AIMS Molecular Science, 7(1): 1–11.
DOI: 10.3934/molsci.2020001
Received: 29 October 2019
Accepted: 06 January 2020
Published: 10 January 2020

http://www.aimspress.com/journal/Molecular

Mini-Review

Single nucleotide polymorphisms Rs1045642 C>T genetic alteration in ATP Binding Cassette Subfamily B Member 1 role in increasing everolimus toxicity in metastatic breast cancer

Simone Leggeri[1] and Navid Sobhani[1,2,*]

[1] Breast Cancer Unit, ASST Cremona, Viale Concordia 1, 26100, Cremona, Italy
[2] Department of Medical, Surgical, & Health Sciences, University of Trieste, Piazza Ospitale 1 34129 Trieste, Italy

* **Correspondence:** Email: navid.sobhani@cantab.net; Tel: 393427227486.

Abstract: Breast cancer is one of the most devastating diseases in the world, the most diffused cancer in women. Despite the incredible progress made in the field, the mortality rate in the metastatic setting is still quite high. Among the different drugs used to treat this disease, the mTOR inhibitor everolimus is one of the most promising ones, that has been approved to be used together with exemestane in the treatment of oestrogen receptor positive/human epidermal growth factor receptor 2 negative BC patients in combination with exemestane in patients who have progressed to anastrozole or letrozole, following the encouraging results coming from BOLERO-2 clinical trial showing a significant increase in progression-free-survival of patients compared to patients treated with exemestane and placebo. In this article we will discuss how the toxicity of this drug could be increased with Rs1045642 C>T genetic alteration in ATP Binding Cassette Subfamily B Member 1 (ABCB1), a pump that expels this drug from the cells, leading to a more inactive ABCB1. With an inactivation of ABCB1 more everolimus would linger within the cancer cells, exerting more of its anti-tumor work. Future diagnosis of genetic alteration of Rs1045642 C>T in ABCB1 could be pivotal for determining if patients would benefit more from everolimus.

Keywords: polymorphism; cytotoxicity; mTOR inhibitor everolimus; breast cancer

1. Introduction

It is known that in humans *ABCB1* (P-glucoprotein (PGY1), Multi-drug Resistance Gene (MDR1), Doxorubicin Resistance, Colchicin Sensitivity) encodes for one of the main cellular membrane drug transporters. Moreover PGY1 is an efflux transporter able to actively move across cell membranes different drugs using ATP [1]. In human, the ABCB1 gene presents elevated number of polymorphisms (SNP) and in fact in literature there are already 50 SNPs situated in its gene coding regions [2]. Two SNPs, rs1128503 e rs1045642, could modify the final conformation of proteins, compromising membrane stability and the recognition of the substrate [3,4]. A clinical study on Georgian patients has demonstrated that SNP rs1045642 and rs1128503 of ABCB1 have resulted in significant association with breast cancer, with p-values of 0.012 and 0.016, respectively [5]. A meta-analysis composed of 10 randomized-to-control studies, totalling to 5,282 breast cancer and 7,730 controls, has demonstrated that single nucleotide rs1045642 C>T is associated with an increased risk of breast cancer (TT *vs.* CC: OR = 1.45, IC 95% = 1.14–1.30, TT *vs.* CT/CC: OR = 1.13, IC 95% = 1.04–1.23, TT/CT *vs.* CC: OR = 1.22, IC 95% = 1.02–1.46) [6].

The single nucleotide polymorphism rs1045642 is important also for its capacity to interfere with the kinetics of numerous antitumor drugs. Therefore it has an important role in avoiding the rising of side effects. In Everolimus-treated MBC patients, it has been demonstrated that polymorphism rs1045642 of ABCB1 is associated with an increased risk to encounter mucositis (OR = 2.30, IC 95% = 1.08–4.77, $P = 0.031$; multivariable analysis) [7]. Different studies have been conducted to define if the presence or absence of SNPs could alter drug metabolism. However, the results from these studies have been often discordant, probably because of different experimental conditions to obtain statistical significance [8].

In this mini-review we will discuss the potential of polymorphism of single nucleotide rs1045642 C>T in ABCB1 and its potential effect on everolimus-induced toxicity in breast cancer patients.

2. Current therapies for metastatic breast cancer

The majority of women diagnosed with breast cancer, undergo a biopsy after surgical intervention to characterize their tumor and therefore understanding its biology. Successively, the biological sample is sent to a laboratory for biological characterization. Particular attention is given to the presence/absence of oestrogen and progesterone receptors. Patients with oestrogen receptor (ER)-positive undergo anti-ER therapies or aromatase inhibition. Usually in women, hormones have many beneficial effects, however in breast cancer, the excessive production of such hormones drives proliferation of malignant cells and therefore their activity must be inhibited.

In pre-menopausal patients, generally tamoxifen is given together with LHRH, which blocks LH release, which is a luteinizing hormone produced by the pituitary gland and it can stimulate the ovary activity, additionally inducing an artificial menopause.

On the other hand, in post-menopause patients' the ovary do not produce enough oestrogens needed for the organism, and androgens produced by the adrenal gland are converted into oestrogens by the aromatase enzyme. Therefore in such case, drugs are used to inhibit aromatase enzymes (anastrozole, letrozole and exemestane).

Another important receptor to consider during tumor characterization is the modified version of the human epidermal growth factor 2 (HER-2), the HER-2/neu. The signalling pathway of this protein leads to cellular growth and differentiation. Overexpression of HER-2/neu is associated with major possibility to encounter regressions. Patients positive for such receptor go through trastuzumab treatment, which can block such receptors impeding tumor growth.

3. PI3K/mTOR/AKT pathway

Phosphatidylinositol-3-kinase (PI3K) has been identified as an important target for cancer research [9]. In fact PI3K is a kinase family, whose main function in the biochemical field is to phosphorylate a hydroxide at position 3 on the inositol ring of Phosphatidylinositol. The PI3K is a heterodimer constituted of a regulatory subunit and a catalytic subunit, existing in different isoforms [10]. There are three different PI3K classes (class I, II e III) on the bases of protein domains that form it and determine their specificity [10].

In human oncology, the PI3K pathway is one of the main pathways showing anomalies. In particular, activation of the PI3K is a frequent event occurring in human tumors, favouring the cellular proliferation, cell survival and resistance to chemotherapy and radiotherapy. It is therefore evident that new treatments capable of acting, alone or in combination, on the PI3K-AKT-mTOR pathway have a high therapeutic potential. The PI3K pathway inhibitors at different stages of development have a high therapeutic potential [10]. The PI3K inhibitors are in different phases of development and are grouped depending on their specificity into the following categories as shown in Figure 1.

(1) Selective PI3K inhibitors
(2) Dual inhibitors (block PI3K and mTOR)
(3) selecting mTOR inhibitors
(4) selective AKT inhibitors

The mammalian target of rapamycin (mTOR), is among one of the most important elements of regulation of protein synthesis [11].

In eukaryotic cells, translation of proteins is a process that has a fundamental role in differentiation, cellular growth and apoptosis [12]. Cell growth is controlled also by the cell cycle. The latter depends on cyclin dependent kinases (CDK), whose function is to phosphorylate factors needed for cell cycle progression. CDK4 and CDK6 phosphorylate tumour suppressor RB1, which must be un-phosphorylated in order for cell cycle to be blocked at G1 in normal cells. In subset of tumors, such as pockets of malignant mesothelioma patients, overexpressing CDK4/6, the CDK4/6 must be inhibited in order to block cancer cells at G1 [13].

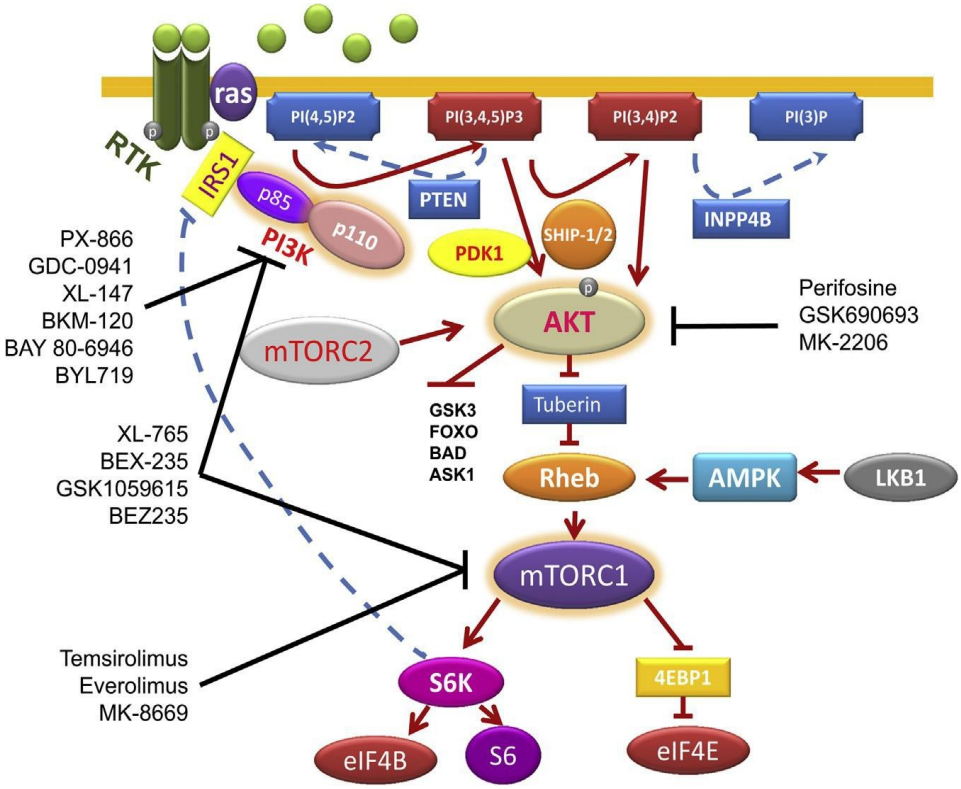

Figure 1. Therapeutic targets of the PI3K-AKT-mTOR pathway.

3.1. mTOR as a protagonist in the cell cycle and growth

The mTOR pathway is regulated by a variety of cellular signals, among which are mitogens and growth factors (such as IGF-1 e IGF-2), hormones like insulin, nutrients (aminoacids, glucose), levels of ATP and stress conditions [11]. mTOR plays a crucial role in protein synthesis and at the beginning of translation [12]. The mTOR pathway controls also transcription of ribosomal proteins and synthesis of ribosomal RNA [14,15]. The main objectives of mTOR are two, ribosomal protein S6 kinase (p70S6K) and the factor of transduction initiation (4E-BP1). The mTOR kinasis, answering to aminoacids and growth factor phosphoryl 4E-BP1 induce its dissociation from eIF4E, which can bind to mRNA enabling the beginning of cap-dependent transcription mechanisms [16].

PI3K/mTOR/AKT pathway depends also on PP2A phosphatases. In fact, it has been demonstrated that mTOR phosphorylates PP2A *in vitro*, inhibiting its activity, while treatment with rapamycine increments activity of phosphatases *in vivo*. Phosphatase and tensin homolog on chromosome 10 (PTEN) counteracts PI3K activity through dephosphorylation of PIP2 and PIP3 generated by PI3K [17].

The pathway involves the serine/threonine protein kinase AKT (also known as protein kinase B or PKB), a downstream effector of PI3K and an upstream regulator of mTOR [17]. The mTOR is phosphorylated by AKT on Ser 2448 *in vitro* and *in vivo* [17–21].

3.2. mTOR complexes

mTOR is a catalytic subunit of two multiprotein complexes, mTORC1 sensitive to rapamycine

and mTORC2 insensitive to rapamycine if not following to a prolonged treatment. mTOR Complex 1 (mTORC1) is constituted of mTOR, regulatory protein G of mTOR called Rheb, a similar protein similar to beta subunit of protein LST8/G of mammals (mLST8/GβL) and of PRAS40 e DEPTOR, recently identified [22]. mTORC1 is found downstream of AKT and its activity is controlled by a series of signals including Ras/Raf/MEK/ERK and a cascade of signals LKB/AMPK. This complex is characterized by interactions between mTOR and its associated regulatory proteins regulating its function, setting a stage to recruit mTORC1 substrates [23]. The activity of such complex is stimulated by insulin, growth factors, serum, phosphatide acid, aminoacids (in particular by leucine) and ossidative stress. mTORC1 is inhibited by low levels of nutrients, low growth factors, reductive stress, caffeine, rapamycine, farnesiltiosalicilic acid and curcumin. The rapamycin and its analogues are allosteric inhibitors of mTORC1; they do not bind to the catalytic units but associate with FKB-12 leading to a disassembly of mTORC1 complex, inhibiting its activity [24].

mTORC1 regulates a series of critical steps involved in protein synthesis. The mTORC1 targets best characterized are kinase proteins p70S6 (p70S6K) and 4E-BP1. Successively P70S6K phosphorylates ribosomal protein p40, S6, which participate in the translation of mRNA, and phosphorylates also eIF4B (eucariotic initiation factor 4B), which is involved in translation. The phosphorylation of 4E-BP1 results in the release of eIF4E, which in association with eIF4G stimulates the beginning of translation. In fact non-phosphorylated 4E-BP1 interacts with eIF-4E factor and avoids the formation of eIF4 complex, blocking interaction between eIF-4G and eIF-4E [25]. mTORC1 regulates different key passages in protein synthesis, controlling the expression of proteins promoting differentiation and cell survival. AKT regulates the mTORC1 complex, phosphorylating and inhibiting the TSC-2 gene (Tuberous Sclerosis 2), which is a GAP protein (GTP-ase activating protein) binding to TSC-1 (Tuberin) forming a complex and blocking the G Rheb protein. The inhibition of TSC-2 enables Rheb protein to accumulate in a state bound to GTP and activate mTORC1 [26]. In addition to amino acids and glucose, also fat acids could regulate the mTORC1 complex. In the heart, for example, free fatty acids are powerful activators of a cascade of events conducing to its activation. In such scenario the activation of mTOR causes inhibition of protein kinase activating monophosphate adenosine (AMPK- alpha), implicated in the control of cellular energy. The mechanism controlling mTORC2 is not yet known; activation of such complex is related to the PI3K signalling [27]. A notorious mTOR inhibitor is everolimus. This drug has been FDA approved after the promising results of BOLERO-2 trial. This clinical investigation was a multicenter phase 3 clinical trial, randomized and double-blinded, which recruited 724 HR+ metastatic breast cancer patients. The clinical investigation evaluated everolimus with aromatase inhibitor (AI) exemestane versus placebo. The patients enrolled in the trial had previously progressed to AI anastrozole or letrozole. This trial demonstrated an improved progression-free survival (PFS) of 11 months in exemestane + everolimus arm in contrast with the 4.1 months of placebo + exemestane [$P < 0.0001$; hazard ratio = 0.38; 95% confidence interval (CI): 0.31–0.48] [28]. Therefore the combination of everolimus plus exemestane doubled the PFS time vs. placebo plus exemestane alone. As an outcome this trial brought to FDA-approval of everolimus with exemestane for the treatment of HR+ MBC patients who did not respond to letrozole nor anastrozole treatments.

In order to improve the efficacy of everolimus treatment it is important to consider toxicity of the drug in order to give to patients the maximum tolerated dose without having serious side effects. A better understanding of mechanisms of toxicity to certain patients is therefore crucial for administering the drug the most efficient way.

The ABCB1 is an ATP binding cassette subfamily B member1, located on 7q21.12 for the membrane glycoprotein with activation of ATP-dependent pump, whose function is that of expel from cell cytoplasm toxic substances of the organism. The presence of functional receptors can have an impact on drug toxicity for cells.

We will end this review by discussing more on how the toxicity of anti-mTOR drug everolimus could be dependent on genetic mutations, with a particular focus on mutations altering the normal function of the ATP pump receptors.

4. Prospective and opinion

When a targeted therapy is formulated, it is necessary to take into consideration a very important factor: the metabolic response. The liver is the main region of drug metabolism. Drugs must be easily metabolized by reactions, such as Redox-reactions, hydrolysis, hydration, conjugations, condensation or isomerization. Intervening enzymes in tissues generally are very concentrated in the liver. The speed of drug metabolism changes within patients. Some patients metabolize a drug so rapidly that hematic and tissue concentrations are not reached. Some patients metabolize; while other patients metabolize so slowly that the normal doses can cause toxicity. Loads of it is due to genetics. One of the factors that are a main influence of drug metabolism is the genotype of those genes belonging to the superfamily of cytochrome p450. There is not only one cytochrome p450, but there are other enzymes belonging to this superfamily: CYP 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, 3A5, 4A11 and 4A7. Among them, CYP 1A2, 2A6, 2C9, 2D6, 2E1 and 3A4 are the major responsible for the hepatic metabolism of drugs and it is therefore necessary to investigate before the presence of gene polymorphism encoding these enzymes involved with metabolism to avoid the occurrence of undesirable and dangerous complications during treatment [29]. Examples of correlations between anti-tumor drugs and cytochromes implied in metabolism are reported in Table 1.

Table 1. Cytochromes involved in breast cancer therapies.

Drug	Involved Cytochromes	Significance Outcome	References
Tamoxifen	Tamoxifen is metabolized through demethylation, catalyzed by enzyme CYP3A4	Non steroidal Oestrogen inhibitor	US9896466B2
Letrozole	Letrozole metabolism is mediated by CYP2A6 and CYP3A4.	Non steroidal Aromatase inhibitor	US7465749B2
Exemestane	*In vitro* studies showed that the drug is metabolized by cytochrome P450 CYP3A4 and aldoketoreductase	Steroidal Aromatase Inhibitor	US4808616A
Everolimus	Inhibitors of CYP3A4 or PgP could increase hematic concentrations of everolimus reducing metabolism or efflux of the everolimus from intestinal cells. Inducers of CYP3A4 or PgP could reduce hematic concentrations of everolimus increasing metabolism or efflux of everolimus from intestinal cells.	Rapamycin Analogue inhibitor of mTOR	US6440990B1

In order for everolimus to be efficient it needs to stay within the cells for an appropriate amount of time. However, an excessive period could lead to serious toxicities of the drug. The drug, after exerting its beneficent functions it must be expelled from the cell. ABCB1 is an important receptor for the liquidation of the drug, as described in the previous chapter. The receptor is therefore needed to avoid drug toxicity. The ABCB1 protein expression has important beneficent implications. It reduces the presence of the drug within the cells, decreasing toxicity and increasing the maximum tolerated dose. Therefore having a functional ABCB1 receptor expressed on the cells would ultimately and indirectly lead to an increase in treatment efficacy. The reduced velocity with which the cells expel the drug can be therefore toxic for the cells. The major side effects correlated with everolimus toxicity are reported below:

- ORAL CABLE DISORDERS

Stomatitis, mucositis, small skin lesions

- RESPIRATORY SYSTEM DISORDERS

Pneumopathies, interstitial pneumonia, dyspnoea

- METABOLIC DISORDERS

Weight loss, hyperglycaemia, transaminitis

- OTHER DISORDERS

Onicopatia, disgeusia, asthenia, skin rash, appetite reduction, headache, epistaxis, pyrexia [30].

Since there is loads of inter-patients toxicity and response to everolimus therapy variation, we have been evaluating in our hospital the polymorphism of *ABCB1* genes as an important factor leading to the toxicity. There are nine common SNPs on ABCB1: rs1045642, rs6949448, rs2235067, rs2032583, rs2032582, rs1922242, rs1128503, rs2520464 and rs3789243 [31].

Pasqual *et al.* measured in 90 post-menopausal women with HR+ HER2- BC treated with examestane and everolimus following progression to non-steroidal inhibitors pharmacokinetics and pharmacodynamics in 37 patients [7]. They found 12 SNPs in genes involved in everolimus pharmacokinetics and pharmacodynamics and discovered an association with clinically relevant toxicities, dose reduction or treatment suspension due to toxicity. They found that ABCB1 Rs1045642 was associated with risk of mucositis ($p = 0.031$). The Rs1045642 C>T is a synonymous mutation (I1145I), present in their population with a minor allele frequency (MAF) of 0.41, with specific frequencies of: C/C 24%, C/T 56%, T/T 21%. The rs1128503 C>T is also a synonymous alteration (G412G), present in their population with a MAF of 0.40, with specific frequencies of: C/C 34%, C/T 45%, T/T 21%. The rs2032582 G>T is a missense mutation (A893S) with a MAF of 0.35, with specific frequencies of: G/G 32%, G/T 53% and T/T of 15% [7].

In our opinion *ABCB1* nucleotide alterations could be significantly associated with toxicity. While rs1045642 C>T and rs1128503 C>T are both synonymous mutations, the rs2032582 G>T is a missense mutation that alters an amino acid in the ABCB1 (Figure 2).

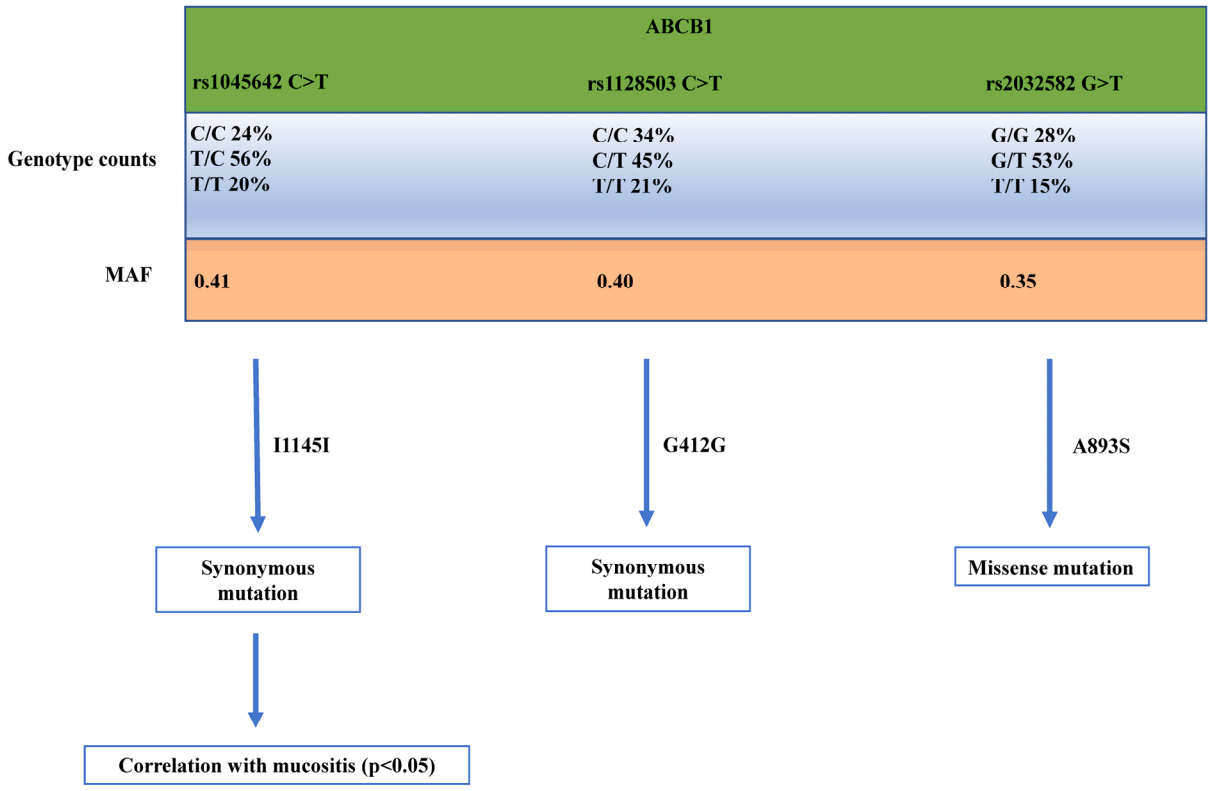

Figure 2. ABCB1 Polymorphisms in metastatic breast cancer.

Accordingly to Pascual *et al.*, polymorphic rs1045642 allele T alters the expression of the gene encoding for the everolimus transporter ABCB1. Polymorphic rs1045642 C>T significantly correlated with risk of mucositis (OR 2.11, 95% CI 1.02–4.37, p = 0.043, according to univariate analysis; OR 2.27, 1.06–4.77, p = 0.031, according to multivariable analysis) [7]. This could be attributable to a fast change by which cells expel everolimus. This means that a therapy able to revert this polymorphism could lead to less everolimus-related side effects, reducing therefore risks to develop stomatitis and small skin injuries.

Personalized medicine aims to use molecular changes within tumors of patients to direct clinical treatment. Besides tumors, personalized medicine can be used to measure thiopurine methyltransferases before administration of azathioprine in intestinal inflammation. In oncology it becomes crucial in determining a maximum tolerated dose for the highest efficacy of the drug, without being toxic to the individual [32].

Personalized medicine in future should involve tailoring of therapy based on biology and genetics of each tumor as well as the clinical characteristics of each patient [33].

Better strategies for ad-hoc treatments of patients, based on the molecular characterization of tumors, will definitely rely on the study of single nucleotide polymorphisms.

The progress that has been achieved in the field is already encouraging and better conclusions from *new* clinical trials are warranted. For example it has been demonstrated that *FGFR4* rs1966265 and FGFR2 rs2981578 have substantially contributed to the clinical outcome of breast cancer treated with chemotherapy, based on docetaxel-epirubicin-ciclophosfamide [34]. Moreover it has been also demonstrated that a polymorphism of uridine glucuronosyltransferase (UDPGT) is able to predict

clearance of dell'epirubicine. In fact, it has been reported that clinical outcomes of early stage breast cancer with UGT2B7-161 C>T SNP correlate with metabolism, toxicity and efficacy of the chemotherapy and epirubicin [35].

Conflict of interest

The authors declare no conflict of interest for the contributions in this manuscript.

References

1. Ambudkar S V, Lelong IH, Zhang J, et al. (1992) Partial purification and reconstitution of the human multidrug-resistance pump: Characterization of the drug-stimulatable ATP hydrolysis. *Proc Natl Acad Sci U S A* 89: 8472–8476.
2. Hoffmeyer S (2000) Functional polymorphisms of the human multidrug-resistance gene: Multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci* 97: 3473–3478.
3. Kimchi-Sarfaty C, Oh JM, Kim IW, et al. (2007) A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science* 315: 525–528.
4. Fung KL, Pan J, Ohnuma S, et al. (2014) MDR1 synonymous polymorphisms alter transporter specificity and protein stability in a stable epithelial monolayer. *Cancer Res* 74: 598–608.
5. Al-Eitan LN, Rababa'h DM, Alghamdi MA, et al. (2019) Role of Four ABC Transporter Genes in Pharmacogenetic Susceptibility to Breast Cancer in Jordanian Patients. *J Oncol* 2019.
6. Wang Z, Wang T, Bian J (2013) Association between MDR1 C3435T polymorphism and risk of breast cancer. *Gene* 532: 94–99.
7. Pascual T, Apellániz-Ruiz M, Pernaut C, et al. (2017) Polymorphisms associated with everolimus pharmacokinetics, toxicity and survival in metastatic breast cancer. *PLoS One* 12: e0180192.
8. Marzolini C, Paus E, Buclin T, et al. (2004) Polymorphisms in human MDR1 (P-glycoprotein): Recent advances and clinical relevance. *Clin Pharmacol Ther* 75: 13–33.
9. Baselga J (2011) Targeting the phosphoinositide-3 (PI3) kinase pathway in breast cancer. *Oncologist* 16 Suppl 1: 12–9.
10. Arcaro A, Guerreiro A (2007) The Phosphoinositide 3-Kinase Pathway in Human Cancer: Genetic Alterations and Therapeutic Implications. *Curr Genomics* 8: 271–306.
11. Saxton RA, Sabatini DM (2017) mTOR Signaling in Growth, Metabolism, and Disease. *Cell* 168: 960–976.
12. Sonenberg N, Hinnebusch AG (2009) Regulation of Translation Initiation in Eukaryotes: Mechanisms and Biological Targets. *Cell* 136: 731–745.
13. Sobhani N, Corona SP, Zanconati F, et al. (2017) Cyclin dependent kinase 4 and 6 inhibitors as novel therapeutic agents for targeted treatment of malignant mesothelioma. *Genes Cancer* 8: 495–496.
14. Pelletier J, Thomas G, Volarevi S (2017) Ribosome biogenesis in cancer: New players and therapeutic avenues. *Nat Rev Cancer* 18: 51–63.
15. Iadevaia V, Liu R, Proud CG (2014) MTORC1 signaling controls multiple steps in ribosome biogenesis. *Semin Cell Dev Biol* 36: 113–120.

16. Qin X, Jiang B, Zhang Y (2016) 4E-BP1, a multifactor regulated multifunctional protein. *Cell Cycle* 15: 781–786.
17. Hay N, Sonenberg N (2004) Upstream and downstream of mTOR. *Genes Dev* 18: 1926–1945.
18. Reynolds IV TH, Bodine SC, Lawrence JC (2002) Control of Ser2448 phosphorylation in the mammalian target of rapamycin by insulin and skeletal muscle load. *J Biol Chem* 277: 17657–17662.
19. Sekulić A, Hudson CC, Homme JL, et al. (2000) A direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. *Cancer Res* 60: 3504–3513.
20. Navé BT, Ouwens DM, Withers DJ, et al. (1999) Mammalian target of rapamycin is a direct target for protein kinase B: Identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. *Biochem J* 344: 427–431.
21. Scott PH, Brunn GJ, Kohn AD, et al. (1998) Evidence of insulin-stimulated phosphorylation and activation of the mammalian target of rapamycin mediated by a protein kinase B signaling pathway. *Proc Natl Acad Sci U S A* 95: 7772–7777.
22. Zhou H, Huang S (2010) The Complexes of Mammalian Target of Rapamycin. *Curr Protein Pept Sci* 11: 409–424.
23. Pópulo H, Lopes JM, Soares P (2012) The mTOR signalling pathway in human cancer. *Int J Mol Sci* 13: 1886–1918.
24. Yang J, Chi Y, Burkhardt BR, et al. (2010) Leucine metabolism in regulation of insulin secretion from pancreatic beta cells. *Nutr Rev* 68: 270–279.
25. Dennis MD, Jefferson LS, Kimball SR (2012) Role of p70S6K1-mediated phosphorylation of eIF4B and PDCD4 proteins in the regulation of protein synthesis. *J Biol Chem* 287: 42890–42899.
26. Huang J, Manning BD (2008) The TSC1-TSC2 complex: A molecular switchboard controlling cell growth. *Biochem J* 412: 179–190.
27. Zaha VG, Young LH (2012) AMP-activated protein kinase regulation and biological actions in the heart. *Circ Res* 111: 800–814.
28. Yardley DA, Noguchi S, Pritchard KI, et al. (2013) Everolimus plus exemestane in postmenopausal patients with HR+ breast cancer: BOLERO-2 final progression-free survival analysis. *Adv Ther* 30: 870–884.
29. Zanger UM, Schwab M (2013) Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* 138: 103–141.
30. Baselga J, Campone M, Piccart M, et al. (2012) Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med* 366: 520–529.
31. Levran O, O'Hara K, Peles E, et al. (2008) ABCB1 (MDR1) genetic variants are associated with methadone doses required for effective treatment of heroin dependence. *Hum Mol Genet* 17: 2219–2227.
32. Jackson SE, Chester JD (2015) Personalised cancer medicine. *Int J Cancer* 137: 262–266.
33. Goutsouliak K, Veeraraghavan J, Sethunath V, et al. (2019) Towards personalized treatment for early stage HER2-positive breast cancer. *Nat Rev Clin Oncol*.

34. Chen L, Qi H, Zhang L, et al. (2018) Effects of FGFR gene polymorphisms on response and toxicity of cyclophosphamide-epirubicin-docetaxel-based chemotherapy in breast cancer patients. *BMC Cancer* 18: 1038.
35. Sawyer MB, Pituskin E, Damaraju S, et al. (2016) A Uridine Glucuronosyltransferase 2B7 Polymorphism Predicts Epirubicin Clearance and Outcomes in Early-Stage Breast Cancer. *Clin Breast Cancer* 16: 139–144.e3.

AIMS Press