

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Macdougall IC, Rossert J, Casadevall N, et al. A peptide-based erythropoietin-receptor agonist for pure red-cell aplasia. *N Engl J Med* 2009;361:1848-55.

Supplementary Table 1: Demographics and Baseline Characteristics

Pt # (Country)	Age Gender	Prior ESA Exposure	Year of PRCA Diagnosis	Prior Immunosuppressive Treatment for PRCA	Pre- treatment Red Cell Transfusions (units/3mo prior to Hematide)	Pre- treatment Hemoglobin (g/dL)	Pre- treatment Reticulocyte Count ($\times 10^9/L$)
1 (GE)	52 F	Epoetin alfa, Epoetin beta	2000	Steroids, IVIG, Cyclophosphamide, Cyclosporine, anti- CD-20 antibodies	6	10.3	1.9
2 (GE)	72 M	Epoetin beta	2005	None	10	10.6	37.8
3 (GE)	54 M	Epoetin beta	2006	None	0	9.8	39.7
4 (GE)	64 F	Epoetin alfa Epoetin beta	2001, 2006	Steroids, Azathioprine, IVIG	1	8.0	21.5
5 (GE)	91 M	Darbepoetin alfa	2005	Steroids	8	8.0	19.3
6 (GE)	80 M	Epoetin beta	2008	Cyclosporine	10	9.9	2.6
7 (FR)	72 M	Epoetin alfa Epoetin beta	2005	Cyclosporine	8	8.2	70.0*
8 (FR)	77 F	Epoetin alfa Epoetin beta	2003, 2006	Cyclosporine	12	9.7	21.1 [†]
9 (FR)	88 M	Epoetin beta	2007	None	0	11.1 [§]	35.0 [§]
10 (FR)	63 F	Epoetin beta Darbepoetin alfa	2008	None	9	8.1	11.0
11 (UK)	38 M	Darbepoetin alfa	2006	None	4	8.1	2.0
12 (UK)	60 M	Epoetin alfa Darbepoetin alfa	2002	Cyclosporine	27	8.2	1.8

13 (UK)	79 M	Epoetin beta	2007	None	6	7.5	28.3
14 (UK)	81 F	Darbepoetin alfa	2007	None	12	10.2	8.5

*Patient 7 received monthly red cell transfusions prior to the study; reticulocyte counts were as low as $2.2 \times 10^9/L$ during the 4 months prior to study enrollment. The baseline value reported here was obtained from a blood sample collected approximately 1 month following a transfusion; the result was high relative to the patient's history.

†The baseline reticulocyte count for Patient 8 reflects a value obtained 1 week after the first Hematide injection; a true baseline value (prior to treatment) was not available.

§At the time of diagnosis, Patient 9 had a hemoglobin value of 6.7 g/dL and a reticulocyte count of $9.5 \times 10^9/L$.

Supplementary Table 2: Adverse Events Reported from April 2006 through March 2009

System Organ Class Preferred Term	No. of Patients		
	Any Adverse Event	Grade 3 or Higher Adverse Event*	Serious Adverse Event†
Blood and Lymphatic System Disorders			
Anemia	2	1‡	1‡
Eosinophilia	1		
Leukocytosis	1		
Leukopenia	1		1§
Lymphadenopathy mediastinal	1		
Neutropenia	1		
Pancytopenia	1		
White blood cell count decreased	1		
Cardiac Disorders			
Atrial fibrillation	1	1	1
Atrial flutter	1		1¶
Bradycardia	1		
Cardiac arrest	1	1	1
Endocarditis	1	1**	1**
Palpitations	1		
Ear and Labyrinth Disorders			
Deafness	1		
Tinnitus	2	1††	
Endocrine Disorders			
Thyroid cyst	1		
Eye Disorders			
Conjunctival haemorrhage	1		
Eyelid oedema	1		
Optic neuropathy	1		1**
Presbyopia	1		
Gastrointestinal Disorders			
Abdominal pain	1		
Constipation	1		
Diarrhoea	3		
Dyspepsia	1		
Nausea	1		
Rectal haemorrhage	1		
Vomiting	1		
General Disorders and Administration Site Conditions			
Asthenia	4		
Catheter site cellulitis	1		
Chills	1		
Drug ineffective	1	1‡	1‡
Fatigue	5	1§	
Influenza like illness	1		
Injection site haematoma	1		
Injection site joint pain	1	1††	

System Organ Class Preferred Term	No. of Patients		
	Any Adverse Event	Grade 3 or Higher Adverse Event*	Serious Adverse Event†
Injection site pruritus	1		
Oedema	1		
Oedema peripheral	3		
Pyrexia	2		
Immune System Disorders			
Milk allergy	1		
Seasonal allergy	1		
Transplant rejection	1		
Infections and Infestations			
Cytomegalovirus infection	1		1 [§]
Escherichia bacteraemia	1		1 [§]
Escherichia urinary tract infection	1		
Gangrene	1	1 ^{‡‡}	1 ^{‡‡}
Influenza	1		
Lower respiratory tract infection	1		
Nasopharyngitis	7		
Peritoneal tuberculosis	1	1 ^{§§}	1 ^{§§}
Pneumocystis jiroveci pneumonia	1		1 [§]
Rhinitis	1		
Sepsis	1		
Staphylococcal infection	2		
Tooth Abscess	1		
Tuberculosis	1	1 ^{§§}	1 ^{§§}
Upper respiratory tract infections	3		
Urinary tract infection	1		
Injury, Poisoning and Procedural Complications			
Contusion	1		
Fall	1		
Forearm fracture	1		
Haemodialysis-induced symptom	2		
Hand fracture	1		
Post-traumatic pain	1		
Procedural hypotension	2		
Scratch	1		
Vascular pseudoaneurysm	1	1 ^{§§}	1 ^{§§}
Wound	1		
Investigations			
Biopsy kidney	1		
Blood creatinine increased	1		
Blood glucose increased	1		
Blood oestrogen decreased	1		
Blood phosphorus increased	1		
Blood potassium increased	1		
Blood pressure increased	2		
Body temperature increased	1		

System Organ Class Preferred Term	No. of Patients		
	Any Adverse Event	Grade 3 or Higher Adverse Event*	Serious Adverse Event [†]
Cardiac murmur	1		
Haemoglobin decreased	5		
Liver function test abnormal	1		
Platelet count decreased	1		
Reticulocyte count decreased	2		
Serum ferritin decreased	1		
Serum ferritin increased	1		
Weight decreased	1		
Metabolism and Nutrition Disorders			
Abnormal loss of weight	1		
Anorexia	1		
Diabetes mellitus	1		
Fluid retention	1		
Hypoglycaemia	1		
Hyponatraemia	1		
Musculoskeletal and Connective Tissue Disorders			
Arthralgia	3		
Arthritis	1		
Intervertebral disc protrusion	1		
Joint swelling	1		
Muscle spasms	1		
Musculoskeletal pain	1		
Pain in extremity	1		
Musculoskeletal and Connective Tissue Disorders NEC			
Back pain	1		
Nervous System Disorders			
Confusional state	1	1	1
Dizziness	1		
Headache	1	1 ^{††}	
Insomnia	1		
Migraine	1		
Restless legs syndrome	1		
Sciatica	1		
Somnolence	1		
Psychiatric Disorders			
Sleep disorder	1		
Renal and Urinary Disorders			
Nephropathy	1		1 [§]
Reproductive System and Breast Disorders			
Hydrocele	1		
Respiratory, Thoracic and Mediastinal Disorders			
Aspiration	1		

System Organ Class Preferred Term	No. of Patients		
	Any Adverse Event	Grade 3 or Higher Adverse Event*	Serious Adverse Event [†]
Bronchial obstruction	1		
Bronchitis	3		
Cough	2		
Dyspnoea	3		
Nasal congestion	1		
Pleural effusion	2		
Productive cough	1		
Pulmonary oedema	1	1 ^{§§}	1 ^{§§}
Skin and Subcutaneous Tissue Disorders			
Alopecia	1		
Eczema	2		
Excoriation	1		
Pruritus	1		
Surgical and Medical Procedures			
Arteriovenous shunt operation	1		1 ^{**}
Cataract operation	1		
Catheter removal	1		
Catheter placement	1		
Central venous catheterization	1		
Dupuytren's contracture operation	1	1	
Gastrointestinal tube insertion	1		
High frequency ablation	1		1 [¶]
Renal transplant	1		
Tooth extraction	1		
Ureteral stent removal	1		
Vascular Disorders			
Femoral artery aneurysm	1	1 [¶]	1 [¶]
Hypertension	5		
Hypotension	1		
Temporal arteritis	1	1 ^{**}	

*Adverse events were Grade 3 unless otherwise noted.

[†]A serious adverse event is defined by the Food and Drug Administration as any untoward medical occurrence that resulted in death, was life-threatening, required in-patient hospitalization or prolonged an existing hospitalization, resulted in persistent or significant disability/incapacity, resulted in a congenital anomaly/birth defect, or required intervention to prevent one or more of these outcomes (FDA Guidance for Industry, Clinical Safety Data Management: Definitions and Standards for Expedited Reporting; ICH-E2A, March 1995).

[‡]Patient #10 had anemia with a lack of response to study drug, both considered medically important, requiring intervention.

[§]Patient #12 was hospitalized four times within 6 months: the first time for cytomegalovirus and nephropathy, a second time for leukopenia, a third time for Pneumocystis jiroveci pneumonia and bacteraemia, and a fourth time for reactivation of cytomegalovirus.

^{||}Patient #8 was hospitalized once for atrial fibrillation and Grade 4 confusional state (vascular dementia). Approximately one year later, the patient died at home (cardiac arrest) approximately 4 months after the last dose of Hematide.

[¶]Patient #7 was hospitalized on separate occasions for atrial flutter, a high frequency ablation, and a femoral artery aneurysm.

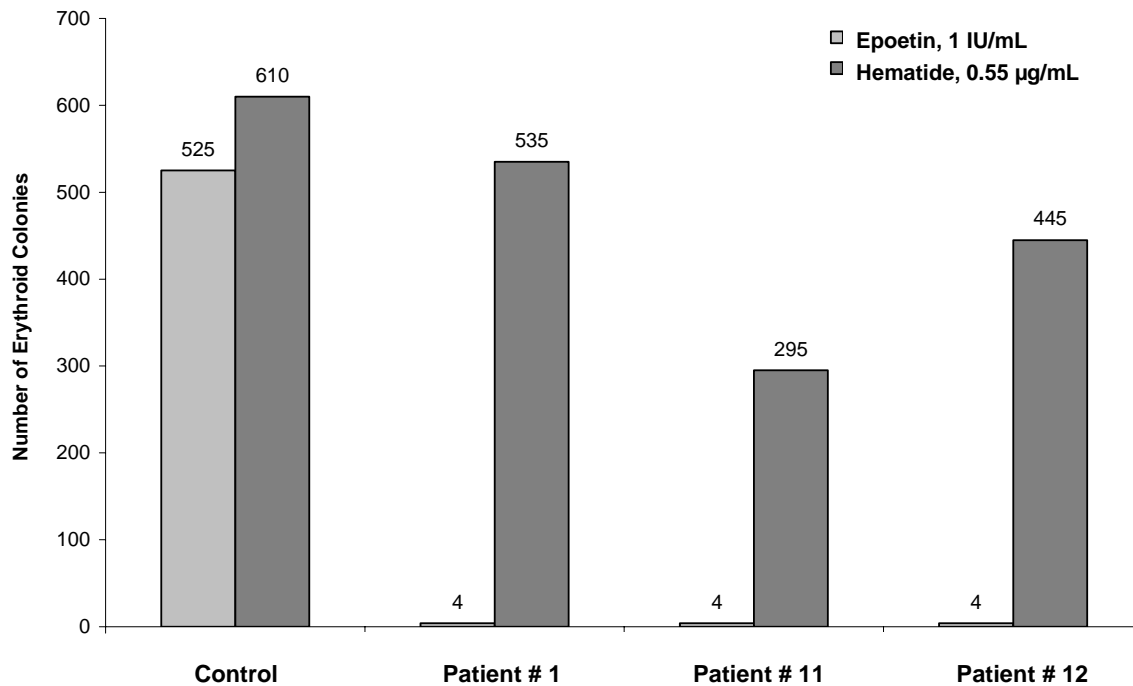
^{**}Patient #5 was hospitalized on two separate occasions: once for an arteriovenous shunt operation and a second time for optic ischemic neuropathy and temporal arteritis. Prior to the hospitalizations, the patient had endocarditis, which was considered medically important, requiring intervention.

^{††}Patient #1 had three Grade 3 adverse events (tinnitus, bone pain, and headache), none were serious.

^{‡‡}Patient #2 was hospitalized for Grade 4 gangrene.

^{§§}Patient #11 was hospitalized on two separate occasions: once for tuberculosis and a second time for peritoneal tuberculosis. During the second hospitalization, vascular pseudoaneurysm and pulmonary oedema were diagnosed; these latter events prolonged the existing hospitalization.

Supplementary Figure 1



In vitro bone marrow culture of erythroid colonies in the presence of epoetin or Hematide.

Results in three patients in this study show marked inhibition of CFU-E growth in the presence of the patients' serum containing anti-erythropoietin antibodies, compared with control serum. This inhibition could, however, be overcome by the addition of 0.55 µg/mL of Hematide to the culture medium. Light gray bars represent the number of in vitro erythroid colonies with control or patient serum in the presence of erythropoietin. Dark gray bars represent the number of in vitro erythroid colonies with control or patient serum in the presence of Hematide.