

- common mutation in hemochromatosis type 4. *Blood* 2002;100:733-4.
- Camaschella C, Roetto A, De Gobbi M. Genetic haemochromatosis: genes and mutations associated with iron loading. *Best Pract Res Clin Haematol* 2002;15:261-76.
 - Cazzola M, Cremonesi L, Papaioannou M, Soriani N, Kioumi A, Charalambidou A, et al. Genetic hyperferritinaemia and reticuloendothelial iron overload associated with a three base pair deletion in the coding region of the ferroportin gene (SLC11A3). *Br J Haematol* 2002;119:539-46.
 - Wallace DF, Pedersen P, Dixon JL, Stephenson P, Searle JW, Powell LW, et al. Novel mutation in ferroportin 1 gene is associated with autosomal dominant hemochromatosis. *Blood* 2002;100:692-4.
 - Vulpe CD, Kuo YM, Murphy TL, Cowley L, Askwith C, Libina N, et al. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. *Nat Genet* 1999;21:195-9.

Induction of γ -globin gene expression by tallimustine analogs in human erythroid cells

Modulation of γ -globin gene expression is of relevance for the treatment of β -thalassemia and sickle cell anemia. We studied the relationship between the structure of DNA-binding tallimustine analogs and their ability to induce erythroid differentiation of K562 cells. One of the most active analogs (compound 10710) was found to efficiently induce fetal hemoglobin (HbF) and γ -globin mRNA production also in normal human erythroid progenitors.

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Drug-mediated modulation of interactions between DNA and nuclear proteins could represent a promising approach to control γ -globin gene expression and fetal hemoglobin (HbF) production.¹⁻³ This is of relevance for the treatment of β -thalassemia and sickle cell anemia,³ since increasing HbF to 30% of the total hemoglobin production leads to an improvement of the clinical status.³

In the present study we first determined whether DNA-binding tallimustine analogs (Figure 1) induce erythroid differentiation of human erythroleukemia K562 cells. K562(S) cells were cultured, treated and analyzed as elsewhere reported.^{1,2} The results (Figure 2A), obtained using compounds at concentrations causing 50% inhibition of cell growth, indicated that among the analogs carrying three pyrrole rings, tallimustine (10655) and 10569 exhibited high induction capacity. Addition of pyrrole rings significantly improved the inducing ability (comparing 10710 to 10569, 10569 to 10709 and 10705 to 10558 yielded p values < 0.03 by the one-way ANOVA test). The effect of the drugs on these cells was associated with production of Hb Portland and an increase in γ -globin mRNA content (*data not shown*).

The most active compound, 10710, was further studied in normal human erythroid precursors cultured in a two-phase liquid culture system.^{2,4,5} Compounds were added on day 4-5 of phase II (when cells started to synthesize Hb). Hb was analyzed by high performance liquid chromatography (HPLC) on day 12 as elsewhere reported.^{4,5} The representative example shown in Figure 2B demonstrates that the percentage of HbF with respect to total Hb (% HbF) increased from 1.8% (untreated cells) to 7.5% and 14.5% in cells treated with 0.5 and 0.75 μ M 10710, respectively. Lower levels of HbF were found with hydroxyurea, tallimustine, and 10569.²

Real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) analysis of the globin mRNA content (Figure 2C)⁶ indicated that 10710 increased both γ -globin and β -globin mRNA using GAPDH mRNA as a reference. However, the fold increase of γ -globin mRNA was significantly higher than that of β -globin mRNA ($p < 0.07$ by the one-way ANOVA test), suggesting a preferential induction of the expression of the γ -globin genes after treatment of human erythroid precursors with 10710. In contrast, the increase in α -globin mRNA was only barely detectable (Figure 2C).

In conclusion, in this study we demonstrated that the number of pyrrole rings and the type of molecular bridge linking the alkylating moiety and the oligopyrrole carboxy-amido backbone affect the differentiation inducing ability of tallimustine analogs. The compound 10710 was the most efficient inducer of γ -globin mRNA synthesis and HbF production

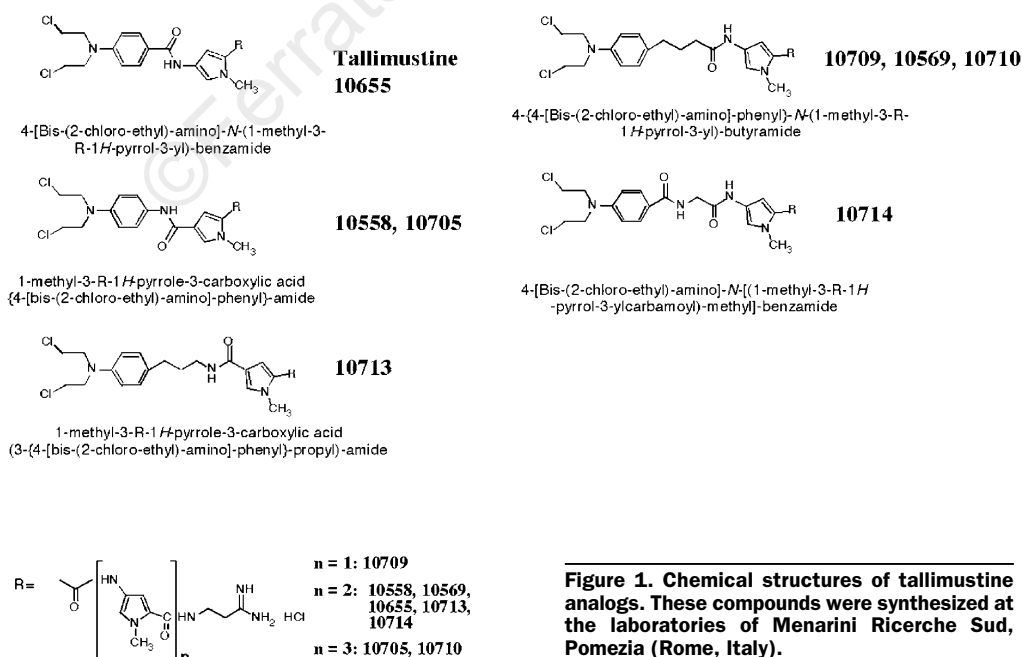


Figure 1. Chemical structures of tallimustine analogs. These compounds were synthesized at the laboratories of Menarini Ricerche Sud, Pomezia (Rome, Italy).

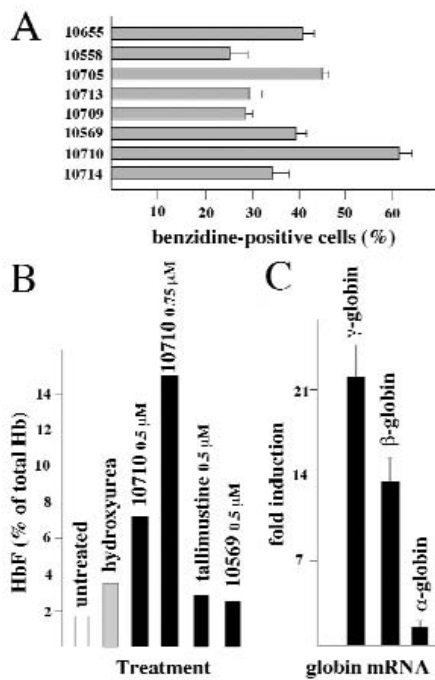


Figure 2. A. Effects of tallimustine analogs on differentiation of K562 cells. K562 cells were cultured in the presence of concentrations of tallimustine analogs that caused 50% inhibition of cell growth; after 6 days the proportion of benzidine-positive (Hb-containing) cells was determined as previously described (results are the means \pm SD of three independent experiments).² **B, C.** Production of HbF (B) and globin mRNA (C) by normal human erythroid cultures. Cells were grown according to the two-phase liquid culture protocol^{4,5} with no drug (untreated), with HU (150 μ M) or with the indicated concentrations of 10710, 10655 and 10569. Hemoglobins were analyzed by HPLC and the % HbF determined (B). Accumulation of mRNA was measured by quantitative real-time RT-PCR assay (C).⁶ The results (mean \pm SD) of the fold increase of globin mRNAs induced by 0.75, μ M 10710 (compared to untreated cells) in three experiments are summarized.

in normal erythroid progenitors. This agent, with its four pyrrole rings, was more active than tallimustine² or hydroxyurea, the drug currently used for HbF enhancement in β -thalassemia and sickle-cell disease.³ In addition to increasing γ -globin mRNA synthesis, this agent also increased β -globin mRNA, but only slightly increased α -globin mRNA.

Since these DNA-binding molecules are cytotoxic,² chemical modifications will be required to reduce their toxicity. In this respect, our study should be considered as a basis for further efforts to develop agents for hematologic diseases in which the induction of both γ -globin and β -globin genes could be clinically relevant.

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References

- Bianchi N, Ongaro F, Chiarabelli C, Gualandi L, Mischiati C, Bergamini P, et al. Induction of erythroid differentiation of human K562 cells by cisplatin analogs. *Biochem Pharmacol* 2000;60:31-40.
- Bianchi N, Chiarabelli C, Borgatti M, Mischiati C, Fibach E, Gambari R. Accumulation of γ -globin mRNA and induction of erythroid differentiation after treatment of human leukaemic K562 cells with tallimustine. *Br J Haematol* 2001;113:951-61.
- Rodgers GP, Rachmilewitz EA. Novel treatment options in the severe β -globin disorders. *Br J Haematol* 1995;91:263-8.
- Fibach E, Manor D, Oppenheim, A, Rachmilewitz EA. Proliferation and maturation of human erythroid progenitors in liquid medium. *Blood* 1989;73:100-3.
- Fibach E, Manor D, Treves A, Rachmilewitz EA. Growth of human normal erythroid progenitors in liquid culture: a comparison with colony growth in semisolid culture. *Intern J Cell Cloning* 1991;9:57-64.
- Gibson UE, Heid CA, Williams PM. A novel method for real time quantitative RT-PCR. *Genome Res* 1996;6:995-1001.

In vitro study of stromal cell defects in myelodysplastic syndromes

The long term bone marrow culture (LTBMC) system was used to examine *in vitro* characteristics of stromal cells in myelodysplastic syndromes (MDS): confluence after four-week culture, activation of caspase-3, production of tumor necrosis- α , interleukin (IL)-1 β and vascular endothelial growth factor (VEGF), and density of endothelial (CD31) cells. Low confluence, associated in some cases with caspase-3 activation and increased angiogenesis, was the most frequent abnormality.

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The ineffective hematopoiesis that characterizes MDS has been attributed to an accelerated apoptosis in hematopoietic progenitors.¹ Altered interactions between stromal and hematopoietic cells have also been reported, but discrepancies exist between studies regarding the implication and functional