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(54) **Biomarkers for cardiovascular side-effects induced by cox-2 inhibitory compounds**

Biomarker für durch cox-2 inhibierende Substanzen verursachte kardiovaskuläre Nebenwirkungen  
Biomarqueurs pour des effets secondaires cardio-vasculaires induits par des composés inhibiteurs de cox-2

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- **GIANNITSIS E: "RATIONALE FOR TESTING THE CARDIOVASCULAR RISK FOR PATIENTS WITH COX-2 INHIBITORS ON THE BASIS OF BIOMARKER NT-PROBNP", CLINICAL LABORATORY, CLIN LAB PUBLICATIONS, HEIDELBERG, vol. 51, no. 1/02, 1 January 2005 (2005-01-01), pages 63-72, XP008056286, ISSN: 1433-6510**

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**EP 2 290 363 B1**

**Description**

## FIELD OF THE INVENTION

5 **[0001]** The invention relates generally to the *in vivo* testing of the efficacy of a compound or composition, and particularly to the testing and biologically functionalizing of cox-2 inhibitory compounds (coxibs) by activity *in vivo*.

## BACKGROUND OF THE INVENTION

10 **[0002]** Use of cox-2 specific inhibitory compounds (coxibs) and some NSAIDs has been associated with an increased risk of cardiovascular events in human including deep venous thrombosis, myocardial infarction, stroke, and sudden death. The current hypothesis is that some of anti-inflammatory compounds inhibit PGI<sub>2</sub> synthesis but not TxA synthesis, altering the homeostatic balance towards the pro-coagulative/pro-thrombotic pathways. Fitzgerald GA. N Engl J Med. 351(17):1709-11 (October 21, 2004). It has been reported that some of anti-inflammatory compounds, mainly cox-2 inhibitors, inhibit PGI<sub>2</sub> synthesis only, resulting in altered homeostatic balance towards the pro-coagulative pathways which in rare cases might lead to the serious cardiovascular side effects in human. Furberg CD, Psaty BM, FitzGerald GA. Circulation 111(3):249 (January 25, 2005).

15 **[0003]** GIANNITSIS EVANGELOS (CLINICAL LABORATORY. 2005, vol. 51, no. 1-2, 2005, pages 63-72) discloses the use of the BNP gene expressed in cardiovascular tissue as biomarker for early detection of cardiovascular risk for patients treated with cox-2 inhibitors.

20 **[0004]** There continues to be a need in the art for additional information about the cardiovascular side effects of the use of cox-2 specific inhibitory compounds.

## SUMMARY OF THE INVENTION

25 **[0005]** The present invention is defined in claims 1 to 3.

30 **[0006]** A 2-week analysis in cynomolgus monkeys (*Macaca fascicularis*) treated with the coxibs COX189 (Lumiracoxib®, Novartis), refocoxib (Vioxx®, Merck), and celecoxib (Celebrex®, Pharmacia/Pfizer), and with the nonselective NSAID, diclofenac (Voltaren®, Novartis) showed that the Vioxx®-treated animals exhibit a specific mRNA expression pattern which shows the presence of an intravascular procoagulative/prothrombotic state particularly in venous vessels of a Vioxx®-treated monkey. The specific genomic pattern includes gene expression changes involved in blood and endothelial cell (EC) activation, interaction of blood cells with EC, activation of INF<sub>γ</sub> pathway, and release of pro-inflammatory cytokines and chemo-attractants. These data together with biochemical and histopathological findings indicate that Vioxx® induces or worsens the pro-coagulative / prothrombotic changes, along with the activation of INF<sub>γ</sub> pathways triggered most probably by an endothelium tropic viral infection (e.g., cytomegalovirus (CMV)) and/or other vascular INF<sub>γ</sub>/TNF inducing situations (e.g., autoimmune vascular disorders).

35 **[0007]** The overall genomic findings show that Cox-2/PGE<sub>2</sub> inhibition results in strong and uncontrolled induction of INF<sub>γ</sub> regulated chemo-attractants, adhesion molecules, and proinflammatory/pro-coagulative molecules which might lead to or increase the risk of cardiovascular adverse events. Histopathological results confirmed the genomic findings showing that the specific genomic pattern is an early signature of vasculitis and is observed only in the animal treated with Vioxx®.

40 **[0008]** Accordingly, the invention provides biomarkers (in the form of serum or plasma proteins) for minimal and early vasculitis or other vasculopathies. In addition, the invention provides biomarkers for predicting potential Vioxx®-induced cardiovascular adverse effects.

45 **[0009]** Identification of biomarkers advantageously allows safe use of cox-2 inhibitory compounds in clinics and selection of cox-2 inhibitory follow-up compounds without cardiovascular toxicity. Indeed, the expression of several genes increased in the vessels of the Vioxx®-treated animal encode for secreted proteins, e.g., chemokine (CXC motif) ligand 10 (CXCL10) and other cytokines, which can be measured in peripheral samples such as blood or urine. Clinical screening of patients prior to, or during administration of Cox-2 inhibitory therapies should increase their safety profile.

50 **[0010]** Monitoring of early changes is predictive of cardiovascular adverse effects in patients treated with compounds exhibiting cox-2 inhibition or increasing the production of molecules induced by interferons, by virus infections, or autoimmune disorders resulting in pro-coagulative / prothrombotic/endothelium changes. These compounds include mainly cox-2 inhibitors, classical NSAIDs, other anti-inflammatory compounds and direct PGE<sub>2</sub>, cAMP and PKA inhibitors.

55 **[0011]** The data identifies another pathway than the PGI<sub>2</sub> synthesis pathway that may be one of the main triggering factors leading to the observed adverse cardiovascular events in human. Alteration in this pathway can be easily monitored in preclinical and clinical studies to avoid such cardiovascular side effects upon cox-2 and/or NSAIDs treatments. Biomarkers or the gene signature identified can also be used to monitor viral infection/INF<sub>γ</sub> pathway activation and some vasculopathies in diverse human diseases including several autoimmune and neurodegenerative disorders with or

without anti-inflammatory and immunosuppressive treatments. Some of the biomarkers can be used for selection of compounds without potential cardiovascular side-effects.

#### BRIEF DESCRIPTION OF THE DRAWINGS

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##### [0012]

FIG. 1. Principal Component Analysis (PCA) of genomic data from six cardiovascular tissues: iliac vein, pulmonary vein, aorta, carotid artery, heart ventricle, and heart atrium. Only genes encoding for MHC molecules and their receptors were included for PCA analysis. The Vioxx®-treated monkey #A60055 (circled) exhibited distinct expression pattern.

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FIG. 2. Specific genomics expression pattern in Vioxx®-treated monkey #A60055. The pattern consisted of transcripts for MHC class I, II & class I, non classical molecules, their receptors (TcRs and NK receptors), chemokines (CXCL9, 110, -11, MCP-1). Overall signature indicating strong INF pathway activation together with IL1/ TNF and coagulation and complement pathways alteration.

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FIG. 3. Histopathological evaluation of samples from different tissues confirms the genomic data showing focal vascular necrosis in the veins of Vioxx®-treated animal #A60055 only. The main findings consisted of EC necrosis, leucocytes/fibrin adhesion to EC surface, fibrinoid degeneration of the media and medial leukocyte infiltration. (A) Iliac vein from vehicle treated animal. (B) Histopathology findings of endothelial cell (EC) necrosis, fibrin leukocyte adhesion to EC surface, fibrinoid degeneration of the media, medial leukocytes infiltration in iliac vein of the monkey #A60055.

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FIG. 4. Strong increase of CXCL10 in veins followed by arteries and heart samples from the Vioxx®-treated monkey #A60055 (indicated by an arrow) only.

FIG 5. Protein profiling in serum and plasma from the monkeys. The monkey #A60055 exhibit a specific protein expression profile: Soluble MHC molecules b2-m, other chemokines, cytokines (INF $\gamma$ , CXCL10, MCP-1, IL18, TNF RII, IL1b), and soluble VCAM-1. Human MAP is used to assess monkey proteins in a Rules-Based Medicine (RBM®) multiplex assay.

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FIG 6. ELISA confirmation of CXCL10 (IP10) protein level in monkey serum samples. The Vioxx®-treated monkey #A60055 exhibits the highest level of CXCL10 protein expression.

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FIG 7. ELISA confirmation of INF $\gamma$  protein level in monkey serum and plasma samples. The Vioxx®-treated monkey #A60055 exhibits the highest level of INF $\gamma$  protein expression.

FIG 8. Localisation of PD-ECGF1 protein at the site of vascular lesion.

#### DETAILED DESCRIPTION OF THE INVENTION

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**[0013]** *Introduction and overview.* The classical discovery process in the pharmaceutical industry is based on targets (enzymes, receptors, cellular assays, animal and disease models, etc.). Chemicals or biological products are tested, in a high-throughput mode, on a battery of pre-selected different targets. The weakness of the classical approach are the "artificially disconnected" *in vitro* target models compared to the tightly interconnected and interdependent relationship of the different targets in a whole organism and the fact that biological activity on all non selected targets is missed.

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**[0014]** By contrast, there is a "non pre-conceived hypothesis" discovery process to rapidly identify and analyze the biological activity of new products in the whole organism, multi-organs and whole transcriptome. All physiological interactions between the different organs or tissues are present and any cellular pathway or any potential targets could potentially be analyzed in a non artificial system.

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**[0015]** The data derived from this comparative multi-organ genomics analysis, coupled with extensive clinical, biochemical and histopathological data, identified a new pathway which may play the major role in the cardiovascular events observed in human treated with cox-2 inhibitors. The mRNA expression changes have been analyzed in several tissue samples from *Macaca fascicularis* following treatment with the Cox-2 specific inhibitors COX189 (Lumiracoxib®, Novartis), Refocoxib (Vioxx®, Merck), and Celecoxib (Celebrex®, Pharmacia/Pfizer), and with the nonselective NSAID, Diclofenac (Voltaren®, Novartis).

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**[0016]** *Administration of compounds.* A two-week oral-gavage treatment with the Cox-2 specific inhibitor COX189 (lumiracoxib®, Novartis) in comparison with refocoxib (Vioxx®, Merck), and celecoxib (Celebrex®, Pharmacia/Pfizer), and with the nonselective NSAID, diclofenac (Voltaren®, Novartis) was performed. All test items were administered to monkeys at doses higher than those used in patients to analyse mRNA expression changes in terms of mechanisms of drug actions and also potential cardiovascular toxic effects. The test items were administered daily at doses of 100 mg/kg/day, except Vioxx® which was administered at 50 mg/kg/day.

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**[0017]** In one embodiment of the invention, the test animal is a vertebrate. In a particular embodiment, the vertebrate is a mammal. In a more particular embodiment, the mammal is a primate, such as a cynomolgus monkey (*Macaca*

*fascicularis*). As used herein, the administration of an agent or drug to a subject includes self-administration and the administration by another.

**[0018]** In more particular embodiments, the "treatment group" of animals received a substance (test item, compound, drug) in a vehicle compound suitable for administration of the substance or the combination of substances, while the "control" (or "baseline") group should receive the vehicle compound only. During the treatment period biological specimen such as tissue pieces (e.g. obtained by biopsy), or body fluids, such as blood, plasma, serum, urine, or saliva, can be sampled. At the end of the treatment time all animals of all groups can be sacrificed and biological specimen such as whole organs or pieces thereof can be sampled. All sampled specimen can be stored as known in the art for further analysis that include, but are not limited to, RT-PCR, Northern blotting, in-situ hybridization, gene expression profiling with microarrays.

**[0019]** In one embodiment, it begins with differentially expressed transcripts in different cardiovascular tissues and proteins in plasma between normal monkeys and cox-2 inhibitory compounds/drugs-treated monkeys with regard to the identification and validation of potential targets and the identification of biomarkers for cardiovascular side effects.

**[0020]** *Gene expression profiles.* After a period of time (e.g., four weeks) of compound/drug administration, the treated animals are necropsied. 120 tissues are dissected and rapidly snap-frozen for genomics analysis. Organ samples are isolated for histopathological examinations and for gene expression localizations, such as by *in situ* hybridization.

**[0021]** In more particular embodiments, the methods of detecting the level of expression of mRNA are well-known in the art and include, but are not limited to, reverse transcription PCR, real time quantitative PCR, Northern blotting and other hybridization methods. A particularly useful method for detecting the level of mRNA transcripts obtained from a plurality of genes involves hybridization of labelled mRNA to an ordered array of oligonucleotides. Such a method allows the level of transcription of a plurality of these genes to be determined simultaneously to generate gene expression profiles or patterns.

**[0022]** As used herein, a gene expression profile is diagnostic when the increased or decreased gene expression is an increase or decrease over the baseline gene expression following administration of a compound.

**[0023]** In one embodiment, the technique for detecting gene expression includes the use of a gene chip. The construction and use of gene chips are well known in the art. See, U.S. Pat Nos. 5,202,231; 5,445,934; 5,525,464; 5,695,940; 5,744,305; 5,795,716 and 6,800,992. See also, Johnston, M. *Curr Biol* 8:R171-174 (1998); Iyer VR et al., *Science* 283: 83-87 (1999) and Elias P, "New human genome 'chip' is a revolution in the offing" *Los Angeles Daily News* (October 3, 2003).

**[0024]** Additional procedures that can be used in the methods of the invention are described in WO 2005/045044, "USE OF FIBROBLAST GROWTH FACTOR", filed November 11, 2004.

**[0025]** Gene expression profiles have been generated using the Affymetrix microarray technology. (i) RNA extraction and purification: Briefly, total RNA was obtained by acid guanidinium thiocyanate-phenol-chloroform extraction (Trizol®, Invitrogen Life Technologies, San Diego, Calif.) from each frozen tissue section and the total RNA was then purified on an affinity resin (Rneasy®, Qiagen) according to the manufacturer's instructions. Total RNA was quantified by the absorbance at  $\lambda = 260 \text{ nm}$  ( $A_{260\text{nm}}$ ) and the purity was estimated by the ratio  $A_{260\text{nm}}/A_{280\text{nm}}$ . Integrity of the RNA molecules was confirmed by non-denaturing agarose gel electrophoresis. RNA was stored at  $-80^\circ\text{C}$  until analysis. One part of each individual RNA sample was kept for the analysis of critical genes by means of Real-time PCR. (ii) GeneChip® experiment: All Genechip® experiments were conducted in the Genomics Factory EU following recommendations by the manufacturer of the GeneChip® system (Affymetrix, Expression Analysis Technical Manual (Affymetrix, Santa Clara, California, 2005). Human U133A genome arrays were used for transcript expression analysis. Double stranded cDNA was synthesized with a starting amount of approximately 5  $\mu\text{g}$  full-length total RNA using the Superscript Choice System (Invitrogen Life Technologies) in the presence of a T7-(dT) 24 DNA oligonucleotide primer. Following synthesis, the cDNA was purified by phenol/chloroform/isoamyl alcohol extraction and ethanol precipitation. The purified cDNA was then transcribed in vitro using the BioArray® High Yield RNA Transcript Labeling Kit (ENZO) in the presence of biotinylated ribonucleotides form biotin labelled cRNA. The labelled cRNA was then purified on an affinity resin (Rneasy, Qiagen), quantified and fragmented. An amount of approximately 10  $\mu\text{g}$  labelled cRNA was hybridized for approximately 16 hours at  $45^\circ\text{C}$  to an expression probe array. The array was then washed and stained twice with streptavidin-phycoerythrin (Molecular Probes) using the GeneChip Fluidics Workstation 400 (Affymetrix). The array was then scanned twice using a confocal laser scanner (GeneArray Scanner®, Agilent) resulting in one scanned image. This resulting ".dat-file" was processed using the MAS5 program (Affymetrix) into a ".cel-file". The ".cel file" was then transferred to an Affymetrix GeneChip Laboratory Information Management System (LIMS) database, which is connected to a UNIX Sun Solaris server through a network filing system that allows for the average intensities for all probes cells (CEL file) to be downloaded into an Oracle database (NPGN). Raw data was converted to expression levels using a "target intensity" of 100. The numerical values displayed are weighted averages of the signal intensities of the probe-pairs comprised in a probe-set for a given transcript sequence (AvgDiff value). The data were checked for quality and loaded in the GeneSpring® software versions 5.0 (Silicon Genetics, Calif., US) for statistical analysis.

**[0026]** *Quality control analysis of transcriptome data:* The following quality measures were analysed for each sample:

Scaling factor, background, percent present calls, AFFX-GAPDH 3': AFFX-GAPDH 5' -ratio, AFFX-GAPDH 3' variance, AFFX-Beta-actin 3': AFFX-Beta-actin 5'-ratio. Biological outliers and tissue contamination were identified using NPGN-database Gene Expression Tools by comparing the average signal intensity per probe set per treatment group to the signal intensity in each sample. Attention was paid to the homogeneity of the data. Average and standard deviation of the background noise level determined the raw data restriction value used in the consequent analysis.

**[0027]** *Principal component analysis of transcriptome data:* Using SIMCA 10.5 software (Umetrics Inc, Kinnelon NJ, USA), Principal Component Analysis (PCA) was performed on all data generated by the microarrays or on genes present at least in 2 out of 4 samples in at least 1 group to determine general expression differences/similarities among the samples and identify potential biological or technical outliers. A projection was made on the first two or three principal components for each tissue. Here, the differences between samples represent differences in the level of expression or in the correlation structure of the genes used for the PCA model.

**[0028]** The information was further refined by the use of complementary techniques. *In situ* hybridization, for example, can indicate precisely which cell type inside an organ is specifically expressing a given gene. This technique based on the detection of RNA is independent of the availability of an antibody. Quantitative PCR has also been used to confirm expression levels of particular genes of interest.

**[0029]** To obtain biomarkers predicting cardiovascular adverse effect of tested compounds/drugs, expression levels of proteins have been analysed in cynomolgus monkey serum and plasma from the present analysis using human Multi-Analyte Profile (MAP) Technology. Human MAP could be used to measure protein levels of more than 80 antigens in monkey serum and plasma (Rules-Based Medicine Inc (RBM®), Austin, Texas USA).

**[0030]** The following EXAMPLE is presented.

#### EXAMPLE

##### IDENTIFICATION OF SPECIFIC GENOMICS SIGNATURE IN VIOXX®-TREATED MONKEY (S)

**[0031]** Overall genomics data obtained for 16 tissues from all monkey groups showed that the Vioxx®-treated animals exhibit a specific pattern of gene expression. This pattern includes significant increases (ANOVA,  $p < 0.05$ ) in the expression of MHC class I classical and non-classical molecules, MHC class II molecules and their respective receptors such as TcRs and Immunoglobulin-like molecules.

**[0032]** Analysis of genomic data from several cardiovascular tissues by Principle Component Analysis (PCA) on the selected genes composed of MHC molecules identified a biological outlier (Animal no: A60055, circled in the FIG. 1) within the Vioxx®-treated group.

**[0033]** Further analysis of all genomic data by PLS-DA provided a list of the most discriminate genes between the animal A60055 and the rest of the animals from Vioxx®, Celebrex®, Cox189 (Novartis), diclofenac and vehicle treated groups (TABLE 1, FIG. 2). The specific gene pattern included mainly interferon inducible genes encoding for Toll like receptors (TLRs), classical and non-classical MHC class I, MHC class II, their respective receptors/ligands such as TcRs and NK receptors, several chemokines such as CXCL10, CCL2, an extensive list of  $INF\gamma$  pathways signalling genes such as Jak1, Stat1, and some IL1/TNF pathway related molecules. In addition, there was strong and significant increases in the expression of coagulation pathways related molecules such as PD-ECGF, coagulation factor II (thrombin) receptor-like 1, Factor 13 A1, several adhesion molecules such as VCAM and ICAM, and a number of genes belonging to the complement activation and other pathways innate immunity pathways. *This genomic expression pattern predominant in the vessels of the Vioxx®-treated monkey (#A60055) indicated development of a potential vasculopathy/vasculitis with strong activation of  $INF\gamma$  pathway suggestively induced by an endothelium tropic infection or reactivation of a vascular autoimmune disorder.*

**[0034]** Interestingly, histopathological evaluation of all tissues showed clear sign of vasculitis in veins only of the Vioxx®-treated animal A60055 (FIG. 3). Thus the specific expression pattern should be a specific genomics signature of minimal vasculitis (see below).

**[0035]** *The role of Vioxx®-induced cox-2 inhibition in the observed genomic and histopathological findings provide a potential link to the increased risks of cardiovascular side effects occurring in patients treated with Vioxx®:* The majority of the observed gene expression changes have been known to be directly involved in the pathogenesis of diverse cardiovascular diseases including atherosclerosis, CAD, thrombosis, autoimmune and neurodegenerative diseases. Among the  $INF\gamma$  inducible gene expression changes, the most striking increase was observed for CXCL10 and other chemokines, e.g., CXCL-9, -11 and MCP-1 (CCL-2) (FIG. 4 and TABLE 1).

TABLE 1

The most discriminant genes for Vioxx animal #A60055 and corresponding genomics expression data from iliac vein samples of monkeys treated with vehicle, Vioxx®, Celebrex®, Cox189 (Novartis), and diclofenac. These results indicated potential vasculopathies in the animal A60055, probably induced by an unknown virus infection together with an exaggerated host immune response against vascular endothelium.

Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
216598_s_at	CCL2	chemokine (C-C motif) ligand 2	6	1	<b>150.9</b>	1.0	2.3	1.6	9.0
202411_at	IFI27	interferon, alpha-inducible protein 27	20	12	<b>20.8</b>	3.8	3.3	4.2	5.6
204533_at	CXCL10	chemokine (C-X-C motif) 10 ligand	74	20	<b>19.8</b>	1.1	1.1	1.4	1.3
209969_s_at	STAT1	signal transducer and activator of transcription 1, 91kDa	50	16	<b>13.5</b>	1.7	2.7	1.4	2.3
212998_x_at DQB2	HLA-DQB2	major histocompatibility complex, class II, DQ beta 2	252	86	<b>10.8</b>	1.2	1.9	1.4	2.2
210163_at	CXCL11	chemokine (C-X-C motif) ligand 11	4	5	<b>9.7</b>	1.5	3.7	2.6	2.1
203915_at	CXCL9	chemokine (C-X-C motif) ligand 9	64	21	<b>9.3</b>	1.6	1.4	1.7	1.3
214038_at	CCL8	chemokine (C-C motif) ligand 8	19	13	<b>8.8</b>	1.8	1.3	1.7	1.8
214453_s_at	IFI44	interferon-induced protein 44	141	24	<b>8.2</b>	2.5	1.3	2.0	1.7
212671_s_at	HLA-DQA1	major histocompatibility complex, class II, DQ alpha 1	445	167	<b>8.2</b>	1.0	2.1	1.6	1.2

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
211654_x_at	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1	544	143	7.7	1.0	2.0	1.8	1.6
AFFX-HUMISGF3A/M979	STAT1	signal transducer and activator of transcription 1, 91kDa	109	25	7.1	1.5	1.2	1.9	1.1
35_MB_at									
213797_at	cig5	viperin	25	17	7.1	1.4	1.3	1.8	2.1
211122_s_at	CXCL11	chemokine (C-X-C motif) ligand 11	10	9	6.6	1.0	1.6	2.3	2.0
210029_at	INDO	indoleamine-pyrrole 2,3 dioxygenase	53	16	6.5	1.1	1.6	1.2	1.4
214567_s_at	XCL1	chemokine (C motif) ligand 1	13	12	5.4	1.1	3.5	1.3	0.5
AFFX-HUMISGF3A/M979	STAT1	signal transducer and activator of transcription 1, 91kDa	99	7	5.3	1.3	1.3	1.2	1.0
35_MA_at									
203153_at	IFIT1	interferon-induced protein with tetratricopeptide repeats 1	100	35	5.0	1.5	0.8	1.1	1.0
217502_at	IFIT2	interferon-induced protein with tetratricopeptide repeats 2	168	62	4.7	1.3	1.1	1.4	0.8
205483_s_at	G1P2	interferon, alpha-inducible protein(clone IFI-15K)	27	13	4.5	3.3	1.4	1.5	2.1
206366_x_at	XCL1	chemokine (C motif) ligand 1	33	15	4.3	1.5	2.0	1.7	1.8

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
AFFX- HUMISGF3A/M979	STAT1	signal transducer and activator of transcription 1, 91kDa	25	16	<b>4.3</b>	1.3	0.9	1.6	1.0
35_5_at									
209823_x_at	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1	233	102	<b>4.3</b>	0.9	2.0	1.0	1.3
204820_s_at	BTN3A3	butyrophilin, subfamily 3, member A3	324	48	<b>4.1</b>	1.6	1.3	1.1	1.3
203868_s_at	VCAM1	vascular cell adhesion molecule 1	285	164	<b>4.1</b>	0.8	1.9	0.9	1.4
211656_x_at	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1	421	132	<b>4.1</b>	1.0	1.6	1.3	1.2
207485_x_at	BTN3A1	butyrophilin, subfamily 3, member A1	55	28	<b>4.0</b>	2.0	1.7	2.1	1.1
202531_at	IRF1	interferon regulatory factor 1	290	49	<b>3.9</b>	0.8	1.1	1.3	1.1
214234_s_at	CYP3A5	cytochrome P450, family 3, subfamily A, polypeptide 5	28	11	<b>3.9</b>	0.9	1.3	1.3	1.2
205114_s_at	CCL3	chemokine (C-C motif) ligand 3	21	12	<b>3.8</b>	1.4	1.3	0.9	1.4
208451_s_at	C4A	complement component 4A	220	121	<b>3.8</b>	1.1	1.6	0.9	2.4
208747_s_at	C1S	complement component 1, s	1786	602	<b>3.6</b>	1.3	1.0	1.0	1.7
205898_at	CX3CR1	subcomponent chemokine (C-X3-C motif) receptor 1	86	36	<b>3.6</b>	1.0	1.1	1.2	1.6

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
208071_s_at	LAIR1	leukocyte-associated Ig-like receptor 1	37	25	<b>3.5</b>	0.5	3.6	1.1	0.8
208436_s_at	IRF7	interferon regulatory factor 7	24	11	<b>3.4</b>	1.6	2.1	1.6	1.2
204858_s_at	ECGF1	endothelial cell growth factor 1 (platelet- derived)	78	30	<b>3.3</b>	1.9	1.1	1.7	1.4
209785_s_at	PLA2G4C	phospholipase A2, group IVC (cytosolic, calcium-independent)	46	17	<b>3.3</b>	1.2	1.4	1.4	1.0
203052_at	C2	complement component 2	205	16	<b>3.3</b>	0.9	1.1	1.1	1.2
204821_at	BTN3A3	butyrophilin, subfamily 3, member A3	40	15	<b>3.3</b>	1.7	1.6	1.5	1.5
213095_x_at	AIF1	allograft inflammatory factor 1	78	62	<b>3.2</b>	0.6	2.0	0.5	1.5
210164_at	GZMB	granzyme B (granzyme 2, cytotoxic T- lymphocyte- associated serine esterase 1)	19	10	<b>3.2</b>	1.6	0.9	0.8	1.8
203882_at	ISGF3G	interferon-stimulated transcription factor 3, gamma 48kDa	402	53	<b>3.1</b>	1.7	1.0	1.5	1.4
209901_x_at	AIF1	allograft inflammatory factor 1	111	74	<b>3.0</b>	0.3	1.5	0.7	1.2
201891_s_at	B2M	beta-2-microglobulin	318	105	<b>3.0</b>	1.4	1.3	1.0	1.4
210072_at	CCL19	chemokine (C-C motif) ligand 19	78	28	<b>3.0</b>	1.2	2.0	1.4	1.6
208893_s_at	DUSP6	dual specificity phosphatase 6	107	40	<b>3.0</b>	1.0	1.2	0.9	1.1

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
217478_s_at	HLA-DMA	major histocompatibility complex, class II, DM alpha	838	145	<b>2.9</b>	1.1	1.4	1.2	1.3
202705_at	CCNB2	cyclin B2	43	14	<b>2.9</b>	1.4	1.2	1.4	1.3
215193_x_at	HLA-DRB1	major histocompatibility complex, class II, DR beta 1	1950	212	<b>2.9</b>	1.2	1.7	1.5	1.3
202687_s_at	TNFSF10	tumor necrosis factor (ligand) superfamily, member 10	533	141	<b>2.9</b>	1.2	1.3	1.2	1.2
1405_i_at	CCL5	chemokine (C-C motif) ligand 5	8	7	<b>2.8</b>	0.5	0.9	0.9	2.2
209619_at	CD74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	922	192	<b>2.8</b>	1.1	1.3	1.0	1.3
202688_at	TNFSF10	tumor necrosis factor (ligand) superfamily, member 10	373	102	<b>2.8</b>	0.9	1.3	1.2	1.1
211367_s_at	CASP1	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	53	13	<b>2.7</b>	1.4	1.2	1.2	1.3
204674_at	LRMP	lymphoid-restricted membrane protein	74	31	<b>2.6</b>	1.7	3.8	1.6	1.6
202436_s_at	CYP1B1	cytochrome P450, family 1, subfamily B, polypeptide 1	171	25	<b>2.6</b>	1.0	1.0	1.3	1.3

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
204006_s_at	FCGR3A	Fc fragment of IgG, low affinity IIIa, receptor for (CD16)	41	19	<b>2.5</b>	0.8	1.3	1.0	1.5
214630_at	CYP11B1	cytochrome P450, family 11, subfamily B, polypeptide 1	25	12	<b>2.5</b>	0.8	1.1	1.0	0.9
210225_x_at	LILRB3	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	98	44	<b>2.5</b>	0.8	1.3	1.1	1.3
206060_s_at	PTPN22	protein tyrosine phosphatase, non-receptor type 22 (lymphoid)	23	11	<b>2.5</b>	1.0	2.5	1.2	0.9
204116_at	IL2RG	interleukin 2 receptor, gamma (severe combined immunodeficiency)	188	27	<b>2.4</b>	1.4	3.8	1.3	1.2
211528_x_at	HLA-A	major histocompatibility complex, class I, A	3314	497	<b>2.4</b>	1.3	1.1	1.3	1.1
209813_x_at	HLA-C	major histocompatibility complex, class I, C	39	22	<b>2.4</b>	0.4	1.2	1.0	1.0
214459_x_at	TRGC2	T cell receptor gamma constant 2	4379	649	<b>2.4</b>	1.4	1.3	1.2	1.3
216920_s_at	HLA-A	major histocompatibility complex, class I, A	72	13	<b>2.4</b>	1.0	1.6	1.1	1.2
211530_x_at	HLA-A	major histocompatibility complex, class I, A	868	214	<b>2.3</b>	1.8	1.5	1.6	1.6

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
208894_at	HLA-DRA	major histocompatibility complex, class II, DR alpha	2704	518	<b>2.3</b>	1.0	1.3	1.2	1.1
38241_at	BTN3A3	butyrophilin, subfamily 3, member A3	36	8	<b>2.3</b>	1.4	1.1	1.0	1.2
205758_at	CD8A	CD8 antigen, alpha polypeptide (p32)	41	21	<b>2.3</b>	1.3	1.0	1.2	1.4
202644_s_at	TNFAIP3	tumor necrosis factor, alpha-induced protein 3	183	50	<b>2.3</b>	1.2	1.7	1.3	1.7
221875_x_at	HLA-F	major histocompatibility complex, class I, F	3883	622	<b>2.3</b>	1.2	1.0	1.2	1.0
209970_x_at	CASP1	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	168	26	<b>2.3</b>	1.1	1.2	1.1	1.5
203020_at	HHL	expressed in hematopoietic cells, heart, liver	575	183	<b>2.2</b>	1.1	1.4	1.0	0.8
217362_x_at	HLA-DRB6	major histocompatibility complex, class II, DR beta 6 (pseudogene)	641	209	<b>2.2</b>	1.2	1.4	1.3	1.0
202465_at	PCOLCE	procollagen C-endopeptidase enhancer	1093	451	<b>2.2</b>	0.5	0.7	0.8	0.3
204057_at	ICSBP1	interferon consensus sequence binding protein 1	67	21	<b>2.2</b>	1.4	1.6	1.2	1.1

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
204890_s_at	LCK	lymphocyte-specific protein tyrosine kinase	42	9	<b>2.2</b>	1.5	3.2	1.4	0.9
205926_at	IL27RA	interleukin 27 receptor, alpha	93	37	<b>2.2</b>	1.1	1.1	1.1	1.3
208200_at	IL1A	interleukin 1, alpha	14	10	<b>2.2</b>	1.1	0.4	1.6	1.2
206541_at	KLKB1	kallikrein B, plasma (Fletcher factor) 1	59	32	<b>2.2</b>	1.2	1.1	1.0	1.2
208791_at	CLU	clustern (complement lysis inhibitor, SP- 40,40, sulfated glycoprotein 2, testosterone- repressed prostate message 2, apolipoprotein J)	3952	905	<b>2.2</b>	0.9	0.8	1.0	1.0
201487_at	CTSC	cathepsin C	333	83	<b>2.1</b>	1.1	1.3	1.1	1.4
207857_at	LILRB1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	30	15	<b>2.1</b>	0.5	1.0	0.7	0.8
201422_at	IFI30	interferon, gamma- inducible protein 30	176	14	<b>2.1</b>	1.0	1.3	1.1	1.4
204806_x_at	HLA-F	major histocompatibility complex, class I, F	3260	520	<b>2.1</b>	1.1	1.2	1.2	1.0
210982_s_at	HLA-DRA	major histocompatibility complex, class II, DR alpha	743	100	<b>2.1</b>	1.0	1.5	1.1	1.0

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
215485_s_at	ICAM1	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	158	30	<b>2.1</b>	0.9	1.1	1.0	1.1
211529_x_at	HLA-A	major histocompatibility complex, class I, A	3620	317	<b>2.1</b>	1.2	1.2	1.3	1.2
214377_s_at	JAK1	Janus kinase 1 (a protein tyrosine kinase)	84	12	<b>2.1</b>	1.3	0.9	1.1	1.1
202446_s_at	PLSCR1	phospholipid scramblase 1	641	187	<b>2.1</b>	1.2	1.1	1.0	1.5
201743_at	CD14	CD14 antigen	179	26	<b>2.0</b>	0.8	1.1	0.7	1.1
216526_x_at	HLA-C	major histocompatibility complex, class I, C	3770	1302	<b>2.0</b>	1.3	1.2	1.4	1.5
202643_s_at	TNFAIP3	tumor necrosis factor, alpha-induced protein 3	109	31	<b>2.0</b>	0.8	1.5	1.2	1.3
206429_at	F2RL1	coagulation factor II (thrombin) receptor-like 1	26	15	<b>2.0</b>	2.0	1.1	1.8	1.5
211144_x_at	TRGC2	T cell receptor gamma constant 2	64	16	<b>2.0</b>	1.3	1.2	1.3	1.0
209924_at	CCL18	chemokine (C-C motif) ligand 18	23	20	<b>2.0</b>	0.7	1.5	2.3	1.6
212067_s_at	C1R	complement component 1, r	654	125	<b>2.0</b>	0.9	0.9	1.0	1.4
214511_x_at	FCGR1A	Fc fragment of IgG, high affinity Ia, receptor for (CD64)	101	30	<b>2.0</b>	0.6	1.1	1.0	1.0
218009_s_at	PRC1	protein regulator of cytokinesis 1	32	14	<b>2.0</b>	0.7	1.1	1.0	0.8

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
220040_x_at	HCA127	hepatocellular carcinoma-associated antigen 127	116	54	<b>2.0</b>	0.6	1.0	0.9	0.5
209365_s_at	ECM1	extracellular matrix protein 1	156	44	<b>2.0</b>	1.3	0.9	1.0	1.1
210571_s_at	CMAH	cytidine monophosphate-N-acetylneuraminic acid hydroxylase	88	17	<b>2.0</b>	1.0	1.0	1.1	1.4
213539_at	CD3D	CD3D antigen, delta polypeptide (TIT3 complex)	85	39	<b>1.9</b>	2.0	3.2	1.7	1.9
209312_x_at	HLA-DRB3	major histocompatibility complex, class II, DR beta 3	3990	399	<b>1.9</b>	1.1	1.5	1.3	1.1
201315_x_at	IFITM2	interferon induced transmembrane protein 2 (1-8D)	1055	139	<b>1.9</b>	1.1	1.0	1.2	1.6
209140_x_at	HLA-B	major histocompatibility complex, class I, B	8146	1478	<b>1.9</b>	1.1	1.1	1.2	1.2
210865_at	TNFSF6	tumor necrosis factor (ligand) superfamily, member 6	56	10	<b>1.9</b>	1.1	1.4	1.3	1.2
206360_s_at	SOCS3	suppressor of cytokine signaling 3	95	30	<b>1.8</b>	0.8	0.9	1.0	1.4
211100_x_at	LILRB1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	77	18	<b>1.8</b>	1.3	1.4	1.0	1.2

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
203305_at	F13A1	coagulation factor XIII, A1 polypeptide	183	27	<b>1.8</b>	0.9	0.9	1.1	1.3
209541_at	IGF1	insulin-like growth factor 1 (somatomedin C)	524	255	<b>1.8</b>	0.9	0.9	0.9	1.2
215313_x_at	HLA-A	major histocompatibility complex, class I, A	5166	264	<b>1.8</b>	1.3	1.2	1.3	1.3
207238_s_at	PTPRC	protein tyrosine phosphatase, receptor type, C	137	75	<b>1.8</b>	1.3	2.5	1.1	1.3
210864_x_at	HFE	hemochromatosis	159	24	<b>1.8</b>	1.2	1.1	1.3	0.9
219059_s_at	XLKD1	extracellular link domain containing 1	286	63	<b>1.8</b>	1.0	1.5	1.0	1.4
211911_x_at	HLA-B	major histocompatibility complex, class I, B	5982	585	<b>1.8</b>	1.4	1.2	1.3	1.2
206584_at	LY96	lymphocyte antigen 96	75	32	<b>1.8</b>	1.1	1.4	1.0	1.4
202953_at	C1QB	complement component 1, q subcomponent, beta polypeptide	227	37	<b>1.8</b>	1.0	1.1	1.0	1.1
211329_x_at	HFE	hemochromatosis	127	29	<b>1.8</b>	0.9	0.7	0.9	0.9
201858_s_at	PRG1	proteoglycan 1, secretory	1003	235	<b>1.8</b>	0.8	1.0	0.9	1.1
208729_x_at	HLA-B	granule major histocompatibility complex, class I, B	5968	985	<b>1.8</b>	1.2	1.1	1.3	1.2
211863_x_at	HFE	hemochromatosis	160	23	<b>1.8</b>	0.8	0.9	1.3	1.0
205859_at	LY86	lymphocyte antigen 86	126	21	<b>1.8</b>	1.5	1.5	0.9	1.2

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
217456_x_at	HLA-E	major histocompatibility complex, class I, E	1205	148	<b>1.8</b>	1.2	1.2	1.4	1.2
203028_s_at	CYBA	cytochrome b-245, alpha polypeptide	173	18	<b>1.8</b>	1.1	1.2	1.2	1.0
208018_s_at	HCK	hemopoietic cell kinase	98	36	<b>1.8</b>	1.2	1.4	1.1	1.2
208812_x_at	HLA-C	major histocompatibility complex, class I, C	4921	1097	<b>1.8</b>	1.3	1.2	1.1	1.2
201508_at	IGFBP4	insulin-like growth factor binding protein 4	2272	954	<b>1.7</b>	0.5	0.6	0.6	0.8
202803_s_at	ITGB2	integrin, beta2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)	114	31	<b>1.7</b>	0.6	1.1	1.0	1.0
204908_s_at	BCL3	B-cell CLL/lymphoma 3	119	19	<b>1.7</b>	0.7	1.0	1.0	1.3
216217_at	PLCL2	phospholipase C-like 2	28	8	<b>1.7</b>	1.0	1.0	0.9	1.0
205270_s_at	LCP2	lymphocyte cytosolic protein 2	73	18	<b>1.7</b>	0.9	1.9	1.2	1.2
210754_s_at	LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	255	48	<b>1.7</b>	1.0	1.5	0.8	1.1
203332_s_at	INPP5D	inositol polyphosphate-5-phosphatase, 145kDa	153	32	<b>1.7</b>	1.2	1.6	1.2	1.2
218232_at	C1QA	complement component 1, q subcomponent, alpha polypeptide	150	26	<b>1.7</b>	0.9	1.5	1.0	1.2

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
208594_x_at	LILRB3	leukocyte immunoglobulin-like receptor, subfamily B, member 3	117	12	<b>1.7</b>	0.9	1.1	1.0	1.4
209348_s_at	MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	280	67	<b>1.7</b>	1.0	1.3	0.9	0.9
201999_s_at	TCTEL1	t-complex-associated-testis-expressed 1-like 1	785	133	<b>1.7</b>	0.9	1.0	0.9	0.9
204924_at	TLR2	toll-like receptor 2	100	26	<b>1.7</b>	0.9	0.8	0.8	1.4
210176_at	TLR1	toll-like receptor 1	68	18	<b>1.7</b>	0.9	1.3	0.8	1.3
202902_s_at	CTSS	cathepsin S	276	38	<b>1.6</b>	1.0	1.2	1.1	1.3
208829_at	TAPBP	TAP binding protein (tapasin)	318	51	<b>1.6</b>	0.9	1.1	1.1	1.0
202638_s_at	ICAM1	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	256	83	<b>1.6</b>	0.8	0.8	0.9	1.5
212203_x_at	IFITM3	interferon induced transmembrane protein 3 (1-8U)	1101	194	<b>1.6</b>	1.4	1.1	1.0	1.3
200905_x_at	HLA-E	major histocompatibility complex, class I, E	1308	239	<b>1.6</b>	1.3	1.1	1.2	1.2
203923_s_at	CYBB	cytochrome b-245, beta polypeptide	183	20	<b>1.6</b>	0.9	1.2	1.0	1.2

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
204747_at	IFIT4	interferon-induced protein with tetratricopeptide repeats 4	123	21	<b>1.6</b>	0.9	0.8	0.9	0.9
209687_at	CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	1071	254	<b>1.6</b>	1.0	1.1	0.8	1.3
211332_x_at	HFE	hemochromatosis	134	13	<b>1.6</b>	1.0	0.8	1.1	0.9
211866_x_at	HFE	hemochromatosis	154	25	<b>1.6</b>	1.0	0.9	1.2	0.9
201859_at	PRG1	proteoglycan 1, secretory granule	683	182	<b>1.5</b>	0.8	1.2	0.9	1.2
203932_at	HLA-DMB	major histocompatibility complex, class II, DM beta	331	43	<b>1.5</b>	1.1	1.4	1.2	1.0
202450_s_at	CTSK	cathepsin K (pyncodysostosis)	415	68	<b>1.5</b>	1.3	1.0	1.2	1.3
203416_at	CD53	CD53 antigen	296	119	<b>1.5</b>	1.2	2.3	1.0	1.4
213932_x_at	HLA-A	major histocompatibility complex, class I, A	1373	131	<b>1.5</b>	1.2	1.3	1.2	1.1
208992_s_at	STAT3	signal transducer and activator of transcription 3 (acute-phase response factor)	698	120	<b>1.5</b>	1.0	1.0	1.0	1.1
219118_at	FKBP11 kDa	FK506 binding protein 11, 19	184	39	<b>1.5</b>	0.6	1.0	0.8	0.8
210559_s_at	CDC2	cell division cycle 2, G1 to S and G2 to M	89	20	<b>1.5</b>	1.0	1.3	1.0	1.2

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
218856_at	TNFRSF21	tumor necrosis factor receptor superfamily, member 21	407	63	<b>1.5</b>	1.2	1.5	1.1	1.2
209049_s_at	PRKCBP1	protein kinase C binding protein 1	314	40	<b>1.5</b>	1.0	1.2	0.9	1.0
213193_x_at	TRB@	T cell receptor beta locus	214	67	<b>1.5</b>	1.2	2.4	1.2	1.2
204118_at	CD48	CD48 antigen (B-cell membrane protein)	225	42	<b>1.5</b>	1.1	1.7	1.0	1.2
209753_s_at	TMPO	thymopoietin	110	41	<b>1.5</b>	0.8	0.9	0.9	1.1
200887_s_at	STAT1	signal transducer and activator of transcription 1, 91kDa	92	12	<b>1.5</b>	1.2	1.1	1.3	1.1
203561_at	FCGR2A	Fc fragment of IgG, low affinity Ila, receptor for (CD32)	128	46	<b>1.5</b>	1.1	1.4	0.8	1.4
209734_at	HEM1	hematopoietic protein 1	196	24	<b>1.5</b>	1.2	1.5	1.0	1.1
AFFX-HUMISGF3A/M979	STAT1	signal transducer and activator of transcription 1, 91kDa	51	11	<b>1.4</b>	1.1	1.1	1.3	1.0
204852_s_at	PTPN7	protein tyrosine phosphatase, non-receptor type 7	39	23	<b>1.4</b>	1.4	2.0	1.0	1.4
211799_x_at	HLA-C	major histocompatibility complex, class I, C	1978	1219	<b>1.4</b>	1.0	1.0	1.5	0.8
204232_at	FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	513	73	<b>1.4</b>	0.9	1.3	1.0	1.5
218831_s_at	FCGRT	Fc fragment of IgG, receptor, transporter, alpha	1066	183	<b>1.4</b>	1.0	1.0	1.0	0.9

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
216231_s_at	B2M	beta-2-microglobulin 9	9970	129	<b>1.4</b>	1.2	1.3	1.1	1.2
219117_s_at	FKBP11	FK506 binding protein 11, 19 kDa	772	157	<b>1.4</b>	0.7	0.8	0.7	0.7
217733_s_at	TMSB10	thymosin, beta 10 0	8296	167	<b>1.4</b>	1.1	1.1	1.1	1.2
203922_s_at	CYBB	cytochrome b-245, beta polypeptide (chronic granulomatous disease)	70	22	<b>1.4</b>	1.2	1.7	0.9	1.1
203729_at	EMP3	epithelial membrane protein 3	400	35	<b>1.4</b>	0.7	0.9	0.8	0.8
205298_s_at	BTN2A2	butyrophilin, subfamily 2, member A2	259	22	<b>1.4</b>	1.1	1.2	1.3	1.1
220336_s_at	GP6	glycoprotein VI (platelet)	39	14	<b>1.4</b>	1.2	1.1	1.2	1.0
200904_at	HLA-E	major histocompatibility complex, class I, E	700	284	<b>1.4</b>	1.1	1.2	1.0	1.2
205831_at	CD2	CD2 antigen (p50), sheep red blood cell receptor	71	18	<b>1.4</b>	1.1	1.5	1.1	1.1
205098_at	CCR1	chemokine (C-C motif) receptor	82	21	<b>1.4</b>	1.1	0.9	0.8	1.3
215990_s_at	BCL6	1B-cell/LL/lymphoma 6 (zinc finger protein 51)	295	32	<b>1.3</b>	1.0	0.9	1.4	1.0
210514_x_at	HLA-A	major histocompatibility complex, class I, A	1046	84	<b>1.3</b>	1.2	1.1	1.2	1.0
213869_x_at	THY1	Thy-1 cell surface antigen	318	97	<b>1.3</b>	0.8	0.8	0.9	0.6

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Systematic Name	SYMBOL	GENENAME	Control -		Vioxx A60055	Vioxx without A60055	Celebrex	Cox189	Voltaren
			Avg	SD					
202637_s_at	ICAM1	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	429	54	<b>1.3</b>	0.8	0.9	1.0	1.0
202957_at	HCLS1	hematopoietic cell-specific Lyn substrate 1	174	17	<b>1.3</b>	1.2	1.5	1.0	1.1
209749_s_at	ACE	angiotensin I converting enzyme 1	76	25	<b>1.3</b>	0.9	0.8	0.8	1.1
210915_x_at	TRB@	T cell receptor beta locus	176	34	<b>1.3</b>	1.3	2.5	1.3	1.0
209048_s_at	PRKCBP1	protein kinase C binding protein 1	176	22	<b>1.3</b>	1.3	1.2	1.1	1.0
221978_at	HLA-F	major histocompatibility complex, class I, F	66	15	<b>1.3</b>	1.3	1.2	1.4	1.4
210904_s_at	IL13RA1	interleukin 13 receptor, alpha 1	354	81	<b>1.2</b>	1.1	0.9	1.1	0.9
203879_at	PIK3CD	phosphoinositide-3-kinase, catalytic, delta polypeptide	160	25	<b>1.2</b>	1.1	1.7	1.1	1.3
204158_s_at	TCIRG1	T-cell, immune regulator 1, ATPase, H+ transporting, lysosomal V0 protein a isoform 3	163	37	<b>1.2</b>	1.0	0.9	1.0	1.0
52940_at	SIGIRR	single Ig IL-1R-related molecule	142	32	<b>1.2</b>	1.1	1.2	0.9	1.0

**[0036]** The strongest increase has been observed in veins (e.g., 20-fold for CXCL10 in pulmonary vein) and adrenal followed by arteries and heart tissues. Much less and irrelevant changes were observed in samples from liver, kidney, GIT, spleen, BM and cartilage. The fact that specific histopathological vascular findings have been observed only in veins and the genomic data show the presence of the specific pattern in all of the CV tissues tested, suggest that the genomic pattern (particularly, some soluble factors e.g., CXCL10 and CCL2) may be considered as early biomarkers for cox-2 inhibition-related CV side-effects or as early biomarkers for minimal (sub-clinical) vasculitis.

**[0037]** *Vioxx® exhibits increased angiostatic and focal inflammatory effects predominantly in veins:* The *in vivo* angiogenic effect of PGE2 is well documented experimentally and in particular by the fact that the EP4 receptor signalling has a major role in regulating closure or maintaining potency of the ductus arteriosus in newborns with congenital heart disease. Apart from this expected inhibition of angiogenic effects of PGE2 by coxibs tested in this analysis, Vioxx® strongly induced the expression of CXCL10, and PD-ECGF (both known anti-angiogenic proteins) mainly in iliac and pulmonary veins which suggests that a strong angiostatic effect occurred in the monkey #A60055.

**[0038]** *The specific gene expression pattern observed in the monkey treated with Vioxx® strongly suggests the involvement of an endothelial cell tropic CMV-like infection or reactivation:* (i) The expression of numbers of genes inducible by INF $\gamma$  was strongly upregulated in most of the tissues from the Vioxx®-treated monkey. According to the literature, the induction of INF $\gamma$  pathway is commonly observed during the first phase of CMV infection or reactivation. It has been shown that CMV antigen-stimulated CD4+ T cells from normal healthy CMV-seropositive donors secreted INF $\gamma$  and TNF alpha, driving chemokines induction in endothelial cells. The strong INF $\gamma$  pathway induction and histopathological findings of focal vasculitis in animal #A60055 together with the literature data indicate that latent endothelial cell tropic CMV infection might induce specific cellular immune responses, resulting in the induction of chemoattractants, leading to inflammation and endothelial cell injury. Bolovan-Fritts CA et al., J Virol. 78(23):13173-81 (December 2004).

**[0039]** (ii) In the vessels of the monkey A60055, expression of chemokines, mainly CXCL10, MCP-1 and at a lesser degree other chemokines e.g., CXCL9 and -11 were significantly upregulated (e.g., 150 fold increase for MCP-1 in pulmonary vein). It has been shown that atheroma-associated endothelial cells express CXCL10, CXCL9 and CXCL11. Their secretion from IFN $\gamma$ -stimulated ECs is increased upon IL-1beta, TNF-alpha, and CD40 ligand treatments and decreased in the presence of nitric oxide. Mach F et al., J Clin Invest. 104(8):1041-50 (October 1999). These data suggest the involvement of these cytokines/chemokines in the pathogenesis/progression of inflammatory vascular changes such as arteriosclerosis or vasculitis. More interestingly, mouse CMV infection in an atherosclerosis animal model and in cholesterol-fed C57BL/6J mice significantly increases atherosclerotic lesion area and aortic expression of CXCL10, MCP-1, and other INF-gamma induced proteins. Burnett MS et al., Circulation. 109(7):893-7 (February 24, 2004). Similarly, mouse CMV infection in the brains of immunodeficient mice, stimulates the production of CXCL10 and MCP-1. Cheeran MC et al., J Neurovirol. 10(3):152-62 (June 2004).

**[0040]** *In light of these data, our results suggest that an endothelial cell tropic CMV-like reactivation might be the main factor involved in the initiation of the observed vascular changes in this analysis.* Interestingly, human CMV encodes four chemokine receptors e.g., US28, which bind many of the human CC-chemokines, including RANTES, MCP-1, CCL3, and CXCL-11. As mentioned above, this class of chemokines contributes to the development of vascular disease such as atherosclerosis, restenosis, and transplant vascular sclerosis. The increased expression of these chemokines genes and/or their respective receptors (TABLE 1) in the monkey treated with Vioxx® raises the question whether they were produced by reactivated CVM virions or by INF $\gamma$  activated endothelial cells as a result of inflammatory reaction to CMV infection.

**[0041]** Literature data also demonstrate that the induction of COX-2 and/or synthesis of PGE2 are essential for efficient CMV replication in human (Zhu H et al., Proc. Natl. Acad. Sci. USA 99:3932-3937 (2002)) and monkey (Rue CA et al., J Virol. 78(22):12529-36 (November 2004)). Interestingly, the rhesus cytomegalovirus (RhCMV) genome encodes a protein homologue to cellular cox-2 (vCOX-2). Experiments with vCOX-2 deleted RhCMV identified vCOX-2 as a critical determinant for endothelial cell tropism. Rue CA et al., J Virol. 78(22):12529-36 (November 2004).

**[0042]** The cPLA2, a key enzyme in arachidonic acid (AA) release, is the primary form of PLA2 responsible for the generation of PGE2, LTB4 and PAF from AA, in response to inflammatory stimuli. It has been established that cPLA2 exhibits antihypertrophic potential probably via signalling pathway of  $\beta$ 2-ARs in heart. Pavoine C & Defer N, Cell Signal. 17(2):141-52 (February 2005). PLA2 signalling pathways has been shown to be involved in human CMV infection in several ways. (i) hCMV infection stimulates arachidonic acid metabolism associated with activation of PLA2 and a cellular cPLA2, (ii) both mRNAs encoding for cPLA2 and COX-2 are increased in infected cells, (iii) blocking the cellular pathway of PLA2 signalling inhibited hCMV infection, and recently (iv) it has been reported that a cPLA2 taken up by virus particles from infected cells plays a role in CMV infection at a post entry step. The inhibition of hCMV-borne cPLA2 had broader consequences on HCMV infection inhibiting the production of key viral antigens IE1, IE2 and pp65. In this monkey analysis, expression of cPLA2 was upregulated in most of the cardiovascular tissues from the Vioxx®-treated monkey only. *Since all other monkeys showed no increase of cPLA2 expression, these data also suggest the presence/reactivation of a CMV infection in the endothelial cell of the Vioxx®-treated monkeys.*

**[0043]** CMV is known as a strictly opportunistic pathogen, in immunocompetent individuals it is easily controlled yet

never eliminated since a robust immune response suppresses persistent viral replication and facilitates a lifelong viral latency. In fact, CMV has several mechanisms to escape diverse host immune responses. CMV encodes for at least four proteins which interfere with classical MHC class I antigen presentation by preventing their cell surface expression, by transporting them to the cytosol, where they are degraded and by competing with TAP for the translocation of antigenic peptides to MHC molecules. However, evasion of MHC I is not perfect, since IFN $\gamma$  activation by CMV can induce the synthesis of large quantities of MHC I and proteasomes that overwhelm viral inhibitory proteins and "rescue" the CTL response. Two CMV-encoded proteins also interact with non-classical MHC class I such as HLA-E, which leads to suppression of NK responses. CMV encode for the UL18 which has homology to MHC I heavy chain and is expressed on the cell surface. Disruption of UL18 severely restricts viral pathogenesis. CMV also interferes with MHC II presentation, which was strongly upregulated in the Vioxx $\text{\textcircled{R}}$ -treated monkey (TABLE 1). Classically, INF-gamma is a potent inducer of MHC II expression in many cell types including endothelial cells. However, some studies showed that in CMV-infected cells, INF-gamma is unable to induce MHC II expression. Recently, MHC class II molecules expressed in EC have been proposed as the entry receptor for CMV. Thus, the protein expression of MHC class II molecules in tissue samples will be tested whether their increased mRNA expression are translated into functional proteins. CMV infection also induces alteration in the expression of important cytokines such as TNF, TGF beta and IL1 and upregulation of the complement control proteins CD46, and CD55. CMV also encodes for a surface Fc-receptor which can bind IgG with high affinity. *Interestingly, expression of most of these genes including MHC molecules, several NK cell receptors, complement proteins, Fc receptors was significantly upregulated in the monkey #A60055. These results support the hypothesis that the specific expression pattern is probably induced by a CMV infection in the animal A60055 (TABLE 1).*

**[0044]** The expression of Toll like receptor 2 and CD14 was significantly increased in several tissues from the Vioxx $\text{\textcircled{R}}$ -treated monkey. Recently, it has been shown that CMV activates inflammatory cytokine responses via TLR2/CD14 during the prereplication phase of the viral life cycle. Indeed, interferon and ISGs are robustly induced by CMV particles during entry via activation of IRF3, one of the key transcription factors for INF $\gamma$  inducible genes. Later during the replication cycle, CMV encodes several chemokines and chemokine receptors that provide potent inflammatory signals. In fact, many of the pathological processes associated with CMV reactivation (including accelerated vascular disease, and graft rejection) appear to be mediated by the release of inflammatory cytokines. Compton T et al., J Virol. 77(8):4588-96 (April 2003). *Even though other viruses (measles virus, and RSV), also activate innate responses in a TLR2/CD14-dependent manner, the overall expression pattern suggests that CMV infection/reactivation is probably responsible for the observed vasculitis in the veins of the Vioxx $\text{\textcircled{R}}$ -treated monkey.*

**[0045]** *CMV reactivation in the vascular system and use of anti-inflammatory compounds including NSAIDs and specific Cox-2 inhibitors:* A number of infectious agents have been associated with atherosclerotic cardiovascular disorders, including CMV, *Helicobacter pylori*, EBV, HIV, HSV1, HSV2, and hepatitis B and C. Rue CA et al., J Virol. 78(22):12529-36 (November 2004). However, several reports in the literature suggest that the CMV infection/reactivation might be one of the major players in the pathogenesis of chronic inflammatory vascular diseases. For examples, rare cases of CMV vasculitis have been described even in healthy individuals, which may be associated with carotid intimal-medial thickening, or development of extensive mesenteric arterial and venous thrombosis. Other studies suggest that CMV infection or reactivation is involved in post-transplant sub endothelium / intramyocardial inflammation, atherogenesis, restenosis, and inflammatory abdominal aortic aneurysm. Koskinen PK et al., Transpl Infect Dis. 1(2):115-26 (June 1999)). Since ECs are one of the major targets for latent CMV infection, CMV induced lytic or inflammatory reaction in ECs may easily result in adherent thrombi formation in vivo. *Thus, infection/reactivation of CMV in endothelial cells may cause vascular injury and promote the development of inflammation, atherosclerotic lesions, and thrombosis. Therefore, the observed vascular findings in this analysis might be the early indicators of a CMV vasculitis.*

**[0046]** In line with our current observations on Vioxx $\text{\textcircled{R}}$  CV effect, Rott D et al., J Am Coll Cardiol. 41(10):1812-9 (May 21, 2003) found that inhibition of Cox-2 aggravated atherosclerosis in the apoE knockout mouse. The authors studied the effect of COX-2 inhibition on infectivity of cytomegalovirus and coincidentally showed increased disease burden in animals treated with the COX-2 inhibitor, including those not infected with the virus. According to the FitzGerald hypothesis (see BACKGROUND OF THE INVENTION), this should reflect selective suppression of PG12 and an unopposed effect of TXA2, however, the authors suggest an alternative hypothesis indicating that the suppression of anti-inflammatory PGs, such as PGJ2, and its metabolite 15-deoxy-delta<sup>12,14</sup>-PGJ2 might also result in this type of vascular changes. Rott D et al., J Am Coll Cardiol. 41(10):1812-9 (May 21, 2003). Another hypothesis might be that Cox-2 specific inhibitors but also NSAIDs can also initiate or aggravate atherosclerotic changes by inhibiting the production of PGE2 leading to the reactivation of latent CMV infection. In fact, it has been clearly documented that PGE2 can inhibit replication of viruses including CMV and HIV-1 through activation of cAMP and PKA which are the key enzymes in the negative regulation of immune responses and a potential target for inhibiting autoreactive T cells. Aandahl EM et al., J Immunol. 169(2):802-8 (July 15, 2002). Other reports support this hypothesis showing that PGE-2 suppresses chemokine production by increasing cAMP through the EP4 receptor. Takayama K et al., J Biol Chem. 277(46):44147-54 (November 15, 2002). It has been shown that PGE2 activated cAMP/PKA inhibits INF $\gamma$  signalling pathway proteins (JAK-1 and STAT1) and consequently decrease chemokine synthesis such as CXCL10. Kanda N et al., J Invest Dermatol. 119(5):

1080-9 (November 2002).

**[0047]** More interestingly, a selective cox-2 inhibitor, NS398, potentiates CXCL10 synthesis upon  $\text{INF}\gamma$  stimulation by preventing PGE2 production and PKA activation. Wright KL et al., Br J Pharmacol. 141(7):1091-7 (April 2004). In our analysis, the significant activation of numbers of  $\text{INF}\gamma$  inducible genes even in vascular tissues where there was no histopathological abnormalities suggest that Vioxx® has similar potentialization effect on the  $\text{INF}\gamma$  pathway activation as described for NS398. Thus, the Vioxx® treatment might lower the threshold for the generation of a chronic vascular inflammation via inhibition of PGE2 and activation of  $\text{INF}\gamma$  pathways triggered by reactivation of a latent CMV infection in endothelial cells. It is noteworthy that the CMV seropositivity has been reported in most of the monkey strains and in about 60-70% of healthy individuals. *Overall, the data suggest that inhibition of Cox-2 and in particular PGE2 by Vioxx® might results in an uncontrollable/continuous production of soluble factors induced by  $\text{INF}\gamma$  pathway activation. The  $\text{INF}\gamma$  pathway is commonly induced in case of endothelial/scular tropic virus infection including some isolates of CMV. As suggested by the presently observed findings, activation of vascular endothelium and attraction of specific blood cells by chemokines (e.g., CXCL10, MCP-1, often activated during a CMV infection) might increase their interaction leading to cardiovascular adverse effects.*

**[0048]** The histopathological examination revealed marginal vascular changes consistent with the genomic findings and suggesting that the specific genomic pattern is an early signature of vasculitis and is observed only in the monkey treated with Vioxx® (FIG 3).

**[0049]** Soluble proteins present in serum and plasma of the same monkeys have been measured using a multiplex assay produced by Rules-Based Medicine (RBM®) of Texas. The results were in line with the genomic results showing the increased level of  $\text{INF}\gamma$  inducible proteins only in the Vioxx®-treated monkey (FIG 5).

**[0050]** Increased expression of CXCL10 chemokine and  $\text{INF}\gamma$  has been confirmed by an ELISA both in serum and plasma from the Vioxx®-treated monkey (FIG 6 and FIG 7). These peripheral biomarkers might allow safe use of cox-2 inhibitory compounds in clinics and selection of cox-2 inhibitory follow-up compounds without cardiovascular toxicity.

**[0051]** Localisation of several proteins (e.g., PD-ECGF1) at the site of vascular lesion indicates the specificity of changes for a vasculopathy (FIG 8). The genomic and serum/plasma protein signature identified in this analysis predicts for a minimal and focal vasculitis and may be used for patient's monitoring of vasculitis induced by different compounds/drugs (e.g., phosphodiesterase inhibitors) or occurring during vascular or autoimmune disorders.

**[0052]** *Conclusion:* Overall genomic data showed that the Vioxx®-treated animals, and in particular the animal #60055 exhibit a specific mRNA expression pattern which strongly suggest the induction of an intravascular procoagulative/prothrombotic state particularly in venous vessels of the Vioxx®-treated animals. The specific genomics pattern includes genes involved in blood and endothelial cell activation, interaction between blood and ECs, strong activation of  $\text{INF}\gamma$  pathway, and release of pro-inflammatory cytokines and chemo-attractants. These data together with biochemical and histopathological findings suggest that Vioxx® may exaggerate host immune response during some/specific viral infection (s) with endothelial tropism, suggestively reactivation of a CMV infection.

**[0053]** Our hypothesis is that the inhibition of Cox-2/PGE2 results in decreased level of cAMP and PKA and consequently in an uncontrollable/continuous production of soluble factors via  $\text{INF}\gamma$  pathways induced by a CMV infection in endothelial/blood cells. Activation of vascular endothelium and attraction of specific blood cells by chemokines should further increase their interaction leading to prothrombotic events and increasing the risk of cardiovascular adverse events. Indeed, the majority of these changes have been shown to be directly involved in the pathogenesis of diverse cardiovascular diseases including atherosclerosis, CAD, and thrombosis. Preliminary histopathological results confirmed the genomic finding showing that the specific genomics pattern is an early signature of vasculitis and observed only in the animal(s) treated with Vioxx®.

**[0054]** Identification of biomarkers might allow the safe use of cox-2 inhibitory compounds in clinics and selection of cox-2 inhibitory follow-up compounds without cardiovascular toxicity. Indeed, several of the gene increases in the vessels of the Vioxx®-treated animal encode for secreted proteins, e.g., CXCL10, other chemokines, which can be measured in peripheral samples such as blood or urine. If a CMV reactivation (or other endothelium tropic virus infection) is confirmed, a vaccination strategy prior to administration of Cox-2 inhibitory therapies might be an alternative approach for improving the CV therapeutic and safety profile of this class of compounds.

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35 **[0056]** All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes. In addition, all Affymetrix identification numbers for each probe set corresponding to each gene changes cited herein (TABLE 1) are incorporated herein by reference in their entirety and for all purposes to the same extent as if each such number was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

**[0057]** The description further encompasses the following items (embodiments):

40 1. A method for the detecting the presence of minimal or early vasculitis or other vasculopathies in a subject, comprising the steps of:

- (a) obtaining a sample from a subject to whom a compound or drug, susceptible to induce cardiovascular pathologies has been administered or a subject with a vascular autoimmune disorder;
- 45 (b) analysing the sample for the presence of a biomarker of minimal or early vasculitis or other vasculopathies; and
- (c) determining whether the subject has minimal or early vasculitis or other vasculopathies based upon the presence of absence of a biomarker of minimal or early vasculitis or other vasculopathies.

50 2. A method for predicting compound or drug-induced cardiovascular adverse effects in a subject to whom a cox-2 inhibitory compound or drug has been administered, comprising the steps of:

- (a) obtaining a sample from a subject to whom a cox-2 inhibitory compound or drug has been administered;
- (b) analysing the sample for the presence of a biomarker of cardiovascular adverse effects; and
- 55 (c) determining whether the subject has cox-2 inhibitor-induced cardiovascular adverse effects based upon the presence of absence of a biomarker of cardiovascular adverse effects.

3. The use of a cox-2 inhibitory compound in the manufacture of an anti-inflammatory medicament with a reduced risk of cardiovascular toxicity, wherein the use comprises the steps of:

monitoring the patient to whom the anti-inflammatory medicament has been administered for the presence or absence of biomarkers predictive of cox-2 inhibitor-induced cardiovascular adverse effects.

5 4. The method of claim 3, wherein cox-2 inhibitory compound is selected from the group consisting of cox-2 specific inhibitors (coxibs), classical NSAIDs, other anti-inflammatory/immunosuppressive/immunomodulatory compounds and direct PGE2, cAMP and PKA inhibitors.

10 5. The method of claim 3, wherein cox-2 inhibitory compound is selected from the group consisting of COX189 (Lumiracoxib®), refocoxib (Vioxx®), and celecoxib (Celebrex®).

6. The method of claim 3, wherein the cox-2 inhibitory compound is the non specific cox-2 inhibitory compound diclofenac (Voltaren®).

15 7. The method of claim 3, wherein the biomarker predictive of cox-2 inhibitor-induced cardiovascular adverse effects is an increase in gene expression of a gene selected from the genes listed in TABLE 1.

20 8. The method of claim 3, wherein the biomarker predictive of cox-2 inhibitor-induced cardiovascular adverse effects is an increase in gene expression of an interferon inducible gene selected from the group consisting of the genes encoding for Toll-like receptors (TLRs), classical and non-classical MHC class I proteins, MHC class II proteins, TcRs, NK receptors, CXCL10, CXCL-9, CXCL 11, MCP-1 (CCL2), Jak1 and Stat1.

25 9. The method of claim 3, wherein the biomarker predictive of cox-2 inhibitor-induced cardiovascular adverse effects is an increase in gene expression of the gene for a coagulation pathways-related molecule selected from the group consisting of PD-ECGF, coagulation factor II (thrombin) receptor-like 1 and Factor 13 A1.

10. The method of claim 3, wherein the biomarker predictive of cox-2 inhibitor-induced cardiovascular adverse effects is an increase in Ccl10 gene expression.

30 11. The method of claim 3, wherein the biomarker predictive of cox-2 inhibitor-induced cardiovascular adverse effects is an increase in the release of pro-inflammatory cytokines and chemo-attractants.

12. The method of claim 3, wherein the biomarker predictive of cox-2 inhibitor-induced cardiovascular adverse effects is an increase in INF $\gamma$  inducible proteins.

35 13. The method of claim 3, wherein the biomarker predictive of cox-2 inhibitor-induced cardiovascular adverse effects is an increase in CXCL10 (IP10) protein levels.

40 14. The method of claim 3, wherein the biomarker predictive of cox-2 inhibitor-induced cardiovascular adverse effects is an increase in PD-ECGF1 protein.

15. The method of claim 3, wherein the biomarker predictive of cox-2 inhibitor-induced cardiovascular adverse effects is an increase in cPLA2 protein.

45 16. The method of claim 3, wherein the sample is a tissue sample.

17. The method of claim 3, wherein the sample is a cardiovascular tissue sample.

50 18. The method of claim 3, wherein the sample is selected from the group consisting of blood, plasma, serum, urine and saliva.

19. A method for the selection of cox-2 inhibitory compounds without cardiovascular toxicity for use in patients, comprising the steps of:

- 55 (a) administering a cox-2 inhibitory compound to a subject;  
(b) monitoring of early changes predictive of cardiovascular adverse effects in patients treated with compounds exhibiting cox-2 inhibition or increasing the production of molecules induced by interferons or by virus infections or vascular autoimmune disorders resulting in pro-coagulative / prothrombotic / endothelium changes;  
(c) selecting the cox-2 inhibitory compounds that do not show cardiovascular toxicity for use in patients; and

(d) selection of sub-population of patients to be treated safely by cox-2 inhibitory compounds/drugs

20. The method of claim 20, wherein the subject is a cynomolgous monkey.

21. A vaccination strategy prior to administration of cox-2 inhibitor to a subject, wherein the vaccination strategy reduces cardiovascular toxicity in the subject to whom the cox-2 inhibitor is administered.

## Claims

1. A method for predicting cox-2 specific inhibitory compound or drug induced cardiovascular adverse effects in a subject to whom a cox-2 specific inhibitory compound or drug has been administered, comprising the steps of:

(a) analysing a sample obtained from a subject to whom a cox-2 specific inhibitory compound or drug has been administered;

(b) analysing the sample for the presence of a biomarker of cardiovascular adverse effects; and

(c) determining whether the subject has cox-2 specific inhibitor-induced cardiovascular adverse effects based upon the presence of a biomarker of cardiovascular adverse effects,

wherein the cardiovascular adverse effect is minimal vasculitis, the biomarker is a soluble protein, the presence of the biomarker is defined by an increase of the biomarker, the sample is blood plasma or blood serum, and the biomarker is CXCL-10.

2. The method according to claim 1, wherein the cox-2 specific inhibitory compound is refocoxib.

3. Use of a peripheral biomarker for predicting cox-2 specific inhibitory compounds-induced minimal vasculitis in clinics, **characterized by** the clinical screening of patients prior to or during administration of cox-2 specific inhibitory therapies, wherein the biomarker is the increased expression of CXCL-10 chemokine in serum and plasma.

## Patentansprüche

1. Verfahren zur Vorhersage von kardiovaskulären Nebeneffekten induziert durch Cox-2 spezifische hemmende Verbindungen oder Medikamente in einem Subjekt, dem eine Cox-2 spezifische hemmende Verbindung oder Medikament verabreicht wurde, wobei das Verfahren die folgenden Schritte umfasst:

a) Analysieren einer Probe, von einem Subjekt gewonnen, dem eine Cox-2 spezifische hemmende Verbindung oder Medikament verabreicht wurde;

b) Analysieren der Probe auf die Anwesenheit eines Biomarkers für kardiovaskuläre Nebeneffekte; und

c) Feststellen, ob das Subjekt durch einen Cox-2 spezifischen Inhibitor induzierte kardiovaskuläre Nebeneffekte hat, auf der Grundlage der Anwesenheit eines Biomarkers für kardiovaskuläre Nebeneffekte,

wobei der kardiovaskuläre Nebeneffekt minimale Vaskulitis ist, der Biomarker ein lösliches Protein ist, die Anwesenheit des Biomarkers als die Erhöhung des Biomarkers definiert ist, die Probe Blutplasma oder Blutserum ist und der Biomarker CXCL-10 ist.

2. Verfahren nach Anspruch 1, wobei die Cox-2 spezifische hemmende Verbindung Refocoxib ist.

3. Verwendung eines peripheren Biomarkers um minimale Vaskulitis, induziert durch Cox-2 spezifische hemmende Verbindungen, in der Klinik vorherzusagen,

**gekennzeichnet durch** das klinische Screening von Patienten vor oder während der Verabreichung von Cox-2 spezifischen hemmenden Therapien, wobei der Biomarker die erhöhte Expression des Chemokins CXCL-10 in Serum oder Plasma ist.

## Revendications

1. Une méthode pour détecter des effets cardiovasculaires adverses induits par un composé ou un médicament inhibant

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spécifiquement la Cox-2 chez un sujet à qui un composé ou un médicament inhibant spécifiquement la Cox-2 a été administré, ladite méthode comprenant les étapes suivantes:

- 5 a) analyser un échantillon obtenu à partir d'un sujet à qui un composé ou un médicament inhibant spécifiquement la Cox-2 a été administré  
b) analyser l'échantillon pour déterminer la présence d'un biomarqueur d'effets cardiovasculaires adverses ; et  
c) déterminer si le sujet présente des effets cardiovasculaires adverses induits par un inhibiteur spécifique de la Cox-2 à partir de la présence d'un biomarqueur d'effets cardiovasculaires adverses,

10 l'effet cardiovasculaire adverse étant une vascularite minimale, le biomarqueur étant une protéine soluble, la présence du biomarqueur étant définie par une augmentation du biomarqueur, l'échantillon étant du plasma ou sérum sanguin, et le biomarqueur étant la CXCL-10..

15 **2.** La méthode selon la revendication 1, **caractérisée en ce que** le composé inhibiteur spécifique de la Cox-2 est le refocoxib.

20 **3.** Utilisation d'un biomarqueur périphérique pour prédire en clinique une vascularite minimale induite par des composés inhibiteurs spécifiques de la Cox-2, **caractérisée par** le criblage clinique de patients avant ou pendant l'administration de thérapies inhibitrices spécifiques de la Cox-2, le biomarqueur étant l'augmentation de l'expression de la chémokine CXCL-10 dans le sérum ou le plasma.

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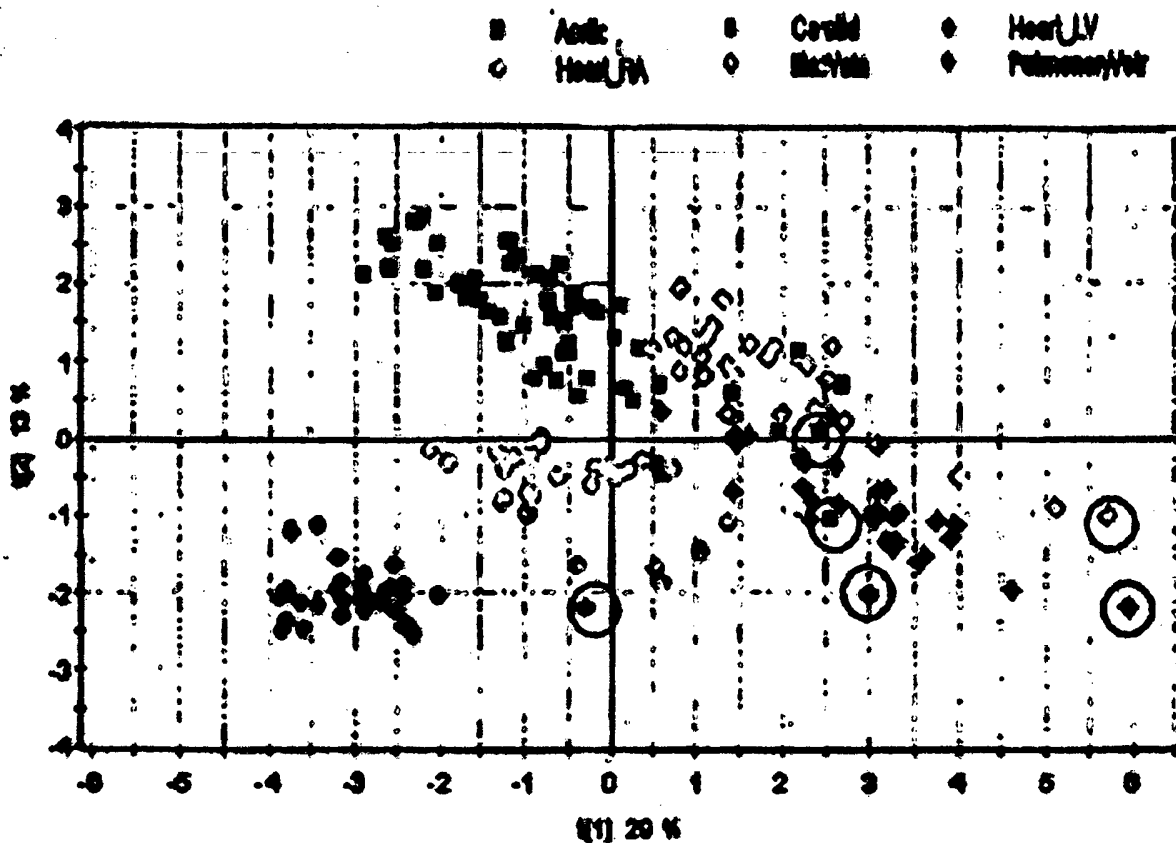


FIG. 1

**PCA analysis for selected genes**

Data from 6 cardiovascular tissues. The Vioxx-treated Monkey #A600055 (circled) exhibited distinct expression pattern.

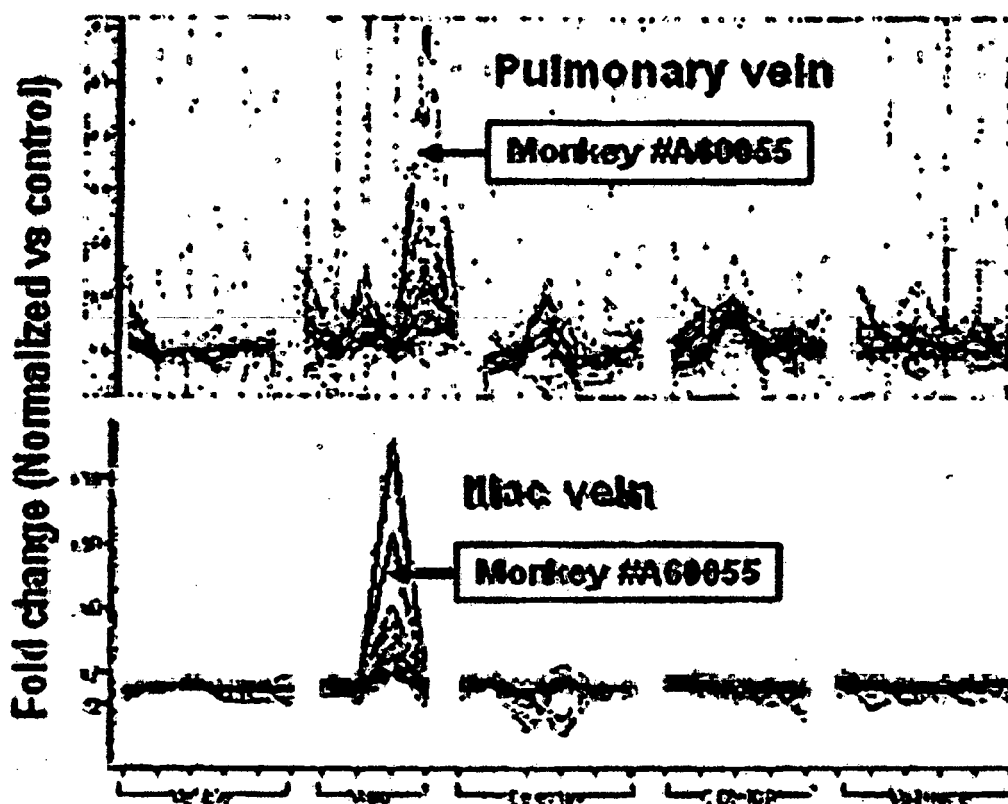
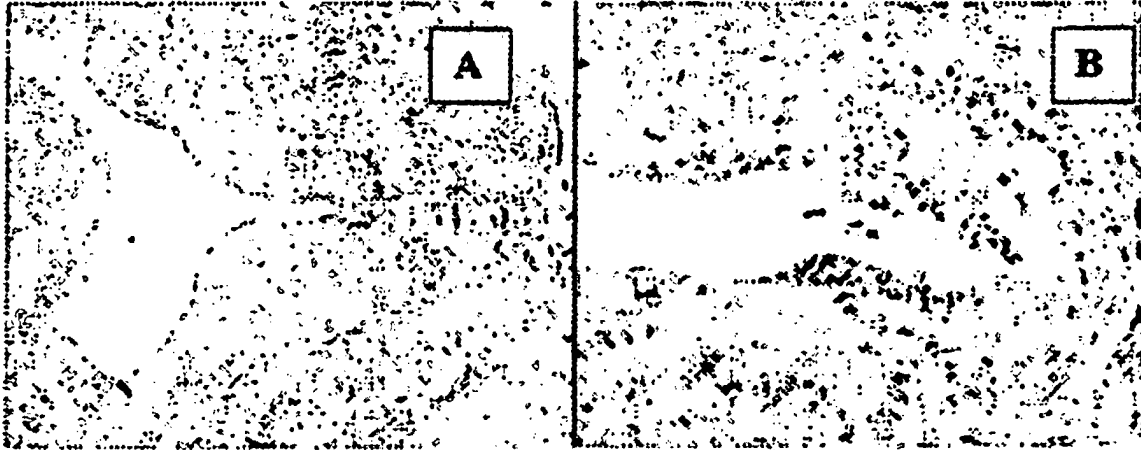


FIG. 2

### Specific mRNA expression pattern in the Monkey #A60055

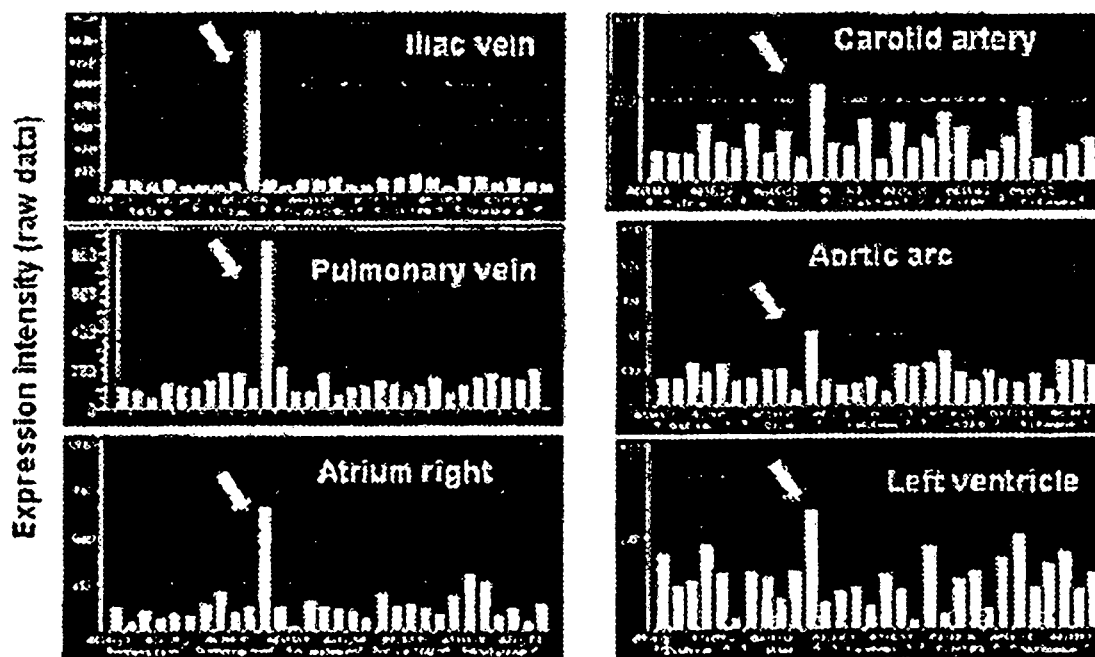
The pattern consisted of transcripts for MHC class I, II & class I, non classical molecules, their receptors (TcRs and NK receptors) and chemokines (CXCL9, -10, -11, MCP-1). Overall signature indicating strong INF pathway activation together with IL1/TNF, and coagulation and complement pathways alteration.



**FIG.3**

**Minimal Focal Vasculitis in the  
Vioxx<sup>®</sup> - treated animal only**

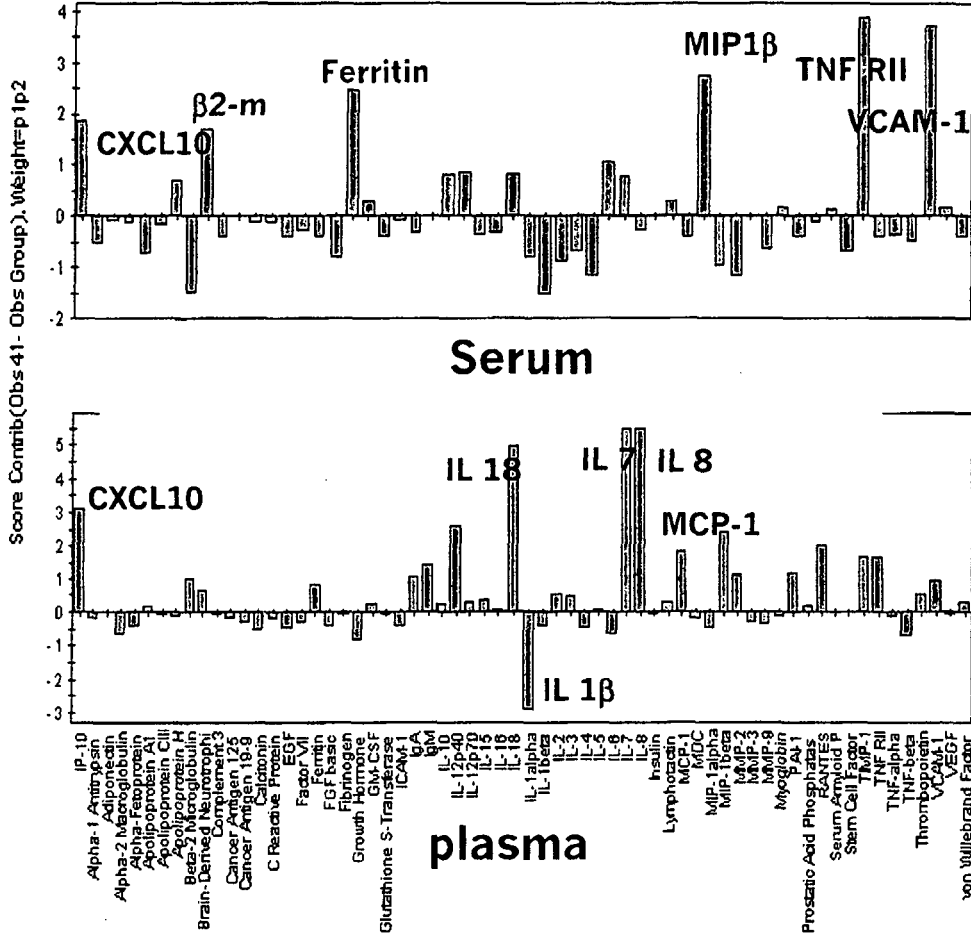
- (A) Iliac vein from vehicle treated animal.
- (B) Histopathology findings of endothelial cell (EC) necrosis, fibrin leukocyte adhesion to EC surface, fibrinoid degeneration of the media, medial leukocytes infiltration in iliac vein of the Monkey #A60055.



**FIG. 4**

Marked increase in the transcript expression of CXCL10 (IP10) in several cardiovascular tissues from the Vioxx<sup>®</sup> - treated Monkey #A60055.

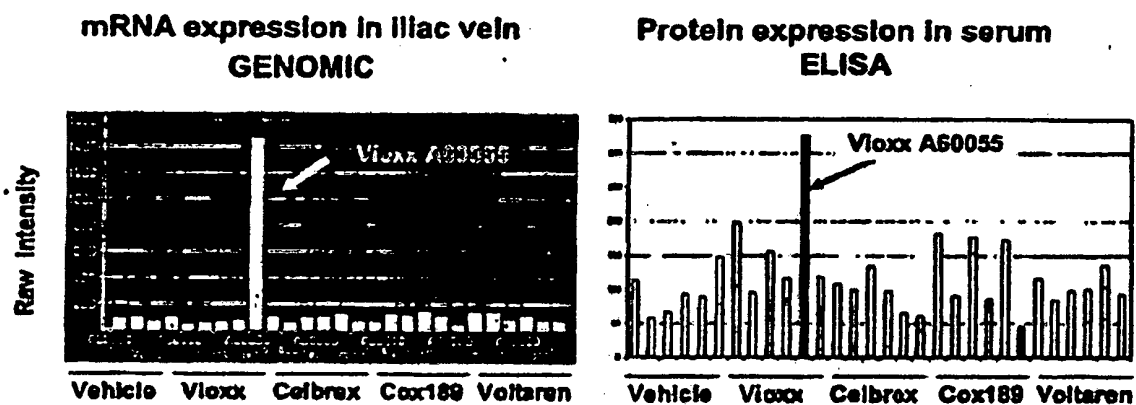
**Score contribution,  
Vioxx monkey A60055 vs all others**



**FIG. 5**

**Protein profiling in serum and plasma using  
RBM® multiplex assay.**

The monkey #A60055 exhibits a specific protein expression profile: soluble MHC molecules, b2-m, other chemokines, cytokines (INFγ, CXCL10, MCP-1, IL18, TNF RII, IL1b), and soluble VCAM-1.



**FIG. 6**

**Elisa confirmation of CXCL10 (IP10) protein level in monkey serum samples**

The Vioxx<sup>®</sup> - treated Monkey #A60055 exhibits the highest level of CXCL10 protein expression.

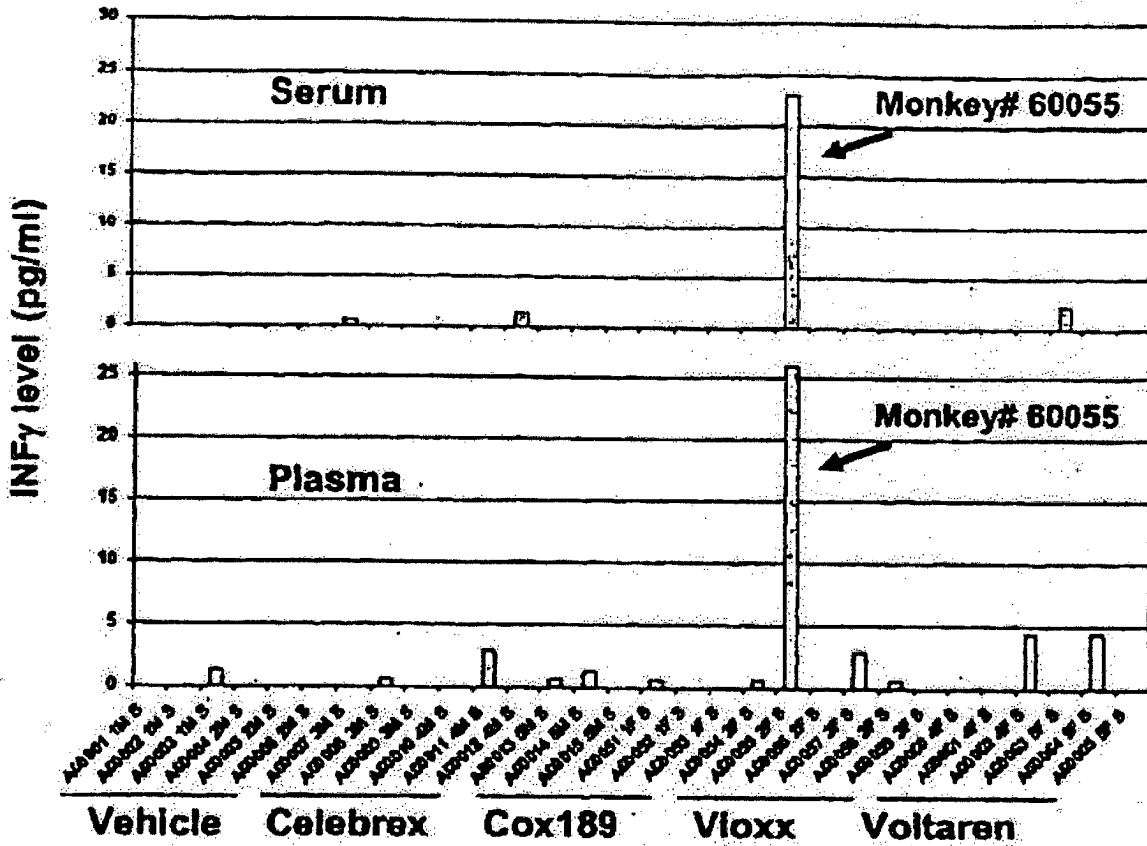
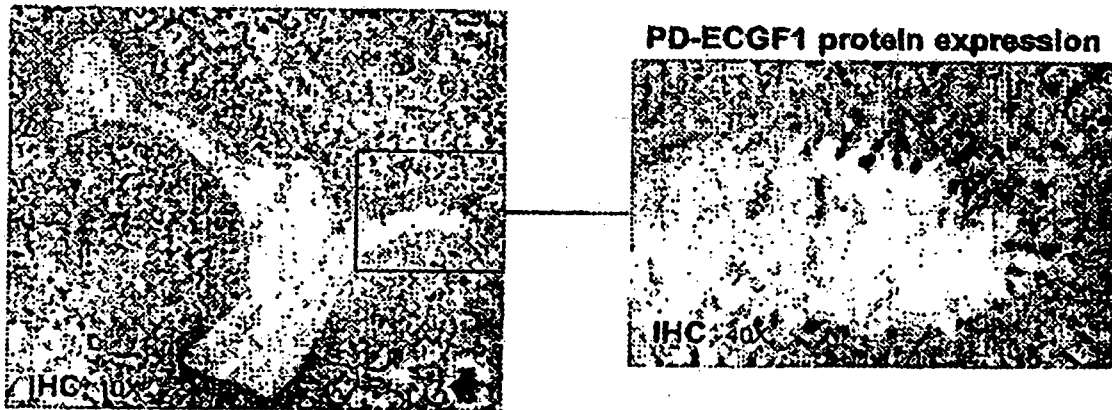


FIG. 7

Elisa confirmation of INFγ protein level in monkey serum and plasma samples. The Vioxx® - treated Monkey #A60055 exhibits the highest level of INFγ protein expression.



**FIG. 8**

Localisation of PD-ECGF1 protein at the site of the vascular lesion

## REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

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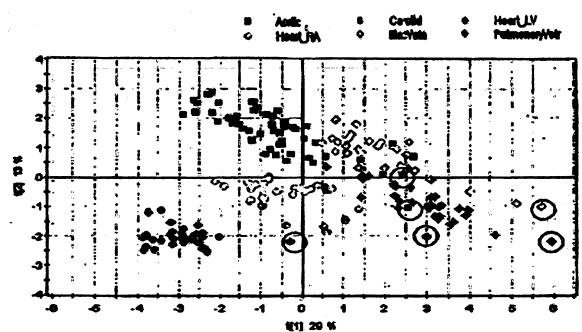
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专利名称(译)	由cox-2抑制性化合物诱导的心血管副作用的生物标志物		
公开(公告)号	<a href="#">EP2290363B1</a>	公开(公告)日	2014-01-08
申请号	EP2010010204	申请日	2006-03-10
[标]申请(专利权)人(译)	FIRALIS		
申请(专利权)人(译)	FIRALIS SAS		
当前申请(专利权)人(译)	FIRALIS SAS		
[标]发明人	FIRAT HUESEYIN BOISCLAIR JULIE GRENET OLIVIER PERENTES ELIAS SCHUMACHER MARTIN M		
发明人	FIRAT, HUESEYIN BOISCLAIR, JULIE GRENET, OLIVIER PERENTES, ELIAS SCHUMACHER, MARTIN M.		
IPC分类号	G01N33/53 A61K45/06 C12Q1/68		
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摘要(译)

分析了用coxibs处理的猴子的心血管组织mRNA表达谱。基因组数据表明显示血管炎的动物表现出特异性mRNA表达模式。该模式包括涉及血液和内皮细胞 ( EC ) 活化的基因表达变化, 血细胞与EC的相互作用,  $INF\gamma$ 途径的激活, 以及促炎细胞因子和化学引诱剂的释放。这些结果提供了最小血管炎的直接证据以及相应的基因组特征和用于最小血管的外周生物标志物。这些结果还表明, 在内皮向性病毒感染和/或自身免疫性血管疾病的情况下, 治疗可能触发/加重临床潜伏性心血管疾病。组织病理学检查显示边缘血管变化与基因组发现一致。使用多重测定法测量血清和血浆中存在的可溶性蛋白质与基因组结果一致, 显示 $INF\gamma$ 诱导蛋白质的水平增加。通过ELISA在血清和血浆中证实CXCL10趋化因子的表达增加。使用这些外周生物标志物可以在临床中安全使用cox-2抑制性化合物, 并选择无心血管毒性的cox-2抑制性随访化合物。这些数据以及生物化学和组织病理学发现表明, 特定的cox2抑制剂可能在某些特定的病毒感染过程中夸大宿主免疫反应, 这些感染伴有内皮向性或相邻的血管自身免疫疾病。



**FIG. 1**  
**PCA analysis for selected genes**  
 Data from 6 cardiovascular tissues. The Vioxx-treated Monkey #A600055 (circled) exhibited distinct expression pattern.