

# EXHIBIT 2

CONFIDENTIAL



Mircera:  
in vivo  
Aktivität

März  
2007

Code  
135 58 30  
4 011462 135519

EXHIBIT  
Cords-10-11  
5/30/07  
LF

Vergleichsstudie

Memo

6-7 mg/ Probe



Pharmaceuticals

To:

Copies:

From: Markus Dembowski TE-DA  
Bldg 221/458  
Tel. +49-8856-607831  
Fax +49-8856-603201

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

1. Samples to be included into the Study:

- |                                |             |
|--------------------------------|-------------|
| 1. <del>Vehicle</del>          |             |
| 2. Epoetin beta (Mock)         | 100ng/mouse |
| 3. Epoetin beta deglycosylated | 100ng/mouse |
| 4. Mircera (Mock)              | 100ng/mouse |
| 5. Mircera deglycosylated      | 100ng/mouse |
- Inkub. ohne ENZYM

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).

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/ $\mu$ L
- adjust the concentration of neuraminidase such that the solution has a concentration/activity of 0.04 U/ $\mu$ L)  $\rightarrow$  Lsg. verwenden (ohne Verdünnung)
- to each 100  $\mu$ L of the EPO or RO0503821 samples, add 1  $\mu$ L of N-glycosidase F solution and 1  $\mu$ L of neuraminidase solution
- incubate over night at 37°C
- per 100  $\mu$ L of sample volume, add 5  $\mu$ L 10 % TFA

1  $\mu$ L in 250  $\mu$ L resuspend. (H<sub>2</sub>O)

## 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 0.20 mg/mL.

## 3. Storage and Shipment

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

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

## 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