

Case Studies

Case study 1: Codine/Morphine

Morphine is a well known analgesic for moderate to severe pain relief. To achieve this pain relief patients are first administered the prodrug codeine (methyldmorphine) which is a naturally occurring opiate. Codeine is metabolised in the liver by a specific enzyme known as cytochrome P450 2D6. This enzyme chemically converts codeine into active drug morphine which induces pain relief around the body (via a demethylation reaction).



Amongst the many factors which affect how well morphine works as an analgesic, genetic variation has been found to contribute to its effectiveness. Patients may have different genetic versions of cytochrome P450 2D6, which in turn will affect the enzymes ability to convert the prodrug codeine to morphine. This may leave some patients having a reduced pain relief response while in others morphine will act as a proficient analgesic. The prospect of pharmacogenetic testing, may allow clinicians to genetically test patients cytochrome P450 2D6 gene to link their variant to their response to morphine.

Case study 2: Warfarin

The anticoagulant Warfarin is one of the world's most widely used oral treatments for a range of thrombotic conditions, including deep vein thrombosis (DVT), pulmonary embolus and atrial fibrillation/stroke. The aim of treatment with Warfarin is to prevent arterial and venous thromboembolism by thinning the blood to a *safe* and *effective* level. This is referred to as 'achieveing anticoagulation': a state that is measured by the International Normalized Ratio (INR) for prothromboin time. Ideally patients recieving Warfarin treatment should have stable INR's within a specified and disease specific range: for example, in order to prevent recurrent myocardial infarction patients should have INR's of between 2.5 to 3.5 ([this](#) article from the website www.anticoagulation.org provides a patient-oriented, introductory-level guide to Warfarin and its use). However a stable and acceptable patient INR is notoriously difficult to acheive, hence Warfarin is said to have a 'narrow theraputic index' (see diagram below).

Despite the longstanding and widespread use of Warfarin, both loading and maintainance dose, for any particular patient, displays wide variability. While the majority of such variation is thought to be due to

lifestyle and environmental factors¹, Warfarin action and metabolism also has a pharmacogenetic component.

Above, in CASE STUDY 1: CODINE/MORPHINE the role of enzymes belonging to the cytochrome P450 family was noted. Here again, in the case of Warfarin, *CYP P450* genes are again important in drug response. However, in this case, it is variation in the *CYP2C9* gene that is important as well Single Nucleotide Polymorphism (SNP, or 'snip') variation in the *VKORC1* gene.

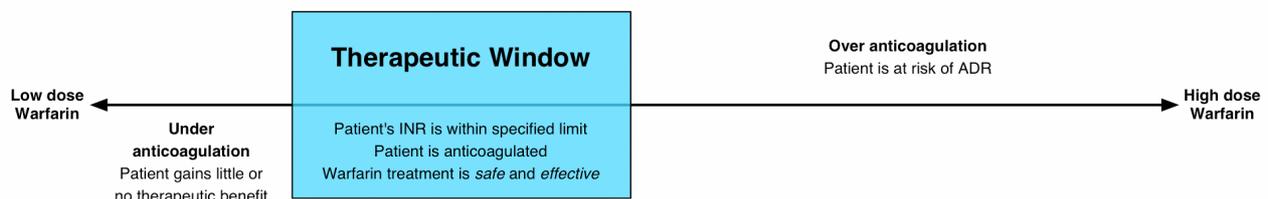
As in the case of thiopurine metabolism, mutations of the *CYP2C9* gene can create phenotypes that are extremely sensitive to Warfarin and, hence, are at an increased risk of ADR (even given a standard loading and/or maintenance dose). Thus, in a similar way to thiopurine treatment, Warfarin pharmacogenetics can be used to improve *safety* in drug prescription. However, in this case, another gene is also implicated in drug metabolism at the other end of the spectrum: here SNP variation in the *VKORC1* gene appears to mediate the *efficacy* of Warfarin treatment.

As noted above Warfarin has a narrow therapeutic index. This means that, even for patients that are 'normal' - in this context, homozygous for the wild-type variants of both *CYP2C9* and *VKORC1* - the difference between being under-anticoagulated (i.e. deriving minimal therapeutic benefit from Warfarin treatment) and being over-anticoagulated (i.e. being at increased bleeding risk - the primary ADR associated with Warfarin therapy) is small.



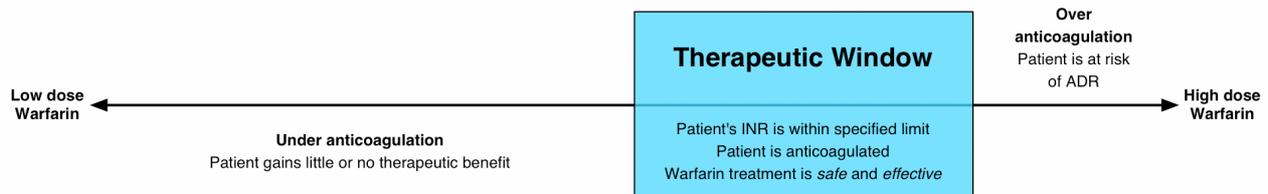
Schematic diagram illustrating the relationship between safety and efficacy in Warfarin treatment for patients who are homozygous for the wild-type variants of both *CYP2C9* and *VKORC1*

However, when pharmacogenetic effects are taken into account, this 'window' can shift significantly up or down the dosage scale.



Schematic diagram illustrating the relationship between safety and efficacy in Warfarin treatment for patients who are either heterozygous or homozygous for mutant variants of *CYP2C9* (most commonly *CYP2C9*2* and/or *CYP2C9*3*) and homozygous for wild-type *VKORC1*

¹ GCSE SCIENCE/BIOLOGY CURRICULUM LINK. Click [here](#) for specification detail from the BioethicsBytes "Bioethics in the UK Curriculum" website



Schematic diagram illustrating the relationship between safety and efficacy in Warfarin treatment for patients who are homozygous for wild-type *CYP2C9* and either heterozygous or homozygous for mutant variants of *VKORC1*

That *CYP2C9* variants mediate the safety of Warfarin treatment, and *VKORC1* - independently - mediate its efficacy, are not the only complications for clinicians in making both, initial, and long-term, prescribing decisions. The effect of Warfarin is heavily influenced by a number of lifestyle and environmental factors, including: age, weight, alcohol consumption, drug regime compliance, and diet. These account for around 80% of variation in Warfarin patient INR and, given the serious consequences of both over- and under-anticoagulation, require that this drug therapy is accompanied by intensive and costly pharmacovigilance regimes. While many hope that pharmacogenetics may help to reduce the need for - and cost of - weekly, or even daily, INR monitoring (which, in the UK, is conducted within the time and budget of NHS hospital or GP surgeries), others believe that scaling back this service may be the ultimate 'false hope' and greatest danger of placing too much certainty in pharmacogenetic information in the future.

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[[BMJ.com](http://www.bmj.com)]

Ingelman-Sundberg M. (2004). "Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future", *Trends Pharmacol Sci.*, 25(4): 193-200. [[PubMed](http://pubmed.ncbi.nlm.nih.gov/)]

Martin, P., Lewis, G., Smart, A. and Webster, A. (2006) False Positive? The commercial and clinical development of pharmacogenetics. Final Report for The Wellcome Trust. [Available online at: <http://www.york.ac.uk/res/pgx/publications/FalsePositive2006.pdf>]

Sanderson S, Emery J, Higgins J. (2005). "CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a HuGENet systematic review and meta-analysis", *Genet Med.*, 7(2): 97-104. [[PubMed](http://pubmed.ncbi.nlm.nih.gov/)]

Case study 3: Thiopurines

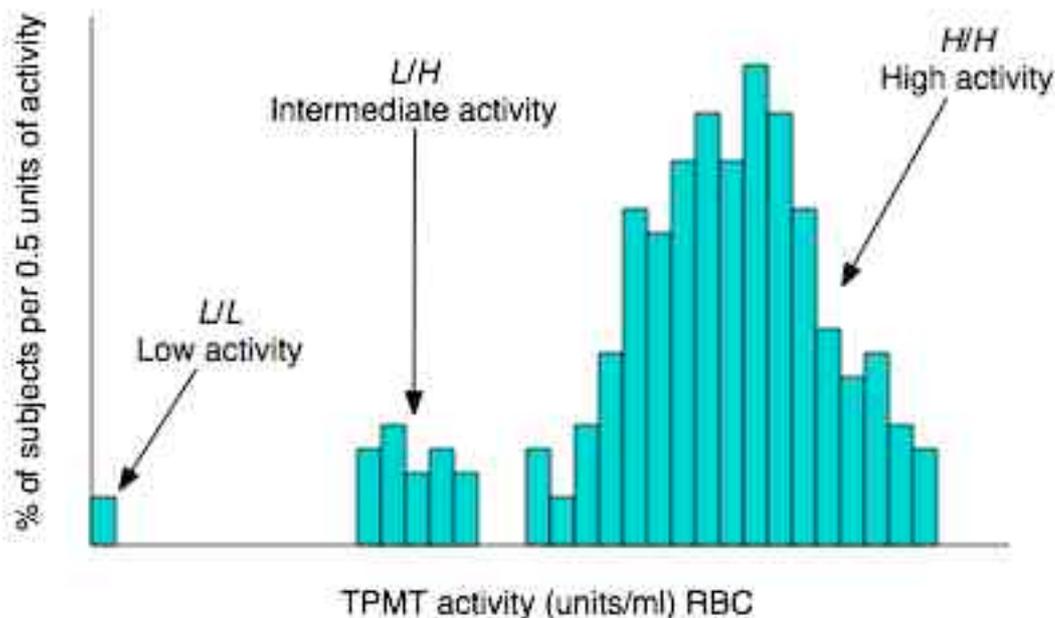
Another example of possible applications of pharmacogenetics is the metabolism of thiopurine drugs such as 6-mercaptopurine and the prodrug azathiopurine. Thiopurines are commonly used as anti-cancer agents and are increasingly used to help treat inflammatory diseases such as inflammatory bowel disease (IBD).

Thiopurines are enzymatically converted into active versions which take action on the cancer or reduce immune response. However levels of these anti-cancer agents are controlled in the body by an enzyme called thiopurine S-methyltransferase or TPMT. This enzyme ensures that levels of the thiopurine products do not rise too high, by metabolising them into inactive forms, which in turn prevents potential toxic damage to the bone marrow, which is known as myelosuppression.

There are two main genetic versions of the TPMT gene which are codominantly inherited, one for low- and high- level enzyme activity. Because every human has two versions of the same gene there are three possible phenotypes or identifiable characteristics¹: those who

- inherit two copies of the gene which indicate low enzyme activity. At risk of myelosuppression. Affects approximately 1/300 patients who receive thiopurine treatment. Very low or no dose administered. Represents 0.3% of population
- inherit one copy of the gene which indicates low enzyme activity and one copy of the gene which indicates high enzyme activity. Intermediate level of enzyme activity. Low dose administered. Represents 11% of population
- inherit two copies of the gene that indicate high enzyme activity. Normal dose administered. Represents 89% of population

TPMT activity relative to genetic composition 298 unrelated adults ²



Implementing a pharmacogenetic test for patients before they receive thiopurine treatment may ensure that the correct drug, in terms of those who have two copies of the low activity gene, may be given alternatives to prevent an adverse drug reaction (myelosuppression) and to ensure the correct dose is given, as those who have one high and one low activity gene may not require the same level of dose when compared to those who have two copies of the high activity gene.

¹ Population figures taken from Karren, P & Natalie, A. (2008). "Thiopurines in current medical practice: molecular mechanisms and contributions to therapy-related cancer", *Nature Review Cancer*, 8:24-36

² Golan, D. E, Tashjian, A. H, Armstrong, E. J & Armstrong, A. W. (2008). *Principles of Pharmacology - The Pathophysiologic Basis of Drug Therapy*. 2nd Edition. Wolters Kluwer Health: USA.

Case study 4: Trastazumab (Herceptin)

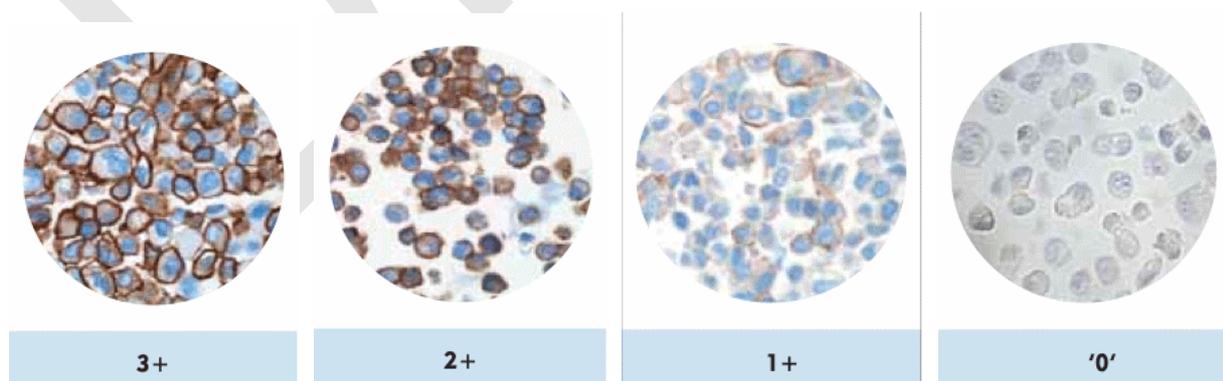
Part 1:

Launched in 1998 by American biotech company Genentech, Trastazumab, better known as Herceptin, is a monoclonal antibody active against the Her-2/neu receptor.

Her-2/neu - or HER2 - is a receptor protein and growth factor, and the gene that codes for it (also called Her-2/neu or HER2, see the [OMIM entry](#) for this gene) is present in every cell of the human body. In normal, healthy tissues it is involved in cell growth and division, however in some cancers - frequently breast cancers - it mutates, such that the gene is amplified and these cancer cells contain many more copies than is usual. Where the extra copies of the HER2 gene are expressed (called overexpression), the cancerous cell grow and divide rapidly and uncontrollably. Although HER2 overexpression is associated with a minority of breast cancers (also some ovarian and prostate cancers) so called HER2-positive cancers tend to be the most aggressive and difficult to treat. Metastases are common, not only in breast tissue, but also in lymph, lung and bone. Where such aggressive, metastatic cancer is recurrent the patient's prognosis is generally poor and usually terminal.

Herceptin acts to block the HER2 receptors on the surface of cancer cells, this not only prevents them from growing and dividing quite so rapidly, as it is usually administered with chemotherapy, it allows the cancer cells to be destroyed by chemical means ([Animation 1](#) illustrates this process graphically).

The HER2 status of a tumour can be clinically ascertained in two ways, only one of which involves a genetic level test for HER2 copy number. Initially biopsied material is subjected to an immunohistochemical assay (IHC) and only if the tumour's HER2 status is ambiguous or borderline is Fluorescence In-Situ Hybridisation (FISH) carried out. These tests classify the tumour into one of four groups according to the level of HER2 (over)expression detected. The groups are termed "0", "+1", "+2" and "+3". The slides below show typical immunohistochemical results for each HER2 status group. The distinction between each of these classifications is made on the basis of intensity of membrane staining¹.



Immunohistochemistry (IHC) slides showing intensity of membrane staining for each HER2 status class.

It is the +2 category that is regarded as equivocal and tumour samples scoring +2 are usually submitted for FISH analysis. Here the levels of HER2 gene amplification are assessed in order that Herceptin treatment is

¹ Slides from Rhodes, A. (2006). "HER2 testing in breast cancer", *The Biomedical Scientist*, June, pp.517. See full article for further details, including percentage staining criteria used in classification.

targeted at only those patients thought to benefit from it: "If the 2+ case shows gene amplification, the patient is currently presumed to benefit from Herceptin therapy, while a patient with a non-amplified 2+ tumour is thought unlikely to benefit." (Rhodes, 2006: 516)

Part 2:

When Herceptin first came onto the market in 1998, it was heralded as a revolution in cancer treatment: it was the first monoclonal antibody to be used successfully in the treatment of cancer and, as a biological rather than chemical treatment, had none of the painful and debilitating side effects associated with aggressive chemotherapy¹. However, Herceptin's development and clinical testing had been a long and costly process for Genentech (see Bazell, 1998, for further details or [this](#) BioethicsBytes post), hence it was initially considered prohibitively expensive for both public and private healthcare providers and patients requiring Herceptin were forced to pay for it themselves². In the UK for example, Herceptin has been licensed as a safe and effective treatment for advanced metastatic breast cancer since 1999, however, it was not until March 2002 that the National Institute for Clinical Excellence (NICE) issued guidance for its provision in these cases on the NHS³. Between these dates (i.e. after the drug had been licensed but before NICE has issued guidance) the decision about whether or not to provide Herceptin - at £20,000 per course - on the NHS was left to individual Primary Care Trusts (PCTs). This situation led to media headlines concerning the Herceptin 'postcode lottery' and stories of neighbouring women who were receiving differential treatment from different PCTs (see for example [this](#) article from the BBC News website).

However, the 'postcode lottery' for Herceptin in the interim between the drug's licensing for use in advanced breast cancer and the publication of the NICE guidance was only the first story of Herceptin 'rationing' to hit the UK headlines: for campaigners the battle to gain NHS access to Herceptin for early-stage breast cancer in 2005 was equally daunting. The BioethicsBytes post [Herceptin: Wanting the wonder drug](#) describes this episode in detail and highlights the central bioethical question as one of resource allocation within the NHS. While many officials and doctors claimed that Herceptin was withheld from patients with all but the most serious cases of advanced metastatic breast cancer (those where chemotherapy had failed to have any impact on the cancer's progression) because the drug's safety and efficacy were as yet unproven⁴ ¹,

¹ Initially Herceptin was thought to have no side effects at all, but more recently it has been suggested that a particular type of heart condition can be aggravated (possibly even caused) by extended use of Herceptin.

² BBC News. (2001). Revolutionary cancer drug arrives in UK. Available online at: <http://news.bbc.co.uk/1/hi/health/503245.stm>

³ NICE. (2002). Breast cancer - trastuzumab: The clinical effectiveness and cost effectiveness of trastuzumab for breast cancer. Available online at: <http://www.nice.org.uk/guidance/index.jsp?action=byID&o=11445>

⁴ Interim results of the BIG/Roche [Herceptin Adjuvant \(HERA\) trials](#) of Herceptin as an adjuvant therapy in early-stage breast cancer only became available in 2005 (see Piccart-Gebhart et al. (2005). "Trastuzumab after Adjuvant Chemotherapy in HER2-Positive Breast Cancer", *N Engl J Med.*, 353(16):1659-72. Available at <http://content.nejm.org/cgi/content/full/353/16/1659>). However, there remained some queries regarding both the safety and efficacy of this use of Herceptin since a number of participants had withdrawn early due to suspected cardiotoxic side-effects, and Roche terminated the placebo arm of the trial early for ethical reasons (see Boseley, 2006). Full results, including the results of 2 year follow-up studies (which are required in phase III clinical trials which compare the new drugs with existing treatments) were not available until early 2007 (see Smith et al. (2007). "2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial", *The Lancet*, 369(9555): 29-36. Available at <http://www.thelancet.com/journals/lancet/article/PIIS0140673607600282/abstract> and/or Untch et al. (2008).

campaigners and other medical professionals suggested that additional numbers of patients, relative success rates and the cost of Herceptin made it uneconomical for NICE to approve Herceptin for early-stage breast cancer².

In August 2006, following political pressure, NICE approved Herceptin as an adjuvant therapy for women with early stage breast cancer whose tumours express HER2 at a high level³. Still, this is a minority of the breast cancer cases that pass through UK oncology clinics. In this sense Herceptin is far from a panacea: for the majority of women whose cancers do not over-express the HER2 protein, Herceptin is ineffective clinically; for the small amount of women whose tumours only over-express the gene weakly (for example those at the lower end of the +2 category) Herceptin - at £20,000 per course - is deemed not cost effective; and, even where Herceptin is effective and the patient's tumours shrink, it is not a cure, since in all but the most exceptional cases, treatment Herceptin and chemotherapy may only add 8-12 months to a patient's predicted life expectancy. Thus, what for many women with breast cancer and their families seems a priceless and invaluable benefit, for others is bought at the expense of patients elsewhere in the NHS. For example, in [this](#) episode of BBC Radio 4's *The Investigation* (first broadcast at 20.00 on November 29 2007), one doctor claims that in 2006 the NHS spent around £100m on Herceptin, though this money only benefited around 500 patients. He believes that if this money were diverted to radiotherapy "it could have a dramatic impact" (for further details see [this](#) article on the programme on the BBC News website).

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Hedgecoe, A. (2004). *The Politics of Personalised Medicine: Pharmacogenetics in the clinic*. Cambridge University Press: Cambridge, UK.

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¹ In addition, some coverage of the controversy over access to Herceptin for early-stage breast cancer implies that its parent company, Roche, also had an economic incentive to expand the market for Herceptin. For example, Boseley (2006) states "Herceptin is ... suitable for only 20% of breast cancer patients. Roche, naturally, having spent many millions of pounds on developing the drug, badly wants as big a slice of this restricted market as it can get".

² See Orr (2006) for some examples of how NICE calculates cost-benefit for the purposes of drug approval.

³ NICE. (2006). Breast cancer (early) - trastuzumab: Trastuzumab for the adjuvant treatment of early-stage HER2-positive breast cancer. Available online at: <http://www.nice.org.uk/guidance/index.jsp?action=byID&o=11586>