PIPADOR REVISION

MRCOG Part 1 Revision Notes

CRISPR and Gene Editing

MOLECULAR BIOLOGY • LECTURE 99

VISUAL EXAMINATION REVISION NOTES

2026 Edition

MRCOG Part 1 Revision Notes

Lecture 99: CRISPR and Gene Editing

Molecular biology • Visual revision edition — follows Lecture 98

COLOUR CODE USED THROUGHOUT THESE NOTES

Cas9 / EX VIVO	TARGET / CUT / RISK	gRNA / BASE EDIT / IN VIVO	HDR / PRIME / O&G	PAM / DIAGNOSTICS

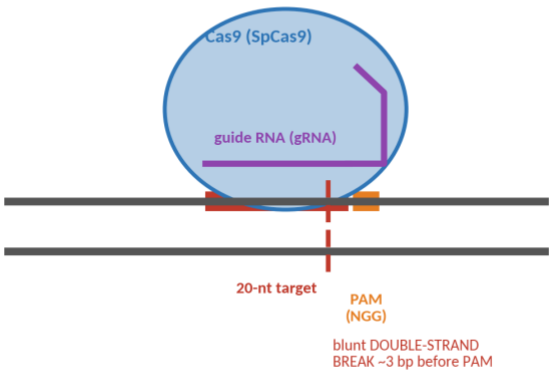
LEARNING OBJECTIVES — tick off as you go

- ☐ Describe CRISPR-Cas9: origin, gRNA, PAM and the double-strand break.
- ☐ Compare NHEJ and HDR repair.
- ☐ Explain dCas9, base editing, prime editing and CRISPR diagnostics.
- ☐ Describe ex vivo and in vivo delivery and clinical applications (Casgevy, NTLA-2001).
- ☐ Discuss safety, germline ethics and fertility/pregnancy counselling.

1 How CRISPR Works

IN ONE LINE: *A guide RNA leads Cas9 to a matching 20-letter DNA sequence next to a PAM; Cas9 cuts both strands; the cell's own repair does the rest.*

CRISPR-Cas9 — a programmable DNA cutter



In bacteria

adaptive immunity: phage DNA saved as 'spacers' in the CRISPR array → crRNA guides Cas to cut the phage next time

Engineered (2012)

Doudna + Charpentier (Nobel 2020): single gRNA (~20 nt + scaffold) + Cas9 = programmable scissors

PAM

Cas9 only cuts next to its motif — NGG for SpCas9 (limits where you can edit)

Variants

SaCas9 (small, fits AAV) • Cas12a (T-rich PAM, staggered cut) • Cas13 (cuts RNA — diagnostics)

Figure 1. Cas9, gRNA and PAM.

MNEMONIC / MEMORY HOOK

'Guide finds, PAM permits, Cas cuts, cell repairs.'

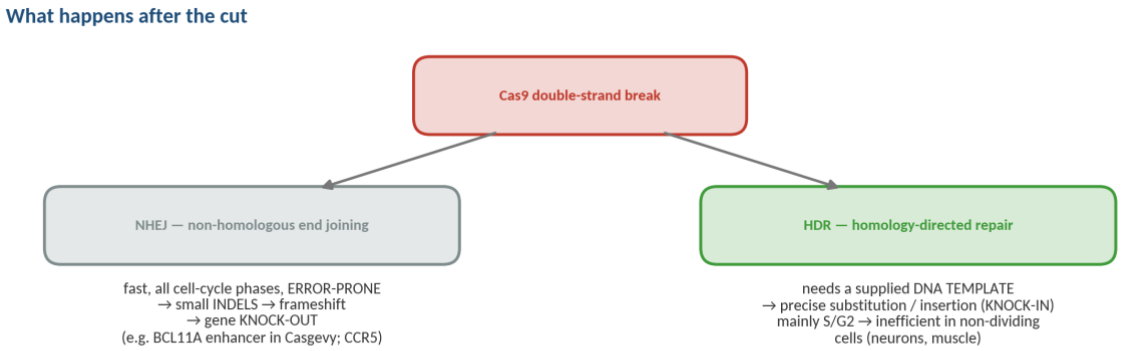


Figure 2. NHEJ vs HDR.

2 Beyond Cutting

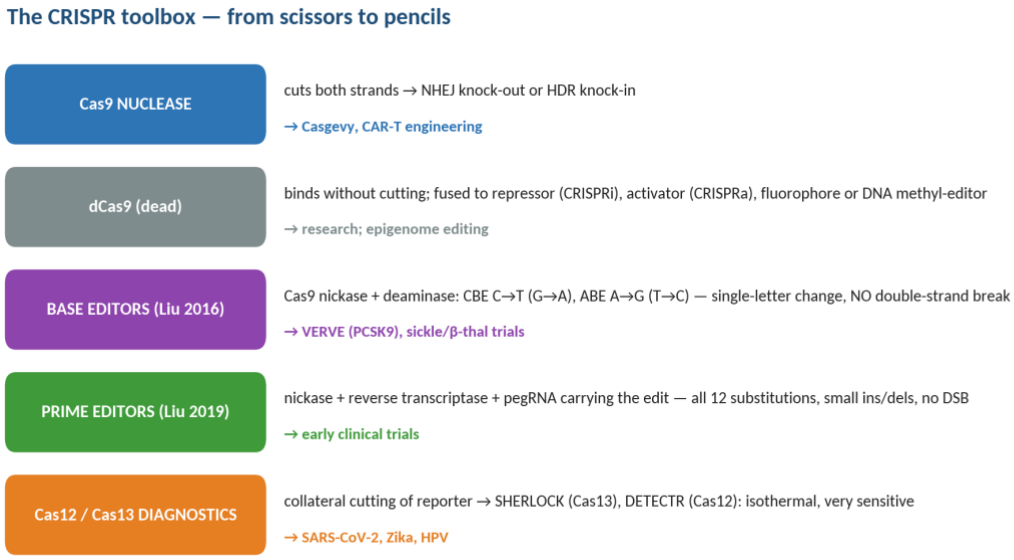


Figure 3. The CRISPR toolbox.

3 Delivery and Applications

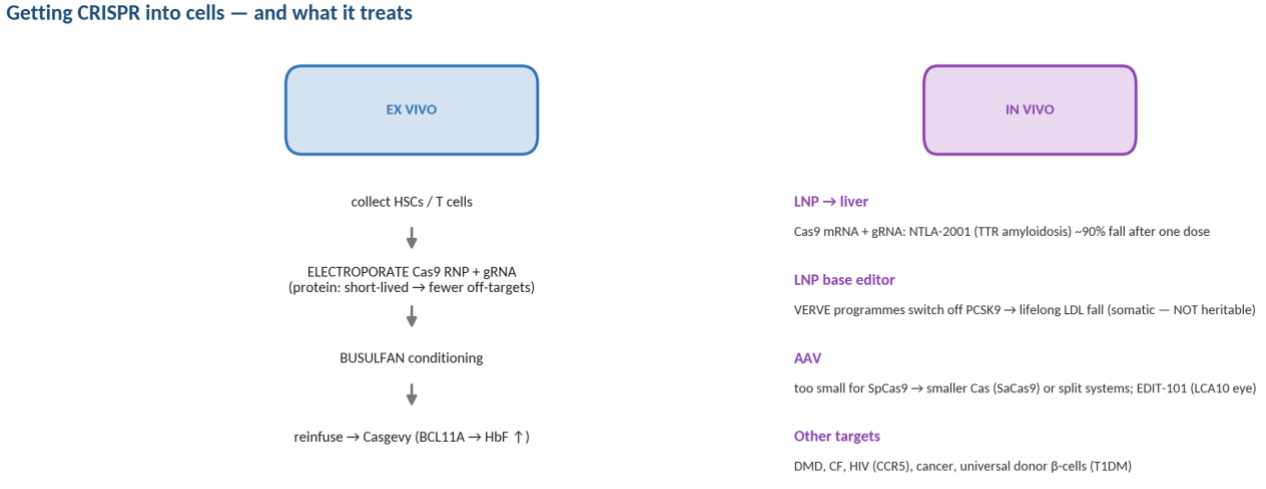


Figure 4. Ex vivo vs in vivo.

Product / trial	Key points
Casgevy (exa-cel)	First licensed CRISPR medicine — MHRA Nov 2023 (world first); sickle cell disease and transfusion-dependent β-thal; BCL11A erythroid enhancer → HbF ↑ (Lecture 98)
NTLA-2001	In vivo LNP Cas9 to liver → ~90% fall in TTR in hereditary ATTR amyloidosis (phase 3 trials)
PCSK9 base editing	VERVE programmes: one infusion → lasting LDL fall in familial hypercholesterolaemia — SOMATIC, so not passed on (first candidate paused for safety review; next-generation versions in trials)
Other trials	LCA10 eye (EDIT-101), DMD, CF, HIV (CCR5), CAR-T enhancement, universal donor β-cells
History	First human CRISPR trial 2016 (China, PD-1 knockout T cells for lung cancer)

4 Safety

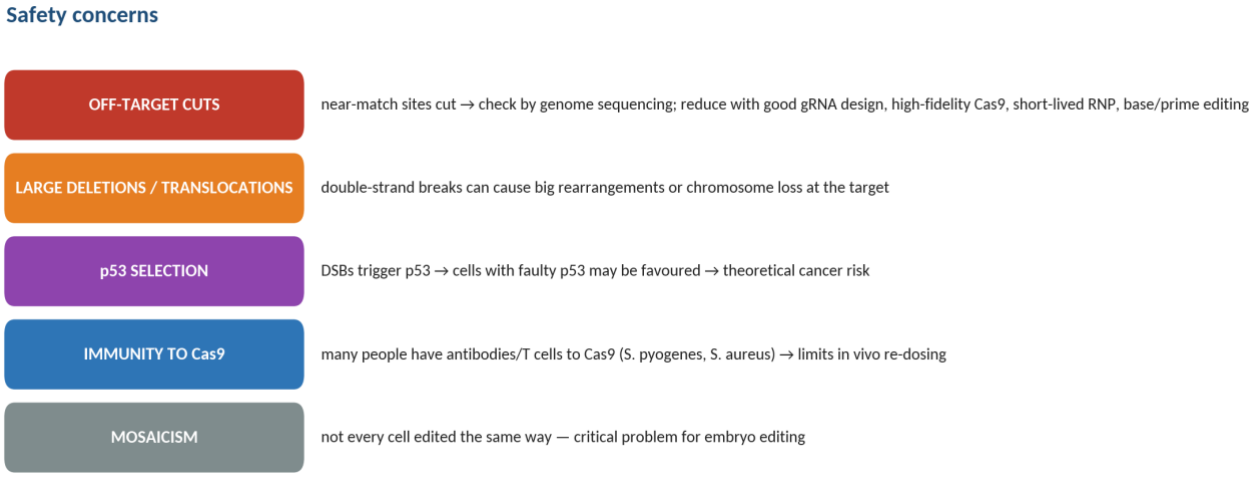


Figure 5. Safety concerns.

5 Ethics and O&G Counselling

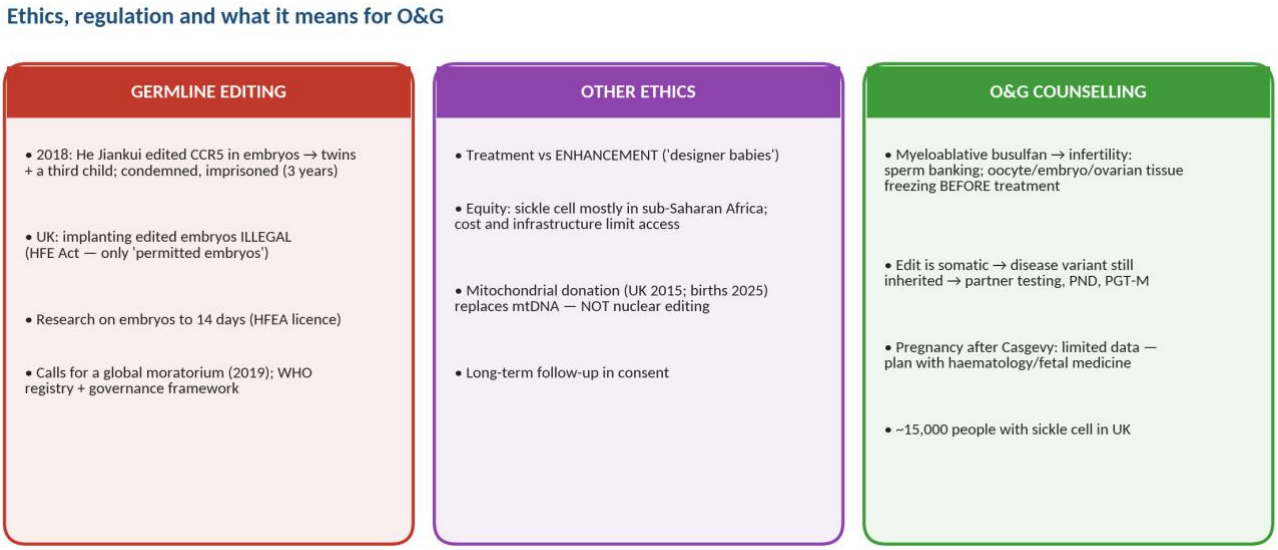


Figure 6. Ethics and counselling.

★ PEARL

Cas9 + gRNA + PAM (NGG) → double-strand break → NHEJ (knock-out) or HDR (template knock-in). Base/prime editing avoid the DSB.

Germline editing for reproduction is illegal in the UK. Before Casgevy: fertility preservation; afterwards the variant is still inherited.

6 MRCOG Exam Pearls — Last-Minute Page

Numbers to know

~20 nt Guide sequence	**NGG** SpCas9 PAM	**2012 / 2020** Discovery / Nobel	**2016 / 2019** Base / prime editing
Nov 2023 Casgevy (MHRA)	**~90%** TTR fall (NTLA-2001)	**2018** He Jiankui embryos	**14 days** Embryo research limit

HIGH-YIELD CHECKLIST

- ☐ Bacterial adaptive immunity → programmable editor (Doudna/Charpentier).
- ☐ Cas9 + ~20-nt gRNA + PAM (NGG) → DSB.
- ☐ NHEJ = indels/knock-out; HDR = template, precise, dividing cells.
- ☐ SaCas9 (AAV-sized), Cas12a, Cas13 (RNA, diagnostics).
- ☐ dCas9: CRISPRi/a, epigenome editing.
- ☐ Base editing C→T / A→G; prime editing (pegRNA + RT) — no DSB.
- ☐ Ex vivo RNP electroporation (Casgevy) vs in vivo LNP (NTLA-2001).
- ☐ Safety: off-target, large deletions, p53, Cas9 immunity, mosaicism.
- ☐ Germline editing illegal in UK; He Jiankui; moratorium.
- ☐ Fertility preservation before busulfan; variant still heritable.

Self-assessment — cover the right-hand column

Question	Answer
What directs Cas9 to its target?	The ~20-nt guide RNA
PAM for SpCas9?	NGG
Repair pathway that creates knock-outs?	NHEJ (indels)
Why is HDR hard in neurons?	It mainly works in dividing (S/G2) cells
Which Cas enzyme targets RNA?	Cas13
Base editor without a double-strand break makes…	C→T (CBE) or A→G (ABE) changes
What carries the edit in prime editing?	pegRNA (+ reverse transcriptase)
Target edited by Casgevy?	Erythroid enhancer of BCL11A
Is PCSK9 base editing heritable?	No — somatic liver cells only
Is germline editing for reproduction legal in the UK?	No

7 References

- Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease. Science 2012.
- Anzalone AV et al. Search-and-replace genome editing without double-strand breaks (prime editing). Nature 2019.
- WHO Expert Advisory Committee. Human genome editing: a framework for governance. 2021.