

EXHIBIT E

KAYE SCHOLER LLP

Dr. Sebastian Jungermann
 Tel. +49 69 25494 300
 Fax +49 69 25494 553
 sjungermann@kayescholer.com

Kaye Scholer LLP
 425 Park Avenue
 New York, New York 10022-3598
 Tel +1 212 836-8000
 Fax +1 212 836-8689
 www.kayescholer.com

Bioassay - Labor für biologische Analytik
 Frau Karin Rominger-Salzmann
 Veilchenweg 1
 D-70839 Gerlingen

Vorab via Email: info@bio-assay.de



27. März 2007

Probenübergabe Epoetin beta, Mircera

Sehr geehrte Frau Rominger-Salzmann,

Sie erhalten auf Trockeneis jeweils 5 Prüfmuster Epoetin beta und Mircera.

<u>Probenbezeichnung</u>	<u>Proteinkonzentration [mg/mL]</u>
1. EPO, Kontrolle (37°C)	0.24
2. EPO, deglykolisiert (37°C)	0.10
3. RO0503821, Kontrolle (37°C)	0.13
4. RO0503821, deglykolisiert (37°C)	0.12
5. EPO, Kontrolle (32°C)	(noch nicht bestimmt)
6. EPO, deglykolisiert (32°C)	(noch nicht bestimmt)
7. RO0503821, Kontrolle (32°C)	(noch nicht bestimmt)
8. RO0503821, deglykolisiert (32°C)	(noch nicht bestimmt)
9. EPO Ausgangsmaterial	8.8
10. RO0503821 Ausgangsmaterial	5.9

Prüfmuster 5 – 8 sind als Backup zu betrachten. Diese Prüfmuster sollen in einem Kinetikversuch geprüft werden. Die Proteinwerte folgen sobald sie vorliegen. Das Protokoll ist in der Anlage.

Mit freundlichen Grüßen

Dr. Sebastian Jungermann

19016331_V2.DOC

Protocol of the Comparison Study for Epoetin beta and Mircera

5 groups of each 20 mice

- | | |
|--------------------------------|-------------|
| 1. Vehicle | |
| 2. Epoetin beta | 100ng/mouse |
| 3. Epoetin beta deglycosilated | 100ng/mouse |
| 4. Mircera | 100ng/mouse |
| 5. Mircera deglycosilated | 100ng/mouse |

Dosage based on protein content OD280nm
Reticulocyte counting after 2, 3, 4 and 5 days, 5 mice/group each

REDACTED

Protocol of Sample Preparation for the Comparison Study for Epoetin beta and Mircera

1. Samples to be included into the Study:

- | | | |
|-----|-----------------------------|-------------|
| 1.1 | Vehicle | |
| 1.2 | Epoetin beta | 100ng/mouse |
| 1.3 | Epoetin beta deglycosilated | 100ng/mouse |
| 1.4 | Mircera | 100ng/mouse |
| 1.5 | Mircera deglycosilated | 100ng/mouse |

2. Sample Preparation and Analytics

Sample preparation will occur based on the protocol applied by the group of Hans Koll in 1999 (main critical point is the solubility of the deglycosylated samples, therefore a protein concentration of 0.25 mg/mL should not be exceeded).

2.1 Deglycosylation

- Dilute protein samples in 10 mM sodium/potassium phosphate containing 100 mM NaCl and 0.01 % Polysorbate 20, pH 7.5 to achieve a concentration of 0.25 mg/mL
- resuspend N-glycosidase F such that the suspension has an activity of 1U/ μ L
- adjust the concentration of neuraminidase such that the solution has a concentration/activity of 0.04 U/ μ L
- to each 100 μ L of the EPO or RO0503821 samples, add 1 μ L of N-glycosidase F solution and 1 μ L of neuraminidase solution
- incubate over night at 37°C
- per 100 μ L of sample volume, add 5 μ L 10 % TFA

2.2 Separation

Separation will be performed by means of reversed phase chromatography, the protocol will be discussed and agreed between the group of Hans Koll and Karin Christa+ Frank Zettl, TE-DR.. After chromatographic separation, the samples are dried in a speed vac and resuspended in dilution buffer. The concentration of the samples should not exceed 0.25 mg/mL.

2.3 Storage and Shipment

Samples are split into different portions and stored/shipped frozen at -70°C or dry ice.

2.4 Analytics

The analytical program includes the following methods:

- Normomouse Bioassay (see Memo from Wulf Pahlke)
- Assay (UV) for protein determination
- SDS PAGE of samples after resuspension in dilution buffer and after freeze thaw (check integrity of the molecule as well as success of deglycosylation)
- RP-HPLC of samples after resuspension in dilution buffer and after freeze thaw (check for aggregates and success of deglycosylation)
- SE-HPLC of samples after resuspension in dilution buffer and after freeze thaw (check for aggregates)
- LysC Peptide Mapping with UV and Mass Detection (identity, check for success of deglycosylation)

Timelines:

To meet the requested timelines, the following schedule is required:

- Deglycosylation from Monday March 19th to Tuesday March 20th (TE-DAC)
- Purification by means of RP-HPLC in TE-RD and TE-DRA from Tuesday, March 20th to Friday, March 23rd
- Shipment to Mannheim on Friday, March 23rd
- Bioassay to be performed from Monday, March 26th to Friday March 30th
- Other analytical methods to be performed starting from Monday, March 26th

Risks/Issues:

- The experiment has not been performed since 1999, there is no possibility for training and test experiments (only one shot!)
- Due to the very low protein concentration of the samples, multiple cycles of chromatographic separations are required to purify the samples
- Availability of more than one RP column needs to be checked