# CORRESPONDENCE

## A Combination of Retinoic Acid and Proteasome Inhibitors for the Treatment of Leukemias Is Potentially Dangerous

*To the Editor:*

Retinoic acid (RA) is used in the treatment of leukemias; however, the phenomenon of RA resistance is often the cause of relapse in some patients. In a recent report, Fanelli et al[1] showed, using an acute promyelocytic leukemia–derived cell line NB4, that the treatment of RA-resistant cells with proteasome inhibitors restores RA sensitivity.

Proteasome inhibitors are already being tested in phase I of clinical trials as anticancer agents[2] and are proposed to be useful in the treatment of chemoresistant and radioresistant leukemias.[3] One might speculate that combination therapy of RA and proteasome inhibitors can be advantageous and may potentially overcome RA-resistant cases. A selective inhibitor of the proteasome [PSI; *N*-benzyloxycarbonyl-Ile-Glu-(*O*-*t*-butyl)-Ala-leucinal] induces apoptosis of murine lymphocytic leukemia L1210 cells,[4] while RA treatment inhibits their proliferation,[5] abolishes clonogenicity, and causes G1 arrest.[6] L1210 is sensitive to RA, regardless of the fact that it does not express PML/RARα or RA binding proteins.[5,7]

We pretreated L1210 cells for 72 hours with either a control solvent (dimethyl sulfoxide [DMSO]) or 5 μmol/L and 50 μmol/L RA. Cells were then further treated for 24 hours with a combination of those agents and 500 nmol/L PSI or DMSO alone as control. Treated cells were fixed in 70% ethanol, stained with acridine orange, and analyzed using a FACSCalibur flow cytometer (Becton Dickinson, San Diego, CA).[8]

We have found that treatment with 5 μmol/L RA, which by itself is not cytotoxic, has a cytotoxic effect when combined with 500 nmol/L PSI (Table 1). However, a combination of 50 μmol/L RA, which is by itself cytotoxic, with 500 nmol/L PSI surprisingly diminishes the cytotoxic effects on L1210 cells, allowing a survival of 21.4% of the cells compared with 12.2% for PSI alone ($\chi^2$ $P < .0001$). Moreover, while RA or PSI alone caused a G0/G1 block, this effect was partially abolished by a combination of both drugs (Table 2). PSI or 5 μmol/L RA treatment did not increase granulosity of the cells, as judged by the flow cytometric SSC-H parameter, while 50 μmol/L RA increased mean granulosity by 62%. The combination of 50 μmol/L RA and PSI increased mean granulosity by 50%.

Although 5 μmol/L RA does not induce differentiation—it causes only a moderate G1 phase block or retardation—50 μmol/L RA treatment most probably induces differentiation of a fraction of L1210 cells, as judged by increased granulosity, and these cells became resistant to proteasome inhibition. Although proteasome inhibitors cause apoptosis of proliferating cells,[4] they prevent apoptosis of some differentiated cells, such as neurons[9] or thymocytes.[10] Our results suggest that any attempt to treat leukemia with a combination of RA and proteasome inhibitors should be made with great caution. It potentially allows the survival of cells, which after the withdrawal of the drugs can cause a fast relapse of the leukemic disease, regardless of the presence of the PML/RMRα fusion protein.

**Table 1. Percentages of Apoptotic, Necrotic, and Living L1210 Cells, After 72 Hours' Pretreatment With Either a Control Solvent or RA at the Indicated Concentrations, Followed by a 24-Hour PSI or Control Treatment**

| Cells | Apoptotic | Necrotic | Living |
|---|---|---|---|
| Control | 12.0 | 4.5 | 83.5 |
| RA 5 μmol/L | 4.2 | 9.0 | 86.8 |
| RA 50 μmol/L | 23.6 | 11.9 | 64.5 |
| PSI 500 nmol/L | 27.2 | 60.6 | 12.2 |
| PSI 500 nmol/L RA 5 μmol/L | 22.0 | 75.2 | 2.8 |
| PSI 500 nmol/L RA 50 μmol/L | 20.6 | 58.0 | 21.4 |

Data were obtained in each group for 10,000 cells separated from other events based on light scatter characteristics. Apoptotic, necrotic, and living cells were discriminated with the use of CELLQuest v.1.2 software system.

**Table 2. Percentages of L1210 Cells in Different Phases of the Cell Cycle, After 72 Hours' Pretreatment With Either a Control Solvent or RA at the Indicated Concentrations, Followed by a 24-Hour PSI or Control Treatment**

| Cells | G0/G1 | S | G2/M |
|---|---|---|---|
| Control | 33.8 | 11.1 | 55.1 |
| RA 5 μmol/L | 43.9 | 11.0 | 45.1 |
| RA 50 μmol/L | 51.5 | 7.2 | 48.3 |
| PSI 500 nmol/L | 49.8 | 4.5 | 45.7 |
| PSI 500 nmol/L RA 5 μmol/L | 55.7 | 2.9 | 41.4 |
| PSI 500 nmol/L RA 50 μmol/L | 44.9 | 7.0 | 48.1 |

Data were obtained for the living cells fraction of the same groups as in Table 1. Cell-cycle percentages were calculated depending on the DNA content with the use of CELLQuest v.1.2 software system.


### ACKNOWLEDGMENT

Supported by KBN Grant No. 4 P05A 084 16.



Cezary Wójcik
*Department of Histology and Embryology*
*Institute of Biostructure*
*Medical University of Warsaw*
*Warsaw, Poland*
Izabella Młynarczuk
*Department of Histology and Embryology*
*Department of Immunology*
*Institute of Biostructure*
*Medical University of Warsaw*
*Warsaw, Poland*
Grażyna Hoser
Jerzy Kawiak
*Department of Clinical Cytology*
*Postgraduate Center of Medical Instruction*
*Warsaw, Poland*
Tomasz Stokłosa
Jakub Gołab
*Department of Immunology*
*Institute of Biostructure*
*Medical University of Warsaw*
*Warsaw, Poland*
Sherwin Wilk
*Department of Pharmacology*
*Mount Sinai Medical Center*
*New York, NY*

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## Double-Homozygosity for Factor V Leiden and the Prothrombin Gene G20210A Variant in a Young Patient With Idiopathic Venous Thrombosis

*To the Editor:*

There is growing evidence that environmental and genetic risk factors often interact to induce clinically manifest venous thromboembolism (VTE). The role of gene-gene interactions, although much rarer, is supported by cosegregation of genetic defects observed in patients with familial thrombophilia. In this context, factor V (FV) Leiden and the prothrombin G20210A gene (FII) mutation are of particular interest because of their high prevalence in the normal population; about 5% and 2%, respectively, in whites.[1,2] The FII mutation has been reported in 10% of FV Leiden carriers with VTE,[3] while FV Leiden is present in 30% to 40% of symptomatic carriers of the FII mutation.[2,4] Homozygosity for these mutations is less common, with a prevalence of 0.02% for FV Leiden and 0.014% for the FII mutation.[1,2] We present here a case of double-homozygosity for these defects.

A 34-year-old man presented with a first episode of idiopathic deep-vein thrombosis. He had never been exposed to environmental risk factors for thrombosis. Treatment consisted of subcutaneous low-molecular-weight heparin for 1 week followed by acenocoumarol for 3 months. At 18 months follow-up, he had experienced no recurrence. Laboratory studies for thrombophilic disorders showed double-homozygosity for FV Leiden and the FII mutation, detected by polymerase chain reaction. None of his 15 relatives had a history of VTE. Four were double-heterozygous carriers, 4 were single carriers of the FII mutation, and 3 were noncarriers (Fig 1). Four relatives, 2 of whom had died, were not tested but they were obligate carriers of at least 1 (N = 1) or both (N = 3) mutations. The patient's father, who had a history of smoking and hypercholesterolemia, died of myocardial infarction at 57 years of age and a son died from cribdeath. His mother experienced a myocardial infarction when she was 61 years old; she had a history of hypertension and diabetes mellitus. Fetal loss had occurred only in 1 noncarrier. Table 1 summarizes characteristics of this family.

Double-homozygosity for FV Leiden and the FII mutation is extremely rare. Theoretically, it is expected in 3 per 100 million whites. The thrombotic risk of this combined abnormality is unknown. Homozygosity for FV Leiden increases the risk of VTE approximately 80-fold compared with noncarriers.[1] The risk in homozygous carriers of the FII mutation has not yet been estimated; only case reports have been published.

Remarkably, only our double-homozygous patient has developed

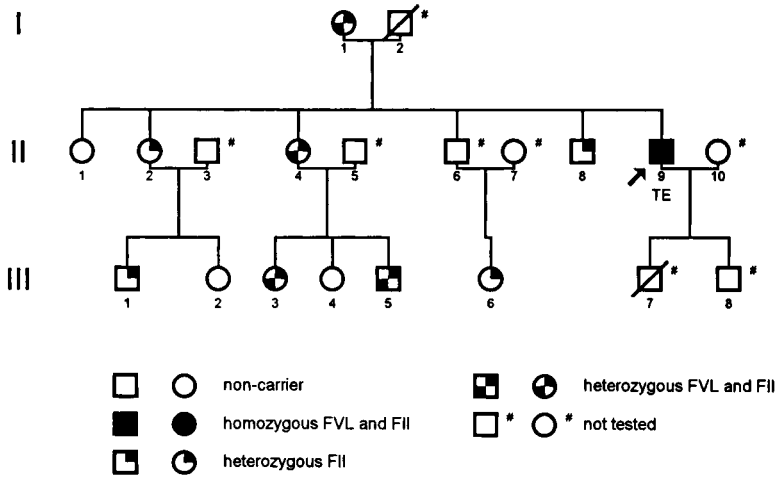


**Fig 1. Pedigree of the reported family. The arrow indicates the propositus, who is a double-homozygous carrier of factor V Leiden (FVL) and the prothrombin G20210A gene mutation (FII). Individuals with a slash through the symbol are deceased. TE, venous thromboembolism.**