disease, according to their effects on how the gene normally works in cells. That meant that conventional small molecule drug treatment needed to deal with different types of molecular pathogenesis.

Recently, there has been a little progress. In 2012 ivacaftor (marketed as Kalydeco by Vertex Pharmaceuticals) became the first drug approved by the US Food and Drug Administration to target a cause of cystic fibrosis rather than the condition's symptoms. It has been targeted to treat patients with the G551D mutation, which causes the chloride channel to fail to open. Ivacaftor works by helping to reopen the chloride channel. While ivacaftor may well result in marked

improvement in longevity, quality of life, treatment burden, and so on, it is directed at just this one mutation, and is applicable to just 4% of cystic fibrosis patients (those who have at least one G551D mutation).

## Question 9.32

There are two broad principles regarding the technological aim of somatic gene therapy: (a) genetically modifying disease cells (without killing them), and (b) killing disease cells either directly or indirectly. Explain what is involved in the two strategies.

**Answer 9.32**

1) The idea is to alter gene expression for therapeutic benefit. That usually means adding a supplementary cloned gene copy to make some desired product (that is lacking in diseased cells of the patient). In principle, it could also mean inactivating or inhibiting how a mutant gene works for therapeutic benefit, or correcting a gene so that it is restored to its normal function.
2) Killing disease cells is particularly appropriate in cancer gene therapies. It can be done directly by transferring into cancer cells a recombinant DNA that can make some cytotoxic agent, such as ricin, or by genetically modifying non-disease cells with the aim of provoking a strong immune response against the disease cells.

## Question 9.33

In mammals, pluripotent cells occur naturally in the early embryo but pluripotent cell lines can also be artificially created. The first approach was to make embryonic stem cell lines from cells isolated from early embryos. More recently, pluripotent cell lines have been made by artificially changing the epigenetic settings of differentiated cells. What is involved in the latter case?

**Answer 9.33**

Artificial epigenetic reprogramming can mean transdifferentiation (when one type of differentiated cell is induced to change into a different type of differentiated cell, such as changing a skin fibroblast to a neuron, for example), or dedifferentiation (when a differentiated cell is induced to change to a less specialized cell). The reprogramming can be carried out by transferring genes encoding the relevant transcription factors needed to induce the desired change, or by providing purified transcription factor proteins or, sometimes, specific chemicals that can induce the production of the required transcription factors.

## Question 9.34

Describe the characteristics of two viral vectors based on RNA genomes and two viral vectors based on DNA genomes that are used in gene therapy.

**Answer 9.34**

- Gammaretroviruses. These are simple, single-stranded RNA viruses that have a reverse transcriptase which allows them to make a cDNA copy that can integrate into chromosomes of the host cell. They cannot pass though the intact nuclear membrane and so can infect dividing cells only (when the nuclear membrane dissolves). Vectors based on gammaretroviruses have a checkered safety record because they quite frequently integrate close to proto-oncogenes causing leukemia.
- Lentiviruses. These are complex single-strand RNA viruses that are also able to make cDNA copies that integrate into chromosomes but they can pass through the nuclear membrane and so can infect both dividing and non-dividing cells. Vectors based on lentiviruses have a good safety profile in gene therapy.
- Adenoviruses. They are complex double-stranded DNA viruses that can infect both dividing and non-dividing cells but are non-integrating. Their big advantage is that they offer high level expression but they are not stably inherited by the daughter cells of dividing cells. Vectors based on adenoviruses have the big disadvantage that they can elicit powerful inflammatory/immune response in patients.
- Adeno-associated viruses. They are comparatively simple, single-stranded DNA viruses. They can infect both dividing and non-dividing cells. They offer high level expression and many strains have useful tropism characteristics. Vectors based on adeno-associated viruses have a much better safety record than adenoviruses.

## Question 9.35

Hematopoietic stem cells can be important target cells in gene therapy for blood disorders and some other disorders, including certain brain disorders. Explain the significance of hematopoietic stem cells and how they are exploited in gene therapy.

**Answer 9.35**

Hematopoietic stem cells (HSC) form in the bone marrow and give rise to all classes of blood cells, including macrophages, monocytes, granulocytes, platelets, erythrocytes, B lymphocytes and T lymphocytes (where precursor cells migrate to the thymus). In addition, HSC give rise to certain tissue immune system cells, such as microglia (the resident macrophages of the brain and spinal cord) and dendritic cells (a class of immune system cells that work in presenting foreign antigens to T cells and that are found in various types of tissue).

Being stem cells, HSC serve as an immortal source of the cells listed above and even a single HSC is believed to be able to re-populate all blood cells. Although the greatest concentration of HSC occurs in the bone marrow they are also found infrequently in peripheral blood. *Ex vivo* gene therapy for recessive blood disorders typically involves using enrichment techniques to obtain cells from the patient that are enriched in HSC. That can involve using monoclonal antibodies specific for the CD34 antigen to select for cells containing this antigen (which is a

marker of HSC). The enriched cell population is then typically transduced with a recombinant retroviral vector such as a lentivirus vector that contains a normal version of the gene that is defective in patients.
Gene therapy has also been possible for certain brain disorders, such as X-linked adrenoleukodystrophy, a lipid storage disorder that primarily affects the brain. That was possible because the transduced HSCs were able to give rise to myelomonocytic cells (with characteristics of both granulocytes and monocytes) that migrated into the central nervous system to replace diseased microglial cells and relieve the lipid storage problem.

## Question 9.36

What possible clinical applications might be derived from induced pluripotent stem cell technologies?

**Answer 9.36**

From a medical perspective, induced pluripotent stem (iPS) cell technologies have two potentially exciting applications as described below.

1) *Provision of human cellular models of disease*. Animal disease models have been very valuable because they allow the use of invasive studies to understand the molecular basis of human disease. But they are only *models*; they quite often show important differences from humans. Accessible skin cells from a patient can now be reprogrammed to become cells that can then be directed to differentiate into cells relevant to the disease process (such as normally inaccessible neurons for a neurodegenerative disorder). The genetically impaired disease cell lines will be useful for drug screening (testing for toxicity, efficacy, and so on) and for studying the molecular basis of disease in human cells.
2) *Therapeutic applications*. Accessible cells from a patient can be artificially dedifferentiated to make iPS cells that are then genetically modified, and then returned to the patient without provoking an immune response. Successful environmentally induced reprogramming of human cells may transform the prospects of using dedifferentiated human cells therapeutically and there is the potential of directing the genetically modified cells to form cells of a desired cell type.

## Question 9.37

Molecular therapeutic strategies sometimes target RNA instead of DNA. What is involved in RNA interference therapy, and how useful has it been?

**Answer 9.37**

RNA interference therapy is a type of artificial gene silencing. Different diseases are potentially amenable to treatment based on gene silencing (in which the expression of the gene is artificially repressed in some way). In some cases, the disease is due to a gain-of-function mutation or to a

dominant-negative effect: the problem is a mutant resident gene that is doing something positively harmful. Here, the strategy must be to selectively inhibit the expression of the mutant gene, without affecting the normal allele too much. (There are therefore parallels with treating infectious diseases, which might be treated by targeting a pathogen-specific gene or gene product).
RNA interference is a natural gene-silencing phenomenon that cells use as a protective mechanism to counteract viruses and to limit the spread of transposons in the germ line. Artificial RNAi-mediated gene silencing usually involves designing two short oligoribonucleotides to have complementary sequences so that they form a siRNA (short interfering RNA) duplex that is transfected into diseased cells. Alternatively, a gene is transfected that can make a short hairpin RNA which is processed in transfected cells to give the desired siRNA duplex. One of the sequences of the siRNA is chosen to be identical to, and specific for, the target RNA that needs to be silenced. In the cell one of the two complementary RNA sequences will bind to the target RNA. The unnatural double-stranded RNA prompts the cell to degrade the RNA.
RNAi therapy is not straightforward: complete gene silencing is difficult to obtain. There can also be the risk of off-target effects, in which very closely related sequences that occur by chance in other genes become collateral targets. A variety of clinical gene trials have been or are being carried out. Although the therapeutic potential of RNAi therapies might be high, the technology needs to be refined.

## Question 9.38

How has exon skipping therapy been applied to treat Duchenne muscular dystrophy?

**Answer 9.38**

Internal deletions are a common cause of pathogenesis in the dystrophin gene, and many deletions that affect central exons result in a frameshift, resulting in severe Duchenne muscular dystrophy (DMD). However, quite large deletions within the central region of the gene are associated with mild Becker muscular dystrophy (BMD) if they do not change the translational reading frame. These large in-frame deletions do not result in severe disease because the sequence of a large central part of the dystrophin protein is unimportant (certainly when compared to the N-terminal and C-terminal regions which are functionally vital components of the protein and are unaffected by a large in-frame central deletion).
Exon skipping therapy seeks to restore the translational reading frame by inducing altered splicing so that at the RNA level a central exon or exons is omitted from the long dystrophin mRNA to produce an effect that is similar to the effect of a central in-frame deletion associated with milder BMD. In total about 25% of DMD-associated deletions might have the translational reading frame restored by inducing skipping of just one internal exon, exon 51, and that would make treatment available to 15% of DMD patients. Skipping of other central exons can further extend this type of treatment.

Local intramuscular injections of an antisense oligonucleotide to induce skipping of exon 51 has been shown to restore dystrophin production in muscle fibers of patients with the appropriate types of dystrophin exon deletion. Follow-up systemic administration of the oligonucleotide (with access to the circulation via abdominal subcutaneous injections) did not elicit any serious adverse reactions and the procedure seems to work quite well. In 10 of the 12 treated boys (aged 7 to 13 years), new dystrophin expression was observed in between about 60% and 100% of muscle fibers, and clinical benefit was significant as measured by improved walking statistics when compared with controls.

The oligonucleotides that are used in therapeutic exon skipping need to be more stable than conventional oligonucleotides. Serepta Therapeutics uses phosphorodiamidate morpholino oligonucleotides; Prosensa uses 2′-*O*-methyl-modified ribose molecules with a full-length phosphorothioate backbone. Even then, the oligonucleotides have a limited half- life after systemic administration (for example, 29 days in the latter case).

## Question 9.39

Genome editing is being used in an attempt to cure HIV-AIDS. What is the experimental strategy towards achieving that goal?

**Answer 9.39**

The human immunodeficiency virus HIV launches its attack on the body by infecting CD4 helper T cells. By attacking and killing helper T cells (regulatory immune system cells with a major role in helping to protect us against viruses), HIV compromises the immune system; people with AIDS are unable to fight off common infections and they develop various virus-induced cancers.

To latch onto a helper T cell, HIV first binds to a CD4 receptor on a T cell and then interacts with a co-receptor—often the chemokine (C–C motif) receptor 5 (CCR5). Unlike CD4, the CCR5 receptor is not so important in T-cell function, and some normal people (about 5–14% of individuals of European descent) have defective CCR5 receptors because they have a *CCR5* allele with an inactivating 32 bp deletion (*CCR5-Δ32*). Heterozygotes with one *CCR5-Δ32* allele are more resistant to HIV infection than the normal population, and homozygotes are highly resistant to HIV infection.

The idea that HIV-AIDS could be cured by artificially inactivating *CCR5* was promoted by the famous ‘Berlin patient’ study. First reported in 2009, an HIV patient with acute myeloid leukemia received allogeneic $CD34^+$ peripheral blood stem cells from an HLA-identical donor who had been screened for homozygosity for the *CCR5-Δ32* allele. Four years later, and after discontinuation of anti-retroviral therapy, the patient appears to be free from HIV, indicating that this could be the first complete cure for HIV infection.

The Berlin patient study was clearly an exceptional situation, and various follow-up studies have sought to extend resistance to HIV by inactivating *CCR5* in autologous T cells. Among them are

phase I and phase II gene therapy trials carried out by Sangamo Biosciences in which genome editing is applied using zinc finger nucleases to target and inactivate the *CCR5* gene.

## Question 9.40

How might a form of germ-line gene therapy be used to treat severe mitochondrial diseases?

**Answer 9.40**

Mutations in mitochondrial DNA (mtDNA) are a significant cause of human disease: pathogenic mutations are found in at least 1 in 200 of the population, and cause severe multisystem disease in approximately 1 in 10,000 of the population. Pathogenic mtDNA can be maternally inherited, but there are no effective treatments for mitochondrial DNA disorders.

In the clinical management of mtDNA disorders, the emphasis has therefore been on prevention. Preimplantation and prenatal diagnosis are well established in clinical genetic practice as a way of selecting unaffected embryos. However, the results can be difficult to interpret for patients with heteroplasmic mtDNA mutations (when there may be variable numbers of mutant and normal mtDNAs in each cell). In addition, an increasingly large group of diseases are recognized to be caused by homoplasmic mtDNA mutations (all the mtDNA molecules are mutant). Here, prevention is not an option—all the offspring would inherit the pathogenic mutation in the maternal egg, and this type of genetic defect can be associated with a very high disease recurrence risk.

An entirely different way of trying to prevent the transmission of homoplasmic mutations is to replace maternal mtDNAs by mtDNAs from an asymptomatic donor. This type of approach has been used in mouse and primate models, with encouraging results. Two recent studies that used slightly different approaches have been carried out in human embryos *in vitro*.

In the pronuclear transfer technique an oocyte with mutant mtDNA is fertilized; the normal karyoplast (combined male and female pronuclei) is isolated and then transferred into an enucleated donor zygote with normal mitochondria. In the metaphase II spindle transfer technique the spindle is transferred from an oocyte that has mutant mtDNA into a mitochondrial donor oocyte followed by intracytoplasmic sperm injection fertilization. Both techniques involve monitoring embryo development following transfer. The resulting human embryos appear to be viable *in vitro*, and the degree of mutant DNA carryover is low or undetectable.

The use of these techniques in a clinical context is currently illegal, and the ethics of this type of disease prevention is currently being debated. If the procedure were to be legalized, it would probably be the first example of germ-line gene transfer in humans.