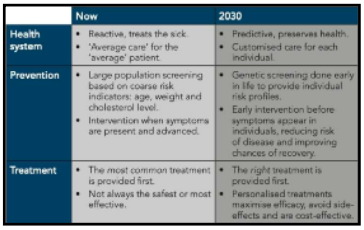
Precision Medicine (W1)



	Now	2030
Health system	• Reactive, treats the sick. • 'Average care' for the 'average' patient.	• Predictive, preserves health. • Customised care for each individual.
Prevention	• Large population screening based on coarse risk indicators: age, weight and cholesterol level. • Intervention when symptoms are present and advanced.	• Genetic screening done early in life to provide individual risk profiles. • Early intervention before symptoms appear in individuals, reducing risk of disease and improving chances of recovery.
Treatment	• The most common treatment is provided first. • Not always the safest or most effective.	• The right treatment is provided first. • Personalised treatments maximise efficacy, avoid side-effects and are cost-effective.

Precision and Personalised Medicine (L1.1)

Precision (and personalised) medicine

- Personalised medicine aims to determine which medical treatments will work best for each patient
- Precision medicine uses medical interventions to alter molecular mechanisms, often genetic, that cause disease or influence a patient's response to certain treatments
- By combining molecular data with an individual's medical history, targeted treatment and prevention plans are developed
- **Precision medicine**: is science — a new wave of evidence-based medicine
- **Personalised medicine**: is a practise — managing a patients care more holistically

Precision medicine is being transformed by genomic approaches

- Decrease in cost
- Moore's Law: describes long-term trend in the computer hardware industry that involves the doubling of 'compute power' every 2 years
- Cost per genome has significantly decreased - ~$1000

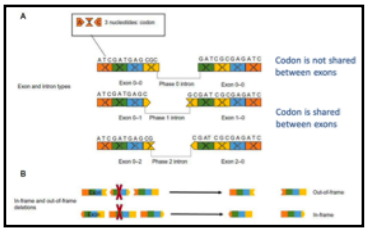
Focus on RNA and DNA targeted therapies

- Modulating gene expression
- Genes transfer
- Gene editing

Therapeutic Approaches using antisense oligonucleotides (ASOs) (L1.2)

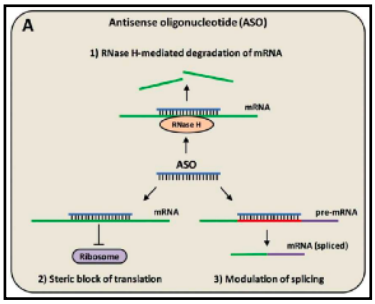
Gene to RNA to protein

- DNA (gene) -> pre-mRNA -> (mature) mRNA -> protein



Correct splicing of introns matters

- Codon could be not shared between exons or could be shared between exons
- Splicing together of different exons (e.g. mutations and/or incorrect exon skipping) can lead to proteins being out of reading frame — non functional

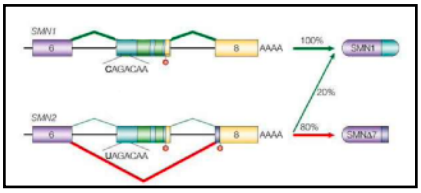


Therapeutic approaches using antisense oligonucleotides

- **Antisense oligonucleotides (ASOs)** = short, single-stranded sequences of DNA or RNA designed to target specific RNAs to modify gene expression
- Exert their **therapeutic effects** in various ways **depending** on their **chemical structure**

1. RNA degradation
   - RNase H-mediated degradation of mRNA
2. Preventing protein translation
   - Steric block of translation
3. Modifying RNA splicing
   - Modulation of splicing - choose spot of splice

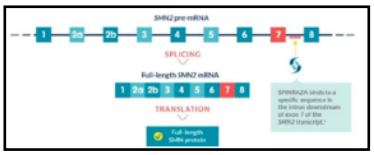
SPINRAZA (Nusinersin) - for **spinal muscular atrophy** (SMA)



- **SMA** = one of the most prevalent genetic disorders in childhood
- Caused by loss-of-function mutations in a **single gene**: **SMN1** (survival motor neuron 1)
- An **SMN2** gene (found as a result of **gene duplication**, unique to humans) is present but this only generates ~**10-20% of the normal levels** of functional SMN protein
- SMN2 differs from SMN1 at 5 bases, and a **C-to-T transition in exon 7 of SMN2** favours skipping of exon 7 during splicing, resulting in the majority of **SMN2 products** being an **unstable, truncated isoform referred as SMNdelta7**

- Exon 7 is skipped when splicing occurs due to different code
- **SMA patients (with no SMN1)** can produce functional SMN protein via SMN2 but at levels that are insufficient for normal function
- This results in the progressive degeneration of spinal-chord motor neurons

SPINRAZA (Nusinersin) - for **spinal muscular atrophy** (SMA)



- A 15-nucleotide sequence in **intron 7** of **SMN2**, termed the intronic splicing silencer N1 **(ISS-N1)** is crucial for **splicing regulation**
- **ISS-N1-blocking ASOs (SPINRAZA)** lead to production of exon 7 and increased expression of SMN2
  - Results in splicing to include exon 7
- Binding of SPINRAZA prevents the splitting at that region to include exon 7
- Overall increases production of functional protein

SPINRAZA (Nusinersin) - Clinical Trial to approved
- Phase I clinical trial showed that intrathecal administration of Spinraza/nusinersen was well-tolerated
- A phase II, a dose-escalation study was performed in 20 infants of Spinraza/nusinersen. Mild improvements in motor function were observed at high dose compared with baseline
- Analysis of post-mortem tissue indicated that intrathecal Spinraza/nusinersen was distributed throughout the spinal cord and brain
- Analysis of patients treated for at least 183 days, Spinraza/nusinersen reduced the risk of death or permanent ventilation by 47% in infantile-onset SMA compared with control
- Now approved by FDA

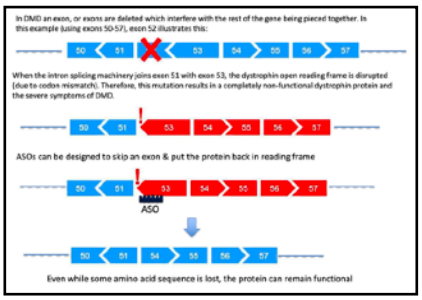
Infantile-onset (Type 1) SMA treated with SPINRAZA (nusinersen)
- SPINRAZA is administered directly to the CNS
- SPINRAZA treatment = 4 loading doses (12mg (5mL) per administration)
- The first 3 are administered at 14-day intervals
- The 4th loading dose should be administered 30 days later
- A maintenance dose should be administered once every 4 months thereafter

Therapeutic Approaches using antisense oligonucleotides (ASOs) (L1.3)

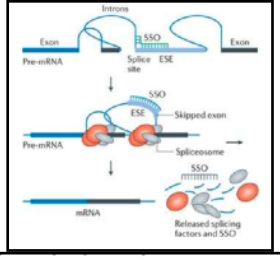
ASOs for Duchenne's muscular dystrophy (DMD)
- X-linked disease, 1 in 3,600 males
- Mutations in dystrophin gene
- Symptoms in early years, worsen with life expectancy ~25 years

ASOs for DMD
- Dystrophin
  - Part of a protein complex that anchors the cytoskeleton in the muscle cell with the extracellular matrix
  - 427 kDa muscle protein (skeletal, muscle, cardiac), long half life (20 weeks)
  - Dystrophin gene 2.4 Mb gene (largest gene known) 79 exons

ASOs for DMD

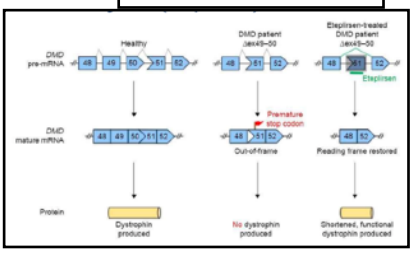


- In DMD an exon, or exons are deleted which interfere with the rest of the gene being pieced together. In this example (using exons 50-57), exon 52 illustrates this:
- When the intron splicing machinery joins exon 51 with exon 53, the dystrophin open reading frame is disrupted (due to codon mismatch). Therefore, this mutation results in a completely non-functional dystrophin protein and the severe symptoms of DMD
- ASOs can be designed to skip an exon and put the protein back in reading frame
- Even while some amino acid sequence is lost, the protein can remain functional



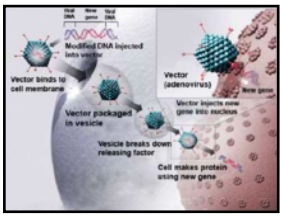
ASOs for DMD block recognition of an intron-exon splice site

- ASO masks the splice site so the splicing machinery moves over this exon to the splice site in the next exon



Exondys 51 (eteplirsen) for DMD

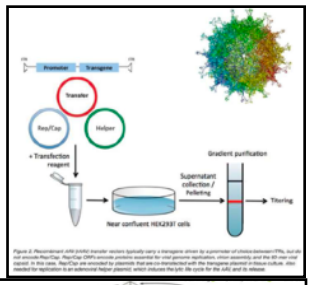
Adeno-associated virus vectors (AAVs) enable gene therapy (L1.4)



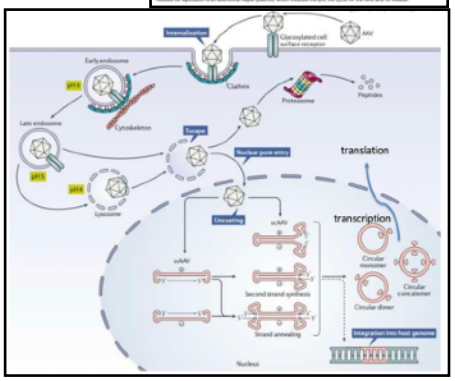
Gene Therapy

- Usually involves viral mediated introduction of defective cells with corrective gene
- ADA (Adenosine deaminase) defect in SCID corrected in T cells by retroviral gene therapy but stopped in 2002 due to development of leukaemia
- **AAV** (Adeno-associated virus) now the voice of expression system

(r)AAV: (Recombinant) adeno-associated virus

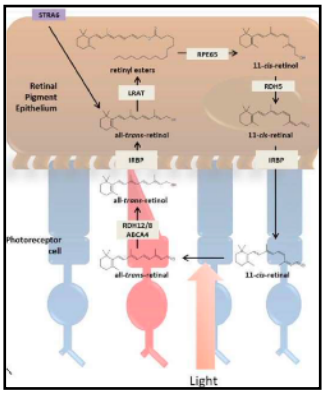


- AAV = single stranded, DNA virus
- Has a “simple” genome packaged in an icosahedral capsid (protein shell)
- For biotech, the **genome** is **typically gutted** so that precious cargo space is for gene delivery, and for safety
- Size of insertion is limiting factor
- AAV vectors **lack the integration-promoting gene** and therefore only rarely and randomly integrate into the human genome
- rAAV can transduce both **dividing and non-dividing cells**, with **stable transgene expression** for years in post-mitotic tissue
- Not pathogenic and very low immunogenicity



rAAV transduction pathway:

Leber Congenital Amaurosis treatment (L1.5)



RPE65 gene therapy for Leber congenital amaurosis

- A common cause of **blindness** in children (2-3 per 100,000 newborns)
- Autosomal recessive mutations in about 13 different genes including RPE65
- RPE65 produced in **retinal pigment epithelium (RPE)** and is required in the visual cycle
- Visual Cycle:
  - Absorption of light causes isomerisation of 11-cis-retinal to all-trans-retinal in photoreceptors for phototransduction
  - Decay of activated rhodopsin yields opsin and all-trans-retinal, which is reduced to all-trans-retinol
  - All-trans-retinol diffuses into the RPE where it is converted back to 11-cis-retinal in a reaction involving RPE65
  - 11-cis-retinal, which then diffuses back into the photoreceptor where it combines with opsin to regenerate visual pigments
- **RPE65** is an **enzyme** that converts **retinol esters to 11-cis-retinol** in RPE

Luxturna: to correct RPE65 deficiency
- The first in vivo gene therapy approved by the FDA (made by Spark Therapeutics)
- Adeno-associated virus vector-based gene therapy indicated for the treatment of patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy
- Injected through the eye to subretinal region

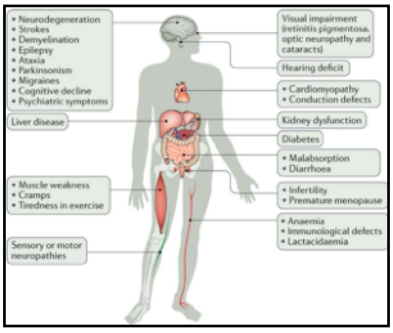
Spinal Muscular Atrophy (SMA) Treatment (L1.6)

Zolgensma - Gene therapy for spinal muscular atrophy (SMA)
- The AAV contains a transgene encoding the human **survival motor neuron (SMN) protein**, under the control of a **cytomegalovirus enhancer** and a **chicken-beta-actin hybrid promoter**
- Single intravenous injection. The drug product has a nominal concentration of 2.0 x 10^{13} vector genomes (vg)/mL

Zolgensma - Gene therapy for SMA
- $2.1 million per patient, Zolgensma is the world's costliest single-dose treatment (half the cost of 10 years of the current therapy Spinraza)
- Novartis will give out up to 100 total doses to be distributed for free through 2021 by a lottery

Germ-line gene therapy for Mitochondrial Disease (L1.7)

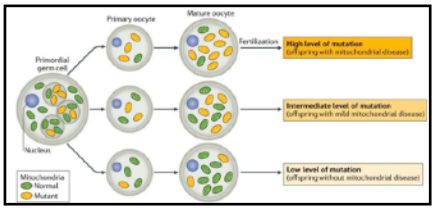
Germ line gene therapy for mitochondrial disease
- 290 genes identified to cause mitochondrial dysfunction and disease (~1 in 5,000 live births)
- Includes:
  - **Numerous nuclear genes** encoding mitochondrial imported proteins involved in OXPHOS, metabolism, mitochondrial DNA maintenance, mitochondrial biogenesis = **Mendelian inherited (father and mother)**
  - 35 of 37 mtDNA genes (~1 in 200 individuals carry a disease-causing mtDNA mutation) = **non-Mendelian** (i.e. **maternally**) inherited
- Mitochondria contain **~1,200 different proteins**
  - ~**99**% of these proteins are encoded by **nuclear** genes (imported into mitochondria)
- **mtDNA**:
  - Encodes only **13 proteins** of respiratory chain plus **22 tRNA and 2 ribosomal RNA genes**



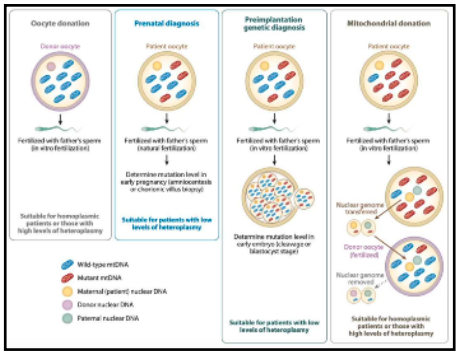
Mitochondrial disease: "any age, any symptom, and mode of inheritance":

mtDNA
- Features
  - 37 genes
  - Maternally-inherited
  - 100s-1000s copies/cell
  - mtDNA repair mechanisms less efficient than nDNA
  - Cells can contain mixed populations of normal and mutant mtDNA = heteroplasmy
- Inherited mtDNA disorders
  - Appear from birth to old age (Depends on mtDNA mutant load in cells and tissues and may change over time)

mtDNA inheritance and disease
- Mother can have both normal and mutatant mtDNA
- Primordial germ cell
- Primary oocyte
- Mature oocyte
- Fertilisation
  1. High level of mutation (offspring with mitochondrial disease)
  2. Intermediate level of mutation (offspring with mild



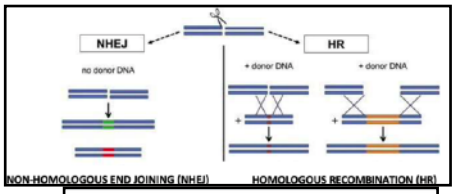
mitochondrial disease)
3. Low level of mutation (offspring without mitochondrial disease)

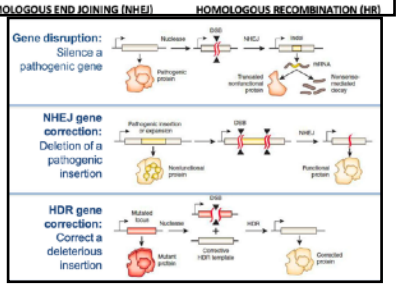


Options for preventing inherited mtDNA disease
- Mitochondrial donation (or mitochondrial replacement therapy):
  - Germ-line gene therapy (heritable, only through maternal line)
  - 'Traditional' forms of gene therapy have tended to target genetic material in the nucleus of a cell, mitochondrial donation aims to substitute all of the mitochondria
- Oocyte donation (IVF), prenatal diagnosis, preimplantation genetic diagnosis, mitochondrial donation

Gene editing methods (L2.1)

Modifying genes following the introduction of double strand breaks
- Non-homologous end joining (NHEJ)
  - Intrinsically error prone and results in formation of **indels** (small insertions/deletions of typically 5-20 nucleotides)
  - Can disrupt reading frame of genes if targeted inappropriately
  - No donor DNA
- Homologous recombination (HR)
  - Requires the addition of **template DNA** with homologous arms but can have variable sequences(mutations, insertions) in between

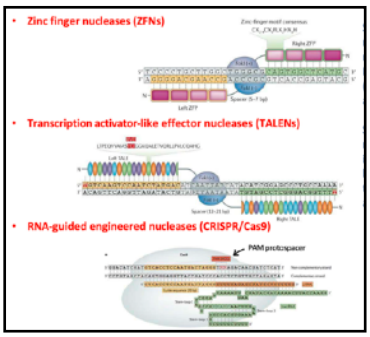




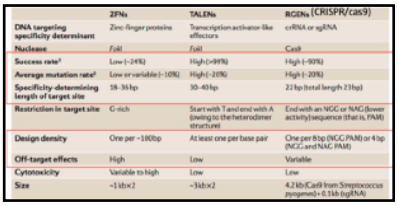
Types of therapeutic gene editing
- **Gene disruption**: Silence a pathogenic gene
- **NHEJ gene correction**: Deletion of a pathogenic insertion
- **HDR gene correction**: correct a deleterious insertion
  - HDR = homology directed repair

Directed nucleases in gene editing (L2.2)

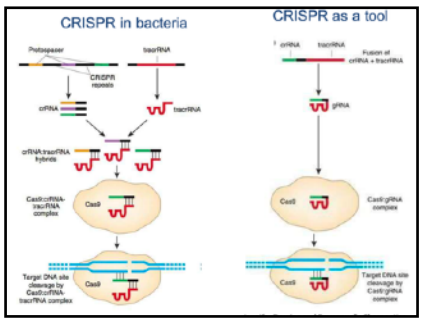
Targeting specific genes using site directed nucleuses
- Zinc finger nucleases (ZFNs)
  - Series of protein modules that **bind 3 nucleotides** bringing **Fok1** nuclease to site (homodimer becomes active)
- Transcription activator-like effector nucleases (TALENs)
  - Series of protein modules that bind individual nucleotides bringing Fok1 nuclease to site (dimer becomes active)
- RNA-guided engineered nucleases (CRISPR/Cas9)
  - RNA molecule (CRISPR) designed that binds nucleotides and targets Cas9 nuclease to site



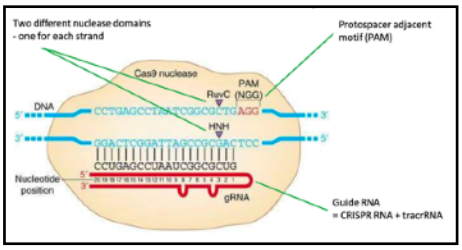
CRISPR/Cas9 vs TALENs & ZFNs
- **Advantages** of CRISPRs over ZFN/TALENs
  - DNA binding is by **classic base pairing rules** (not protein:DNA interactions)
  - Making the custom sgRNA used by CRISPRs is much easier, cheaper, quicker
- **Disadvantages** of CRISPRs over ZFN/TALENs
  - Overall recognition sequence is small, making off-target effects a potentially issue



CRISPR in bacteria vs as a tool
- Bacteria
  - Remember previously encountered sequences
  - Used as defence if re-encountered
  - Target DNA site cleavage by Cas9:crRNA-tacrRNA complex
- Tool

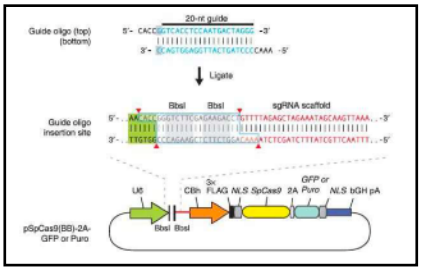


- Target double stranded DNA and create specific breaks



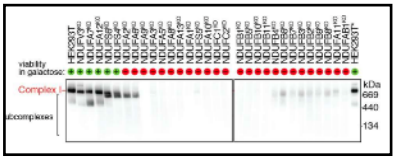
The CRISPR/Cas9 complex
- Two different nuclease domains
  - One for each strand
- Protospacer adjacent motif (PAM)
- Guide RNA = CRISPR RNA + tracrRNA
- Target sequence complementary to gRNA
- To target a gene in cells, you need to express the genes encoding the gRNA and Cas9 (or inject pre-made gRNA and protein directly)

Applications of gene therapy (L2.3)



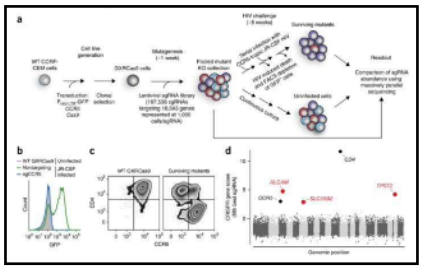
What is genome editing being used for?
- **Discovery Research**: making cell and animal models (knocking out/ modifying genes)
- Transfect cells with plasmid (Express gRNA and Cas9)
- Not all cells are targeted, so selection required
  - GFP fluorescing cells can be FACS sorted and cloned into 96-wells
  - Individual cells are screened and targeted gene sequenced to confirm indels
- Also CRISPR gene editing in **agricultural research**

What is genome editing being used for?



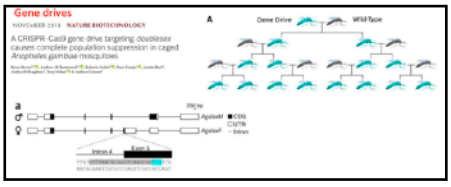
- **Basic Research**: making cell and animal models (knocking out/ modifying genes)
- Creation of 30 human (HEK293T) cell lines each with a knockout (NHEJ) of a gene encoding one of 30 subunits of respiratory chain complex I in human cells (TALENs and CRISPR/Cas9) and analysis of defects/ proteomes
- Simple knockouts (NHEJ) or mutations (HR)
- Genetically modify the zygote and screen the progeny

What is genome editing being used for?



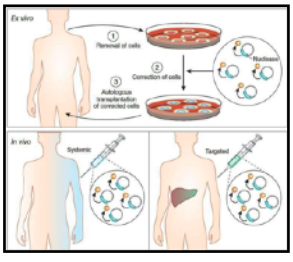
- Screens to identify **new therapeutic targets**
- In addition to genes encoding the HIV co-receptors CD4 and CCR5, new genes - TPST2, SLC35B2 and ALCAM were identified to be required for HIV infection
  - These results indicate that HIV relies on a limited number of non-essential host proteins for replication and suggest pathways for potential therapeutic intervention
  - Targeted 18,543 genes

What is genome editing being used for?

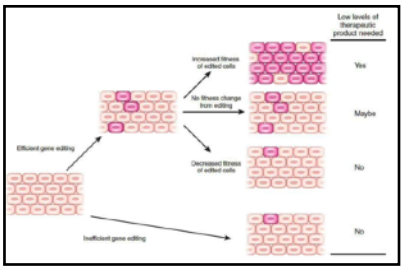


- **Gene drives**
- The gene **doublesex** is **responsible** for **female development**
- They targeted the **intron 4-exon 5 splice site** with a gRNA and introduced DNA with homologous arms and containing a germline expression cassette for Cas9 and same gRNA in between
- During meiosis the Cas9-gRNA complex **cleaves** the **wild-type allele** at the target sequence and the CRISPR/Cas9 cassette is copied into the wild-type locus by HDR ('homing'), **disrupting exon 5** in the process (95% penetration)
- Female Anopheles gambiae mosquitoes with **2 copies of the altered doublesex gene did not lay eggs**
- After 8 generations, the drive had spread through the **entire population**, such that no eggs were laid

Genome editing for treating disease (L2.4)



Potential therapeutics to treat disease
- Somatic cell editing (not heritable, similar to gene therapy)



- **Ex vivo** limitations
  - Isolations and culture/maintenance of specific cells
  - Potential for mutations in cell culture
- **In vivo** limitations
  - Targeting the right organ
  - Life span of cells targeted
- Other **general problems**
  - Homology directed repair doesn't take place in non dividing cells

Comparison of different platforms:
- Zn finger nucleases vs TALENs vs CRISPR/Cas9

	Zn finger nucleases	TALENs	CRISPR/Cas9
Recognition site	Typically 9–18 bp per ZFN monomer, 18–36 bp per ZFN pair	Typically 14–20 bp per TALEN monomer, 28–40 bp per TALEN pair	22 bp (20-bp guide sequence + 2-bp protospacer adjacent motif (PAM) for *Streptococcus pyogenes* Cas9); up to 44 bp for double nicking
Specificity	Small number of positional mismatches tolerated	Small number of positional mismatches tolerated	Positional and multiple consecutive mismatches tolerated
Targeting constraints	Difficult to target non-G-rich sequences	5′ targeted base must be a T for each TALEN monomer	Targeted sequence must precede a PAM
Ease of engineering	Difficult; may require substantial protein engineering	Moderate; requires complex molecular cloning methods	Easily re-targeted using standard cloning procedures and oligo synthesis
Immunogenicity	Likely low, as zinc fingers are based on human protein scaffold; FokI is derived from bacteria and may be immunogenic	Unknown; protein derived from *Xanthamonas* sp.	Unknown; protein derived from various bacterial species
Ease of *ex vivo* delivery	Relatively easy through methods such as electroporation and viral transduction	Relatively easy through methods such as electroporation and viral transduction	Relatively easy through methods such as electroporation and viral transduction
Ease of *in vivo* delivery	Relatively easy as small size of ZFN expression cassettes allows use in a variety of viral vectors	Difficult due to the large size of each TALEN and repetitive nature of DNA encoding TALENs, leading to unwanted recombination events when packaged into lentiviral vectors	Moderate: the commonly used Cas9 from *S. pyogenes* is large and may impose packaging problems for viral vectors such as AAV, but smaller orthologs exist

Gene editing approaches to HIV and acute B-cell lymphoblastic leukaemia treatment (L2.5)

Therapy: e.g. Targeting CCR5 for HIV therapy
- CCR5 = T cell co-receptor for HIV-1
- Individuals with loss of 32 aa in CCR5 are resistant to HIV-1 infection
  - Prevents viral binding, virus-cell fusion and entry

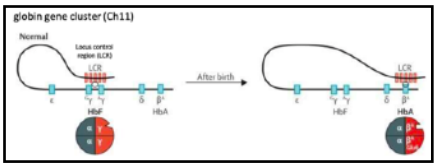
Targeting CCR5 for HIV therapy
- 12 people with HIV on antiretroviral drugs
- T-cells from each participant transfected with a ZFN target CCR5 gene - ~25% efficiency
- Modified cultured cells transfused back into the participants
- Results:
  - All had elevated levels of T cells in their blood
  - 6 participants stopped anti-retrovirals; HIV levels rebounded more slowly than normal, and their T-cell levels remained high for weeks
  - Researchers suspect that the virus was unable to infect and destroy the altered cells enabling modified T-cells to proliferate

TALENs for B-cell acute lymphoblastic leukaemia therapy
- CD54 gene loci

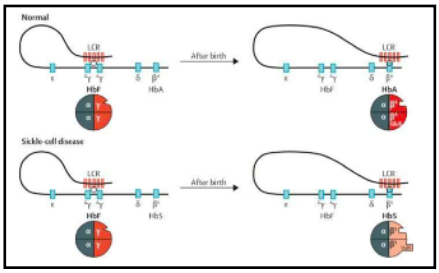
Gene editing approaches to Sickle Cell Anaemia treatment (L2.6)

Sickle cell anaemia
- Monogenic disorder caused by the autosomal recessive inheritance of an A->T transversion of the beta-globin gene (beta-s [HBB]). Forms haemoglobin polymers/aggregates
  - Sickle-cell anaemia
  - Haemolytic
  - Anaemia, ischaemia (blocking of blood flow)
  - Inflammation
  - Susceptibility of infection
  - And organ injury

Haemoglobin (Hg)

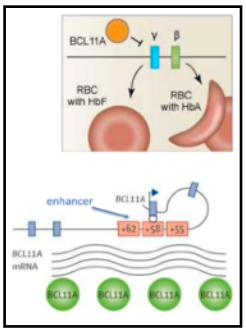


- Adult haemoglobin tetramer contains two alpha and two beta-globins (alpha2beta2)
- Both alpha-globin and beta-globin genes are encoded from a cluster of similar genes, on chromosome 16 and 11
- The globin gene clusters undergo developmental regulation
  - In the latter 2 trimesters of gestation, foetal haemoglobin is prevalent
  - Foetal haemoglobin contains two alpha and two gamma-globins (alpha2gamma2)
  - Only after birth, foetal haemoglobin is replaced by adult haemoglobin

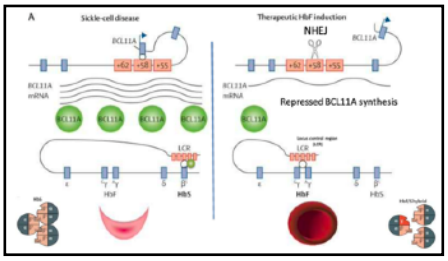


Foetal Hg protects against sickle cell disease
- Newborn babies do not manifest sickle-cell disease complications until they are ~6 months of age
- **Asymptomatic** patients with **sickle-cell disease** who co-inherited a Hereditary Persistence of Foetal Haemoglobin (**HPFH**) have a **benign** phenotype
  - HPFH is typically caused by either **large deletions of the beta-globin gene cluster** or **point mutations** in the **promoters of the gamma-globin genes** and is characterised by **high concentrations** of **foetal haemoglobin** throughout life

Tetramers with foetal haemoglobin are protectives
- Foetal haemoglobin tetramers containing **gamma-subunits** have much **lower probability** of co-polymerising with the sickle haemoglobin tetramers containing two beta-S peptides
- Gamma-chains have a **less hydrophobic patch** for making lateral interactions with Val6 of the beta-subunit
- Can foetal Hg be turned on in sickle-cell individuals?

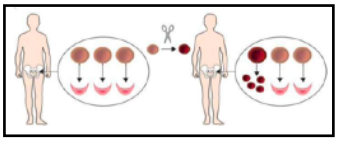


BCL11A negatively regulates foetal Hg production
- BCL11A encodes a zinc-finger transcription factor = a negative regulator of gamma-globin gene expression
- Patients with BCL11A haploinsufficiency have an HPFH-like phenotype
  - But neurodevelopmental problems since BCL11A also has important roles in neuron development and B-cell lymphopoiesis (so we can't simply turn it off everywhere)
- But BCL11A has an **enhancer** in one of its **intron** that drives its expression in **erythroid cells**

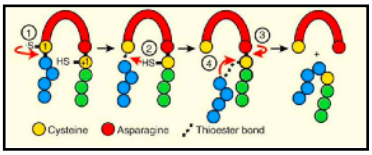


A novel genetic therapy for sickle-cell disease
- Deletion of the BCL11A erythroid enhancer be genome editing results in specific loss of BCL11A expression in erythroid precursors (not in neurons or B lymphocytes)
- This increases foetal haemoglobin production

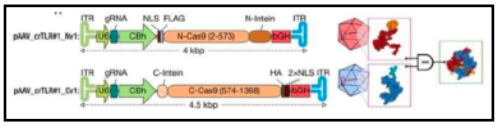
A novel genetic therapy for sickle-cell disease
- Isolation of haemopoietic stem cells (CD34+) from patients
- Delivery of genome editing technology to disrupt the BCL11A enhancer
- Autologous re-infusion of modified cells
- Correction of the beta-S mutation would be the definitive genome-editing approach to cure sickle-cell disease, but this requires robust HDR in haematopoietic stem cells
- HDR is only active at exceedingly low levels in these quiescent haemopoietic stem cells. The NHEJ pathway is present



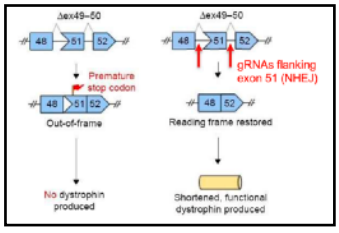
Future Challenges (L2.7)



Inteins to split Cas9 for packaging into AAVs
- **Inteins** — "protein introns" that **splice out autocatalytically** from **host polypeptides** to generate a **functional protein**
- The **N-terminal half** of Cas9 is **fused** upstream of the **N-intein** and packaged in **one AAV** and the **C-terminal half** of Cas9 **fused** downstream of the **C-intein** is packaged into **another AAV**
- When expressed together, the inteins undergo post-translational autocatalytic excision while ligating the two Cas9 portions together



Somatic gene editing on a pig model of DMD
- Removing exon 51 to restore the DMD reading frame
- In DMD-delta-52 pigs, intramuscular injection of adeno-associated viral vectors carrying an intein-split Cas9 and a pair of guide RNAs targeting sequences flanking exon 51 (**AAV9-Cas9-gE51**) induced expression of a



shortened dystrophin (DMD-delta-51-52) and improved skeletal muscle function
- AAV9-Cas9-gE51 application snipped exon 51 in vitro and in vivo restoring the DMD reading frame
- In vivo, intramuscular application yielded high local rates of gene editing
- The shortened DMD-delta-51-52 improved muscle function and mobility preventing malignant arrhythmias, prolonging lifespan
- This i.v. AAV9-Cas9 genome editing approach may prove clinically useful for the treatment of patients with DMD in the future

Drugs and Therapy 1 (W2)

Drug Discovery and Development (L1.1)

Drug Discovery and Development

- Discovery phase
  - Target identification
  - Lead compound
  - Candidate drug
- Development phase
  - Preclinical and clinical development
  - Regulatory approval
  - Registration

"Nature" of a drug?

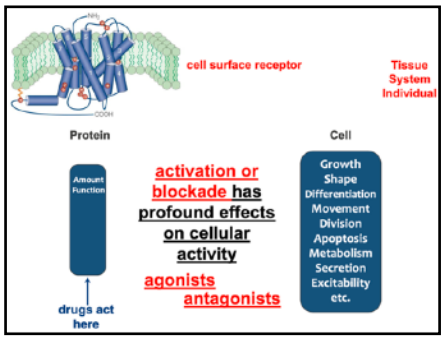
- Drug
  - Any chemical substance (other than a nutrient or essential dietary ingredient) that brings about a change in biological function
    - Natural products
    - Synthetic chemicals
- Chemical name - a-[[(1,1 dimethylethyl) amino] methyl]-4-hydroxyl-1,3-benzenedimethanol
- **Generic** name: salbutamol (medicines active ingredient)
- **Trade** name: ventolin (FSK)

Targets for drug action

- "A drug will not work unless it is bound"
- most* drugs exert their effects by binding to protein molecules
  - Receptors
  - Ion channels
  - Enzymes
  - Transporters

Proteins = primary targets of exisiting drug molecules

- G-protein coupled receptors
  - e.g. D2 receptor
  - Indirectly activated by levodopa and used in Parkinson's disease
- Ion channels
  - Voltage gated Na+ channel
  - Inhibited by local anaesthetics (i.g. lignocaine)
- Enzymes
  - Cyclo-oxygenase
  - Irreversibly inhibited by aspirin, reducing production of prostaglandins
- Transporters
  - 5-HT uptake
  - Blocked by fluoxetine (Prozac; used as an anti-depressent)

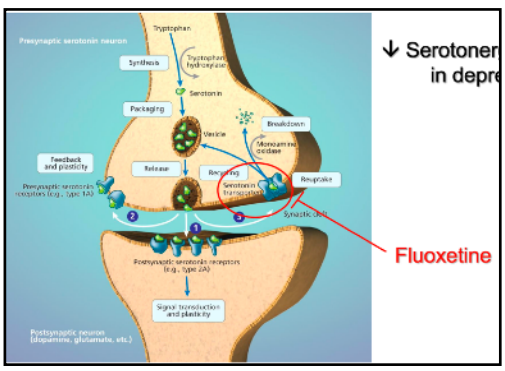


Strategies for finding new drug targets

1. Analysis of pathophysiology
   - Understanding of pathways involved in disease determines novel target
2. Analysis of mechanism of action of existing therapeutic drugs
   - Work 'backwards' from known action to mechanism
3. Genomic approaches
   - Most drug targets are proteins, so encoded in genome
   - Disease genes (mutated); disease-modifying genes; druggable genes

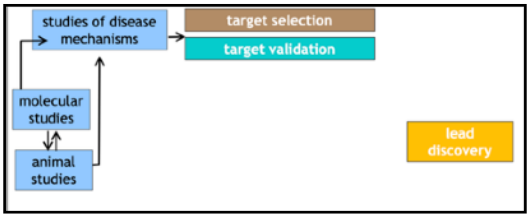
Depression and 5-HT (1)

- Decrease serotonergic function in depression
- Fluoxetine blocks uptake serotonin transporter and inhibits reuptake



Targets identified via drug effect (2):

Drug	Disease	Target
Benzodiazepines	Anxiety; sleep disorders	$GABA_A$ Receptor (allosteric site)
Aspirin-like drugs	Inflammation; pain	COX Enzymes
Cyclosporin; FK506	Transplant rejection	Immunophilins
Vinca alkaloids	Cancers	Tubulin
Dihydropyridines	Cardiovascular disease	L-type Calcium Channels
Sulphonylureas	Diabetes	K_{ATP} Channels
Typical antipsychotics	Schizophrenia	Dopamine D_2 Receptors
Tricyclic antidepressants	Depression	Monoamine Transporters
Fibrates	Raised blood cholesterol	PPARα



How can we validate a target:
- Animal studies
- Molecular studies
- Studies of disease mechanism
  - Target selection and target validation

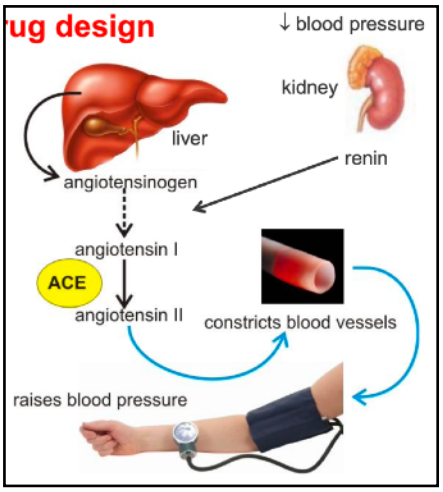
Sourcing and creating new drugs (L1.2)

What are the steps involved in identifying a new lead compound?
- Target selection and target validation
- **Screen compounds** (10^5 - 10^6)
  - Large number of related chemicals derived from
    - Natural sources, rational drug design or chemical libraries
  - Primary Hit Candidate (10^2 - 10^3)
  - Validated Hit Candidate (~10 hit series)
  - Lead compounds (3 lead series)

Sources of new drugs
1. Screening of natural products (50%)
   - Plants
   - Animals (venoms/toxins/secondary metabolites)
   - Microbes
2. Serendipity (by chance)
3. Rational design (most new drugs are synthetic produced from rational design)
4. Screening of chemical libraries

Serendipity
- Sildenafil (Viagra)
  - Developed as a dilator of coronary arteries
  - During clinical trials male patients reported effect on sexual activity
  - Marketing strategy shifted to anti-impotence drug

Rational drug design
- ACE (angiotensin converting enzyme inhibitors (captopril))
- Peptide in pit viper (Bottrop's jararaca) venom which was a inhibitor of angiotensin II production



Captopril
- Captopril was developed from this peptide after it was found that modifications of the terminal sulfhydryl moiety of the peptide provided a high potency of ACE inhibition

Other approaches to rational drug design - exploiting a side effect
- Sulphanilamide
  - An antibacterial with the side effect of **lowering glucose levels** in the blood and also **diuretic** activity
- Tolbutamide
  - A compound which has been optimised to only **lower blood glucose levels**. Useful in the treatment of type II diabetes