5. Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of mammalian iron metabolism. Cell. 2010;142(1): 24-38.
6. Babitt JL, Huang FW, Wrighting DM, Xia Y, Sidis Y, Samad TA, et al. Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. Nat Genet. 2006;38(5):531-9.
7. Nemeth E, Roetto A, Garozzo G, Ganz T, Camaschella C. Hepcidin is decreased in TFR2 hemochromatosis. Blood. 2005;105(4):1803-6.
8. van Dijk BA, Laarakkers CM, Klaver SM, Jacobs EM, van Tits LJ, Janssen MC, Swinkels DW. Serum hepcidin levels are innately low in HFE-related haemochromatosis but differ between C282Y-homozygotes with elevated and normal ferritin levels. Br J Haematol. 2008;142(6):979-85.
9. Tjalsma H, Laarakkers CMM, van Swelm RPL, Theurl M, Theurl I, Kemna EH, et al. Mass spectrometry analysis of hepcidin peptides in experimental mouse models. PLoS ONE 2011;6:e16762.
10. Piperno A, Girelli D, Nemeth E, Trombini P, Bozzini C, Poggiali E, et al. Blunted hepcidin response to oral iron challenge in HFE-related hemochromatosis. Blood. 2007;110(12):4096-100.
11. Roe MA, Collings R, Dainty JR, Swinkels DW, Fairweather-Tait SJ. Plasma hepcidin concentrations significantly predict interindividual variation in iron absorption in healthy men. Am J Clin Nutr. 2009;89(4):1088-91.
12. Waheed A, Parkkila S, Zhou XY, Tomatsu S, Tsuchihashi Z, Feder JN, et al. Hereditary hemochromatosis: effects of C282Y and H63D mutations on association with beta2-microglobulin, intracellular processing, and cell surface expression of the HFE protein in COS-7 cells. Proc Natl Acad Sci USA. 1997;94(23):12384-9.
13. Lee PL, Beutler E. Regulation of hepcidin and iron-overload disease. Annu Rev Pathol. 2009;4:489-515.
14. European Association For The Study Of The Liver. EASL clinical practice guidelines for HFE hemochromatosis. J Hepatol. 2010;53(1):3-22.
15. van Bokhoven MA, van Deursen CT, Swinkels DW. Diagnosis and management of hereditary haemochromatosis. BMJ. 2011;342:c7251.
16. Swinkels DW, Jorna AT, Raymakers RA. Synopsis of the Dutch multidisciplinary guideline for the diagnosis and treatment of hereditary haemochromatosis. Neth J Med. 2007;65(11):452-5.
17. Fischer R, Harmatz PR. Non-invasive assessment of tissue iron overload. Hematology Am Soc Hematol Educ Program. 2009;215-21.
18. Njajou OT, Vaessen N, Joosse M, Berghuis B, van Dongen JW, Breuning MH, et al. A mutation in SLC11A3 is associated with autosomal dominant hemochromatosis. Nat Genet. 2001;28(3):213-4.
19. Kuhlenbaumer G, Hullmann J, Appenzellerm S. Novel genomic techniques open new avenues in the analysis of monogenic disorders. Hum Mutat. 2010 Nov 18.
20. Kroot JJ, Kemna EH, Bansal SS, Busbridge M, Campostrini N, Girelli D, et al. Results of the first international round robin for the quantification of urinary and plasma hepcidin assays: need for standardization. Haematologica. 2009;94(12):1748-52.

The rationale for using hydroxycarbamide in the treatment of sickle cell disease

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The modern management of sickle cell disease (SCD) is based on three therapeutic approaches: blood transfusion (first used successfully in 1818), penicillin (discovered in 1928) and hydroxycarbamide (first synthesized in 1869).[1] Dresler and Stein made this simple molecule from hydroxylamine, hydrochloric acid and potassium cyanide as a technical exercise in organic chemistry, as part of a series of experiments generating derivatives of urea. Hydroxycarbamide lay dormant for more than fifty years until it was studied as part of an investigation into the toxicity of protein metabolites and found to produce a megaloblastic anemia, which was thought to mimic pernicious anemia.[2] In the early 1960s further *in vitro* studies showed that hydroxycarbamide had activity against leukemia cell lines and some tumors[3] and this led to clinical studies showing particular activity in myeloproliferative disorders.[4]

Increased fetal hemoglobin (HbF) production has long been recognized as one of the key factors which can ameliorate SCD[5] and in the 1970s 5-azacytidine was investigated as an HbF promoting agent because of its potential ability to reactivate silenced γ-globin genes by inhibiting the methylation of deoxycytidine. Although 5-azacytidine successfully increased HbF levels in baboons, and subsequently in patients with SCD and thalassemia, it was relatively toxic.[6] Hydroxycarbamide was also used in the early baboon experiments, partly as a cytotoxic control because it was known to have no effect on methylation and, perhaps surprisingly, was also found to promote HbF synthesis. Because of concerns about the toxicity of 5-azacytidine, hydroxycarbamide was developed as a safer alternative and an initial study in 2 adults with sickle cell anemia (HbSS) (SCA) showed significant increases in both HbF and total hemoglobin.[7] Further observational studies followed, before the Multicenter Study of Hydroxyurea (MSH) study was published in 1995. In this double-blind randomized controlled study, 152 adult patients with SCA were assigned to hydroxycarbamide and 147 given placebo; the hydroxycarbamide group showed reductions in the rate of acute pain (median 2.5 *vs*. 4.5 episodes per year, $P<0.001$), acute chest syndrome (25 *vs.* 51, $P<0.001$) and blood transfusion (48 *vs.* 73, $P<0.001$).[8] The study was stopped early because of the reduction in acute pain in the hydroxycarbamide arm. The only other published randomized controlled study involved a single-blind crossover study of 25 children and young adults with SCA treated for six months with hydroxycarbamide and for six months with placebo; hydroxycarbamide showed a treatment effect on reducing the number of hospitalizations ($P=0.0016$) and total days in hospital ($P=0.0027$).[9] Since then a steady stream of registry, observational and follow-up studies have followed, all showing similar beneficial effects with increases in HbF levels and reductions in some acute complications. Most notably, two observational studies have suggested increased survival associated with long-term hydroxycarbamide use. A follow-up study of the patients in the original MSH study showed a 40% reduction in mortality in those who chose to continue hydroxycarbamide after nine years ($P=0.04$);[10] a non-randomized study of patients in Greece with SCA, HbS/β^0thalassemia and HbS/β^+thalassemia showed the probability of 10-year survival was 86% for those taking hydroxycarbamide and 68% for those not taking it ($P=0.001$).[11]

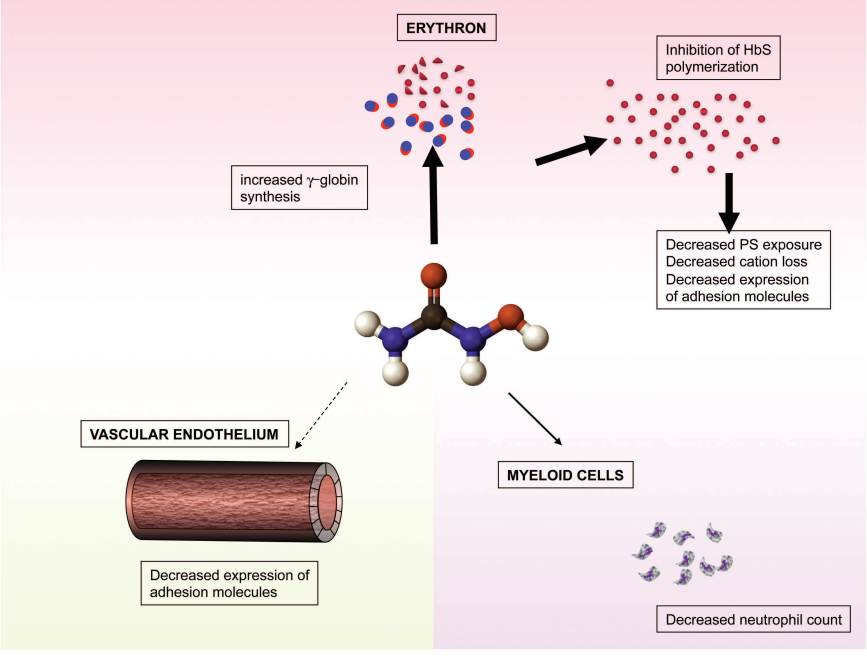

Figure 1. Diagram showing three independent actions of hydroxycarbamide which are potentially of therapeutic benefit in preventing vaso-occlusion and vasculopathy. Most evidence exists for hydroxycarbamide's action on erythroid cells (thick arrow), while observational studies have suggested that the reduced neutrophil count is also of therapeutic benefit (thin arrow). Laurance *et al.* in this issue showed *in vitro* evidence of hydroxycarbamide altering adhesion molecule expression in the vascular endothelium[17] (dotted arrow) although the clinical significance of this is not yet known.

In parallel with clinical trials, laboratory studies have tried to identify how hydroxycarbamide works, although precise mechanisms of HbF promotion have not been defined. Hydroxycarbamide is an S-phase cytotoxic agent which does not demethylate DNA; it is thought to directly reduce DNA synthesis by inhibiting ribonucleotide reductase activity. This non-specific interruption of the cell cycle probably accounts for most of the HbF promoting activity. In cell culture systems hydroxycarbamide acts on both early and late erythroid progenitors to increase the total intracellular hemoglobin, γ-globin mRNA and HbF levels.[12] There is also evidence that hydroxycarbamide can act as a nitric oxide donor and increase cGMP levels which accelerates translation of the γ globin gene.[12] Despite uncertainty about its molecular mechanism of action, the *in vivo* effects of hydroxycarbamide on the blood in SCA are fairly well defined. The changes are dose-dependent and in addition to higher HbF levels include increased erythrocyte volume and hemoglobin content, decreased reticulocyte count, decreased white cell count and increased segmentation of the neutrophil nucleus. Further potentially beneficial erythrocyte changes have been noted, including reduced dehydration,[13] reduced phosphatidylserine exposure, and reduced expression of adhesion molecules.[14] These secondary erythrocyte changes, and most of the clinical benefit, seem likely to be directly related to the increased HbF levels and reduced hemoglobin polymerization within the red cell, resulting in reduced red cell damage (Figure 1); this interpretation is supported by studies in a mouse model of sickle cell disease in which HbF induction did not occur in response to hydroxycarbamide, and no hematologic or clinical benefit was seen.[15] However, some therapeutic effects seem to occur independently of increases in HbF. The inevitable fall in white cell count which accompanies hydroxycarbamide use may be of rheological benefit, being significantly linked to clinical improvement in the MSH study, whereas HbF increase was not.[16] Similarly, in this issue Laurance, *et al.* show that hydroxycarbamide acts directly on vascular endothelium to decrease the expression of some adhesion molecules, which is clearly independent of any effects on the β-globin gene family.[17] Interestingly, Laurance *et al.* also show that the effects of hydroxycarbamide on adhesion molecules differed depending on

Table 1. Definite, probable and possible indications for hydroxycarbamide in sickle cell disease.

Definite
More than three episodes of severe acute pain per year
Two or more episodes of acute chest syndrome
Probable
Frequent episodes of acute pain requiring analgesia
Persistent albuminuria or other evidence of renal disease
Hypoxemia or other evidence of lung disease
Sickle hepatopathy
Tricuspid jet velocity >2.5m/s on echocardiography or pulmonary hypertension
Primary or secondary stroke prevention when blood transfusions are unacceptable
Hemoglobin <7g/dL
Possible
Poor growth
Conditional transcranial Doppler velocities
Patient or family request
Elevated steady state white cell count
Pre-operative management
Significant coexistent disease likely to exacerbate SCD, such as asthma, SLE
Strong wish to avoid blood transfusions, including Jehovah's Witnesses
Living in low-income country with greatly increased early mortality

whether the endothelial cells were from large or small blood vessels. This may be an important observation in explaining why hydroxycarbamide is more beneficial for some complications of SCD than others; for example, hydroxycarbamide may be more effective in preventing acute pain caused by microvascular complications than cerebrovascular disease in large blood vessels. Although the results of this study are potentially important, it is not clear how these findings will translate into clinical effects, and it seems likely that the effect of hydroxycarbamide on vascular endothelium is much less important than its promotion of γ-globin gene expression.

New clinical applications are beginning to emerge for hydroxycarbamide in SCD beyond its established use in reducing the frequency of acute pain and acute chest syndrome. There is observational evidence that hydroxycarbamide may improve hypoxemia, prevent cerebrovascular disease and reduce proteinuria.[18] The evidence for these applications is provisional, although encouraging in some cases. However, a randomized controlled trial assessing hydroxycarbamide and venesection as an alternative to blood transfusion for secondary stroke prevention was stopped early last year with an excess of strokes in the hydroxycarbamide arm.[5,18] A trial of hydroxycarbamide as primary stroke prevention in children with abnormal transcranial Doppler blood velocities is currently on-going.[18] The suggested indications for hydroxycarbamide continue to expand, possibly ahead of the available supporting evidence (Table 1).

Approximately 13% patients with SCA on the UK National Haemoglobinopathy Registry take hydroxycarbamide, with estimates in other countries varying from 10% to 30%,[5] although precise figures are not available. In the USA, there is an emerging consensus that hydroxycarbamide is underused, with some suggesting that everyone with SCA should take hydroxycarbamide unless there is a definite contraindication. In Europe, there has generally been more caution. Short-term side-effects are few, but the theoretical possibilities of an increased risk of malignancy and reduced fertility continue to cause concern, particularly when prescribing the drug for young children who may take it for many decades. Anecdotal evidence suggests no increased risk of malignancy, and there is no convincing evidence of subfertility, although a small retrospective study found reduced sperm counts in men who had previously taken hydroxycarbamide.[19]

There is strong and increasing evidence that hydroxycarbamide is beneficial for many people with SCA, and probably also other types of sickle cell disease. It is certainly a much better drug than might have been expected when it was initially used to promote HbF in early studies. It has the great assets of being cheap, orally active and relatively free of side-effects, and also probably has some incidental therapeutic actions beyond its effects on HbF, as shown by Laurance *et al.*[17] However, whilst it has been shown to alter the natural history of SCD, it is not in any sense curative and it is to be hoped that better drugs with more HbF promoting ability and other specific benefits are developed soon. For example, in this issue of Haematologica, Canalli *et al.* show interesting evidence that simvastatin may reduce the adhesiveness of leukocytes in SCD,[20] and could potentially be useful as part of a multi-drug approach. Just as imatinib is now established as the treatment of choice in chronic myeloid leukemia, hopefully a new drug will be designed which specifically inhibits HbS polymerization and relegates hydroxycarbamide to the footnotes.

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References

1. Dresler WFC, Stein R. Ueber den Hydroxylharnstoff. Justus Liebigs Ann Chemie. 1869;150:1317-22.
2. Rosenthal F, Wislicki L, Kollek L. Uber die Bezeihungen von schwersten Blutgiften zu Abbauprodukten des Eiweisses. Ein Beitrag zum Entstehungsmecanismus der perniziosen Anamie. Klin Wochenschr. 1928;7:972-7.
3. Stearns B, Losee KA, Bernstein J. Hydroxyurea. A new type of potential antitumor agent. J Med Chem. 1963;6:201.
4. Kennedy BJ, Yarbro JW. Metabolic and therapeutic effects of hydroxyurea in chronic myelogenous leukemia. Trans Assoc Am Physicians. 1965;78:391-9.
5. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. Lancet. 2010;376(9757):2018-31.
6. Charache S, Dover G, Smith K, Talbot Jr CC, Moyer M, Boyer S. Treatment of sickle cell anaemia with 5-azacytidine results in increased fetal hemoglobin production and is associated with nonrandom hypomethylation of DNA around the γ–δ–β globin gene complex. Proc Natl Acad Sci USA. 1983;80:4842-6.
7. Platt OS, Orkin SH, Dover G, Beardsley GP, Miller B, Nathan DG. Hydroxyurea enhances fetal haemoglobin in sickle cell anaemia. J Clin Invest. 1984;74(2):652-6.
8. Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, et al. Effect of hydroxyurea on frequency of painful crises in sickle cell anemia. N Engl J Med.1995;332(20):1317-22.
9. Ferster C, Vermylen G, Cornu M, Buyse F, Corazza C, Devalck P, et al. Hydroxyurea for the treatment of severe sickle cell anaemia: a pediatric clinical trial. Blood. 1996;88:1960-4.
10. Steinberg MH, Barton F, Castro O, Pegelow CH, Ballas SK, Kutlar A, et al. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. JAMA. 2003;289(13):1645-51.
11. Voskaridou E, Christoulas D, Bilalis A, Plata E, Varvagiannis K, Stamatopoulos G, et al. The effect of prolonged administration of hydroxyurea on morbidity and mortality in adult patients with sickle cell syndromes: results of a 17-year, single-center trial (LaSHS). Blood 2010;115(12):2354-63.
12. Cokic VP, Smith RD, Beleslin-Cokic BB, Njoroge JM, Miller JL, Gladwin MT, Schechter AN. Hydroxyurea induces fetal hemoglobin by the nitric oxide-dependent activation of soluble guanylyl cyclase. J Clin Invest. 2003;111(2):231-9.
13. Bridges KR, Barabino GD, Brugnara C, Cho MR, Christoph GW, Dover G, et al. A multiparameter analysis of sickle erythrocytes in patients undergoing hydroxyurea therapy. Blood. 1996;88(12):4701-10.
14. Styles LA, Lubin B, Vichinsky E, Lawrence S, Hua M, Test S, et al. Decrease of very late activation antigen-4 and CD36 on reticulocytes in sickle cell patients treated with hydroxyurea. Blood. 1997;89(7): 2554-9.
15. Lebensburger JD, Pestina TI, Ware RE, Boyd KL, Persons DA. Hydroxyurea therapy requires HbF induction for clinical benefit in a sickle cell mouse model. Haematologica. 2010;95(9):1599-603.
16. Charache S, Barton FB, Moore RD, Terrin ML, Steinberg MH, Dover GJ, et al. Hydroxyurea and sickle cell anemia. Clinical utility of a myelosuppressive "switching" agent. The Multicenter Study of Hydroxyurea in Sickle Cell Anemia. Medicine (Baltimore). 1996;75(6):300-26.
17. Laurance S, Lansiaux P, Pellay F-X, Hauchecorne M, Benecke A, Elion J, and Lapoumeroulie C. Differential modulation of adhesion molecule expression by hydroxycarbamide in human endothelial cells from the micro- and macrocirculation: potential implications in sickle

cell disease vasoocclusive events. Haematologica 2011;96(4):534-42.
18. Ware RE. How I use hydroxyurea to treat young patients with sickle cell anaemia. Blood. 2010;115(26):5300-11.
19. Berthaut I, Guignedoux G, Kirsch-Noir F, de Larouziere V, Ravel C, Bachir D, et al. Influence of sickle cell disease and treatment with hydroxyurea on sperm parameters and fertility of human males. Haematologica. 2008;93(7):988-93.
20. Canalli AA, Proença RF, Franco-Penteado CF, Traina F, Sakamoto TM, Saad STO, et al. Participation of the Mac-1, LFA-1 and VLA-4 integrins in the in vitro adhesion of sickle cell disease neutrophils to endothelial layers, and reversal of adhesion by simvastatin. Haematologica 2011;96(4):526-33.

Acute myeloid leukemia with monosomal karyotype at the far end of the unfavorable prognostic spectrum

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(Related Original Article on page 631)

The treatment of acute myeloid leukemia (AML) is among the most dose-intensive approaches in clinical oncology and involves variable therapeutic options with highly diverse consequences in terms of toxicities and anti-leukemic effects. One illustrative example is the choice between consolidation chemotherapy and stem cell transplantation in first remission and also the choice among highly diverse types of stem cell transplantation such as autologous, allogeneic-sibling, haplo-identical, unrelated donor or umbilical cord blood grafting. Prognostic factors provide guidance in clinical practice in these complex treatment management dilemmas. An average 40% of adult patients up to the age of 60 will have long-term survival prospects; for older patients this is only 10-15%. Among these estimates there is considerable variation in outcome between individual patients. Patient related factors (e.g. age, comorbidity conditions) and hematologic factors (e.g. '*de novo*' *vs.* secondary AML) impact on individual treatment outcome. Most prominently, particular leukemia-specific somatic genetic alterations furnish essential prognostic determinants. These genomic abnormalities in the leukemic blasts are assessed with classical cytogenetic techniques (banding, fluorescence *in situ* hybridization) or a range of molecular methods. There is no question that cytogenetics, more than any other genetic source of information, has become solidly established in the diagnostic work up of patients with AML.[1-3] Cytogenetics unravels the highly variable clinical biology of AML and thus allows for sharp clinically useful diagnostic and prognostic distinctions. Recent studies have revealed that AML with so called monosomal karyotypes are at the extreme unfavorable end of the prognostic spectrum and predict one of the worst possible outcomes. This issue of the journal contains a report by Xie *et al.* that examined the significance of residual karyotypically normal cells in monosomal karyotype AML (MK-AML).[4]

Monosomal karyotype AML: what is it about?

During the past 25 years several large clinical trial groups, such as the Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON) and the Swiss Group for Clinical Cancer Research (SAKK), have collected cytogenetic diagnostics at baseline in patients with AML enrolled in their treatment protocols. This has generated data sets in large series of comparatively homogeneously treated patients in whom the prognostic contribution of various cytogenetic abnormalities such as complex karyotypes (i.e. multiple chromosomal aberrations) could be evaluated. Statistical analysis revealed that loss of a complete autosomal chromosome conferred profound negative prognostic impact (Figure 1A), whereas structural abnormalities negatively influenced prognosis in association with an autosomal monosomy.[5] Extra chromosomes

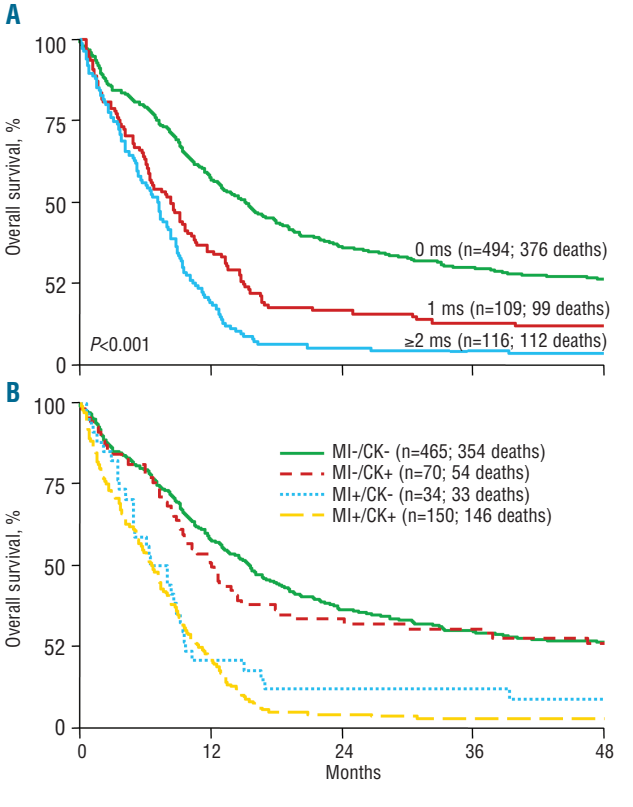

Figure 1. Overall survival of patients with acute myeloid leukemia (AML) and non-core-binding-factor chromosomal abnormalities. (A) Survival in relation to numbers of autosomal chromosomal monosomies (none, 1, and ≥2 ms). (B) Survival in relation to 'monosomal karyotype' (in figure designated as MI) as defined by Breems *et al.*[5] and/or 'complex karyotype with ≥3 cytogenetic clonal abnormalities' (CK). *Reprinted with permission. ©2008 American Society of Clinical Oncology. All rights reserved."* Breems D et al. J Clin Oncol 2008;26(29):4791-7.