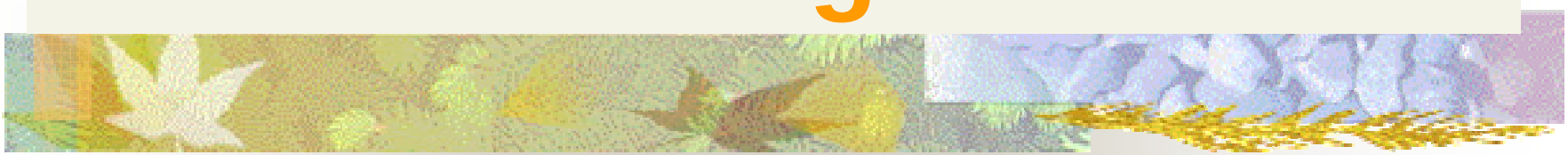
Erythropoietin

cure or nightmare



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

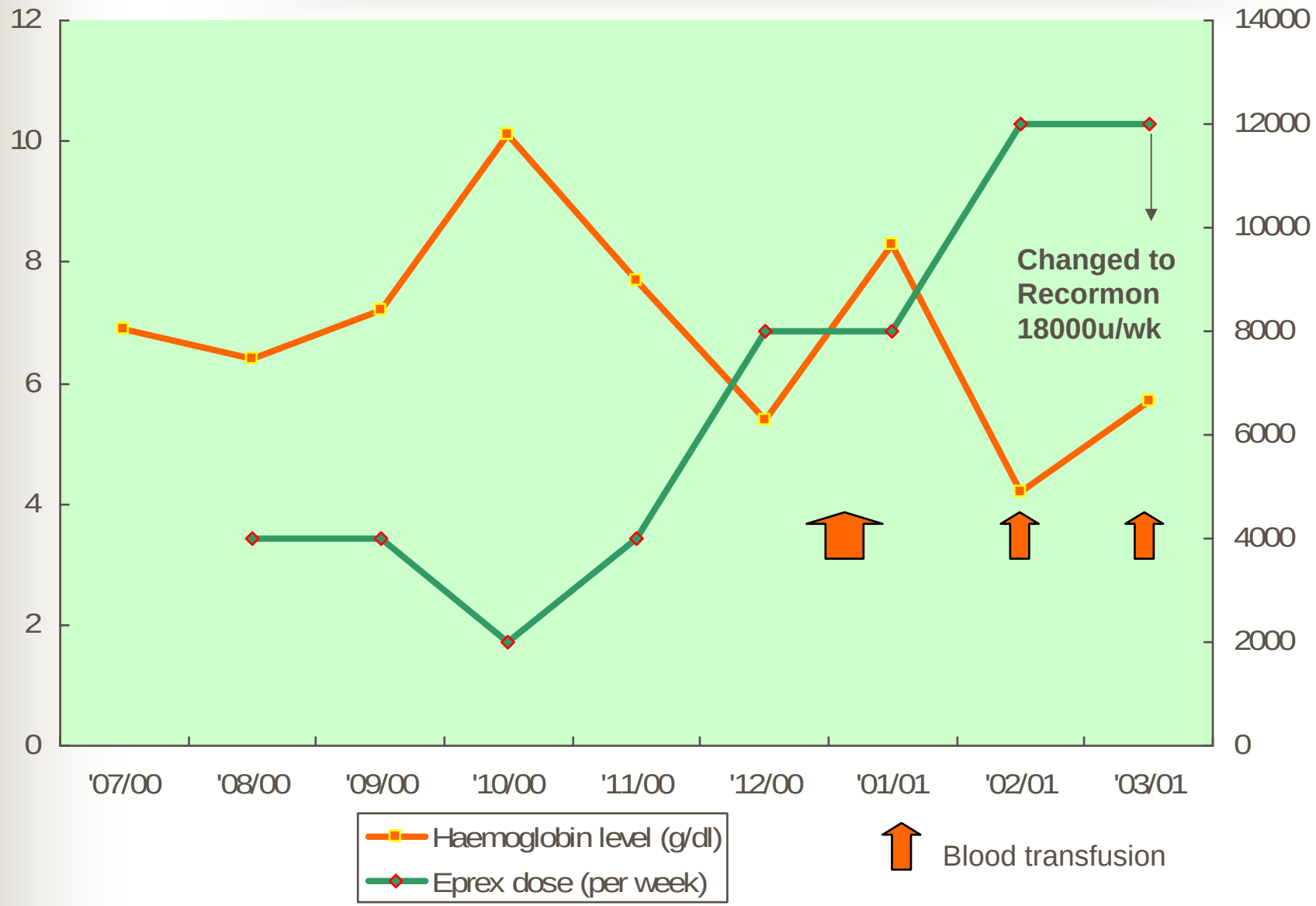
PYNEH

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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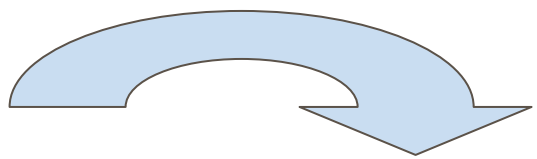
Changed to
Recormon
18000u/wk
Haemoglobin level (g/dl)
Eprex dose (per week)
Blood transfusion

Epo resistance

- Major causes:
  - Iron deficiency
  - Chronic inflammation/infection
  - Underdialysis
- Minor causes:
  - Hyperparathyroidism
  - Aluminium toxicity
  - Vit B12/folate deficiency
  - Haemolysis
  - Blood loss

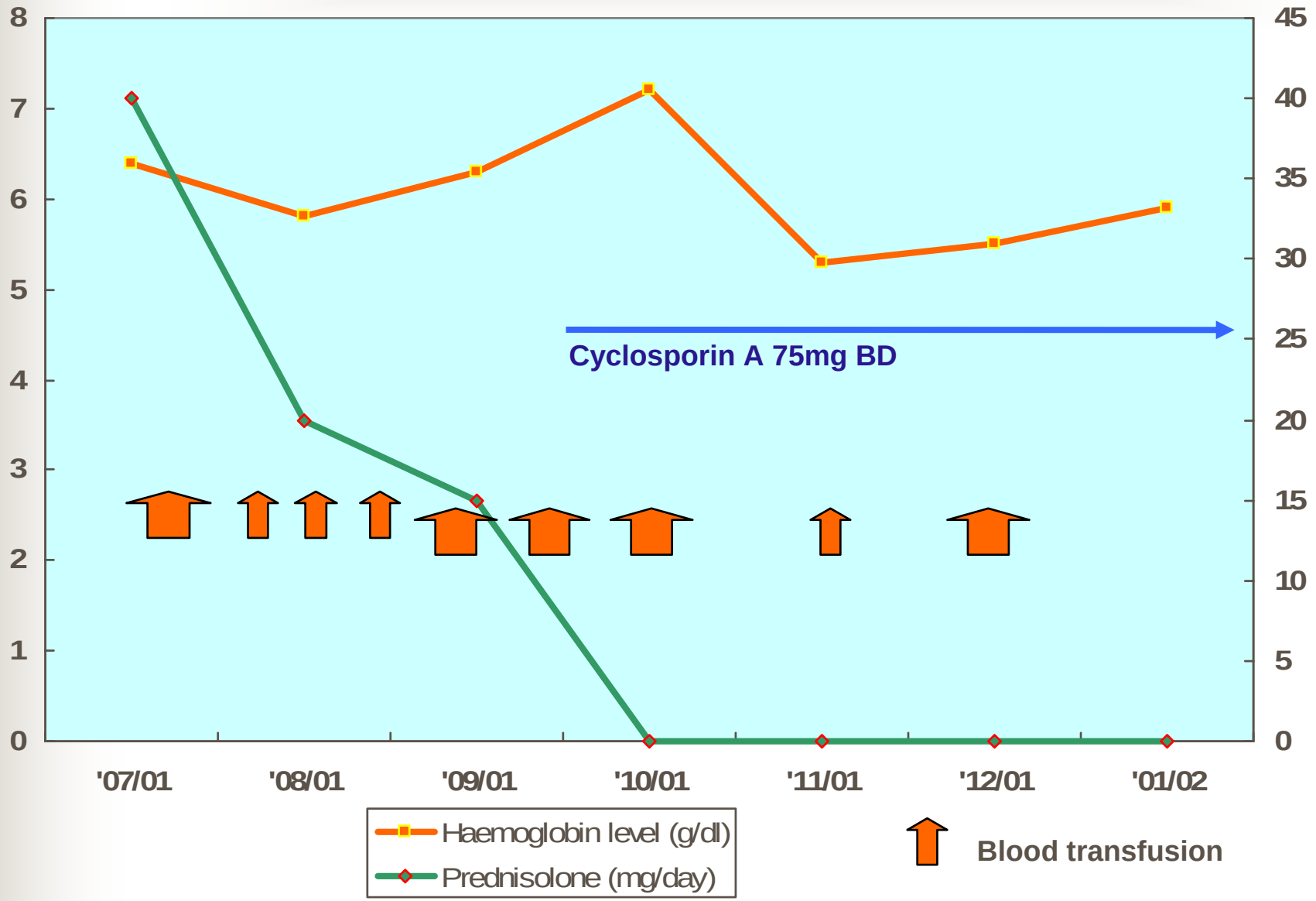
- Further work up
  - Feb 01 OGD Esophagitis
  - Mar 01 Blood test C3, ANA normal, CRP slightly elevated
  - Mar 01 reti count <1.0%
  - Mar 01 BMA Inadequate tissue
  - Apr 01 Colonoscopy NAD
  - May 01 Ba enema 1cm Diverticulum
  - May 01 US abdomen gall stone
  - May 01 KT/V 2.3
  - Nov 01 CT thorax 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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Cyclosporin A 75mg BD
Haemoglobin level (g/dl)
Prednisolone (mg/day)
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 Normal Values ≤0.6% cpm	Test 1 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 ≥0.9% cpm in the above assay.
A negative antibody is ≤0.6% cpm in the above assay.
Results between positive and negative are borderline.
N/A = not applicable
*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

- 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

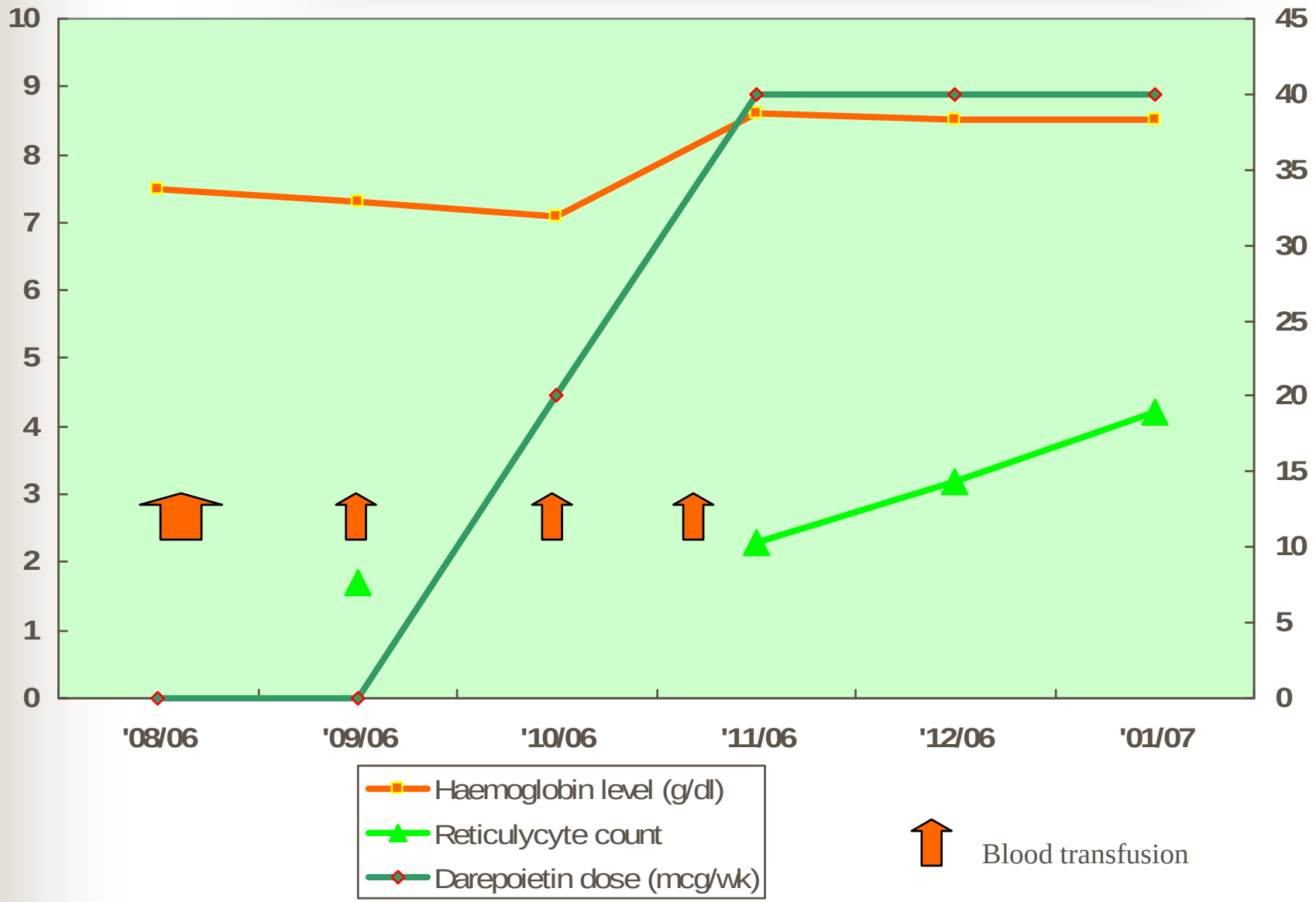
RESULTS OF CIRCULATING EPO CONCENTRATION (ELISA Testing Method)

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 Normal Values ≤0.6% cpm	Test 1 Date: 27 Sep 02	Test 1 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 ≥0.9% cpm in the above assay.
A negative antibody is ≤0.6% cpm in the above assay.
Results between positive and negative are borderline.
N/A = not applicable
*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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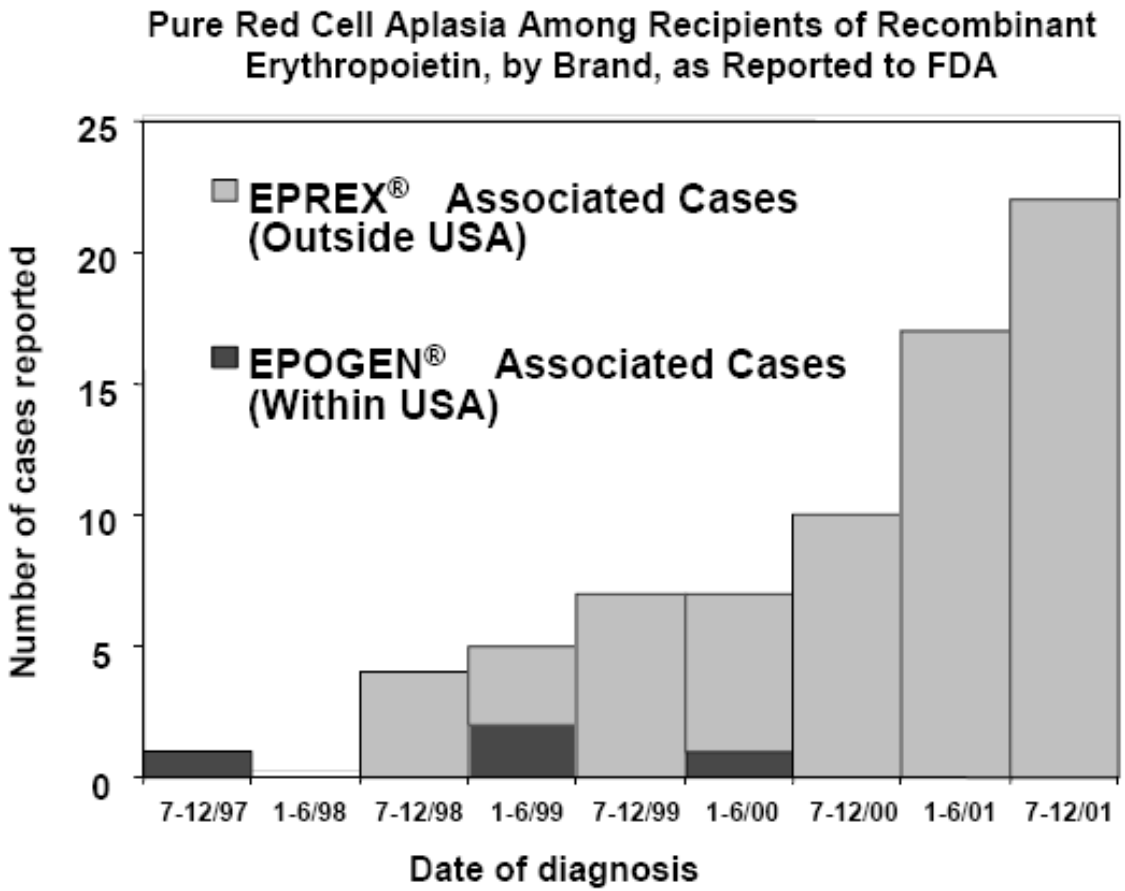
Haemoglobin level (g/dl)
Reticulycyte count
Darepoietin dose (mcg/wk)
Blood transfusion

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



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 assoicated 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 subcutanous route of administration

Incidence

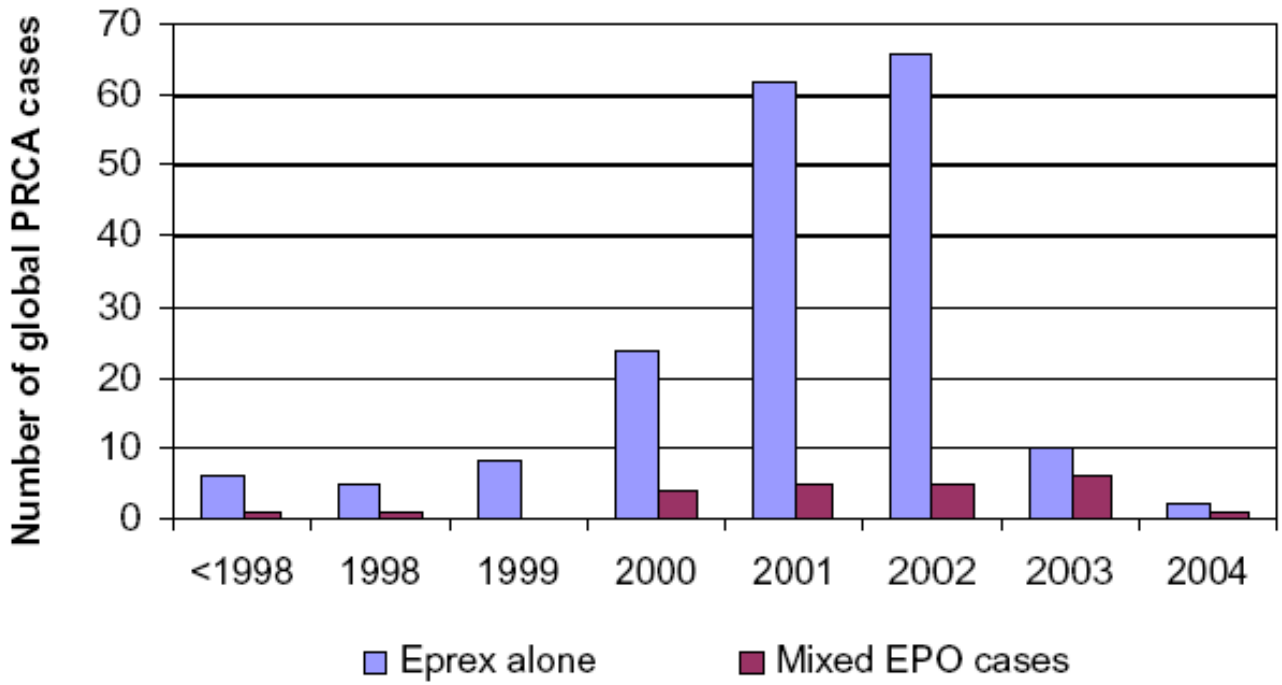


Fig. 1. Global case numbers of antibody-mediated PRCA from 1988 until August 31, 2004.

Ian Macdougall NDT 2005

Incidence

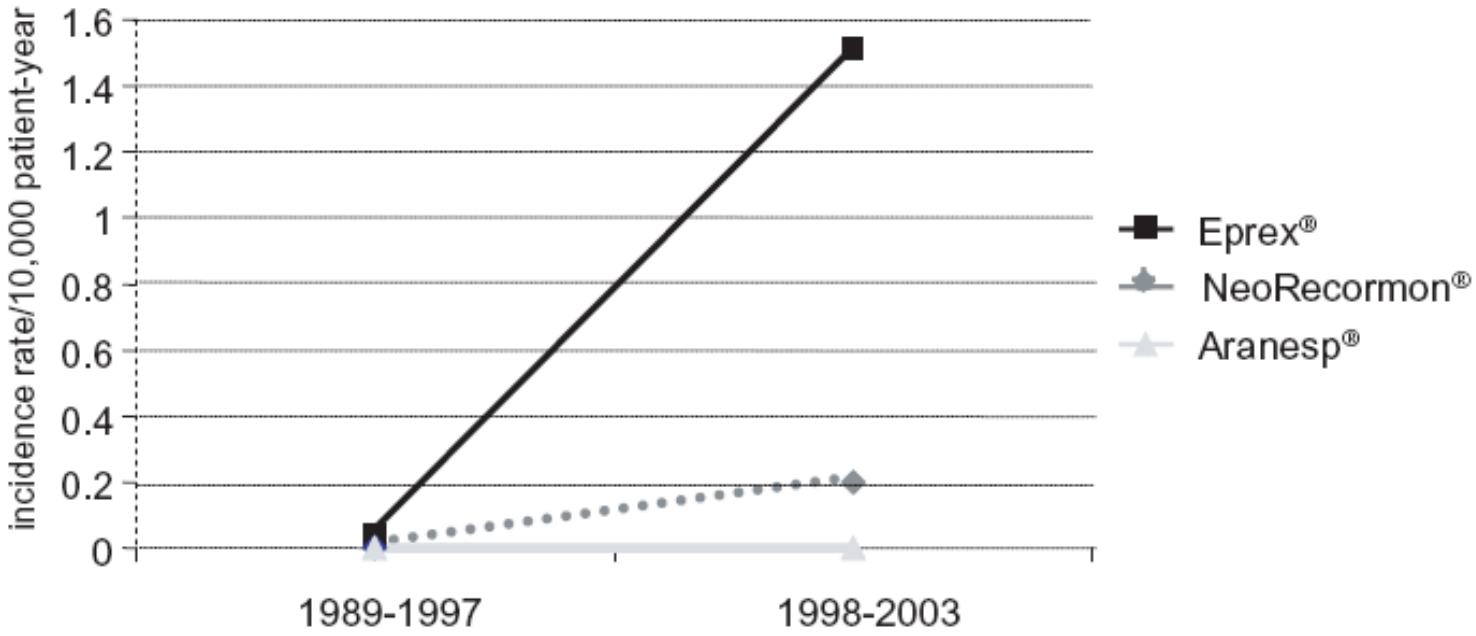


Fig. 2. Global incidence rate of antibody-mediated PRCA by product, 2001–2003 [9,18,19].

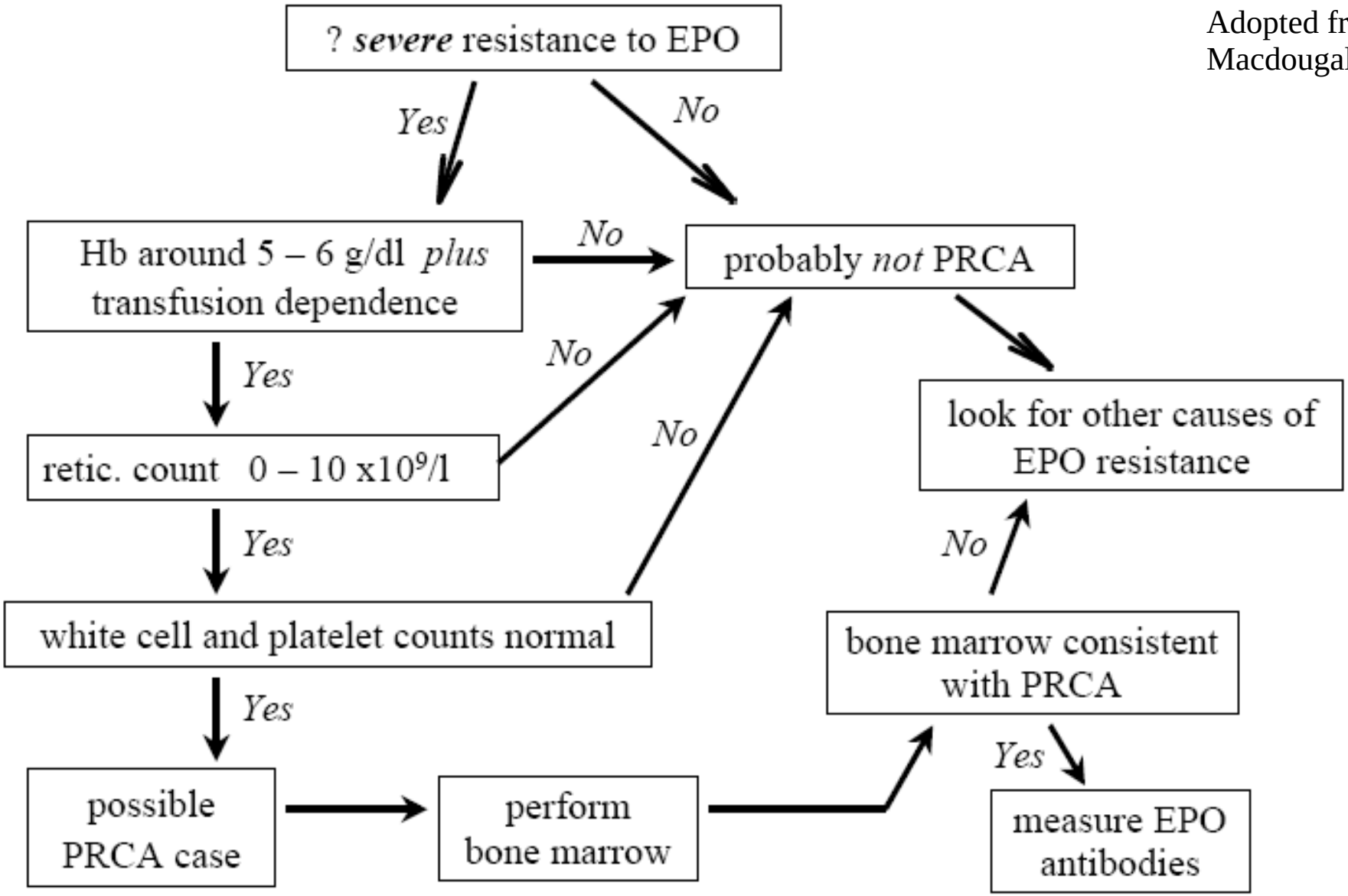
Ian Macdougall NDT 2005

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 $10x10^9/L$
  - 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 neurtralizing ability

Casadevall at al Eur J Haematol. 2004

Suspected case of EPO-associated PRCA – clinical algorithm



Adopted from Macdougall

Diagnosis

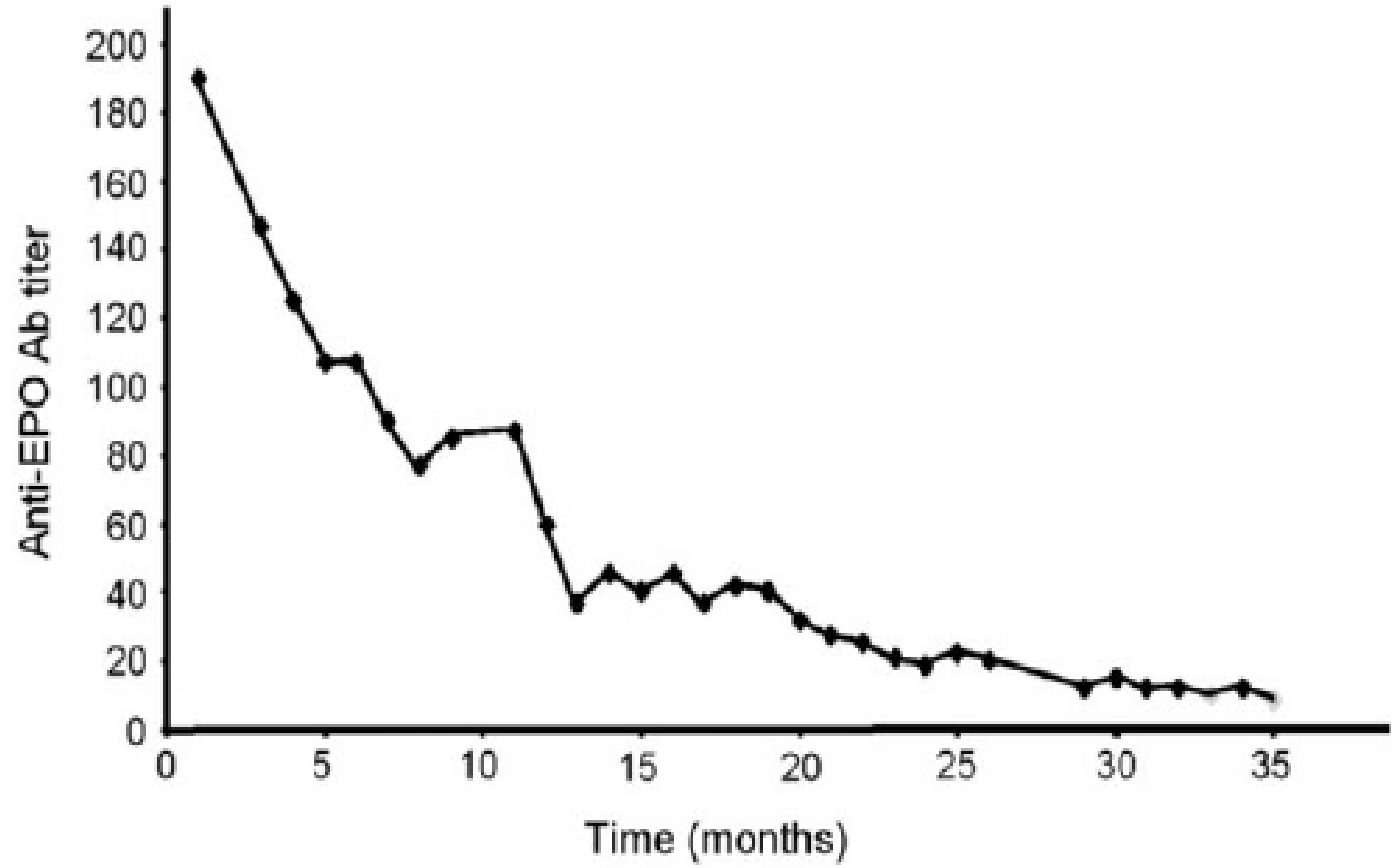
- Other causes of PRCA
  - Acute self-limited PRCA due to viral infections
    - Mumps, infectious mononucleosis, viral hepatits, Parvovirus B19 infection
  - Acute self-limited PRCA due to drugs
    - Antiepileptics, azathioprine, chloramphenicol, sulfonamides, isoniazid , procainamide
  - Chronic parvovirus 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

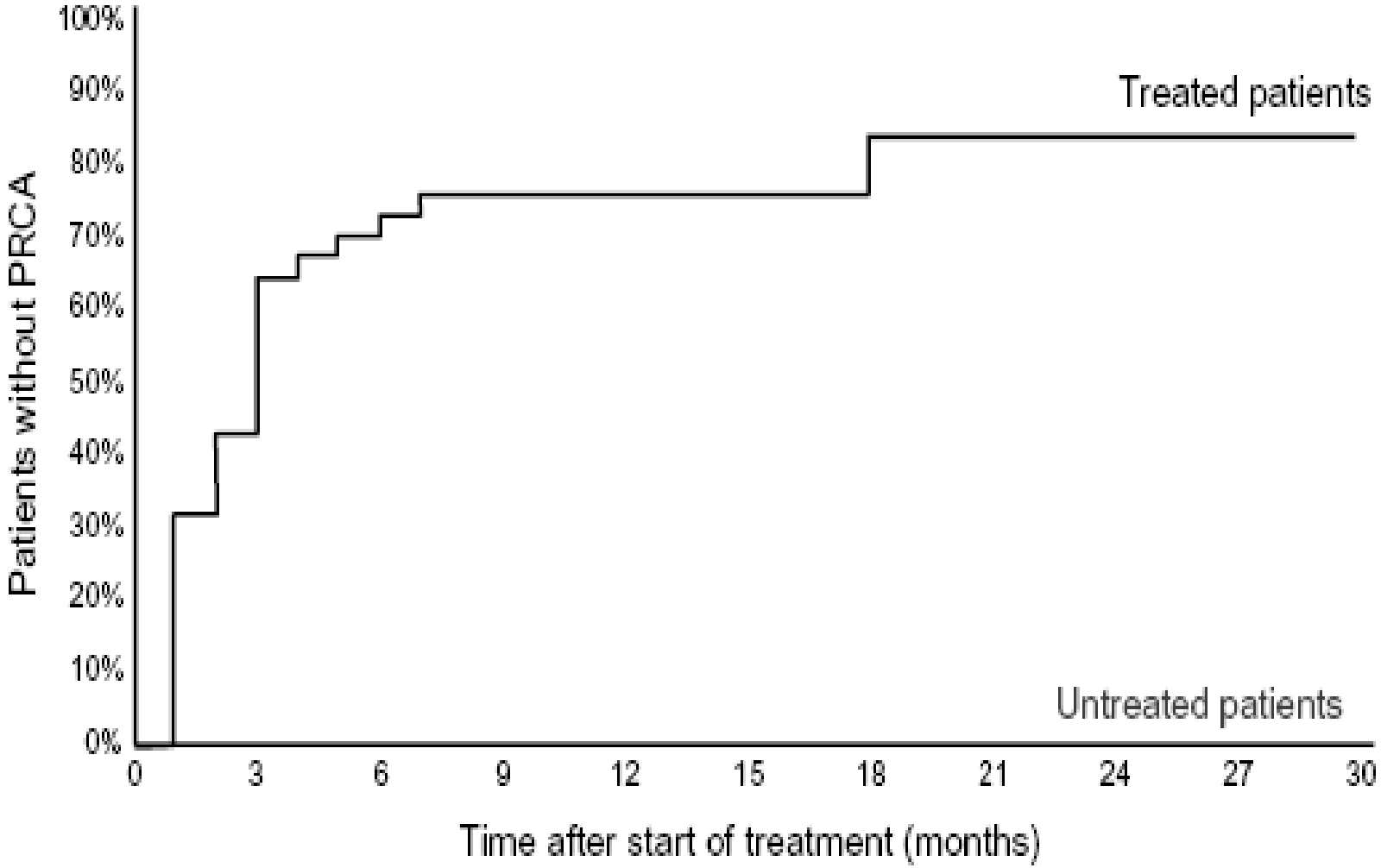


Protracted waning of anti-EPO Ab titres in a patient with Ab-mediated PRCA. Month 0 cooresponds to the occurrnce o PRCA

J. Rossert et al. NDT 2005

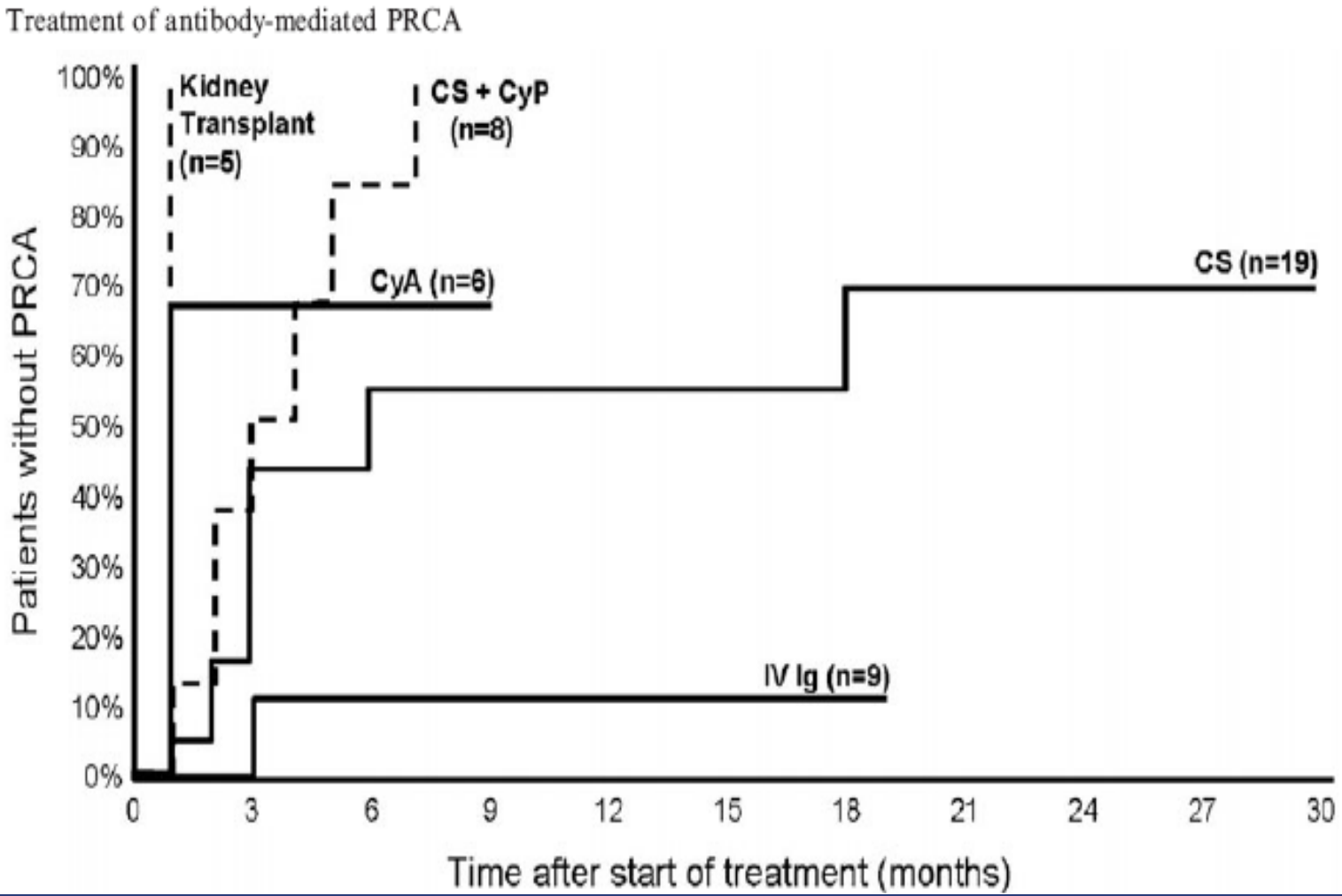
Immunosuppressive therapy

- Data available so far—retrospective case reports or case series only
- Appear more effective
- Immunosuppresants used
  - Cortocosteroid 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 treatement and in 37 patients with Ab-mediated PRCA who were treted with immnosupressive drugs.

Verhelst et al Lancet 2004



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

Verhelst et al Lancet 2004

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

Verhelst et al Lancet 2004

Immunosuppressants	Dosage	Route	Recovery, %
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

Immunosupppressive 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 rechallange 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 Eprex 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.*	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.

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

Median follow up of 9 months

Outcome study in RADAR Bennet et al BLOOD 2005

The RADAR Project

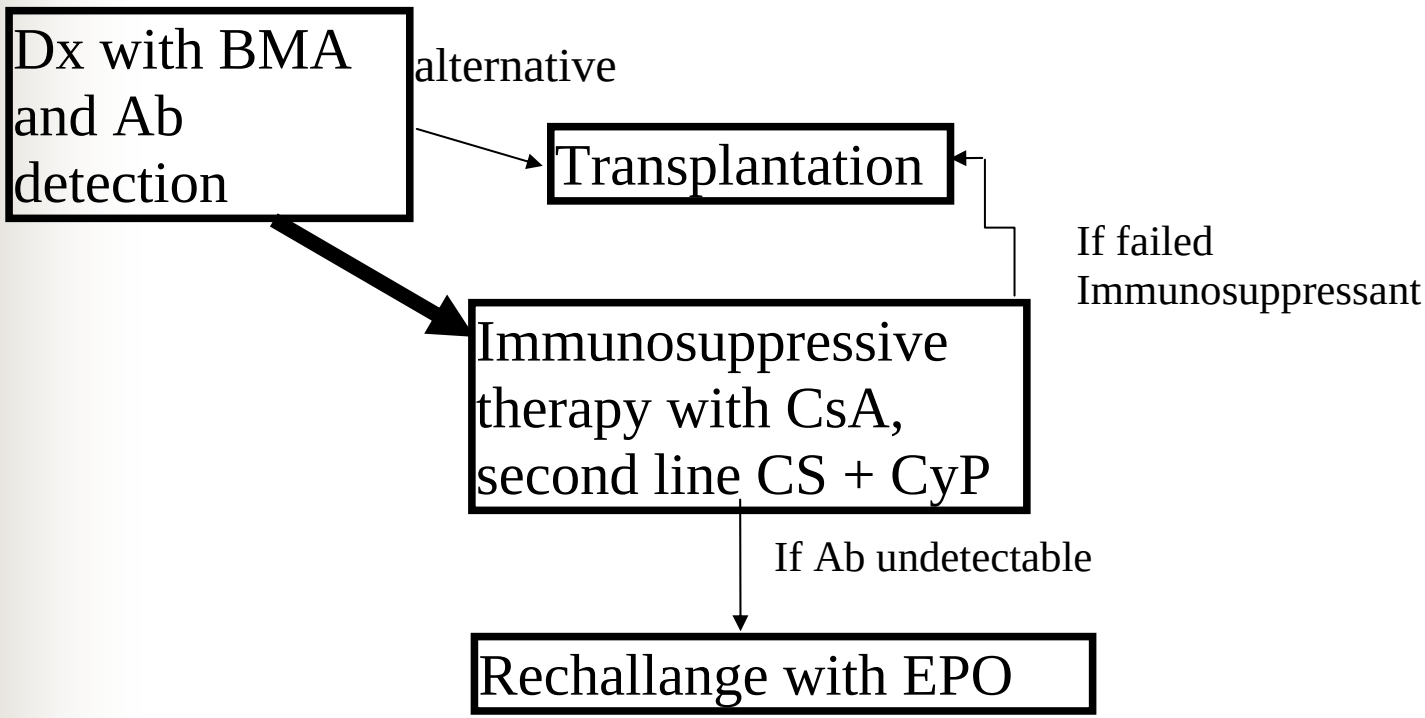
Outcome study of individuals with anti-EPO antibodies from the PRCA; Research on Adverse Drug Events and Reports (RADAR) Blood 2005

- 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

- 82 yr old retired medical practitioner
- ESRF on CAPD, with anaemia Hb 8g/dl
- Eprex s.c. since Sep 1998

Table 1. Table 1 shows good initial response to EPREX

Date	01/98	03/98	06/98	09/98	12/98	05/99	08/99	11/99	12/99
EPO dose IU/week	No	6000	6000	3000	3000	3000	4000	8000	12000
Hb g/dl	8.0	9.0	12.3	14.2	13.2	12.6	11.5	6.6	8.1
EPO antibodies								positive	positive
Transfusion	No	No	No	No	No	No	No	Yes	Yes

Table 2. Table 2 showing EPO resistance and Blood Transfusion Dependency

Date	Jan/00	Apr/00	Aug/00	Oct/00	June/01	July/01	Sept/01	Nov/01
EPO dose IU/week	3000	No	No	No	No	No	No	Aranesp
Hb g/dl	6.5	7.6	8.4	7.9	9.0	8.6	9.4	9.2
EPO antibodies	—	positive	—	—	positive	—	positive	—
Transfusion	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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