Pharmacogenomics suggests new treatment approach for leukaemia

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Tyrosine kinase inhibitors could provide a new approach for treating an aggressive form of acute myeloid leukaemia (AML), according to research published recently [1]. Donald Small's group at Johns Hopkins University School of Medicine (http://www.med.jhu.edu/) have shown that a new drug, CEP701, inhibits the tyrosine kinase activity that occurs in up to 41% of AML patients who have mutations in the gene for receptor tyrosine kinase FLT3. This gene normally has an important role in the development of haematopoietic stem cells, dendritic cells, B progenitor cells and natural killer (NK) cells but is abnormally expressed on the malignant cells of some patients with AML.

FLT3 mutations and prognosis

Patients with internal tandem duplication (ITD) mutations or point mutations at an aspartate residue within the kinase domain of FLT3 have a much worse prognosis. 'In one study, the cure rate for AML in patients with an FLT3 mutation was reported to be as low as 7%, compared to a cure rate of 44% in those patients without a mutation,' says Small. The search for new therapies to improve this includes developing drugs to selectively target the tyrosine kinase activity, effectively eliminating the impact of the mutation. 'There is a good precedent for this approach in AML,' comments Brian Druker (Oregon Health and Science University Cancer Institute; http://www.ohsu.edu/), 'since this approach is proving successful in treating chronic myeloid leukaemia,' [2]. Gleevac™, a tyrosine inhibitor developed by Druker and colleagues in association with Novartis (http://www.novartis.com) received FDA approval for use in patients with chronic myeloid leukaemia (CML) in May 2001. It is approved for use in three phases of CML: the chronic phase that no longer responds to standard therapy with interferon, the accelerated phase and a myeloid blast crisis.

Targeting tyrosine kinase activity

The recent clinical successes of Druker's group suggest that targeting a mutated tyrosine kinase implicated in a disease is a promising new strategy. 'We decided that FLT3 fulfilled the criteria of a good rational potential target in AML, and we began to home in on this target by generating FLT3-carrying cell lines with which to screen a library of small-molecule tyrosine kinase inhibitors,' explains Small. The assay system picked out one compound, the indolocarbazole derivative, CEP701. Dose response experiments using CEP701 with mouse cells expressing constitutively phosphorylated FLT3 showed that the drug was a potent inhibitor of FLT3 autophosphorylation (Fig. 1). 'This inhibition was selective, as CEP701 was only able to inhibit other, closely related tyrosine kinases, at concentrations 500 times greater,' explains Small [1].

Characterizing the tyrosine kinase inhibitor

'This study demonstrates that CEP701 inhibits wild type and both types of mutant FLT3 in AML samples from patients. It also shows that the drug induces cytotoxicity in a number of these leukemic cell samples,' observes Small. CEP701 was also able to inhibit FLT3 phosphorylation in several myeloid leukaemia-derived human cell lines. There were some differences, however, in the cytotoxic effect of CEP701. Although some of the cell lines tested did exhibit cell death, some did not. The cell lines and patient samples in which FLT3 phosphorylation was inhibited by CEP701, but in which no cytotoxicity was observed, probably do not rely on FLT3 signalling alone for survival. 'This variable cytotoxic response raises important issues regarding the use of FLT3 inhibitors to treat leukaemia,' stresses Small. His theory is that the inhibition of FLT3 phosphorylation probably results in the withdrawal of a strong anti-apoptotic signal, but it does not introduce a pro-apoptotic signal. 'It may be that FLT3 inhibition needs to be carried out in conjunction with standard chemotherapy; the two treatments might be synergistic and could result in the killing of leukaemia cells,' he adds.

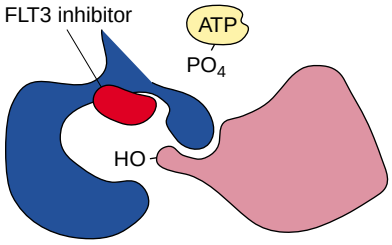

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Figure 1. The figure shows how an FLT3 inhibitor is likely to work. CEP701 (or other FLT3 inhibitors) binds to the active site of FLT3 that is normally bound by ATP. This blocks the binding of ATP and prevents FLT3 from transferring a phosphate group to target proteins. This in turn blocks the ability of FLT3 to transmit its signal, which tells the cell to grow and not to die. Figure kindly supplied by Donald Small (Johns Hopkins University School of Medicine; http://www.med.jhu.edu/).

1359-6446/02/$ – see front matter PII: S1359-6446(02)02394-2

Clinical trials

In vivo experiments in mice with FLT3–ITD leukaemia showed that a single dose of CEP701 could completely inhibit FLT3 phosphorylation for several hours, and dosing 2–3 times a day improved survival rates; 50% of control mice died by day 16 whereas 50% of the group given 10 mg kg^{-1} CEP701 every 8 hours survived until day 27. Analysis of the result showed that the overall difference in survival was significant ($P<0.001$). The drug was shown to be well tolerated in a previous Phase I trial (Small and colleagues, unpublished data). A Phase II trial for AML patients expressing FLT3 mutations is now under way at the Johns Hopkins Kimmel Cancer Center (http://www.hopkinskimmelcancercenter.org). 'We have just started recruiting patients with relapsed AML into an open-label study to see if they will respond to CEP701 with a reduction in percentage blast cells; we hope that some may even go into remission,' reports Small. If results are promising, the group hopes that the next step will be to combine CEP701 treatment with standard chemotherapy. There is a precedent for this: previously, M3 AML had a low cure rate [3], but after retinoic acid therapy was combined with chemotherapy, the cure rate increased to 70–80%. Small hopes that 'a similar synergistic result may be achieved with CEP701, transforming AML with FLT3 mutations from a worse prognosis to a good prognosis.'

References

1 Levis, M. *et al.* (2002) An FLT3-targeted tyrosine kinase inhibitor is cytotoxic to leukaemia cells *in vitro* and *in vivo*. *Blood* 99, 3885–3891

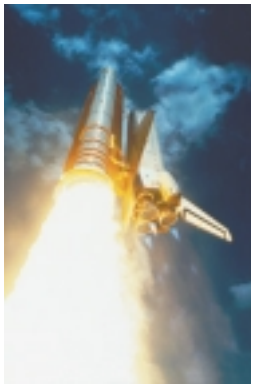
2 Druker, B.J. *et al.* (2001) Activity of a specific inhibitor of BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukaemia and acute lymphoblastic leukaemia with the Philadelphia chromosome. *New Engl. J. Med.* 344, 1038–1042

3 Randolph, T.R. (2000) Acute promyelocytic leukemia (AML-M3) Part 1: Pathophysiology, clinical diagnosis and differentiation therapy. *Clin. Lab. Sci.* 13, 98–105

News in brief

Neurodegenerative diseases

Mouse and computer models of Parkinson's disease



Two separate reports have shed light on how genes misfire in Parkinson's disease (PD), and the origin of the tremors associated with the disorder [1,2]. The first report [1], conducted by researchers at the University of California, Los Angeles (UCLA; http://www.ucla.edu/), produced a method of imaging the misfiring of thousands of genes in a mouse model. This could lead to a research blueprint to pinpoint abnormal regions in the brain linked to autism or schizophrenia.

Desmond Smith, UCLA Assistant Professor of Molecular and Medical Pharmacology, said: 'This approach identifies which genes play a role in abnormal brain function and where they are located. We can use this information to narrow down the brain regions linked to genetic disorders and pinpoint the genes responsible for causing them.'

The study involved the technique of voxelation – involving the analysis of cubes of brain by DNA chip technology – to compare the gene expression in mice brains, half of which were treated with drugs to induce PD. The brains were then 'voxelated' to track the expression of 9000 genes simultaneously. Upon comparing healthy and diseased mice, Smith and co-workers found that mice with PD had an abnormal shift in gene activity and, in particular, the upregulation of genes involved in cell–cell interactions was observed in the PD brains.

The second study, which could explain the origin of the debilitating tremors of PD, was conducted by scientists at the Department of Mathematics, Ohio State University (http://www.osu.edu/), using a computer model of electrochemical activity in a brain affected by PD [2]. The research showed unusual patterns in the way the brain cells fired signals.

David Terman, Professor of Mathematics at Ohio State University, said: 'In a normal brain, every cell is doing its own thing, and the signals create a random pattern. But in our model, we saw cells firing together in lockstep, creating a synchronized pattern that matched the timing of Parkinson's tremors.'

This research could help to elucidate a mystery of the medical community; that is, how the loss of the neurotransmitter dopamine leads to the tremors of PD. In the past, researchers have thought that an increase in the frequency of neural signals was the cause. Although this occurrence of neurons firing almost twice as fast as normal during Parkinson's episodes could explain other symptoms of the disease, such as slowness of movements and stiffness, it could not explain the tremors. 'Our computer model shows that the pattern of signals is important – not just the frequency,' said Terman.

Terman and colleagues hope to extend this research to include other regions of the brain, but these initial results could provide researchers with new directions for therapies for PD.

1 Brown, V.M. *et al.* (2002) Multiplex three-dimensional brain disease expression mapping in a mouse model of Parkinson's disease. *Genome Res.* 12, 868–884

 1359-6446/02/$ – see front matter PII: S1359-6446(02)02397-8