UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

AMGEN INC., Plaintiff, v. F. HOFFMANN-LA ROCHE LTD., a Swiss Company, ROCHE DIAGNOSTICS GmbH, a German Company and HOFFMANN-LA ROCHE INC., a New Jersey Corporation, Defendants.	**Exhibit C** C. A. No.: 05-CV-12237-WGY

PUBLIC VERSION

Exhibit 48

Declaration of Robert M. Galvin in Support of Amgen Inc.'s Reply in Support of its Motion for Summary Judgment of Infringement of '422 Claim 1, '933 Claim 3, and '698 Claim 6

ELUCIDATION OF STRUCTURE

SUMMARY: EVIDENCE OF STRUCTURE

In the current study, the RO0503821 registration batches G004.05E to G008.05E as well as the batches G011.05E, G013.05E and G014.05E (produced with EPO from building 354, denoted as EPO/354) were analyzed using a set of different analytical methods to investigate the structure of the RO0503821 molecule. The methods were chosen to characterize the RO0503821 molecule with respect to the molecular mass, the positional isomer distribution, the glycostructures and the higher order structure. The purpose of the study was to demonstrate the consistency of the RO0503821 manufacturing process and to compare RO0503821 drug substance produced with the 3F process (registration batches and the three qualification batches for EPO/354) to drug substance produced previously according to the preliminary process, the 1F process and the 2F process. For details regarding the developmental history of the process refer to 3.2.S.2.6 "Developmental History".

Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) was used to determine the molecular mass of the different RO0503821 batches. The signals of the mass spectra obtained from the RO0503821 registration batches and the RO0503821 EPO/354 batches correspond to the signals that are also present in the spectra obtained from the measurements of the previous and current RS. The molecular masses of the main component are in accordance to the molecular mass expected from RO0503821. In addition, it was found that the molecular masses of the RO0503821 molecule of the registration batches as well as of the RO0503821 batches (EPO/354) are highly consistent and comparable to the molecular masses of the previously used RS (R204421) as well as to the current RS (782 386 00).

The sites as well as the degree of pegylation of the RO0503821 batches were analyzed by means of LysC-peptide mapping. The amino terminus, Lys 45 and Lys 52 constitute the major pegylation sites of the RO0503821 molecule exhibiting a degree of pegylation of approximately 40 %, 15 % and 25 %, respectively. Lys 20, Lys 116 and Lys 154 are also significantly pegylated (degree of pegylation between approximately 5 % and 10 %). The remaining Lys 97, Lys 140 and Lys 152 are either not affected by pegylation or exhibit a degree of pegylation close to the detection limit (DL) of the method (detection limits calculated for different positional isomers range from 1 to 5 %). With the analysis of the registration batches it was demonstrated that with respect to the positional isomer distribution, the 3F process is performed with a high degree of consistency. The positional isomer distributions determined are within the range defined by RO0503821 batches produced previously using the preliminary, 1F and 2F production processes. In addition, the positional isomer distributions of the RO0503821 batches (EPO/354) were found to be comparable to the

registration batches as well as to the database derived from previously produced batches.

Besides the analysis of the positional isomers, the carbohydrate part of the RO0503821 molecule was investigated and compared to the carbohydrate structures of EPO used for pegylation. The assessment was made with respect to the asialo N-linked oligosaccharides, the O-linked oligosaccharides and the total sialic acid content. The purpose of the analysis was to investigate the influence of the RO0503821 manufacturing process on the carbohydrate structure of the EPO protein used for pegylation as well as to demonstrate the consistency of the RO0503821 manufacturing process.

The N-linked glycans were enzymatically released from the protein by means of N-glycosidase F treatment, the sialic acids were cleaved off simultaneously by neuraminidase treatment. The released asialo N-linked oligosaccharides were analyzed using high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). With the analyses performed it was found that for the registration batches as well as for the batches produced with EPO/354, the relative amount of tetraantennary and lactosamine structures is slightly decreased in RO0503821 as compared to EPO used for pegylation, whereas the amount of biantennary and triantennary structures is slightly increased. The differences in N-linked glycosylation observed between RO0503821 and the respective EPO used for pegylation are within the range defined by RO0503821 batches produced previously with the preliminary process, the 1F process and the 2F process. This also holds true for the RO0503821 registration batches and the batches produced with EPO/354.

The O-linked oligosaccharides of the RO0503821 registration batches and the RO0503821 batches produced with EPO/354 as well as of the respective EPO samples used for pegylation were investigated by means of LysC peptide mapping with online mass detection, the O-linked glycosylation of the samples was assessed using specific ion current (SIC) chromatograms of the LysC peptide K6 carrying the O-linked oligosaccharides. The data demonstrated that for the registration batches as well as for the batches produced with EPO/354, the relative ion count of the peptide K6_NANA2 is slightly decreased in RO0503821 as compared to EPO used for pegylation, whereas the relative ion count of the peptides K6_NANA0 and K6_NANA1 is slightly increased. The differences in O-linked glycosylation observed between RO0503821 and the respective EPO used for pegylation are within the range defined by RO0503821 batches produced previously with the preliminary process, the 1F process and the 2F process. This also holds true for the RO0503821 registration batches and the batches produced with EPO/354.

The sialic acids were enzymatically released from the protein and analyzed by means of high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). It was found that for the registration batches as well as the batches produced with EPO/354, a slight difference in the total sialic acid content was observed as compared to EPO used for pegylation.

The differences observed are within the range defined by RO0503821 batches produced previously with the preliminary process, the 1F process and the 2F process.

Taken together, it is concluded that with respect to N-linked glycosylation, O-linked glycosylation and the total sialic acid content of RO0503821 as compared to EPO used for pegylation, RO0503821 is manufactured with a high degree of consistency. For the registration batches as well as for the batches produced with EPO/354, the slight quantitative differences in glycosylation observed between RO0503821 and EPO are well within the range defined by previous batches.

The higher order structure of the RO0503821 registration batches and the RO0503821 batches (EPO/354) was investigated by circular dichroism (CD) spectroscopy. The spectra of the batches were compared to the spectra of the reference standard 782 386 00 acquired concurrently. The CD spectra of the RO0503821 solutions were acquired in the far-UV (185 nm – 260 nm) as well as in the near-UV (250 nm – 350 nm) spectral regions. The far-UV CD of proteins is due to chirally asymmetric relative orientations of neighboring peptide bonds that are different for the different classes of secondary structures (e.g. α-helix, β-sheet, β-turns, random). Therefore, the far-UV CD spectrum reflects the composite secondary structure of the protein. The intensities as well as the locations of the near-UV CD bands are dependent on the structural and electric environment of the side chains of the individual aromatic amino acids immobilized within the tertiary structure of the folded protein. As a consequence, the near-UV CD spectrum constitutes a fingerprint of the tertiary structure. With the analyses performed it was found that the far UV and near UV CD spectra of the registration batches as well as of the RO0503821 (EPO/354) batches are comparable to the spectra obtained for the reference standard. The slight differences observed are within the limits of precision of the methodology. It is concluded that with regard to the higher order structure, the RO0503821 registration batches and the batches (EPO/354) were produced with a high degree of consistency.

As a summary, it can be concluded that with respect to the molecular mass, the positional isomer distribution, the glycostructures and the higher order structure the RO0503821 registration batches as well as the batches (EPO/354) were produced in a highly consistent manner.

ELUCIDATION OF STRUCTURE

ANALYSIS OF THE PEGYLATION SITES

1. SUMMARY

The RO0503821 registration batches, RO0503821 batches produced with Epoetin beta (EPO) from building 354 (denoted as EPO/354) as well as the former reference standard (RS) R204421 (derived from batch G001.03E produced according to the 1F process) and the current RS 782 386 00 (derived from batch G002.04E produced with the 2F process) were analyzed by means of LysC Peptide mapping of RO0503821 in order to determine the positional isomer distributions.

It was demonstrated that with respect to the positional isomer distribution, RO0503821 drug substance is produced with a high degree of consistency. In addition, the positional isomer distributions obtained from the RO0503821 registration batches G004.05E to G008.05E produced with the 3F process (60 g scale manufacturing process transferred to the commercial facility) and the batches G011.05E, G013.05E and G014.05E produced with EPO/354 were found to be comparable to the data obtained from RO0503821 batches produced previously using the 1F and 2F production processes.

2. OBJECTIVE

The production of RO0503821 includes a pegylation step of EPO with the MSBA30K PEG reagent. This pegylation is the result of the reaction of the succinimidyl ester group of the MSBA30K PEG reagent with a free amino group of EPO forming an amide bond. As a consequence, the RO0503821 molecule can theoretically be pegylated at the N-terminus or at the ε-amino group of the different lysine residues. The RO0503821 variants where pegylation occurred at different amino groups are denoted as positional isomers. The name of a positional isomer indicates the amino group to which the PEG chain is attached to the EPO molecule. The main principle of this test method is based on the fact that a pegylated lysine prohibits cleavage at this site by the LysC protease.

It was the objective of the current study to determine the positional isomer distributions of the RO0503821 registration batches and batches produced with EPO/354, and the RS (former RS R204421 derived from batch G001.03E, 1F process and current RS 782 386 00 derived from G002.04E, 2F process) in order to demonstrate consistency of the production process as well as comparability of the registration batches and the batches produced with EPO/354 to the 1F and 2F processes.

3. MATERIALS AND METHODS

The data given in this report were obtained with the registration batches G004.05E to G008.05E and the RO0503821 batches (EPO/354) G011.05E, G013.05E and G014.05E. In addition, the former reference standard RS R204421 (derived from G001.03E, 1F) or the current RS 782 386 00 (derived from G002.04E, 2F) were analyzed concurrently with the RO0503821 batches. For calculation of the positional isomer distributions, the analysis of the EPO RS G030.06 was also included in each series of measurements.

Testing was performed according to the procedure described in report 3.2.S.4.2 "Peptide Mapping (Lys-C) and Polyethylene Glycol (PEG) Positional Isomer Distribution of RO0503821 Drug Substance", the characteristics of the method are also described in the respective validation report 3.2.S.4.3.

4. RESULTS

Four series of measurements were performed in order to analyze the pegylation of the RO0503821 registration batches and the RO0503821 (EPO/354). The positional isomer distributions obtained for these batches are compiled in Table 1. As can be derived from Table 1, the RO0503821 registration batches and the batches produced with EPO/354 are produced with a high degree of consistency, the positional isomer distributions determined are comparable to the previous reference standard RS R204421 and the current RS 782 386 00 measured concurrently with the registration batches.

Table 1 Pegylation Analysis of RO0503821 Batches

Series	Batch	Site of Pegylation								
		NH_2	Lys 20	Lys 45	Lys 52	Lys 97	Lys 116	Lys 140	Lys 152	Lys 154
1	RS R204421 [1]	Redacted								
1	G004.05E									
2	RS 782 386 00 [2]									
2	G005.05E									
2	G006.05E									
3	G007.05E									
3	G008.05E									
3	RS 782 386 00 [2]									
4	RS 782 386 00 [2]									
4	G011.05E									
4	G013.05E									
4	G014.05E									
4	RS 782 386 00 [2]									

[1] Reference Standard derived from batch G001.03E produced according to the 1F process
[2] Reference Standard derived from batch G002.04E produced according to the 2F process

To facilitate comparison of the positional isomer distribution of batches from the 3F process to the positional isomer distributions obtained previously with the preliminary process, the 1F and the 2F processes, the data obtained from the

registration batches and the RO0503821 batches (EPO/354) were compared to the ranges observed with previous batches.

Table 2 summarizes the statistics of positional isomer distributions of RO0503821 batches produced previously. The statistics are also illustrated in Figure 1 for the registration batches and in Figure 2 for the RO0503821 batches produced with EPO/354. The data demonstrate that the positional isomer distributions of RO0503821 batches produced according to the 3F process are within the ranges observed previously with batches derived from the preliminary process as well as from the 1F and 2F processes.

Therefore it is concluded that with respect to the positional isomer distribution of the RO0503821 drug substance, the 3F process is comparable to the previously used production processes of RO0503821.

5. CONCLUSION

It is concluded that with regard to the positional isomer distribution of the PEG-chain, RO0503821 is produced with high consistency. The data obtained for the registration batches G004.05E through G008.05E as well as for the RO0503821 batches (EPO/354) are within the range defined by the batches produced with previously used production processes.

Table 2 **Statistics of the Positional Isomer Distribution of RO0503821 Batches Produced with Different Production Processes**

	Batch	Site of Pegylation								
		NH_2	Lys 20	Lys 45	Lys 52	Lys 97	Lys 116	Lys 140	Lys 152	Lys 154
Statistics of RS 204421	Mean Standard Deviation LL [1] UL [2]	Redacted								
Statistics of RS 782 386 00	Mean Standard Deviation LL [1] UL [2]									
Statistics of Batches from the Preliminary Process	Mean Standard Deviation LL [1] UL [2]									
Statistics of Batches from the 1F-Process [3]	Mean Standard Deviation LL [1] UL [2]									
Statistics of Batches from the 2F-Process [4]	Mean Standard Deviation LL [1] UL [2]									
Statistics of Registration Batches	Mean Standard Deviation Minimum Maximum									
Statistics of RO0503821 produced with EPO/354	Mean Standard Deviation Minimum Maximum									

[1] Lower Confidence Limit, calculated using 3 standard deviations
[2] Upper Confidence Limit, calculated using 3 standard deviations
[3] The mean of RS R201440 derived from batch G001.03E was included into the statistical analysis
[4] The mean of RS 782 386 00 derived from batch G002.04E was included into the statistical analysis

Figure 1 **Degree of Pegylation of the Amino Terminus (NH_2) and the Lysine Residues of RO0503821 Registration Batches as Compared to RO0503821 Derived from Different Production Processes**

RS R204421 mean of all measurements performed

Gnnn.02E mean of the RO0503821 batches G008.02E through G013.02E from the preliminary process

Gnnn.03E mean of the RO0503821 batches G001.03E through G005.03E from the 1F process

Gnnn.04E mean of the RO0503821 batches G001.04E through G004.04E from the 2F process

Registration batches G004.05E through G008.05E from the 3F process

Error bars indicate the confidence limits calculated with 3 standard deviations

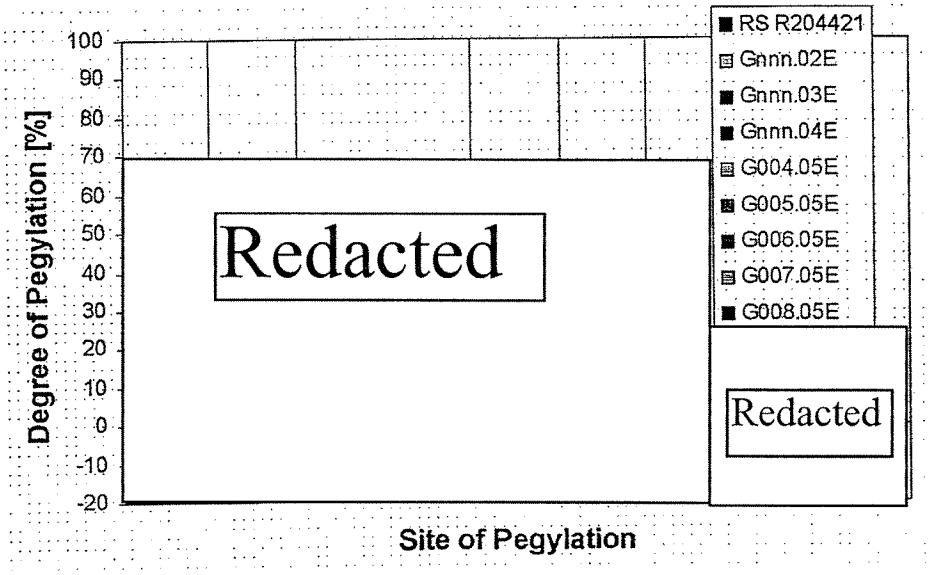

Figure 2 **Degree of Pegylation of the Amino Terminus (NH_2) and the Lysine Residues of RO0503821 Batches Produced with EPO/354 as Compared to RO0503821 Derived from Previous Production Processes**

RS 782 386 00 mean of all measurements performed

Gnnn.02E mean of the RO0503821 batches G008.02E through G013.02E from the preliminary process

Gnnn.03E mean of the RO0503821 batches G001.03E through G005.03E from the 1F process

Gnnn.04E mean of the RO0503821 batches G001.04E through G004.04E from the 2F process

Gnnn.05E Registration batches G004.05E to G008.05E from the 3F process

G011.05E, G013.05E and G014.05E (EPO/354) from the 3F process

Error bars indicate the confidence limits calculated with 3 standard deviations