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Surface and Interacellular Targets of Acute Myeloid Leukemia Therapy Detectable at Leukemic Stem Cell level

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Review Article

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Abstract

For over 50 years investigators have considered a malignant stem cell as the potential origin of and a key therapeutic target for acute myeloid leukaemia (AML) and other forms of cancer. Leukaemic stem cells (LSCs) have been identified in acute myeloid leukaemia (AML). Similar to haemopoietic stem cells, these LSCs are able to self-renew, differentiate, and proliferate extensively. Recent studies suggest that LSCs are critical for the initiation and maintenance of leukaemia. Exciting new insights into the fundamental underpinnings of LSCs are now being made in an era in which drug development pipelines offer the potential to specifically target pathways of significance.

This mini review will describe the possible targets expressed on the surface of AML, the intracellular targets and the novel molecular and flow cytometry methodologies being used to particularly ablate the LSC population.

Keywords: Acute Myeloid Leukemia; Leukemic stem cell; Malignant stem cell

Abbreviations: AML: Acute Myeloid Leukaemia; LSCs: Leukaemic Stem Cells; AB: Antibiodies; BM: Bone Marrow; SIRPα: Signal Regulatory Protein; ADCC: Antibody Dependent Cell-mediated Cytotoxicity; ScFv: Single Chain Fragment of the Variable Regions; RTK: Receptor Tyrosine Kinases

Targets of AML Therapy Detectable in Leukaemic Stem Cells

Molecular constructions in AML cells that may serve as targets of specific therapy are located in various compartments of the leukaemic cell. Generally, such targets are either detectable on the cell surface or within the cytoplasm [1,2].

Targets Expressed on the Surface of AML Cells

Based on target molecules expressed on the surface of leukaemic cells, a number of new treatment strategies have recently been established [3-8]. One of these concepts employs humanized antibodies (Ab) conjugated with a cytostatic drug. These conjugates bind to leukaemic cells through an interaction of the Ab with the target structure on the surface of AML blasts.

Consecutively, the Ab–drug conjugate is internalized by the leukaemic cells. After internalization, the drug is released from the Ab and inhibits critical cell functions (depending on the nature of the cytostatic drug) and eventually leads to cell death [5,6,9,10].

An important example for such conjugates is Mylotarg (gemtuzumab/ozogamicin), which consists of a humanized anti-Siglec-3 Ab (CD33) and the highly potent (toxic) cytostatic drug calicheamicin [5,6,9,11]. However, it has been found that patients are prone to relapse despite being effective at inducing remission in some patients, which raises the question of the resistance of LSCs to the toxin [12].

Another treatment concept for surface antigen targeted anti-leukaemic therapy is based on conjugates consisting of (humanized) antibodies and radio-isotopes [8,13-15]. One example is 131I-anti-CD45 [13,14,16]. However, it remains unclear whether this therapy will result in an improvement in the disease-free survival of patients with AML.

Furthermore, anti-interleukin-3 (IL-3) receptor alpha chain (CD123)-neutralizing antibody (7G3) targeted AML-LSCs, impairing homing to bone marrow (BM) and activating innate immunity of NOD/SCID mice. 7G3 treatment profoundly reduced AML-LSC engraftment and improved mouse survival. Mice with pre-established disease showed reduced AML burden in the BM and periphery and impaired secondary transplantation upon treatment, establishing that AML-LSCs were directly targeted. 7G3 inhibited IL-3-mediated intracellular signaling of isolated AML CD34 (+) CD38 (-) cells in vitro and reduced their survival. These results provide clear validation for therapeutic MoAbs targeting of AML-LSCs and for translation of in vivo preclinical research findings toward a clinical application [17].

CD47 (cytokine receptor like CD123), a transmembrane protein that serves as a ligand for signal regulatory protein (SIRPα), is upregulated on AML LSCs than on HSCs. An increased expression of CD47 on LSC contributed to pathogenesis by inhibiting phagocytosis though the interaction of the CD47 with an inhibitory receptor on phagocytes. Thus, increased CD47 expression proved to be an independent poor prognostic factor. Targeting of human AML stem cells by blocking MoAbs directed against CD47 preferentially enabled phagocytosis of AML LSC. Elimination of human cancer cells in xenograft models of AML by this targeting method was also shown. The level of expression of CD47 on AML blasts is also associated with poor prognosis [18,19].

CD96, a member of the Ig gene superfamily, has been shown to be expressed in a majority of the LSC population and at a much lower frequency in HSC's. CD96+ AML cells are highly enriched for LSC activity compared to CD96- AML cells. The presence of CD96 expression allows AML-LSC can be distinguished from normal HSC. Thus, CD96 is a cell surface marker which can serve as an LSC-specific therapeutic target [20].

Also recently, it was shown that CD96-specific antibodies can efficiently activate ADCC (Antibody dependent cell-mediated cytotoxicity) which is an important Fc receptor mediated effector mechanism for the in vivo activity of therapeutic antibodies. However, in a clinical setting, future studies are yet to determine whether or not the single chain fragment of the variable regions fusion proteins (scFv-based mini-antibodies) will be able to eradicate AML LSCs [21].

((for info on CD96 by Hosen et al 2007 [22]: i) Two clones, clone G8.5 and clone TH-111 were used to analyse CD96 expression in normal adult HSCs and progenitor cells by flow cytometry. Only ≈ 5% of BM cells in HSC-enriched region (Lin-CD34+CD38-CD90+) expressed CD96 weakly. ii) In ≈ 66% of samples, the percentage of CD96-positive cells in the $CD34^{+}CD38^{-}$ AML-LSC-enriched fraction was significantly higher than in normal human BM $CD34^{+}CD38^{-}$ cells. CD96 is expressed almost exclusively in the $CD90^{-}$ subset. Thus, CD96 is frequently expressed on $CD34^{+}CD38^{-}Lin^{-}CD90^{-}$ AML blasts, which are enriched for LSC activity and exclude HSC. iii) FACS was used to fractionate human AML specimens into CD96+ and CD96- populations and then transplanted into mice. CD96+ AML cells uniquely showed engraftment of human CD45 positive cells. The confirmation that the engrafted hCD45+ were human myeloid leukemia blasts was done by measuring CD13/CD14/CD33 expression and/or Wright-Giemsa stain of peripheral blood or BM cells. >> demonstrate that CD96 is expressed on functional LSC in human AML.)).

((for info on CD96 from Nodehi S, et al. [21]: Strategies are being developed to increase the antitumor efficacy of monoclonal antibodies by enhancing ADCC activity. Binding affinity to the target antigen as well as Fc binding to activating Fc-receptors has been identified as critical parameters for the ADCC activity of monoclonal antibodies. Single chain fragment of the variable regions (ScFv) -Fc fusion proteins (mini-antibodies) may represent one promising new molecule format)).

CD32 and CD25 are cell surface markers which were found to be highly expressed in human AML LSCs and not expressed in normal HSCs. They were also found to be present in the all-important cell cycle-quiescent, AML initiating cells in the endosteal niche that might be the cause for AML relapse. This could facilitate the development of therapeutic strategies in AML because they were present in a significant number AML patients, especially the poor-risk population [23].

CXC chemokine receptor (CXCR4) which is a cell membrane receptor is found on stem cells [24-26]. It has been found that SDF-1α (stromal cell-derived factor-1) /CXCR4 interactions contribute to the resistance of LSCs to apoptosis in the microenvironment. Effective targeting of CXCR4 and its interactions paves the way to eliminate and target cells that are usually protected by the bone marrow microenvironment [27].

Very recently, it was suggested that CXCR4 expression is associated with poor prognosis in AML patients and a marker of more aggressive disease in a normal karyotype AML population [28]. This can even be incorporated into risk assessment of AML patients [28,29]. In addition, cycling LSC population, characterized by CD93 expression (which is a marker for non- quiescent LSC population), have been shown in AML with an MLL gene rearrangement [30].

Intracellular Targets of AML Therapy

Over the past few years, a large number of cytoplasmic and nuclear target structures in AML cells have been identified [13,31-33]. Among these are DNA-methylating enzymes, histone deacetylases, leukaemia-specific fusion gene-products (such as PML/RARα), pro-oncogenic transcription factors (STAT-family, Ets, c-Myb, HOX, NFκB, others) and critical elements in pro-oncogenic signal transduction cascades (RTKs, mutated oncogenic forms of Ras, others) [33].

Recent reports suggest that nuclear factor κB (NF-κB) is constitutively expressed in blast cells in a majority of patients with AML [13,34-36]. Additionally, NF-κB-activity is detectable in the (quiescent) LSC population in these patients, whereas normal unstimulated HSC do not express NF-κB activity [13,35]. Thus, trying to eradicate LSC's by direct targeting using NF-κB pathway could be a potential therapeutic strategy.

A number of different tyrosine kinase inhibitors have recently been identified and applied in clinical trials in leukaemic patients [37-48]. Important stem cell receptor tyrosine kinases (RTK) expressed in AML cells are the SCF receptor KIT, M-CSF receptor FMS, PDGFRβ, FLT1 and FLT3 [33,47,49,50]. At least some of these tyrosine kinases are also expressed in LSC [37]. Several previous and more recent observations suggest that these molecules do play an important role in leukaemogenesis [33,47,49,51,52]. Similarly, the FLT3 gene is the most frequently mutated gene in patients with AML [33,49,50,52,53]. These mutations lead to ligand-independent dimerisation of the receptor and its auto-phosphorylation with consecutive activation of multiple signal transduction pathways including the STAT5-, RAS/MAPK- and PI3K/AKT-pathway [54]. Since these mutations apparently act pro-oncogenically, it is appealing to speculate that they all take place and are detectable at the stem cell level in patients with AML.

A number of drugs targeting RTKs have recently been applied to AML cells in clinical and/or pre-clinical trials. Likewise, the inhibition of FLT3 by AG1296 or Herbimycin A in AML cells in mice was found to counteract the progression of leukaemia [33,49]. In addition, a number of targeting drugs directed against RTKs have been developed in recent years, including CEP701, CEP751, SU5614, SU5416, SU11248 and PKC412 [37,42-48,55]. These inhibitors may act on several RTKs including FLT3, thus inhibiting proliferation of leukaemic cells. Additionally, some of these inhibitors have already been evaluated in vivo. Likewise, CEP701 has been reported to induce responses in AML patients refractory to conventional chemotherapy [45]. Interestingly, at least some of these RTK-type receptors are known to be expressed in LSC.

A novel and promising therapeutic strategy to preferentially target human AML cells was uncovered by lysosome disruption. An important finding of this study was that AML LSCs were found to be enriched in bulk AML cells and their subsets which showed increased lysosomal size and biogenesis after lysosome disruption in human AML cells. This research study goes to show that some common biological features and mechanisms remain open to selective targeting even though AML biology is so widely known as heterogeneous [56].

Aurora A kinases are a family of mitotic serine/threonine kinases that play a role in cytokinesis during mitosis and cell division. AurA showed a significant higher level of expression in AML LSCs than in HSCs and can be used as a marker. The study showed that specific AurA inhibitors could be used to reduce LSCs. The study also further found that the reduction of

LSC could be enhanced with stimulation with G-CSF and the use of AurA inhibitors [57].

Mcl—1 has been found to be up-regulated during AML relapses in FLT3/ITD AML LSCs (probably due to the fact that Mcl-1 confers some sort of resistance to chemotherapy) [58]. Furthermore, deletion of Mcl-1 led to induced death of transformed AML and eradicated disease in the AML NOD/SCID mice [59]. So, the lowering of Mcl-1 through a variety of approaches such as disruption or degradation of Mcl-1 by Bcl-2 inhibitors and other inhibitors and interfering with transcription and translation processes makes Mcl-1 a favourable therapeutic target in AML. The same study suggests that combination approaches which disrupt multiple pro-survival pathways and activate their pro-apoptotic pathways could show promise for targeting [60].

Inhibition of the PI3K-Akt-mTOR pathway seems to be a therapeutic strategy in human AML. Ryningen, et al. suggests that a combined targeting of different stages in the pathway could be investigated as a possible therapeutic strategy [61].

A recent report showed that AML cells rely on a tumor-specific heat shock protein species (teHsp90) that is selectively activated under conditions of cell stress and signalosome activity [62]. Additionally, a central component of stress response pathways, activation of NF-kB, is evident in LSCs, but not in normal resting HSCs [63]. Challenge of LSCs with agents that inhibit NF-kB, proteasome activity, HSP function, and glutathione balance have all been demonstrated to selectively target leukemic stem/progenitor cells in comparison to normal HSC controls [13,64,65].

A recent study indicates that the physical transfer of mitochondria from mesenchymal cells can influence leukemic cells, making them more resistant to chemotherapy, a phenomenon also described for ovarian cancer cells [66,67]. Collectively, these findings indicate that modulation of niche interactions may serve to increase the sensitivity of LSCs to therapeutic intervention.

Finally, there is also evidence that a pro-inflammatory state can influence LSC growth/survival. An intriguing study by Kagoya, et al. has recently shown that autocrine secretion of TNF-alpha drives the constitutive activation of NF-kB, a property of LSCs that is presumably associated with their intrinsic biology and malignant transformation [63]. Further, a number of mutations associated with myeloid malignancy and stem cell transformation support a pro-inflammatory milieu that likely favors growth of LSCs [68]. Conversely, chronic inflammation has been shown to degrade the potential of normal HSC [69,70]. Hence, inhibition of pro-inflammatory factors may serve the dual function of inhibiting LSC activity and creating an environment more favorable for normal stem cells. This type of intervention could be particularly interesting in the context of post chemotherapy treatment, where the need for suppression of residual disease and promotion of normal cell regeneration is perhaps most acute.

Summary and Future Directions

AML populations are consisted of hierarchical structure and in recent years it has been possible to begin analyzing individual constituents of the leukaemic clone. Although, varying AML subtypes differentiate to differing levels, it has become increasingly evident that important similarities exit at the top of the developmental hierarchy.

Given the quiescent status of LSCs and their relatively low frequency, ablation of this population is likely to be a significant challenge. Despite the fact that a variety of LSC characteristics are almost identical to normal HSCs, recent studies of AML molecular biology suggest that some differences between normal and leukaemic cells are apparent in the stem cell/progenitor cell pool [71]. From a therapeutic perspective, this observation is extremely important because it suggests LSCs do have unique characteristics that may make them preferentially sensitive to apoptosis /ablation. This information also serves to emphasize the importance of better understanding LSCs and how they differ from normal HSCs.

By establishing general parameters for induction of LSC apoptosis, it should be potential to develop more effective clinical therapies. Given the heterogeneity of mutations that give rise to these malignancies, the ability to target the malignant population is not likely to be achieved by a single specific inhibitor. To this end, it is fundamental to completely understand the signaling pathways that regulate survival and death in LSC populations. Current studies have started to characterize molecular mechanisms that may be relevant to LSC survival. However, more comprehensive methodologies using multiparameter or combined approaches should be the priority for future studies [72-74]. Well-designed studies incorporating elements such as randomized discontinuation [75], as has been done in other oncology studies [76], may be required to answer these questions.

We look forward to the day when these questions become relevant in the drug development process for AML, and have reason to hope that they are around the corner.

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