Chronic myeloid leukemia: the basis of treatment for tomorrow

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Recent years have seen dramatic advances in deciphering the molecular pathogenesis of chronic myeloid leukemia (CML). This has resulted in the rapid development of many BCR-ABL1-specific tyrosine kinase inhibitors (TKI) which have improved 10-year survival to more than 80%. In this paper we focus on some future directions for CML biology and therapy.

Targeting CML stem cells

Work from Tessa Holyoake's lab in Glasgow showed that the majority of CML progenitor ($CD34^+$) cells undergo division in culture in the presence of growth factors.[1] When imatinib, nilotinib or dasatinib were added to the culture, the proliferating cells were killed while cells that do not divide (i.e. are dormant) were completely refractory to the drugs.[2] These dormant or quiescent cells are probably responsible for 'molecular persistence', namely, the residual low level of BCR-ABL1 transcript positivity detected by quantitative PCR in many cases. Since the available anti-BCR-ABL1 TKIs seem unable to eliminate dormant cells, the question is how to devise alternative biological strategies to eradicate them. Recent studies from different groups have provided encouraging potential therapeutic approaches.

Protein phosphatase 2A (PP2A) activation

PP2A is a tumor suppressor whose activity is inhibited in Philadelphia (Ph)-positive leukemias but not in normal hematopoietic stem/progenitor cells.[3] Drugs such as FTY720 (fingolimod) and its non-phosphorylatable derivative[4] re-activate PP2A, thereby limiting the adverse effect that other types of drugs might exert on normal cells. FTY720, an immunosuppressive synthetic sphingosine analog,[5] shows anti-leukemic activity on $CD34^+$ cells from TKI–sensitive and TKI–resistant CML progenitors.[6] FTY720 acts as an anti-leukemic agent in its non-phosphorylated form without exerting toxicity on normal myelopoiesis.[6-9] The question arises whether BCR-ABL1 is necessary for this drug to work. In CML progenitors, FTY720 induces apoptosis because of the ability of active PP2A to simultaneously impair BCR-ABL1 activity/expression.[6] Recent evidence suggests that FTY720 also targets other kinases through PP2A.[8] In primitive CML cells, the apoptotic effect of FTY720 might not require BCR-ABL1 activity[7] which, as reported, is not essential for their survival.[10] Indeed, it seems that alternative signaling pathways which require the expression of BCR-ABL1 for their activation and/or maintenance are necessary for the effect of FTY720 in the most primitive CML cells. Overall, FTY720 can kill primitive and mature progenitor cells without showing any type of toxicity other than its possible immunosuppressive activity. Because FTY720 has the property of acting on different oncogene-driven pathways while preserving normal cells, it is not surprising that it is active in different leukemias.[9,11]

Farnesyl transferase inhibition

Copland, Holyoake and colleagues reported that BMS215662, a farnesyl transferase inhibitor, showed preferential cytotoxicity against non-proliferative (quiescent) cells, different from most drugs. It eradicates Ph^+ primitive long-term culture initiating cells (LTC-ICs), either alone or in combination with imatinib or dasatinib.[12] *In vivo*, it has little effect on the engraftment of K562 cells in mice when used as a single agent, but there is virtually no K562 tumor formation when it is combined with dasatinib. It selectively increases caspase 3, thus causing apoptosis in CML $CD34^+CD38^-$ ('stem') cells but in not their normal counter-

parts. The mechanism of apoptosis seems to be triggered by aberrant phosphorylation of CDK2 in CML cells which, in its turn, leads to a conformational change in the anti-apoptotic protein BAX, significant release of cytochrome c from the mitochondria, mitochondria swelling, and eventual activation of the caspase pathway.

Autophagy inhibition

CML cells which survive TKI treatment show reduced size and significant increase in cytoplasmic vacuoles, an effect similar to growth factor deprivation.[13] This is the phenotype of cells undergoing autophagy, a biological response to nutrient shortage. Once the autophagy process starts, the enzyme LC3 is converted from a cytosolic to a membrane-bound form. Treatment of CML stem ($CD34^+CD38^-$) cells with dasatinib causes such LC3 conversion and increase of autophagy. This observation provided the rationale for combining a TKI, which kills only more mature BCR-ABL1$^+$ cells, with an autophagy inhibitor (like chloroquine) to eliminate primitive CML cells. A clinical trial based on this principle is ongoing in the UK; patients who achieve major cytogenetic responses after one year on imatinib are randomized to continue imatinib alone or imatinib plus chloroquine. Follow-up data are not yet available, but it will be interesting to see if there is any major advantage in the combination arm.

Inhibition of CXCR4

BCR-ABL1 specifically inhibits CXCR4, the receptor for SDF1 (CXCL12). This is a chemokine produced by stromal cells that mediates the chemotaxis of $CD34^+$ progenitor cells, thus playing a critical role in their homing to the bone marrow microenvironment.[14] Imatinib restores CXCR4 expression, which promotes the migration of CML cells to the bone marrow stroma.[15] This is associated with G_0-G_1 cell cycle arrest, inhibition of their proliferation and enhanced survival in a quiescent state, a phenotype attributed to the refractoriness of primitive CML cells to TKI therapy. Therefore, pharmacological inhibition of CXCR4 might be expected to reverse this mechanism of primary resistance, a phenomenon indeed seen with plerixafor (previously AMD3100), a CXCR4 antagonist. If these *in vitro* findings are successfully translated into clinical practice by combining inhibitors of CXCR4 with TKIs, the treatment of CML could be greatly improved.

Inhibition of Hedgehog (Hh) signaling

The Hh pathway is very important both in embryonic development and in adult cells, as it is involved in chromatin remodeling, cell cycle control, and apoptosis.[16] Expression of the Hh effectors Gli-1 and, in particular, Gli-2, are significantly increased in both chronic and accelerated phase CML progenitors.[17] A Novartis compound (LDE225) has been used to inhibit this pathway in *in vitro* assays. In combination with nilotinib, it significantly reduced the clonogenic potential of primitive CML LTC-ICs, but not of normal LTC-ICs.[18] This compound is now making its way into a clinical trial.

IL1 receptor accessory protein

IL1-RAP is a membrane marker which is up-regulated in very primitive ($CD34^+CD38^-$) CML cells, with negligible expression in their normal counterparts.[19] This is an important observation because it is the first example of a surface marker that can distinguish BCR-ABL1-negative from BCR-ABL1-positive cells. Thus the production of antibodies that can target IL1RAP and selectively kill CML Ph$^+$ cells may be of clinical value. In practice, the specially derived polyclonal KMP1 antibody is cytotoxic, and can bind to and kill primitive $CD34^+CD38^-$ CML cells, but spares the corresponding normal bone marrow cells.

New drugs in development

The T315I BCR-ABL1-mutant protein is highly resistant to imatinib, dasatinib, nilotinib and bosutinib, and remains a big concern for clinicians. Among the new TKIs in development that may be active against the T315I, ponatinib (ariad, previously AP24534) and DCC-2036 (deciphera) are of interest, as they represent the prototypes of a novel approach to kinase inhibition. Ponatinib is considered a pan-BCR-ABL1 inhibitor which potently inhibits the T315I mutant and overcomes mutation-based resistance.[20,21] This agent is currently part of clinical trials in major centers worldwide. Another possible target in clinical development is the angiopoietin 1 receptor Tie 2. The deciphera compound (DCC-2036)[22] is a very good candidate to inhibit the Tie 2 receptor.

Conclusions

It has been suggested by some, perhaps mischievously, that the problems of CML are now essentially solved and it is time to move to other fields. TKIs are not perfect, and we have to predict and devise strategies for managing the minority of patients who will respond poorly. There is still much work to be done; work that should benefit patients unfortunate enough to have CML in the future and, hopefully, also other patients whose management may be based on principles derived from pioneering work in CML research.

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