**Chapter 39**

***Personalized Medicine in Cancer Treatment***

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**CASE 1 - *DPYD***

***Introduction*** – Dihydropyrimidine dehydrogenase (DPD) deficiency is an autosomal recessive disorder that is characterized by a wide range of severity, with neurological problems in some individuals and no signs or symptoms in others. In individuals with severe DPD deficiency, the disorder becomes apparent in infancy with recurrent seizures, intellectual disability, microcephaly, hypertonia, delayed development of motor skills such as walking, and autistic behaviors that affect communication and social interaction. Other affected individuals are asymptomatic and may be identified only by laboratory testing. More than 50 mutations in the *DPYD* gene have been identified in people with DPD deficiency.

***Primary Question*** – ***What is the estimated carrier frequency in the Caucasian population of DPD deficiency?***

1. 0.5-1%
2. 1-3%
3. 3-5%
4. 5-7%
5. 7-10%

***Correct Response*** – c

***Explanation of Correct/Incorrect Response*** – It is estimated that 3-5% of the Caucasian population has partial DPD deficiency. Heterozygotes (carriers) have intermediate DPD activity and may have partial DPD deficiency. Common causes of partial DPD deficiency include DPYD \*1/\*2 and \*1/\*13.

***First Reply Question*** – Approximately 10-40% of individuals who receive treatment develop severe toxicity such as neutropenia, nausea, vomiting, severe diarrhea, stomatitis, mucositis, hand-foot syndrome, and neuropathy. ***For which drug are individuals at increased risk for adverse drug reactions if they have DPD deficiency?***

1. 5-fluorouracil
2. codeine
3. irinotecan
4. rasburicase

***Correct Response*** – a

***Explanation of Correct/Incorrect Response*** – The rate-limiting step of 5-fluorouracil (5-FU) catabolism is dihydropyrimidine dehydrogenase (DPD) conversion of 5-FU to dihydrofluorouracil (DHFU). Importantly, several germline genetic variants in the *DPYD* gene on chromosome 1p21.3 result in deficient DPD activity, and increased drug half-life that can translate to severe and even fatal 5-FU toxicity. In addition, the FDA label for the fluoropyrimidines indicates that variants in *DPYD* are associated with increased risk for adverse and potentially toxic events, and therefore is contraindicated in patients with known DPD deficiency.

**CASE 2 - *UGT1A1***

***Introduction*** – Gilbert syndrome is an autosomal recessive unconjugated hyperbilirubinemia. This mild disorder affects the metabolism of several substances, which can present with jaundice among affected individuals, with mild abdominal pain or nausea triggered by fasting or infections. Individuals with Gilbert syndrome have normal liver function tests and typically require no treatment. *UGT1A1\*28* allele and other *UGT1A1* missense variants have been implicated in Gilbert syndrome.

***Primary Question*** – ***What is the most common method to test for UGT1A1\*28 allele?***

1. Methylation-specific PCR
2. Multiplex ligation-dependent probe amplification (MLPA)
3. PCR and size separation by capillary electrophoresis
4. Realtime quantitative PCR
5. Southern analysis

***Correct Response*** – c

***Explanation of Correct/Incorrect Response*** – Genetic testing for *UGT1A1* can be performed from DNA extracted from whole blood or other tissues, which typically involves targeted testing of the *UGT1A1\*28* [(TA)7TAA] repeat polymorphism (*UGT1A1\*1* is [(TA)6TAA] repeats). The most common assay is a laboratory-developed test involving fluorescent PCR amplification and size separation by capillary electrophoresis. Of note, this assay will also detect the five [(TA)5TAA] (\*36) and eight [(TA)8TAA] (\*37) repeat alleles. The five TA repeat allele is assumed to maintain efficient transcription, while the uncommon eight TA repeat allele is similar to the seven TA repeat allele (*\*28*).

***First Reply Question*** – *UGT1A1\*28* heterozygotes and homozygotes have an approximate 25% and 70% reduction in enzyme activity, respectively, and individuals who are homozygous for *UGT1A1\*28* are at increased risk for myelosuppression, diarrhea, and neutropenia due to the build-up of the active metabolite. ***For which drug are individuals at increased risk for adverse drug reactions if they have UGT1A1\*28?***

1. 5-fluorouracil
2. codeine
3. irinotecan
4. rasburicase

***Correct Response*** – c

***Explanation of Correct/Incorrect Response*** – Irinotecan is used to treat metastatic colorectal cancer, typically given in combination with other anticancer agents (e.g., 5-FU, leucovorin). It is also used in combination with cisplatin for the treatment of extensive small cell lung cancer. Irinotecan works by binding to the topoisomerase I-DNA complex and preventing DNA replication, and thus causes double-strand DNA breakage and cell death. The active form of irinotecan is SN-38, which is glucoronized to SN-38 glucoronic acid (SN-38G) and detoxified in the liver via conjugation by the uridine diphosphate glucuronosyltransferase (UGT) 1A1 enzyme, which releases SN-38G into the intestines for elimination. Notably, impaired elimination of the cytotoxic SN-38 metabolite can result in severe toxicities, including myelosuppresion, diarrhea and neutropenia.

**CASE 3 - *G6PD***

***Introduction*** – Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked disorder that affects over 400 million people worldwide. It occurs most frequently in the malarial endemic regions of Africa, Asia, and the Mediterranean due to the protection it provides against malaria infection. Of note, different racial and ethnic groups have predominant founder mutations such as the G6PD Mediterranean (c.563C>T) variant, which has important implications when considering genetic testing. The G6PD enzyme catalyzes the first step in the pentose phosphate pathway, which produces antioxidants to protect cells against oxidative stress. Triggers that heighten oxidative stress in red blood cells result in hemolytic anemia and symptom onset in patients with G6PD deficiency.

***Primary Question*** – ***What is the incidence of G6PD deficiency in African-American males in the United States?***

1. 1 in 500
2. 1 in 100
3. 1 in 50
4. 1 in 10
5. 1 in 5

***Correct Response*** – d

***Explanation of Correct/Incorrect Response*** – G6PD deficiency is an X-linked disorder that affects over 400 million people worldwide and ~1 in 10 African-American males in the United States. G6PD variants that result in enzyme deficiency confer a G6PD deficient phenotype in hemizygous males and homozygous or compound heterozygous females. It is difficult to diagnose G6PD deficiency in heterozygous females due to random X chromosome inactivation.

***First Reply Question*** – Without enough functional G6PD, red blood cells are unable to protect themselves from the damaging effects of reactive oxygen species and subsequent hemolysis. Factors such as infections, certain drugs, and ingesting fava beans can increase the levels of reactive oxygen species, thus causing red blood cells to undergo hemolysis faster than the body can replace them. The loss of red blood cells causes the signs and symptoms of hemolytic anemia such as dark urine, enlarged spleen, fatigue, rapid heart rate, shortness of breath and jaundice, which are the characteristic features of G6PD deficiency. ***For which drug are individuals at increased risk for adverse drug reactions if they have G6PD deficiency?***

1. 5-fluorouracil
2. codeine
3. irinotecan
4. rasburicase

***Correct Response*** – d

***Explanation of Correct/Incorrect Response*** – Rasburicase is a drug approved by the FDA for prophylaxis and treatment of hyperuricemia during chemotherapy in adults and children with lymphoma, leukemia, and solid tumors. When chemotherapy is administered, cancer cells are destroyed, releasing large amounts of uric acid into the blood. Rasburicase is a recombinant urate oxidase enzyme that works by breaking down uric acid to allantoin and hydrogen peroxide, which is eliminated from the body by the kidneys. The pegylated form of urate oxidase, pegloticase, is also FDA approved for the treatment of refractory gout. Notably, both rasburicase and pegloticase carry an FDA boxed warning and are contraindicated for use in patients with known glucose-6-phosphate dehydrogenase (G6PD) deficiency due to mutations in the *G6PD* gene on chromosome Xq28.

**CASE 4 - *CYP2D6***

***Introduction*** – One of the most notable discoveries in the field of pharmacogenetics was the 1977 identification of polymorphic debrisoquine metabolism, and the subsequent realization that the ‘poor metabolism’ trait was inherited in an autosomal recessive fashion due to variant alleles encoding a hepatic cytochrome P450 oxidase. The responsible enzyme, cytochrome P450-2D6 (CYP2D6), was purified and characterized, and is now believed to be directly involved in the metabolism of ~25% of all commonly used drugs. The *CYP2D6* gene on chromosome 22q13.2 is highly polymorphic, with over 100 variant star (\*) alleles catalogued by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee, many of which encode reduced or no enzyme activity. Importantly, *CYP2D6* is also prone to copy number variation (CNV), including both gene duplication and deletion, which can significantly influence the interpretation of *CYP2D6* genotyping, sequencing, and phenotype prediction.

***Primary Question*** – A targeted clinical *CYP2D6* genotyping test reports a \*4/\*4 genotype for your patient. The \*4 allele is a common loss-of-function allele that encodes an enzyme with no activity. ***What is the most likely predicted CYP2D6 metabolizer phenotype for this patient?***

1. Ultrarapid metabolizer
2. Extensive metabolizer
3. Intermediate metabolizer
4. Poor metabolizer

***Correct Response*** – d

***Explanation of Correct/Incorrect Response*** – Ultrarapid metabolizers have greater than two functional copies of the CYP2D6 gene, extensive metabolizers have two functional copies, intermediate metabolizers have one reduced and one non-functional *CYP2D6* allele (or two reduced function alleles), and poor metabolizers have two non-functional copies. Because this patient has two *CYP2D6\*4* loss-of-function alleles, the most appropriate answer is that this patient is a poor metabolizer.

***First Reply Question*** – ***Interpatient variability has been associated with CYP2D6 genotype for which of the following medications?***

1. 5-fluorouracil
2. codeine
3. tamoxifen
4. rasburicase
5. all of the above
6. both b and c

***Correct Response*** – f

***Explanation of Correct/Incorrect Response*** – The rate-limiting step of 5-fluorouracil catabolism is dihydropyrimidine dehydrogenase (DPD) conversion of 5-fluorouracil to dihydrofluorouracil, and several germline genetic variants in the *DPYD* gene have been implicated in severe 5-fluorouracil toxicity. Rasburicase is a drug approved by the FDA for prophylaxis and treatment of hyperuricemia during chemotherapy in adults and children with lymphoma, leukemia, and solid tumors. Both rasburicase and pegloticase carry an FDA boxed warning and are contraindicated for use in patients with known glucose-6-phosphate dehydrogenase (G6PD) deficiency due to mutations in the *G6PD* gene. Both codeine and tamoxifen are metabolized, in part, by CYP2D6. CYP2D6 poor metabolizers are unable to efficiently convert codeine to morphine and as a consequence may not experience pain relief, whereas ultra-rapid metabolizers can metabolize codeine too efficiently leading to morphine intoxication and toxicity. In addition, although there is conflicting literature, CYP2D6 loss-of-function alleles have been associated with poor prognosis among patients treated with tamoxifen.

**CASE 5 - *TPMT***

***Introduction*** – Thiopurine S-methyltransferase (TPMT) inactivates azathioprine, mercaptopurine and thioguanine, which are used to treat acute lymphoblastic leukemia, autoimmune diseases, and inflammatory bowel disease, as well as to prevent rejection of solid organ transplants. About 10% of the population have intermediate levels of TPMT activity and 0.3% have low or undetectable enzyme activity and are considered TPMT deficient. Individuals with low TPMT activity are at risk for 6-thioguanine-mediated toxicity and myelosuppression with standard dosing; however, at risk individuals can be treated with decreased doses of thiopurine drugs. Identifying at-risk individuals prior to treatment can be accomplished by genotyping the most common *TPMT* variant alleles. A CPIC guideline with dosing recommendations for azathioprine, mercaptopurine, and thioguanine based on *TPMT* genotype is available

***Primary Question*** – A targeted clinical *TPMT* genotyping test reports a \*1/\*3A genotype for your patient that is about to undergo treatment with 6-mercaptopurine. The \*3A allele is a known loss-of-function allele that encodes aTPMT enzyme with no activity. ***What is the most likely predicted TPMT phenotype for this patient?***

1. Increased activity
2. Normal activity
3. Intermediate activity
4. Low/deficient activity

***Correct Response*** – c

***Explanation of Correct/Incorrect Response*** – Patients with homozygous wild-type/normal/high activity have two functional \*1 alleles, those with heterozygote or intermediate activity have one functional allele (\*1) plus one nonfunctional allele (\*2, \*3A, \*3B, \*3C, or \*4), and those with homozygous variant/mutant/low/ deficient activity have two nonfunctional alleles (\*2, \*3A, \*3B, \*3C, or \*4). Given that this patient is heterozygous for a \*3A loss-of-function allele, the correct predicted TMPT phenotype is ‘intermediate activity’.

***First Reply Question*** – ***Based on the CPIC recommendations for TPMT-directed azathioprine dosing, what is the therapeutic recommendation for the \*1/\*3A patient noted above?***

a. Start with normal starting dose (e.g., 2-3 mg/kg/d) and adjust doses of azathioprine based on disease-specific guidelines.

b. Consider starting at 30-70% of target dose (e.g., 1-1.5 mg/kg/d), and titrate based on tolerance.

c. Consider alternative agents. If using azathioprine start with drastically reduced doses and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines.

***Correct Response*** – b

***Explanation of Correct/Incorrect Response*** – Patients with reduced function or non-functional *TPMT* alleles are at high risk of bone marrow toxicity and require significant dose reduction (10-fold lower than the standard dose). One copy of a non-functional or reduced function allele may be associated with an increased risk of toxicity, with possible dose reductions of 30 to 50%. Therapeutic drug monitoring may help optimize dosing for individuals with one or two *TPMT* mutations; however, myelosuppression may be due to other factors, such as drug-drug interactions. When none of the targeted *TPMT* mutations are detected, patients are presumed to have normal TPMT activity, be at low risk of bone marrow toxicity, and treated with the standard dose.