

Unit 4: Pharmacogenomics and Immunotherapeutics

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4.1 Gene mapping and cloning of a disease gene

Unit 1 mapped genes in principle; this section is the hunt itself, because most pharmacogenomic targets began as disease genes. The classical method is **positional cloning**: first, linkage analysis in affected families (Unit 1.6) narrows the gene to a chromosomal region, without any knowledge of what the gene does; then the region is searched candidate by candidate until mutations are found that segregate with the disease and are absent from healthy controls; the cloned gene is sequenced, its protein deduced, and the disease mechanism can be worked out from the protein. The landmark hunts still organise how the method is taught: the **CFTR** chloride channel gene for cystic fibrosis (1989), found by positional cloning and immediately explaining the disease as a channel defect (and, decades later, giving the target for the CFTR modulator drugs), the **huntingtin** gene (1993), whose expanded CAG repeat causes Huntington disease, and **BRCA1** (1994), which turned breast cancer risk into a testable, manageable trait. Modern sequencing (Unit 3.4) has shortened the hunt from years to weeks, but the logic is unchanged: position first, identity next, mechanism and drug target last.

4.2 Genetic variation and its role in health and pharmacology

No two genomes are identical. Human genetic variation comes in graded sizes: **single nucleotide polymorphisms (SNPs)**, one base changed, are the most common, numbering in the millions across each genome; small insertions and deletions; variable repeats; and **copy number variations**, whole stretches present in differing numbers of copies, which for drug metabolism genes can mean zero, one, two or many gene copies. A variant matters when it changes a protein's sequence or amount, or alters a regulatory region; most variants do neither, so variation studies must prove function, not just association. In health, variation underlies susceptibility to common disease, which genome wide association studies map by comparing variant frequencies between patients and controls. In **pharmacology**, the same variation, landing in the genes for drug metabolising enzymes, transporters and targets, is the main inherited reason patients differ in drug response: the same dose can be ineffective in one patient, standard in a second and toxic in a third. The discipline that studies this is pharmacogenomics (gene by gene, pharmacogenetics in the older term), and the rest of this unit works through its three classical territories: metabolism, transport and receptors. A special case is immune mediated reaction, where HLA variants decide who develops severe hypersensitivity: abacavir is prescribed only after excluding HLA-B57:01, and carbamazepine induced Stevens-Johnson syndrome is strongly linked to HLA-B15:02 in patients of Asian ancestry.

4.3 Polymorphisms affecting drug metabolism

This is the most clinically mature part of pharmacogenomics, because the enzymes of Unit 1's smooth endoplasmic reticulum are highly polymorphic. Patients fall into metaboliser classes for each enzyme: poor (little or no activity), intermediate, normal (extensive) and ultrarapid (extra gene copies or high activity).

Gene and enzyme	Drug affected	Consequence of the variant
CYP2D6	Codeine (and tramadol)	Ultrarapid metabolisers convert codeine to morphine dangerously fast (fatal respiratory depression has occurred, including in breastfed infants); poor metabolisers get no analgesia. Poor metabolisers also fail to activate tamoxifen to endoxifen

Gene and enzyme	Drug affected	Consequence of the variant
CYP2C19	Clopidogrel	Loss of function alleles (such as *2) leave the prodrug unactivated, platelet inhibition fails and stent thrombosis risk rises; testing now guides antiplatelet choice
CYP2C9 and VKORC1	Warfarin	Slow CYP2C9 variants plus sensitive VKORC1 variants cut the required dose several fold; genotype guided dosing algorithms exist
TPMT	Azathioprine, mercaptopurine	Deficient patients cannot detoxify the drugs and develop life threatening bone marrow suppression; testing or dose reduction precedes therapy
UGT1A1	Irinotecan	The *28 variant reduces glucuronidation of the active metabolite SN-38, increasing severe diarrhoea and neutropenia
NAT2	Isoniazid, hydralazine, procainamide	Slow acetylators accumulate the parent drugs: more isoniazid neuropathy, more drug induced lupus
G6PD (enzyme deficiency)	Primaquine, rasburicase, some sulfonamides	Deficient red cells cannot withstand oxidative stress and haemolyse; an enzyme defect, screened before these drugs where risk is high

Guideline bodies (the CPIC consortium) now publish genotype to prescription tables for dozens of drug and gene pairs, which is the practical form in which this section reaches the bedside.

4.4 Genetic variation in drug transporters

ABCB1 encodes P glycoprotein, the efflux pump of the gut wall, blood brain barrier, liver and tumour cells; its common variants have been studied against substrates from digoxin to anticancer drugs, with real but often modest and context dependent effects, an honest contrast with the metabolism genes. **SLCO1B1** encodes OATP1B1, the transporter that pulls statins from blood into hepatocytes; a reduced function variant raises simvastatin blood levels and with them the risk of muscle injury, demonstrated in the SEARCH genome wide study, and regulatory guidance now caps simvastatin dose or advises testing in carriers. **ABCG2** (the breast cancer resistance protein, BCRP) limits rosuvastatin absorption and raises its levels in variant carriers. In the kidney, variants of the organic cation transporter OCT2 alter the handling of metformin and the nephrotoxicity of cisplatin. The teaching point is comparative: transporter pharmacogenomics is genuine and occasionally decisive, but its predictive value today is generally weaker than that of the metabolic enzymes, and claims should be scaled accordingly.

4.5 Genetic variation in G protein coupled receptors

Unit 2.4 established GPCRs as the largest drug target family, so variation in their genes is the natural third territory. The best studied example is the beta2 adrenoceptor gene **ADRB2**: common variants at positions 16 (arginine or glycine) and 27 change how the receptor downregulates, and early studies reported different bronchodilator responses to salbutamol between genotypes; larger trials have softened the claim, and routine genotyping before inhaler therapy is not standard practice. The beta1 receptor gene **ADRB1** variant Arg389Gly changes coupling efficiency, and the heart failure drug bucindolol showed genotype dependent benefit in a dedicated trial, with metoprolol response also reported to vary. The mu opioid receptor gene **OPRM1** variant A118G modestly alters opioid dose requirements in several studies. The balanced summary: GPCR pharmacogenomics is mechanistically sound and occasionally important in trial design and drug labelling, but unlike CYP2C19 and clopidogrel or TPMT and azathioprine it has not yet produced many bedside tests, because receptor variants usually shift response by small amounts against a background of disease and adherence variation. That calibration, knowing which genetic claims are

practice ready, is itself a pharmacogenomic skill.

4.6 The omics family: genomics, proteomics, metabolomics, functionomics and nutrigenomics

Pharmacogenomics sits inside a family of whole system sciences that the syllabus lists together, each defined by the level of the cell it surveys:

Science	What it surveys	Core methods	Pharmacological use
Genomics	The whole DNA sequence and its variation	Sequencing, microarrays, association studies	Finding disease genes and response variants
Proteomics	The whole protein complement of a cell or fluid	Two dimensional gels, mass spectrometry	Section 4.7
Metabolomics	The whole set of small molecule metabolites	Mass spectrometry, NMR spectroscopy	Reading the biochemical state a drug leaves behind; toxicity signatures
Functionomics (functional genomics)	What genes do, across the genome	Knockout and knockdown screens (including siRNA, Unit 1.5), expression profiling	Assigning function to new genes and targets
Nutrigenomics	Interactions between diet and genes	Genotyping with dietary intervention studies	Explaining individual responses to nutrients, for example folate handling with MTHFR variants and the inherited trait of adult lactose intolerance

The levels are increasingly close to the clinical phenotype as the table descends from DNA to metabolites: the genome says what could happen, the metabolome reports what is happening now, which is why the omics are usually combined rather than played against each other.

4.7 Applications of proteomics in pharmacology and drug discovery

Proteomics, the large scale study of proteins, earns its place in this unit through applications rather than technique. Its first application is **biomarker discovery**: comparing the protein pattern of blood or tissue between disease and health, or before and after treatment, nominates proteins that can diagnose or stage disease, or report early whether a drug is working; a biomarker that predicts response (HER2 for trastuzumab, section 4.10) converts directly into a patient selection test. Its second is **target identification**: proteins overexpressed or modified specifically in diseased tissue become candidate targets, and chemical proteomics, using drug like probes to fish out every protein a compound touches, maps a drug's true target spectrum, including the off targets behind its adverse effects. Its third is **toxicity prediction**: protein signatures of liver or kidney injury in preclinical samples flag dangerous compounds earlier than waiting for organ damage. Proteomics is technically the hardest omics, because proteins span a far wider concentration range than transcripts and cannot be amplified like DNA; its findings pass through the validation tools of Unit 3 (ELISA and western blotting) before they are believed.

4.8 Immunotherapeutics: types

Immunotherapeutics treats disease by directing the immune system rather than by acting on the diseased tissue directly, and it divides by strategy. **Passive** immunotherapy supplies ready made immune effectors: monoclonal antibodies (the dominant class, sections 4.9 and 4.10) and adoptive cell therapies such as CAR-T cells. **Active** immunotherapy provokes the patient's own immune system: therapeutic cancer

vaccines and the **checkpoint inhibitors**, antibodies that release the brakes (the PD-1, PD-L1 and CTLA-4 checkpoints) which tumours exploit to switch T cells off. **Cytokine therapy** doses the immune system's own messengers, interferon alpha and high dose interleukin 2 being the historical examples, potent but toxic. The same toolbox inverted gives immunosuppressive immunotherapy for transplantation and autoimmune disease: antibodies against CD25 on activated T cells (basiliximab), against TNF (infliximab, adalimumab), against the B cell marker CD20 (rituximab) and against IgE (omalizumab). Immunotherapeutics is therefore not a drug class but a direction of aim, and its adverse effects mirror its mechanism: immune activation brings inflammatory, autoimmune like toxicity, and immune suppression brings infection.

4.9 Humanisation of antibody therapy

The first therapeutic antibodies were mouse proteins, and the human immune system read them as foreign: **muromonab**, the mouse antibody against CD3, provoked human anti mouse antibody responses that neutralised it and caused reactions on repeat dosing. **Humanisation** is the engineering ladder built to escape that problem, and antibody names record the rung: the international naming stems mark the source, -o- for mouse, -xi- for chimeric, -zu- for humanised and -u- for human, before the -mab ending.

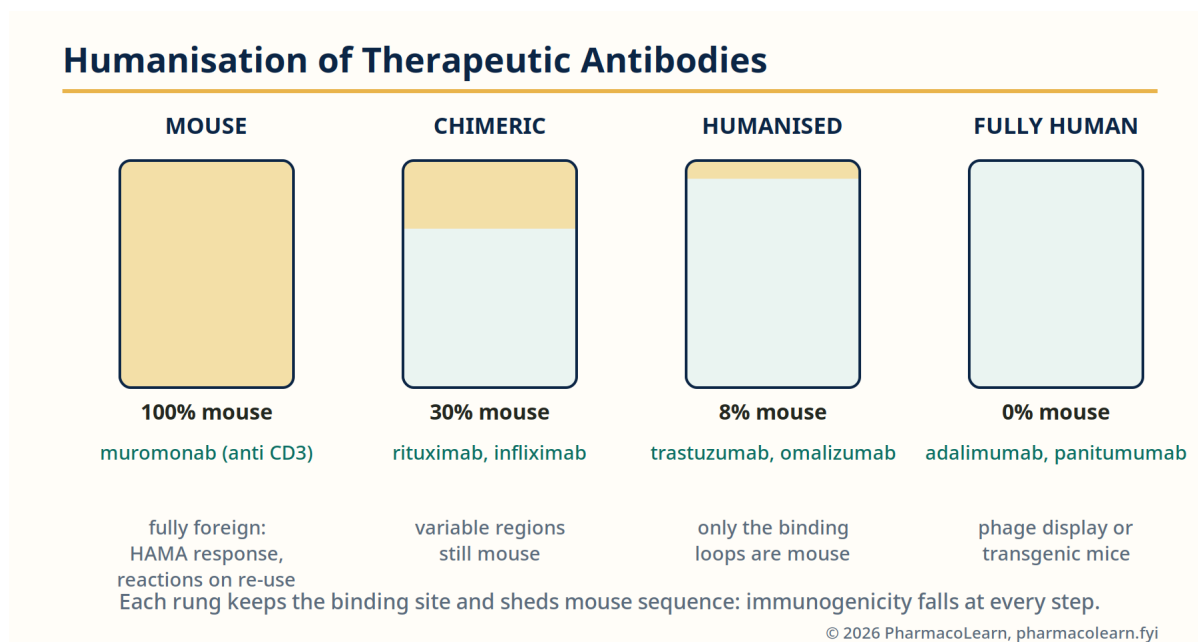


Figure: The humanisation ladder. Each rung replaces more mouse sequence with human, cutting immunogenicity while keeping the binding site that makes the drug.

A **chimeric** antibody (rituximab, infliximab) grafts the whole mouse variable regions onto human constant regions, leaving about a third of the molecule mouse. A **humanised** antibody (trastuzumab, omalizumab) grafts only the complementarity determining regions, the binding loops themselves, into a human antibody framework, leaving only about 5 to 10 percent mouse sequence. **Fully human** antibodies come from phage display libraries or from transgenic mice carrying human antibody genes (adalimumab, panitumumab). Humanisation reduces but does not abolish immunogenicity: patients can still raise anti drug antibodies, especially against chimeric molecules, losing response or reacting at infusion, which is why the antidrug antibody assays of Unit 3.7 are part of biologic therapy monitoring.

4.10 Immunotherapeutics in clinical practice

In the clinic, immunotherapeutics is organised around targets, and target testing comes first. **Trastuzumab** treats only the minority of breast and gastric cancers that overexpress HER2, so the HER2 test is the prescription's first half; its characteristic toxicity, cardiac dysfunction, follows from HER2 signaling in heart

muscle. **Rituximab** deletes CD20 positive B cells and serves both B cell lymphomas and autoimmune diseases such as rheumatoid arthritis. The **TNF blockers** (adalimumab, infliximab, etanercept) transformed rheumatoid arthritis and inflammatory bowel disease, at the price of reactivating latent tuberculosis, so screening precedes treatment. The **checkpoint inhibitors** (pembrolizumab and nivolumab against PD-1, ipilimumab against CTLA-4) produce durable remissions in melanoma and lung cancer in a minority of patients, and their immune related adverse events, colitis, pneumonitis, thyroiditis and others, are managed by stopping the drug and suppressing the immune system the treatment released. Bevacizumab (against VEGF) starves tumours of blood vessels, and omalizumab (against IgE) treats severe allergic asthma. The practice principles are uniform: confirm the target, screen for the infections and organ risks the mechanism predicts, expect infusion and immune reactions rather than classical dose related toxicity, and plan for costs that reflect cell based manufacture, the problem the biosimilars of Unit 5.12 exist to soften.

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