Genome Engineering

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A Brief History of Genetic Modification

- The first GMO was made in 1973
  - Bacteria carrying DNA of another species

- Most manipulation of DNA has been done using short fragments in test tubes and bacterial systems

- This DNA can be transferred to human / animal cells to add something new to their genome
  - Naked DNA or packaged into a virus ‘vector’ for delivery
  - Insertion is random → unexpected consequences
  - Could be inserted into fertilized ovum to make an animal

- First targeted transgenic animal (mouse) made in 1987
  - Genes could be removed or changed in an animal
A brief history of genetic modification II

• Polymerase chain reaction (PCR) and chemical synthesis of DNA made things faster

• Methods like polymerase chain reaction (PCR) and chemical synthesis of DNA → synthetic biology
  › Still a long way off making mammals

• RNAi allowed genes to be turned down
  › Has to be a constantly present

• Achieving a desired genetic change - even in cells in tissue culture remained practically impossible
  › This is the first step towards therapeutic genome modification
Gene therapy – could only add new DNA

Kaufmann KB et al, EMBO Mol Med, 2014
Key limitations

• Most cutting and modification done *in vitro* not in cells
  › Have to get DNA out of cells and back in again
  › Limits the length that can be easily modified

• The enzymes used to cut had defined recognition sites
  › E.g. GAATTC
  › They rarely cut exactly where you want
  › They cut at many other places

• PCR and synthetic DNA methods were helping
  › But did not overcome these basic problems

• We all wanted a DNA cutting enzyme (a nuclease) that:
  › Cuts at a sequence of our choosing
  › Would work inside living cells
Enter targeted nucleases

Zinc finger nucleases and TALENs

- A new protein required for each site to be cut
- Proprietary technology
- Expensive
- Efficiency limited
CRISPR/Cas9 (and variants)

Lives up to the hype!

- **Open source** (for research)
- **Cheap**
- **Highly efficient**

### Clustered Regularly Interspaced Short Palidromic Repeats (CRISPR)

**gRNA**

**Cas9**

CRISPR in Action

Two ways to edit DNA with CRISPR/Cas9

Genome to be edited

gRNA/Cas9
Why is it so easy?

• There are genetic constructs that make Cas9 and have a site to add DNA for any gRNA you like
  › [https://www.addgene.org/CRISPR/](https://www.addgene.org/CRISPR/)

• If this is put into cells, it just goes to work…
So very easy

• You can buy Cas9 protein and any gRNA you like
• Mix them in a tube
• ‘Transfect’ them into a cell
  › with or without a repair DNA
• If there is no DNA, is it a GMO?

$200

$600
The catch: Specificity and ‘off-target effects’

- Off-target effects are considered the main limitation
  - Cutting at undesired locations
  - Partly addressed by combining two Cas9 ‘nickases’

Gene therapy can now include gene correction

Targeting the CRISPR/Cas9 is still a major hurdle for in vivo gene therapy
### ‘CRISPR’ clinical trials on the US NIH database

**ClinicalTrials.gov Search Results 09/18/2017**

<table>
<thead>
<tr>
<th>Title</th>
<th>Recruitment</th>
<th>Study Results</th>
<th>Conditions</th>
<th>Interventions</th>
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| A Safety and Efficacy Study of TALEN and CRISPR/Cas9 in the Treatment of HPV-related Cervical Intraepithelial Neoplasia# | Not yet recruiting | No Results Available | • Human Papillomavirus-Related Malignant Neoplasm | • Biological: TALEN  
• Biological: CRISPR/Cas9                           |
| Safety of Transplantation of CRISPR CCR5 Modified CD34+ Cells in HIV-infected Subjects With Hematological Malignancies | Recruiting        | No Results Available | • HIV-1-infection                              | • Genetic: CCR5 gene modification                |
| Examining the Knowledge, Attitudes, and Beliefs of Sickle Cell Disease Patients, Parents of Patients With Sickle Cell Disease, and Providers Towards the Integration of CRISPR in Clinical Care | Not yet recruiting | No Results Available | • Sickle Cell Disease                          | • Biological: UCART019                           |
| A Study Evaluating UCART019 in Patients With Relapsed or Refractory CD19+ Leukemia and Lymphoma | Recruiting        | No Results Available | • B Cell Leukemia • B Cell Lymphoma            | • Biological: UCART019                           |
| PD-1 Knockout Engineered T Cells for Advanced Esophageal Cancer       | Recruiting        | No Results Available | • Esophageal Cancer                            | • Drug: Cyclophosphamide  
• Drug: Interleukin-2  
• Other: PD-1 Knockout T Cells                                      |
| PD-1 Knockout Engineered T Cells for Muscle-invasive Bladder Cancer   | Not yet recruiting | No Results Available | • Invasive Bladder Cancer Stage IV             | • Biological: PD-1 Knockout T Cells  
• Drug: Cyclophosphamide  
• Drug: IL-2                                                      |
| PD-1 Knockout Engineered T Cells for Castration Resistant Prostate Cancer | Not yet recruiting | No Results Available | • Hormone Refractory Prostate Cancer           | • Biological: PD-1 Knockout T Cells  
• Drug: Cyclophosphamide  
• Drug: IL-2                                                      |
| PD-1 Knockout Engineered T Cells for Metastatic Renal Cell Carcinoma  | Not yet recruiting | No Results Available | • Metastatic Renal Cell Carcinoma              | • Biological: PD-1 Knockout T Cells  
• Drug: Cyclophosphamide  
• Drug: IL-2                                                      |
Gene drives
Gene drives

- Genes that are inherited at greater than Mendelian rates
Normal (Mendelian) inheritance

• There are two copies of the genome
• One copy of each gene comes from each parent

The gene will be in \( \frac{1}{4} \) of next generation (assuming most are grey)
Gene drives

• A gene drive can cause a gene to be duplicated
What does CRISPR have to do with gene drives?

CRISPR/Cas9 Gene drive

1. **TARGET**
   - Sg RNA
   - Cas9
   - Cargo

   Transgene/gene drive cassette

2. Sg RNA guides
   - Cas9 protein to cleave target DNA

3. Cleaved DNA gets repaired using transgene as template

4. Both chromosomes passed to next generation will have transgene/gene drive cassette
Applications of gene drives

• Inhibiting / controlling insect vectors of disease
  › Health benefits, especially for developing nations

• Pest animal control
  › Potential environmental benefit

• Controlling pests of agriculture
  › Commercial benefit
  › Improved food security

• Any release requires enormous care and consideration

• Public scrutiny required
  › Very substantial dread
Strategic issues

• How does Australia ensure we are active participants and not bystanders?

• How will we decide when genome engineering is considered safe for human medicine?
  › What will be the quality benchmarks for certification?

• How will we balance potential health benefits against environmental concern (e.g. gene drives)?

• How will we ensure that policy and the public are informed by science?

• How will we respond to international regulatory moves (e.g. licencing or moratoria)