Intravenous Iron Carbohydrate Nanomedicines

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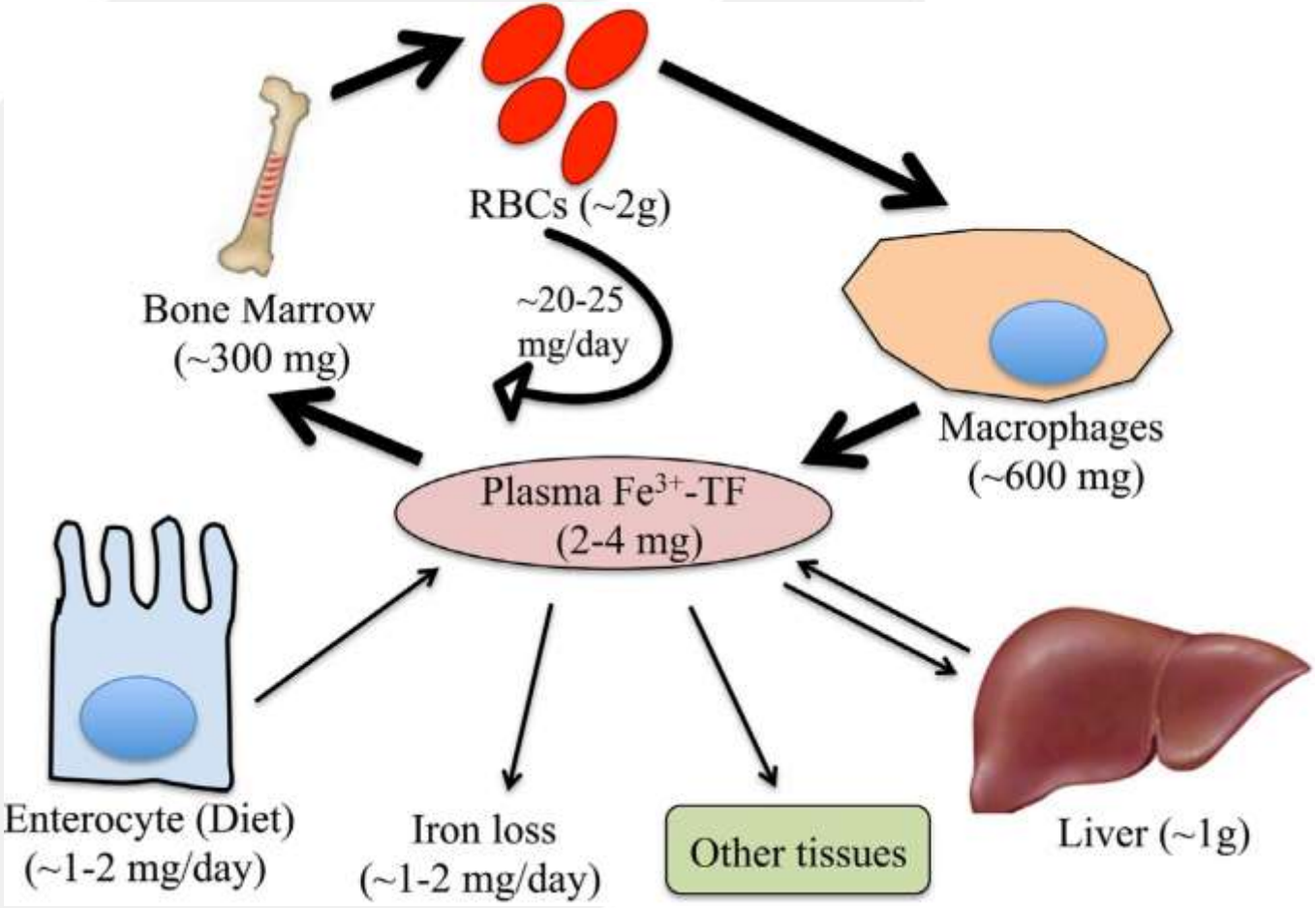
IV Iron-Carbohydrate Nanomedicines

- to treat iron deficiency (anemia, chronic heart failure, chronic kidney disease, inflammatory bowel disease, malignancy and gynecologic etiologies)
- preparations require removal of the iron-carbohydrate complex circulating in plasma and subsequent uptake by resident macrophages in the RES to then release iron to transferrin for delivery to the site of action, the bone marrow
- class of complex drugs with heterogeneity in biodistribution due to the different physicochemical characteristics (particle core size, structure and robustness of the carbohydrate shell, hydrodynamic diameter and geometry of the nanoparticles)

Systemic Iron Hemeostasis



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Test Items

- FCM - ferric carboxymaltose
- IS - iron sucrose
- IIM - iron isomaltoside 1000
- ID - iron dextran
- consist of a polynuclear iron(III)-oxyhydroxide core surrounded by a carbohydrate shell

Diagnostics vs Toxicologic Pathology

Active ingredient	Ferric carboxymaltose (FCM)	Iron sucrose (IS)	Iron isomaltoside 1000, Iron derisomaltose (IIM)	Low molecular weight iron dextran (ID)
Carbohydrate	Carboxymaltose	Sucrose	isomaltoside 1000, derisomaltose	Dextran
Molecular weight	145-155 kDa	42-44 kDa	150 kDa	165 kDa

Pilot study - Design

- 16 male SD rats in two groups
- diet: low iron diet (LID, group 1) or standard diet (group 2) for 51 days
- 10 µl of blood (tail vein) to evaluate base level and once to twice weekly during the induction phase for assessment of Hb
- clinical observations at least once daily
- body weight at study start and once weekly during the induction phase and at necropsy
- at sacrifice, blood sampling from the abdominal aorta for haematology and clinical chemistry including serum iron, unsaturated iron binding capacity (UIBC), serum ferritin and transferrin
- no pathology evaluation

Pilot study - Results

- all animals survived
- Group 1 at LID with slightly lower body weights
- **Hb concentrations**:

  Group 1 (LID):

  Hb decreased from 11.1 g/dL on Day 1 to 6.5 g/dL on day 23 (thereafter stable until Day 51)

  Group 2 (standard diet):

  Hb steadily increased from 11.4 g/dL on Day 1 to 16.7 g/dL on Day 51

Pilot study – Model successful

- iron deficiency anemia (IDA) model successful in SD rats
- low Hb, Hkt, and low erythrocyte parameters (MCV, MCH, and MCHC, high reticulocytes, high platelet count)
- high transferrin
- low serum iron
- high unsaturated and total iron binding capacity
- low transferrin saturation

Control study - Design

- 2 groups each of 7 male SD rats fed with LID to induce anemia for approximately 5 weeks
- 1 group of 7 males fed with standard diet (non-anemic controls)
- Hb determined once weekly via the tail vein
- non-anemic and anemic control animals received a single intravenous dose of normal saline or 30 mg/kg ferric carboxymaltose (FCM, Ferinject®, diluted with saline to 15 mg iron/mL) at a dose volume of 2 mL/kg, respectively
- observed daily for signs of toxicity during treatment period

Control study - Design

- body weight recorded once before induction of anemia and weekly thereafter during whole induction phase
- body weight in addition before assignment to experimental groups, on Day 1 and necropsy day (Day 3)
- blood samples (500 µL) collected from tail vein at 0, 1, 4, and 48 hours post-administration for pharmacokinetic parameters including serum iron, serum transferrin and unsaturated iron binding capacity (UIBC)
- at end of study period (Day 3), blood collection by cardiac puncture for haematologic and clinical biochemistry pharmacodynamic parameters (including Hb, serum ferritin and complete blood count

Control study - Design

- **transferrin saturation (TSAT) calculated by serum iron/ total iron binding capacity (TIBC) X 100**
- **TIBC calculated by:**
  Method 1:
  calculated by the equation TIBC (µg/dL) = serum iron (µg/dL) + UIBC (µg/dL)
  Method 2:
  calculated by the equation TIBC (µg/dL) = 1.41 x serum transferrin (mg/dL).
- **blood sampling before perfusion**
- **necropsy after perfusion by 0.9% NaCl for ~20 min**

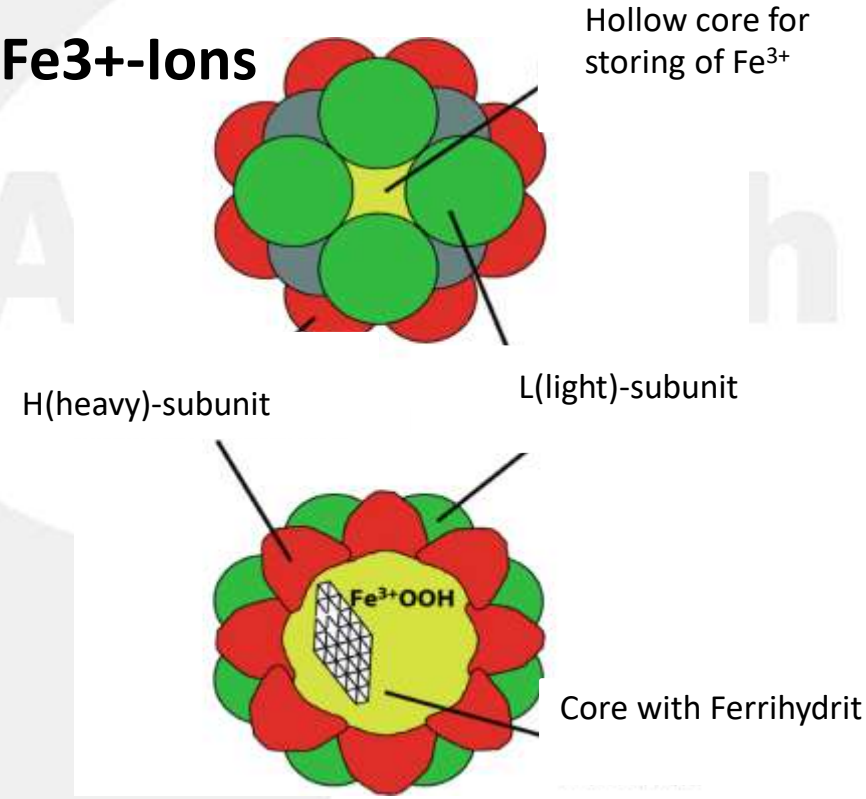
Control study - Design

- collection of brain, heart, liver, kidneys, spleen, femur and skeletal muscle (4% NF)
- iron quantification by ICP-OES in liver, spleen, kidneys, heart, bone (femur) and skeletal muscle
- bone marrow differentiation (May-Grünwald): at least 500 cells
- tissue samples from all animals trimmed, processed, embedded in paraffin wax, cut at 4 µm thickness and stained by HE and Prussian Blue (Perls' stain)
- immunohistochemistry with antibodies against ferritin heavy (FH) and light (FL) chains in all available tissues

  anti-ferritin heavy chain antibody (abcam ab183781, clone no. EPR18878, Lot no. GR239188-2) and anti- ferritin light chain antibody (abcam ab69090, Lots no. GR3204764-2 and GR278011-2)

Ferritin

- **Fe3+ as oxyhydroxid-phosphate-complex (bound to gluta-minic acid)**
- **up to 4500 Fe3+-Ions**



- **Most important iron depositing protein**
- **Serum concentration correlates with cellular iron homeostasis**

Control study – Results (clinic, weights)

- all animals survived
- no adverse clinical signs
- animals at LID at the end of anemia induction phase at significantly lower body weight (compared to non-anemic controls)
- Organ weight changes in anemic rats:
  - reduced absolute liver and increased lung weights
  - increased relative spleen, heart and lung weights
- Organ weight changes in anemic rats under FCM:
  - significant increase in absolute and relative spleen weight (extramedullary hematopoiesis).

Control study – Serum iron, TK, iron biodistribution

- serum iron concentrations significantly lower in anemic controls
- significant increase in serum iron 1-hour post FCM (remained until 4 hours, returned to normal values at 48 hours)
- FCM treatment led to a significant decrease in UIBC at 1 and 4 hours followed by an increase back up to normal values at 48 hours
- FCM administration resulted in approximately 1.5 to 2.5 larger AUC values than observed in the normal rats receiving vehicle.

Control study – Serum iron, TK, iron biodistribution

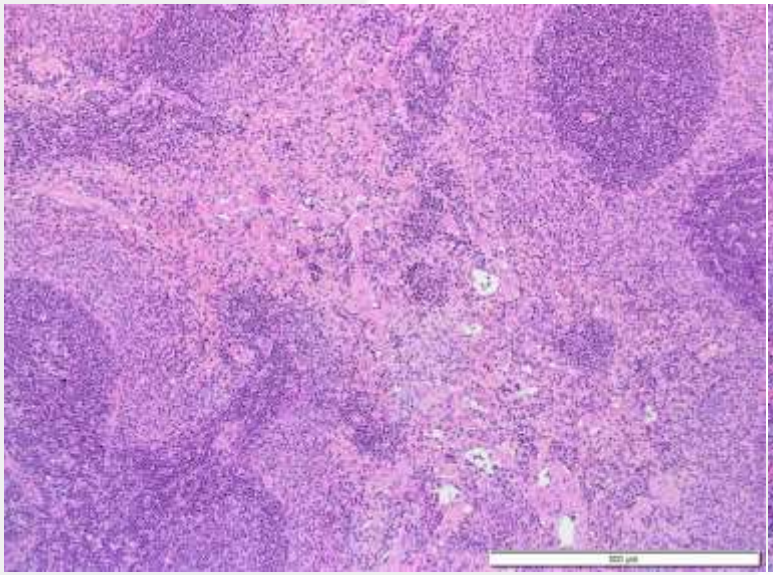
- **after FCM, iron rapidly distributed to tissues within the pharmacological target compartments; liver, spleen and femur (surrogate for bone marrow)**
- **biodistribution of iron in the liver lower than iron distributed to the spleen**
- **iron in femur after FCM significantly higher than in anemic and non-anemic controls**
- **iron in heart markedly reduced in the anemic controls**
- **after FCM rapid restoration of heart iron (similar in skeletal muscle)**
- **no significant difference in total organ iron concentration in the kidneys**

Control study - Hematology

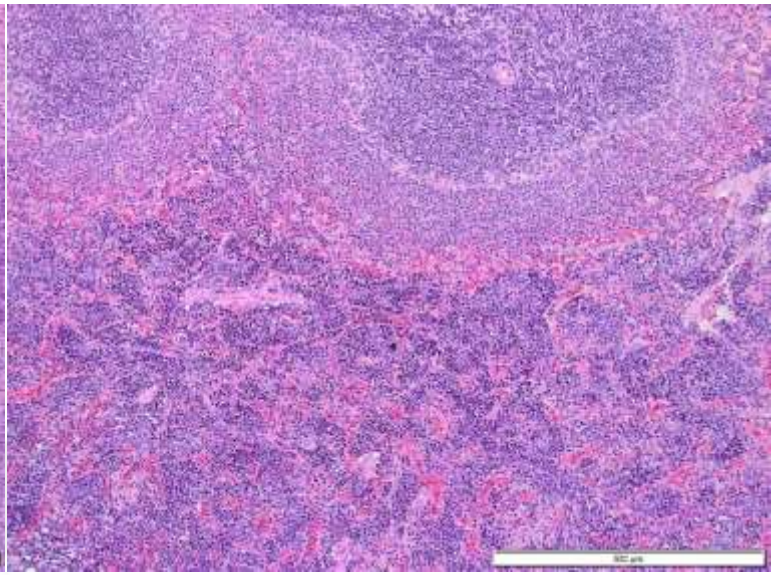
- **Anemic animals: marked decreases in Hb, Hkt, MCV, MCH, MCHC, and reticulocytes, while platelets increased**
- **FCM-treated animals: after 48 hours amelioration in most of the parameters**

Control study – Spleen (HE)

- **Erythropoiesis increased in anemic animals, however, much higher under FCM animals**



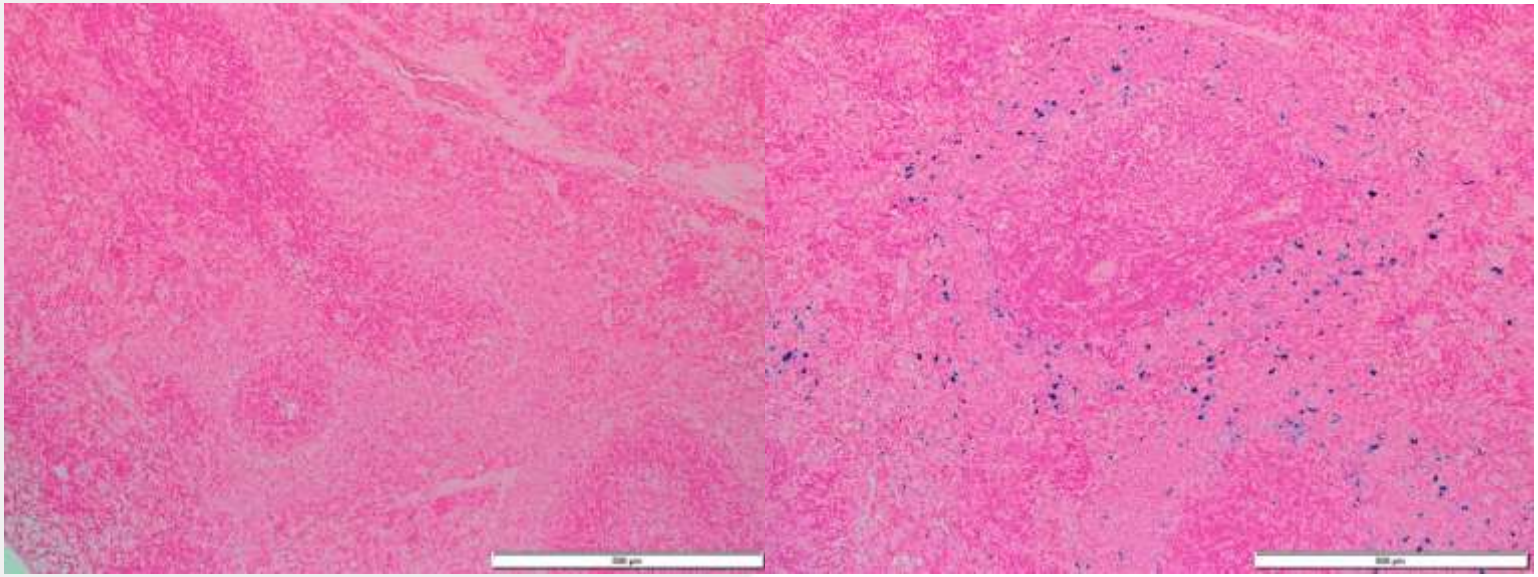
Naive



FCM

Control study – Spleen (Perl's)

- **Hemosiderin in the red pulp adjacent to the mantle zone was noted in all naïve and FCM animals but not in anemic controls.**

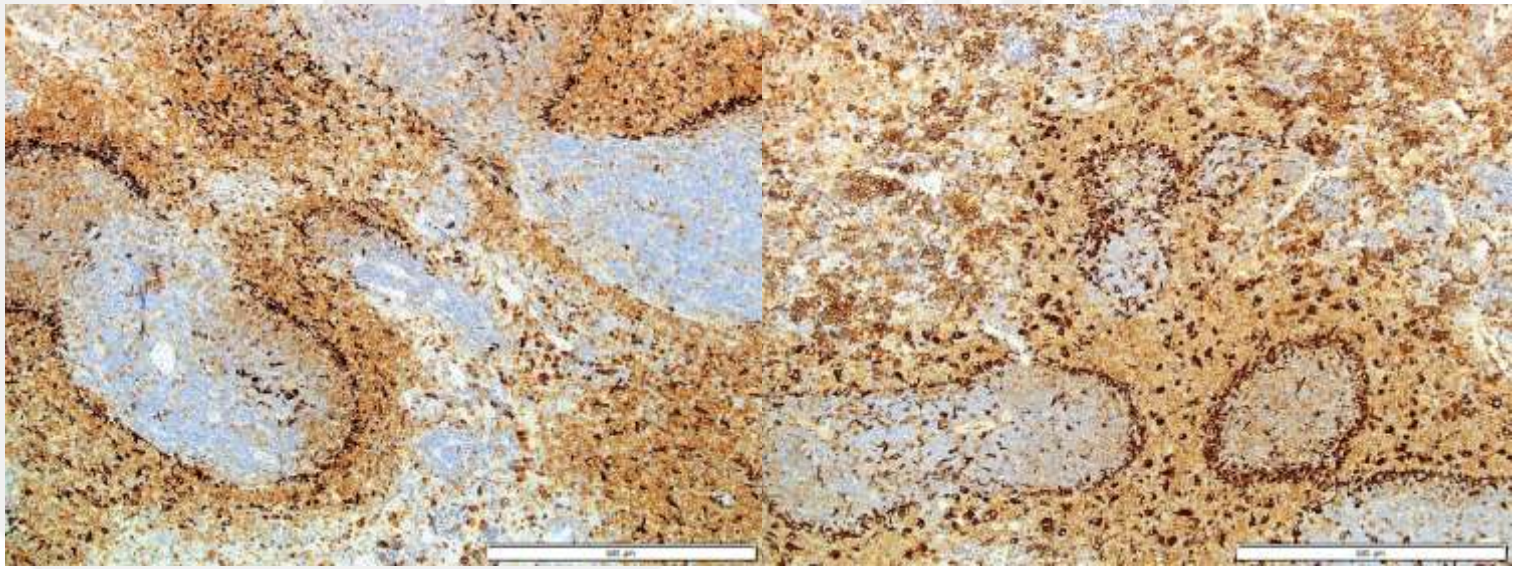


Anemic

FCM

Control study – Spleen (Ferritin Heavy)

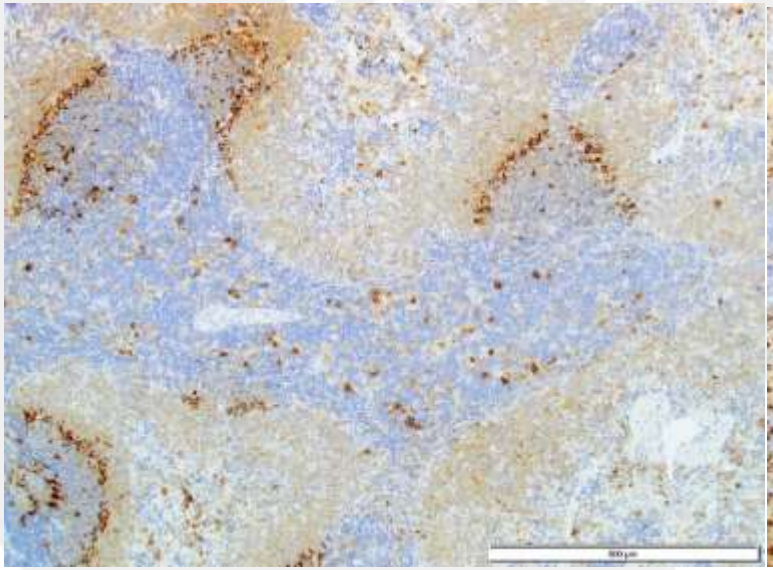
- **lower severity in PALS of anemic controls and higher in FCM-treated animals compared to naïve controls.**
- **lower in marginal sinus, marginal zone, follicles and red pulp, in anemic and FCM-treated (close to naïve controls) animals**



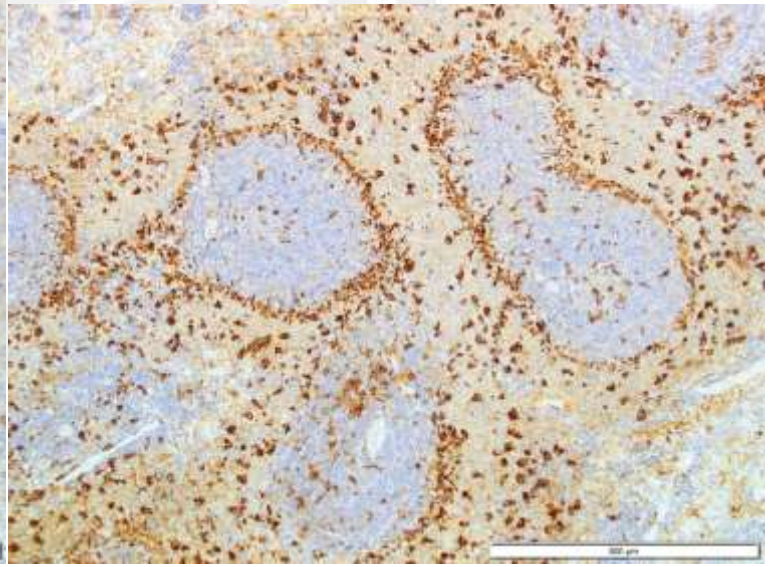
Anemic **FCM**

Control study – Spleen (Ferritin Light)

- **in PALS similar to ferritin heavy chains**
- **in the marginal sinus and the marginal zone, the values in anemic controls were lower than in naïve controls but increased under FCM**



Anemic



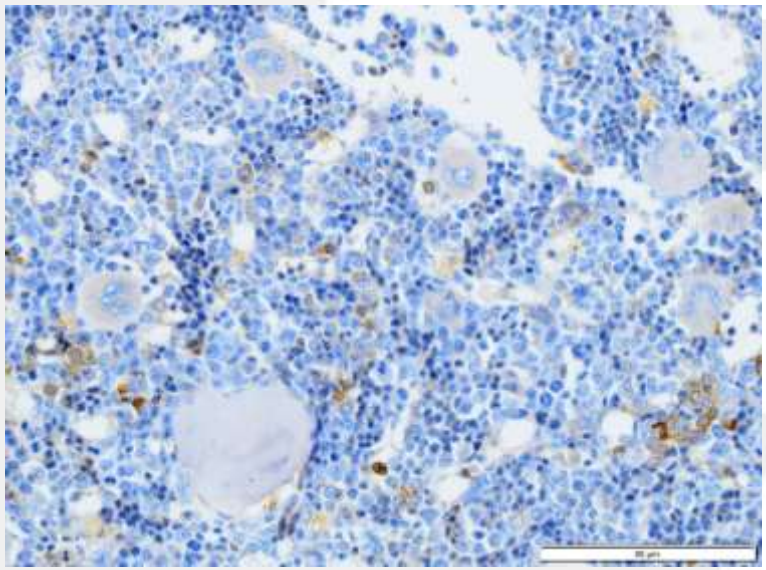
FCM

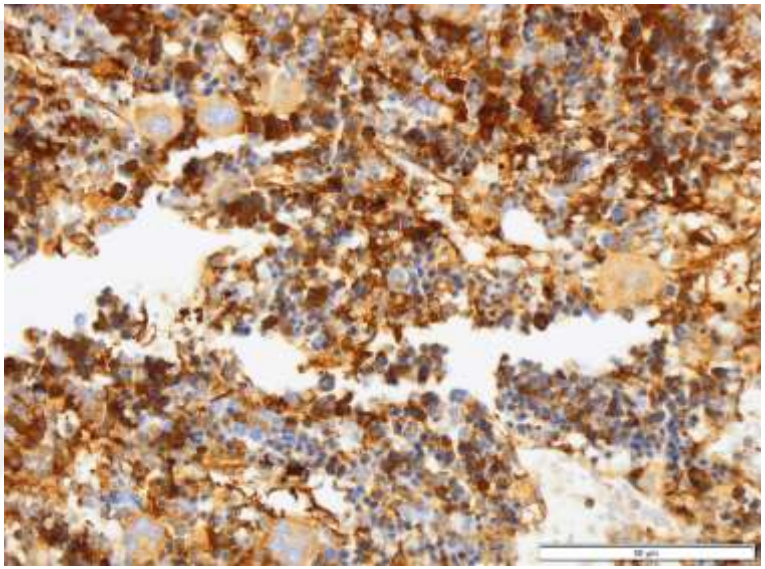
Control study – Bone Marrow (HE)

- presence of blast cells forming groups within the bone marrow in two animals each from anemic control and FCM group
- HE-stained sections: nature of the blasts could not be established.
- erythropoiesis increased under FCM
- megakaryocytes increased in anemic control and partially in FCM group
- cellularity of bone marrow increased significantly in FCM group

Control study – Bone Marrow (IHC)

- **ferritin heavy and light chains at similar distributions within the marrow**
- **very low severity in anemic controls**

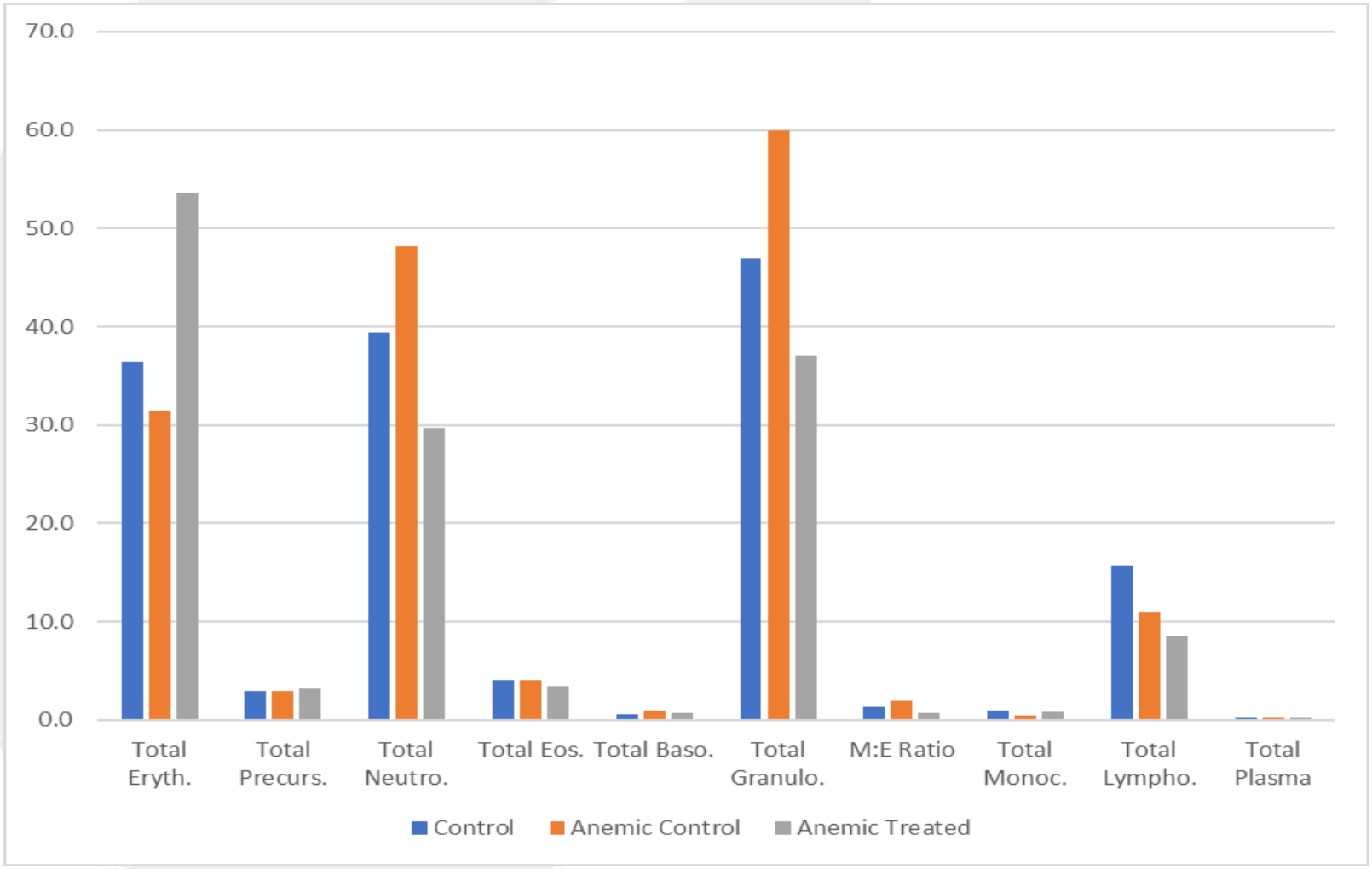




Ferritin heavy chain

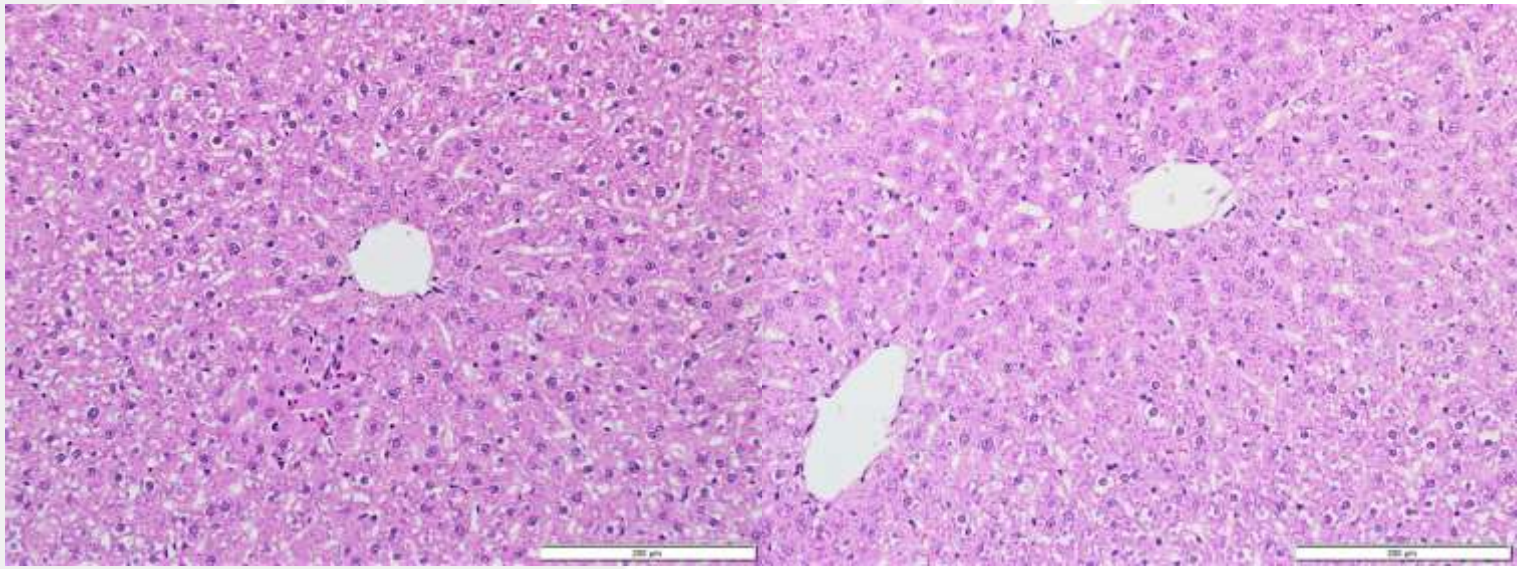
Anemic **FCM**

Control study – Bone Marrow (BMD)



Control study- Liver (HE)

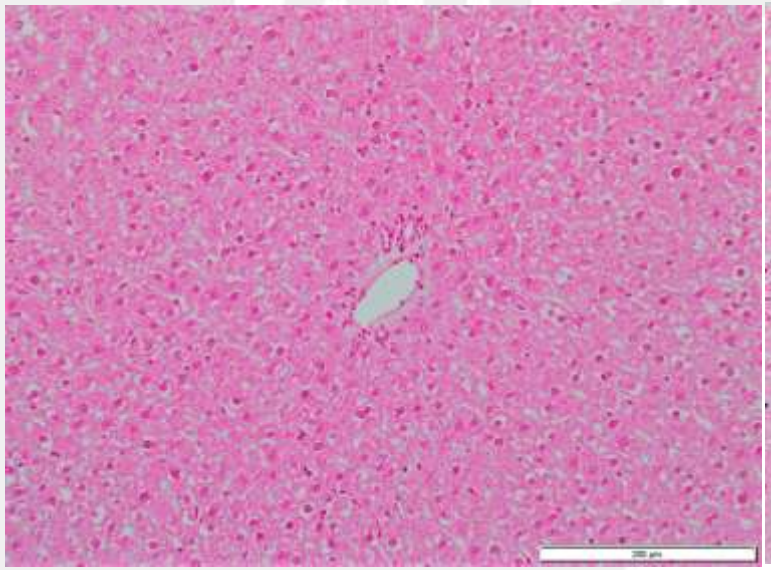
- **hemopoietic cells increased in incidence and severity under FCM treatment**
- **minor hepatocellular hypertrophy (centrilobular) was noted in anemic controls and FCM animals**



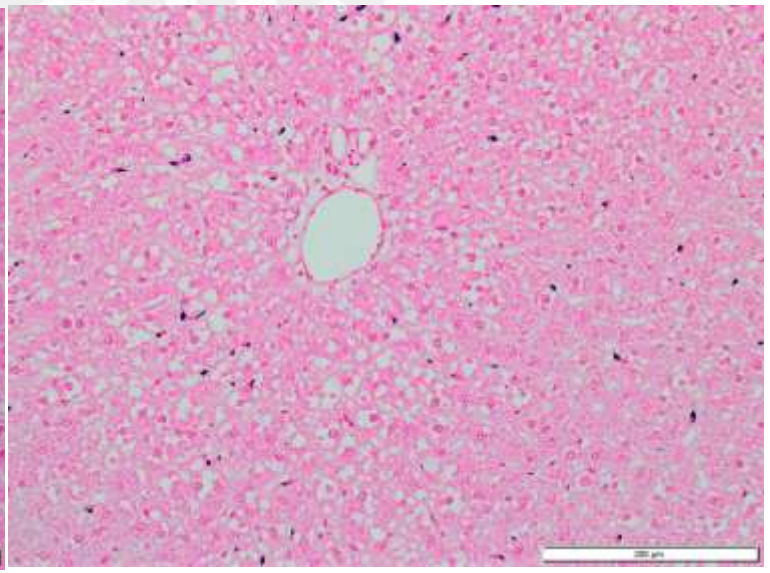
Anemic **FCM**

Control study- Liver (Perl's)

- **hemosiderin in Kupffer's cells (mainly located within the sinusoids) under FCM**



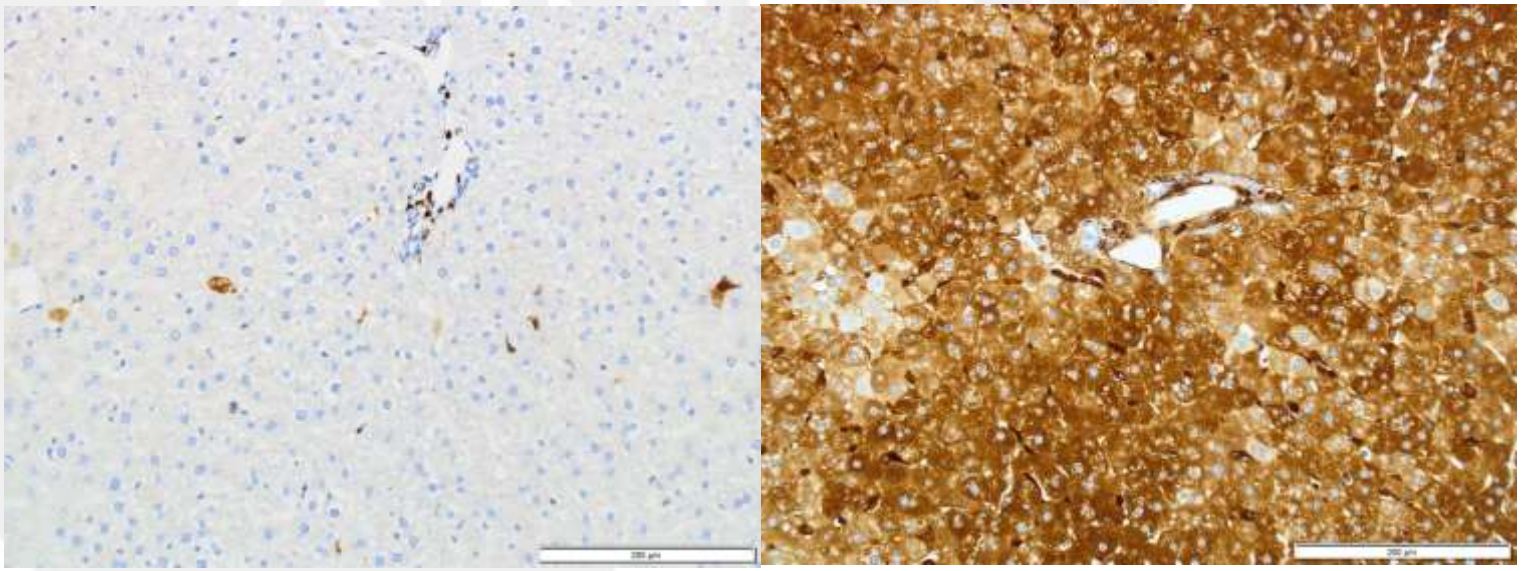
Anemic



FCM

Control study- Liver (IHC)

- ferritin heavy and light chains in hepatocytes of naïve controls at highest severity followed by FCM-treated animals
- almost no hepatocellular expression in anemic animals
- in Kupffer's cells, FCM caused a huge increase

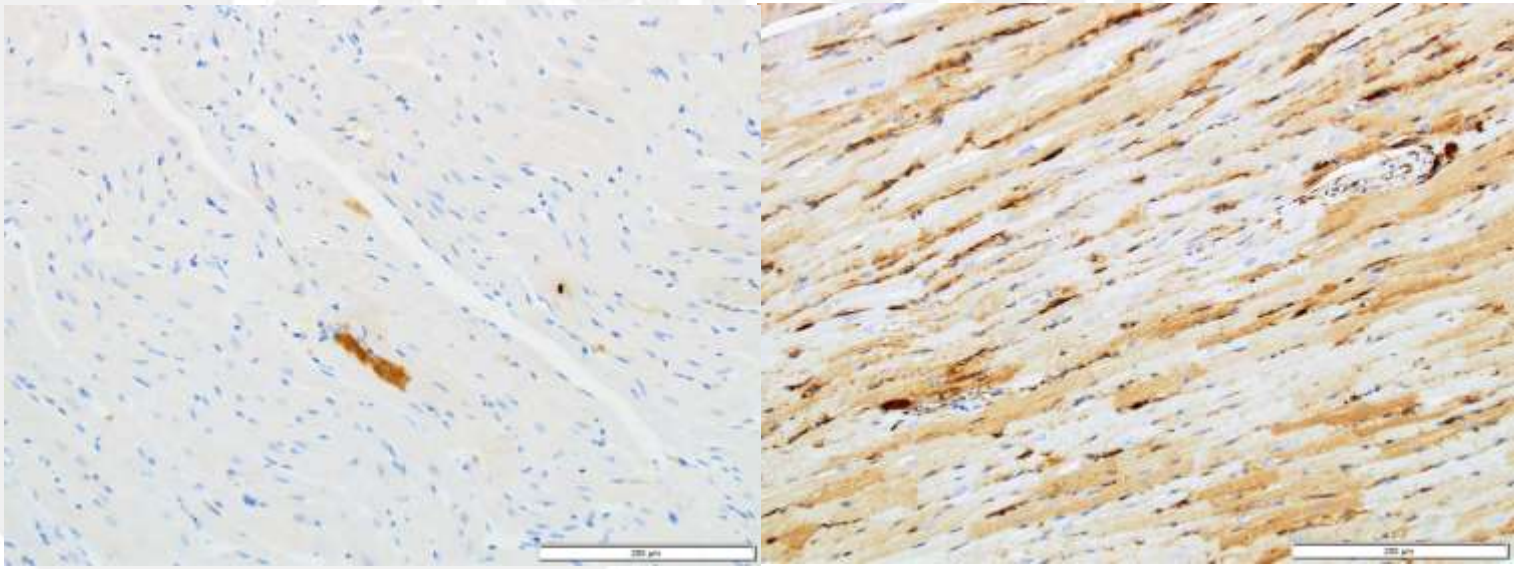


Anemic FCM

Ferritin light chain

Control study – Heart (IHC)

- no hemosiderin
- ferritin heavy and light chains diffusely expressed in naïve control and under FCM
- ferritin heavy chains much more expressed than for light chains
- decreased chain distribution in anemic controls

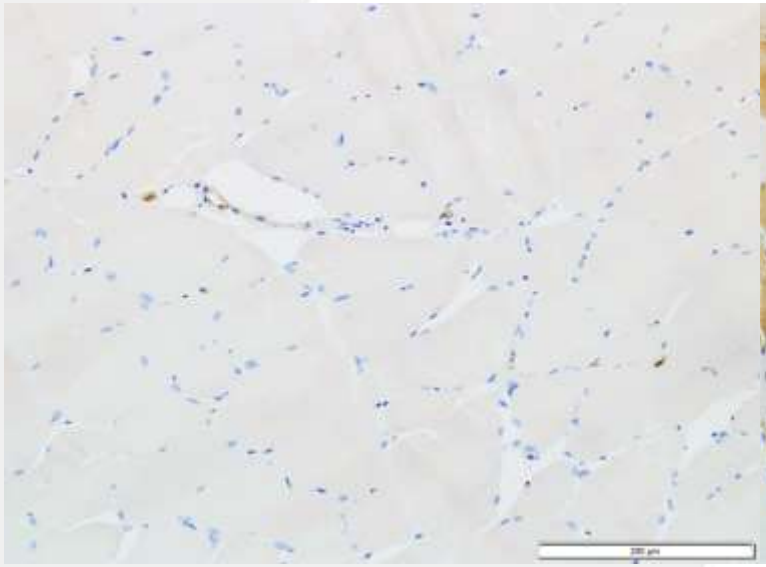


Anemic FCM

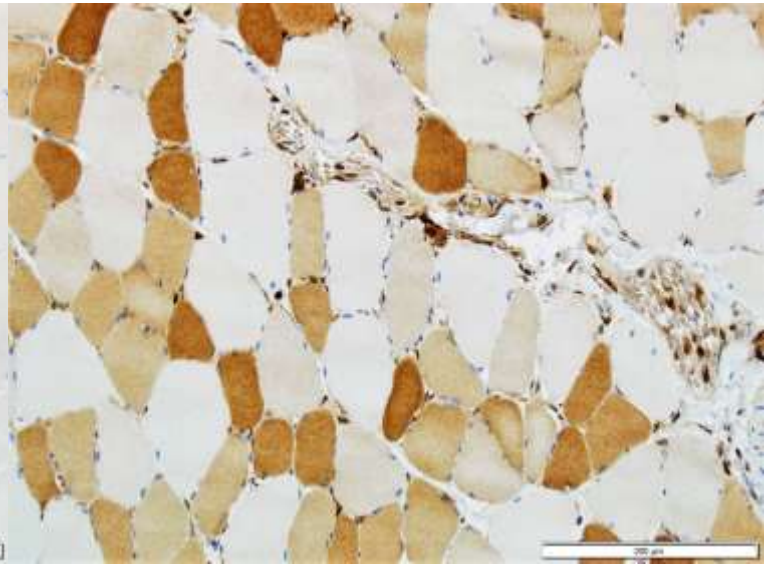
Ferritin light chain

Control study – Skeletal Muscle

- **Similar than in heart muscle**



Anemic



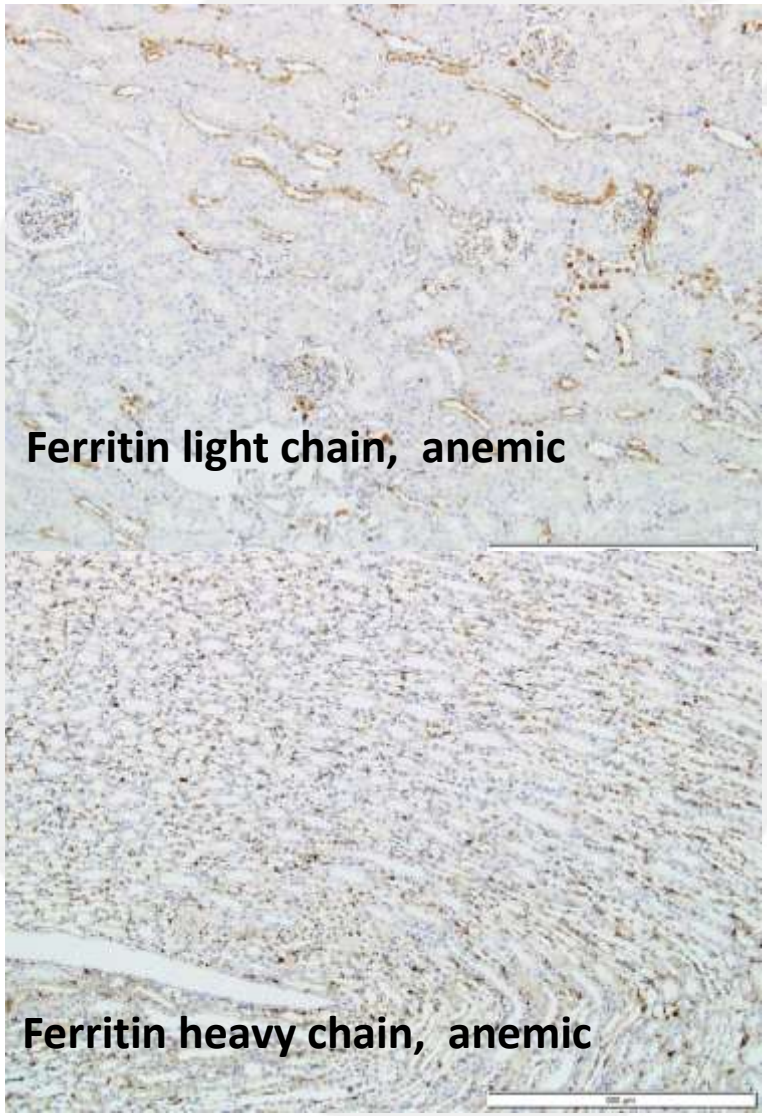
FCM

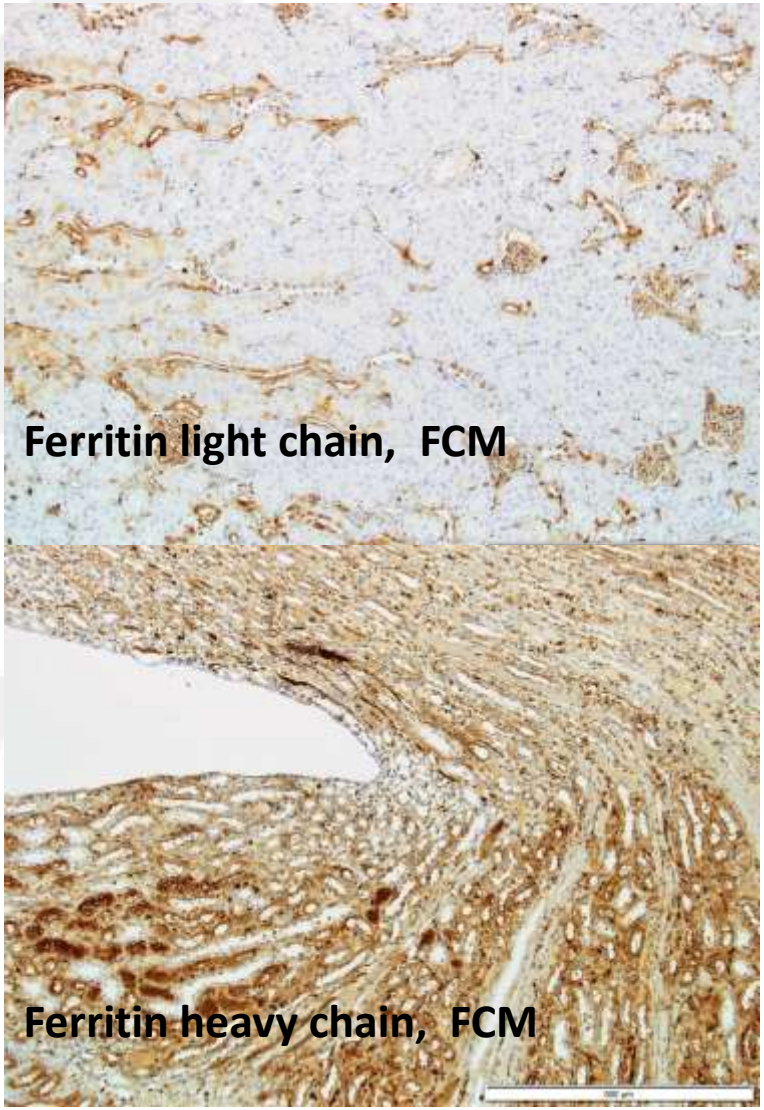
Ferritin heavy chain

Control study - Kidneys (IHC)

- **Heavy and light chains of ferritin in naïve control animals in the glomeruli, proximal, distal and rectal tubules, and collecting ducts**
- **heavy chains more expressed than light chains**
- **in anemic controls, values dropped significantly for any structure for both ferritin chains**
- **ferritin heavy and light chain expression increased in all structures under FCM**

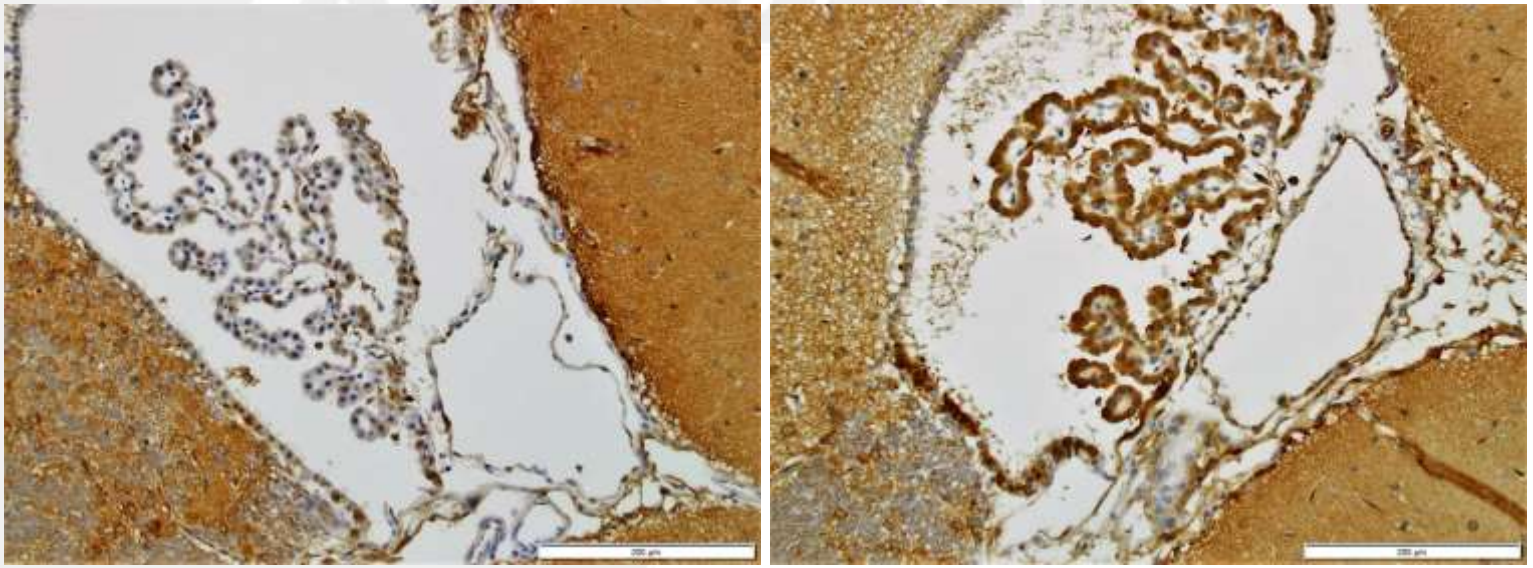
Control study Kidneys (IHC)





Control study – Plexus (IHC)

- **ferritin light and heavy chains were expressed in all visible structures from naïve control animals.**
- **Under FCM, the major change was noted in the plexus**



Ferritin heavy chain

Anemic **FCM**

Comparative study

- 105 anemic rats (5 groups each 21 males)
- fed with LID for approximately 5 weeks to induce anemia
- only animals with sufficient induction of anemia (Hb)
- single intravenous dose of normal saline (control), or 30 mg/kg of FCM, IS, IIM or ID
- dose volume of 5 mL/kg
- daily observed for signs of toxicity
- body weight recorded once before the induction of anemia and weekly thereafter, including days before the assignment to the experimental groups and necropsy

- FCM - ferric carboxymaltose
- IS - iron sucrose
- IIM - iron isomaltoside 1000
- ID - iron dextran

Comparative study

- **blood samples (500 µL) from the jugular vein at 0 and 1, 2, 4, 6, 8, 12, 24 and 48 hours post-administration for pharmacokinetic parameters (including serum iron, serum transferrin and unsaturated iron binding capacity (UIBC)**

- **from the jugular vein at 24, 48, 96, 168, and 336 hours post-administration blood sampling (300 µL) for pharmacodynamics (Hb, serum ferritin, complete blood count with differential)**

- **subgroups of animals (n=7) were sacrificed on Day 3, 8 and 15.**

Comparative study

- necropsy after perfusion by 0.9% NaCl for ~20 min
- blood sampling prior to perfusion
- transferrin measured with immune complexes of transferrin with goat anti-transferrin antibodies
- ferritin was determined by immune complex aggregation of reaction of ferritin with rabbit anti-ferritin antibody coated polystyrene latex particles
- necropsy and follow-up procedures were conducted as described for the control study.

Control study – Serum iron, TK, iron biodistribution

- C_{max} values for serum iron similar for FCM, IIM and ID.
- FCM had the lowest serum iron AUC_{0-48} which may be indicative of more rapid clearance from plasma by macrophages
- mean C_{max} serum iron highest with IS (formulation with smaller carbohydrate coating)
- serum iron decreased to levels similar to the vehicle group within 48 hours after administration of each of the tested preparations.

Control study – Serum iron, TK, iron biodistribution

- **FCM: highest biodistribution to the liver (followed by IIM, IS, ID)**
- **ID: highest biodistribution to the spleen (followed by FCM, IIM and IS)**
- **bone: iron distribution was 14% and 23% higher with FCM and IS compared to IIM and 23% and 31% higher compared to ID**
- **heart: increased iron most efficient with FCM followed by IIM**
- **IS: iron distribution to the kidney markedly higher compared to other formulations**

- **FCM - ferric carboxymaltose**
- **IS - iron sucrose**
- **IIM - iron isomaltoside 1000**
- **ID - iron dextran**

Control study – Pharmacodynamics

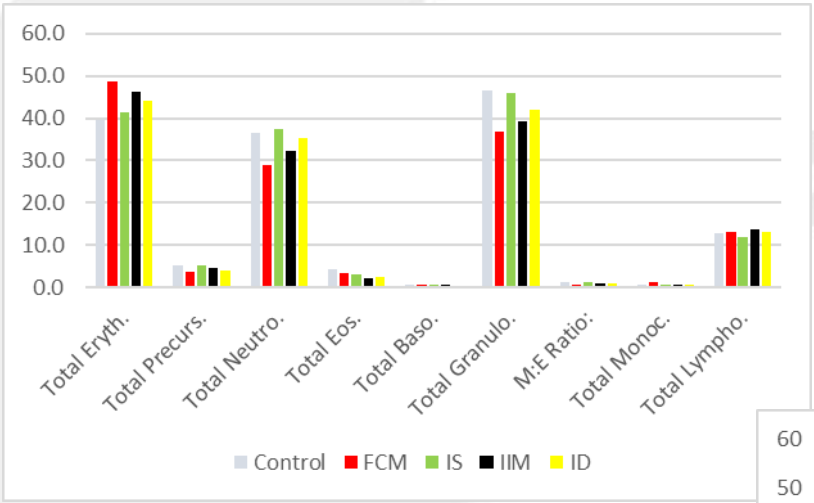
- **Hb concentrations rose rapidly by Day 3 and mean concentrations were not statistically different between iron preparations with the exception of FCM treated animals compared to ID treated animals on Day 5**
- **increases in serum ferritin were not significantly different among the IV iron preparations.**
- **FCM and ID produced the highest increases in serum ferritin at Day 3**
- **Day 15, serum ferritin was similar to vehicle control for FCM, IS and IIM treated groups.**
- **Transferrin saturation (TSAT) did not vary significantly between the preparations**

- **FCM - ferric carboxymaltose**
- **IS - iron sucrose**
- **IIM - iron isomaltoside 1000**
- **ID - iron dextran**

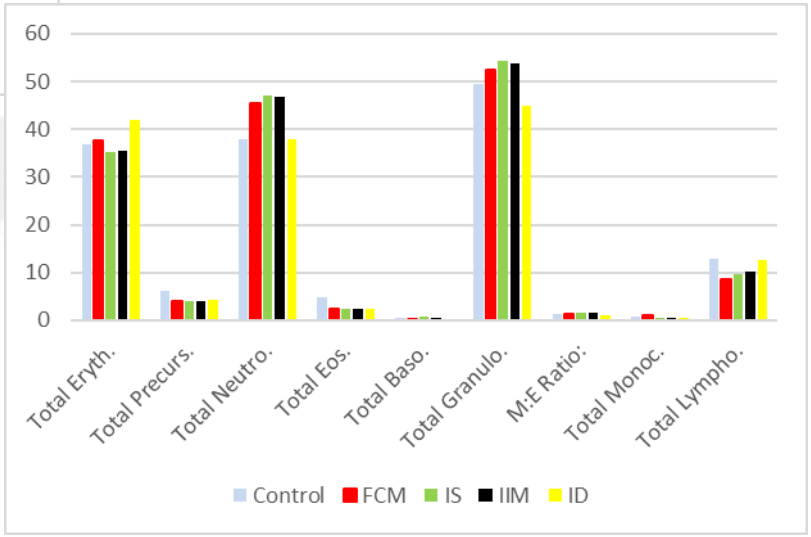
Control study – Pathology, BMD

- **Similar pathology as described for control study**
- **BMD (Days 3 and 15)**

Day 3



Day 15



Mechanism

- anemic rat model induced by a low iron diet over a period of several weeks successfully establishe (previously published studies performed using normal SD rats)

- iron deficiency affects hemostasis, especially of erythrocytes
- adaptive mechanisms include hepcidin, ferroportin, iron regulatory proteins and other regulators.
- liver-derived hepcidin as key hormone binds to ferroportin (FPN: unique iron exporter in mammals) blocking iron export

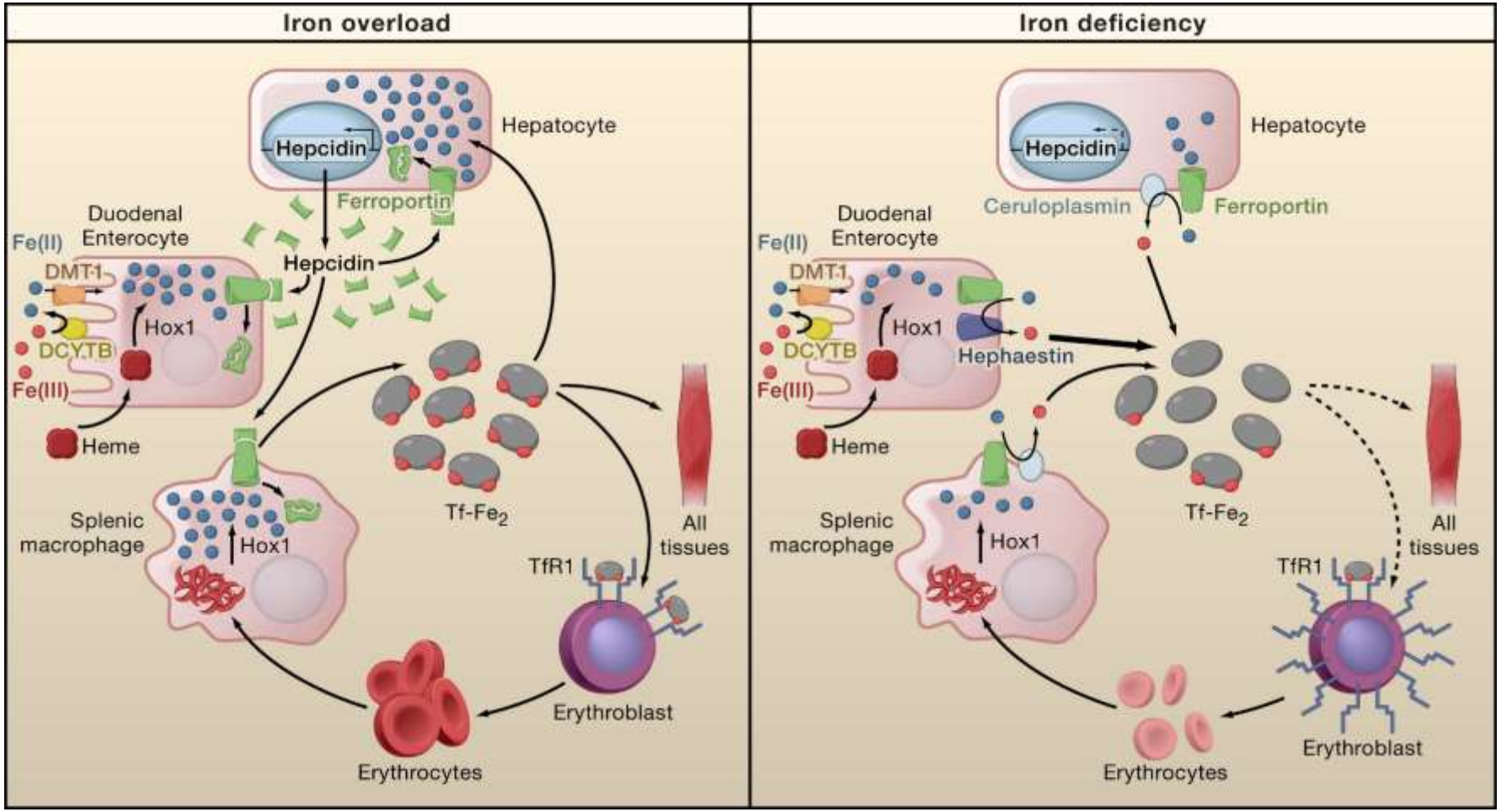
Mechanism

- FPN is highly expressed under iron deficiency in different cells, e.g., enterocytes ad macrophages, whilst hepcidin is downregulated and local mechanisms increase the intestinal iron absorption
- iron regulatory protein (IRP) control the cellular iron content
- other mechanisms of iron control involve mTOR inhibition and ferritin, i.e., cells may recover iron stored in ferritin
- macrophages involved in iron recycling by phagocytosis of senescent red cells

Mechanism

- IV iron may increase Hb or iron storage
- available in different forms and can be administered at high doses
- recovering iron levels noted by Perls' stain in the spleen, but also in the liver
- predominance of FCM associated iron deposition in Kupffer cells versus spleen might be related to different plasma protein adherence be different coating and charge differences unique to FCM that may preferentially drive the signal for Kupffer's cell recognition and opsonization versus uptake into other tissues

Mechanism



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Mechanism

- FCM caused increased heavy and light chain ferritin increased in Kupffer cells compared to normal and anemic controls
- low amount of both ferritin chains in hepatocytes of anemic controls may indicate sensitivity of hepatocyte iron stores to iron deficiency.
- increased expression of ferritin chains in renal proximal tubules indicate low overall risk for nephrotoxicity
- heart and skeletal myofibers and bone marrow showed rapid restoration of heavy and light ferritin subunits
- reason for increased ferritin chains in the plexus remains unknown.