Universitat de les Illes Balears

Facultat de Ciències

Memòria del Treball de Fi de Grau

Evaluation of personalized medicine as possible therapeutical approach in oncology

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Any acadèmic 2018-19

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Paraules clau del treball:
Personalized medicine, chemotherapy, cancer, pharmacogenomics, monoclonal antibodies, breast cancer, CART

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Abbreviations

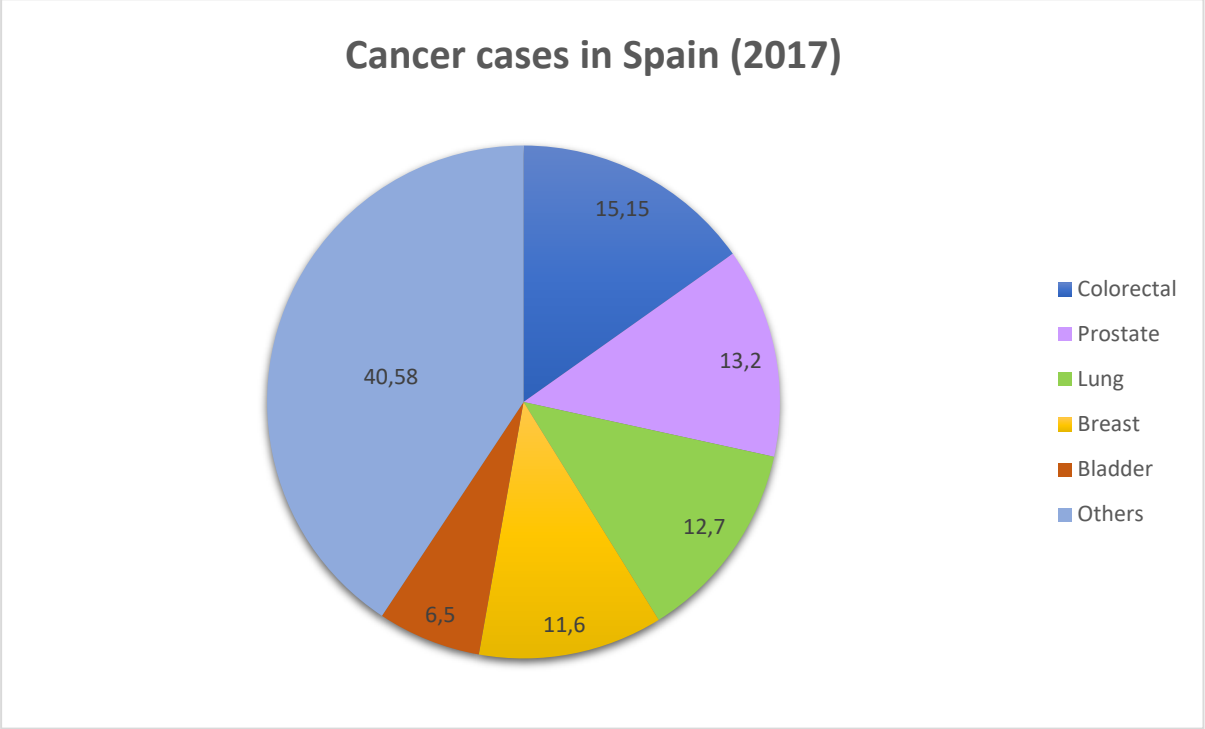
ATP	Adenosine triphosphate
B-ALL	B-cell acute lymphoblastic leukemia
B-NHL	B-cell non-Hodgkin lymphoma
CART	Chimeric antigen receptor T cell therapy
C-KIT	Receptor tyrosine kinase inhibitor
CYP	Cypriot pound
DNA	Deoxyribonucleic acid
FDA	Food and Drug Administration
GCO	Global Cancer Observatory
GISTs	Gastrointestinal stromal tumors
IM	Intermediate metabolizer
MABs	Monoclonal antibodies
NCBI	The National Centre for Biotechnology Information
NIH	National Institutes of Health
NLM	The National Library of Medicine
NM	Normal metabolizer
PDGFRA	Growth factor receptor alpha
PM	Poor metabolizer
PREPARE	Preventing Adverse Drug Reactions
R/R	Relapsed/refractory
RM	Rapid metabolizer
RNA	Ribonucleic acid
scFv	Single chain variable fragment
SEOM	Sociedad Española de Oncología
TCR	T cell receptor
U-PGx	Ubiquitous Pharmacogenomics
UR	Ultrarapid metabolizer
WHO	World Health Organization

Summary

Different types of cancers can be quite heterogenous from a molecular point of view but have been treated with similar chemotherapeutical drugs in the past. Such classical chemotherapy may cause damage to both tumoral and healthy cells, because its molecular mechanism, intercalation between the two strands of the DNA, does not distinguish between these cells. Recent advances in technology have been the key to initiate a novel therapeutical approach called personalized medicine, which tries to take the genetic and molecular differences of each patient into account. In this bachelor thesis PubMed has been the primary source of information to evaluate the possible use of this novel approach in oncology. As a result, various promising strategies could be found such as pharmacogenomics, targeted drugs (receptor tyrosine kinase inhibitors), monoclonal antibody and Chimeric Antigen Receptor of T cell treatment. In addition, an estimate of the economic impact on Public Health Systems and the possibility to implement personalized medicine has been realized. Although a solid conclusion could no be achieved, some unexpected concepts have been found, for example that the creation of Big Data based on molecular screening of cancer samples may be the key not only to establish an effective and side effect free treatment for every patient but also to lower the general costs and to successfully implement personalized medicine.

Introduction

Cancer is known as one of the most mortal human diseases and caused by the mutation of one or several genes that subsequently induce the uncontrolled growth of cells and, consequently, the formation of tumors. The last official register is from 2012 and was realized by the Global Cancer Observatory (GCO). The result of this project, which was sponsored by the World Health Organization (WHO), showed about 14.1 million of new cases and 8.2 million of cancer-related deaths worldwide in 2012. In Spain there were an estimated of 228,482 new cases in 2017 in both sex and the most diagnosticated tumors were from colorectal (15.15%), prostate (13.2%), lung (12.7%), breast (11.6%) and bladder (6.5%) origin (Sociedad Española de Oncología, SEOM). Therefore, lung and breast cancer are on the top of major global health problems and many of the studies related with cancer are focused on both types, with the aim to decrease their worldwide incidence and to establish novel therapeutic approaches (Chan et al., 2017).



Graph 1. Representation in per cent of cancer incidence in Spain the year 2017, where colorectal, prostate, lung, breast and bladder are considered of high incidence with >14,000 cases. Others are referred to tumors were incidence is <14,000 cases.

The conventional therapies used by now, like chemotherapy, are relatively unspecific and based on cytotoxic drugs causing damage to both tumoral and normal cells. For example, doxorubicin (also called adriamycin) is an anthracycline, a compound class with antibiotic

and antitumoral properties. Its isolation was first described in 1969 (Arcamone et al., 1969) and the result of the mutation of a *Streptomyces* strain, thus, generating a new variety called *Streptomyces peucetius* var. *caesius*. Indeed, experiments demonstrated that doxorubicin was more effective against mouse tumors than daunorubicin found in the original *Streptomyces* strain.

During the process of mitosis, doxorubicin inhibits tumor cell proliferation by intercalating between the base pairs of the double strand of DNA and blocking the enzyme topoisomerase II. Both molecular mechanisms cause a disruption of the DNA and finally lead to cell death. Inside the cell, doxorubicin may be chemically reduced or oxidized by enzymes of the cytochrome P450 system and the mitochondrial electron transport chain, usually referred to as redox cycling. In this case doxorubicin is oxidized to a metabolite called semiquinone that can be transformed back to doxorubicin. The process liberates free radicals in the cell that are responsible of unspecific membrane damage and oxidative stress.

In clinical settings doxorubicin is used in combination with other medications to treat certain types of cancer like breast, lung, stomach, bladder and ovarian, as well as certain types of leukemia, thyroid and some types of soft tissue or bone sarcomas, non-Hodgkin's and Hodgkin's lymphoma and sarcoma (Thorn et al., 2012). However, the molecular mechanism of doxorubicin is non-selective meaning that it acts on all proliferating cells because all dividing cells are prone to the intercalation of the drug between the separated DNA strands. In this way doxorubicin affects both tumoral cells and healthy cells and produces severe side effects in patients which on the clinical level manifest as physiological reactions like alopecia, nausea, vomiting, mucositis, and consequently cachexia, stomatitis and cardiotoxicity (Denard et al., 2012) all involving continuously dividing cells.

Although the molecular mechanism of doxorubicin (and other classical chemotherapeutics) is rather general, the clinical results of this treatment are still variable and vary from patient to patient (Schilsky, 2010). These facts have been one of the reasons to initiate further research on new antitumoral treatments focused on interindividual differences of the mutational pattern of genes with the aim to find an optimized treatment for each patient (Ogion et al., 2012).

In the past decade, significant advances in molecular biology techniques, such as microarrays, next-generation sequencing, and whole exome sequencing, have allowed researchers to better

understand the details of tumor cell biology, to identify complex genomic abnormalities and to define specific biomarkers involved in tumor growth. The results of such studies can improve general clinical practice by allowing a more personalized prognostic and predictive approach to patient management (De Abreu et al., 2014).

This novel clinical concept has led to the generation of several new technical terms, such as, for example, precision medicine, stratified medicine or personalized medicine, which are generally interchangeable but have been defined in many ways. According to the U.S. National Institutes of Health (NIH) personalized medicine is “an emerging practice of medicine that uses an individual’s genetic profile to guide decisions made in regard to the prevention, diagnosis, and treatment of disease” (Cho et al., 2012).

Hypothesis

In conventional medicine, therapies are based on statistical terms, ignoring the interindividual variability of patients. Personalized medicine aims to overcome this limitation by dividing patients of a certain pathology into subgroups by means of an extended diagnosis, going beyond the classic "signs and symptoms" approach. In this context, some clinical fields may benefit more than others from this novel strategy and the molecular pathophysiology of cancer cells makes oncology especially susceptible for a renovation of its rather classical treatment schemes.

Objectives

1) Evaluate the possibility to use personalized medicine as a therapeutical approach in oncology by examining distinct clinical strategies and their related molecular and cellular context

2) Evaluate the economic impact that personalized medicine in oncology may have on the Public Health Systems.

Materials and Methods

In this bachelor thesis the database PubMed (Public Medline) maintained by The National Library of Medicine (NLM) has been the principal scientific source used to search for information about personalized medicine in oncology because it contains the highest number of clinical articles. The National Centre for Biotechnology Information (NCBI) is the responsible institution of this archive that in total includes more than 29 million Manuscripts because of the access to MEDLINE and includes references about science journals and abstracts related to medicine, nurseling, health and biochemistry. Publications mentioned in this study have been found in PubMed using key words related to the subject studied.

Journals listed in this database are publishing scientific manuscripts related to health topics that have passed a peer-review process, which is a system to approve the validity of a manuscript before it is published. This procedure requires one or more experts in the subject or the area of study, which will evaluate and comment the scientific soundness and the applied methods of the study, importance of the findings, and judge the originality of the article. These so-called reviewers usually remain anonymous in order to guarantee a correct and honest judgement, which is unbiased by conflict of interests due to personal relationships. This procedure is crucial and helps to improve the quality of published research, to validate academic work and to increase the credibility of the conclusions.

Several keywords have been used to search for manuscripts about the topic personalized medicine in oncology and other actual applications with the aim to obtain the maximum information and to understand the evolution of these techniques in comparison to classical chemotherapy. Double quotes have been used to search words in the same manuscript in the order of writing. In case of not using double quotes, results will provide manuscripts were the group of words inserted appears without order. A plus sign is used to find a group of words that appears in the same manuscripts.

Doxorubicin	Cancer + biomarkers
Cancer + chemotherapy	Chemotherapy
Monoclonal antibodies	Genetic analyses + cancer
“Personalized medicine” + oncology	“Breast cancer” + chemotherapy

“Personalized medicine” + chemotherapy	“Pharmacogenomic biomarkers”
“Tyrosine kinase receptor inhibitor” + oncology	“Breast cancer + molecular subtypes”
“Monoclonal Antibody treatment” + oncology	“Chimeric antigen receptor” + oncology
“Drug metabolism” + biotransformation	“Ubiquitous pharmacogenomics”
“Future personalized medicine”	“Pharmacogenomics + oncology
“Gastrointestinal stromal tumors” + oncology	“Personalized medicine” + economies

Table 1. List of words used for acquiring the information treated in this bachelor’s thesis.

Another important tool provided by PubMed is the option to download an excel in csv format of the number of publications per year that contain the words searched on this platform, which is interesting additional information to see how the topic evolves in the past years. Because personalized medicine is quite a new science this publication count has been considered when searching the words: personalized medicine oncology (without using quotes, which means that these words can appear separated and in any order in the manuscript).

Results and Discussion

It is known that drug efficacy is different for each patient and this observation constitutes the basis of personal medicine. These differences have often been related with mutations and polymorphisms and of genes and are also addressed in the actual definition of the technical term personalized medicine by the NIH (see page 8). However, today the recent discovery of epigenetics has made it even more complex to determine what exactly is the genetic profile of a person. In this context, epigenetics is the regulation of hereditable phenotype by changing chromosomal expression but maintaining DNA sequence without modifications. These alterations in the chromosome are mostly by DNA methylation, histone modifications and non-coding RNA, all which may lead to the alteration of molecular processes. An example is the locus where molecules should bind at the chromosome and altering the transcription of proteins and their regulation without changing gene sequences (Roberti et al., 2019).

Drug Metabolism

Drug metabolism is an essential part of clinical therapies as biotransformation of active ingredients directly affects the amount of drug in the body. In general terms, only this unaltered part of the inactive ingredient is pharmacologically effective, although in some cases pharmacologically active metabolites exist or the so called active pharmaceutical ingredient is an inactive precursor and has first to be metabolically activated. An example for such an inactive precursor is Tamoxifen, which binds to the estrogen receptor and is used to treat breast cancer in women with tumors that are estrogen-receptor positive (Ahern et al., 2017).

In this context, genetic differences between individuals can lead to differences in metabolic rates, thus determining the results of clinical therapies with pharmaceutical drugs. The metabolic pathway can be divided into two steps that take place subsequently:

Phase 1 Reactions	Oxidation, Reduction, Hydrolysis
Phase 2 Reactions	Conjugation with hydrophilic compounds

The most important enzymes of phase I reaction are members of the CYP family (cytochrome P450) whereas in phase II reactions transferase enzymes play a pivotal role. It has been found

that the metabolic reactions regulated by cytochrome P450 enzymes are the most important ones for the biotransformation of drugs. This biotransformation activity refers to the metabolic capacity of an enzyme and can be modified by two important variants: polymorphisms, which include the level affluence of gene expression for each enzyme and their enzymatic efficacy, or by the variants on the alleles, involved in changes on genes able to alter this activity. Thus, a classification has been established to differentiate different levels of enzyme activity of biotransformation processes (Bishop, 2018):

Metabolizer classification	Enzyme Activity	Effect on Drug Treatment
Poor metabolizers (PM)	not detectable or small	Very strong pharmacological and side effects; lower dose is imperative
Intermediate metabolizers (IM)	attenuated	Increased risk of side effects, lower dose is required
Normal metabolizers (NM)	unaltered	Typical drug and side effects
Rapid metabolizers (RM)	increased	Increased risk of lower drug effects, higher dose required
Ultrarapid metabolizers (UM)	very high	Very low drug effect, higher dose imperative

Table 2. Classification of the different types of metabolizers by their enzyme activity.

An example of a polymorphic gene involved in biotransformation is CYP2D6 and their 63 allelic variants included all types of enzyme activity, from PM to UM including IM, NM and RM. These metabolizer activities are observed when a group of population is exposed to a probe substrate. However, it is important to know that in personalized medicine phenotypes may be analysed for each patient and a right dose may be given to finally obtain reliable data. Nowadays, commercial determination of metabolic phenotypes only exists where the economic status of the population is high enough to permit diagnostic analysis of the genetic profile. Nevertheless, various studies have demonstrated that it is a challenge to predict phenotype with the genotype data because of the complexity of allelic variant combinations. An example is the phenotype CYP2D6*1/*2 which is normally classified as a poor metabolizer and less likely as an intermediate metabolizer. As more allele variants are discovered, more analysis may be done, which leads to a more complex genotype (Gaedigk et al., 2007).

Regarding personalized medicine, it is important to take these differences in drug metabolism into account because they are one of the reasons to understand why different patients with the same pathology do not show the same desired effect to drug treatment. For example, an ultrarapid metabolization leads to very low drug levels because the administrated substance is rapidly cleared from the body. This in turn results in the necessity of higher drug dosage for this patient in order to achieve the desired clinical effect. On the other hand, a poor metabolizer will need much more time to metabolize the same drug than a normal metabolizer, leading to abnormal high drug levels, which increases the possibility of adverse drug reactions. In such a case a reduction of the applied drug dose is indispensable (Ahmed et al., 2016).

Pharmacogenomics

Classical chemotherapy treatment is based on a simplified concept by categorizing cells in rapidly proliferating and non-dividing, fully differentiated. The rapidly proliferating cells are defined as tumoral according to this concept and targeted by the classical chemotherapeutics, whose molecular mechanism is usually by the intercalation of DNA, using ligand molecules able to stop DNA replication opening the double strand, causing changes on the pair of bases by binding on one of the strands and ending on functional alterations, inhibition of replication and transcription, which stops cells proliferation. This chemotherapy treatments act on cells with a higher level of reproduction, like tumoral cells do.

Nevertheless, in a healthy organism exist also physiologically normal, non-tumoral cells with a naturally high proliferation rate, such as hair follicles, intestinal mucous or hematological progenitor cells of the bone marrow. Classical chemotherapeutics are not able to distinguish between these normal cells and cancer cells and affect all dividing cells in the relatively non-specific, same way. Therefore, adverse drug reactions are also similar for all active ingredients of this chemotherapeutic type because they are linked to tissues with a higher cell division rate. In this context, typical symptoms are losing hair (hair follicles) - diarrhoea, nausea, vomiting (mucosa of the gastrointestinal tract) and alterations of the blood panel (haematological precursor cells). For this reason, the therapeutic margin of drug dosage is very limited in the case of classical chemotherapeutics as even slight overdosing is rapidly highly toxic for the patient.

In this context, the observation of variances in drug metabolism has led to a new scientific field called pharmacogenomics, whose aim is to detect all mutations in the genome, which are related to drug efficacy in order to guarantee an optimal efficacy and to prevent adverse drug reactions by stablishing specific selection of drugs and their dose. However, using pharmacogenomics as a guide to select the optimal drugs and their dose is being difficult by missing connections between gene-drug interaction. Genetic analyses have been the key to predict drug actions by the improvement of molecular diagnostics and which include the screening for certain biomarkers that regulate tumoral cell reaction to drugs (Lauschke et al., 2017). As result of all this progress, a several projects have been initiated, for example, the Preventing Adverse Drug Reactions (PREPARE), which is conducted by U-PGx (Ubiquitous Pharmacogenomics) to apply pharmacogenomics as a part of clinical standard therapy in Europe. Cecchin et al. 2019 cited "U-PGx is a randomized clinical trial with the aim to test the effectiveness of implementing pre-emptive PGx testing in real world clinical setting, to primarily provide evidence of its value in improving patients' outcomes, being easy to be used in the clinical practice, and cost-effective", were pre-emptive PGx means the obtaining of genetic variants involved on the first drug prescription that have been already used in US clinics but it's quite new in Europe. This study begun on January the 1st of 2017 and is expected to be analysed on 2020 with the aim to create an individual genetic profile for patients monitoring biomarkers to determinate which drugs or dose is better for each patient before the medic prescribe. The PREPARE study will evaluate 40 clinically relevant biomarkers and moreover analyse the effects on 13, so called, pharmacogenes by pre-emptively testing patients. During the experiment a group of patients will be guided by the results obtained for these 40 drugs and a second one will be the control group, which will be prescribed with standard care. The expected results will be an important step to prevent diseases like cancer by obtaining a clinical profile by genotyping polymorphisms that are constant on our whole life and are involved on drugs metabolism, so will be analysed once and will be usable for lifetime like an everyday clinical practice, therefore, an individual drug response profile for each patient is generated. Although an important dare of this project will be the implication of other countries evaluating their own culture and healthcare systems to finally expand a common model of caring from Europe to the rest of the world. Another important challenge that this project will take on note is the economics; an evaluation will be done on the countries implicated on this U-PGx quality to previously create an estimate of costs to facilitate the future implementation on other regions by their financial funds.

Receptor Tyrosine Kinase Inhibitors

Some enzyme-coupled plasma membrane receptors are involved in the transduction of extracellular signals stimulating cell metabolism and proliferation. One example of these receptors is the receptor tyrosine kinase (C-KIT). The molecular mechanism of signal transduction is the phosphorylation of target proteins using ATP. These proteins are activated by the phosphorylation and induce a signalling cascade, which finally leads to cell proliferation. Thus, KIT plays an important role for cell functions and recent studies have related their uncontrolled activity to the development of cell mutations by overexpression, which leads on the activation of oncogenes and the induction of cancer (Balachandran et al., 2015). However, selective tyrosine kinase inhibitors can block this receptor, which stops the malignancy and, in a future, could be considered as a therapy to treat cancer by knowing the expression of this enzymes on individual genome for each patient. Imatinib mesylate (Gleevec® or Glivec®) is a targeted cancer drug and was the first KIT inhibitor discovered in 2000. It is used to treat some types of blood cancers and gastrointestinal stromal tumors (GISTs). GIST are mesenchymal tumors derived from intestinal cells of Cajal and these are induced by the expression of c-KIT (CD117) in 85-95% of patients and about 3-5% of the remainder of KIT-negative GISTs contain platelet-derived growth factor receptor alpha (PDGFRA) mutations (Zitvogel et al., 2016). Imatinib mesylate targets KIT by blocking its ATP cassette and, consequently, blocking their ability to make tumor cells grow and divide. Under normal conditions KIT activation requires binding of its ligand stem cell factor, which induces structural changes in the KIT receptor. The fully activated kit receptor then directs phosphorylation and activation of targets in several downstream signalling pathways, which influence processes such as cell proliferation, growing and apoptosis. If ligand is absent, wild-type KIT will remain inhibited. However, KIT mutations can release this inhibition in the absence of ligand binding allowing for constitutive oligomerization and activation of the KIT receptor. These mutant KIT proteins can drive continuous oncogenic signalling leading to an increase in cell proliferation growth and survival which can ultimately lead to the formation of a gastrointestinal stromal tumor or GIST. Most of the GIST have constitutive KIT activity which can be inhibited by the tyrosine kinase inhibitor Imatinib mesylate. In vitro data demonstrates that Imatinib competitively binds to the ATP binding pocket of both active and inactive KIT preventing or inhibiting activation. This is followed by inhibition of related downstream signalling. As a result, Imatinib induces apoptosis in many KIT positive gist cells (Iqbal et al., 2014).

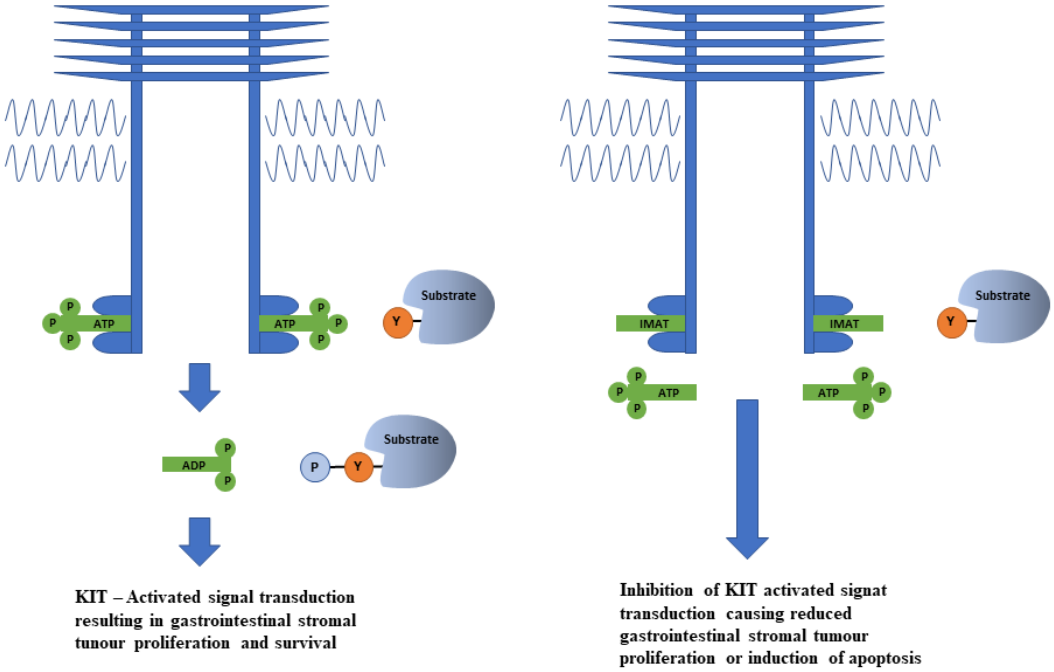


Figure 1. Image A, the KIT activated in normal conditions / Image B, represents KIT inhibition by Imatinib. In purple the Tyrosine Kinase domain, in green ATP molecules and in grey Imatinib occupying ATP's place. Image adapted from Quek et al., 2010.

In comparison to chemotherapy this drug has improved the progression of cancer, being free on patients that are under this treatment and the survival have been rise as well. Imatinib acts against mutations of KIT present in patients with GISTs and responses to this drug depend on the functional domain affected (Bethany et al., 2017).

Breast Cancer

Breast cancer is considered as one of the malignancies with more cases per year. In 2012 as much as 1.6 million cases and 500,000 deaths worldwide were registered in the same year (Chan et al., 2017). However, because of the huge number of genes the expression of specific genes involved in the development of cancer has been extensively studied and subsequently used to classify breast cancer by molecular subtypes. An important point is that this molecular classification is only valid for breast cancer as the observed differences are locally restricted. Such tumors that depend on their location in the body are usually defined as locoregional tumors. The treatment of locoregional tumors with locoregional characteristics opens the possibility for a more specific treatment, which only affects the tissues of this zone. This fact

may improve the life quality of the patient as the antitumoral effects will focused only on that zone and probably cause less adverse drug effects.

Breast cancer can be divided into five subtypes depending on different molecular markers, i.e., progesterone and estrogen receptor (PR/ER), human epidermal growth factor receptor (HER2) and protein Ki-76, which all are important regulators of cell proliferation in breast tissue (Prat et al., 2017).

Molecular subtype	PR	ER	HER2	Ki-76
Luminal A	+	+	-	
Luminal B	+	+	+ or -	High levels
Triple-negative/basal-like	-	-	-	
HER2-enriched	-	-	+	
Normal-like	+	+	-	Low levels

Table 3. Classification of molecular subtypes depending on the + or – receptors expression and the levels of Ki-76 protein.

The aim of this classification is to create groups reflecting the expression or non-expression of these genes and the level of protein Ki-76 because with this knowledge, treatments can be focused depending on each molecular subtype. The relationship between the expression of these genes, proliferation and the classification of breast cancer is not a precise overlap, but it helps to considerate them as different pathologies (Taherian-Fard et al., 2015).

Another relevant group of genes in the development of breast cancer are the BRCA genes, which encode tumor suppressor genes. The related proteins are involved in the activation of reparation processes of the DNA and are therefore essential to maintain cell functionality intact. BRCA1 is directly involved in the repair of the DNA whereas BRCA2 is an important transcriptional regulator and is involved in the induction of binding the single strand DNA for the post interaction with other proteins.

Both genes have been associated to the development of cancer because mutations may block physiological function of the proteins and by this promote the development of breast cancer. It has been clinically observed that the loss of BRCA1 expression accelerates the division of normal and malignant mammary cells and is therefore considered as a possible risk factor of

developing breast cancer. Other cases showed that reduction levels of BRCA2 domains allow the accumulation of damage of the DNA

Nowadays patients with BRCA mutations are challenging for clinical management because there are no guidelines for standard testing (Paul et al., 2014). Mutated BCRA 1/2 genes can be inherited to the next generation and are considered as one of the major risk factors of developing breast cancer in women, which usually occurs before the age of 50 years. Studies have demonstrated that at an age of 70 years about 60-70% of BRCA1 mutation and a 45-55 of BRCA2 mutation carriers have suffered from breast cancer and 40% BRCA1 and 20% of BRCA2 mutation carriers will develop ovarian cancer (Clark & Domchek, 2011).

However, molecular subtypes have been essential for breast cancer, it shows the variability of this malignancy depending on gene expression, which is what personalized medicine is looking for, medicine is taking a personalized way where targeted therapeutics will be the future for cancer prevention (Fragomeni et al., 2018). In a future it may have to be considered to establish a partnership on women with family planning and the development of a guideless testing for BRCA1 and BRCA2 by sharing data globally of testing procedures providing essential information for clinicals and care provides for the patients.

Monoclonal Antibody Treatment

Classical chemotherapy acts on tumoral cells because they proliferate in a faster way than normal cells do. However, cancer cells are not the only cells that proliferate rapidly, and an exception to the rule, are for example progenitor cells of blood cells, epithelial cells and mucosa cells, which are for this reason also sensitive to classical chemotherapy. Hematological toxicity is the most relevant adverse drug reaction of such treatment as it decreases the production of red blood cells and the number of other bone marrow cells. This alteration can then lead to anaemia, bleeding and increased sensitivity to infections. The latter is caused by a decrease of the number of leukocytes which play an important role for the immune system. Neutrophils for example are generated in the bone marrow and responsible of detecting and attacking to bacterial infections via phagocytosis. Low levels of neutrophils produce neutropenia and when this happens chemotherapy treatments are stopped, or the dose is reduced. A decrease of leukocyte levels is always alarming and could end deadly, and that is why those levels are monitored (Testard-Paillet et al., 2007).

Cancer cells have the capacity to proliferate and grow without the activation of the immune system, which means that the tumor may be extended to other organs through metastasis depending on the cellular migration activity. In order to prevent this situation action must be taken during the initial period of this illness, which is crucial for the possibility to achieve a complete tumor eradication. In this context, a novel approach has been developed in the last decade, which focuses on the involvement of the immune system of the patient during the therapy. The centrepiece of this idea is to use monoclonal antibodies (MABs). In this therapy antibodies are produced by single clones of B cells, which are monospecific targets and have a long half-life (Rogers et al., 2014). These characteristics have been the reason of its triumph as drugs and the use of this treatment to combat cancer. The number of monoclonal antibodies treatments has been increased in oncology because of the low toxicity, the specificity and the implication in immunology (Henricks et al., 2015). The only negative point is that MABs require an intravenous treatment. This is because antibodies are glycoproteins and in case of taking those orally their structure would be altered in contact with the very low, acidic pH of the stomach, which would denaturalize the antibodies so that their function won't be realized. This treatment is not used only for cancer, but also other diseases are clinically treated by MABs because of its capacity of activating the immune system.

The procedure to create antibodies is initiated by the exposure of an animal, normally a rat or a rabbit, to an antigen which activates the immune system and triggers its response. B lymphocytes are the cells that generate the antibodies after their transformation to plasmocytes. Epitopes are the region of the antigen molecules were antibodies attach by binding with the region of the antibody called paratope. As a result, monoclonal and polyclonal antibodies are interesting as treatment and for clinics applications. Both antibodies bind to the antigen, the difference is that monoclonal antibodies bind to a unique epitope because those were created by the same B cell clones. In contrast polyclonal antibodies are produced by different B cells, and their paratope will bind to different epitopes of the antigen. That is why depending on the exposed antigen, specific B cells will produce specific antibody, and finally those monoclonal antibodies will be recollected in the spleen of the animal, because it where antibodies are produced. This B cells will be mixed and fused with myeloma cells, which have the capacity of growing continuously. The resulting hybridomas are cells with both characteristics: constant growing and producing antibodies.

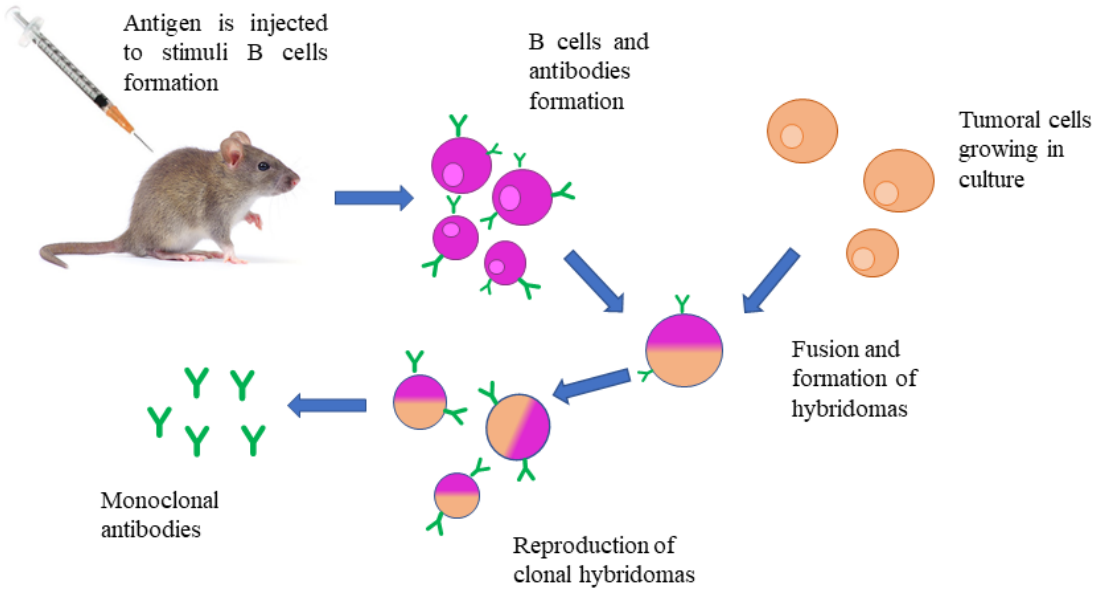


Figure 2. Procedure to obtain monoclonal antibodies by the injection of an antigen, isolation of B lymphocytes, formation of hybridomas and selection of a single hybridoma cell clone expressing the desired monoclonal antibodies. Adapted from Pandey 2010.

Antibodies can target tumoral cells by specific membrane receptors like EGFR, HER-2, HER-3 or HER-4, which are expressed by tumoral cells and responsible of the uncontrolled cell growth (Simpson & Caballero, 2014). Trastuzumab was the first humanized monoclonal anticancer antibody approved by the US Food and Drug Administration (FDA) in 1998 and used against HER2-positive breast cancer. However, trastuzumab is not the only monoclonal antibody used to treat cancer, a few examples are shown in the following table:

Drug	Molecular Target	Antibody format	Application
Cetuximab	EGFR	Chimeric	Colorectal, breast, lung cancer
Panitumumab	EFGR	Human	Colorectal cancer
Pertuzumab	HER-2	Humanized	Breast cancer
Alemtuzumab	CD52	Humanized	Chronic lymphocytic leukaemia

Table 4. Examples of MABs and their use for different cancer depending on the location and the targeting receptor. Table adapted from Chiavenna et al., 2017.

Cetuximab can block the receptor of the epidermal growth factor receptor (EGFR), which stops the growth of epidemical cells and its proliferation. MABs are often limited by drug resistance of the malignancy or by partial responses, but often this problem can be overcome by the combination of two or more MABs (Wheeler et al., 2013). Because tumoral cells are constantly dividing they are prone to develop new mutations, which may lead to resistance of these cells to anticancer treatments. However, those mutated cells also may produce news antigens, which results in the creation of new MABs, so that they can be rapidly recognised by those monoclonal antibodies, It is clearly a big step for medicine, from the unspecific classical chemotherapy that defines tumoral cells only via the speed of cell division to a cancer therapy that targets tumoral cells via specific, molecular surface markers.

Chimeric Antigen Receptor T Cell Therapy

Classical chemotherapy treatments are based on relatively unspecific diagnostics focused rather on the tissue origin of the cancer than on the complex mutational pattern of the cancer. The latter would require a personalized knowledge about the patient's cancer in order to control and cure his individual malignancy. Immunotherapy has been one of the research results that promote a more personalized medicine with the aim to improve patient's probability of survival. As a result, chimeric antigen receptor T cell therapy (CART) have emerged as a possibility to cure cancer, concretely hematological malignancies, which begin in marrow bone tissue and therefore involve also cells of the immune system (Ruella et al., 2017). In contrast to conventional therapies CART is based on genetic engineering of chimeric antigen receptor that are introduced in the genome of T cells. Once expressed in the T cell, those chimeric receptors provide the capacity target molecular surface markers of the tumor cells. Such recognition of tumoral cells by their antigens leads to a high-level, thus efficient, immune response being able to eliminate tumors in a relatively short time and is as well able to prevent because the modified immune cells remain in the body for years.

Chimeric antigen receptors (CAR) are generated by the fusion of single chain variable fragment (scFv) originated on monoclonal antibody with the signalling and stimulation machinery of the T cell receptor (TCR), resulting in a special receptor that binds to specific proteins expressed on the cancer cells of a specific patient. The recognition in the patient organism is possible by extracting T cells from his blood and activating them in the laboratory by adding the CAR. Once the fusion is done, a huge number of CART cells are produced *ex*

vivo in the cell culture laboratory (massive cell expansion) in order to finally give them back to the patient via intravenous transfusion and to induce an efficient immune response.

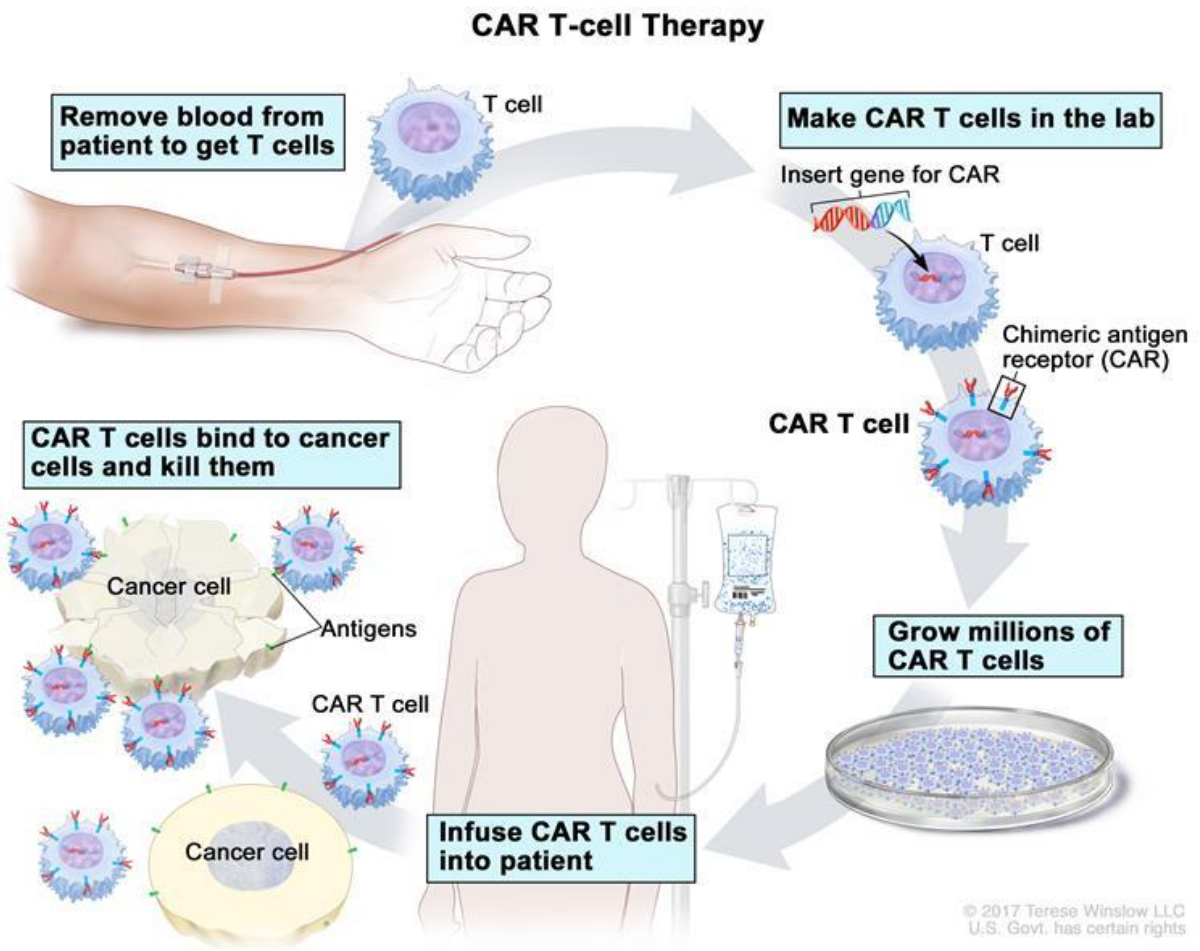


Figure 3. Procedure to obtain CART by removing white blood cells and isolating T cells from patients for then insert CAR to these cells in the laboratory, which will activate and recognize cancer cells on patients' body. Once the fusion is done, a huge number of CART cells are creating in the laboratory and infuse to patients.

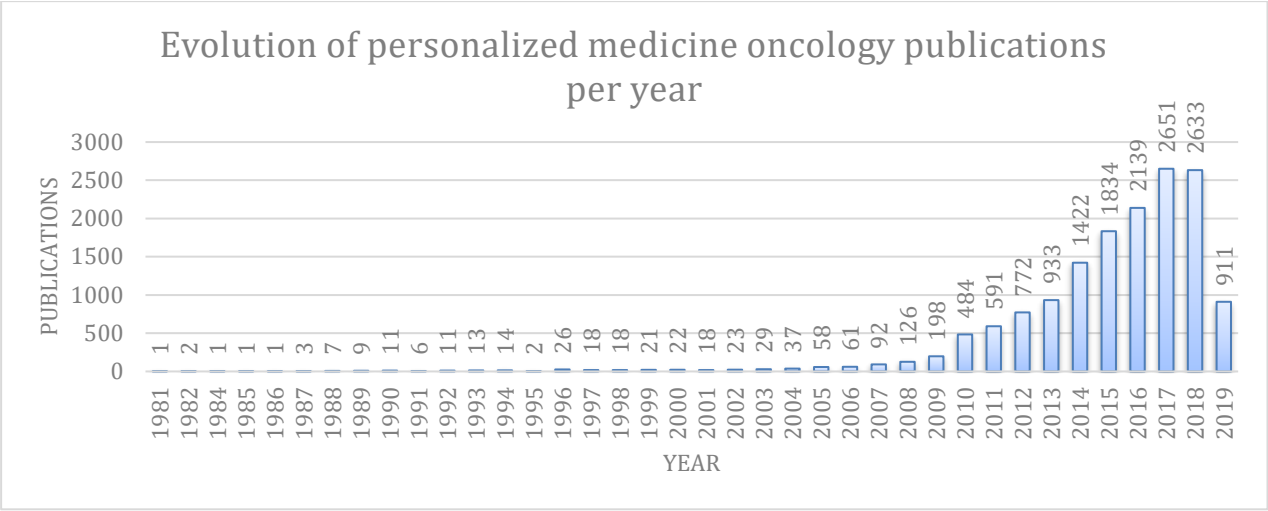
The first CART therapy accepted by the Food and Drug Administration (FDA) was Kymriah® (tisangenlecleucel) in 2017, which is used to treat paediatric and younger patients that suffer CLL using the B-cell acute lymphoblastic leukemia (B-ALL). Another CART that has been approved by the FDA, Yescarta® (axicabtagene ciloleucel), was discovered a few months after the Kymriah® and is used to treat patients with relapsed/refractory (R/R) B-cell non-Hodgkin lymphoma (B-NHL). However, it seems realistic that CART therapy can be also the key to treat other diseases than hematologic cancers, being an interesting clinical approach that requires further research. However, also this treatment has its limitations because of the needed of transfer a massive number of cell production and clinical management which must

be supported by economic resources. In addition, this CART therapy is not successful for all patients because of rapid disease progression during T cell production.

Economic Impact of Personalized Medicine on Public Health Systems

Public health systems are alarmed by the worldwide increase of new cases of cancer each year. Considering this situation oncologists are looking for new ways to treat cancer considering classical chemotherapy as an obsolete treatment with limited clinical outcomes. This change of paradigm has led to search for a more individual and personalized medicine, which has been made possible by the increasing speed of technological development and the specificity of the new techniques with the capacity to recognize molecular properties of malignant tumoral tissues. Another important factor is also the increased number of researchers involved in search for new approaches of personalized medicine in oncology (Tannock et al., 2016).

Clearly, personalized medicine is still at its beginning, on several years the number of publications that contain the words “personalized medicine oncology” shown an exponential increase (Graph 2). This huge increment of publications in the last 10 years can be related with the growing research about new clinical techniques such as the before mentioned pharmacogenetics, MABs, CART, and the increase in new essential knowledge. This knowledge requires the continuous actualization of what is considered personalized medicine, which is defined by the aim to find an individual clinical profile where genomics will reveal how a patient should be treated avoiding side effects and improving their wellbeing during these treatments.



Graph 2. Representation of the increase evolution of personalized medicine in oncology in the last 38 years. Numbers on the top of the data bar represents the exact number of publications in that year.

Longevity in our society has increased and the worldwide birth rate is also still growing exponentially. This results in the need to create a management plan that considers this increase in people in our healthcare system and prevent the lack of resources.

In fact, this new scientific and clinical situation has recently induced a political decision about the future of this potential therapeutic strategy. On the 13th of February of 2019, the "Boletín Oficial de las Cortes Generales del Senado" published the adoption of the report by the presentation of the genomic study, were as a conclusion of the fast evolution of clinical and technological techniques in genomics, is was found necessary to take advantage of this situation to investigate a personalized profile and the incorporation of a genomic medicine at the "Sistema Nacional de Salud". A total of 13 points were established as conclusion of the report with the aim to improve the Spanish Health System. The establishment of a specific budget, using resources and creating connections between entities and scientific experts in the subject, sharing knowledge of sequencing and services of genomic medicine has been proposed. Diagnostic information might be registered as part of an electronic profile of each patient, to create a genetic patient profile, which is relevant for personalized medicine as a process of recollection of individual data. Subsequently, the recompilation of all this information will be useful to create also Big Data as a steering tool for our Health System, which is a complex task and requires an enormous capacity to store information and handle diverse types of data, for example, from laboratories, pharmacies, clinical imaging data, prescriptions and detailed diagnosis,. All this data will have to be organized and processed with the final aim to a maximum improvement of patient's healthcare. Big Data is a new

challenge in medicine because it hasn't been used yet as a tool and is limited also by the confidentiality of diagnostic data of patients that can't be easily shared. However, because the recollection of information grows rapidly in medicine, Big Data will be indispensable in any Health System as a priority tool that could prevent diseases and, thus, lower costs (Adibuzzaman et al., 2018). As a result, big data applications have been created, for example, Flatiron, that was launched in New York in 2012 and pretends to create the biggest database about cancer including clinical data. Regarding the interconnectivity its platform is also called OncologyCloud platform. This software is connected to different cancer centres and permits to share information and to establish communication between oncologists in the United States (Österreich 2016).

All this leads to a future vision where patients will be treated based on their personal genetics, leaving therapies behind where the cancer type was only defined through the tissue and cell origin form which it developed. An important fact of the creation of a genetic profile for each patient is that this data will be more exploited if the samples are recollected from newborn. This will provide valuable information of specific genes of the patient and in a future it would be interesting to distinguish between the first newborn genetic profile and the state of adult genetic profile to see and identify the mutation and changes the DNA suffered. Such before and after comparison is especially valuable in oncology as the development of cancer is trigger through mutations, thus, by changes on molecular level. Due to the variability of cancer specific information and analysis was required to create those new treatments that are already used at the moment. However, in the future a huge number of samples will have to be collected and analysed in order to find new biomarkers of tumoral cells, promoting novel targeted treatments and bringing patient care to the level of an individual genetic diagnostic. Although such a development would be considered positive form a patient point of view, the budget of Public Health Systems is limited, and the costs of such personalized treatments are difficult to estimate. Therefore, its general implementation is being limited and the increase of the costs may perhaps not be assumed entirely by public health systems. This would be creating a two-class health system where patients without monetary resources or with a poor economic situation won't be able to get these novel treatments. It's noteworthy to mention that some techniques are protected by patents owned by multinational pharmaceutical companies which derails the possibility to get the licence to reproduce the mechanism of action of some effective antitumoral drugs.

One of the novel techniques explained in this bachelor thesis is the targeted therapy with MABs, like Trastuzumab, which is used to treat breast cancer when tumoral cells express the receptor HER-2. The Swiss pharmaceutical company F. Hoffmann-La Roche is one of the biggest multinationals of pharmaceutics of the world and a lot of patents are part of the company's intellectual property, including Trastuzumab, which is also called Herceptin. The corresponding patent was bought in 2009 by this company for 36.000 million euros and has already returned 60.000 million euros since its commercialization. However, this high price of MABs therapy is in part caused by the complicated production process, which cannot be easily upscaled to an industrial production scale. In fact, other targeted oncological treatments are cheaper than classical chemotherapy, which is in line with the goal of a generalized implementation of personalized medicine: facilitate the monetary accessibility by cheaper drugs to combat tumors. On the other hand, classical chemotherapy, radiology or surgery requires different exposures to every type of cancer and the maintenance of a medical supervision, indirect costs and direct costs. Arrospide and colleagues (Arrospide et al., 2015) made a price breakdown of breast cancer at the Basque Country by the evaluation of chemotherapy prices from 1996 to 2011. Also, in this study costs have been calculated for different stages of cancer, that requires different treatments, diagnosis, surgery and number of sessions. The obtained results show an annual total cost of 17.879 euros per year, if chemotherapy is included, and a cost of 13.313 euros per year, if not.

The repercussion of this monetarily situation affects the Health System through increasing pharmaceutic costs and as well limited accessibility to this treatment due to the impossibility to assume them. Oncological drugs represent the hugest market in pharmaceutic contents and in the last 10 years their cost have soared because the demand has increased as well. Based on the data that is available today the economic impact that personalized medicine may have on our Health System in the future is uncertain. Nevertheless, some aspects are relevant for an economic evaluation such as limitations in clinical evidences due to low patient numbers of studies, the used methodological procedures and the effectiveness to discover new oncological biomarkers. Another point that could be relevant in the distant future is the increased longevity through the improvement of health care that may indirectly rise the general costs. Still, at least the population of western countries is not increasing considerably at the moment, and it is questionable if developing countries will be able to implement an effective Health System at all. Thus, costs and consequences that personalized medicine implies should be considered separately for each country depending on its financial resources.

National economic plans should not be based on a one-time alone activity, but rather must be an interactive process on a long-term basis. Analytics, technical procedures and collection of data are the key to foresee the exact cost and health results. However, the economic impact is easier to establish for theory procedures, but practise procedures are challenging to demonstrate (Gavan et al, 2018).

Personalized medicine is starting to recollect data from tumors with the aim to create a database with genetic sequences from a multitude of cancer patients. Such a database will allow the comparison of many cancers of the same region and to detect similarities or differences of its mutational patterns. Variability in such patterns may lead to the creation of subgroups of patients that will be treated with different, specific oncologic treatments, avoiding undesired side effects or the administration of non-effective drugs. It can be supposed that such information may evolve into prescribing algorithms, which take multiple predictive biomarkers of specific oncogenes into account that will appear on the clinical genetic profile of each patient. However, the generation of recollecting such an enormous set of data and the organization of the related database will take many years until significant results can be expected.

Conclusions

1) Personalized medicine is a valid approach in oncology as a new way to treat cancer. A variety of very different clinical strategies based on the specific molecular and cellular context, such as pharmacogenomics, targeted drugs, MABs and CART are at the moment being developed. The current results are promising and due to the diversity of mutations other strategies can be expected.

2) A final statement about the economic impact of personalized medicine in oncology on our public health systems cannot be given because there is not enough data publicly available for a solid conclusion. Nevertheless, it can be stated that personalized medicine is not by default more expensive than classical chemotherapy. Moreover, the creation of detailed individual genetic information may no only be a prerequisite for personalized treatments but also makes the generation of Big Data possible that in turn could be able to lower the treatment costs for the public health systems.

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