THE UNIVERSITY of ADELAIDE

# Investigating Drugs that Enhance Imatinib Uptake and Factors which Contribute to the Functional Activity of OCT-1 CML Cells

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# TABLE OF CONTENTS

**TABLE OF CONTENTS** .......... **I**

**LIST OF FIGURES AND TABLES** .......... **VI**

**ABBREVIATIONS** .......... **X**

**PUBLICATIONS** .......... **XIII**

*Manuscripts* .......... *xiii*

*Conference Abstracts* .......... *xiii*

**SCHOLARSHIPS & AWARDS** .......... **XV**

**ACKNOWLEDGEMENTS** .......... **XVI**

**ABSTRACT** .......... **XVIII**

**DECLARATION** .......... **XX**

**CHAPTER I INTRODUCTION** .......... **1**

1.1. CHRONIC MYELOID LEUKAEMIA .......... 2

*1.1.1. Clinical features of CML* .......... *2*

*1.1.2. Pathophysiology of CML* .......... *3*

1.2. CML THERAPIES IN PRE-TKI ERA .......... 5

1.3. CML THERAPIES IN THE ERA OF TYROSINE KINASE INHIBITORS .......... 8

*1.3.1. First generation TKI: imatinib* .......... *8*

*1.3.2. Resistance to imatinib* .......... *12*

*1.3.3. Second generation TKIs: dasatinib and nilonitib* .......... *15*

*1.3.4. Third generation TKI: ponatinib* .......... *18*

*1.3.5. Transplantation in the era of TKIs* .......... *19*

1.4. DRUG TRANSPORTERS FOR IMATINIB .......... 20

*1.4.1. Efflux transporters: ABCB1 and ABCG2* .......... *20*

*1.4.2. Influx transporters for imatinib: OCT-1* .......... *20*

1.5. DRUG INTERACTIONS WITH IMATINIB VIA TRANSPORTERS .......... 24

1.6. HYPOTHESIS AND AIMS .......... 26

**CHAPTER II MATERIALS AND METHODS** .......... **28**

2.1. COMMONLY USED REAGENTS ......29
2.2. SOLUTIONS, BUFFERS AND MEDIA ......31
*2.2.1. Cell culture media* ......*31*
*2.2.2. Complete (25x stock)* ......*31*
*2.2.3. Flow cytometry fixative (FACS Fix)* ......*31*
*2.2.4. Hanks balanced salt solution (HBSS)* ......*31*
*2.2.5. Freeze Mix* ......*31*
*2.2.6. Laemmli's buffer* ......*32*
*2.2.7. 1 x Red Cell Lysis buffer:* ......*32*
*2.2.8. MACS buffer* ......*32*
*2.2.9. TBS* ......*33*
*2.2.10. TBST* ......*33*
*2.2.11. Membrane blocking solution (2.5%)* ......*33*
*2.2.12. Pefabloc stock solutions* ......*33*
*2.2.13. PhosSTOP (10x stock)* ......*33*
*2.2.14. Prazosin hydrochloride* ......*33*
*2.2.15. RIPA Buffer* ......*34*
*2.2.16. SDS-Polyacrylamide Gel* ......*34*
*2.2.17. 2xSDS load buffer* ......*34*
*2.2.18. Thaw solution* ......*35*
*2.2.19. Tyrosine kinase inhibitors* ......*35*
*2.2.20. White cell fluid* ......*36*
2.3. CELL LINES ......36
*2.3.1. K562* ......*36*
*2.3.2. KU812* ......*36*
*2.3.3. HL60* ......*36*
*2.3.4. HeLa* ......*36*
2.4. PRIMARY CELLS FROM CML PATIENTS OR HEALTHY DONORS ......37
2.5. GENERAL TECHNIQUES ......37
*2.5.1. Bradford protein assay* ......*37*
*2.5.2. Cell counts and cell viability determination* ......*38*
*2.5.3. Cryopreservation of cells* ......*38*
*2.5.4. Tissue culture* ......*38*
*2.5.5. Thawing cells* ......*39*
*2.5.6. Lymphoprep isolation of mononuclear cells (MNC)* ......*39*

2.6. SPECIALISED TECHNIQUES ........................................................................................40
*2.6.1. Imatinib intracellular uptake and retention (IUR) assay ....................................40*
*2.6.2. Western blot for phosphorylated Crkl (p-Crkl) and IC50 ..................................41*
*2.6.3. Real time quantitative PCR (RQ-PCR)..........................................................42*
*2.6.4. Gene expression profiling analysis...........................................................45*
*2.6.5. Enzyme immunoassays for prostaglandin E2 (PGE2).........................................46*
*2.6.6. Enzyme immunoassays for 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2)........................47*
*2.6.7. Western blotting for protein of interests.............................................48*
*2.6.8. Preparation of nuclear extract and cytoplasmic fraction...................................49*
*2.6.9. Peroxisome proliferator agonist receptor γ transcription factor assay ..............50*
*2.6.10. $CD14^+$ and $CD15^+$ cells isolation .......................................................51*
2.7. STATISTICS ........................................................................................54

**CHAPTER III IDENTIFICATION AND VALIDATION OF OCT-1 ACTIVITY ENHANCERS BY BIOINFORMATICS AND EXPERIMENTAL APPROACHES......55**

3.1. INTRODUCTION ........................................................................................56
3.2. METHODS ........................................................................................59
*3.2.1. Selection of candidate drugs as functional OA enhancers ..................................59*
*3.2.2. Drugs preparation ........................................................................................60*
*3.2.3. Validation of functional OA enhancer candidates...............................................60*
3.3. RESULTS ........................................................................................63
*3.3.1. The Connectivity Map (CMAP) as a tool to select candidate drugs as functional OA enhancers........................................................................................63*
*3.3.2. Experimental validation of OCT-1 enhancer candidates with IUR assay...........71*
*3.3.3. Effects of CMAP candidates on IUR and OA in K562 cell lines .........................71*
*3.3.4. Effects of NSAIDs on IUR of imatinib and OA in K562 cell lines .......................73*
*3.3.5. Effects of clinically applicable drugs on IUR of imatinib and OA in K562 cell lines.....................................................................................................75*
*3.3.6. Effects of clinically applicable drugs on IUR and OA in KU812 cell lines..........75*
*3.3.7. Effects of temperature on the regulation of IUR by diclofenac and ibuprofen in KU812 cells........................................................................................78*
*3.3.8. Effects of diclofenac and ibuprofen on $IC50^{imatinib}$ of BCR-ABL-positive cell lines.....................................................................................................80*
*3.3.9. Effects of diclofenac and ibuprofen on viable cell counts when co-administrated with imatinib in BCR-ABL-positive cells ..........................................................82*

*3.3.10. Effects of diclofenac and ibuprofen on the OA of primary cells*..........................*84*
3.4. DISCUSSION ..........................................................................................87

**CHAPTER IV DICLOFENAC REGULATES OCT-1 ACTIVITY AT THE TRANSCRIPTIONAL LEVEL..........................................................................93**

4.1. INTRODUCTION ..........................................................................................94
4.2. METHODS ..........................................................................................96
*4.2.1. IUR and OA assessment in the presence and absence of Actinomycin D............96*
*4.2.2. Gene expression profiling analysis..........................................................97*
4.3. RESULTS ..........................................................................................97
*4.3.1. Effect of transcriptional inhibition on the ability of diclofenac to increase OA in KU812 cell line ..........................................................................................97*
*4.3.2. Identification of candidate gene for OA regulation by diclofenac using gene expression profiling with GeneChip Human gene 1.0ST Array ......................................98*
4.4. DISCUSSION ..........................................................................................119

**CHAPTER V THE ASSOCIATION BETWEEN OCT-1 ACTIVITY AND COX-2 IN CP-CML PATIENTS ..........................................................................................124**

5.1. INTRODUCTION ..........................................................................................125
5.2. METHODS ..........................................................................................130
*5.2.1. Examination of PTGS2 gene expression in primary samples............................130*
*5.2.2. Examination of plasma levels of PGE2 and 15d-PGJ2.....................................131*
5.3. RESULTS ..........................................................................................131
*5.3.1. PTGS2 expression in CML patients..........................................................131*
*5.3.2. PGE2 and 15d-PGJ2 levels in CML patients..................................................133*
*5.3.3. Correlation of PTGS2 expression and PGE2/15d-PGJ2 plasma levels............135*
*5.3.4. Correlation of OCT-1 Activity with PTGS2 expression, PGE2 and 15d-PGJ2 levels... ..........................................................................................135*
*5.3.5. Correlation of Molecular Response to imatinib and PTGS2 expression, PGE2 and 15d-PGJ2..........................................................................................143*
5.4. DISCUSSION ..........................................................................................143

**CHAPTER VI THE ASSOCIATION BETWEEN OCT-1 ACTIVITY AND PPARΓ IN CP-CML PATIENTS ..........................................................................................151**

6.1. INTRODUCTION ..........................................................................................152
6.2. METHODS ..........................................................................................156

*6.2.1. PPARγ expression levels after treatment with ligands in CML cell line* ............156
*6.2.2. PPARγ transcriptional activity in CML MNC samples* ....................................156
*6.2.3. PPARγ in isolated CD14$^+$ and CD15$^+$ cells from CML patients and healthy donors.* ....................................158
6.3. RESULT ....................................158
*6.3.1. The IUR assay in the presence of PPARγ ligands* ....................................158
*6.3.2. PPARγ expression and its subcellular localization in CML cells by Western Blotting* ....................................162
*6.3.3. Correlation of OCT-1 activity with PPARγ transcriptional activity in CP-CML MNCs..* ....................................166
*6.3.4. Correlation between PPARγ transcriptional activity in MNCs and 15d-PGJ2 plasma levels in CP-CML samples* ....................................171
*6.3.5. Isolation of CD14$^+$ cells and CD15$^+$ cells from CML patients and normal donors...* ....................................171
*6.3.6. Protein and transcriptional activity levels of PPARγ in isolated CD14$^+$ and CD15$^+$ isolated cells* ....................................176
6.4. DISCUSSION ....................................179
**CHAPTER VII DISCUSSION** ....................................**185**
7.1. INTRODUCTION ....................................186
7.2. MAJOR FINDINGS ....................................187
*7.2.1. Diclofenac and ibuprofen have opposite effects on functional OCT-1 activity in CML cells* ....................................187
*7.2.2. Diclofenac regulates OA at the transcriptional level but not directly via SLC22A1 gene expression* ....................................188
*7.2.3. The role of the COX pathway in OA regulation* ....................................189
*7.2.4. PPARγ negatively regulates OA in CML cells* ....................................190
7.3. SUMMARY ....................................191
7.4. FUTURE DIRECTIONS ....................................192
7.5. CONCLUSION ....................................194
**APPENDIX A PUBLICATION ARISING FROM THIS THESIS** ....................................**195**
**APPENDIX B SUPPLEMENTARY FIGURES AND TABLES** ....................................**204**
**REFERENCES** ....................................**211**

# List of Figures and Tables

Figure 1.1 The Philadelphia Chromosome and BCR-ABL oncogenes ..........................4
Figure 1.2 BCR-ABL signal transduction pathways .....................................................6
Figure 1.3 Mechanism of imatinib...................................................................................10
Figure 1.4 Mechanisms of imatinib resistance ............................................................13
Figure 1.5 Mechanism of action of nilotinib and dasatinib..........................................16
Figure 1.6 Secondary structure and alignment of OCT1 with coding region SNPs......22
Figure 1.7 OCT-1 activity is associated with molecular response to imatinib therapy.25
Figure 3.1 Detailed results for one instance in Connectivity Map ...............................64
Figure 3.2 Summary of Connectivity Map Compounds identified with different criteria to enhance OCT-1 activity........................................................................................66
Figure 3.3 Bar plot for eight candidate drugs selected from Connectivity Map ...........69
Figure 3.4 Intracellular uptake of imatinib and OCT-1 activity in K562 cells in the absence or presence of CMAP candidates..............................................................72
Figure 3.5 OCT-1 activity in K562 cells in the absence or presence of NSAIDs .........74
Figure 3.6 OCT-1 activity in K562 cells in the absence or presence of 11 clinically applicable drugs ........................................................................................................76
Figure 3.7 Intracellular uptake of imatinib and OCT-1 activity in KU812 cells in the absence or presence of diclofenac, fenbufen, ibuprofen and paromomycin............77
Figure 3.8 The effects of temperature on the IUR of imatinib in KU812 cells and the OA in the presence of diclofenac and ibuprofen ....................................................79
Figure 3.9 The $IC50^{imatinib}$ results in the presence or absence of diclofenac or ibuprofen ..................................................................................................................81
Figure 3.10 The effects of diclofenac and ibuprofen on the number of viable cells after 72 hours co-incubation with imatinib. .................................................................83
Figure 3.11 The effects of diclofenac or ibuprofen on OCT-1 activity in primary MNCs ..................................................................................................................85
Figure 4.1 Intracellular uptake of imatinib and OCT-1 activity in untreated and ActD pre-treated KU812 cells in the absence or presence of diclofenac ..........................99
Figure 4.2 Quality control of RNA and microarray samples......................................100
Figure 4.3 Unsupervised clustering for the KU812 cells treated with imatinib and/or diclofenac for 2 hours .............................................................................103

Figure 4.4 *PTGS2* mRNA expression levels in KU812 after treatment with imatinib and/or diclofenac for 2 hours ....................110
Figure 4.5 *SLC22A1* expression levels in KU812 after treatment with imatinib and/or diclofenac for 2 hours ....................111
Figure 4.6 Identification of candidate genes based on fold change greater than 1.5 and *p*-value less than 0.05 in KU812 cells ....................114
Figure 4.7 Gene expression levels of five candidate genes after the treatment with KU812 cells with imatinib and/or diclofenac for 2 hours ....................117
Figure 4.8 Gene expression levels of four candidate genes in KU812 after the treatment with imatinib and/or diclofenac for 2 hours....................120
Figure 5.1 An overview of Cyclooxygenase pathway ....................126
Figure 5.2 *PTGS2* expressions in normal donors and CP-CML patients at diagnosis 132
Figure 5.3 Plasma levels of PGE2 and 15d-PGJ2 in normal donors and CP-CML patients at diagnosis ....................134
Figure 5.4 Correlation of *PTGS2* expression and plasma levels of PGE2/15d-PGJ2 in CP-CML patients at diagnosis ....................136
Figure 5.5 Relationship between OCT-1 activity and *PTGS2* expression levels in total white cells in CP-CML patients at diagnosis....................138
Figure 5.6 Relationship between OCT-1 activity and *PTGS2* expression levels in mononuclear cells in CP-CML patients at diagnosis....................140
Figure 5.7 Relationship between PGE2 and OCT-1 activity in CP-CML patients at diagnosis ....................141
Figure 5.8 Relationship between 15d-PGJ2 and OCT-1 activity in CP-CML patients at diagnosis ....................142
Figure 5.9 Relationship between the molecular response and *PTGS2* expression, PGE2 and 15d-PGJ2....................144
Figure 6.1 The *PPARG* gene expression in cancer cell lines....................157
Figure 6.2 Pre-treatment with PPARγ antagonists diclofenac and GW9662 resulted in an increase in Imatinib IUR and OA in KU812 cells ....................160
Figure 6.3 Pre-treatment with PPARγ agonists GW1929, rosiglitazone, or troglitazone resulted in a decrease in Imatinib IUR and OA in KU812 cells....................161
Figure 6.4 *PPARG* expression levels remained unchanged after treatment with PPARγ ligands in CML cell lines....................163
Figure 6.5 PPARγ ligands did not affect PPARγ protein expression in KU812 cells after a 3-hour incubation....................164

Figure 6.6 No significant change observed in the subcellular localization of PPARγ protein after the treatment with PPARγ ligands for 3 hours in KU812 cells..........167
Figure 6.7 PPARγ transcriptional activity in mononuclear cells was negatively associated with OCT-1 activity in CP-CML patients at diagnosis .........................169
Figure 6.8 PPARɣ activity in CP-CML MNCs had no correlation with the 15d-PGJ2 plasma levels.........................................................................................172
Figure 6.9 Procedure for iIsolation procedures for of neutrophils and monocytes .....174
Figure 6.10 Purity of isolated neutrophils, monocytes and lymphocytes....................175
Figure 6.11 PPARγ protein expression and activity in $CD14^+$ cells and $CD15^+$ from normal donors and CP-CML patients at diagnosis ................................................177
Figure 6.12 PPARɣ activity in CP-CML MNCs had no correlation with the PPARɣ activity of $CD14^+$ cells or $CD15^+$ ..........................................................178
Figure 6.13 Postulated interactions between OCT-1 and diclofenac or other NSAIDs in CP-CML MNCs..........................................................................................182
Figure 7.1 Working model of OCT-1 activity regulation by PPARγ in CP-CML patients..................................................................................................193

Supplementary Figure 1 The heat map of SLC superfamily transporters in KU812 after treatment with imatinib and/or diclofenac for 2 hours ..........................................207
Supplementary Figure 2 The heat map of ABC superfamily transporters in KU812 after treatment with imatinib and/or diclofenac for 2 hours ..................................208
Supplementary Figure 3 The heat map showing genes with fold change greater than 1.5 in dual treatment compared with imatinib only in KU812 cells.............................209
Supplementary Figure 4 The heat map showing genes with *p*-value<0.05 in KU812 cells ......................................................................................................210

Table 3.1 NSAID agents selected in this study ..........................................................61
Table 3.2 Clinically applicable drugs selected in this study ........................................62
Table 3.3 Top 25 Connectivity Map compounds identified using an input signature weighted to OCT-1 as the primary up-regulated gene ............................................68
Table 3.4 Candidate compounds selected from CMAP ................................................70
Table 4.1 The ratio of 28S:18S rRNA and RNA integrity number (RIN) value of the samples for the Affymetrix GeneChip® Human gene 1.0 ST array .......................102
Table 4.2 Top 15 up-regulated or down-regulated genes ranked based on the fold change between the control and imatinib alone in KU812 cells ............................104
Table 4.3 Top 15 up-regulated or down-regulated genes ranked based on the fold change between the control and diclofenac alone in KU812 cells .........................105
Table 4.4 Top 15 up-regulated or down-regulated genes ranked based on the fold change between the control and co-administration of imatinib and diclofenac in KU812 cells ..........................................................................................................106
Table 4.5 Top 15 up-regulated or down-regulated genes ranked based on the fold change between imatinib alone and co-administration of imatinib and diclofenac in KU812 cells ..........................................................................................................107
Table 4.6 Top 25 genes ranked based on the *p*-value when comparing the conditions of control and imatinib alone to the conditions of diclofenac alone and dual treatment of two drugs ...................................................................................................108
Table 4.7 List of candidate genes that meet the criteria of fold change >1.5 and *p*-value<0.05 .........................................................................................................116
Table 4.8 Direct target genes of PPARγ identified using Chip Enrichment Analysis.118

Supplementary Table 1 Primer sequences for RQ-PCR ............................................205

# ABBREVIATIONS

| | |
|---|---|
| **15d-PGJ2** | 15-deoxy-Δ12,14-PGJ2 |
| **ABCB1/ABCG2** | ATP binding cassette (ABC) transporter proteins B1 and G2 |
| **ABL** | Abelson kinase |
| **ACD** | Anticoagulent Citrate Dextrose Solution Formula A |
| **AP** | Accelerated phase |
| **ATP** | Adenosine triphosphate |
| **BC** | Blast crisis |
| **BCR** | Breakpoint cluster region |
| **BCR-ABL** | Breakpoint cluster region-Ableson kinase fusion transcript/protein |
| **BM** | Bone marrow |
| **BSA** | Bovine serum albumin |
| **C** | Celcius |
| **CCyR** | Complete cytogenetic response |
| **CD** | Cluster of differentiation |
| **cDNA** | Complementary deoxyribonucleic acid |
| **CHIP** | Microarray chip |
| **CHR** | Complete haematologic response |
| **CMAP** | Connectivity map |
| **CML** | Chronic myeloid leukaemia |
| **CMR** | Complete molecular response |
| **COX-1/2** | Cyclooxygenase-1/2 |
| **CP** | Chronic phase |
| **Crkl** | Crk-like protein |
| **DDI** | Drug-drug interaction |
| **DEPC** | Diethyl pyrocarbonate |
| **DMSO** | Dimethyl sulphoxide |
| **DNA** | Deoxyribonucleic acid |
| **dNTPs** | Deoxynucleotide triphosphates |
| **DTT** | Dithiothreitol |
| **EDTA** | Ethylene diamine tetraacetate |
| **ERK** | Extracelullar signal-regulated kinase |
| **FBS** | Foetal Bovine Serum |
| **FC** | Fold change |
| **FITC** | Fluorescein isothiocyanate |
| **GAPDH** | Glyceraldehyde 3-phosphate dehydrogenase |
| **GUSB** | Beta-glucuronidase |
| **HBSS** | Hanks Balanced Salt Solution |

| | |
|---|---|
| **HCL** | Hierarchical Clustering |
| **IC50** | 50% inhibitory concentration |
| **IFN-α** | Interferon alpha |
| **IgG** | Immunoglobulin G |
| **IRIS** | International randomised study of interferon versus STI571 |
| **IUR** | Intracellular uptake and retention |
| **kD** | Kilo Dalton |
| **L** | Litre |
| **M** | Molar |
| **MACS** | Magnetically activated cell sorting |
| **MAPK** | Mitogen activating protein Kinase |
| **MCyR** | Major cytogenetic response |
| **mM** | Milli Molar (10-3 Molar) |
| **MMR** | Major molecular response |
| **MNC** | Mononuclear cells |
| **mRNA** | Messenger ribonucleic acid |
| **NF-κB** | Nuclear factor of kappa light polypeptide gene enhancer in B-cells |
| **ng** | Nano gram ($10^{-9}$ gram) |
| **nM** | Nano molar ($10^{-9}$ Molar) |
| **NSAIDs** | Non-steroidal anti-inflammatory drugs |
| **OA** | OCT-1 activity |
| **OAT-1** | Organic anion transporter-1 |
| **OCT-1** | Organic cation transporter 1 |
| **PB** | Peripheral blood |
| **PBS** | Phosphate Buffered Saline |
| **PCR** | Polymerase chain reaction |
| **p-Crkl** | Phosphorylated Crk-like protein |
| **PE** | Phycoerythrin |
| **PGE2** | Prostaglandin E2 |
| **Ph** | Philadelphia chromosome |
| **PI3K** | Phosphatidylinositol3 kinase |
| **PPARγ** | Peroxisome Proliferator Agonist Receptor |
| **PTGS** | Prostaglandin-endoperoxide synthase (COX) |
| ***p*-value** | Probability value |
| **PVDF** | Polyvinylidene fluoride |
| **RANKL** | Receptor activator of nuclear factor kappa-b ligand |
| **RNA** | Ribonucleic acid |
| **ROC** | Receiver operating characteristic |
| **RPMI** | Roswell Park Memorial Institute (media) |

| | |
|---|---|
| **RQ-PCR** | Real-time quantitative polymerase chain reaction |
| **SDS-PAGE** | Sodium dodecyl sulphate-polyacrylamide |
| **SEM** | Standard error of the mean |
| **STI571** | Signal transduction inhibitor 571 (imatinib) |
| **TBP** | TATA-binding protein |
| **TBS** | Tris buffered saline |
| **TBST** | Tris buffered saline with 0.1% Tween20 |
| **TKI** | Tyrosine kinase inhibitor |
| **TWC** | Total white cells |
| **U** | Units |
| **v/v** | Volume per volume |
| **vs.** | versus |
| **w/v** | Weight per unit volume |
| **µg** | Micro gram ($10^{-6}$ gram) |
| **µM** | Micro molar ($10^{-6}$ molar) |

# PUBLICATIONS

***Manuscripts***

**Wang J**, Hughes TP, Kok CH, Saunders VA, Frede A, Groot-Obbink K, et al. Contrasting effects of diclofenac and ibuprofen on active imatinib uptake into leukaemic cells. British Journal of Cancer. 2012;106(11):1772-8 **Impact Factor 5.04**

***Conference Abstracts***

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# SCHOLARSHIPS & AWARDS

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# ABSTRACT

In CML cells, the functional OCT-1 activity (OA) in mononuclear cells (MNC) from *de novo* chronic myeloid leukaemia patients in chronic phrase (CP-CML) is significantly associated with imatinib-mediated *in vitro* tyrosine kinase inhibition and is a strong indicator of imatinib response.

Here we identified candidate drugs as potential OA enhancers using Connectivity Map analysis (CMAP). Their effects on OA were extensively validated in CML cell lines and patient samples, together with 12 NSAIDs and 11 commonly prescribed drugs. A significant enhancement of OA was observed after the treatment with diclofenac. Importantly this increase in OA translated to a significant increase in BCR-ABL kinase inhibition. Additionally, the long-term co-administration of diclofenac sensitized CML cells to imatinib on cell proliferation. In CML patients' mononuclear (MNC) samples, diclofenac significantly increased OA especially in patients with low OA. In contrast, ibuprofen significantly decreased the OA in CML cell lines and primary samples. This effect on OA also translated into a reduction in BCR-ABL kinase inhibition and an increase in cell growth in the presence of imatinib. Unlike diclofenac, the inhibitory effect of ibuprofen is also observed in normal cells most likely in an independent manner from OCT-1 protein.

The enhancement of OA by diclofenac could be eliminated in the presence of Actinomycin D (a transcription inhibitor), indicating that diclofenac regulated OA at a transcriptional level. However, the expression of *SLC22A1* (*OCT-1*) remained unchanged after treatment with diclofenac. Since diclofenac is an inhibitor for cyclooxygenase-2 (COX-2), the potential involvement of COX-2 in OA regulation was investigated. The plasma concentration of 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2), a

prostaglandin product by COX-2, was significantly associated with OA in CP-CML. Given 15d-PGJ2 is a potent agonist for peroxisome proliferator-activated receptor γ (PPARγ) and diclofenac is also a known PPARγ ligand, whether PPARγ had a role in OA regulation was then investigated. Significant increase in OA in KU812 cells was observed after treatment with PPARγ antagonist GW9962, while treatment with PPARγ agonists (GW1929, troglitazone, or rosiglitazone) significantly decreased OA. In addition, there was a significant negative association between OA and the PPARγ transcriptional activity in CP-CML MNC collected at diagnosis. Further identification of the key factor contributing to high PPARγ activation in patients with low OA may provide better understanding of intrinsic OA interpatient variability, as well as the clinical and biological relevance of PPARγ in CML.

# DECLARATION

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