

Epidemiological and nonclinical studies investigating effects of iron in carcinogenesis—A critical review[☆]

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Accepted 31 October 2013

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Abstract

The efficacy and tolerability of intravenous (i.v.) iron in managing cancer-related anemia and iron deficiency has been clinically evaluated and reviewed recently. However, long-term data in cancer patients are not available; yet, long-term i.v. iron treatment in hemodialysis patients is not associated with increased cancer risk. This review summarizes epidemiological and nonclinical data on the role of iron in carcinogenesis. In humans, epidemiological data suggest correlations between certain cancers and increased iron exposure or iron overload. Nonclinical models that investigated whether iron can enhance carcinogenesis provide only limited evidence relevant for cancer patients since they were typically based on high iron doses as well as injection routes and iron formulations which are not used in the clinical setting. Nevertheless, in the absence of long-term outcome data from prospectively defined trials in i.v. iron-treated cancer patients, iron supplementation should be limited to periods of concomitant anti-tumor treatment.

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Keywords: Iron deficiency; Anemia; Carcinogenesis; Tumor progression; Nonclinical; Intravenous iron; Parenteral iron

1. Introduction

Iron-containing proteins participate in many essential biological processes such as oxygen transport, cellular respiration and redox reactions. However, ferrous iron (Fe^{2+}) can catalyze the production of hydroxyl radicals ($\bullet\text{OH}$) [1] which are stronger oxidants than the antimicrobial superoxide radicals ($\text{O}_2^{\bullet-}$), and therefore can exert oxidative damage to nearby lipids, carbohydrates, proteins or DNA. Since iron is not actively secreted from the body, systemic iron levels are regulated by the liver-derived peptide hepcidin, whereas cellular iron levels are controlled by iron-regulatory proteins (IRPs) that bind to iron-response elements (IREs) in the messenger RNA of iron-related genes (Fig. 1) [2,3]. These highly conserved mechanisms of iron sequestration also provide protection against infectious diseases by depriving invading pathogens of this essential nutrient [4,5]. However, these mechanisms are also activated by inflammatory processes associated with chronic diseases such as cancer.

Interleukin (IL)-6 and IL-1 are the main inflammatory effectors that increase the expression of hepcidin [2], which in turn deactivates ferroportin, the iron export protein on the surface of enterocytes, hepatocytes and macrophages [6,7]. The reduced absorption and release of iron leads to an imbalance between iron requirements for erythropoiesis in the bone marrow and the iron supply from macrophages. This functional iron deficiency (FID) is believed to be one of the major causes of the anemia of chronic disease (ACD). Conversely, low hepcidin activity results in increased dietary absorption and mobilization of iron from cellular stores, which can result in iron overload.

In cancer patients, anemia is associated with shorter survival time [8], and symptoms of iron deficiency and anemia (e.g. weakness and fatigue) affect patients' quality of life [9,10]. Intravenous (i.v.) iron in conjunction with erythropoiesis-stimulating agents (ESAs) has been shown

to improve hemoglobin status and reduce blood transfusion needs in anemic cancer patients [9,11–17]. In contrast, oral iron has very limited efficacy in this patient population, and therefore, treatment guidelines recommend i.v. iron supplementation [18,19].

Although i.v. iron preparations passed genotoxicity testing (e.g. Ames) as part of the development and approval process, an open question remains whether iron supplementation of cancer patients might influence tumor progression. One preliminary study with long-term follow-up showed no effect of i.v. iron on 3-year progression-free survival in anemic patients with lymphoid malignancies [20,21]. However, there are insufficient data from prospectively defined studies to address this question. Several prior reviews have outlined mechanisms how elevated iron levels may influence signaling pathways and tumor progression or, vice versa, how signaling through certain pathways may contribute to altered iron metabolism in cancer patients [1,22–24]. In order to facilitate informed decisions on the clinical use of i.v. iron supplementation, the review presented here evaluates how the designs and results of published epidemiological and nonclinical studies compare to the clinical use of i.v. iron, which is intended to provide sufficient iron availability and normalization of hemoglobin levels in patients with cancer-related iron deficiency or anemia.

2. Clinical data

2.1. Hereditary hemochromatosis

The most common cause of iron overload is hereditary hemochromatosis (HH), a genetic condition with inappropriately low hepcidin levels or activity. This results in accumulation of iron in the liver and other organs and can be diagnosed by transferrin saturation >45% and elevated

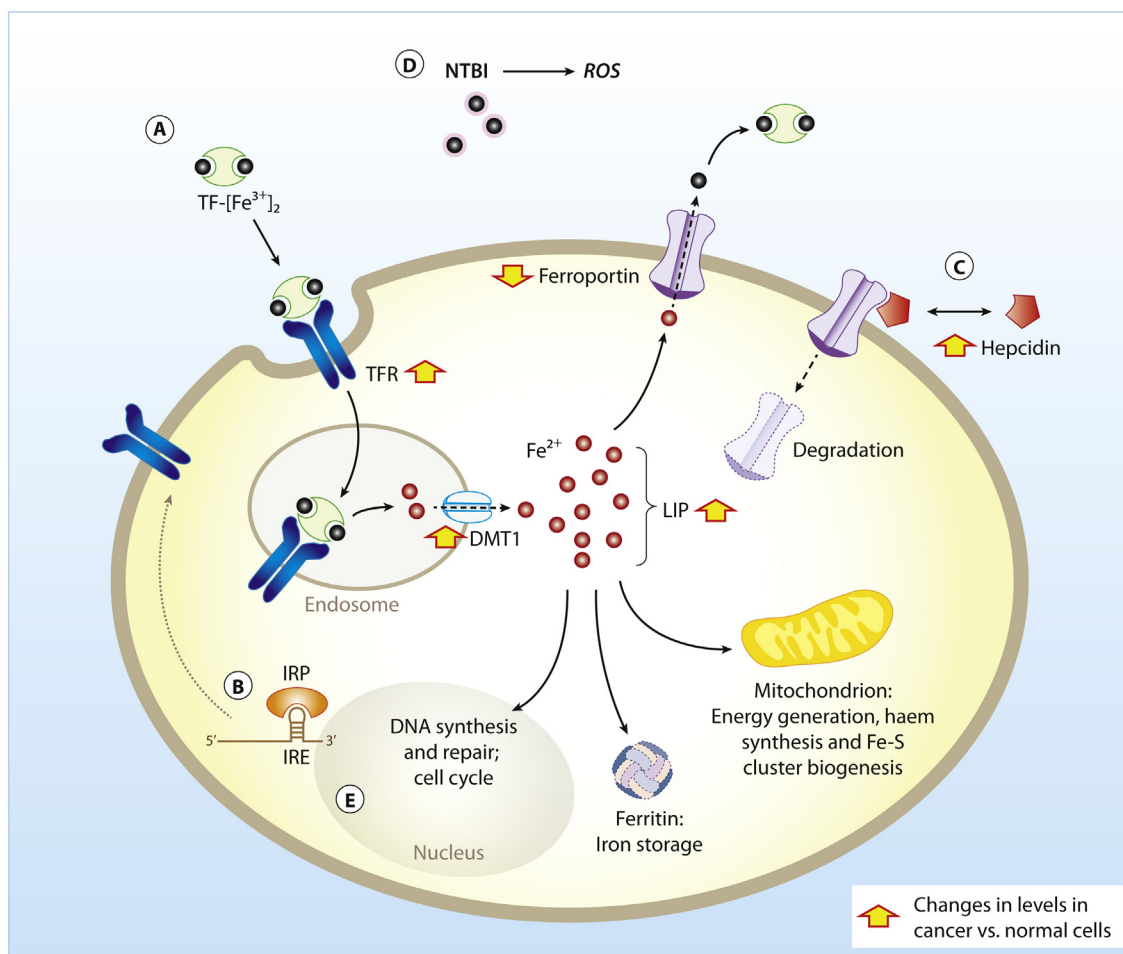


Fig. 1. Cellular iron metabolism – iron uptake and efflux in normal and cancer cells. (A) Circulating iron is generally bound to transferrin (TF). TF-bound iron binds to transferrin receptor (TFR) on the plasma membrane of most cells, and the TF- $[\text{Fe}^{3+}]_2$ -TFR complex is internalized by endocytosis. In the endosome, Fe^{3+} is reduced to Fe^{2+} and transported into the cytosolic labile iron pool (LIP) that harbors the metabolically active forms of cellular iron. From the LIP, iron is delivered to intracellular compartments such as the nucleus and mitochondria (for metabolic utilization), to cytoplasmic ferritin (for storage in a bioavailable, non-toxic form) or exported from the cell by ferroportin, an iron efflux pump. (B) On the cellular level, iron metabolism is controlled by iron-regulatory proteins (IRP). Under conditions of low intracellular iron levels, iron-regulatory proteins (IRP) bind to iron-response elements (IRE) present in the untranslated regions of mRNAs encoding ferritin subunits, ferroportin, isoforms of divalent metal transporter 1 (DMT1) and TFR. Binding stabilizes the mRNAs that encode TFR and DMT1, and represses the translation of ferritin and ferroportin. The net result is an increase in iron uptake and a decrease in iron storage and efflux. (C) Systemic iron homeostasis is largely controlled by hepcidin, a key iron regulatory hormone. Hepcidin downregulates ferroportin and thereby inhibits iron efflux from duodenal enterocytes, macrophages, and hepatocytes into the circulation. (D) High amounts of systemic iron may lead to saturation of TF and subsequent formation of non-transferrin bound iron (NTBI). NTBI is taken up non-selectively by tissues and can lead to the formation of reactive oxygen species (ROS), causing oxidative stress and ultimately cell damage. (E) In cancer cells, genes encoding proteins that increase intracellular iron (TFR, DMT1, hepcidin) are generally upregulated, whilst those decreasing iron levels (ferroportin) are downregulated. DMT1, divalent metal transporter 1; IRE, iron-response element; IRP, iron regulatory protein; LIP, labile iron pool; NTBI, non-transferrin bound iron; ROS, reactive oxygen species; TF, transferrin; TFR, transferrin receptor.

serum ferritin levels whereupon a ferritin >1000 together with elevated aminotransferase levels may be indicative of a risk of cirrhosis [25]. Typical complications associated with HH are liver cirrhosis, diabetes, hypogonadism, and cardiomyopathy [26]. Complications are avoidable if serum iron levels are successfully managed by phlebotomy before cirrhosis develops [27]. Individuals with HH were originally thought to have an up to 200-fold higher risk of hepatocellular carcinoma (HCC), but more conservative estimates suggest that a 20-fold increase may be more realistic [28]. Since HCC occurs almost exclusively in HH patients who have developed cirrhosis, there is limited

evidence of a direct role for excess iron in carcinogenesis; instead, cirrhosis may provide the link for progression to HCC.

Increased risk of developing HCC also exists with a disorder called African iron overload (AIO), which most probably arises through interaction between environmental factors (e.g. consumption of iron-rich beverages brewed in non-galvanized steel drums) and potential genetic factors [29,30]. Several studies and a large meta-analysis investigating the risk of non-hepatic cancers in patients with HH or with HH-predisposing genotypes have provided conflicting results [27,31–36] considerable.

Table 1

Epidemiological studies investigating iron status and risk of cancer.

Study design, population, study name, follow-up (FU)	Key outcomes	Ref.
Prospective cohort study, adults, AMORIS, 10.6 y FU	High TIBC (i.e. low TSAT) associated with increased cancer risk	[58]
Retrospective cohort, adults	Iron overload as well as iron deficiency affect PFS and OS	[146]
Prospective cohort, adults, NHANES I, 10–13 y FU	Higher TSAT in men who developed cancer, no effect of dietary iron	[54]
Prospective cohort, adults, NHANES I, 13–17 y FU	Higher TSAT in men and women who developed cancer	[55]
Prospective cohort, males, 12 y FU	No relationship between dietary iron intake and bladder cancer	[51]
Prospective cohort, adults, NHANES II, 12–16 y FU	High serum iron or TSAT associated with increased risk of cancer death	[57]
Prospective cohort, adults, NHANES I, 18–21 y FU	High TSAT and high iron intake associated with high cancer risk	[53]
Prospective cohort, adults, Framingham Offspring Study, 14 y FU	Elevated serum iron associated with development of cancer	[52]
Prospective cohort, adults, NHANES I, 18–21 y FU	Elevated serum iron and cholesterol in subjects who developed cancer	[56]
Prospective cohort, adults, SU.VI.MAX, 7.5 y FU	High ferritin in women who developed cancer, no effect of dietary iron	[59]
Prospective cohort, women, CNBSS, 16.4 y FU	No association of iron or heme iron intake with breast cancer risk	[47]
Prospective controlled trial, chronic hepatitis C, up to 12 y FU	Phlebotomy and low iron diet lowered the risk of HCC	[62]
Retrospective nested case-control study, blood donors, 3–12 y FU	Repeated blood donation had no effect on cancer risk	[60]
Prospective cohort, endometrial cancer cases, CNBSS, 16.4 y FU	No effect of meat or dietary iron intake	[50]
Retrospective nested case-control study, Barrett's esophagus	No difference in TSAT and serum ferritin vs. matched controls	[31]
Randomized controlled single-blinded trial, PAD, 4.5 y mean FU	Lower risk of new visceral malignancy in the phlebotomy arm	[61]
Retrospective, population-based case-control study	High daily intake of animal-derived iron associated with breast cancer	[49]
Prospective nested case-control study, breast cancer, 9–11 y FU	No effect of either dietary iron intake or increased serum ferritin levels	[48]
Retrospective nested case-control study, blood donors	Depending on tumor type, Hb decline started 1–3 y before diagnosis	[156]

AMORIS, Apolipoprotein Mortality Risk Study; CNBSS, Canadian National Breast Screening Study; FU, follow-up; Hb, hemoglobin; HCC, hepatocellular carcinoma; NHANES, National Health and Nutrition Examination Survey; OS, overall survival; PAD, peripheral arterial disease; PFS, progression-free survival; SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants; TIBC, total iron binding capacity; TSAT, transferrin saturation.

2.2. Epidemiology of iron intake, iron status and cancer risk

Many epidemiological studies have searched more broadly for a link between cancer and environmental exposure to iron, dietary iron intake, or iron status (relevant studies since a 1998 review [22] are summarized in Table 1). Studies exploring excessive environmental exposure to iron are often limited by poor characterization of the environmental factors and causal relations to effects other than the chemical properties of iron [37]. Population studies relating to gastrointestinal (GI) cancers have been reviewed recently in detail elsewhere [38,39].

Although most population studies support an association between colorectal cancer (CRC) risk and iron exposure [40–42], there are others that do not [41,43,44]. In fact, iron deficiency might also be associated with an increased risk of GI malignancies [39], particularly in *Helicobacter pylori*-infected patients [45], and iron may modulate cancer risk differently in different regions of the GI tract. A case-controlled study suggested that increased CRC risk is related to luminal exposure to excessive iron (combined with a high fat diet) rather than increased iron stores (based on serum ferritin levels) [44]. In addition to geographic region-specific differences, other factors such as different dietary habits, iron status assessment or iron intake/supplementation complicate interpretation or comparison of epidemiological studies.

Where correlations between high red meat consumption and CRC risk have been identified [40,42], it is difficult to distinguish whether the heme iron or saturated fat constituents have contributed to the associated risk [38]. Moreover, considering the contribution of iron alone, in one study fecal levels of carcinogenic N-nitrosated products were higher in

healthy volunteers fed a diet high in red meat or the equivalent amount of heme, than in those whose diet was supplemented with ferrous salts [46].

Compared to the well-explored epidemiology of CRC, few population studies have focused on the role of iron in other cancers. Two studies on breast cancer did not demonstrate a strong link between iron status and cancer risk [47,48]. In a Chinese cohort study, women with elevated serum ferritin levels had more fibrocystic changes, but these were non-proliferative, and no link between iron and breast cancer was observed [48]. High intake of animal-derived iron (mainly heme) and fat were both associated with an increased risk of primary breast cancer [49]. Studies investigating endometrial cancer [50], bladder cancer [51] or the transition from Barrett's esophagus to esophageal adenocarcinoma [31] showed no link between iron status, dietary iron intake and cancer risk.

Several studies have evaluated data of the large US National Health and Nutrition Examination Surveys (NHANES I and II), and found higher transferrin saturation (TSAT), partly in combination with elevated cholesterol or high dietary iron intake, as risk factor of developing cancer [52–57]. Conversely, the very large Swedish Apolipoprotein Mortality Risk study (AMORIS; 220,642 individuals with iron status assessment of whom 9269 developed cancer more than three years after the test) found a correlation between high total iron binding capacity (i.e. low TSAT) and cancer risk [58]. Serum iron did not correlate with cancer risk. Analysis of middle-aged people in France found a higher risk of developing cancer only in women with elevated serum ferritin [59]. However, since serum ferritin is an acute phase protein, it is possible that elevated serum ferritin levels observed in such studies are secondary to an underlying disease.

2.3. Phlebotomy and blood transfusion

Based on long-term data from national blood banks, a nested case-control study showed no clear association between the risk of cancer and the number of blood donations that were taken as a measure of iron depletion. However, sub-analysis revealed a trend toward reduced risk of cancers of the liver, lung, colon, stomach and esophagus in men with increasing number of blood donations [60]. Among patients with peripheral arterial disease (PAD) who were randomized to phlebotomy or no iron reduction, significantly less visceral malignancies and lower mortality were observed in the phlebotomy group [61]. The results were particularly striking because cancer incidence in treatment and control arms began to diverge as early as 6 months after the study started. Similar treatment, phlebotomy combined with low-iron diet, has been shown to reduce the risk of HCC in patients with chronic Hepatitis C virus (HCV) infection [62].

3. Nonclinical models and data

In a simplified model, carcinogenesis can be described as a multi-step process of induction, promotion and progression [63]. Accordingly, substances involved in carcinogenesis can be categorized as cancer inducers if they initiate the transformation of normal into cancer cells or as cancer promoters if they are not inherently carcinogenic but enhance the activity of a cancer inducer. Substances that enhance tumor progression typically support the proliferation and spread of existing cancer cells.

3.1. Can high iron load induce tumors?

Concerns that parenteral iron might be carcinogenic originally stem from sporadic cases of sarcoma in patients treated with intramuscular (i.m.) iron dextran in the 1960s [23]. Animal studies investigating the tumor induction potential of iron compounds [22,23] (Table 2) used mainly subcutaneous (s.c.) or i.m. application of iron dextran or intraperitoneal (i.p.) application of ferric nitrilotriacetate (Fe-NTA) or ferric saccharate.

Initial experiments investigated sarcoma formation in rodents receiving iron dextran s.c. or i.m. over a period of 3–17 months (total iron doses of 1583–18,200 mg/kg bodyweight) [64,65]. Depending on the dosing regime and cumulative iron dose, 55–80% of animals developed tumors. Differing degrees of tumorigenicity were observed in other species, with hamsters, rabbits, guinea pigs, dogs and squirrel monkeys being less susceptible to iron-related cancer induction than rats or mice [64,66–68]. Varying the number of injection sites suggested that high local iron concentration was critical for tumor induction [69]. This particular series of animal studies used administration routes and iron doses that are not comparable to i.v. iron administration in current clinical practice. Compared to relevant clinical i.v. iron doses

(750–3000 mg cumulative dose; i.e. 10–40 mg/kg in a 75 kg patient) [9,12–16,70], 10- to 450-fold higher cumulative iron doses were administered.

Lower but still high amounts of i.p. injected Fe-NTA were required to produce renal cell carcinoma (RCC) in rodents [71,72]. Animals received daily Fe-NTA injections over 12 weeks to achieve the observed 60–80% incidence of RCC after 1 year. Notably, Fe-NTA is severely nephrotoxic and 53% of animals died within 2 weeks in some experiments. In addition to dissociated iron, the Fe-NTA complex itself can undergo redox cycling under physiological conditions [73], and can pass the glomeruli into proximal renal tubes more readily than the larger and more stable iron-carbohydrate complexes [74]. Also noteworthy, NTA alone (albeit at high doses) is carcinogenic [75] by mechanisms involving the Fenton reaction [76] and acts as a tumor promoter [77]. Therefore, studies involving Fe-NTA have limited relevance to the therapeutic use of clinical iron preparations in cancer patients.

In accordance with the large iron doses used, several of these nonclinical studies reported iron deposits in organs and tissues. Rats receiving ferric saccharate i.p. acquired brownish peritoneal serosa [68]. In iron dextran treated animals, pigmented phagocytes were localized at the site of injection, and hemosiderin iron deposits were detected in the liver and kidneys [64,68]. It seems likely that in these animal models, transfer of iron from the injection site to body iron stores could not keep pace with the rate and amount of administered iron. These, high, persistent local concentrations of iron have been shown to induce tumor formation in some animal models, but not others. In contrast to i.p. administration, i.v. administration facilitates rapid dispersal of iron throughout the circulation and subsequent uptake by the reticuloendothelial system (RES); this administration route was not tested with any animal models so far reported.

3.2. Can iron promote the activity of known tumor inducers?

In a variety of animal models (Table 3), cancer was induced by different chemical, genetic or surgical means, and the effect of iron on tumor promotion was investigated.

3.2.1. Liver cancer models

Several environmental pollutants including hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs) and diethylnitrosamine (DEN) have been shown to induce liver cancer in animals. Due to the association of iron overload with HCC in humans, the possibility that iron overload might promote the effects of these chemical carcinogens has been investigated [78–81]. All the reviewed studies used massive single or multiple doses of s.c. iron dextran (cumulative dose of 600–4000 mg iron/kg body weight) in combination with the chemical inducers. The effects of iron-loading ranged from increased pre-neoplastic changes [81] to development of HCC in the long term [78].

Table 2
Parenteral iron administration in models of cancer induction.

Tumor type, species	Iron complex, route	Cumulative iron dose	Key outcome	Ref.
Sarcoma, rat	Iron dextran, i.m.	1583–4622 mg/kg	55–70% developed tumors	[65]
Sarcoma, mouse, rat, guinea pig, hamster, rabbit	Iron dextran, s.c. or i.m.	Mice: 5200–18200 mg/kg Rats: 4333 mg/kg	Tumors in 76% of mice, sarcoma in 80% of rats, low tumorigenicity in hamsters, and none in guinea pigs or rabbits.	[64]
Sarcoma, mouse, rat, rabbit, dog	Iron dextran, s.c.	Mice: up to 4000 mg/kg Rats: up to 833 mg/kg	Sarcomas only in mice and rats and after multiple injections	[66]
Sarcoma, rabbit	Iron dextran, i.m.	933 mg/kg	Sarcoma in 33% of rabbits (follow-up study to [64])	[68]
Sarcoma, rat	Iron dextran, s.c.	2000 mg/kg	Tumor incidence decreased with increased number of injection sites	[69]
Sarcoma, squirrel monkey	Iron dextran, i.m.	500 mg/kg	No sarcoma or non-injection site tumors	[67]
RCC, rat	Fe-NTA, i.p.	360–504 mg/kg	RCC in 78% of mice (43% metastases, 8% died from nephrotox)	[71]
RCC, mouse	Fe-NTA, i.p.	130–194 mg/kg	RCC in 60% of male mice (53% of males died from nephrotox), female mice less susceptible to nephrotox and carcinogenicity	[72]
Mesothelioma, male rat	Ferric saccharate and NTA, i.p.	390 mg/kg	Mesothelioma in 47% Fe-saccharate only and 68% Fe-saccharate + NTA rats	[157]
RCC, male rat	Fe-NTA, i.p.	130–182 mg/kg	RCC in 53–56% of rats, tumors larger in non-phlebotomized rats	[74]
Skin, female mouse	Iron dextran, i.m.	600 mg/kg	Papillomas in 50% of mice	[87]
Mesothelioma, rat	Ferric saccharate and NTA, i.p.	500 mg/kg	Mesothelioma in 66.7% of male but only 3.3% of female rats	[158]

i.m. intramuscular; i.p., intraperitoneal injection; NTA, nitrilotriacetic acid; RCC, renal cell carcinoma; s.c., subcutaneous.

For comparison purposes, the weight of a mouse was estimated to be 25 g, a rat 300 g, a rabbit 3 kg, and a squirrel monkey 1 kg.

Table 3
Administration of oral and parenteral iron in models of cancer promotion.

Tumor type, species	Iron complex, route	Cumulative iron dose	Key outcome	Ref.
HCC, mouse	Iron dextran, s.c.	600 mg/kg	Increased incidence of liver hyperplastic nodules and HCC	[78]
HCC, mouse	Iron dextran, s.c.	600 mg/kg	Increased incidence of HCC	[80]
HCC, female rat	Iron dextran, s.c.	600 mg/kg	Increased incidence and number of hepatic nodules	[79]
HCC, female rat	Iron dextran, s.c.	4000 mg/kg	Increased number and size of GST-P expressing liver foci	[81]
HCC, rat	Dietary iron, oral	N/A	All rats on the normal diet developed hepatic adenocarcinomas	[159]
Skin, female mouse	Iron dextran, i.m.	560 mg/kg	Higher incidence and number of tumors and earlier appearance	[82]
Skin, female mouse	Iron dextran, i.m.	560 mg/kg	Increased frequency of papillomas and skin carcinomas	[83]
Skin, female mouse	Iron dextran, i.m.	600 mg/kg	Higher incidence and number of tumors per mouse	[86]
Skin, female mouse	Iron dextran, i.m.	300–900 mg/kg	Higher incidence and number of tumors and earlier appearance	[160]
Skin, female mouse	Iron dextran, i.m.	600 mg/kg	Higher conversion rate from benign to malignant disease	[85]
Skin, female mouse	Iron dextran, i.m.	600 mg/kg	Lower incidence of tumors in pregnant vs. virgin mice	[84]
EAC, rat	Iron dextran, i.p.	144 mg/kg	Increased incidence of CLE. CLE with dysplasia and EAC	[88]
UC and CRC, mouse	Iron dextran, i.p. Dietary iron, oral	i.p.: 216 mg/kg dietary: N/A	Tumors in 73% of mice on oral iron vs. no tumors in i.p. group	[90]
UC and CRC, mouse	Dietary iron, oral	N/A	88% vs. 19% CRC with iron-enriched vs. control diet	[89]
CRC, mouse	Dietary iron, oral	N/A	42% vs. 31% tumors in high iron vs. low iron group	[91]
GI tract tumors, rat	Dietary iron, oral	N/A	Higher number of duodenal tumors in iron-deficient rats	[93]
CRC, male rat	Iron dextran, i.p.	75 mg/kg	Similar incidence but more tumors per animal in iron group	[92]
CRC, male mouse	Iron dextran, s.c.		No influence on intestinal tumorigenesis	[136]
Breast cancer, mouse	Dietary iron, oral	N/A	Lower tumor growth rate in low vs. normal iron group	[94]
Breast cancer, female rat	Dietary iron, oral	N/A	Moderate iron deficiency increased tumor incidence Severe iron deficiency resulted in earlier onset	[95]
Breast cancer, female rat	Dietary iron, oral	N/A	Low tumor incidence with low iron diet Increased tumor incidence with excessive iron diet	[96]
Renal, Male Syrian Hamsters	Dietary iron, oral	N/A	70% vs. 50% with renal tumors after high vs. normal iron diet	[97]
Oral, rat	Dietary iron, oral	N/A	Similar incidence but earlier development of squamous cell carcinomas in the iron deficient group	[100]
Lung tumors, mice	Dietary iron, oral	N/A	Similar tumor incidence but more lung adenomas in iron deficient mice	[101]

CLE, columnar-lined esophagus; CRC, colorectal cancer; EAC, esophageal adenocarcinoma; GI, gastrointestinal; GST-P, placental form of glutathione-S-transferase; HCC, hepatocellular carcinoma; i.m. intramuscular; i.p., intraperitoneal; N/A, not available; s.c., subcutaneous; UC, ulcerative colitis.

For comparison purposes, the weight of a mouse was estimated to be 25 g and a rat 300 g.

Table 4
Administration of oral or parenteral iron in models of progression of established tumors.

Tumor type, species	Iron complex, route	Cumulative iron dose	Key outcome	Ref.
Leukemia, mouse	Iron dextran, i.p.	25 or 250 mg/kg	More tumor cells recovered from iron-treated animals	[102]
CRC, SW480 and Caco-2 cells	Ferrous sulfate, in vitro	N/A	Significant increase in cellular proliferation	[103]
EAC, OE33 and SEG-1 cells	Ferrous sulfate, in vitro	N/A	Less TfR1 and DMT1 mRNA, more H-ferritin protein	[104]
Hepatoma, colon AC, mammary AC, mouse	Dietary iron, oral	N/A	Tumors grew slower in the low iron group	[105]

AC, adenocarcinoma; CRC, colorectal cancer; DMT, divalent metal transporter; EAC, esophageal adenocarcinoma; TfR, transferrin receptor. For comparison purposes, the weight of a mouse was estimated to be 25 g.

3.2.2. Skin cancer models

Animal models of skin cancer typically perform a two-step process involving topically applied tumor inducers followed by tumor promoters. Inducers, such as 7, 12-dimethylbenz(a)anthracene (DMBA), cause genetic alterations, while promoters such as topical peroxide, TPA (12-O-tetradecanoylphorbol-13-acetate), BPO (benzoylperoxide) or croton oil induce inflammation and oxidative stress to favor tumor formation. The effect of iron overload as a tumor promoter in animals which have received tumor initiators only [82] or both initiators and promoters [83–86] has been studied. In all cases, except in pregnant female mice [84], severe iron overload promoted tumor formation. Experiments typically used high cumulative doses of i.m. iron dextran (300–900 mg iron/kg body weight). The absence of tumors in iron-treated animals not receiving tumor initiators demonstrates that parenteral iron overload can promote but does not induce skin cancer in these models [87].

3.2.3. GI tract

In rats with surgically induced esophageal adenocarcinoma (EAC), tumor formation was significantly promoted in animals receiving only moderately excessive doses of i.p. iron dextran [88]. Similar to observations in other tumor induction models, no tumors formed with i.p. iron alone.

In a mouse dextran sulfate sodium (DSS) model of ulcerative colitis (UC), increased dietary iron intake was accompanied by increased tumor incidence [89]. However, further experiments showed that this phenomenon was specific for dietary iron, while parenteral iron did not promote tumor formation in the same model [90]. In a second model, using IL-2 knockout mice that develop spontaneous inflammation, parenteral iron replenished splenic iron and significantly reduced inflammation in the colon without increasing hyperplastic lesions [90].

In a model of CRC using the colonotropic carcinogen, azoxymethane (AOM), tumor incidence increased in AOM-treated mice placed on a high iron diet compared to those placed on a low iron diet; dietary iron alone did not initiate tumors [91]. In another CRC model using the carcinogenic inducer dimethylhydrazine (DMH), parenteral iron increased the tumor burden without significantly increasing tumor incidence [92]. Authors of both studies concluded that in these

carcinogen-induced CRC models, iron exerted its effects as a tumor promoter but not as a tumor inducer. In contrast, in another study of DMH-induced CRC, duodenal but not colonic tumors were more frequent in iron deficient than iron replete rats [93], suggesting that tumor promotion in these CRC models varies with the type of chemical inducer and iron supplementation.

3.2.4. Breast and estrogen-related cancers

In animal models of breast cancer [94–96], a trend-wise correlation of tumor incidence and/or tumor growth with dietary iron uptake was observed. However, in one study, elimination of confounding factors such as anemia and low body weight abolished differences between animals on low and normal iron diet [96]. In another study, a moderately iron deficient diet increased both tumor incidence and tumor burden, whereas severe iron deficiency resulted in rapid onset of tumor formation but ultimately lower tumor incidence and burden [95]. In this particular study, increased tumor development in iron deficient rats was attributed to decreased natural killer (NK) cell cytotoxicity.

In male Syrian hamsters that develop renal cancer following 17 β -estradiol treatment, tumor incidence was increased in animals on high versus low iron diet [97]. The mechanism of estrogen-induced tumor formation may involve redox cycling of catecholesterogen metabolites such as 4-hydroxyestradiol and superoxide radical formation. In the presence of Fe²⁺, conversion of superoxide to H₂O₂ by SOD could lead to hydroxyl radicals via the Fenton reaction. Interestingly, expression of enzymes required to produce catecholesterogen metabolites in the organs of rodents correlate with their susceptibility to estrogen-induced tumors [98]. Additionally, there may be a synergistic effect between 17 β -estradiol and dietary iron on iron homeostasis, since animals treated with 17 β -estradiol alone accumulated iron in the liver and kidneys [97]. Accordingly, an estrogen-responsive region in the transferrin promoter that upregulates transferrin expression in a 17 β -estradiol-incubated breast cancer cell line has been identified [99].

3.2.5. Other cancers

Two additional models of chemically induced carcinogenesis, one a rat model of oral cancer and the other a mouse model of lung cancer, support the notion that iron deficiency

can increase tumor incidence or promote tumor development [100,101]. In the second study, no increase in lung adenoma incidence was found in mice with elevated systemic iron levels due to diets containing up to 5000 ppm iron.

3.3. Can iron influence the progression of established tumors?

In a mouse model of leukemia, mice received an i.p. injection of 25 or 250 mg iron/kg body weight (iron dextran) 6 h prior to i.p. injection of tumor cells. In this study, leukemia cell proliferation was greater in iron-treated animals than in controls without iron treatment [102]. The timing and anatomical proximity of iron and tumor cell injections in this model suggests a direct effect of iron on tumor proliferation, as observed with in vitro studies [103,104] (Table 4).

In a model using three different tumorigenic cell lines transplanted into severely iron deficient mice, tumor sizes were smaller than in iron replete controls [105]. However, in these anemic mice, negative effects of iron deficiency on nutritional status may have also contributed to reduced tumor growth.

3.4. The role of iron chelators in tumor promotion models

An alternative approach to study the role of iron in cell proliferation is to reduce available iron by systemically applied iron chelators (e.g. deferoxamine [DFO]) [106,107] that enhance renal iron excretion. Furthermore, iron chelators are investigated as potential new anti-cancer therapies that exert various modes of action beyond sequestration of iron from rapidly proliferating cancer cells [108,24]. Iron chelators affect expression of diverse genes, including those involved in cell cycle control such as p53, cyclins, cyclin dependent kinases, GADD45 and WAF1, but also the tumor suppressor gene Ndr1 as well as p38 MAPK, ERK and HIF-1 α [109,110].

DFO was initially found to reduce the size of rat mammary adenocarcinomas and human HCC tumor transplants in athymic nude mice [111,112]. DFO also reduced the number of preneoplastic hepatic lesions in rats prone to severe liver fibrosis and HCC due to excessive lipid peroxidation after a choline- and L-amino acid-defined diet [113]. However, iron chelators had no effect on xenograft models of human neuroblastoma, cervical carcinoma or AIDS-related Kaposi's sarcoma, despite varying effects on reduction of systemic iron [114–116]. In the Kaposi's sarcoma model, DFO actually enhanced tumor growth, which could be attenuated by administration of iron-saturated DFO. Possibly, DFO promoted proliferation via anti-apoptotic mechanisms since the apoptotic index of tumor cells in DFO-treated animals was significantly decreased. Conversely, DFO or low iron diet increased in situ apoptosis in the rat 1376NF mammary adenocarcinoma model [117].

A panel of different iron chelators including di-2-pyridyl-derived chelators such as Dp44mT have been developed that induce tumor cell apoptosis in a range of xenograft models [118,119]. Pyridoxal isonicotinoyl hydrazone (PIH) derivatives, containing di-pyridyl ketone isonicotinoyl hydrazone (PKIH) and its analogs, showed high antiproliferative activity with more specificity toward neoplastic SK-N-MC neuroepithelia cells than untransformed MRC-5 fibroblasts [120]. Notably, iron-loaded PKIH analogs demonstrated the same anti-proliferative activity as the iron-free chelator. It has been shown that PKIH increases the generation of ROS through redox cycling of the iron complex [121].

Recently published experiments with eltrombopag, a small-molecule thrombopoietin receptor antagonist and metal chelator, showed a dose-dependent reduction of free intracellular iron and cell division rates, a G(1) cell cycle arrest and increased differentiation in human and murine leukemia cells (HL60, U937, URE) [122]. Furthermore, eltrombopag prolonged survival in murine leukemia transplantation models. Preloading cells with ferric ammonium citrate (500 μ g/mL, 24 h) to increase intracellular iron abrogated the in vitro anti-proliferative and differentiation-inducing effects. Notably, cells with increased iron but no eltrombopag showed no increase in proliferation rate, differentiation and morphological changes compared to untreated controls.

4. Involvement of iron in mechanisms of carcinogenesis

Mechanisms how iron may contribute to tumor induction or progression in clinical and nonclinical models as outlined above have been reviewed extensively [1,22–24]. Such mechanisms include oxidative DNA damage by iron-catalyzed free radical production, alterations in gene expression consistent with increased iron requirements in proliferating cells (Fig. 1) as well as decreased immune surveillance against cancer.

4.1. Oxidative damage to cellular constituents

Under conditions of iron overload, levels of non-transferrin bound iron (NTBI) and intracellular labile iron increase, and can catalyze the production of reactive oxygen species (ROS) which may lead to organ dysfunction [123,124]. An increase of DNA breaks was found in leukocytes obtained from rats chronically fed a diet containing excess FeSO₄ as well as after incubation of human leukocytes, primary colonocytes or tumor cell lines with Fe-NTA [125–127]. Interestingly, a comparison of Fe-NTA with organic iron (heme and hemin) and inorganic iron (FeSO₄), showed that organic, rather than inorganic iron compounds may promote carcinogenic events [128]. In non-neoplastic rat liver epithelial cells that were previously initiated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), ferric ammonium citrate enhanced neoplastic colony formation in

a dose-dependent manner, but cell proliferation was substantially inhibited at the iron concentrations used [129].

4.2. Altered gene expression patterns of proteins involved in iron homeostasis and proliferation

In general, genes encoding proteins that increase intracellular iron (TRFC [transferrin receptor], SLC11A2 [divalent metal transporter 1, DMT1] and HAMP [hepcidin]) are upregulated in tumor cells, whilst those decreasing iron levels (SLC40A1 [ferroportin]) are downregulated [103,104,24,130,131]. Among breast cancer patients, lower ferroportin levels in tissue biopsies correlated with severity of histological grade, molecular subtype and worse long-term prognosis [131]. In two mouse cancer models, tumor growth rates and formation of metastases could be reduced by increasing ferroportin and decreasing TfR expression levels in transplanted tumor cells [130,131]. In line with these observations, the induction of the tumor suppressor p53 in lung and colorectal cancer cell lines increased ferritin and decreased TfR protein levels [132], and p53 may induce cell cycle arrest via restricted availability of intracellular iron to iron-dependent enzymes (cyclin, cyclin-dependent kinases, ribonucleotide reductase) [133]. In addition, hepcidin levels may be influenced via a p53 response element in the *HAMP* promoter [134].

Involvement of the Wnt signaling pathway has been proposed for iron-mediated proliferation of colorectal cancer (CRC) cell lines [135]. In this model, iron loading enhanced Wnt signaling activity only in cells mutant for Adenomatous Polyposis Coli Protein (APC) or cells expressing constitutively active β -catenin. However, in mice carrying an Apc-germline mutation that mimics familial adenomatous polyposis, a hereditary form of CRC [136], administration of high iron doses (2000 mg/kg body weight s.c. on two consecutive days followed by once monthly), did not promote intestinal tumorigenesis.

In lymphoma cell lines with translocated copies of c-myc, iron can inhibit proliferation via free radical-mediated DNA damage and downregulation of c-myc expression [137,138].

4.3. Iron and immune surveillance of cancer

Iron can modulate activity of the innate and adaptive immune system, including NK cells, monocytes, macrophages and lymphocytes. As noted earlier, iron sequestration (principally by monocytes and macrophages of the reticuloendothelial system) has been argued to represent a mechanism by which the body withholds iron from invading pathogens and virus-infected cells [133]. Consistent with this idea, low iron status has been associated with enhanced immunoprotection against certain infections such as malaria and tuberculosis [139,140]. However, innate and cell-mediated immunity can also be impaired by iron deficiency [141]. For example, in iron-deficient but otherwise healthy elderly women, T-cell proliferation in response to

mitogenic stimuli was reduced compared to the response in iron-replete women [142]. Similarly, rats fed a moderately iron-deficient diet and challenged with the carcinogen DMBA had a higher tumor burden and reduced NK cell activity compared to rats fed a normal iron diet [95]. In T-lymphocytes, iron reduces activation of the pro-apoptotic/anti-proliferative interferon- γ (IFN- γ)/STAT1 pathway and favors the differentiation of T helper 1 (T_H1) cells, which could favor cytotoxic anti-microbial and anti-tumor responses. However, in a different context, iron supplementation was also shown to protect malignant T cells from IFN- γ induced apoptosis in vitro, while in vivo, iron chelation plus IFN- γ treatment inhibited malignant T cell growth in a severe combined immunodeficient (SCID) mouse model [143]. Furthermore, in macrophages, high intracellular iron concentration can inhibit the IFN- γ -stimulated release of oxygen radicals and nitric oxide (NO) [144], which are key effector pathways in innate immunity. In monocytes, iron reduces surface expression of cell adhesion molecules such as intracellular cell adhesion molecule 1 (ICAM-1) and human leukocyte antigen (HLA)-DR, which are important in leukocyte migration, T-cell mediated killing and other T- and B-cell responses contributing to adaptive immunity [145]. Hence, the interplay of iron metabolism and regulation of immune responses is complex and context dependent [145].

5. Discussion

Intravenous iron supplementation has proven its efficacy and tolerability, and is recommended in guidelines on the management of chemotherapy-induced anemia [11,17]; yet, some uncertainty remains about long-term effects in cancer patients since long-term follow-up data have not been recorded. Therefore, human epidemiological data or non-clinical studies have to be evaluated for evidence or hints on a potential role of iron in carcinogenesis. However, these studies often do not reflect the clinical setting in oncology patients and their results are thus inconclusive. Overall, the data suggest that chronic iron overload (e.g. in hereditary hemochromatosis; TSAT >45%, serum ferritin >1000 ng/mL suggesting cirrhosis) increases the risk of cancer. However, iron deficiency can also negatively affect clinical outcomes and immune surveillance. Thus, maintenance or achievement of a normal iron status with appropriate use of i.v. iron at a rather wide TSAT target range of 20–50% [18] seems to be reasonable in cancer patients receiving chemotherapy.

5.1. Human data

While iron overload in hereditary hemochromatosis (caused by *HFE* mutations) is associated with higher risk of liver cancer, the influence of *HFE* mutations on risk of other cancers remains under debate. Epidemiological studies on other risk factors for cancer showed links with high dietary iron intake or iron status, particularly for CRC. It appears

that the intraluminal milieu can trigger oxidative/genotoxic changes in gut epithelial cells that may lead to GI tract cancers. Importantly, cells exposed to the gut lumen are not subject to the same control of iron metabolism as reticuloendothelial cells.

In patients with multiple myeloma, deviation from normal TSAT (<20% or >45%) was associated with reduced progression-free and overall survival as well as markers of impaired organ function (i.e. increased prohormone of brain natriuretic peptide [proBNP], and reduced estimated glomerular filtration rate [eGFR]) [146]. However, it cannot be clearly distinguished whether the abnormal TSAT was the reason for impaired survival or caused by already more advanced malignancy. Notably, 47% of multiple myeloma patients with available iron status were either iron-deficient (32%) or had iron overload (15%) which may have resulted from the high transfusion frequency in this subgroup.

Intentionally reduced iron status (phlebotomy) in PAD patients showed a striking reduction in cancer risk already after 6 months on the trial; yet, confirmatory studies are warranted since the study participants' demographics may have confounded the results [147].

5.2. Nonclinical data

Data on the role of iron as a tumor inducer derive mainly from nonclinical studies involving either iron dextran (i.m. or s.c.) or Fe-NTA (i.p.). The carcinogenicity of the highly redox active Fe-NTA precludes meaningful extrapolation of nonclinical data with this agent to clinical studies employing non-genotoxic iron-carbohydrate formulations approved for clinical use. Numerous animal studies, particularly in rodents, have shown sarcoma development after s.c. or i.m. delivery of iron dextran at cumulative doses at least 1–2 orders of magnitude higher than those employed in the clinical setting. As noted previously, tumor induction in this setting is likely related to persistent and localized iron overload.

As with the population studies [38], cancer models suggest that excessive iron within the gut luminal environment may promote GI tract tumors. However, considerable differences in experimental outcomes may reflect different susceptibility of various GI tract regions to the tumor promoting effects of either iron deficiency or iron overload. Some studies provided evidence for enhanced growth rate of implanted [105] or spontaneous tumors [94] and increased incidence of estrogen-induced tumors [97] in animals receiving high iron diets. Conversely, no evidence was found for a role of dietary iron in the promotion of oral and lung cancers [94–96,100,101], and one study demonstrated increased tumor incidence in iron deficient animals [95].

Only one chemical carcinogen model and one tumor implantation model used iron doses similar to those indicated for parenteral iron replacement therapy [92,102]. Both studies involved i.p. administration of iron dextran, close to or directly at the site of subsequent tumor formation. In these instances, the high MW iron-dextran complexes may

have been retained in the peritoneal cavity and exposed cells lining the cavity to excessive iron levels. In the clinical setting of i.v. administration, a comparable situation is unlikely since iron is rapidly targeted to the RES [148]. To the best of our knowledge, no nonclinical cancer model using the clinically relevant i.v. administration route has been published to date.

In vitro studies investigating the genotoxicity and cytotoxicity of iron (mainly as ferrous sulfate, hemoglobin and hemin rather than clinically used parenteral iron preparations) should be interpreted within the context of the limited in vivo evidence for potential tumorigenic activity. In vitro studies may present several possible mechanisms by which iron could induce cancer, including oxidative DNA damage; however, the contribution of these mechanisms can only be estimated when the data are taken in conjunction with in vivo data from animal studies and human epidemiology.

5.3. Iron in the clinical setting

In oncology patients, i.v. iron therapy is indicated for the restoration of normal iron status and correction of anemia. International anemia treatment guidelines recommend administration of i.v. iron if functional iron deficiency is present, and repletion of iron stores if absolute iron deficiency is the underlying cause of anemia [18]. Clearance of i.v. iron complexes from the circulation, uptake by the RES, appropriate iron deposition in physiological iron stores and the generation of ROS are influenced by the physicochemical properties of iron complexes including their reduction potential, the strength of iron binding and the molecular weight of the complex [124,149,150]. Inefficient delivery of iron to physiological iron stores and excessive transferrin saturation after i.v. or oral iron supplementation may result in iron deposition in liver cells where it is associated with histopathology in animals and in mesenchymal cells [151–155].

6. Conclusions

Human data, mainly based on cases after i.m. injection of iron dextran and populations with chronic iron overload, suggest a correlation between chronically increased iron levels and increased cancer risk. Animal models, mainly based on non-intravenous administration of extremely high cumulative iron doses in iron-replete recipients, suggest that iron overload can promote tumor growth. Overall, data from epidemiological and nonclinical studies are often conflicting and extrapolation to the clinical setting aiming for normalization of hemoglobin (around 12 g/dL) is difficult. In the absence of long-term pharmacovigilance studies, iron treatment to prevent or manage chemotherapy-induced anemia should be limited to the time of cytotoxic anti-tumor treatment and iron status should be closely monitored (target TSAT range 20–50%).

Funding

This work was supported by an unrestricted grant from Vifor Pharma Ltd. (Switzerland) funding medical writing support. Interpretation of the data as well as the review and decision to submit the manuscript for publication have been performed by all authors independently.

Conflicts of interest statement

Y. Beguin disclosed membership in the speaker bureau and advisory board of Vifor Pharma Ltd.

M. Aapro disclosed membership in speaker bureaus and/or advisory boards, and/or receipt of study grants from Vifor Pharma Ltd., Sandoz, Amgen, Roche, Hexal and Hospira.

H. Ludwig disclosed consulting and speaker honoraria from Vifor Pharma Ltd.

L. Mizzen is employee of Vifor Pharma Ltd.

A. Österborg disclosed receipt of membership in the advisory board of Vifor Pharma and conduct of clinical trials with Vifor Pharma Ltd.

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Acknowledgements

All authors contributed equally to the literature search, data interpretation and review of the manuscript. Medical writing support was provided by Sarah Krebs and Walter Fürst (SFL Regulatory Affairs & Scientific Communication, Switzerland). The manuscript was reviewed and commented by Beate Rzychon (Vifor Pharma Ltd., Switzerland).

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Heinz Ludwig is director of the Centre for Oncology and Hematology at the Wilhelminen Hospital in Vienna, Austria. He received his medical degree in 1971 from the University of Vienna before becoming a research fellow at the Institute of Immunology, Vienna. Dr Ludwig's major research interests are in medical oncology/hematology, in particular multiple

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Lee Mizzen is executive director of Nonclinical Development at Vifor Pharma in Victoria, British Columbia, Canada. He received his PhD in 1986 from the University of Western Ontario followed by postdoctoral research at Cold Spring Harbor Laboratory, New York and the University of San Francisco, California. He was a research fellow at the Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, University of Toronto before joining StressGen Biotechnologies, Victoria, where he held positions as Director of Research and Vice President Scientific Affairs.

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Dr Österborg is a member of several international scientific organizations and is past-coordinating investigator in numerous international collaborative clinical trials. He lectures widely at international conferences and has organized many global continuing medical education courses discussing the role of immunotherapy in treating lymphoproliferative disorders. He is the author of over 190 original research articles and textbook chapters in the fields of hematology and oncology, and is Co-Editor-in-Chief of *Medical Oncology*.