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Title

Bedside to bench: interfering with leukemic stem cells.

Permalink

<https://escholarship.org/uc/item/3x54f2j9>

Journal

Nature medicine, 14(5)

ISSN

1546-170X

Authors

Krause, Daniela S
Van Etten, Richard A

Publication Date

2008-05-01

Peer reviewed

■ BEDSIDE TO BENCH

Interfering with leukemic stem cells

Daniela S Krause & Richard A Van Etten

Kinase inhibitors such as imatinib (Gleevec) have improved the outlook for many people with chronic myeloid leukemia and related blood disorders. But such drugs do not target the leukemia stem cell population and may not be curative. Krause and Van Etten discuss several clinical studies that suggest that interferon- α may provide a solution by selectively eliminating leukemic stem cells—although only more basic research will tell us whether this is true and how it may happen.

Recombinant interferon- α (IFN- α), an immunomodulatory cytokine, is used to treat chronic hepatitis C virus infection and as a therapy for several cancers, such as melanoma and Kaposi's sarcoma. IFN- α also has potent therapeutic activity in the myeloproliferative diseases (MPDs), such as chronic myeloid leukemia (CML), polycythemia vera and essential thrombocythemia. MPDs are clonal hematopoietic stem cell disorders characterized by overproduction of mature myeloid or erythroid cells, which share a common pathophysiology involving dysregulated tyrosine kinase signaling¹.

Despite the effectiveness of IFN- α , its mechanism of action in the MPDs is poorly understood. Interest in the topic is now rekindled by several recent clinical studies^{2–5} that hint that IFN- α may target leukemic stem cells.

Clinicians of a certain age will recall when medical therapy of CML was palliative. Busulfan and hydroxyurea, once the drugs of choice, suppressed production of myeloid cells but did not

selectively target the malignant clone containing the Philadelphia chromosome, the CML-specific translocation product that creates the *BCR-ABL* fusion gene. Nor did these drugs interrupt the inexorable progression of CML from chronic phase, in which myeloid differentiation is preserved, to blast crisis, a terminal condition resembling acute leukemia.

In 1986, IFN- α was tested in CML subjects and was found to induce cytogenetic and even molecular remissions in which *BCR-ABL* mRNA transcripts became undetectable. In some subjects, these remissions were maintained when treatment was discontinued⁶. IFN- α was also found to normalize blood counts in people with other MPDs, including some subjects with polycythemia vera and essential thrombocythemia⁷. Cytogenetic studies of selected subjects suggested that IFN could specifically suppress malignant stem cells in MPDs that lack a Philadelphia chromosome, but it was impossible to prove such effects in the absence of a broadly applicable molecular marker.

In 1990, the demonstration that the product of the Philadelphia chromosome, the *BCR-ABL* fusion tyrosine kinase, could induce CML-like disease in mice accelerated the search for drugs that could block its enzymatic activity¹. In 2001, the ABL kinase inhibitor imatinib mesylate abruptly supplanted IFN- α as front-line therapy for patients newly diagnosed with CML. Treatment with imatinib results in vastly superior cytogenetic and molecular responses⁸, but imatinib and other second-line ABL kinase inhibitors are plagued by the problem of acquired resistance and their inability to eliminate quiescent *BCR-ABL*⁺ stem and progenitor cells. These drawbacks have revived the search for treatment strategies that can eradicate leukemic stem cells in CML and other MPDs⁹.

In 2005, the discovery of a somatic mutation (V617F) in the *JAK2* tyrosine kinase in nearly every individual with polycythemia vera and about half of the individuals with essential thrombocythemia¹⁰ provided a molecular

marker for these diseases that was analogous to *BCR-ABL* in CML. With this marker in hand, researchers have provided clinical evidence suggesting that IFN- α may specifically target leukemic stem cells in these MPDs.

In a study published in *Blood*, Kiladjan *et al.*² treated a cohort of individuals afflicted with polycythemia vera with a pegylated formulation of IFN- α 2a. They observed complete hematological remission (normalization of erythrocyte and leukocyte counts) in 83% of subjects. In 24 of 27 evaluable subjects, remission was accompanied by a decrease in the mutant *JAK2* allele in granulocytes from a mean of 49% to 27%², and, in one subject, the mutant *JAK2* became undetectable, consistent with a molecular remission. Similar results have been obtained in an ongoing study by Quintás-Cardama *et al.*³ examining PEG-IFN- α 2a treatment in individuals with polycythemia vera and essential thrombocythemia.

A third report describes 12 subjects with CML who achieved molecular remission on imatinib and who subsequently discontinued kinase inhibitor therapy⁴. Half of the subjects promptly relapsed with detectable *BCR-ABL* mRNA transcripts, whereas the others remained in molecular remission without imatinib, with a median follow-up of 18 months. Interestingly, all six of the latter subjects had been previously treated with IFN- α ⁴. Another recent study found that imatinib can induce molecular remission in more than half of subjects who have prior cytogenetic remissions in response to IFN- α ⁵, a much higher rate than in subjects with CML that had not been previously treated with IFN- α ⁸.

Collectively, these studies provide strong but indirect evidence that IFN- α preferentially targets the mutant clone in CML, polycythemia vera and essential thrombocythemia and might act to decrease or eliminate the malignant stem cell population in these MPDs.

The relative kinetics of the molecular responses to imatinib and IFN- α in CML also support this hypothesis. *BCR-ABL* mRNA

Daniela S. Krause is at the Massachusetts General Hospital, Department of Pathology, 55 Fruit Street, Boston, Massachusetts 02114, USA. Richard A. Van Etten is at the Molecular Oncology Research Institute and Division of Hematology/Oncology, Tufts Medical Center, 800 Washington Street, Boston, Massachusetts 02111, USA.
e-mail: rvanetten@tuftsmedicalcenter.org

transcripts show an initial rapid decline in imatinib-treated patients that may be the result of elimination of committed progenitors. This decline is followed by a plateau—reflecting the persistence of resistant leukemic stem cells¹¹, whereas molecular responses in IFN-treated people with CML require much longer treatment periods⁶. The fact that IFN- α can induce molecular responses in both CML and polycythemia vera further suggests that leukemic stem cells expressing dysregulated tyrosine kinases might be uniquely sensitive to this cytokine.

These clinical ‘bedside’ findings argue for additional basic and translational ‘bench’ research into the molecular mechanisms of IFN action in the MPDs. Twenty years after the introduction of IFN therapy for MPD, little is known about how it operates or why some patients respond to it while others do not.

The possibilities are numerous: some actions of IFN may work directly on the malignant stem cell, such as induction of interferon regula-

tory factor-8 (ref. 11) and Fas and inhibition of *BCR-ABL* transcription¹³. IFN- α also selectively impairs proliferation of primitive CML progenitors¹⁴. Moreover, both *BCR-ABL* and *JAK2 V617F* promote hematopoietic cell proliferation and survival through pathways involving the cell cycle regulator p27 and Foxo transcription factors—providing a potential common mechanism for IFN. In addition to direct effects, IFN- α may also target malignant stem cells through its ability to restore normal β_1 -integrin-mediated adhesion to the bone marrow niche. IFN- α also has pleiotropic immunological actions, including increasing the cytotoxicity of T and NK cells and inducing cell-mediated and humoral immune responses to candidate MPD antigens¹⁵.

More work at the bench may illuminate the basic mechanisms of IFN- α in CML and polycythemia vera and thereby offer new approaches to eradicate malignant stem cells in MPDs, resulting in permanent cure. On the clinical side, randomized studies of IFN- α in combination

with kinase inhibitors and with vaccination are warranted in people with CML who have not attained molecular remission on imatinib.

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■ BENCH TO BEDSIDE

BRCA: From therapeutic target to therapeutic shield

Neil P Shah

Three studies examine how resistance to chemotherapy develops in cancers deficient in *BRCA1* and *BRCA2*. The mechanism involves restoration of *BRCA1* and *BRCA2* activity. Shah examines the implications for the clinic, such as the potential value of continuing treatment with cisplatin and similar agents even after drug resistance develops.

Our knowledge of how cancer cells respond and subsequently develop resistance to chemotherapy is far from complete. Two recent studies in *Nature*^{1,2} and one in *Cancer Research*³ unravel how such resistance develops in ovarian cancers associated with mutations in the *BRCA1* and *BRCA2* genes. These mechanistic findings may have implications for how chemotherapeutic agents are prescribed for cancer patients and demonstrate the need for understanding

whether targets of chemotherapy are necessary for cancer cell maintenance.

The FANC-*BRCA* pathway consists of a number of proteins that are required for an appropriate cellular response to DNA cross-linking, a form of DNA damage. An inherited mutation inactivating either the *BRCA1* gene or the *BRCA2* gene can be found in people with familial breast and ovarian cancer. In the tumor tissue itself, the remaining normal copy of the *BRCA*-encoding gene is typically lost. *BRCA1* and *BRCA2* are therefore classified as ‘tumor suppressor genes’, because their loss results in the development of cancer. The *BRCA1* and *BRCA2* genes encode proteins that are involved in homologous recombination, a process that repairs double-stranded DNA breaks and stalled DNA replication forks. As a result of *BRCA* deficiency, mutations that lead to the development of cancer can accumulate. The lifetime risk of developing ovarian cancer approaches 40% for women with *BRCA1* mutations and is 10–20% for women with *BRCA2* mutations⁴.

Owing to a lack of both symptoms and effective screening tools, most individuals with ovarian cancer are diagnosed with advanced-stage disease, which requires chemotherapy for disease control. Encouragingly, cells that are deficient in repairing double-stranded DNA breaks

are particularly sensitive to chemotherapeutic agents that work by inducing such breaks, such as cisplatin, presumably because accumulating mutations eventually have deleterious consequences leading to cell death. A new class of drugs undergoing early clinical development inhibits poly(ADP-ribose) polymerase (PARP), a protein required for repairing single-stranded breaks. Without PARP, these single-stranded breaks also stall DNA replication forks; PARP inhibitors therefore selectively kill *BRCA*-deficient cells^{5,6} (Fig. 1) and may be a less toxic form of effective therapy.

The long-term success of drugs such as cisplatin is severely limited by incomplete disease eradication (resulting in the need to repeatedly retreat patients who relapse) and, more importantly, by the eventual development of drug-resistant disease; as a result, most advanced-stage patients are not cured.

The three new studies examine how resistance to cisplatin or PARP inhibitors develops in primary ovarian cancers and pancreatic cancer cell lines associated with mutations in *BRCA1* or *BRCA2*. Remarkably, resistance correlated with restoration of detectable levels of *BRCA1* and *BRCA2* protein as a result of secondary mutations that restore the reading frame of the proteins. The new findings dovetail with previous

Neil P. Shah is in the Department of Hematology/Oncology, University of California, San Francisco, 505 Parnassus Avenue, Suite M1286, Box 1270, San Francisco, California 94143, USA.
e-mail: nshah@medicine.ucsf.edu