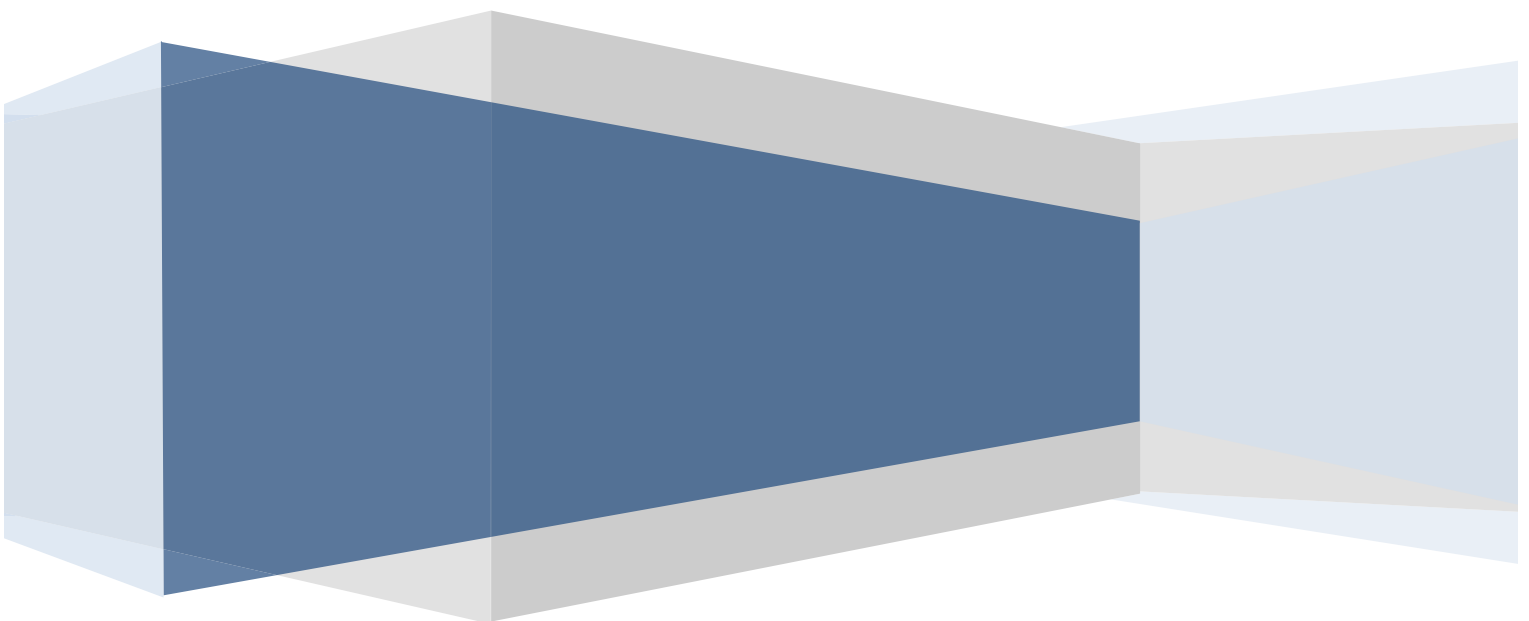




**Comparing Functions and Pathways Affected
by the COX-2 Inhibitors Celecoxib (Celebrex)
and Rofecoxib (Vioxx): Search & Explore in IPA**



EXECUTIVE SUMMARY

IPA's Search and Explore enables researchers to generate targeted search results, and then act on those results to create relevant biological models.

The Search feature in Ingenuity Pathways Analysis (IPA) provides users with direct access to [Ingenuity's Knowledge Base](#). The Ingenuity Knowledge Base is a database that contains detail-rich information on genes, chemicals, normal cellular and toxicity phenotypes, diseases, pathways, and their interrelationships. The Search capability generates relevant lists of genes and chemicals associated with specific biological functions, pathways, or annotations. Unlike traditional solutions, the resulting lists can then be transformed into fully interactive, literature-supported graphical models of experimental systems using IPA Explore tools such as Grow, Connect, and Path Explorer.

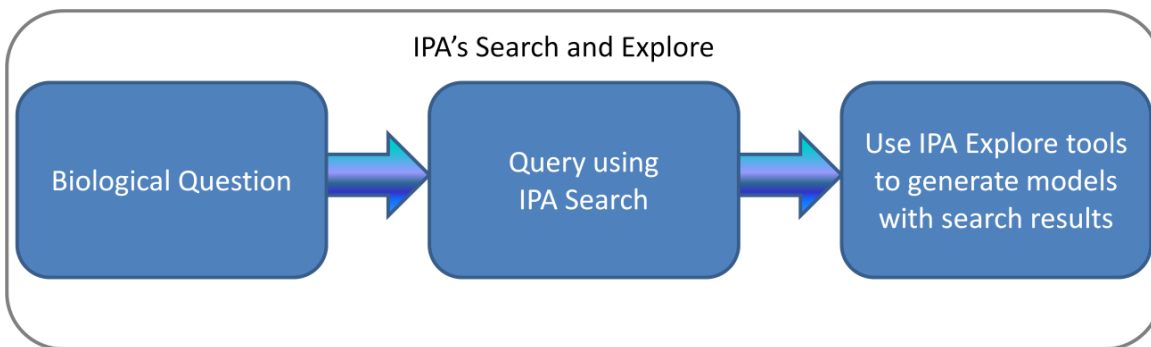


Figure 1: IPA's Search and Explore workflow

In this white paper, IPA's Search and Explore functions are used to retrieve and explore drug-related information from the literature – specifically to understand and identify the key mechanistic differences between Celebrex (celecoxib) and Vioxx (rofecoxib). Celebrex is on the market today; however, Vioxx has been withdrawn due to cardiovascular side effects. Both drugs inhibit the same target, COX-2 (or PTSG2), and are NSAIDs (nonsteroidal anti-inflammatory drugs) used for the treatment of rheumatoid arthritis. This example will illustrate how IPA's Search and Explore tools can be applied to understand where these drugs differ in terms of the pathways they impact, the cellular and toxicity phenotypes they play a role in, and the downstream genes they impact. The resulting comparisons can be used to generate testable hypotheses around drug mechanism of action and mechanism of toxicity.

STEPS

STEP 1: GAIN DEEP KNOWLEDGE OF THE DRUG TARGET COX-2

In IPA there are three main approaches used to retrieve and explore drug-related information from the literature:

- a. Simple Query on the target or drug
- b. Chem View
- c. Gene View

In this example, both drugs have the same target, so a simple query (using IPA Search) on the target is a good first step. Once in IPA, enter Cox-2 in the search box. PTGS2, the official symbol for Cox-2, is returned by the search. From there, click on the gene symbol to access the Gene View.

Gene View: PTGS2 (Neighborhood Explorer)

Review the categorized literature findings and database information for this node.

PTGS2 Human Mouse Rat

Entrez Gene Name: prostaglandin-endoperoxide synthase 2 (prostaglandin/GH synthase and cyclooxygenase)

Synonyms: COX-2, gpIqHS, NCO-2, MTCO2, PGGMS, PqHS-2, PqH synthase 2, PqG2, PqG, PMS-2, Prostaglandin endoperoxide synthase 2, TSS10

Source ID: --

Protein Family, Domain: EGF-like domain, enzyme, lipid binding, peroxidase, Prostaglandin-endoperoxide synthase, protein binding

Subcellular Location: cell body, Cytoplasm, cytosol, dendrite, dendritic branches, dendritic spine, dendritic tree, Endoplasmic Reticulum, endoplasmic reticulum lumen, endoplasmic reticulum membrane, inner nuclear membrane, lipid rich fraction, membrane fraction, mitochondrial fraction, mitochondrial membrane, nuclear envelope, nuclear fraction, outer nuclear membrane, perikaryon, perinuclear region, perinuclear space, rough endoplasmic reticulum, soluble fraction

Canonical Pathway: Arachidonic Acid Metabolism; Eicosanoid Signaling

Member Of: Cyclooxygenase, Prostaglandin-endoperoxide synthase

Top Findings from Ingenuity Knowledge Base (Show all 6266 categorized literature findings)

regulates: prostaglandin E2, PTGS2, PPSG1, VEGF, PTGER4, RLT1, BCL2, prostaglandin, epiprostanal, arachidonic acid, GSK3, HRT10, WDR24, AMPK2, PPI6B2

regulated by: lipopolysaccharide, IL1B, TNF, rhubarb myristate acetate, PD8059, IFNG, dexamethasone, SB203580, 15-deoxy-delta-12,14-PGJ 2, indomethacin, IL1A, prostaglandin E2, F38 MAPK, ERK, EGF

binds: CSN3B, JUB, IKB1, IKK1A, FOX, NFKB1, CREB1, ATF2, CEBPD, EP300, AP-1, Ap1, HDAC3, HDAC1 (includes EG-3065), HDAC2

role in cell: apoptosis, proliferation, invasion, quantity, survival, colony formation, differentiation, migration, cell movement, drug resistance

disease: cancer, neoplasia, tumorigenesis, pain, osteoarthritis, hyperplasia, inflammation, rheumatoid arthritis, dysmenorrhea, pulmonary fibrosis, alopecia, prostate cancer, lung cancer, squamous-cell carcinoma, colorectal cancer, neurodegeneration, breast cancer, eosinophilia, polynia, hypercalcaemia, Alzheimer's disease, adhesion, schizophrenia, fibrosis, thrombosis, colon cancer, coronary artery disease, hyperkeratosis, thrombocytopenia, thrombocytopenic purpura, allergic rhinitis, atherosclerosis, cough, migraine, osteoporosis, burkitts, active ulcerative proctitis, colorectal carcinoma, pathological myopia, actinic keratosis, allergic rhinitis, allergic rhinosinusitis, postmenopausal osteoporosis, congestion, prothrombotic, proctitis, juvenile rheumatoid arthritis, myocardial infarction, familial adenomatous polyposis, hypercholesterolemia, the common cold, choroidal neovascularization, tension headache, hyperlipidemia, gout, ulcerative colitis, macular degeneration, encephalomyelitis, cervical cancer, non-small cell lung cancer, non-small-cell lung carcinoma, leiomyosarcoma, tumor, lung adenocarcinoma, Waldenström's macroglobulinemia, colitis, stroke, strusis ovarian carcinoma, ovarian neoplasm, dysplasia, polyarticular juvenile rheumatoid arthritis, heart failure, adenocarcinoma, head and neck cancer, thyroid cancer, familial medullary thyroid cancer, medullary thyroid carcinoma, diabetic atherosclerosis, ovarian cancer, ovarian adenocarcinoma, hepatocellular carcinoma, adenoma

Descriptions from External Databases

Entrez Gene Summary: Prostaglandin-endoperoxide synthase (PTGS), also known as cyclooxygenase, is the key enzyme in prostaglandin biosynthesis, and acts both as a dioxygenase and as a peroxidase. There are two isoforms of PTGS: a constitutive PTGS1 and an inducible PTGS2, which differ in their regulation of expression and tissue distribution. This gene encodes PTGS2, which shows 98% - 99% amino acid sequence identity with mouse, rat, sheep, bovine, horse and rabbit PTGS2 proteins, respectively. Human PTGS2 is expressed in a limited number of cell types and regulated by specific stimulatory events, suggesting that it is responsible for the prostaglandin biosynthesis involved in inflammation and mitogenesis. The expression of this gene is deregulated in epithelial tumors.

GO Annotations

Molecular Function: peroxidase activity; prostaglandin-endoperoxide synthase activity; iron ion binding; protein binding; oxidoreductase activity; oxidoreductase activity, acting on single donors with incorporation of molecular oxygen; incorporation of two atoms of oxygen; heme binding; metal ion binding

Biological Process: electron transport; fatty acid biosynthetic process; cell motility; response to oxidative stress; blood pressure regulation; negative regulation of cell proliferation; cyclooxygenase pathway; anagen; regulation of inflammatory response

Cellular Component: nucleus; cytoplasm; endoplasmic reticulum; membrane; membrane; caveolar membrane; protein complex

6266 Categorized Literature Findings (Show details)

Figure 2: Gene View for PTGS2 in IPA

The Gene View for PTGS2 (COX-2) (see Figure 2) reveals a total of 6,266 categorized findings from the literature describing PTGS2's role in the cell, membership in pathways, expression and localization patterns, relevance to disease, mutations, and more. Key findings include:

- COX-2 is one of the key enzymes in prostaglandin biosynthesis pathways.
- COX-2 is involved in inflammation and mitogenesis.
- COX-2 is associated with many diseases including pain, osteoarthritis, rheumatoid arthritis, and several types of cancer.
- COX-2 is a target for Vioxx and Celebrex. It is also a target for other NSAIDs such as aspirin. However, these drugs target both COX-1 (PTSG1) and COX-2 (PTSG2).
- Both Vioxx and Celebrex act selectively on COX-2

STEP 2: EXPLORE VIOXX

After gaining background knowledge of the drug target (COX-2), the next step in this workflow is to understand the drugs of interest, Vioxx and Celebrex, and compare their effects on biological systems. To perform this comparison, search for Vioxx, then add it to a new pathway. Build out this pathway using the Grow tool to find all genes and gene products affected by that drug. This customized pathway now reflects the immediate molecular neighborhood around Vioxx. To understand what pathways, phenotypes and toxicity events are relevant to this drug and its neighborhood these molecules can be selected, added to a list, and then analyzed using IPA's core analysis. The same set of actions can be applied for the second drug, and then use IPA's Comparison Analysis

function to understand which pathways and phenotypes are unique or commonly affected by each drug. The following section illustrates these steps in more detail (see also Figure 3).

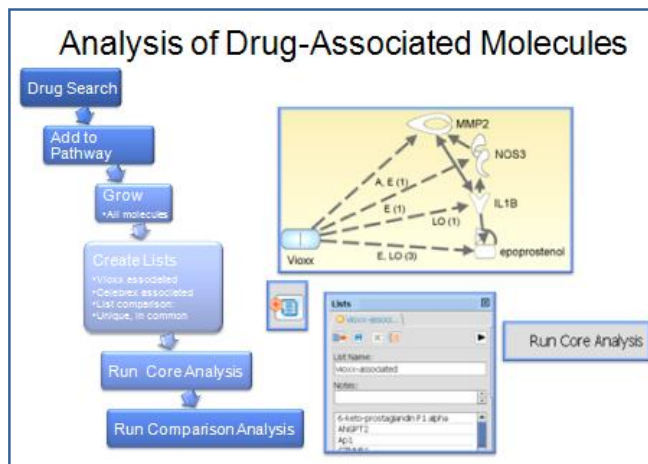


Figure 3: Schematic showing how to find molecules affected by the drugs of interest and then compare their biological effects

Start by searching on Vioxx in IPA. Results will be presented in a table (see Figure 4). Select Vioxx (synonym rofecoxib) by clicking the checkbox on the left, and then add this to a new pathway by clicking on the “Add to Pathway” button.

#	Name	Matched Term	Synonyms	Description	Location	Type	Drugs	
<input checked="" type="checkbox"/>	1	rofecoxib	Vioxx	162011-90-7, 3-phenyl-4-(4-methylsulfonyl)phenyl-4-(4-methylsulfonyl)phenyl-2-phenyl-1,3,4-oxadiazole, C17H14O4S, HS: 0966, HS: 966, Vioxx		Unknown	chemical drug	

Figure 4: Search results table in IPA

Once rofecoxib has been added to a new pathway, choose the Build tools, select rofecoxib, and then use the Grow function to bring in all genes and gene products affected by rofecoxib. Add all of the molecules in this pathway to a list and understand the key functions and pathways associated with rofecoxib-responsive genes by running a core analysis (see Figure 5).

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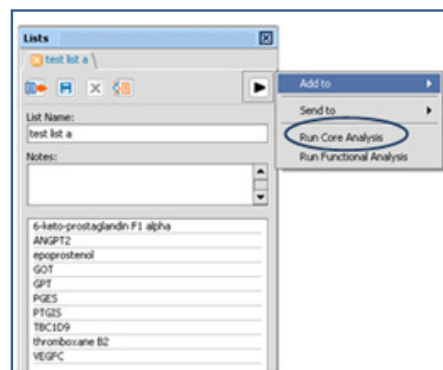
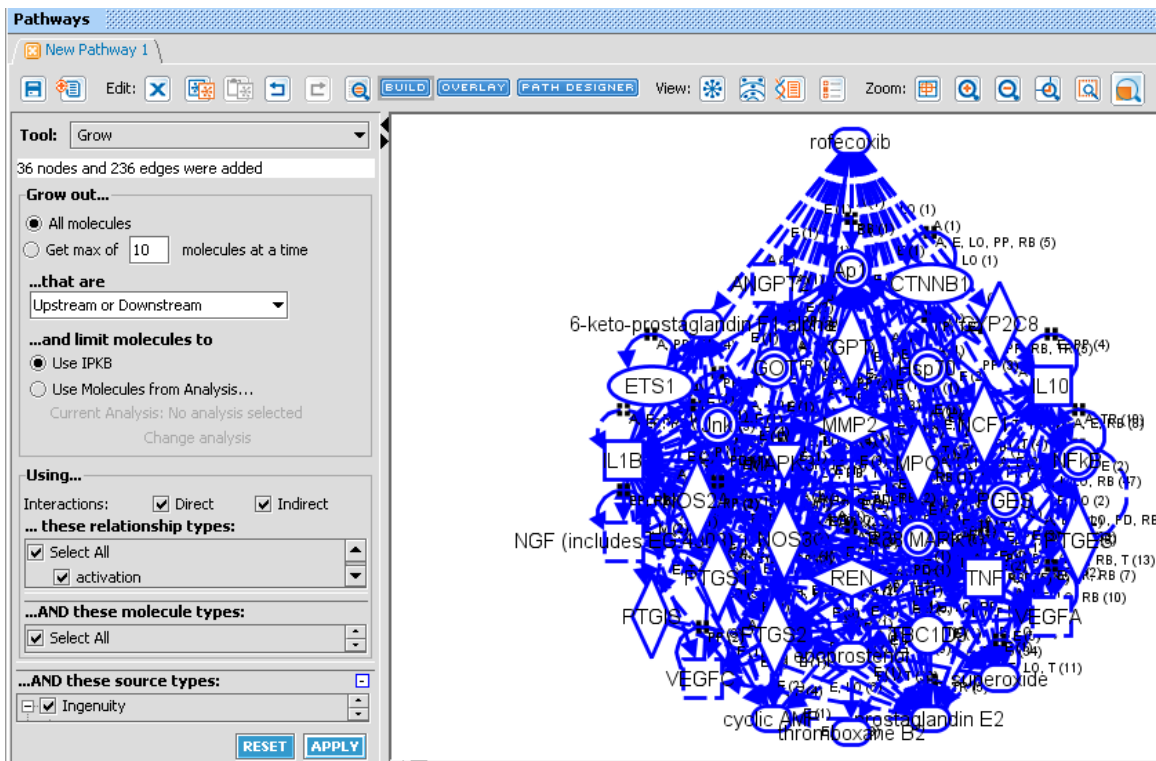


Figure 5: Running a core analysis on a list of molecules

A review of the Functional Analysis results indicates that cardiovascular disease is the most significant Function/Disease associated with rofecoxib-responsive genes. Further examination illustrates that many of these genes are associated with vascular lesions, hypertension, and thrombosis. While many of these disease associations are significant to understanding the biological effects of rofecoxib, the remainder of this workflow will focus on the association with vascular lesions (see Figure 6).

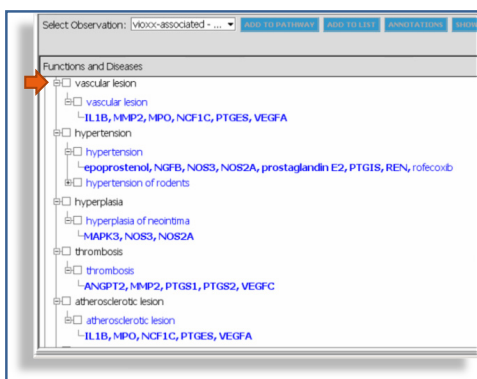


Figure 6: Functions and Diseases tab results reveal a strong association with vascular lesions

The rofecoxib-responsive genes associated with vascular lesion (IL1B, MMP2, MPO, etc.) can further be understood by looking at the Effect on Function feature, which indicates that several of these molecules increase atherosclerotic lesion, while IL1B KO decreases atherosclerotic lesion. This feature enables the researcher to infer the predicted effect that a drug of interest may have on a particular biological function. For example, an IL1B knockout decreases the size of atherosclerotic lesions (acting together with ApoE), suggesting that an increase in plasma levels of IL1B would be detrimental to cardiovascular health. Exploration of these molecules in a My Pathway highlights their relationships to Vioxx, enabling researchers to infer the impact of drug treatment on the atherosclerotic lesion phenotype.

To explore Drug and Function/Disease relationships, add Vioxx to vascular lesion-associated molecules. Select Connect, and then Overlay (see Figure 7).

Path Designer can be applied to color the molecules according to disease phenotype, membership in list, and other criteria in order to better highlight the relationships between molecules.

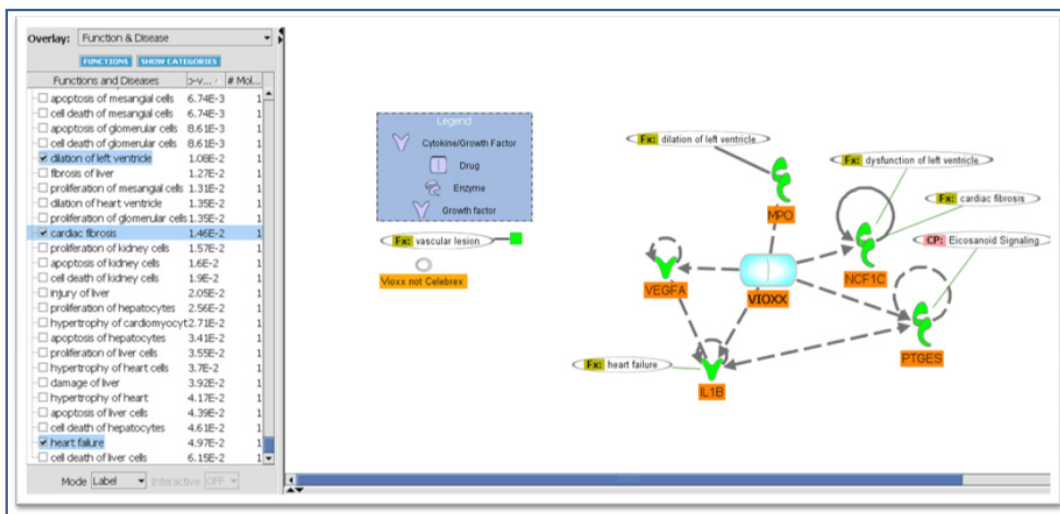


Figure 7: Exploration of drug and function/disease relationships in My Pathways enables inferring the impact of Vioxx treatment on disease phenotypes

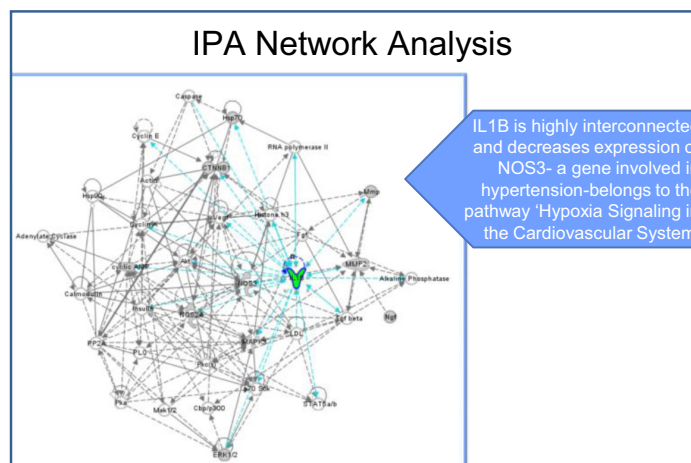


Figure 8: Through IPA network analysis IL1B is interconnected and decreases NOS3

After running the network analysis, it can be seen that exploration of molecular networks centered on IL1B reveals that IL1B is highly interconnected to other Vioxx-responsive molecules and that it decreases expression of NOS3 (see Figure 8). NOS3 is a gene involved in hypertension, and participates in the pathway “Hypoxia Signaling in Cardiovascular System.”

SUMMARY: VIOXX

In IPA, several different capabilities were utilized to learn more about Vioxx (see Figure 9).

1. The Functions and Diseases results from the core analysis of Vioxx-responsive genes identified cardiovascular disease as the most significant function.
2. Vioxx-to-gene relationships were examined, revealing that Vioxx increases plasma IL1B, which is associated to heart disease and vascular lesions.
3. Network Analysis revealed that ILB is highly interconnected to other Vioxx-associated molecules, that it decreases NOS3, and that ILB may increase hypertension.
4. From these steps, it can be hypothesized that Vioxx increases IL1B in the plasma, which may then lead to negative cardiovascular effects.

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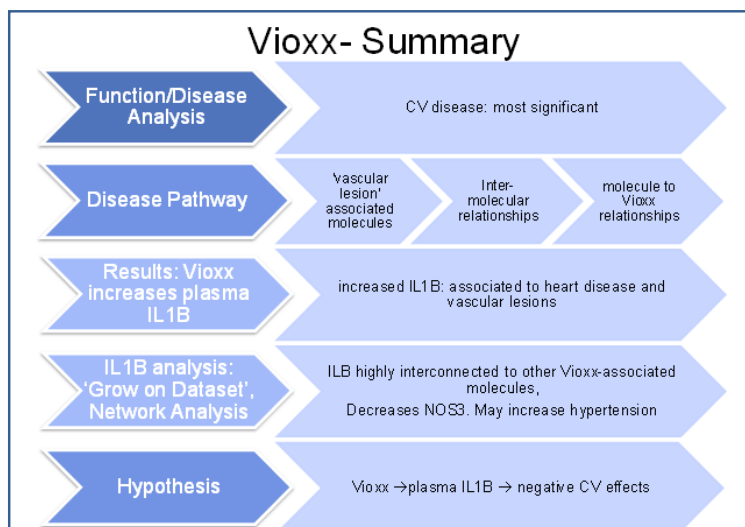


Figure 9: Summary of what can be discovered about Vioxx using IPA

STEP 3: EXPLORE CELEBREX

To understand what is known about Celebrex, mimic the steps taken to explore Vioxx in IPA.

Select Celebrex (celecoxib) and then add this to a new pathway clicking on the Add to Pathway button.

Choose the Build tools, select celecoxib and then use the Grow function to identify genes affected by celecoxib. Add these celecoxib-responsive genes to a list and run a core analysis to identify the key functions, pathways, and networks relevant to this gene set (see Figure 10). Review of this Functional Analysis results reveal that cell death is the most significant Function/Disease associated with this gene set.

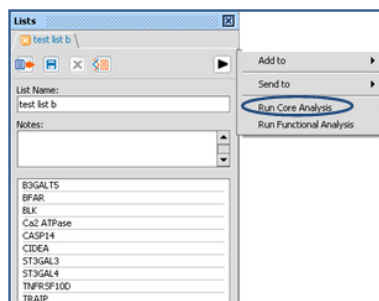


Figure 10: Running an analysis on a list of molecules

To compare the biological processes, pathways, and phenotypes associated with Vioxx-responsive genes versus Celebrex-responsive genes, run a Comparison Analysis on the original core analyses. From the Quick Start Screen, click Compare Analyses under the Core Analysis section and individually select each of these single analyses to compare. One function that appears to be significant is apoptosis (see Figure 11).



Figure 81: Significance of apoptosis to Celebrex and Vioxx related molecules. Vioxx analysis in green. Celebrex analysis in yellow.

The Celebrex-specific association with apoptosis is confirmed in the Canonical Pathways analysis, which reveals a strong association between Celebrex-responsive genes and p53 signaling, cell death receptor signaling, and apoptosis signaling (see Figure 12).

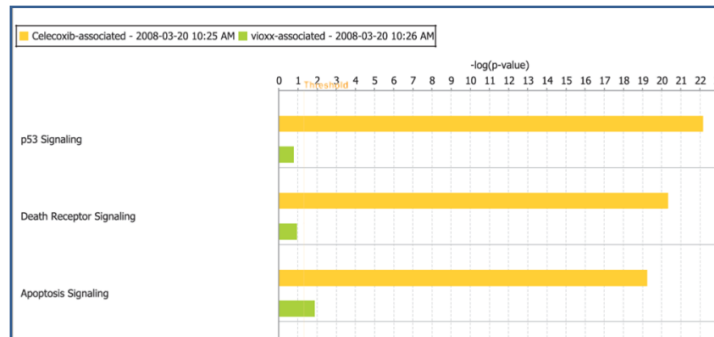


Figure 12: Comparison of canonical pathways associated with Celebrex versus Vioxx-responsive genes

To visualize where the molecules are associated in the Apoptosis Signaling pathway, double-click on the bar, and then click the View Pathway link. The associated molecules will be colored gray by default, and re-colored using the Path Designer feature (see Figure 13).

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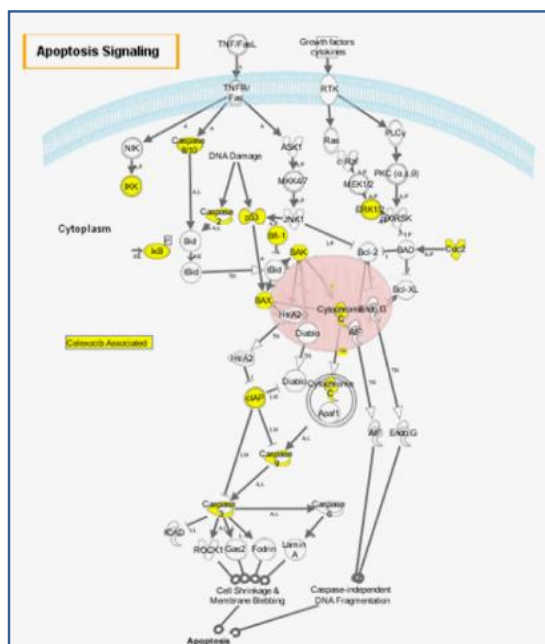


Figure 93: Apoptosis signaling pathway: Celebrex association

A better understanding of the Celebrex-apoptosis relationship can be gained from IPA's Chem View for this drug (see Figure 14).

Chem View: celecoxib (Neighborhood Explorer)	
Review the categorized literature findings and database information for this node.	
<p>Synonyms: 169590-42-5, 184007-95-2, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide, C17H14F3N3O2S, Celebrex, H5DB 7038, SC 58636, YH 177</p> <p>Systematic Name: 4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide</p> <p>IUPAC Name: 4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide</p> <p>CAS Registry Number: 169590-42-5, 184007-95-2</p> <p>SMILES: CC1=CC=C(C=C1)C2=CC(=NC23=CC=C(C=C3)S(=O)(=O)N4C=CC=C4)N5C=CC=C5</p> <p>InChI: [CH]1=C17H14F3N3O2S[C]1-11-2-4-12[S]-9-11[S]-10-14(17)18,19[20]22-23(15)3-4-6-14(9-7-13)24(21,24)25/N3-104,1H3,(H2,21,24,25)/H21H2</p> <p>Chemical Formula: C₁₇H₁₄F₃N₃O₂S</p> <p>Molecular Weight: 381.373</p> <p>PubChem Link: 2662</p> <p>Canonical Pathways: --</p>	
Top Findings from Ingenuity Knowledge Base (show all 647 categorized literature findings)	
regulates:	PTGS2, MCL1, F3, FAS, CASP9, PTGS1, prostaglandin E2, PCNA, CCND1, CPLA1, BRCA1, TNFRSF10B, BAK1, VEGF
regulated by:	CYP2C9
binds:	PTGS2
role in cells:	apoptosis, proliferation, growth, survival, G1 phase, cell death, invasion, cell viability, adhesion, infiltration
diseases:	tumorigenesis, neoplasia, inflammation, cancer, collapsing glomerulopathy, proteinuria, mesangiolysis, alopecia, arthritis, diarrhea, mesangial sclerosis, pain, experimentally induced inflammation, lung cancer
Group/Family of Compound	
Member of Groups:	sulfonamide

Figure 104: Chem View for Celebrex

From the Chem View summary under "Top Findings," key information about Celebrex can be quickly ascertained, such as its role in the cell (proliferation) and in diseases (arthritis). There are also links to clinicaltrials.gov. (see Figure 15) that show ongoing clinical trials for use of Celebrex for treating different types of cancer.



Figure 115: From Chem View in IPA, link to ClinicalTrials.gov for additional information

Additional cardiovascular-related information can be found under Tox Functions. By drilling down to look at molecules associated with cardiac infarction, it can be seen that CRP, an inflammatory marker for myocardial infarction, is associated with Celebrex (see Figure 16).

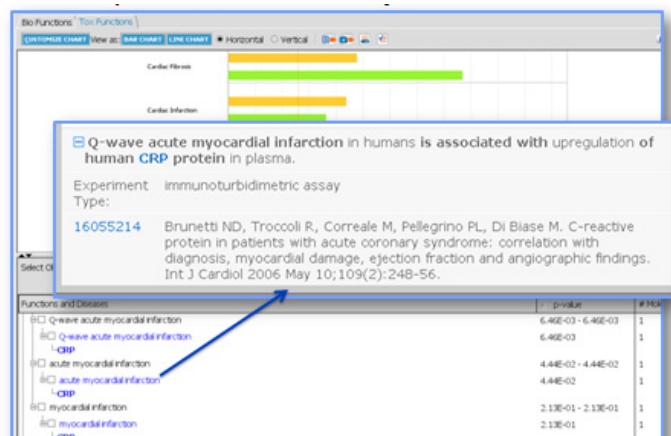


Figure 16: Comparison: Cardiotoxicity related functions

Click on CRP to open its Gene View. Scroll down to the Biomarker Status section to see that CRP is a potential biomarker. Specifically, CRP is an inflammatory marker for myocardial infarction and stroke (see Figure 17).

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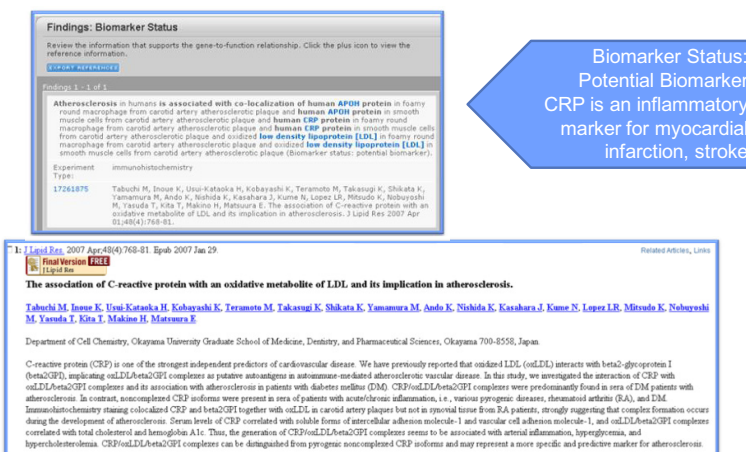


Figure 17: Gene View for CRP

To explore Celecoxib effects on CRP (myocardial infarction marker), a Grow from CRP on Celebrex Analysis can be performed (see Figure 18).

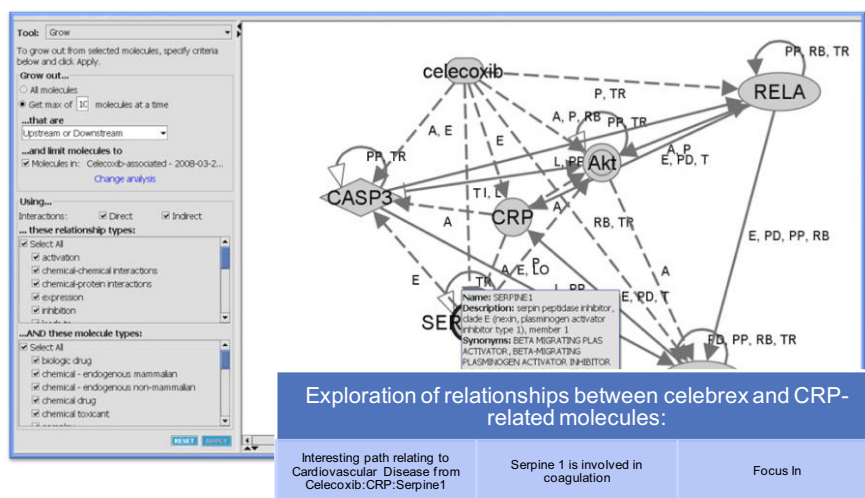


Figure 12: Exploring Celecoxib effects on CRP (myocardial infarction marker)

The association of CRP and Celebrex can be explored in a My Pathway. By drilling down to understand the relationships between Celebrex, CRP, and stroke, it can be seen that Celebrex decreases expression of CRP. A decrease in CRP has been found to alleviate hypertension in a rat model.

Expanding this model will look for additional ways that Celebrex may have cardiovascular effects via its effect on CRP. Additional molecules from the Celebrex analysis related to CRP are uncovered by using the Grow tool's Grow on Analysis feature. In this analysis it is found that Serpine1, a molecule associated with the coagulation system, is related to Celebrex and CRP.

SUMMARY: CELEBREX

By exploring these findings, reiterated below, a hypothesis can be formulated and then represented as a pathway (see Figures 19 and 20).

1. Celebrex decreases CRP expression.
2. CRP is known to activate and increase expression of Serpine1. Therefore, decreases in CRP are expected to decrease Serpine 1.
3. Knockout of serpine1 is known to decrease thrombosis.

Hypothesis: Celecoxib decreases expression of CRP, resulting in decreased activation and expression of Serpine 1. Reduced levels of Serpine1, in turn, decrease thrombosis.

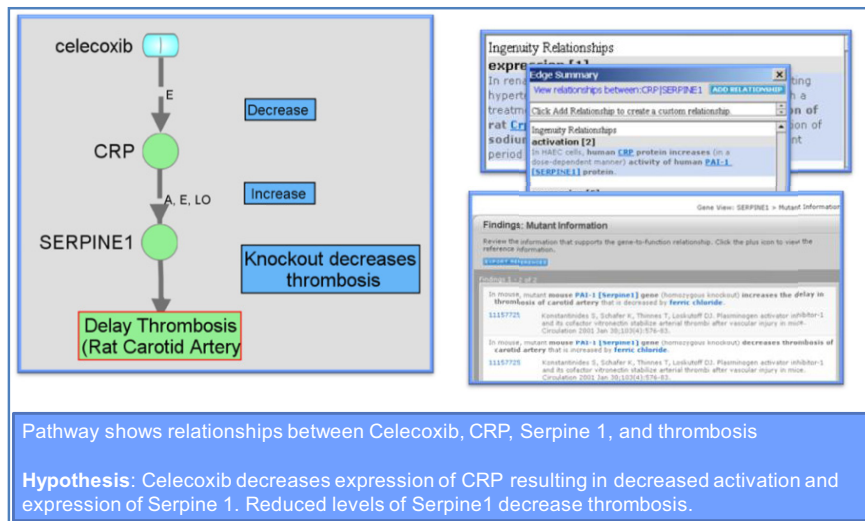


Figure 19: Exploring Celecoxib effects on myocardial infarction and thrombosis

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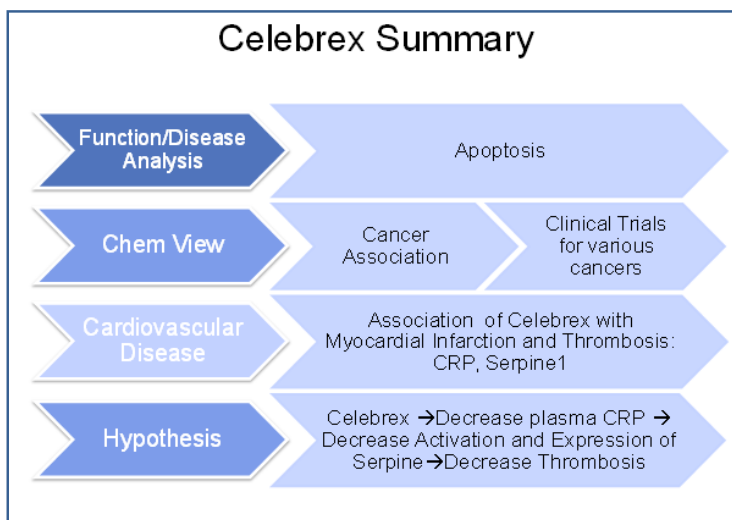
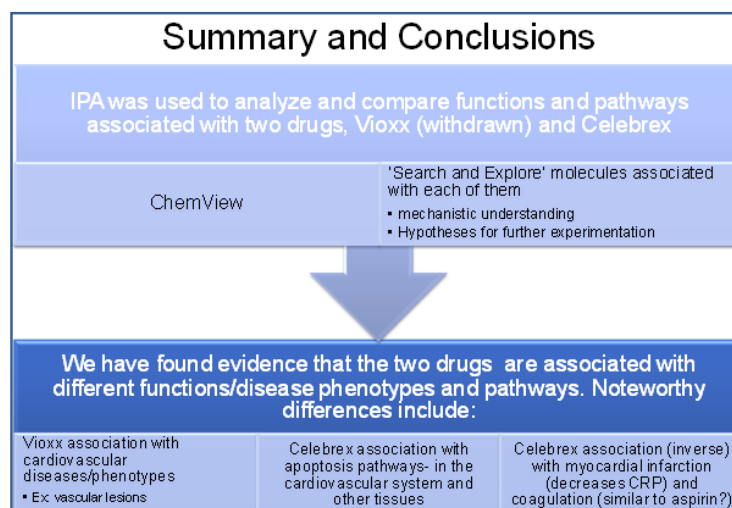


Figure 13: Summary of what can be discovered about Celebrex using IPA

CONCLUSION

By applying the Search and Explore workflow within IPA, the biological effects of Celebrex and Vioxx were examined and compared. Different pathways, functions, and disease phenotypes are associated with the genes that are affected by these two drugs. Vioxx-responsive and Celebrex-responsive molecules identified using the Grow tool were analyzed with an IPA Comparison Analysis, which identified the functions and diseases most significantly associated with these two gene sets. The most significant function associated with the Vioxx gene set is cardiovascular disease. Further exploration of the associated molecules reveals disease pathways by highlighting molecular interactions with Vioxx (see Figure 21 below). Green highlighting indicates drug-responsive molecules that have been linked to vascular lesions, while orange indicates the molecules are uniquely Vioxx-responsive. Examination of Celebrex specifically shows a strong association with cell death and apoptosis. Exploration at a detailed level of molecules associated with cardiovascular disease uncovered an association with decreased thrombosis and myocardial infarction.



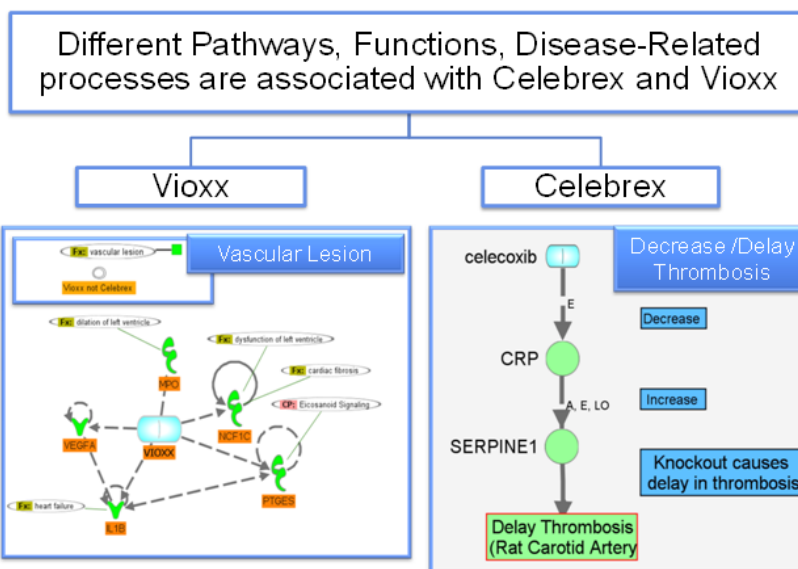


Figure 21: Clear mechanistic differences between Celebrex and Vioxx

The Search & Explore workflow outlined in this whitepaper is just one of many possible avenues within IPA for gaining valuable knowledge about genes, chemicals, and the biological pathways and processes they impact, and for building relevant, testable models of experimental systems. IPA's technology facilitates biomarker discovery, target identification, elucidation of drug mechanism of action and mechanism of toxicity, and detailed understanding of the molecular underpinnings of disease. IPA Search & Explore combines effective searching over relevant, detailed biological and chemical knowledge with powerful tools for transforming that knowledge into pathways that integrate multiple layers of context. These capabilities address an unmet need within the biomedical research community and establish IPA as an essential component of life science researchers' toolkit.

Learn more about IPA Search & Explore with a free 2-week trial of IPA. Click [here](#) for more information.

For additional information on all of the features and benefits of IPA, as well as a full bibliography (searchable by keyword) describing the more than 1000 peer-reviewed articles that cite IPA results, please visit the Ingenuity website at www.ingenuity.com.