sanofi aventis

Because health matters

2354 7 JUL 29 P2:22

June 29, 2007

BY HAND DELIVERY

Dockets Management Branch (HFA-305)
Food and Drug Administration
Department of Health and Human Services
5630 Fishers Lane, Room 1061
Rockville, Maryland 20852

CITIZEN PETITION SUPPLEMENT No. 4
(2003P-0064)

Sanofi-aventis US LLC, a subsidiary of sanofi-aventis, and successor in interest to Aventis Pharmaceuticals, S.A. ("sanofi-aventis") submits this Supplement No. 4 to its Citizen Petition filed February 19, 2003 (2003P-0064/CPl) (hereafter the "Citizen Petition"). This Citizen Petition Supplement No. 4 is submitted under Sections 505(b) and 505(j) of the Federal Food, Drug, and Cosmetic Act ("FDCA") (21 U.S.C. §§ 355(b) and (j)) and 21 C.F.R.§ 10.30. This Supplement No. 4 is also supported by a signed Declaration of Jeffrey Ian Weitz, M.D., which is attached hereto in Appendix A.

I. This Supplement Provides Important New Data

This Supplement No. 4 describes additional, important experimental work on distinctive and process-dependent structures within enoxaparin that lead to its biological and clinical properties. The experimental studies described herein are provided in Appendices B-F.

Enoxaparin acts as an anti-inflammatory agent.[1] Inflammation is an important contributor to arterial thrombosis in acute coronary syndromes for which enoxaparin is approved for use, such as myocardial infarction and unstable angina.[2] Data reported herein for the first time demonstrate that subtle differences in structure and relative abundance of different components may render generic enoxaparin products pro-inflammatory and thus less safe.

II. Background

The Citizen Petition requests that: (1) until such time as enoxaparin has been fully characterized, FDA refrain from approving any ANDA citing Lovenox® as the reference listed drug unless (a) the manufacturing process used to create the generic product is determined to be equivalent to sanofi-aventis's manufacturing process for enoxaparin, or (b) the application is supported by proof of equivalent safety and

2003P-0064

Sanofi-aventis U.S., PO Box 5925, Bridgewater, NJ 08807
Tel: (908) 981.5000 – www.sanofi-aventis.com

SUP 5

effectiveness demonstrated through clinical trials; and (2) FDA refrain from approving any ANDA citing Lovenox® as the reference listed drug unless the generic product contains a 1,6 anhydro ring structure at the reducing ends of between 15% and 25% of its polysaccharide chains.[3]

As explained in the prior Citizen Petition Supplement No. 3, enoxaparin is a complex mixture of polysaccharide components. The polysaccharides found in enoxaparin range in length from about 2 to over 26 sugar units (i.e. 2mers to 26mers or larger)[4] and contain many different kinds of component sugar units, some natural and some created by the manufacturing process.[5] In consequence, each different polysaccharide in the enoxaparin mixture contains a unique sequence of individual sugar components from one end of the chain to the other. The concentration of each of those different polysaccharides in enoxaparin also varies, further contributing to the complexity of the enoxaparin polysaccharide mixture.

The length and sequence, as well as the relative abundance, of the different polysaccharide chains in the enoxaparin mixture, in turn, dictate enoxaparin's complex biological and clinical activities. For example, enoxaparin is an anticoagulant, affecting targets that include antithrombin III ("ATIII"), an inhibitor of thrombin, factor Xa and other clotting enzymes, as well as tissue factor pathway inhibitor (TFPI), and others.[6] Significantly, enoxaparin is also an anti-inflammatory agent,[7] for example, in the acute coronary indications of unstable angina and myocardial infarction.[8] Enoxaparin's anti-inflammatory activity results, at least in part, from its capacity to modulate components of the contact and complement systems, as well as the expression of various cell adhesion molecules.[a,9]

Indeed, as demonstrated in the studies described below, a generic product that does not have all the same polysaccharide structures and their relative proportions present in enoxaparin may not possess the same level of anti-inflammatory activity as enoxaparin, and could dangerously even introduce pro-inflammatory activities.

However, there are no reliable, published structural comparisons that a generic drug manufacturer can use to determine whether its product has the same spectrum of anti-inflammatory activities as enoxaparin, and accordingly, the same effectiveness and safety as enoxaparin in patients. That is because the specific polysaccharide sequences responsible for enoxaparin's anti-inflammatory effects are poorly understood in the published literature, and, in many cases, unknown. In fact, even

---

[a] Enoxaparin's anti-inflammatory activity involves interactions with a number of pathways that are independent of ATIII. Those pathways include interactions with P-selectin, proteins of the complement system, and the contact-kinin system. *See* Stevenson JL, et al., Differential metastasis inhibition by clinically relevant levels of heparins – correlation with selectin inhibition, not antithrombotic activity. *Clin. Cancer Res.* 2005; 11(19):7003-7011; Gikakis N., et al. Effect of factor Xa inhibitors on thrombin formation and complement and neutrophil activation during in vitro extracorporeal circulation. *Circulation* 1996; 94(Suppl. II):II-341-II-346; Bergamaschini L, et al. Peripheral treatment with enoxaparin, a low molecular weight heparin, reduces plaques and ß-amyloid accumulation in a mouse model of Alzheimer's disease. *J. Neurosci.* 2004; 24(17):4181-4186.

the specific polysaccharide sequences responsible for the anticoagulant activity of enoxaparin are not fully published nor completely understood.

The data presented below further demonstrate that the only methods that can be used to ensure that a generic product has the same biological and clinical effects as enoxaparin are to characterize all of the different polysaccharide sequences and to establish that these sequences and their relative amounts are the same as in enoxaparin,[b] demonstrate the use of an equivalent manufacturing process, or perform appropriate clinical trials.

III. Enoxaparin Modulates Expression of CD54, a Pro-Inflammatory Molecule, via Unknown Polysaccharide Structures

Inflammation is an important contributor to arterial thrombosis in conditions such as myocardial infarction and unstable angina,[10] for which enoxaparin is approved for use.[11] Dr. Ilka Ott breaks new ground by demonstrating that subtle differences in structure and relative abundance of different components may disadvantageously make generic enoxaparin products pro-inflammatory and thus less safe, as her report illustrates. (Appendix C.)

Specifically, Dr. Ott's report presents for the first time a model system showing that clinically observed concentrations of enoxaparin inhibit thrombin-induced expression of CD54 (also called ICAM-1) on the surface of cultured endothelial cells. CD54 is an adhesion molecule that tethers inflammatory cells onto the surface of endothelial cells.[12] The clotting factor thrombin induces cells to express CD54 on their surface, initiating a local inflammatory process that contributes to the acute coronary syndromes for which enoxaparin is indicated.[13] A portion of CD54 can be cleaved from the endothelial cell surface and can enter the circulation. High levels of this circulating form of CD54 are found in the blood of patients suffering from those coronary diseases.[14]

Dr. Ott tested whether enoxaparin, as well as fractions and polysaccharides derived from enoxaparin, inhibit that CD54 over-expression, using a cultured human endothelial cell model. She found that, at clinically relevant concentrations, enoxaparin indeed inhibits CD54 expression on those cells, which may account for some of enoxaparin's anti-inflammatory effects in patients. (*See* Appendix C, at Fig. 3.) That inhibitory activity is retained, at least in part, in ATIII-depleted

---

[b] As previously explained in Citizen Petition Supplement No. 3, at 14, there is no published evidence that a complete characterization of a low molecular weight heparin is possible at the present time, and it is the position of sanofi-aventis that such characterization cannot presently be achieved. In addition, a complete characterization of enoxaparin cannot be achieved by digesting enoxaparin with enzymes to disaccharide or other small sugar fragments and then determining the types and concentrations of such fragments. Such a method is inherently flawed because the disaccharides or other so-called "building blocks" obtained after breaking apart the enoxaparin polysaccharides provide no information about the sequences and relative abundances of the original polysaccharides. Such a building block analysis is akin to attempting to determine the meaning of a page of text by determining the total number of each of the 26 letters of the alphabet the text contains. It is not the type and number of the individual letters, but the arrangement of those letters into words and sentences that provides meaning to the text.

systems, indicating that this activity is mediated via both ATIII-dependent and ATIII-independent mechanisms. (*See Id.*, at Figs. 6-19.)

Appendix B presents the list of samples Dr. Ott tested in this model. (Samples 1, 4, 5, 8-13, 15, and 16 of those listed in Appendix B.) Those include enoxaparin, containing 15-25% 1,6 anhydro ring structure content,[c] and two other LMWHs with < 7% or 40-50% 1,6 anhydro group content.[d] (Samples 9, 10, and 11, respectively; *see* Appendix B.) Dr. Ott also tested fractions of 6mers, 7mers, and 12mers taken from enoxaparin (samples 5, 15, and 8, respectively; *see* Appendix B) as well as 6mer and 12mer fractions taken from the LMWH with < 7% 1,6 anhydro group content. (Samples 1 and 4, respectively; *see* Appendix B.)

Dr. Ott's tests with enoxaparin and < 7% 1,6 anhydro group content LMWH fractions demonstrate that enoxaparin's effect on CD54 expression is not dependent on the 1,6 anhydro group content, but does depend on other structures within the enoxaparin mixture. The 6mer fraction from enoxaparin inhibits CD54 expression in Dr. Ott's model, demonstrating an anti-inflammatory effect. (Appendix C, Figs. 4-5.) Unexpectedly, and in contrast, the enoxaparin 7mer and 12mer size fractions actually promote CD54 expression, and thus are pro-inflammatory in this model. (*Id.*)

The 1,6 anhydro group content does not explain the differences in CD54 expression among enoxaparin's polysaccharides because the 6mer, 7mer, and 12mer fractions each affect CD54 expression differently even though their 1,6 anhydro group content is similar. In addition, the 6mer and 12mer fractions from LMWH with < 7% 1,6 anhydro group content exhibit activities similar to the respective enoxaparin 6mer and 12mer fractions, which contain 15-25% 1,6 anhydro group content. (*See id.*)

These findings reveal that there must be other structures within the 6mer fraction that endow it with the capacity to inhibit CD54 expression, and still others within the 7mer and 12mer fractions that disadvantageously enhance CD54 expression. The identity of those structures remains to be elucidated and published.

Without complete characterization, demonstrating an equivalent manufacturing process, or performing clinical trials, one could not know whether a proposed generic has an equivalent mix of anti-inflammatory, neutral, and pro-inflammatory structures as enoxaparin. Under such circumstances, there is a risk that the generic product would have no anti-inflammatory activity or anti-inflammatory activities that are less than those of enoxaparin. It is even possible that the generic product could have pro-inflammatory activities. A generic product with no anti-inflammatory activity

---

[c] This structure is a non-natural modification at one end of certain enoxaparin polysaccharides caused by the beta-elimination depolymerization process involved in the manufacture of enoxaparin. The 1,6 anhydro ring structure is described in more detail in the Citizen Petition.

[d] Those two other LMWHs were prepared by duplicating the sanofi-aventis manufacturing process, but introducing a discrete change in certain parameters. The LMWHs with < 7% or 40-50% 1,6 anhydro group content have similar anti-Xa activity, anti-IIa activity, anti-Xa/anti-IIa ratio, and molecular weight average and distribution as enoxaparin, as described in the Citizen Petition, at 14-15 and Appendix A.

or with anti-inflammatory activity less than that of enoxaparin could be ineffective in unstable angina and myocardial infarction patients because of inflammation's key role in the development of those conditions. A generic product with pro-inflammatory activity would be unsafe and ineffective in such patients.

IV. Enoxaparin Inhibits Complement Activation Through As Yet Undiscovered Polysaccharide Structures

One of enoxaparin's other anti-inflammatory targets is the contact-kinin system,[15] which is a complex collection of proteins that links blood coagulation with inflammation.[16] When the contact-kinin system is activated, another protein system, called the complement system, is also activated, which can further contribute to inflammation.[17]

Studies of enoxaparin's effect on the contact-kinin system conducted by Professor L. Bergamaschini of Maggiore Hospital at the University of Milan were reported in Citizen Petition Supplement No. 3,[18] and are provided again in Appendix D herein. Appendix D also presents new work from Professor Bergamaschini shedding further light on the anti-inflammatory action of enoxaparin. The new work shows that enoxaparin not only inhibits the contact-kinin system, but also blocks the complement system *in vitro*.

In particular, the attached data demonstrate that enoxaparin inhibits beta-amyloid-mediated activation of C4, an important component of the complement system. Furthermore, the new data reveal that the inhibitory activity of enoxaparin in that *in vitro* assay depends not only on enoxaparin's characteristic 1,6 anhydro ring structure, but also on other enoxaparin structures that are yet to be elucidated and published. Inhibition of C4 activation by enoxaparin also appears to be, at least in part, independent of inhibition of the contact-kinin system because the different enoxaparin fractions showed different effects on the inhibition of C4 and high molecular weight kininogen (HK) activation by beta-amyloid. (*See* Appendix D, at 35 for comment.)

Professor Bergamaschini tested the ability of fifteen different samples to inhibit beta-amyloid-induced activation of C4. (*See* Appendix B; samples 1-15.) Among the tested samples are enoxaparin, containing 15-25% 1,6 anhydro ring structure content, and the < 7% and 40-50% 1,6 anhydro group-containing LMWHs. (Samples 9, 10, and 11, respectively; *see* Appendix B.) Enoxaparin is a more potent inhibitor of C4 activation by beta-amyloid than either the <7% 1,6 anhydro group-containing LMWH or the 40-50% 1,6 anhydro group-containing LMWH. (Appendix D, at 31.)

Professor Bergamaschini also tested 6mer, 7mer, 8mer, 10mer, and 12mer fractions taken from enoxaparin as well as 6mer, 8mer, 10mer, and 12mer fractions taken from the < 7% 1,6 anhydro group-containing LMWH. (Samples 5, 15, 6-8, and 1-4, respectively; *see* Appendix B.) Unexpectedly, the enoxaparin 6mer fraction (with 15-25% 1,6 anhydro group content) almost completely inhibits C4 activation by beta-amyloid, while the 6mer fraction from the < 7% 1,6 anhydro group-containing LMWH

has considerably less activity (Appendix D, at 27.). Those data as a whole show that the 15-25% range of 1,6 anhydro group content found in enoxaparin correlates with enoxaparin's ability to inhibit C4 activation *in vitro*, and accordingly, may contribute to enoxaparin's anti-inflammatory effects in arterial thrombosis patients.

Moreover, Professor Bergamaschini's data also show that the 1,6 anhydro group is not the only structural fingerprint within enoxaparin that correlates with inhibition of C4 activation. Specifically, the 6mer and 7mer polysaccharide fractions from enoxaparin strongly inhibit beta-amyloid-induced activation of C4 protein in this *in vitro* model. (Samples 5 and 15; Appendix D, at 27 and 33.) In contrast, the 12mer fraction from enoxaparin has little inhibitory activity, while the 8mer and 10mer fractions have intermediate activity. (Samples 6-8; Appendix D, at 28-30.) Each of those enoxaparin size fractions has the same range of 1,6 anhydro group content, demonstrating that structures other than the 1,6 anhydro group are responsible for the more potent inhibitory activities of the 6mer and 7mer fractions relative to the fractions that contain longer polysaccharide chains.[e] Those specific structures remain to be elucidated and published.

A generic product that does not contain the same set and concentration of those, as yet unpublished, enoxaparin structures may not modulate the complement system to the same extent as enoxaparin, even if its 1,6 anhydro group content is the same as it is in enoxaparin. Yet, as discussed in Supplement No. 3, there are no current structural assays that could determine whether a proposed generic has the same anti-inflammatory structures as enoxaparin, and hence, the same anti-inflammatory activities. In fact, there is a risk that a generic product, in view of both Dr. Ott's and Professor Bergamaschini's new findings, might have no anti-inflammatory activity or might have less anti-inflammatory than enoxaparin. Dr. Ott's data go further and indicate that a generic product could even promote inflammation. Such uncertainty in the anti-inflammatory properties of a generic product would pose a safety risk to patients with acute coronary diseases who are switched to the generic drug.

V. Enoxaparin Suppresses Conversion of Cultured Endothelial Cells from an Antithrombotic to Prothrombotic Phenotype Via a Structure Distinct From the 1,6 Anhydro Group

Patients with coronary artery disease have circulating pro-inflammatory substances.[19] When cultured endothelial cells are exposed to these substances, they undergo a switch in phenotype.[20] Normal endothelial cells resist thrombosis, at least in part, because they express anticoagulant molecules on their surface. In contrast, when activated by pro-inflammatory mediators, expression of these anticoagulant molecules is suppressed and clotting is promoted on the cell surface.[21]

---

[e] In addition, enoxaparin's inhibition of C4 activation appears to be at least partly independent of the contact-kinin system inhibition because the different samples showed different effects on the inhibition of C4 and HK activation by beta-amyloid. (*See* Appendix D, at 35 for comment.)

Clot formation in those circumstances is enhanced via at least two mechanisms. First, exposure of endothelial cells to inflammatory mediators induces the expression of tissue factor (TF), a substance that initiates blood clotting.[22] Second, inflammatory mediators cause a flip-flop in the cell membrane such that negatively charged fatty substances that are normally at the inner surface of the cell membrane appear on the outer surface.[23] Clotting factors bind to the negatively charged cell surface where they are activated. This process disadvantageously promotes clot formation.[24]

Using cultured endothelial cells that had been stimulated with pro-inflammatory mediators for 4 hours, Professor Pierre Sié examined the capacity of enoxaparin and its components to attenuate TF expression and subsequent activation of coagulation on the cell surface. (*See* Appendix E, *and see* Appendix B for the identities of the tested samples.) After ten minutes exposure, enoxaparin reduced factor X activation on the cells. That activity is not dependent on the presence of the 1,6 anhydro group because similarly sized fractions with a 1,6 anhydro group content of < 7% had the same activity as those with a 1,6 anhydro group content of 15-25%. (Appendix E, Fig. 2, compare sample 1 to sample 5, 2 to 6, 3 to 7, and 4 to 8.) Those results indicate that the 1,6 anhydro group is not an important determinant of enoxaparin's activity in this assay.

Therefore, structures yet to be elucidated and published are mediating enoxaparin's capacity to modulate cell-based activation of coagulation. Because those structures necessarily cannot be assayed by any published means in a generic product, there is no way to know, absent complete characterization or clinical trials, whether a generic product will possess the same cell-based anticoagulant activity or a disadvantageously reduced activity.

VI. Even One of Enoxaparin's Most Studied Biological Activities Is Influenced by Subtle Structural Features Dependent Upon the Enoxaparin Manufacturing Process

As described above, the structural features of enoxaparin that mediate its anti-inflammatory activities and its cell-based anticoagulant activity are poorly understood. In contrast, perhaps enoxaparin's best understood biological activity is its binding to ATIII, an interaction that is an important contributor to enoxaparin's anticoagulant activity in the blood stream.

In its Citizen Petition, sanofi-aventis noted that LMWHs inhibit the coagulation cascade in part by binding to ATIII via a particular pentasaccharide sequence, herein termed "the classical ATIII-binding sequence," which is present in some of the enoxaparin polysaccharide chains.[25] That classical ATIII-binding pentasaccharide sequence is certainly an important contributor to ATIII binding. However, the data presented in Supplement No. 3 demonstrate that enoxaparin's interactions with ATIII are much more complex than previously thought. In particular, other structural features of enoxaparin also contribute to ATIII binding.[26] In Supplement No. 3, sanofi-aventis described the ATIII binding properties of twenty-one process-dependent ATIII-binding polysaccharides purified from enoxaparin, which range in size from 6mers to 10mers.

Dr. C. Boudier of Université Louis Pasteur de Strasbourg in Illkirch, France determined the affinities of those polysaccharides for ATIII and made two important observations.

First, ten of the enoxaparin polysaccharides contain the classical ATIII binding sequence as part of their overall sequence, but bind to ATIII with affinities that vary over an approximately 500-fold range.[27] That result demonstrates that the sugar structures that flank the classical ATIII binding sequence within each of the polysaccharides also influence ATIII binding.[28]

Second, and unexpectedly, Dr. Boudier discovered that eleven of the polysaccharides do not even contain the classical ATIII-binding sequence, but still bind to ATIII with affinities in the same range of those of the ten polysaccharides that do contain that classical ATIII-binding sequence.[29] In other words, Dr. Boudier surprisingly found that the classical ATIII-binding sequence is not essential for high affinity binding of enoxaparin to ATIII.

Dr. Boudier's results illustrate that a published method of assessing the ATIII binding activity of enoxaparin is not an acceptable characterization method because it ignores other structural features that contribute to ATIII binding.[30] That reported method involves determining the concentration of a 4-sugar surrogate that measures only 3 of the 5 saccharide residues comprising the classical ATIII-binding pentasaccharide sequence.[31] The method is unacceptable because it (a) measures the concentration of only part of the classical ATIII-binding sequence, (b) does not consider the ATIII binding contributions of saccharide residues flanking the classical ATIII-binding sequence in enoxparin polysaccharides, and (c) fails to account for the enoxaparin polysaccharide sequences that do not contain the classical ATIII-binding sequence, but nonetheless bind ATIII with high affinity.[32]

This Supplement No. 4 provides new data from Dr. Boudier elucidating the mechanism by which five process-dependent enoxaparin polysaccharide samples interact with ATIII. (Appendix F.) The five samples are labelled A, B, B2, C, and D, and represent four different octasaccharide species. Samples B and B2 are two different purifications of the same octasaccharide species, but sample B contains a 5% impurity from a second octasaccharide species. (*See* Table 1 below.) All of the octasaccharides contain the classical ATIII-binding pentasaccharide sequence. However, the three flanking sugar residues in the four different octasaccharides differ.

Theoretically, both ionic and non-ionic chemical interactions between ATIII and the enoxaparin polysaccharides might contribute to the binding of the octasaccharides to ATIII.[33] Dr. Boudier addressed the relative contributions of each of those chemical mechanisms by measuring the binding affinity at two different salt concentrations. Increasing the salt concentration separates the effects of ionic and non-ionic components of binding, because ionic interactions are weakened at higher salt concentrations while non-ionic interactions are generally unaffected.[34]

Dr. Boudier's results illustrate that the relative importance of ionic and non-ionic interactions with ATIII depends on the sequence of the octasaccharide. For instance, the affinity of octasaccharide A for ATIII changes less when the salt concentration is increased than do the affinities of octasaccharides B, B2, C, and D. (*See* Table 1, below, showing a 10-fold reduction in affinity for octasaccharide A compared with a 18-30-fold decrease for octasaccharide samples B, B2, C, and D.) Thus, non-ionic interactions play a more important part in the binding of octasaccharide A to ATIII than they do in the binding of the other octasaccharides to ATIII. In addition, octasaccharide D has a much higher affinity for ATIII than the others under all conditions and increasing the salt concentration has less of an effect on its affinity for ATIII than it does on the affinities of octasaccharide samples B, B2, and C. Those results indicate that non-ionic interactions are important in the binding of octasaccharide D with ATIII.

Table 1

[NaCl] mM	Octa A[a] K_d (μM)	Octa B K_d (μM)	Octa B2[b] K_d (μM)	Octa C K_d (μM)	Octa D K_d (μM)	pentasaccharide control[c] K_d (μM)
100	--	--	--	--	--	0.0264
250	0.279	0.112	0.103	0.112	0.0134	0.242
500	2.73	2.55	3.08	3.35	0.243	4.75
Fold change in K_d from 250 to 500 mM NaCl[d]	9.8	22.8	29.9	29.9	18.1	19.6

Experiments were performed in 50 mM HEPES buffer having the noted concentration of NaCl. See Appendix B for details.

[a] K_d is the dissociation constant of the polysaccharide for ATIII, which is a measure of binding affinity. The lower the number, the tighter the ATIII binding.

[b] Octasaccharides B and B2 are two different purifications of the same octasaccharide sequence. The octasaccharide B sample contains 5% octasaccharide D as an impurity.

[c] A synthetic pentasaccharide with the sequence of fondaparinux (Arixtra®; GlaxoSmithKline) is used as a control. That sequence is a natural variant of the classical ATIII-binding sequence, having an N-sulfated glucosamine moiety instead of an N-acetylated glucosamine moiety.

[d] Calculated by dividing the K_d value determined at 500 mM NaCl by that measured at 250 mM NaCl.

Moreover, the only difference in sequence between octasaccharide D and octasaccharides B and B2 is the configuration of an uronic acid residue located just outside of the classical ATIII-binding sequence. That difference is sufficient to cause a 10-fold difference in ATIII binding affinity and a somewhat stronger non-ionic interaction with ATIII at 250 mM and at 500 mM salt. That result further supports the

conclusion of Supplement No. 3 that small changes in sugar structures flanking the classical ATIII-binding sequence can have large effects on ATIII binding.

In summary, Dr. Boudier's new data re-emphasize that the enoxaparin manufacturing process generates a complex and process-dependent array of ATIII binding polysaccharides. That new data further show that the mode of interaction of those polysaccharides with ATIII differs depending on their sequence.

Nonetheless, even if enoxaparin's ATIII binding profile were simpler and even if the currently reported techniques could adequately characterize enoxaparin's ATIII binding and compare it with that of a proposed generic, that would still be inadequate to ensure the effectiveness and safety of that generic. That is because enoxaparin has other biological activities that are independent of ATIII binding, some of which are discussed in the sections above. The enoxaparin structural components that influence those other targets remain largely unreported.

Sanofi-aventis notes that octasaccharides A and B have the sequences ΔIIa-IIs-Is-Is and ΔIs-IIa-IIs-Is, respectively, and were previously described in Citizen Petition Supplement No. 1, at 6 and Appendix B. Supplement No. 1 reported a K_d for octasaccharide A of 0.034.1 μM (SE = 58%) and of 0.013 μM (SE = 70%) in 50 mM Tris buffer with 100 mM NaCl, at pH 7.5 and reported a K_d for octasaccharide B of 0.334 μM (SE = 82%) and of 0.120 μM (SE = 18%) under the same conditions. Octasaccharide D was previously described in Supplement No. 3, as polysaccharide no. 21, and was reported therein to bind to ATIII with a K_d of 0.00149 μM in 50 mM HEPES buffer with 100 mM NaCl. Based on those data, reported under two different buffer conditions but the same 100 mM NaCl salt concentration, the difference in K_d between octasaccharides B and D is about 100-fold at 100 mM NaCl.[35]

VII. Conclusion

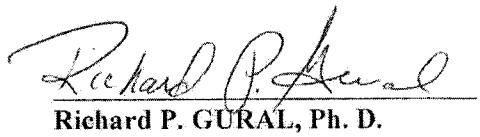
The new data described herein further demonstrate that identification of the presence of a limited number of structural signatures in a generic enoxaparin product is not sufficient to show that that product is equivalent to Lovenox®. Specifically, the presence of a similar proportion of polysaccharides with a 1,6 anhydro group at their end, or a similar number of canonical ATIII binding pentasaccharide sequences, does not provide a basis to predict how that generic product will perform when administered to patients.

Importantly, Dr. Ott's breakthrough studies demonstrate that enoxaparin contains different polysaccharides with anti-inflammatory, neutral, and pro-inflammatory activities. If a generic product does not provide the correct balance of those different polysaccharides it may have no anti-inflammatory activity, it may have less anti-inflammatory than enoxaparin, or it may even be pro-inflammatory. That uncertainty poses a serious risk to patient safety, particularly for those patients with acute coronary diseases for which Lovenox® is approved for use.

Critically, because the structural features underlying those activities are not identifiable by published methods, there are no published assays a generic manufacturer can use to ensure that all those structural features are present in their product and that those structural features are present in the same amounts as in approved enoxaparin. Thus, unless and until such time as enoxaparin can be fully characterized,[36] FDA should refrain from approving any ANDA citing Lovenox® as the reference listed drug unless: (a) the manufacturing process used to create the generic product is determined to be equivalent to sanofi-aventis's manufacturing process for enoxaparin, or (b) the application is supported by proof of equivalent effectiveness and safety demonstrated through clinical trials.

CERTIFICATION

The undersigned certifies that, to the best knowledge and belief of the undersigned, this supplement includes all information and views on which the supplement relies, and that it includes representative data and information known to the petitioner which are unfavorable to the petition.



Richard P. GURAL, Ph. D.
Vice President
Regulatory Affairs
sanofi-aventis US LLC
a subsidiary of sanofi-aventis

---

[1] Citizen Petition at 15-17; *and see, e.g.*, Gikakis N., et al. Effect of factor Xa inhibitors on thrombin formation and complement and neutrophil activation during in vitro extracorporeal circulation. *Circulation* 1996; 94(Suppl. II):II-341-II-346; Wan MX, et al. Low molecular weight heparin inhibits tumor necrosis factor α-induced leukocyte rolling. *Inflammation Res.* 2001; 50:581-584; Turpie AGG and Antman EM. Low-molecular-weight heparins in treatment of acute coronary syndromes. *Arch. Internal Med.* 2001; 161:1484-1490; Manduteanu I, et al. A novel attribute of enoxaparin: inhibition of monocyte adhesion to endothelial cells by a mechanism involving cell adhesion molecules. *Pharmacology* 2002; 65:32-37; Bergamaschini L, et al. Peripheral treatment with enoxaparin, a low molecular weight heparin, reduces plaques and ß-amyloid accumulation in a mouse model of Alzheimer's disease. *J. Neurosci.* 2004; 24(17):4181-4186; Stevenson JL, et al., Differential metastasis inhibition by clinically relevant levels of heparins – correlation with selectin inhibition, not antithrombotic activity. *Clin. Cancer Res.* 2005; 11(19):7003-7011.

[2] *See, e.g.*, Libby P. Molecular basis of the acute coronary syndromes. *Circulation* 1995; 91:2844-50; Cohen M, et al. A comparison of low-molecular-weight heparin with unfractionated heparin for unstable coronary artery disease. *N. Engl. J. Med.* 1997; 337(7):447; Ross R. Atherosclerosis: an inflammatory disease. *N. Engl. J. Med.* 1999; 349:115-26; Hope SA and Meredith IT. Cellular adhesion molecules and cardiovascular disease. Part II. Their association with conventional and emerging risk factors, acute coronary events and cardiovascular risk prediction. *Internal Med. J.* 2003; 33:450-462; Mousa S. and Kaiser B. Tissue factor pathway inhibitor in thrombosis and beyond: role of heparin. *Drugs of the Future* 2004; 29(7):751-766; Lovenox® Prescribing Information, 2004, NDA 20-164/S-055, under "Indications and Usage."

[3] *See* Citizen Petition, at 1.

[4] Citizen Petition Supplement No. 3, at 2.

[5] *See Id.*, at 2, note b.

[6] *See* Citizen Petition, at 4 and 19-21. *See also*, Mousa S and Kaiser B. Tissue factor pathway inhibitor in thrombosis and beyond: role of heparin. *Drugs of the Future* 2004; 29(7):751-766; Rao LVM and Rapaport SI. Activation of factor VII bound to tissue factor: a key step in the tissue factor pathway of blood coagulation. *Proc. Natl. Acad. Sci., USA* 1988; 85:6686-91; Gerotziafas GT, et al. Comparative effects of synthetic pentasaccharide, low-molecular weight heparin, unfractionated heparin and

recombinant hirudin on the generation of Factor VIIa and prothrombin activation after coagulation of human plasma. *Blood. Coag. Fibrinol.* 1998; 9:571-580.
[7] *See* note 1 above.
[8] *See* note 2 above.
[9] *See* Citizen Petition, at 10 and 15-16; *and see* note 1 above.
[10] *See* notes 1 and 2 above; *and see also* Viles-Gonzales JF, et al. Thrombin/inflammation paradigms: A closer look at arterial and venous thrombosis. *Am. Heart. J.* 2005; 149(1 Suppl):S19-31; Fox EA and Kahn SR. The relationship between inflammation and venous thrombosis. A systematic review of clinical studies. *Thromb. Haemost.* 2005; 94(2):362-5; Buffon A, et al. Widespread coronary inflammation in unstable angina. *N. Engl. J. Med.* 2002; 347(1):5-12; Busch G, et al. Coagulation factor Xa stimulates interleukin-8 release in endothelial cells and mononuclear leukocytes: implications for acute myocardial infarction. *Arterioscler. Thromb. Vasc. Biol.* 2005; 25:461-466; Wasserman EJ and Shipley NM. Atherothrombosis in acute coronary syndromes: mechanisms, markers, and mediators of vulnerability. *Mt. Sinai J. Med.* 2006; 73(1):431-9; Benson V, et al. ICAM-1 in acute myocardial infarction: A potential therapeutic target. *Curr. Mol. Med.* 2007; 7:219-227.
[11] Lovenox® Prescribing Information, 2004, NDA 20-164/S-055, under "Indications and Usage."
[12] *See, e.g.*, Niessen HWM, et al. Intercellular adhesion molecule-1 in the heart. *Annals N.Y. Acad. Sci.* 2002; 973:573-585.
[13] *See, e.g.*, Shyu KG, et al. Circulating intercellular adhesion molecule-1 and E-selectin in patients with acute coronary syndrome. *Chest* 1996; 109:1627-30; Haught WH, et al. Alterations in circulating intercellular adhesion molecule-1 and L-selectin: further evidence for chronic inflammation in ischemic heart disease. *Am Heart J.* 1996; 132:1-8; Ghaisas NK, et al. Elevated levels of circulating soluble adhesion molecules in peripheral blood of patients with unstable angina. *Am. J. Cardiology* 1997; 80:617-19; O'Malley T, et al. Early increase in levels of soluble inter-cellular adhesion molecule-1 (sICAM-1): Potential risk factor for the acute coronary syndromes. *European Heart J.* 2001; 22: 1226-34.
[14] *See* notes 12 and 13 above; *and see, e.g.,* Li Y-H, et al. Elevation of soluble adhesion molecules is associated with the severity of myocardial damage in acute myocardial infarction. *Am. J. Cardiology* 1997; 80:1218-1221; Ogawa H, et al. Plasma soluble intercellular adhesion molecule-1 levels in coronary circulation in patients with unstable angina. *Am. J. Cardiol.* 1999; 83:38-42; Mulvihill N, et al. Early temporal expression of soluble cellular adhesion molecules in patients with unstable angina and subendocardial myocardial infarction. *Am. J. Cardiol.* 1999; 83:1265-1267.
[15] Bergamaschini L, et al. Peripheral treatment with enoxaparin, an LMWH, reduces plaques and beta-amyloid accumulation in a mouse model of Alzheimer's disease. *J. Neurosci.* 2004; 24 (17):4181-4186.
[16] *See* Heller W and Wendel HP. Clivarin® and other LMWHs in a cardiopulmonary bypass model: studies on contact activation. *Blood Coag. Fibrinol.* 1993; 4(Suppl. 1):S45-S54.
[17] *See* Appendix D herein; *see also* Bergamaschini L, et al. *J. Neurosci.* 2004; 24 (17):4181-4186; Heller W and Wendel HP. *Blood Coag. Fibrinol.* 1993; 4(Suppl. 1): S45-S54.
[18] Citizen Petition Supplement No. 3, at 13-14.
[19] *See, e.g.*, Shyu KG, et al. *Chest* 1996; 109:1627-30; Ghaisas NK, et al. *Am. J. Cardiology* 1997; 80:617-19; O'Malley T, et al. *European Heart J.* 2001; 22: 1226-34.
[20] *See, e.g.*, Cadroy Y, et al. Heparin reverses the procoagulant properties of stimulated endothelial cells. *Thromb. Haem.* 1996; 75:100-105.
[21] *See Id.*
[22] *See, e.g.,* Taubman MB, et al. Tissue factor in the pathogenesis of atherosclerosis. *Thromb. Haemost.* 1997; 78(1):200-204; Luther T and Mackman N. Tissue factor in the heart. *Trends Cardiovasc. Med.* 2001; 11(8):307-312.
[23] Tedgui A & Mallat Z. Apoptosis as a determinant of atherothrombosis. *Thromb. Haemost.* 2001; 86:420-426.
[24] *See Id.*
[25] *See* Citizen Petition, at 6.
[26] *See* Citizen Petition Supplement No. 3, at 3-9.
[27] *Id.*, at 5, Table 1.
[28] *See also* Citizen Petition Supplement No. 1, at 4-7.
[29] Citizen Petition Supplement No. 3, at 7, Table 2.
[30] *Id.*, at 6-9, *and see* Figure 1 therein.

[31] *id.*
[32] *id.*
[33] Hjelm R and Schedin-Weiss S. High affinity interaction between a synthetic, highly negatively charged pentasaccharide and α- or β-antithrombin is predominantly due to nonionic interactions. *Biochemistry* 2007; 46:3378-3384; *and see* Appendix B attached hereto.
[34] *See, e.g.*, Record T, et al. Ion effect on ligand-nucleic acid interactions. *J. Mol. Biol.* 1976; 107:145-158.
[35] *See* Citizen Petition Supplement No. 3, at 5-6; Citizen Petition Supplement No. 1 at 6.
[36] *See* Citizen Petition Supplement No. 3.