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Arthur J. Sytkowski, M.D.

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November 9, 1990

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A.J. Melaragno  
Captain, Medical Corps  
United States Navy  
Director of Research and Development  
National Naval Medical Center  
Bethesda, Maryland 20814-5044

RE: Status Report for Grant # N00014-90-J-1847  
Entitled "Development of Hematopoietic Growth Factors  
for Use in Military Personnel"

Dear Dr. Melaragno:

This report delineates our accomplishments from June 1, 1990  
through September 30, 1990.

Project I: Human Erythropoietin

This project is designed to develop second generation erythropoietin molecules with more favorable pharmacologic properties that can be applied to military personnel. Part of our strategy has been to study the structure of the molecule using a combination of chemical and molecular biologic techniques in order to rationally engineer new bio-molecules. When this grant began, we had evidence that amino acids 99-109 of the hormone were important for its biological activity. In the last four months, we have completed our initial series of experiments and have found that the amino acids in this domain are important both for structural stability and receptor recognition. Mutation of these amino acids to others which significantly alter the structure of this area of the molecule result in recombinant erythropoietins that are more susceptible to proteolytic cleavage inside the secreting cell. This information is extremely important, since it has identified a critical portion of the molecule that we can now begin to engineer rationally in order to increase stability beyond that expressed by the native hormone.

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Preliminary results from this study will be presented at the American Society of Hematology meeting next month. The abstract that was submitted is enclosed.

In order to focus on each amino acid specifically, we have begun to use site-directed mutagenesis and convert each amino acid in this domain to alanine, thereby permitting us to study the role of each amino acid side chain individually. We anticipate that in the next four to six months, we will have these mutants sequenced and expressed in mammalian cells and will have begun to study their biological activity and compare it to that of the native molecule. Additionally, in the next four months, we will initiate our proposed studies of erythropoietin chemical modification and cross-linking, which will allow us to begin in vivo animal studies of molecules with potentially increased serum half-lives.

#### Project II: Erythroid Burst Promoting Activity

We have established an in vitro bioassay using murine erythroid cells. Using this assay we have demonstrated that erythroid burst promoting activity is produced by murine lymphocytes and rabbit lymphocytes. This important discovery confirms the feasibility of using animal models for the purification and sequencing of the BPA protein. In this regard, we have established a collaboration with Dr. Claude Veillon, Vitamins and Minerals Branch, United States Department of Agriculture, Beltsville, Maryland. Dr. Veillon is a highly skilled, analytical chemist and has access to animals at the United States Department of Agriculture campus in Beltsville as large as cattle. We expect that in the next four months, we will be able to harvest a bovine spleen and determine whether we are able to prepare erythroid burst promoting activity from this highly abundant lymphocyte source.

We have initiated our studies of the synergistic activity of erythropoietin and burst promoting activity as outlined in our original proposal. Preliminary results of these experiments using murine erythroleukemia tissue culture cells as targets have demonstrated that the combination of erythropoietin and erythroid burst promoting activity result in massive proliferation of erythroid target cells. Moreover, differentiation (hemoglobin synthesis) appears to be correspondingly increased when these two agents are used simultaneously. We have also found, using normal human erythroid cells in culture, that BPA markedly increases the erythropoietin sensitivity of these cells. Thus, it is clear that erythropoietin and BPA have a pronounced synergistic activity, a finding that should be of great value in our proposed

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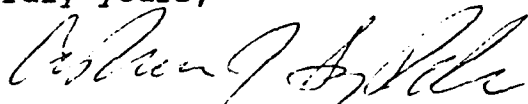
in vivo studies later in this grant. A preliminary report of some of these findings will be presented in the American Society for Cell Biology meetings and the American Society of Hematology meetings later this year. The relevant abstracts that have been submitted are enclosed.

In the next four months, in addition to studies of bovine spleen lymphocytes, we plan to begin screening lymphocyte cell lines for the production of BPA. Identification of such lines would make it possible to generate large amounts of the molecule in the laboratory, thereby averting the need for animal sources.

### Summary

Our progress on both Project I and Project II has been significant. We anticipate that peer reviewed publications supported by this grant will appear and be published by our laboratory within the next six to nine months. Our stated goals of using these hematopoietic growth factors in vivo to amplify red cell production seems achievable.

Truly yours,



Arthur J. Sytkowski, M.D.  
Director, Laboratory for Cell  
and Molecular Biology

AJS:rck

enclosures

cc: Dr. Laurie Feldman  
Research Administration

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