Umeå University Medical Dissertations
New Series No 1069 ISSN 0346-6612 ISBN 91-7264-214-9

Department of Radiation Sciences
Umeå University, SE-901 87 Umeå, Sweden

Clinical impact of epoetins in the treatment of anemia with special emphasis on patients with lymphoid malignancies.

Dosing, iron supplementation and safety.

Michael Hedenus

2007

Department of Radiation Sciences
Umeå University, Sweden

Department of Radiation Sciences
Umeå University
SE-901 87 Umeå, Sweden

© Michael Hedenus 2007
Printed by Print & Media, Umeå University, Umeå 2007

Nog finns det mål och mening i vår färd -
men det är vägen, som är mödan värd.

Karin Boye ur "I rörelse"

List of original papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I Hedenus M., Hansen S., Taylor K., Arthur C., Emmerich B., Dewy C., Watson D., Rossi G. and Österborg A.; Randomized dose-finding study of darbepoetin alfa in anaemic patients with lymphoproliferative malignancies: *Br J Haematol* 2002; **119**, 79-86.

II Hedenus M, Adriansson M, San Miguel J, Kramer M, Schipperus M, Juvonen E, Taylor K, Belch A, Altés A, Martinelli G, Watson D, Matcham J Rossig G and Littlewood T; Efficacy and Safety of Darbepotein alfa in Anaemic Patients with Lymphoproliferative Malignancies: a Randomised, Double-blind, Placebo-controlled Study: *Br J Haematol* 2003; **122**(3):394-403.

III Hedenus M, Birgegård G, Näsman P, Ahlberg L, Karlsson T, Lauri B, Lundin J, Lärfars G and Österborg A: Addition of intravenous iron to epoetin beta increases hemoglobin response and decreases epoetin dose requirement in anemic patients with lymphoproliferative malignancies: a randomized multicenter study: *Leukemia*, 2007 Jan 25 (Epub ahead of print).

IV Hedenus M, Vansteenkiste J, Kotasek D, Austin M, Amado R.; Darbepoetin alfa for the treatment of chemotherapy-induced anemia: disease progression and survival analysis from 4 randomized, double-blind, placebo-controlled trials. *J Clin Oncol* 2005; **23**:6941-6948.

The papers I-IV are reprinted with the permission of the publishers

List of contents

ABBREVIATIONS 9

INTRODUCTION AND AIMS 10

BACKGROUND 12

- Definition and prevalence of anemia in cancer patients 12
- Pathophysiology of cancer-related anemia and iron deficiency 12
- Assessment of iron deficiency 14
- Cancer-related anemia and changes in quality of life 15
- Human erythropoietin and recombinant erythropoiesis stimulating agents 16
- How to treat cancer-related anemia? 17
- When to start and stop epoetin treatment? 18
- How to dose epoetins? 19
- What are the major goals of epoetin treatment? 20
- Prediction of hemoglobin response 21
- The role of iron supplementation during epoetin treatment 22
- Is treatment with epoetins hazardous? 22
- Is treatment with intravenous iron hazardous? 23

PATIENTS AND METHODS 25

- Paper I Dose finding of darbepoetin alfa 25
- Paper II Dose confirmation of darbepoetin alfa 26
- Paper III Intravenous iron supplementation 27
- Paper IV Disease progression and survival 28

RESULTS 31

- Paper I 31
- Paper II 32
- Paper III 33
- Paper IV 34

DISCUSSION 38

- Dosing of darbepoetin alfa 38
- Impact of intravenous iron-supplementation – the NIFe study 39
- Prediction of response 40
- Safety of treatment with darbepoetin alfa 42

CONCLUSIONS 44

FUTURE PERSPECTIVES 45

ACKNOWLEDGEMENTS 46

DISCLOSURE OF INTEREST 48

REFERENCES 49

PAPERS I – IV

ABBREVIATIONS

ACI	=	Anemia of chronic inflammation
AE	=	Adverse event
ASCO	=	American Society of Clinical Oncology
ASH	=	American Society of Haematology
BM	=	Bone marrow
CHr	=	Reticulocyte hemoglobin content
CIA	=	Chemotherapy induced anemia
CLL	=	Chronic lymphocytic leukemia
CRD	=	Chronic renal disease
DA	=	Darbepoetin alfa
ECAS	=	European Cancer Anemia Survey
EOS	=	End of study
EOT	=	End of treatment
EPO	=	Erythropoietin
EPO-R	=	EPO-receptor
EORTC	=	European Organisation for Research and Treatment of Cancer
ESA	=	Erythopoeisis stimulating agents
FACT-An	=	Functional Assessment of Cancer-Therapy-Anemia
FID	=	Functional iron deficiency
Hb	=	Hemoglobin
HE	=	Hypochromic erythrocytes
HR	=	Hazard Ratio
ITT	=	Intention to treat (analysis)
IU	=	International units
IV	=	Intravenous
K-M	=	Kaplan-Meier
LPD	=	Lymphoproliferative disease
MM	=	Multiple myeloma
NCCN	=	National Comprehensive Cancer Network
NCI	=	National Cancer Institute
NHL	=	Non-Hodgkin's lymphoma
OS	=	Overall survival
PFS	=	Progression-free survival
PK	=	Pharmacokinetic
PP	=	Per protocol
PS	=	Performance status
QoL	=	Quality of life
RBC	=	Red blood cells
RES	=	Reticulo-endothelial system
rHuEPO	=	Recombinant human erythropoietin
SC	=	Subcutaneously
sTfR	=	Soluble transferrin receptor
TSAT	=	Transferrin saturation
WHO	=	World Health Organization

INTRODUCTION AND AIMS

Anemia is a common complication in patients with cancer (*Caro JJ 2001*) leading to an impaired quality of life (*Cella D 1998*). Treatment of chemotherapy induced anemia (CIA) with recombinant erythropoietic stimulating agents (ESAs) is fairly efficient and improves quality of life, even in patients with mild to moderate anemia *(Crawford J 2002).*

Administration of recombinant human epoetins (rHuEPO) alfa and beta is indicated subcutaneously three times per week, although it has more recently been shown that epoetin alfa once weekly may be possible in patients with CIA in solid tumors *(Witzig TE 2004)* and epoetin beta in lymphoproliferative disorders (LPD) *(Cazzola M 2003)*. It has been demonstrated that in vivo activity of erythropoietin (EPO) increases with increasing sialic acid content on the carbohydrate portion *(Egrie JC 2003)* and is associated with a longer serum half-life *(Mcdougall IC 1999)*. Darbepoetin alfa (DA) contains two extra N-linked carbohydrate chains and appears to be effective for the treatment of anemia when administered less frequent than rHuEPO to patients with solid tumors receiving chemotherapy *(Glaspy JA 2002)*. However the adequate dose of DA in patients with LPD and CIA was not assessed at the time this thesis was planned.

In contrast to oral iron supplementation, the intravenous (IV) route has been shown to reverse functional iron deficiency (FID) in patients with chronic renal diseases (CRD) receiving ESAs and thereby reduce the weekly ESA requirement *(Lotacelli F 2004)*. Preliminary data from two randomized studies in patients with various types of malignancies with CIA, indicate that the response to ESA significantly improved when IV iron was given to correct FID compared to patients who received oral iron supplement or epoetin alfa alone *(Auerbach M 2004, Henry D 2006)*. In moderately anemic patients with clinically stable LPD not receiving chemotherapy during ESA treatment the clinical impact of concomitant IV iron supplementation has not yet been studied.

Reduced survival in mainly non-anemic patients with solid tumors receiving epoetin alfa and beta has been reported by two trials *(Henke M 2003, Leyland-Jones B 2005)*. Although both of these studies were investigational in nature, neither used epoetins within currently approved indication. However the possibility of an adverse treatment effect of ESAs within approved indication cannot be excluded.

Treatment with ESAs is expensive and only about 40-70% of patients with CIA will respond *(Mano M 2005)*. The identification of factors that could enable the clinician to predict the hematological response to ESA therapy in individual patients would be of great value. A variety of variables have been suggested as predictors of good response in anemic patients with mainly solid tumors but none established (*Littlewood T 2003)*. It remains to assess parameters that might be predictive of Hb response in anemic patients with LPD.

The specific aims of this thesis were:

- To determine the relevant dose of DA in patients with LPD and CIA (paper I, II).
- To study the clinical value and impact on laboratory iron variables of intravenous iron supplementation combined with epoetin-beta treatment in mild or moderate anemic iron repleted patients with clinically stable LPD (paper III).
- To assess new or evaluate previously proposed factors that might predict hemoglobin response to treatment with ESAs in anemic patients with LPD (paper I, II).
- To investigate short-term and long-term safety of DA in anemic patients with LPD and solid tumors (paper IV).

BACKGROUND

Definitions and prevalence of anemia in cancer patients

Definitions of anemia vary internationally (Table 1), with the World Health Organization (WHO) and the US National Cancer Institute (NCI) classifying anemia by grade (0-4, with 0 representing "normal" and 4 the most "severe"). In these classifications schemes, more severe anemia grades are identical in terms of Hb thresholds, but less severe grades are identified by slightly different Hb levels.

Table 1. Anemia classifications schemes

	EORTC	NCI	WHO	CCCN
MILD	10.0-11.9	10.0-WNL	9.5-10.9	10-11
MODERATE	8.0-9.9	8.0-10.0	8-0-9.4	8-10
SEVERE	<8.0	6.5-7.9	6.5-7-9	<8
LIFE-THREATING	NA	<6.5	<6.5	NA

NA=not applicable; WNL=within normal limits (12-16 g/dL for women, 14-18 g/dL for men).

Anemia (Hb < 10.5 g/dL) exists in more than 50% of patients with multiple myeloma (MM) at the time of diagnosis *(Kyle RA 1975)* or other lymphoproliferative disorders (LPD) *(Moullet I 1998)*. The incidence of anemia, taking into account all grades, is even more frequent in patients receiving chemotherapy. Seventynine percent of lymphoma patients receiving CHOP treatment develop mild/moderate anemia and up to 49 % grade 3 or 4 *(Groopman JL 1999)*.

A more recent prospective European Cancer Anemia Survey (ECAS) exploring anemia in 14 367 patients with cancer of any kind reported that prevalence of anemia at enrollment and during the 6 months survey was 39% and 67% respectively and specifically for lymphoid malignancies even higher, 49 % and 73 % respectively. At enrollment mild anemia was recorded for 29.3%; moderate anemia for 8.7% and severe anemia for 1.3% of patients. When analyzed by cancer treatment status at enrollment, overall 31.7% of patients not receiving cancer treatment were anemic versus 50.5% of patients receiving chemotherapy (*Ludwig H 2004)*.

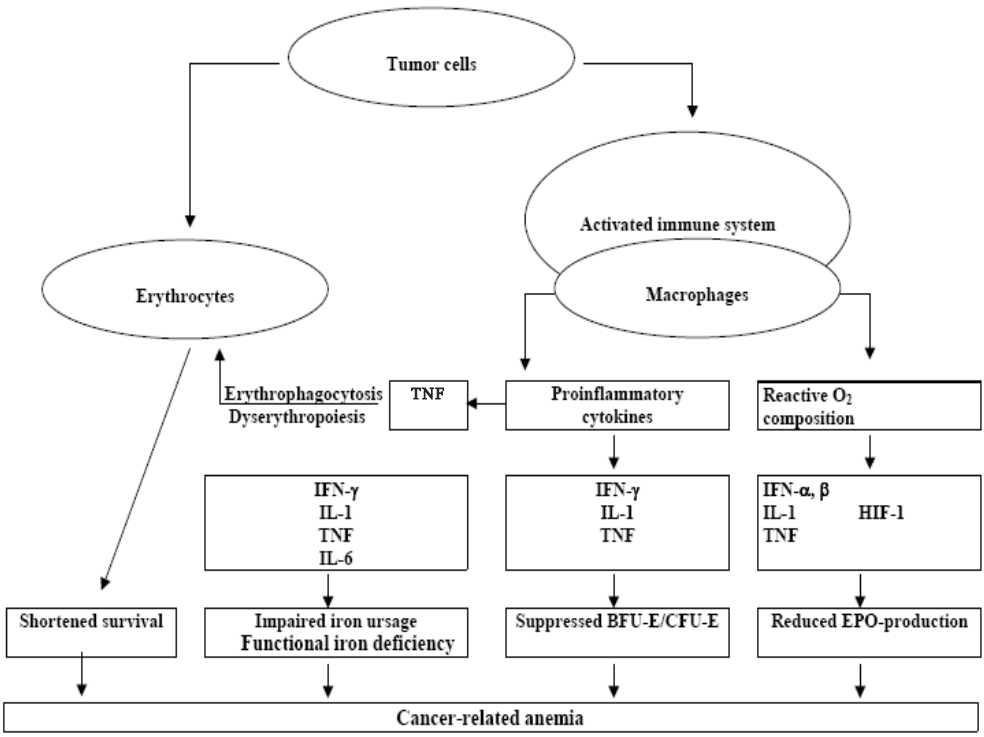
Pathophysiology of cancer-related anemia and functional iron deficiency

A variety of factors contribute to anemia in cancer patients including chronic bleeding, haemolysis, nutritional deficiency and bone marrow (BM) invasion by tumor cells. Chemotherapy and radiotherapy cause proliferation arrest and death of erythroid precursor cells *(Spivak JL 1994, Nowrousian MR 2002)*.

However major contributors to cancer-related anemia is depression of hemoglobin (Hb) values, a condition termed the anemia of chronic inflammation (ACI) or anemia of chronic disease caused by inflammatory cytokines (figure 1a). Interleukin-1β, interferon gamma, tumor necrosis factor-α and interleukin-6 play prominent roles in this respect (*Nowrousian MR 2002, Weiss G 2005)*. In patients with ACI the proliferation and differentiation of erythroid precursors

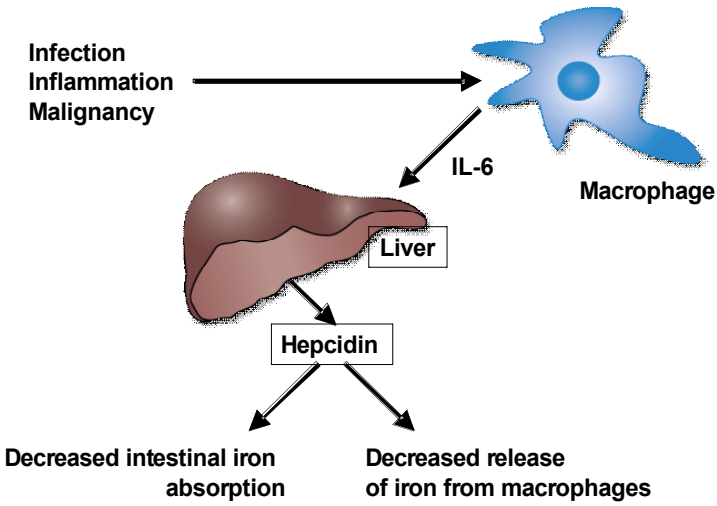
are impaired by cytokine mediated down-regulation of the expression of erythropoietin (EPO) receptors, induction of apoptosis (*Means RT 1999)* and direct toxic effects *(Maciejewski 1995)*. Also impaired production and activity of EPO has been demonstrated *(Miller CB 1990, Faquin WC 1992)* and increased erythrophagocytosis during inflammation leads to a decreased erythrocyte half-life *(Spivak JL 2002)*.

Figure 1a. Pathophysiology of cancer-related anemia



Moreover inflammatory cytokines interfere with Hb synthesis because of dysregulation of iron metabolism (*Nemeth E 2004)* commonly called functional iron deficiency (FID). This has been reported in patients with different chronic diseases and ACI *(Nemeth E 2004, Viatte L 2006, Rivera S 2005, Weiss G 2005)*. Inflammatory cytokines (especially IL-6) induce the synthesis of hepcidin (figure 1b). Hepcidin is a peptide hormone secreted by the hepatocytes, which regulates cellular iron efflux and distribution in the body. By degrading ferroportin, the main exporter of iron from cells, hepcidin decreases the availability of iron to the developing normoblasts by decreasing accessibility of storage iron from the reticuloendothelial system (RES) and from enterocytes of the gastrointestinal tract *(Weinstein DA 2002, Roy and Andrews 2005, Nemeth and Ganz 2006)*.

Figure 1b. Pathophysiology of Functional Iron Deficiency (FID)



FID may be described as a situation in which the individual has adequate iron stores, but insufficient available iron at the site of erythroblast production resulting in an iron restricted erythropoiesis. This can be encountered in two different situations corresponding to either decreased iron supply, as in ACI, or when iron needs are increased by stimulation of BM erythroid activity; the best example being provided by therapy with Erythropoietic Stimulating Agents (ESAs).

FID has been shown to occur in many different populations with ACI receiving ESA therapy but has been best described in chronic renal disease (CRD) *(Lotacelli F 2004)* and is proposed to be one of the major reasons for not responding to ESA treatment *(Glaspy J and Cavill I 1999; Glaspy and Beguin 2005, Cavill I 2002, 2006*).

Assessment of Iron Deficiency

In routine clinical practice, iron status is commonly assessed using ferritin, serum iron and transferrin saturation (TSAT). Serum ferritin indicates the amount of iron stored in RES. In healthy subjects, absolute iron deficiency occurs when iron stores are depleted (serum ferritin <12-30 ug/L) and iron delivery to the erythroid BM is impaired (TSAT <15 %).

Serum iron decreases during inflammation but TSAT is usually normal *(Elin RJ 1997)*. However approximately 20 % of patients with ACI have low TSAT (as low as 10 percent), even though only about one-quarter of such patients are truly iron deficient *(Schilling RF 1991).*

Also serum ferritin increases independently of iron stores during states of inflammation or infection *(Elin RJ 1997)* and due to release of intracellular ferritin from damaged cells (e.g. liver disease). Thus ferritin values in serum of >100 ug/L are commonly found in cancer patients.

Despite the limitations of measuring these iron parameters FID has been proposed to be characterized by serum ferritin <100 ug/L or TSAT <20 % (*Henry D 1998, Lotacelli F 2004).* But as inflammation has an influence on ferritin and TSAT, other more specialized markers have come

into use, such as the percentage of hypochromic erythrocytes (HE), reticulocyte Hb concentration (CHr) and soluble transferrin receptor in serum (sTfR) .

HE is an indirect measurement of how much iron is actually entering the red blood cell and like CHr is measured by an automated blood cell analyzer. Both have been established useful in anemia related to CRD *(Mast AE 1999, Lotacelli F 2004, Brugnara C 2006)*.

Levels of sTfR in serum are helpful in identifying healthy subjects with subclinical iron deficits *(Souminen P 1998)*. sTfR is increased in iron depleted subjects but is also an indirect measure of erythropoietic activity, reflects the erythroid mass and thus is increased in polycythemia vera, hemolysis and during treatment with ESAs *(Skikne BS et al 1990, Punninen K 1997; Junca J 1998)*. TfR expression is reported to be down-regulated by inflammatory cytokines. Therfore the ratio of sTfR/ log ferritin has been proposed to differentiate patients with ACI and iron-restricted erythropoiesis but normal iron stores from patients who also are iron depleted *(Punninen K 1997, Souminen P 1998, Thomas C 2000).*

However even with these new markers the evaluation of iron status is problematic in patients with ACI or cancer *(Junca J 1998; Wians FH 2001, Mast AE 2002).* Therefore, the gold standard is still considered to be the assessment of iron in a BM aspirate stained with Prussian blue, although unreliable when negative *(Barron BA 2001)*. In ACI macrophages in BM will contain normal or increased amounts of storage iron, while erythroid precursors usually show decreased or absent staining for iron *(Ellis LD 1964).*

Cancer-related anemia and changes in quality of life

Cancer-related fatigue is a multifactorial and prevalent problem (*Portenoy RK 1994, Cella D 2004).* In fact fatigue has been reported by the majority of cancer patients to have a more significant impact on their lives than pain *(Vogelzang NJ 1997, Stone P 2000)*. Patients also report that fatigue interferes with their own and their caregivers' careers or economic status *(Curt G 2000, 2003, StoneP 2003)*.

Cancer-related anemia is a major cause of fatigue with a powerful negative impact on patient quality-of-life (QoL) *(Portenoy RK 1994, Stone P 2000, Cella D 2002)*. Results of ECAS clearly show that Hb levels significanctly correlated with mean performance status (PS). Poor PS (scores of 3-4) was uncommon in patients with higher Hb values, but much more frequent in patients with lower Hb levels *(Ludwig H 2004).*

To aid in the interpretation of QoL results of outcomes in cancer anemia from clinical trials the most used tool is the Functional Assessment of Cancer-Therapy-Anemia (FACT-An) scale *(Cella D 1997)*. FACT-An, a part of the functional assessment of chronic illness therapy (FACIT) measurement system for patients with cancer and other chronic illnesses has been validated in studies involving cancer *(Cella D 1997, Littlewood T 2001)* and in a nationally representative sample from a general US population *(Cella D 2003)*.

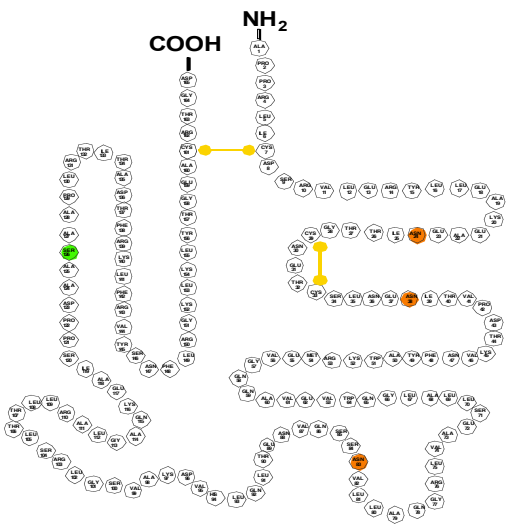
The FACT-An subscale is a 55-item cancer-specific questionnaire measuring physical, social, emotional and functional wellbeing as well as an additional 20-item questinonnaire (Anemia subscale) that measures 13 fatigue-associated items (Fatigue subscale) and seven non-fatigue-associated items. Each of these measures is scaled with low scores indicating poor QoL and high scores indicating good QoL.

The Fatigue subscale scores from 0-52. Substantial fatigue has been defined as a score below 30 points. Linking QoL score changes to Hb levels results in clinically meaningful improvement in fatigue has been defined as an increase of at least 3 points (*Cella D 2002*).

Human erythropoietin and recombinant erythropoiesis stimulating agents

EPO is a 165-amino acid glycoprotein with a ~40% carbohydrate content and a molecular weight of 30,400 daltons (figure 2), that stimulates red blood cell (RBC) production and is produced primarily in the kidney in adults.

Figure 2. Molecule of rHuEPO



EPO synthesis increases in response to tissue hypoxia and is likely to be metabolised in the kidney, liver and in the BM. The human homeostatic mechanism results in an increase in EPO production as Hb levels fall below 12 g/dL, suggesting an importance for maintaining normal Hb-levels *(Finch CA 1982)*. Chemotherapy also influences endogenous EPO production. After a course of chemotherapy a transient increase of serum EPO has been observed *(Birgegård 1989, Glaspy J 2005)*.

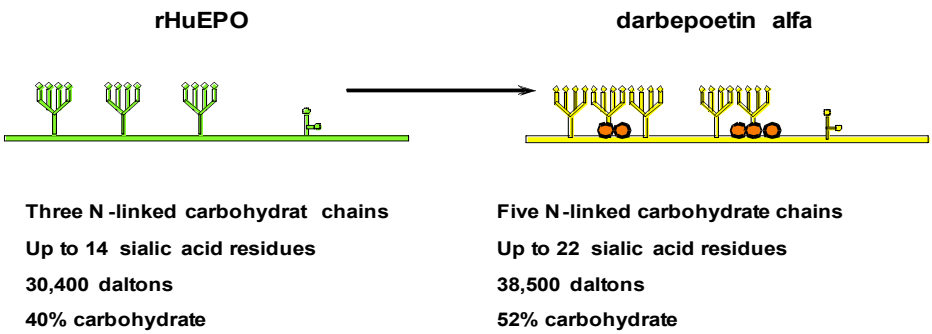
EPO regulates proliferation, apoptosis and differentiation of committed erythroid progenitors in the BM by binding to the cell surface EPO-receptor (EPO-R) and activating an intracellular phosphorylation cascade (*Mulcahy L 2001)*. JAK2, a tyrosine kinase, is activated and this results in the production of reticulocytes which are released into the blood to mature into RBC (*Syed RS 1998)*. Expansion of the erythropoietic BM in response to EPO is very gradual and it requires several weeks for maximum activity to be achieved *(Beguin Y 1995)*.

Utilizing DNA technology, recombinant human EPO (rHuEPO) can be produced by mammalian cells into which the human EPO gene has been introduced. rHuEPO has the same molecular structure, weight, carbohydrate content (that binds sialic acid), amino acid sequence, and receptor binding affinity as endogenous EPO. rHuEPO stimulates erythropoiesis in precisely the same

way *(Jelkmann W 1990, Darling RJ 2002)* and has the same pharmacokinetic (PK) properties as endogenous EPO *(Ramakrishnan R 2004)*.

It has been demonstrated that in vivo activity of EPO increases with increasing sialic acid content *(Egrie JC 2003)* with a theoretical maximum content of 14 sialic acid molecules *(Elliot S 2003)*. Structural modifications by two additional carbohydrate chains were made to rHuEPO to produce darbepoetin alfa (DA), which resulted in an increased serum half-life and decreased receptor-binding affinity (*Egrie JC 2001, 2003, Elliot S 2003*) (figure 3).

Figure 3. Comparison of the physiochemical properties of rHuEPO and darbepoetin alfa.



Based on equivalent peptide mass it has been proposed that therapeutically equivalent doses of epoetin alfa or beta can be determined by using a ratio of 200 IU to 1 µg DA. Using this ratio the DA doses (2.25-4.5 µg/kg) correspond to weekly doses of 450-900 IU/kg of rHuEPO alfa or beta or approximately a total of 30,000 - 60,000 IU weekly *(Macdougall IC 1999; Lotacelli F 2004)*.

PK studies with DA have been completed in a range of patient populations and healthy volunteers. In those studies in which comparisons to rHuEPO have been done, DA was consistently cleared more slowly suggesting that dosing with DA subcutaneously (SC) less frequently than the 3-times-weekly doses might be effective (*Heatherington AC 2001; Allon M 2002*; *Glaspy J 2002*).

How to treat cancer-related anemia?

First and foremost, other causes of anemia (e.g. bleeding, haemolysis, nutritional deficiency) and hereditary causes should be ruled out. According to the ECAS only about 50 % of anemic cancer patients receive any kind of treatment for their anemia. The mean trigger threshold for ESA therapy is 9.4 g/dL but for blood transfusions as low as 8.3 g/dl *(Ludwig H 2004)*.

Although there is extensive experience with bloodtransfusions and this is the most rapid means to treat anemia, safety considerations may have dampened the appeal. Blood transfusions pose several adverse effects to the recipient including immunological reactions to the blood group, leukocytes, platelets, serum antigens, transmissible infectious agents, immune suppression, graft-vs-host disease and iron overload *(Walker RH 1987, Lawrence T 1999)*. Also it is difficult to obtain a stable Hb as the increased Hb levels achieved with blood transfusion return to baseline values

within several days or a few weeks. The cost for one blood unit in Sweden has been estimated to about 200-400 Euros *(Glenngård AH 2005, Jidell E 2006)*. Although randomized prospective cost effectiveness studies comparing optimal transfusion therapy with ESA treatment have not yet been conducted.

With the exception of life-threatening or severe anemia (Hb <8 g/dL), ESA therapy has been included in all important guidelines and has increasingly been recognized as an alternative to blood transfusions as the standard of care for cancer patients in many countries. Treatment with recombinant ESAs is well tolerated and leads to increased Hb levels (albeit not in more than 50–70% of patients) over an extended time, thereby diminishing the need for blood transfusion in about 50% of patients with solid tumors (*Demetri GD 1998, Seidenfeld J 2001*, *Littlewood T 2001, Gabrilove J 2001, Bohlius J 2004*). Similar results have been reported in anemic patients with LPD (*Ludwig H 1990, Cazzola M 1995, 2003; Österborg A 1996, 2002, Glaspy J 1997, Dammacco F 1998, 2001).*

When to start and stop epoetin-treatment?

Although in severe cases immediate blood transfusion may be necessary, once the patient has been diagnosed with chemotherapy induced anemia (CIA), therapy with EASs should be considered based on both degree of anemia and clinical symptomatology according to published guidelines by cancer organisations in the US (*Rizzo JD 2002, NCCN 2006*) and Europe *(Bokemeyer C 2004, 2006*) summarised in table 2.

Table 2. Comparison of existing guidelines on the use of ESAs in cancer

	ASCO/ASH 2002	**EORTC 2006**	**NCCN 2006**
Hb value to ***start*** treatment with ESAs **Recommendation**	ESAs is recommended for patients with chemotherapy-associated anemia and **Hb declined to a level ≤ 10 g/dL**.	In cancer patients receiving **chemotherapy and/or radiotherapy or <u>not</u>**, treatment with ESAs should be initiated at **Hb 9-11 g/dL, based on anemiarelated symptoms**	Strongly consider ESAs **in symptomatic patients with Hb < 10 g/dL;**
Hb value to ***start*** treatment with ESAs **Consideration**	For patients with declining Hb but less severe anemia (Hb < **12** g/dL), the decision of whether to use ESAs immediately or to wait until Hb fall closer to 10 g/dL should be determined by **clinical circumstances**.	ESAs may be considered in **asymptomatic, anemic patients with Hb ≤11,9 g/dL**, to prevent a further decline in Hb, according to individual factors (e.g., type/intensity of chemotherapy, baseline Hb)	Consider ESAs in **symptomatic patients with Hb 10 – 11 g/dL;** Consider ESAs in **asymptomatic patients with Hb 10 –11 g/dL, with risk factors for symptomatic anemia**
Hb value to ***stop*** treatment with ESAs	**Hb levels can be raised to (or near) 12 g/dL**, at which time the dosage of epoetin should be titrated to maintain that level or restarted when the level falls to near 10 g/dL.	Treatment should be continued **as long as Hb level remains ≤12-13 g/dL**, and patients show symptomatic improvement.	ESAs dosage should be titrated to maintain **optimal Hb value (12 g/dL)**

The guidelines of the American Society of Clinical Oncology (ASCO) and the American Society of Hematology (ASH) advocate that physicians caring for patients with LPD are advised to begin treatment with chemotherapy and/or corticosteroids and observe haematologic outcomes achieved solely through tumor cell reduction before considering treatment with ESAs *(Rizzo JD 2002)*. Although the level of evidence regarding this specific recommendation for lymphoid malignancies was regarded as low.

Since the ASH/ASCO guidelines were published, several large randomized, placebo-controlled, double-blind trials only in patients with lymphoid malignancies receiving concurrent chemotherapy demonstrated that treatment with ESAs significantly increased Hb levels, improved QoL and reduced transfusion need (*Damacco F 2002, Österborg A 2002, Cazzola M 2003)*. This has been acknowledged in the more recent National Comprehensive Cancer Network (NCCN) and European Organisation for Research and Treatment of Cancer (EORTC) guidelines.

Recent reported studies have suggested that there might be clinical benefits with early intervention with ESA treatment for CIA *(Lyman GH and Glaspy J 2006)*. Thus use of ESAs, according to the EORTC panel, can be considered also in asymptomatic patients with Hb value less than 12 g/dL to prevent further decline *(Bokemeyer C 2006)*. Also the NCCN guidelines state that these patients should be further evaluated for risk factors for developing symptomatic anemia. The risk factors listed are: transfusion in past 6 months, history of prior myelosuppressive therapy or radiotherapy to >20% of the skeleton, myelosuppressive potential of current therapy (taking into account duration, schedule, and agents), advanced age and low Hb level.

Present guidelines are based on studies where chemotherapy was given both prior and concomitant with treatment with ESAs. However some studies indicate that treatment with epoetins is at least as effective in increasing Hb concentration and reducing the need for blood transfusion *(Ludwig H 1995, Quirt I 2002, Smith R 2002)* or significantly increasing QoL *(Quirt I 2002)* in anemic cancer patients not receiving chemotherapy as compared with those undergoing chemotherapy. This also has been acknowledged in the EORTC-guidelines (evidence grade B).

According to the guidelines ESA treatment should be suspended at Hb level of >12-13g/dL and reinitiated if the Hb value falls to < 12 g/dL, but with a 25 -50 % reduction of the dose. The dosing may then be titrated to maintain Hb level of 12-13 g/dL (Table 2).

How to dose epoetins?

In two studies in anemic patients with LPD where patients were randomized to receive different doses of rHuEPO beta the investigators were able to confirm that a dose of 150 IU/kg 3 times SC per week produced a superior response in about 60% compared with a lower starting dose *(Cazzola M 1995, Österborg A 1996)*. The approval of epoetin beta for CIA in LPD (administered three times per week) was obtained in 2001, based on a phase III trial *(Österborg A 2002)*. In 2003, the regimen "once weekly" 30,000 IU epoetin beta per week was approved for lymphoid malignancies, based on an open label, randomized study of two parallel groups, comparing once versus thrice weekly dosing *(Cazzola M 2003)*. Likewise more recently 40,000 IU once weekly dosing of rHuEPO alfa has been proven to be similar efficient in CIA in patients with solid tumors *(Witzig TE 2005)*.

According to some recommendations the epoetin dose should be doubled, if the Hb value has not increased by 1.0 g/dL after 4 or more weeks of treatment in anemic patients with chronic

lymphocytic leukemia (CLL) and MM *(Ludwig H 2002)* or any non-myeloid malignancy (*NCCN 2006)*. As there is a lack of studies comparing dose doubling versus a maintained dose level in non-responding patients the EORTC and the ASH/ASCO guidelines advocate cessation of ESA therapy.

In anemic patients with solid cancer, DA can be administered once weekly or every two or even every three weeks SC *(Vansteenkiste J 2002, Glaspy J 2002, Kotasek D 2003)*. In order to both obtain a more rapid and higher response rate an upfront dose escalation of DA has been tested ("frontloading" concept) followed by lower maintenance schedules *(Glaspy J 2003)*. These promising findings were further explored in a double-blind, randomised phase III study in patients with non-myeloid malignancies and CIA. However the expected advantage of front-loaded dosing could not be confirmed *(Kotasek D 2007)*.

In 1999 when the dose-finding study (paper I) was planned the relevant dose of DA for CIA in patients with LPD was unkown. Until then epoetin alfa had been evaluated in a broad range of solid tumor types. Studies in the LPD setting had mainly included patients with MM *(Ludwig H 1990; Barlogie and Beck 1993; Garton JP 1995; Silvestris F 1995, Mittelman M 1997)*. In these studies epoetin alfa or beta was generally administered three times per week.

What are the major goals of epoetin treatment?

The ASCO/ASH report *(Seidenfeld J 2002*) did not find sufficient data to perform a meta-analysis with regards to the effect of ESAs and QoL. Clinical trials suitable for inclusion for such an analysis have recently emerged and two meta-analyses have been published *(Jones M 2004, Bohlius J 2004)*. The larger meta-analysis performed by Bohlius et al of data from 11,459 anaemic cancer patients in 23 trials found that ESAs significantly (P=0.05) improved QoL scores.

Also a retrospective analysis of over 4000 patients treated with ESAs in two open-label, non-randomized studies showed a correlation between Hb levels and QoL scores. The trials involved patients in community practice groups, emphasizing that the benefits might be applicable to "routine" cancer patients and not simply those in trials performed in academic centers *(Demetri G 1998, Glaspy J 1997)*.

The mitigation of fatigue with ESAs also is suggested to be associated with other beneficial outcomes including reductions in depression, anxiety (*Kallich J 2002*) and improvements in productivity (*Cremieux PY 1999)*.

Once anemia treatment is initiated subsequent considerations include target Hb level. The two studies by Demetri and Glaspy on community practice groups permitted analysis of the impact of rising Hb levels on QoL. An increase in Hb value that occurred anywhere in the range between 7.5 and 14 g/dL correlated with a rise in QoL score. The most pronounced increases in QoL scores were observed when the Hb value ranged between 11 and 12 g/dL *(Crawford J 2002)*. This finding has been questioned as another trial failed to identify an optimal Hb level for improvement of QoL *(Österborg A 2002)*. In this study an increase in Hb of at least 2 g/dL from baseline (without transfusion) correlated significantly with improvements in QoL. This is in general agreement with another study where increases in Hb concentration (as opposed to reaching a predefined target Hb level) produced the greatest improvements in QoL *(Glimelius B 1998)*.

Thus many published studies report that ESA therapy improves patient QoL by increasing Hb level and consequently current guidelines advocate that the overall goals of treating anemia in cancer patients should include correction of anemia to target Hb levels of up to 12-13 g/dL (table 2). No such data are yet available from studies focusing on blood transfusion management targeting normal Hb-levels.

Prediction of hemoglobin response

Treatment with ESAs is expensive, the median time to elevation of Hb levels is approximately 4-8 weeks and only about 50-70% of patients with CIA will respond with an increase in Hb level of ≥2.0 g/dL *(Mano M 2005)*. Thus the identification of factors that could enable the clinician to predict the hematological response to epoetin therapy in individual patients would be of great value clinically and economically.

Prognostic baseline factors such as age, gender, inflammatory cytokines and variables related to iron metabolism have not been reported to be of sufficient prognostic value *(Littlewood T 2003*). Also other baseline parameters studied such as tumor mass or BM infiltration, white blood cell count, platelet count or serum folate concentration have also not been proven to be clinically useful *(Platanias LC 1991, Barlogie and Beck 1993)*.

Particularly high Hb response rates with epoetin alfa have previously been reported in early studies in patients with MM, with response rates in excess of 75 % *(Ludwig H 1990, Österborg A 1998, Damacco F 1998)*. Consequently it has been suggested that patients with MM might respond somewhat better to ESAs than anemic patients with other lymphoid malignancies *(Marsh WA 1999)*.

Most focus has been directed towards relative endogenous EPO deficiency. This has been observed in about 75% of patients with LPD by some investigators (*Cazzola M 1995*) and has been suggested as a useful predictor of response to treatment with ESA (*Ludwig H 1990; Barlogie and Beck 1993, Cazzola M 1995; 2003; Österborg A 1996, 2002*) and a cut off level of 100 IU/mL has been proposed in patients with moderate/mild anemia (*Österborg A 2002*). However this has been questioned as the predictive power seems insufficient for clinical use *(Littlewood T 2003)*.

Until now an early Hb-increase seems to be the most widely used predictor for response to ESA treatment. An early Hb increase has been reported to be associated with higher overall haematopoietic response rates in patients with or without chemotherapy *(Glaspy J 1997)*. According to one study patients that showed a ≥1 g/dL increase in Hb levels in the first 4 weeks of treatment, 78% went on to be responders *(Quirt I 2001)*. However, a meta-analysis of data from four randomized trials in 604 patients with non-myeloid malignancies concluded that up to 46 percent of those not showing a rise in Hb by two or four weeks may ultimately respond *(Littlewood T 2003)*.

Also it has been suggested that an increase of 15-25 % in serum sTfR from baseline may have predictive value for response to ESA treatment in the LPD setting *(Cazzola M 1996, 2003)*. This interesting finding needs confirmation.

Overall, none of the parameters and models that have been tested for their potential value to predict a response or lack of response has proven useful in clinical practice *(Littlewood T 2003)*. More research to identify early predictors with greater predictive value seems warranted, unless more effective ESAs or treatment modalities are emerging.

The role of iron supplementation during epoietin treatment

Numerous studies have shown that oral iron therapy fails to maintain adequate iron stores in hemodialysis patients receiving ESA therapy *(Macdougall IC 1999, Cavill I 2002, 2006)*. This may be because oral iron is poorly absorbed in these patients, especially when the serum ferritin level is normal or elevated *(Goch J 1996)*.

In contrast several studies have shown that intravenous (IV) iron therapy effectively provides sufficient iron for optimal erythropoiesis in ESA-treated uremic patients. Moreover, in these studies IV iron supplementation has been found to reduce the weekly ESA requirement by 30 % to 50 % in patients with renal anemia (*Fishbane S 1995, Macdougall IC 1999, Besarab A 1999, Cavill I 2002, Lotacelli F 2004)*. In patients with CRD FID has been defined as serum ferritin < 100 ng/mL and TSAT of < 20 % and IV iron supplementation to ESA treatment is recommended in international guidelines *(Lotacelli F 2004)*.

In anemic cancer patients no consensus exists about the route of iron supplementation, whether oral or IV *(NCCN 2006, Bokemeyer C 2004)*. Two recent randomized studies in anemic patients with various types of malignancies treated with chemotherapy indicated that the response to epoetin alfa was improved significantly when IV iron was given simultanously compared with patients who received oral iron or epoetin alone *(Auerbach M 2004, Henry D 2006)*. However the conclusion drawn from the results has been questioned as at least a proportion of the included patients might have been iron depleted *(Beguin Y 2004)*.

Nevertheless IV iron supplementation during ESA-therapy of CIA has been recommended *(Glaspy J 1999, Beguin Y 2005, Cavill I 2006)*. When the NIFe-study was planned in 2003 the only available data were those preliminary reported by M. Auerbach in 2001.

Is treatment with epoetins hazardous?

The possible risk of increased thrombotic events with use of ESAs to raise Hb to standard target up to 12-13 g/dL has been observed in many studies (~6% epoetins vs. ~4% placebo/control). Thus it was not a surprise when the recent uppdated Cochrane meta-analysis (35 trials, n = 6769) confirmed that thrombo-embolic complications were more frequent in patients receiving ESA treatment with a relative risk of 1.67 (95% CI: 1.35-2.06) *(Bohlius J 2006)*. However there was no correlation of increased risk for thrombo-embolic events and different Hb-levels or other studied factors at baseline. Thus ESAs may be thrombogenic by mechanisms that are independent of Hb levels.

Anemia has been associated with a poor prognosis in multiple cancer settings *(Moullet I 1999)* and may even be an independent prognostic factor for survival according to a meta-analysis of 60 published studies *(Caro J 2001)*. Tumor hypoxia correlates with increased tumor aggressiveness, resistance to radiotherapy or chemotherapy and decreased patient survival *(Vaupel P 2001)*. Epoetins have been shown to stimulate non-haematopoietic tissues including physiological angiogenesis during wound healing *(Haroon ZA 2003)*. Thus decreasing tumor hypoxia by increasing Hb-concentrations or angiogenesis with ESA treatment might have a positive impact concomitant with anti-tumor therapy.

And indeed several studies, including randomized clinical trials and retrospective analyses, have reported an association between anemia correction with ESA treatment and improved outcomes (*Thomas GM 2002, Van Belle SJ 2003)* in head and neck cancer *(Becker A 2000, Glaser CM 2001)*

cervical cancer *(Höckel M 1993)*, ovarian cancer *(Munstedt K 2003)* or non-small cell lung cancer *(Langendijk H 2003)*. Moreover Littlewood et al. (2001) and Vansteenkiste et al. (2002) found in post hoc subgroup analyses a trend for progression-free survival (PFS) benefit in ESA-treated patients with mixed tumors or lungcancer respectively compared with placebo.

On the other hand there is concern that ESA treatment might have a negative impact on tumor growth. Expression of the EPO receptor (EPO-R) has been demonstrated in a variety of human cancers, including breast, prostate, colon, ovary, uterine, cervical, glioblastoma and head and neck squamous cell carcinoma *(Hardee M 2006, Österborg A 2007)* and a tumor-stimulating effect of EPO *in vitro*.has been reported in some tumor cell-lines *(Acs G 2001; Yasuda Y 2003)*.

In contrast a number of different tumor cell-lines do not demonstrate any proliferation response to ESAs *(Westphal G 2002)*. Moreover the specificity of available antibodies for identifying the presence and functionality of EPO-R at the protein level has been questioned *(Eliott S 2006, Österborg A 2007)*.

Two randomized, placebo-controlled studies conducted in metastatic breast cancer (*Leyland-Jones 2005)* and head and neck cancer (*Henke M 2003*) patients with epoetin alfa and epoetin beta respectively, have suggested increased tumor progression and decreased survival outcomes associated with epoetin treatment. But these two studies were performed in mainly non-anemic patients, were investigational in nature with neither using rHuEPO within currently approved indication. Also they have been intensively discussed regarding study design and methodology *(Kaanders and van der Kogel 2004, Leyland-Jones 2004, Österborg A 2006)*.

A recent meta-analysis of 42 trials with 8167 patients suggested that treatment of anemia with an ESA may have no impact on OS in patients with cancer (*Bohlius J 2006)*. Still the possibility of a negative treatment effect of ESAs within approved indication cannot be excluded.

Is treatment with intravenous iron hazardous?

In contrast to IV dextran iron that poses anaphylactic reactions iron sucrose is safe and no drug related deaths have been reported *(Besarab A 1999)*.

It has been shown that IV iron might increase oxidant stress (*Schreiber-Mojdehkar 2004)*. However no increased mortality has been seen in hemodialysis patients (*Feldman HI 2004*) and the available evidence does not suggest that any additional cardiovascular risk accrues in patients with CRD receiving IV iron *(Besarab A 1999, Lotacelli F 2004)*.

The reduction in relative iron availability in the presence of an infection has been interpreted as a protective reaction that retards of microbe growth by iron. In hemodialysis patients there is no evidence of increased infections rates when IV iron supplementation is administered (*Hoen B 1998*).

In vitro data indicate that iron might be carcinogenic due to its catalytic effect on the formation of hydroxyl radicals, suppression of the activity of host defence cells and promotion of cancer cell multiplication *(Weinberg ED* 1996). It has been observed in rodents, rabbits and humans that injections of iron may result in sarcoma at the sites of deposition (*Weiss G and Gordrdeuk VR 2005*).

Also hyperferremia might theoretically promote evolution of cancer as cancer cells have a higher iron requirement than their normal counterpart. This has impact on the expression of critical molecules involved in cell cycle progression and proliferation such as p53, c-myc, N-myc

and others (*Le N and Richardson DR 2002)*. As each transfused unit of blood contains about 200 mg iron the potential adverse impact on tumor progression with blood transfusions might be extrapolated to the IV iron setting. Perioperative transfusion of multiple units of blood in patients undergoing cancer surgery with no evidence of metastatic disease have been reported to be associated with enhanced risk of tumor recurrence although a survey of published reports on this matter indicated that 14 studies found an increased risk but 13 did not *(Tartter PI 1989)*.

In noncirrhotic persons with hereditary hemochromatosis the risk of hepatic carcinoma is at least 200 times greater than in normal persons *(Kew MD 1990, Weinberg ED 1996*). However of seven epidemiological studies in individuals with increased iron burden by other unspecified routes only four have reported a positive association with an increased risk of various kinds of neoplasms *(Weinberg ED 1996*).

Hypoferremia might theoretically prevent tumor evolution. In a study of almost 38.000 normal blood donors, the relative risk of developing cancer as compared with non-donors was 0.79 (P<0.001). But although reduction in body iron could be responsible for this observation the authors suggested that blood donors may lead a more healthy life than the rest of the population *(Merk K 1990)*.

Thus the relationship between IV iron or iron-overload and cancer promotion remains unconclusive and there is no consensus for an upper safety limit for serum ferritin levels for IV iron treatment. Although to avoid toxicity from iron excess in patients with CRD it is recommended that IV iron should be withheld when TSAT exceeds 50 % and/or serum ferritin is greater than 800 mg/L *(Lotacelli F 2004)*.

PATIENTS AND METHODS

All statistical analyses were carried out by use of the SAS system, version 8.2 (SAS Institute, NC, US)

Paper I (Dose finding of darbepoetin alfa)

Aim

The objectives were to assess the dose-response relationships of DA with respect to Hb and RBC transfusions and to determine a reasonable dose of DA for patients with LPD and chemotherapy induced anemia (CIA). Although not powered for such an analysis we decided to identify promising predictors of Hb response.

Patients

66 patients with indolent non-Hodgkin`s lymphomas (NHL), CLL or MM and CIA were randomized. 73 % had lymphoma and 27 % MM. Demographic characteristics were generally similar between groups, except in three patients (all in the 2.25 μg/kg cohort) with severe anemia and high baseline serum EPO levels well over 600 IU/L. Baseline serum ferritin and TSAT values indicated that included patients were not iron depleted.

The Intention to Treat (ITT) population consisted of all randomized patients who received at least one dose of study drug.

Study Design

This was a multicentre randomized, double blind, placebo-controlled, dose-finding phase II study. Inclusion critera were anemia Hb ≤11 g/dL and ongoing chemotherapy. Key exclusion critera were anemia of any other cause than cancer, ongoing inflammation or infection and impaired renal or hepatic function. Patients were not allowed to have received any RBC transfusion within 2 weeks of randomization.

Patients were randomized into four cohorts in a 1:2:2:1 ratio to receive DA 1.0 μg/kg (n=11), 2.25 μg/kg (n=22), 4.5 μg/kg (n=22) or placebo (n=11) administered SC once weekly for 12 weeks. No dose-increase was allowed. The study was stratified for MM vs lymphoma to balance the treatment groups.

The primary end point was to assess the Hb response (increase of Hb ≥2 g/dL in the absense of RBC transfusion in the previous 28 days) for the different dosing cohorts compared to placebo.

Secondary endpoints were haematopoietic response (either Hb response or an increase in Hb concentration to ≥12 g/dL in the absence of RBC transfusion), sustained Hb response (Hb response that was maintained for 28 days), the incidence of RBC transfusions from week 5 to the end of treatment (EOT) and safety of DA. The mean change from baseline in Hb was measured after 4 and 12 weeks of treatment in the abscense of a RBC transfusion in the previous 28 days

Statistics

In order to detect a dose-response relationship to Hb response with 80 % power using a significance level of 5% (one sided) a sample size of 66 patients is needed considering a drop out rate

of 20%. This study was not designed to have adequate power to make formal comparisons of the primary endpoint between the treatment groups.

Hb response proportions were estimated using the Kaplan-Meier (K-M) method and differences were evaluated with the log rank test. Cox regression models were used to assess the treatment effect of DA versus placebo, dose-response relationships and the effects of covariates (myeloma vs lymphoma; different baseline Hb levels, prior RBC transfusion or not; and baseline serum-EPO level < 100 IU/L versus > 100 IU/L) on endpoints. Effect of treatment and covariates were considered statistically significant if the p-value < 0.05.

Paper II (Dose confirmation of darbepoetin alfa)

Aim

The aim was to confirm that DA that the dose 2.25µg/kg once weekly was appropriate and safe. This study was designed and powered to evaluate the treatment effect of DA relative to placebo separately for patients with lymphoma (Hodgkin`s disease, NHL or CLL) or MM.

Patients

A similar setting was used as in the previous study (paper I), although patients with aggressive NHL were allowed to be included. 349 patients with LPD and CIA were randomized. Baseline characteristics were well balanced in the two arms, although a higher proportion of patients with high-risk NHL were included in the DA group than in the placebo group (44% vs 33%, respectively). Additionally this group contained a higher proportion of patients with Binet stage C CLL (59% vs 42%). Half of the patients had a diagnosis of lymphoma and half of MM. Seventyfive percent of the patients had a baseline serum EPO-level below 100 IU/L.

293 patients (84 %) completed the study with a similar number in both study arms. 322 patients were incluced in the ITT population (receiving at least one dose of the study drug) and 180 patients in the completers analysis (subset of patients who completed 12 weeks of treatment).

Study Design

This was a pivotal phase III multicentre, randomized, double-blind, placebo-controlled study. Patients were randomized in a 1:1 allocation to receive either DA 2.25 µg/kg or placebo, administered SC once weekly for 12 weeks. Randomization was stratified to balance the treatment groups with respect to malignancy type (lymphoma versus MM) and prior chemotherapy (heavy versus not heavy pretreated). Heavy pretreatment was defined as two or more lines of chemotherapy or one line of chemotherapy and a stemcell transplant. The dose was to be doubled for patients who had an inadequate increase in Hb (< 1g/dl) at week 4 and withheld if a patients Hb value exceeded 14-15 g/dL and reinstated at 50 % of the previous dose once Hb concentration decreased under 13 g/dL.

Primary end point was the proportion of patients achieving Hb response. The main secondary efficacy endpoints were the incidence of RBC transfusions from week 5 to end of treatment (EOT), the mean Hb-change from baseline to EOT and QoL assessment.

The primary QoL scale used in this study was the FACT-Fatigue subscale (page 15). Patients included in the analysis had to have received the study drug for at least 4 weeks, and completed

the questionnaire at baseline and at least once during the treatment phase. Patients were asked to fill in the questionnaire every 4 weeks before bloodsampling for Hb assessment and before beeing informed of their current Hb level. For patients without an EOT assessement, the last recorded data were carried forward.

Statistics

The sample size was selected to allow for the detection of Hb response rates of 25% in the placebo group and of 50 % in the DA group within each malignancy stratum with 90 % power at a two-sided significance level of 0.05. An estimated withdrawal rate of 10 % was taken into consideration.

The K-M method was used to estimate the proportion of patients with Hb-response or receiving RBC transfusion. Statistical comparisons of proportions between treatment groups were based on the chi-square test.

The mean change in Hb concentration for the ITT population was assessed by subtracting the baseline Hb value from the last value during the treatment phase. Hb values within 28 days after a RBC transfusion were excluded from the analysis.

Cox proportional regression analysises were performed to evaluate the effect of those promising co-variates on Hb responses identified in the previous study (paper I).

Paper III (Intravenous iron supplementation)

Aim

The aim was to study the correction of mild or moderate anemia and the effect on iron kinetics by rHuEPO beta treatment with or without IV iron supplementation in truly iron-repleted patients with clinically stable LPD.

Patients

Eligible were patients with a diagnosis of LPD (indolent NHL, CLL or MM) not requiring chemotherapy or blood transfusions, cancer related anemia at Hb level of 9-11 g/dL (measured on two different occasions within one month and an interval of at least 2 weeks) and with positive stainable iron in a bone marrow aspirate performed within one month before inclusion.

Baseline demographic characteristics were similar between the two treatment groups. Forty two (63%) patients had a confirmed diagnosis of indolent NHL or CLL and 25 patients (37%) had MM. The mean Hb level at baseline was 10.3 g/dL. Median serum ferritin level was 128 µg/L and median TSAT 22%.

The ITT population was defined as all randomized patients. The per protocol (PP) population was defined as patients who completed the study and received treatment for 16 weeks without receiving any RBC transfusion or chemotherapy.

Study design

This was a Swedish multicentre randomized and open label study. Stratification according to malignancy type (MM versus NHL/CLL) and baseline serum-EPO level (< 100 IU/L versus > 100 IU/L), in order to balance the two treatment groups was performed.

All patients received 30 000 IU epoetin beta SC once weekly for 16 consecutive weeks and at inclusion were randomized to receive no iron or IV iron. Iron sucrose was administered 100 mg weekly from week 0 to 6, followed by every 2 weeks from weeks 8 until 14.

Primary efficacy criteria were to compare the mean change in Hb concentrations from baseline to EOT at week 15 between the two treatment groups. Secondary endpoints were to evaluate and compare in the two treatment groups the effect of IV iron treatment on other Hb-parameters, the dose of rHuEPO beta used and the effect on iron-status.

Statistics

The size of the study population (30 patients in each group) had been chosen to detect a difference in Hb level increase of 1 g/dL, with a significance level of 5% (2-sided), and a statistical power of 80%.

To evaluate the endpoint-differences between the two groups the two sample t-test was used. The within-group analysis was performed using the paired sample t-test for correlated means. Repeated measurements analysis was used to analyze time-dependent data and multiple comparisons of continuous data were performed by analysis of variance. To evaluate variables the chi-square test was used or, in the case of small expected frequencies, Fisher's Exact Test. 5%, 1%, and 0.1% levels of significance were considered. For all end points efficacy analysises were performed in both the ITT and PP populations.

Paper IV (Disease progression and survival)

Aim

The main aim was to investigate the impact of DA on PFS and OS in cancer patients with CIA. We also wanted to assess the effect of different levels and changes in Hb concentrations and their association with PFS and OS.

Patients

Four published double blind, randomized and placebo-controlled CIA studies were included in these analyses (Table 3). Studies A and B contained long term follow up. Two of the studies (study B and D) are described in paper I and II.

Table 3. Study characteristics

Study	Reference	Tumor Type	Follow up	Number of Patients		
			Median (months)	Darbepo alfa N=708	Placebo N=421	Total N=1129
A	Vansteenkiste 2002	Lung cancer	15.8	155	159	314
B	Hedenus 2003	LPD	32.6	175	169	344
C	Kotasek 2003	Solid tumors	4.0	323	82	405
D	Hedenus 2002	LPD	4.0	55	11	66

Long-Term Follow-up: Studies A and B.

In both studies, patient baseline demographics and tumor type distribution were well balanced between groups. However, some imbalances for prognostic factors within individual tumor types were observed in Study B as described in paper II (page 26).

Short-Term Follow-up: Studies C and D.

For the short term analysis we increased the pooled sample size (n = 1,129) by including studies A, B, C and D to provide a more than 80% power to detect an effect on survival of the magnitude seen in the previous reports by Henke and Leyland Jones.

Study C included mainly anemic patients with solid tumors. In both studies, baseline demographics and disease characteristics were similar between the DA and placebo groups, except for a higher proportion of women in the placebo group in study D.

Method

In studies A and B that included long term follow-up, survival and disease status were reported by the investigators at 3 months intervals.

We performed a pooled analysis of all four studies evaluating PFS and OS in 3 different patient subsets: patients with baseline Hb concentrations < 9.0 g/dL, 9.0 to 10.5 g/dL, and ≥10.5 g/dL (table 4). These categories were selected to allow for groups of sufficient size.

Table 4. Baseline Hb subsets (Sample Size, No, % deaths and median FU)

	< 9.0 g/dL			9.0 to 10.5 g/dL			> 10.5 g/dL			Overall		
	N	Deaths (%)	Median FUP (Days)	N	Deaths (%)	Median FUP (Days)	N	Deaths (%)	Median FUP (Days)	N	Deaths (%)	Median FUP (Days)
Darbepo	149	47 (32%)	108	356	99 (28%)	107	203	57 (28%)	120	708	203 (29%)	113
Placebo	91	52 (57%)	198	225	86 (38%)	266	105	47 (45%)	204	421	185 (44%)	230

FUP = follow-up period (Note: The median follow-up time is longer in the placebo arm because fewer patients were randomized to placebo in the shorter dose finding studies. As a result, when combined with studies A and B that contained long-term follow-up, the median time of follow-up for the placebo group is longer).

We examined the potential effects of to rapid Hb increases and high Hb concentrations on PFS and OS. Because long-term mortality is unlikely to be influenced by these factors we limited the analyses to the first 12 months follow up (Table 5).

Table 5. Follow up and mortality for patients Hb ≥ 13 g/dL or rapid Hb increase

	Maximum Hb ≥ 13 g/dL			≥ 1 g/dL Rise in 14 Days*		
	N	Deaths (%)	Median Follow up (Days)	N	Deaths (%)	Median Follow up (Days)
Darbepoetin	241	60 (25%)	120	527	134 (25%)	117
Placebo	37	10 (27%)	120	249	100 (40%)	266

*The minimum Hb value among all values in the specified interval was compared with the current value to determine increases in Hb concentration in 14 days. Hb measurements on the day of a transfusion and for the next 28 days thereafter were excluded from the analysis to eliminate transfusion as the cause of Hb increases.

Statistics

The effect of DA on PFS and OS was evaluated with K-M estimates. Only patients that received at least one dose of study drug were included in the analyses. PFS and OS were measured from the time of first administration of DA until progression or death from any cause. Patients were censored at the date of last contact.

Cox regression analyses stratified by study protocols examined the relationship between treatment (DA compared with placebo) and time-dependent Hb-related covariates (baseline Hb, maximum Hb achieved, and rate of Hb increase) and their association with PFS and OS. For each stratum a distinct baseline hazard function was calculated.

RESULTS

Paper I (Dose finding of darbepoetin alfa)

Of a total of 78 screened patients from 15 centers in Europe and Australia 66 patients were randomized. All patients received at least one dose of study drug and were included in the safety and ITT analysis. Three patients (2 patients in the 4.5 µg/kg group and one in the placebo group) discontinued the study prematurely but were included in the efficacy analyses.

The median weekly weight adjusted doses administered were 0.98 µg /kg, 2.20 µg /kg and 4.40 µg /kg respectively for the three different DA cohorts. The proportion of patients requiring a dose reduction (because of an >2.0 g/dL increase in Hb during any 28 days period in the absence of RBC transfusion) was 27 % in the 1.0 µg/kg group, 23 % in the 2.25 µg/kg group and 41% in the 4.5 µg/kg group.

We observed a trend towards a linear dose-response effect for all endpoints although not statistically significant. The small number of patients in each treatment group and the heterogeneity of the population may have affected the ability to detect such an effect.

The proportion of Hb responders was 45 %, 55 % and 62 % in the three different DA dose cohorts respectively. Only 10% of patients responded in the placebo group. A higher proportion of patients achieved Hb response, haematopoietic response or sustained Hb response (definitions page 25) in the combined DA groups than in the placebo group (P<0.001 for all parameters). The shortest median time to Hb response was observed at week 8 in the 4.5 µg/kg cohort.

Also the proportion of patients receiving RBC transfusion from week 5 until week 13 was lower for the different DA dose cohorts (27%, 27% and 15% respectively) compared with the placebo group (45%).

The mean change in Hb from baseline to week 13 was 1.56 g/dl, 1.64 g/dL and 2.46 g/dL for the different dose cohorts compared with a mean change of only 1.00 g/dL in the placebo group.

A Cox regression analysis to identify possible baseline predictors of endpoints was performed (table 6).

Table 6. Effects of baseline covariates on end-points

Covariates	Hb Response	Hb Correction Hb ≥12 g/dl	RBC transfusion Wk 5-EOT
Hb > 10 vs < 10g/dL	NS	P=0.001	P=0.004
S-EPO >100 vs <100IU/L	NS	P=0.002	P=0.013
Prior RBC transf. Yes vs No	P=0.001	P=0.001	P<0.0001

NS=not significant EOT=end of treatment RBC=red blood cell

The median serum ferritin value decreased with increasing DA dose, suggesting an increased mobilization of iron for Hb synthesis. The median minimum TSAT value was 14 % in the DA group and 18.5% in the placebo group during the study course.

Increases in Hb concentrations were well controlled in patients receiving DA. 40 % of patients receiving different doses of DA experienced a rapid rise in Hb (≥ 2 g/dL within a 28 days period); 27 %, 32% and 55% respectively. Additionally three patients exceeded maximum Hb

concentration (15 g/dL for men and 14 g/dL for women), although all Hb concentrations for all patients remained within normal limits. No obvious clinical sequelae were observed in these individuals. The safety profile of DA was similar to placebo.

Paper II (Dose confirmation of darbepoetin alfa)

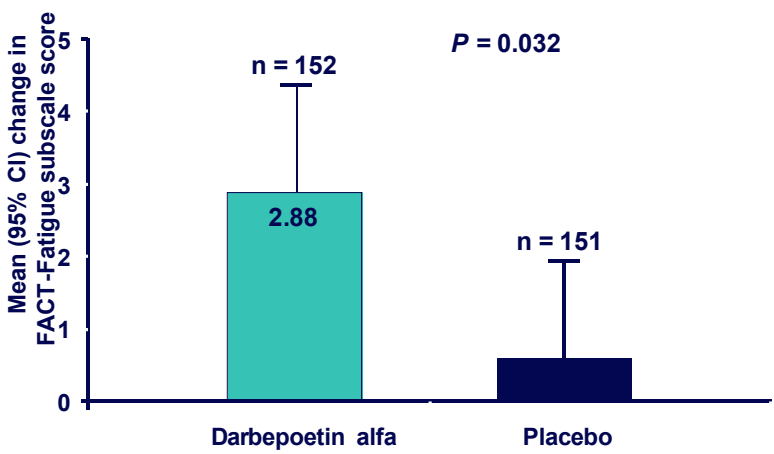
Fortynine recruiting centers in Europe, Australia and Canada randomized 349 patients. Five patients withdrew from the study before receiving the first dose of study drug. Dose-doubling was performed according to protocol in 43 % of the patients receiving DA compared to 64 % in the placebo group.

The KM proportion of patients achieving Hb response was 60% in the DA group compared to only 18% in the placebo group (P<0.001). DA also resulted in higher mean changes in Hb from baseline than placebo according to the "completers" analysis (patients receiving study drug for 12 weeks) 2.66 g/dL versus 0.69 g/dL. Also a significantly (P<0.001) lower percentage of patients in the DA group (31%) received RBC tranfusion than in the placebogroup (48%).

The efficacy of DA was consistent for all end points independent of malignancy type. 64 % of patients with lymphoma and 56 % with MM achieved Hb response (P =0.926). Only a trend (P=0.163) towards increased proportion of patients achieving Hb response was observed in patients with serum EPO levels less than 100 IU/L compared to patients with higher levels (69 % versus 44 %).

A significant improvement in QoL was also observed with the study drug compared to placebo (Figure 4).

Figure 4. Mean change from baseline to EOS in FACT-F subscale score



Patients who were most fatigued at baseline reported the largest improvement of fatigue. For every 1 g/dL increase in Hb, the estimated mean increase in FACT Fatigue subscale score was 1.39 (95% CI: 0.83-1.94). More detailed QoL data from this study has recently been reported elsewhere *(Littlewood T 2006).*

The safety of DA with the tested dose was confirmed. Adverse events (AEs) were evenly distributed between the two treatment arms. Fourteen patients died during the study or within 30 days after the last dose of study drug and none were considered to be related to the study drug by the investigators. Twelve patients (5%) in the DA group and three patients (2%) in the placebo group ha a thrombotic event. No relationship was observed between the incidence of thrombotic events and high or increasing Hb concentrations.

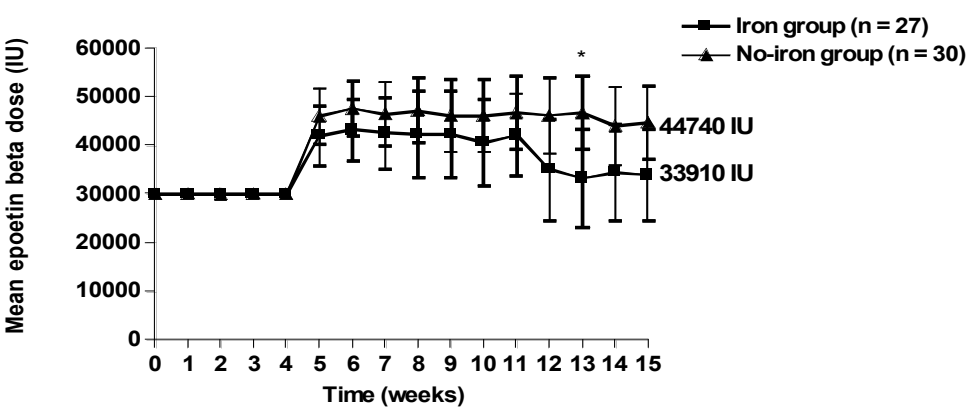
Paper III (Intravenous iron supplementation)

Mean change in Hb from baseline to EOT in the PP population was 2.91 versus 1.50 g/dL (P <0.0001). Also the increase of mean Hb from baseline and during the study was more rapid and significantly greater from week 8 and onwards in the iron group than in the no-iron group. At EOT there was a significant difference (P <0.0001) in mean Hb between the two groups of 1.24 g/dL (95% CI : 0.65 - 1.82) in favour of the iron group.

Also Hb response was achieved more rapidly and in significantly (P = 0.0012) more patients in the iron group than in the no-iron group (93% versus 53%, PP population). Median time to achieve Hb response was 6 weeks in the iron group compared with 12 weeks in the no-iron group.

Epoetin beta dose doubling was more common in the no-iron group compared with the iron group and dose reductions or doses withheld were more common in the iron group. Due to these dose modifications in the PP population there was an increasing difference from week 5 and onwards in the mean weekly epoetin patient dose in favor of the iron group group (Figure 5). The difference between the two groups was statistically significant at week 13 (P =0.029). At week 15, the average difference was more than 10 000 IU or approximately 25% lower in the iron group than in the no-iron group. Also the mean total cumulative patient dose of epoetin was greater, 626 600 IU in the no-iron group versus 511 400 IU in the no-iron group (P =0.051) for the PP population and 629 000 IU and 532 000 IU for the ITT population (P =0.059).

Figure 5. Mean (95% CI) weekly patient epoetin dose in each treatment group.



*Significant difference (p = 0.029) between treatment arms

In the no-iron group, there was a rapid decrease in mean serum ferritin and the level continued to decrease until at the end of study. In contrast, in the iron group there was an increase in the mean ferritin concentration and TSAT levels during the study. The mean serum level of sTfR increased similar in both groups. There was a very rapid increase in mean reticulocyte count and the levels remained similar elevated throughout the study in both groups.

There appeared to be a significant ($P = 0.004$) association between low TSAT (<20%) or low serum ferritin (<100 µg/L) at baseline and a subsequent Hb response, although Hb response rates were high irrespective of ferritin or TSAT values (Table 7).

Table 7. Association between baseline TSAT or ferritin values and Hb response in each treatment group (PP).

	Percentage responders (%)		
Baseline value	No-iron group	Iron group	P value
TSAT <20%	46	100	0.0041
TSAT ≥20%	58	86	0.0856
Ferritin <100 µg/L	50	100	0.0040
Ferritin ≥100 µg/L	55	85	0.0778

There were no unexpected untoward events in either study group. The incidence of thromboembolic (one suspected fatal pulmonary embolism and one thrombophlebitis related to iron injection) and serious cardiovascular events (6 events) were as expected in this elderly patient population studied. 21% of patients in the no-iron group and 39% in the iron group exceeded Hb >14 g/dL during the study ($P = 0.090$). The highest Hb level 15.8 g/dL was recorded in the iron group.

Paper IV (Disease progression and survival)

Follow up. Study A

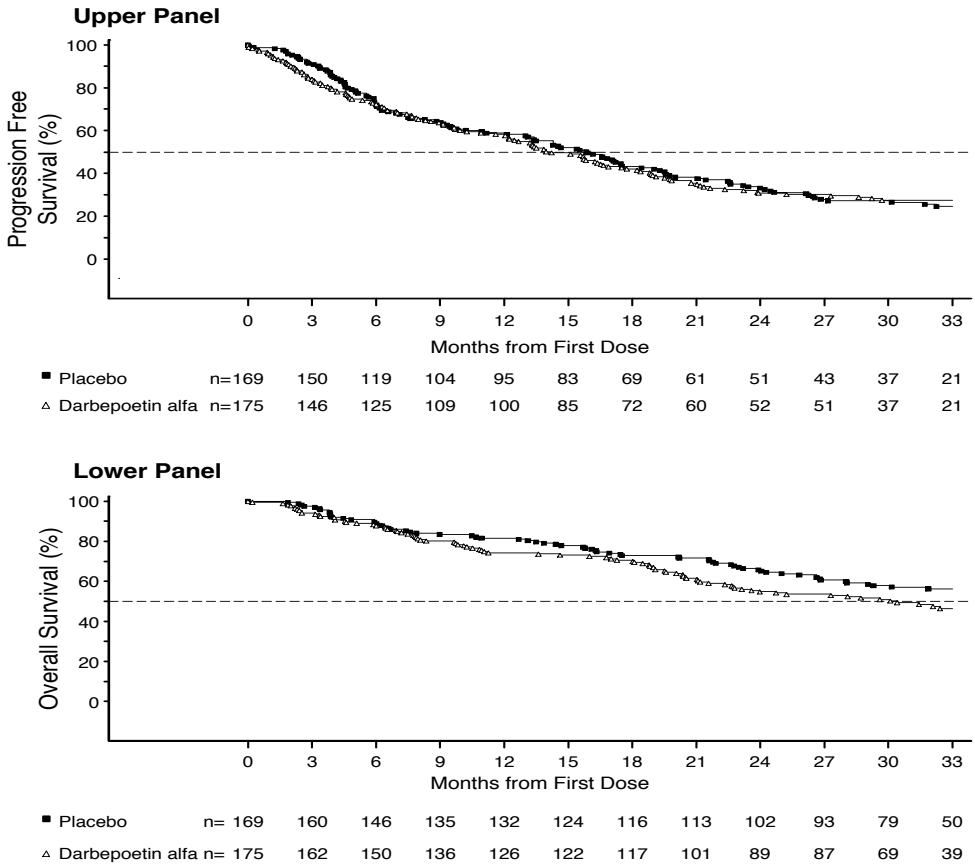
Disease progression or death for study A (lung cancer patients) in the DA group was not significantly different from that of patients in the placebo group.

The median (95% CI) duration of PFS was 5.1 (4.1 - 6.9) months and 4.4 (3.7 - 5.3) months for DA and placebo. Hazard ratio (HR) was 0.79 (95% CI: 0.62 -1.00; $P = 0.051$). The median (95% CI) OS time was 10.4 (8.8 -12.0) and 7.8 (6.6 to 9.0) months. HR was 0.77 (95% CI: 0.59 - 1.01; $P = 0.060$). Progression and survival endpoints analyzed by histology (non-small cell lung cancer or small cell lung cancer) revealed no significant differences comparing DA and placebo.

Follow up. Study B

Disease progression or death was also similar for LPM patients in the DA and placebo groups (figure 6). The median (95% CI) OS time was 30.4 (22.7 -NE [not estimable]) months for DA and 36.6 (30.2 -NE) months for placebo. HRs for OS and PFS were 1.26 (95% CI: 0.92 - 1.71; $P = 0.152$) and 1.03 (95% CI: 0.80 - 1.32; $P = 0.803$) respectively.

Figure 6. K-M curve of PFS (upper panel) and OS (lower panel) for LPM.

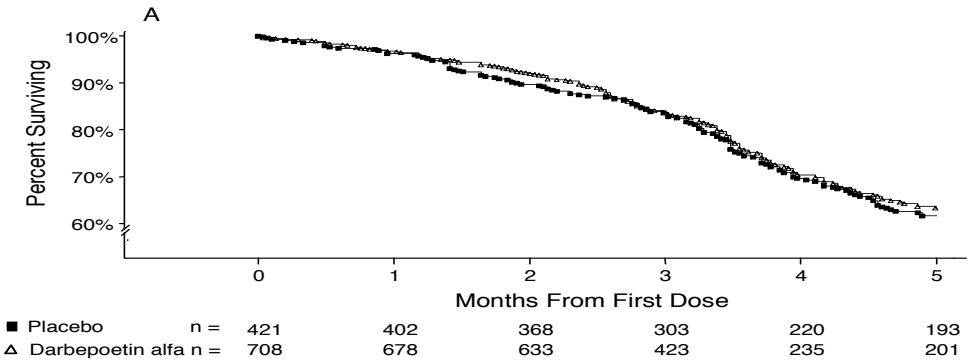


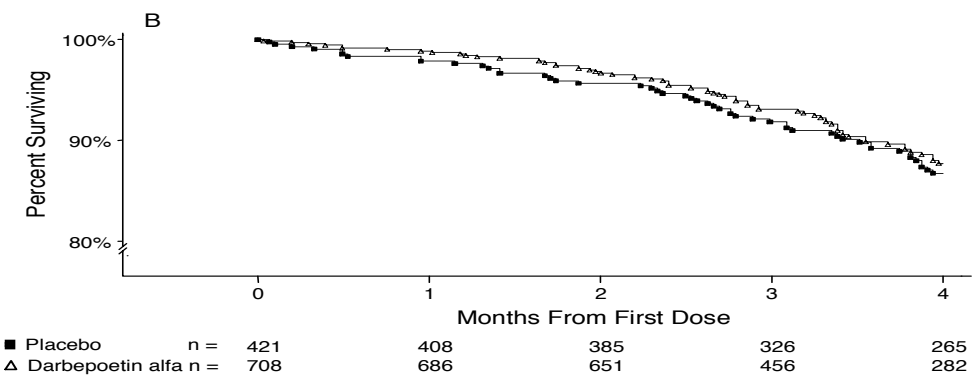
Progression and survival endpoints analyzed by histology (aggressive and indolent lymphomas, CLL, and MM) or disease stage also revealed no significant differences between patients receiving DA and placebo (data not shown).

Follow up. Pooled analysis (studies A, B, C and D)

Also the pooled analysis of PFS and OS, demonstrated no difference between DA and placebo groups (figure 7). The median (95% CI) duration of PFS was 8.0 (7.2 - 9.3) months for DA and 6.8 (5.9 - 8.0) months for placebo. The median (95% CI) OS time was 19.0 (14.6 - 22.3) months for DA and 16.4 (14.6 - 22.6) months for placebo. The estimated HR (95% CI) related to DA use was 0.92 (0.78 - 1.07) (P = 0.280) for PFS and 0.95 (0.78 - 1.16) (P = 0.619) for OS.

Figure 7. K-M curve of (A) PFS and (B) OS for the pooled data set.





Effects on PFS and OS of baseline Hb categories (studies A, B, C and D)

Sample size, deaths rates and median follow up by baseline Hb categories (<9 g/dL; 9.0 - 10.5 g/dL; >10.5 g/dL) are shown in table 4 (page 29).

Relative to patients receiving placebo with Hb concentrations of 9 to 10.5 g/dL at baseline the HR for PFS for patients with a baseline Hb concentration < 9 g/dL was 2.05 (95 % CI: 1.09 - 2.82) for patients receiving placebo and 1.51 (95 CI:1.09 - 2.10) for patients receiving DA. Similarly, an association between anemia and adverse OS was observed for patients receiving placebo (HR 2.08; 95% CI: 1.38 - 3.14) and DA (HR 1.86, 95%: CI 1.20 - 2.89).

Comparisons of OS and PFS between patients receiving DA and placebo analysed for each of the baseline Hb categories mentioned above, did not indicate any adverse effect of treatment with DA. For patients with baseline Hb < 9 g/dL the estimated HR was 0.66 (95% CI: 0.45 to 0.98; $P = 0.037$) for OS and 0.62 for PFS (95% CI: 0.45 - 0.86; $P = 0.004$). No differences between DA and placebo patient groups were observed for either endpoint for the other baseline Hb categories 9-10.5 g/dL or higher.

Effects on PFS and OS of Hb rise ≥ 1 g/dL and maximum Hb ≥ 13 g/dL

Sample size, deaths rates and median follow up are shown in table 5 (p. 30). As shown in table 8 Cox regression models clearly indicated that an achievement of the Hb parameters Hb ≥ 13 g/dL and ≥ 1 g/dL rise in 14 days had no adverse effect on OS or PFS.

Table 8. Analysis of patients by rate of Hb increase and maximum Hb.

Model	*P*-value	HR	95% CI
≥ 1 g/dL Hb increase in 14 days			
Progression-free Survival	< 0.001	0.51	0.42 to 0.62
Survival	< 0.001	0.43	0.34 to 0.56
Achieved Hb of ≥ 13 g/dL			
Progression-free Survival	0.001	0.66	0.51 to 0.84
Survival	0.001	0.56	0.40 to 0.79

DISCUSSION

Dosing of darbepoetin alfa (Paper I and II)

After analysis of the dose-finding study (paper I) and data from other studies we decided to proceed with a confirmative phase III trial (paper II) of the efficacy and safety of DA dosing 2.25 µg /kg once weekly in lymphoid malignancies as this seemed justified by serveral considerations discussed below.

Firstly we observed a trend towards a dose-response effect for all end points although not statistically significant in the three DA dose cohorts (1.0 µg /kg, 2.25 µg /kg and 4.5 µg /kg). There was no difference in mean change in Hb from baseline to end of the study (EOS) in the two cohorts with lower doses of DA (1.56 g/dl and 1.64 g/dL respectively). However this might be because the 2.25 µg /kg cohort was confounded by three patients with severe anemia and baseline serum EPO levels well over 600 IU/mL, patients that have been reported to be less prone to respond to ESA-treatment *(Cazzola M 1996; Österborg A 1996).* None of these three patients achieved Hb response.

Secondly in this study of the twenty-two patients who experienced a rapid rise in Hb (≥ 2 g/dL within a 28 days period) more than 50 % were observed in the 4.5 µg /kg cohort. There was concern that the higher dose might pose a potential risk for thrombo-embolic complications due to rapid rise of Hb concentrations.

Thirdly a large phase III randomized trial in lung cancer patients *(Vansteenkiste J 2002)* receiving platinum-based chemotherapy reported that a DA starting dose of 2.25 µg /week had similar response rates as reported for comparable doses of rHuEPO alfa in similar settings. Additionally, in an almost identically designed but larger dose-finding study in anemic patients with solid tumors receiving chemotherapy the group receiving 5.0 µg /kg every second week (consistent with 2.25 µg /kg once weekly) did much better (mean Hb increase 2.50 g/dL) compared to the 2.25 µg /week cohort in our study *(Glaspy J 2002)*. Also a similar dose-response curve was observed in the Glaspy study and in our LPD dose finding study *(Hedenus M 2001).*

The Hb responses we observed in our confirmative phase III trial (mean Hb increase 2.66 g/dL) dosing 2.25 µg /kg once weekly are very similar to those reported in the Vansteenkiste and Glaspy studies. The results are also comparable with those reported from other trials with rHuEPO beta dosing 150 IU/kg three times weekly *(Österborg A 2002)* or 30 000 IU once weekly of rHuEPO beta (*Cazzola M 2004)* in the LPD setting and 40 000 IU rHuEPO alfa once weekly in anemic patients with solid tumors (*Witzig TE 2004).*

Congruent with some recommendations *(Ludwig H 2002, NCCN 2006)* dose-doubling in our phase III trial was performed (if the Hb value had not increased by 1.0 g/dL after 4 weeks of treatment) in 43 % of the patients receiving DA compared to 64 % in the placebo group. However the role of dose-doubling in this study is unclear, as late response of dosing DA 2.25 µg /kg once weekly cannot be ruled out. Consequently as there is a lack of studies comparing dose doubling versus a maintained dose level in non-responding patients the EORTC and the ASH/ASCO guidelines do not advocate dose doubling in ESA therapy.

Based on these two studies (paper I and II) all recent important guidelines *(NCNN 2006; Bokemeyer C 2004 and 2006)* have recognized 150 µg once weekly dosing of DA in the treatment of CIA in patients with LPD and has also been approved by the Food and Drug

Association (FDA) and the European Agency for the Evaluation of Medicinal Products (EMEA).

Impact of intravenous iron-supplementation – the NIFe study (Paper III)

We observed that the mean change in Hb from baseline to EOT in the PP population was almost twice as high in the iron group compared to the no iron group (2.91 g/dL vs 1.50 g/dL). This difference was achieved in the iron group despite less dose doubling and a greater number of patients had epoetin doses withheld or reduced. This probably reduced a potentially even greater difference in Hb level between the two groups.

To our knowledge the Hb response rate of 93% in the iron group is the highest ever reported and higher than that observed by Auerbach (68 %) and Henry (73 %). This might be because different patient populations were studied and the Auerbach study contained a treatment phase of six weeks only. On the other hand interpretation of the study by Auerbach et al might be limited by the possibilty that a proportion of patients may have been iron depleted before the start of treatment (*Beguin Y 2005*). This is suggested by the relatively low percentage of patients in the Auerbach study with hematopoietic responses in the groups not treated with iron (25 %) or treated with oral iron (37 %).

Due to different patient populations studied there may be a wide variation in Hb response to ESA treatment in different trials (43-75%) (*Bokemeyer C 2004, 2006*) corresponding with the Hb response rate in the no-iron arm in our study (53%). It was lower than that reported (72-75%) in a previous study of epoetin beta administration in a similar patient population with CIA *(Cazzola M 2003*). However this might have been because, in the latter study, approximately 40% of patients received IV iron supplementation during the trial.

Dose doubling at week 5 was performed in 57 % of the patients in the no-iron group compared to 41 % in the iron arm (congruent with 43 % of the patients receiving DA in the pivotal study reported in paper II). Nevertheless the mean weekly epoetin beta dose after 13 weeks of treatment was significantly lower and at week 15 at least 10 000 IU (25%) lower in the iron group than in the no-iron group, despite the occurrence of more missed epoetin beta doses in the no-iron group. In patients with CRD concomitant administration of IV iron has been shown to allow ESA dose reduction *(Macdougall 1999; Locatelli F 2004)*. To our knowledge our study is the first to report that addition of IV iron to epoetin treatment in cancer patients reduces the dose of epoetin. This might be of economic significance and warrants carefully conducted pharmaeconomic prospective studies.

Despite a third of the patients in the iron group having doses withheld or reduced after five weeks of treatment with epoetin beta the mean weekly patient dose at EOT was 34 000 IU; thus higher than the dose given at the start of the study (30 000 IU). This might indicate that at least in this patient population a higher weekly dose of epoetin beta is needed; e.g. 40 000 IU as recommended with epoetin alfa.

Iron restricted erythropoiesis may be present in epoetin treatment of cancer-related anemia by two different mechanisms. Iron deposits may become depleted, and secondly iron may be trapped in the macrophages due to FID. Chemotherapy increases endogenous serum levels of EPO *(Birgegård G 1989, Glaspy J 2005)* which has impact on iron metabolism caused by increasing hepcidin concentration. This is also seen in increased serum iron levels induced by RBC transfusions *(Nehmet E 2006)*.

We therefore focused on transfusion independent patients with stable disease not requiring treatment with chemotherapy and with proven iron stores in the BM. Hereby we were able to study iron kinetics in the absence of major confounding factors. As for the treatment of FID and maintenance of adequate iron stores during ESA therapy in anemic patients with CRD, the recommended regimen is 25 to 100 mg of IV iron every week for 10 weeks *(Lotacelli F 2004)*. We chose a similar iron supplementation regimen in our study. FID was common at baseline as indicated by the low mean TSAT (22 %) and mean ferritin in serum of about 200 µg/L in presence of stainable iron in the BM.

Ferritin in the non-iron group fell rapidly during the first week and then steadily declined to about 110 µg/L at the EOS. In some patients the iron deposits were exhausted by the increased erythopoiesis as shown by subnormal S-ferritin levels and FID evolved in the majority (26 out of 30 patient) as indicated by TSAT<20 % during more than 75 % of the treatment period. Only 54% of these patients responded with an increase in Hb >2 g/dL

In contrast in the iron treated patients S-ferritin showed no initial fall and continued to increase and almost doubled. TSAT increased from baseline and stabilized at 30 %. All patients with TSAT <20% at any time in this treatment arm presented Hb response. Compared with the no-iron group this was a significantly difference (P<0.001).

Interestingly there was a similar increase in mean sTfR level from baseline in both groups. The reason may be that the increase was caused by a lack of iron in the no-iron group but increased erythropoiesis in the iron group. Thus sTfR assessment does not seem to discriminate between FID and increased erythroid marrow activity.

Our observations indicate that FID (low TSAT and iron-restricted erythropoiesis despite demonstrated iron stores) is common in patients with anemia of lymphoid malignancies and an important reason for lack of response to epoetin treatment. Moreover IV iron supplementation to ESA therapy improves Hb response significantly both by overcoming FID and by avoiding iron-depletion caused by increased erythropoiesis.

Our data, along with those of Auerbach, Henry and Lerchenmueller clearly suggest that IV iron supplementation should be included in clinical guidelines. And indeed in the recent uppdated EORTC guidelines concomitant IV iron supplementation (but not oral) in the ESA treatment of anemia in cancer is recommended *(Bokemeyer C 2006)*.

Prediction of response (paper I and II)

In the dose-finding study (paper I) we performed a posthoc analysis to identify promising predictors of Hb response (table 6). These were tested in the larger phase III pivotal trial (paper II).

Previous reports indicated that anemic patients with MM might respond better to ESAs than patients with other lymphoid malignancies *(Ludwig H 1990, Damacco 1998, Marsh WA 1999)*. The analysis in the dose-finding study (paper I) gave some support for this suggestion. To investigade this potential effect the pivotal trial (paper II) was designed to evaluate the treatment effect of DA relative to placebo separately for patients with lymphoma (Hodgkin`s disease, NHL or CLL) or MM. We observed similar Hb response rates with DA in patients with lymphoma or MM (64% versus 56 %). This finding is congruent with other randomized, placebo-controlled studies conducted in patients with MM that did not report higher response rates than those

expected for other LPD *(Dammacco 2001, Österborg A 2002*). Thus different types of lymphoid malignancies respond similar to ESA therapy.

Relative endogenous EPO deficiency has been reported to be present in up to 75% of patients with LPD (*Cazzola M 1995*) and has been suggested as a useful predictor of response to treatment with ESA (*Ludwig H 1990; Cazzola M 1995; Österborg A 1996*) with a proposed cut-off level of EPO < 100 IU/mL in patients with mild/moderate anemia (*Österborg A 2002)*. In the dose-finding study we observed that patients with low endogenous EPO concentration in serum (< 100 IU/mL) at baseline were more likely to achieve Hb correction and were less likely to receive RBC-transfusions compared to patients with higher serum levels. In the larger phase III trial (paper II) the difference in Hb response was statistically significant independent (P=0.16) of endogenous serum EPO level at baseline, although an increased effect was observed in patients with values < 100 IU/mL than in patients with values > 100 IU/mL (69 % versus 44 %). In contrast to previous reports in our phase III trial (paper II) only 35 % (in the NIFe-study even less, 12 %) of the patients revealed baseline serum EPO levels > 100 IU/mL and importantly more than 40 % of these achieved a Hb response. This clearly indicates that assessment of baseline endogenous EPO level is not a very useful predictor for Hb response in anemic patients with LPD. Thus if at all, the adequate cut off level in LPD remains to be defined. It has been stated (but not shown) that patients with baseline EPO levels > 500 IU/mL are less likely to respond to treatment with EASs (*Ludwig H 2002*).

A lower response rate has been observed in patients with a blunted residual BM function as indicated by severe anemia, dependence on blood transfusions or a platelet count < 100×10^9/L *(Cazzola M 1995, Österborg A 2002, Beguin Y 2002)*. In the dose-finding study patients with indications of impaired BM function at baseline (transfusion dependent patients with severe anemia) were less likely to achieve Hb-response or Hb correction and were more likely to receive RBC transfusions. Therefore it seems likely that patients with impaired BM are less prone to respond to ESA treatment.

The NCCN guidelines provide a preliminary recommendation that iron supplementation may be considered in patients with serum ferritin levels <100 µg/L or TSAT <20% indicating FID. Although the NIFe-study confirmed a significant association between low TSAT (<20%) or low serum ferritin (<100 µg/L) at baseline and Hb response (table 8) most patients gained from IV iron supplementation independently of ferritin and TSAT levels at baseline as shown by the high proportions of patients achieving Hb response. Thus our data do not support the NCCN recommendations but rather indicate that IV iron supplementation to ESA therapy should be given independently of TSAT and serum ferritin levels at baseline. Although iron overload must be taken into consideration.

In summary the trials included in this thesis were not able to identify or confirm previously proposed clinically useful and reliable predictors for response to ESA treatment.This is congruent with published reviews that overall, none of the parameters and models that have been tested for their potential value to predict a response or lack of response before treatment starts has proven useful in clinical practice *(Glaspy J 1999, Littlewood T, 2003).*

Prediction of response might be deemed unnecessary if it can be confirmed that Hb response rates in about 90 % patients can be achieved with concomitant IV iron supplementation. This has recently been reported in another study with a similar design as the NIFe-study (*Lerchenmueller C*

2006). Thus maybe focus should rather be on iron parameters indicating FID and consequently need for IV iron administration concomitant with ESAs as is routinely performed in the CRD setting *(Lotacelli F 2004)*.

Safety of treatment with darbepoetin alfa (Paper IV)

Our analyses demonstrated a nearly identical progression-free survival (PFS) and and overall survival (OS) for DA and placebo after long term follow-up in patients with lung cancer and LPD. Although it is important to stress that the analyses were performed on safety data and the primary endpoints of the two studies were not PFS or OS.

The observation in the LPD population is supported by a long-term follow-up analysis of a similar large study that clearly indicated that epoetin beta had no significant effect on survival compared with placebo in anemic patients with LPD *(Österborg A 2005)*. In the latter study median survival was similar in both treatment groups (17 months with epoetin beta and 18 months with placebo). However median survival of patients with LPD included in our study were almost twice as long as reported by Österborg et al, indicating that different patient populations have been studied rather than impact of the different ESAs (DA and epoetin beta) used in these two trials.

Because the detrimental effect on survival reported in the Leyland Jones study seemed to be due to an increase in mortality in the epoetin alfa arm during the first 16 weeks of the study, we performed a pooled analysis with two additional studies with short-term follow up, and analyzed PFS and OS over 16 weeks. The size of the pooled patient sample (n = 1,129) and estimated hazard ratios (HRs) corresponding to decreases in PFS and OS provided more than 80% power to detect an effect of DA on survival of the magnitude seen in the reports by Henke and Leyland Jones. Also this pooled analysis of short term follow up demonstrated identical PFS and OS for DA and placebo.

Two Cochrane meta-analyses have been performed. The first review involving 1,624 patients suggested possible benefits in OS (HR 0.81; 95%-CI 0.67-0.99; 19 trials, n = 2,865) for ESA therapy compared to placebo *(Bohlius J 2004)*. A more recent updated meta-analysis however reported no difference on OS (HR 1.08; 95%-CI 0.99-1.18; 42 trials, n = 8,167) or tumor progression (HR 1.12; 95%-CI 1.01-1.23; 13 trials, n = 2,833) *(Bohlius J 2006)*. It should be noted that the studies published by Henke and Leyland-Jones were included in the second analysis. Moreover Leyland-Jones reported a more recent long term follow up of the patients in his study and found no difference in OS between the two study groups after 19 months.

However it was recently reported significantly decreased locoregional progression-free survival due to treatment with epoetin-beta only in patients with head and neck cancer that expressed EPO-R but not in patients who had tumors that did not *(Henke M 2006)*. Even more recently an interim analysis of a randomized study on radiotherapy in non-anemic patients (target Hb interval 14.5-15.5 g/dL) with primary squamous cell carcinoma of the head and neck treated with DA indicated a significant adverse locoregional failure outcome and of disease-specific death. Consequently the study was closed (*www.dahanca.dk*).

Although our analysis do not support concerns of impaired OS or PD in cancer patients with CIA treated with DA the recommendation by EORTC of 2004 and 2006 remains that the use of ESAs with the aim of improving survival or response to treatment should only be evaluated

in prospective properly designed studies. Treatment of patients beyond the correction of anemia has to be regarded as experimental and is potentially harmful *(Bohlius J 2006)*.

In comparison with the results reported by Leyland-Jones and Henke our combined analyses were limited by the fact that high Hb levels and fast rates of Hb increases could not be studied due to the more conservative titration rules employed in our trials. Therefore in our combined analyses we explored whether these factors were associated with an adverse outcome for patients treated with DA versus placebo.

These analyses first confirmed multiple previous reports indicating an association between anemia and adverse tumor and survival outcomes (*Shasha D 2001)*. HR for PFS and OS for patients with a baseline Hb concentration < 9 g/dL were significanctly greater but not for patients with higher baseline Hb levels and treatment with DA treatment had no adverse effect on any of the different Hb categories.

Moreover we observed that an increase in Hb concentrations >13 g/dL and a rapid increase of Hb concentration had no negative impact on OS or PFS but rather seemed represent markers of reduced risk (HR~0.5) in DA treated patients. Although it is important to point out that these analyses do not address causality. A possible interpretation of these observations is that patients with more advanced cancer and consequently worse prognosis are less responsive to DA treatment. Our findings are in line with the recent uppdated Cochrane meta-analysis where no correlation of increased risk for thrombo-embolic events and different Hb-levels was found in cancer patients treated with ESAs *(Bohlius J 2006)*.

CONCLUSIONS

The major conclusions of this thesis are:

1. The darbepoetin alfa (DA) dosing of 2.25 µg/kg (~150 µg) once weekly for patients with lymphoid malignancies and chemotherapy induced anemia (CIA) is appropriate and safe and has similar effects on hemoglobin (Hb) response as has been reported with rHuEPO alfa and beta dosing 150 IU/kg three times weekly.

2. Concomitant intravenous iron administration with rHuEPO beta significantly increased Hb levels and proportion of Hb responders, and produced more rapid Hb responses in a well defined population of mild/moderate anemic patients with lymphoid malignancies and proven iron deposits in the BM not receiving chemotherapy.

3. The NIFe study is the first to demonstrate that in cancer patients the weekly rHuEPO-dose requirement might be decreased with concomitant IV iron supplementation.

4. Our analyses do not support concerns of impaired overall survival or progressive disease in cancer patients with CIA treated with DA when used within approved label indications.

5. We were not able to confirm previous proposed predictors as reliable for Hb response to epoetin treatment.

6. FID (low TSAT and iron-restricted erythropoiesis despite demonstrated iron stores) is common in patients with anemia of lymphoid malignancies and an important reason for lack of response to epoetin treatment.

7. The effects of concomitant intravenous iron administration with rHuEPO beta on Hb concentrations seems to be accomplished both by overcoming FID and by avoiding iron-depletion caused by increased erythropoiesis.

8. We were able to confirm previous reports of an association between severe anemia and adverse survival outcome.

9. Patients treated with DA and with a rapid Hb increase or achieving normal Hb concentration do not seem to have impaired survival outcomes.

FUTURE PERSPECTIVES

The optimal iron dose or schedule in the maintenance phase of ESA treatment has not been assessed.

No studies have yet been performed comparing IV iron alone versus iron concomitant or preceding ESA-treatment.

In patients with CRD concomitant administration of IV iron has been shown to allow ESA dose reduction. Our results indicate that these findings can be extended to the cancer patient population. This observation must be confirmed by future studies including pharmaeconomic considerations.

An important question is the potential harm of the administration of IV iron or RBC transfusions to cancer patients, who often have elevated total-body iron stores due to iron overload.

The ideal Hb range to start ESAs for a cancer patient with anemia has not yet been defined and the question whether there is an optimal target Hb concentration remains open.

Both epoetin (alfa and beta) and darbepoetin are approved specifically for anemia associated with chemotherapy, but further evidence in terms of safety and cost-effectiveness of the different drugs, different doses and schedules should be useful to guide clinical practice. Adequately powered comparative studies could contribute to this question.

QoL-studies comparing optimal transfusion therapy to obtain a close to normal Hb concentration with ESA treatment have not yet been conducted.

The real impact of ESA therapy on tumor behaviour and patient survival has not yet been clarified. Results of more ongoing trials will soon be available. These must be awaited before any similar trials can be launched.

New ESAs are emerging. An even longer serum half-life than that of DA can be obtained by PEGilation of the EPO molecule. CERA (Roche) is a PEGilated rHuEPO beta and Hematide a pegilated semisyntethic EPO molecule. A new and exciting concept is orally bioavailable inhibitors of hypoxia inducing factor prolyl hydroxylase leading to an increase in the production of endogenous EPO production.

ACKNOWLEDGEMENTS

This thesis was written thanks to many friends in the Haematology world who, in contrast to the author, did not think it would be pathetic to perform such an effort at my age. With time I became convinced that it might be important if not for me but for younger collegues; to prove that clinical research is worthwhile and can be performed concomitant with ordinary clinical work at a non-academic Swedish County hospital in fruitful and cheerful cooperation with many people on a local and international basis.

The studies presented in this thesis were made possible solely through the help, enthusiasm and encouraging attitudes of many collegues and study nurses at all participating institutions and members of the medical research departments of the Amgen and Roche companies. I am indebted to all the patients for consenting to participate in the studies included in this thesis. And of course my collegues and the large number of medical, nursing and laboratory staff who provided help, service and care for the patients included in the studies over all the years at the Section of Haematology, Sundsvall Hospital to whom I am most grateful.

In particular, I wish to express my sincere gratitude to

My super-ego that made this thesis necessary.

Jack Lindh, my supervisor for the last couple of years, for encouraging me to write this thesis, your constant questioning but constructive criticism and for never-ending patience while gently guiding his somewhat unpleasant feeling doctorial student through the obscure academic labyrinth of obstacles towards the completion of this work.

Anders Österborg, my co-supervisor and co-author for not only introducing me into but also sharing your knowledge and experiences in the field of clinical research of erythropoetins, for your believing in my capacity and your constant and constructive feed-back.

Karin Larsson, my study nurse. Whithout your continuing help, skill, enthusiasm and commitment this thesis had not been possible.

All my co-authors, particularly **Gunnar Birgegård,** for sharing his unchallenged knowledge in the field of iron and **David Watson and Raphael Amado** from the Amgen company, for fruitful collaboration and for having confidence in my ability to lead the studies.

My statisticians Lisa Hendricks, Per Näsman and Anna Törner for their unrewarding attempts to introduce me into the mysterious world of statistics.

My colleagues Maria Strandberg, Anders Själander and Birgitta Goine at the Section of Haematology in Sundsvall for your help with patients included in these studies, excellent teamwork and much fun in the clinical work.

Ulf Jansson for excellent co-operation and laboratory work.

Staffan Magnusson, head of the Department of Medicine, Sundsvall Hospital, for your silent support and agreeing to create informal opportunities for clinical research.

Åsa Elf-Klackenberg, at the time for the NIFe-study working at Roche A.B. Sweden, for your growing confidence in my ability and your cheerful and unconditioned support for this project.

Donald Stanley, my friend and co-lover of opera music, for your wise comments and kind assistance to correct my Swenglish in the thesis.

Lars Franzén, Lars Beckman och Jonas Wallvik, my collegues and pre-opponents.

And at last but not at least, **my beloved wife, coach and manager Kerstin and my wonderful ever growing family,** for your love and trust in me, though I have been absent for quite a while, if not always physically but almost constantly mentally. I promise to join the family again as soon as possible.

This thesis was in part supported by unrestricted grants from Roche A.B., Sweden, and the Research and Development Centre, Sundsvall Hospital, Sundsvall, Sweden

DISCLOSURE OF INTEREST

I have received unrestricted research grants from Roche A.B, Sweden and the Research and Development Centre, Sundsvall Hospital, Sundsvall, Sweden.

My hospital and the staff at the clinic have been economically refunded by Amgen and Roche for costs these projects generated.

The clinical trials as well as the statistical analysis and the manuscript in Papers I, II and IV have been performed in cooperation with Amgen. However I had input on the design of the studies II and IV, had access to all data for the statistical analyses and moreover made a major contribution to the manuscripts and the conclusions drawn in paper I, II and IV. The trial reported in paper III was designed and conducted by the investigators only.

REFERENCES

Acs G, Acs P, Beckwith et al. Erythropoietin and erythropoietin receptor expression in human cancer. *Cancer Research,* 2001**; 61**: 3561-65.

Allon M, Kleinman K, Walczyk M, et al. Pharmacokinetics and pharmacodynamics of darbepoetin alfa and epoetin in patients undergoing dialysis. *Clin Pharmacol Ther*; 2002; **72**: 546-555.

Auerbach M, Barker L, Bahrain H et al: Intravenous iron optimizes the response to erythropoetin in patients with anemia of cancer and cancer chemotherapy: Results of a multicenter, open-label, randomized trial: *Proc Am Soc Haemotol* 2001; 3323 (abstract).

Auerbach M, Ballard H, Trout RJ, et al. Intravenous iron optimizes the response to recombinant human erythropoietin in cancer patients with chemotherapy-related anemia: a multicenter, open-label, randomized trial. *J Clin Oncol*. 2004; **22**: 1301-1307.

Barlogie B, Beck T. Recombinant human erythropoietin and the anemia of multiple myeloma. *Stem Cells*. 1993; **11**(2): 88-94

Barron BA, Hoyer JD, Teffery A: A bone marrow report of absent stainable iron is not diagnostic or iron deficiency. *Ann Hematol* 2001; **80**: 166-9.

Becker A,Stadler P, Lavey RS. et al. Severe anemia is associated with poor tumor oxegenation in head and neck squamos cell carcinomo.*Int J Rad Oncol Biol Phys;* 2000; **46**: 459-66.

Beguin , Loo M, R`Zik S et al. Quantitative assessment of erythropoiesis in haemodialysis patients demonstrates gradual expansion of erythroblasts during constant treatment with recombinant human erythropoietin.1995. *Br J Haematol*; **89**: 17-23.

Beguin Y: Prediction of response and other improvements on the limitations of recombinant human erythropoietin therapy in anemic cancer patients. *Haematologica* 2002; **87**: 1209-1221.

Beguin Y: Iron supplement in cancer patients receiving erythropoietin. *J Clin Oncol* 2004; **22** (21) 4428-9.

Beguin Y. Intravenous iron and recombinant human erythropoietin in cancer patients. *J Clin Oncol.* 2005; **23**: 651-653.

Besarab A, Frinak S, Yee J. An indistinct balance: the safety and efficacy of parenteral iron therapy. *J Am Soc Nephrol.* 1999; **10:** 2029-2043

Birgegård G, Wide L, Simonsson B. Marked erythropoietin increase before fall in Hb after treatment with cytostatic drugs suggests mechanism other than anaemia for stimulation. *Br J Haematol* 1989; **72**: 462-66.

Bohlius J, Langensiepen S, Schwarzer G, et al. Erythropoietin for patients with malignant disease (Cochrane Review). In *The Cochrane Library,* Issue 3, 2004. Chichester, UK: John Wiley and Sons Ltd

Bohlius J, Wilson J, Seidenfeld J, et al. Recombinant Human Erythropoietins and Cancer Patients: Updated Meta-Analysis of 57 Studies Including 9353 Patients. *JNCI.* 2006; **98**: 707-714

Bohlius J, Weingart O, Trelle S, Engert A. Cancer-related anemia and recombinant human erythropoietin – an updated overview. *Nat Clin Pract Oncol*. 2006; **3**: 152-164.

Bokemeyer C, Aapro MS, Courdi A, et al. EORTC guidelines for the use of erythropoietic proteins in anaemic patients with cancer. *Eur J Cancer*. 2004; **40**: 2201-2216.

Bokemeyer C, Aapro MS, Courdi A, et al. EORTC guidelines for the use of erythropoietic proteins in anaemic patients with cancer: 2006 update. *Eur J Cancer*. 2007; **43**(2): 258-70.

Brugnara C, Schiller B, Moran J:Reticulocyte haemoglobin equivalent (Ret He) and assessment of iron-deficient states; *Clin.Lab.Haem.*2006; **28:** 303-308.

Caro JJ, Salas M, Ward A, et al: Anemia as an independent prognostic factor for survival in patients with cancer: a systemic, quantitative review. *Cancer* 2001**; 91**: 2214-2221

Cavill I: Iron and erythropoietin in renal disease. *Nephrol Dial Transplant.* 2002; **17** Suppl 5: 19-23.

Cavill I, Auerbach M, Bailie GR, et al. Iron and the anaemia of chronic disease: a review and strategic recommendations. *Curr Med Res Opin*. 2006; **22**: 731-737.

Cazzola M, Messinger D, Battistel V et al. Recombinant human erythropoietin in the anemia associated with multiple myeloma or non-Hodgkin lymphoma: dose finding and identification of predictors of response. *Blood* 1995; **86**: 4446-53.

Cazzola M, Ponchio L, Pedrotti C, et al. Prediction of response to recombinant human erythropoietin (rHuEpo) in anemia of malignancy. *Haematologica* 1996; **81**: 434-41.

Cazzola M, Beguin Y, Kloczko J, et al. Once-weekly epoetin beta is highly effective in treating anaemic patients with lymphoproliferative malignancy and defective endogenous erythropoietin production. *Br J Haematol.* 2003; **122**: 386-393.

Cella, D.The Functional Assessment of Cancer Therapy-Anemia (FACT-An) Scale: a new tool for the assessment of outcomes in cancer anemia and fatigue. *Semin Hematol,* 1997; **34,** 13-19.

Cella D. Factors influencing quality of life in cancer patients; anemia and fatigue. Semin Oncol 1998; 25 (suppl 17): 43-46. Cella D. Factors influencing quality of life in cancer patients; anemia and fatigue. *Semin Oncol* 1998; **25** (suppl 17): 43-46.

Cella, D., Lai, J-S., Chang, C-H., et al: Fatigue in cancer patients compared with fatigue in the general United States population. *Cancer* 2002; **94**, 528-538.

Cella D, Dobrez D and Glaspy J; Control of cancer-related anemia with erythoietic agens: a rewiew of evidence for improved quality of life and clinical outcomes*; Annals of Oncology* 2003; **14**: 511-519.

Cella D, Zagari M, Vondoros C et al; Epoetin alfa treatment results in clincically significant improvements in quality of life in anemic cancer patients when referenced to the general population; *J Clin Oncol* 2003; **21**, No 2, 366-373.

Cella D, Hahn EA, Dineen K. Meaningful change in cancer-specific quality of life scores with chemotherapy-induced anemia treated in clinical practice. *Oncologist* 2004**; 9**(4): 451-458.

Crawford J, Cella D, Cleeland CS, Cremieux PY, Demetri GD, Sarokhan BJ et al. Relationship between changes in hemoglobin level and quality of life during chemotherapy in anemic cancer patients receiving epoetin alfa therapy. *Cancer* 2002; **95**(4): 888-95.

Cremieux PY, Finkelstein SN, Berndt ER et al B: Cost effectiveness, quality-adjusted life-years and supportive care. Recombinant human erythropoietin as a treatment of cancer-associated anaemia. *Pharmacoeconomics*. 1999; **16**(5 Pt 1): 459-72.

Curt G, Breitbart W, Cella D, et al. Impact of cancer-related fatigue on the lives of patients: new findings from the Fatigue Coalition. *Oncologist*. 2000; **5**: 353-360

Curt G, Johnston P. Cancer fatigue: the way forward. *Oncologist* 2003; **8** (Suppl. 1): 27-30

Dammacco F, Silvestris F, Castoldi GL, et al. The effectiveness and tolerability of epoetin alfa in patients with multiple myeloma refractory to chemotherapy. *Int J Clin Lab Res.* 1998; **28**: 127-134.

Dammacco F, Castoldi G, Rödjer S. Efficacy of epoetin alfa in the treatment of anemia of multiple myeloma. *Br J Haematol*. 2001**; 113**: 172-179.

Darling RJ, Kuchibhotla U, Glaesner W, et al. Glycosylation of erythropoietin affects receptor binding kinetics: role of electrostatic interactions. Biochemistry 2002; **41**: 14524-14531.

Demetri, G.D., Kris, M., Wade, J., Degos, L., Cella, D. and Procrit Study Group. Quality-of-life benefit in chemotherapy patients treated with epoetin alfa is independent of disease response or tumor type: results from a prospective community oncology study. *Journal of Clinical Oncology*, 1998; **16**: 3412-3425

Egrie JC, Browne JK: Development and characterization of novel erythropoiesis stimulating protein (NESP). *Br. J. Cancer,* 2001; **84**(Suppl 1): 3–10.

Egrie JC, Dwyer E, Browne JK, et al: Darbepoetin alfa has a longer circulating half-life and greater in vivo potency than recombinant human erythropoietin. *Exp Hematol* 2003; **31**: 290-299,

Elin RJ, Wolff SM, Frinch CA: Effect of induced fever on serum iron and ferritin concentrations in man. *Blood* 1997; **49**: 147-53

Elliott S, Lorenzini T, Asher S, et al: Enhancement of therapeutic protein in vivo activities through glycoengineering. *Nat Biotechnol*, 2003; **21**: 414-421.

Elliott S, Busse L, Bass MB et al: Anti-Epo receptor antibodies do not predict Epo receptor expression. *Blood*, 2006; **107:** 1892-1895.

Ellis LD, Jensen WN, Westerman MP. Marrow iron. An evalutation of depleted stores in a series of 1,332 needle biopsies. *Ann Intern Med*, 1964; **61:** 44.

Faquin WC, Schneider TJ, Goldberg MA. Effect of inflammatory cytokines on hypoxia-induced erythropoetin production. *Blood* 1992; **79:** 1987-1994.

Feldman HI, et al: Association between intravenous iron and mortality in hemodialysis patients. *JASN*, 2004; **15**: 1623-1632

Finch CA. Erythropoiesis, erthropoietin and iron. *Blood* 1982; **60**: 1241-46.

Fischbane S, Frei GL, Maesaka J: Reduction in recombinant human erythropoietin dose by the use of chronic intravenous iron supplementation: *Am J Kidney Dis*,1995; **26**: 41-46.

Gabrilove J, Cleeland CS, Livingston RB et al. Clinical evaluation of once-weekly dosing of epoetin alfa in chemotherapy patients: improvements in hemoglobin and quality of life are similar to three weekly dosing. *J Clin Oncol* 2001; **19**: 2875-2882.

Garton JP, Gertz MA, Witzig TE et al. Epoetin alfa for the treatment of the anemia of multiple myeloma. A prospective randomized placebo-controlled, double blind trial.*Arch Int Med* 1995; **155:** 2069-2074.

Glaser CM, Millesi W, Kornek GV, et al: Impact of hemoglobin level and use of recombinant erythropoietin on efficacy of preoperative chemoradiation therapy for squamous cell carcinoma of the oral cavity and oropharynx. *Int J Radiat Oncol Biol Phys* 2001; **50**: 705-715

Glaspy J, Bukowski R, Steinberg D et al. Impact of therapy with epoetin alfa on clinical outcomes in patients with nonmyeloid malignancies during cancer chemotherapy in community oncology practice. *J Clin Oncol* 1997; **15**: 1218-34

Glaspy J, Demetri G, Cremieux P. Does baseline endogenous erythropoietin level impact hemoglobin (Hb) response to rHuEPO in cancer patients? *Blood* 1999; **94** Suppl 1: 15b.

Glaspy J, Cavill I.Role of iron in optimizing responses of anemic cancer patients to erythropoetin. *Oncology* 1999; **10**: 4: 461-473

Glaspy, J.A., Jadeja, J.S, Justice, G et al: Darbepoetin alfa given every 1 or 2 weeks alleviates anaemia associated with cancer chemotherapy. *British Journal of Cancer* 2002; **87**, 268-276.

Glaspy JA, Jadeja JS, Justice G, et al: A randomized, active-control, pilot trial of front-loaded dosing regimens of darbepoetin-alfa for the treatment of patients with anemia during chemotherapy for malignant disease. *Cancer* 2003; **97**: 1312-20.

Glaspy J, Henry D, Patel R, et al: Darbepoetin Alfa 20010162 Study Group. Effects of chemotherapy on endogenous erythropoietin levels and the pharmacokinetics and erythropoietic response of darbepoetin alfa: a randomized clinical trial of synchronous versus asynchronous dosing of darbepoetin alfa. *Eur J Cancer*. 2005; **41**: 1140-1149.

Glaspy J and Beguin Y:Anaemia management strategies: optimising treatment using epoetin beta (NeoRecormon). *Oncology.* 2005; **69** Suppl 2: 8-16. Review.

Glenngård AH, Persson U, Söderman C. Cost associated with bloodtransfusions in Sweden-The societal cost of autologous, allogenic and perioperative RBC transfusion. *Transfusion Med;* 2005; **15**: 295-306.

Glimelius B, Linne T, Hoffman K, et al. Epoetin beta in the treatment of anemia in patients with advanced gastrointestinal cancer. *J Clin Oncol.*1998; **16**: 434-440

Goch J, Birgegård G, Danielson BG, Wikström B. Iron absorption in patients with chronic uremia on maintenance hemodialysis and in healthy volunteers measured with a simple oral iron load test. *Nephron* 1996; **73**: 403-406.

Groopman JE, Itri LM. Chemotherapy-induced anemia in adults: incidences and treatment. *J Natl Cancer Inst* 1999; **91**: 1616-1634.

Hardee M, Arcasoy M, Blackwell K, et al: Erythropoietin biology in cancer. *Clin Cancer Res* 2006; **12**: 332-39.

Haroon ZA, Amin K, Jiang X et al; A novel role for erythropietin during fibrin-induced wound-healing response.*Am J Pathol* 2003; **163**: 993-1000.

Heatherington AC, Schuller J, Mercer AJ. Pharmacokinetics of novel erythropoiesis stimulating protein (NESP) in cancer patients: preliminary report. *Br J Cancer* 2001; **84** Suppl 1: 11-16.

Hedenus M, Glaspy J, Dewey C et al. Dose response of darbepotein alfa is similar in anemic patients with solid tumors or lymphoproliferative malignancies: *Blood* 2001; 98: 297a (Abstract 1255).

Henry DA. Supplemental iron: A key to optimizing the response of cancer related anemia to rHuEPO. *Oncologist* 1998; 275-278.

Henry DH: The role of intravenous iron in cancer-related anemia. *Oncology*, 2006; **20** (Suppl 6): 21-28.

Henke M, Laszig R, Rube C, *et al.*: Erythropoietin to treat head and neck cancer patients with anaemia undergoing radiotherapy: randomised, double-blind, placebo-controlled trial. *Lancet* 2003; **362** (9392): 1255-1260.

Henke M, Mattern D, Pepe M et al: Do Erythropoietin Receptors on Cancer Cells Explain Unexpected Clinical Findings? *J Clin Oncol* 2006; **24**: 4708-13.

Hoen B, et al: Risk Factors for Infecctions in Hemodialysis Patients. *JASN*,1998**; 9**: 869-76

Höckel M, Knoop C, Schlenger K, et al. Intratumoral pO2 predicts survival in advanced cancer of the uterine cervix. *Radiotherapy Oncol* 1993**; 26***:* 45-50.

Jelkmann W, Wolff M, Fandry J, Modulation of the production of erythropoetin by cytokines. In vitro studies and their clinical implications. *Contrib Nephrol* 1990**; B7**: 68-77.

Jidell E, Norda R, Berséus O, Swärd-Nilsson AM. Regarding the article"Costs associated with bloodtransfusion in Sweden"(letter to the editor). *Transfus Med* 2006; **16**: 151-2.

Jones M, Schenkel B, Just J, Fallowfield L. Epoetin alfa improves quality of life in patients with cancer, results of metaanalysis. *Cancer* 2004; **101**: 1720-32.

Junca J, Fernandez-Aviles F, Oriol A. et al. The usefulness of the serum transferrin receptor in detecting iron deficiency in the anemia of chronic disorders. *Haematologica* 1998; **83**: 676-80.

Kaanders JHAM and van der Kogel AJ. Erythropoietin to treat anemia in patients with head and neck cancer (Letter); *Lancet.* 2004*;* **363**: 78-79.

Kallich JD, Tchekmedyian NS, Damiano AM, et al.Psychological outcomes associated with anemia-related fatigue in cancer patients. *Oncology* (Williston Park). 2002; **16**(9 Suppl 10): 117-24. Review.

Kew MD. Pathogenesis of hepatocellular carcinoma in hereditary hemochromatosis:Occurrence in noncirrhotic patients. *Hepatology* 1990; **11**: 1086-7.

Kyle RA. Multiple myeloma: review of 869 cases. *Mayo Clin Proc* 1975; **50**: 29-40.

Kotasek, D., Stegerg, Faught W et al. Darbepoetin alfa administered every 3 weeks alleviates anaemia in patients with solid tumours receiving chemotherapy; results of a double-blind, placebo-controlled, randomised study, *Eur J Cancer* 2003; **39**: 2026-34.

Kotasek D, Canon J-L, Mateos MV, Hedenus M, et al :A randomized, controlled trial comparing darbepoetin alfa correction/maintenance dosing with weekly dosing for treating chemotherapy-induced anemia; *Current Medical Research and Opinion* 2007; in press.

Langendijk H, de Jong J, Wanders R, et al. The importance of pretreatment haemoglobin level in inoperable non-small cell lung carcinoma treated with radical radiotherapy; *Radiotherapy Oncol* 2003; **67:** 231-5.

Lawrence T, Goodnough MD, Brecher M et al. Transfusion medicine-Review. *N Engl J Med* 1999; **340**: 438-447.

Le N, Richardsson DR: The role of iron in cell cycle progression and the proliferation of neoplastic cells. *Biochim Biophys Acta* 2002; **160,**3: 31-46.

Lerchenmueller C, Husseini F, Gaede B et al: Intravenous iron supplementation in patients with chemotherapy-induced anemia receiving darbepoetin alfa every 3 weeks, *Blood* 2006; **108** (11), abstract 1552.

Leyland-Jones B , Semiglazov V, Pawlicki M et al; Maintaining Normal Hemoglobin Levels With Epoetin Alfa in Mainly Nonanemic Patients With Metastatic Breast Cancer Receiving First-Line Chemotherapy: A Survival Study. *Journal of Clinical Oncology* 2005; **23,** 5960-68.

Leyland-Jones B, Mahmud S: Erythropoietin to treat anemia in patients with head and neck cancer. *Lancet* 2004; **363**: 80.

Littlewood, T.J., Bajetta, E., Nortier, J.W.R. et al: Epoetin Alfa Study Group. Effects of epoetin alfa on hematologic parameters and quality of life in cancer patients receiving nonplatinum chemotherapy: results of a randomized, double-blind, placebo-controlled trial. *Journal of Clinical Oncology* 2001; **19**: 2865-2874.

Littlewood TJ, Zagari M., Pallister C, Perkins A: Baseline and early treatment factors are not clinically useful for predicting individual response to erythropoietin in anemic cancer patients. *Oncologist* 2003; **8**(99): 99-107.

Littlewood TJ, Kallich JD, San Miguel J, Hendricks L, Hedenus M: Efficacy of darbepoetin alfa in alleviating fatigue and the effect of fatigue on quality of life in anemic patients with lymphoproliferative malignancies. *J Pain Symptom Manage*. 2006; **31**(4): 317-25.

Locatelli F, Aljama P, Barany P, et al. European Best Practice Guidelines Working Group. Revised European best practice guidelines for the management of anaemia in patients with chronic renal failure. *Nephrol Dial Transplant*. 2004; **19** (Suppl 2): ii1-47.

Ludwig H, Fritz E, Kotzmann H, et al. Erythropoietin treatment of anemia associated with multiple myeloma. *N Engl J Med.* 1990; **322:** 1693-1699.

Ludwig H, Sundal E, Pecherstorfer M, et al: Recombinant human erythropoietin for the correction of cancer associated anemia with and without concomitant cytotoxic chemotherapy. *Cancer* 1995; **76**: 2319-2329.

Ludwig H, Kanti Rai, Joan Blade et al. Management of disease-related anemia in patients with multiple myeloma or chronic lymphocytic leukemia:epoetin treatment recommendations. *The Hematology Journal,* 2002; **3:** 121-130.

Ludwig H, Van Belle S, Barett-Lee P, et al; The European Cancer Anaemia Survey (ECAS): a large, multinational prospective survey defining the prevalence, incidence, and treatment of anaemia in cancer patients; *Eur J Cancer*. 2004; **40**(15): 2293-306.

Lyman GH, Glaspy J. Are there Clinical Benefits with Early Erythropoietic Intervention for Chemotherapy-Induced Anemia? Cancer 2006; **106**(1): 223-233

Macdougall IC. Strategies for iron supplementation: oral versus intravenous. *Kidney Int.* 1999; **69** suppl: S61-S66.

Macdougall IC, Gray SJ, Elston O, et al: Pharmacokinetics of novel erythropoiesis stimulating protein compared with epoetin alfa in dialysis patients. J *Am Soc Nephrol* 1999; **10**: 2392-2395.

Maciejewski JP, Selleri C, Sato T et al; Nitric oxide supression of human hematopiesis in vitro: contribution to inhibitory action of interferon gamma andd tumor necrosis factor-alpha; *J Clin Invest* 1995; **96**: 1085-92.

Mano M, Butzberger P, Reid A, Rodger A, Soutar R, Welsh J. Current role of erythropoietin in the management of patients with haematological and solid malignancies. *Cancer Ther*. 2005; **3**: 41-56.

Marsh WA, Rascati KL: Meta-analyses of the effectiveness of erythropoietin for end-stage renal disease and cancer. *Clin Ther* 1999; **21**: 1443-55.

Mast AE, Blinder MA, Lu Q, Flax S, Dietzen DJ. Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency. *Blood.* 2002; **99**: 1489-1491

Means RT, Jr. Advances in the anemia of chronic disease. *Int J Haematol* 1999; **70**: 7-12

Merk K, Mattson B, Mattson A et al. The incidence of cancer among blood donors. *Int J Epidemiol* 1990; **19:** 505-9.

Miller CB, Jones RJ, Piantodosi S, et al. Decreased erythropoetin response in patients with the anemia of cancer. *N Engl J Med* 1990; **322**: 1689-1692.

Mittelman M, Zeidman A, Fradin A et al; Recombinant human erythropoietin in the treatment of multiple myeloma-associated anemia. *Haematologica,* 1997; **98:** 204-10.

Moullet I, Salles G, Ketterer N, et al: Frequency and significance of anemia in non-Hodgkin's lymphoma patients. *Ann Oncol* 1998; **9**: 1109-1115.

Mulcahy L: The erythropoietin receptor. *Semin. Oncol.* 2001; **28** (2 Suppl 8): 19-23

Munstedt K, Kovacic M, Zygmunt M, et al: Impact of hemoglobin levels before and during chemotherapy on survival of patients with ovarian cancer. *Int J Oncol* 2003; **23:** 837-843,

National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology. Cancer- and treatment-related anemia. V.2.2006. http://www.nccn.org/professionals/physician_gls/PDF/anemia.pdf

Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin Regulates Cellular Iron Efflux by Binding to Ferroportin and Inducing Its Internalization. *Science* 2004; **306**: 5704.

Nemeth, E. et al. Il-6 mediates hypoferremia in inflammation by inducing the synthesis of the iron regulatory hormone, hepcidin. *J. Clin. Invest.* 2004; **113**: 1271-1276

Nemeth E, Ganz T. Hepcidin and iron-loading anemias. *Haematologica.* 2006; **91**: 727-732.

Nowrousian MR, Pathopysiology of cancer-related anemia. Recombinant Human Erythropoietin (rhEPO) in Clinical Oncology – Scientific and Clinical Aspects of Anemia in Cancer. Wien, New York; Springer 2002: 39-62

Österborg A, Boogaerts MA, Cimino R et al: Recombinant human erythropoetin in transfusion-dependent anemic patients with multiple myeloma or non-Hodgkin´s lymphoma: A randomized multicenter study-The European Study Group of Erythropoetin (Epoetin beta) Treatment in Multiple myeloma and Non-Hodgkin´s lymphoma. *Blood* 1996; **87**: 2675-2682

Österborg A, Brandberg Y, Molotova V. et al: Randomized, double-blind, placebo-controlled trial of recombinant human erythropoetin, epoetinepoietinepoetin beta, in hematologic malignancies. *J Clin Oncol* 2002; **20**: 2486-2494.

Österborg A, Brandberg Y, Hedenus M: Impact of epoetin beta on survival of patients with lymphoproliferative malignancies: Long-term follow-up of a large randomized study: *Br J Haematol* 2005; **129**, 206-209.

Österborg A, Aapro M, Cornes P et al: Review: Preclinical studies of erythropoietin receptor expression in tumour cells: Impact on clinical use of erythropoietic proteins to correct cancer-related anaemia. *Eur J Cancer* 2007; **(43)**: 510-519.

Platanias JC, Miller CB, Mick R et al:Treatment of chemotherapy-induced anemia with recombinant human erythropoietin in cancer patients. *J Clin Oncol.* 1991; (**11**): 2021-6.

Portenoy RK, Thaler HT, Korblith AB et al. Symptom prevalence, characteristics, and distress in a cancer population. *Qual Life Res* 1994; **3**: 183-189

Punnonen K, Irjala K, Rajamäki A. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron ddeficiency. *Blood* 1997; **89**: 1052-7.

Quirt I, RobesonC, Lau CY et al: Epoetin alfa therapy increases hemoglobin levels and improves quality of life in patients with cancer-related anaemia who are not receiving chemotherapy and patients with anemia who are receiving chemotherapy. *J Clin Oncol* 2001; **19(**21): 4126-4134

Ramakrishnan R, Cheung WK, Wacholtz MC, et al: Pharmacokinetic and pharmacodynamic modeling of recombinant human erythropoietin after single and multiple doses in healthy volunteers. *J. Clin. Pharmacol.* 2004; **44** (9): 991-1002

Rivera S, Liu L, Nemeth E, et al. Hepcidin excess induces the sequestration of iron and exacerbates tumor-associated anemia. *Blood.* 2005; **105**(4): 1797-1802

Rizzo JD, Lichtin AE, Woolf SH, et al. Use of epoetin in patients with cancer: evidence-based clinical practice guidelines of the American Society of Clinical Oncology and the American Society of Hematology. *Blood.*2002; **100**: 2303-2320.

Roy CN, Andrews NC. Anaemia of inflammation: the hepcidin link. *Curr. Opin. Haematol,* 2005; **12**(2): 107-111.

Schilling RF. Anemia of chronic disease: a misnomer. *Ann Intern Med,* 1991; **115:** 572

Schreiber-Mojdehkar B et al: Intravenous, oxidant stress, non-transferrin bound iron and saturation of transferrin in hemodialysis patients. *JASN* 2004**; 15**: 1648-1655.

Seidenfeld J, Piper M, Flamm C, et al. Epoetin treatment of anemia associated with cancer therapy: a systematic review and meta-analysis of controlled clinical trials. *J Natl Cancer Inst* 2001; **93**: 1204-14.11.

Shasha D: The negative impact of anemia on radiotherapy and chemoradiation outcomes. *Semin Hematol* 2001; **38**: 8-15.

Silvestris F, Romito A, Fanella P et al; Long-term therapy with rHu-EPO in progressing multiple myeloma. *Haematology* 1995; **70,** 313-18.

Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood.* 1990; **75**(9): 1870-1876

Smith R, Tchekmedyian NS, Richards D et al. Darbepoetin alfa effectively alleviates anemia in patients with chronic anemia of cancer: efficiacy and pharmacokinetic results of a dose-escalation study. *Proc Am Soc Clin Oncol* 2002; **21**: 367a (Abstract 1465).

Souminen P, Punnonen K, Rajamäki A et al; Serum Transferrin Receptor and Transferrin-Ferritin Index Identify Healthy Subjects With Subclinical Iron Deficits. *Blood,*1998; **92**: 2934-39.

Spivak JL: Cancer-related anemia: its causes and characteristics. *Semin. Oncol.,*1994**; 21** (2 Suppl 3): 3-8.

Spivak JL: Iron and the anemia of chronic disease. *Oncology* 2002; **16.** Suppl 10: 25-33.

Stone P, Richardson A, Ream E, et al. Cancer-related fatigue: Inevitable, unimportant and untreatable? Results of a multi-centre patients survey. *Ann Oncol* 2000; **11**: 971-975

Stone P, Ream E, Richardson A, et al. Cancer-related fatigue- a difference of opinion? Results of a multicenter survey of healthcare professionals, patients and caregivers. *Eur J Cancer Care* 2003; **12**: 20-27.

Syed RS, Reid SW, Li C et al: Efficiency of signalling through cytokine receptors depends critically on receptor orientation.*Nature* 1998; **395** (6701): 511-6.

Tartter PI. Does blood transfusion predispose to cancer recurrence? *Am J Clin Oncol.* 1989*;* **12**: 169-171.

Thomas ML: Impact of anemia and fatigue on quality of life in cancer patients: A brief review. *Med Oncol* 1998; **15**: S3-S7.

Thomas C; Thomas L: Biochemical markers and haematological indicies in the diagnosis of functional iron deficiency. *Clin Chem* 2000; **48**: 7, 1066-76.

Thomas GM: Raising hemoglobin level: an opportunity for increasing survival? *Oncology* 2002; **63** (Suppl 2): 19-28.

Van Belle SJ, Cocquyt V: Impact of hemoglobin levels on the outcome of cancers treated with chemotherapy. *Crit. Rev. Oncol. Hematol.* 2003; **47**: 1-11.

Vansteenkiste J, Pirker R, Massuti B et al. Darbepoetin alfa decreases transfusions and fatigue in lung caner patients receiving chemotherapy. Results of a double blind, placebo controlled, randomised phase 3 study. *J Natl Cancer Inst.* 2002**; 94**(16): 1211-1220

Vaupel P, Thews O, Hoeckel M: Treatment resistance of solid tumors: Role of hypoxia and anemia. *Med Oncol* 2001; **18**: 243-259.

Viatte L, Nicolas G, Lou DQ, et al. Chronic hepcidin induction causes hyposideremia and alters the pattern of cellular iron accumulation in hemochromatotic mice. *Blood*, 2006; **107**(7): 2952-2958

Vogelzang NJ, Breitbart W, Cella D, *et al*, For The Fatigue Coalition: Patient, caregiver, and oncologist perceptions of cancer-related fatigue: results of a tripart assessment survey. *Semin. Hematol.* 1997; **34** (3 Suppl 2): 4–12.

Walker RH: Special report: transfusion risks. *Am J Clin Pathol* 1987; **88**: 374-378.

Weinberg ED: The role of iron in cancer. *Eur J Cancer Prev* 1996; **5**: 19-36.

Weinstein DA, et al. Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease. *Blood* 2002; **100**: 3776-3781.

Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med*. 2005; **352**: 1011-1023.

Weiss G, Gordeuk VR: Benefits and risks of iron therapy for chronic anaemias. *Eur J Clin Invest* 2005; **35** (Suppl.3): 36-45

Westphal G, Niederberger E, Blum C et al: Erythropoietin and G-CSF receptors in human tumor cells: Expression and aspects regarding functionality. *Tumori* 2002; **88**: 150-9.

Wians FH, Urban JE, Keffer JH et al. Discriminiating between iron deficiency anemia and anemia of chronic disease using traditional indices of iron status vs transferrin receptor concentration. *Am J Clin Pathol* 2001; **115:** 112.

Witzig TE, Silberstein PT, Loprinzi JA et al: Phase lll, Randomized.Doubleblind Study of Epoetin Alfa versus Placebo in Anemic Patients with Cancer undergoing Chemotherapy. *J Clin Oncol* 2004; **23**: 2606-17.

Yasuda Y, Fujita Y, Matsuo T, *et al*.: Erythropoietin regulates tumor growth of human malignancies. *Carcinogenesis* 2003; **24** (6): 1021-1029.