



## Early View

Original article

# **Pulmonary Arterial Hypertension associated with Protein Kinase Inhibitors: A pharmacovigilance-pharmacodynamic study**

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## **Pulmonary Arterial Hypertension associated with Protein Kinase Inhibitors: A pharmacovigilance-pharmacodynamic study**

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### **"Take home" message**

In the WHO pharmacovigilance database, a signal of PAH was found for dasatinib, bosutinib, ponatinib, ruxolitinib and nilotinib. The potential role of SRC protein kinases family and TEC in PAH induced by protein kinase inhibitors was further highlighted.

## **Abstract**

The pathophysiology of pulmonary arterial hypertension (PAH) induced by protein kinase inhibitors (PKI) remains unclear. To gain knowledge into this rare and severe pathology we performed a study combining a pharmacovigilance approach and the pharmacodynamics properties of PKI.

A disproportionality analysis on the WHO pharmacovigilance database using the Reporting Odds Ratio (ROR) and 95% confidence interval was first performed. Then, we identified the most relevant cellular targets of interest through a systematic literature review and correlated the pharmacovigilance signals with the affinity for the different PKI. We further performed a hierarchical cluster analysis to assess patterns of binding affinity.

A positive disproportionality signal was found for dasatinib, bosutinib, ponatinib, ruxolitinib and nilotinib. Five non-receptor protein kinases significantly correlate with disproportionality signals: c-src ( $r= 0.79$  and  $p= 0.00027$ ), c-yes ( $r= 0.82$  and  $p= 0.00015$ ), Lck ( $r= 0.81$  and  $p= 0.00046$ ), Lyn ( $r= 0.80$  and  $p= 0.00036$ ), all belonging to the SRC protein kinase family; and TEC ( $r= 0.85$  and  $p= 0.00006$ ). Kinases of the BMP signaling pathway also seem to play a role in the pathophysiology of PKI-induced PAH. Interestingly, dasatinib affinity profile seems different from that of other PKIs in the cluster analysis.

The study highlights potential role of SRC protein kinases family and TEC in PAH induced by PKI. This approach combining pharmacovigilance and pharmacodynamics data allowed us to generate some hypothesis about the pathophysiology of the disease, however the results have to be confirmed by further studies.

## **Introduction**

Pulmonary hypertension (PH) is defined as an increase in mean pulmonary arterial pressure (PAPm)  $\geq 25$  mmHg assessed by right heart catheterization [1]. The pathophysiology is characterized by an increased migration and proliferation of pulmonary arterial smooth muscle cells, leading to vascular remodeling [2]. The classification proposed by ERS/ESC guidelines defines five groups of different pathological features which characterize the diverse clinical PH groups[3]. Group 1 characterizes pulmonary arterial hypertension (PAH), a rare and life-threatening condition characterized by the remodeling of pulmonary arteries [4], and associated with various etiologies. Indeed, PAH may be idiopathic, heritable or associated with conditions like connective tissue disease, HIV infection, congenital heart disease, schistosomiasis or drug and toxin induced with worldwide heterogeneity.

Among drug-induced PAH, the multi Protein Kinase Inhibitor (PKI) dasatinib had been increasingly linked to PAH since 2009 [5, 6]. More recently, several cases have reported potential association with or deterioration of pre-existing PAH with other PKIs such as bosutinib, ponatinib and lapatinib [7–9].

Since these compounds inhibit multiple kinases, the identification of a target responsible for such a rare adverse event is challenging. We thus mixed pharmacovigilance data mining with the pharmacodynamic properties of PKIs, to gain knowledge into potential mechanisms underlying this rare and severe adverse event.

## **Methods**

### *Study design*

We first performed a disproportionality analysis from the World Health Organization (WHO) pharmacovigilance database Vigibase®. Disproportionality analyses are largely used by regulators to generate “pharmacovigilance signals” aiming at assessing putative links between drugs and adverse drug reaction (ADR)[10]. Such methods compare the reporting proportion

between a studied drug and all other drugs in the database for a given ADR. Several measures of disproportionality have been developed but there is no recognized gold standard [11]. They do not provide risk quantification but could be used as a proxy of the risk of adverse drug reaction when no other estimate is available (i.e. for extremely rare ADR) [12–15]. In a second step we identified cellular targets of interest through a systematic literature review. Finally, we evaluated the association between the pharmacovigilance disproportionality signals and the affinity for different PKI.

#### *Pharmacovigilance database*

VigiBase® is the WHO global database of individual case safety reports (ICSRs). This database contained at the time of extraction approximatively 16 million reports of suspected adverse effects of medicines, from more than 150 countries, collected since 1968. VigiBase® provides ICSR with patient information such as gender, age, medical history, country; suspected and concomitant drugs taken with chronological information, as well as drug indication and dosage; a description of the adverse effect with its severity and outcomes.

#### *Selection of cases*

We use the standardized High Level Term (HLT) “Pulmonary Hypertensions” of MedDRA (Medical Dictionary for Regulatory Activities) terminology to identify PH cases from VigiBase®. To select drug induced type 1 PAH we excluded all ICSR of PH associated with cardiac, pulmonary or thrombotic disorders, connective tissue diseases, HIV infection, congenital heart disease or schistosomiasis. Details are available on supplementary appendix 1.

Then, ICSR containing drugs or toxins known to induce PAH (aminorex, fenfluramine, dexfenfluramine, benfluorex, amphetamines (dexamfetamine), phentermine, mazindol) were also excluded [16].

### *Selection of protein kinase inhibitors*

To select PKIs with a reasonable level of information to calculate accurate Reporting Odds Ratio (ROR), we included in the analysis only PKI with more than 100 suspect ICSRs reported in the WHO pharmacovigilance database between January 1<sup>st</sup> 2002 and December 31 2017 [17]. We therefore selected 28 drugs: Afatinib, Alectinib, Axitinib, Bosutinib, Cabozantinib, Ceritinib, Cobimetinib, Crizotinib, Dabrafenib, Dasatinib, Erlotinib, Gefitinib, Ibrutinib, Lapatinib, Lenvatinib, Lestaurinib, Osimertinib, Nilotinib, Palbociclib, Pazopanib, Ponatinib, Regorafenib, Ruxolitinib, Sorafenib, Sunitinib, Trametinib, Vandetanib and Vemurafenib.

To avoid confounding in the pharmacovigilance signal interpretations, nintedanib and imatinib were excluded *a priori* from the selection because of suspected protopathic and indication bias. Indeed, it is impossible to distinguish reports of PAH induced by pulmonary fibrosis in nintedanib treated patients and drug inefficacy in imatinib treated patients from adverse events [18, 19].

### *Identification of protein kinases involved in PAH and affinity between PKIs and these targets*

Cellular targets of interest involved in PAH pathophysiology were identified through a systematic literature review in Medline with the Medical Subject Headings ("Familial Primary Pulmonary Hypertension"[Mesh]) AND "Protein Kinases"[Mesh].

Affinity data for the targets of interest were extracted from the IUPHAR/BPS Guide to PHARMACOLOGY 2018 developed by the « International Union of Basic and Clinical Pharmacology » and the « British Pharmacological Society » [20].

### *Disproportionality analysis*

We first performed a disproportionality analysis with the ROR method for each PKI of interest considered as suspect [21]. We compared the proportion of PAH reported for each PKI with the proportion of PAH associated with all other drugs used as non-cases. The cut-off

for signal detection was defined as a ROR lower boundary 95% confidence interval greater or equal to 1 and number of cases (n) greater or equal to 3 [22]. We also performed a temporal analysis to assess the influence of media safety alerts on reporting rate of PAH among reported adverse events, as previously described [23].

### *Statistical analyses*

To assess the link between the identified cellular targets of interest and pharmacovigilance signals, we calculated the Pearson correlation coefficients (r) between the negative logarithm of the dissociation constant  $K_d$  (pK<sub>d</sub>) and the ROR.

We hypothesized that the higher the affinity for the cellular target, the higher was the 'risk' of notification of suspected drug-induced PAH. In order to take into account the multiplicity of comparisons, the statistical significance threshold for all p-values was adapted using a Bonferroni correction [24].

Sensitivity analyses were performed to assess the robustness of the results: i) excluding PKIs which had less than 3 cases of PAH; ii) standardizing the time on the market for the different PKIs at six years after FDA approval date, corresponding to the time between dasatinib approval and the first published safety alert; iii) performing the correlation using other affinity data, extracted from Davis et al [25].

We further performed a hierarchical cluster analysis, through hierarchical k-means clustering method, to assess the similarity among receptor binding affinity profile of the included PK [26].

Lastly, for the PKI associated with a significant pharmacovigilance disproportionality signal we studied the influence of media safety alerts on the reporting rate of PAH. Moreover, as suggested by a reviewer we performed a multinomial regression analysis to assess the influence of dose and duration of exposure on the PAH cases outcomes (recovered/not recovered/died)

Descriptive results are expressed as mean  $\pm$  standard deviation (SD) or median (Interquartile range (IQR)).

All analyses were performed using R statistical software (version 3.2.3).

## **Results**

### *Selection of cases*

Until December 31<sup>st</sup> 2017, a total of 286 834 ICSRs were related to the 22 selected PKI. Among them, 733 cases of PH were extracted. The exclusion of cases associated with other PAH etiologies and concomitant drugs led to 442 ICSRs included in the final analysis (Figure S1).

### *Description of PAH cases*

Among the 442 cases of PAH, 193 were women (43.7%), 202 men (45.7%) and for 47 gender was unknown (10.6%), and mean age was  $57.6 \pm 15.8$  years. A pleural effusion was associated with PAH in 75 cases (17.0%). The average delay between PAH and PKI introduction is 23 (IQR 6.3-41.3) months (data available for 206 ICSRs), with substantial heterogeneity: 2.9 (IQR 1.7-12.8) months for bosutinib, 27.9 (IQR 11.5-45.0) months for dasatinib, 11.7 (IQR 2.6-22.0) months for nilotinib, 10.7 (IQR 8.1-11.4) months for ponatinib and 12.0 (IQR 3.9-49.1) months for ruxolitinib.

### *Identification of protein kinases involved in PAH*

Thirty-five PKs involved in PAH pathophysiology were identified through the literature review (Figure S2): ALK1/5, AMPKa1/2, BMPR-1/2, B-Raf, c-yes, DDR1, EIF2K4, ERB-b1, FAK, FGFR1/2, HER2, IGF-1R, JAK1/2, JNK1/2, KIT, Lck, Lyn, HGF, PDGFR $\alpha/\beta$ , PKG, RAF1, ROCK-2, Src, TEC, TIE2, and VEGFR-1/2/3. Most relevant references about target of interest are reported in Appendix 2.

### *Disproportionality analysis*

Among the 28 selected PKI, at least one PAH case was reported for 22. A positive disproportionality signal was found for dasatinib, bosutinib, ponatinib, ruxolitinib and nilotinib, with a ROR of 28.64 (25.53, 31.93), 13.43 (8.65, 20.87), 3.88 (1.86, 7.46), 3.71 (2.44, 5.65) and 3.39 (2.43, 4.73) respectively. ROR are represented in Figure 1. Results of the sensitivity analysis (standardizing on time on the market) were consistent with the main analysis, except for nilotinib which became non-significant. Results are presented in supplementary material (Appendix 3. Figure S3).

Drug dosage was available for 295 cases and are represented in Figure 2. Among the 170 PAH cases associated with dasatinib, only 2 were reported with a dosage higher than recommended. No correlation was found between PKI dosage, duration of exposure and outcome severity (data not shown).

### *Correlation analysis*

Among the 22 PKI identified in Vigibase, affinity data for the target of interest were available for 16 [20]. Five protein kinases are significantly correlated with disproportionality signals: c-src ( $r= 0.79$  and  $p= 0.00027$ ), c-yes ( $r= 0.82$  and  $p= 0.00015$ ), Lck ( $r= 0.81$  and  $p= 0.00046$ ), Lyn ( $r= 0.80$  and  $p= 0.00036$ ) and TEC ( $r= 0.85$  and  $p= 0.00006$ ). Proportion of variance (r-squared) explained by the model were respectively 0.72, 0.67, 0.64, 0.64 and 0.72 for c-src, c-yes, Lck, Lyn and TEC. The results of the correlation analysis for each target classified according to its main cellular function are presented in Figure 3.

Results for c-yes, c-src and TEC remain significant in all 3 sensitivity analyses, while results for Lck and Lyn remain significant in 2 of them. Furthermore, two other targets became significantly associated with disproportionality signals in the sensitivity analysis excluding PKI with less than 3 PAH cases, ALK1 and ALK5 ( $r=0.9$  and  $0.98$  respectively). Results are available in supplementary material (Appendix 4).

### *Cluster analysis*

We performed a hierarchical clustering based on the affinity data of each TKI. Results are presented in Figure 4, which represents the degree of PKI affinity for the identified PK involved in PAH. The dasatinib affinity profile differs from that of bosutinib, ruxolitinib and nilotinib.

### *Time trend analysis*

We studied the association between PAH reports and media safety alerts by a temporal analysis of the annual proportion of PAH reports for 1000 reported adverse events for each TKI with a significant pharmacovigilance disproportionality signal. Notably, an important increase in the rate of notification for dasatinib and bosutinib can be seen after first media alert. Results are presented in Figure 5.

## **Discussion**

To our knowledge this is the first pharmacovigilance analysis assessing the reporting risk of PAH associated with PKI use. Among more than 16 million ADR reported in the WHO pharmacovigilance database Vigibase® at the date of the extraction, 286 834 ICSRs were associated the 28 selected PKI including 442 PAH cases. Disproportionality analysis showed that dasatinib, bosutinib, ponatinib, ruxolitinib and nilotinib displayed a significant pharmacovigilance signal. Those results are consistent with the literature, with dasatinib being the most widely implicated PKI in induction or aggravation of PAH (6, 21–24). More recently, bosutinib, ponatinib and ruxolitinib were also linked to PAH [7, 27]. Results for nilotinib seems less robust because the pharmacovigilance disproportionality signal disappeared in sensitivity analysis, and high dosages were used for a third of the cases. Moreover, well documented cases reports are still lacking in the literature for nilotinib. The pharmacovigilance signal found for ruxolitinib could also be questioned because ruxolitinib is prescribed in the treatment of polycythemia vera and essential thrombocythemia that are

recognized cause of PH. Otherwise, a published case series suggested that lapatinib, a PKI used in breast cancer with human epidermal growth factor receptor mutations, might also cause PAH, but only one of the six patients presented in this article had right heart catheterization confirming precapillary PAH [28]. In our study, lapatinib showed a weak, non-significant disproportionality signal with a ROR of 1.13 (0.61, 2.10). Whereas not included in our study because of a lack of reported ICSRs in Vigibase, lorlatinib has recently been linked to PAH [8]. Further studies are needed to confirm these first reports.

The correlation analysis showed that c-src, c-yes, lck, lyn and TEC were highly correlated to PAH reporting risk. The Src tyrosine kinase family contains nine members: three of them (Src, Fyn and Yes) are ubiquitously distributed and six (Blk, Yrk, Fgr, Hck, Lck and Lyn) are variously expressed depending on the tissue. Src tyrosine kinases are crucial for TWIK-related acid sensitive potassium 1 (TASK-1) potassium channel functioning, acting as a cofactor [29]. Mimicking hypoxia condition, inhibition of SRC kinases decrease TASK-1 activity result in intracellular calcium level increase thus enhancing vasoconstriction and vascular remodeling [29]. However, these findings have to be balanced by the studied dasatinib dosage which corresponded to 500 times the clinical dose. Beyond inhibition of such protein kinases, dasatinib might induce apoptosis and endothelial cell dysfunction through an increase of mitochondrial reactive oxygen species that is independent from Src family kinases inhibition [30]. However, there is no significant changes in pulmonary hemodynamic parameters in rats daily treated with high doses of dasatinib (10× clinical doses) for 4 weeks [30]. Given the probable influence of the PKI dosage on the onset of PAH, secondary targets may also have an important contribution that should be further elucidated in preclinical research [30–32]

TEC and Lyn have been linked to pleural effusions through an immune-mediated mechanism and could represent a common signaling pathway explaining the high proportion of such disorder in TKI related PAH cases [15, 34]. Consistent with the high incidence of dasatinib-induced pleural effusion, rats treated with high doses of dasatinib developed pleural effusion following a period of at least 5 weeks, supporting a direct link between high doses of dasatinib and the development of pleural effusion [35]. Interestingly, this work highlights that high circulating levels of dasatinib alter pulmonary endothelial permeability in a ROS-dependent manner *in vitro* and *in vivo*, leading to pleural effusion.

Members of the BMP signaling pathway showed heterogeneous results in our study. Whereas ALK1, ALK5 and BMPR-1 showed positive correlation in the main or in sensitivity analysis, BMPR-2, the first cause of heritable PAH, did not show any correlation in our study. The BMP signaling pathway is involved in cell proliferation, mitochondrial dysfunction and inflammation [19]. Mutation of *BMPR2* the gene coding for the receptor BMPR2 accounts for 70-80% of heritable PAH, furthermore BMPR2 concentration has also been shown to be reduced in lung tissue from patients with PAH [36]. However, estimates indicate that only approximately 20% of individuals with a known genetic mutation in BMPR2 will develop PAH during their life, thus, *BMPR2* mutation is required but is not sufficient alone for phenotypic expression and increases an individual's chance of developing PAH [37][19]. Interestingly, it has recently been shown that BMPR2 reduction, through micro-RNA 124, leads to a mitochondrial Warburg phenotype and may explain the mitochondrial increase of ROS found by Guignabert et al [30, 38].

The absence of association between PDGF and VEGF protein kinases reinforces the fact that vascular remodeling is not a major component of PAH induced by PKI; which is consistent with the observations of PAH reversal upon PKI discontinuation. Despite some evidence suggesting that irreversible PAH should occur through ROS generation [27, 39].

Genetic mutations are considered to be permissive of disease, and require additional epigenetic, inflammatory or environmental factors for the development of PAH in people with those mutations [40]. Similarly and based on *in vitro* and *in vivo* findings, PKI increase the risk of developing PAH but require a comparable genetic, epigenetic or environmental “second hit” which remains to be identified [30]. According to published case series, a higher proportion of men may develop PKI-induced PAH, indeed the incidence of PAH is fourfold higher in women than in men in the general population [19]. It is known that men have worse prognosis mainly because of a maladaptive response of the right ventricle to PAH, we thus cannot exclude a participation of hormones and sex in triggering PAH [41].

In the cluster analysis, we tried to identify a PKI family specifically involved in PAH. The results are mainly in accordance with the literature and consistent with the PKI chemical structure [42]. Interestingly, the dasatinib affinity profile for PKs involved in PAH seems unique among the drug class. On the other hand, PKIs such as vandetanib or crizotinib, which share a similar affinity profile to that of bosutinib, nilotinib and ruxolitinib, but which are used in solid organ malignancies, are not associated with an reporting of PAH (Figure 4). This observation may enlighten the role of the underlying hematological disease in the genesis of PAH beyond inhibition of PK.

Given that pharmacovigilance notifications are based on a spontaneous reporting system, the number and proportion of cases reported for a medicinal product may vary depending on many factors such as media safety alerts, time since marketing, or selective notification. Thus, the exact exposed population to a given drug is unknown. Illustrating this variability, the time trend analysis showed a large increase in the rate of reporting after the firsts case-series and case-reports publications. However, despite those biases, a correlation between relative risks and measure of disproportionality was found [12]. Moreover, while we retrieved all cases for selection in this study we cannot exclude that spurious PAH were included, indeed only two

cases reported abnormal right heart catheterization results. Unfortunately, the medications introduced after the onset of the adverse event are not fulfilled in the database to avoid spurious pharmacovigilance signal, thus they could not be used for case selection. In 2 cases (one with dasatinib and one with ruxolitinib) a previous exposition to interferons was found; but the link with PAH onset was not considered strong enough to be excluded. Furthermore, new onset and aggravation of PAH were considered similar. Unfortunately, our study of co-mediations, associated pathologies and drug dosages was limited by the high rate of missing data on the ICSRs reported in Vigibase. This reinforces the importance to report all suspected ADR on pharmacovigilance systems to improve their efficiency [43].

In the present pharmacovigilance/pharmacodynamics analysis, we assumed that PAH was caused by a single PK and we did not account for co-inhibition of multiple PKs. However, we tried to address this limitation in performing a cluster analysis to identify at risk group of PKI. Lastly, our study was not able to detect inhibition/activation of non-PK cellular targets (e.g. proteasomes, G protein-coupled receptors, voltage-gated ion channels or ligand-gated ion channels). Therefore, the implication of other target in the pathogenesis of PKI-induced PAH cannot be ruled out.

## **Conclusion**

This is to our knowledge, the first pharmacovigilance analysis to investigate the risk of PAH associated with PKI. The disproportionality analysis showed that dasatinib, but also bosutinib, ponatinib, ruxolitinib and nilotinib had a significant disproportionality signal. This study highlights potential roles of SRC protein kinases family and TEC in PAH induced by PKI. Overall, this study contributes to a better understanding of PAH induced PKI and to identify potential target of interest that needs to be further explored.

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## Figure legend

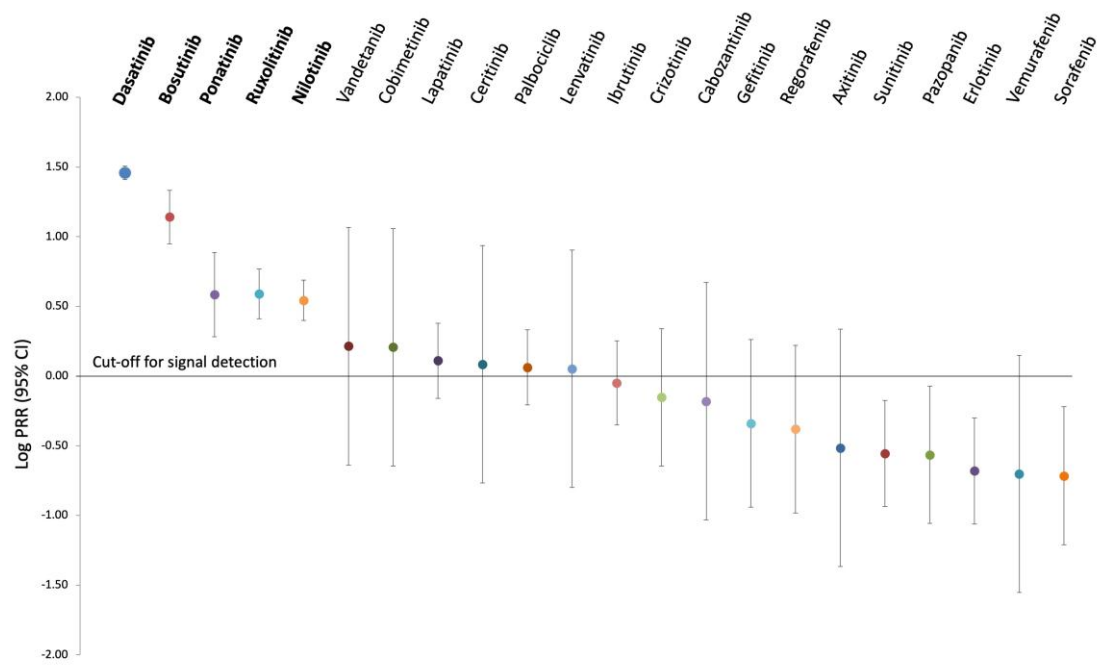
**Figure 1.** Forest plot of the ROR (95% CI) values of PKI related PAH. PKI associated with positive disproportionality signals are in bold.

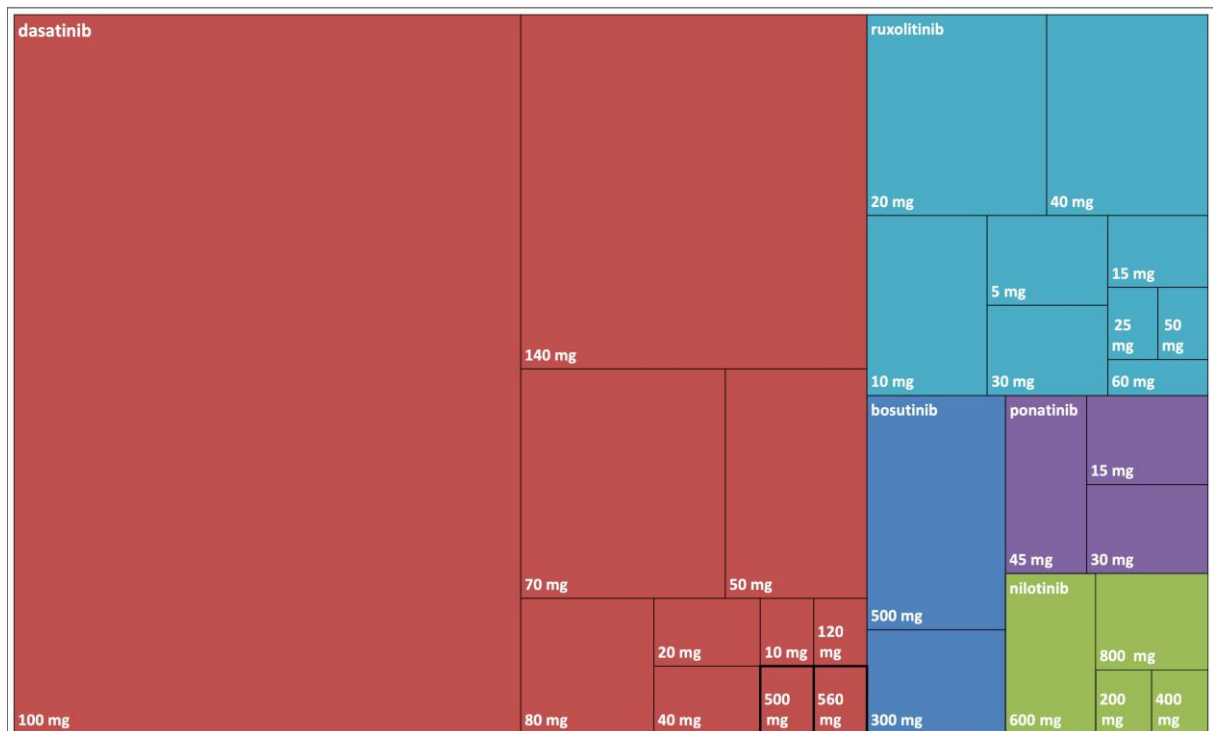
**Figure 2.** Tree map of drug dosages for the 5 most reported PKI, i.e. dasatinib (n=170), bosutinib (n=13), ruxolitinib (n=36), nilotinib (n=9) and ponatinib (n=10). The surface area is proportional to the number of reported cases for each dosage/drug combination. Dosages beyond recommended doses are also highlighted.

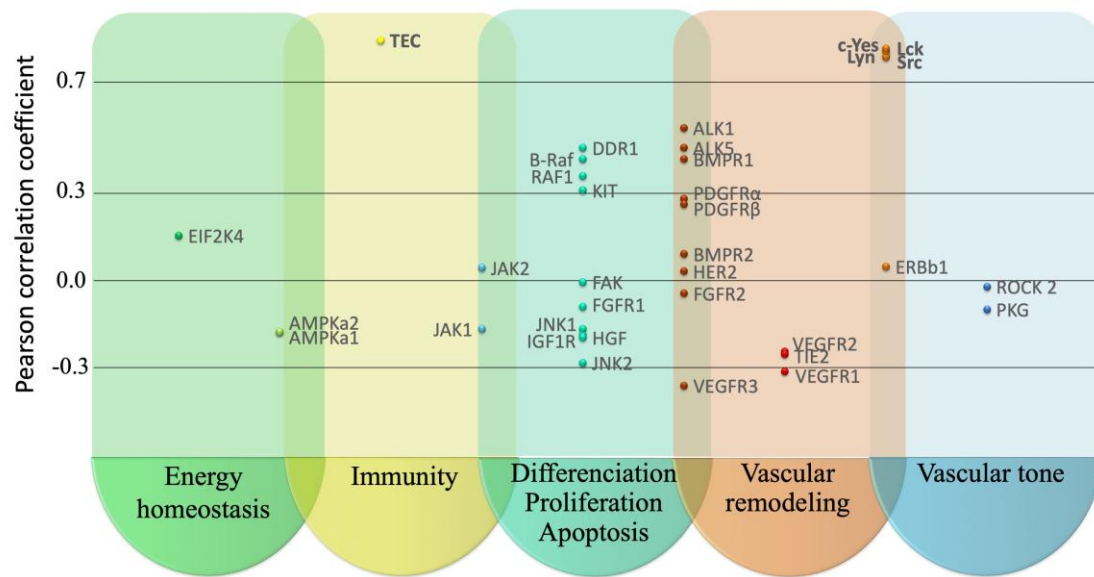
**Figure 3.** Manhattan plot synthesizing the correlation analysis, with significant correlations in the initial analysis highlighted in bold. Pearson correlation coefficients of each target are classified according to their main cellular function

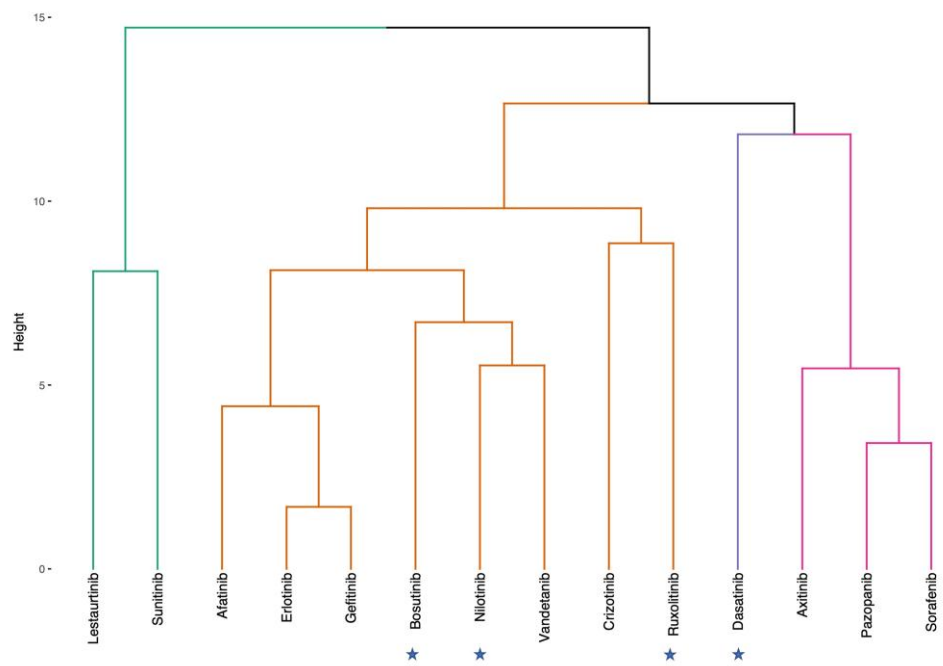
**Figure 4.** Clusters dendrogram of protein kinases inhibitors based on their affinity profile. Protein kinase inhibitors with a significant pharmacovigilance disproportionality signal are underlined by adding a star.

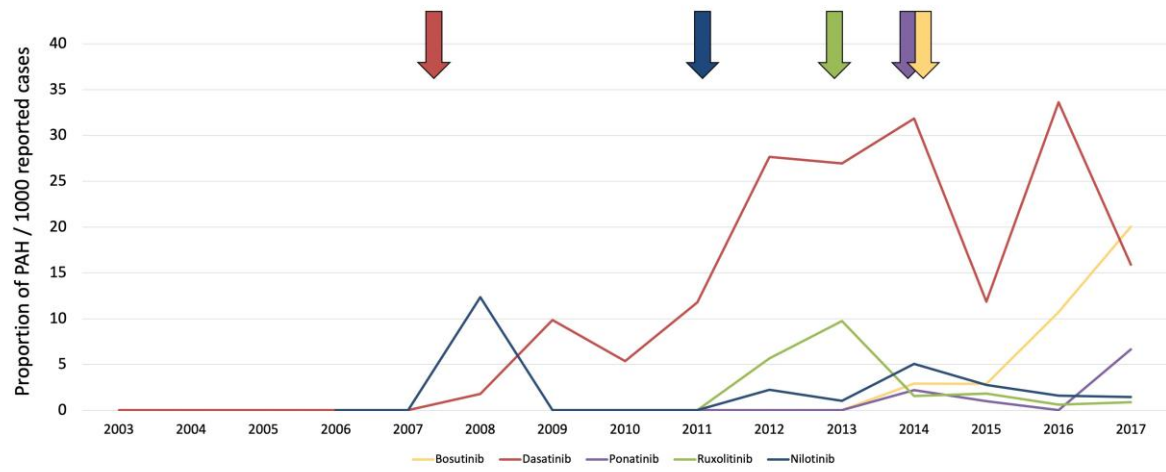
**Figure 5.** Proportion of reported PAH cases for 1000 reported adverse events per year for the 5 PKI with a significant disproportionality signal. The arrows indicate the first published cases reports in Medline for each drug.











## SUPPLEMENTARY MATERIAL

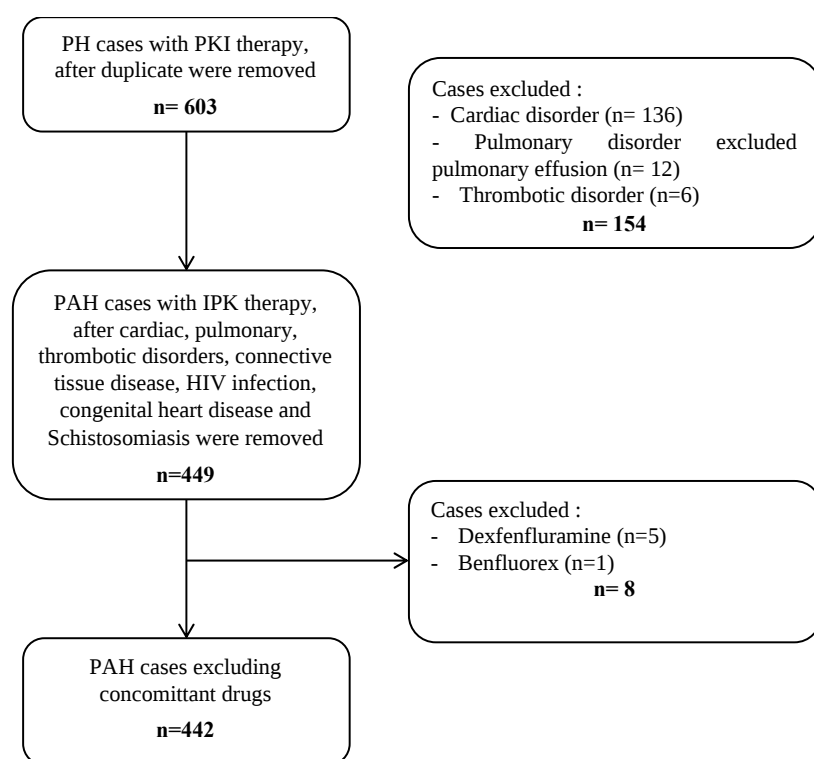
### **Appendix 1. Details of co-reported MedDRA term excluded from the analysis**

-**cardiac disorders** (from MedDRA classification: Cardiac disorders SOC - Cardiac and vascular disorder congenital HGLT - Cardiac and vascular investigation HGLT).

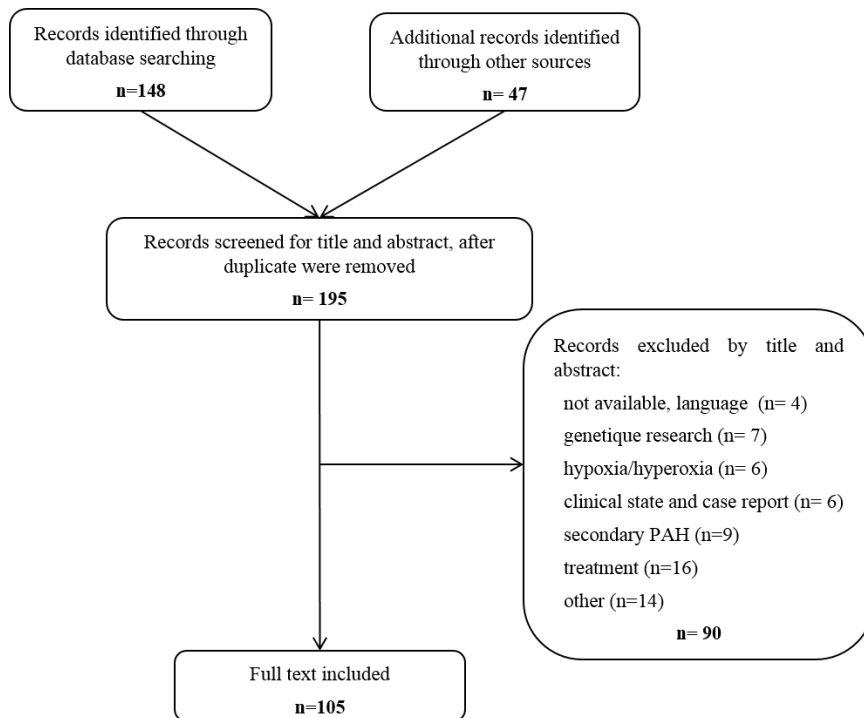
- **pulmonary disorders** (respiratory and mediastinal neoplasms malignant and unspecified HGLT - bronchial disorders (excl neoplasms) HGLT - lower respiratory tract inflammatory and immunologic conditions HLT– parenchymal lung disorders HLT - pulmonary thrombotic

and embolic conditions HLT - respiratory tract disorders NEC HLT – tumour embolism /  
tumour thrombosis PT) and  
**-thrombotic disorders** (embolism and thrombosis HGLT).

**Figure S1. Flow chart of PAH cases selection for analysis**



**Figure S2. Flow chart of the literature review aiming to identify protein kinases involved in pulmonary function.**



## Appendix 2. Selected protein kinases and most relevant references.

| Target involved in pulmonary pathophysiology                                        | Sources                                                                    |
|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| <b>ALK1 Activin receptor-like kinase-1</b>                                          | (Star et al., 2010) ; (Girerd et al., 2017) ; (Gore et al., 2014)          |
| <b>ALK5 transforming growth factor-<math>\beta</math>1 (TGF<math>\beta</math>1)</b> | (Tojais et al., 2017)<br>(Upton and Morrell, 2013)                         |
| <b>AMPKa1 (AMP-activated protein kinase)</b>                                        | (Ibe et al., 2013)<br>(Omura et al., 2016)                                 |
| <b>AMPKa2</b>                                                                       | (Ibe et al., 2013)                                                         |
| <b>BMPR-1 = ALK6</b>                                                                | (Chida et al., 2012)                                                       |
| <b>BMPR-2</b>                                                                       | (Tojais et al., 2017)                                                      |
| <b>B-Raf (Rapidly Accelerated Fibrosarcoma)</b>                                     | (Awad et al., 2016)                                                        |
| <b>C-Raf = Raf1</b>                                                                 | (Hopper et al., 2015)                                                      |
| <b>DDR1<br/>Discoidin domain receptor</b>                                           | (Sakamoto et al., 2001)                                                    |
| <b>EIF2AK4<br/>eukaryotic translation initiation factor 2<br/>alpha kinase 4</b>    | (Tenorio et al., 2015)<br>(Eichstaedt et al., 2016)<br>(Best et al., 2017) |
| <b>ERB-b1 = EGFR = Her1</b>                                                         | (Dahal et al., 2010)                                                       |
| <b>ERB-b2 = HER2</b>                                                                | (Dahal et al., 2010)                                                       |
| <b>focal adhesion kinase FAK</b>                                                    | (Paulin et al., 2014)                                                      |
| <b>FGFR1</b>                                                                        | (Zheng et al., 2015)<br>(Kim, 2014)<br>(Izikki et al., 2009)               |
| <b>FGFR2</b>                                                                        | (Schermuly et al., 2011)                                                   |
| <b>IGF-1R (insulin like growth factor)</b>                                          | (Sun et al., 2016)<br>(Baumgart et al., 2017)<br>(Dewachter et al., 2014)  |
| <b>JAK 1</b>                                                                        | (Lachmann et al., 2017)                                                    |
| <b>JAK2</b>                                                                         | (Mattar et al., 2016)                                                      |
| <b>JNK1/2 (c-Jun N-terminal kinase) =<br/>mitogen-activated protein kinase 9</b>    | (Wilson et al., 2015)<br>(Das et al., 2016)                                |
| <b>c-kit = KIT<br/>stem cell growth factor receptor (SCFR)</b>                      | (Montani et al., 2014)<br>(Farha et al., 2014)                             |

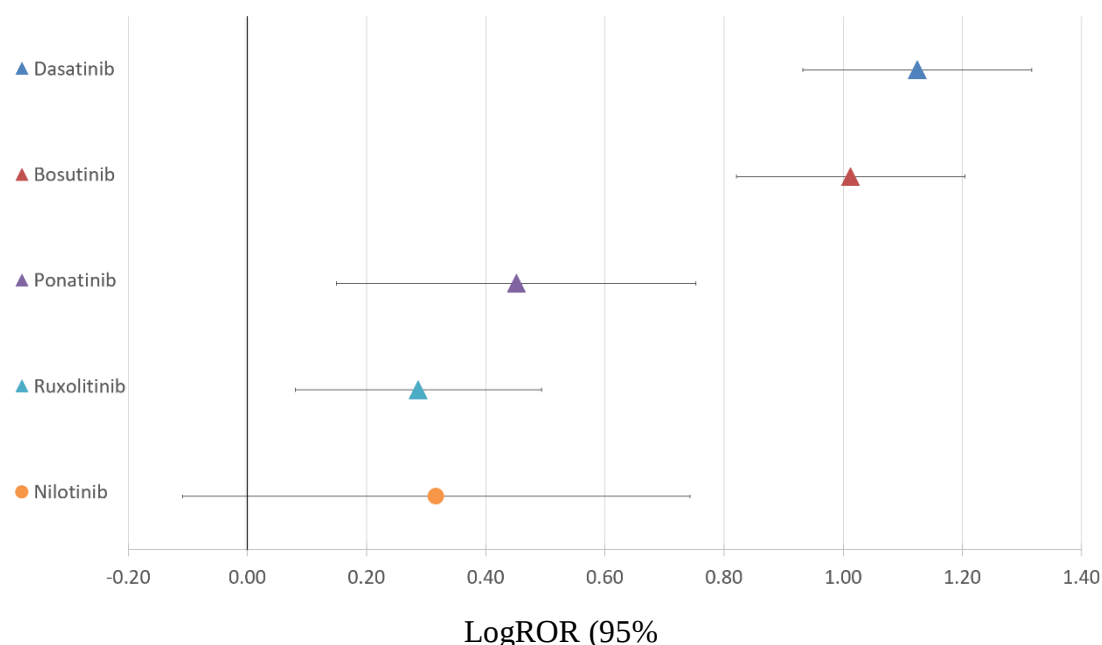
|                                            |                                                 |
|--------------------------------------------|-------------------------------------------------|
| <b>Lck Leukocyte C-terminal Src kinase</b> | (Andruska et al., 2017)                         |
| <b>lyn</b>                                 | (Pullamsetti et al., 2012a)                     |
| <b>c MET = HGF</b>                         | (Schermuly et al., 2011)                        |
| <b>PDGFR<math>\alpha</math></b>            | (Berghausen et al., 2013)<br>(Schermuly, 2005)  |
| <b>PDGFR<math>\beta</math></b>             | (Cai et al., 2017)<br>(Weatherald et al., 2017) |
| <b>PKG cGMP-dependent protein kinase</b>   | (Patel et al., 2014)                            |
| <b>ROCK-2</b>                              | (Shimizu et al., 2013)                          |
| <b>Tyrosine-protein kinase c-Src</b>       | (Guignabert et al., 2016)                       |
| <b>TEC</b>                                 | (de Lavallade et al., 2008)                     |
| <b>TEK receptor tyrosine kinase TIE2</b>   | (Guignabert et al., 2016)                       |
| <b>VEGFR-1</b>                             | (Derrett-Smith et al., 2013)                    |
| <b>VEGFR-2</b>                             | (Nicolls et al., 2012)                          |
| <b>VEGFR-3</b>                             | (Hwangbo et al., 2017)                          |
| <b>c-yes</b>                               | (Pullamsetti et al., 2012b)                     |

### Appendix 3. Results of ROR sensitivity analysis, standardizing on time on the market.

Sensitivity analysis were performed to compare the proportion of PAH reported for each PKI with the proportion of PAH reported for all other PKI.

We performed an analysis using only reported cases from the first six years after the FDA approval. A positive disproportionality signal was found for dasatinib with a ROR of 13.32 (8.56; 20.72), bosutinib 10.30 (6.63; 16.00), ponatinib 2.83 (1.41; 5.66), ruxolitinib 1.94 (1.20; 3.12) and nilotinib 2.07 (0.78; 5.53). Logarithmic value are represented in Figure S3.

**Figure S3. Disproportionality signal of PAH induced by PKI six years after FDA approval versus all medication in pharmacovigilance database. ROR and 95% CI were log transformed.**



**Appendix 4. Results of correlations sensitivity analysis standardizing on time on the market, including only PKI with more the 3 PAH cases and using affinity data from Davis et al.**

| Target    | 6 year after approval |                 | More than 3 cases |                 | Affinity data from Davis et al. |                 |
|-----------|-----------------------|-----------------|-------------------|-----------------|---------------------------------|-----------------|
|           | r                     | p-value         | r                 | p-value         | r                               | p-value         |
| ALK1      | 0.47                  | 0.079           | <b>0.9</b>        | <b>0.0058</b>   | 0.54                            | 0.036           |
| ALK5      | 0.42                  | 0.12            | <b>0.98</b>       | <b>0.000069</b> | 0.4                             | 0.14            |
| AMPKa1    | -0.17                 | 0.55            | -0.26             | 0.57            | -0.18                           | 0.53            |
| AMPKa2    | -0.2                  | 0.47            | -0.3              | 0.52            | -0.18                           | 0.51            |
| B_Raf     | 0.37                  | 0.17            | 0.68              | 0.093           | 0.43                            | 0.11            |
| BMPR_1    | 0.3                   | 0.28            | 0.85              | 0.015           | 0.56                            | 0.031           |
| BMPR_2    | 0.2                   | 0.47            | -0.12             | 0.8             | 0.095                           | 0.74            |
| c_yes     | <b>0.84</b>           | <b>0.000079</b> | <b>0.87</b>       | <b>0.0011</b>   | <b>0.82</b>                     | <b>0.00018</b>  |
| c-src     | <b>0.89</b>           | <b>0.000007</b> | <b>0.9</b>        | <b>0.0054</b>   | <b>0.86</b>                     | <b>0.000042</b> |
| DDR1      | 0.48                  | 0.068           | 0.66              | 0.11            | 0.47                            | 0.075           |
| EIF2K4    | 0.2                   | 0.47            | 0.31              | 0.5             | 0.16                            | 0.56            |
| ERB_b1    | 0.053                 | 0.85            | 0.044             | 0.93            | 0.052                           | 0.85            |
| FAK       | 0.084                 | 0.76            | -0.046            | 0.92            | -0.0045                         | 0.99            |
| FGFR1     | -0.2                  | 0.48            | 0.0069            | 0.99            | -0.17                           | 0.54            |
| FGFR2     | -0.091                | 0.75            | 0.29              | 0.53            | -0.044                          | 0.88            |
| HER2      | -0.012                | 0.97            | 0.012             | 0.98            | 0.027                           | 0.92            |
| HGF       | -0.2                  | 0.47            | -0.0081           | 0.99            | -0.2                            | 0.48            |
| IGF_1R    | -0.22                 | 0.44            | -0.3              | 0.52            | -0.19                           | 0.49            |
| JAK1      | -0.21                 | 0.46            | -0.31             | 0.49            | -0.17                           | 0.55            |
| JAK2      | 0.014                 | 0.96            | -0.041            | 0.93            | 0.046                           | 0.87            |
| JNK1      | -0.2                  | 0.48            | -0.2              | 0.67            | -0.17                           | 0.55            |
| JNK2      | -0.31                 | 0.26            | -0.41             | 0.37            | -0.32                           | 0.25            |
| KIT       | 0.3                   | 0.28            | 0.44              | 0.32            | 0.33                            | 0.23            |
| Lck       | <b>0.84</b>           | <b>0.00016</b>  | 0.86              | 0.028           | <b>0.78</b>                     | <b>0.00057</b>  |
| Lyn       | <b>0.83</b>           | <b>0.00012</b>  | 0.83              | 0.02            | <b>0.8</b>                      | <b>0.00036</b>  |
| PDGFRalfa | 0.22                  | 0.44            | 0.44              | 0.32            | 0.29                            | 0.29            |

|           |             |                |             |                |             |                 |
|-----------|-------------|----------------|-------------|----------------|-------------|-----------------|
| PDGFRbeta | 0.25        | 0.38           | 0.39        | 0.39           | 0.27        | 0.32            |
| PKG       | -0.14       | 0.63           | -0.15       | 0.75           | NA          | NA              |
| RAF1      | 0.33        | 0.22           | 0.85        | 0.016          | 0.37        | 0.18            |
| ROCK_2    | 0.038       | 0.89           | -0.19       | 0.68           | -0.02       | 0.94            |
| TEC       | <b>0.77</b> | <b>0.00082</b> | <b>0.96</b> | <b>0.00069</b> | <b>0.88</b> | <b>0.000015</b> |
| TIE2      | -0.22       | 0.43           | -0.056      | 0.91           | -0.25       | 0.36            |
| VEGFR_1   | -0.32       | 0.24           | -0.26       | 0.58           | -0.32       | 0.24            |
| VEGFR_2   | -0.29       | 0.29           | -0.26       | 0.58           | -0.29       | 0.29            |
| VEGFR_3   | -0.37       | 0.18           | -0.39       | 0.39           | -0.37       | 0.18            |

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