

Unit 4: Recombinant DNA Technology, Drug Discovery and Indigenous Crude Drugs

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4.1 Recombinant DNA technology: the toolkit

Recombinant DNA technology joins DNA from different sources into one molecule and maintains it in a host. The toolkit elements, in the order a practical exam expects:

- **Restriction endonucleases** cut DNA at specific short sequences, many leaving staggered (sticky) ends that anneal to matching ends from any other DNA cut with the same enzyme.
- **DNA ligase** seals the joins. **Vectors** carry the recombinant molecule into cells: plasmids for bacteria, bacteriophage and cosmids for larger inserts, yeast and viral vectors for other hosts.
- **Gene sources:** genomic DNA, or **cDNA** copied from messenger RNA by reverse transcriptase, which for expression in bacteria is essential because bacteria cannot remove introns.
- **Selection and screening:** antibiotic resistance markers on the vector identify cells that took it up; probes, antibodies or activity assays identify the clone carrying the wanted gene.
- **Amplification and sequencing:** the polymerase chain reaction (PCR) amplifies a chosen sequence in vitro with a thermostable polymerase; Sanger and later high throughput sequencing read the result, so the construct is verified, not assumed.

4.2 rDNA in drug discovery and production

The first recombinant pharmaceutical, **human insulin** (1982), set the pattern: the human gene (as separate A and B chain genes in the first process, later as proinsulin) expressed in *Escherichia coli*, the product purified and folded to a protein identical to the human hormone, ending dependence on pig and cattle pancreas with their allergy and supply problems. The procession since includes human growth hormone (ending cadaver derived supply and its prion risk), erythropoietin, interferons, hepatitis B surface antigen vaccine (a recombinant subunit vaccine made in yeast), tissue plasminogen activator and the coagulation factors.

Making a Medicine with rDNA Technology



First success of the route: recombinant human insulin, 1982.

THE SAME TOOLKIT ALSO SUPPLIES

Recombinant receptors and enzymes as targets for screening assays
Hepatitis B surface antigen vaccine, made in yeast
Engineered animal models that test whether a target matters

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Figure: The recombinant production route from human gene to medicine, with recombinant human insulin as its first success.

For **drug discovery** the technology works at three further levels: it supplies pure human targets, receptors and enzymes, for screening assays (the kinases and GPCRs of the MPC103T syllabus are screened as recombinant proteins); it supplies disease proteins and antigens for structural studies that feed structure based design; and animal models engineered with human genes or gene deletions test whether a target matters in a whole organism before chemistry money is spent. The subject's earlier units isolated what organisms happened to make; this unit is about instructing organisms to make what is needed.

4.3 Hybridoma technology and monoclonal antibodies

A B lymphocyte makes one antibody but will not grow indefinitely in culture; a myeloma cell grows indefinitely but makes no useful antibody. **Köhler and Milstein (1975)** fused the two: immunise a mouse, fuse its spleen B cells with myeloma cells using polyethylene glycol, and select in **HAT medium**, in which the myeloma partner (deficient in the enzyme HGPRT) dies and unfused B cells die naturally, leaving only hybridomas. Screening and cloning of the survivors yields a **monoclonal antibody**: one specificity, one sequence, unlimited supply.

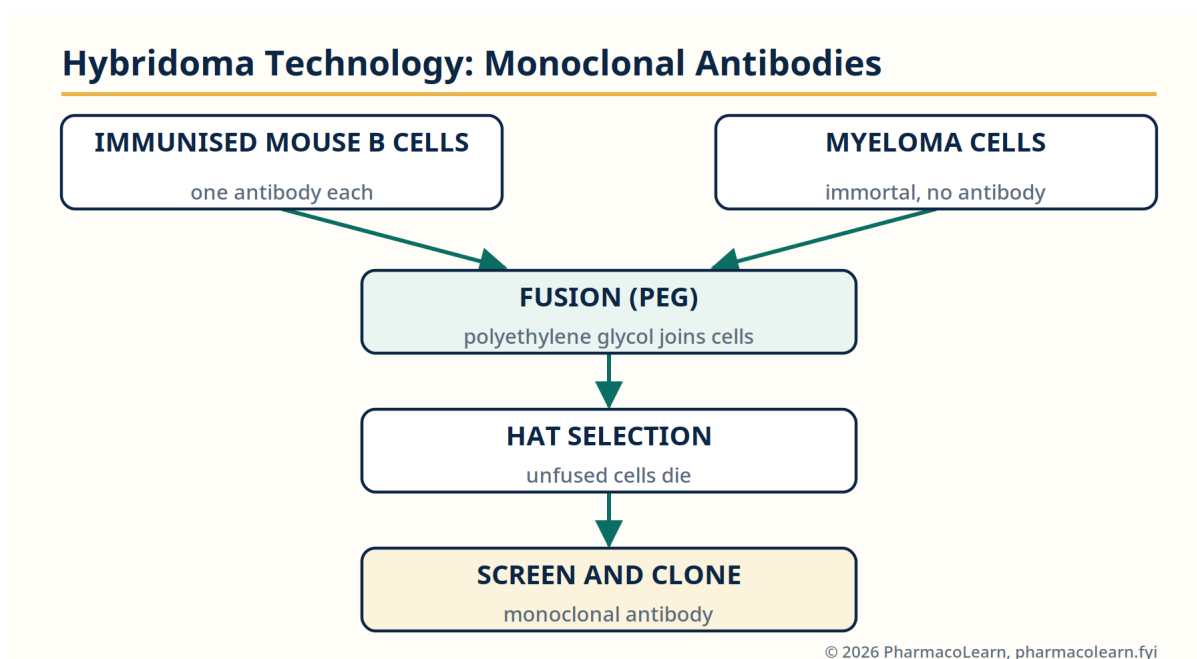


Figure: Hybridoma production: fusion, HAT selection and cloning convert a mouse immune response into an unlimited supply of one antibody.

Pharmaceutical consequences: monoclonal antibodies became a drug class in their own right (the trastuzumab, rituximab and adalimumab generation, progressively humanised and then fully human to escape the immunogenicity of mouse protein), and became the discovery tools of the trade: assay antibodies, target validation reagents, antibody drug conjugates that deliver cytotoxic payloads to antigen bearing cells, and the purification handles of downstream processing.

4.4 Oligonucleotide therapy

Oligonucleotide drugs are short synthetic DNA or RNA like strands, chemically stabilised, that act on nucleic acids rather than proteins:

- **Antisense oligonucleotides** bind a target messenger RNA by base pairing and either recruit RNase H to destroy it or block its processing. They must be protected against nucleases, hence backbone modification (phosphorothioate linkages) and sugar modifications (2 prime-O-methoxyethyl and relatives). Fomivirsen, against cytomegalovirus retinitis, was the first approved antisense drug (1998); nusinersen, which corrects the splicing of the SMN2 gene in spinal muscular atrophy, shows the splice switching branch of the family.
- **Small interfering RNAs (siRNAs)** load into the RISC complex and direct it to cleave a complementary mRNA; patisiran, delivered in a lipid nanoparticle to the liver, was the first approved siRNA (2018).
- **Aptamers** are folded oligonucleotides selected to bind a protein target in three dimensions, pegaptanib (anti-VEGF) being the approved example.

The class-wide obstacles are the delivery and stability problems of large charged molecules, which is why the liver, the eye and the spinal canal, all reachable compartments, host most successes so far.

4.5 Gene therapy: introduction, clinical applications and recent advances

Gene therapy treats disease by delivering a functional gene, or gene editing machinery, to the patient's cells. **Ex vivo** therapy removes cells, corrects them in the laboratory and returns them (the controllable route); **in vivo** therapy delivers the vector to the patient directly. Delivery vehicles divide into viral vectors, retrovirus and lentivirus (integrating, durable, with insertional mutagenesis risk that early trials tragically confirmed), adenovirus (efficient, transient, immunogenic) and adeno-associated virus (AAV, largely non integrating, the current in vivo favourite, limited cargo size), and non viral systems, chiefly lipid nanoparticles.

Clinical applications with regulatory approvals include: ex vivo correction of ADA deficient severe combined immunodeficiency; AAV delivered RPE65 gene for inherited retinal dystrophy (voretigene neparvovec), the first in vivo gene therapy for an inherited disease; AAV delivered SMN1 for spinal muscular atrophy (onasemnogene abeparvovec); lentiviral beta globin for transfusion dependent beta thalassaemia; and the CAR T cell therapies, in which a patient's T cells are engineered ex vivo with a chimeric antigen receptor against CD19, gene therapy wearing an immunotherapy uniform.

Recent advances are dominated by **gene editing**: CRISPR Cas9 editing of a patient's own blood stem cells ex vivo, reactivating fetal haemoglobin, won the first CRISPR approvals in 2023 for sickle cell disease and beta thalassaemia (exagamglogene autotemcel). Base editing and prime editing, which rewrite single letters without double strand breaks, in vivo editing trials, and steadily better capsids and lipid nanoparticles for tissue targeting mark the current frontier. The syllabus phrase recent advances should always be answered with the editing story plus the standing problems: durability, immune responses to vectors, manufacturing cost and equitable access.

4.6 Principles of RNA and DNA estimation

Nucleic acid estimation is a Unit 1 practical skill of this course (the MPC105P practical list includes RNA and DNA estimation), and its principles are chemical:

- **Ultraviolet absorption**: nucleic acids absorb maximally near 260 nm because of their bases. One absorbance unit at 260 nm corresponds to about 50 microgram per ml for double stranded DNA, 40 for RNA and 33 for single stranded DNA. The A260/A280 ratio (about 1.8 for pure DNA, 2.0 for pure RNA) reports protein contamination; it measures purity and quantity together in one reading, and it cannot tell DNA from RNA in a mixture.

- **Colour reactions for specific sugars:** the **diphenylamine (Dische) reaction** gives a blue colour with the deoxyribose of DNA under hot acid conditions, and the **orcinol (Bial) reaction** gives a green colour with the ribose of RNA; these classical methods estimate one nucleic acid in the presence of the other.
- **Fluorescent dyes:** modern quantitation uses dyes whose fluorescence rises sharply on binding double stranded DNA (ethidium bromide historically, safer intercalating and groove binding dyes now), giving sensitivity far beyond UV absorption for dilute samples.

4.7 Indigenous crude drugs I: the diabetic therapy group

The indigenous system (Ayurveda and allied practice) carries a large antidiabetic materia medica; the syllabus names five. In each case the active constituents, not vague virtue, are the chemistry:

Crude drug	Principal active constituents	Chemical note
<i>Gymnema sylvestre</i> (gurmar)	Gymnemic acids, a family of oleanane triterpene saponins	The leaf famously abolishes sweet taste temporarily (gymnemic acids block sweet receptors); saponin chemistry of section 2.12 on an oleanane frame; intestinal glucose uptake effects reported
<i>Salacia reticulata</i>	Salacinol and kotalanol, sulfonium ion glycosidase inhibitors	Potent alpha glucosidase inhibitors of unusual sulfonium sulfate structure; the acarbose mechanism from a root bark
<i>Pterocarpus marsupium</i> (vijaysar)	(Minus)-epicatechin (a flavanol, section 2.10 chemistry), pterostilbene, marsupsin	Heartwood constituents; epicatechin is the constituent most discussed in beta cell studies
<i>Swertia chirata</i> (chirayata)	Amarogentin and swertiamarin, secoiridoid glycosides; xanthenes such as mangiferin	Amarogentin is among the most bitter substances known; the drug is the classical intensely bitter tonic of the group
<i>Trigonella foenugraecum</i> (fenugreek, methi)	The unusual amino acid 4-hydroxyisoleucine; galactomannan soluble fibre; steroidal saponins (diosgenin)	4-hydroxyisoleucine is reported to support glucose dependent insulin release; the fibre slows carbohydrate absorption; the seed also links back to diosgenin, section 2.12

The measured professional position, which examiners credit: these are chemically defined constituents with plausible and in several cases demonstrated mechanisms in vitro and in animal models, and variable, usually modest human trial evidence; they are adjuncts studied alongside, not replacements for, standard diabetic therapy.

4.8 Indigenous crude drugs II: liver dysfunction and antitumor

Phyllanthus niruri (bhumyamalaki), the syllabus drug for liver dysfunction, is a small herb of the Euphorbia family carrying lignans (phyllanthin and hypophyllanthin), flavonoids including quercetin itself (section 2.11), and ellagitannins. It is a classical Ayurvedic drug for jaundice and liver complaints, and the related species *Phyllanthus amarus* drew wide attention from trials in chronic hepatitis B carriage; the chemical teaching points are the lignan skeleton (two phenylpropane units joined beta to beta, the same dimer family as podophyllotoxin, section 1.3) and the discipline of species identity, since the *Phyllanthus* species are easily confused and chemically distinct.

Curcuma longa Linn. (turmeric, haldi), the syllabus antitumor drug, owes its colour and activity to the curcuminoids of the rhizome, chiefly **curcumin**, a diarylheptanoid: two ferulic acid like aromatic rings joined by a seven carbon chain that exists largely as its conjugated enol, with demethoxy and bisdemethoxycurcumin beside it. Curcumin modulates several signalling pathways in cell studies (NF-kB among them) and shows antitumor activity in vitro and in animal models; its notorious limitation is pharmacokinetic: very poor aqueous solubility and rapid conjugation, so blood levels after oral dosing are low, and the literature must be read with that in mind. Formulation science (piperine combinations,

liposomes, nanoparticles) is the attempt to close that gap, and the examinable verdict is research promise with unproven clinical benefit, on a molecule whose chemistry is thoroughly characterised.

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