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Unravelling the Role of Stem Cells in Chronic Myeloid Leukaemia and Potential Treatments

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Abstract

Chronic Myeloid Leukaemia (CML) is a disorder of the haematopoietic system characterised by the malignant clonal growth of bone marrow cells, specifically haematopoietic stem cells (HSCs), which are capable of giving rise to all lineages of blood cells. In essence, CML is a condition where transformed HSCs give rise to cells of myeloid lineage, e.g. platelets, neutrophils etc, causing them to accumulate in the circulating blood. The aim of this paper was to understand and review the role of stem cells (SCs) and Cancer Stem Cells (CSCs) in CML, current strategies for targeting CSCs, potential targets for CML CSC treatment such as histone deacetylase inhibition, autophagy inhibitors, p53 activation by SIRT1 inhibitors, HIF inhibition, and the regulation of c-myc.

Keywords: Chronic Myeloid Leukaemia (CML); Stem cells; Cancer

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Introduction

Chronic Myeloid Leukaemia (CML) is a disorder of the haematopoietic system characterised by the malignant clonal growth of bone marrow cells, specifically haematopoietic stem cells (HSCs), which are capable of giving rise to all lineages of blood cells. In essence, CML is a condition where transformed HSCs give rise to cells of myeloid lineage, e.g. platelets, neutrophils etc, causing them to accumulate in the circulating blood. Around 6,000 people are currently living with CML in the UK, and 4 people per week die because of it [1]. CML has a median presentation age of 53 [2], however, all age groups, including children, are affected. It usually presents with symptoms of fatigue, anorexia, weight loss, splenomegaly and hepatomegaly, however approximately 40% of patients are asymptomatic [2].

CML is triphasic. The disease first has a benign chronic phase in which cells will mature from stem cells and slowly accumulate into tumours. It then progresses into accelerated phase, and then into blast phase, which is rapidly fatal. In blast phase, the cells begin to multiply at a faster rate and are not as differentiated from stem cells as those that you would see in the chronic phase. The movement from chronic to blast phase usually occurs within 3-5 years [2].

Literature Search Strategy

CML is unique from other human cancers in that the pathology of the disease is shown to be mostly attributed to the product of a single oncogene, BCR-ABL (breakpoint cluster region - Ableson leukemia virus) [3]; it has the effects of making cells able to grow and survive independently of cytokines. This protects cells against apoptotic signals from cytokines; increasing the action of integrin to reduce cell adhesion; and phosphorylating substrates which activate pathways involving Ras, Raf, phosphatidylinositol-3 kinase (PI3K) and Myc, to induce growth and proliferation [2]. Overall, the existence of this fusion oncogene produces the hallmarks of cancer, that are characteristic for all cancers, specifically for CML.

The Cochrane Library, PubMed and Medline were searched with key terms such as *chronic myeloid leukaemia* or shortened to *CML* paired with *cancer stem cells*. Appropriate terms such as *treatments* or *TKI resistance* etc. were used to narrow down the search. Boolean operators such as AND and OR were used to narrow the search.

CML is caused by, and the diagnosis usually based upon, detection of the Philadelphia (Ph) chromosome. The Ph chromosome was first described as a shortened version of chromosome 22 in 1960 [2] and then redefined as a t (9;22) translocation in 1973, in which the translocation creates a 9q+ and small 22q- (Ph) chromosome [2]. 95% of patients have this exact t (9;22) translocation and another 5% have other various similar translocations [2], however, they all have the same end product: fusion of the *BCR* (breakpoint cluster region) gene on 22q- to the *ABL* (Ableson leukemia virus) gene on 9q+.

The fusion on chromosome 22 results in the creation of the fusion protein BCR-ABL; a constitutively active cytoplasmic

tyrosine kinase [2]. Dependant on the exact point of the fusion patients may produce different sizes of BCR-ABL (all existing in the range of approximately 185-230kd). Patients with larger BCR-ABL molecules are shown to progress through CML more slowly [2].

When CML progresses from chronic phase to blast phase secondary step-by-step chromosomal changes occur; duplication of the Ph chromosome; trisomy 8; and, mutations/deletions of tumour-suppressor genes [2]. These presumably contribute to the secondary, more aggressive, malignant phenotype.

CML was universally fatal before the 1990s when Imatinib mesylate (also known as Imatinib, brand name Glivec©), a tyrosine kinase inhibitor (TKI), was developed. This medication has enabled patients to live with CML in remission, but it is by no means a cure. After stopping therapy, relapse occurs and, with time, CML may also become TKI resistant [4]. The driver behind this relapse of cancer is thought to be leukaemic stem cells (LSCs) that are reversibly quiescent, meaning they do not progress through the cell cycle, making them immune to TKI therapy such as imatinib-induced apoptosis [4]. These so-called "cancer stem cells" (CSCs) are a minority of all cancer cells which make up a pool of self-sustaining cells and are the only cells in a tumour which can self-renew, thereby maintaining the tumour.

CSCs have the ability to grow and divide into all of the cell types in a heterogeneous lineage that constitutes a tumour and are resistant to cancer therapies, such as chemotherapy, which targets fast-growing cells. CSC's can only be seen experimentally, as the cells that are able to recreate a growing tumour [5].

Unlimited potential to proliferate and self-renew are characteristics shared by CSCs and HSCs, both exhibiting similar genetic expressions of self-renewal [6]. For instance, activation of c-Myc reactivates an embryonic stem cell (ESC)-like programme in normal and cancer cells, c-Myc functioning as a master regulatory gene. Some example pathways shared by ESCs and CSCs are Notch, Sonic hedgehog, Wnt-βcatenin, and fibroblast growth factor-2 [6].

Evidently, stem cells and CSCs share a lot of similarities, however, stem cells keep a balance to maintain the adult cell pool compared to the deregulation of stem cells that causes cancerous stem cells leading to cancer.

Current Treatments

The current treatment of choice for CML is tyrosine kinase inhibitors (TKI), the classic drug used is Imatinib. These work by targeting tyrosine kinase produced by the BCR-ABL fusion gene, the oncogene expressed in CML. The development of Imatinib represented a new era of cancer therapy, as most patients with CML went into remission following TKI treatment.

It was found, however, that once Imatinib was withdrawn, patients relapsed and some even went on to develop TKI resistance. This is due to leukaemic stem cells (LSCs) emerging from quiescence and developing resistance to Imatinib-induced apoptosis. This resistance has reduced the possibility of treatment-free remission to just 10-20% [7].

Additionally, a quarter of CML patients fail TKI therapy for other reasons; alternative oncogene activation, BCR-ABL kinase mutations or disease progression to accelerated phase or blast crisis [7]. These failures in TKI therapy highlight the need for continued research into potential new treatments and the significant possibility of relapse has led to patients being treated indefinitely with Imatinib. This indefinite treatment plan comes with disadvantages such as: adverse effects from long-term treatment; non-compliance and the expense associated with continuous drug treatment.

The effectiveness of Imatinib is shown to be increased when it is used in combination with other treatments such as; histone deacetylase inhibitors, autophagy inhibitors, sirtuin 1 inhibitors, and hypoxia-inducible factors.

Chemotherapy and radiotherapy also have limited benefits as treatments, as they do not target LSCs, and spare cell-cycle arrested differentiated cells, which, due to plasticity, often go on to replace any lost stem cells [8].

The limited effectiveness of current treatments against LSCs has led to much research and the development of new therapies. So far, lineage ablation has proven to be a good technique to determine whether the loss of cancer stem cells causes the loss of non-stem cells or if the differentiated cells simply become new stem cells that go on to sustain tumour growth [9]. It causes the selective destruction of certain cell types in a cell population, enabling the modelling of pathologies with a specific cell type population from a large sample cohort.

LSCs have certain unique properties that are not found in normal human stem cells. Specific LSC markers have been identified, such as the active form of NF-κB, a DNA transcription factor involved in the regulation of inflammation and cell signalling. The active form of nuclear factor-κB (NF-κB) is associated with anti-apoptotic activity in cancer and is absent in other stem cells. *In vitro* experiments carried out show that if the NF-κB marker is inhibited it induces apoptosis in LSCs [9]. Second generation autophagy inhibitors such as hydroxychloroquine also target LSCs, however, bivalent aminoquinoline Lys05 (a dimeric analogue of chloroquine) has been found to be up to 10 times more effective in cancer cell lines and a double transgenic model of CML [7].

Potential Targets for CML Treatment

Histone deacetylase inhibition

Histone deacetylase inhibitors (HDACis) are a class of drugs that have the potential to be used as an effective treatment for a range of cancers, with CML being one of these [10-16].

DNA, in the form of chromatin, forms nucleosomes by wrapping around histone octamers. The access of transcription factors to DNA is modulated by chemical changes to the histones. One method of modifying histones is by acetylation. Acetylation of the positively charged N-terminal lysine residues of the histones inhibits binding to the negatively charged chromatin, allowing for a more open structure which enables transcription factor access to DNA, and therefore, gene transcription. It follows that histone

deacetylation results in more tightly packed chromatin, usually resulting in gene silencing. Histone acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylase enzymes (HDACs).

Regulation of gene expression by epigenetic histone modification such as acetylation/deacetylation is known to contribute significantly to the development of cancer.

Cell proliferation, apoptosis and metabolism are also controlled by deacetylation of histone and non-histone proteins [5], which may contribute to cancer development. HDACs are often dysregulated in cancer [17].

HDACis increase histone lysine acetylation, therefore modulating gene expression. HDACis may also modulate the acetylation of non-histone proteins which could contribute to the efficacy of the agents for cancer therapy [18]. Hence the ability of HDACis to inhibit proliferation and induce apoptosis shows them to be a promising potential cancer therapy.

The role of HDACis in the treatment of CML has been widely investigated, alone or in combination with TKIs. It has been found that treating CML with HDACis, in combination with the TKI imatinib mesylate (IM), was significantly more effective in inducing apoptosis in chronic phase (CP) CML progenitor cells, than treatment with IM alone. However, HDACis alone have little effect on CP CML cell apoptosis; HDACis targeting of CP CML cells is potentiated by inhibition of BCR-ABL tyrosine kinase by TKIs. The HDACis in combination with IM inhibits CML progenitor proliferation and CML committed progenitor growth in colony assays. In addition, combination treatment with HDACis and IM results in significant reduction of LSCs, which have the potential to cause relapse in transgenic BCR-ABL mice models and prevented leukaemia relapse after discontinuation of treatment in these mice. However, this treatment combination may not be effective in patients with IM-resistance related BCR-ABL mutations due to poor efficacy of IM in these patients [19].

Clinical trials have been initiated to determine the efficacy, safety and tolerability of HDACis therapy in combination with TKIs, in patients in cytogenetic remission with residual BCR-ABL cells. The success of this treatment will be determined by the ability to produce long-term remission after cessation of treatment. It is yet to be seen if targeting of LSCs in CML by HDACis has a future in targeting primitive quiescent cancer stem cells in other leukaemias and cancers [19].

Autophagy inhibitors

Autophagy is a catabolic process by which substances or damaged organelles are degraded, to prevent the accumulation of harmful molecules and obtain nutrients in adverse environments. Initiation of autophagy is dependent on signalling, activated by adverse cellular conditions such as hypoxia, cytotoxic molecules accumulation, fasting and nutrient depletion [20,21].

There are main two mechanisms for the uptake of molecules into the phagosome; non-selective pathway and selective pathway. The non-selective pathway is Implicated in situations of nutrient depletion such as glucose starvation or low amino acid levels. Any material present in the cytoplasm is taken up for recycling in the phagosome. The selective pathway is involved in the disposal of harmful molecules and damaged organelles. The most common mechanism used to tag proteins for disposal is the ubiquitin pathway, however, other mechanisms do exist. This pathway becomes dysfunctional in cancer biology and neurodegenerative conditions [22].

In the normal cell, autophagy is a crucial process for preventing mutations in DNA by enabling adaptation to cellular stress and removing cytotoxic species, thus, autophagy prevents cancer development. However, in CML, and other solid tumours, autophagy has a cytoprotective role which enables the progression of disease [23]. Autophagy has been implicated in TKI treatment failure whereby molecular stress placed on CD34+ BCR-ABL expressing CSCs by treatment results in the upregulation of autophagy pathways. Furthermore, autophagy is an essential mechanism for driving tumour growth as it enables cancer cells to survive in hypoxic and nutrient deficient conditions [24-26]. Relapse often occurs due to the inadequate clearance of these CSCs.

Current Stage of Research (development *in vivo* and model and preliminary results): Inhibition of autophagy has been suggested to target the elimination of CSC. Autophagy inhibitors which prevent the fusion of the phagosome with the lysosome such as hydroxychloroquine have mainly been used in preclinical and clinical trials. However, novel autophagy inhibitors such as methyladenine which target phagosome nucleation and expansion have also been described [25,26]. Regardless of the mechanism, the main aim of autophagy inhibitors is to increase the vulnerability of CSCs to TKIs. Autophagy inhibitors must be used in tandem with TKIs to have clinical efficacy [23]. So current pre-clinical and clinical trials have combined TKI therapy with autophagy inhibitors to simultaneously target CML cells and CML CSCs to reduce the likelihood of relapse of disease [27].

The initial success of autophagy inhibitors *in vitro* testing has led to these compounds being tested *in vivo* [23]. Novel inhibitors Lys05 (a more potent form of hydroxychloroquine) and PIK-111 (prevents phagosome expansion) have been demonstrated be very effective at clearing CSCs in mouse models. The use of these compounds resulted in the pool of CSCs being diminished and a simultaneous increase in the numbers of susceptible progenitor CML cells was seen, no toxicity has been reported. These inhibitors were shown to be more potent than hydroxychloroquine [28].

Hydroxychloroquine which is indicated for the treatment of rheumatic arthritis has been shown to be effective at depleting reservoir of CML CSCs *in vivo*. However, phase 1 trials have yielded disappointing results for hydroxychloroquine combo therapy with TKIs. Vogl *et al* described mixed results for hydroxychloroquine/bortezomib (proteasome inhibitor) in a phase 1 trial with few patients showing clearance of CSCs. Analysis of patient tumour cells showed arrest of autophagy, however, this did not translate to positive clinical results. The authors suggested higher doses of hydroxychloroquine or more potent inhibitors were needed to induce CSC death. Additionally, many patients involved in this

trial had previously been exposed to bortezomib which may have elicited mutations in tumour cells giving rise to drug efflux pumps and other resistance mechanisms. Thus, impairing response to the combined therapy. No major toxicity was associated with hydroxychloroquine in this trial [29]. Similar results have been seen across trials using hydroxychloroquine for CML and other solid tumours in combined therapy [30-33].

p53 Activation by SIRT1 Inhibitors

Another possible target of stem cell therapy is activating p53 by Sirtuin 1 (SIRT1) inhibitors. Sirtuins are NAD-dependent histone deacetylases, and SIRT1 is one molecule in this family. SIRT1 has been linked to many regulatory processes in mammals, such as the cell cycle, metabolism and DNA repair [34,35].

SIRT1 has been demonstrated to play an important role in murine stem cells and HSC self-renewal and differentiation in stressful conditions [25,36]. SIRT1, importantly, is linked to regulation of p53 meaning it has a potential role in pathogenesis of leukaemia and tumour formation [37,38]. A study by Li et al, 2012 demonstrated that SIRT1 has an important role specifically for CML LSC survival and proliferation.

Primitive, quiescent CML $CD34^+$ cells are particularly resistant to apoptosis induced by Imatinib. Li et al, 2012 showed that CML $CD34^+$ cells that had undergone SIRT1 knockdown had increased apoptosis. Apoptosis of these cells was then further increased when treated with Imatinib; again, demonstrating the efficacy of combination treatment compared to non-combination [35].

One method of why SIRT1 inhibition affects CML LSC is its effect on p53; SIRT1 inhibition increases the acetylation of p53. In SIRT1 knockdown CP CML $CD34^+$ cells, levels of acetylated p53 protein were increased. Importantly, acetylated and total p53 levels were not increased in normal $CD34^+$ cells. Production of SIRT1 downregulates the acetylation of p53, preventing effective cell cycle regulation, potentially leading to malignant mutations continuing through the cell cycle [35].

CML cells being specifically targeted by SIRT1 also depends on p53 acetylation and expression. P53 is required in CML cells for growth inhibition and apoptosis following SIRT1 inhibition in BCR-ABL expressing cells. It is not enough to inhibit SIRT1, potential treatments must ensure p53 is acetylated in order to positively affect p53 transcriptional activity in CML progenitor cells [35].

SIRT1 expression is activated in haematopoietic progenitor cells by the BCR-ABL oncogene; this is the gene causing CML to develop from HSCs [35]. SIRT1 inhibition also suppresses the effects of this BCR-ABL gene on bone marrow progenitor cells and leukemogenesis [35].

It is also important to keep in mind that SIRT1 also has a potential tumour suppressing effects; demonstrated in a study, where its expression was shown to reduce tumour formation and cell proliferation in a murine colon cancer model [39-41]. SIRT1 not only has a role to play in CML, but also acute myeloid leukaemia (AML), where SIRT1 overexpression has been seen [42], showing SIRT1 inhibition treatments have wider implications than CML.

Research into potentially using SIRT1 inhibitors as a treatment has already taken place in murine models. One experiment reported weight loss in the murine phase of drug development; yet it was unclear if this was a direct effect of SIRT1 inhibition [39]. More research is required to fully appreciate the effects SIRT1 has on CML pathogenesis before a treatment could be marketed.

Hypoxia induced factors and LSCs – HIF inhibition

HSCs reside in the bone marrow and favour regions of low oxygen tension. This is also true of the LSCs in CML, and these regions can be referred to as the stem cell niches [43]. The environment of low oxygen promotes the survival of stem cells to divide and produce progeny in both health, with HSCs and in disease, with LSCs [44,45].

Curiously, the function of the BCR/ABL oncogene is diminished in areas of low oxygen (Guintolis). This helps explain as to why LSCs seem to evade destruction by TKIs and promote relapse of CML. The reduction in the BCR/ABL oncogene means that, unlike with other CML cells, it is not vital for LSC survival and so inhibition of the oncogene with TKIs will not lead to the elimination of the LSCs.

Hypoxia-inducible factor-1 (HIF-1) is a transcription factor which has a role in the survival of LSCs in these areas of low oxygen tension [46]. It is a dimer with an alpha subunit (HIF-1α) and beta subunit (HIF-1β) and can found in both normal and low oxygen conditions [47]. The HIF-1β subunit is consistently active whereas the HIF-1α subunit is inducible under certain circumstances. The HIF-1α subunit when in normal oxygen conditions becomes hydroxylated by prolyl hydroxylase which marks it for degradation by a proteasome. This stops the dimer coming together and so transcription cannot occur [48]. In hypoxic conditions, the HIF-1α subunit becomes more active than when it is in normal oxygen concentrations [49]. When there is less than 7% oxygen (hypoxic conditions), the alpha subunit has the ability to bind to the beta subunit. This induces the transcription of genes that control metabolism, cell growth and division, and the growth of new blood vessels - all of which have the potential to contribute to the progression of CML (Majmundar). Not only is there evidence that HIF-1 controls these particular gene functions which could be involved in any cancer progression, but evidence also exists that HIF-1 promotes CML disease advancement specifically (Semenza). Hence why the targeting of this factor has such therapeutic potential in CML management.

Acriflavine (ACF) is a HIF-1 inhibitor and has the potential to target TKI-resistant LSCs and decrease the likelihood of CML relapse. Already ACF has been approved for non-oncological use which means that it's toxicity and potential side effects have already been explored, reducing the time it would take to approve this drug for oncological use in comparison to a completely newly produced drug. It has been shown for the majority of time to not significantly affect the CD34+ cell population from disease-free healthy donors again eluding towards the fact that it has a good therapeutic index which is very beneficial when looking for new drugs [49]. ACF has been shown in a number of ways to affect both LSCs and other CML cells.

It has been shown that the KCL22 and K562 cell line populations in low oxygen were essentially obliterated upon treatment of ACF. This is probably due to the cells being induced to apoptose. ACF also ceases the potential of KCL22 or K562 cells to repopulate after cessation of treatment. The combination of ACF and imatinib (IM) has been tested on these cells also. It has been shown that ACF and IM had a synergistic effect in decreasing the number of viable cells after treatment. However, after between 7-13 days of incubation, the number of viable cells increased again in the cell lines treated with only IM. Regardless of whether IM was present or not in combination with ACF the number of viable cells is non-existent after treatment with ACF, suggesting that ACF targets cells that acquire resistance to TKIs. These cells are postulated to be the LSCs and so these results are promising for the use of ACF [49].

The effects of ACF has also been tested in primary CML cell lines that have come directly from patients suffering from CML. The ability of the cells to repopulate after ACF treatment has been shown to be diminished by the use of colony formation assays (CFAs), this is something that has not been shown consistently with IM treatment. Long-term culture initiating cell (LTC-IC) assays have shown the same results. All results from these assays elude to the inhibitory effects of ACF in stem cell potential [49].

BCR-ABL-transduced bone marrow (BM) cells were transplanted into mice to produce CML mice. Again, a good therapeutic index was shown as when ACF was given to the healthy mice without the BCR/ABL-transduced BM cells as they experienced no serious side effects such as a change in weight, or performance. The CML mice that were treated with ACF experienced less infiltration of myeloid cells in the lungs, reduced splenomegaly (increase in spleen size) and closer to normal levels of white blood cells in their peripheral blood in comparison to the CML mice which were not treated. In addition, the population of GFP+/CD34-/LSK cells, the LSCs that are long-term survivors and have the ability to incite CML in mice that they are transplanted into, is diminished on treatment with ACF [49]. Again, these are promising results for the treatment of CML and prevention of relapse caused by LSCs.

Regulation of c-myc

Myc is a family of regulator genes and proto-oncogenes that code for transcription factors. Most proto-oncogenes become an accessory of cancer due to a mutation or truncation of its genes. However, c-Myc becomes a risk factor due to an over-expression of itself caused by a loss of transcriptional control. c-Myc is essentially a nuclear phosphoprotein and helix-loop-helix leucine zipper (HLHLZ) protein that manages the expression of transcription in cell proliferation, apoptosis and metabolism [50].

Expression of c-Myc proteins results in the expression of numerous pro-proliferative genes which form a heterodimer with the transcription factor, MAX, via binding to enhancer box sequences (E-boxes) and engaging with histone acetyltransferases (HATs). Consequently, myc proteins can be over-expressed in over 80% of cancers. This happens due to transcribed genes being regulated by Myc, which results in the recruitment of elongation factors. This directly influences DNA replication. Down-regulation of c-Myc allows cells to be more sensitive to apoptosis by amplifying the intrinsic mitochondrial pathway and triggering or amplifying death receptor pathways. c-Myc overexpression supports the need of cancers to get more nucleic acid, nutrients etc. [51,52].

Normally (non-transformed cells), c-Myc is regulated by developmental or mitogenic signals. c-Myc mRNA is usually very short and therefore comes with a very short half-life. Therefore, in the absence of positive regulatory signals c-Myc transcription decreases which result in a lower proliferative drive [15]. Thus, reducing the risk of CML or cancers alike.

Genetically engineering alleles to remove c-Myc results on the cessation of cell proliferation and arrest in G_0/G_1 of the cell cycle. A correlation also exists between the level of c-Myc in cells and the cell's' rate of proliferation [42]. C-Myc is the key substrate targeted by Fbw7 in CML-initiating cell populations. Human CML Leukemia-Initiating Cells require Fbw7 function as its substrate is c-Myc. A study found that patients treated with TKI displayed normal levels of c-Myc. Fbw7 silencing led to the accumulation of both c-Myc and phospho-c-Myc protein in total progeny derived from BCR-ABL+ infected CD34+ cells [37].

The study also found that the deletion of c-Myc during disease progression of CML leads to an almost complete absence of BCR-ABL cells from the peripheral blood and an absence of infiltration into secondary tissue. This could pave way for potential clinical studies as to how to genetic therapies may aid in curing CML. Studies have also shown that too much or too little c-Myc could have detrimental effects on the body; instead, c-Myc should be kept in a very specific range to prevent toxicity [37].

Studies have also identified a ubiquitin ligase-substrate pair regulating LSC activity, suggesting that targeting of the Fbw7-c-Myc is an attractive therapeutic target in resistant CML strains [37].

Discussion

Histone Deacetylase Inhibition

Clinical trials have been undertaken to establish the tolerability and safety of HDACis in combination with IM in patients with CML who are in cytogenetic remission with evidence of residual BCR-ABL+ cells. As a result, HDACis have recently been licensed for use in many oncological malignancies. The mechanism of action of HDACis are complex and are a result of many different genes and pathways. Although these mechanisms are unknown, new nuclear functions of cytoplasmic tyrosine kinases have been identified relating to histone and transcription factor modulation have been identified [13]. More research is required to determine broader application of HDACis in other malignancies.

Autophagy Inhibitors

Autophagy inhibiting compounds are already licensed for use for other clinical indications. This will enable fast tracking of drug development as the toxicity profiles of the drugs will already be known. Hydroxychloroquine which is a licensed anti-rheumatic drug has not proven efficacious in Phase 1 and 2 trials. As such

novel autophagy inhibitors are being developed. Many of these compounds are based on hydroxychloroquine and have been shown to be more potent in *in vivo* preclinical trials [38-45].

SIRT1 Inhibitors

SIRT1 inhibitors are not currently undergoing any clinical trials, they are in the very early stages of research. Weight loss was a potential adverse effect of this therapy reported in mice models, yet it was unclear if this was a direct effect of SIRT1 inhibition or not [26]. No other research papers into SIRT1 inhibition could be found, suggesting continuing research may be happening [46-50].

HIF-1 Inhibition

ACF has already been approved for non-oncological use which means that it's toxicity and potential side effects have already been explored, reducing the time it would take to approve this drug for oncological use in comparison to a completely newly produced drug. It has been shown for the majority of time to not significantly affect the CD34+ cell population from disease-free healthy donors again eluding towards the fact that it has a good therapeutic index which is very beneficial when looking for new drugs [11]. However, there is little research into the effects of the drug specifically on LSCs, rather than just CML cells in general and so we have to be hesitant about how valuable and significant some of the results from the research have been. Further, there has been little *in vivo* research comparing ACF's effects with the effects of IM on CML and so we cannot say that ACF is actually a better drug than IM for treating CML or not.

c-myc Regulation

The presence of c-myc in over 80% of cancers makes it a well-studied protein that can be altered to treatment cancers, and CML in specific. Studies on c-myc and BCR/ABL shows that c-myc levels have be restricted within both upper and lower limits to prevent toxicity either way. Since most CSCs are not destroyed in relapsing cancers, the c-myc/Fbw7 pathway seems to be allured by investigators to prevent cancer relapse. Genetic therapies focusing on the combinatorics of the Fbw7 pathway, BCR/ABL gene and the c-myc pathway should be tested in order to suppress CSCs in CML to prevent relapse [51-54].

Conclusion

From our research, we put forward that targeting regulation of DNA expression, essential self-protection mechanisms, apoptotic mechanisms, transcription factors and cell regulator genes are plausible options for eliminating CML stem cells. These approaches are encouraging, however targeting stem cells for CML treatment is still a very new field and more research is needed, especially into how these targets affect chronic phase cells and specifically in human CML.

When considering new therapies, it is important to make sure that they inhibit CSC growth without diminishing the pool of normal SCs. In order to increase our chance of treating CML more successfully, it would be beneficial to develop novel assays in order to forecast and monitor the CML's response to therapy. This is especially important if new advantageous mutations arise in the CSCs which then make them resistant to CSC targeted therapy, this would then allow a different CSC targeted therapy to be initiated alongside TKI therapy.

We also have to be careful not to assume that simply targeting CSCs would cure CML, remembering the influence that the micro-environment has on the cancer cells. Further, research needs to be done into what the response is to CSC loss due to targeted therapy and the attempts of regeneration of these CSCs as this area could also be a potential target.

Limitations

Many articles fail to describe the rationale behind drug dosing regimens in pre-clinical models. The behaviour of cells in after cell lineage tracing and ablation is very different to how they act once transplanted into *in vivo* models. This may raise some concerns about the validity of results obtained from *in vitro* experiments. Additionally, the use of cell lines derived from one patient carries the risk of cross contamination. Single cell lines increase the risk of bias. Furthermore, many cell lines used in CML research are derived from Blast crisis lines. This contrasts with the population group (chronic phase) as treatments are targeted for which may affect the validity of pre-clinical models.

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