Choline kinase as a precision medicine target for therapy in cancer, autoimmune diseases and malaria

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Cancer cells have an altered metabolism that provides advantages to support unregulated growth and higher duplication rates. Among these critical changes, energy generation through aerobic glycolysis as well as increased glutamine catabolism, are essential components associated with altered levels of specific metabolites. Up-regulation of lipid metabolism also occurs frequently in cancer cells. Increased fatty-acid biosynthesis as well as their elongation are among recognized events that define cancer cells. Not surprisingly, the CDP-choline and CDP-ethanolamine pathways responsible for the generation of membrane phospholipids is a major alteration frequently found in human tumours. Choline kinase \( \alpha \) (ChoK\( \alpha \)) plays a critical role in this latter metabolic route and is the focus of a targeted therapeutic strategy. Small molecule inhibitors of this enzyme are effective and selective anticancer drugs that have recently entered clinical trials. More recently, Cho K\( \alpha \) inhibitors have been proposed as a novel therapeutic approach against malaria and rheumatoid arthritis. Here, the evidence that support the use of ChoK\( \alpha \) as a novel drug target for precision medicine approaches is discussed.

Keywords: phospholipids metabolism; choline kinase; choline kinase inhibitors; anticancer drugs; rheumatoid arthritis

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Introduction

Targeted therapy is one of the most active areas of research in modern medicine. This is the result of a boost in the understanding of the molecular basis of many dreadful diseases that emerges from a dramatic increase in the information generated by international consortia. As an example, the availability of massive sequencing information on the genetic alterations that take place in the onset and progression of 50 different types of cancer provided by The International Cancer Genome Consortium (ICGC), is making it possible to identify potential candidates of genes responsible for each particular pathology (https://icgc.org). The expectation of this huge international effort is to be able to identify some of the critical genes (the “drivers”) involved in the onset and progression of those 50 cancer types, pinpointing novel potential candidate targets for therapy [1]. This information may promote the unexpected use of already approved drugs for cancer pathologies to which they were not originally intended and developed.

It is well established that cancer is a group of diseases that results from the accumulation of genetic and epigenetic alterations in a set of genes involved in the regulation of critical cellular functions [2]. However, more than 200 cancers exist, each one with specific and diverse sets of alterations, which in principle makes rather difficult, if not impossible, to design a universal strategy to treat all these pathologies. This is of particular relevance in tumours where genetic and clinical complexity has been demonstrated as in
the case of breast cancer \[3\]. Furthermore, not all patients with similar pathologies respond equally to same treatments depending upon genetic and epigenetic diversity of each individual \[4, 5\]. Thus, personalized and targeted therapies must be designed for each specific type of cancer and, within each disease, for each patient itself. Therefore, the right treatment must be provided to the right patient, and also to the right cancer, to increase the probability of a successful outcome.

Since similar pathologically classified cancers may bare a set of different alterations defining their differential clinical behaviour, it imposes the requirement of improved diagnostic tools and specific treatments \[4, 5\]. These personalized therapies must be based on the use of drugs designed to efficiently modulate the identified molecular targets for each particular patient. Interestingly, there may be available drugs already approved for other diseases, acting against the specific targets responsible for the pathological behaviour of cancer cells. These drugs could be also indicated to treat specific cancers harbouring those alterations.

Besides all these complexities and specificities for the more than 200 pathologies grouped under the designation of cancer, all cancer cells may require a small set of common alterations to make it possible to support their uncontrolled and unrestricted growth \[2\]. Therefore, there may be room for a reasonable optimism in our battle against cancer. Among these essential alterations, metabolism, and in particular phospholipid metabolism, may emerge as a rather universal requirement that may be used for the design of successful broad-spectrum anticancer strategies. Phosphatidylcholine (PC) is the most abundant phospholipid required for the construction of membranes in all eukaryotic cells. This essential lipid is regulated mostly by the CDP-choline pathway, known as the Kennedy pathway, of which choline kinase (ChoK) \[1\] is one of its critical enzymes (Figure 1). In a wider perspective, choline metabolism is emerging as a critical metabolic hallmark of a large variety of tumours and some of the enzymes involved in its modulation has attracted great attention \[6\]. The best known enzyme involved in choline metabolism related to cancer onset and progression is ChoK.

**ChoK**

ChoK is presented in humans as two genes (Figure 2), CHKA and CHKB, located in chromosomes 11q13.1 and 22q13.33 respectively and coding for proteins of ca.50 kDa (ChoK\(\alpha\)) and 45 kDa (ChoK\(\beta\)) 395 amino acids. Two
differential splicing mRNAs species are generated from the CHKA gene, rendering two proteins, ChoKα1 (52 kDa, 457 amino acids) and ChoKα2 (50 kDa, 439 amino acids) with ChoKα1 harboring an extra domain of 18 amino acids as their only difference.

Despite an overall sequence identity of 56% between ChoKα and ChoKβ, evidence for different metabolic and biological functions has been reported. The most striking difference has been found in KO mice studies. ChoKα is essential for mouse embryo development since its removal results in embryonic lethality [9]. By contrast, ChoKβ is dispensable for mouse embryo development and ChoKβ KO mice are viable but acquire rostrocaudal muscular dystrophy [10]. A similar disease has been found in humans linked to mutations of the CHKB gene [11]. These results support differential roles for both ChoKα and ChoKβ isoforms in vivo.

Reinforcing the essential role of ChoK activity in development, a recent report indicates that CEK4, a member of the Choline/Ethanolamine kinase family, is also essential in embryo development in plants [12].

That ChoKα and ChoKβ may have differential roles is further supported by direct enzymatic measurement since both have choline kinase and ethanolamine kinase activity when purified. However, in whole cells assays, overexpression of ChoKα displays both activities while overexpression of ChoKβ has only ethanolamine kinase activity [13]. Furthermore, while ChoKα KO mice are not viable, ChoKα+/− heterozygous mice shows a 30% decrease in ChoK activity, and shows an accumulation of Cho and a reduction of PCho in liver and testis. However, PC levels and expression of other enzymes involved in PC biosynthesis are unaffected. These results altogether suggest that ChoKβ does not compensate for the levels of PC biosynthesis under in vivo conditions.

In the ChoKβ KO mice, total ChoK activity is greatly decreased, but no significant change in the total amount of PC or PE is detected in several tissues such as liver, brain, kidney, and heart. However, and in keeping with its effects, a 30% decrease in PC was found in forelimb and hindlimb muscles, suggesting that residual ChoKα is sufficient to maintain normal PC levels in most tissues, but not in limb muscles. Finally, reduced PE levels observed in ChoKα+/− heterozygous mice provides evidence for a role of ChoKα in the biosynthesis of PC and PE, confirming previous studies in whole-cells systems [13]. This is in keeping with the observation that PE levels are unaffected in ChoKβ KO mice, and suggest that PE homeostasis is fully maintained when the ChoKα protein is intact [10].
The crystal structure of human ChoK proteins demonstrates that is a dimeric protein \([14, 15]\). The ratio of homo- (\(\alpha/\alpha\), \(\beta/\beta\)) or hetero- (\(\alpha/\beta\)) dimer populations seems to be tissue-specific \([16]\). Combination of the two isoforms renders different levels of activity under cell-free system conditions being the \(\alpha/\alpha\) homodimer the most active combination, the \(\beta/\beta\) homodimer the less active, and the \(\alpha/\beta\) heterodimer showing an intermediate phenotype \([16]\). This ability for dimerization may have important biochemical and biological consequences since it has been proposed that a balance of expression of both isoforms results in a differential effect on the regulation of cell cycle \([17]\), introducing a potential rather complex system for the regulation of its function, based on epigenetic and transcriptional regulation.

**Role of ChoK\(\alpha\) in cancer**

ChoK\(\alpha\) is overexpressed in a large diversity of human tumours including breast \([18]\), lung \([19, 20]\), colorectal \([19]\), bladder \([21]\), prostate \([19, 22]\), ovary \([23]\), endometrial \([24]\), and T-cell lymphoma \([25]\). Increased levels of ChoK\(\alpha\) expression have been reported also in osteosarcoma tumour-derived cancer cells \([26]\), HBV-induced hepatocarcinomas \([27]\), breast, colon- and liver-derived cancer cells \([28]\), glioblastoma-derived cell lines \([29]\) and pancreatic tumour-derived cell lines \([30]\). In addition, ChoK\(\alpha\) expression levels have been proposed to be a marker of lung \([20]\), breast \([31, 32]\), bladder \([21]\), ovary \([22]\) and liver \([33]\) cancer aggressiveness and metastasis, and is associated in breast cancer with an ER- status \([18, 32]\), increased invasiveness and drug resistance \([31, 32, 34]\). All these findings identified ChoK\(\alpha\) as a novel therapeutic target in cancer \([35]\), although no evidence suggests a differential role for ChoK\(\alpha\) 1 or ChoK\(\alpha\)2.

Indeed, both small molecules \([36-43]\) and siRNAs \([44-46]\) that modulate ChoK\(\alpha\) activity have been designed and proved to be potent antitumour strategies. In further support of this, siRNA knockout of ChoK\(\alpha\) is lethal for HeLa cells, but ChoK\(\beta\) alone, or simultaneous silencing of ChoK\(\alpha\) and ChoK\(\beta\) has no effect on cell viability, suggesting that an adequate balance of both isoenzymes is required for cell viability \([17]\).

All these results taken together identifies ChoK\(\alpha\) as a bonafide new target in oncology, and as a consequence, several groups have made attempts to design small molecules as new inhibitors of its enzymatic activity and characterize their activity as anticancer drugs under in vitro and in vivo conditions.

We developed the first family of ChoK\(\alpha\) inhibitors (ChoKIs) with proven antitumour activity as Hemicolinium-3 (HC3) derivatives. MN58b, a first generation ChoK\(\alpha\) competitive inhibitor, showed potent antiproliferative and antitumoural activity \([36-43]\). RSM-932A, a second generation of ChoKIs \([47]\), has completed a first-in-human, Phase I clinical trial (http://clinicaltrials.gov/ct2/show/NCT01215664). After this initial fundamental finding, several families of ChoKIs have been demonstrated to be potent antitumour drugs \([48-52]\). Alternatively, siRNA have been used successfully as a further strong proof of concept evidence \([44-46]\).

While the implication of ChoK\(\alpha\) in human cancer is now well recognized, the role of ChoK\(\beta\) is still not fully resolved. Genetic evidence suggests a different role of ChoK\(\beta\) than cancer affection \([9-11]\) and inhibition of ChoK\(\beta\) expression by siRNA does not affect cell viability \([17]\). However a full analysis of the involvement of ChoK\(\beta\) in different tissues and tumours may be needed to definitively discard any involvement of this isoenzyme in cell cycle regulation and human cancer onset and progression.

**Mechanism of Action of ChoKIs and ChoK\(\alpha\) Silencing**

Several works study the consequences of interfering with ChoK\(\alpha\) activity that could explain the mechanism of action of ChoK\(\alpha\)-specific inhibitors (ChoK\(\alpha\)Is) and ChoK\(\alpha\) silencing with specific siRNA. Chemical or siRNA inhibition of ChoK\(\alpha\) renders a variety of effects including loss of mitochondria potential and release of cytochrome c \([53]\), increased ceramides production \([54]\), ER stress \([55]\), unfolded protein response (UPR) \([55]\), and ROS homeostasis through alteration of glutathione levels \([56]\). All these effects end with the subsequent activation of cell death including apoptosis or necrosis with an exquisite specificity towards cancer cells \([53-57]\).

Although most of these effects could be attributed to the direct effects on PC metabolism by interfering with ChoK\(\alpha\) activity, evidence has been provided to implicate ChoK\(\alpha\) in pathways other that PC metabolism. Some signalling pathways involved in proliferation in many cell systems are affected by ChoKIs and ChoK\(\alpha\) siRNA. In breast cancer cells and HeLa cells, both ERK and AKT phosphorylation are reduced by either chemical inhibition or siRNA silencing \([46, 58]\). Furthermore, in breast cancer cells, ChoK\(\alpha\) is phosphorylated by c-Src and associates with the EGFR \([59]\), a key component in the regulation of cell proliferation in this system. Pharmacological inhibition or molecular silencing of ChoK\(\alpha\) in T-cell lymphomas decreased Ras-GTP activity, attenuates AKT and ERK phosphorylation, and reduces expression of MYC. All these events results in cell death by apoptosis or necrosis \([25]\). In keeping with these observations, there is evidence that MYC and HIF1 are involved in the
regulation of ChoKα expression [25, 60, 61], suggesting that overexpression of ChoK may be a consequence of the acidification that is produced in many solid tumors. By contrast, neuronal differentiation of Neuro-2a cells induced by retinoic acid, a process that is associated with inhibition of proliferation and extension of neurite outgrowth, is linked to the upregulation of ChoKα expression [62]. This process is under control of CCAAT/Enhancer binding Protein-β (C/EBPβ) that binds to the CHKA promoter with the involvement of Ras oncoproteins in both cellular proliferation and differentiation processes [63-67]. In keeping with this, ChoKα regulation has been positioned downstream of Ras and regulated by PI3K and Ral-GDS, two well known direct effectors of the Ras oncoprotein [68].

A drastic and sustained alteration of the levels of expression of proteins involved in the regulation of endoplasmic reticulum (ER) stress and the UPR such as GRP78, PDI, IRE1α, CHOP, C/EBPβ and TRB3 occurs after ChoKαsi treatment and siRNA silencing in breast, lung and colon-derived tumour cells [55]. By contrast, in non-tumourigenic cells, treatment with ChoKαIs induce a drastic increase in ATF4 that generates a transient and attenuated ER stress response. These alterations are selective resulting in cell death in cancer cells through a CHOP dependent process, but triggers G1 cell cycle arrest in non-tumourigenic mammary and colon cells. This differential effect can be explained since ChoKαIs induced in tumour cells a drastic reduction in cyclin D1, RB and E2F1α, all well known G1 to S phases checkpoint mediators, while the levels of E2F1α and total RB are not significantly affected in non-tumourigenic cells [55]. These results suggest that maintenance of the RB-E2F1α complex in non-tumourigenic cells induces arrest in G0/G1 but not cell death in these cells [55].

siRNA interference drives HeLa cells to apoptotic death, but treatment with the specific ChoKα inhibitor V-11-0711 (IC_{50}=20 nM), causes reversible growth arrest, suggesting that catalytic inhibition of ChoKαIs not sufficient for the induction of cell death in HeLa cells, and that this enzyme plays a role in cancer cell survival other than regulation of PC synthesis [169]. These results can be reconciled with all previous observations when analysing the effects of different ChoKαIs. When these inhibitors also promote ER stress and UPR, a strong apoptotic effect is observed [55]. However if a partial reduction in the levels of ChoKα protein is achieved, there is a weak induction of ER stress and UPR and, as a consequence, a weak induction of apoptosis in cancer cells [55]. Thus, inhibition of the catalytic activity of ChoKα may not be sufficient for the induction of cell death in tumour cells and a drastic reduction of the ChoKα content may be also required. These results are also in keeping with the proposed balance between ChoKα and ChoKβ as a mechanism for regulation of cell proliferation where the specific reduction of the levels of ChoKα is required to trigger cell death [17]. Thus, inhibitors of ChoKα must be designed that induce both a reduction of its enzymatic activity and a drastic reduction of its intracellular levels. A prototype for these inhibitors is RSM-932A (also named TCD-717) that has recently entered clinical trials (http://clinicaltrials.gov/ct2/show/NCT01215864) and has been shown to be able to both, inhibit ChoKα enzymatic activity and reduce ChoKα levels by UPR most likely due to induction of a conformational change exposing the unfolded structure of the enzyme to endogenous proteases [55].

The above results linking ChoKα in the regulation of cell proliferation and apoptosis are further supported by gene expression studies. Thus, alteration of the transcriptional activity of some critical pathways involved in cell cycle regulation and apoptosis response [70] or nucleotide metabolism [71] have been reported by interference with ChoKα activity.

In epithelial ovarian cancer (EOC) cells, interference with ChoKα induce a reduction in the tumourigenic properties of these cells [56]. This antitumour activity was related to a specific altered ROS homeostasis induced by a reduction in cysteine and glutathione (GSH) levels in ChoKα-depleted cells. This effect was observed in tumour cells but not in non-tumourigenic cells, and is mediated by a decrease of the trans-sulphuration pathway [55]. In contrast with other cellular systems, no significant effect on ERKs and AKT signalling was observed in the ChoKα-depleted EOC cells [46, 56, 58]. This is in keeping with the colon cancer-derived cells SW620, where no significant effect on AKT-Ser473 phosphorylation or increase in ceramides production are observed under similar conditions [71]. These results indicate that although increased expression of ChoKα is a frequent event in many types of tumour cells that make them addicted to its activity, the mechanism for acquisition of this dependency and therefore generation of resistance, diverges from cell to cell type.

Attempts have been made to investigate the metabolomic and transcriptomic characteristics of human breast cancer xenografts by high-resolution magic angle spinning (HR-MAS) MR spectroscopy [72, 73]. The expression levels of CHKA and GDPDP5 in breast cancer xenografts derived from luminal-like, basal-like and HER2 enriched breast cancer tissues, strongly correlated to their corresponding human samples. Differential patterns were observed for metabolic profiles and levels of expression of genes involved
in choline metabolism when luminal B and basal-like xenografts were compared, suggesting its usefulness for the classification of subtype-specific tumours providing a very elegant model for the analysis of the relevance of the CDP-choline pathway in the onset of breast cancer. This model could be useful also to design more precise targeted therapies and follow up of patients sensitive or resistant to therapy [3].

On this regard, the use of abnormal choline metabolism as a diagnostic tool and for follow up of response in cancer treatment is being explored by means of 1H magnetic resonance spectroscopy (MRS) and positron emission tomography (PET) imaging. The different imaging techniques in choline metabolism that are useful for diagnosis of cancer and follow up of response to treatment has been recently reviewed [74].

**Resistance to ChoKIs Treatment**

Resistance to chemotherapy agents is a common clinical problem in the treatment of cancer [75-77]. In fact, the appearance of chemotherapy-resistant cancers is one of the most significant obstacles for the cure of cancer. It is estimated that almost 50% of all tumours are intrinsically resistant to chemotherapy, and most of the remaining will develop resistance after treatment. Resistance may occur in primary therapy (innate resistance) or appear during treatment (acquired resistance). Information on the mechanisms of action of conventional cancer drugs and cancer drugs under development, along with their corresponding mechanisms of resistance, is needed for a better use of proper combination schedules [75-77]. With this knowledge, we may anticipate and prevent a lack of response to specific agents or its innate or acquired resistance, and circumvent the problem. It will be also possible to design specific strategies for an adequate follow up of patients depending upon each treatment, and accommodate treatment to the most convenient combination of drugs.

The main goal of cancer treatment is to trigger cell death of tumour cells (apoptosis, necrosis, autophagy, etc). However, chemotherapeutics and radiotherapy activates signalling pathways that turns on both apoptotic and cell survival mechanisms [77]. Some of these pathways can be affected by genetic alterations that are found in specific types of tumours, and can diverge between patients within the same kind of tumour [4]. Among them, survival signals involve the MAPKs (ERK, JNK, p38), PI3K/AKT and NFκB pathways while those related to cell death are mostly driven by inhibiting these survival networks or up-regulating some members of the MAPKs superfamily.

Our recent studies demonstrate that the antitumour effects of ChoKαIs in primary NSCLC tumours and in lung cancer-derived cells are overcome by overexpression of acid ceramidase (ASAH1), an enzyme involved in the metabolism of ceramides [78]. These results imply that both innate and acquired resistance to ChoKα inhibitors is linked to overexpression of ASAH1, and are in keeping with the known mechanism of action of ChoKα that induce increased levels of ceramides [54] and allows the identification of tumours that will be resistant to ChoKα even prior to start treatment. As a consequence, combination of acid ceramidase inhibitors with ChoKα could be a powerful strategy to overcome resistance to ChoKα inhibition, and as recently demonstrated, this strategy works under in vitro conditions in NSCLC-derived cells [78]. Alternative mechanisms for acquisition of resistance to ChoKα in pancreatic (Mazarico *et al*, unpublished) and breast cancer cells (Alvarez *et al*., unpublished) have been identified indicating that each specific type of tumour may develop specific resistance mechanisms to ChoKα inhibition that deserve further investigation.

**Combinatorial Strategies with ChoKI**

A synergic effect of ChoKα inhibition and 5FU has been reported in breast cancer cells [78] and colon cancer cells [71]. Alteration of levels of expression of enzymes involved in the metabolism of 5FU such as TS and TK1, is related to the mechanism of action of ChoKα, enhancing its antitumour effects and preventing the generation of 5FU resistance [71]. This effect seems to be executed at transcriptional level and mediated by down modulation of E2F1, a transcriptional factor that control transcription levels of TS and TK1 [71]. Similar effects have been observed for deoxyuridine triphosphatase (dUTPase) and ribonucleotide reductase M2 (RRM2), two additional enzymes involved in the mechanism of action of 5-FU and also under control of E2F1 (de la Cueva *et al*, unpublished). Therefore combination of ChoKα with 5FU increases its antitumour effects and avoids generation of resistance to 5FU. This finding may have an impact in improving the management of colorectal cancer patients.

Recently it has been reported that CK37, a specific ChoKα, is synergic with gemcitabine or cisplatin using Jurkat cells as a model, but failed to synergize with doxorubicine or cyclophosphamide [24]. CK37 was also found synergic with valproic acid (histone deacetylase inhibitor) but not with bortezomid (proteasome inhibitor). The molecular mechanisms to explain these differential effects have not been explored but deserve further consideration for a more rational use of ChoKα inhibitors.

Finally, similar synergistic effects have been obtained when lung cancer cells were treated with cisplatin and
ChoKαIs (Cebrian et al., unpublished observations) and when pancreatic ductal adenocarcinoma-derived cells were treated with ChoKαIs along with gemcitabine, 5-FU and oxaliplatín (Mazarico et al. unpublished). These results provide further evidence that the use of novel, previously unexplored strategies such as interfering with phospholipid metabolism to selectively eliminate cancer cells is a very promising approach to improve the outcome of actual cancer treatments and overcome resistance to chemotherapy.

Thus, a better knowledge of the mechanism of action of drugs directed towards enzymes involved in lipids metabolism such as ChoKα and their mechanism of resistance may facilitate the design of more efficacious combinatorial therapies along with novel or existing drugs. The evidence provided by several groups indicate that ChoKα could be used in a broad spectrum of combinatorial regimes against several types of human cancers.

**ChoKIs, More that Anticancer Drugs**

Since PC synthesis is a general requirement of cancer cells, the therapeutic effects of inhibiting ChoKα has been demonstrated towards a large variety of tumour-derived cancer cells and tumour xenografts [35, 47-49, 53-60]. Surprisingly, blockade of this metabolic pathways has proven to be non deleterious to normal, primary cells and to immortalized non-tumourigenic cells, suggesting that different responses are triggered in tumourigenic and non-tumourigenic cells as previously shown in a variety of cell systems [53-60].

Recently, ChoK dysfunction has been involved in other pathological circumstances. As indicated above, lack of ChoKα in mice is lethal at early embryo development [9], while ChoKβ is not required for embryo viability but is necessary for the proper development of forelimbs [10, 11]. The effects of lack of ChoKβ in mice are due to differential expression patterns [80, 81], affect mitochondrial function [82, 83] and alter neuromuscular junctions [84]. Several recent reports demonstrate that inactivating mutations at the CHKB gene are responsible of human muscular dystrophy and myopathy [11, 82-92]. Therefore, since the lack of ChoKβ activity is the cause of muscular dystrophy, any intervention restoring its activity may serve to reinstate normal muscle development in this pathological setting.

ChoK has been shown to play a critical function in the life cycle of human pathogens such as *Plasmodium falciparum*, since ChoKIs are effective against this parasite under in vitro and in vivo assays [93, 94]. This effect is due to the fact that both the primary sequence and the tertiary structure of the catalytic site of *P. falciparum* ChoK are similar to that of the human ChoKα. ChoKIs interfere with *P. falciparum* viability both in *in vitro* studies or in *in vivo* mouse models of malaria [95, 96]. HC-3, the progenitor structure of MN58b and RSM-932A, two potent specific inhibitors of the human ChoKα, inhibits the *P. falciparum* ChoK and is lethal to the parasite by interfering with choline transport [96, 97]. Other effective inhibitors for *P. falciparum* ChoK have been reported recently [98, 99]. Of interest, MN58b and RSM-932A, two potent anticancer drugs, also have potent antimalarial activity with inhibitory concentrations in the low nanomolar range, blocking the parasite’s intraerythrocytic development, which may further effect on its egress or invasion [94]. These compounds are effective against both sensitive and resistant strains, a relevant finding due to the emergence of resistance to artemisin derivatives, the actual treatment of choice in countries where the disease is endemic [100]. The availability of new targets for development of new drugs against *P. falciparum* is of great interest, and ChoK constitutes a novel target with a promising future.

ChoKα are also very efficient for the treatment of rheumatoid arthritis in animal models, indicating that these drugs have the potential to be used as potent therapeutics also in inflammatory diseases [101]. MN58b, a potent specific ChoKα inhibitor, suppressed cell migration and resistance to apoptosis of cultured fibroblast-like synoviocytes (FLS), involved in cartilage destruction in rheumatoid arthritis (RA). Support to this has been provided using the K/BxN arthritis mouse model, since ChoK inhibition with MN58b significantly decreased FLS migration and proliferation, and abrogated joint inflammation and damage in either pretreatment or established disease protocols [101].

This is not surprising but in keeping with previous observations that establish synovial inflammation, hyperplasia and joint destruction as hallmarks of RA [102]. Phosphoinositide 3-kinase (PI3K)/Akt and Mitogen-activated protein kinase (MAPK) are involved in the regulation of FLS function in RA, including regulation of matrix metalloproteinases (MMP) expression and synoviocyte growth and survival, and due to this critical role are the focus of therapeutic intervention in RA [103]. Indeed, as indicated above, selective inhibition of ChoKα by MN58b attenuates MAPK and PI3K/Akt signalling [104, 58] and inhibition of PI3K has been shown to affect ChoK activity [104]. ChoKα is a potent modulator of these signalling pathways in FLS, and its inhibition blocks phosphorylation of Rb, downregulates cyclin D1, and interferes with PDGF signalling, as previously shown in other cell systems [69].

**Concluding Remarks**
Cancer is a compendium of more that 200 diseases, each with specific genetic and epigenetic alterations that engage a number of signalling pathways and induce metabolic disturbances. Similar pathologically classified cancers behave in a very distinct manner due to the accumulation of a set of different genetic and metabolic alterations that define their differential clinical behaviour. These genetic differences impose the requirement of specific treatments that will need to be combinatorial to be most effective. Personalized therapies must be based on proper combination of available drugs that efficiently modulate the specific molecular targets altered in each tumour. The identification of new potential targets is the driving force for the development of novel effective drugs. Some metabolic alteration, such as increased phospholipid demand, may be a rather common characteristic effective drugs. Some metabolic alteration, such as increased phospholipid in eukaryotic membranes, required is a critical enzyme for the generation of PC, the most phospholipid abundant phospholipid in eukaryotic membranes, required for cell proliferation of cancer cells. ChoKα is a critical enzyme for the generation of PC, the most abundant phospholipid in eukaryotic membranes, required for cell proliferation of cancer cells. ChoKα is frequently overexpressed in a large number of human tumours and its inhibition specifically induce cell death of cancer cells but a reversible cell cycle arrest of non-tumourigenic cells. It may constitute a strategy for the treatment of many cancer types since it executes a novel mechanism of action and can be combined successfully with a number of current therapeutic approaches. ChoKα inhibitors have been found effective as well for the treatment of other diseases, including malaria and rheumatoid arthritis. RSM-932A (also known as TCD-717) is the first ChoKα inhibitor that has reached clinical trials as a new anticancer drug leading the path to new effective drugs against cancer, malaria and rheumatoid arthritis.

Abbreviations

(ChoKα, ChoKβ): Choline Kinase α or β; (PC): phosphatidylcholine; (PCho): phosphocholine; ChoK inhibitor; (ChoKαI): ChoKα-specific inhibitor; (NMR): nuclear magnetic resonance; (siRNA): small interfering RNA; (5-FU): 5-Fluorouracil; (PI3K): phosphatidylinositol 3 kinase; (MAPK): mitogen activated protein kinase; (EGRF): epidermal growth factor receptor; (dUTPase): deoxyuridine triphosphatase; (RRM2): ribonucleotide reductase M2; (MRS): magnetic resonance spectroscopy; (PET): positron emission tomography; (HR-MAS MRS): high-resolution magic angle spinning magnetic resonance spectroscopy; (FLS): fibroblast-like synoviocytes; (RA): rheumatoid arthritis.

Disclosure of Potential Conflicts of Interest

JC Lacal holds patents for the medical use of choline kinase inhibitors.

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Although the official name for choline kinase is CHK, I prefer to name it as ChoK to avoid confusion with the checkpoint kinase, also designated as CHK. Some authors use CK for choline kinase, but unfortunately it is the traditional name of casein kinase and I will also avoid its use.

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