

6/5/08

Date Prepared: \_\_\_\_\_

**REQUEST FOR EC/BSA CONCEPT APPROVAL  
REQUESTS FOR APPLICATIONS (RFAs)/CONTRACTS (RFPs)**

**Title: ESAs and Tumor Growth**

PA \_\_\_ RFA x Coop. Ag. \_\_\_ Activity Code (e.g. R01) RFP \_\_\_

New \_\_\_ Reissue \_\_\_

Division: **Cancer Biology (DCB)**

Program Director: R. Allan Mufson, Ph.D.

Division Director: Dinah Singer, Ph.D.

Length of Award (Yrs.):  
5

Source of Funds: RPG \_\_\_ Control \_\_\_ Centers \_\_\_  
Other X

Anticipated Award Date: **TBD**

Other Res. X Construct. \_\_\_ NRSA

RFAs (Set Aside): \$5 M

Amount of Set Aside 01 Year: \$1M

Est. Number of Awards: \_\_\_ 2-3

Est. Cost for Project Period:

Justification for Use of RFA/RFP Mechanism:

Attached: \_\_\_ Very little funded research in area important to patient quality of life.

Congressional Mandate:

Other:

## Erythropoietic Stimulating Agents (ESAs) and Tumor Growth

### **Background:**

#### **1. Scientific Opportunity and Priority for the NCI.**

Erythropoietin (EPO) is a glycoprotein hematopoietic growth factor essential for red blood cell production. Its peptide backbone consists of a single chain of 165 amino acids with specific residues that recognize an EPO receptor protein on erythroid progenitor cells. Approximately 40% of the molecular weight of the molecule is carbohydrate. The protein was originally purified from urine by Miyake and Goldwasser in 1977. This was followed by two groups, one led by Jacobson and the other by Lin, cloning the gene coding for EPO almost simultaneously in the mid 1980s. The availability of the cDNA sequence allowed the expression and production of recombinant EPO by the biotechnology industry. Thus, erythropoietin became available for clinical use in medical practice.

EPO is synthesized physiologically by cells in the kidney cortex. Chronic kidney disease impairs EPO synthesis and leads to severe anemia. Thus, patients with renal disease were the initial population to benefit from the availability of recombinant EPO to relieve them of the requirement for repeated transfusions. A mutated and hyperglycosylated form of EPO termed darbepoetin  $\alpha$  was also developed to treat these patients. Recombinant EPO or its derivatives, also termed ESAs, were also approved in 1993 and 2002 by the Food and Drug Administration (FDA) to increase hemoglobin concentrations and decrease the need for transfusions, in the anemia that occurs in patients with non myeloid malignancies undergoing chemotherapy. However concern over the use of ESAs in these patients developed in 2003, when it was observed in clinical trials that patients with mammary carcinoma treated with ESAs had poorer survival, as well as patients with head and neck cancer who received radiotherapy. This poorer survival was attributed to enhanced local disease progression. *These and subsequent clinical trials involving the administration of ESAs to cancer patients have raised the questions about how ESAs might affect tumor progression. Fundamental questions about the effects of EPO on tumor cells themselves and on the tumor microenvironment must be addressed to answer this question.*

The NCI convened a workshop entitled Erythropoietin and Tumor Growth in December 2007. That workshop convened clinicians and basic scientists, who conducted clinical trials and basic research on ESAs, to review both the clinical trials basic research data on the effects of these agents on tumors in patients as well as the effects on *in vivo* and *in vitro* model systems. At the conclusion of the workshop, it was determined that there were serious gaps in our knowledge about the effects of ESAs on tumor cell biology relevant to its effects on tumors in patients. It is necessary to fill these gaps in order to better understand the effects of ESAs on tumor progression in patients. *As ESAs may contribute to the quality of life of cancer patients with anemia due to chemotherapy and are widely prescribed in this setting, a workshop consensus determined that a mechanism to stimulate research in this area should be of high priority to the NCI.*

### **2. Purpose of RFA:**

#### Context of Scientific Knowledge in this Area

Erythropoietin promotes proliferation, survival, and ultimately the differentiation of erythroid progenitors through the enhanced expression of anti apoptotic proteins. EPO binds to a dimeric receptor protein (EPO-R) on the surface of erythroid progenitors and activates a series of downstream signaling molecules including Janus kinase 2 (JAK2), and the signal transducer and activator of transcription (STAT5). In addition, EPO can activate the phosphoinositide 3-kinase/AKT pathway, protein kinase C

isoforms, and the Ras-Raf-Mek pathway. It was initially believed that only hematopoietic progenitor cells would express EPO receptors; however, beginning in the mid 1990s evidence began accumulating for EPO receptor expression outside of erythroid lineage cells. The EPO receptor has now been putatively detected on neurons, endothelial cells, cardiac myocytes, mammary and gastric epithelial cells. Engagement of the EPO-R on these cells has been shown to induce mitogenesis, angiogenesis, mobilization of intracellular calcium ions, and protection from apoptosis in some systems.

In addition to their well-established roles in erythropoiesis, EPO and EPO-R have been reported to be associated with a number of tumor cells including mammary carcinoma and melanoma. Some of the work identifying EPO-R on normal and tumor cells has depended on a commercially available polyclonal antibody (C20), but the data has been questioned due to the lack of specificity of the antibody. A small number of studies have actually measured EPO binding to identify EPO-R on tumor cells. These studies have reported extremely low receptor numbers (<100 per cell) on mammary carcinoma or pheochromocytoma cells. Despite the difficulties in identifying cell surface receptors on non-hematopoietic lineage cells, functional effects of EPO have been reported for non-erythroid tumor cells. The effect of EPO on tumor cell proliferation has been conflicting, although some positive effects on proliferation have been documented. For example, an ovarian tumor cell line (A2780) has been shown to express both EPO and EPO-R. Neutralizing anti-EPO antibody reduces cell growth and the reduction can be reversed with the addition of exogenous EPO. Other investigators have had problems replicating *in vitro* results with this cell line, but have observed, in nude mice, that inhibition of EPO-R expression resulted in reduced *in vivo* tumor growth and invasiveness. Knock down of EPO-R in ovarian carcinoma cells by shRNA resulted in decreased invasiveness and *in vitro* cell proliferation. EPO-R knock down in melanoma cells reduced invasiveness, and such cells did not develop visible tumors *in vivo* compared to control counterparts. These findings suggest that there may be EPO independent constitutive EPO-R signaling pathways operating in some tumor cells. Local administration of EPO has been reported to increase angiogenesis and tumor cell growth in tumor explant models *in vivo*, although systemic EPO administration did not have this effect. *In vitro* EPO has also been reported to stimulate proliferation of primary endothelial cells, suggesting that a role for EPO in tumor vascularization remains to be considered.

EPO has been reported to have anti-apoptotic and injury protective effects on normal neural tissue and myocardium and on some tumor cells. It has been suggested that these anti-apoptotic effects occur through a trimeric heterodimer consisting of the EPO-R dimer and the  $\beta_c$  cytokine R subunit. The downstream signaling mechanisms underlying these anti-apoptotic effects in non-erythroid cells are not known. In murine myeloid leukemia cell line 32D, 5U/ml of human EPO were reported to suppress cisplatin induced growth arrest and apoptosis. Systemic treatment of rats bearing mammary adenocarcinoma R3220 with EPO induced a decrease in the frequency of apoptotic cells, but did not significantly affect growth rate. EPO also induced an increase in AKT activation in these cells. AKT phosphorylation, which is closely associated with EPO's anti-apoptotic activity, has also been reported in melanoma cells treated with EPO. EPO has also been reported to inhibit staurosporine induced apoptosis in SH-SY5Y neuroblastoma cells. In these cells, EPO also induced expression of the anti-apoptotic Bcl-2 family member Bcl-xl at the mRNA and protein levels. In glioma cells in culture, EPO induced the expression of Bcl-2 and Bcl-xl, increased the phosphorylation of ERK and JNK and c-jun signaling. EPO induction of Jak-2 phosphorylation has been reported in head and neck tumor cell lines; STAT-5 phosphorylation in prostate tumor cells; and finally JAK-2, STAT-5, and PI3 kinase/Akt pathways in small cell lung carcinoma cell lines.

Despite numerous preliminary reports suggesting that ESAs affect tumor growth, there is relatively little mechanistic understanding of their effects on tumor cell biology. This initiative is intended to stimulate research in critical areas of ESA biology: Examples of the range of questions that remain to be addressed include, but are not limited to:

1. How is the erythropoietin receptor expression regulated in non-hematopoietic tissues both malignant and benign? Such investigations can and should involve improved methods for detecting EPO receptors on cells beyond the use of the currently available antibody.
2. Can the effects of ESAs on cell proliferation in appropriate cell culture models (e.g., mammary tumor and head and neck tumor cells) be determined and related to our understanding of the effects of EPO on normal erythroid progenitor cells?
3. Is ESA induction of potentially anti-apoptotic proteins (e.g. Bcl-2, Bcl-xl) in appropriate tumor cell models related to increases in resistance to apoptosis in response to therapeutic agents or physiological stimuli?
4. What signal transduction pathways can be definitively identified as regulating apoptosis or cell proliferation in EPO responsive tumor cell lines?
5. Can animal models of human malignant disease (e.g. genetically engineered mice) be identified in which the pharmacological effects of ESAs on tumor growth and progression can be determined and studied systematically? Such studies can utilize genetically engineered malignancies bred on to mice of varying genetic backgrounds to reveal sources of patient to patient variation in response to ESAs.
6. What are the effects of ESAs on tumor vasculogenesis or angiogenesis in appropriate tumor cell or animal model systems?
7. What are the effects of ESAs on tumor invasiveness and migration in both in vitro and in vivo model systems?
8. Does constitutive signaling through a dysregulated EPO receptor contribute to tumor growth or survival in vivo or in vitro?

Although there are fragmentary research reports relating to all of these questions in the literature as discussed above, there is a dearth of in depth and coherent research in any or all of these areas to allow valid in vivo and in vitro correlations and/or conclusions to be drawn. Focused and in depth research is needed in both animal and cell culture models to facilitate our understanding of clinical results with ESA administration to patients. It is expected that the studies designed in response to this RFA will approach these questions with the clinical implications in mind, and that the studies will

emphasize both in vivo and in vitro research on appropriate model systems. The research areas outlined here are not to be deemed inclusive or exclusive. They are merely meant to suggest areas of research of particular interest.

Investigators are encouraged to submit proposals in any research area that will facilitate our understanding of the biology of ESAs and EPO receptors and their effects on tumor progression.

### **3. Current Portfolio Analysis.**

The NCI grant portfolio contains only 2 currently funded R01 research grants related to the effects of EPO or related molecules on tumor growth. Both grants are focused on mammary carcinoma. In fiscal year '08 there are presently 3 R01 applications on EPO and tumor growth that were scored, but none will currently be within the NCI payline. For fiscal year '08, there are also currently 3 R01 applications on EPO that were not scored. There is 1 application related to this research area currently funded through NIDDK

### **4. Justification for Use of RFA Mechanism:**

Applications in this research area are sparse, and those received have not done well in peer review. An RFA targeting this biologically and medically important research area is expected to stimulate the submission of high quality research applications, from outstanding applicants who may not have been previously motivated to enter this field. In addition, the NCI DEA will be able to empanel a review panel with a specific understanding of the research needs in the field.

**5. Budget:** \$5.0M dollars (total) will be available from the NIH Foundation to support this RFA. The funds derive from unrestricted donations by industry to support research into the biology of ESAs; donors will have no involvement in any aspect of this initiative. Available funds should support 2-3 grants at around \$350K in total costs (including F&A costs) for each year of a five year award.

**6. Evaluation Criteria for RFA:** Program Staff will convene a panel of expert extramural clinicians and basic research scientists, not funded by the RFA, who will meet with the grantees for a mid-course assessment. The meeting will determine progress of the award recipients towards answering the most significant questions regarding the role of ESAs in tumor cell biology. We will also ask our extramural advisors to help use evaluate the findings of the research at the end of the RFA award period. They will be asked to advise us whether or not significant progress has been made in understanding the effects of ESA on tumor cells, and if that data can be successfully applied to clinical situations.