

# EXHIBIT

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## **Suggested Elimination Pathways of CERA in CKD Patients**

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## Elimination Pathways of EPO & PEG

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## Elimination Pathways of EPO

- **Major Elimination Pathways** are within the "Bone Marrow & Spleen" that include,
  - EPO Receptor-mediated Internalization, and
  - Proteolysis
- **Those minors** are within the "Kidney & Liver" that include,
  - Glomerular Filtrations in the Kidney, and
  - Peptidase-mediated Catabolism in the Liver

(Adopted from Jelkmann. W., Eur. J. Hematol., 69 (2002) 265-274)

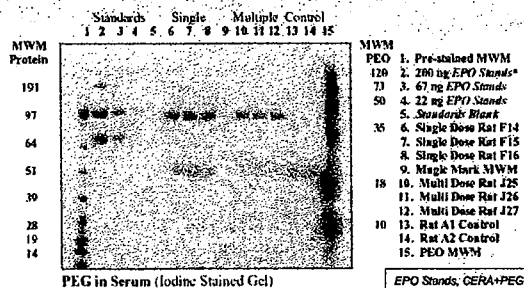
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## Part 1 Elimination Patterns of CERA *in Rats*

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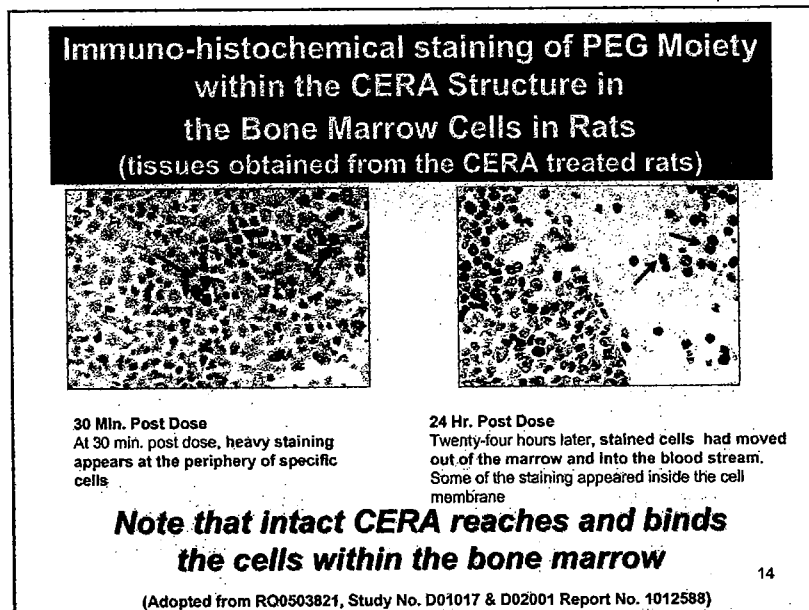
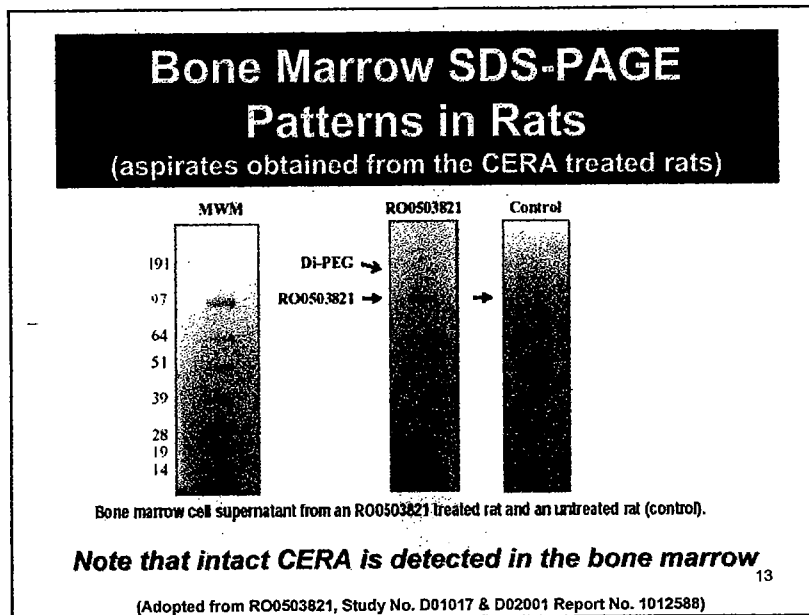
## Serum SDS-PAGE Patterns in Rats (serum obtained from the CERA treated rats)



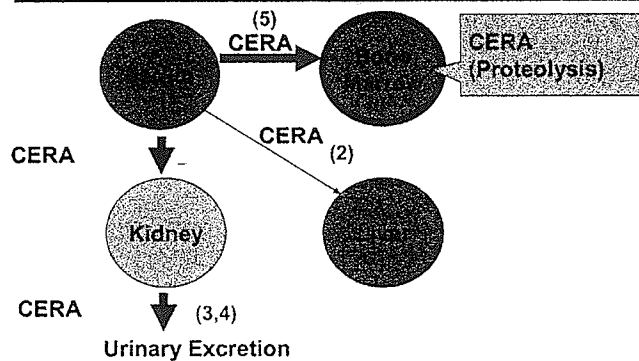
**Note that CERA remains intact in the serum**

(Adopted from RO0503821, Study No. D01017 & D02001 Report No. 1012588)

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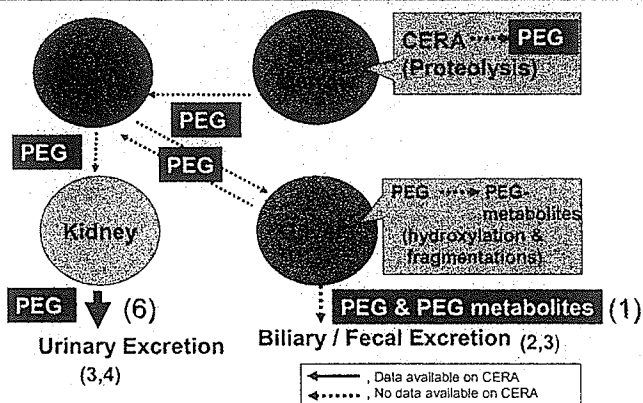
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## Suggested Elimination Pathways of CERA in Rats



The numbers in the parenthesis represent those appeared in the "Summary of Non Clinical DMPK Results" (p.15).

## Suggested Elimination Pathways of PEG in Rats



The numbers in red in the parenthesis represent the excretion rate.

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## Urinary Excretion of CERA in Healthy Volunteers (HVs)

### ➤ *Routes of CERA Administrations*

S.C. administrations of CERA ( $3.2 \mu\text{g/kg}$ ) to the HVs., and the urine was collected at an interval of every 12 hours for successive 3-days after the dosing.

### ➤ *Results*

No CERA was apparently detected in the HVs urine (No data available on the PEG moiety elimination patterns)

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(Adopted from RO0503821, Protocol No. BP16198 Report No. 1004598)

## The immune system related to CERA elimination in Human

### ➤ *Clinical Data*

No Anti-CERA Antibodies detected in the Serum of CERA Treated HVs and Pts.

The concentrations of TNF- $\alpha$  and IL-6, which are cytokines reflecting activated macrophage, in the serum showed no relationship to AUC in CERA Treated HVs

### ➤ *Speculative Synthesis*

The immune system *per se* does not seem to play crucial roles in the elimination of CERA

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(Adopted from RO0503821 all clinical studies and Protocol No. BP17570, Report No. 1017562)

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## **Summary of Clinical DMPK Results**

- 1) *Intact CERA detected in the serum.*
- 2) *No intact CERA was apparently detected in the HVs urine.*
- 3) *No Anti-CERA Antibodies was detected in the Serum of CERA Treated HVs and Pts.*
- 4) *No distinct relationship shown between AUC and kidney/liver functions.*

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## **Speculative Diagrams (HV's)**

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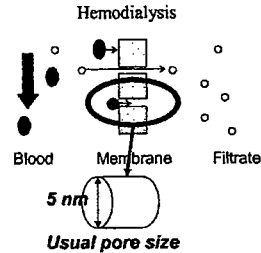
## Glomerular Filtrations vs. Hemodialysis

### ● Glomerular Filtrations

- 30 kDa of PEG is filtrated via glomerular apparatus within the kidney.
- 30 kDa of PEG has a diameter of about 8 nm, whereas the pore size of the glomerular basement membrane is within the range of 3-5 nm.
- Nevertheless, the PEG flexibility and deformability together with its rod-like conformation allow for the filtration of PEG.

(Adopted from Nakaoka R. et. al., J. Control. Release 46 (1997) 253-262)

### ● Hemodialysis



- The usual pore size of the HD membrane is similar to that of the glomerular basement membrane.
- However, the pore size of all dialysis membranes are not always available.

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## PEG Elimination in Hemodialysis Patients

- **The PEG moiety of 30 kDa is thought to be filtrated through the dialysis membrane possessing the usual pore size.**
- **As to the elimination of the PEG moiety, if the dialysis membrane whose pore size is smaller than that of the glomerular basement membrane were to be employed, however the contribution of the hepatic metabolism and the fecal excretion of PEG would be much greater.**

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## **Summary of Suggested Elimination Pathways of CERA**

- *CERA is noticeably taken up by the EPO receptor localizing the bone marrow cells.*
- *The EPO moiety within the CERA structure is undergone by the proteolytic reactions in the bone marrow cells, resulting in PEG moiety formation.*
- *The residual PEG moiety within the CERA structure predominantly is excreted into the urine.*
- *The residual PEG moiety within the CERA structure is suggested to cross the dialysis membrane of the normal pore size employed for the dialysis patients.*
- *If the dialysis membrane whose pore size is smaller than that of the glomerular basement membrane were to be employed, however the contribution of the metabolism and the fecal excretion would be much greater in the elimination of the PEG moiety.*

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## **Points-to-Consider**

- *We have suggested several elimination pathways of CERA based on the circumstantial evidences reported by EPO & PEG.*
- *While showing the suggested elimination pathways of CERA to the regulatory agency is important, the agency may require the study data in which CERA is employed.*
- *Therefore, the willingness of Chugai is that a small scale additional PK studies after administrations of CERA will have to be done.*
- *The rationale behind this idea is that the urinary excretion and the filtration of PEG are thought to be dependent upon the disease status, i.e., PreD, HD or CAPD, and upon the pore size of hemodialysis membrane.*

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## **Proposal to Roche**

- *To investigate the metabolism and excretion patterns of  $^{14}\text{C}$ -CERA in nephrectomized rats.*
- *"The mass-balance studies using  $^{14}\text{C}$ -CERA" to be performed in PreD patients & HV's.*

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***"Thank you for your attention"***

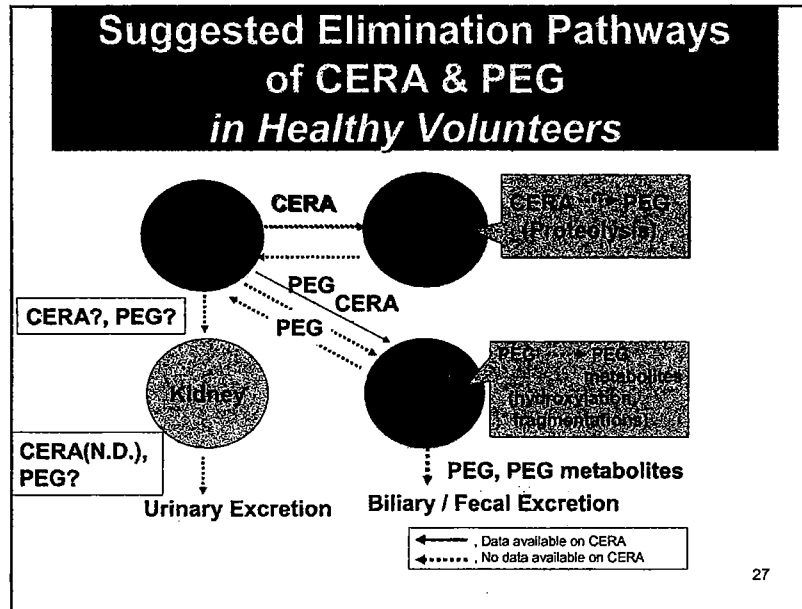
***Kiyo***

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### Why do we concern about the elimination of PEG in Hemodialysis Patients?

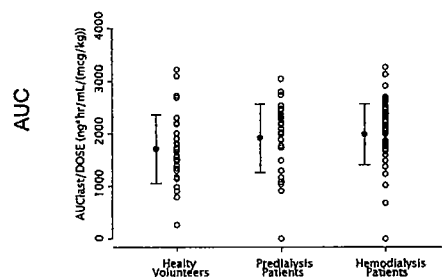
- *The major target population of CERA is CKD patients undergoing hemodialysis.*
- *In both EU and US, the average hemodialysis period is known to be 2- or 3-years, because kidney transplantations are being frequently carried out for the patients.*
- *Unfortunately, however, as far as the medical practices in Japan are concerned, the kidney transplantations are rarely done, and consequently the hemodialysis period is longer than 10-years.*

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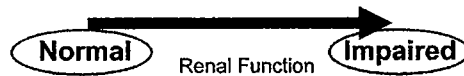
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## Relationships between Serum AUC and Renal Functions in Human

(i.v. administrations of CERA to HVs & Pts)



- No distinct relationship between serum AUC and renal functions were manifested
- These data strongly suggest that the kidney is not the main site by which the elimination of CERA is mediated

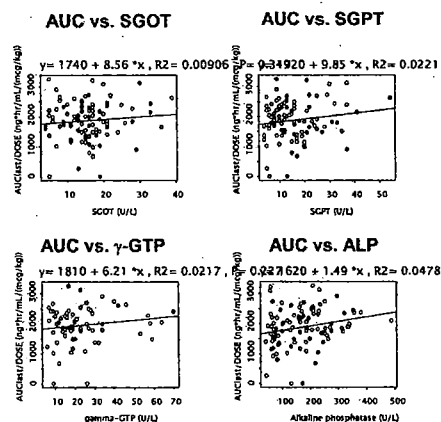


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(Adopted from RO0503821 clinical studies, the integrated analysis of 5 (five) clinical studies in Japanese)

## Relationships between Serum AUC vs. Hepatic Functions in Human

(after i.v. administrations of CERA to HVs & Pts)



- No distinctive relationship between serum AUC and hepatic functions

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(Adopted from RO0503821 clinical studies, the integrated analysis of 5 (five) clinical studies in Japanese)



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## **Summary of Non Clinical DMPK Results**

- 1) *Only intact CERA detected in the serum.***
- 2) *Radioactivities detected in the bile and the liver.***
- 3) *In both urine and the feces, radioactivities also detected.***
- 4) *Both intact CERA and PEG moiety within the CERA structure detected in the urine.***
- 5) *Intact CERA reaches and binds cells in the bone marrow.***

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## **Speculative Diagrams (Rats)**

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### Distribution & Excretion Patterns of CERA after Single IV Injection to Rats (data obtained from studies using radioactive CERA)

➤ **Tissues with high radioactivities examined were,**

blood, bile, adrenal grand medulla,  
lymph nodes, lung, testis, and adrenal gland

➤ **% radioactivities detected after CERA administrations were,**

urine (58 %), feces (8.6 %), and  
carcass (33 %), respectively.

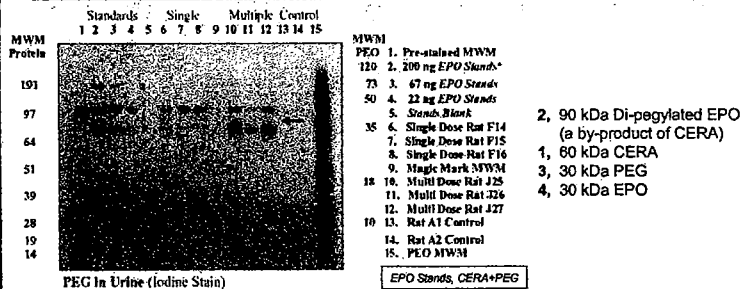
(% of CERA administered)

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(Adopted from RO0503821, Study No. 08223 Report No. 1014641)

### Urine SDS-PAGE Patterns in Rats

(urine obtained from the CERA treated rats &  
iodine staining of PEG-moiety within  
the CERA structure)



**Note that both intact CERA and 30kDa PEG  
are detected in the urine**

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(Adopted from RO0503821, Study No. D01017 & D02001 Report No. 1012588)

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## Elimination Pathways of PEG

- **Major Elimination Pathways** are within the "Kidney & Immune System" that includes,
  - Glomerular Filtrations in the kidney, and
  - Phagocytosis via the Immune System, particularly in the case of PEG m.w. is over 100 kDa
- **That minor** is within the "Liver" that include,
  - Metabolisms, i.e., Hydroxylation and Fragmentations, in the Liver, followed by
  - Biliary Excretion

(Adopted from Caliceti P. et. al., Advanced Drug Delivery Reviews., 55 (2003) 1261-1277 7  
and in house report in Chugai)

## Suggested Elimination Pathways of CERA

1. Rats
2. Healthy volunteers
3. Hemodialysis patients

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## **Proposal to Roche**

- *To investigate the metabolism and excretion patterns of  $^{14}\text{C}$ -CERA in nephrectomized rats.*
- *"The mass-balance studies using  $^{14}\text{C}$ -CERA" to be performed in PreD patients & HVs.*

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## **Special Remarks**

- As far as the protein-based medicines are concerned, the Japanese Agency has never ever requested the metabolism patterns of the substance *per se* at the time of submissions.
- Essentially the same conditions can be applied to those in overseas.
- In the case of CERA, however, not a few investigators reported that the major elimination pathway of PEG is via an urinary excretion at least in the healthy volunteers (HVs).
- Due to the hypouresis manifested in CKD, it seems very likely that the PEG cannot be efficiently excreted into the urine.
- Therefore, the Japanese Agency will request the elimination patterns of the PEG moiety in CERA to Chugai.
- We believe it quite reasonable to prepare the answers as to the elimination patterns of CERA from the body.

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