RECOMBINANT HUMAN ERYTHROPOIETIN: ARE THERE CLINICAL APPLICATIONS BEYOND CHRONIC RENAL DISEASE?

Medical Grand Rounds

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Recombinant DNA technology has led to an increasing array of therapeutic agents for clinical application. Presently, three hematopoietic growth factors are released for general use (erythropoietin, G-CSF, GM-CSF). More are in clinical trials as well as other cytokines that have hematopoietic stimulatory effects. Potentially, they can lead to significant advances in the management of certain hematological disorders and other related medical problems. However, because of the enormous cost of these products, indiscriminant use will be devastating to the cost of medical care. The precise role of these agents has not been completely defined. Erythropoietin was the first hematopoietic growth factor to "reach the shelf." The rationale for its use and the important impact on the treatment of patients with chronic renal failure has been clearly documented. This subject was reviewed by Dr. Cronin at these exercises in May 1994. However, erythropoietin is employed in a variety of other clinical settings. There has been a lesser degree of documentation of indications and benefits. The purpose of this presentation is to review pertinent facts about erythropoietin production and function and to critically examine the present state of our knowledge of additional potential therapeutic applications.

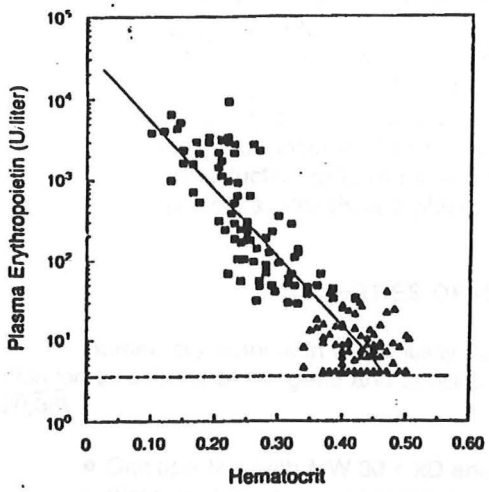
PHYSIOLOGIC DETERMINANTS OF ERYTHROPOIETIN PRODUCTION

- Oxygen carrying capacity
  - Anemia (1-4)
  - Erythrocytosis (5-8)
  - Carboxyhemoglobin (9,10)

- Blood oxygen tension (hypoxemia)
  - Ambient pO_2 (6,9)
  - Cardiopulmonary diseases (7,8,11)
- Hemoglobin oxygen affinity
  - Abnormal hemoglobins (12)
  - Carboxyhemoglobin (9,10)
- O_2 requirement or consumption (13)
  - Hypothyroidism and panhypopituitarism
  - Protein malnutrition

Quantitative and qualitative differences in response to these varied stimuli are well recognized. eg hypoxemia greater stimulus than anemia

- Erythropoietin behaves as both a hormone and growth factor (14)
- Erythropoietin production independent of its plasma level (14)
- Plasma clearance independent of plasma concentration and size of bone marrow erythroid compartment (14-16)
- Broad range of normal plasma level (4-26 mU/ml) (4)
- Within reference range, no correlation of plasma erythropoietin and Hgb or Hct (4)
- In iron deficiency and hypoplastic anemias, negative correlation between log of erythropoietin level and degree of anemia (4)
- Unequivocal rise above normal requires Hgb <10.5 (Hct <32) (4) (Fig 1)



Plasma Erythropoietin Levels in 175 Normal Blood Donors and Patients with Anemia, According to Hematocrit. Triangles denote normal blood donors, and squares patients with various anemias (those with anemia due to renal disease, rheumatoid arthritis, or solid tumors are excluded). The dashed line represents the limit of detection of the assay.

Figure 1 Reference 4

REGULATION OF ERYTHROPOIETIN PRODUCTION

Cloning of the mouse and human erythropoietin genes, located on chromosome 7 (7pter-q22) in man, has permitted the study of the control of erythropoietin production. (14,17-20)

- Single copy of erythropoietin gene per haploid genome
- Contained in a 5.4 kb region; has 4 introns and 5 exons
- Human and mouse genes have 90% homology in 5' sites preceding transcription start site, 80% in coding regions and 65% in first intron; conservation of locations of introns and splice donor and acceptor sites

- Hypoxia responsive cells are predominantly in the kidney (liver is primary site in fetal life and minor site post-natally) ((21-23)
  - subset of peritubular interstitial cells in the cortex and outer medulla of rodents; (? fibroblast-like type I interstitial cells) (24-26)
- Basal level of production by all cells (14,20)
- Erythropoietin production increases exponentially with hypoxia and magnitude is function of severity (27)
- Expanding areas of focal hypoxia results in increasing recruitment of erythropoietin producing cells rather than graded production per cell (27)
- Regulated both through transcription of erythropoietin gene and subsequent post-translational increased erythropoietin mRNA stabilization (14,28)

- The sensor of hypoxia may be a heme protein (20,29)
  - Heme containing proteins associated with nitrous oxide/c-GMP system proposed (30)
  - Its activity may be mediated via upregulation of members of jun and fos protooncogene families (31)
- Transcription of erythropoietin gene appears to require binding of factor(s) to enhancer region located 3' to structural sequences (32-33a)
  - Production of DNA binding factor(s) requires de-novo DNA transcription, protein synthesis and phosphorylation

PROPERTIES OF HUMAN ERYTHROPOIETIN

Human erythropoietin was initially purified and partially sequenced from urine (34,35). This led to cloning of the gene and subsequent production of the recombinant protein (14,17-20,36).

- Glycoprotein with MW 30.4 kD and 35% carbohydrate (37-39)
- Sialic acid residues necessary for in vivo activity (desialized causes rapid hepatic clearance) (40,41)
- Translated fragment has 27 aa leader sequence cleaved during secretion yielding 166 aa mature protein (35)
- Specific activity 210,000 U/mg protein and 129,000 U/total weight (42)
- Among species, translated protein highly conserved with 151 minimum residues (177 between monkey and man) (14)
- Proposed tertiary structure of an α-helical fold and loop topology suggests erythropoietin belongs to family of hormones, hematopoietic growth factors and cytokines:
  - eg GRH, prolactin, IL-2 thru 7, G-CSF, GM-CSF (43)
- Definitive identification of receptor binding and active site not accomplished
  - possibly closest helix to C terminus, aa 152-160 (14,39,44-48)

TRANSDUCTION OF ERYTHROPOIETIN ACTION

Erythropoietin action is accomplished by activation of its specific receptor (Epo-R). The gene has been cloned (49).

- Erythroid progenitor cells express Epo-R; (50)
  - both high and low affinity (51)
  - former physiologically important kd$\approx$100 pmol/L
- 507 a.a. protein MW 65 Kd; 82% homology with mouse Epo-R (52)
- Belongs to cytokine receptor superfamily characterized by four conserved Cys residues in extracellular domain eg
  - GH, IL-2 thru 7, GM-CSF, G-CSF, LIF, prolactin, c-mpl (49)

- 5 residue motif near transmembrane domain (WSXWS) ? essential for erythropoietin binding (53)
- Cytoplasmic signal transduction domains: (49,54,55)
  - membrane proximal site transduces proliferative signal
  - carboxy terminal region down modulates growth signal
  - proliferation and differentiation (and viability) signals may be separate
- Erythropoietin-receptor complex internalized by endocytosis (56)

- Mechanism of transmission of growth-regulatory signal poorly understood
  - tyrosine phosphorylation involved in signal transduction (57-59)
    Epo-R has no intrinsic protein kinase activity
  - no cytoplasmic domain homologies among superfamily members
  - ligand binding to Epo-R induces receptor clustering with non-receptor tyrosine kinase JAK2 (60)
    Epo-R and other proteins tyrosine phosphorylated
  - erythropoietin-activated tyrosine phosphorylated proteins specifically recognize well-defined enhancer (61)
    enhancer has features similar to known rapid response genes

- Epo-R gene located on chromosome 19p (62-64)
  - highly conserved - 82% homology with mouse
  - 5-6.5 kb, 8 exons 7 introns

- Initial stage when Epo-R gene transcription is activated is unknown (49,65-67)
  - transcription is tissue specific and temporally regulated
  - increased transcription preceding increased Epo-R density on CFU-E
  - minimum promoter, enhancer and inhibitor regulatory sequences mapped
  - Epo-R may participate in positive feed-back loop

THE PHYSIOLOGIC RESPONSE TO ERYTHROPOIETIN

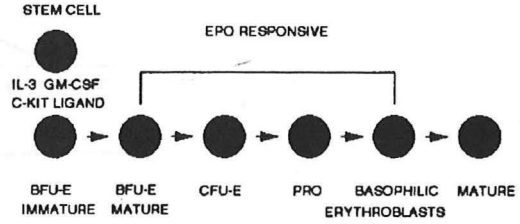
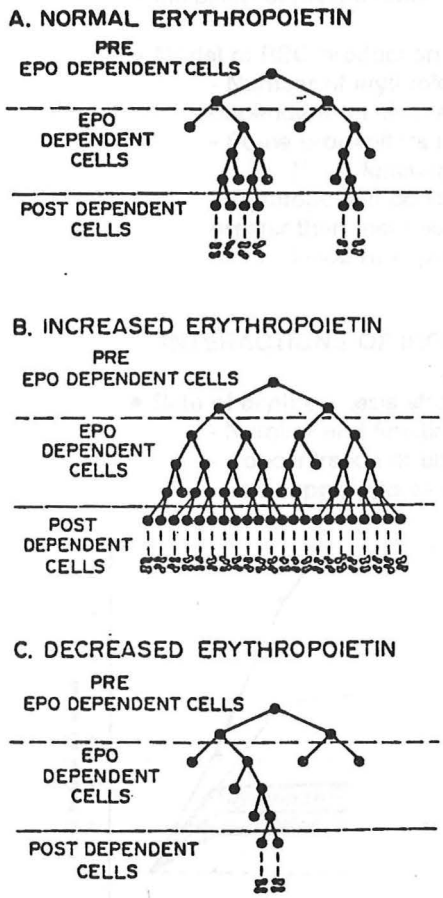


Figure 2 Stages of Erythroid Differentiation

- Several stages of erythroid development are recognized (Fig 2)

- Three are identified by in vitro culture techniques (14,49,68,69)
  - Immature BFU-E (burst forming unit-erythroid): few or no erythropoietin receptors; proliferate in response to other cytokines eg. IL-3, GM-CSF, Kit ligand (Steel factor, mast cell growth factor) (70-71)
  - Mature BFU-E: erythropoietin receptors; give rise to CFU-E (72)
  - CFU-E (colony forming unit-erythroid): highest density of erythropoietin receptors; highly proliferative and differentiate to pro-erythroblasts
- Later stages (erythroblasts) are distinguished morphologically; differentiation erythropoietin independent after basophilic erythroblast stage



Model of erythropoiesis based on EPO suppression of programmed cell death (apoptosis). Erythroid-progenitor cells enter a period of development in which they are dependent upon EPO for survival (EPO DEPENDENT CELLS). See Fig. 3 for the relationship to the EPO-dependent period and the stages of erythroid differentiation. (●) Surviving viable cells; (⊗) cells undergoing programmed cell death owing to insufficient EPO. Before entering the EPO-dependent period. the progenitors can survive without EPO (PRE EPO DEPENDENT CELLS). Cells surviving transit through the EPO-dependent period can complete maturation into reticulocytes without EPO and ultimately become red cells (POST EPO DEPENDENT CELLS). Only one division appears to occur in murine erythropoiesis in the post-EPO-dependent stages. Reproduced with slight modification from Koury and Bondurant (1990b).

Figure 3 Reference 77

- Mechanisms of commitment to erythroid lineage from hematopoietic stem cells are

poorly understood

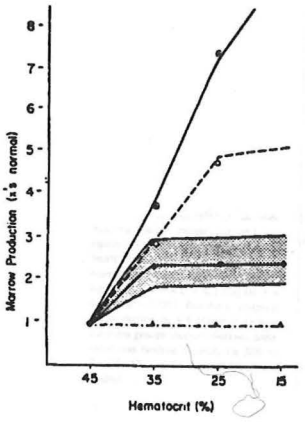
The principal consequences of the action of erythropoietin on the erythron are:

- Proliferation (mitogenic signal) (14,49)
  - Late BFU-E and CFU-E stages
- Differentiation eg.: (14,49)
  - Hemoglobin synthesis
  - Synthesis of erythroid specific membrane proteins
  - Not clear whether erythropoietin induces differentiation or is permissive by sustaining viability
- Inhibition of programmed cell death (apoptosis) (73-76)

- Model of RBC production in vivo: (77) (Fig 3)
  - Number of erythroid progenitors that survive and become reticulocytes dependent on circulating erythropoietin concentrations
  - Some progenitors require less erythropoietin than others to survive (78)
    Not a function of erythropoietin receptor number, affinity or structure
  - Erythropoietin concentration necessary to stimulate proliferation may be greater than that necessary to sustain inhibition of apoptosis (7,79)
    Once RBC production increased lower levels sustain it

INTERACTIONS OF IRON, ERYTHROPOIETIN AND ERYTHROPOIESIS

- Rate of erythropoiesis strongly dependent upon: (80)
  - Number and function of erythroid progenitors
  - Concentration of erythropoietin
  - Iron supply (stores and saturation of TIBC)



Marrow production rates were characterized in normal individuals subjected to prolonged periods of phlebotomy-induced anemia[3]. Subjects relying upon a normal distribution of reticuloendothelial iron stores were able to increase marrow production to a maximum of three times normal (shaded area) at hematocrit of 35%. With lower hematocrit levels, marrow production did not increase further. Iron deficient individuals were unable to increase marrow production significantly above the basal level despite major reductions in their hematocrit (triangles). When iron was supplied from more than one source, as in the patient with hemochromatosis (open circles) or normal subjects receiving oral iron supplements at a time when reticuloendothelial iron stores were adequate, marrow production increased to between four and five times normal at hematocrit of 25%. This was significantly better than that seen with normal individuals relying upon reticuloendothelial iron stores alone. Finally, individuals receiving daily infusions of senescent red cells (circled dots) were able to deliver significantly larger amounts of iron to the marrow to support marrow production levels of seven to eight times normal at hematocrit of 25%.

Figure 4 Reference 81

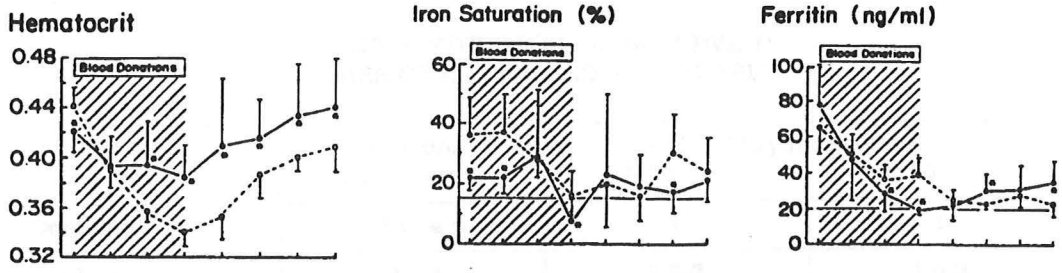
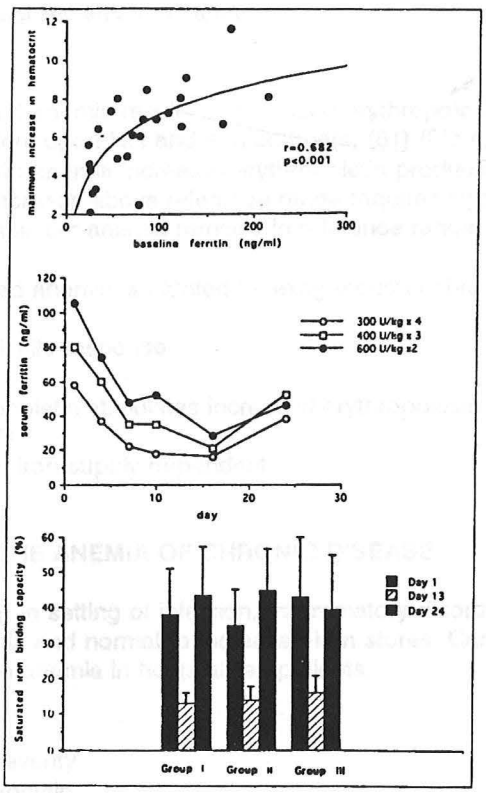


Figure 5 Reference 84

Hematocrit and iron values in normal subjects undergoing phlebotomy with (•) and without (○) exogenous erythropoietin



Top. Correlation between baseline serum ferritin (ng/mL) and maximum increase in hematocrit in 24 healthy men taking recombinant human erythropoietin. Correlation parameters are: $y = -3.0 \pm 5.1 \text{ Log}(x)$, $r = 0.682$, $p < 0.001$. **Middle.** Changes of serum ferritin as a function of time in the three groups studied. **Bottom.** Saturated iron binding capacity (± SD) on Days 1, 13, and 24 in the three groups studied.

Figure 6 Reference 85

Table 1

RATE OF ERYTHROPOIESIS RELATIVE TO DEGREE OF ANEMIA AND IRON SUPPLY

Iron Stores	Additional Iron Source	Rate of Erythropoiesis Hct 35	Hct 20
Depleted	None	1	1
Replete	None	1.5-3	1.5-3
Replete	Oral	2-3	4-5
Overload	None	2-3	4-5
Replete	Hemoglobin	3-4	7-8

Erythropoietic rate with normal Hct and iron stores = 1

- Phlebotomy induced anemia results in increased erythropoiesis
  - rate dependent upon Hct and iron source(s) (81) (Fig 4, Table 1)
- Phlebotomy induced anemia increases erythropoietin production (82,83)
  - Significant increase above reference range requires Hgb ≤ 10.5 (Hct ≤ 32)
  - Increment at lesser anemia remains in reference range

- Phlebotomy induced anemia is blunted by exogenous erythropoietin administration (84) (Fig 5)
  - Iron supply limits response

- Exogenous erythropoietin stimulates increased erythropoiesis in dose dependent fashion (85,86) (Fig 6)
  - Magnitude is iron supply dependent

THE ANEMIA OF CHRONIC DISEASE

Definition: Anemia occurring in setting of infection, inflammatory disorders and malignancy characterized by hypoferremia and normal to increased iron stores. Can also be seen in other diseases (87). Most common anemia in hospitalized patients.

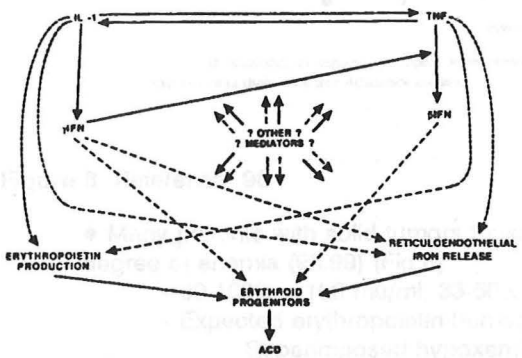
Characteristics: (88,89)

- Mild to moderate severity
- Normocytic to microcytic
- Hypoferremia and, usually, decreased saturation of iron binding capacity (<20%)
- Normal to decreased transferrin (TIBC)
- Normal to increased serum ferritin and marrow iron

Pathogenesis: Several abnormalities contribute, often of variable importance, in a given disease setting or individual patient.

- **Shortened erythrocyte survival** (90)
  Mild and variably present
- **Diminished supply of iron for erythropoiesis** (91)
  Diminished reutilization of senescent RBC iron
  Impaired release of storage iron
  Hypoferremia may be cytokine mediated (TNF, IL-1, IFN-γ)
  Role as causal factor of anemia debated
- **Cytokine mediated impairment of erythropoiesis** (92,93) (Fig 7)
  Both cellular and humoral mediated inhibition shown
  Candidate cytokines:
  - Tumor necrosis factor alpha
    Increased serum concentration in diseases with ACD
    Directly inhibits BFU-E growth
    Indirectly inhibits CFU-E via β interferon
  - Interleukin-1
    Increased serum concentration in diseases with ACD
    Indirectly inhibits erythroid colony growth via T cell release of γ-IFN
  - Interferon-γ
    Increased in disorders with ACD
    Directly or indirectly inhibits erythroid colony growth

  Synergistic inhibition of erythroid progenitors between cytokines demonstrated.
- **Impaired erythropoietin production and responsiveness** (94,95) (Fig 7)
  - Erythropoietin production increased in ACD but inappropriate for degree of anemia ie relative deficiency
    - In vitro, cytokines impair erythropoietin production (IF-γ, IL-1, TNF-α)
      Additive or synergistic
      ↓ mRNA: ? synthesis or stability
  - Increasing erythropoietin concentration can overcome cytokine mediated impairment of erythroid colony growth



Schematic diagram representing effects of inflammatory cytokines on processes involved in the impairment of erythropoiesis in ACD. Positive regulatory effects are indicated by solid lines and negative effects by broken lines.

Figure 7 Reference 92

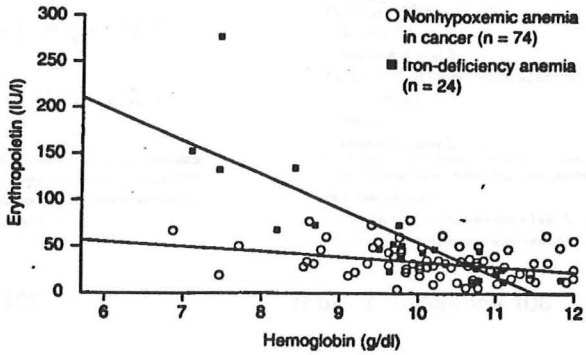
ERYTHROPOIETIN THERAPY IN CANCER

Anemia is common in cancer and multiple potential mechanisms exist: (96,97)

- Blood loss
- Iron deficiency
- Nutritional deficiencies (eg folate, B_{12})
- Hemolysis
- Hemodilution/splenomegaly
- Myelophthisis
- Paraneoplastic RBC aplasia
- Diagnostic phlebotomy
- Chemotherapy
- Radiation therapy
- Anemia of chronic disease

Observation Relevant to Erythropoietin Therapy in Anemic Cancer Patients:

- Experimental observations of ACD suggest impaired erythropoiesis, erythropoietin production, and response to erythropoietin may exist (see above)

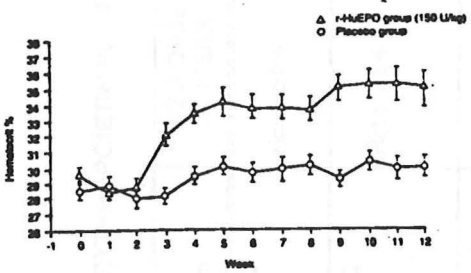


Relationship of serum erythropoietin concentrations and hemoglobin concentrations in patients with cancer and in those with iron deficiency anemia.

Figure 8 Reference 98

- Many patients with solid tumors have inappropriately low erythropoietin levels for degree of anemia (98,99) (Fig 8)
  - 80-100% <100 mu/ml; 33-50% <50 mu/ml
  - Expected erythropoietin-hemoglobin relationship absent
    Superimposed hypoxemia may trigger further erythropoietin synthesis
- Erythropoietin production reduced without prior chemotherapy

- Experimentally and clinically, cis-platinum may further decrease erythropoietin production without measurable effects on other parameters of renal function (100-104)
- Chemotherapy often causes anemia without effect on erythropoietin response
- Anemia in multiple myeloma appears more complex (105)
  - Decreased erythropoietin can result from renal disease
  - Decreased erythropoiesis can occur with adequate erythropoietin response to anemia
  - Inadequate erythropoietin response can occur with normal renal function (as in solid tumors) - stage related



Mean weekly hematocrit values (±SE) for patients administered recombinant human erythropoietin (r-HuEPO) or placebo injections in the population of patients administered chemotherapy that included cisplatin.

Figure 9 Reference 106

Results in Patients Administered Chemotherapy Including Cisplatin by Treatment Group

Parameter	Treatment Group: r-HuEPO (n = 64)	Treatment Group: Placebo (n = 61)
Hematocrit (%)		
Baseline	29.4	28.4
Final	35.4	29.7
Change	6.0*	1.3
Correctors (% of patients with Hct ≥ 38%)	35.9*	1.6
Responders (% of patients with Hct increase ≥ 6%)	48.4*	6.6
Patients transfused (%)	53.1	68.9
Month 1	43.8	44.3
Months 2 and 3	26.8*	56.4
Mean units of blood transfused per patient	3.56	4.01
Month 1	1.71	1.20
Months 2 and 3	1.20	2.00

Abbreviations: r-HuEPO, recombinant human erythropoietin; Hct, hematocrit.

* Difference between the r-HuEPO and placebo groups reached statistical significance; see text for P values.

Table 2 Reference 106

Results of Clinical Trials of Erythropoietin in Cancer Patients (Excluding acute leukemia, myelodysplasia and bone marrow transplantation) (Page 12)

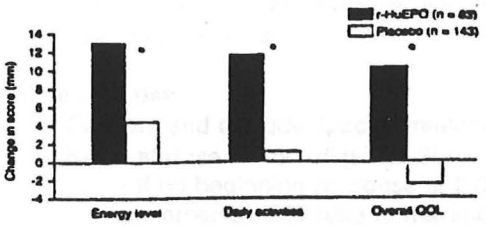
- Approximately 1/3 of patients with anemia, not on chemotherapy, will respond to 100 u/kg TIW SQ (106,115)
  - Many had prior chemo and/or radiotherapy
  - Higher doses not tested

- Approximately 50-75% of anemic patients receiving chemotherapy will respond to 150-300 u/kg TIW SQ (106-115) (Fig 9, Table2)
  - Decline of Hgb with prior chemotherapy frequent (108)

RESULTS OF TRIALS OF ERYTHROPOIETIN IN CANCER PATIENTS

TUMOR TYPE	# PTS	MEAN Hgb/Hct	DOSE (Duration)	RESPONSE CRITERIA	% RESPONSE	COMMENTS	Ref
Solid 2/3 Lymphoma 1/3	63 55	29 28	100 u/kg TIW SQ (8 weeks) Placebo	Hct ↑ ≥6%	32 p< 0.008 11	Randomized; No chemo; QOL measured	106
Solid 2/3 Lymphoma 1/3	79 74	29 29	150 u/kg TIW SQ (12 weeks) Placebo	Hct ↑ ≥6%	58 p< 0.008 14	Randomized; Non-platinum CT; Transfusion ↓ QOL measured	107
Solid	30	9.3 (28)	25-300 u/kg 5x/w IV (4 weeks)	Hgb ↑ ≥10%	85 @ 200-300 u/kg	Non-platinum CT Dose escalation; Prior CT Hgb ↓	108
Solid 2/3 Lymphoma 1/3	64 61	29 28	150 u/kg TIW SQ (12 weeks) Placebo	Hct ↑ ≥6%	48 p< 0.008 7	Randomized; Platinum CT Transfusion ↓ QOL measured	106
Solid	21	10 (31)	25-200 u/kg 5x/w IV (4 weeks)	Hgb ↑ ≥10%	70 @ 100-200 u/kg	Dose escalation; Platinum CT; =150-300 TIW	109
Solid	50 49	8.6	100 u/kg TIW SQ (9 weeks) Placebo	Hgb ≥ 10 gm	82 p< 0.0001 2	Randomized Platinum CT Transfusion ↓	109a
Solid	38	5.3-10.9	150-300 u/kg TIW SQ	Hgb ↑ ≥2 gm	50	Dose Escalation Squamous better	110-112
Myeloma	18	8.7-10.9	150-300 u/kg TIW SQ	Hgb ↑ ≥2 gm	78	Dose escalation; 17/18 N Cr; PS ↑	113
Myeloma	28	8.7	150 u/kg TIW SQ	Hgb ↑ ≥2 gm	75	N Cr; Dose Escalation	114

- May prevent further decline or eliminate anemia (108)
- Applies to chemotherapy with or without platinum
- Transfusion requirements during later cycles of chemotherapy may be reduced (106,107,115)



Change in energy level, ability to engage in daily activities, and overall quality of life (QOL) (in mm) from baseline to final evaluation for r-HuEPO–treated responders (hematocrit value increased by 6% or more) and placebo-treated patients pooled across all trials. *Difference between r-HuEPO and placebo groups reached statistical difference ($P < .05$).

Figure 10 Reference 106

- Energy level, activity limits and overall quality of life may be enhanced (106,115,115a) (Fig 10)
- Dose-response relationship may exist (108, 109)
- Erythroid marrow response documented (116)

- Anemic patients with multiple myeloma may be more responsive and require relatively lower doses (150 u/kg TIW) (113,114)
- Use of erythropoietin to minimize or prevent anemia during radiotherapy being explored (117,118)
  - Question of relationship of Hgb and outcome of RT in respect to response and survival

Additional Observations From Clinical Trials

- Beginning response usually seen by 2-3 weeks
  - Incremental doses may be necessary
- Significant utilization of iron stores occurs
  - Iron deficient erythropoiesis may blunt magnitude of response
  - Iron supplementation recommended during therapy
- Subcutaneous route preferred - provides more sustained blood level of erythropoietin
- No correlation of response to pre-treatment serum erythropoietin level
  - May not apply to multiple myeloma
- Minimal toxicity noted
  - Occasional development or worsening of hypertension
  - Higher doses (300 u/kg) occasionally associated with venous thrombosis
  - Questionable cause/effect relationship
- Lower maintenance doses may suffice after therapy goal achieved

Provisional Recommendations for Erythropoietin Therapy in Cancer

- Establish a goal for therapy
  - Improved functional status
  - Reduce transfusion dependence
  - Prevent symptomatic anemia during chemotherapy

Consider Using In:

- Patients who have required RBC transfusions
- Patients not on therapy who have symptoms possibly attributable to anemia
- Patients on chemotherapy who have developed anemia that may explain symptoms
- Patients receiving chemotherapy regimens with a high probability of producing symptomatic anemia

Guidelines for use:

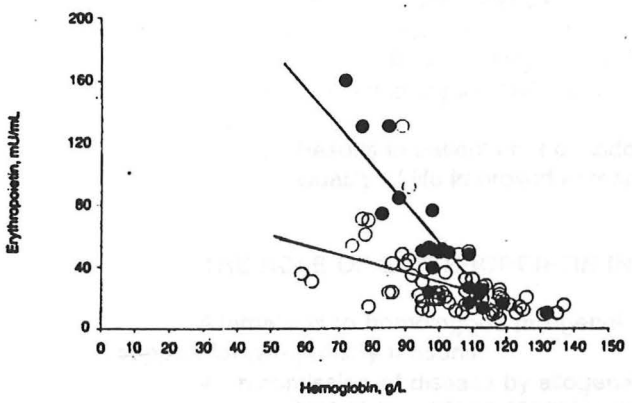
- Evaluate and exclude specific treatable cause of anemia
- Begin at dose of 150 u/kg TIW SQ
  - If no beginning response at 2-3 weeks, consider increasing dose at increments of 50 u/kg to maximum of 300 u/kg
  - If response occurs, continue dose until goal achieved, then attempt to maintain with lower doses
- TID oral iron supplementation recommended (monitor SI, TIBC and serum ferritin)
  - Consider parenteral iron supplements only if oral iron does not prevent iron deficient erythropoiesis
- Monitor BP, especially in patients with history of hypertension

ERYTHROPOIETIN THERAPY IN AIDS

- Anemia common in HIV infected patients (119)
  - 70-100% with AIDS develop anemia (122)
    Correlated with stage of infection

- Multiple potential mechanisms exist - often interacting and additive (119-122)
  - Nutritional deficiencies (eg folate, B_{12})
  - Hemolysis
  - Hemodilution/splenomegaly
  - Myelophthisis from disseminated opportunistic infections
  - Diagnostic phlebotomy
  - Chemotherapy of AIDS related neoplasms
  - ? Direct effect of HIV on hematopoietic progenitors (123)
  - Drugs used to treat HIV - especially zidovudine (120)
  - Anemia of chronic disease
    - Majority anemic AIDS patients have iron metabolism parameters of ACD (119,122)
    - Mean serum erythropoietin similar to patients with ACD secondary to infections (122)
    - Erythropoietin response blunted relative to degree of anemia (121,122) (Fig 11)
      Erythropoietin-hemoglobin relation maintained
      Erythropoietin response often significant with added zidovudine induced anemia

Erythropoietin production suppressed in vitro by HIV-1 (124)

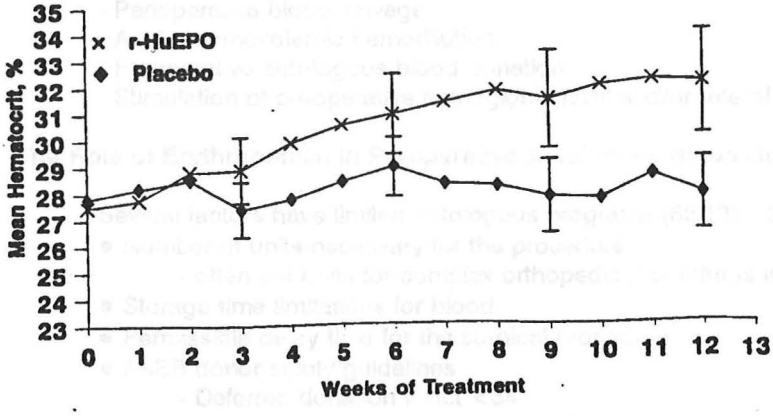


...Relationship between hemoglobin and serum immunoreactive erythropoietin in 65 anemic, untreated patients with the acquired immunodeficiency syndrome (AIDS) (open circle) and 23 patients with iron deficiency anemia (black circle). For the AIDS patients, the equation for the regression line was $y = -6.8x + 98.1$ ($r = .50$, $P < .01$). For iron deficiency, the equation for the regression line was $y = -25.8x + 318$ ($r = .90$, $P < .01$). The difference between the slopes was significant ($P < .01$).

Figure 11 Reference 121

Results of Trials of Erythropoietin in AIDS

- 4 prospective, placebo controlled, double blinded randomized trials of erythropoietin in anemic AIDS patients on zidovudine (125,126) (Fig 12)



Mean weekly hematocrit for patients with erythropoietin levels less than or equal to 500 IU/L. Bars represent 95% CIs. r-HuEPO = recombinant human erythropoietin.

Figure 12 Reference 126

- Erythropoietin increases hemoglobin level in patients with endogenous serum erythropoietin <500 u/ml
- Transfusion requirement significantly decreased in patients with prior transfusion dependence
- Dose of 100 u/kg TIW SQ equivalent to higher doses and IV administration
- No significant toxicity compared to placebo

• Open labelled study in 1900+ anemic AIDS patients confirmed these observations (127)

- Results in patients not on zidovudine pending
- Quality of life improved in responders (127a)

THE ROLE OF ERYTHROPOIETIN IN SURGICAL TRANSFUSION MEDICINE

Alternatives to homologous (allogeneic) transfusion in surgery has had increasing support for two primary reasons:

• Transmission of disease by allogeneic blood eg:
- HIV (risk ≈ 62500-225000/unit) (128,128a)
- Hepatitis B (risk ≈ 1:1250/unit) (129)
- Hepatitis C (risk ≈ 1:3333/unit) (130)
- EBV
- CMV
- GVH disease

• Increasingly limited blood supply

• Also, immunomodulating effects of allogeneic transfusion may exist (131)

These alternatives include:
- Education of physicians in blood ordering practices
- Perioperative blood salvage
- Acute normovolemic hemodilution
- Preoperative autologous blood donation
- Stimulation of preoperative hemoglobin level and/or rate of erythropoiesis

The Role of Erythropoietin in Preoperative Autologous Blood Donation

Several factors have limited autologous programs (82,132-136)

• Number of units necessary for the procedure
- often ≥4 units for complex orthopedic procedures and CABG

• Storage time limitations for blood

• Permissible delay time for the surgical procedure

• AABB donor safety guidelines
- Deferred donation if Hct <34
- No greater frequency than q 3-4 days

This has resulted in a rate of failure to achieve the desired number of autologous units .and the resultant need for allogeneic transfusion:

- ≤ 3 units requested: 6-13% fail; 9-13% receive allogeneic blood
- ≥ 4 units requested 40-60% fail; 30-43% receive allogeneic blood

Several factors identified as related to inability to donate necessary number of units (82,83,132)

- Initial Hct and red cell mass
- Iron availability despite oral iron supplements
  - initial iron stores
  - Rate of mobilization of iron stores
- Inadequate erythropoietin response to phlebotomy (82,83)
  - Increase occurs but remains in normal range
  - Hgb < 10 gm/dl necessary to trigger significant elevation of erythropoietin above reference range (4)
- Female gender - primarily due to higher frequency of Hct, red cell mass and iron store limitations

- The rate of increased red cell production due to phlebotomy in an autologous donor setting can be expanded by 50% with erythropoietin and oral iron administration (137)

Results of trials utilizing erythropoietin in autologous blood donations for surgical procedures: (138-143)

- Randomized trial of placebo or recombinant human erythropoietin with autologous donations in **non-anemic** orthopedic surgery candidates: (139) (Table 3)
  - increased the average number of units donated
  - Did **not** reduce the number of patients receiving allogeneic transfusion

- Randomized trial of placebo or recombinant human erythropoietin with autologous donations in **anemic** orthopedic surgery candidates: (140) (Table 3)
  - increased the average of units donated
  - decreased the percentage of patients receiving allogeneic transfusion
- Dose of 250 u/kg BIW (500 u/kg/week) maximum necessary for Hgb repair after 2 unit phlebotomy (144)
- Dose of 800-1600 u/kg/week minimum for optimal repair with 4-6 unit phlebotomy (84,139,143,143a)
- Iron stores are rate limiting for response to erythropoietin

- Randomized trial of placebo or recombinant human erythropoietin with autologous donations in **non-anemic** cardiac surgery candidates: (143) (Table 4)
  - Decreased the percentage of patients receiving allogeneic transfusions

Role of Erythropoietin Alone in Surgical Patients

- Rationale Includes:
  - Increase in preoperative red cell mass
  - "Priming" erythropoiesis for more prompt recovery from blood loss

Table 3

EFFECT OF ERYTHROPOIETIN ON AUTOLOGOUS BLOOD DONATION FOR ORTHOPEDIC SURGERY

Treatment	Pre-donation Hct	Iron Supplement	Mean Units Donated	% Patients Requiring Allogeneic Blood
Placebo	<40	Oral	2.6	50
Placebo	<40	IV	3.3	50
Erythropoietin	<40	Oral	3.8*	25*
Erythropoietin	<40	IV	5.1**	16*
Placebo	≥40	Oral	4.6	9
Erythropoietin	≥40	Oral	5.6	9

Goal of donations was 1 unit twice weekly for 3 weeks; Donated if Hct ≥34
Erythropoietin administered at each visit - Total 6 doses
***p < 0.05 versus placebo; **p <0.05 versus placebo and oral iron**

Table 4

EFFECT OF ERYTHROPOIETIN ON AUTOLOGOUS BLOOD DONATION FOR OPEN HEART SURGERY

Erythropoietin Dose (units)	# Patients	Mean Change Hgb (gm/dl)[a]	% Donated 2 units[b]	% Allogeneic Transfusion[c]
Placebo	28	-1.1	79	38
12,000	28	-0.9	93	11
24,000	30	+0.1	100	10

Erythropoietin given subQ days 21, 14, 7 pre-surgery
1 unit donated days 14 and 7 pre-operatively
[a] p<0.001; [b] p = 0.018; [c] p = 0.013

Results of Trials of Perioperative Erythropoietin

- Randomized trial of perioperative erythropoietin versus placebo in hip replacement: (145) (Table 5)

- Increased preop Hct
- Decreased percentage of patients transfused
- Benefit related to pretreatment Hgb (<13.5)

- Preliminary data in open heart surgery:
  - One of two trials suggests reduction in transfusion (141,146)

- Iron stores are rate limiting for response to erythropoietin

Conclusions: Use of erythropoietin in surgical setting remains investigational but appears promising in selected groups

Table 5

RESULTS OF RANDOMIZED TRIAL OF PREOPERATIVE ERYTHROPOIETIN FOR HIP REPLACEMENT SURGERY

Regimen	# Patients	Time (days)	Mean ↑ Hgb Preop	% Patients Transfused *
Placebo	78	14	-.26	44
Erythropoietin	77	14	.88	23
Placebo-Erythropoietin	53	5 9	.22	30

Treatment begun 10 days preoperatively
*** p = 0.007 for erythropoietin versus placebo**

MYELODYSPLASTIC SYNDROMES
(Pre-leukemia, smoldering acute leukemia)

Definition: Group of clonal hematopoietic stem cell disorders characterized by: (147)

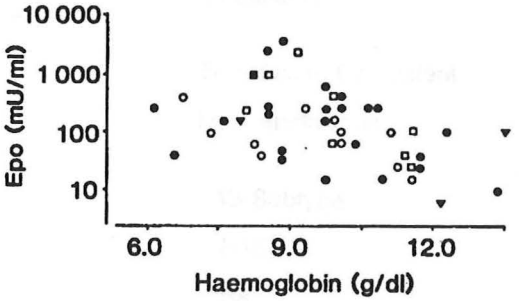
- One or more peripheral cytopenias
  - Anemia frequent and often transfusion dependent
- Blood cell functional defects
- Abnormal bone marrow differentiation (dysplasia)
- Abnormal bone marrow maturation
- Potential conversion to acute leukemia
- No consistently effective therapy

Classification: Subclassified by blood and bone marrow morphology: (148)

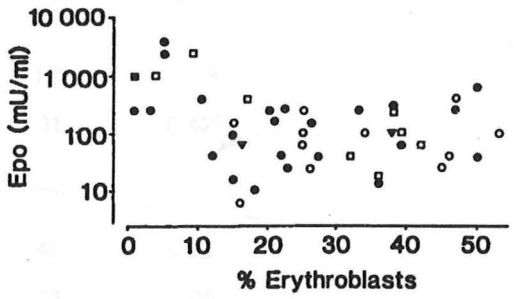
- Refractory anemia (RA)
- Refractory anemia with ringed sideroblasts (RARS)
- Chronic myelomonocytic leukemia
- Refractory anemia with excess blasts (RAEB)
- RAEB in transition (RAEB-T)

Erythropoiesis: Characterized by significant **differences** between patients in: (149,150)

- Numbers of blood and bone marrow BFU-E
- Numbers of recognizable erythroid precursors
- Reduced production due to
  - Ineffective erythropoiesis or
  - Hypoproliferative state
- Reduced RBC lifespan (hemolysis)



Serum Epo and peripheral blood haemoglobin concentrations in 46 myelodysplastic patients: ●, RA; ○, SA; □, RAEB; ■, RAEBT; ▼, CMML.



. Serum Epo concentrations and percent bone marrow erythroblasts in 46 patients with myelodysplastic syndromes. Symbols as in Fig

Figure 13 Reference 150

- Variable serum erythropoietin levels relative to severity of anemia
  - (eg 32-4900 u/L with Hgb 8-9) (Fig 13)
  - Moderate correlation with Hgb level
  - Weak correlation with marrow erythroid activity

Clinical Trials of Erythropoietin in MDS

Interpretation of data from published trials of erythropoietin therapy in MDS has been difficult due to:

- Heterogeneity of mechanisms of anemia
- Small numbers of patients per series
- Variable doses of erythropoietin (300-3000 u/kg/week)
- Variable schedules of therapy (Twice weekly-daily)
- Different routes of administration (subQ or IV)
- Different criteria for response
  - 1-2 gm/dl increase in Hgb without transfusion
  - Decrease or elimination of transfusion requiremen

Eligibility for treatment has been consistent (Hgb <10.0 gm/dl or transfusion dependence)

Some conclusions or trends can be observed from studies with adequate data: (151-167) (Table 6)

Table 6

RESULTS OF ERYTHROPOIETIN TREATMENT OF MDS

Patient Group	# Patients	Response Rate
All patients	190	0.22
Transfusion Dependent	159	0.18
No Transfusions	31	0.42[a]
FAB Subtype		
RAEB	42	0.38[b]
RA	73	0.23
RARS	66	0.12
Other	8	0.00
Maximum weekly Dose		
≥ 1000 u/kg	98	0.29[c]
< 1000 u/kg	92	0.14

Pre-treatment erythropoietin levels of responders

(mu/ml)	% of responders
≤ 200	43
> 200-500	27
> 500	30

[a] p = 0.003 vs transfusion dependent; [b] p = 0.002 vs RARS;
[c] p = 0.016 vs lower doses

- 20-25% response rate overall (confirmed in a multi-institutional trial) (168)

- Transfusion dependent patients have lower response rate
- Differences in responses seen by FAB sub-category
  - RARS particularly refractory to erythropoietin alone
- Response rates appear greater with higher doses
- Previously recommended cutoffs for serum erythropoietin levels (<200 or <500 mu/ml) only relative. Some with higher levels will respond
- Side effects have been infrequent and rarely required discontinuation; most seen with higher doses
  - Local and systemic reactions
  - Splenic pain
  - Hypertension has been rare
  - Progression to acute leukemia does not appear to be greater than expected frequency
- Attempts to delineate clinical predictive factors for response not sufficiently specific to be applicable (169-171)

Combination of erythropoietin with other growth factors:

Rationale for combined growth factor therapy of anemia: (172)

- GM-CSF acts on progenitor cells more proximal than erythropoietin; could expand erythropoietin responsive late BFU-E (recruitment)
- In vitro, G-CSF acts additively or synergistically with erythropoietin to promote erythroid colony growth (synergy of growth factors)

Table 7

RESULTS OF ERYTHROPOIETIN TREATMENT OF MDS WITH OTHER GROWTH FACTORS

INVESTIGATOR	OTHER FACTOR	MAXIMUM EPO DOSE u/kg/week	PATIENT GROUP	# PATIENTS	RESPONSE RATE
Hansen	GM-CSF	840	All	11	0.45
			Tx Dependent	8	0.25
			No Transfusion	3	1.00
Negrin	G-CSF	2100	All	24	0.42
			Tx Dependent	22	0.36
			No Transfusion	2	1.00

Limited non-randomized clinical trials of erythropoietin with G-CSF and GM-CSF have been reported to produce a higher response than erythropoietin alone (173,174) (Table 7)

- Applies to both transfusion and non-transfusion dependent anemia
- Potentially higher response in RA and RARS

Conclusion: Patients with MDS who have symptomatic anemia and/or a transfusion requirement should be considered for erythropoietin therapy after other treatable causes of anemia have been excluded. Non-responders may be candidates for combined growth factor therapy. Cost is a major determinant.

ERYTHROPOIETIN IN RHEUMATOID ARTHRITIS
(and other chronic inflammatory states)

RA represents the prototype of chronic inflammatory states associated with anemia of chronic disease

- 40-80% RA patients anemic (175-178)
  - Presence and severity correlate with disease activity
  - 50-70% have anemia chronic disease
  - 25-50% have iron deficiency anemia (often with ACD also)
- Erythropoietin response to anemia exists (177,178)
  - Magnitude of response blunted relative to degree of anemia (Fig 14)

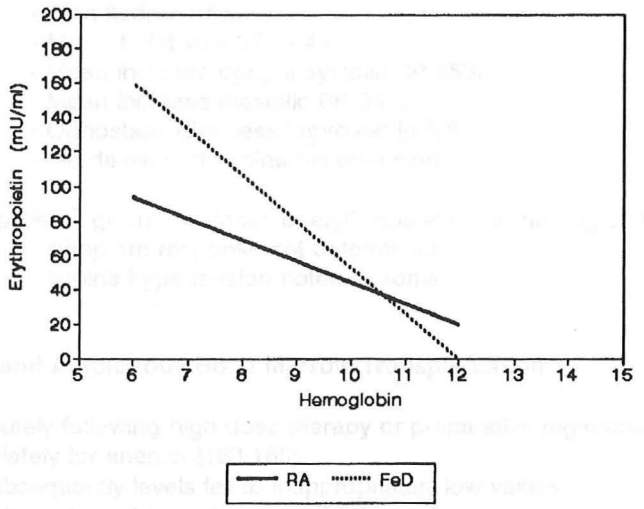


Figure 14 Serum Erythropoietin in RA vs Iron Deficiency

Treatment of Anemia in Rheumatoid Arthritis with Erythropoietin

- Interpretation of clinical trials complicated by:
  - Frequent coexistence of iron deficiency
  - Small numbers of patients with moderate to severe anemia

Conclusions:

- Most patients will respond to erythropoietin with minimum increase of Hct of ≥ 5% (179,180)
  - Doses of 100-300 u/kg subQ 3x/week necessary
  - Applies to patients with no evidence of iron deficiency
  - Patients with iron deficiency also respond but role of iron therapy versus erythropoietin not clear
- Patients with systemic onset juvenile rheumatoid arthritis and severe anemia have high response rate to erythropoietin (181)

ADDITIONAL AREAS OF POTENTIAL ERYTHROPOIETIN APPLICATION

Orthostatic Hypotension

- Some patients with primary (182a) and secondary (182) autonomic neuropathy and orthostatic hypotension have decreased RBC mass and anemia
- Erythropoietin levels inappropriately low in the most anemic subjects
- Possibly reflects effect of sympathetic nervous system on erythropoietin production

- 8 patients with this problem, given erythropoietin (182)
  - All had Hct ≤ 40 pre-treatment
  - 7 on fludrocortisone
  - Mean ↑ Hct was 34 → 45
  - Mean increase upright systolic BP 25%
  - Mean increase diastolic BP 33%
  - Orthostatic dizziness improved in 6/8
  - 3/8 developed supine hypertension

- 5 patients given low doses of erythropoietin had hemoglobin increases (182a)
  - symptom response not determined
  - supine hypertension noted in some

Allogeneic and Autologous Bone Marrow Transplantation

- Acutely following high dose therapy or preparative regimens, erythropoietin levels rise appropriately for anemia (183-185)
- Subsequently levels fall to inappropriately low values
- Universal need for autologous RBC transfusions

Allogeneic Transplantation:

- Treatment strategies:
  - Erythropoietin to recipient post transplant (186,187)
    2 of 2 prospective studies showed decreased transfusion requirement and reduced time to transfusion independence

  - Erythropoietin to donor with autologous RBC storage pre-transplant and to recipient post-transplant (188)
    1 study showed decreased transfusion requirement
    5/11 patients required no autologous RBC

Autologous Transplantation:

- Treatment strategies:
  - Erythropoietin with G or GM-CSF pre and post-transplant (189,190)
    2 of 2 studies showed no benefit
  - Erythropoietin post-transplant (191)
    1 study showed no decrease in transfusion requirement but shortened time to recovery of anemia

Anemia of Prematurity

- Transfusion requiring anemia is common in premature infants
- Erythropoietin production is commonly deficient (192)
- Preliminary comparative trials suggest a reduction in transfusion requirement with erythropoietin therapy (193-194a)

Aplastic Anemia

- Anectdotal experiences suggest some patients benefit from erythropoietin (151,152,195,196)

Primary Myelofibrosis

- Anectdotal experiences suggest some patients benefit from erythropoietin (159,161,163,167,197)

Sickle Cell Anemia

- Rationale: increase intra-erythrocytic Hgb F to reduce sickle hemoglobin polymerization with resultant decrease in hemolysis and pain crises (197a)
  - Hydroxyurea increases intracellular Hgb F
  - Erythropoietin increases F containing reticulocytes (198)
  - Erythropoietin augments hydroxyurea effect (199)

Gaucher's Disease (200)

Thalassemia (201,201a)

Burn Patients (202)

ECONOMIC CONSIDERATIONS

The therapeutic use of recombinant proteins such as growth factors represents a notable addition to total health care costs. "This conflict between cost and quality identifies a larger conflict between the provision of individual therapeutic benefit and the societal cost of that benefit" (203).

Cost-Benefit Concerns for Erythropoietin:

- Chronic anemia infrequently impacts on survival due to ready availability of RBC transfusions (204)
- Treatment or prevention of chronic anemia impacts significantly on quality of life (psychologic, social and physical function) (115a,127a,205,206)
- Therapeutic risks of transfusion greater than erythropoietin therapy
- Societal cost of 1 unit RBC transfusion - estimated $460 (203)
  - $250-300 direct costs (product, administrative costs)
  - ~$200 indirect costs (treatment of transmitted diseases and non-medical costs to patient)
- Cost of erythropoietin in 70 kg person receiving 150u/kg TIW
  - $200 for indigent to $400 for full-pay patient per week
- Estimated cost of erythropoietin versus transfusion therapy only in anemic cancer patient for three months based on data from randomized trial outcomes (106)
  - Erythropoietin therapy (erythropoietin plus transfusion cost)
    ~$4000 for indigent patient
    ~$5700 for full pay patient
  - No erythropoietin therapy (transfusion costs)
    ~$1300
- Quality of life improved for erythropoietin responsive patients (106)

This type of comparison will undoubtedly lead to confrontations between some health care providers and policy makers.

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