Professor Kearkiat Praditpornsilpa, MD, Thailand

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Clinician's experience of biocopy EPO in Thailand

Professor Kearkiat Praditpornsilpa, MD

23 July 2017

Clinician's Experience of Biocopy EPO in Thailand

Kearkiat Praditpornsilpa MD

Division of Nephrology, Chulalongkorn University,
King Chulalongkorn Memorial Hospital, Bangkok, Thailand

Biotherapeutic/Biopharmaceutical Agents/Biologics

- **Biosimilars**
- **Follow-on biologics**
- **Subsequence entry biologics**
- **Intended copy product**

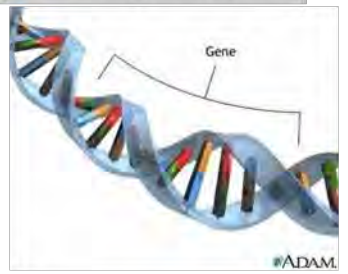
- **Innovator Products**

Highly regulated market
VS
Less regulated market or Non-regulated market

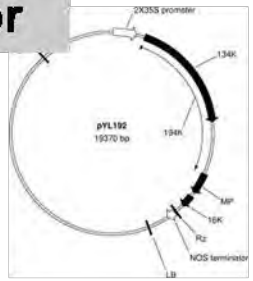
- Biocopy Products

Complexity of Biotherapeutic Products Manufacturing

1) Genetic sequence



2) DNA vector

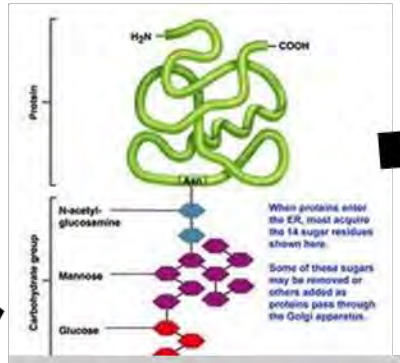


3) Recombinant cell system

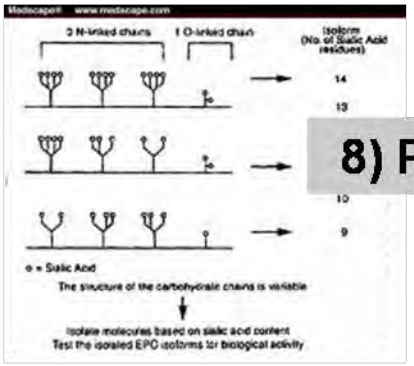
4) Fermentation condition

5) Purification protocol

6) Therapeutic protein product



7) Formulation,

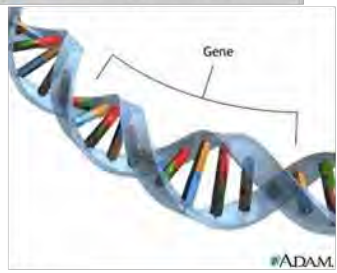


8) Product

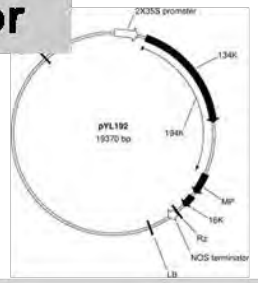
Package, preservation

Complexity of Biotherapeutic Products Manufacturing

1) Genetic sequence



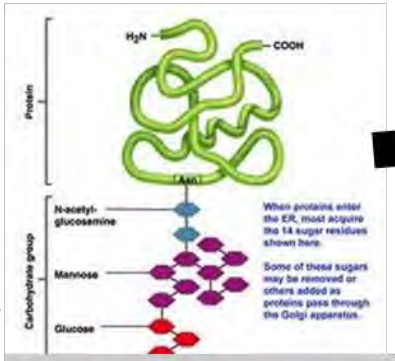
2) DNA vector



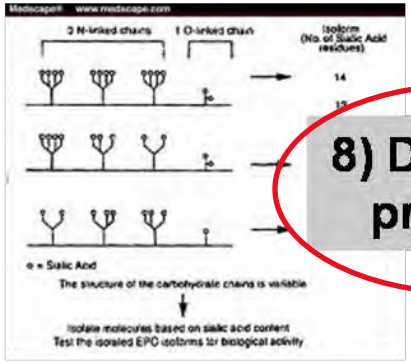
3) Recombinant cell system

Biosimilar Product
or
Intended Copied Product

6) Therapeutic protein product



7) Formulation,



8) Different product

Biologicals 39 (2011) 346–347

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Towards regulation of similar biotherapeutic products: Thailand's perspective

Prapassorn Thanaphollert [a,*], Kriang Tungsanga [b]

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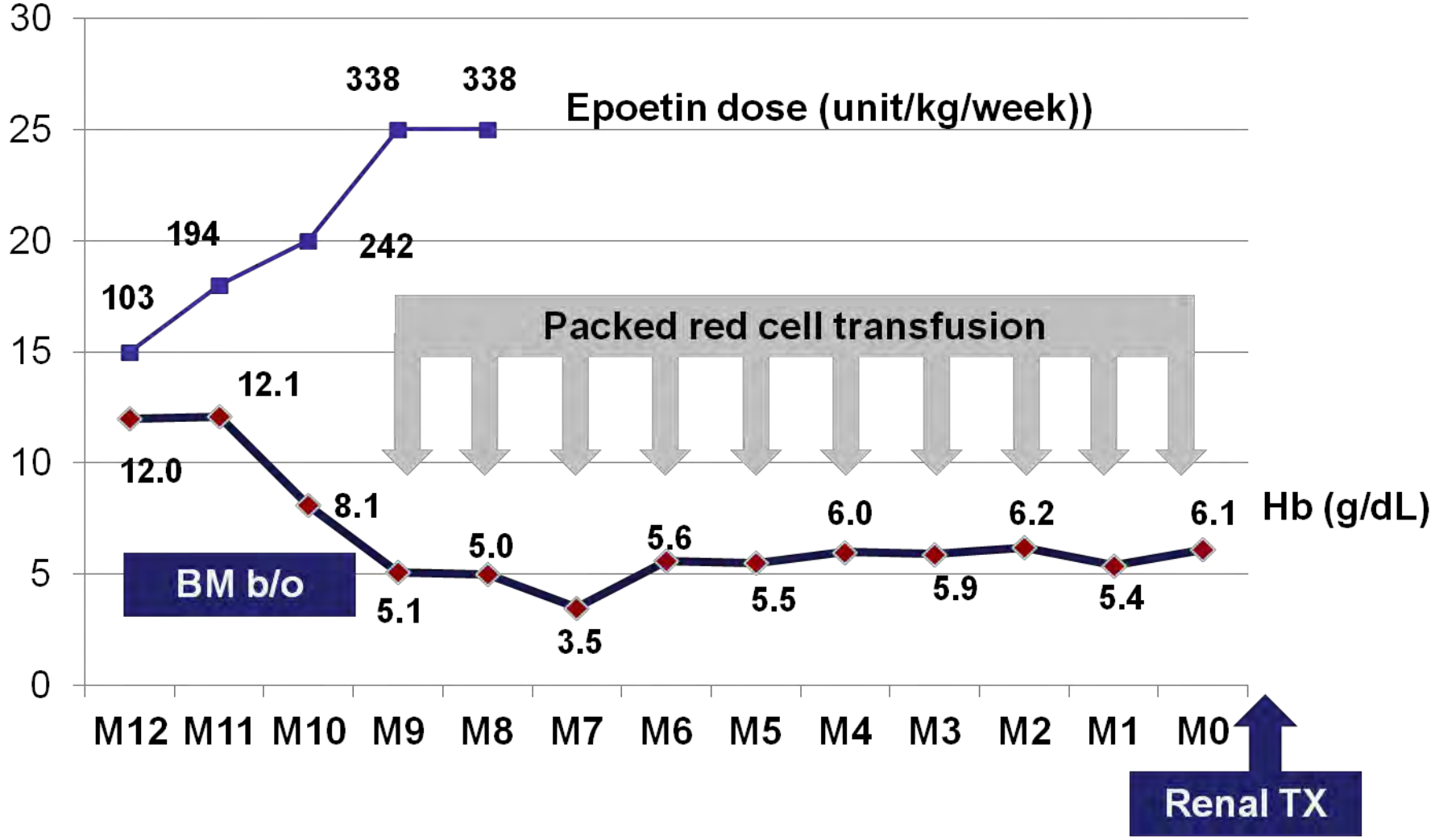
Keyword:
SBP in Thailand

A B S T R A C T

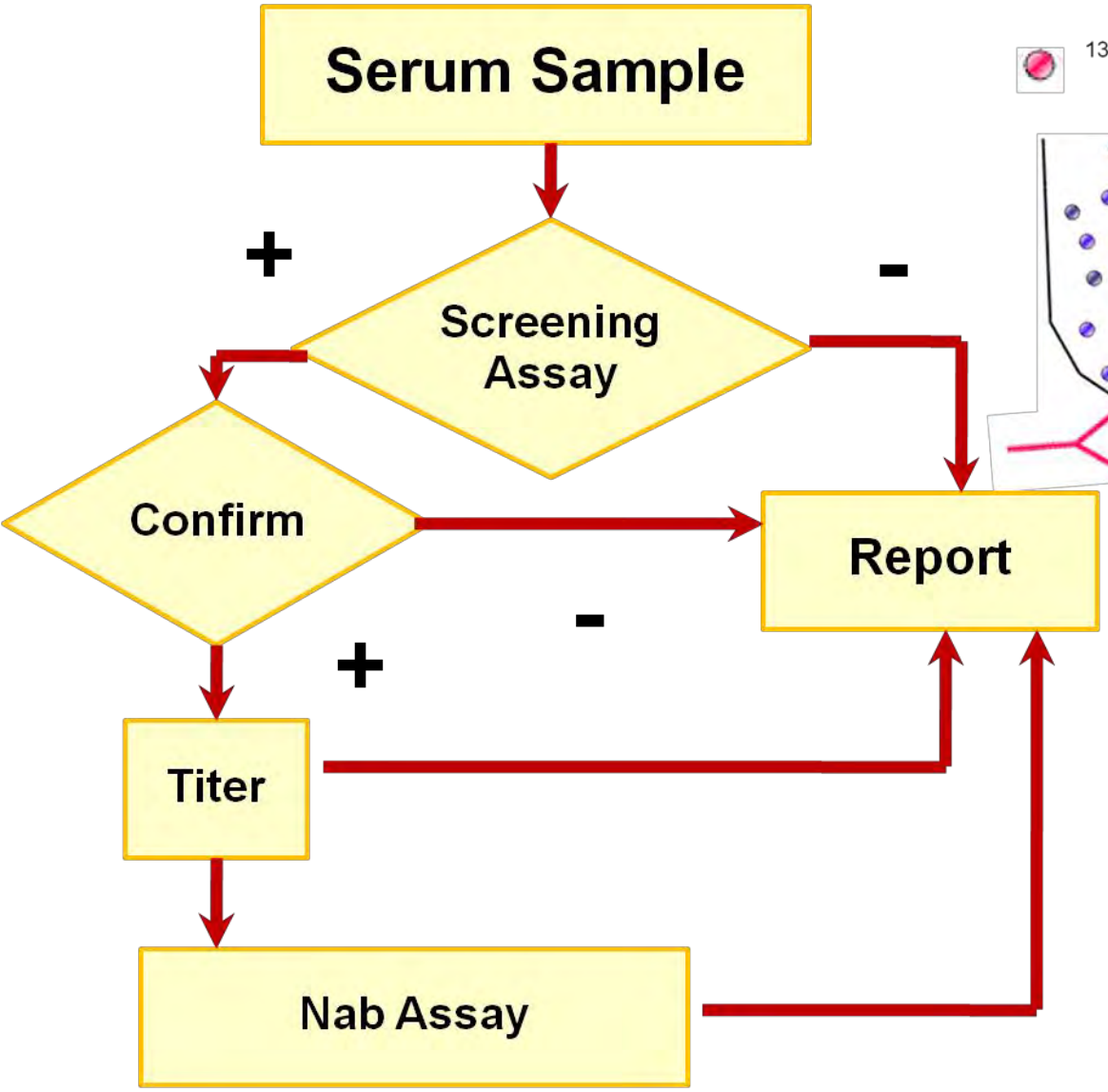
The implementation of universal health coverage scheme in Thailand allows quality, equitable and accessible health care for all. Patients with life threatening and chronic diseases can get access to biotherapeutic products to treat their ailments. This triggered a major impact on the need for specific guidelines in evaluation of similar biotherapeutic products in order to standardize the regulatory pathway to license this class of products ensuring that the products meet acceptable levels of quality, safety and efficacy. The development of similar biotherapeutic products (SBP) should be considered to ensure therapeutic equivalence of biotherapeutics products at more affordable prices. This will lead to greater ease and speed of approval and assurance of the quality, safety and efficacy of these products. Therefore, we report herein the SBP situation in Thailand.

Recovery from anti-Epo associated PRCA after renal Tx

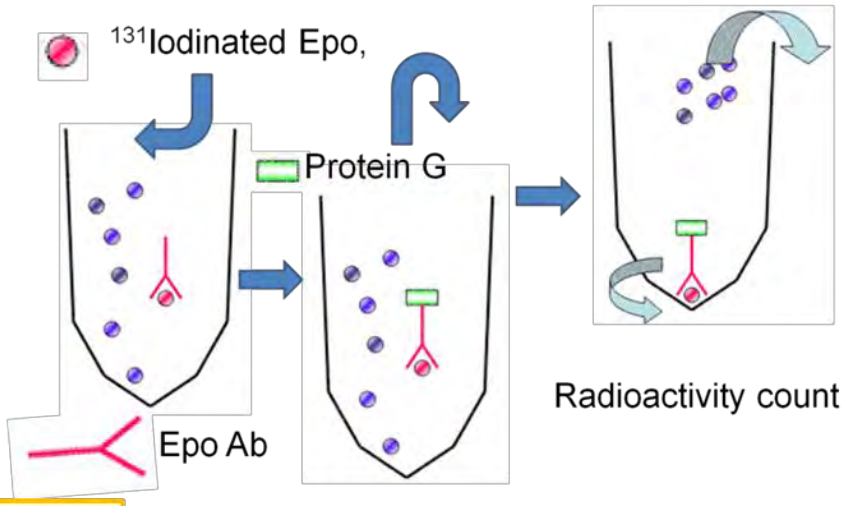


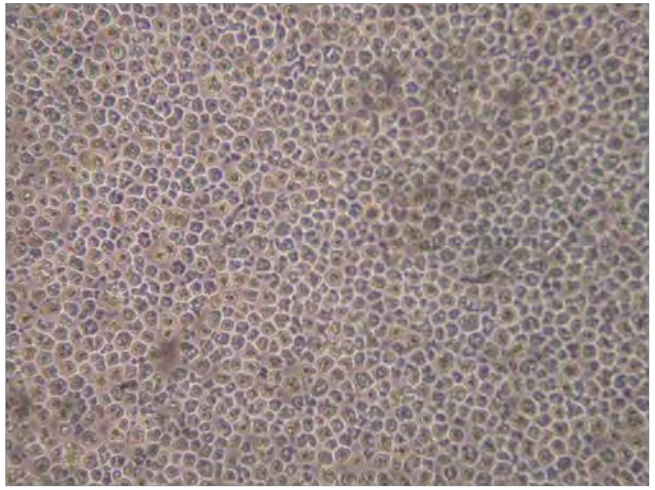
Praditpornsilpa K et al. Nephrol Dial Transplant 2005;20:626-630

Tier Approach to Neutralizing Ab



Immunoprecipitation Test for Anti-Epo





UT-7/EPO cell line

Subcutaneous Injection of Epo and Risk of Anti-r-HuEpo

Clinical criteria
• Treatment with recombinant Epo for average of 9-12 months
• Loss of efficacy defined by drop of Hb 1 g/L/day or need blood Tx 1 u/week
• Reticulocutopenia (less than 10,000/µL)
• Normal white blood cell count/platelet count
Confirmation investigation
• Positive serum assay for anti-erythropoietin Ab
• Evidence of neutralizing Ab
• Normal cellularity bone marrow and < 5% erythroblast with maturation block

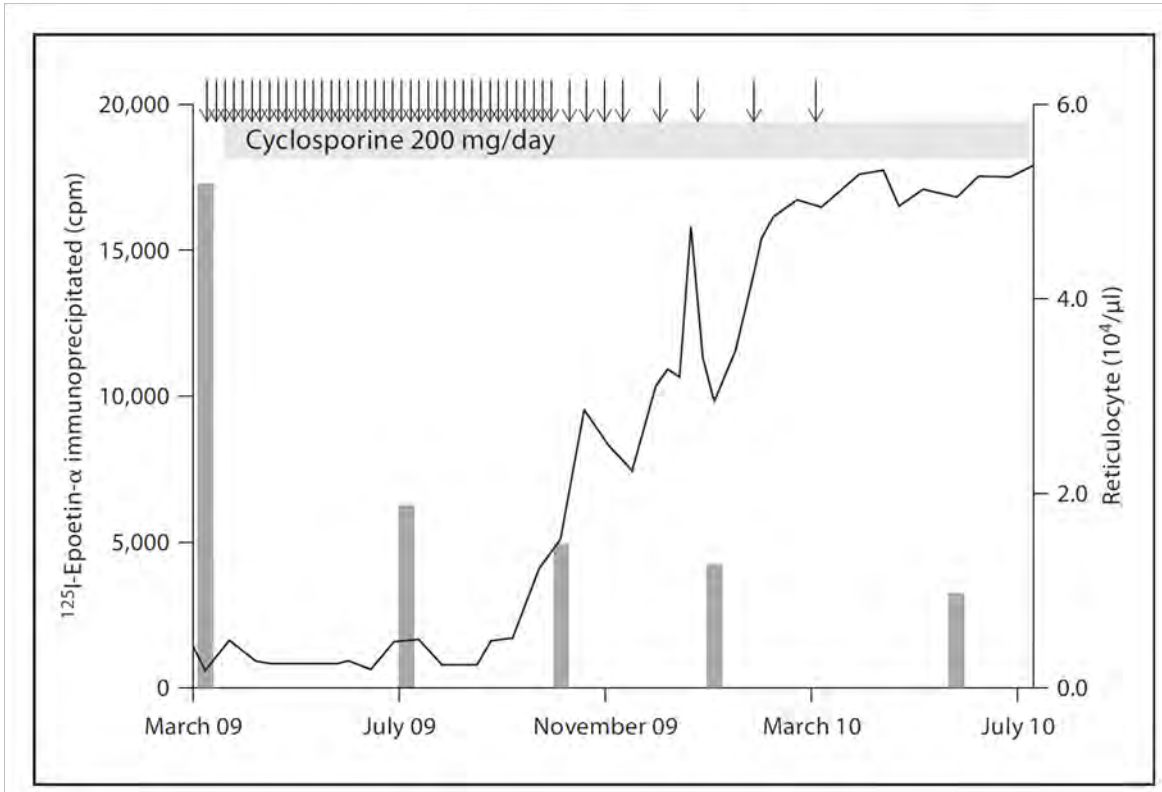
Case Report

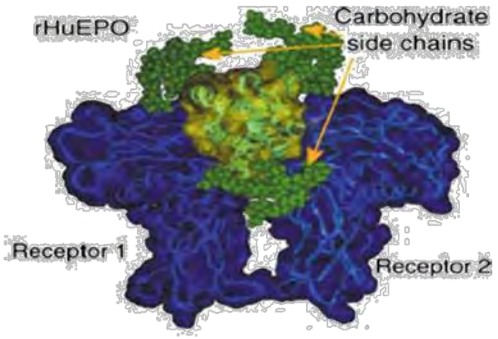
Acta Hæmatologica

Acta Haematol 2011;126:114–118
DOI: 10.1159/000328041

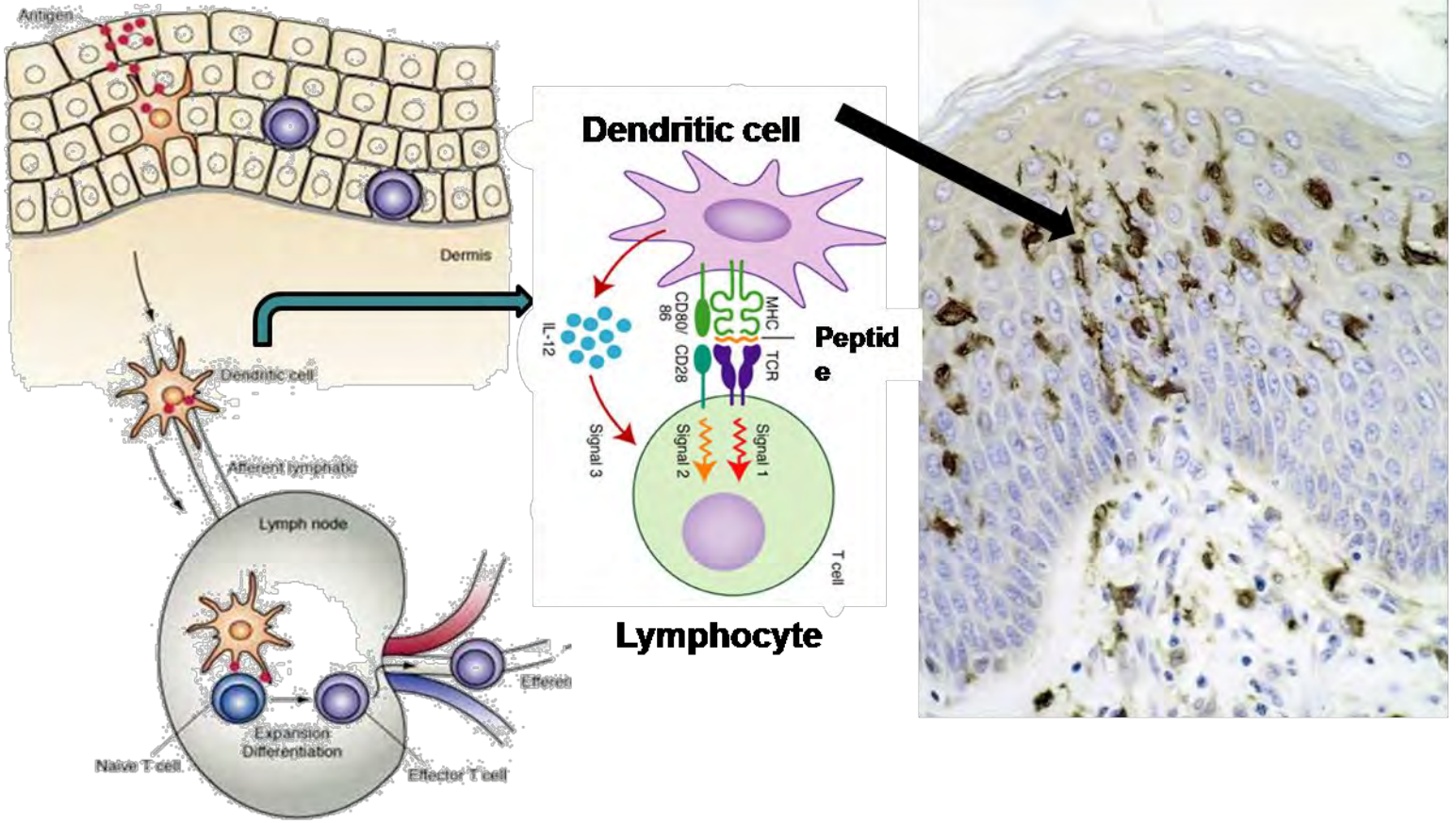
Received: January 31, 2011
Accepted after revision: March 22, 2011
Published online: June 7, 2011

Pure Red Cell Aplasia Induced Only by Intravenous Administration of Recombinant Human Erythropoietin



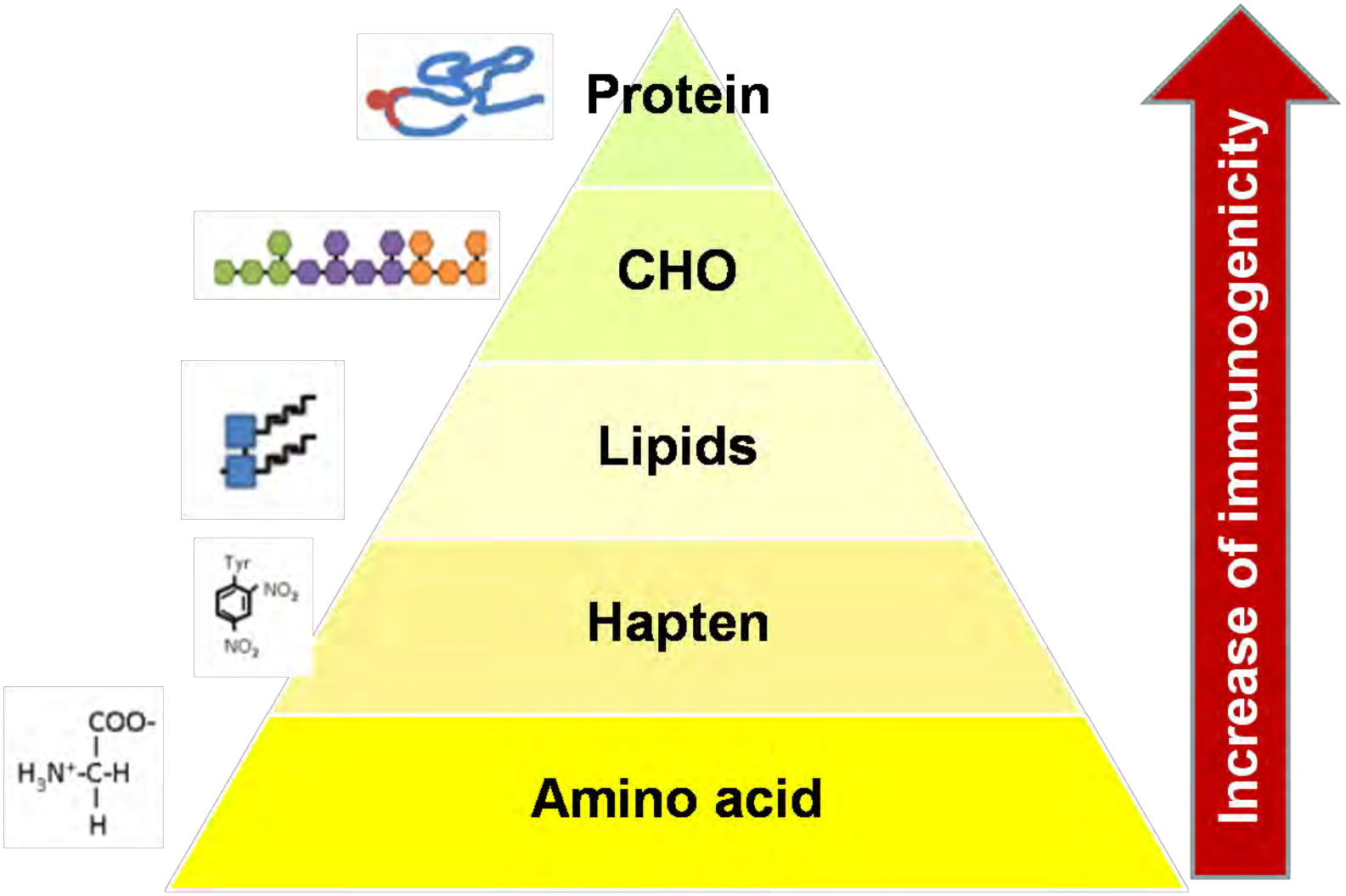


Immunogenicity of Biotherapeutic Products: Anti-Drug Antibody (ADA)



Nature Reviews Immunology 2005:5,617-628

Immunogenicity

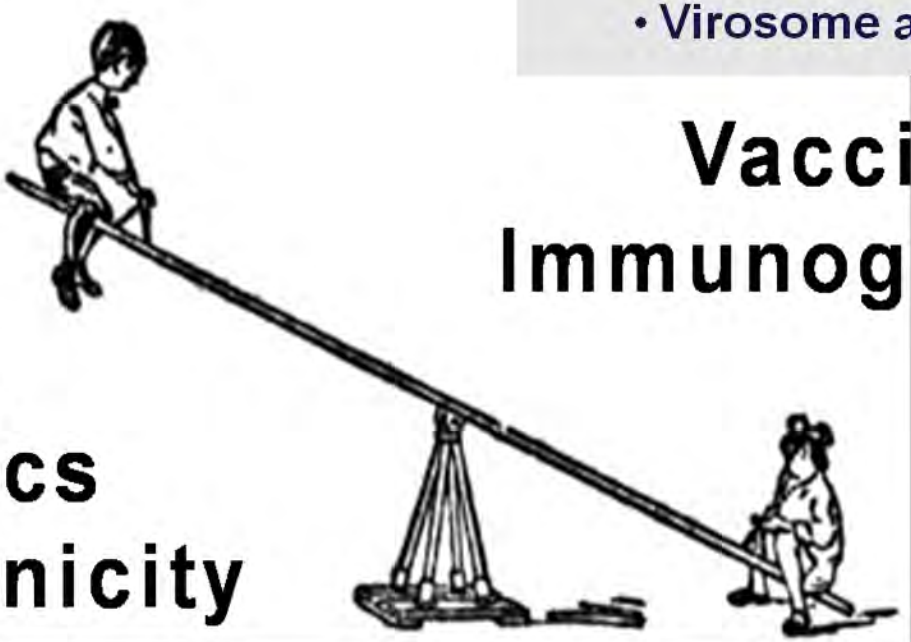


Balance of Immunogenicity

Immunological adjuvant

- Freund's adjuvant
- Aluminium salts
- Oil based adjuvant
- Virosome adjuvant

Product quality



Vaccine Immunogenicity

Biologics Immunogenicity

http://www.kidney-international.org

Biosimilars need comparative clinical data

To the Editor: We have read with great interest the study by Praditpornsilpa *et al.*[1] in *Kidney International* about the association between antibody-associated pure red cell aplasia (PRCA) and the use of copies of epoetins alpha and beta, for which the marketing authorization was based on the generic regulatory approach used for small molecules, which does not

see original article on page 88

Erythropoiesis-stimulating agents and pure red-cell aplasia: you can't fool Mother Nature

Jay B. Wish[1]

Subtle alterations in the properties of biopharmaceutical agents may increase their immunogenicity and lead to the production of

http://www.kidney-international.org

original article

go the same quality
nd distribution as their
milar erythropoiesis-
l-cell aplasia in
a model for other

Biosimilar recombinant human erythropoietin induces the production of neutralizing antibodies

Kearkiat Praditpornsilpa[1], Khajohn Tiranathanagul[1], Pawinee Kupatawintu[2], Saengsuree Jootar[3], Tanin Intragumtornchai[4], Kriang Tungsanga[1], Tanyarat Teerapornlertratt[5], Dusit Lumlertkul[6], Natavudh Townamchai[1], Paweena Susantitaphong[1], Pisut Katavetin[1], Talerngsak Kanjanabuch[1], Yingyos Avihingsanon[1] and Somchai Eiam-Ong[1]

[1]Division of Nephrology, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; [2]National Blood Center, Thai Red Cross Society, Bangkok, Thailand; [3]Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [4]Division of Hematology, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; [5]Division of Nephrology, Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand and [6]Division of Nephrology, Department of Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

Biosimilar recombinant human erythropoietin induces the production of neutralizing antibodies

	Anti-r-HuEpo positive	Anti-r-HuEpo negative
Numbers of patients (cases)	23	7
Gender, male/female (case/case)	13/10	3/4
Age, years ± s.d.	61.1 ± 21.4	52.8 ± 4.8
CKD status, cases (%)		
Predialysis	8 (34.8)	2 (28.6)
Hemodialysis	14 (60.9)	4 (57.1)
Peritoneal dialysis	1 (4.3)	1 (14.3)
Etiology of CKD, cases (%)		
Diabetic nephropathy	5 (26.1)	3 (42.9)
Chronic glomerulonephritis	3 (13.0)	0
Unknown	14 (60.9)	4 (57.1)
r-HuEpo exposure duration, months ± s.d. (range in months)	12.1 ± 7.8 (3–36)	22.3 ± 19.8 (6–60)
r-HuEpo dose, U/kg/week ± s.d.	149 ± 82	171 ± 91
Hb before LOE, g/dl ± s.d.	10.8 ± 1.6	11.4 ± 0.7
Hemoglobin by the time of LOE, g/dl ± s.d.	5.6 ± 0.9	7.3 ± 0.7
Reticulocytes, cell/mm^3 ± s.d.	5978 ± 1217	13,128 ± 3,456
Serum ferritin, ng/ml ± s.d.	368.6 ± 83.1	370.3 ± 93.7
Transferring saturation, % ± s.d.	28.3 ± 6.6	28.8 ± 5.2
Serum folate, pg/ml ± s.d.	12.8 ± 4.5	12.5 ± 4.3
Serum B_{12}, pg/ml ± s.d.	258.2 ± 189.2	177.1 ± 84.4
CRP, mg/l ± s.d.	4.22 ± 2.98	3.62 ± 3.56
iPTH, pg/ml ± s.d.	241.4 ± 127.1	284.0 ± 151.6

Praditpornsilpa K et al. Kidney Int 2011; 80:88-92

Table 2 | Total c.p.m. (%) by different sera dilution to detect anti-r-HuEpo-associated PRCA cases in patients using subcutaneous biosimilar r-HuEpo: antibody-positive and antibody-negative cases, negative control, and pure negative control

	Mean of percent c.p.m. ± s.d.					
	1:20 dilution	1:50 dilution	1:100 dilution	1:1000 dilution	1:10,000 dilution	1:20,000 dilution
Anti-r-HuEpo-positive cases (*N*=23)	18.2 ± 8.8	12.7 ± 9.7	10.5 ± 9.2	3.5 ± 5.0	1.0 ± 1.0	0.3 ± 0.8
Anti-r-HuEpo-negative cases (*N*=7)	0.2 ± 0.1	NA	NA	NA	NA	NA
Negative control (*N*=30)	0.2 ± 0.1	NA	NA	NA	NA	NA
Pure negative control (*N*=30)	0.2 ± 0.1	NA	NA	NA	NA	NA

Abbreviations: c.p.m., counts per minute; NA, not applicable; PRCA, pure red-cell aplasia; r-HuEpo, recombinant human erythropoietin.

Estimation of risk

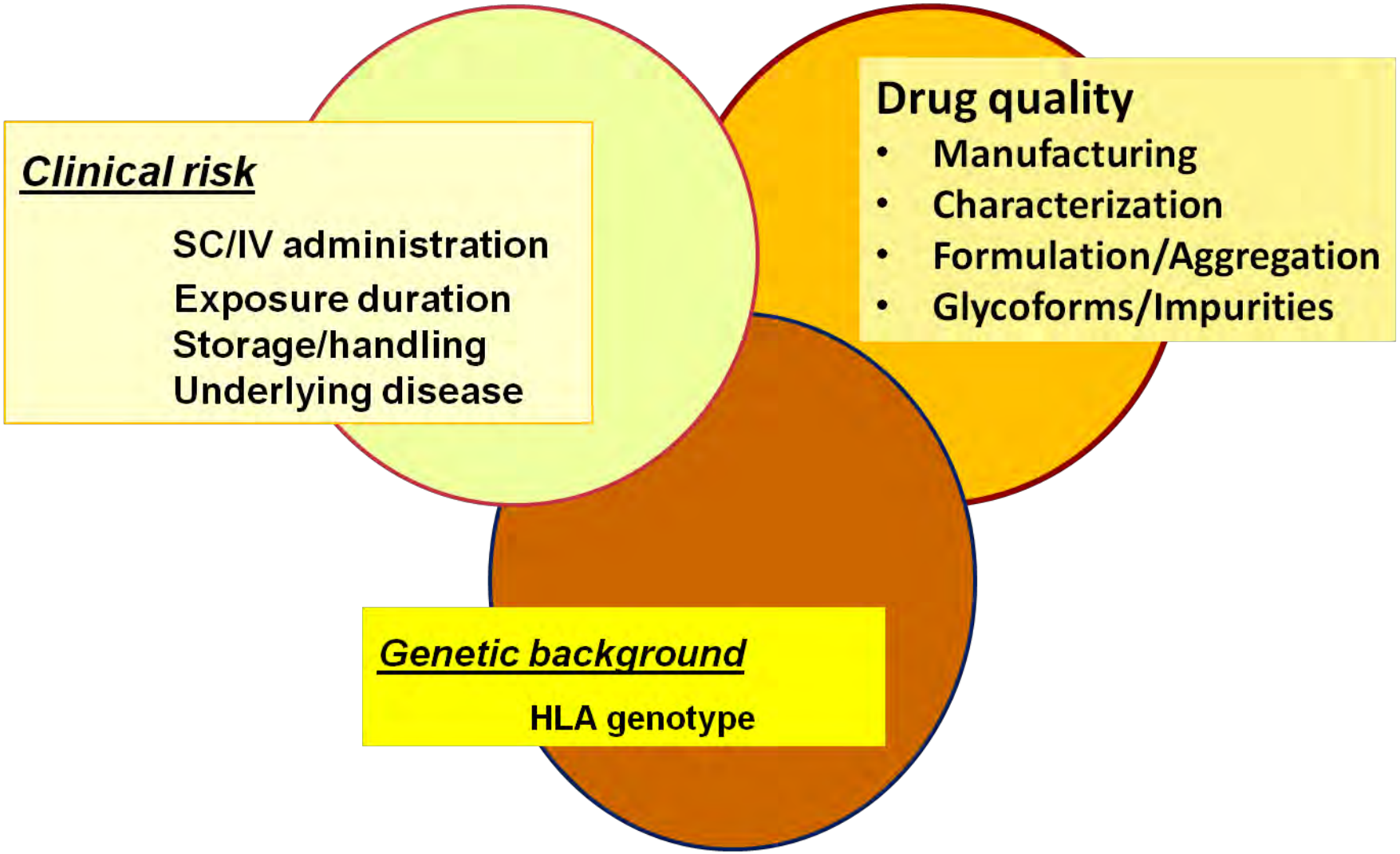
Yearly prevalence of dialysis patients in 2008 receiving r-HuEpo was obtained from the Thai Renal Replacement Therapy Registry data. In 2008, there were a total of 26,511 chronic dialysis cases receiving r-HuEpo.[8] The prevalence of CKD stage IV (predialysis) was obtained from a recent epidemiologic survey conducted in Thailand.[9] From 63 million people, the prevalence of CKD stage IV was 0.2%. Out of this 0.2%, about 40% had Hb below 10 g/dl and needed r-HuEpo treatment. From the Thai ESA registry data, 78% of the patients had received biosimilar r-HuEpo.

Estimation of risk for anti-r-HuEpo-associated PRCA was calculated by $23/(26{,}511 + (63{,}000{,}000 \times 0.002 \times 0.4)) \times 0.78 = 23/59{,}990$.

Thus, an estimation of the actual cases using biosimilar r-HuEpo denominator with this complication was 1:2608. This indicated that 1 out of 2608 patients using biosimilar r-HuEpo would develop PRCA.

Praditpornsilpa K et al. Kidney Int 2011; 80:88-92

What are the risk factors/pathogenesis of anti-r-HuEpo associated PRCA ?



Nephrol Dial Transplant (2008) 1–5
doi: 10.1093/ndt/gfn450

NDT
Nephrology Dialysis Transplantation

Original Article

The association of anti-r-HuEpo-associated pure red cell aplasia with HLA-DRB1*09-DQB1*0309

Table 1. Patients' characteristics of anti-r-HuEpo-associated PRCA cases

Numbers of patients (cases)	22
Age, years ± SD (range)	56.9 ± 16.9 (17–82)
Gender: male/female (case/case)	11/11
r-HuEpo exposure duration: months ± SD (range)	9.9 ± 3.2 (7–17)
r-HuEpo form at the time of diagnosis of PRCA	
Total alpha-r-HuEpo (cases)	19
Teflon-coated stopper pre-filled syringes	11
Uncoated stopper pre-filled syringes	7
Biosimilar alpha-r-HuEpo (cases)	1
Beta-r-HuEpo (cases)	3

r-HuEpo, recombinant human erythropoietin; PRCA, pure red cell aplasia.

HLA-A, -B, -DR haplotype frequencies in the Thai Stem Cell Donor Registry

P. Kupatawintu[1], S. Pheancharoen[1], A. Srisuddee[1], H. Tanaka[2], K. Tadokoro[2] & O. Nathalang[3]

N = 16,807

Most common haplotypes: HLA-A33-B58-DR17 (4.54%)

HLA-A		HLA-B		HLA-DR	
Antigen	Frequency (%)	Antigen	Frequency (%)	Antigen	Frequency (%)
33	13.80	13	7.90	15	17.50
2	29.24	35	4.50	12	16.90
11	27.70	44	4.50	9	11.50
24	17.34	18	3.90	4	11.40
1	2.20	27	3.30	7	8.20
30	2.00	13	7.90	14	7.60
26	1.90	18	3.90	17	6.50
23	0.10	27	3.30	11	5.70
31	1.34	39	2.70	16	4.70
3	0.90	15	0.50	8	4.20
29	0.60	14	0.10	13	3.60
32	0.05	41	0.10	10	1.70
25	0.01	42	0.01	1	0.50

Tissue Antigen 2010; 75:730-736

Nephrol Dial Transplant (2008) 1–5
doi: 10.1093/ndt/gfn450

NDT
Nephrology Dialysis Transplantation

Original Article

The association of anti-r-HuEpo-associated pure red cell aplasia with HLA-DRB1*09-DQB1*0309

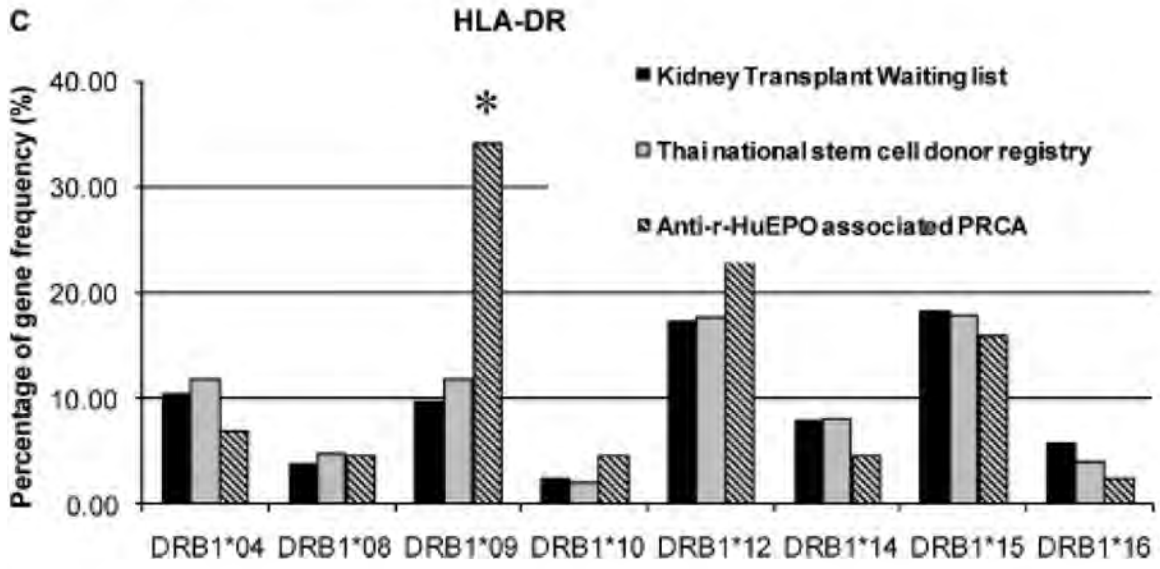
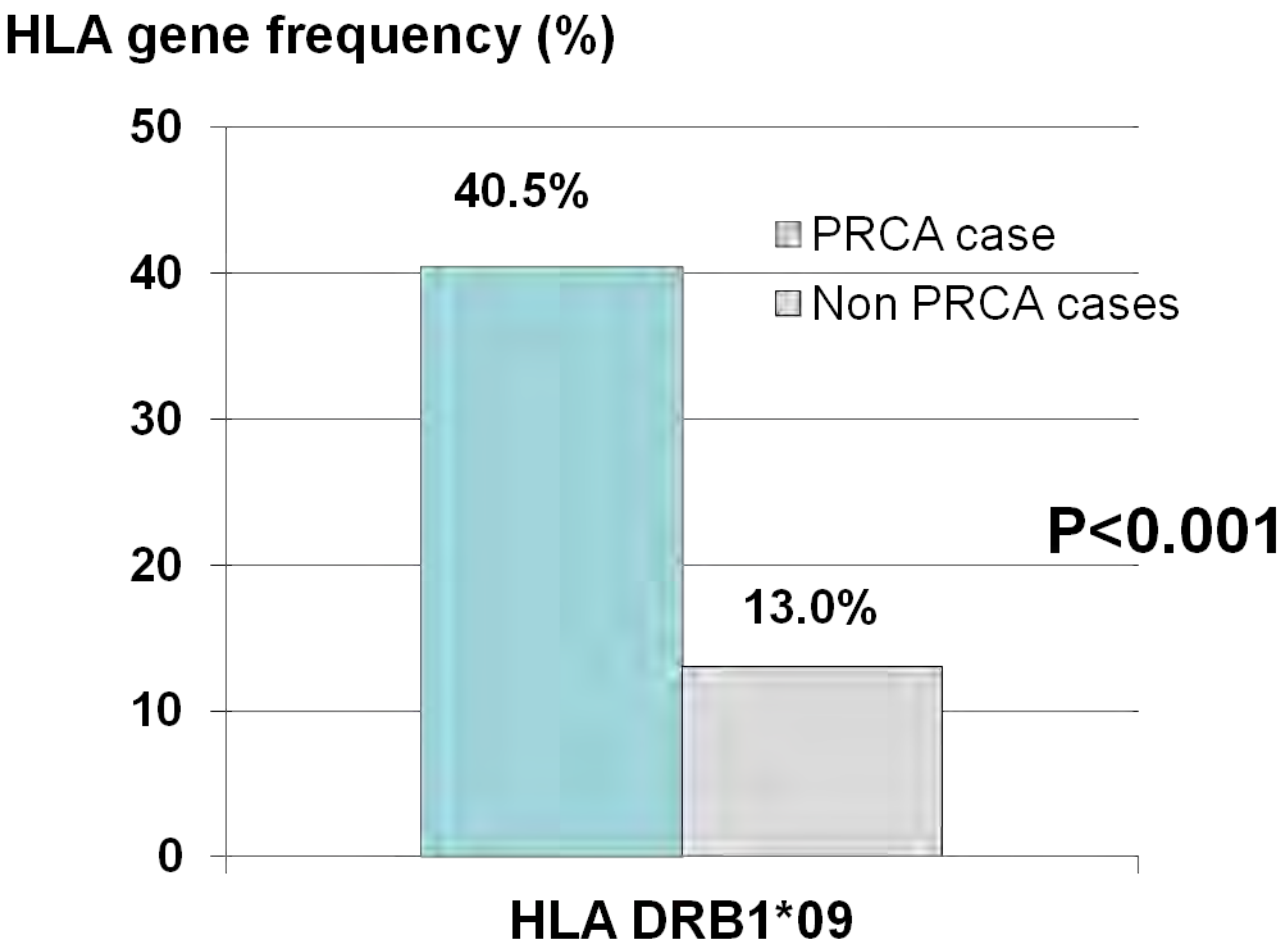


Table 4. Odds ratio for HLA alleles identified as potential associated HLA gene with anti-r-HuEpo-associated PRCA

	Anti-r-HuEpo-associated PRCA cases	Thai national stem cell donor	Odds ratio (95% CI)	*P*
HLA-DRB1*09	15 (34.1%)	354 (11.8%)	2.89 (1.88–4.46)	<0.001

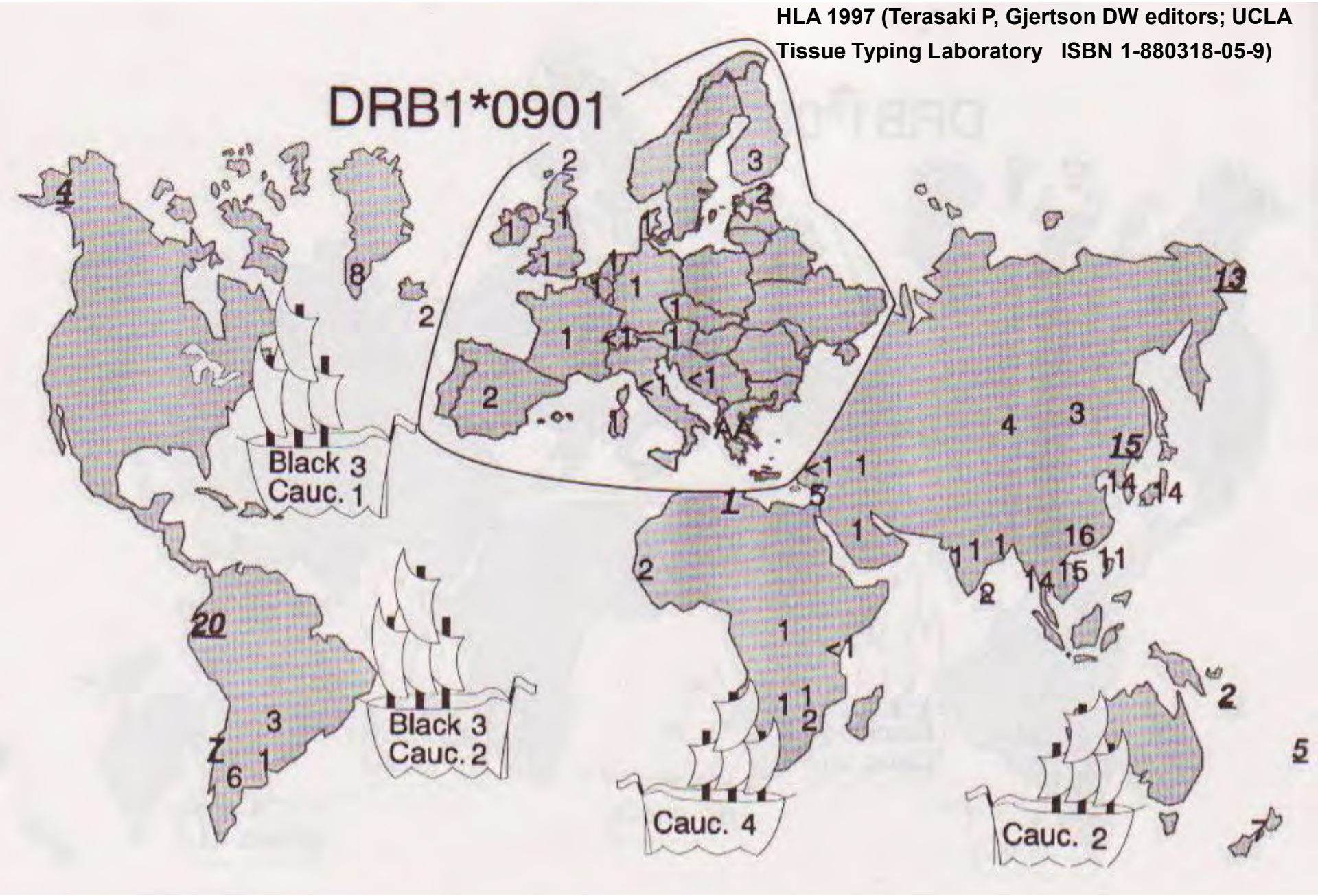
HLA gene frequency of anti-rHuEpo PRCA cases: N =75

CKD-ESRD receiving epoetin



Unpublished data

HLA 1997 (Terasaki P, Gjertson DW editors; UCLA Tissue Typing Laboratory ISBN 1-880318-05-9)



Demographic characteristics	Cases	Controls
n	24	81
Age (years), mean (±SD)	59.5 (±18.6)	56.8 (±16.0)
Sex, frequency (%)		
Male	17 (71)	48 (59)
Female	7 (29)	33 (41)
Race, frequency (%)		
Asian	1 (4)	4 (5)
Black	0 (0)	2 (2)
Other	0 (0)	1 (1)
Caucasian	23 (96)	74 (91)
Country, frequency (%)		
Canada	3 (13)	12 (15)
France	8 (33)	9 (11)
Germany	1 (4)	0 (0)
UK	7 (29)	44 (54)
Italy	1 (4)	3 (4)
Netherlands	0 (0)	4 (5)
Spain	2 (8)	0 (0)
Sweden	2 (8)	9 (11)
*Disease history, frequency (%)**		
Glomerulonephritis	7 (47)	19 (28)
Diabetic nephropathy	1 (7)	14 (20)
Renal vascular disease	6 (40)	8 (12)
Interstitial nephritis	0 (0)	4 (6)
Poly/multicystic kidney disease	2 (13)	4 (6)
Obstructive/reflux nephropathy	0 (0)	4 (6)
Congenital renal hypoplasia/dysplasia	0 (0)	4 (6)
Renal neoplasms	0 (0)	1 (1)
Renal failure metabolic disease	1 (7)	2 (3)
Toxic nephropathy	0 (0)	1 (1)
Paraproteinemia	0 (0)	1 (1)
Other	1 (7)	16 (23)
n	0 (0)	5 (7)

A Case-Control Study of the Association Between Select HLA Genes and Anti-Erythropoietin Antibody Positive Pure Re Cell Aplasia

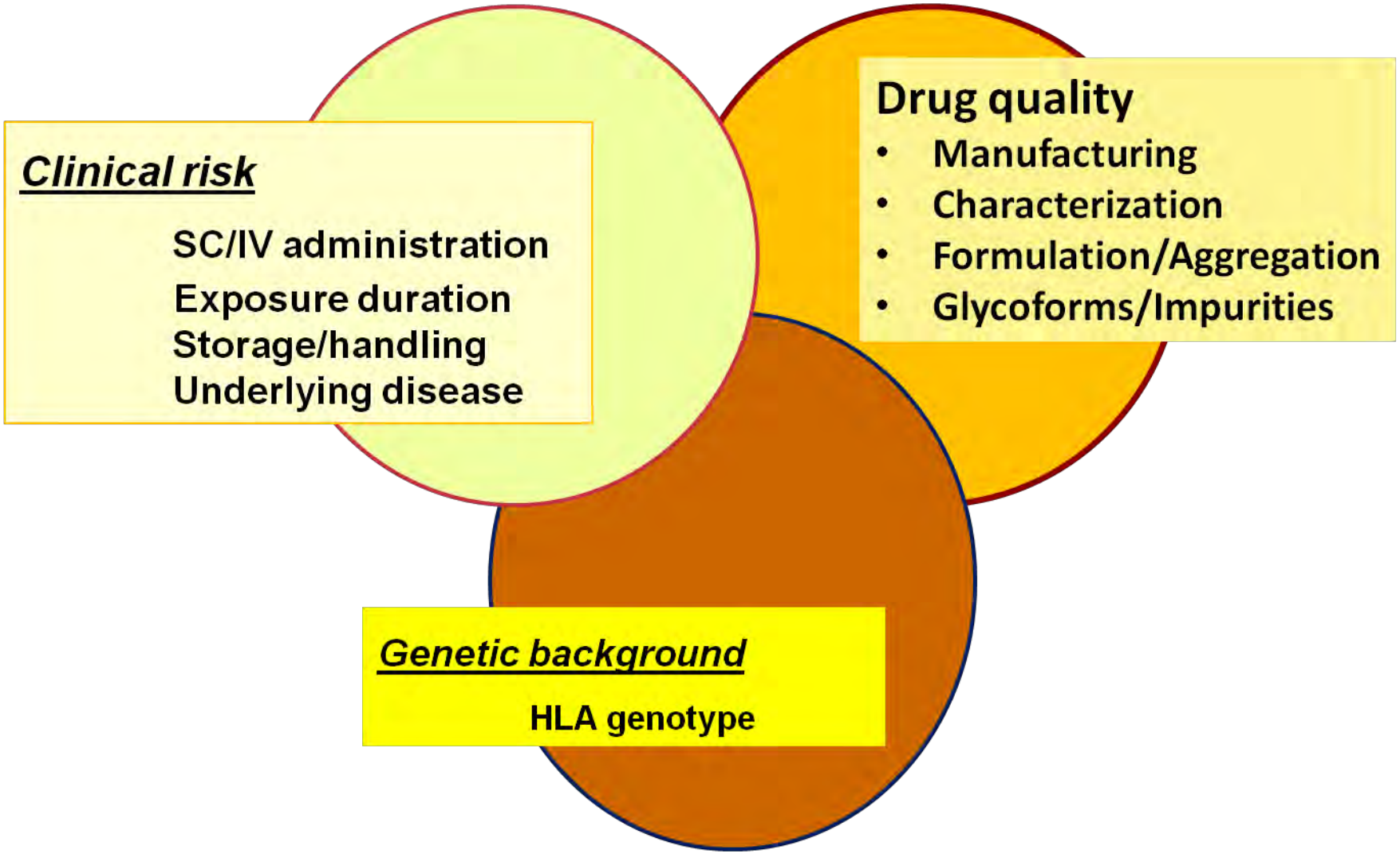
Fijal B. Pharmacogenomics 2008;9:157-67

Case-Control of HLA, PRCA Association

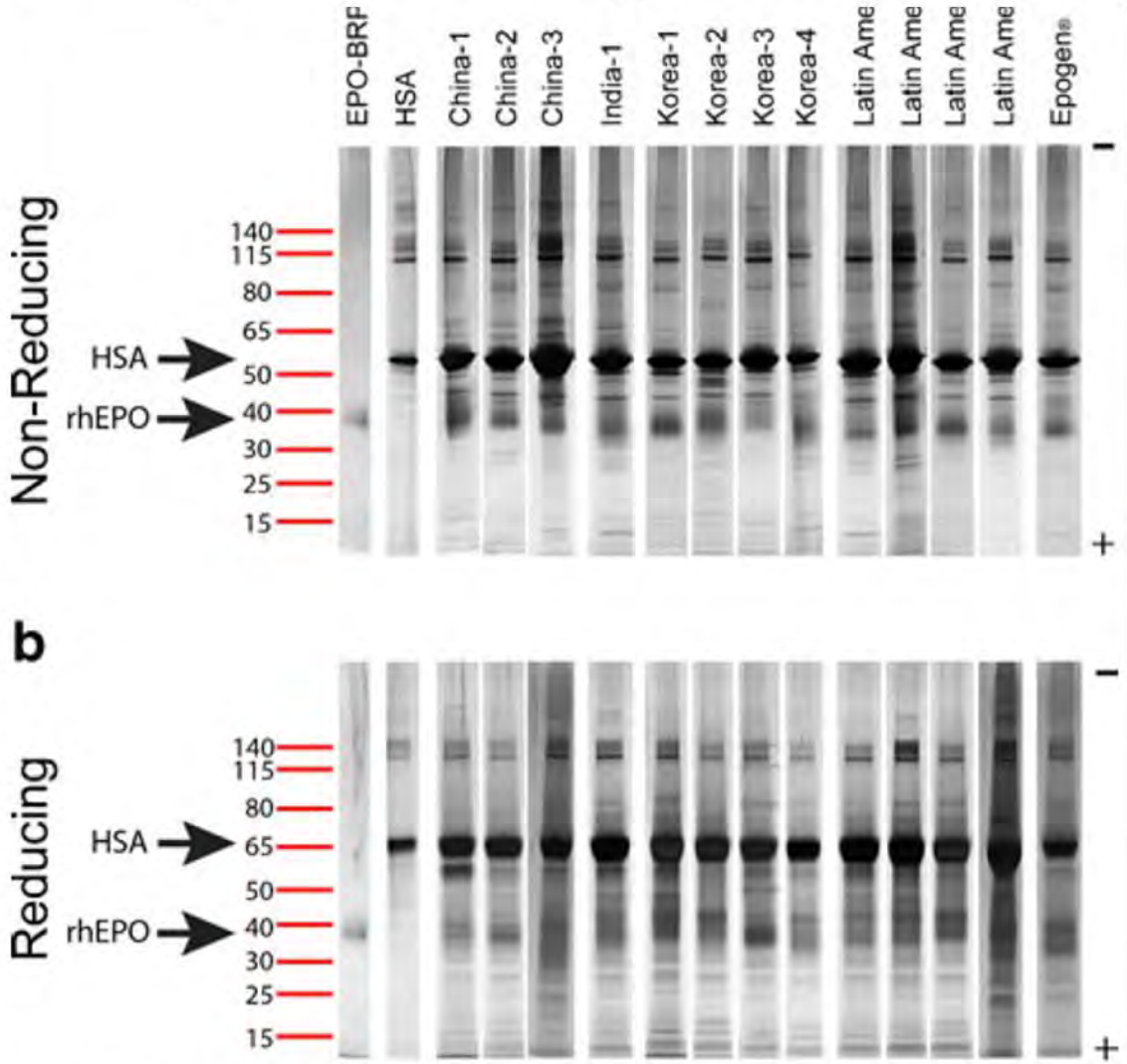
Race	Frequency (%)		OR	*95% CI*
	Cases	Controls		
• All matched subjects				
N	24	81		
DR B1*9/-	6 (25.0%)	2 (2.5%)	10.8	(2.2-53.7)
Other/other	18 (75.0%)	79 (97.5%)	1.0	--
• Asian				
N	1	4		
DR B1*9/-	1 (100.0%)	1 (25.0%)	NA	NA
Other/other	0 (0.0%)	3 (75.0%)	NA	NA
• Caucasian				
N	23	74		
DR B1*9/-	5 (21.7%)	1 (1.3%)	20.3	(2.2-184.5)
Other/other	18 (78.3%)	73 (98.7%)	1.0	--

Figal B et al. Pharmacogenomic 2008;9: 157-167

What are the risk factors/pathogenesis of anti-r-HuEpo associated PRCA ?



How Bio-questionable are the Different Recombinant Human Erythropoietin Copy Products in Thailand?



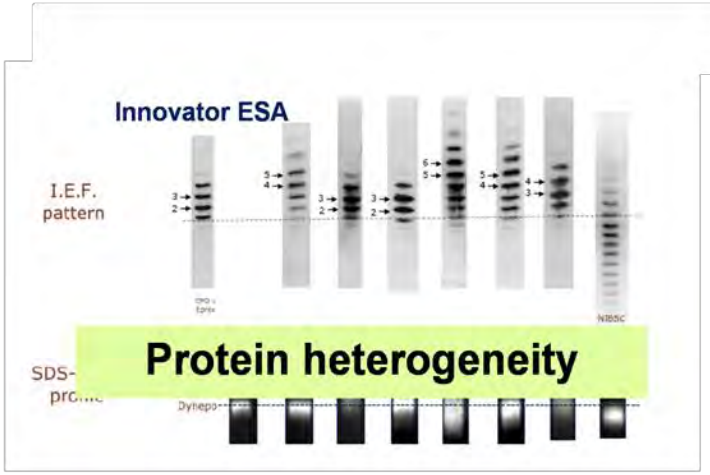


Fig. 1 SDS-PAGE gel of different rhEPO copy products (2 IU/lane) stained with SilverQuest™ Silver Staining under (**a**) non-reducing conditions and (**b**) reducing conditions. *Arrows* indicate the expected molecular weights of rhEPO or HSA.

Pharm Res. 2014 May;31(5):1210-8

How Bio-questionable are the Different Recombinant Human Erythropoietin Copy Products in Thailand?

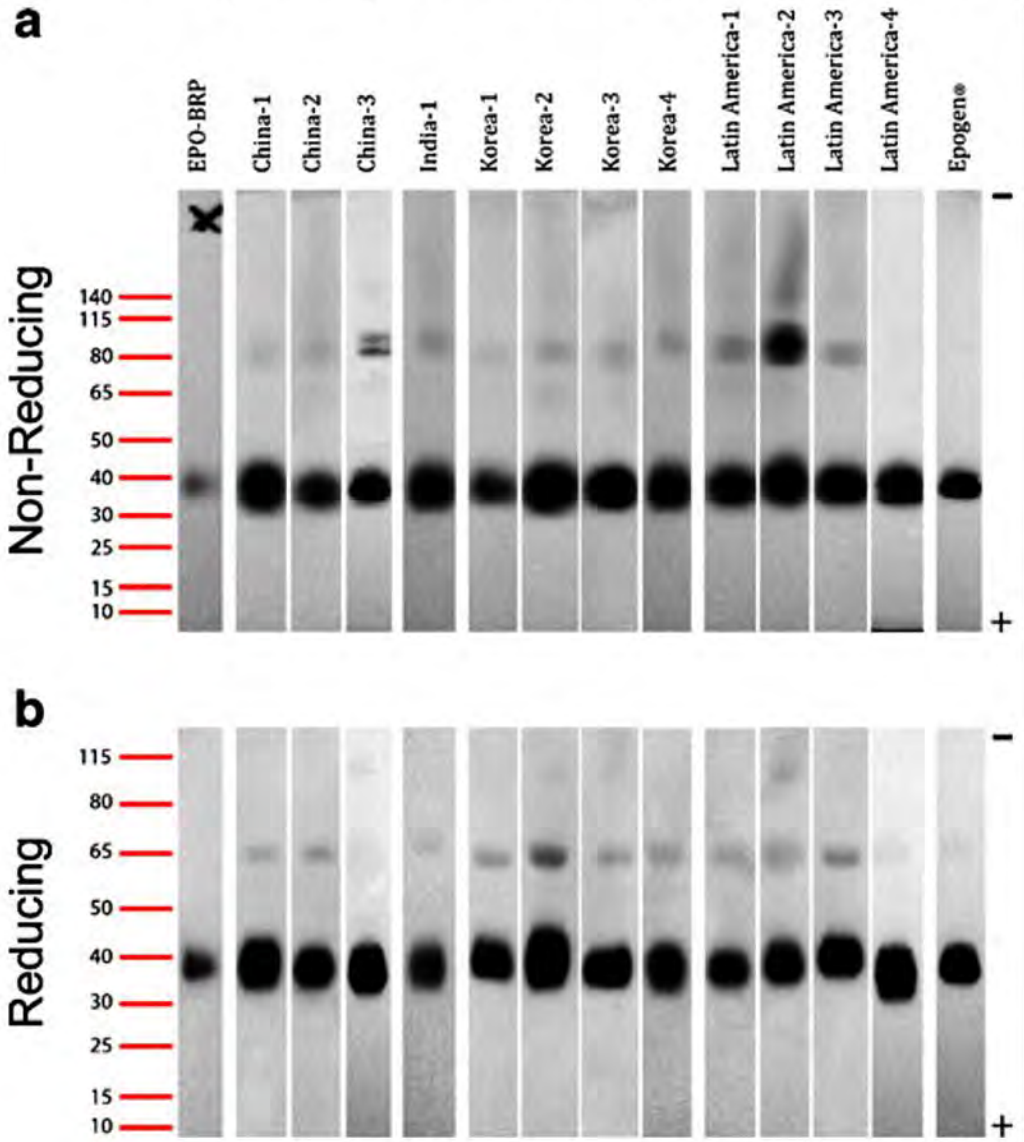


Fig. 2 Performance of various rhEPO copy products on SDS-PAGE (2 IU/lane) detected with Western blot using anti-EPO antibody under (**a**) non-reducing and (**b**) reducing condition.

Pharm Res. 2014 May;31(5):1210-8

How Bio-questionable are the Different Recombinant Human Erythropoietin Copy Products in Thailand?

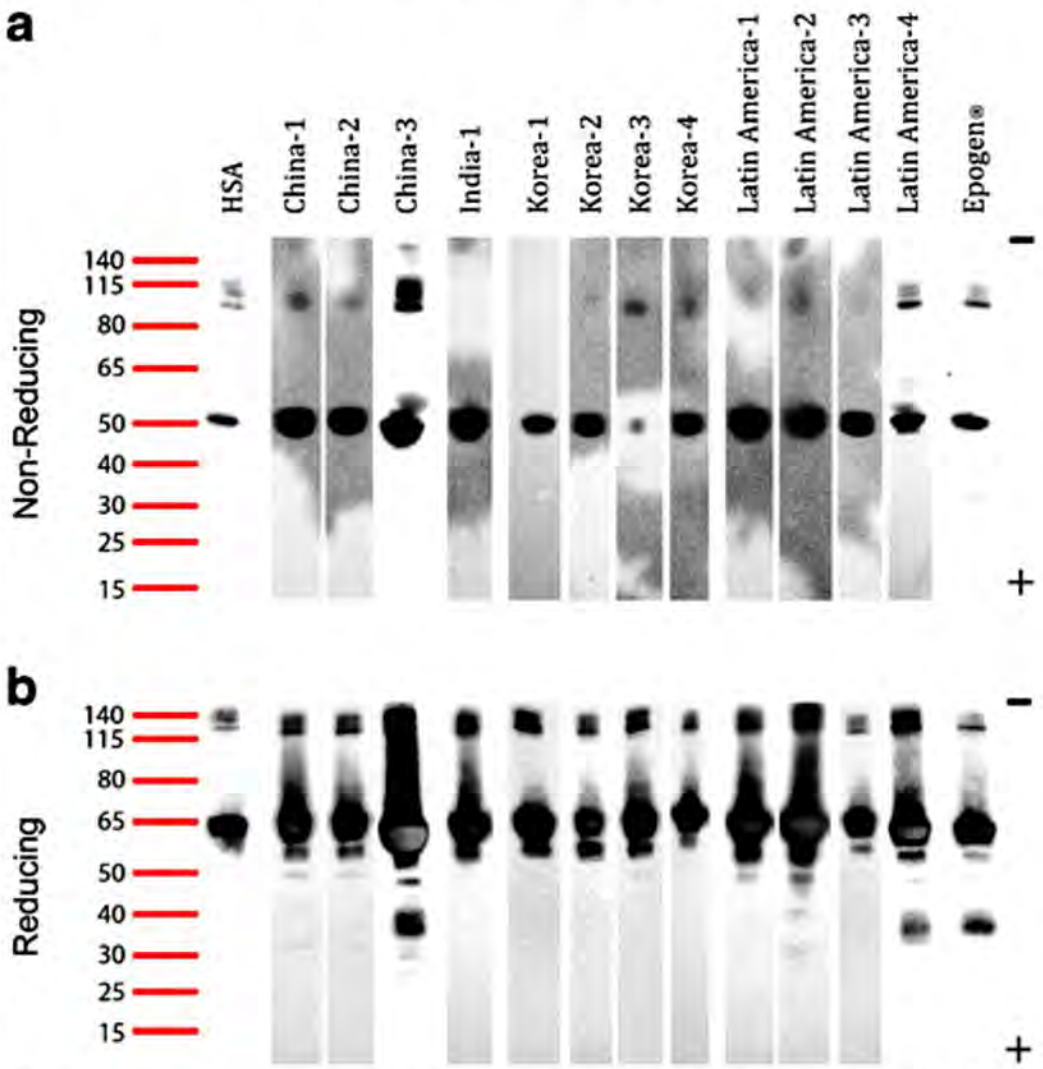


Fig. 3 Performance of various rhEPO copy products on SDS-PAGE (2 IU/lane) detected with Western blot using anti-HSA antibody under (**a**) non-reducing and (**b**) reducing condition.

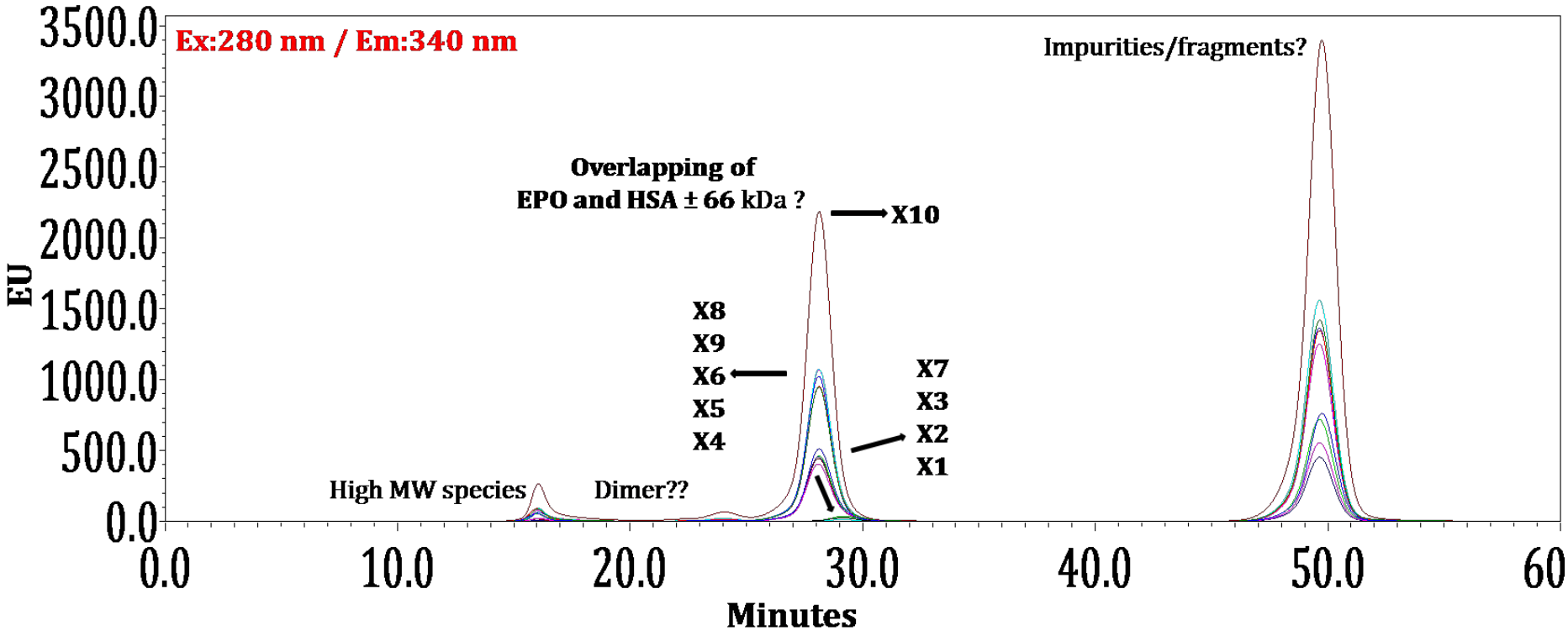
EPO and HSA present in all rhEPO products on Thai market

- **Monomer**
- **Dimer**
  - **EPO / EPO + HSA / HAS + others**
- **High molecular weight species**
  - **Combined EPO and HSA or aggregates???**
- **Fragments**

Pharm Res. 2014 May;31(5):1210-8

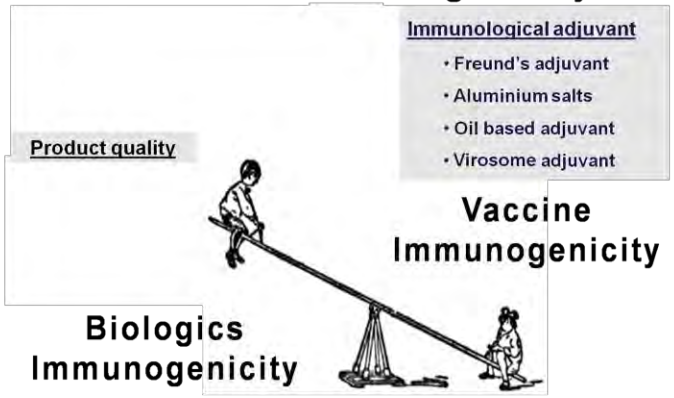
Chromatogram of EPO products

- Loaded on the same IU



Pharm Res. 2014 May;31(5):1210-8

Balance of Immunogenicity



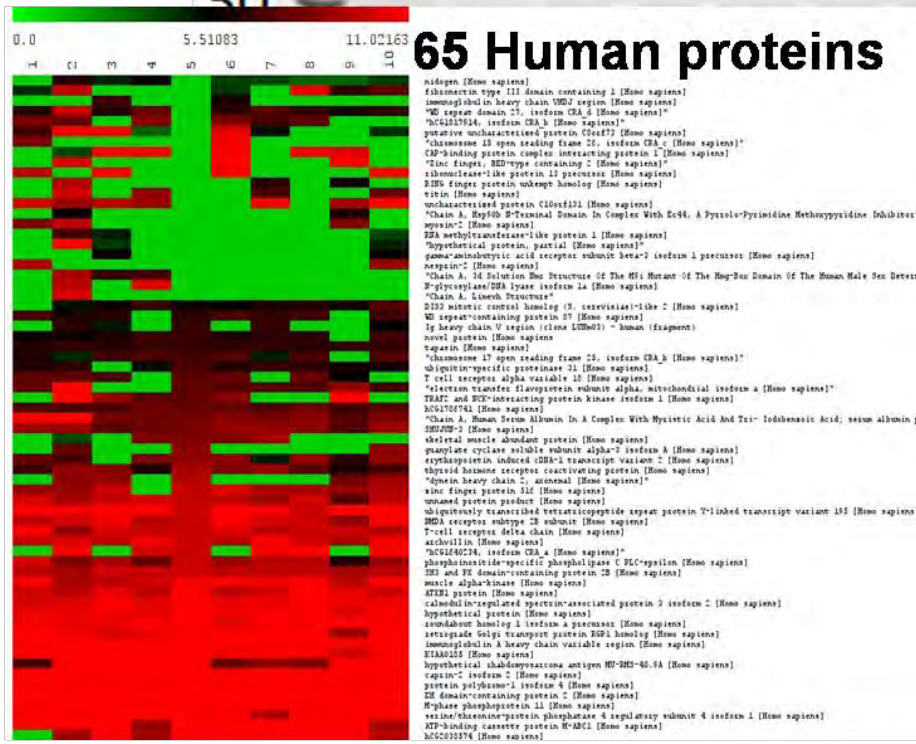
• HSA

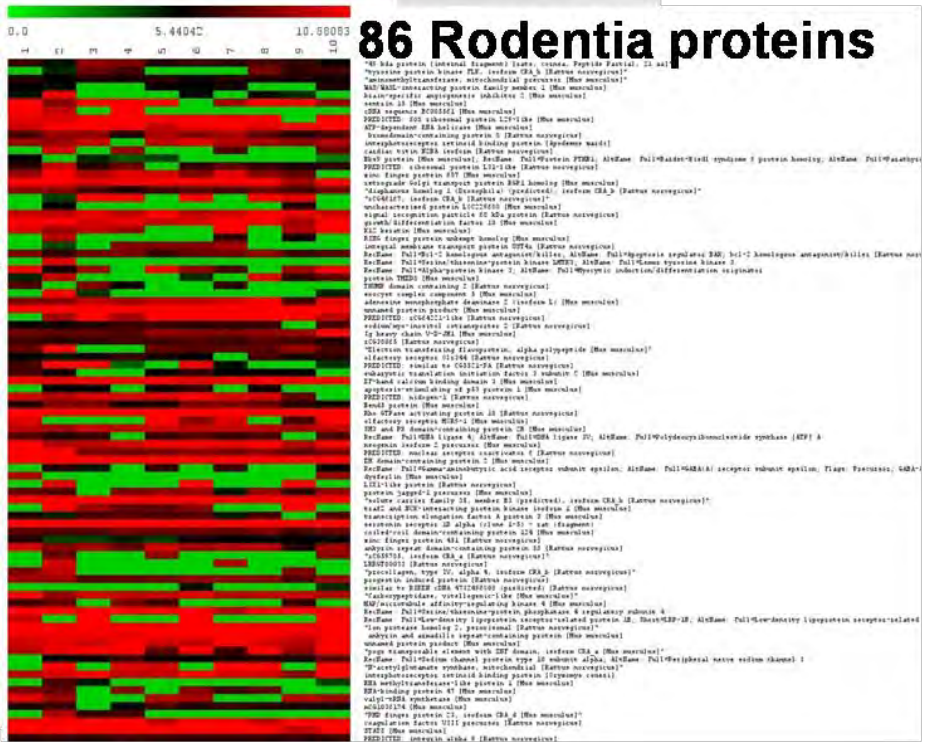
- HSA and EPO peak are "inseparable"
- The presence of dimer???
- Traces of impurities/fragments
- Presence of higher molecular weight species
  - EPO within aggregates?

• Polysorbate + Glycine

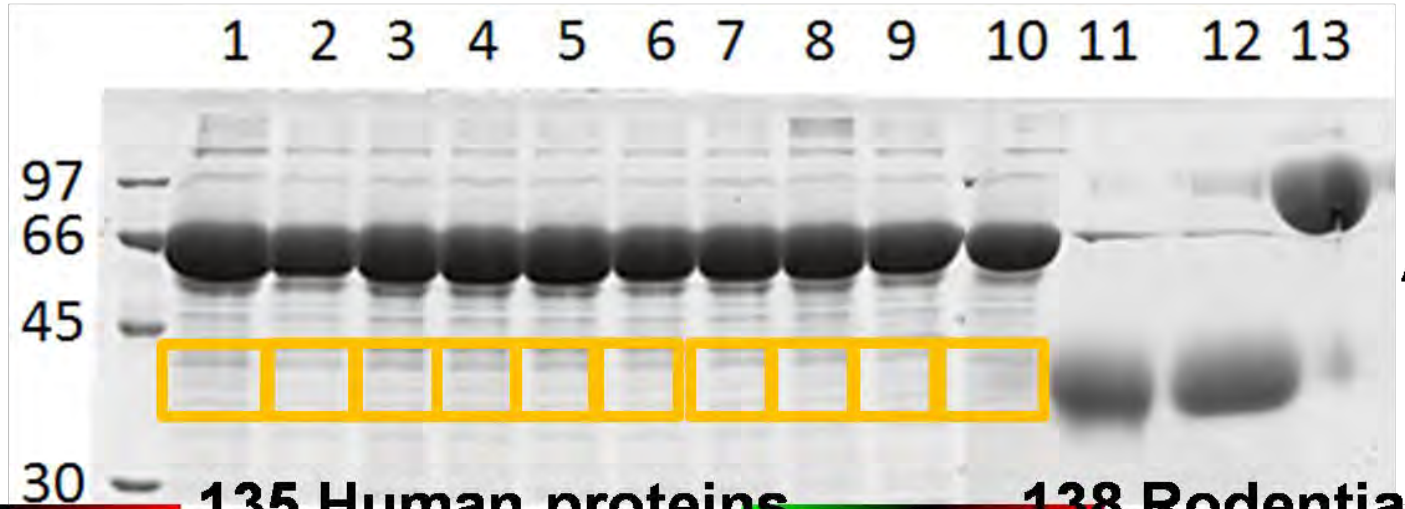
- EPO peak is detected
- No dimer
- No traces of impurities/fragments
- Absence of higher molecular weight species

GeLC-MS analysis of impurities in Epoetin



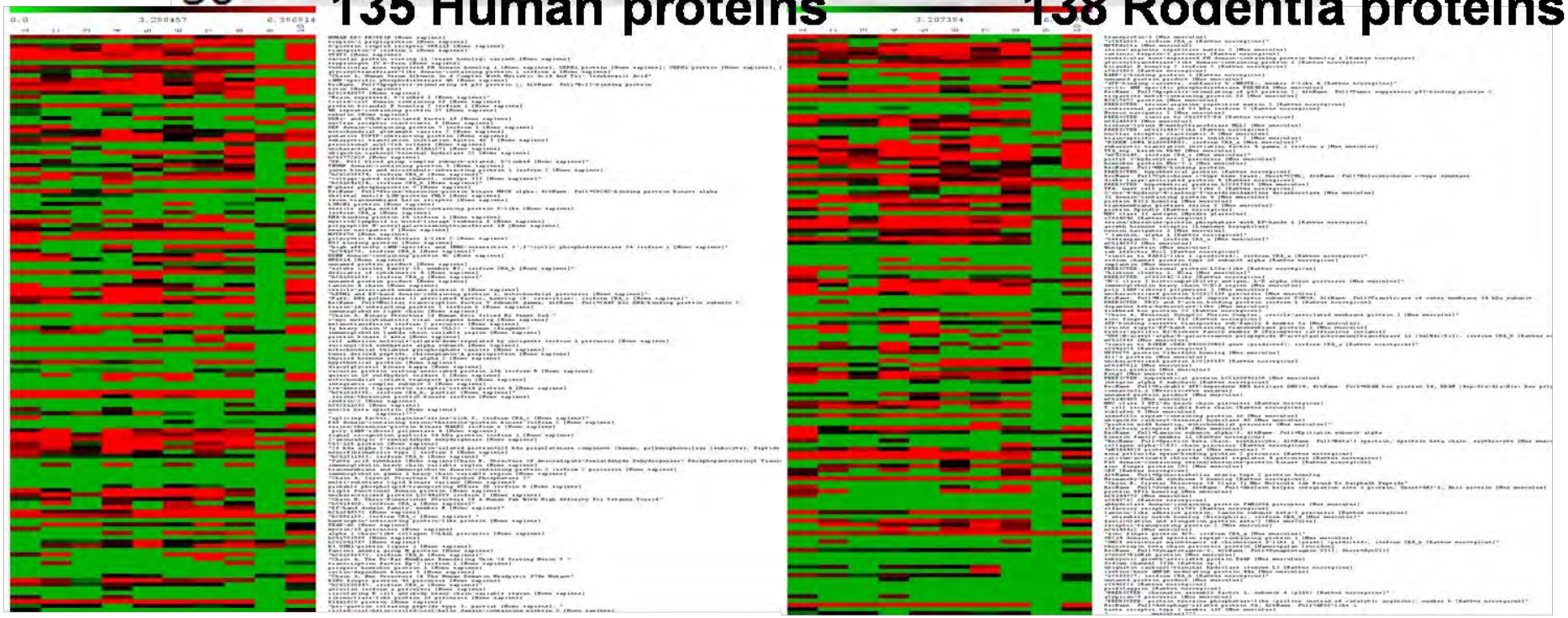


GeLC-MS analysis of impurities in Epoetin

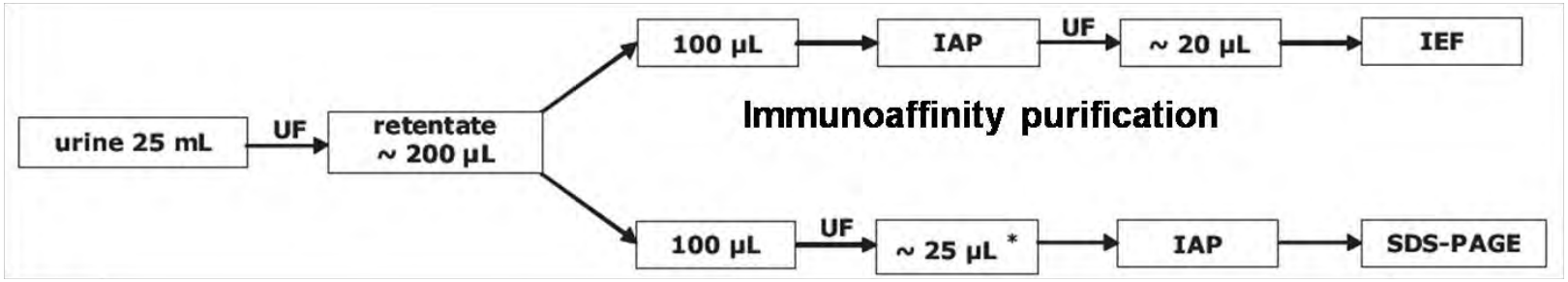


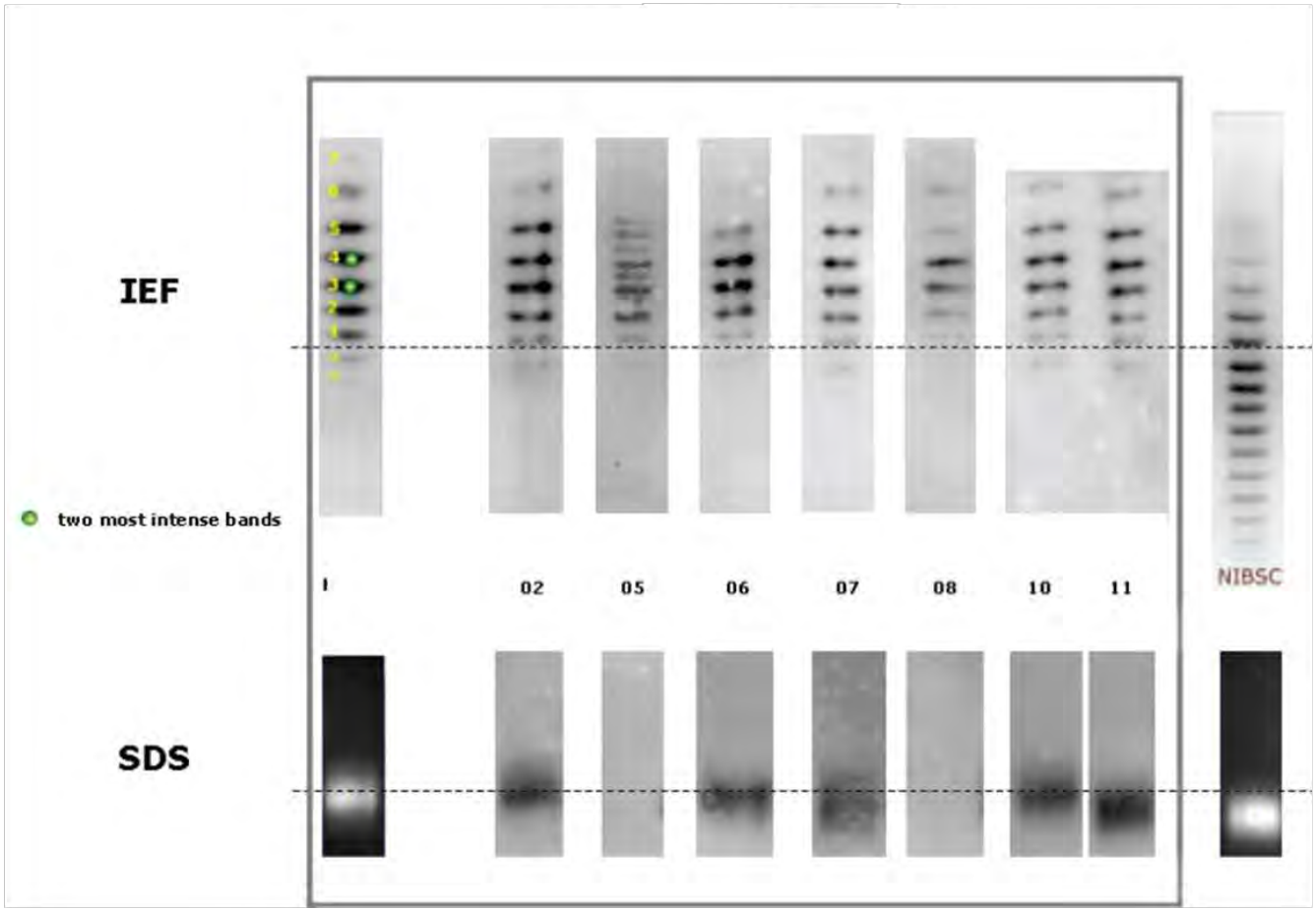
135 Human proteins

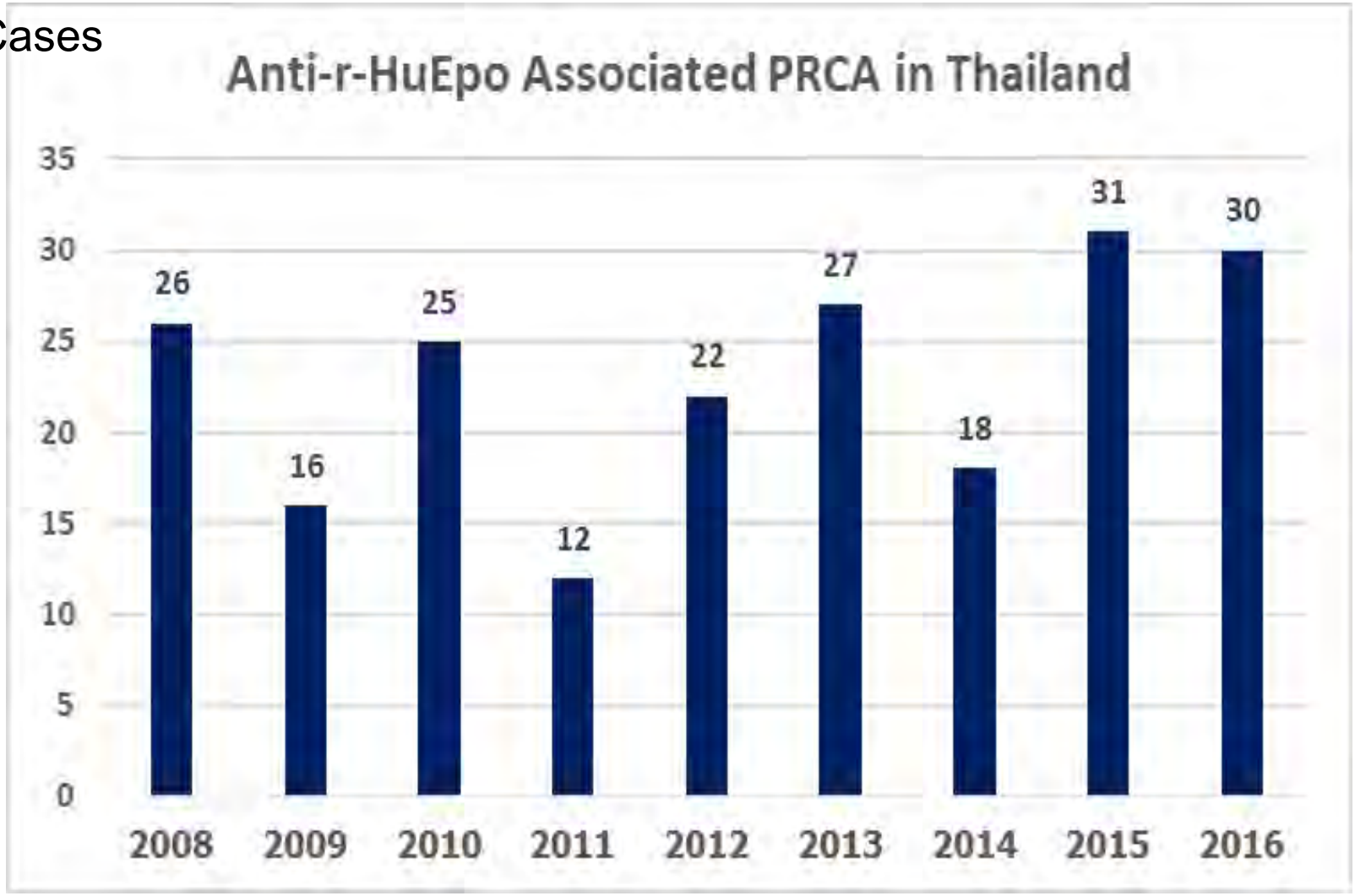
138 Rodentia proteins



Urine Profiles of Individual Epo







Mean = 23 cases/year
Total = 207 cases

Up to 6 Jan 2017

Clinical Nephrology, Vol. 81 – No. 5/2014 (355-358)

Rechallenge with intravenous recombinant human erythropoietin can be successful following the treatment of anti-recombinant erythropoietin associated pure red cell aplasia

Kearkiat Praditpornsilpa[1], Khajohn Tiranathanakul[1], Saengsuree Jootar[2], Kriang Tungsanga[1], and Somchai Eiam-Ong[1]

Table 1. Clinical and laboratory data of 5 patients re-challenged with intravenous r-HuEpo after the reversal of anti-r-HuEpo antibody.

Case	Age (years)	CKD stage	Duration of subcutaneous r-HuEpo (month)	Dose of subcutaneous r-HuEpo before PRCA	Hb during loss of efficacy (g/dL)	Duration of immunosuppression (month)	Anti-r-HuEpo titer	Interval between reversal of antibody and re-challenge (month)	Dose of intravenous r-HuEpo after the antibody reversal	Anti-r-HuEpo at 6th month after r-HuEpo re-challenge
1	55	IV	9	125 u/kg/week	6.5	6	1:10,000	5	140 u/kg/week	negative
2	49	V	18	120 u/kg/week	6.8	5	1:10,000	1	142 u/kg/week	negative
3	53	V	11	160 u/kg/week	5.7	4	1:10,000	6	160 u/kg/week	negative
4	48	IV	8	107 u/kg/week	5.8	6	1:10,000	0	140 u/kg/week	negative
5	39	V	9	125 u/kg/week	5.9	6	1:10,000	0	166 u/kg/week	negative

Final Report

A Prospective, Immunogenicity Surveillance Registry of Erythropoiesis Stimulating Agent (ESA) with Subcutaneous Exposure in Thailand

Prepared by

Center of Excellence for Biomedical and Public Health Informatics
Faculty of Tropical Medicine
Mahidol University

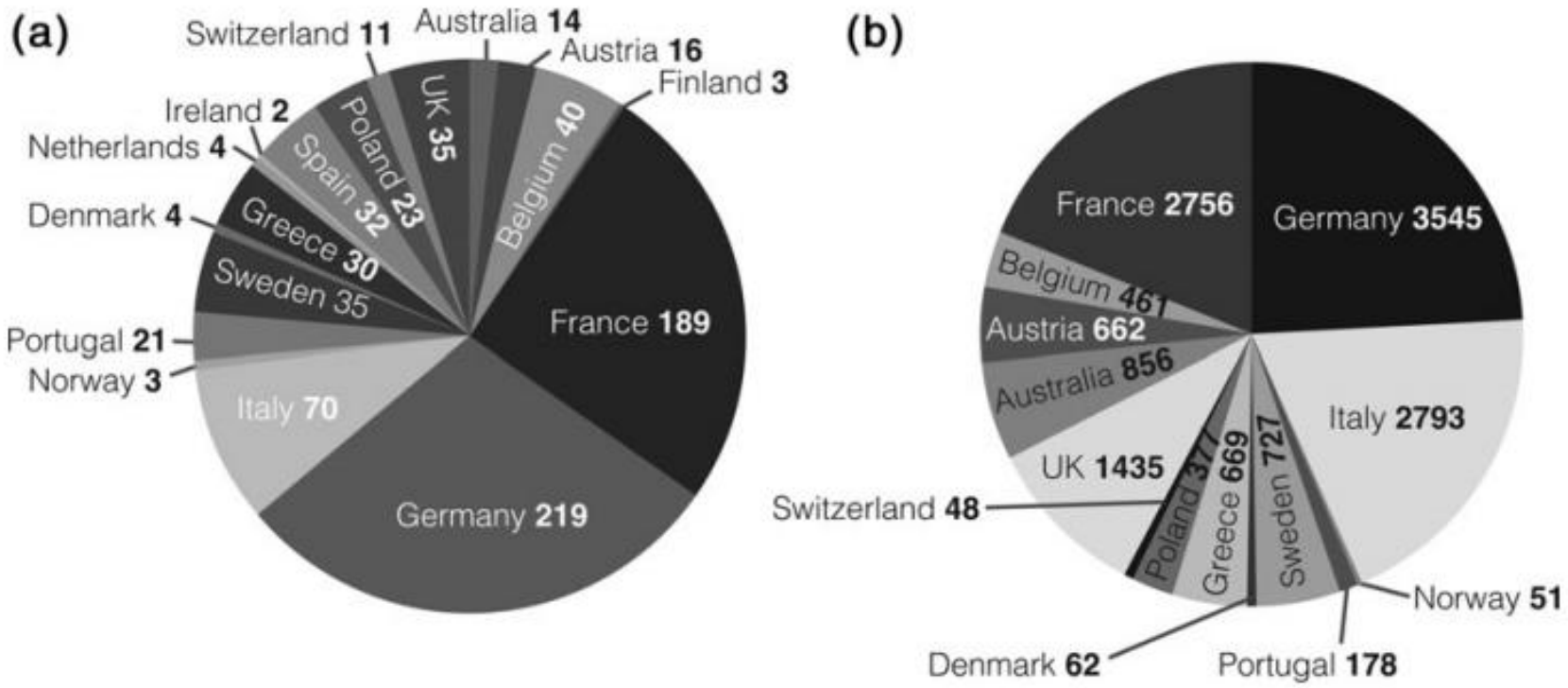
Table 1: Demographic data of the registry patients (n = 4018)

Characteristics	
• Male/Female (%/%)	49.9/50.1
• Age: Mean(SD) (Year)	57.4 (15.3)
• CKD Vintage: Mean (SD) (%)	18.6 (45.3)
• History of kidney transplantation (%)	1.28
• Clinical indication of ESA (%)	
Renal anemia	99.8
Myelodysplastic syndrome	0.2

Table 1: Demographic data of the registry patients (n = 4018)

• Stages of CKD (%)	
CKD stage 1	0.1
CKD stage 2	0.1
CKD stage 3	2.6
CKD stage 4	13.1
CKD stage 5	84.3
• Serum creatinine: Mean (SD) (mg/dL)	9.02 (5.67)
• Estimated GFR: Mean (SD) (mL/min/1,73m^2)	8.8 (7.9)

Incidence of erythropoietin antibody-mediated pure red cell aplasia: the Prospective Immunogenicity Surveillance Registry (PRIMS)



Nephrol Dial Transplant (2015) 30: 451–460

Case	Age at onset of LOE (years)/ gender	Race	Primary CKD cause	CKD stage	Product	ESA storage	Duration of exposure up to LOE
1	76/Male	White	Renovascular disease and hypertension	4	Aranesp®	Home	14 months
2	63/ Female	White	Renovascular disease and hypertension	5	NeoRecormon®	Home	6 months
3	92/Male	White	Unspecified	3	Eprex®	Home	11 months
4	66/Male	White	Unspecified	5	Eprex®	Home	21 months
5	85/Male	White-Indonesian	Polycystic kidney disease, renovascular disease and hypertension	4	Eprex®	Home	11 Months

Nephrol Dial Transplant (2015) 30: 451–460

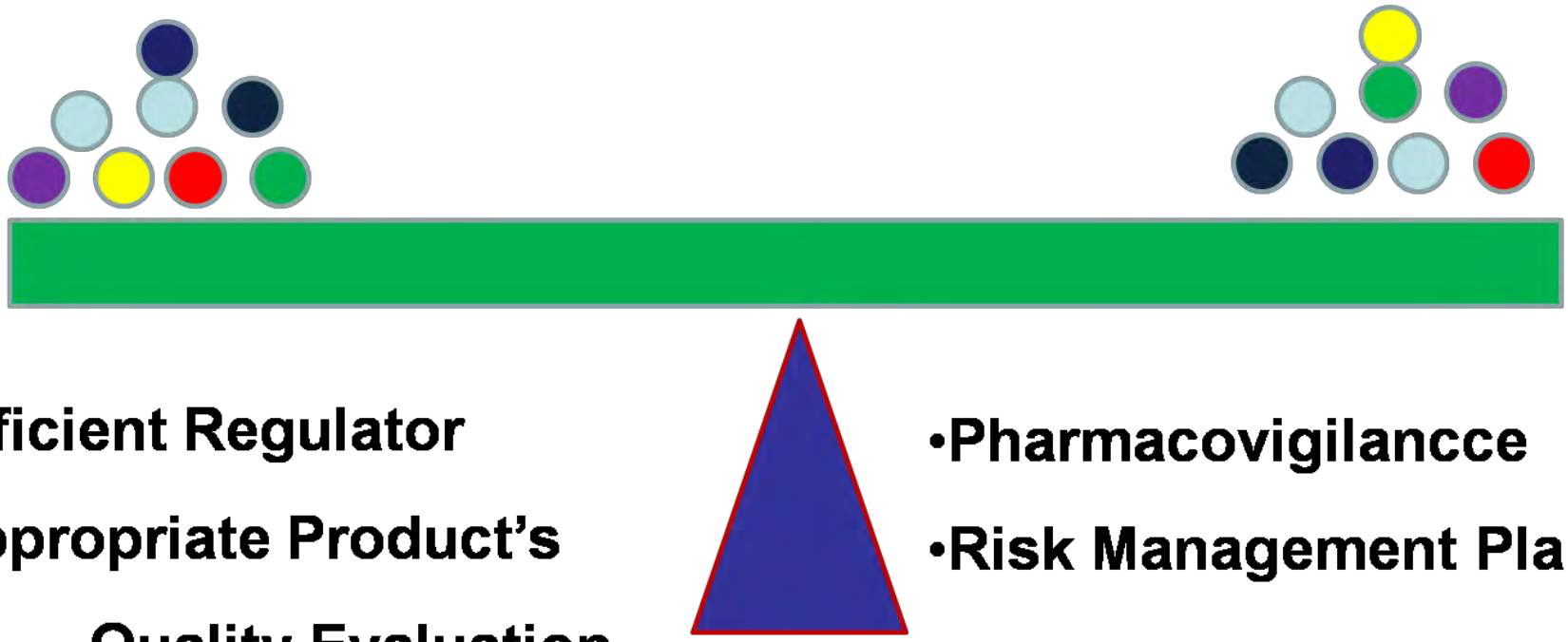
•R&D

•Patient's Safety

•Efficacy

•Access to Treatment

•National Financial Burden



•Efficient Regulator

•Appropriate Product's Quality Evaluation

•Effective National AE Report

•Pharmacovigilancce

•Risk Management Plan