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INNOVATIONacademy

5-Fluorouracil Pharmacogenetics

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Introduction

- Fluoropyrimidine drug 5-fluoropyrimidine wasdeveloped in the 1950s.
- First line treatment for solid tumours including colorectal and breast cancer
- Approximately 2 million patients pa receive these antimetabolites worldwide
- Approximately 25% of patients will develop severe (Common Terminology Criteria for Adverse Events (CTCAE) grade ≥3 toxicity
- Toxicity leads to delays of subsequent cycles or termination of treatment.

Toxicity impacts negatively on efficacy and prognosis as well as posing a significant cost burden to health care providers.

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Estimated 5FU grade 3-4 toxicity cost in bed days to Guy's and St Thomas' Hospitals

	% suffering toxicity	Hospital stay (days)	% admitted to ITU	Intensive care stay (days)
Diarrhea	10	5	1	2
Neutropenia	5	5	rare	
Hand foot syndrome	Rare, except capecitabine (40-50%)	none	none	
Mucositis	5	5	1	2
Cardiac toxicity	1, probably under-recognised	7	none	
Nausea, vomiting	2	5	rare	2

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Is there a case for pharmacogenetic testing?

- Dihydropyrimidine dehydrogenase deficiency (DPD) recognised as a cause of 5FU toxicity – first death due to DPD deficiency reported in 1985 (Tuchman et al *N Engl J Med* 313(4), 245-249 (1985).
- The FDA warned in 2003 that 5FU and capecitabine are contra-indicated in patients with DPD deficiency
- 5FU and the pro-drug capecitabine are extensively metabolised and the metabolic pathways are relatively well understood – multiple pharmacogenetic targets
- Predicting and avoiding severe toxicity would benefit patients

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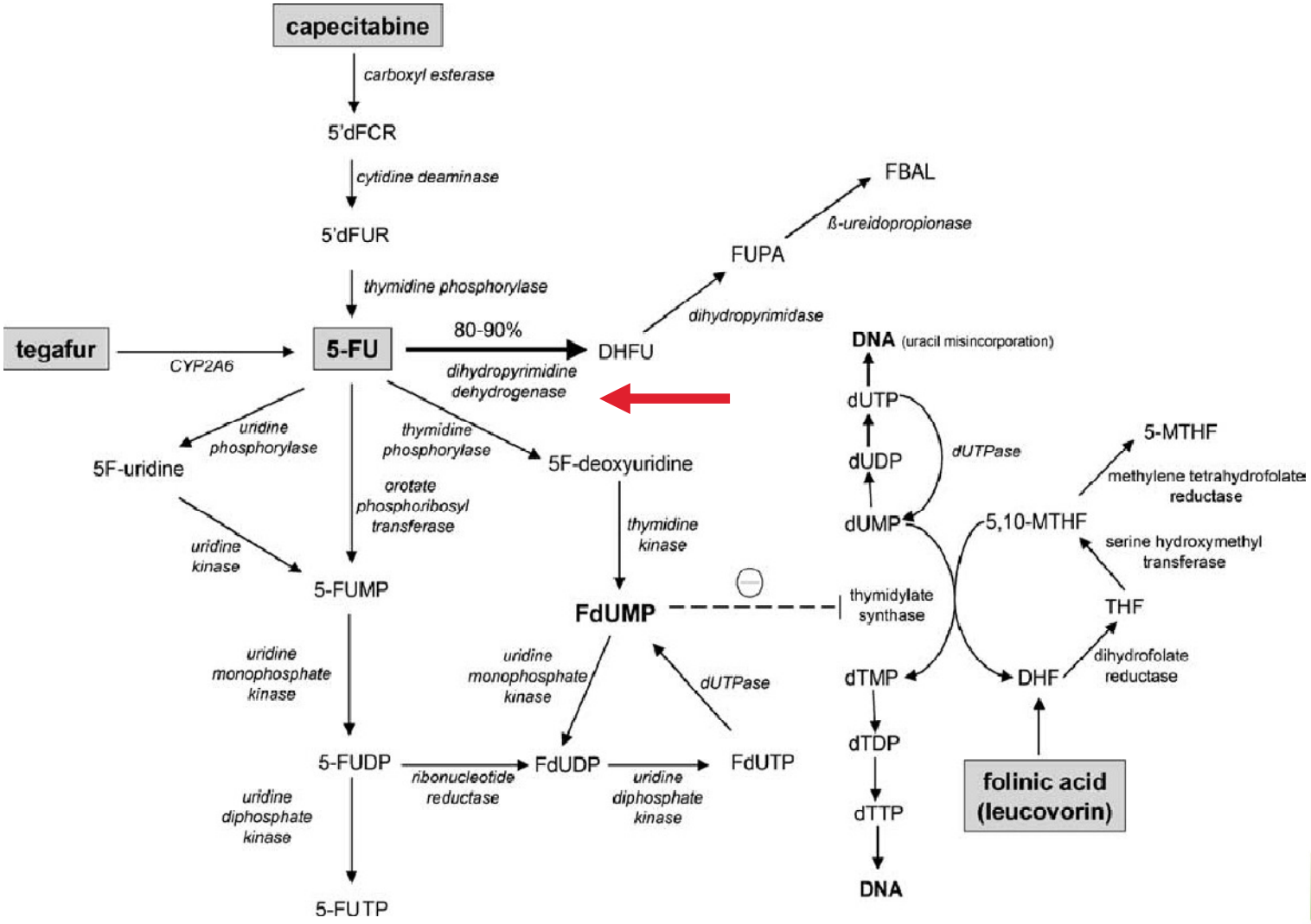
Fluoropyrimidine metabolism and DPD



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Dihydropyrimidine dehydrogenase (DPD) deficiency

- 5FU has a narrow therapeutic window with 80-90% of 5FU catabolised through DPD
- Complete DPD deficiency is a rare metabolic disorder characterised by increased thymine and uracil in urine – fatal toxicity if treated with 5FU.
- Partial (heterozygous) DPD deficiency is asymptomatic, occurs in 4-6% of the population and is associated with severe Grade 3-4 toxicity to 5FU

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Testing for DPD deficiency by enzyme assay

- Unlike TPMT, DPD cannot be assayed in red cells
- White cell radiochemical assay not suitable for high throughput screening or referred samples
- Assay is not linear at low protein concentrations
- Genotype-phenotype correlation in the carrier range is very poor.

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DPYD sequencing of 47 cancer patients referred for toxicity

DYPD variant	n	Neutro-paenia	Diarrhoea	Mucositis	Male/female
IVS14+1G>A c.1905+1G>A	4/47	1	3	-	0/4
D949V c.2846A>T	4/47	1	2	1	3/1
I560S c.1679T>G	1/47	1	-	-	0/1
Total	**8/47**	**3**	**5**	**1**	**3/6**

Loganayagam A et al. Cancer Chemother Pharmacol. 2010 Jan;65(2):403-6.

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Series of 430 colorectal cancer patients

Grade 3-4 toxicity experienced in the first four cycles of treatment

Toxicity type	**Grade 0-2** n (%)	**Grade 3-4** n (%)
Diarrhoea	362 (84)	68 (16)
Mucositis	415 (97)	15 (4)
Neutropenia	387 (90)	43 (10)
All toxicity	326 (76)	104 (24)

Loganayagam et al. Br J Cancer. 2013 Jun 25;108(12):2505-15.

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DPD mutations: four deleterious variants in 430 patients

Nine variants tested, four *DPYD* variants found in 6% of the cohort explain 23% of toxicity cases ($p<10^{-16}$, logistic regression)

	Wildtype	*DPYD* heterozygous (4 variants)	Total
Side effects **(diarrhoea, mucositis, neutropaenia)**	80	24	104
Tolerant	326	0	326
Total	406	24	430

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Variant *DPYD* genotypes and grade 3-4 toxicity

Variant genotype	Cycle 1-2 toxicity (n)	Cycle 1-2 dose reduction	Cycle 3-4 toxicity (n)	Cycle 3-4 dose reduction
c.1905+1G>A heterozygous	2	dose reduced by 50% and 25%	1	Withdrew from therapy
c.1905+1G>A/ c.1601G>A	1	Grade 4 toxicities, withdrew from therapy	-	-
c.2846A>T heterozygous	3	1 patient withdrew from therapy, 2 patients tolerated 25% dose reduction	1	25% dose reduction
c.2846A>T / c.1601G>A	1	Withdrew from therapy	-	-
c.1601G>A heterozygous	10	8 patients dose reduced by 25%, two patients withdrew	4	3 patients 25% dose reduction 1 patient discontinued
c.1679T>G	-	-	1	25% dose reduction

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Cost of toxicity in bed days

- 326 patients with grade 0-2 toxicity: 65 days in hospital
- 104 patients experiencing grade 3-4 toxicity: 423 hospital days
- The 24 patients carrying a *DPYD* variant comprised just 6% of the cohort, but accounted for 171 admission days or 35% of the total

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DPD and 5FU toxicity summary

- Four *DPYD* variants predict severe toxicity and were present 6% of the study population
- All patients with these variant genotypes experienced Grade 3-4 toxicity
- These *DPYD* variants explained ~25% of cases of grade 3-4 toxicity

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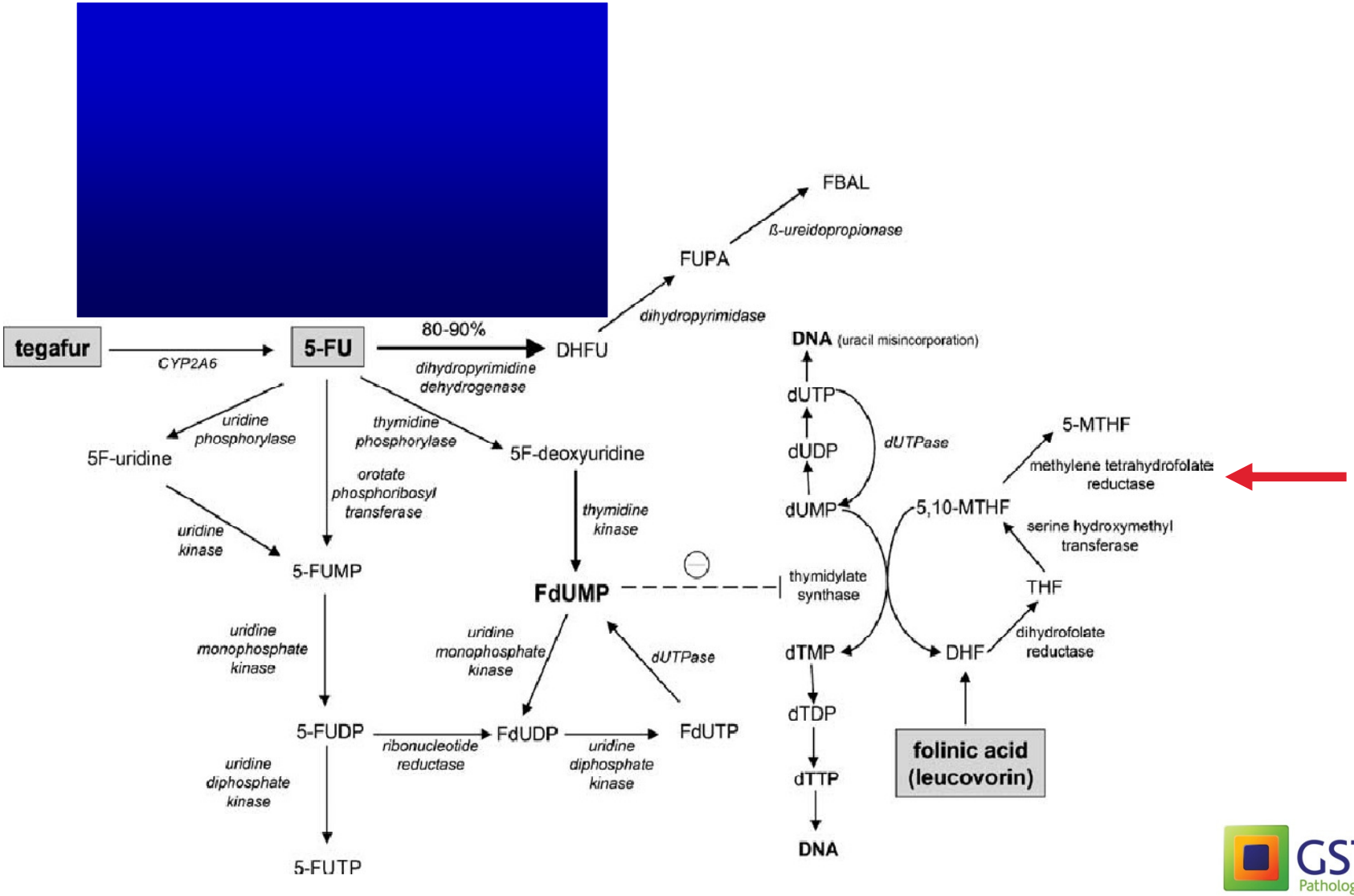
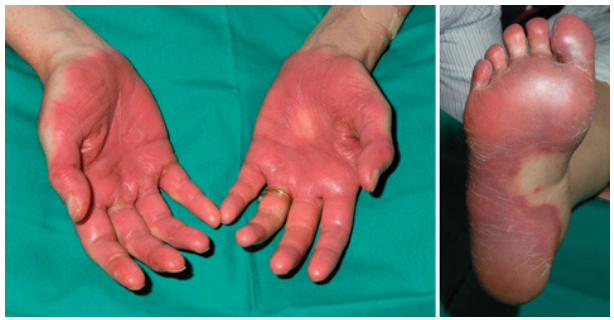
Capecitabine activation to 5FU



Capecitabine (n=244) – handfoot syndrome

Grade 2-3 hand foot syndrome (HFS) occurred in 23% of patients

- HFS associated with MTHFR 1298CC homozygous variant genotype (logistic regression, $P=4.1x10^{-6}$, OR=9.99, 95% CI: 3.84–27.8).
- Pyridoxine may ameliorate severity of HFS, although evidence is contradictory (Chen et al PLoS One. 2013 Aug 20;8(8))
- Consider dose reduction or alternative 5FU formulations



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Capecitabine: cytidine deaminase promoter SNPs

	-92 AG or -92 GG or -451 CT or -451 TT genotype	Wild type
Grade 2-4 diarrhea	43	22
Grade 0/1 diarrhea	75	99

Disequilibrium between the -92 and -451 alleles. Alleles have an additive effect with each allele doubling the risk of toxicity

p= 0.0055, OR 2.3, 95% CI 1.3 to 4.2

? Dose reduction

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Barriers to uptake of pharmacogenetic testing

- *DPYD* genotyping - concern that a dose reduction strategy will compromise efficacy – evidence in the literature suggests this is not the case
- Capecitabine markers must be replicate before being brought into the diagnostic service
- Testing must be perceived as cost effective.
- Each Trust will require a business case for testing
- Requires a change in clinical practice

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Conclusions

- Pharmacogenetics is a ‘new’ diagnostic area with considerable potential for cost savings to the NHS.
- Patients should be tested prior to therapy rather than seeking an explanation for side effects after these have occurred
- Pharmacogenetics plus pharmacokinetic testing may be the future
- The main barrier to uptake of a service is the need to change clinical practice.

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Current research (funded by GSTT Charity)

Exome chip genotyping:

- Cisplatin and carboplatin pharmacogenetics – lung and ovarian cancer
- Pemeterexed – lung
- Oxaliplatin and 5FU – colorectal
- Thiopurines – gastroenterology
- Biologics - gastroenterology

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