Check for updates

The FEBS Journal

FEBS PRESS science publishing by scientists

STATE-OF-THE-ART REVIEW

Autophagy and mitochondrial metabolism: insights into their role and therapeutic potential in chronic myeloid leukaemia

Pablo Baquero[1], Amy Dawson[1] and Gudmundur Vignir Helgason[1,2]

1 Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow, UK
2 Paul O'Gorman Leukemia Research Centre, Institute of Cancer Sciences, University of Glasgow, UK

Keywords
autophagy; chronic myeloid leukaemia; metabolism; mitochondria; oxidative phosphorylation; therapeutics

Correspondence
G. V. Helgason, Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow, G61 1QH, UK
Fax: +44 141 330 5021
Tel: +44 141 330 7245;
E-mail: Vignir.Helgason@Glasgow.ac.uk

(Received 9 June 2018, revised 7 August 2018, accepted 14 September 2018)

doi:10.1111/febs.14659

Despite the development of selective BCR-ABL-targeting tyrosine kinase inhibitors (TKIs) transforming the management of chronic myeloid leukaemia (CML), therapy-resistant leukaemic stem cells (LSCs) persist after TKI treatment and present an obstacle to a CML cure. Recently, we and others have made significant contributions to the field by unravelling survival dependencies in LSCs to work towards the goal of eradicating LSCs in CML patients. In this review, we describe these findings focusing on autophagy and mitochondrial metabolism, which have recently been uncovered as two essential processes for LSCs quiescence and survival respectively. In addition, we discuss the therapeutic potential of autophagy and mitochondrial metabolism inhibition as a strategy to eliminate CML cells in patients where the resistance to TKI is driven by BCR-ABL-independent mechanism(s).

Introduction

Chronic myeloid leukaemia (CML) is a clonal myeloproliferative disease that arises following transformation of a haemopoietic stem cell (HSC) by the chimeric oncogene *BCR-ABL*. *BCR-ABL* expression results from a reciprocal translocation between chromosome 9 and 22 leading to the generation of the abnormal Philadelphia chromosome [1]. In turn, the expression and translation of *BCR-ABL* give rise to a constitutively active nonreceptor tyrosine kinase, which is the main driver of the chronic phase (CP) of the disease [2]. The majority of newly diagnosed CML patients are in CP, which if not treated, can progress to accelerated phase followed by a blast crisis over a 5-year time frame [3]. The development of selective BCR-ABL tyrosine kinase inhibitors (TKIs), represented by the first-generation TKI imatinib and subsequent second- and third-generation TKIs (i.e. dasatinib, nilotinib, bosutinib, ponatinib), have significantly improved the life expectancy of CP CML patients, making CML a clinically manageable disorder in the majority (50–70%) of patients [4,5]. TKIs are highly effective at eliminating dividing cells, yet they fail to eradicate the most primitive, quiescent CML leukaemic stem cells (hereafter referred as LSCs). This may ultimately promote acquired TKI resistance and drive relapse or disease progression [6]. It has been estimated that more than 80% of CML patients who respond to TKI maintain minimal residual disease (MRD) due to the presence of therapy-resistant LSCs [7]. Even in

Abbreviations
ATG, autophagy genes; CML, chronic myeloid leukaemia; CP, chronic phase; LSC, leukaemic stem cell; OXPHOS, oxidative phosphorylation; TKI, tyrosine kinase inhibitors.

patients in deep molecular response, where BCR-ABL expression is undetectable by quantitative PCR, the existence of LSCs capable of reinitiating the disease has been demonstrated, highlighting the relevance of targeting LSCs in order to achieve cure [8]. Furthermore, around 25% of all CML patients are refractory to all available TKIs, mainly due to TKI intolerance or resistance [9]. In this context, resistance to TKI treatment can be classified in BCR-ABL-dependent or BCR-ABL-independent resistance; depending on the mechanism, the cells obtain to overcome the effects of the drugs. Although BCR-ABL-dependent resistance is caused by mutations that affect the binding of the TKI to the kinase pocket of BCR-ABL, BCR-ABL-independent mechanisms are less well understood. The understanding of these mechanisms, together with the development of approaches for LSCs elimination, is currently a major challenge for researchers working on CML [10].

In recent years, autophagy and mitochondrial metabolism have emerged as two crucial processes that cancer cells can use to promote resistance to anticancer therapy in several settings, including haematological tumours [11–14]. Furthermore, recent studies have uncovered that inhibition of either of these processes in combination with TKIs is an effective strategy to eliminate therapy-resistant LSCs. Here, we discuss the role of autophagy and mitochondrial metabolism in the regulation of LSCs and their utility as therapeutic targets in CML.

Role and regulation of autophagy in cancer

Cellular homoeostasis involves strict regulation of coordinated mechanisms in order to sustain cell health. Autophagy is one of these mechanisms and plays an important role in the recycling of intracellular components [15]. The best characterized variant of autophagy, macroautophagy (hereafter referred as autophagy), involves the formation of the autophagosome, a double membrane structure that engulfs the cargo and delivers it into the lysosomes for degradation. One important step in the initiation of autophagy is the activation of ULK1, a serine/threonine protein kinase that forms a complex with ATG13 and FIP200 and drives autophagosomes formation [16]. Under nutrient replete conditions, mTORC1 phosphorylates ULK1 and ATG13, inhibiting autophagy initiation [17]. On starvation, ULK1 is dephosphorylated followed by autophosphorylation and phosphorylation of ATG13 and FIP200, leading to a rapid induction of autophagy [18]. Furthermore, autophagy can be enhanced as well by an energetic stress response mediated by the AMP-activated protein kinase (AMPK). AMPK is activated in response to a drop in the intracellular levels of ATP and stimulates autophagy by inhibiting mTORC1, and also by phosphorylating ULK1, as well as class III phosphatidylinositol-3-kinase (PI(3)K/Vps34) and Beclin 1 (BECN1) [19,20]. The complex VPS34-BECN1-ATG14-p150 promotes elongation of forming autophagosomes following synthesis of phosphatidylinositol 3-phosphate. Furthermore, autophagosome completion is mediated by two ATG7-dependent ubiquitin-like conjugation systems: (a) the ATG12/ATG5/ATG16 and (b) the conjugation of phosphatidylethanolamine to the microtubule-associated protein 1 light chain 3 beta, also known as LC3B [21]. Finally, the outer membrane of the autophagosome fuses with the lysosomal membrane to form the autolysosome, where the degradation of the autophagic body together with its cytoplasmic content occurs [18].

Although autophagy has long been considered a non-selective catabolic process, more recently it was shown to selectively degrade potentially harmful intracellular material. For instance, autophagy can eliminate protein aggregates and damaged mitochondria by two processes known as aggrephagy and mitophagy respectively [22,23]. These mechanisms can prevent the development of a pro-tumourigenic environment characterized by an accumulation of reactive oxygen species (ROS), DNA damage, and genomic instability [24,25]. In line with these effects, conditional deletion of the autophagy-related (*ATG*) genes *Atg5* or *Atg7* in murine models promotes tumour initiation in different tissues such as liver and pancreas [25–27]. Conversely, several studies demonstrate that autophagy deficiency prevents tumour progression towards a malignant phenotype, indicating that cancer cells can take advantage of autophagy to develop into a more aggressive tumour [28,29]. Indeed, the oncogenes *H-ras*^{V12}, *K-ras*^{V12}, and *B-raf*^{V600} have been shown to upregulate autophagy, providing cancer cells with higher levels of basal autophagy than their normal counterparts [29–31]. These results support the concept of a dual role of autophagy in cancer, depending on the type or stage of the disease.

Although mTORC1 is constitutively activated in > 70% of all cancers [32], it has been reported that mTOR activation and high basal autophagy levels can coexist in the same tumour. A possible mechanism involved in this apparent paradox relies on the phosphatase PP2A, capable of dephosphorylating ULK1, hence activating autophagy bypassing mTOR-mediated inhibition of ULK1 [33]. In addition, mTORC1 regulates autophagy at a transcriptional level by phosphorylating and inactivating the family of MiT/TFE transcription factors [34]. These proteins promote the expression of *ATG* genes [35,36]. Interestingly, tumour

cells can also bypass mTOR-mediated negative regulation on MiT/TFE proteins activating autophagy and lysosome gene expression [37]. This way, cancer cells can benefit from both the biosynthetic effects mediated by mTORC1 and the metabolic changes associated with autophagy-dependent catabolic processes. These data, together with the fact that mutations in *ATGs* are rare in human cancer [38], indicate that a functional process of autophagy is required for cancer progression and support the use of autophagy inhibitors as a therapeutic approach for cancer treatment. However, as previously discussed, autophagy appears to have a temporal or context-dependent role in different cancers. For example, Rosenfeldt *et al.* [27] showed that, in mice bearing oncogenic *Kras* and lacking *p53*, autophagy deficiency does not block tumour progression but, in contrast, accelerates tumour growth. These results highlight the importance of studying the role of autophagy in different genetic contexts and robust preclinical models before perusing with autophagy inhibition as a therapeutic intervention.

Role and regulation of autophagy in CML and LSCs

In CML, the role BCR-ABL plays in the regulation of autophagy is controversial. It has been shown that autophagy is induced in both CML cell lines and LSCs following *in vitro* treatment with imatinib, suggesting that BCR-ABL is a negative regulator of autophagy [12,39]. However, other studies demonstrate that expression of this oncogene promotes autophagosomes formation and that autophagy is essential for BCR-ABL-dependent leukaemogenesis [40,41]. In agreement with the latter, CML cell lines that express high levels of BCR-ABL (e.g. K562, KCL22) have a very active autophagy flux, suggesting the existence of mechanisms involved in bypassing the negative effects on autophagy mediated by BCR-ABL-dependent mTOR activation. Since BCR-ABL$^+$ cells exhibit higher levels of ROS than normal cells [42], one possibility is that elevated oxidative stress enhances autophagy, directly or indirectly through AMPK activation, similar to previous studies performed with other models [43] (Fig. 1). Interestingly, AMPK is active at basal levels in LSCs in acute myeloid leukaemia (AML) [44], and its activation leads to autophagy induction in normal HSCs [45]. Future studies will need to assess whether this important metabolic sensor is constitutively active and regulates autophagy in CML LSCs as well.

Rothe *et al.* [46] reported that haematopoietic stem/progenitor cells (CD34$^+$ cells) from CML patients express higher levels of key *ATG* genes compared with CD34$^+$ cells from healthy donors. Intriguingly, we have preliminary *in vitro* data suggesting the TKI treatment induces expression of *ATG* genes in CD34$^+$ cells, although interrogation of the expression of *ATG* genes in CD34$^+$ cells from CML patients, before and after 7 days of imatinib treatment, indicated no significant changes in the expression signature of *ATG* genes [47]. These data suggest that BCR-ABL may regulate autophagy at a transcriptional level in CML progenitor cells; however, since autophagy is a very dynamic process, which can be rapidly regulated by environmental conditions, further experiments measuring the expression of *ATG* genes following TKI treatment at different time points are required to support this conclusion. In this regard, *in vivo* models have recently been generated to measure autophagy flux, using mice expressing the autophagy marker LC3 tagged with GFP [48]. Using this system, our data demonstrate increased autophagy flux in the stem/progenitor cells of BCR-ABL-harbouring mice compared to their wild-type counterparts, with the former showing increased sensitivity to autophagy inhibitors [48]. Further utilization of this system will provide a suitable model to study the regulation of autophagy in CML LSCs at both transcriptional and post-translational levels.

In CML, the expression of *ATG* genes has been shown to be upregulated in more primitive populations compared to more mature cells [46]. In line with this, our results obtained from leukaemic mice expressing GFP-LC3 revealed that LSCs have higher autophagy levels compared to more differentiated cells [48]. Similarly, Warr *et al.* [49] have shown that normal HSCs are poised to rapidly use autophagy as an adaptive stress response and that this pro-autophagic programme is driven by the transcription factor Forkhead box protein O3 (FOXO3A). In CML, BCR-ABL activates AKT which in turn phosphorylates FOXO3A promoting its cytoplasmic retention and inactivation. TKI treatment downregulates AKT signalling localizing FOXO3A to the nucleus leading to cell cycle arrest [50]. Nevertheless, in a fraction of LSCs, FOXO3A is in the nucleus even in the absence of TKIs due to the negative regulation of AKT by TGFβ [51]. Therefore, another possible mechanism to explain the higher autophagy levels in LSCs compared to normal cells would be the activation of FOXO3A mediated by TGFβ, which might bypass the TKI-dependent effects on autophagy mediated by BCR-ABL/PIK3/AKT/mTORC1 signalling (Fig. 1).

Mice lacking *FOXO3A* show abnormally high ROS levels, impaired leukaemia initiating capacity and myeloproliferation [51,52]. Similarly, recent results from *in vivo* studies indicate that potent lysosomal

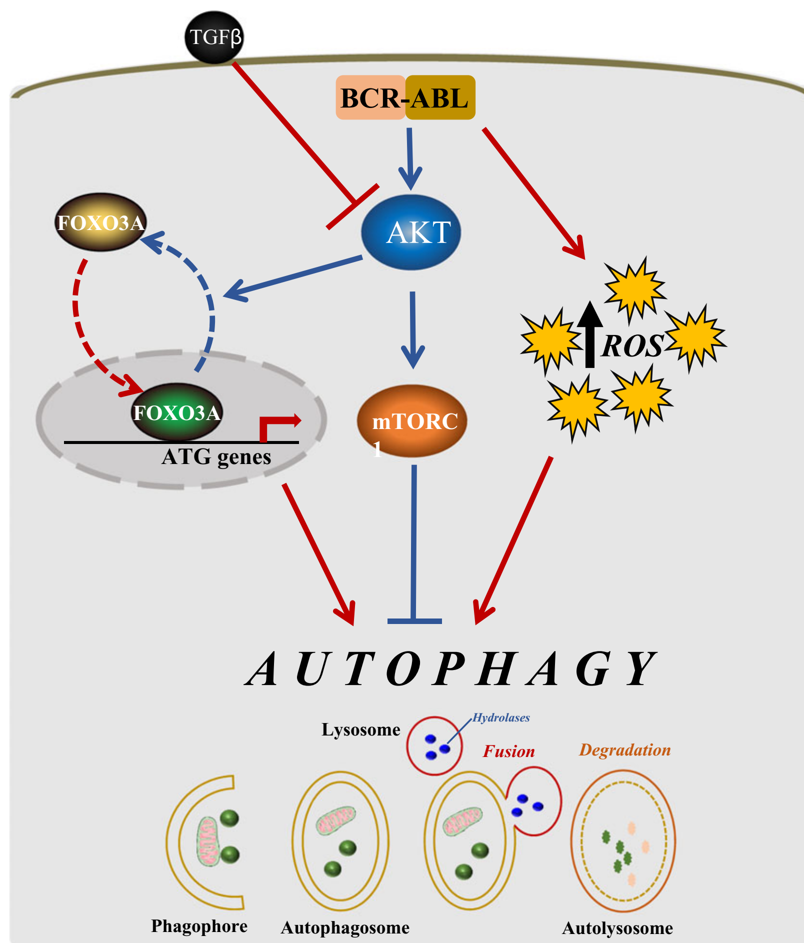

Fig. 1. Potential mechanisms regulating autophagy in LSCs. BCR-ABL-driven canonical signalling results in the activation of AKT and mTOR complex 1 (mTORC1). mTORC1 activation phosphorylates ULK1 leading to the inhibition of autophagy. Additional mechanisms are in place to allow autophagy activation in the presence of activated mTORC1. High levels of reactive oxygen species (ROS) driven by BCR-ABL potentially results in an oxidative stress-mediated activation of autophagy. ROS can enhance autophagy directly or indirectly through AMPK activation. Furthermore, TGFβ signalling blocks AKT inactivation of FOXO3A, leading to their relocalization from the cytoplasm (inactive) to the nucleus (active). Nuclear FOXO3A allows transcription of autophagy genes and a resultant activation of the autophagy process. Blue lines indicate inhibition and red lines indicate activation of autophagy.

inhibition drives LSCs out of quiescence leading to an expansion of myeloid progenitors [48]. In addition, these results are in line with previous studies in normal HSCs, showing that autophagy deficiency increases oxidative stress and rapidly reduces the stem cell pool [45,53,54]. For future studies, it would be informative to investigate whether FOXO3A promotes LSCs maintenance through autophagy and whether the exit of quiescence induced by autophagy inhibition is due to an accumulation of ROS in LSCs. Interestingly, recent work from Thorburn *et al.* shows that autophagy can regulate apoptosis by degrading cytoplasmic FOXO3A [55]. Therefore, these results regarding the link between autophagy, FOXO3A and the maintenance of LSCs warrant further investigation.

Interplay between autophagy and oxidative stress in LSCs

Our recent work, using an isotope-assisted metabolomics-based approach, has uncovered an increase in mitochondrial oxidative metabolism in LSCs compared to their normal counterparts. These results were linked to an increase in mitochondria content and membrane potential indicating that LSCs are respiring at a higher level than HSCs [56]. In line with these results, Giustacchini *et al.* [57] recently reported that LSCs displayed a significant enrichment for genes associated with oxidative phosphorylation (OXPHOS) and fatty acid metabolism when compared with normal HSCs. Interestingly, since mitochondria are a major source of

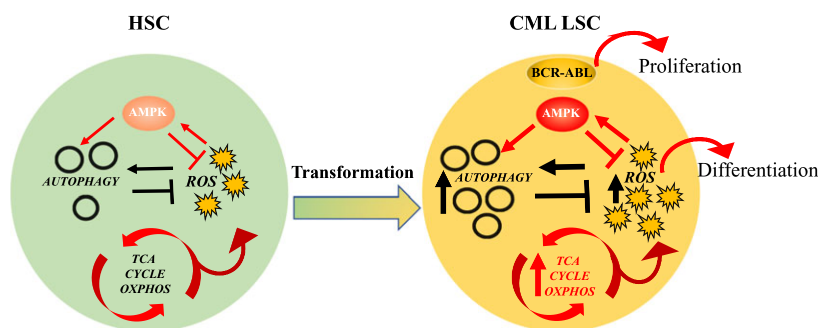

Fig. 2. Interplay between autophagy, mitochondrial metabolism, and ROS in HSCs and LSCs. Higher levels of reactive oxygen species (ROS) in leukaemic stem cells (LSC), compared with normal haematopoietic stem cells (HSC), might derive from increased mitochondrial metabolism in LSCs. Autophagy can be upregulated by an increase in ROS, maintaining oxidative stress below a certain level, in order to prevent ROS-induced differentiation. Additionally, AMPK can be activated by ROS, resulting in a cycle where AMPK might be modulating ROS levels directly and/or indirectly through autophagy induction.

ROS [58], these results support a model in which the elevated levels of ROS in LSCs compared to normal HSCs [42] might derive from an increase in mitochondria respiration. As aforementioned, autophagy has been shown to be upregulated when levels of ROS are high [59]; thus, it is possible that elevated levels of autophagy in LSCs are due to an increment in ROS that in turn derives from an increased mitochondrial respiration. Finally, high levels of autophagy might be preventing differentiation of LSCs by maintaining the levels of ROS below a certain threshold (Fig. 2). Importantly, *AMPK* deletion leads to an increase in ROS levels and depletion of AML LSCs [44]. Hence, AMPK might be another critical antioxidant factor to sustain the LSC pool. In a similar way to these results described in AML LSCs, AMPK could be exerting its antioxidant effects in CML LSCs by generating the reducing agent NADPH through upregulation of the pentose phosphate pathway, following increased glucose uptake [44]. In addition, mitochondrial ROS have been shown to be required for AMPK activation, which triggers a PGC-1α-dependent antioxidant response [43]. Therefore, we can speculate that in LSCs, AMPK activity might be enhanced by an increase in ROS levels and, in turn, AMPK can halt the oxidative stress by both autophagy-dependent mechanism and transcriptional regulation of antioxidant genes (Fig. 2).

One important question that arises from the proposed model would be whether autophagy is regulating the levels of ROS in LSCs by degrading mitochondria via mitophagy. In line with this, de Almeida *et al.* demonstrated that HSCs have higher mitochondrial mass and lower mitochondrial turnover than mature haematopoietic cells, a contradiction to previously established literature [60]. These results suggest that the elevated levels of autophagy reported in HSCs compared to their more differentiated counterparts [49] are not indicative of mitophagy upregulation. Given that the higher mitochondrial content and activity in LSCs compared to HSCs are critical for their survival [56], it is unlikely that the increase in ROS generated by mitochondrial respiration in LSCs is counteracted by mitophagy alone. Since several studies have previously demonstrated the role of autophagy as a ROS scavenger in HSCs and in CML cells [54,61], this suggests a model in which autophagy regulates oxidative stress mainly by other mechanisms than mitochondrial degradation. Such potential mechanisms include autophagy modulating the internal supply of amino acids such as glutamine [62], which is the main source of the antioxidant glutathione (GSH), and regulating the redox balance by degrading kelch-like ECH-associated protein 1 (KEAP1). Under homoeostatic conditions, KEAP1 mediates the ubiquitination of the nuclear factor erythroid 2-related factor 2 (NRF2) leading to its inactivation by proteasomal degradation. Following oxidative stress, KEAP1 is sequestered by the autophagy cargo receptor SQSTM1/p62 leading to the inhibition of the degradation of NRF2 and to its activation. This way, NRF2 can translocate to the nucleus and upregulate the expression of antioxidant defence genes, such as glutathione peroxidase, superoxide dismutase, and thioredoxin [59]. Interestingly, the KEAP1-NRF2 system can also regulate oxidative stress by affecting mitochondrial dynamics, as active NRF2 has been shown to induce mitophagy through PINK1 (PTEN-induced kinase 1) transcriptional upregulation [63]. Upon mitochondrial damage, PINK1 is accumulated on the mitochondrial surface and recruits PARKIN to ubiquitinate several mitochondrial proteins. Next, these ubiquitinated proteins

interact with LC3, coupling the autophagy machinery with the damaged or unwanted mitochondria due to be degraded [64]. Furthermore, NRF2 also induces mitochondrial biogenesis *in vivo* [65], and cells from KEAP1-knockout mice or treated with KEAP1 inhibitors display an increase in oxidative metabolism and ATP levels [66–68], indicating that the NRF2-KEAP1 system is vital to maintain the integrity of mitochondria, both at catabolic and anabolic level. If NRF2 proves to be upregulated in LSCs, this could potentially be the reason of the higher mitochondrial content and respiration in LSCs compared to their normal counterparts. Interestingly, two studies have recently uncovered different mechanisms for mitophagy in AML LSCs and HSCs [69,70], suggesting that this process might be regulated in a context-dependent manner. Although future studies will help elucidating these puzzling mechanisms in different cell types, it is now clear that CML and AML LSCs share a common dependency on OXPHOS, which may be a therapeutically exploitable vulnerability.

Targeting therapy-resistant CML cells with second-generation autophagy inhibitors

It has been previously shown that in CML, TKI treatment enhances autophagy that promotes survival and TKI resistance in LSCs [12]. This study led to the development of the randomized Phase II clinical trial CHOICES (ClinicalTrials.gov Identifier: NCT01227135) where the efficacy of imatinib versus imatinib plus 800 mg·day^{-1} HCQ was evaluated. However, results from this trial, and others recently conducted in other types of cancers, suggest that it is unlikely that consistent autophagy inhibition is achieved following HCQ treatment, even with the maximum tolerated dose given to patients [71–74]. These results highlight the need to develop and investigate more potent and/or selective autophagy inhibitors using robust preclinical models. In recent investigations, second-generation autophagy inhibitor, Lys05, appears to be a promising clinical alternative to HCQ [75]. These compounds accumulate within and deacidify the lysosome (lysosomotropism), resulting in impaired autophagy flux. Lys05 is a dimeric analogue of HCQ, which has shown 3- to 10-fold more potent effects than HCQ in cancer cell lines. Our recent data show that Lys05 achieves autophagy inhibition in LSCs and effectively sensitizes them to TKIs *in vitro* and *in vivo* [48]. Importantly, combination of TKI and HCQ did not add any effects to TKI as a single treatment on LSCs survival. More strikingly, the combination of Lys05 and nilotinib showed more selectivity for BCR-ABL$^+$ cells than for wild-type cells suggesting the existence of a potential therapeutic window.

Importantly, *in vivo* treatment of leukaemic mice with Lys05 results in a reduction in LSCs followed by an increase in progenitor cells [48]. From a clinical perspective, these results highlight the importance of combining lysosomotropic agents with TKIs. In this context, while the lysosomal inhibitor is promoting the exit of quiescence in LSCs leading to a more differentiated status, the TKI prevents an accelerated myeloproliferation by exerting its pro-apoptotic effects in progenitor and mature leukaemic cells (Fig. 3).

One of the major concerns about the clinical use of HCQ and its derivatives is the high risk of

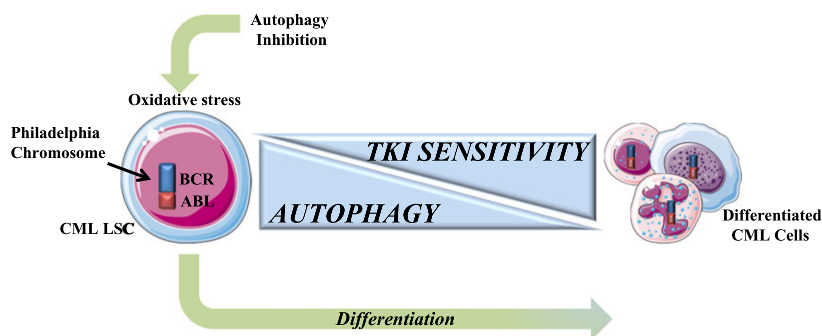

Fig. 3. Targeting LSCs with second-generation autophagy inhibitors. Chronic myeloid leukaemia stem cells (LSCs) have high levels of autophagy compared to normal haematopoietic stem cells. The use of second-generation autophagy inhibitors selectively targets LSCs driving them into differentiation, potentially by increasing oxidative stress. Tyrosine kinase inhibitors (TKI) target the more differentiated, proliferating cell population. Therefore, using second-generation autophagy inhibitors to drive cells towards a more differentiated phenotype should allow these cells to be targeted by TKI.

retinopathy due to a long-term exposure to the drug [76]. Given that this adverse effect has been directly associated with high doses and long duration of the treatment, the use of more potent autophagy inhibitors might help achieve the desired effects with lower doses and in a shorter time period, potentially mitigating such side effects. Furthermore, we and our collaborators have observed that mice treated with high doses of Lys05 developed Paneth cell dysfunction characterized by intestinal obstruction [75]. Interestingly, this toxicity recapitulates the intestinal phenotype of mice and humans with genetic defects in *Atg16L1* [77] providing an additional evidence that Lys05 targets autophagy *in vivo*. Such side effects must be considered against the potential benefit to patients when considering the introduction of second-generation autophagy inhibitors into the clinical setting.

A relevant clinical question is whether more selective autophagy inhibitors than HCQ and Lys05 could provide an effective therapeutic strategy to eliminate LSCs. PIK-III is a recently developed autophagy inhibitor, which inhibits the kinase activity of VPS34 leading to a defective autophagosomes formation [78]. Results using PIK-III demonstrate that *in vitro* treatment of CML $CD34^+$ cells with this compound reduces the number of cells with capacity to engraft and leads to an almost complete elimination of engrafted primitive Philadelphia positive cells when combined with TKI [48]. Although the chemical properties of PIK-III are not suitable for *in vivo* studies, new derivatives have already been developed with oral bioavailability and remain to be tested in CML models [79].

As previously mentioned, around 25% of CML patients develop mechanisms of resistance to TKI that are independent on BCR-ABL activity, many of which remain poorly understood. Of note, combined mTOR and autophagy inhibition has shown to be effective in targeting TKI-resistant CML cells [80]. Interestingly, Rebecca *et al.* recently identified palmitoyl-protein thioesterase 1 (PPT1), as a molecular target for dimeric quinacrines, which are more potent analogues of HCQ and Lys05 [81]. Strikingly, the action of these compounds is dual; inactivates mTORC1 by disrupting its lysosomal localization and deacidifies the lysosomes, which leads to a block in the autophagic flux. Since inhibition of mTOR with single agents induces cytoprotective autophagy and has largely failed as an efficient therapy for cancer, targeting PPT1 with dimeric quinacrines may offer a new and effective way to overcome BCR-ABL-independent resistance in CML patients.

Targeting therapy-resistant CML cells with inhibitors of oxidative phosphorylation

Cancer cells reprogramme their metabolic pathways to adapt to their high energy demands and sustain their uncontrolled proliferation [82]. Thus, identifying metabolic differences between normal and transformed cells provides an attractive opportunity to selectively target cancer cells. As previously discussed, LSCs display a higher mitochondrial respiration and have a greater expression of OXPHOS-related genes compared with normal HSCs [56,57]. We have recently exploited this OXPHOS-dependent metabolic vulnerability in LSCs by inhibiting mitochondrial metabolism using the antibiotic tigecycline [56]. Of clinical relevance, the combination of tigecycline and imatinib was able to eradicate LSCs and prevent disease relapse in preclinical patient-derived xenograft (PDX) model. Importantly, a minimal effect was found in normal HSCs following *in vivo* treatment indicating the existence of a therapeutic window. These results, together with the fact that tigecycline has already been approved by the FDA, point to the combination of tigecycline and imatinib as a suitable clinical approach to eliminate disease persistence in CML patients. However, results from an already conducted phase I clinical trial with tigecycline in AML patients were discouraging [83], as no significant clinical response was observed in any of the patients at the end of the trial. Tigecycline treatment did not affect the expression of mitochondrial-encoded proteins in 24 of 27 patients, indicating that the concentration achieved in AML patients was not sufficient to be effective and hit its target. Accordingly, the steady-state levels of tigecycline reported were around 1μM, a concentration that has minimum effect on mitochondrial metabolism *in vitro* [84]. The development of a more stable formulation [85] to sustain an effective plasma concentration support further investigations with tigecycline in the near future. Of note, the outcome of a study using tigecycline in combination with TKI and in a different population of patients might be different to the previously mentioned clinical trial, as tigecycline was used as a single agent in highly refractory AML patients.

Importantly, both CML blast crisis cells and AML stem cells exhibit increased fatty acid oxidation and high OXPHOS gene signature that provides resistance to chemotherapy [11,86]. Since both of these diseases have a 'high mutator' phenotype and are highly resistant to standard therapies, the reliance on oxidative metabolism provides a rational to test OXPHOS inhibitors as a strategy for targeting leukaemic cells with

alternative resistant mechanisms, including BCR-ABL-independent mechanisms. Moreover, these studies open up new opportunities to test other OXPHOS inhibitors that could be useful in targeting therapy-resistant CML cells such as VLX600 [87], Gamitrinib [88], and Etomoxir [89].

Future questions and concluding remarks

Inhibiting autophagy or mitochondrial metabolism in combination with TKI treatment represents potential clinical strategies to eliminate therapy-resistant LSCs in CML patients with MRD. Therefore, understanding the role of these two processes in LSCs and in normal HSCs is essential to develop effective and safe therapeutic approaches.

Although it has been shown that autophagy functions as a protective mechanism for cancer cells following therapy, it is important to consider that many of these studies were performed *in vitro* and the effect on autophagy was often measured after short time points. In LSCs, autophagy levels are upregulated compared to more mature cells suggesting that basal autophagy is a critical regulator of LSCs maintenance. Such evidence provides a rationale to test novel autophagy inhibitors in both preclinical and clinical settings in an attempt to eliminate LSCs. Of note, in our *in vivo* studies, we observed a reduction in LSCs following BCR-ABL and autophagy inhibition compared to TKI as a single agent. As mice were treated for 3 weeks, these results suggest that the eradication of LSCs might be progressive and not due to a rapid cytoprotective response mediated by BCR-ABL inhibition. Whether more selective autophagy inhibitors would achieve more potent effects than Lys05 in relation to inducing *in vivo* differentiation of LSCs is a question that remains to be fully answered. In addition, it is very likely that, inside the bone marrow, other survival mechanisms might be protecting LSCs from therapeutic insults. LSCs reside within an adapted niche that renders them resistant to therapy [90]. Interestingly, autophagy is currently emerging as an important process for the interaction between cancer cells and the stroma [91,92]. The role autophagy plays in mesenchymal cells in the leukaemic niche would be an important factor to consider in the context of future studies that aim to elucidate the mechanisms underlying LSCs maintenance. In this regard, the antitumour effects of systemic loss of autophagy is greater than specific autophagy loss in tumour cells, suggesting that both host autophagy and cell-autonomous autophagy contribute to tumour growth [93].

Importantly, an alternative process of autophagy can occur independently of the hierarchical activity of *ATG* proteins, named noncanonical autophagy [94]. This indicates that therapeutic strategies targeting canonical autophagy proteins might be bypassed by noncanonical mechanisms. As both forms of autophagy rely on the lysosomes for the degradation of cytoplasmic material, the use of lysosomotropic agents such as Lys05 or dimeric quinacrines could be the best choice as an autophagy targeting approach, despite their broad effect.

In addition, the metabolic effects on LSCs following TKI treatment would be particularly interesting to address in future studies. Although studies have provided some indications about this regulation, all of these experiments were performed *in vitro*, and further investigations should take advantage of the state-of-the-art Scl-tTa–BCR-ABL model [95] to compare the metabolic differences between stem cells isolated from long-term TKI-treated and untreated mice. Likewise, the contribution of the bone marrow microenvironment to the aberrant metabolism of LSCs remains an avenue for further investigations. Such questions can be addressed using relevant coculture and 3D *in vitro* culture systems; however, directly measuring aberrant metabolism *in vivo* as a result of an altered microenvironment remains technically challenging. Interestingly, recent studies showed that LSCs can uptake mitochondria from stromal cells by endocytosis, leading to an increase in chemotherapy resistance [96]. Given that BCR-ABL$^+$ cells can alter neighbouring BCR-ABL$^-$ haematopoietic progenitor cells [97], studying whether leukaemic cells are modulating the metabolism of BCR-ABL$^-$ cells through mitochondria transfer represents another attractive area for further investigation.

In conclusion, recent preclinical CML studies are paving the way to use second-generation autophagy inhibitors and OXPHOS inhibitors in CML clinical trials. However, our understanding of the role and connection of autophagy and mitochondrial metabolism in the regulation of LSCs is in its infancy. Future preclinical and clinical studies will provide further information about the therapeutic value of targeting autophagy and mitochondrial metabolism in CML.

Acknowledgements

We thank K. Rattigan for proofreading the article. This work was supported by Kay Kendall Leukemia Fund (KKL1069); Leuka; the Howat Foundation, Lady Tata Memorial Trust and Friends of Paul O'Gorman Leukaemia Research Centre. GVH is a

KKLF Intermediate Research Fellow, Leadership Fellow and John Goldman Fellow.

Author contributions

This review was written and edited by Pablo Baquero. The manuscript was edited by Amy Dawson and G. Vignir Helgason. Figures were generated by Pablo Baquero and Amy Dawson.

References

1 Heisterkamp N, Stephenson JR, Groffen J, Hansen PF, de Klein A, Bartram CR & Grosveld G (1983) Localization of the c-ab1 oncogene adjacent to a translocation break point in chronic myelocytic leukaemia. *Nature* **306**, 239–242.

2 Daley GQ, Van Etten RA & Baltimore D (1990) Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science* **247**, 824–830.

3 Sawyers CL (1999) Chronic myeloid leukemia. *N Engl J Med* **340**, 1330–1340.

4 Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, Deininger MW, Silver RT, Goldman JM, Stone RM *et al.* (2006) Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med* **355**, 2408–2417.

5 O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T *et al.* (2003) Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med* **348**, 994–1004.

6 Holyoake T, Jiang X, Eaves C & Eaves A (1999) Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. *Blood* **94**, 2056–2064.

7 Holyoake TL & Vetrie D (2017) The chronic myeloid leukemia stem cell: stemming the tide of persistence. *Blood* **129**, 1595–1606.

8 Chomel JC, Bonnet ML, Sorel N, Sloma I, Bennaceur-Griscelli A, Rea D, Legros L, Marfaing-Koka A, Bourhis JH, Ame S *et al.* (2016) Leukemic stem cell persistence in chronic myeloid leukemia patients in deep molecular response induced by tyrosine kinase inhibitors and the impact of therapy discontinuation. *Oncotarget* **7**, 35293–35301.

9 Holyoake TL & Helgason GV (2015) Do we need more drugs for chronic myeloid leukemia? *Immunol Rev* **263**, 106–123.

10 Jabbour E, Parikh SA, Kantarjian H & Cortes J (2011) Chronic myeloid leukemia: mechanisms of resistance and treatment. *Hematol Oncol Clin North Am* **25**, 981–995, v.

11 Ye H, Adane B, Khan N, Sullivan T, Minhajuddin M, Gasparetto M, Stevens B, Pei S, Balys M, Ashton JM *et al.* (2016) Leukemic stem cells evade chemotherapy by metabolic adaptation to an adipose tissue niche. *Cell Stem Cell* **19**, 23–37.

12 Bellodi C, Lidonnici MR, Hamilton A, Helgason GV, Soliera AR, Ronchetti M, Galavotti S, Young KW, Selmi T, Yacobi R *et al.* (2009) Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells. *J Clin Invest* **119**, 1109–1123.

13 Zhang G, Frederick DT, Wu L, Wei Z, Krepler C, Srinivasan S, Chae YC, Xu X, Choi H, Dimwamwa E *et al.* (2016) Targeting mitochondrial biogenesis to overcome drug resistance to MAPK inhibitors. *J Clin Invest* **126**, 1834–1856.

14 Ma XH, Piao SF, Dey S, McAfee Q, Karakousis G, Villanueva J, Hart LS, Levi S, Hu J, Zhang G *et al.* (2014) Targeting ER stress-induced autophagy overcomes BRAF inhibitor resistance in melanoma. *J Clin Invest* **124**, 1406–1417.

15 Amaravadi R, Kimmelman AC & White E (2016) Recent insights into the function of autophagy in cancer. *Genes Dev* **30**, 1913–1930.

16 Yang Z & Klionsky DJ (2010) Mammalian autophagy: core molecular machinery and signaling regulation. *Curr Opin Cell Biol* **22**, 124–131.

17 Nazio F, Strappazzon F, Antonioli M, Bielli P, Cianfanelli V, Bordi M, Gretzmeier C, Dengjel J, Piacentini M, Fimia GM *et al.* (2013) mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. *Nat Cell Biol* **15**, 406–416.

18 Dikic I & Elazar Z (2018) Mechanism and medical implications of mammalian autophagy. *Nat Rev Mol Cell Biol* **19**, 349–364.

19 Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, Vasquez DS, Joshi A, Gwinn DM, Taylor R *et al.* (2011) Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* **331**, 456–461.

20 Kim J, Kim YC, Fang C, Russell RC, Kim JH, Fan W, Liu R, Zhong Q & Guan KL (2013) Differential regulation of distinct Vps34 complexes by AMPK in nutrient stress and autophagy. *Cell* **152**, 290–303.

21 Rybstein MD, Bravo-San Pedro JM, Kroemer G & Galluzzi L (2018) The autophagic network and cancer. *Nat Cell Biol* **20**, 243–251.

22 Chourasia AH, Boland ML & Macleod KF (2015) Mitophagy and cancer. *Cancer Metab* **3**, 4.

23 Liu Z, Chen P, Gao H, Gu Y, Yang J, Peng H, Xu X, Wang H, Yang M, Liu X *et al.* (2014) Ubiquitylation of autophagy receptor Optineurin by HACE1 activates

selective autophagy for tumor suppression. *Cancer Cell* **26**, 106–120.

24 Mathew R, Karp CM, Beaudoin B, Vuong N, Chen G, Chen HY, Bray K, Reddy A, Bhanot G, Gelinas C *et al.* (2009) Autophagy suppresses tumorigenesis through elimination of p62. *Cell* **137**, 1062–1075.

25 Takamura A, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguri S, Eishi Y, Hino O, Tanaka K & Mizushima N (2011) Autophagy-deficient mice develop multiple liver tumors. *Genes Dev* **25**, 795–800.

26 Yang A, Rajeshkumar NV, Wang X, Yabuuchi S, Alexander BM, Chu GC, Von Hoff DD, Maitra A & Kimmelman AC (2014) Autophagy is critical for pancreatic tumor growth and progression in tumors with p53 alterations. *Cancer Dis* **4**, 905–913.

27 Rosenfeldt MT, O'Prey J, Morton JP, Nixon C, MacKay G, Mrowinska A, Au A, Rai TS, Zheng L, Ridgway R *et al.* (2013) p53 status determines the role of autophagy in pancreatic tumour development. *Nature* **504**, 296–300.

28 Yang A, Herter-Sprie G, Zhang H, Lin EY, Biancur D, Wang X, Deng J, Hai J, Yang S, Wong KK *et al.* (2018) Autophagy sustains pancreatic cancer growth through both cell-autonomous and nonautonomous mechanisms. *Cancer Discov* **8**, 276–287.

29 Strohecker AM, Guo JY, Karsli-Uzunbas G, Price SM, Chen GJ, Mathew R, McMahon M & White E (2013) Autophagy sustains mitochondrial glutamine metabolism and growth of BrafV600E-driven lung tumors. *Cancer Discov* **3**, 1272–1285.

30 Guo JY, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, Kamphorst JJ, Chen G, Lemons JM, Karantza V *et al.* (2011) Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev* **25**, 460–470.

31 Yang S, Wang X, Contino G, Liesa M, Sahin E, Ying H, Bause A, Li Y, Stommel JM, Dell'antonio G *et al.* (2011) Pancreatic cancers require autophagy for tumor growth. *Genes Dev* **25**, 717–729.

32 Xie J, Wang X & Proud CG (2016) mTOR inhibitors in cancer therapy. *F1000Res* **5**, F1000 Faculty Rev-2078.

33 Wong PM, Feng Y, Wang J, Shi R & Jiang X (2015) Regulation of autophagy by coordinated action of mTORC1 and protein phosphatase 2A. *Nat Commun* **6**, 8048.

34 Ferguson SM (2015) Beyond indigestion: emerging roles for lysosome-based signaling in human disease. *Curr Opin Cell Biol* **35**, 59–68.

35 Martina JA, Diab HI, Lishu L, Jeong AL, Patange S, Raben N & Puertollano R (2014) The nutrient-responsive transcription factor TFE3 promotes autophagy, lysosomal biogenesis, and clearance of cellular debris. *Sci Signal* **7**, ra9.

36 Ploper D, Taelman VF, Robert L, Perez BS, Titz B, Chen HW, Graeber TG, von Euw E, Ribas A & De Robertis EM (2015) MITF drives endolysosomal biogenesis and potentiates Wnt signaling in melanoma cells. *Proc Natl Acad Sci USA* **112**, E420–E429.

37 Perera RM, Stoykova S, Nicolay BN, Ross KN, Fitamant J, Boukhali M, Lengrand J, Deshpande V, Selig MK, Ferrone CR *et al.* (2015) Transcriptional control of autophagy-lysosome function drives pancreatic cancer metabolism. *Nature* **524**, 361–365.

38 Lebovitz CB, Robertson AG, Goya R, Jones SJ, Morin RD, Marra MA & Gorski SM (2015) Cross-cancer profiling of molecular alterations within the human autophagy interaction network. *Autophagy* **11**, 1668–1687.

39 Sheng Z, Ma L, Sun JE, Zhu LJ & Green MR (2011) BCR-ABL suppresses autophagy through ATF5-mediated regulation of mTOR transcription. *Blood* **118**, 2840–2848.

40 Colecchia D, Rossi M, Sasdelli F, Sanzone S, Strambi A & Chiariello M (2015) MAPK15 mediates BCR-ABL1-induced autophagy and regulates oncogene-dependent cell proliferation and tumor formation. *Autophagy* **11**, 1790–1802.

41 Altman BJ, Jacobs SR, Mason EF, Michalek RD, Macintyre AN, Coloff JL, Ilkayeva O, Jia W, He YW & Rathmell JC (2011) Autophagy is essential to suppress cell stress and to allow BCR-Abl-mediated leukemogenesis. *Oncogene* **30**, 1855–1867.

42 Bolton-Gillespie E, Schemionek M, Klein HU, Flis S, Hoser G, Lange T, Nieborowska-Skorska M, Maier J, Kerstiens L, Koptyra M *et al.* (2013) Genomic instability may originate from imatinib-refractory chronic myeloid leukemia stem cells. *Blood* **121**, 4175–4183.

43 Rabinovitch RC, Samborska B, Faubert B, Ma EH, Gravel S-P, Andrzejewski S, Raissi TC, Pause A, St.-Pierre J & Jones RG (2017) AMPK maintains cellular metabolic homeostasis through regulation of mitochondrial reactive oxygen species. *Cell Rep* **21**, 1–9.

44 Saito Y, Chapple Richard H, Lin A, Kitano A & Nakada D (2015) AMPK protects leukemia-initiating cells in myeloid leukemias from metabolic stress in the bone marrow. *Cell Stem Cell* **17**, 585–596.

45 Ho TT, Warr MR, Adelman ER, Lansinger OM, Flach J, Verovskaya EV, Figueroa ME & Passegué E (2017) Autophagy maintains the metabolism and function of young and old stem cells. *Nature* **543**, 205.

46 Rothe K, Lin H, Lin KB, Leung A, Wang HM, Malekesmaeili M, Brinkman RR, Forrest DL, Gorski SM & Jiang X (2014) The core autophagy protein ATG4B is a potential biomarker and therapeutic target in CML stem/progenitor cells. *Blood* **123**, 3622–3634.

47 Bruennert D, Czibere A, Bruns I, Kronenwett R, Gattermann N, Haas R & Neumann F (2009) Early in vivo changes of the transcriptome in Philadelphia chromosome-positive CD34+ cells from patients with chronic myelogenous leukaemia following imatinib therapy. *Leukemia* **23**, 983–985.

48 Baquero P, Dawson A, Mukhopadhyay A, Kuntz EM, Mitchell R, Olivares O, Ianniciello A, Scott MT, Dunn K, Nicastri MC *et al.* (2018) Targeting quiescent leukemic stem cells using second generation autophagy inhibitors. *Leukemia*. [Epub ahead of print]. https://doi.org/10.1038/s41375-018-0252-4
49 Warr MR, Binnewies M, Flach J, Reynaud D, Garg T, Malhotra R, Debnath J & Passegue E (2013) FOXO3A directs a protective autophagy program in haematopoietic stem cells. *Nature* **494**, 323–327.
50 Pellicano F, Scott MT, Helgason GV, Hopcroft LE, Allan EK, Aspinall-O'Dea M, Copland M, Pierce A, Huntly BJ, Whetton AD *et al.* (2014) The antiproliferative activity of kinase inhibitors in chronic myeloid leukemia cells is mediated by FOXO transcription factors. *Stem Cells* **32**, 2324–2337.
51 Naka K, Hoshii T, Muraguchi T, Tadokoro Y, Ooshio T, Kondo Y, Nakao S, Motoyama N & Hirao A (2010) TGF-beta-FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. *Nature* **463**, 676–680.
52 Miyamoto K, Araki KY, Naka K, Arai F, Takubo K, Yamazaki S, Matsuoka S, Miyamoto T, Ito K, Ohmura M *et al.* (2007) Foxo3a is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell* **1**, 101–112.
53 Liu F, Lee JY, Wei H, Tanabe O, Engel JD, Morrison SJ & Guan JL (2010) FIP200 is required for the cell-autonomous maintenance of fetal hematopoietic stem cells. *Blood* **116**, 4806–4814.
54 Mortensen M, Soilleux EJ, Djordjevic G, Tripp R, Lutteropp M, Sadighi-Akha E, Stranks AJ, Glanville J, Knight S, Jacobsen SE *et al.* (2011) The autophagy protein Atg7 is essential for hematopoietic stem cell maintenance. *J Exp Med* **208**, 455–467.
55 Fitzwalter BE, Towers CG, Sullivan KD, Andrysik Z, Hoh M, Ludwig M, O'Prey J, Ryan KM, Espinosa JM, Morgan MJ *et al.* (2018) Autophagy inhibition mediates apoptosis sensitization in cancer therapy by relieving FOXO3a turnover. *Dev Cell* **44** (555–565), e3.
56 Kuntz EM, Baquero P, Michie AM, Dunn K, Tardito S, Holyoake TL, Helgason GV & Gottlieb E (2017) Targeting mitochondrial oxidative phosphorylation eradicates therapy-resistant chronic myeloid leukemia stem cells. *Nat Med* **23**, 1234–1240.
57 Giustacchini A, Thongjuea S, Barkas N, Woll PS, Povinelli BJ, Booth CAG, Sopp P, Norfo R, Rodriguez-Meira A, Ashley N *et al.* (2017) Single-cell transcriptomics uncovers distinct molecular signatures of stem cells in chronic myeloid leukemia. *Nat Med* **23**, 692–702.
58 Suda T, Takubo K & Semenza GL (2011) Metabolic regulation of hematopoietic stem cells in the hypoxic niche. *Cell Stem Cell* **9**, 298–310.
59 Poillet-Perez L, Despouy G, Delage-Mourroux R & Boyer-Guittaut M (2015) Interplay between ROS and autophagy in cancer cells, from tumor initiation to cancer therapy. *Redox Biol* **4**, 184–192.
60 de Almeida MJ, Luchsinger LL, Corrigan DJ, Williams LJ & Snoeck HW (2017) Dye-independent methods reveal elevated mitochondrial mass in hematopoietic stem cells. *Cell Stem Cell* **21** (725–729), e4.
61 Karvela M, Baquero P, Kuntz EM, Mukhopadhyay A, Mitchell R, Allan EK, Chan E, Kranc KR, Calabretta B, Salomoni P *et al.* (2016) ATG7 regulates energy metabolism, differentiation and survival of Philadelphia-chromosome-positive cells. *Autophagy* **12**, 936–948.
62 Guo JY, Teng X, Laddha SV, Ma S, Van Nostrand SC, Yang Y, Khor S, Chan CS, Rabinowitz JD & White E (2016) Autophagy provides metabolic substrates to maintain energy charge and nucleotide pools in Ras-driven lung cancer cells. *Genes Dev* **30**, 1704–1717.
63 Murata H, Takamatsu H, Liu S, Kataoka K, Huh N-H & Sakaguchi M (2015) NRF2 regulates PINK1 expression under oxidative stress conditions. *PLoS ONE* **10**, e0142438.
64 Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, Burman JL, Sideris DP, Fogel AI & Youle RJ (2015) The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature* **524**, 309–314.
65 Merry TL & Ristow M (2016) Nuclear factor erythroid-derived 2-like 2 (NFE2L2, Nrf2) mediates exercise-induced mitochondrial biogenesis and the anti-oxidant response in mice. *J Physiol* **594**, 5195–5207.
66 Holmström KM, Baird L, Zhang Y, Hargreaves I, Chalasani A, Land JM, Stanyer L, Yamamoto M, Dinkova-Kostova AT & Abramov AY (2013) Nrf2 impacts cellular bioenergetics by controlling substrate availability for mitochondrial respiration. *Biol Open* **2**, 761–770.
67 Georgakopoulos ND, Frison M, Alvarez MS, Bertrand H, Wells G & Campanella M (2017) Reversible Keap1 inhibitors are preferential pharmacological tools to modulate cellular mitophagy. *Sci Rep* **7**, 10303.
68 East Daniel A, Fagiani F, Crosby J, Georgakopoulos Nikolaos D, Bertrand H, Schaap M, Fowkes A, Wells G & Campanella M (2014) PMI: A ΔΨm independent pharmacological regulator of mitophagy. *Chem Biol* **21**, 1585–1596.
69 Jin G, Xu C, Zhang X, Long J, Rezaeian AH, Liu C, Furth ME, Kridel S, Pasche B, Bian X-W *et al.* (2018) Atad3a suppresses Pink1-dependent mitophagy to maintain homeostasis of hematopoietic progenitor cells. *Nat Immunol* **19**, 29–40.
70 Pei S, Minhajuddin M, Adane B, Khan N, Stevens BM, Mack SC, Lai S, Rich JN, Inguva A, Shannon KM *et al.* (2018) AMPK/FIS1-mediated mitophagy is

required for self-renewal of human AML stem cells. *Cell Stem Cell* **23** (86–100), e6.
71 Rangwala R, Leone R, Chang YC, Fecher LA, Schuchter LM, Kramer A, Tan KS, Heitjan DF, Rodgers G, Gallagher M *et al.* (2014) Phase I trial of hydroxychloroquine with dose-intense temozolomide in patients with advanced solid tumors and melanoma. *Autophagy* **10**, 1369–1379.
72 Rosenfeld MR, Ye X, Supko JG, Desideri S, Grossman SA, Brem S, Mikkelson T, Wang D, Chang YC, Hu J *et al.* (2014) A phase I/II trial of hydroxychloroquine in conjunction with radiation therapy and concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme. *Autophagy* **10**, 1359–1368.
73 Mahalingam D, Mita M, Sarantopoulos J, Wood L, Amaravadi RK, Davis LE, Mita AC, Curiel TJ, Espitia CM, Nawrocki ST *et al.* (2014) Combined autophagy and HDAC inhibition: a phase I safety, tolerability, pharmacokinetic, and pharmacodynamic analysis of hydroxychloroquine in combination with the HDAC inhibitor vorinostat in patients with advanced solid tumors. *Autophagy* **10**, 1403–1414.
74 Horne GA, Mukhopadhyay A, Latif A, Stobo J, Kelly C, Dixoon-Hughes J, Mcmahon L, Cony-Makhoul P, Byrne J, Helgason GV *et al.* (2018) Choices: A randomized phase II trial for imatinib vs hydroxychloroquine plus IM for patients with CML in MCYR with residual disease detectable by Q-PCR EHA Abstract, Abstract S810.
75 McAfee Q, Zhang Z, Samanta A, Levi SM, Ma XH, Piao S, Lynch JP, Uehara T, Sepulveda AR, Davis LE *et al.* (2012) Autophagy inhibitor Lys05 has single-agent antitumor activity and reproduces the phenotype of a genetic autophagy deficiency. *Proc Natl Acad Sci USA* **109**, 8253–8258.
76 Shi TT, Yu XX, Yan LJ & Xiao HT (2017) Research progress of hydroxychloroquine and autophagy inhibitors on cancer. *Cancer Chemother Pharmacol* **79**, 287–294.
77 Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK, Kishi C, Kc W, Carrero JA, Hunt S *et al.* 4th (2008) A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature* **456**, 259–263.
78 Dowdle WE, Nyfeler B, Nagel J, Elling RA, Liu S, Triantafellow E, Menon S, Wang Z, Honda A, Pardee G *et al.* (2014) Selective VPS34 inhibitor blocks autophagy and uncovers a role for NCOA4 in ferritin degradation and iron homeostasis in vivo. *Nat Cell Biol* **16**, 1069–1079.
79 Honda A, Harrington E, Cornella-Taracido I, Furet P, Knapp MS, Glick M, Triantafellow E, Dowdle WE, Wiedershain D, Maniara W *et al.* (2016) Potent, selective, and orally bioavailable inhibitors of VPS34 provide chemical tools to modulate autophagy in vivo. *ACS Med Chem Lett* **7**, 72–76.
80 Mitchell R, Hopcroft LEM, Baquero P, Allan EK, Hewit K, James D, Hamilton G, Mukhopadhyay A, O'Prey J, Hair A *et al.* (2017) Targeting BCR-ABL-independent TKI resistance in chronic myeloid leukemia by mTOR and autophagy inhibition. *J Natl Cancer Inst* **110**, 467–478.
81 Rebecca VW, Nicastri MC, McLaughlin N, Fennelly C, McAfee Q, Ronghe A, Nofal M, Lim CY, Witze E, Chude CI *et al.* (2017) A unified approach to targeting the Lysosome's degradative and growth signaling roles. *Cancer Discov* **7**, 1266–1283.
82 Pavlova NN & Thompson CB (2016) The emerging hallmarks of cancer metabolism. *Cell Metab* **23**, 27–47.
83 Reed GA, Schiller GJ, Kambhampati S, Tallman MS, Douer D, Minden MD, Yee KW, Gupta V, Brandwein J, Jitkova Y *et al.* (2016) A Phase 1 study of intravenous infusions of tigecycline in patients with acute myeloid leukemia. *Cancer Med* **5**, 3031–3040.
84 Skrtic M, Sriskanthadevan S, Jhas B, Gebbia M, Wang X, Wang Z, Hurren R, Jitkova Y, Gronda M, Maclean N *et al.* (2011) Inhibition of mitochondrial translation as a therapeutic strategy for human acute myeloid leukemia. *Cancer Cell* **20**, 674–688.
85 Jitkova Y, Gronda M, Hurren R, Wang X, Goard CA, Jhas B & Schimmer AD (2014) A novel formulation of tigecycline has enhanced stability and sustained antibacterial and antileukemic activity. *PLoS ONE* **9**, e95281.
86 Farge T, Saland E, de Toni F, Aroua N, Hosseini M, Perry R, Bosc C, Sugita M, Stuani L, Fraisse M *et al.* (2017) Chemotherapy-resistant human acute myeloid leukemia cells are not enriched for leukemic stem cells but require oxidative metabolism. *Cancer Discov* **7**, 716–735.
87 Zhang X, Fryknas M, Hernlund E, Fayad W, De Milito A, Olofsson MH, Gogvadze V, Dang L, Pahlman S, Schughart LA *et al.* (2014) Induction of mitochondrial dysfunction as a strategy for targeting tumour cells in metabolically compromised microenvironments. *Nat Commun* **5**, 3295.
88 Kang BH, Siegelin MD, Plescia J, Raskett CM, Garlick DS, Dohi T, Lian JB, Stein GS, Languino LR & Altieri DC (2010) Preclinical characterization of mitochondria-targeted small molecule hsp90 inhibitors, gamitrinibs, in advanced prostate cancer. *Clin Cancer Res* **16**, 4779–4788.
89 Estan MC, Calvino E, Calvo S, Guillen-Guio B, Boyano-Adanez Mdel C, de Blas E, Rial E & Aller P (2014) Apoptotic efficacy of etomoxir in human acute myeloid leukemia cells. Cooperation with arsenic trioxide and glycolytic inhibitors, and regulation by

oxidative stress and protein kinase activities. *PLoS ONE* **9**, e115250.

90 Schepers K, Campbell TB & Passegue E (2015) Normal and leukemic stem cell niches: insights and therapeutic opportunities. *Cell Stem Cell* **16**, 254–267.

91 Sousa CM, Biancur DE, Wang X, Halbrook CJ, Sherman MH, Zhang L, Kremer D, Hwang RF, Witkiewicz AK, Ying H *et al*. (2016) Pancreatic stellate cells support tumour metabolism through autophagic alanine secretion. *Nature* **536**, 479–483.

92 Katheder NS, Khezri R, O'Farrell F, Schultz SW, Jain A, Rahman MM, Schink KO, Theodossiou TA, Johansen T, Juhasz G *et al*. (2017) Microenvironmental autophagy promotes tumour growth. *Nature* **541**, 417–420.

93 Guo JY, Karsli-Uzunbas G, Mathew R, Aisner SC, Kamphorst JJ, Strohecker AM, Chen G, Price S, Lu W, Teng X *et al*. (2013) Autophagy suppresses progression of K-ras-induced lung tumors to oncocytomas and maintains lipid homeostasis. *Genes Dev* **27**, 1447–1461.

94 Codogno P, Mehrpour M & Proikas-Cezanne T (2011) Canonical and non-canonical autophagy: variations on a common theme of self-eating? *Nat Rev Mol Cell Biol* **13**, 7–12.

95 Koschmieder S, Gottgens B, Zhang P, Iwasaki-Arai J, Akashi K, Kutok JL, Dayaram T, Geary K, Green AR, Tenen DG *et al*. (2005) Inducible chronic phase of myeloid leukemia with expansion of hematopoietic stem cells in a transgenic model of BCR-ABL leukemogenesis. *Blood* **105**, 324–334.

96 Moschoi R, Imbert V, Nebout M, Chiche J, Mary D, Prebet T, Saland E, Castellano R, Pouyet L, Collette Y *et al*. (2016) Protective mitochondrial transfer from bone marrow stromal cells to acute myeloid leukemic cells during chemotherapy. *Blood* **128**, 253–264.

97 Welner RS, Amabile G, Bararia D, Czibere A, Yang H, Zhang H, Pontes LL, Ye M, Levantini E, Di Ruscio A *et al*. (2015) Treatment of chronic myelogenous leukemia by blocking cytokine alterations found in normal stem and progenitor cells. *Cancer Cell* **27**, 671–681.