Hydroxychloroquine for Chronic Myeloid Leukaemia: Complete cure on the horizon?

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Chronic Myeloid Leukaemia (CML), a cancer with a known driver

CML is characterised by a reciprocal translocation [t(9;22)(q34;q11)] between chromosomes 9 and 22, producing the Philadelphia chromosome. This abnormal chromosome carries a fusion gene, BCR-ABL that is translated into the constitutively active BCR-ABL protein tyrosine kinase (TK). In haemopoietic stem cells this protein activates a cascade of signalling pathways resulting in increased survival, proliferation, [(1)] altered adhesion [(2)] and limited growth factor dependence. The progeny of these cells outgrow the normal cells to propagate the disease, CML. Functionally, protein kinases interact with ATP and the introduction of an ATP competitive inhibitor of BCR-ABL kinase was a major milestone in CML therapy. In 1998, the first TK inhibitor (TKI) imatinib was introduced to the clinic. Consequently, ABL kinase targeted [(3)] small molecule inhibitors captured the market with consecutive development of broad spectrum inhibitors like dasatinib and more potent inhibitors like nilotinib.

CML therapeutics: Good, better, best. Never let it rest.

TKI is the drug class of choice in chronic phase CML, but these drugs are less effective in accelerated phase or blast crisis. Even in chronic phase drug resistance due to kinase domain mutations, overexpression of BCR-ABL and other mechanisms has been problematic. Newer TKIs, such as dasatinib and nilotinib, were developed to circumvent this issue and recent TKIs, including danusertib and ponatinib, are in clinical trial for the most resistant mutation, T315I. In addition to drug resistance, quiescent CML stem cells are insensitive to all TKIs thus far tested and are thought responsible for disease persistence [(4)]. The scientific community is concerned that persistent CML stem cells may be independent of BCR-ABL activity for survival and proliferation and thus cannot be eradicated by TKI treatment alone. This has led the pharmaceutical industry to try to synthesise new classes of drugs to combat CML, seeking cure.

Autophagy: 'Live and let die'

As has been demonstrated in solid neoplasms, TKI treatment initiates the process of autophagy in primary CML cells [5]. Autophagy is a recycling process of vital cellular constituents, damaged organelles and harmful materials that is initiated following enclosure in double membrane bound autophagy vacuoles (AV) and delivery to lysosomes for proteolytic degradation. Autophagy can be initiated in starvation due to withdrawal of growth factor signalling. In CML, BCR-ABL mimics growth factor stimulation [6] thus its inhibition by TKI may induce autophagy in a similar fashion. Physiological autophagy is essential for cellular homeostasis and the death signal can promote cellular demise through autophagy, either independent of, or linked to apoptosis [7]. Though suppression of autophagy may be associated with cell transformation and carcinogenesis, malignant cells can also induce autophagy as a protective way to relieve therapy induced stress as evidenced in CML after TKI treatment [5]. The decisions between 'survival' or 'death' through induction of autophagy in malignancy likely depends on cellular context and signalling intensity [8]. Tackling 'survival' autophagy in CML [5] may therefore provide new hope for eradication of the disease. CHOICES (**CH**lor**O**quine and **I**matinib **C**ombination to **E**liminate **S**tem cells), a phase II randomised clinical trial (ISCRTN No. 61568166) is currently completing a safety run-in period combining TKI with Hydroxychloroquine (HCQ) before being opened to further patients in the UK.

Hydroxychloroquine: A new spin on an old concept

HCQ is a derivative of the antimalarial drug chloroquine with significantly reduced toxicity. These drugs have been prescribed for rheumatic diseases, several inflammatory conditions and immune related skin disorders [9]. Most recently these drugs have been used in conjunction with anti-neoplastic chemo-or radiotherapies, where induction of autophagy has been shown to function as an escape mechanism for neoplastic cells to evade death. These quinolone derivatives are lysosomotropic agents with weak DNA intercalating properties. The cytotoxic and anti-inflammatory effects of these drugs are exerted mainly through their lysosomal function. Lysosomes or 'suicide bags' are the key component of final degradation of AV. These weak bases (quinolones) penetrate the plasma membrane and get concentrated in the lysosome, where protonation of the drug increases the pH of the lysosomal environment. This in turn results in inhibition of fusion between lysosome and AV, preventing further proteolytic degradation, thus working as an autophagy inhibitor. Apart from accumulation of AV, this process instigates a series of events which may occur collectively or selectively in a tissue specific manner. The increased pH inhibits Toll Like Receptor (TLR) activities, receptor recycling, alteration of MHC assembly and

sequestration of membrane ceramides which limits membrane turnover [10]. These are probably the critical mechanisms for the anti-inflammatory actions of the drug. The consequent expansion of lysosome and increased permeabilisation of lysosomal membrane can trigger release of enzymes like cathepsin B and insertion of Bax into mitochondrial membrane, followed by release of cytochrome C and loss of mitochondrial membrane potential [11]. Eventual cell death can ensue by p53 dependent pathways, either by caspase mediated apoptosis [12] or in a caspase independent manner [13].

The chemo-sensitising properties of these drugs can also be attributed to inhibition of drug transport by ATP binding cassette (ABC) transporters. ABC transporters, like MultiDrug Resistance protein (MDR) or Multidrug Resistance-associated Protein 1 (MRP1), have been implicated in imatinib resistance [14] and therefore this plausible influence of HCQ on TKI treatment requires further investigation.

Complete cure of CML: hope and despair

HCQ is not a 'clean' autophagy inhibitor as it possesses multiple mechanisms of action. In addition long term use of HCQ can rarely cause retinopathy, myopathy and neuropathic complications, leading to caution with its use in CML patients. The drug can be used at up to 1200 mg/day [15] and for CML patients in the CHOICES trial is being used at 800 mg/day for 12 months, which should provide a steady state blood concentration of approximately 6μM [16], well within the autophagy inhibitory range *in vitro*. Other presently available inhibitors of autophagy, like, 3 methyladenine, wortmannin and bafilomycin $A_{1,}$ are quite non-specific and unsuitable for clinical use. Moreover, the potential to inhibit autophagy is likely to prove critical as the majority of alternative therapies for CML, including proteosome inhibitors [17], protein phosphatase 2A activator [18], farnesyl transferase inhibitors [19] and histone deacetylase inhibitors, are all capable of autophagy induction. Pharmaceutical companies are actively exploring development of specific autophagy inhibitors.

Concluding remarks

The mechanism of the survival/death effect of autophagy induced by chemotherapeutic agents in CML cells needs to be elucidated. Whilst HCQ with TKI in CML patients should provide proof of concept, it is hoped that in the future "cleaner" autophagy inhibitors may enhance eradication of CML and other forms of cancer with less off target toxicity.

The authors declare no competing financial interests.

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