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PHARMACOGENETICS IN MOROCCO
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THESIS

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JURY

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PLAN

LIST OF ABBREVIATIONS

5FU : 5 Fluorouracil

ABC : ATP Binding Casette

ADME :Absorption Distribution Metabolism Excretion

BCR-ABL : Breakpoint Cluster Region Abelson

CD : Cluster of Differentiation

COA : Coumarinic Oral Anticoagulants

CRC : Colorectal Cancer DME

DME : Drug Metabolizing Enzymes

DNA : Deoxyribonucleic Acid

DPD : Dihydropyrimidine dehydrogenase

DNA : Deoxyribonucleic acid

EGFR : Epidermal Growth Factor Receptor

EM : Extensive Metabolizer

GST : Glutathione S Transferases

HER : Human Epithelial Growth Factor Receptor

IM : Intermediate Metabolizer

KRAS : Kirsten Rat Sarcoma

LV : Leucovorin

MSI : Microsatellite Instability

PGx : Pharmacogenetics

PM : Poor Metabolizer

SNP : Single Nucleotide Polymorphism

dTMP : deoxythymidine monophosphate

TP : Thymidine Phosphorylase

TPMT : Thiopurine Methyl Transferase

UGT : UDP glucurosyltransferase

UM : Ultra rapid Metabolizer

VEGF : Vascular Endothelial Growth Factor

VEGFR : Vascular Epidermal Growth Factor Receptor

INTRODUCTION

The aim of drug treatment is to administer the appropriate drug in the correct dose to produce the desired therapeutic effect and minimum toxicity. In most cases, the choice of the drug and dose are based on the 'trial and error' basis and there is a wide range of efficacy and side effects. Although in most patients, the desired therapeutic effect may be achieved and they may benefit from treatment, there may be others that do not respond to the treatment or may even suffer an adverse effect with little or no benefit from the treatment.

There is extensive inter-individual variability in drug response [1]. Factors that affect drug response are multifold and complex, some of which concern fundamental aspects of human biology, because drug response affects well being and survival (Table 1) **[24].** These factors include the demographic characteristics of the patient such as age, sex and ethnicity; the nature of the disease, concomitant diseases, patients' diet, alcohol consumption, cigarette smoking; co-treatment with other drugs and others. It is estimated that genetic factors account for 15-30% of the variability in drug response, however for some drugs, this may be the major determinant in drug response. It has been recognised from clinical observations for more than 50 years that genetic differences between people contribute to the inter-individual differences in the response to many commonly prescribed drugs **[2, 3]** In these cases, patients with very high or low plasma or urinary drug concentrations that correspond to a specific phenotype of a drug response were identified, and the biochemical traits leading to the variation of drug concentrations were found to be inherited. The observation that the individual variation of drug response is often larger among members in a population (population variability) than within the same person at different times (intrapatient variability) further supports inheritance as a major determinant of drug response **[4].** These clinical and population-based

findings fostered the formation of pharmacogenetics to specifically address genetic contribution to individual variability in drug therapy.

Pharmacogenetics is the science that studies genetic differences in metabolic pathways which can affect an individual's response to drugs [5]. The term pharmacogenetics comes from the combination of the two words: pharmacology and genetics. Pharmacology is the study of how drugs work in the body and genetics is the study of how characteristics that result from the action of a single gene or of several genes acting together are inherited and how they affect cells in the body.

Unlike pharmacogenetics which studies the influence of genetics on the fate of drugs, pharmacogenomics studies the effect of the whole genome on drug response. Pharmacogenomics is a new science about how the systematic identification of all the human genes, their products, inter-individual variation, intra-individual variation in expression and function over time may be used both to predict the right treatment in individual patients and to design new drugs. Both pharmacogenetics and pharmacogenomics concern the effect of genetic variations on drug metabolism and response, but these terms differ [6], however used interchangeably.

The differences between the two are the initial approach of the science:

- Pharmacogenetics starts with an unexpected drug response and looks for a genetic cause.
- Pharmacogenomics on the other hand begins with looking for genetic differences within a population that explain certain observed responses to a drug or susceptibility to a health problem.

Traditionally pharmacogenetics has focused on the role of genetic variation in pharmacokinetics [7] (e.g. absorption, distribution, metabolism, excretion of drugs)

and pharmacodynamics (e.g., drug response proteins, such as receptors, channels and transporters) .

The variability of the pharmacokinetics of a molecule between two individuals depends on various factors: genetic polymorphism, environmental factors (nutrition, co-administration of drugs or smoking), the physiological condition and the existence of concomitant diseases.

The objectives of this work are:

- To report the Experience of the Medical Genetics Department of the Hassan II University Hospital, Fes;
- To study the genetic polymorphisms of certain drug metabolizing enzymes and targets and to know how these polymorphisms influence drug response,
- To evaluate the challenges of this field in Morocco and propose some recommendations.

GENERALITIES

I. History of pharmacogenetics

The field of pharmacogenetics was not officially recognized till late 1950s, although early observations of unusual drug reactions based on biochemical individuality were noted in the 1930s.

The term 'pharmacogenetics was first published by the German physician Friedrich Vogel in 1959 **[9].** This was in response to earlier observations of inter-individual variability in phenylthiocarbamide taste perception and isolated cases of drug-induced porphyria. Additional landmark scientific discoveries in the 1950s included:

- the identification of primaquine-induced hemolytic anemia among African-Americans (which was later shown to be due to glucose-6-phosphate dehydrogenase [*G6PD*] variant alleles), [**10**].
- succinylcholine-induced prolonged apnea during anesthesia (due to autosomal recessive butyrylcholinesterase deficiency) **[11],**
- and severe adverse effects after antituberculosis treatment with isoniazid (later shown to be due to *N*-acetyltransferase [*NAT2*] variant alleles) [12].

In addition to the article by Vogel, two other similar publications at that time included the American Medical Association-initiated review of available pharmacogenetic studies by Arno Motulsky **[13]** and the first textbook dedicated to the discipline in 1962 by Werner Kalow **[14].**

In 1977, hepatic cytochrome P450 oxydase was identified. This enzyme controls the metabolism of debrisoquine(an antihypertensive drug) and sparteine (anti arrythymic) and it is known to be one of the most influential discoveries in pharmacogenetics and its potential clinical utility. The genetic polymorphism of this drug metabolizing enzyme, was identified after adverse drug reactions such as

nausea, diplopia, and blurred vision occurred after the administration of sparteine, or incapacitating orthostatic hypotension after debrisoquine was given to some patients. Adverse drug reactions were also the clinical events that revealed genetic variants of other drug-metabolising enzymes or drug targets. Thus, genetic polymorphisms were discovered by incidental observations that some patients or volunteers experienced unpleasant and disturbing adverse drug reactions when given standard doses of drugs (Table 1) **[15].**

Table 1: common adverse drug reactions in some commonly used drugs in different population and the genes responsible [15]

	Adverse Drug Reaction			*Genetic Risk Factor*				
Drug	*Type*[a]	*Reaction*	*Prevalence*	*Risk Allele*	*Freq.*[b]	*Effect*[c]	*Population*[d]	*Ref.*
Clopidogrel	F	Cardiovascular events	0.13	*CYP2C19*2/3/4/5*	0.03	3	European	40
Ximelagatran	B	Hepatotoxicity	0.08	*HLA-DRB1*0701*	0.08	4	European	31
Gefitinib	A	Diarrhea	0.28	*ABCG2* Q141K	0.07	5	European	41
Isoniazid	A	Hepatotoxicity	0.15	*CYP2E1*1* & *NAT2*[d]	0.13	7	European	42
Amoxicillin-clavulinate	B	Hepatotoxicity	<0.001	*HLA-DRB1*1501*	0.15	10	European	43
Lumiracoxib	B	Hepatotoxicity	0.013	*HLA-DRB1*1501*	0.15	13	European	44
Simvastatin (high dose)	A/B	Myopathy	0.02	*SLCO1B1*	0.15	17	European	34
Irinotecan	A	Neutropenia	0.20	*UGT1A1*28*	0.32	28	European	45, 46
Ticlopidine	B	Hepatotoxicity (cholestatic)	<0.001	*HLA-A*3303*	0.14	36	Japanese	47
Tranilast	A	Hyperbilirubinemia	0.12	*UGT1A1*28*	0.30	48	European	36
Mercaptopurine	A	Neutropenia, other toxicity	0.12	*TPMT*2/3A/3B/3C*[f]	0.05	49	European	48
Flucloxacillin	B	Hepatotoxicity	<0.001	*HLA-B*5701*	0.04	81	European	32
Allopurinol	B	Severe cutaneous reaction	<0.001	*HLA-B*5801*	0.15	678	Han Chinese	49
Abacavir	B	Hypersensitivity reaction	0.08	*HLA-B*5701*	0.04	>1000	European	50
Carbamazepine	B	Stevens-Johnson syndrome	0.003	*HLA-B*1502*	0.04	>1000	Han Chinese	51

In Morocco, the first study in this domain was published by Bouayad et al. in 1982 on the acetylation of isoniazid **[16, 17].** The study was aimed at determining the phenotype of 100 patients to isoniazid. The results showed that the majority were rapid metabolizers, which explains the good tolerance of the administered dose (10mg/Kg). But since then, there have been no published studies in this direction. It was not until 2011 that new studies emerged **[18].** The first study focuses on the effect of genetic variants of cytochrome 2C9 (CYP2C9) and vitamin K peroxidase (VKORC1) on the sensitivity of the Moroccan patients to acenocoumarol. The evaluation of allelic frequencies of *CYP2C9*2* and *CYP2C9*3*, VKORC1 '1639 G>A shows that these alleles modulate the sensitivity to acenocoumarol, hence the necessity of predictive tests for these polymorphisms before each prescription [19].

The second study is focused on cytochrome 3A5 (CYP3A5) with the determination of the frequency of allele *CYP3A5*1* and *CYP3A5 * 3* in the Moroccan population and assessing their impact on the daily dose of tacrolimus for patients with kidney transplant **[20].**

These studies are the first to provide genetic data related to the frequency of polymorphisms of CYP2C9, CYP3A5 and VKORC1 in the Moroccan population, which offers the prospect of developing new pharmacogenetic studies.

The domain of pharmacogenetics therefore, seeks to establish the relationship between the therapeutic effect and individual genetic variations and their consequences which comprises:

- the metabolic pathways of administered treatment(pharmacokinetics)
- the therapeutic targets of administered molecules (pharmacodynamics).

Combining the pharmacokinetics and pharmacodynamics may explain the significant variation which exists between individuals with regards to the therapeutic effect.

II. Principles of pharmacogenetics /pharmacogenomics

1. Molecular basis of the variability in the human genome

Genetic variability is the consequence of the variation of DNA sequence in the genome. Due to DNA sequence variation, genes may exist in alternative forms called alleles. Specific sets of alleles forming the genome of an individual are called its genotype, the observable characteristics such as the capacity to to metabolize a drug or drug response are called phenotype. Since autosomal chromosomes are paired, each position (locus) is represented twice. If both chromosomes have the same allele occupying its locus, the condition is called homozygous; if the alleles at the two loci are different, the individual is heterozygous for this allele.

Genetic polymorphism is the occurrence of two or more alleles at a given locus of which the rare allele has a frequency of at least 1% or more in a given population.

Pharmacogenomics focuses on variation within the human genome. The human genome is composed of 3.1 billion nucleotides bases and the number of genes is about 26,000. Every person inherits two copies of most genes, one from each parent. Although any two individuals' DNA is over 99 percent identical, the number of nucleotides is so large, approximately 3 billion that millions of variant sequences still occur across the human population. The most abundant type of variant is single nucleotide polymorphism (SNP) **[25]**; other common types are deletions, insertions, and tandem repeats. Each gene's nucleotide sequence encodes a molecular product, usually a protein. Sequence variation may result in alterations in the gene's product, which in turn may have an effect on the phenotypes that the product influences. Genetic researchers use several types of studies to establish and explore gene-phenotype relationships. Heritability studies can indicate the relative contributions of genetic and non genetic (e.g. environmental) influences to a

particular phenotype. Linkage studies analyse pedigrees of related individuals and genetic markers to hone in on regions in the genome that may harbour genes associated with phenotypes of interest. Candidate genes association studies can be used to investigate gene-phenotype relationships suggested by linkage studies as well as to focus on genes selected for their physiological or pharmacologic relevance to a phenotype.

2. Single nucleotide polymorphisms

Genetic variations in drug metabolizing enzymes, transporters, receptors and drug targets have significant effects on the efficacy and toxicity of many drugs. Pharmacogenomics combines traditional pharmaceutical sciences such as biochemistry with annotated knowledge of genes' protein and SNPs. It is believed that drugs might one day be tailor-made and adjusted to each individual's genetic make-up. Although environmental factors such as diet, age, lifestyle and state of health can influence an individual's response to medicines, their genetic make-up is the key to creating personalised drugs with greater efficacy and safety.

SNPs are the most frequently found DNA sequence variations in the human genome **[27],** compared with non frequent variants, the primary cause of genetic disorders. It is believed that SNPs may contribute significantly to genetic risk for common diseases. Approximately 1 million SNPs are likely to occur in human genes, with approximately 500,000 being non-coding SNPs, 200,000 being replacement coding SNPs. SNPs found in the coding and regulatory regions of genes are likely to be the most relevant variants. Efforts to identify all SNPs and their relevance to disease (cancer) susceptibility and treatment outcome are continuous and may take several more years. However, the approach taken by many scientists at present is the candidate gene approach in which one examines SNPs of the chosen gene that are likely to have an effect.

III. Benefits of pharmacogenetics

The main aim of pharmacogenetics is to improve the healthcare of patients through personalized medicine. Personalized medicine broadly defined, is health management informed by knowledge of the underlying genetics of each individual [21]. This is done by looking for individual characteristics which will enable physicians to adapt treatment in the most specific way for a given patient. Thus the field of pharmacogenetics helps to improve the safety of patients through tests that identify patients who are likely to develop dangerous reactions to drugs, enabling physicians to monitor them closely and adjust the dosing or change treatment. [22]

Moreover, this field helps to develop more accurate methods of determining dosage. Thus, instead of the dose of the drug being based on patient's body weight and age, it would be based on an individual's genetics thereby reducing the likelihood of an overdose.

Another benefit of this field is the development of better vaccines. Vaccines made of genetic material could activate the immune system to have all the benefits of existing vaccines but with reduced risk of infections [23].

This science also helps to detect specific mutations in tumors and to prescribe the necessary therapy possible.

IV. Factors influencing the response to treatment

Both genetic and non-genetic factors influence an individual's response to drugs by modulating the dose-response curves of drug efficacy and drug toxicity of patients. This can alter clinical outcome if the drug dose is not adjusted accordingly. Genetic factors cause a permanent change in protein functions, whereas environmental and physiological factors and their influence on drugs are transient in most cases (Table 2) **[24].**

Genetic polymorphisms of proteins involved in drug targeting (i.e. pharmacodynamics) and drug metabolisms and transport (i.e. pharmacokinetics) are likely to be the most important sources of individual drug efficacy.

Pharmacokinetics is defined as the time course of drug concentrations in the body, and can be separated into individual components such as absorption, distribution, metabolism and elimination abbreviated ADME. Drugs are xenobiotics, that is to say a compound that is foreign to us. Its introduction into the organism is followed by two stages:

- the first stage is processing, often by the liver,
- and a second effect on the target, in a variable order.

These two steps are preceded by a phase of intestinal absorption, for drugs administered orally. All these steps are performed by carriers and enzymes whose expression can vary depending on the polymorphism of the gene concerned. In addition, the effects of drugs can be influenced by changes in the metabolism of foreign genes and the target speaker in the response of the organism.

Table 2: Major factors affecting individual drug response

Factors	Effects
Genetic factors	Major variables; stable and inherited
Therapeutic targets	Drug efficacy(pharmacodynamics)
Drug metabolizing enzymes	Drug metabolism(pharmacokinetics)
Drug transporters	Drug disposition(pharmacokinetics)
Targets of adverse drug reactions	Drug toxicity(pharmcodynamics and pharmakinetics)
Factors with indirect effects	Drug efficacy pharmacokinetics and toxicity
Other factors	Mostly transient
Environmental factors:	Drug efficacy, pharmacokinetics and toxicity
Environmental chemicals, co-administered drugs, tobacco smoking, alcohol drinking, dietary constituents	
Physiological factors	
Age ,sex, disease state, pregnancy; circadian rythm, exercise ,starvation	Drug efficacy, pharmacokinetics and toxicity

V. Genetic polymorphisms of drug metabolizing enzymes

A large number of enzymes most of which are polymorphic participate in metabolism of xenobiotics such as drugs and carcinogens. These enzymes are termed drug metabolizing enzymes, DMEs. In general,drug metabolism occurs in the liver in two phases (Figure 1).

- Phase I DMEs, mostly cytochromes P450 (CYPs) metabolically activate xenobiotics to reactive electrophilic forms.
- Phase II DMEs, catalyze the conjugation reactions with various radicals (UDP glucuronosyltransferases, (UGTs), N-acetyl-transferases (NATs), glutathione S-transferases (GSTs), or other...) and thus reinforce their hydrophilic nature and their solubility in bile and urine.

In addition to these enzymes are the Phase III transport proteins which ensure the transfer of metabolites in the cell or outside the cell, e.g. P-gp protein and the proteins of the ABC Family ("ATP binding cassette") [28].

Genetic polymorphism of many enzymes involved in this process leads to inter-individual variations in metabolism and pharmacokinetics of drugs and could therefore influence drug response.

About 40 % of phase I metabolism of clinically used drugs is affected by polymorphic enzymes. As these genetic polymorphisms alter enzyme activity, they may change the rate of drug metabolism and influence drug plasma levels.

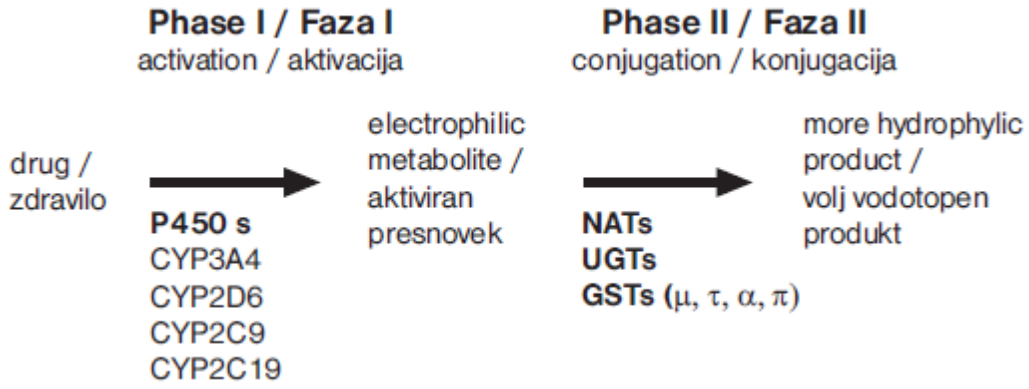


Fig 1: phase I and phase II of drug metabolism. P450s -Cytochromes P450s, NATs-N acetyl-transferases, UGTs (UDP-glucuronyltransferase), GSTs (Glutathione S transferases)...[29].

1. Cytochromes P450s (CYPs)

Cytochromes P450s are hemoproteins, i.e they belong to a family of proteins containing a heme cofactor. Most, if not all clinical drugs are metabolized by cytochromes P450. CYPs catalyse the mono-oxygenation of lipophilic dugs to give rise to metabolites with altered activity and increased water solubility or metabolites more suitable to further metabolism by other enzymes. **[30].** P450 is a major variable affecting drug plasma concentration, drug detoxification and drug activation in the case of a prodrug.

In humans sixty genes have been identified, but only a small number of proteins, numbering twenty, encoded by these genes (CYP1, CYP2 and CYP3) contribute to drug metabolism **[31].** They account for about 75- 80% of phase 1 metabolism and 65-70% of the clearance of clinically used drugs. The result is the frequency of drug or food interactions by competition of substrates which represent a major problem in the dose optimization. These interactions can also identify other mechanisms such as induction or repression of genes. The majority of the CYPs

isoforms are expressed in the liver, while some are expressed in other tissues such as the central nervous system, gastrointestinal tract, lung, trachea, nasal and olfactory mucosa and adrenal gland.

The most important CYPs involved in drug metabolism include CYP2D6, CYP3A4, CYP2C9, and CYP2C19.

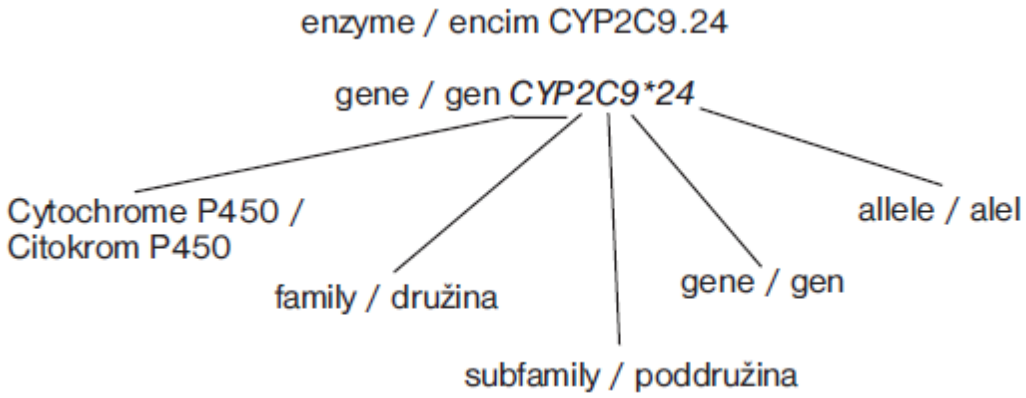


Fig 2: nomenclature of cytochromes P450s [29]

1.1. CYP2D6

One of the most studied polymorphisms involves the gene CYP2D6. CYP2D6 is a polypeptide of 497 amino acids. The CYP2D6 gene is located on chromosome 22q13.1. The locus contains two neighbouring pseudogenes, CYP2D7 and CYP2D6. The enzyme accounts for only a small percentage of all hepatic P450s, but its role in drug metabolism is extensively higher than its relative content. This gene is responsible for the metabolism of approximately 20 to 25% of all marketed drugs **[32].** CYP2D6 polymorphism is one of the best studied among P450s. This polymorphism is to be considered for two reasons:

- despite its low expression in the liver, the enzyme recognizes as substrates of many drugs (β-blockers, tricyclic antidepressants,

antiarrhythmics class I, antipsychotics ...), in total 25% of the drugs used in clinical practice

- The frequency of the different variants is far from negligible and is different depending on the population concerned. (Table 3)(33). For example, the *CYP2D6 *17* is found mainly in blacks (20-35% of the population), *CYP2D6 * 10* is common in Southeast Asia (50% of the population) and *CYP2D6 * 4* is common in Caucasians (12-21% of the population). These three variants are associated with reduced or no activity and thus to a slower metabolism leading to drug overdose **[33]** or to inefficacy if the enzyme converts a prodrug to an active drug **[34].**

The frequency and genetic basis of major variants of *CYPD26* are well documented. (Table 3) **[33].**

If CYPD26 is mainly responsible for the blood level of a drug and the genetic polymorphism of drug target is not an issue, knowing the CYPD26 phenotype of an individual patient would allow physicians to prescribe a safe and effective dose of the drug to the patient.

A patient's CYP2D6 phenotype is often clinically determined via the administration of debrisoquine (a selective CYP2D6 substrate) and subsequent plasma concentration assay of the debrisoquine metabolite (4-hydroxydebrisoquine). **[35].**

CYP2D6 shows the largest phenotype variability among the CYPs, largely due to its genetic polymorphism. The genotype accounts for normal, reduced, and non-existent CYP2D6 function in subjects. Pharmacogenomic tests are now available to identify patients with variations in the CYP2D6 allele and have been shown to have widespread use in clinical practice. **[36]**. The CYP2D6 function in any particular subject may be described as one of the following. Table 4 **[24]**:

- poor metabolizer – little or no CYP2D6 function
- intermediate metabolizers – metabolize drugs at a rate somewhere between the poor and extensive metabolizers
- extensive metabolizer – normal CYP2D6 function
- ultrarapid metabolizer – multiple copies of the *CYP2D6* gene are expressed, and therefore greater-than-normal CYP2D6 function.

Subjects with multiple gene copies will metabolize drugs more rapidly and therapeutic plasma levels will not be achieved at ordinary drug dosages. Individuals lacking functional *CYP2D6* genes metabolize selective CYP2D6 substrates at a lower rate, and the risk for adverse drug reactions is higher.

Table 3. Major human polymorphic variant *CYP2D6* alleles and their global distribution [33]

MAJOR VARIANTS alleles	mutation	consequence	Caucasians	Asians	Black Africans	Ethiopians and Saudi Arabians
CYP2D6 * 2xn	Gene duplication/multiduplication	Increased enzyme activity	1−5	0−2	2	10−16
CYP2D6 * 4	Defective splicing	Inactive enzyme	12−21	1	2	1−4
CYP2D6 * 5	Gene deletion	No enzyme	2−7	6	4	1−3
CYP2D6 * 10	P34S, S486T	Unstable enzyme	1−2	51	6	3−9
CYP2D6 * 17	T107I, R296C, S486T	Altered affinity for substrates	0	0	20−35	3−9

Table 4: CYP2D6 functions

Phenotype	Characteristics	Clinical consequence
PM	Major variants: CYP2D6*3, -*4,-*5,-*6 Enzyme inactive 5-10% white; 1-2% Chinese and Japanese	High plasma drug level Risk of drug-related side effects Use of reduced drug dose
IM	Major variants: CYP2D6*9, -*10, -*41 Low residual enzyme activity	Lower dose for some patients
EM	Not a uniform group	

Knowing the genetic polymorphism of CYP2D6 has a lot of clinical implications; in the treatment of cancer, by metabolising the prodrug tamoxifene into its active metabolites endoxifen and 4-hydroxytamoxifen by N demethylation and 4- hydroxylation. Both metabolites are known to have a higher affinity for the drug target (oestrogene factor ER), and the greater ability to inhibit cell proliferation in endocrine therapy for prevention and treatment of ER-positive breast cancer than the parent drug in the treatment of cardiovascular diseases, in the treatment of pain, etc.III

1.2. CYP3A5

CYP3A subfamily accounts for more than 50% of all CYP-dependant drug metabolism and substantial inter-individual variability in CYP3A activity was

observed. Polymorphisms identified so far did not explain this variability as no correlation was found between the genotype and the phenotype. However, induction and inhibition by drugs and food variants seem to be clinically more relevant, as they increase or decrease CYP3A drug metabolism. To date, 23 variants have been identified. *3 is the most common variant allele and it's responsible for non-functional CYP3A5.

1.3. CYP2C9

Another polymorphism to consider is that of CYP2C9 whose several variants have a reduced activity and affects a significant fraction of white people 20.4% *CYP2C9 *1/*2* and 11.6% for the *CYP2C9*1/*3* [37]. CYP2C9 is involved in the metabolism of many clinically important drugs, including hypoglycemic agents (tolbutamide, glipizide), non steroid anti inflammatory agents (Ibuprofen), anticoagulants (S warfarin), and antiepileptics (phenytoin).

1.4. CYP2C19

CYP2C19 catalyzes the metabolism of many commonly used drugs, including (*S*)-mephenytoin (anticonvulsant), omeprazole (antiulcerative), and diazepam (anxiolytic). CYP2C19 plays an important role in the proton-pump inhibitor therapy for peptic ulcer and gastroesophageal reflux diseases. More than 20 polymorphisms of *CYP2C19* have been reported [38]. Most "poor metabolization" of CYP2C19 is attributable to the *CYP2C19*2* and -*3* genotypes, which are null alleles.

2. Other drug metabolizing enzymes

Many non-P450 drug metabolizing enzymes also play critical roles in the metabolism of a variety of drugs.

Polymorphisms of these enzymes influence the metabolism and therapeutic effects of the drugs, some of which are clinically significant.

2.1. Thiopurine methyl-transferase (TPMT)

TPMT is an enzyme that is encoded in humans by the TPMT gene. A pseudogene for this locus is located on the chromosome 6q **[39].**

TPMT catalyzes the S-methylation of 6-mercaptopurine, azathioprine, and thioguanine, to inactivate the thiopurine drugs, which are used for the treatment of leukemia and autoimmune diseases.

More than 20 variant alleles of the *TPMT* gene have been identified, among which *TPMT*2*, *TPMT*3A*, and *TPMT*3C* are defective alleles that produce poor enzymatic activities [39]. Approximately 90% of white persons inherit high enzyme activity, 10% inherit intermediate activity (heterozygous), and 0.3% inherit low or no activity. Persons carrying defective TPMT alleles accumulate higher levels of cytotoxic thiopurine nucleotides than those with the wild-type alleles after receiving standard dose of the drugs, leading to severe hematological toxicity by the parent drugs. In these scenarios, a reduced drug dose should be prescribed.

2.2. Butyrylcholinesterase

The serum butyrylcholinesterase hydrolyzes the muscle relaxant succinylcholine and thereby determines the serum concentration of the drug and the duration of muscle relaxation. A variant allele of the gene encodes an atypical form of the enzyme that is not active in hydrolyzing succinylcholine. Approximately 1 in 3500 white persons is homozygous for the variant allele. Patients with the atypical butyrylcholinesterase but receiving a normal dose of the muscle relaxant have higher serum levels of the drug and develop prolonged muscle paralysis and apnea **[40].**

2.3. UDP (UGT1A1)

Another example of polymorphism on a transferase is that of UDP-glucuronosyltransferase UGT1A1, well known because its endogenous enzyme substrate is bilirubin.

The increase from six to seven tandem repeats of TA in the "TATA box" sequence of the gene promoter is accompanied by decreased expression of the enzyme, characteristic of the Gilbert's disease. This polymorphism is also involved in the biliary excretion and SN-38, the active metabolite of irinotecan, which must, like bilirubin be conjugated to a radical glucuronyl before being eliminated. SN-38 accumulates in patients carrying the mutation resulting, thereby, in diarrhea and leucopenia.

More than 100 UGT1A1polymorphisms have been identified. The frequency of UGT1A1*6 polymorphism is high among Japanese and Chinese(16–23%), whereas it is low (<1%) in whites. The high frequency of the UGT1A1*6 variant allele may contribute to the high incidence of neonatal hyperbilirubinemia in Asian populations, consistent with a major role of UGT1A1 in the glucuronidation of bilirubin. UGT1A1 polymorphisms cause three forms of inherited, unconjugated hyperbilirubinemia in humans **[41, 42]**. However, no such studies have yet been reported among Moroccans. The Criggler- Najjar syndrome types I and II are caused by variant alleles in the UGT1A1 coding region and the Gilbert's syndrome by polymorphisms in the promoter of the UGT1A1 gene. The severity of the hyperbilirubinemia correlates with the enzymatic activities of the polymorphic UGT1A1. Patients with the Criggler-Najjar type I syndrome completely lack bilirubin glucuronidation, which results in very high serum levels of unconjugated bilirubin and early childhood death.

VI. Genetic polymorphisms of drug targets

It is relatively frequent that the effects of a drug depends both on the polymorphism of its metabolic enzymes and that of target enzymes or receptors. In other cases, only the target polymorphisms have been identified.

The first example is that of such as coumarin (warfarin and Coumadine®acenocoumarol or Sintrom®). More than 500,000 patients receive in France this type of treatment which 17,000 are hospitalized for bleeding or thrombotic event, representing 13% of hospital admissions for iatrogenic injury related to drugs **[43].** Warfarin doses to achieve a satisfactory state of coagulation ("International Normalized Ratio "or INR between 2 and 3) ranges from 7-10 to 50-60 mg per week. The drug is metabolized in the liver by CYP2C9 and has as target the vitamin K epoxide reductase (VKORC1) whose gene has recently been identified **[44].** Each of genes for these two enzymes present polymorphisms varying drug response. The wild form of CYP2C9 is predominantly CYP2C9 * 1 / *1. There are in Caucasian populations, two allelic variants of the CYP2C9, CYP2C9 *2 and CYP2C9 *3 that cause a decrease in the activity of the enzyme mostly seen in the in the case of the CYP2C9* 3 allele. Their presence is associated with increased risk of hemorrhage, maximum homozygous *3 / *2 and * 2 / * 2, and also observed in heterozygous * 2 / * 3. Knowing that about 30% of Americans are carriers of at least one of these two variants whose presence double or triple the risk of hemorrhagic stroke, it would certainly be helpful to base the first prescription of warfarin on genetic analysis. The mechanism of susceptibility to bleeding is because CYP2C9 metabolizes the S-enantiomer of warfarin much more active than the R, these two enantiomers are present in the product administered. The VKORC1 is the key enzyme of the vitamin K cycle regenerating reduced active form from epoxy. This reduced form is an essential cofactor for γ-carboxylation residues glutamic acid

present on many coagulation proteins (prothrombin, factors VII, IX and X). The gene VKORC1 located on chromosome 16 is the seat of mutations associated with case sensitivity or resistance warfarin. Fifty percent of the variability in response to acenocoumarol is related to these two polymorphisms, one on the CYP2C9 gene, the other on VKORC1.

VII. Technologies and methods used in pharmacogenetics

1. PCR

The polymerase chain reaction (PCR) is a technique in molecular biology to amplify a single or few copies of DNA across several orders of magnitude of a DNA sequence. Developed in 1984 by the American biochemist, Kari Mullis, (45) it is now a common and often an indispensable technique used in medical and biological research labs for a variety of applications. These include DNA cloning for sequencing, DNA-based phylogeny or functional analysis of genes diagnosis of hereditary diseases, identification of genetic fingerprints (used in forensic and DNA paternity testing) and diagnosis of infectious diseases.

A basic PCR set up require several components and agents:

- DNA template: the sample DNA that contains the target sequence.
- DNA polymerase: a type of enzyme that synthesizes ne strands of DNA complementary to the target sequence. The first and the most commonly used is the Taq polymerase.
- Primers: short pieces of single stranded DNA about 20-30nt long that can hybridize to one strand of template DNA. Primers are complementary to the target sequence. Two primers are needed in the PCR reaction. A forward primer and a reverse primer.
- Water
- Buffer solution
- Deoxunucleotide triphosphates(dNTPs)

1.1 Steps in PCR (7)

There are three major steps involved in the PCR technique:

- denaturation,

- annealing and,
- extension.

In step one; the DNA is denatured at high temperatures (from 90-97 degrees Celsius). In step two, primers anneal to the DNA template strands to prime extension. In step three, extension occurs at the end of the annealed primers to create a complementary copy strand of DNA using the DNA polymerase or the Taq polymerase. This effectively doubles the DNA quantity through the third steps in the PCR cycle. To amplify a segment of DNA using PCR, the sample is first heated so the DNA denatures, or separates into two pieces of single-stranded DNA. Next, an enzyme called "Taq polymerase" synthesizes two new strands of DNA, using the original strands as templates. This process results in the duplication of the original DNA, with each of the new molecules containing one old and one new strand of DNA. Then each of these strands can be used to create two new copies, and so on.The annealing phase happens at a lower temperature, 50-60°C. This allows the primers to hybridize to their respective complementary template strands, a very useful tool to forensic chemistry. The newly-formed DNA strand of primer attached to template is then used to create identical copies off the original template strands desired. Taq polymerase adds available nucleotides to the end of the annealed primers. The extension of the primers by Taq polymerase occurs at approx 72°C for 2-5 minutes. DNA polymerase I cannot be used to elongate the primers as one would expect because it is not stable at the high temperatures required for PCR. The beauty of the PCR cycle and process is that it is very fast compared to other techniques and each cycle doubles the number of copies of the desired DNA strand. After 25-30 cycles, whoever is performing the PCR process on a sample of DNA will have plenty of copies of the original DNA sample to conduct experimentation.

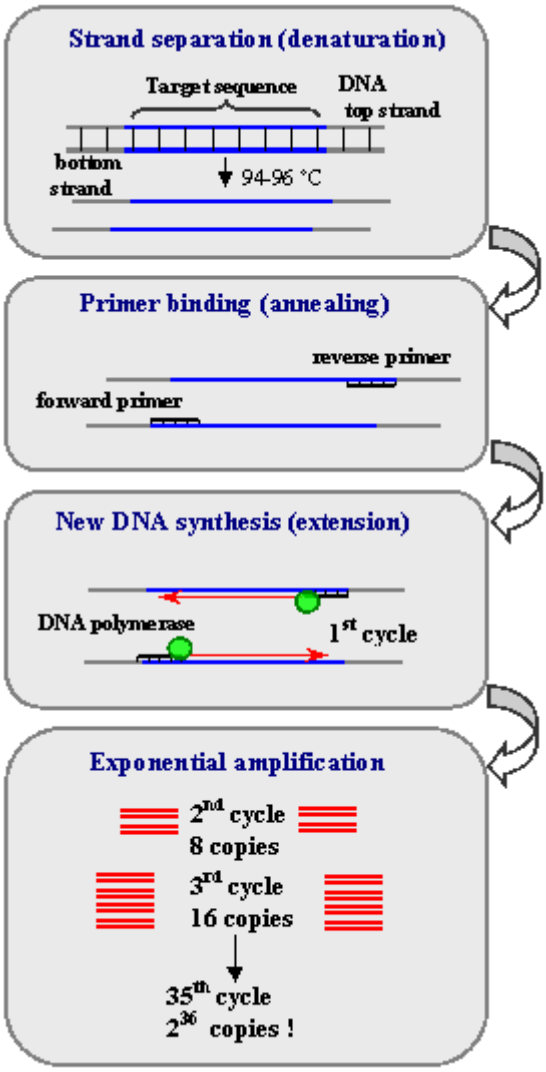


Fig 3: Basic steps in PCR [46]

2. Other Methods

Other methods used in pharmacogenetics include:

- **DNA sequencing**: DNA sequencing is the process of determining the precise order of nucleotides in a DNA molecule. It includes any method or technology that is used to determine the order of the four bases, Adenine, guanine, cytosine and thymine in a strand of DNA.
- **DNA microarray**
- **Mass spectrometry**
- **Fluorescence-based platform.**

DISCUSSION

I. PHARMACOGENETICS OF ORAL ANTICOAGULANTS

Anticoagulants, in simple terms, are chemical substances that are used to prevent or limit the coagulation of blood, thereby reducing its viscosity. They are used in the treatment and prevention of thrombosis (formation of blood clots) in blood vessels, as well as the prevention of other complications, notably, emboli.

The major anticoagulants used in medicine are heparins (injectable), mainly used in emergency cases, due to their rapid effect, for a short period of time, and the Vitamin K antagonists (AVK), oral anticoagulants ,which have a slower effect, and used for long-term treatment.

Oral anticoagulants are widely used in the prevention and treatment of thromboembolic events such as deep vein thrombosis, atrial fibrillation,recurrent stroke or pulmonary embolism, mechanical heart valve replacement, and their use in clinical practice is on the increase.

1. Vitamin K Antagonists (VKA)

Vitamin K is essential for the hepatic synthesis of factors II (prothrombin), VII, IX, X as well as protein C and S. AVK deplete the active form of Vitamin K by inhibiting the Vitamin K epoxide reductase (VKOR).

AVK are the most frequently used oral anticoagulants worldwide. They are administered orally for long –term anticoagulation therapy. The major AVK used in medicine are: coumarin derivatives 4 hydrodroxy coumarins with aromatic properties at the 3' position and collectively called coumarinic oral anticoagulants. (COAs). The commercially available coumarins are warfarin, acenocoumarol, phenprocoumon. In Morocco, the most frequently used COA is acenocoumarol (Sintrom). These medications have a narrow therapeutic index and an unpredictable dose-response relationship giving rise to frequent complications such as bleeding,

when the dose is supratherapeutic and clot formation when it is infratherapeutic. [46]. The management of oral anticoagulation is challenging because of the large variability of dose-response relationship which is in part caused by genetic polymorphism. The safe and effective use of these oral anticoagulants is closely monitored by maintaining prothrombin time within a therapeutic range range. Prothrombin time is expressed as International Normalized Ratio (INR). The allelic variants of genes like cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase subunit 1(VKORC1) are closely associated with the maintenance of dose of oral anticoagulants. However, the final dosage in an individual is based on a complex set of genetic and environmental factors.

In the case of warfarin, the required doses to achieve a satisfactory state of coagulation ("International Normalized Ratio "or INR between 2 and 3) ranges from 7-10 to 50-60 mg per week. The drug is metabolized in the liver by CYP2C9 and has as target the vitamin K epoxide reductase (VKORC1) whose gene has recently been identified [50]. Each of genes for these two enzymes present polymorphisms varying drug response. The wild form of CYP2C9 is predominantly CYP2C9 *1/*1. There are, in Caucasian populations, two allelic variants of the CYP2C9, CYP2C9*2 and CYP2C9 *3 to cause a decrease in the activity of the enzyme still more marked in the case* 3 allele. Their presence is associated with increased risk of haemorrhage, maximum homozygous *3/*3 and *2 / *2, and also observed in heterozygous *2/*3. Knowing that about 30% of Americans are carriers of at least one of these two variants presence whose double or triple the risk of hemorrhagic stroke, it would certainly be helpful to base the first prescription of warfarin on genetic analysis. The mechanism of susceptibility to bleeding is because CYP2C9 metabolizes the S-enantiomer of warfarin much more active than the R, these two enantiomers are present in the product administered. The VKORC1 is the key

enzyme of the vitamin K cycle regenerating reduced active form from epoxy. This reduced form is an essential cofactor for γ-carboxylation residues glutamic acid present on many coagulation proteins (prothrombin, factors VII, IX and X). The gene VKORC1 located on chromosome 16 is the seat of mutations associated with case sensitivity or resistance warfarin. Fifty percent of the variability in response to acenocoumarol is related to these two polymorphisms, one on the CYP2C9 gene, the other on VKORC1.

II. PHARMACOGENETICS OF THIOPURINE DRUGS: TPMT

As described earlier, the *TPMT gene* provides instructions for making an enzyme called thiopurine S methyltransferase (TPMT). This enzyme is located in the cytoplasm and present in a number of tissues (blood cells, heart, intestines). TPMT carries out the S-methylation of thiopurine drugs: 6-thioguanine, 6-mercaptopurine and azathioprine. It is located on the short arm (p) of chromosome 6 at position 22.3 (6p22.3).

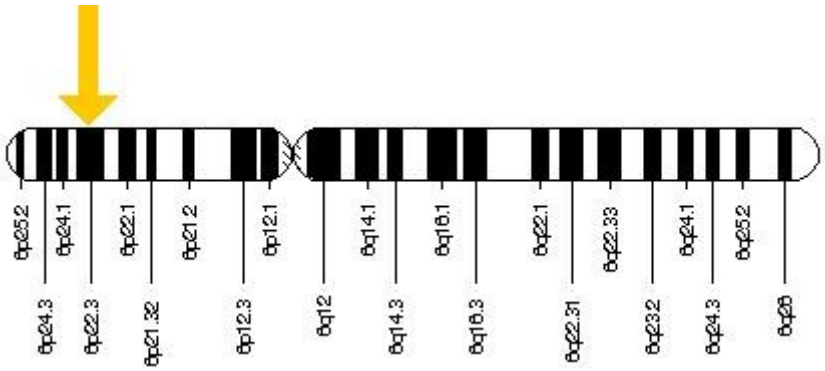


Fig 4: location of the TPMT gene on chromosome 6 [68]

Genetic polymorphisms that affect this enzymatic activity are correlated with variations in toxicity and sensitivity to thiopurine drugs in individuals, causing thiopurine S-methyl transferase deficiency. As a result, patients undergoing treatment with thiopurine drugs need to be tested for genetic polymorphisms in *TPMT gene.* If these polymorphisms exist, the dose of the drug must be decreased to lower the risk of toxicity.

We report a study conducted by Janati Idrissi M et al., of the Medical Genetics Department of the Hassan II University Hospital, Fes aimed at determining the genetic variations of the *TPMT* gene responsible for common adverse drug reactions observed in patients who are given thiopurine drugs. The study was done after it was observed that majority of Moroccan patients show little or no severe adverse reactions to thiopurine drugs.

103 unrelated individuals were included in this study. All the patients gave their informed written consent and the protocol was validated by the Ethical committee of the Hospital.

Blood samples from the patients were used for DNA extraction. The three common TPMT polymorphisms, G238C (TPMT*2), G460A (TPMT*3B) and A719G (TPMT*3C) were determined using previously described PCR with minor modifications. The G238C polymorphism was analysed using an allele-specific PCR method, while PCR-RFLP was used to detect the G460A and A719G polymorphisms using the restriction enzymes *MwoI* and *AccI* (New England Biolabs), respectively.

The PCR reactions were performed in a volume of 25µ/l with 100ng/µl as a final concentration of DNA for all reactions. PCR amplification consisted of an initial denaturing step at 94°C for 5 min followed by 30 cycles of denaturing at 94°C for 30 seconds, annealing at 58°C (TPMT*2) or 53°C(TPMT*3B) or 57°C (TPMT*3C) for 30 seconds and extension at 72°C for 30 seconds. The final extension step was done at 72°C for 7 min. Samples of each genotype were sequenced.

The results showed that, in the Moroccan population, the genotype analysis of the three TPMT mutant alleles, *TPMT*2, *3B and *3C* showed no mutant alleles among the 206 alleles tested. However, it was discovered that the samples had a wild type allele, *TPMT*1.*

The clinical relevance of TPMT genetic variants is well established, and it is important, not only for toxicity but efficacy of therapy as well. TPMT has been studied at the genetic level in a variety of populations around the world **[69]**. It is clinically used before treatment with drugs from the thioguanide family in many leading institutions. **[70, 71].**

The thiopurine drugs are used for their cytotoxic and immunosuppressive properties. They are known for their adverse drug reactions. **[72]**. Defects in the

TPMT gene lead to decreased methylation and decreased inactivation of thiopurine drugs , leading to enhanced bone marrow toxicity which may cause myelosuppression, anemia, leukopenia, bleeding and infection. [73, 74].

In this study, the most common variant alleles of TPMT in the Moroccan population were determined and compared to other studies reported in the literature. It was found that, among the 206 alleles tested, *TPMT*2 TPMT*3A, TPMT*3C* showed no mutant alleles as we considered that the samples showed a wild type allele *TPMT*1.*

TPMT*3C is the most prevalent TPMT mutant allele in Africans (Table 5) [75] with an allele frequency of 5.4% in Kenyans [76], 7.6% in Ghanaians [77], 1.3% in Egyptians [78] and 2.4% in Tunisians [79]. But this mutant allele is not detected in Moroccans.

TPMT*3A (a mutant allele which contains both G460A and A719G) is the second TPMT mutant allele in Africans, and it was detected only in the Egyptians and Tunisians; with an allele frequency of 0.3% and 1.68% respectively [78, 80]. One mutant TPMT allele, the TPMT*2 was not detected in any African population, which suggests that this allele is rare in Africans.

The general pattern of TPMT allele frequency in Moroccans is not similar to any other population; even though the geographical proximity and the African origins of the Moroccans suggest that TPMT allele frequency should be similar or somehow approaching the frequency in Tunisians. TPMT*2,*3B and *3C were not detected among 206 studied alleles, which indicates that the Moroccans population is different and it might have another genetic variation pattern.

Even though the results of the study did not show the presence of the most common TPMT variant alleles, more studies are needed to investigate TPMT in Moroccans with a much larger population, taking into consideration patients from

different sub –regions and also from the same family as well as investigating other genetic polymorphisms. The genotype of the Moroccan population did not reveal any of the tested polymorphisms; and so the results may be explained by the limited size of the population studied.

Since the major variant alleles responsible for toxicity are not found in the Moroccan population, normal doses of thiopurine drugs can be administered to these patients without any fear of toxicity.

Table 5: Allele frequencies of TPMT in Moroccan population compared to Africans and Caucasians [75]

Populations	N(subjects number)	TPMT*2	TPMT*3A	TPMT*3C
Caucasians				
British	191	0.5	4.5	0.3
French	199	0.5	5.7	0.8
Italian	206	0.5	3.9	1
Swedish	800	0.1	3.8	0.4
Norvegian	66	0	3.4	0.3
Semi-norvegian	194	0	0	3.3
West/east Africa				
Kenyan	101	0	0	5.4
Ghanaian	217	0		7.6
North-Africans				
Egyptians	200	0	0.3	1.3
Tunisians(2011)	119	0	1.68	0
Tunisians(2012)	208	0	0	2.4
Moroccans(present study)	103	0	0	0

III. PHARMACOGENETICS IN THE DIAGNOSIS AND TREATMENT OF CANCER

1. The role of genetics in the development of cancer.

Several factors b oth internal and external may contribute to the development of cancers [47].In light of the growing prevalence of cancer and its implications to modern healthcare, more work is being done by the scientific community to research on the disease. However, whilst most of the research have been dedicated to studying the environmental influences on cancer, genetic factors (or determinants) have been overlooked.

Presently the role of genetics in the development of cancer is being unearthed. Virtually every cancer occasionally runs in families (which could reflect host or environmental factors, or both); and each cancer type is an occasional complication of some hereditary condition usually, a rare one. So, while, most cancers have some genetic determinants, few common cancers can be largely attributed to a single major mutant gene, but for breast colon, ovary, prostate and lung cancers rare mutant genes that enormously increase the risk have been discovered.

Knowledge of genetic predisposition to cancer and corrective lifestyle changes can help an individual avoid the chances of developing the disease, and in some cases, avoiding it all together.

Recently, the role of genetics in the development of cancer is widely being recognised. The advancement in genetic research and technologies is providing new possibilities in the screening; management and treatment of cancer.

1.1 Genes involved in the development of tumors

A tumour is an abnormal cell growth. Cancer (malignant tumor) is a disease characterised by abnormal cell with the potential to spread to other parts of the

body [48]. Cancer is caused by accumulation of genetic and epigenetic mutations in genes that normally play a role in the regulation of cell proliferation, thus leading to uncontrolled cell growth. Cells acquire these mutations as a result of spontaneous or environmentally-induced DNA damage. Genes involved in mutations include those whose products;

1) directly regulate cell proliferation (either inhibiting or promoting cell growth)
2) control programmed cell death or apoptosis
3) are involved in the repair of damaged DNA

Depending on how they affect these processes, these genes can be grouped into two categories; **tumor suppressor genes and proto-oncogenes .**

1.1.1 Tumor Suppressor Genes

Tumor suppressor genes are defined as genes which encode proteins that inhibit the formation of tumors. Their normal function is to inhibit the development of tumors and therefore act as 'brakes' that control the cell cycle. Mutations in tumor suppressor genes contribute to the development of cancer by inactivating their inhibitory function. These mutations are termed **loss-of-function** mutations. Inactivation of both pairs of tumor suppressor genes is required before their function can be eliminated. Since one copy of tumor suppressor genes is enough to control cell proliferation, both alleles of a tumor suppressor gene must be lost or inactivated in order to promote cell development. Therefore mutations in tumor suppressor genes are recessive at the level of an individual cell. Five broad classes of proteins are generally recognised as being encoded by tumor suppressor genes [49]*:*

- intracellular proteins such as p16 cyclin kinase inhibitor that regulate or inhibit progression through a specific stage of the cell cycle.

- Receptors for secreted hormones (e.g. tumor derived growth factor) that inhibit cell proliferation.
- Checkpoint –control proteins that arrest the cell cycle if DNA is damaged or abnormal.
- Proteins that promote apoptosis.
- Enzymes that participate in DNA repair.

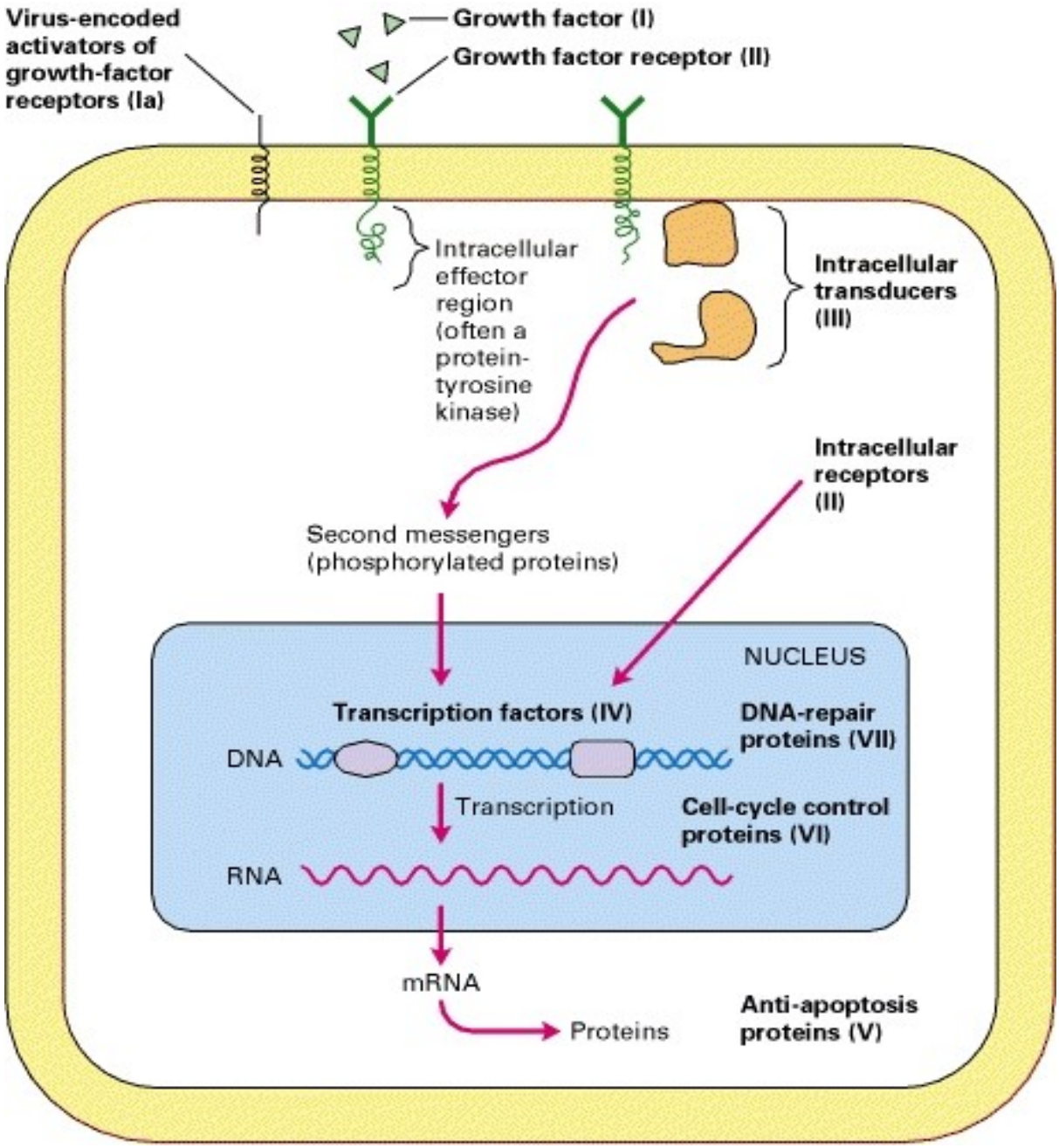


Fig 5.[50]

Although DNA repair do not directly function to inhibit cell proliferation, cells that have lost their ability to repair errors, gaps or broken ends in DNA accumulate mutations in many genes, including those that are critical in controlling cell growth and proliferation. Thus loss-of function in DNA repair enzymes contribute to the inactivation of tumor suppressor genes and activation of oncogenes.

Some examples of tumor suppressor genes are:

- Retinoblastoma protein (RB) implicated in human retinoblastoma.
- p53 encoded by Tp53 protein. Homozygous loss of p53 is responsible for 65% of colon cancers, 30%-50% breast cancers and 50% lung cancers. Mutated p53 is also involved in the pathophysiology for lymphomas , sarcomas and neurogenic tumors.
- others include APC, CD95, ST14, ST7, ST5

1.1.2 Oncogenes

An oncogene is any gene that encodes a protein able to transform cells in culture or to induce cancer in animals. Cells contain many normal genes that are involved in regulating cellular proliferation. Some of these genes can be mutated to forms that promote uncontrolled cell proliferation. The normal forms of these genes are called proto-oncogenes, while the mutated, cancer-causing forms are called oncogenes. Whilst tumor suppressor genes inhibit cell proliferation, oncogenes actively promote cell growth. Mutations that convert proto-oncogenes to oncogenes typically increase the activity of the encoded protein or increase the expression of the normal gene. Such mutations are dominant or **gain-of function** mutations. Hence mutation in only one of the two alleles is needed for the induction of cancer.

Some examples of oncogenes are indicated in Table 6.

Table 6: Examples of oncogenes and the types of cancers they cause

CATEGORY	EXAMPLES	CANCERS	GENE FUNCTIONS
Regulatory GTPases	Ras protein	Colorectal cancers, pancreatic cancer, cancer of the thyroid, myeloid leukemia	Involved in signalling a major pathway leading to cell proliferation
Transcription factors	Myc gene	Malignant T cell lymphomas, acute myeloid leukemia, breast cancer, pancreatic cancer	Regulate transcription of genes that induce cell proliferation
Receptor tyrosine kinase	EGFR, VEGFR, PDGFR, HER2/neu	Breast cancer, gastrosintestinal stromal tumors, pancreatic cancer, non small cell lung cancer	Transduce signals for cell growth and differentiation
Growth factors, mitogens	C -sis	Glioblastomas, fibrosarcomas, breast carcinomas, melanomas	Induces cell proliferation

1.1.2.1 Ras family of proteins

Ras proteins are small GTPases(guanidine triphosphates) and are involved in transmitting signals within cells. When Ras is switched on, it subsequently switches on other proteins which activate genes involved in cell growth, differentiation and survival. As a result mutation in *Ras* gene can lead to permanently activated Ras proteins which cause overactive and unintended cell signalling. Because these signals lead to cell growth and division, overactive Ras signalling can ultimately lead to cancer [51].

The most studied members of **RAS** (derived from **RA**t **S**arcoma virus), the most common oncogenes in human cancer are **NRAS KRAS and HRAS.** Mutations that permanently activate Ras genes are found in 20% to 25% of human tumors and 90% of certain types of cancer.

Oncogenic mutations in the Ras gene prevent the protein from hydrolyzing GDP to GTP. As a result the protein always remains in its active GTP –bound form, continually activating the MAP kinase cascade,leading to proliferation.

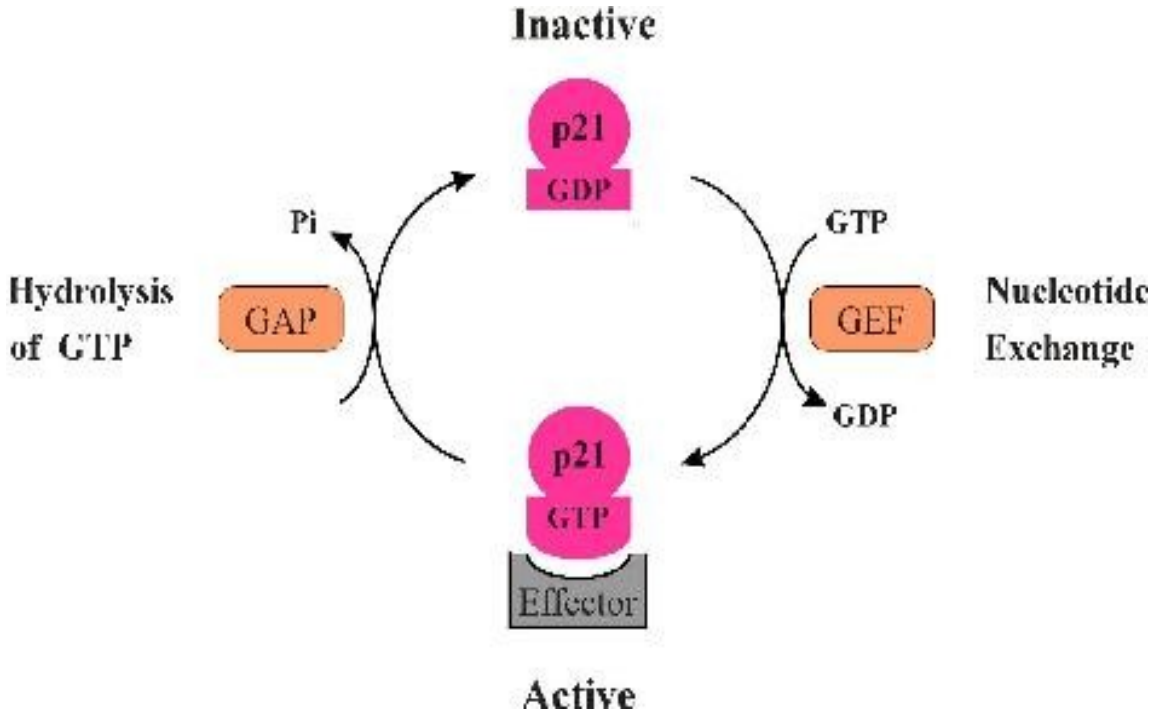


Fig 6: How mutations in the ras oncogene promote cancer [52]

1.1.2.1.1 K RAS

The KRAS gene, encodes the human cellular homolog of a transforming gene isolated from the Kirsten rat sarcoma virus. It is located on chromosome 12(12p11.22). It encodes for a 21kD protein located on the inner surface of the plasma membrane and has a GTPase activity. This protein is an essential component of the signal transduction cascade downstream the EGFR membrane receptor. Fig 6 [53].

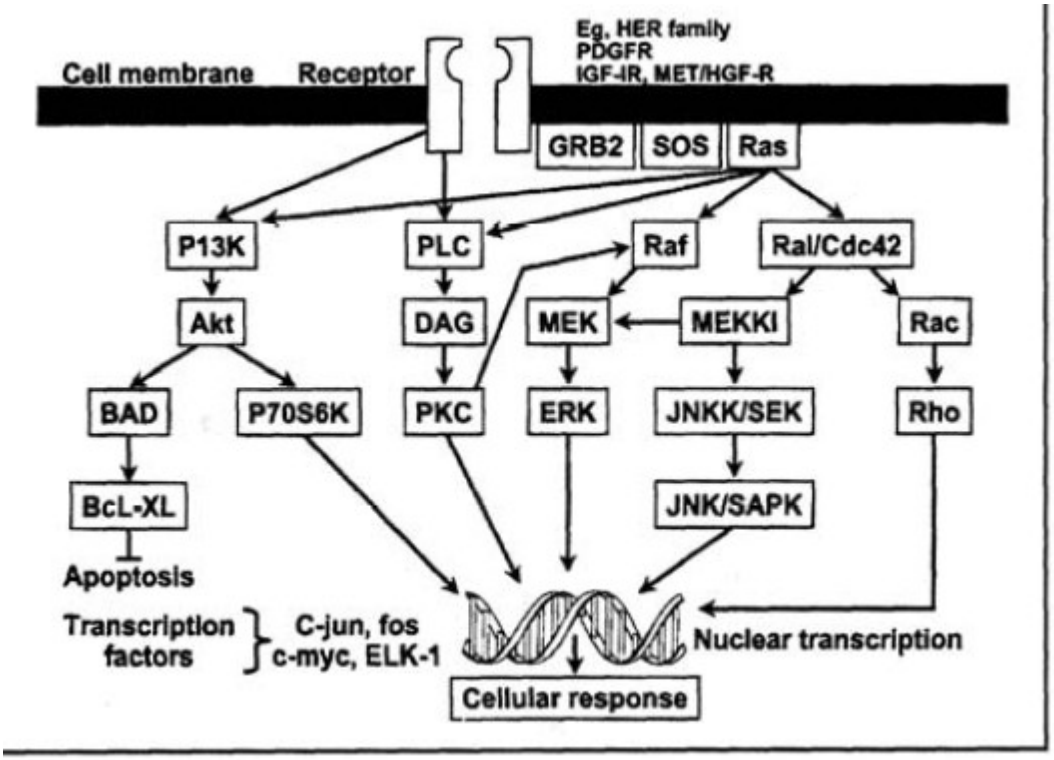


Fig 7: schematic representation of the activation pathways controlled by EGFR and KRAS

Kras is one of the first oncogenes to be discovered. Mutations in this gene have been detected in a large number of of human cancers notably in lung cancer, pancreatic cancer and thyroid cancer [54].Kras acquires activating mutations in about 30% of colorectal cancers [55]. These somatic mutations cause constitutive activation of EGFR pathway (independent of ligand binding to its receptor),below the receptor and therefore not adjustable by antiEGFR agents.The most frequent

mutations were detected in codons 12 (~82% of KRAS mutations) and 13 (~17%) of exon 2 of KRAS gene. Mutations in codon 61 and 146 have been reported but represent only a minor prortion of all KRAS mutations and their clinical relevance in colon cancer is not very clear.

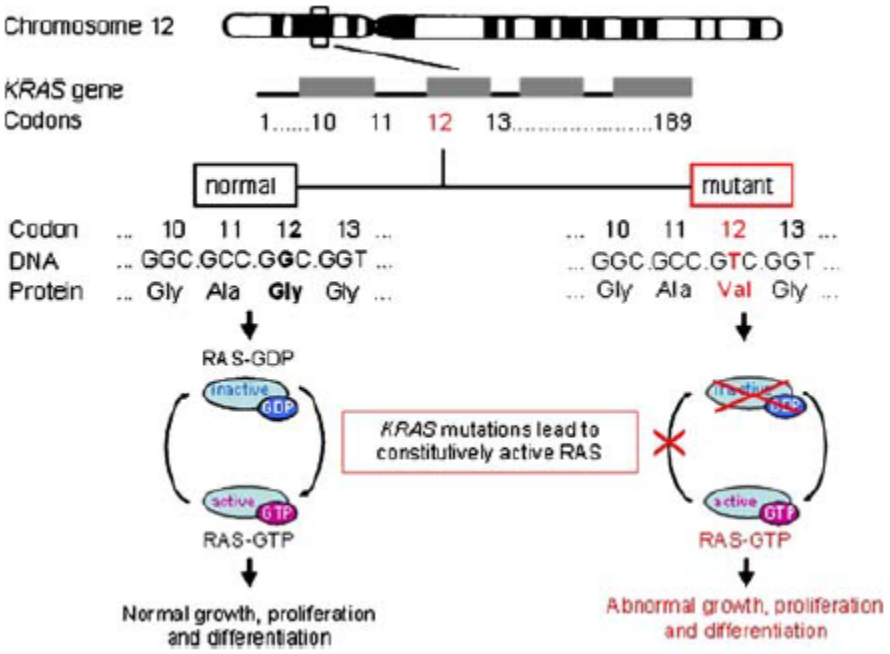


Fig. 8: Role of KRAS mutations in oncogenic activation of intracellular signalling [56].

2. Targeted therapy

Targeted therapy refers to drugs or other substances that block the growth and spread of cancer by interfering with specific molecules (molecular targets) that are involved in the growth, progression and spread of cancer, instead of simply interfering with all rapidly developing cells (e.g. as in the case of traditional chemotherapy). This is an aspect of personalised medicine that seeks to treat patients based on the unique characteristics of the tumor or the patient (genetic profile).

For decades, the hallmark of medical treatment of cancer has been intravenous cytotoxic chemotherapy. These drugs target rapidly dividing cells, including cancer cells and certain normal tissues. As a result, many patients experience many side effects such as alopecia, gastrointestinal symptoms, and myelosuppression. Even though targeted therapies are technically considered chemotherapy, and present their own adverse effects such as acneiform rash **(Fig 9)** [58], cardiac dysfunction , thrombosis, hypertension and proteinuria, they are however, better tolerated than traditional chemotherapy. These drugs are currently used solely or in association with chemotherapy, surgery or radiation therapy **[57]** in the treatment of many malignant tumors, including breast, lung, colon and pancreatic cancers, as well as lymphoma, leukemia and multiple myeloma. Some differences between target therapies and chemotherapy are listed in Table 4.

Table 4: differences between targeted therapy and standard chemotherapy

Targeted therapy	Standard (traditional) chemotherapy
Act on specific molecular targets that are associated with cancer	Act on all rapidly dividing normal and cancerous cells
Deliberately chosen to interact with their target	Identified because they kill cells
Often cytostatic i.e. they block tumour cell proliferation	Cytotoxic in nature i.e. they kill tumour cells
Less harmful to normal cells and more effective	Harmful to normal cells. Lead to destruction of DNA

2.1 Biology of targeted therapy

Traditional cytotoxic chemotherapy works primarily through the inhibition of cell division. [58]. In addition to cancer cells, other rapidly dividing cells (e.g., hair, gastrointestinal epithelium, bone marrow) are affected by these drugs. In contrast, targeted therapy blocks the proliferation of cancer cells by interfering with specific molecules required for tumor development and growth [58]. Some of these molecules may be present in normal tissues, but they are often mutated or overexpressed in tumors. Among the earliest targeted therapies were antibodies directed against the cell surface markers cluster of differentiation 20 (CD20), CD33, and CD52, which are present on lymphoma and leukemia cells. Because CD20 is also present on normal lymphoid cells, targeting of this molecule affects overall immune function. This observation has led to the use of the anti-CD20 monoclonal antibody rituximab (Rituxan) for the treatment of autoimmune diseases such as rheumatoid arthritis [59,60] in addition to non-Hodgkin's lymphoma.[61].

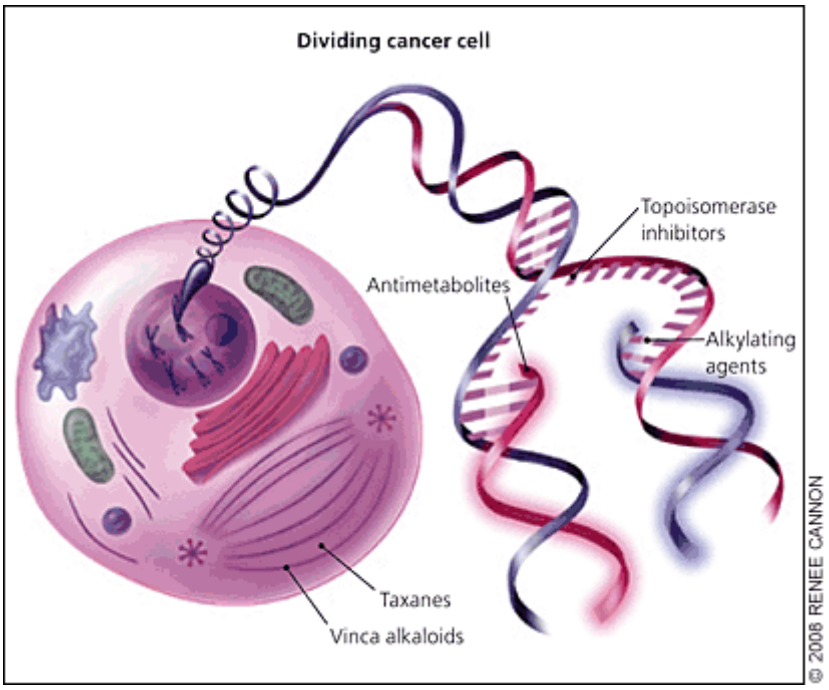


Figure 9:[58]

Mechanisms of traditional chemotherapy. These drugs act on rapidly dividing cells, which include normal tissues (e.g., hair, gastrointestinal epithelium, bone marrow) in addition to cancer cells. Alkylating agents interfere with DNA base pairing, leading to strand breaks and arresting DNA replication. Topoisomerase inhibitors prevent DNA uncoiling. Taxanes and vinca alkaloids interfere with micro-tubule function required for cell mitosis. Antimetabolites block the formation and use of nucleic acids essential for DNA replication.

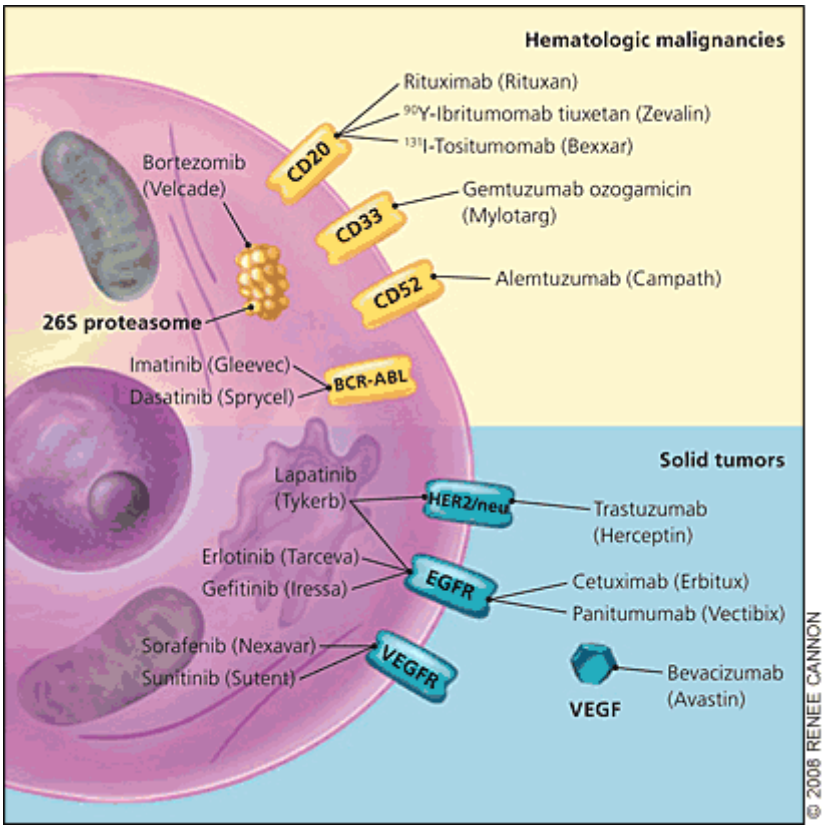


Figure 10:[58]

Mechanisms of targeted therapies. The molecular targets in this figure are not over-expressed in a single cell type, but rather on various malignant and normal tissues. For example, CD20 is present on lymphoma and normal lymphoid cells, HER2/neu is present on 25 percent of breast cancer cells, and VEGFR is present on normal and tumor-associated vasculature. Downstream intracellular signaling molecules, some of which are targeted by small molecule inhibitors, are not depicted. Some drugs (e.g., sorafenib [Nexavar], sunitinib [Sutent], imatinib [Gleevec], dasatinib [Sprycel]) have multiple targets, most of which are not depicted.

The molecular pathways most often targeted in the treatment of solid tumors are those of the epidermal growth factor receptor (EGFR, also known as HER1), vascular endothelial growth factor (VEGF), and HER2/neu. Such pathways can be inhibited at multiple levels: by binding and neutralizing ligands (i.e., molecules that bind to specific receptor sites on cells); by occupying receptor-binding sites (thereby preventing ligand binding); by blocking receptor signaling within the cancer cell; or by interfering with downstream intra-cellular molecules.

In some instances, targeted therapy has led to truly tailored therapy. Trastuzumab (Herceptin) is a monoclonal antibody directed against HER2/neu, a molecular target related to EGFR that is over-expressed in approximately 25 percent of patients with breast cancer. Because trastuzumab is ineffective in the 75 percent of patients with breast cancers that do not overexpress HER2/neu, it is used only if HER2/neu overexpression is documented in tumor tissue **[62].** Similarly, targeting of EGFR in patients with non-small cell lung cancer is most effective against cancers that are highly dependent on the EGFR signaling pathway **[63].**

2.2 Types of targeted therapy

Many different types of targeted therapies are used in the treatment of cancer. Targeted therapies come in two main forms **[65,66,67]**:

- Monoclonal antibodies
- Small molecule inhibitors.

Table 8: Differences between small molecule inhibitors and monoclonal antibodies [65,66,67]

	Monoclonal antibodies	Small molecule inhibitor
Mode of administration	Intravenous	Orally
Metabolism		Cytocrome P450
Efficacy	Highly effective.target-specific	Achieve less specific targets
Mode of manufacturing	Bioengineered, hence more expensive	Chemically produced and therefore less expensive
Half-life	Ranges from days to weeks	A few hours

2.3 Candidates for targeted therapy

Not all cancer patients benefit from targeted therapy. The criteria for selecting patients for a particular targeted therapy depends on the type of cancer .

For example, patients with certain types of cancer should have an appropriate target before given a particular targeted therapy. In chronic myelocytic leukemia, the ABL BCR gene is the required target.

In other cases, the tumor tissue must have a particular gene mutation that encodes the target. Patients who do not have the mutation will not be candidates because the therapy will have nothing to target.

Sometimes a patient is selected only when their tumor is inoperable or when they do not respond to other treatments e.g. traditional chemotherapy.

2.4 Limitations and side effects of targeted therapy

It has been known that some patients develop resistance to targeted therapy drugs in a variety of ways. Resistance can occur in two ways:

- the target changes through mutation so it no longer interacts with the the drug,
- or the tumor finds a new pathway in such a way that in no longer depends on the target.

For this reason it is always advisable to combine two targeted therapy drugs. Another way to prevent the development of resistance is to combine targeted therapy drugs with traditional chemotherapy drugs.

Patients on targeted therapy drugs are known to present certain side effects. The side effect may depend on the particular drug the patient is taking in association with other drugs. In some cases, the presence of side effects has been an indicator of the efficacy of the drug. Some of the most common side effects of targeted therapy are:

- toxicity of the liver, causing hepatitis and elevated liver enzymes
- diarrhea
- hypertension
- proteunuria
- skin problems

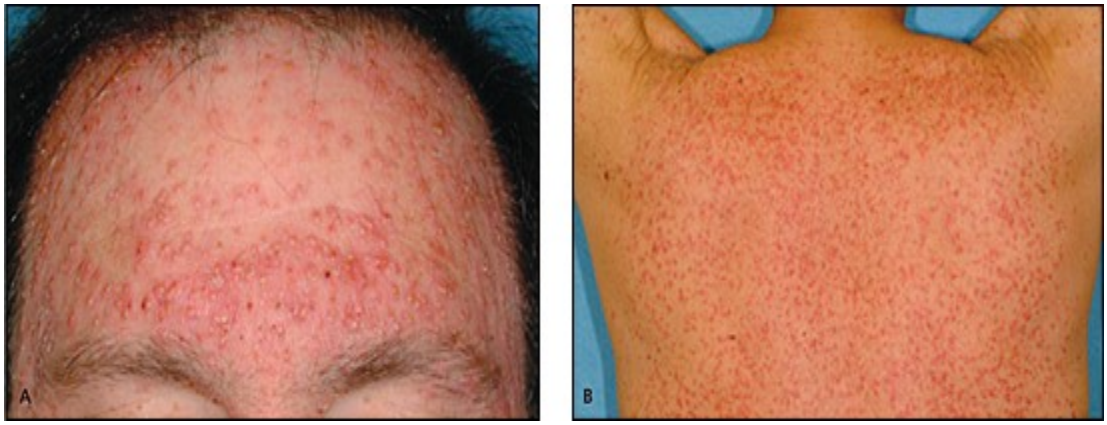


Figure 9 :[58]

Acneiform rash on *(A)* the face and *(B)* back of patients treated with cetuximab (Erbitux), a monoclonal antibody targeting epidermal growth factor receptor.

3. The role of pharmacogenetics in the treatment of colorectal cancer.

Colorectal cancer is the third most commonly diagnosed cancer and the first leading cause of cancer death in both men and women in the United States. In Morocco, it is the second cause of cancer of the digestive tract after that of stomach cancer. It is one of the best examples of the different stages of carcinogenesis process. Pharmacological treatment in addition to surgical resection of CRC patients has increased survival rates over the past several years. Inherited individual genetic variation in key genes that are associated with survival, tumor recurrence or progression, response to treatment, and frequency and severity of chemotherapy-related toxicities can have a great impact on treatment outcome.

The role of pharmacogenetics in the treatment of metastatic colorectal cancer depends on the identification of molecular predictors of response and toxicity. The goal is to use pharmacogenetics to develop tailored therapeutic strategies for individuals with the hope of maximising benefit and reducing toxicity.

Over the past decade, significant advances have been made in the treatment of this cancer with the introduction of 3 cytotoxic agents, [5-

fluorouracil(capecitabine), oxaliplatin and irinotecan] and 3 target agents,(bevazimumab, cetuximab, panitumumab) as clinical agents. Each of these agents have a high degree of variation to toxicity and anti-tumor activity.

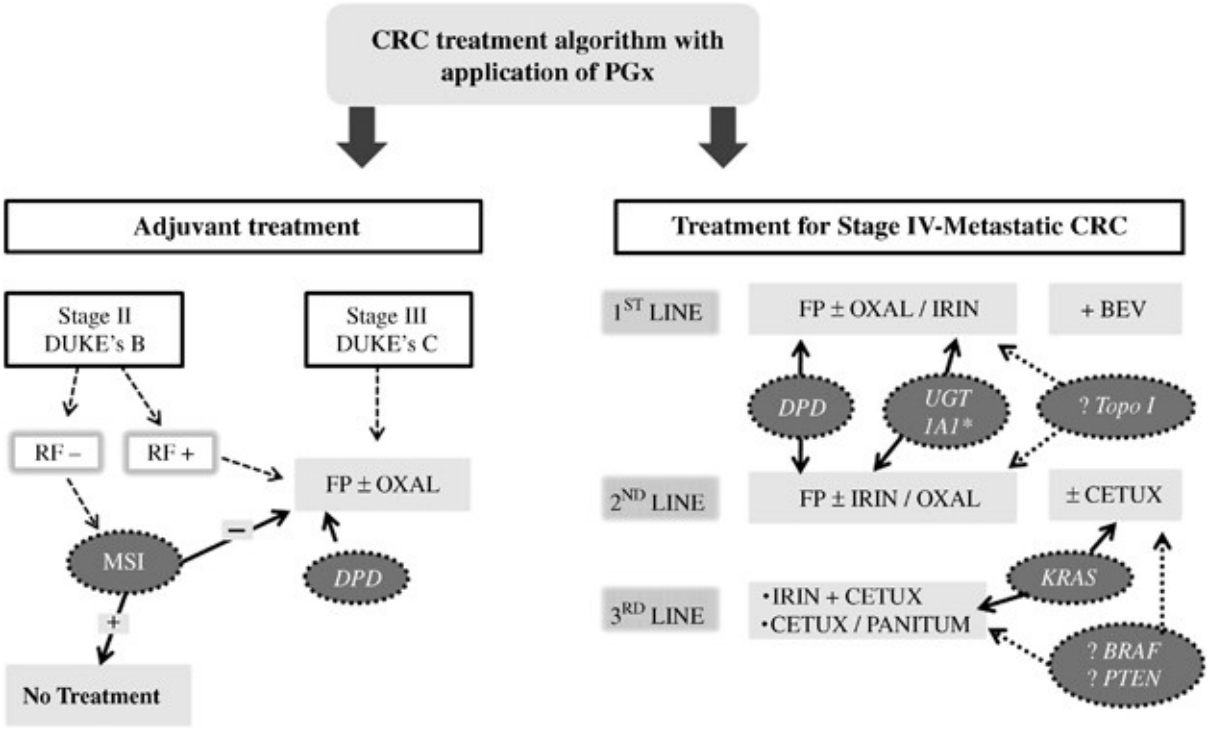


Fig 10 [121].

Incorporation of pharmacogenetics in the treatment of CRC. Treatment algorithm with potential PGx applications. Patients with early stage CRC may avoid unnecessary or even harmful chemotherapy by determining their MSI and/or their DPD proficiency status. Patients with advanced disease may be selected for appropriate first-, second- or third-line chemotherapy according to their tumor KRAS status, but may also avoid toxicities by determining the presence of UGT1A1*28 mutation or DPD deficiency. There is growing evidence on the role of BRAF and PTEN alterations and topoisomerase I expression before the use of EGFR antibodies and irinotecan or oxaliplatin; a validation by large studies is underway.

3.1 Oxaliplatin

Oxaliplatin is a relatively new platinum analogue that is currently used in pharmacotherapy of metastatic colorectal cancer (CRC). It is a third-generation platinum compound that was licensed for adjuvant treatment in combination with 5-fluorouracil (5FU) and leucovorin (LV) in metastatic CRC in the EU since 1999 and in the USA since 2002.

Research shows that oxaliplatin has greater efficacy on metastatic CRC with little toxic effects as compared to the first platinum analogue, cisplatin. Patients receiving oxaliplatin should have experienced recurrence or progression of metastatic disease within 6 months of completion of first-line 5-FU/ LV + irinotecan combined therapy.

Oxaliplatin shows synergistic cytotoxic effects with 5-FU and LV. Combination therapy (85 mg/m2 every 2 weeks or 130 mg/m2 every 3 weeks) has a two fold higher response rate compared to 5-FU/LV therapies and also improves progression free survival (PFS) in chemotherapy-naive patients. The main dose-limiting toxicity of oxaliplatin is a peripheral neuropathy that affects 85-95% of all treated patients, and is reversible after treatment discontinuation.

Oxaliplatin underlies non-enzymatic biotransformation processes that form reactive intermediates. It exerts its anti-tumor effects by formation of intra- and inter-strand platinum-DNA adducts between the aquated oxaliplatin derivative and a DNA base, leading to apoptosis, inhibiting cellular replication and possibly interfering with RNA synthesis. These can be counteracted by cellular defense mechanisms preventing DNA damage through either increased detoxification of platinum-DNA adducts catalyzed by glutathione-*S*-transferases (GSTs), or via DNA-repair pathways leading to increased DNA-repair activity and improved removal of

platinum-DNA adducts, finally resulting in a reduced anti-tumor acting capacity of oxaliplatin.

Genetic polymorphisms affect the activity of cellular DNA repair and platinum conjugation. There is growing evidence that polymorphisms in genes coding for DNA repair enzymes and metabolic inactivation routes contribute to the inter-individual differences in anti-tumour efficacy and toxicity of oxaliplatin.

Glutathione-S-transferases (GSTs) are phase II metabolizing enzymes involved in the cellular detoxification of electrophilic xenobiotics, including platinum derivatives, by catalyzing the conjugation with glutathione. GSTs support inactivation and excretion of platinum compounds, and therefore prevent cells from DNA damage, but also lead to decreased efficacy of oxaliplatin treatment.

Several polymorphisms in the GST subclasses (*GSTP1*, *GSTT1* and *GSTM1*) that may alter GST activity have been studied in association with oxaliplatin therapy. Decreased or abolished enzyme activity has been linked to a reduced detoxification capacity, leading to an increased efficacy of platinum compounds. A single nucleotide polymorphism (SNP) in exon 5 at position 313 (A to G) in the GSTP1 gene results in a valine being incorporated into this enzyme at site 105 instead of the usual isoleucine (Ile105 fi Val). The mutant GSTP1 enzyme is less potent in detoxification of carcinogens **[122]** and individuals with two mutant alleles have shown a significant survival benefit from combined oxaliplatin/5-FU treatment with a median survival of 24.9 months compared to only 7.9 months for metastatic colorectal cancer patients with two wild-type alleles.

Other common polymorphisms in the GSTT1 and GSTM1 genes include deletions that result in complete loss of enzyme activity in homozygous individuals. However, no association with altered survival or clinical response in patients with advanced colorectal cancer treated with oxaliplatin/5-FU was observed for the

GSTT1 and GSTM1 genotypes. It seems therefore likely that the GSTT1 and GSTM1 subclass enzymes play a less important role in colorectal tissue (cancer) cells as compared to the p subclass. Recent findings confirm that p subclass enzymes are over expressed in colorectal cancer tissues relative to normal mucosa (123).

DNA-repair genes that participate in the repair of damaged nucleotides, such as platinum-induced DNA adducts, include genes of the nucleotide-excision repair (NER) pathway (e.g., *ERCC1*, *XPD* and *XPA*) and genes of the base-excision repair (BER) pathway (e.g., *XRCC1* and *XRCC3*) (124). Genetic polymorphisms in theses genes are responsible for low DNA repair capacity leading to efficacy of oxaliplatin or higher DNA and hence little or decrease response to oxaliplatin.

F.Z Hijri et al. of the department of medical oncology of the Hassan II University Hospital, Fes report a case of a 70 year old man diagnosed with a stage IIIb (UICC 2010 classification) colon adenocarcinoma who presents cytotoxicity induced by oxaliplatin after the administration of adjuvant chemotherapy. The cause of the hearing loss is unknown. However it is observed that the patient presents an improvement in his hearing when oxaliplatin was substituted with irinotecan and bevacizumab. No tests were however, performed to show if there is a genetic cause to this rare toxicity. Studying the genetic polymorphisms of genes may account for the cause of the ototocity in this patient.

3.2 Irinotecan

Irinotecan is a topoisomerase I inhibitor that has been approved for treatment in metastatic CRC patients either alone or in combination with 5-FU/leucovorin. Prolonged overall survival and increased response rates have been described for irinotecan/5-FU combination therapy compared with the single treatment with 5-FU/leucovorin (LV). The main dose-limiting toxicities often resulting in discontinuation of this effective treatment are severe diarrhea and

neutropenia. Irinotecan itself acts as an inactive prodrug; it can either be converted to its active metabolite SN-38 by carboxylesterase enzymes or metabolized to inactive metabolites by CYP isoforms.

The UDP-glucuronosyltransferase (UGT1A1) enzyme further conjugates and detoxifies SN-38 into inactive SN-38 glucuronide. P-glycoprotein encoded by ATP-binding cassette transporter B1 (ABCB1) is an important efflux pump for irinotecan.

Genetic polymorphisms associated with UGTs, CYP isoforms such as CYP3A4 and CYP43A5 has an effect on treatment outcome, either by reducing the effect of the drug or increasing its effect, thereby leading to toxicity.

3.3 5-fluorouracil

5-Fluorouracil (5-FU) continues to be the backbone of CRC treatment, even after more than 40 years of clinical use. 5-FU is converted into specific nucleotides, which results in the antitumor effect. Toxicities attributed to the drug include neutropenia, stomatitis, and diarrhea **[125]**. Thymidine phosphorylase (TP) is the enzyme responsible for conversion of 5-FU to fluorodeoxyuridine, which is then converted to fluorodeoxyuridine monophosphate, the active metabolite. The mechanism of 5-FU toxicity is primarily through inhibition of thymidylate synthase (TS), the rate-limiting enzyme in the pyrimidine nucleotide synthesis. Deoxyuridine monophosphate is normally converted to deoxythymidine monophosphate (dTMP) via TS. Inhibition of TS results in less dTMP and ultimately inhibition of DNA synthesis and repair.

3.4 Epidermal Growth Factor Receptor Targeted Monoclonal antibodies

Epidermal growth factor receptor (EGFR) has been validated as a therapeutic target in several human tumors, including colorectal cancer (CRC) **[81].**

Ligand occupancy of the EGFR activates the RAS/RAF/MAPK, STAT, and PI3K/AKT signalling pathways, which together modulate cellular proliferation, adhesion, angiogenesis, migration, and survival [82, 83].

In human colorectal cancer, EGFR is also associated with tumor development and progression. The mechanisms underlying the role of EGFR in colorectal cancer are not entirely clear. EGFR is over-expressed in up to 82% of colorectal cancers [84].

Mutations in the EGFR gene are however, rare in colorectal cancer but occur regularly in other types of cancer, such as lung cancer [85, 86]. Based on the importance of the EGFR axis in colorectal cancer, drugs that interfere with various functional domains of the receptor have been developed. Currently two anti-EGFR monoclonal antibodies have been approved in several countries for the treatment of colorectal cancer [87].

Cetuximab, a human-mouse chimeric IgG1 monoclonal antibody, was the first EGFR targeted agent approved for treatment of colorectal cancer [88]. Panitumumab, a fully human IgG2K monoclonal antibody, was recently approved in the US and Europe as third-line treatment of metastatic colorectal cancer [89]. Both antibodies have been shown to reduce the risk of tumor progression and to improve overall survival (OS), progression-free survival (PFS) and quality of life in patients with refractory colorectal cancer. [91-92].

The anti-EGFR targeted antibodies, cetuximab and panitumumab administered as monotherapy in CRC have shown response and disease stabilization rates of approximately 10% and 30%, respectively. Although EGFR expression is used for patient selection, clinical experience shows that the level of EGFR expression as measured by immunohistochemistry does not predict clinical benefit.

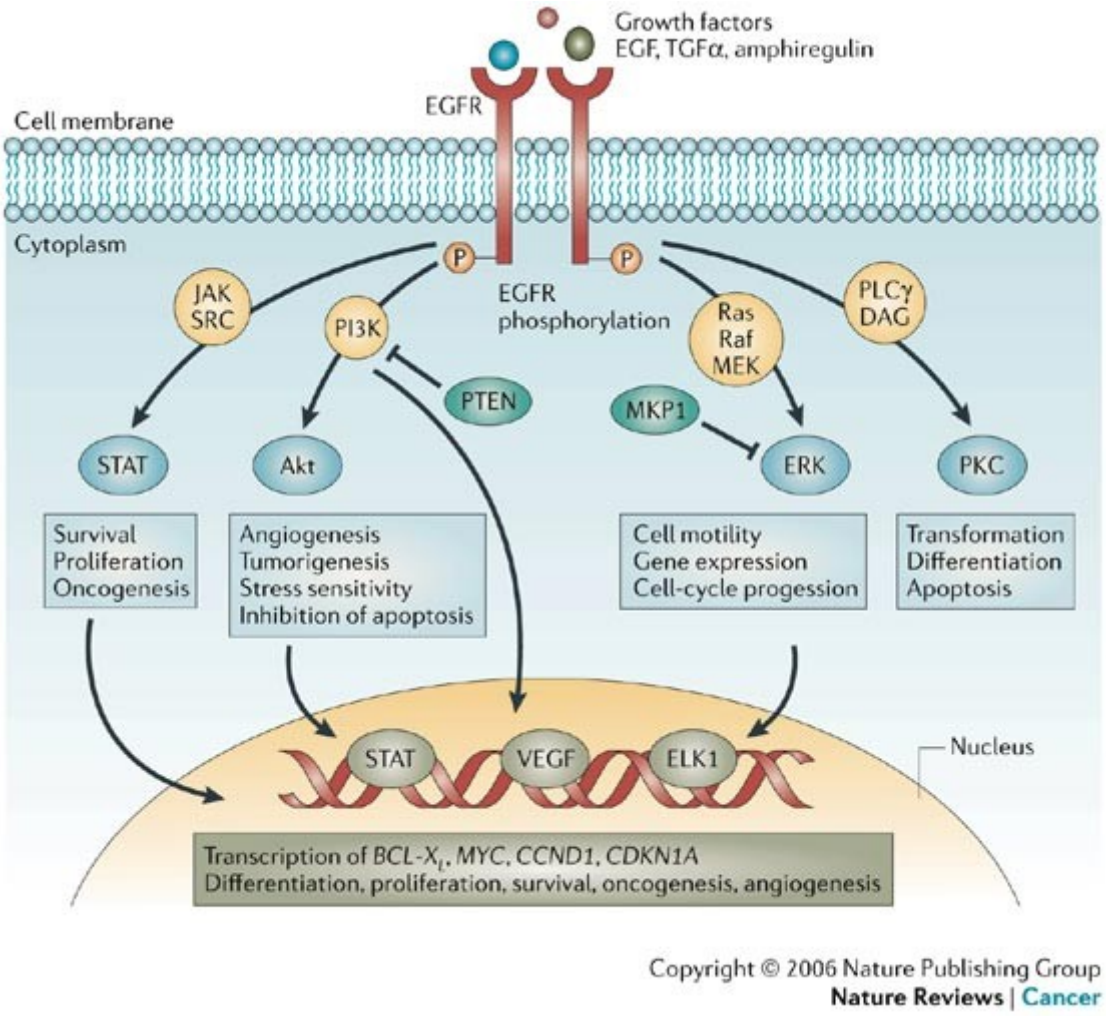


Fig 11: EGFR signalling pathways (90)

Numerous studies have proven that only a subset of patients with advanced colorectal cancer respond to anti-EGFR therapy. The human KRAS oncogene is mutated in over 30% of colorectal cancers. Over 3,000 KRAS point mutations in colorectal cancer have been reported thus far. Even though mutations in the KRAS gene have been confirmed as negative predictors to the response of anti-EGFR therapies, not all KRAS (wt-KRAS) wild type patients will respond to treatment. [93].

Recent studies have demonstrated that additionally wild type BRAF (wt-BRAF) genotype is required for response to panitumumab or cetuximab, suggesting that BRAF genotype criteria should be used together with KRAS genotype for selecting patients who are about to benefit from anti EGFR therapy [94].

BRAF and KRAS are two important members of the MAP kinase pathway. (MAPK) and are mutated in 30 to 40 % and 5 to 10% in CRC respectively [95]. It should be noted that the MAP kinase pathway is a fundamental signal transduction pathway with impact on cellular functions such as proliferation, differentiation and apoptosis, and is hyper activated in about 30% of human cancers [96].

To some extent, BRAF mutations and KRAS mutations can be considered as equivalent in their tumorigenic effect [97], and at least the T1799A transversion seems to be inversely correlated with the frequency of KRAS mutations [98,99].

KRAS mutations are, in most cases an early event in the development and progression of colorectal cancers. [100].

Consistent with this concept, several studies have demonstrated that KRAS mutation status is an important prognostic factor in colorectal cancer [101,102]. KRAS mutations are associated with tumors of more advanced stage, increased metastatic potential, poor prognosis, and decreased PFS and OS of patients [102,103]. The prognostic value of KRAS mutations in colorectal cancer is presently controversial and warrants further confirmation.

A study of Marchoud et al, aimed to study KRAS mutations in codons 12 and 13 which are located in exon 2 and to estimate the V600E BRAF in 92 patients with advanced colorectal cancer. [104].

The results of their study showed that, out of the 92 patients tested, 70 patients had the wild type KRAS gene, and 22 (23.91%) mutations were found were found in 92 patients. Only one patient exhibited more than one mutation, a codon 12KRAS mutation and a BRAF V600E mutation.

As the activating mutations of the KRAS gene are found in 30 to 40% of colorectal tumors, the KRAS status (wild-type versus mutated type) has been shown to predict the response to EGFR-targeted therapies with monoclonal antibodies in patients with metastatic CRC. In their study, among the 92 successfully tested Moroccan patients with advanced CRC, the ratio of mutated (23.91%) versus non-mutated KRAS patients (76.09%) was lower than described in other studies [105,106]. The distribution of the seven tested KRAS mutations among the mutated KRAS patients was in concordance with the distribution reported from other countries [107,108]. Studies from various countries have analysed the frequency of the type of KRAS point mutation in CRC. Most of authors have identified the G>A transition as the most frequently found type of KRAS mutation [109,110]. In the current study, the G>A transition appeared also to be the predominant mutation.

This oncogenic BRAF activation therefore, the downstream of EGFR affects the response to anti-EGFR inhibitors. As studies show, the presence of the V600E mutation in BRAF correlates with the lack of response to cetuximab and panitumumab and reduces the progression-free survival and overall survival compared to the treated wt-BRAF patients [112,113]. Although the mutations in KRAS are considered to be a highly specific negative biomarker of response to cetuximab and panitumumab (nearly 95% of the patients with mutations fail to

respond to treatment), the selection of patients for anti-EGFR treatment on such basis is not sensitive enough.

Namely, as much as 40 to 60% of the patients with wt-KRAS fail to respond to treatment with anti-EGFR-targeted monoclonal antibody therapy **[114].** BRAF mutational status is of utmost importance to be verified as another molecular determinant of response to anti-EGFR-targeted monoclonal antibody therapy.

In their group, they found 5.43% patients with the V600E mutation in BRAF which is similar to the published by Di Nicolatino et al. 2008 data reporting the BRAF V600E mutation in the range of 3 to 10% **[115,116].** One patient with the V600E mutation in BRAF was mutated-KRAS. However, most of authors reported that mutations in KRAS and BRAF are mutually exclusive **[117**]. While summing up the mutational status of KRAS and BRAF in terms of responsiveness to anti-EGFR treatment, the two markers identified up to 28.26% of non-responders.

Several studies have also reported that KRAS mutations confer resistance to anti-EGFR monoclonal antibodies [118,119]. KRAS mutations are associated with poor responses to therapy, reduced PFS and shorter OS in colorectal cancer patients treated with cetuximab alone or in combination with chemotherapy **[118].** Similarly, an analysis of KRAS mutations in tumor samples from 92% of patients by Amado RG et al. in a registrational clinical trial of panitumumab for the treatment of metastatic colorectal cancer predicted a lack of efficacy of panitumumab on PFS and OS in patients with KRAS mutant tumors.

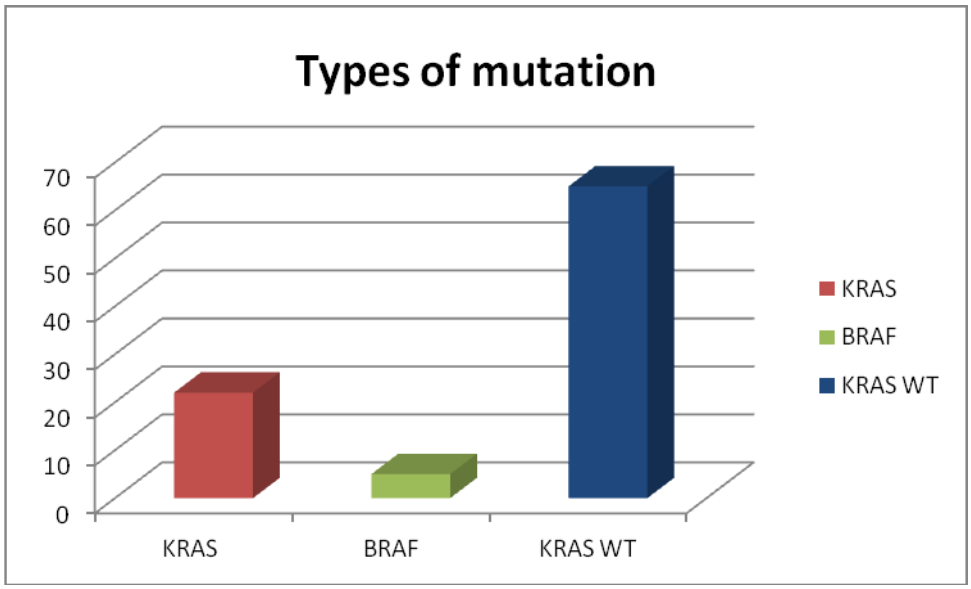
Taken together, these results indicate that KRAS mutation status is an important parameter for selecting patients for therapy: patients with mutant tumors will not benefit from EGFR-targeted therapies. On the basis of these data, the European Medicines Agency (EMEA) has approved the use of cetuximab and

panitumumab for the treatment of metastatic colorectal cancer in patients who carry a normal, wild-type KRAS gene [120].

However, as only a fraction of patients with colorectal tumors that carry a wild-type KRAS allele can achieve a clinical response with EGFR-targeted therapies, the search for additional predictive parameters remains an important tool in the treatment of patients without this mutation. Furthermore, more research need to be done on the multiple genetic variations involved in the carcinogenesis of colorectal cancer. This will better modulate therapeutic choices, allowing more patients to benefit from new targeted therapies.

Table 6: Distribution of KRAS and BRAF mutations in 92 Moroccan patients.

MUTATION	N° of Patients	Percentage (%)
KRAS	22	23.9
BRAF	5	5.4
NONE	65	70.7
TOTAL OF PATIENTS	92	



PERSPECTIVES AND RECOMMENDATIONS

Pharmacogenomics promises to unravel the genetic variability in drug response. This is built on the success of pharmacogenetics in establishing causal relations between single-gene polymorphisms and some individual drug responses.

The importance of pharmacogenetics in drug therapy also manifests in its potential to translate into individualized medicine, drug development and drug regulation which need to cope with individual variability in drug therapy and are only at the beginning of meeting this difficult and complex challenge The achievements of personalized/individualized medicine so far has been limited. Good clinical data to support the use of genetic testing for the treatment of most diseases are still not available. At present, predictive genotyping for drug metabolizing enzymes does not occur routinely in clinical practice. This could be due to the lack of awareness and knowledge about pharmacogenetic variability among healthcare professionals and patients, but also due to lack of prospective studies that will show that pharmacogenetic testing contributes to treatment efficacy. Even in the case of some commonly used drugs, such as warfarin, there have been very few attempts to assess the benefits of pharmacogenetic testing for genetic polymorphism involved in its metabolism.

The early knowledge as to whether a polymorphic pathway is involved in drug metabolism/action will lead to a reduction in time and costs used in the development of a new drug. The knowledge on genes involved in drug response has already helped to develop new cancer treatment.

Even though the French National Academy of Medicine proposed the following recommendations, it will in no doubt also benefit the Moroccan population if applied:

1. The search for allelic variations in genes concerned should as soon as possible, be the rule before the prescription of a high-risk drug, that is to

say a drug that causes severe side effects, especially when the genetic polymorphisms involved affect a greater fraction of the population. This is the case, of vitamin K Antagonists, many immunosuppressors and chemotherapy drugs, among others. Hence the need to make these routine exams accessible in genetics laboratories, pharmacology and molecular biology care facilities, especially if they include departments for treatment of tumors or organ transplantation.

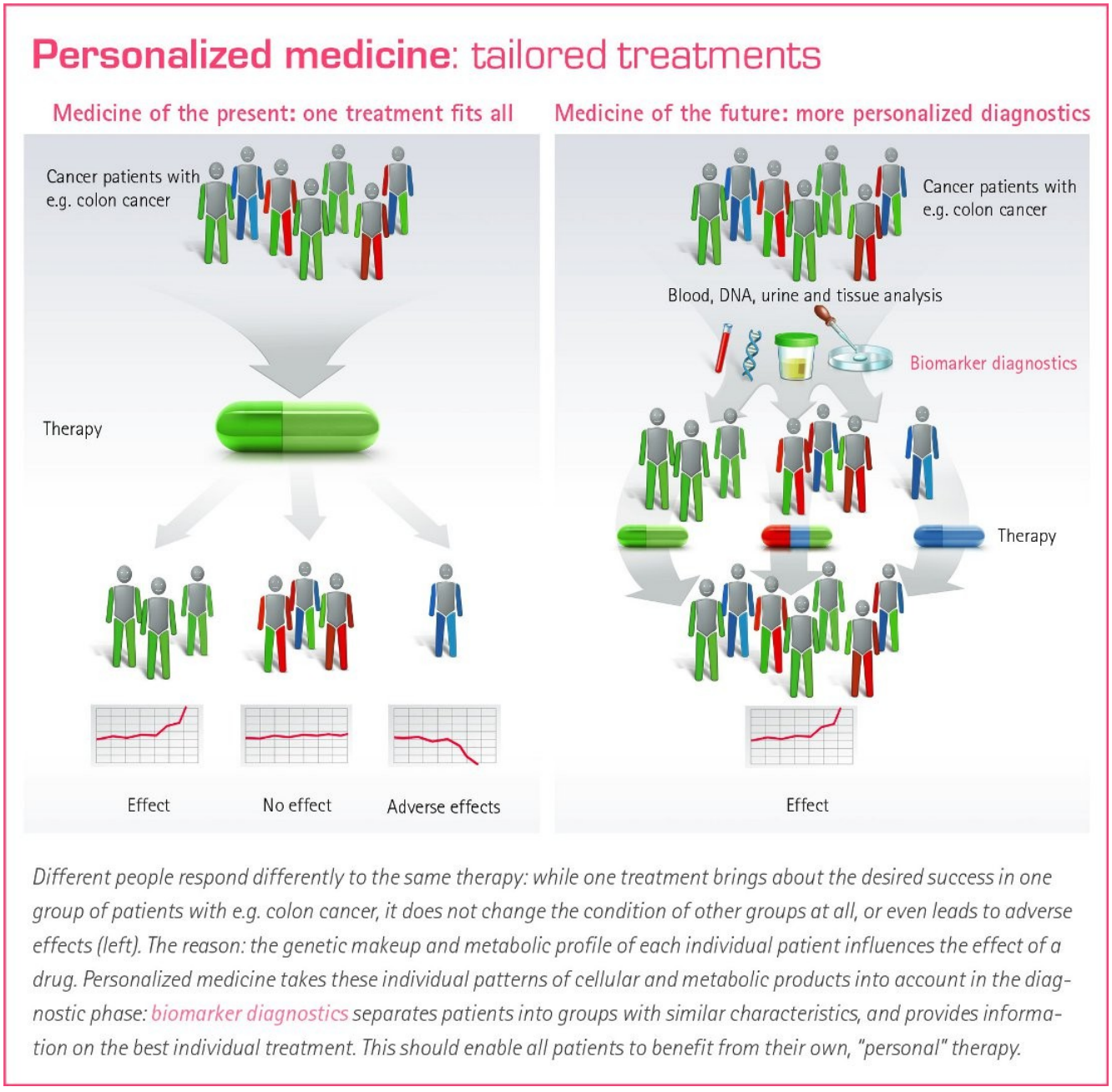
2. The significance of polymorphisms found in the genes of molecular targets or drug metabolizing enzymes should be validated by the study of phenotyping and the influence of these polymorphisms on drug efficacy as well as the occurrence of complications.
3. Laboratory tests covering polymorphisms of the most common drug metabolizing and transport enzymes should be available in the country and tested in targeted populations to see if such genotyping reduces the cost / benefit ratio.
4. The discovery of polymorphism known to cause an inappropriate response (hypersensitivity, resistance or severe side effects) when taking a particular drug must be communicated to the patient carefully and only if it has practical consequences (drug to avoid or use under supervision or associations to avoid). This discovery should then lead to a family survey to detect and inform exposed subjects.
5. The identification of the metabolic pathways of a drug under study by a pharmaceutical company must be systematic.
6. Also, it is important to create biological resource centres where tumor samples are grouped and studied, thereby establishing the relationship between the identity of the tumor and the effectiveness of various

therapies. Such centres should also gather DNA samples of the subjects included in pharmaco-epidemiology studies in order to take into account the allelic variants in drug response.

CONCLUSION

There is conclusive evidence that genetic variability of drug metabolizing enzymes and transporters or drug targets influence drug metabolism and disposition. There is also increasing evidence that genotyping for polymorphic drug metabolizing enzymes, in particular CYPs, has the potential to improve drug therapy and achieve higher response rates and reduced adverse effects. Many open questions still remain regarding the relevance of the knowledge of pharmacogenetic information for clinical end points and cost-benefit aspects of pharmacogenetic based dosing. These questions need to be answered by prospective randomized clinical trials.

Nevertheless pharmacogenetics offers a promise of personalized medicine in the future. Testing for multiple common genetic polymorphisms which can modify the efficacy or the adverse effects of treatment holds the promise of the individualization of treatment according to the individual's genetic background.



Different people respond differently to the same therapy: while one treatment brings about the desired success in one group of patients with e.g. colon cancer, it does not change the condition of other groups at all, or even leads to adverse effects (left). The reason: the genetic makeup and metabolic profile of each individual patient influences the effect of a drug. Personalized medicine takes these individual patterns of cellular and metabolic products into account in the diagnostic phase: biomarker diagnostics separates patients into groups with similar characteristics, and provides information on the best individual treatment. This should enable all patients to benefit from their own, "personal" therapy.

ABSTRACTS

ABSTRACT

Pharmacogenetics is the study of genetic differences in metabolic pathways that influence an individual's response to drug. Genetic factors account for 15-30% of the variability in drug response, however for some drugs this could be the major determinant in drug response.

Pharmacogenetics aims to identify genetic sources of variability in response to drugs by studying genetic variations affecting drug metabolizing enzymes, transporters and drug targets thus causing inter-individual variability in drug levels (pharmacokinetics), drug response (pharmacodynamics) and side effects.

The objectives of our work are: to report the Experience of the Hassan II University Hospital, Fes; to evaluate the challenges of this field in Morocco and propose some recommendations.

We report a study of the Medical Genetics Department of the Hassan II University Hospital, Fes aimed at studying the most common mutant alleles of the Thiopurine Methyl Transferase (TPMT) gene*; TPMT*2; TPMT*3A ,TPMT*3C* in 103 Moroccan patients. The results showed no mutant alleles among the 206 alleles tested, thereby explaining why Moroccans show little or no toxicity to thiopurine drugs.

We also report the different therapeutic choices in colorectal cancer and how the genetic polymorphisms coding for the metabolizing enzymes affect the response to these drugs.

It is important to develop this field in Morocco, by developing the concept of personalized medicine where treatment is tailored to suit the genetic make-up of each individual with the aim of improving treatment efficacy while reducing toxicity.

RÉSUMÉ

La pharmacogénétique est l'étude des variations génétiques qui influencent génétiques qui la réponse d'un individu aux médicaments. Les facteurs génétiques représentent 15 à 30% de la variabilité dans cette réponse. La pharmacogénétique vise à identifier les sources génétiques de cette variabilité en étudiant les polymorphismes génétiques intervenant dans la pharmacocinétique et la pharmacodynamique des médicaments.

Les objectifs de notre étude sont de rapporter l'expérience du CHU Hassan II de Fès, évaluer les limites de ce domaine et proposer des recommandations.

Nous rapportons une étude du service de génétique médicale du Centre Hospitalier Hassan II de Fès visant à étudier les allèles mutants les plus courants du gène TPMT*; TPMT * 2; TPMT * 3A, TPMT * 3C ,* chez 103 patients marocains. Les résultats ont montré qu'aucun allèle mutant n'a été retrouvé parmi les 206 allèles testés, ce qui explique pourquoi les Marocains montrent peu ou pas de toxicité aux médicaments thiopurines.

Nous présentons également les différents traitements adjuvants dans le cancer colorectal et les polymorphismes génétiques intervenants dans la réponse à ces médicaments.

Il est important de développer ce domaine au Maroc en développant le concept de la médecine personnalisée où le traitement est adapté en fonction du profil génétique de chaque individu dans le but d'améliorer l'efficacité du traitement tout en réduisant des toxicités.

ملخص

علم الصيدلة الجينية هو دراسة الاختلافات الجينية التي تؤثر على استجابة الفرد للأدوية. وتساهم العوامل الوراثية بنسبة 15٪ إلى 30٪ من تباين هذه الاستجابة.

تهدف الصيدلة الجينية إلى تحديد الأسباب الوراثية لهذا التباين من خلال دراسة الأشكال الجينية التي تساهم في حركية و حيوية العقاقير.

أهداف دراستنا هي استحضار تجربة المستشفى الجامعي الحسن الثاني بفاس لتقييم أوجه العقبات في هذا المجال واقتراح توصيات.

تمت هذه الدراسة بقسم علم الوراثة الطبية بالمستشفى المذكور من خلال معاينة الحليلات الطافرة الأكثر شيوعا للمورثة عند مائة وثلاثة (103) مريض مغربي. TPMT

من خلال النتائج لم يتم العثور على أي حليل طافر من بين 206 حليل مدروس، وهذا يفسر قلة سمية أدوية التيوبيرينات عند المغاربة. thiopurine

نستعرض أيضا مختلف العلاجات المساعدة في سرطان القولون والمستقيم وتعدد الأشكال الجينية التي تساهم في الاستجابة لهذه الأدوية.

يلزم النهوض بهذا المجال في المغرب عن طريق تطوير مفهوم الطب الشخصي حيث يقدم العلاج اعتمادا على البيانات الشخصية الجينية لكل فرد بهدف تحسين فعالية العلاج وتقليل سميته.

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