APPENDIX B

Supplemental Declaration of Stephen G. Chaney, Ph.D.

I, Stephen Chaney, Ph.D., hereby make the following declaration in support of that Supplemental Position Paper submitted by sanofi-aventis S.A.:

I. Background and Qualifications

I obtained my B.S. degree in Chemistry from Duke University, my PhD degree in Biochemistry from UCLA and completed my postdoctoral training in the Department of Microbiology at Washington University in St. Louis. I have been at the University of North Carolina for 34 years where I am currently Medical Alumni Distinguished Teaching Professor of Biochemistry and Biophysics. For the past 25 years, my research has focused on platinum anticancer agents. I have extensively studied cisplatin (*cis*-diamminedichloroplatinum(II)), Pt(dach)Cl_2 (*cis*-1,2-diaminocyclohexanedichloroplatinum(II)), malonatoplatinum (*cis*-1,2-diaminocyclohexanemalonatoplatinum(II)), ormaplatin (tetraplatin, (*trans-RR*)1,2-diaminocyclohexanetetrachloroplatinum(IV)), oxaliplatin ((*trans-RR*)1,2-diaminocyclohexaneoxalatoplatinum(II)) and their biotransformation products. I developed a new HPLC methodology to separate the chemical biotransformation products of Pt(dach)Cl_2, malonatoplatin, tetraplatin and oxaliplatin[1,2] and have studied the biotransformations of those compounds *in vitro*,[2-8] in cell culture,[9-12] in rats[13-15] and in conjunction with human clinical trials.[16-18] I have studied the effect of the carrier ligand (*cis*-diammine and dach (1,2-diaminocyclohexane)), leaving ligands (chloride, malonate, oxalate, water and methionine) and oxidation state (platinum (IV) or platinum (II)) on the cellular uptake,[4,10,11,19,20] cytotoxicity in cell culture,[10,11,19,20] pharmacokinetics,[15,16,18] toxicity in dorsal root ganglia explant cutures[6] and toxicity in animals.[21] I have also characterized the effect of the DNA adducts formed by cisplatin and oxaliplatin on the molecular processes related to the efficacy, toxicity and mutagenicity of those adducts.[22-40] I have over 100 refereed (peer-reviewed) papers and review articles and I have been invited to speak at the last four International Symposia on Platinum Compounds in Cancer Chemotherapy. I am the same Stephen Chaney who authored a declaration in support of the original Position Paper submitted by sanofi-aventis.

II. Solutions of Oxaliplatin With Added Sugar

I have been asked to consider whether or not oxaliplatin might form byproducts if stored in sugar solutions for extended periods of time and whether any of the byproducts that do form could be cytotoxic or have undesirable toxicity. I have reviewed the Report from Sanofi-Synthelabo Recherche (August 2004) of a study wherein lactose, maltose, glucose and sucrose were added to solutions of oxaliplatin in water. That Report shows that the addition of sugars to oxaliplatin increases the formation of certain complexes during storage as compared to a solution of oxaliplatin in water (without added sugars) under the same storage conditions. In particular, the complexes that formed in increased amounts were Pt(IV)(DACH)$(OH)_2$(oxalato), a dimer formed from Pt(II)(DACH)$(H_2O)_2$, and a number of unidentified complexes derived from both Pt(IV)(DACH)(oxalato)(X_2) (where X_2 = unidentified ligands) and Pt(II)(DACH)$(H_2O)_2$.

The Pt(IV)(DACH) and Pt(II)(DACH) complexes that are increased by the addition of sugars to oxaliplatin are likely to be active in humans. Although the structures of some of the particular complexes resulting from the reactions of sugars with oxaliplatin and its related chemical compounds (Pt(IV)(DACH)$(OH)_2$(oxalato) and Pt(II)(DACH)$(H_2O)_2$) are currently unknown, it is well established that Pt(II)(DACH)$(H_2O)_2$ and Pt(IV)(DACH) derivatives are active and potentially toxic.[6,11,21,58,59,62]

Moreover, the type and severity of toxicity caused by these complexes may well be different from oxaliplatin. For example, we have shown that Pt(II)(DACH)(H_2O)(Cl) and Pt(II)(DACH)$(H_2O)_2$ are more cytotoxic than oxaliplatin in HT-29 cells[11] and are more neurotoxic than oxaliplatin in a rat dorsal root ganglia neurite outgrowth assay[6]. With respect to the Pt(IV)(DACH) compounds Yamashita *et al*[58] and Siddik *et al*[59] have shown that while Pt(IV)(DACH)$(OH)_2$(oxalato) is less cytotoxic than oxaliplatin in L1210 cells, other closely related Pt(IV)(DACH)(oxalato)$(X)_2$ compounds have equal or greater cytotoxicity than oxaliplatin in L1210 cells. The neurotoxicity of these Pt(IV)(DACH) compounds relative to oxaliplatin has not been determined, but we have shown that ormaplatin (tetraplatin, (*trans-RR*)1,2-diaminocyclohexanetetrachloroplatinum(IV)) is more neurotoxic than oxaliplatin both in dorsal root ganglia explant cultures[6] and in a Wistar rat model *in vivo*.[21] Ormaplatin has also been shown to be more neurotoxic than oxaliplatin in human clinical trials.[62]

When evaluating the suitability of a formulation of oxaliplatin in a solution containing a sugar it is important to evaluate the stability of oxaliplatin over the expected shelf life of that formulation and to identify any new Pt(DACH) complexes that form. Furthermore, when evaluating the potential efficacy and toxicity of the Pt(DACH) complexes that do form, such as those identified in the sanofi-aventis Report, it is important to understand that the efficacy and toxicity of such contaminants cannot easily be predicted on the basis of their similarity to oxaliplatin or other prior tested compounds. While some properties of platinum complexes (*e.g.* cellular uptake, cross-resistance and mutagenicity) can be explained on the basis of their hydrophobicity, aqueous stability and carrier ligand (*e.g.* *cis*-diammine vs. 1,2-diaminocyclohexane), other important characteristics are very difficult to predict on the basis of structural similarities. Efficacy, tumor range, and the extent and type of toxicity are determined by a combination of pharmacokinetics, pharmacodynamics, biotransformations and other factors that are not clearly understood. In the case of the Pt(dach) platinum complexes, it is quite clear that their characteristics are not solely dependent on the diaminocyclohexane carrier ligand. For example, of the Pt(dach) complexes evaluated to date, only oxaliplatin has been shown to display clear efficacy in the treatment of metastatic colon cancer[60,61] and only oxaliplatin and ormaplatin have been reported to display neurotoxicity as the dose-limiting toxicity[60-62].

III. Conclusions

Solutions containing oxaliplatin and a sugar can undergo chemical reactions leading to increases in the formation of certain Pt(DACH) complexes, which may have unexpected toxicity and may not retain the same tumor specificity as oxaliplatin.

Dated: March 1, 2007

Stephen D. Chaney

Stephen Chaney

References

1. Mauldin SK, et al. High-performance liquid chromatographic separation of platinum complexes containing the cis-1,2-diaminocyclohexane carrier ligand. *Anal. Biochem.* 1986; 157:129-143.

2. Luo FR, et al. High-performance liquid chromatographic separation of the biotransformation products of oxaliplatin. *J. Chromatography B.* 1999; 724:345-356.

3. Mauldin SK, et al. Displacement of the bidentate malonate ligand from (*d,l-trans*-1, 2-diaminocyclohexane)malonatoplatinum(II) by physiologically important compounds *in vitro*. *Biochem. Pharmacol.* 1988; 37:3321-3333.

4. Luo F, et al. *In vitro* partitioning and biotransformations of oxaliplatin in rat blood and RPMI-1640. *Proceedings of the Am. Assoc'n. for Cancer Res.* 1997; 38:311.

5. Luo F, et al. Biotransformations of oxaliplatin in rat blood *in vitro*. *J. Biochem. Molec. Toxicol.* 1999; 13:159-169.

6. Luo F, et al. Comparative neurotoxicity of oxaliplatin, ormaplatin, and their biotransformation products utilizing a rat dorsal root ganglia *in vitro* explant culture model. *Cancer Chemotherapy & Pharmacol.* 1999; 44:29-38.

7. Gibbons GR, et al. Rapid reduction of tetrachloro(*d,l-trans*)1,2-diaminocyclohexaneplatinum(IV) (tetraplatin) in RPMI 1640 tissue culture medium. *Cancer Res.* 1989; 49:1402-1407.

8. Chaney SG, et al. *In vitro* biotransformations of tetrachloro(*d,l-trans*)-1,2-diaminocyclohexaneplatinum(IV) (tetraplatin) in rat plasma. *Cancer Res.* 1990; 50:4539-4545.

9. Mauldin SK, et al. Intracellular biotransformation of platinum compounds with the 1, 2-diaminocyclohexane carrier ligand in the L1210 cell line. *Cancer Res.* 1988; 48:5136-5144.

10. Mauldin SK, et al. Effects of the bidentate malonate ligand on the utilization and cytotoxicity of platinum compounds in the L1210 cell line. *Cancer Res.* 1986; 46:2876-2882.

11. Luo FR, et al. Cytotoxicity, cellular uptake, and cellular biotransformations of oxaliplatin in human colon carcinoma cells. *Oncology Research.* 1998; 10:595-603.

12. Chaney SG, et al. An unexpected biotransformation pathway for tetrachloro-(*d,l-trans*)-1,2-diaminocyclohexaneplatinum(IV) (tetraplatin) in the L1210 cell line. *Cancer Res.* 1991; 51:969-973.

13. Thompson DC, et al. Effect of the chemoprotective agent WR-2721 on disposition and biotransformations of ormaplatin in the Fischer 344 rat bearing a fibrosarcoma. *Cancer Res.* 1995; 55:2837-2846.

14. Thompson DC, et al. Organ specific biotransformation of ormaplatin in the Fischer 344 rat. *Cancer Chemother. Pharmacol.* 1995; 36:439-447.

15. Luo FR, et al. Pharmacokinetics and biotransformations of oxaliplatin in comparison with ormaplatin following a single bolus intravenous injection in rats. *Cancer Chemotherapy & Pharmacol.* 1999; 44:19-28.

16. Petros WP, et al. Pharmacokinetic and Biotransformation Studies of Ormaplatin in Conjunction with a Phase-I Clinical Trial. *Cancer Chemother. Pharmacol.* 1994; 33:347-354.

17. Sakata M, et al. Possible correlation between ormaplatin biotransformations and neurotoxicity. *Oncol. Res.* 1995; 7:67-71.

18. Shord SS, et al. Oxaliplatin biotransformation and pharmacokinetics: a pilot study to determine the possible relationship to neurotoxicity. *Anticancer Res.* 2002; 22:2301-2309.

19. Gibbons GR, et al. Role of carrier ligand in platinum resistance in L1210 cells. *Cancer Res.* 1990; 50:6497-6501.

20. Schmidt W and Chaney SG. Role of carrier ligand in platinum resistance of human carcinoma cell lines. *Cancer Res.* 1993; 53:799-805.

21. Holmes J, et al. Comparative neurotoxicity of oxaliplatin, cisplatin, and ormaplatin in a Wistar rat model. *Toxicolog. Sci.* 1998; 46:342-351.

22. Page JD, et al. *In vitro* repair of cisplatin-DNA adducts by a defined enzyme system. *In* M. Nicolini (ed.), Platinum and other metal coordination compounds in cancer chemotherapy, pp. 115-126. *Boston*: Matinus Nijhoff Publishing, 1988.

23. Husain I, et al. Repair of cis-platinum-DNA adducts by ABC excinuclease *in vivo* and *in vitro*. *J. Bacteriol.* 1985; 163:817-823.

24. Gibbons GR, et al. Role of DNA replication in carrier-ligand-specific resistance to platinum compounds in L1210 cells. *Carcinogenesis* 1991; 12:2253-2257.

25. Page JD, et al. Effect of the diaminocyclohexane carrier ligand on platinum adduct formation, repair, and lethality. *Biochemistry* 1990; 29:1016-1024.

26. Mamenta EL, et al. Enhanced replicative bypass of platinum-DNA adducts in cisplatin-resistant human ovarian carcinoma cell lines. *Cancer Res.* 1994; 54:3500-3505.

27. Petersen LN, et al. Increased gene specific repair of cisplatin induced interstrand crosslinks in cisplatin resistant cell lines, and studies on carrier ligand specificity. *Carcinogenesis* 1996; 17:2597-2602.

28. Vaisman A, et al. Cell cycle changes associated with the formation of Pt-DNA adducts in human ovarian carcinoma cells with different cisplatin sensitivity. *Cytometry* 1997; 27:54-64.

29. Delmastro DA, et al. DNA damage inducible-gene expression following platinum treatment in human ovarian carcinoma cells. *Cancer Chemother. Pharmacol.* 1997; 39:245-253.

30. Vaisman A, et al. Cisplatin-induced alterations in the expression of the mRNAs for UV-damage recognition protein. *Oncol. Res.* 1996; 8:7-12.

31. Vaisman A, et al. The role of hMLH1, hMSH3, and hMSH6 defects in cisplatin and oxaliplatin resistance: Correlation with replicative bypass of platinum-DNA adducts. *Cancer Res.* 1998; 58:3579-3585.

32. Vaisman A, et al. Effect of DNA Polymerases and High Mobility Group Protein 1 on the Carrier Ligand Specificity for Translesion Synthesis past Platinum-DNA Adducts. *Biochemistry* 1999; 38:11026-11039.

33. Reardon JT, et al. Efficient nucleotide excision repair of cisplatin, oxaliplatin, and bis-aceto-amine-dichloro-cyclohexylamine-platinum(IV) (JM216) platinum intrastrand DNA diadducts. *Cancer Res.* 1999; 59:3968-3971.

34. Vaisman A and Chaney SG. The efficiency and fidelity of translesion synthesis past cisplatin and oxaliplatin GpG adducts by human DNA polymerase beta. *J. Biol. Chem.* 2000; 275:13017-13025.

35. Vaisman A, et al. Efficient translesion replication past oxaliplatin and cisplatin GpG adducts by human DNA polymerase eta. *Biochemistry* 2000; 39:4575-4580.

36. Vaisman A, et al. The effect of DNA structure on the catalytic efficiency and fidelity of human DNA polymerase beta on templates with platinum-DNA adducts. *J. Biol. Chem.* 2001; 276:18999-19005.

37. Havener JM, et al. Translesion synthesis past platinum DNA adducts by human DNA polymerase mu. *Biochemistry* 2003; 42:1777-1788.

38. Bassett E, et al. Efficiency of extension of mismatched primer termini across from cisplatin and oxaliplatin adducts by human DNA polymerases beta and eta *in vitro*. *Biochemistry* 2003; 42:14197-14206.

39. Bassett E, et al. The role of DNA polymerase η in translesion synthesis past platinum-DNA adducts in human fibroblasts. *Cancer Res.* 2004; 64:6469-6475.

40. Wu Y, et al. NMR solution structure of an oxaliplatin 1,2-d(GG) intrastrand cross-link in a DNA dodecamer duplex. *J. Mol. Biol.* 2004; 341:1251-1269.

41. Kartalou M and Essigmann JM. Mechanisms of resistance to cisplatin. *Mutat. Res.* 2001; 478:23-43.

42. Safaei R and Howell SB. Copper transporters regulate the cellular pharmacology and sensitivity to Pt drugs. *Crit Rev. Oncol. Hematol.* 2005; 53:13-23.

43. Safaei R, et al. The role of copper transporters in the development of resistance to Pt drugs. *J. Inorg. Biochem.* 2004; 98:1607-1613.

44. Liang XJ, et al. A pleiotropic defect reducing drug accumulation in cisplatin-resistant cells. *J. Inorg. Biochem.* 2004; 98:1599-1606.

45. Liang XJ, et al. Trafficking and localization of platinum complexes in cisplatin-resistant cell lines monitored by fluorescence-labeled platinum. *J. Cell Physiol.* 2005; 202:635-641.

46. Pinto AL and Lippard SJ. Binding of the antitumor drug *cis*-diamminedichloroplatinum(II) (cisplatin) to DNA. *Biochim. Biophys. Acta* 1985; 780:167-180.

47. Chaney SG. The chemistry and biology of platinum complexes with the 1, 2-diaminocyclohexane carrier ligand. *Int. J. Oncol.* 1995; 6:1291-1305.

48. Christen RD, et al. Signaling and drug sensitivity. *Cancer Metastasis Rev.* 1994; 13:175-189.

49. Treiber DK, et al. Cisplatin-DNA adducts are molecular decoys for the ribosomal RNA transcription factor hUBF (human upstream binding factor). *Proc. Natl. Acad. Sci. USA* 1994; 91:5672-5676.

50. Zhai X, et al. Cisplatin-DNA adducts inhibit ribosomal RNA synthesis by hijacking the transcription factor human upstream binding factor. *Biochemistry* 1998; 37:16307-16315.

51. Bancroft DP, et al. 195Pt NMR kinetic and mechanistic studies of *cis*- and *trans*-diamminedichloroplatinum(II) binding to DNA. *J. Am. Chem. Soc.* 1990; 112:6860-6871.

52. Johnson NP, et al. Kinetic analysis of the in vitro binding of radioactive *cis*- and *trans*-dichlorodiammineplatinum(II) to DNA. *Chem. Biol. Interact.* 1980; 30:151-169.

53. Butour JL, et al. Kinetics of the reaction of *cis*-platinum compounds with DNA in vitro. *Biochem. Biophy. Res. Commun.* 1985; 133:347-353.

54. Macquet JP, et al. Pharmacological and preclinical toxicology studies of 1,2-diaminocyclohexane(isocitrato)platinum(II). *Cancer Res.* 1984; 44:3736-3743.

55. Schwartz P, et al. Preparation and antitumor evaluation of water soluble derivatives of dichloro(1,2-diaminocyclohexane)platinum(II). *Cancer Treat. Rep.* 1977; 61:1519-1525.

56. Speer RJ, et al. Preclinical testing of some cisplatin cogeners as potential antitumor agents. *J. Clin. Hemat. Oncol.* 1980; 10:9-13.

57. Chaney SG, et al. Carrier ligand effects in platinum resistant cell lines. *In* S. B. Howell (ed.), Platinum and other metal coordination compounds in cancer chemotherapy, pp. 269-283. *New York*: Plenum Press, 1991.

58. Yamashita T, et al. Cytotoxicity of platinum(IV) and platinum(II) complexes containing *1R*, *2R*-cyclohexanediamine as a ligand. *Biol. Pharm. Bull.* 1993; 16:1014-1018.

59. Siddik ZH, et al. Role of p53 in the ability of 1,2-diaminocyclohexane-diacetato-dichloro-Pt(IV) to circumvent cisplatin resistance. *J. Inorg. Biochem.* 1999; 77:65-70.

60. Wiseman LR, et al. Oxaliplatin - A review of its use in the management of metastatic colorectal cancer. *Drugs & Aging* 1999; 14:459-475.

61. Mani S, et al. Oxaliplatin: a review of evolving concepts. *Cancer Invest.* 2002; 20:246-263.

62. O'Rourke TJ, et al. Phase I clinical trial of ormaplatin (tetraplatin, NSC 363812). *Anti-Cancer Drugs* 1994; 5:520-526.