

Erythropoietin cure or nightmare



**Dr. SK CHAN, Dr. CK CHAN, Dr. CC CHOW
PYNEH**


HISTORY

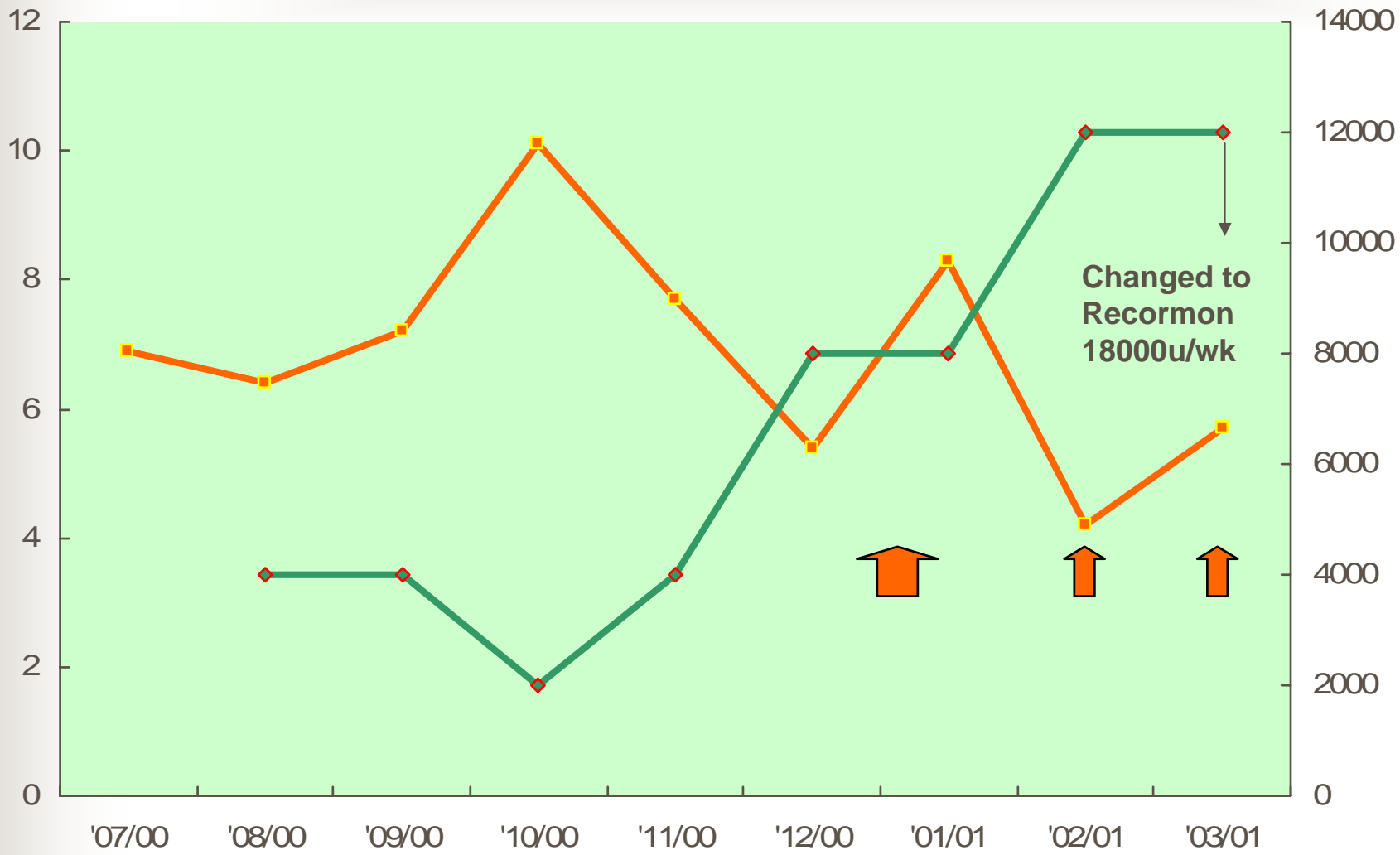






History

- Madam Chu, F/61
- Beta Thal Trait
- Peptic ulcer, uterine fibroid with TAHBSO
- 1994: Proteinuria 1.3gm/day; elevated IgA; creatinine 118 umol/L
- Refused renal biopsy
- Treated as IgA nephropathy with ACEI and ARB
- Progressive renal failure, started CAPD since May 2000 (creatinine 853umol/L, Hb 7.8g/dL)

- 
- Hb level ~ 6.4-7g/dL
 - Work up for anaemia/starting EPO (between July-Oct 2000)
 - Fecal occult blood –ve
 - PTH 53ug/ml
 - Iron saturation 49%
 - Started Erythropoietin Alpha (Eprex) s.c. since Aug 2000, dose 4000u/wk



 Haemoglobin level (g/dl)
 Eprex dose (per week)

 Blood transfusion



Epo resistance

- Major causes:

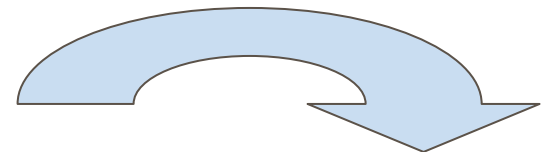
- Iron deficiency
- Chronic inflammation/infection
- Underdialysis

- Minor causes:

- Hyperparathyroidism
- Aluminium toxicity
- Vit B12/folate deficiency
- Haemolysis
- Blood loss

■ Further work up

- | | | |
|----------|-------------|---------------------------------------|
| ■ Feb 01 | OGD | Esophagitis |
| ■ Mar 01 | Blood test | C3, ANA normal, CRP slightly elevated |
| ■ Mar 01 | reti count | <1.0% |
| ■ Mar 01 | BMA | Inadequate tissue |
| ■ Apr 01 | Colonoscopy | NAD |
| ■ May 01 | Ba enema | 1cm Diverticulum |
| ■ May 01 | US abdomen | gall stone |
| ■ May 01 | KT/V | 2.3 |
| ■ Nov 01 | CT thorax | no thymoma |
| ■ Jun 01 | BMA | |




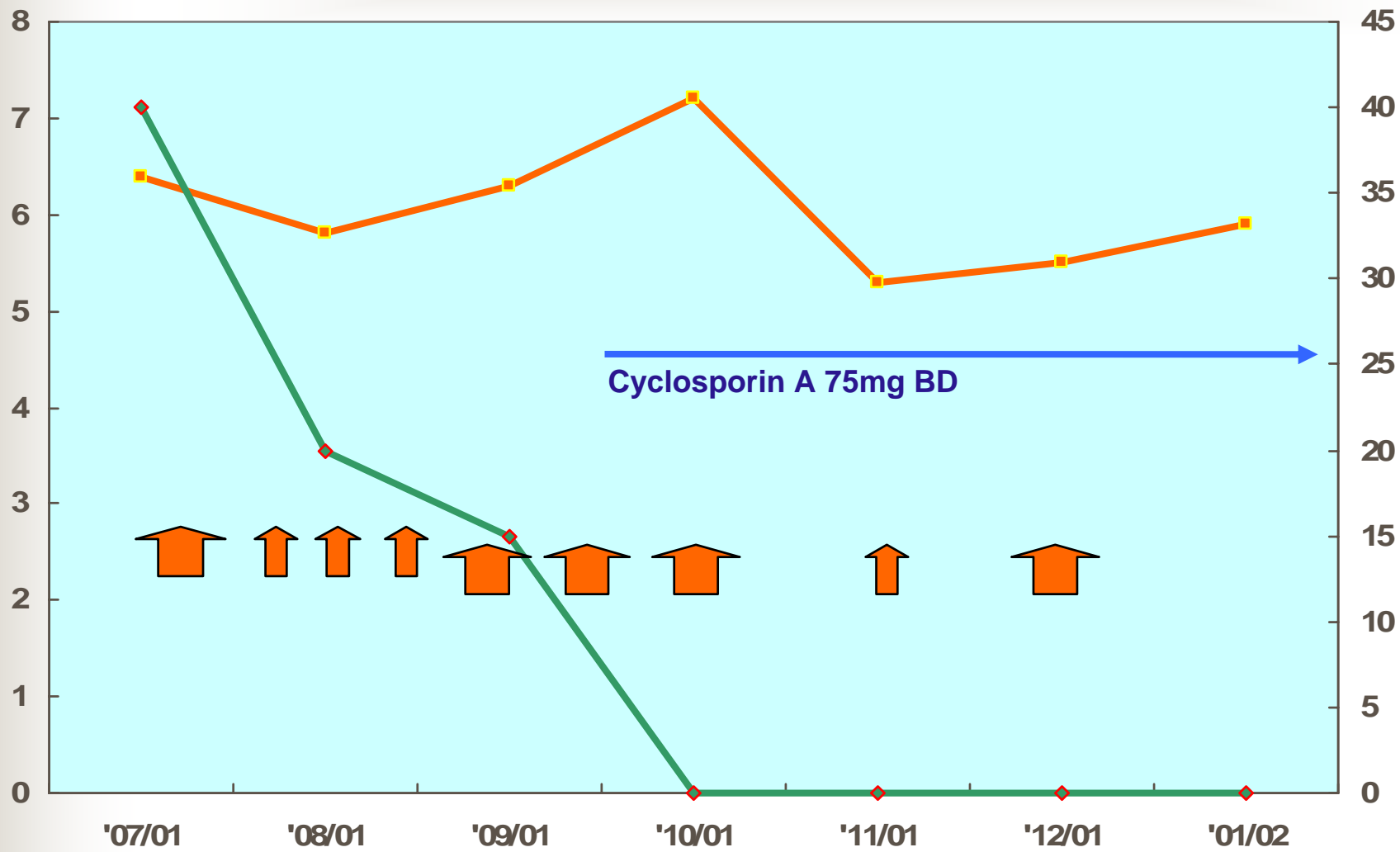


■ Result of BMA on June 2001:


- Megakaryocytes are adequate in number and show normal morphology
- Granulopoiesis is normal in activity and shows orderly maturation
- Erythropoiesis is markedly depressed with essentially no erythroblasts seen
- Conclusion: compatible with PURE RED CELL APLASIA

- 
- What further work-up you would like to proceed?

- 
- Recormon was stopped on Jun 2001
 - Further Ix with Parvovirus B19 IgG –ve (Sept 2001)
 - Parvovirus PCR –ve (Jan 2002)
 - Prednisolone 40mg daily was started



↑ Blood transfusion

- 
- Cyclosporin A was continued for total 1 year. Stopped afterward due to poor response
 - Reticulocyte count before and after immunosuppressive therapy: both <1.0%
 - Anti-EPO antibody titre on Sep 2002
 - Borderline

Global BRM AE number:

APCDSS2002000099 Protocol:

EPO-IMU-001

Physician Name:

Kit, Chan Ching/Shing, Wong Kin

Site:

HONG KONG

RESULTS OF CIRCULATING EPO CONCENTRATION (ELISA Testing Method)

Results in mU/mL of different blood draws			
PAL ID Number:	18		
Date of Last EPO Dose:	Unknown		
Sample Collection Date:	Unknown		
Sample Collection Time:	Unknown		
Result Release Date:	01 Oct 02		
Reference Range 10-30 mU/mL (Healthy Normal)	Test 1 Date: 01 Oct 02		
	21.4		

RESULTS OF IMMUNOPRECIPITATION TEST FOR PRESENCE OF ANTI-EPO ANTIBODIES

Results in %cpm of different blood draws			
PAL ID Number:	18		
Date of Last EPO Dose:	Unknown		
Sample Collection Date:	Unknown		
Sample Collection Time:	Unknown		
Result Release Date:	01 Oct 02		
Dilutions of sera tested	Test 1		
Normal Values $\leq 0.6\%$ cpm	Date: 27 Sep 02		
1:20	0.6		
1:50	0.3		
1:100	0.2		
1:1,000	0.1		
1:10,000	0.0		
1:20,000	0.0		
Overall Result	Borderline*		

A positive antibody is $\geq 0.9\%$ cpm in the above assay.A negative antibody is $\leq 0.6\%$ cpm in the above assay.

Results between positive and negative are borderline.

*Borderline range = 0.4-0.8% cpm in September 2002; Borderline range has been revalidated to 0.7-0.8%cpm in August 2003.

The result reported here should not be used alone for the diagnosis or treatment of clinical disease.

Sample 735: PAPERWORK: AE NUMBER=20060806400; TUBE: BARCODE=1045; TIME COLLECTED=09:00

N/A = not applicable



■ Diagnosis

- Anti-EPO associated PRCA
- Failed to response to immunosuppressive treatment

■ Progress

- Transfusion dependent
 - Required ~0.6 unit of pack cell/week from year 02 to 06
- Reticulocyte count: <1.0% - 1.7%
- Prevention of iron overload
 - Iron chelating agent was given
 - Ferritin level ~13000



We know where we are But where should we go?

- Continue regular blood transfusion
- Rechallenge with another EPO
- Renal transplantation



Our choice and patient's choice

- Other preparation of recombinant erythropoietin may help??
- After discussion with Madam Chu, Darbepoietin IVI, since Oct 2006
- Reason behind:
 - Risk of ongoing iron overload with transfusion
 - ? Safer to use other preparation of EPO
- Anti-EPO titre repeated on Sep 2006:
 - 0.1%--negative

Global BRM AE number:

APCDSS2002000099 Protocol:

EPO-IMU-001

Physician Name:

Kit, Chan Ching/Shing, Wong Kin

Site:

HONG KONG

RESULTS OF CIRCULATING EPO CONCENTRATION (ELISA Testing Method)

Results in mU/mL of different blood draws			
PAL ID Number:	18	735	
Date of Last EPO Dose:	Unknown	27 Jun 01	
Sample Collection Date:	Unknown	05 Sep 06	
Sample Collection Time:	Unknown	10:00	
Result Release Date:	01 Oct 02	19 Sep 06	
Reference Range 10-30 mU/mL (Healthy Normal)	Test 1 Date: 01 Oct 02	Test 2 Date: 15 Sep 06	
	21.4	15.0	

RESULTS OF IMMUNOPRECIPITATION TEST FOR PRESENCE OF ANTI-EPO ANTIBODIES

Results in %cpm of different blood draws			
PAL ID Number:	18	735	
Date of Last EPO Dose:	Unknown	27 Jun 01	
Sample Collection Date:	Unknown	05 Sep 06	
Sample Collection Time:	Unknown	10:00	
Result Release Date:	01 Oct 02	19 Sep 06	
Dilutions of sera tested	Test 1	Test 1	
Normal Values $\leq 0.6\%$ cpm	Date: 27 Sep 02	Date: 15 Sep 06	
1:20	0.6	0.1	
1:50	0.3	0.1	
1:100	0.2	0.1	
1:1,000	0.1	0.1	
1:10,000	0.0	0.1	
1:20,000	0.0	0.1	
Overall Result	Borderline*	Negative	

A positive antibody is $\geq 0.9\%$ cpm in the above assay.A negative antibody is $\leq 0.6\%$ cpm in the above assay.

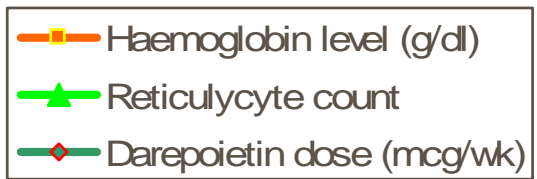
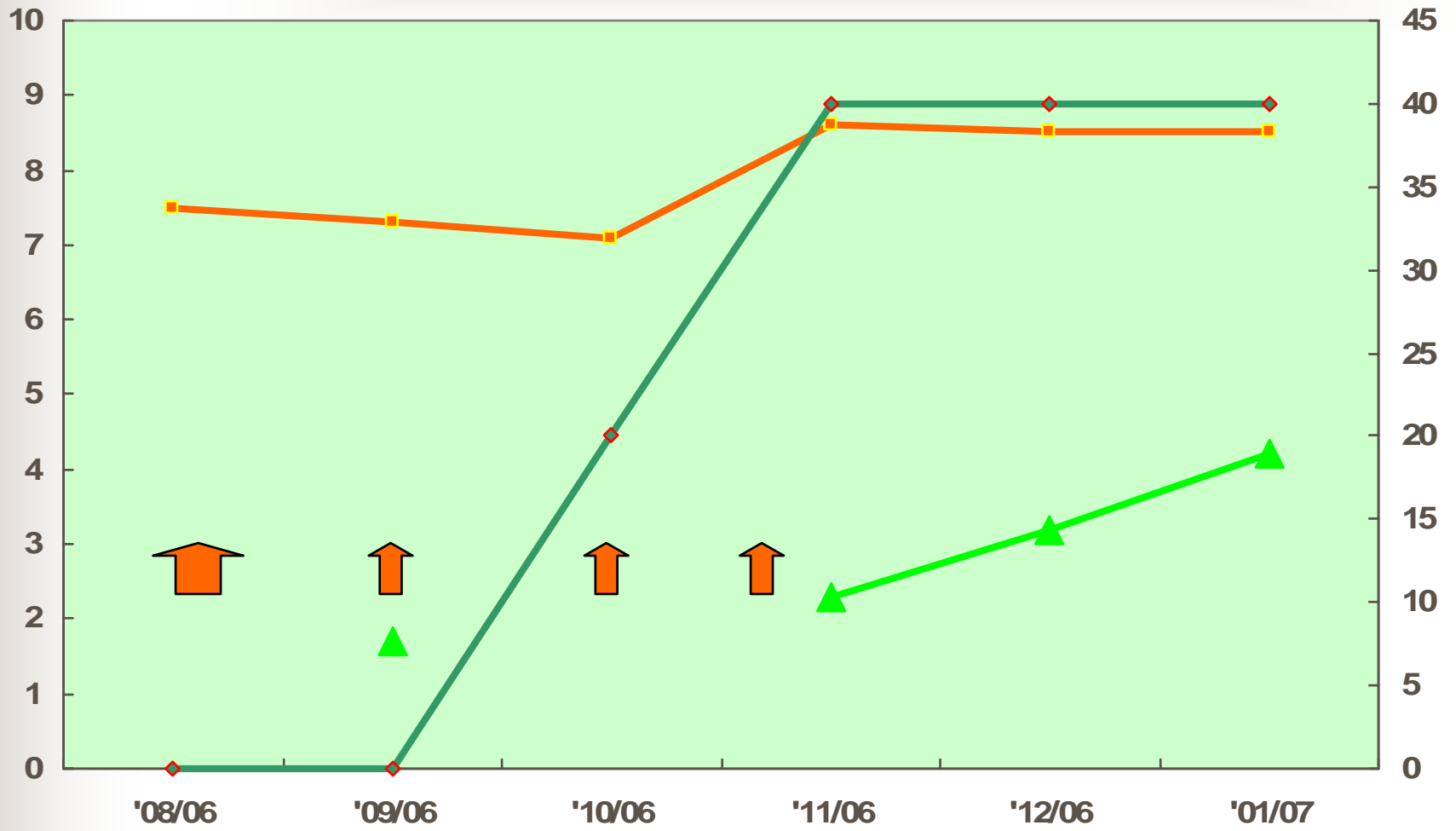
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 Blood transfusion

Anti-erythropoietin

antibody associated



PRCA

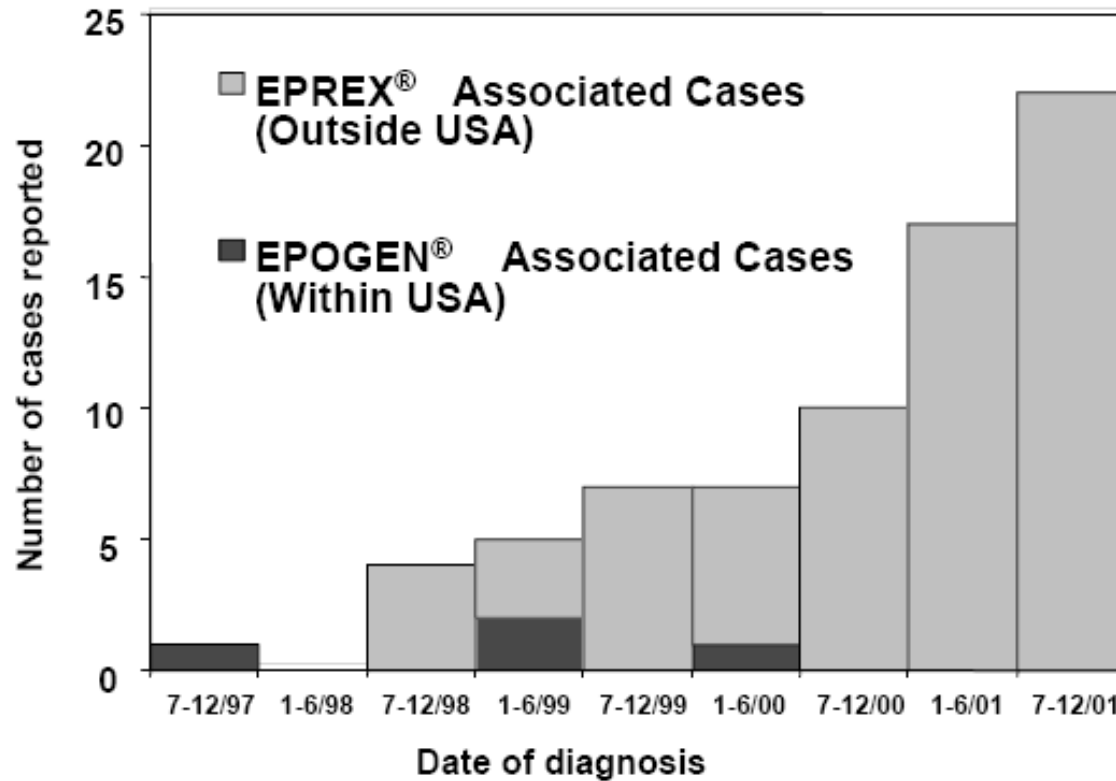


Observation

- Casadevall reported 13 possible cases in *NEJM* on Feb 2002
- FDA letter in *NEJM*, 141 possible cases
- Memo sent to doctors by the drug company concerned
- Erythropoietin alpha (Eprex) *s.c.* was announced to be contraindicated in renal failure patients in the same year

Increase in PRCA since 1999

Pure Red Cell Aplasia Among Recipients of Recombinant Erythropoietin, by Brand, as Reported to FDA



Gershon et al. *NEJM* 346:1584, 2002



Observation

- (Epo alpha; Eprex) launched 1989; Epo beta 1990; Darbepoietin alpha in 2001
- Upsurge of cases since 1998
- From 1998 to 2004, nearly 200 erythropoietin associated PRCA were reported
- Most cases reported are outside USA



Observation

- A change in formulation of Eprex since 1998 to comply with European concerns (removal of Human serum albumin, replaced by polysorbate 80)
- Change in package of pre-filled syringe: coated vs uncoated rubber stopper
- All Eprex cases utilized subcutaneous route of administration

Incidence

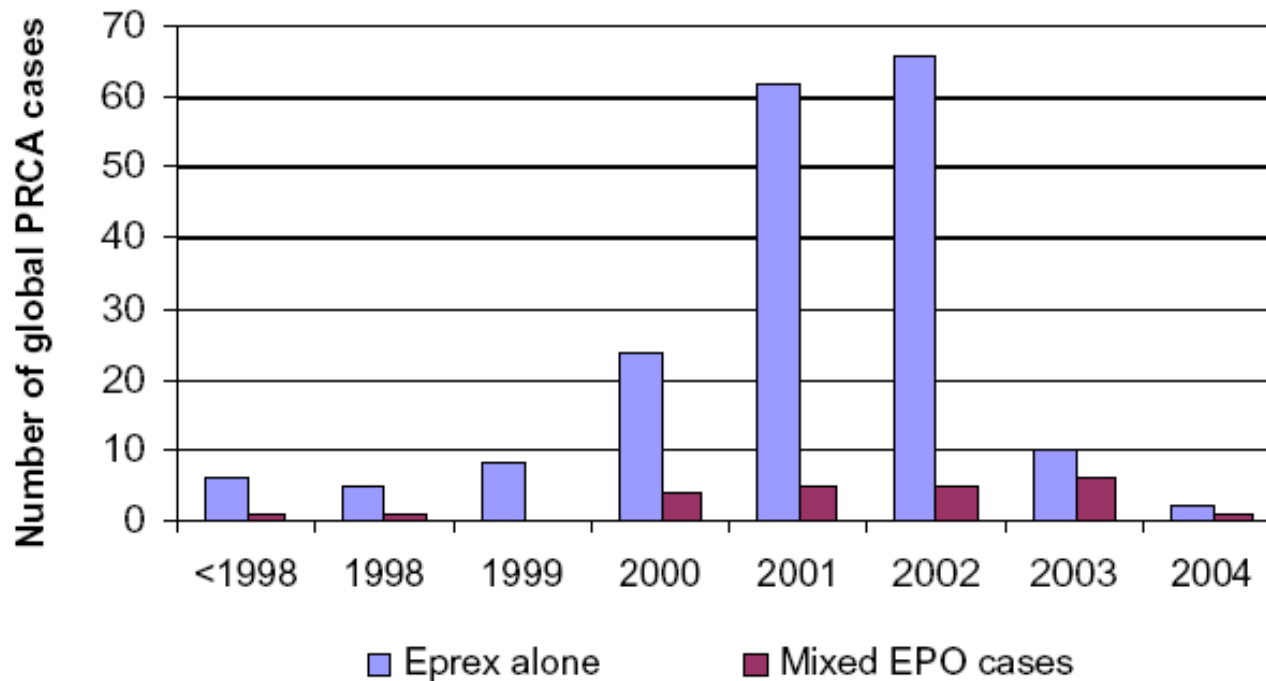


Fig. 1. Global case numbers of antibody-mediated PRCA from 1988 until August 31, 2004.

Incidence

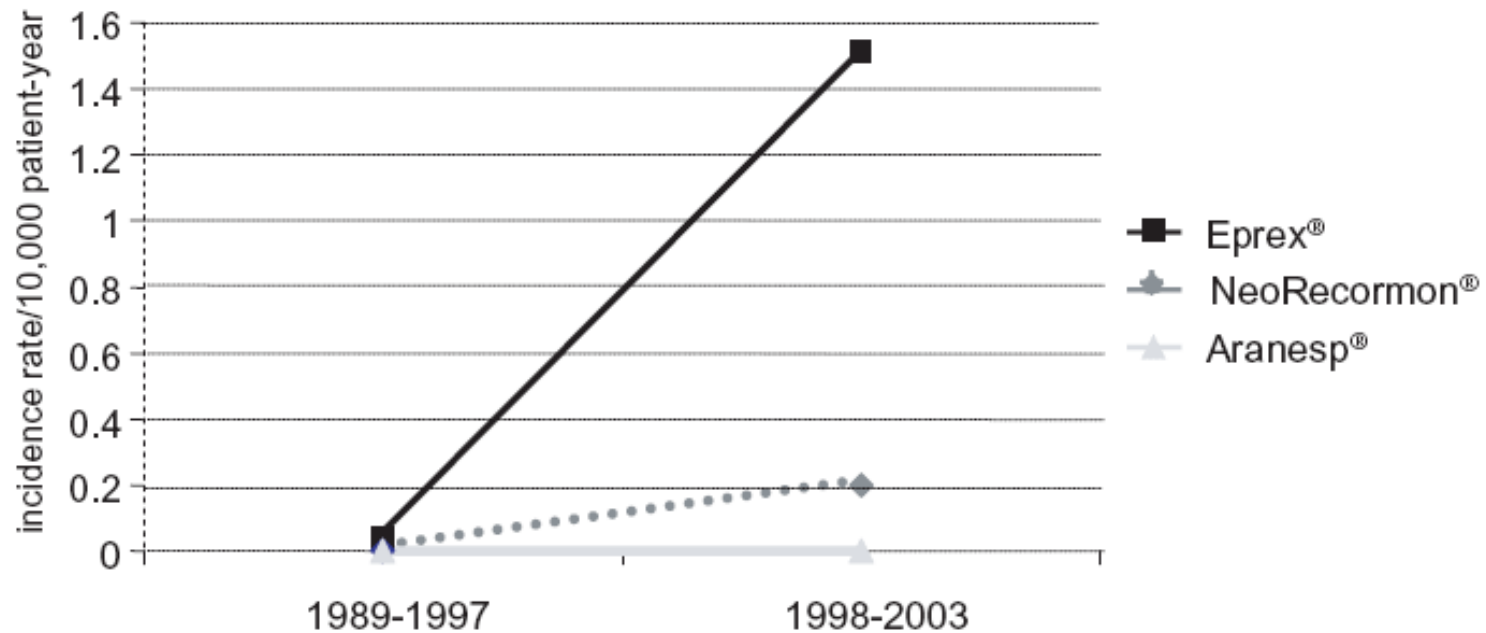


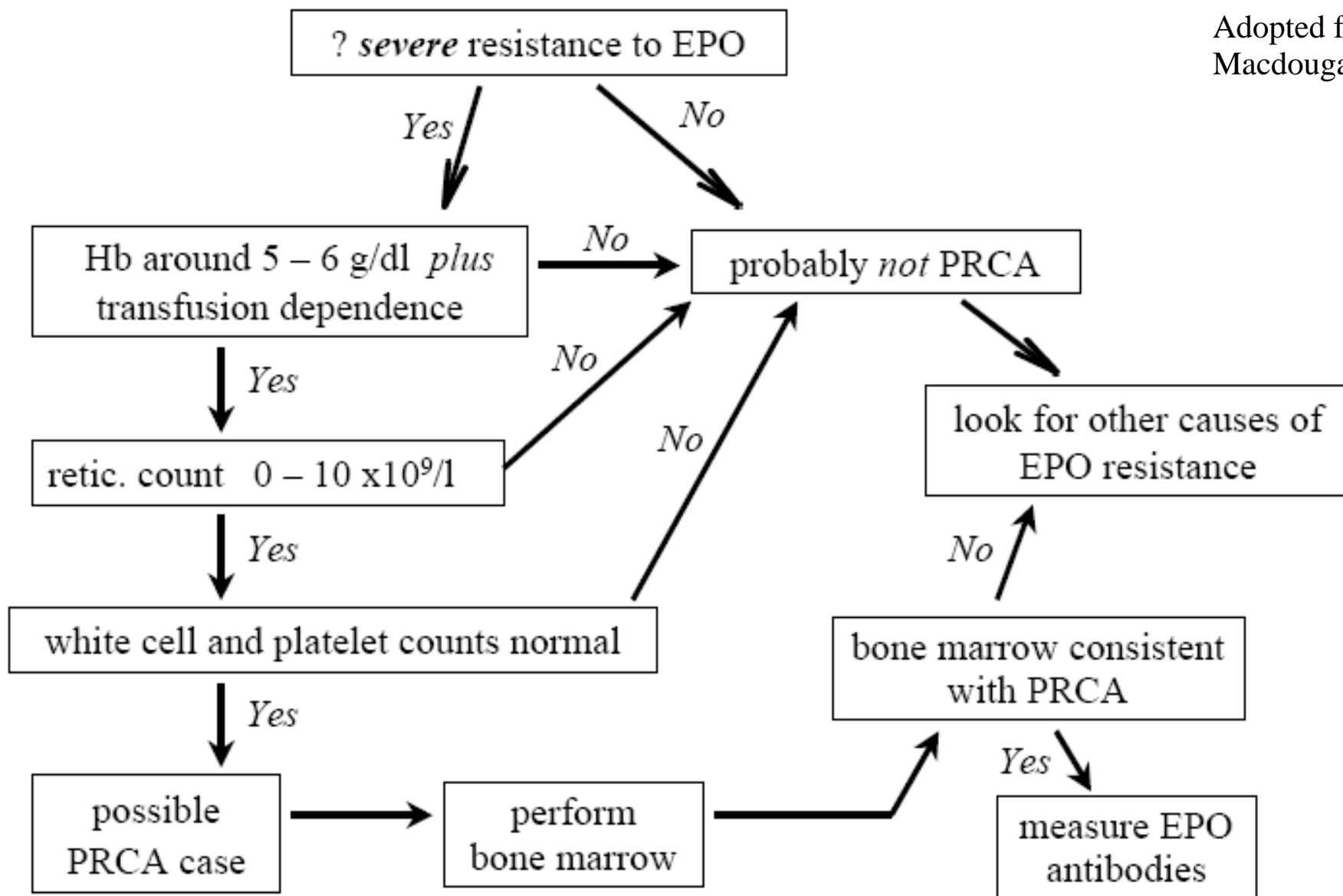
Fig. 2. Global incidence rate of antibody-mediated PRCA by product, 2001–2003 [9,18,19].

Diagnosis

- *Major criteria (each of the major criteria should be identified in all cases)*
 - Treatment with erythropoietin for at least 3 weeks
 - Drop of haemoglobin level of about 1g/L/day without transfusion or transfusion need of about 1 unit/week to keep haemoglobin level stable
 - Reticulocyte count less than $10 \times 10^9/L$
 - No major drop of white blood cell or platelet counts
- *Minor features*
 - Skin and systemic allergic features
- *Confirmational investigations*
 - Bone marrow aspirate with normal cellularity and less than 5% erythroblasts with evidence of maturation block
 - Serum assay shows presence of antierythropoietin antibodies and evidence of neutralizing ability

Suspected case of EPO-associated PRCA – clinical algorithm

Adopted from
Macdougall





Diagnosis

- Other causes of PRCA
 - Acute self-limited PRCA due to viral infections
 - Mumps, infectious mononucleosis, viral hepatitis, Parvovirus B19 infection
 - Acute self-limited PRCA due to drugs
 - Antiepileptics, azathioprine, chloramphenicol, sulfonamides, isoniazid , procainamide
 - Chronic parvovirus infection in immunocompromised subjects
 - Acquired chronic PRCA
 - Autoimmune disease like RA, SLE



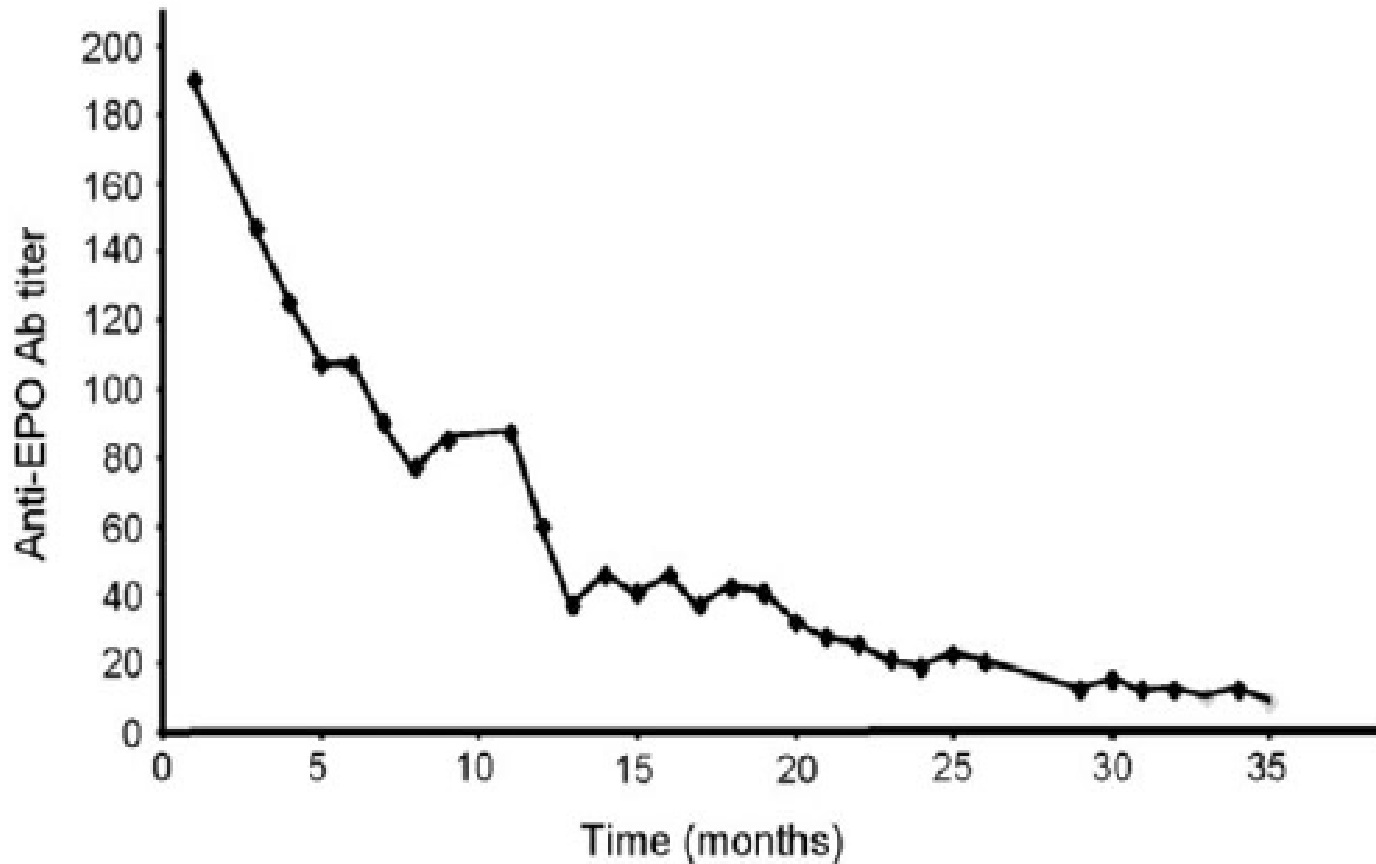
Treatment of anti-EPO PRCA

- EPO discontinuation
- Immunosuppressive therapy
 - Steroid + cyclophosphamide
 - Cyclosporin
- Kidney transplantation
- Rechallenge with EPO



EPO discontinuation

- Majority of cases did not respond to simple discontinuation
- Anti-EPO disappear slowly
- No recovery as long as the presence of anti-EPO



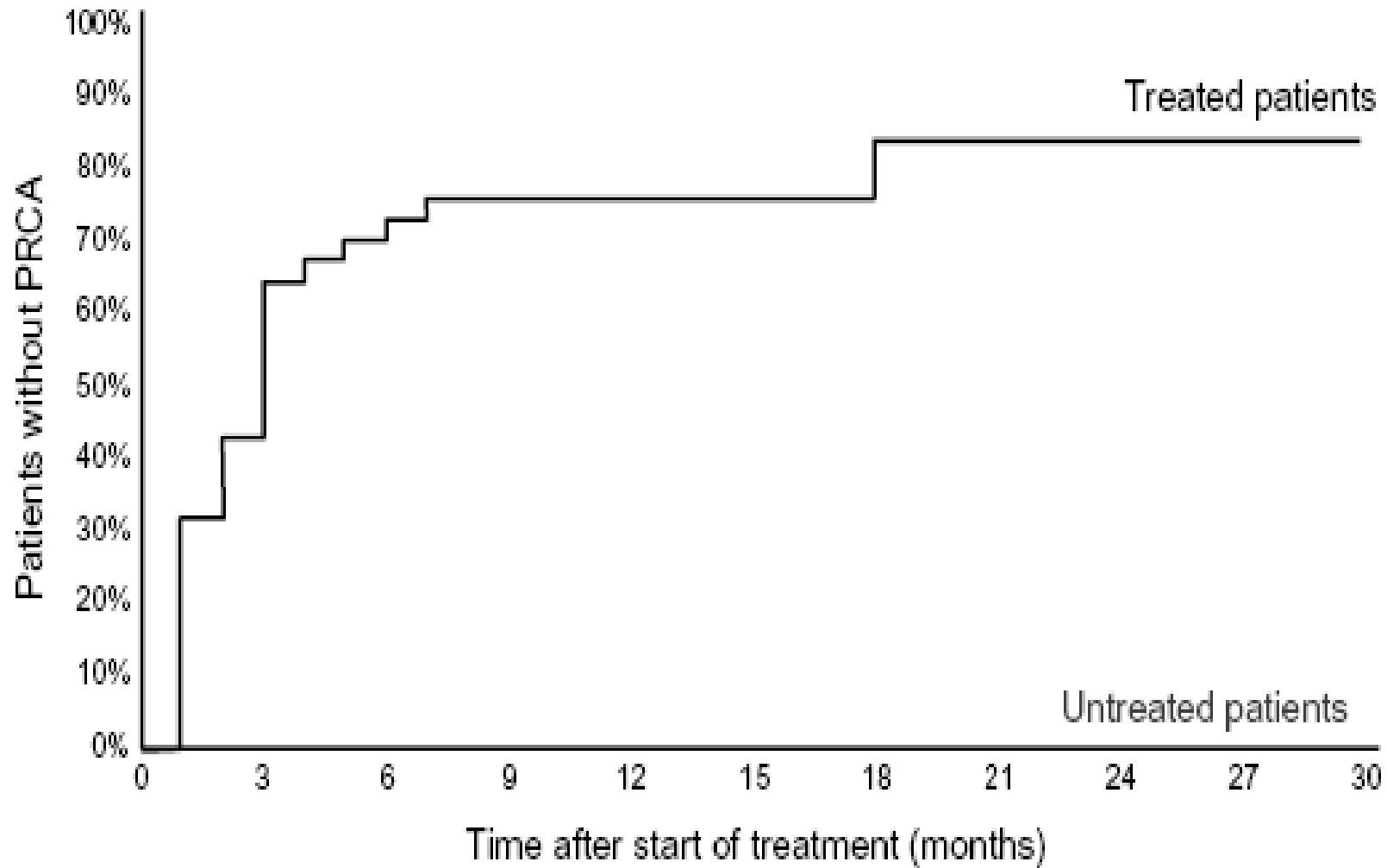
Protracted waning of anti-EPO Ab titres in a patient with Ab-mediated PRCA. Month 0 corresponds to the occurrence of PRCA

J. Rossert et al. NDT 2005



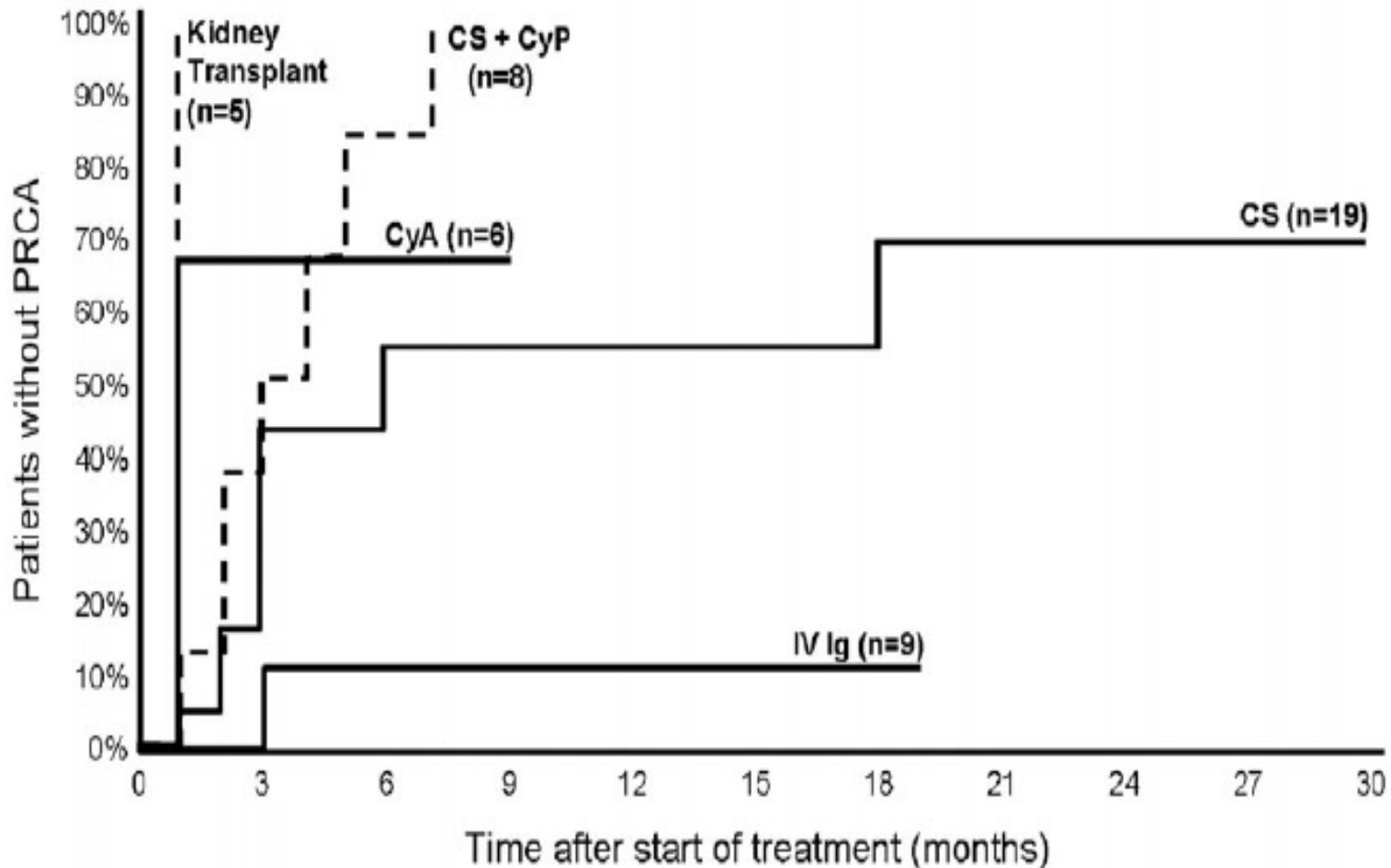
Immunosuppressive therapy

- Data available so far—retrospective case reports or case series only
- Appear more effective
- Immunosuppressants used
 - Corticosteroid alone
 - Corticosteroid + cyclophosphamide
 - Cyclosporin A
 - IVIG
 - Rituximab (anti-CD 20)
 - Difference combination



Rate of recovery in 10 patients with Ab-mediated PRCA who did not receive an immunosuppressive treatment and in 37 patients with Ab-mediated PRCA who were treated with immunosuppressive drugs.

Treatment of antibody-mediated PRCA



Rate of recovery following various immunosuppressive therapy regimens, in 37 patients with confirmed Ab-mediated PRCA. Some patients received more than one treatment
CS=corticosteroids; CyP=cyclophosphamide; CyA=cyclosporin A; IVIg=intravenous immunoglobulins

Treatment	No. of patients who recovered (%)
No therapy	0/10 (0%)
Rituximab (anti-CD20 mAb)	0/2 (0%)
Mycophenolate mofetil	0/1 (0%)
I.v. Ig	1/9 (11%)
Cs ± i.v. Ig	10/18 (58%)
CyA	4/6 (67%)
Cs + i.v. Ig + plasmapheresis	1/1 (100%)
Cs + cyclophosphamide	7/8 (87%)
Kidney transplant	6/6 (100%)

Cs = corticosteroids; CyA = cyclosporine A; IVIg = intravenous immunoglobulins; mAb = monoclonal antibody.

Outcome of immunosuppressive therapy, in a series of 37 patients with Ab-mediated PRCA

<u>Immunosuppressants</u>	<u>Dosage</u>	<u>Route</u>	<u>Recovery, %</u>
CyP + Pred	50-100mg/day + 1mg/kg/day	oral	87
Cyclosporine	100mg bd or 5-8mg/kg/day	oral	67
Pred	1mg/kg/day	oral	56
IVIg	2g/kg over 2-5 days	IV	11

Haemoglobin level, reticulocyte counts, and transfusion interval should be monitored over a 4-8 weeks interval. If no haematologic response occurs within 3-4 months with initial therapy, a therapeutic trial of a second-line therapy should be considered

Recovery rates are based on long-term follow-up reported by the European PRCA Study Group for 47 patients with complete follow-up data

Immunosuppressive regimens used for treatment of epoetin-associated pure red cell aplasia
Bennet et al BLOOD 2005



Kidney transplantation

- Almost all Kidney transplant recipient recovered from Ab-mediated PRCA
- ? due to immunosuppressants used during and after transplantation
- ? due to small antigenic differences between endogenous EPO and recombinant EPO

rHuEPO Rechallenge

- Rossert, Macdougall and Casadevall summarised 8 case reports which showed successful outcome in EPO rechallenge after **recovery** from Epo-associated PRCA

No. of patients	Immunosuppressants used	EPO of rechallenge
2	CSA	Darbepoetin alpha IV
1	CSA	Epoetin beta SC
1	anti-CD20	Epoetin alpha IV
1	CS + CyP	Epoetin alpha IV
1	CS + CyP	Epoetin beta IV
1	CS	Epoetin alpha IV
1	CS	Epoetin beta SC



The RADAR Project

Outcome study of individuals with anti-EPO antibodies from the PRCA;
Research on Adverse Drug Events and Reports (RADAR) Blood 2005

- Subjects:
 - All reported cases from Jan 1998 to Apr 2004 to the authority
 - Include both Eporex and Recormon
- 191 patients world wide, 170 had at least 3 months follow up

Table 2. Epoetin rechallenge cases (n = 34 individuals) and development of epoetin responsiveness

Case no. ^a	RIPA or ELISA antibody status at rechallenge	RIPA or ELISA antibody assay	Concomitant immunosuppression	Neutralizing assay results at time of rechallenge	Epoetin responsiveness
Group 1					
1	-	RIPA	+	Unknown	+
2	-	RIPA	+	-	+
3	-	RIPA	+	-	+
4	-	RIPA	-	-	+
5	-	ELISA	-	Unknown	-
6	-	ELISA	-	-	+
7	-	Unknown	+	Unknown	+
8	-	Unknown	+	Unknown	+
9	-	Unknown	-	Unknown	+
Group 2					
10	+	RIPA	+	Unknown	-
11	+	RIPA	+	Unknown	-
12	+	RIPA	+	Unknown	-
13	+	RIPA	+	Unknown	+
14	+	RIPA	+	Unknown	+
15	+	RIPA	+	-	+
16	+	RIPA	+	-	+
17	+	ELISA	+	Unknown	-
18	+	Unknown	+	Unknown	+
19	+	Unknown	+	Unknown	+
20	+	Unknown	+	Unknown	+
21	+	Unknown	+	Unknown	-
22	+	Unknown	+	-	+
23	+	Unknown	+	+	-
Group 3					
24	+	RIPA	-	Unknown	-
25	+	RIPA	-	Unknown	-
26	+	RIPA	-	Unknown	-
27	+	RIPA	-	Unknown	-
28	+	RIPA	-	Unknown	-
29	+	RIPA	-	Unknown	-
30	+	RIPA	-	Unknown	-
31	+	RIPA	-	Unknown	+
32	+	RIPA	-	Unknown	+
33	+	RIPA	-	-	+
34	+	ELISA	-	Unknown	-

The 3 groups were defined as follows: Group 1, no evidence of antierythropoietin ELISA or RIPA antibody at the time of rechallenge; Group 2, antibodies detected and concomitant immunosuppression was administered; Group 3, antibodies detected and concomitant immunosuppression was not administered.

^aThe percentage epoetin responsive were 89% for Group 1, 57% for Group 2, and 27% for Group 3.

Median follow up of 9 months



The RADAR Project

Outcome study of individuals with anti-EPO antibodies from the PRCA;
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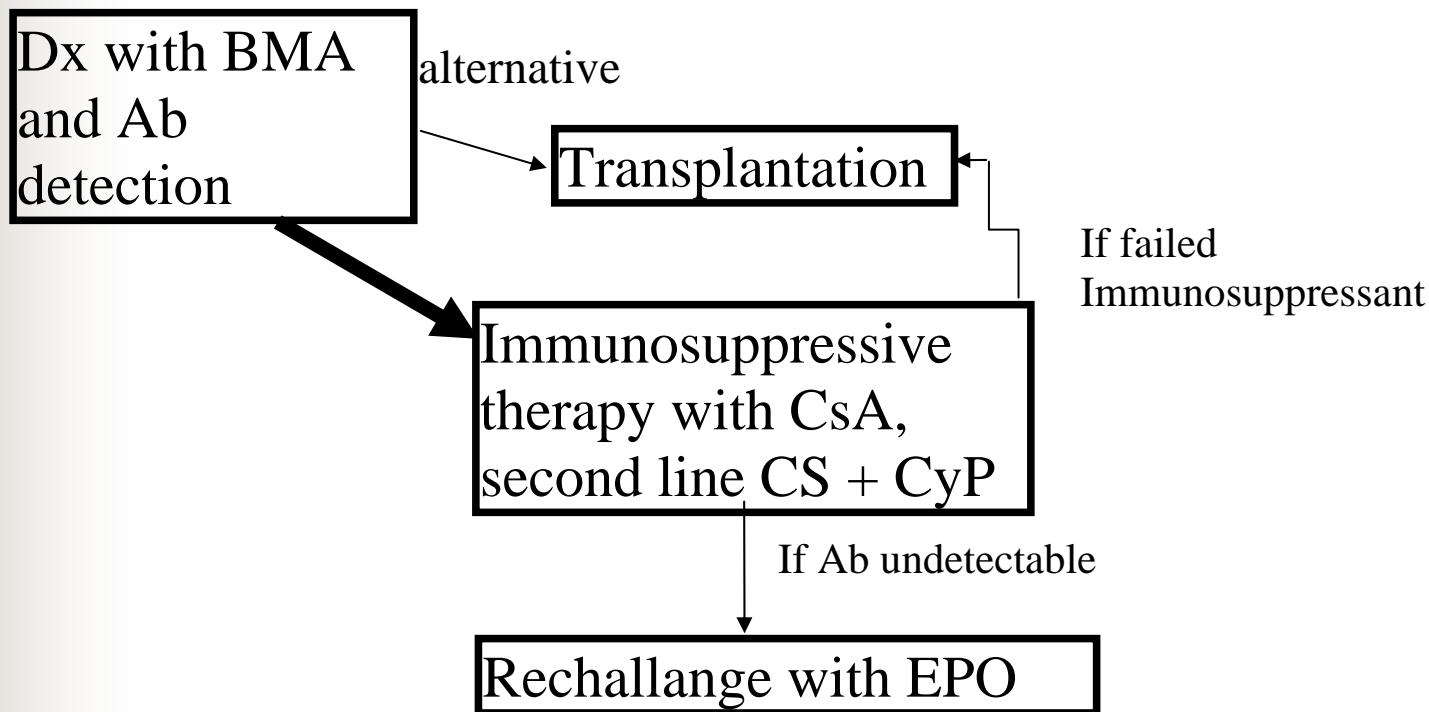
- 34 received rechallenge of EPO after onset of PRCA, response rate are:
 - Group 1 (no antibody): 89%
 - Group 2 (presence of antibody, concomitant immunosuppressants): 57%
 - Group 3 (presence of antibody, no immunosuppressants): 27%
- Those who failed rechallenge may receive another form of immunosuppressive therapy to clear anti-EPO antibody



rHuEPO Rechallenge

- Possibility of publication bias in individual case reports
- Difficult to conduct randomized trial due to limited number of patients in each centre
- Difficult to compare effectiveness on different preparation of EPO
- Possibility of cross-reactivity should raise serious concern, causing severe immune response

Proposed treatment protocol for anti-EPO PRCA





Questions to be answered...

- Follow-up period of 4 months after rechallenge with EPO; will the patient develop another episode of Antibody associated PRCA?
- Route of Injection; can the route be changed to S.C. for this patient in the future?



Looking forward...

- Further study on the susceptibility of this condition
- Further follow up whether rechallenge of EPO could induce another episode of ab-associated PRCA
- To compare effectiveness of different preparations of EPO during rechallenge study



Thank you

Case report from ASARI and GOKAL J Am Soc Nephrol 2004

■ Started Darbepoetin since Nov 2001

Table 3. Aranesp dose and haemoglobin response in spite of persistence of EPO antibodies and without the need of blood transfusions

Date	08/11/01	03/01/02	18/02/02	19/06/02	22/07/02	23/08/02	16/10/02
Aranesp mcg/wk	40	40	40	40	20	20	30
Hb g/dl	9.4	10.7	9.7	14.3	15.2	14.4	13.1
EPO antibodies	—	—	positive	positive	positive	—	—
Transfusion	Yes	No	No	No	No	No	No

Table 4. The levels of anti-EPO antibodies from patient's serum to different EPO preparations

Collection Date	Anti-Eprex	Anti-EPOGEN	Anti-Aranesp	Anti-Neorecornm
06/18/01	0.72 mcrg/ml	0.87 mcrg/ml	0.61 mcrg/ml	0.68 mcrg/ml
09/17/01	0.54 mcrg/ml	0.60 mcrg/ml	0.41 mcrg/ml	0.49 mcrg/ml
02/18/02	0.30 mcrg/ml	0.38 mcrg/ml	0.20 mcrg/ml	0.25 mcrg/ml
05/27/02	0.21 mcrg/ml	0.26 mcrg/ml	NEGATIVE	0.14 mcrg/ml
10/07/02	0.19 mcrg/ml	0.25 mcrg/ml	0.12 mcrg/ml	0.17 mcrg/ml



Case report from ASARI and GOKAL J Am Soc Nephrol 2004

■ Conclusion

- Cross reactivity present among different preparations of EPO
- Systemic reaction and effectiveness may depends on the level of antibodies
- Trial of Darbepoetin may be worthwhile