BIOGRAPHICAL SKETCH

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NAME: FLEMING, MARK D, MD, DPHIL

eRA COMMONS USER NAME (credential, e.g., agency login): fleming_m

POSITION TITLE: Pathologist-in-Chief, Boston Children's Hospital and S. Burt Wolbach Professor of Pathology, Harvard Medical School

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Princeton University, Princeton, NJ	AB	06/1987	Molecular Biology
University of Oxford, Oxford, UK	DPhil	01/1990	Organic Chemistry
Harvard Medical School, Boston, MA	MD	06/1993	Medicine
Brigham and Women's Hospital, Boston, MA		12/1996	Resident and Clinical Fellow in Anatomic Pathology and Hematopathology
Boston Children's Hospital, Boston, MA		07/2001	Research Fellow in Hematology

A. Personal Statement

I am a clinically active hematological pathologist whose research focuses on the genetics and genomics of hematological diseases, with emphasis on diseases of red blood cells. My laboratory works in three general areas: 1) discovering the pathways of iron metabolism in red blood cells, 2) the genetics and pathogenesis of inherited anemias, congenital marrow failure disorders, and myelodysplastic syndromes in children, 3) the ubiquitin-proteasome system in erythropoiesis. Our particular focus in iron metabolism is to understand how red blood cell precursors take up and utilize iron for heme synthesis, including the congenital sideroblastic anemias and erythropoietic protoporphyria. To do so we use primarily two approaches: next generation sequencing of novel anemia phenotypes in humans to define new genes, as well as targeted mutagenesis in mice to functionally characterize these novel loci as well as others implicated in human genetic disorders of iron metabolism and erythropoiesis. My role in this project will be as a mentor to the PI, Dr. Sarah Ducamp.

a. Han AP, **Fleming MD**, and Chen JJ. Heme-regulated eIF2alpha kinase modifies the phenotypic severity of murine models of erythropoietic protoporphyria and beta-thalassemia. J Clin Invest. 2005;115(6):1470-1473. PMCID: PMC1136998.

b. Lichtenstein DA, Crispin AW, Sendamarai AK, Campagna DR, Schmitz-Abe K, Sousa CM, Kafina MD, Schmidt PJ, Niemeyer CM, Porter J, May A, Patnaik MM, Heeney MM, Kimmelman A, Bottomley SS, Paw BH, Markianos K, **Fleming MD**. A recurring mutation in the respiratory complex 1 protein NDUFB11 is responsible for a novel form of X-linked sideroblastic anemia. Blood. 2016 Oct 13;128(15):1913-1917. PMCID: PMC5064715.

c. Berhe S, Heeney MM, Campagna DR, Thompson JF, White EJ, Ross T, Peake RW, Hanrahan JD, Rodriguez V, Renaud DL, Patnaik MS, Chang E, Bottomley SS, **Fleming MD**. Recurrent heteroplasmy for the MT-ATP6 p.Ser148Asn (m.8969G>A) mutation in patients with syndromic congenital sideroblastic anemia of variable clinical severity. *Haematologica*. 2018 Jul 13. doi: 10.3324/haematol.2018.199109. [Epub ahead of print] PMCID: PMC6269299.

d. Crispin A, Guo C, Chen C, Campagna DR, Schmidt PJ, Lichtenstein D, Cao C, Sendamarai AK, Hildick-Smith GJ, Huston NC, Boudreaux J, Bottomley SS, Heeney MM, Paw BH, **Fleming MD**, Ducamp S. Mutations in the iron-sulfur cluster biogenesis protein HSCB cause congenital sideroblastic anemia. J Clin Invest. 2020 Oct 1;130(10):5245-5256. doi: 10.1172/JCI135479.

B. Positions and Honors

Positions and Employment

1998- 2000	Instructor in Pathology, Harvard Medical School, Boston, MA
1997-2009	Assistant Pathologist, Hematopathology, Brigham and Women's Hospital,
1998	American Board of Pathology, Diplomate, Anatomic Pathology and Hematology
1999-	Pathologist, Hematopathology, Boston Children's Hospital
2000-2006	Assistant Professor of Pathology, Harvard Medical School
2006-2009	Associate Professor of Pathology, Harvard Medical School
2007-2009	Interim Pathologist-in-Chief and Interim Chair of Pathology, Boston Children's Hospital
2009-	Pathologist-in-Chief and Chair of Pathology, Boston Children's Hospital
2010-	S. Burt Wolbach Professor of Pathology, Harvard Medical School, Boston, MA
2015-	Associate Member, Broad Institute of Harvard and MIT, Cambridge, MA

Other Experience and Professional Memberships

1998-	American Society of Hematology, Member
2001-2002	Scientific Subcommittee on Heme and Iron, American Society of Hematology
2002	Red Cells, Iron and Heme Abstract Review Committee [Chairperson], American Society of Hematology
2003	Scientific Subcommittee on Heme and Iron [Chairperson], American Society of Hematology
2003-	International BioIron Society, Member
2006	Iron in Hematology Education Committee [Chairperson], American Society of Hematology
2008-	American Society of Clinical Investigation, Member
2008-2011	Scientific Committee on Hematopathology and Clinical Laboratory Hematology, American Society of Hematology

Honors

1987	Marshall Scholarship, British Marshall Aid Commemoration Commission
1987	Senior Prize for Academic Excellence, Department of Molecular Biology, Princeton University
1987	Phi Beta Kappa, Princeton University
1987	summa cum laude, Princeton University
1993	Dr. Harold Lamport Biomedical Research Prize, Harvard Medical School
1993	magna cum laude, Harvard Medical School
1998	American Liver Foundation Postdoctoral Research Fellowship
1999	American Society of Hematology, Fellow Scholar Award
2000	Pew Scholar in the Biomedical Sciences

C. Contribution to Science

1. I began in the field as a medical student, pathology resident, and subsequently post-doctoral fellow working with Dr. Nancy Andrews, when very little was known about how iron is trafficked within the body, and much less was understood concerning how systemic iron metabolism was regulated. At the time, a comprehensive genomic map was beginning to facilitate the technique of positional cloning in the mouse, allowing me to approach the cloning of several historically important rodent iron metabolism mutants. The first target was the *microcytic anemia* (*mk*) mutant, which physiological studies had suggested was a key component of intestinal iron absorption and red blood cell precursor iron uptake. Indeed, we demonstrated that the 12 transmembrane protein now known as DMT1 (also known as Nramp2) was mutated in *mk* mice. Simultaneous and subsequent studies from two laboratories, including our own, showed that DMT1 was a transmembrane iron transporter. Remarkably, another rodent mutant, the *Belgrade* (*b*) rat, with a similar phenotype proved to have the identical mutation. These two mutants, however, were quite complementary with one-another, as the intestinal iron uptake and red blood cell defects had been uniquely well characterized in the *mk* mouse and *b* rat, respectively. Taken together, the historical physiological data, functional data and genetics conclusively demonstrated that DMT1 was the primary iron importer responsible both for apical intestinal epithelial cell iron absorption as well as transferrin cycle iron uptake. In the same period, I played a significant collaborative role in the discovery and characterization of the only known cellular iron exporter, ferroportin 1 (FPN1).

a. **Fleming MD**, Trenor CC, Su M, Foernzler D, Beier D, Dietrich W, and Andrews NC. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. Nature Genetics 1997; 16: 383-386. PMID: 9241278.
b. Su MA, Trenor CC, Fleming JC, **Fleming MD**, and Andrews NC. The G185R mutation disrupts function of the iron transporter Nramp2. Blood. 1998;92(6):2157-2163. PMID: 9731075.
c. **Fleming MD**, Romano MA, Su MA, Garrick LM, Garrick MD, and Andrews NC. Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. Proc Natl Acad Sci USA. 1998;95(3):1148-1153. PMCID: PMC18702.
d. Donovan A, Brownlie A, Zhou Y, Shepard J, Pratt SJ, Moynihan J, Paw BH, Drejer A, Barut B, Zapata A, Law TC, Brugnara C, Lux SE, Pinkus GS, Pinkus JL, Kingsley PD, Palis J, **Fleming MD**, Andrews NC, and Zon LI. Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. Nature. 2000;403(6771):776-781. PMID: 10693807.

2. In my own laboratory, continued to use positional cloning as a method of gene discovery. Most significant is our work on the *nm1054* mutant. Like *mk* mice and *b* rats, *nm1054* animals suffer from a hypochromic microcytic anemia. We comprehensively characterized the pathophysiology of the anemia, demonstrating that it was due to a selective red blood cell precursor iron uptake defect. We went on to show that *nm1054* was a large genomic deletion, and using bacterial artificial chromosome (BAC) transgenic and retrovirus-mediated cDNA phenotypic complementation, as well as targeted mutagenesis, demonstrated that the gene responsible for the anemia was the previously known protein of unknown function, six-transmembrane epithelial antigen of the prostate 3 (Steap3). Because Steap3 was highly and selectively expressed in erythroid precursors and had an oxidoreductase domain, we hypothesized and then demonstrated that it was a ferrireductase localized to the transferrin cycle endosome. This observation lead to the conclusion that Steap3 was almost certainly the endosomal reductase required to reduce ferric (Fe^{3+}) iron bound to transferrin to ferrous (Fe^{2+}) iron, which can be transported by DMT1. In subsequent studies, we showed that not only does Steap3 function as a ferrireductase, but that it, as well as its homologues Steap2 and Steap4, are metalloreductases that can also reduce copper from Cu^{2+} to Cu^{1+}. We have subsequently collaborated to describe the crystal structure of the Steap3 and Steap4 oxidoreductase domain, and, employing an ethylnitrosourea (ENU) induced Steap3 mutant, *Steap3*^{Y288H}, collaborated to define sequences important for targeting Steap3 to the endosome.

a. Ohgami RS, Campagna DR, Antiochos B, Wood EB, Sharp JJ, Barker JE, and **Fleming MD**. nm1054: a spontaneous, recessive, hypochromic, microcytic anemia mutation in the mouse. Blood. 2005;106(10):3625-3631. PMCID: PMC1819405.
b. Ohgami RS, Campagna DR, Greer EL, Antiochos B, McDonald A, Chen J, Sharp JJ, Fujiwara Y, Barker JE, and **Fleming MD**. Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. Nat Genet. 2005;37(11):1264-1269. PMCID: PMC2156108.
c. Ohgami RS, Campagna DR, McDonald A, **Fleming MD**. The Steap proteins are metalloreductases. Blood. 2006;108(4):1388-1394. PMCID: PMC1785011.
d. Lambe T, Simpson RJ, Dawson S, Bouriez-Jones T, Crockford TL, Lepherd M, Latunde-Dada GO, Robinson H, Raja KB, Campagna DR, Villarreal Jr G, Ellory C, Goodnow C, **Fleming MD**, McKie AT, and Cornall RJ. Identification of a Steap3 endosomal targeting motif essential for normal iron metabolism. Blood. 2009;113(8):1805-8. PMCID: PMC2947353.

3. Over the course of two decades we have amassed a large repository of patient DNAs and clinical data, including many patients with a clinical phenotype of “iron-refractory, iron deficiency anemia (IRIDA),” We showed the fundamental pathophysiology of IRIDA is inappropriate overproduction of the iron regulatory hormone hepcidin, which negatively regulates iron egress from cells. The coincident descriptions of mapping of the IRIDA locus to human chromosome 22q and the description of mouse mutants in the transmembrane protease Tmprss6 with an IRIDA-like phenotype by us and other investigators, allowed us to identify biallelic mutations in *TMPRSS6* in our IRIDA patients. Subsequently, others have shown in genome-wide association studies (GWAS) that common polymorphisms in TMPRSS6 are can account for ~5% of the variability in serum iron in adults, indicating that “genetic susceptibility” may underlie even seemingly simple acquired nutritional disorders such as iron deficiency. We have subsequently gone on to demonstrate in collaboration with Alnylam Pharmaceuticals that siRNA-mediated suppression of Tmprss6 in mice can modulate the anemia as well as secondary iron overload seen in a mouse model of □-thalassemia intermedia.

a. Finberg KE, Heeney MH, Campagna D, Aydýnok Y, Pearson HA, Hartman KP, Mayo MM, Samuel SM, Strouse JJ, Markianos K, Andrews NC, **Fleming MD**. Mutations in TMPRSS6 cause iron-refractory, iron deficiency anemia (IRIDA). Nat Genet. 2008;40:569-571. PMCID: PMC3104019.

b. Schmidt PJ, Toudjarska I, Sendamarai AK, Racie T, Milstein S, Bettencourt BR, Hettinger J, Bumcrot D, **Fleming MD**. An RNAi therapeutic targeting Tmprss6 decreases iron overload in *Hfe*$^{-/-}$ mice and ameliorates anemia and iron overload in murine β-thalassemia intermedia. *Blood*. 2013 Feb 14;121(7):1200-08. PMCID: PMC3655736.
c. Schmidt PJ, Racie T, Westerman M, Fitzgerald K, Butler JS, **Fleming MD**. Combination therapy with a Tmprss6 RNAi-therapeutic and the oral iron chelator deferiprone additively diminishes secondary iron overload in a mouse model of β-thalassemia intermedia. *Am J Hematol*. 2015 Apr;90(4):310-3. PMCID: PMC4403964.
d. Heeney MM, Guo D, De Falco L, Campagna DR, Olbina G, Kao PP-C, Schmitz-Abe K, Rahimov F, Gutschow P, Westerman K, Ostland V, Jackson T, Klaassen R, Markianos K, Finberg K, Iolascon A, Westerman M, London WB, **Fleming MD**. Normalizing hepcidin predicts *TMPRSS6* mutation status in patients with chronic iron deficiency. Blood. 2018 Jul 26;132(4):448-452. doi: 10.1182/blood-2017-03-773028. Epub 2018 Jun 12. Erratum in: Blood. 2018 Sep 20;132(12):1355. PMCID: PMC6071554.

4. Erythroid precursors use iron largely to make heme for hemoglobin production. Germline mutations in the first enzyme in erythroid heme biosynthesis, aminolevulinic acid (ALA) synthase 2 (ALAS2), however, result in an unusual form of hypochromic, microcytic anemia termed a sideroblastic anemia (SA), in which iron is pathologically deposited within the mitochondria of erythroid precursors. The molecular genetic basis of many other congenital sideroblastic anemias, CSAs, is obscure. Because of the potential to understand more fully the pathways of iron utilization in mitochondria, we have collected and studied the largest cohort of patients with CSA ever assembled—more than 180 probands. Using a combination of candidate gene sequencing, positional cloning and next generation whole exome/genome sequencing, we can now explain more than 60% of cases of CSA. Selected studies not included in section 1 are listed below.

a. Guernsey DL, Jiang H, Campagna DR, Evans SC, Ferguson M, Kellogg MD, Lachance M, Matsuoka M, Nightingale M, Rideout A, Saint-Amant L, Schmidt PJ, Orr A, Bottomley SS, **Fleming MD**, Ludman M, Dyack S, Fernandez CV, Samuels ME. Mutations in mitochondrial carrier family gene SLC25A38 cause nonsyndromic autosomal recessive congenital sideroblastic anemia. Nat Genet. 2009 Jun;41(6):651-3. PMID: 19412178.
b. Campagna DR, de Bie CI, Schmitz-Abe K, Sweeney M, Sendamarai AK, Schmidt PJ, Heeney MM, Yntema HG, Kannengiesser C, Grandchamp B, Niemeyer CM, Knoers NVAM, Swart S, Marron G, van Wijk R, Raymakers RA, May, Markianos K, Bottomley SS, Swinkels DW, and **Fleming MD**. X-linked sideroblastic anemia due to ALAS2 intron 1 enhancer element GATA binding site mutations. Am J Hematol, 2014 Mar 89(3):315-9. PMCID: PMC3943703.
c. Riley LG, Heeney MM, Rudinger-Thirion J, Frugier M, Campagna DR, Zhou R, Hale GA, Hilliard L, Kaplan JA, Kwiatkowski JL, Sieff CA, Steensma DP, Rennings AJ, Simons A, Schaap N, Roodenburg RJ, Kleefstra T, Arenillas L, Fita-Torró J, Ahmed R, Abboud M, Bechara E, Farah R, Tamminga RYJ, Bottomley SS, Sanchez M, Swinkels DW, Christodoulou J, **Fleming MD**. The phenotypic spectrum of germline *YARS2* variants: from isolated sideroblastic anemia to mitochondrial myopathy, lactic acidosis and sideroblastic anemia 2. *Haematologica*. 2018 Dec;103(12):2008-2015. PMCID: PMC6269294.
d. Ducamp S, **Fleming MD**. The molecular genetics of sideroblastic anemia. Blood. 2019 Jan 3;133(1):59-69. PMCID: PMC6318428.

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