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**Title: In vitro assessment of anti-fibrotic activity does not predict their in vivo efficacy in murine models of Duchenne muscular dystrophy**

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**Abstract**

**Aim:** Fibrosis is the most common complication from chronic diseases, and yet no therapy capable of mitigating its effects is available. Our goal is to unveil specific signalling regulating the fibrogenic process and to identify potential small molecule candidates that block fibrogenic differentiation of fibro/adipogenic progenitors.

**Method:** We performed a large-scale drug screen using muscle-resident fibro/adipogenic progenitors from a mouse model expressing EGFP under the *Collagen1a1* promotor. We first confirmed that the EGFP was expressed in response to TGF $\beta$ 1 stimulation *in vitro*. Then we treated cells with TGF $\beta$ 1 alone or with drugs from two libraries of known compounds. The drugs ability to block the fibrogenic differentiation was quantified by imaging and flow

cytometry. From a two-rounds screening, positive hits were tested *in vivo* in the mice model for the Duchenne muscular dystrophy (mdx mice). The histopathology of the muscles was assessed with picrosirius red (fibrosis) and laminin staining (myofiber size).

Key findings: From the *in vitro* drug screening, we identified 21 drugs and tested 3 *in vivo* on the mdx mice. None of the three drugs significantly improved muscle histopathology.

Significance: The *in vitro* drug screen identified various efficient compounds, none of them strongly inhibited fibrosis in skeletal muscle of mdx mice. To explain these observations, we hypothesize that in Duchenne Muscular Dystrophy, in which fibrosis is a secondary event due to chronic degeneration and inflammation, the drugs tested could have adverse effect on regeneration or inflammation, balancing off any positive effects and leading to the absence of significant results.

Keywords: drug screening, fibro/adipogenic progenitors, fibrosis, repair, skeletal muscle.

## Introduction

Acute tissue injury generates transient inflammation and extracellular matrix (ECM) deposition which return to basal levels after the regenerative process is complete. However, under certain pathologic conditions, persistent damage and inflammation within the tissue generates excessive and chronic deposition of ECM components. This condition of persistent inflammation and elevated ECM is known as fibrosis [1,2]. Fibrosis hinder tissue regeneration and contributes to organ malfunction in different pathologies such as liver and kidney diseases, idiopathic pulmonary fibrosis, heart failure, and muscular dystrophies. Although fibrosis contributes to 45% of mortality in developed countries, the mechanisms regulating the initiation and the establishment of fibrosis have not yet been completely elucidated [3,4]. Despite the extensive study of fibrogenesis in response to injury, essentially no effective anti-fibrotic therapy is yet

available. A better understanding of the cellular effectors and the molecular signals regulating this pathological condition is necessary.

In skeletal muscle, an organ with a high regeneration potential, fibrosis is a hallmark of severe muscular dystrophies. This is the case of the incurable Duchenne muscular dystrophy (DMD), where the lack of dystrophin protein leads to cycles of impaired regeneration, resulting in chronic degeneration of the tissue [5–7]. At the cellular level, fibroblasts are the master mediators of tissue fibrosis [8]. In the past 10 years, a group of tissue-resident multipotent mesenchymal progenitors have been described as precursors of fibroblasts. In adult muscles, these cells were named fibro/adipogenic progenitors (FAPs), based on their spontaneous potential to differentiate into myofibroblasts and adipocytes, both *in vivo* and *in vitro* [9–11]. Damage induces FAP activation and expansion which are modulated by inflammatory signals, including Interleukin-4 and 13 (IL-4, IL-13), Tumor Necrosis Factor  $\alpha$  (TNF $\alpha$ ), and Transforming Growth Factor  $\beta$  (TGF $\beta$ ) [9,12–17]. Following acute damage, activated FAPs provide trophic support to satellite cells, required for efficient normal regeneration [18–21]. However, in degenerative condition such as in chronically damaged muscles of DMD patients, tissue clearance of FAPs fails. Chronic damage primes FAPs to differentiate towards both adipocytes and fibroblasts, leading to fibrofatty infiltration, and eventually loss of muscle function [15,22,23]. Due to their dual role in skeletal muscle regeneration/degeneration, FAPs are an ideal cellular target to improve regeneration and prevent fibrofatty deposition [19]. Thus, interventions targeting molecular pathways involved in FAP activation and differentiation are an attractive strategy to combat fibrotic diseases successfully.

Inhibition of the TGF $\beta$  signaling pathway during skeletal muscle regeneration reduces FAP number and down-regulates fibrillar collagen type 1 deposition, a hallmark of fibrosis

[10,15,24,25]. TGF $\beta$  plays an essential role in tissue modeling and remodeling, and therefore, it is considered a determinant molecule in the initiation and establishment of fibrosis [26]. The canonical TGF $\beta$  pathway classically transmits extracellular signals via transmembrane serine/threonine kinase receptors and intracellularly via SMAD2/3/4 proteins. The non-canonical TGF $\beta$  pathways include a variety of intracellular cascades activated by TGF $\beta$  independent of SMAD2/3/4. These include the molecules TGF $\beta$ -activated kinase 1 (TAK1), mitogen-activated protein kinase (MAPK) such as P38, extracellular signal-regulated kinases (ERK), and JUN N-terminal kinase (JNK), as well as nuclear factor kappa-light chain enhancer of activated B cell (NF $\kappa$ B), among others [27,28]. Therapeutic manipulation of the canonical and non-canonical TGF $\beta$  pathways have been shown to be beneficial in multiple myopathic states and fibrosis of various tissues [29].

Here, we used the Collagen1a1\*3.6 EGFP mice to isolate and culture FAPs and analyze their differentiation into collagen producing cells, by following the expression of the EGFP reporter [30]. We performed a drug screening on freshly isolated FAPs, which allowed us to investigate the role of critical signalling pathways in regulating their fibrogenic differentiation. Mdx mice (mouse model for the DMD) were treated with compounds that were found to strongly inhibit Collagen expression *in vitro*. However, none of the drugs reduced fibrosis *in vivo*. Here, we report that using an *in vitro* drug screening approach is a feasible technique to delineate pathways leading to the activation of a fibrogenic program. However, in the context of the DMD fibrosis cannot be specifically targeted independently of the other disease components: inflammation and muscle regeneration.

## Results

### *1. Collagen1a1\*3.6 EGFP transgenic mice is a reliable and powerful tool for analyzing*

### ***collagen expression induced by TGF $\beta$ 1***

We implemented an *in vitro* model to test compounds for the ability to block or interfere with FAP differentiation along the fibrogenic lineage. Knowing that increased collagen type 1 expression and deposition is a major hallmark of fibrosis [31], we took advantage of the Collagen1 $\alpha$ 1\*3.6 EGFP transgenic mouse [30]. These mice express the EGFP gene under the control of a 3.6 kb fragment of rat procollagen type 1 alpha 1 (*Colla1*) regulatory sequences (upstream promoter sequence). The use of this model enables the direct identification of any cell type that actively expresses high levels of *Colla1* type 1 based on EGFP fluorescence.

To establish a suitable model capable of tracking FAP differentiation *in vitro*, EGFP negative FAPs were isolated from *Tibialis Anterior* (TA) of the Collagen1 $\alpha$ 1-3.6\*EGFP mice 3 days after NTX damage and placed in culture [15,32]. Once they reached 60% confluency, FAPs were treated with 1 ng/ml of TGF $\beta$ 1 for 72 hours, and the percentage of EGFP positive cells was quantified using flow cytometry (Figure 1A). As suggested by previous studies [15], the physiological concentration of 1 ng/ml was sufficient to increase EGFP expression by 350% (Figure 1B and C). These results confirm that FAPs from the Collagen1 $\alpha$ 1\*3.6 EGFP transgenic mice can be used as a robust *in vitro* model for screening potential blockers of *Colla1* expression.

### ***2. Drug screening in FAPs reveals potential targets for muscle fibrosis therapy***

To evaluate the specific molecular pathways regulating FAP fibrogenic differentiation, we performed a drug screening using the *in vitro* model previously described and validated (Figure 1). To do so, EGFP negative FAPs were sorted from TAs and treated with 1 ng/ml of TGF $\beta$ 1 alone or with a set of 722 compounds organized into two libraries, a tyrosine kinase inhibitor library (KIL, 481 compounds) and a TOOL compound library (TOOL, 241 compounds) (Figure

S1A, Table 1, and Table 2). Then, the percentage of EGFP positive cells was quantified by using a Cellomics Array scan (Figure S1A). Non-toxic compounds (see methods) with the ability to reduce the expression TGF $\beta$ 1-induced EGFP expression by 50% and with a p-value < 0.05 were selected as positive hits (Tables 1 and 2, Figure 2A, B and S1A). Although 141 compounds met all the selection criteria, only 60 were selected for further assessment as these were compounds available in the market or in advanced phases of clinical development (Tables 1 and 2). For the second round of screening, EGFP negative FAPs were treated as previously described with the selected compounds, and the percentage EGFP positive cells was quantified by flow cytometry (Figure S1A). Figures 2C and 2E show the most potent hits of both libraries that were then further tested in dose-response experiments. A total of 21 compounds showed a dose-dependent decrease in the percentage of EGFP positive cells induced by TGF $\beta$ 1 ( $p < 0.05$ , Figure 2C and E, Table 3). In the KIL library, MAPK inhibitors (VX702 and VX745), tyrosine kinase inhibitors (TyrK) (Nilotinib and Sorafenib), and the Farnesyltransferase inhibitor Tipifarnib significantly reduced the percentage of EGFP positive cells ( $p < 0.05$  or below, Figure 2C). In the TOOL library, the Bromodomain inhibitor (BRDI) I-BET151, the histone deacetylase (HDAC) inhibitor PCI-24781 and the PKC activator Indolactam V produced statistically significant reductions in EGFP positive cells ( $p < 0.05$  or below, Figure 2E). The most potent hits, with a translational potential from both libraries, were further tested at different doses and dose-response relationships were established (0.1-5  $\mu$ M) (Figure 2D and F). These results show that targets of the TyrK inhibitor and the BRDI families regulate the establishment or maintenance of collagen expression and therefore could be investigated as potential therapies for fibrotic diseases.

Interestingly, epigenetic modulators such as the BRDI I-BET151, and the HDAC inhibitor PCI-24781, were among the strongest inhibitors of *Colla1* induction in our screen (as evidenced by

EGFP expression) [33]. Based on these results, we tested a drug library composed of epigenetic regulators was tested by following the same procedure described above (Figure S1A). Among 26 epigenetic regulators (Table 4), the only group of compounds able to reduce the fluorescence induced by TGF $\beta$ 1 were the members of the BRDI family: JQ1, and PFI-1 (Figure 2G). As already demonstrated in the heart, we confirm that the BRDI family members are essential regulators of FAP differentiation into fibroblasts [34].

Several transcriptional modifiers and regulators have been described as final effectors of the TGF $\beta$  signaling as well as mediating its involvement in fibroblast activation and scar deposition. These include serum responsive factor (SRF) [35], C-ets-1 (ETS1) [36], and NF $\kappa$ B [37]. NF $\kappa$ B pathway modulation has been associated with improvement in muscle health in mdx mice [38–40]. These results suggest that NF $\kappa$ B might be modulating FAP fibrogenic differentiation and warranted further screening for non-toxic NF $\kappa$ B inhibitors such as Withaferin [41]. Thus, we tested the ability of this compound to reduce collagen expression *in vitro* by using our previously described method (Figure S1A). Withaferin (WWA) and two of its synthetic analogues (M4 and M27) reduced the percentage of EGFP positive FAPs *in vitro* at a 0.5  $\mu$ M dose (respectively -87%, -88%, and -90%,  $p < 0.05$ ) (Figure 2H). Overall, these results suggest that FAP differentiation into collagen-producing cells can be regulated by multiple signaling pathways, some of which could be independent of the canonical TGF $\beta$  signaling pathway. These results add layers of complexity and suggest that compensatory pathways could influence the results while testing drugs *in vivo*.

### **3. Non-canonical TGF $\beta$ pathway regulates FAPs fibrogenic differentiation.**

To validate that the candidate drugs act by another signaling than inhibiting the TGF $\beta$  pathway, we measured the level of activation of the downstream protein p38 MAPK (p-p38) in

C3H10T1/2 mesenchymal progenitor cells (Figure 3A and B). As previously described [12,15], the treatment with TGF $\beta$ 1 increased the phosphorylation levels of p38 by 2.48-fold. Interestingly, while Nilotinib and Sorafenib are known as TyrK inhibitors, co-treatment of C3H10T1/2 cells with TGF $\beta$ 1 and these inhibitors blocked p38 phosphorylation, suggesting that their action works through the p38 MAPK signaling pathway (Figure 3B, respectively -56% and -59%). On the other hand, Masitinib, another well-known TyrK inhibitor (targeting Stem Cell Factor (SCF), c-Kit or PDGFR $\beta$ ) did not reduce p38 phosphorylation in our system. One of the possibilities is that the dose used in this assay was too low to induce any effect, as a decrease in EGFP positive FAP is noticeable only at 5  $\mu$ M for Masitinib (Figure S2). Lastly, while knocking-out BRD4 or using JQ1 *in vitro* on LPS-stimulated microglia is known to induce dephosphorylation of p38 [42], TGF $\beta$ -induced p38 MAPK phosphorylation was not affected by JQ1 in our system (Figure 3A and B).

In the context of fibrosis, the importance of the SMAD-independent TGF $\beta$  signaling pathway has been highlighted in various fibrosis models and the involvement of TAK1, a common upstream component of this pathway, as a key regulator of fibrosis has been shown in several tissues including kidney [43], skin [44], liver [45], and heart [46,47]. In muscle, TAK1 has been implicated in the regulation of MuSC fate and myofiber maintenance [48–50]. Moreover, TAK1 is a known activator of the p38 MAPK pathway [51–53], and indeed, the use of a pharmacological inhibitor (5Z-7-Oxozeaenol) decreased TGF $\beta$ -induced phosphorylation of p38 MAPK ( $p < 0.01$ ; Figure 3A and B). To evaluate the participation of TAK1 in FAP differentiation towards fibroblasts, we evaluated the expression of fibrogenic genes by muscle FAPs *in vitro*. Sorted FAPs were treated with TGF $\beta$ 1 (1ng/ml) with or without 5z-7-Oxozeaenol (1  $\mu$ M) for 6 hours (Figure 3C). In accordance with our previous results, the increase in gene

expression of Collagen 1a1 (*Colla1*), connective tissue growth factor (*CTGF*), periostin (*Postn*), Smooth muscle actin (*Acta2*), and fibronectin (*Fn1*) induced by TGF $\beta$ 1 was attenuated in FAPs incubated with the TAK1 inhibitor 5Z-7-Oxozeaenol ( $p < 0.05$  or below, Figure 3C).

Taken together, these results suggest that non-canonical TGF $\beta$  signaling pathways participate in regulating the fibrogenic differentiation of FAPs. Also, we established that central components of this pathways such as TAK1 are playing an important role in modulating FAP fate, which may have significant translational potential for future clinical use.

#### **4. Testing anti-fibrotic drugs candidates in vivo**

To further validate *in vivo* our *in vitro* screening results, we tested the ability of JQ1, Withaferin, Masitinib, and Sorafenib to inhibit fibrosis deposition *in vivo* in the mouse model for DMD: the mdx mice. First, mdx mice were fed with a diet containing JQ1 from 4-5-weeks of age for one year (Figure 4A). Overall, the growth rate of treated animals was similar to that of untreated controls, suggesting the absence of systemic toxicity (Figure S3A). However, we noticed that mice fed with JQ1 display muscle mass loss specifically in the Quadriceps (-18%,  $p < 0.09$ ; Figure S3B). While collagen deposition (Picrosirius Red coloration (PSR)) in the diaphragm was not affected by the JQ1 diet, the size of myofibers was strongly decreased (-23%,  $p < 0.05$ , Figure 4B to F). JQ1 has previously been demonstrated to have antifibrotic activity in the heart in both aortic constriction and post-myocardial infarction settings [34,54], as well as in bleomycin-injured lungs [55]. A possible reason for the discrepancy in the results we obtained from skeletal muscle is that while the studies describing the effect of JQ1 in the heart involved acute injury models, the model we used in this study is a chronic fibrotic disease with a strong inflammatory component. One alternative possibility is that our experimental animal models may not be representative of the clinical picture in patients with fibrosis. In contrast, the mdx:utr<sup>+/-</sup> mouse

has been described as a better model that develop more severe fibrosis earlier after disease onset, including in the limb musculature [59,60]. As a result, we took advantage of the mdx:utr<sup>+/-</sup> mouse model to further test the effect of JQ1 on muscle fibrosis. We performed continuous infusion of JQ1 (30mg/kg/day) by implanting mice with subcutaneous osmotic minipumps for 4 weeks (from 6 to 10-weeks-old, Figure 4G). The continuous delivery of JQ1 reduced post-natal growth, with 9-weeks-old mouse weight at decreased by 10% compared to the control group ( $p < 0.05$ , Figure S3C). Concomitant to this, muscle mass of both gastrocnemius and quadriceps were reduced (respectively -11%  $p < 0.01$  and -11.3%  $p < 0.001$ ) (Figure S3D). Moreover, while the diaphragm's histopathology was not affected (Figure 4H to L), collagen deposition of TA and gastrocnemius was decreased by 15% and 10% ( $p < 0.05$ ; Figure S3E to G). However, this was not associated with changes in myofiber size (Figure S3H to J).

We also tested a natural compound, Withaferin A, known for its inhibitory effect on the transcription factor NFkB [92,93]. Withaferin A is known to negatively act on the development of myocardial fibrosis *in vivo* [94] and treatments with NFkB inhibitors improved muscle function in mdx mice [39,40]. The use of Withaferin A has been associated with decreased inflammation in several models [95]. However, in our hands, mice treated with Withaferin *in i.p* (4 mg/kg/day), displayed swelling of the abdominal area and peritoneal adhesions, resulting in the interruption of its testing *in vivo* after 2 weeks of treatment (data not shown, Table S3).

We then decided to test the TyrK inhibitors Nilotinib and Sorafenib. While we previously demonstrated that Nilotinib strongly inhibits fibrosis in the muscle and the heart after acute damage [15,61], Nilotinib administration has been associated with adverse effects such as hyperglycemia, increased LDL and HDL, vascular and cardiovascular toxicity when used in the context of long-term treatment [62]. Very recently, White et al., demonstrated that DMD and

Becker muscular dystrophy (BMD) patients displayed plasma lipid abnormalities early in the onset of the disease [63]. In addition, *in vitro*, Nilotinib inhibits C2C12 myoblast differentiation [64]. Thus, Nilotinib is unlikely to be viable as drug for long-term treatment of DMD patients.

In contrast, Masitinib, which is active on a similar range of substrates than Nilotinib and Sorafenib, does not demonstrate toxicity when tested *in vivo* and *in vitro* [65] (Table S2). To note, while Masitinib demonstrated great inhibitory activity during our first screening, it did not pass our second screening as it was not able to decrease the percentage of EGFP positive cells at concentrations in the therapeutic range (Figure 2A and S2). Masitinib was injected i.p. every day for four weeks (from 8- to 12- week-old), followed with 1 rest week in mdx:utr<sup>+/+</sup> mice at 60 mg/kg/day (Figure 5A). No differences in mouse weight were noticed, confirming the absence of toxicity (Figure S4A). However, only a slight decrease in total collagen content was detected by PSR in the diaphragm (-12.8%,  $p < 0.05$ ; Figure 5B and C, Figure S4B). This was associated with no changes in myofiber size in the diaphragm, while a 16% decrease was observed in the TA ( $p < 0.05$ ; Figure 5D to F and S4C). Finally, to determine whether the results we observed were influenced by the route of drug administration, we delivered Masitinib for 8 weeks (from 6- to 14-weeks-old) by osmotic minipump implantation (Figure 5G). However, despite this method, no improvement of fibrosis deposition and muscle histopathology was observed (Figure 5H, I, J and N, Figure S4D to F). Lastly, mice were treated with Sorafenib, another promising TyRK inhibitor similar to Nilotinib, by using the osmotic pump delivery method for 8 weeks (Figure 5G). Overall, no differences were noticeable on mouse body weight, muscle mass, collagen deposition and myofiber size (Figure 5H, K, L, M and N; Figure S4G to I). A summary of the results can be found Table S3.

## Discussion

There is currently no cure for fibrosis, mainly because this condition is a multifactorial factors and likely multiple molecular pathways are involved in triggering, establishing, and maintaining scar-forming disorders and related pathologies. Therapeutic strategies to reduce fibrosis in different tissues have been attempted with limited success [2]. Most of the information that we know about the fate of FAPs and their contribution to muscle regeneration and repair has been done by using skeletal muscle as a model to study fibrosis [9–12,15,20,21,23,66]. Here, we established a screening system using primary FAP cell culture to: i) define the intracellular pathways leading to the activation of a fibrogenic program in response to TGF $\beta$ 1; ii) identify a set of drugs capable of interfering with the fibrogenic differentiation of FAPs, and iii) test their therapeutic potential. We found that compounds such as Nilotinib, Sorafenib, JQ1, I-BET151, and Withaferin were able to decrease the expression of Collagen type 1 induced by TGF $\beta$ 1 *in vitro*.

Using muscle resident FAPs, we first confirmed that TGF $\beta$ 1 upregulates the expression of *Colla1*. (Figure 1A-C). To note, collagen expression might not be representative of the other ECM genes such as CTGF/CCN2 or fibronectin, which actively participate in the installation of fibrosis [67,68]. The expansion of resident FAPs is closely associated with elevated TGF $\beta$  levels and increased ECM deposition during regeneration and repair [22,69,70]. In addition, we previously showed a temporal correlation between the peak of TGF $\beta$  expression in the tissue and the *Colla1* levels after skeletal muscle acute damage at day 7 [15,20].

For some of the compounds emerging from our screen, an anti-fibrotic effect had previously been demonstrated in different organs, including lung, kidney, liver, heart, and skeletal muscle. Members of the TyrK inhibitor family such as Imatinib [25,71,72], Nilotinib [15,20,73–75], Sorafenib [76], Sunitinib [77]; and epigenetic regulators such as BRD4 and HDAC inhibitors

have been effective in blocking fibrosis [55,78–81]. BRD regulators specifically recognize acetylated lysine residues, which act as scaffolds and attract components of the transcriptional machinery to the acetylated lysine residues of histones, resulting in modulation of gene transcription. This finding is particularly interesting because the role(s) of epigenetic regulators in fibrosis and their therapeutic potential is still poorly understood [82]. Pharmacologic modulation of epigenetic readers is emerging as a novel therapeutic approach for the treatment of inflammatory diseases [82].

Here, we identify FAPs as pharmacological targets for the action of TyrK, BRD and p38 MAPK inhibitors. The TGF $\beta$  pathway is a shared molecular pathway regulating TyrK, BRD and p38 MAPK signaling. Growing evidence supports the role of TAK1 as a significant regulator of TGF $\beta$  signaling through the regulation of the profibrotic response in several systems [43–45,83–87]. Very recently, it has been shown that the action of Catalpol (an anti-inflammatory and antioxidant drug from Chinese medicinal herb *Rehmannia*) on DMD histopathology was due to its binding to TAK1 [88,89]. Besides its role in fibrosis, TAK1 also has been described as an important mediator of carcinogenesis and cell survival [90]. In all these settings, TAK1 regulates inducible transcription factors such as NF $\kappa$ B, and other kinases such as p38 MAPK and c-Jun N-terminal kinases (JNKs) [38,51–53,91]. In our initial drug screening, all the TAK1 inhibitors tested were found to be toxic, thus confirming its central role in cell survival. For this reason, we did not pursue the use of the TAK1 inhibitors *in vivo*.

The mdx mouse is the most widely used murine model of DMD; however, it does not entirely recapitulate the fibro-fatty progression observed in humans. Indeed, mdx mice develop less fibrosis and with a later onset compared to human patients. In addition, in the murine model the fibrosis is primarily confined to the diaphragm muscle [56]. Other murine models, such as the

mdx:utr<sup>+/-</sup>, which also lacks one allele of utrophin, a functional analog of dystrophin, have been proposed as a better alternative [57,58]. mdx:utr<sup>+/-</sup> mice mimic the human disease more closely and develop more severe fibrosis earlier after disease onset, including in the limb musculature [59,60]. For this reason, we decided to use the mdx for long-term experiments (JQ1 diet) and the mdx:utr<sup>+/-</sup> for the short-term experiments (i.p. and pumps).

JQ1 is an inhibitor of the BET family of proteins which includes BRD2, 3 and 4 [96]. Its action as an antifibrotic has been demonstrated in various tissues, including the heart, lungs, kidneys, and the liver [34,54,78,97,98]. In this study, we tested the action of JQ1 on muscle fibrosis with long-term treatment (medicated food for one year) and a short-term treatment (osmotic minipump for 4 weeks). While we did detect a small decrease in fibrosis in TA and GC with the short-term treatment, we also detected a substantial decrease in myofiber size in the diaphragm of the mice treated with JQ1 for a year, suggesting that the drug affects fiber maintenance or metabolism (Figure 4 and S3). We speculate that the absence of effect on matrix deposition commensurate with that observed *in vitro* may be due to an unbalance between the anti-fibrotic and the anti-inflammatory function of JQ1 [99,100].

Lastly, the use of TyrK inhibitors, especially Nilotinib, *in vitro* and *in vivo* has shown promising results in various settings and fibrotic diseases [15,61,76,101,102]. In the past our lab successfully used Nilotinib in acute muscle regeneration and heart failure [15,61], demonstrating that the *in vitro* screening is a powerful tool able to highlight anti-fibrotic drugs. However, Nilotinib has been shown to induce side effects in long-term treated patients [62], and off-target effects in skeletal muscle myogenic progenitors [64]. Because of that, and despite the fact that it reduces Col1a1-EGFP levels only at high doses (5  $\mu$ M), we focused on the TyrR inhibitor Masitinib as a candidate to replace Nilotinib. Currently, Masitinib is used in 31 clinical trials

(NIH clinicalTrials.gov). However, while it also induced a slight decrease in fibrosis content in the diaphragm, it did not improve the overall histopathology of the disease (Figure 5, S4 and Table S3). Similar results were found with the TyrK inhibitor Sorafenib (Figure 5, S4 and Table S3). It is of interest to note that in our *in vitro* experiment, we used the Col1a\*3.6-eGFP, which contain only the 3.6kb promoter. Differences in the response to the drug *in vivo* could be the consequence of the action of other factors acting on different elements than the 3.6kb. On top of that, one of the other many possible explanations would be that the drugs, as does Nilotinib, also interact with immune cells, endothelial cells, and muscle cells (progenitors and myofibers) [64]. Indeed, MAPK p38, NFkB, KIT, PDGF, and FGF signaling pathways regulate immune cell functions [103,104], vascularization [105,106], or myogenic cell proliferation, differentiation, and myofiber growth [107–109]. This project demonstrates the importance of dual read-out drug assay, where FAP and myoblast, myofiber, or immune cell behaviour should be tested *in vitro* before to move on *in vivo*.

In conclusion, here we show that in a novel system in which fibrosis is a secondary event caused by dysfunction of the parenchymal muscle stem cells and fibers, inhibitors capable of preventing the activation of a fibrogenic transcriptional programme seem to be inefficient in protecting against the disease.

## Materials and methods

### *Animals*

Mice were maintained in an enclosed and pathogen-free facility. Mice were housed in standard cages under 12 h light–dark cycles and fed *ad libitum* with a standard chow diet. All experimental procedures were approved by the University of British Columbia Animal Care Committee. Transgenic mice expressing the enhanced green fluorescent protein (EGFP) under a

*Collagen1a1* enhancer, *Colla1*\*3.6-eGFP, were a gift from Pr. D.W. Rowe (Center for Regenerative Medicine and Skeletal Development, University of Connecticut Health Center, USA). mdx:utr<sup>+/-</sup> mice were a gift from Dr. Lisa Hoffman (Western University, London, ON, Canada). Adult mice >8-weeks-old, both male and female, were used unless otherwise specified. Acute muscle damage was induced by intramuscular injection of 0.15 µg notexin (NTX) snake venom (Latoxan), into the tibialis anterior muscle (TA).

Mice were fed from 4- to 53-week-old with control diet (Research Diet, #D111112201) or JQ1 diet (control diet supplemented with JQ1 at 5 mg/kg (Cayman Chemical #11187, or Selleckchem #S7110)).

Mice were daily treated for 4 weeks (from 8- to 12-week-old) by intraperitoneal (i.p.) injection with the vehicle (DMSO/ETOH=1:1), Masitinib (AB SCIENCE, #AB1010, 60 mg/kg/day); or Withaferin (Imstar, #IMS-008, 4mg/kg/day).

### ***Mini osmotic pump***

The empty mini osmotic pump (ALZET; model 2002) was filled with the vehicle (DMSO/ETOH=1:1) or drug solution: JQ1 (Cayman Chemical #11187 or Selleckchem #S711, 30 mg/kg/day, for 4 weeks (5- to 10-week-old)); Masitinib (ABSCIENCE #AB1010, 60 mg/kg/day, from 6 to 14 weeks); and Sorafenib (BAYER #BXA5X4R, 6 mg/kg/day, for 8 weeks (6- to 14- week-old) in sterile conditions. In order to allow the pump to equilibrate and reach its steady-state pumping rate, the filled pumps were primed overnight in sterile saline at 37°C. Animals were anesthetized and shaved. A 1 cm incision was performed in the skin on the caudal ventral abdomen at midline. Small sterile curved haemostats were used to tunnel dorsally and laterally into the subcutaneous space to create a pocket size of the pump. The osmotic pump was slowly inserted into the pocket, and the catheter was inserted into the lower ventral abdominal

cavity through an opening created by poking a hole into the muscle layer via 18 G needle. 6-0 monocryl suture was used to tighten and tied the muscle around the opening via purse string suture with square knots. The pump was adjusted, so the catheter sits naturally on the abdominal muscle. Finally, the 6-0 monocryl suture was used to close the skin layer.

### ***Histology***

Before tissue collection, animals were perfused transcardially with 20 ml of 1X PBS 4% PFA. Tissues were processed for paraffin-embedding using standard methods. Sections of muscle tissues were stained with picrosirius red (PSR) in order to quantify collagen deposition (Wax-it Histology Service Inc.).

For laminin staining, sections were deparaffinized and antigen retrieval performed in proteinase K buffer (Abcam, #ab64220) for 20 min at room temperature (RT). Slides were then washed in 1X PBS and then incubated in a blocking solution containing 3% normal goat serum and 0.3% triton X-100 in 1X PBS for 60 mins at RT prior to incubation with Laminin (Abcam #ab11575; 1:200) at 4°C overnight. Slides were then washed with 1X PBS and incubated in blocking solution containing the secondary antibodies (ThermoFisher) for 2 hours at RT. Following antibody incubation,  $3 \times 5$  min PBS washes were performed, and sections were stained with DAPI for 10 min (ThermoFisher, #D3571, 0.6  $\mu$ M) before being mounted with fluorescent mounting medium (Dako).

### ***Imaging***

PSR and laminin images were acquired at 10X magnification using a Nikon Eclipse Ni equipped with a device camera (Nikon Digital Sight DS-U3 for brightfield, Qimaging Retiga EXi for fluorescence) and operated via NIS software. Collagen deposition and CSA were calculated using Fiji (ImageJ, version 2.0.0-rc/69/1.52n, NIH, MD) and Open-CSAM [112]. Images were

assembled using Adobe Illustrator CS6 (Adobe)

### ***FAP culture***

FAP were isolated and cultured as described in [32] with some modifications. Damaged TAs were carefully dissected and gently torn with tissue forceps. Enzymatic digestion was performed with Collagenase D (Roche Biochemicals; 1.5 U/ml) and Dispase II (Roche Biochemicals; 2.4 U/ml), at 37 °C for 60 min. Preparations were passed through 70 µm and 40 µm cell strainers (Becton Dickinson), and washed in 1X PBS containing 2 mM EDTA and 2% FBS (FACS buffer). Resulting single cells were collected by centrifugation at 300g for 5 min. Cell homogenate was incubated with primary antibodies for 30 min at 4°C in FACS buffer. Monoclonal primary antibodies were used as following: anti-CD31 (eBioscience, clone: 390), anti-CD45 (AbLab, clone: I3/2), anti-Sca-1 (eBioscience, clone: D7) and anti-α7 integrin (AbLab, clone: R2F2). Cells were stained with Hoechst 33342 (2.5 µg.ml<sup>-1</sup>, Sigma) and resuspended in FACS buffer immediately before sorting. Sorting was performed on FACS Aria II (Becton Dickinson) or Influx (Becton Dickinson). Gates were defined based on fluorescence minus one (FMO) controls and EGFP negative FAPs were sorted as CD31/CD45/α7int- Sca1+ GFP-. FAPs were seeded at a density of 10.000 cell/cm<sup>2</sup> in high-glucose Dulbecco's modified eagle medium (DMEM) (Invitrogen) supplemented with 10% FBS and 1.5 ng/ml bFGF (Invitrogen).

### ***In vitro drug screening***

EGFP negative FAPs were plated at 10,000 cells/cm<sup>2</sup> in 384 well-plates (Falcon) and treated at 50-60% confluence, either with rh-TGFβ<sub>1</sub> (1 ng/ml) (eBioscience) or rh-TGFβ<sub>1</sub>+ drugs in DMEM 5% FBS.

For the first screening, 6 to 8 mice were pooled before sort and four to nine technical replicates

were performed. 722 chemical compounds (Table 1 and 2) organized into two libraries (KIL and TOOL, donated by Dr. Rima Al-awar (Ontario Cancer Institute, Toronto) were tested. After a 72h treatment period, cells were fixed with paraformaldehyde (PFA) 4% for 10 min, and Hoechst 33342 (Sigma) was used for nuclei staining. By using the Cellomics Array scan (Thermo Fisher Scientific), viability and GFP expression were analyzed. For the viability, a decrease of 50% in the number of cells per well compared to the TGF $\alpha$  treatment was considered as non-survival (TGF $\alpha$ -treated wells contain  $3,139 \pm 178$  cells/well, data not shown).

For the second screening, 6 to 8 mice were pooled before sort, triplicate were performed and the experiment was repeated at least 2 times. screening was performed on 60 compounds in the same condition as previously described at a 1  $\mu$ M dose. After 72 hours, cells were detached from the plate using trypsin and resuspended in FACS buffer and Hoechst 33342 (Sigma). Percentage of EGFP positive cells was calculated on the total event that were Hoechst+. The most significant drugs were finally tested in a dose response (0.01 to 5  $\mu$ M) and the percentage of FAP EGFP positive was analyzed by FACS.

An epigenetic library of 26 compounds was obtained from Chemical Probes and was screened by flow as previously described (concentrations of 0.25 and 1  $\mu$ M). Withaferin and its derivatives compounds (Imstar Therapeutics) were tested at a range of 0.06 to 0.5  $\mu$ M concentrations. Percentage of EGFP positive FAPs was quantified by flow cytometry as described above.

Lastly, once FAP reached 60% confluence, the cells were incubated in 5% FBS, no bFGF prior to being stimulated with rf-TGF $\alpha$  (1 ng/ml) and later with or without 5-z-7Oxozeanol at 1 nM for 6 hours. RNA isolation was performed using RNazol reagents as per the supplier's instructions.

### ***RT-PCR and ddPCR***

Reverse transcription was performed using 100 ng of RNA and Superscript Reverse Transcriptase according to the supplier's instructions (Applied Biosystems). The cDNA was diluted ten times in RNase free water (ThermoFisher) and 2.5  $\mu$ l was used in a reaction mix containing Droplet Digital PCR Supermix (Bio-Rad), TaqMan assay and RNase free water. The Taqman probes used are listed in Table S1. Droplets were generated with a QX100 droplet generator (Bio-Rad). After mixing 20  $\mu$ l of reaction mix and 70  $\mu$ l of droplet generator oil (Bio-Rad). The emulsified samples were loaded onto 96-well plates and endpoint PCRs were performed in C1000 Touch thermal cycler (Bio-Rad) at the following cycling conditions: 95 °C for 10 min, followed by 45 cycles at 94 °C for 30 s and 60 °C for 1 min, followed by 98 °C for 10 min. The droplets from each sample were read through the QX200 droplet reader (Bio-Rad). Resulting PCR-positive and PCR-negative droplets were counted using QuantaSoft software (Bio-Rad). Data for each gene were normalized to *hprt* expression.

### ***Cell culture***

C3H10T1/2 is a cell line of mesenchymal progenitors (Clone 8, American Type Culture Collection, Manassas, USA), that need to be maintained at 50-70% confluence. C3H10T1/2 cells were grown at 37 °C in 5% CO<sub>2</sub> in growth medium; high-glucose DMEM (Invitrogen), supplemented with 10% (v/v) FBS and Penicillin/streptomycin. For western blot analysis, cells were serum-starved for 1 hr before being treated with 1 ng/ml of rh-TGF $\beta$ 1 (e-Bioscience) in DMEM supplemented with 2% (v/v) FBS. Specific treatments the following inhibitors were also used at 1  $\mu$ M final concentration: Nilotinib (Tasigna®, AMN107; Novartis), Sorafenib (Bayer), JQ-1 (Cayman Chemical), 5Z-7-Oxozeaenol (Cayman Chemical). Protein extracts were done after 30 min of incubation at 37 °C in 5% CO<sub>2</sub>.

### ***Protein extraction and western blot***

Protein extracts from cells were obtained using RIPA 1X lysis buffer (#9806 Cell signaling, MA, USA) plus protease/phosphatase inhibitors (#P8340 and #P0044, Sigma-Aldrich, USA). Cells were sonicated for 10 s and centrifuged at 9000g. Proteins were quantified with the Micro BCA assay kit following the manufacturer's instructions (Pierce, IL, USA). Extracts were subjected to SDS-PAGE electrophoresis in precast NuPAGE™ 4 to 12%, Bis-Tris, transferred to PDVF membranes (Millipore, CA, USA), and probed with primary antibodies: rabbit anti-phospho-p38 (Thr180/Tyr182) (1:500, #9211S, Cell Signaling, USA), rabbit anti-p38 (1:500, #9212, Cell Signaling, USA) and mouse anti- $\beta$ -Actin (1:5000, #21001901, AbLab, BC, CA). Then, primary antibodies were detected with IRDye® Infrared Dye labeled secondary antibodies (LI-COR). All immunoreactions were visualized by with an Odyssey® imaging system. Western blot densitometry quantification was done using Fiji (ImageJ, version 2.0.0-rc/69/1.52n, NIH, MD) software. Protein levels were normalized with the levels of the loading control ( $\beta$ -Actin).

### ***Statistical analysis***

Graph and statistical tests were performed using Prism 8 (GraphPad Software, La Jolla California, USA). Depending on the experiment, one-way or two-way ANOVA were performed, corrections were applied, followed by post-hoc test. Gaussian distribution was not assumed. A probability of <5% ( $p < 0.05$ ) was considered statistically significant. Sample size and/or technical replicate number for each experiment is indicated in the figure legend. Graphs are represented as mean  $\pm$  standard error of the mean. Figures were assembled using Adobe Illustrator CS6 (Adobe).

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### **Author contributions**

M.T., M.L., C.C., L.R., F.L., O.C., L.W.T., and A.W. were responsible for performing and analyzing experiments. M.T., M.L., H.S., and F.M.V.R. were involved in experimental design, data interpretation, and preparation of the manuscript. All authors were involved in editing the manuscript.

### **Competing interests**

The authors declare no competing or financial interests.

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Table 1. KIL library

Full_ Reg_ Num ber	Synonyms	Targets	Fold change (TGFb/sample)	p value	Cell number	Output	Selected for second screen
OIC R000 3189 B01	(+)-P276, P276-00	CDC2 (CDK1, p34cdc2, CDC28p)/Cyclin B, CDK4/Cyclin D1	1.46	0.27011	1310	NEG	
OIC R000 0805 A01	(5Z)-7-Oxozeaenol	TAK1 (TGF1a, MAP3K7)	2.18	0.00331	1578	NEG	
OIC R000 0789 A01	1-Naphthyl PP1, 1-NA- PP 1	SRC (c-SRC), FYN (p59fyn), ABL1 (ABL)	6.93	0.00023	1423	SURV	
OIC R000 0533 B01	10-DEBC hydrochloride	AKT1 (PK3a)	1.76	0.07089	1697	NEG	
OIC R000 0480 B01	2-(p-Hydroxyanilino)- 4-(p-chlorophenyl) thiazole, HCl, SKI-II, ABC-294640	SPK1 (SPHK, SK1, Sphingosine Kinase)	4.67	0.00234	2081	POS	
OIC R000 0480 A01	2-(p-Hydroxyanilino)- 4-(p-chlorophenyl) thiazole, SKI-II, ABC- 294640	SPK1 (SPHK, SK1, Sphingosine Kinase)	1.06	0.81000	2494	NEG	
OIC R000 0481 A01	2-Dimethylamino- 4,5,6,7-tetrabromo-1H- benzimidazole, DMAT, Casein Kinase II	CK1a (Casein kinase 1, CK1)	0.91	0.74226	2179	NEG	

OIC R000 0280 A01	2-Thio(3-iodobenzyl)- 5-(1-pyridyl)-[1,3,4]- oxadiazole	GSK3B	1.11	0.69980	1650	NEG
OIC R000 0482 A01	5-(3-Methoxy-4-((4- methoxybenzyl)oxy)ben zyl)-pyrimidine-2,4- diamine	FMS (c-FMS, CSF- 1R, CSF1R)	1.29	0.60553	2100	NEG
OIC R000 0791 A01	5-Iodotubercidin	AK (Adenosine Kinase), CK1a (Casein kinase 1, CK1)	1.53	0.37648	2816	NEG
OIC R000 0342 A01	6-[4-(2-Piperidin-1- ylethoxy)phenyl]-3- pyridin-4- ylpyrazolo[1,5- a]pyrimidine, Dorsomorphin, BML- 275, AMPK Inhibitor, Compound C	AMPK, ALK2, BMPR1A (ALK3), BMPR1B (ALK6)	2.81	0.00437	1510	SURV
OIC R000 0323 A01	6-bromoindirubin-3'- oxime, BIO	GSK3	1.27	0.31127	3355	NEG
OIC R000 0509 A01	7-Cyclopentyl-5-(4- phenoxyphenyl)-7H- pyrrolo[2,3- d]pyrimidin-4-ylamine	LCK (p56lck), SRC (c-SRC)	6.30	0.00103	2208	POS
OIC R000 8756 A01	A 1070722, A-1070722	GSK3	0.82	0.28322	2385	NEG
OIC	A 769662, A-769662	AMPK	1.76	0.00787	3498	POS

R000 8706 A01						
OIC R000 0792 A01	A 83-01	TGFbR1 (ALK5)	7.70	0.00018	1573	SURV
OIC R000 0536 B01	A-443654, A-654	AKT1 (PKBa)	0.67	0.38335	162	SURV
OIC R000 7449 A01	AB12134C3, PNK inhibitor	PNK (PNKP)	0.91	0.64848	1648	NEG
OIC R000 0790 B01	ABT-702 dihydrochloride	AK (Adenosine Kinase)	1.18	0.47932	3518	NEG
OIC R000 1138 A01	ABT-869, Linifanib, AL-39324, RG-3635	KDR (VEGFR2 VEGFR, FLK1), PDGFRb (PDGFR, FDCFR), FMS (c- FMS, CSF-1R, CSF1R), FLT3 (STK1, FLK2)	1.93	0.00612	1393	SURV
OIC R000 1166 A01	AC220, AC-220, Quizartinib, AC010220	FLT3 (STK1, FLK2), FMS (c-FMS, CSF- 1R, CSF1R), KIT (c- KIT), PDGFRb (PDGFR, PDGFR1), RET	3.55	0.00034	1289	SURV
OIC	AEE 788, AEE-788,	EGFR (ERBB1,	3.42	0.00003	1453	SURV

R000 8728 A01	NVP-AEE-788	HER1), ErbB2 (TKR1, HER2, NEU), KDR (VEGFR2, VEGFR, FLK1)				
OIC R000 0345 A01	AEG 3482	JNK1 (JNK)	1.77	0.04674	2158	POS
OIC R000 0778 A01	AG 18, RG-50810, TyrphostinA23	EGFR (ERBB1, HER1), PDGFRb (PDGFR, PDGFR1)	2.27	0.00734	3522	POS
OIC R000 0779 A01	AG 213, Tyrphostin AG 213	EGFR (ERBB1, HER1), PDGFRb (PDGFR, PDGFR1)	1.15	0.50776	3832	NEG
OIC R000 0776 A01	AG 490, Tyrphostin AG 490	EGFR (ERBB1, HER1), JAK2, JAK3/STAT, JAK3/AP-1, JAK3/MAPK	1.46	0.10701	3298	NEG
OIC R000 0780 A01	AG 494	EGFR (ERBB1, HER1)	1.56	0.07194	3631	NEG
OIC R000 0781 A01	AG 825, Tyrphostin AG 825	ErbB2 (TKR1, HER2, NEU)	1.34	0.20138	3780	NEG
OIC R000 1190	AG13958, AG-013958	PDGFRb (PDGFR, PDGFR1), KDR (VEGFR2, VEGFR,	2.10	0.06268	1850	NEG

A01		FLK1)				
OIC						
R000						
0644		AKT1 (PKBa),				
D02	Akt 1/2 Kinase inhibitor	AKT2 (PKBb)	1.48	0.10256	2989	NEG
OIC						
R000						
1150						
A01	Akt-I-1	AKT1 (PKBa)	1.61	0.05252	2295	NEG
OIC						
R000						
1151		AKT1 (PKBa),				
B01	Akt-I-1,2	AKT2 (PKBb)	2.25	0.00364	3055	POS
OIC		CDC2 (CDK1,				
R000		p34cdc2,				
0751		CDC28p)/Cyclin B,				
A01	Alsterpaullone	CDK2/Cyclin A,	3.19	0.00180	1576	SURV
		CDK2/Cyclin E,				
		CDK5/p35, GSK3				
OIC		CDC2 (CDK1,				
R000		p34cdc2,				
1118		CDC28p)/Cyclin B,				
A01	Alvocidib, HMR-1275,	CDK2/Cyclin A,				
	L-868275, MDL-	CDK2/Cyclin E,				
	107826A, NSC-649890,	CDK4/Cyclin D1,				
	L-868276, Flavopiridol	CDK6/Cyclin D3,				
		CDK7, CDK9/Cyclin				
		T1, Bcl-2, Mcl-1,	0.13	0.00000	69	SURV
		Survivin, XIAP				
OIC		LCK (p56lck), SRC				
R000		(c-SRC), KDR				
1172		(VEGFR2, VEGFR,				
A01	AMG-47a	FLK1), p38a	1.55	0.12178	1302	SURV

OIC R000 7447 A01	AMG-900	AurA (AuroraA), AurB (AuroraB), AurC (AuroraC)	0.93	0.62919	2820	NEG
OIC R000 1189 A01	AMG-Tie2-1	TIE2 (TEK)	3.12	0.00061	1577	SURV
OIC R000 8753 A01	AMG458	MET (c-MET, HGFR)	1.64	0.00024	2988	POS
OIC R000 1141 A01	AP-24534, Ponatinib	BCR, ABL1 (ABL), SRC (c-SRC), FLT3 (STK1, FLK2), KIT (c-KIT), RET, PDGFRb (PDGFR, PDGFR1), FGFR1 (FLT2), FGFR2, FGFR3	1.27	0.29139	326	SURV
OIC R000 8766I 01	Apatinib mesylate, YN- 968D1	RET, KDR (VEGFR2, VEGFR, FLK1), FLT1 (VEGFR1), SRC (c- SRC), KIT (c-KIT)	1.75	0.00829	2882	NEG
OIC R000 0535 A01	API-2, Triciribine, NSC154020, TCN, Tricibine, VQD-002	AKT1 (PKBa)	0.18	0.00165	222	SURV
OIC R000 0324 A01	AR-A014418	GSK3	1.13	0.57700	2974	NEG

OIC R000 0813 A01	Arctigenin, (-)- Arctigenin	MAP2K1 (MEK1), DNA Topoisomerase II, HIV Integrase, NFKB, AP-1	1.62	0.09098	3681	NEG
OIC R000 0798 A01	Arcyriaflavin A	CDK4/Cyclin D1, CaMK2b (CaMK2)	0.91	0.66006	3067	NEG
OIC R000 7900 A01	ARQ-197, Tivantinib	MET (c-MET, HGFR)	1.38	0.14152	655	SURV
OIC R000 0762 A01	AS-252424	PI3K	1.60	0.05573	3427	POS
OIC R000 0281 A01	AS-601245	JNK1 (JNK)	1.37	0.20541	1227	SURV
OIC R000 3201 A01	AS-703026, MSC1936369B	MAP2K1 (MEK1), MAP2K2 (MEK2)	0.74	0.04494	1509	SURV
OIC R000 0298 A01	AS604850, AS-604850, AS 604850	PI3K	1.39	0.23965	1901	NEG
OIC R000 8722 A01	AS605240, AS-605240	PI3K	0.92	0.72046	2733	NEG
OIC	ASC-033, APY 33	IRE1 (IRE1a, ERN1)	1.32	0.16699	3220	NEG

R000 4079 A02						
OIC R000 4080 A02	ASC-069, APY 69	IRE1 (IRE1a, ERN1)	0.28	0.00000	1825	NEG
OIC R000 4081 A02	ASC-081, APY 81	IRE1 (IRE1a, ERN1)	1.42	0.12567	141	SURV
OIC R000 4082 A02	ASC-082, APY 82	IRE1 (IRE1a, ERN1)	0.35	0.00395	2643	NEG
OIC R000 4083 A02	ASC-086, APY 86	IRE1 (IRE1a, ERN1)	1.79	0.01600	362	SURV
OIC R000 8697 A01	ASP-3026	ALK, EGFR (ERBB2, HER1)	1.24	0.09435	2766	NEG
OIC R000 1160 A01	AT-7519 derivative, AT-7519M derivative	Unknown	2.05	0.07255	3282	NEG
OIC R000 1160 B02	AT-7519 hydrochloride, AT7519 hydrochloride	CDC2 (CDK1, p34cdc2, CDC28p)/Cyclin B, CDK2/Cyclin A, CDK3/Cyclin E, CDK4/Cyclin D1,	3.84	0.00001	1521	SURV

		CDK5/p35, CDK6/Cyclin D3				
OIC R000 8727 A01	AT7867, AT-7867	AKT1 (PKBa), AKT2 (PKBb), AKT3 (PKBg), p70S6K (S6K, S6K1)	1.28	0.00003	1315	SURV
OIC R000 1155 A01	AT9283, AT-9283	AurA (AuroraA), AurB (AuroraB), JAK2, JAK3, ABL1 mutant (ABL mutant), FLT3 (STK1, FLK2)	0.68	0.00351	450	SURV
OIC R000 8717 A01	Aurora A Inhibitor I	AurA (AuroraA)	1.00	0.76602	928	SURV
OIC R000 1163 A01	AV-412, MP-412	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	1.48	0.08671	1054	SURV
OIC R000 1191 A01	AV951, KRN951, Tivozanib	FLT1 (VEGFR1), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3), KIT (c-KIT), PDGFRb (PDGFR, PDGFR1), p38 MAPK	1.47	0.03054	2708	NEG
OIC R000 0302 A01	Axitinib, AG-013736	PDGFRb (PDGFR, PDGFR1), FLT1 (VEGFR1), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3), FMS (c-	0.73	0.08181	711	SURV

		FMS, CSF-1R, CSF1R)				
OIC R000 7882 A01	AZ 3146, AZ-3146	TTK (ESK, MPS1L1, Monopolar spindle 1 (Mps1) kinase)	1.41	0.06961	2384	NEG
OIC R000 8739 A01	AZ TAK1 Inhibitor	TAK1 (TGF1a, MAP3K7)	0.91	0.48922	654	SURV
OIC R000 1168 A01	AZ-960	JAK2	1.71	0.01864	1237	SURV
OIC R000 7472 A01	AZ628, AZ-628	BRAF (B-raf), RAF1 (c-Raf)	1.60	0.04568	4107	POS
OIC R000 1145 B01	AZD 7762, AZD-7762 hydrochloride	CHK1, CHK2	0.90	0.44362	521	SURV
OIC R000 1145 B02	AZD 7762, AZD-7762 hydrochloride	CHK1, CHK2	1.76	0.01324	299	SURV
OIC R000 1194 A01	AZD-1152-HQPA, Barasertib	AurB (AuroraB)	1.30	0.18920	923	SURV
OIC R000 1139	AZD-1152, Barasertib	AurB (AuroraB)	1.38	0.20052	1135	SURV

A01							
OIC							
R000							
7902							
A01	AZD-1480, AZD1480	JAK1, JAK2, JAK3	0.81	0.26610	1587	SURV	
OIC							
R000		FGFR1 (FLT2),					
7901		FGFR2, FGFR3,					
A01	AZD-4547	FGFR4	0.67	0.04845	1006	SURV	
OIC							
R000		AKT1 (PKBa),					
8729		AKT2 (PKBb),					
A01	AZD-5363, AZD5363	AKT3 (PKBg),	1.87	0.00858	3388	POS	X
OIC							
R000		FRAP (MTOR,					
3206	AZD-8055, AZD 8055,	FRAP1), mTORC1,					
A01	AZD8055	mTORC2	3.23	0.00035	1408	SURV	
OIC							
R000		ABL1 (ABL), SRC					
0540	AZD0530, Saracatinib,	(c-SRC), KIT (c-					
A01	NSC-735464	KIT)	1.20	0.38920	501	SURV	
OIC							
R000		CDC2 (CDK1,					
7890		p34cdc2					
A01	AZD5438, AZD-5438	CDK28p)/Cyclin B,	0.91	0.52694	1373	SURV	
OIC							
R000		MAP2K1 (MEK1),					
0497	AZD6244, ARRY-	MAP2K2 (MEK2),					
A01	142886, AZD-6244,	Erk2 (ERK, p38)	2.06	0.04377	2501	POS	X
OIC							
R000							
8724	AZD6482, AZD-6482	PI3K	1.44	0.01050	2848	NEG	

A01					
OIC					
R000	AZD8330, AZD-8330,	MAP2K1 (MEK1),			
7894	ARRY-424704, ARRY-	MAP2K2 (MEK2)	0.97	0.84617	523
A01	704				SURV
		KDR (VEGFR2,			
		VEGFR, FLK1),			
OIC		FLT4 (VEGFR3),			
R000		KIT (c-KIT),			
8755	BAY 57-9352,	PDGFRb (PDGFR,	0.96	0.70354	2852
A01	Telatinib	PDGFR1)			NEG
OIC					
R000	BAY 61-3606				
0325	hydrochloride, sc-				
B01	202351	SYK	2.50	0.00686	1662
					NEG
		KIT (c-KIT),			
		PDGFRb (PDGFR,			
		PDGFR1), RET,			
		RAF1 (c-Raf), P21AF			
OIC		(B-raf), TIE2 (TIEK),			
R000		KDR (VEGFR2,			
7903	BAY 73-4506,	VEGFR, FLK1), p38	1.39	0.10227	1844
A01	Regorafenib	MAPK			NEG
OIC					
R000		FGFR1 (FLT2),			
8699	BGJ-398, NVP-BGJ-	FGFR2, FGFR3,	0.80	0.18353	1788
A01	398, NVP-BGJ398	FGFR4			NEG
OIC					
R000					
7463	BGT-226, NVP-BGT-	PI3K, FRAP (MTOR,	0.63	0.00518	861
A01	226, NVP-BGT226	FRAP1)			SURV
OIC					
R000		PLK1 (PLK,	0.81	0.50937	295
	BI 2536	STPK13)			SURV

0544 A01						
OIC R000 0797 A01	BI 78D3	JNK1 (JNK)	1.69	0.05152	2581	NEG
OIC R000 1149 A01	BI-6727, volasertib	PLK1 (PLK, STPK13)	4.47	0.00010	715	SURV
OIC R000 1188 A01	BI-D1870	RSK1 (RSK, S6Ka, MAPKAPK1C)	2.89	0.00090	3186	POS
OIC R000 1143 A01	BIBF-1120, Intedanib, Vargatef	KDR (VEGFR2, VEGFR, FLK1), PDGFRb (PDGFR, PDGFR1), MET (c-MET, HGFR)	1.49	0.02881	2239	NEG
OIC R000 0346 B01	BIBU 1361 dihydrochloride	EGFR (ERBB1, HER1)	0.36	0.00001	2290	NEG
OIC R000 0498 A01	BIBW-2992, Tovok, Afatinib	EGFR (ERBB1, HER1)	2.43	0.05641	1620	SURV
OIC R000 0348 B01	BIBX 1382 dihydrochloride, Faldidamol	EGFR (ERBB1, HER1)	1.84	0.03716	2827	POS
OIC R000	BIRB 796, Doramapimod	p38 MAPK	1.47	0.01682	3099	NEG

3192 A02						
OIC R000 0549 B01	Bisindolylmaleimide I hydrochloride, GF 109203X HCl, Go 6850 HCl	PKC	2.96	0.00643	1608	POS
OIC R000 1185 B01	Bisindolylmaleimide X, HCl salt	PKC	2.11	0.00655	3350	POS
OIC R000 7885 A01	BIX 02189, BIX-02189	MAP2K5 (MEK5), Erk5 (BMK1, PRKM7)	2.03	0.00896	2489	POS
OIC R000 8759 A01	BIX-RSK2 Inhibitor	RSK1 (RSK, S6Ka, MAPKAPK1C), RSK2 (S6K-a3, p90- RSK2)	10.17	0.00006	715	SURV
OIC R000 7448 A01	BKM120, BKM-120, NVP-BKM-120	PI3K	1.57	0.05329	755	SURV
OIC R000 8700 A01	BMS 777607, BMS- 777607	MET (c-MET, HGFR), AXL (Ark), RON (MST1R), TYRO3 (RSE, SKY, TIF, BYK)	0.91	0.40257	2788	NEG
OIC R000 7883 A01	BMS 794833, BMS- 794833	MET (c-MET, HGFR), KDR (VEGFR2, VEGFR, FLK1)	1.17	0.45808	2713	NEG
OIC R000	BMS-2	MET (c-MET, HGFR)	2.49	0.00147	1342	SURV

1179							
A01							
OIC							
R000							
1174							
A01	BMS-3	LIMK1 (LIMK)	0.93	0.63912	553	SURV	
OIC							
R000							
0326							
B01	BMS-345541	IKKb (IKK2)	1.43	0.15877	2341	NEG	
OIC							
R000							
1175							
A01	BMS-5	LIMK1 (LIMK)	2.30	0.00144	1419	SURV	
OIC							
R000							
0758							
A01	BMS-536924	IGF1R (JTK13, IGFIR)	1.43	0.15517	2606	NEG	
		FGFR1 (FLT2), FGFR2, FGFR3					
OIC		FLT1 (VEGFR1), KDR (VEGFR2, VEGFR3), FLK1, FLT4 (VEGFR3)					
R000							
8730	BMS-582664, Brivanib alaninate		2.01	0.00059	3165	POS	X
A01							
		EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU), ErbB3 (HER3), ErbB4 (HER4)					
OIC							
R000							
0546							
A01	BMS-599626, AC-480		1.77	0.06487	2432	NEG	
OIC							
R000							
7460	BMS-754807	IGF1R (JTK13, IGFIR)	1.43	0.00522	3815	NEG	

A01							
OIC R000 0504 A01	Bosutinib, SKI-606	BCR, ABL1 (ABL), SRC (c-SRC), FGR (SRC2), LYN	0.53	0.18338	248	SURV	
OIC R000 1119 A01	Brivanib, BMS-540215	KDR (VEGFR2, VEGFR, FLK1), FGFR1 (FLT2), FGFR3, FLT4 (VEGFR3), FGFR2	1.34	0.11377	3094	NEG	
OIC R000 7467 B01	BS-181 hydrochloride	CDK7	1.53	0.03426	2753	POS	
OIC R000 0283 A01	BX-795	PDK1 (PDPK1), TBK1, IKKe	1.24	0.43337	415	SURV	
OIC R000 1181 A01	BX912, BX-912	PDK1 (PDPK1)	2.59	0.00149	3162	POS	
OIC R000 8760 A01	BYL-719, NVP-BYL-719	PI3Ka	1.49	0.02497	2003	NEG	
OIC R000 1129 A01	C-1	PKC	1.48	0.01757	2869	NEG	
OIC R000 7897	CAL-101, GS-1101	PI3Kd	1.73	0.03685	2637	POS	X

A01						
OIC						
R000						
1171						
A01	CC-401, JNK-401	JNK1 (JNK), JNK2	1.60	0.03095	3449	POS
OIC						
R000						
8716	CCT129202, CCT-	AurA (AuroraA),				
A01	129202	AurB (AuroraB),	0.45	0.00155	628	SURV
		AurC (AuroraC)				
OIC						
R000						
8752		AurA (AuroraA),				
A01	CCT137690	AurB (AuroraB),	1.26	0.00098	421	SURV
		AurC (AuroraC),				
		FLT3 (STK1, FLK2)				
OIC						
R000						
7465		CDC2 (CDK1,				
A01	Cdk1/2 Inhibitor III	p34cdc2,	2.09	0.00278	1472	SURV
		CDC28p)/Cyclin B,				
		CDK2/Cyclin A				
OIC						
R000						
1133	Cediranib, AZD-2171,	FLT1 (VEGFR1),				
A01	Recentin	KDR (VEGFR2)	2.95	0.00045	1544	SURV
		VEGFR, FLK1,				
		FLT4 (VEGFR3),				
		KIT (c-KIT)				
		PDGFR (PDGFR,				
		PDGFR1)				
OIC						
R000						
0794						
A01	CGK 733	ATR, ATM	1.29	0.25008	3704	NEG
OIC						
R000						
0347	CGP 57380, CGP-					
A02	57380	MNK1	7.01	0.00092	2414	POS
OIC	CGP-57380, CGP	MNK1	1.29	0.27700	3162	NEG

R000 0347 A01	57380					
OIC R000 0327 B01	CGP-74514A hydrochloride	CDC2 (CDK1, p34cdc2, CDC28p)/Cyclin B	2.39	0.00831	1124	SURV
OIC R000 8737 A01	CH5424802, AF-802, CH-5424802	ALK	0.83	0.34953	1186	SURV
OIC R000 0555 B01	Chelerythrine chloride	PKC	0.30	0.01899	55	SURV
OIC R000 7880 A01	CHIR-124	CHK1	2.81	0.00080	1434	SURV
OIC R000 0284 A01	CHIR-98014 isomer, CT-98014	CSF 3	0.65	0.03881	946	SURV
OIC R000 0343 A01	Chk2 Inhibitor II, 339253	CHK2	1.41	0.19500	3273	NEG
OIC R000 0501 A01	CI-1033, Canertinib, PD-183805, SN-26606	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU) - Irreversible	1.44	0.33080	641	SURV
OIC R000	CID 755673	PKD1 (PKD)	3.81	0.00366	2209	POS

0515 A01						
OIC R000 0771 A01	Compound 401	DNAPK (DNA-PKcs), FRAP (MTOR, FRAP1)	1.81	0.03360	3336	POS
OIC R000 0344 A01	Compound 52, NG-52	CDC2 (CDK1, p34cdc2, CDC28p)/Cyclin B	2.07	0.02010	2430	POS
OIC R000 0503 A01	CP-690550, CP-690,550, Tasocitinib, Tofacitinib	JAK3	1.18	0.49561	485	SURV
OIC R000 1164 A01	CP-724714	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	2.37	0.00319	3056	POS
OIC R000 7470 A01	CP466722, CP-466722	ATM	0.91	0.65596	2903	NEG
OIC R000 8764 A01	Crenolanib, ARO-002, CP-868596	PDGFRa (PDGFR2), PDGFRb (PDGFR, PDGFR1)	3.10	0.00002	635	SURV
OIC R000 0285 A01	CT-99021, CHIR-99021	GSK3	0.99	0.96092	1345	SURV
OIC R000 3190	CX-4945	CK2a1 (CK2, CKII, Casein kinase2)	0.67	0.00564	809	SURV

A02						
OIC R000 1156 A01	CYC-116	AurA (AuroraA), AurB (AuroraB), KDR (VEGFR2, VEGFR, FLK1)	1.38	0.16595	797	NEG
OIC R000 1128 A01	Cyclapolin 9, Cyclapolin9	PLK1 (PLK, STPK13)	1.36	0.11931	2241	NEG
OIC R000 1128 A03	Cyclapolin 9, Cyclapolin9	PLK1 (PLK, STPK13)	1.03	0.83656	3740	NEG
OIC R000 1169 A01	CYT11387, CYT387, CYT-387, Momelotinib	JAK2	1.83	0.01044	1435	SURV
OIC R000 0510 A01	D4476	CK1a (Casein kinase 1, CK1)	3.53	0.00356	2043	POS
OIC R000 8757 A01	Dacomitinib hydrate, PF-00299804, PF- 00299804-03, PF-299, PF-299804	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU), ErbB4 (HER4)	4.75	0.00001	1518	SURV
OIC R000 0500 A01	Dasatinib, BMS- 354825, Sprycel, NSC- 732517	BCR, ABL1 (ABL), SRC (c-SRC), EphA1 (EPH), FYN (p59fyn), LCK (p56lck), PDGFRb (PDGFR, PDGFR1), YES, Erk2 (ERK,	1.65	0.08302	723	SURV

p38)						
OIC R000 0803						
R01	DCA, Dichloroacetate	PDK1 (PDPK1)	2.46	0.00526	3171	POS
OIC R000 3191						
A02	Dianilinopyrimidine_01	Pan kinase	0.94	0.66305	3146	NEG
OIC R000 8768	Dinaciclib, MK-7965, NSC-727135, SCH- 727965	CDK2/Cyclin A, CDK5/p25, CDC2 (CDK1, p34cdc2, CDC28p)/Cyclin B, CDK9/Cyclin T1	0.28	0.00001	119	SURV
OIC R000 0774		DAGK (DGK, Diacylglycerol kinase)	1.00	0.98617	3385	NEG
OIC R000 1130		PDGFRb (PDGFR, PDGFR1)	1.37	0.00681	2466	NEG
OIC R000 0342	Dorsomorphin dihydrochloride, BML- 275, AMPK Inhibitor, Compound C	AM, PK, ALK2, BMPR1A (ALK3), BMPR1B (ALK6)	1.46	0.03790	2589	NEG
OIC R000 0765	Dovitinib, CHIR-258, TKI-258, GFKI-258	KDR (VEGFR2, VEGFR, FLK1), FLT3 (STK1, FLK2), KIT (c-KIT), PDGFRb (PDGFR, PDGFR1)	2.54	0.03431	682	SURV
OIC	E7080, E-7080, ER-	FGFR1 (FLT2), KIT	3.10	0.00126	1558	SURV

R000 1193 A01	203492-00, Lenvatinib	(c-KIT), PDGFRb (PDGFR, PDGFR1), FLT1 (VEGFR1), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3)				
OIC R000 0756 A01	EKI-785, CL-387785	EGFR (ERBB1, HER1) - Irreversible	0.98	0.92561	3189	NEG
OIC R000 0800 A01	Ellagic acid	CK2a1 (CK2, CKII, Casein kinase2)	1.51	0.08675	3322	NEG
OIC R000 3196 A02	EMD-1214063	MET (c-MET, HGFR)	1.27	0.22380	2326	NEG
OIC R000 3198 K01	ENMD-2076, ENMD- 981693	AurA (AuroraA)	1.20	0.32800	1560	SURV
OIC R000 1140 A01	Enzastaurin, LY- 317615	AKT1 (PKBa), PKCb, p70S6K (S6K, S6K1), GSK3B	1.57	0.06325	3285	NEG
OIC R000 0817 A01	EO-1428	p38a, p38b	1.87	0.02634	4163	POS
OIC R000 0514	ER 27319 maleate	SYK	0.20	0.00060	209	SURV

M01						
OIC						
R000						
1195						
A01	ERK2 inhibitor	Erk2 (ERK, p38)	2.25	0.00650	1264	SURV
OIC	Erlotinib HCl, CP-					
R000	358774, OSI-774,	EGFR (ERBB1,				
0320	Tarceva, NSC-718781,	HER1), ErbB2				
B01	RG-1415, Ro-50-8231	(TKR1, HER2, NEU)	1.10	0.69744	2799	NEG
OIC		FRAP (MTOR,				
R000		FRAP1), FKBP12				
0309	Everolimus, RAD-001,	(Rotamase),				
A01	Certican	Angiogenesis inhibitor	1.28	0.28647	1632	NEG
OIC						
R000						
0526						
B01	FAK Inhibitor 14	FAK (FAK1)	1.64	0.21998	1663	NEG
OIC						
R000		ROCK1, ROCK2				
0310	Fasudil HCl, AT-877,	(ROCKa), Calcium				
B01	HA-1077 (diHCl), Eril	sensitizer	1.10	0.69744	2348	NEG
OIC		Calcineurin (CN, PP-				
R000		2B), I $\kappa$ S expression				
0319	FK-506, Tacrolimus,	inhibitor, FKBP12				
A01	Fujimycin, Prograf	(Rotamase)	1.22	0.38975	2880	NEG
OIC						
R000						
0534						
A01	FPA 124	AKT1 (PKBa)	1.60	0.15469	1933	NEG
OIC						
R000	GDC-0879, AR-					
1137	00341677	BRAF (B-raf)	1.36	0.13560	3074	NEG

A01						
OIC						
R000						
0286						
A02	GDC-0941	PI3K	2.00	0.00034	1462	SURV
OIC						
R000						
0286I	GDC-0941 bismesylate,					
01	RG-7321, Pictilisib	PI3K	3.58	0.00202	752	SURV
OIC						
R000						
0303	Gefitinib, Iressa,	EGFR (ERBB1,				
A01	ZD1839	HER1), ErbB2 (TKR1, HER2, NEU)	3.23	0.00260	1515	SURV
OIC						
R000						
0782		EGFR (ERBB1,				
A01	Genistein	HER1)	1.74	0.03944	3541	POS
OIC						
R000						
8696	GLPG-0259,	MAPKAPK5				
A01	Compound A	(PRAK)	1.50	0.02044	206	SURV
OIC						
R000						
0315						
A01	Go 6976, PD-406976	PRC, JAK2	1.15	0.56992	751	SURV
OIC						
R000						
8745	GSK 2334470, GSK-					
A01	2334470	PDK1 (PDPK1)	1.44	0.00054	2524	NEG
OIC						
R000						
8740						
A01	GSK PERK Inhibitor	PEK (PERK)	1.48	0.00226	2695	NEG

OIC R000 8702 A01	GSK-1059615, GSK- 615	PI3K	1.52	0.00564	1194	SURV
OIC R000 7462 A01	GSK-1120212, Trametinib	MAP2K1 (MEK1), MAP2K2 (MEK2)	0.84	0.04568	3425	NEG
OIC R000 1122 A01	GSK-1904529A, GSK1904529A	IGF1R (JTK13, IGFIR)	1.27	0.29093	2829	NEG
OIC R000 8736I 01	GSK-2118436B, GSK2118436B, Dabrafenib mesylate	RAF1 (c-Raf), BRAF (B-raf)	1.60	0.03796	294	SURV
OIC R000 0287 A01	GSK-269962A	ROCK1, ROCK2 (ROCKa)	1.61	0.07002	472	SURV
OIC R000 1148 A01	GSK-461364, GSK461364	FLK1 (FLK, STK13)	1.45	0.05671	928	SURV
OIC R000 0802 A01	GSK-650394	SGK1 (SGK), SGK2	1.74	0.03799	3254	POS
OIC R000 1152 A01	GSK690693, GSK- 690693	AKT1 (PKBa)	2.52	0.00147	1536	SURV
OIC GTP 14564		FLT3 (STK1, FLK2)	1.31	0.28065	3252	NEG

R000 0349 A01						
OIC R000 8747 A01	GW 788388, GW- 788388	TGFbR1 (ALK5)	6.11	0.00000	1572	SURV
OIC R000 0350 A01	GW-5074	RAF1 (c-Raf)	2.09	0.05985	2094	NEG
OIC R000 0288 A01	GW-843682X	PLK1 (PLK, STPK13), PLK3 (PRK, FNK, CNK)	1.17	0.61564	663	SURV
OIC R000 0328 A01	GW2974	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	1.28	0.30885	3072	NEG
OIC R000 0329 B01	GW441756 hydrochloride	TRKA (TRK)	1.19	0.43473	3111	NEG
OIC R000 0330 B01	GW583340 dihydrochloride	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	2.16	0.01363	2593	POS
OIC R000 0331 A01	GW8510	CDK2/Cyclin A	1.29	0.27768	3135	NEG
OIC R000	H 1152, Glycyl dihydrochloride	ROCK1, ROCK2 (ROCKa)	1.76	0.05676	2626	NEG

0822 B01							
OIC R000 0513 B01	H 89 dihydrochloride, H-89 dihydrochloride	PKACa (PKA)	4.00	0.00324	2278	POS	X
OIC R000 1127 B01	HA 1100 hydrochloride, Hydroxyfasudil	ROCK1, ROCK2 (ROCKa)	2.62	0.00160	1366	SURV	
OIC R000 0783 A01	HDS 029	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU), ErbB4 (HER4)	1.20	0.34990	3667	NEG	
OIC R000 8718 A01	Hesperadin	AurB (AuroraB), Tyrosine Kinase inhibitor	0.86	0.55599	156	SURV	
OIC R000 7892 A01	HMN-214, IVX-214	PLK1 (P <sup>1</sup> K, STP K13)	1.87	0.00909	869	SURV	
OIC R000 0487 A01	IC-261, SU-5607	CK1a (Casein kinase 1, CK1)	0.52	0.05470	258	SURV	
OIC R000 3178 A02	IC86621, IC-86621	DNAPK (DNA- PKcs)	0.79	0.18497	3468	NEG	
OIC R000 87114	IC87114, D-030, IC- 87114	PI3Kd	2.41	0.00650	3192	POS	X

0631 A01						
OIC R000 0814 B01	IKK 16	IKKa (IKK1), IKKb (IKK2)	2.26	0.05734	1213	SURV
OIC R000 7452 A01	ILK inhibitor	ILK	1.09	0.57585	4037	NEG
OIC R000 0311 A01	Imatinib (free base), Gleevec, Glivec, CGP- 57148B, STI-571	PDGFRb (PDGFR, PDGFR1), KIT (c- KIT), ABL1 (ABL)	1.52	0.13862	3354	NEG
OIC R000 0311 01	Imatinib Mesylate, Gleevec, Glivec, CGP- 57148B, STI-571	BCR, ABL1 (ABL), PDGFRb (PDGFR, PDGFR1), KIT (c- KIT)	1.99	0.02411	1823	NEG
OIC R000 0815 A01	IMD 0354, IMD-0354	IKKb (IKK2),	2.71	0.00365	1533	SURV
OIC R000 0769 A01	Imidazolo-oxindole PKR inhibitor C16	PKR (EIF2AK1, EIF2AK2)	1.07	0.78009	1122	SURV
OIC R000 7458 A01	INCB018424, INC-424, INC-018424, INCB- 424, INCB-18424, Ruxolitinib, Jakafi, Jakavi	JAK1, JAK2, JAK3, TYK2	1.27	0.24037	3206	NEG
OIC R000	Indirubin, NSC-105327, Couroupitine B, Indigo	CDC2 (CDK1, p34cdc2,	1.35	0.06366	3475	NEG

8041 A02	red, Indigopurpurin	CDC28p)/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK4/Cyclin D1, CDK5, GSK3B				
OIC R000 0804 A01	IPA 3	PAK1 (PAKa)	2.65	0.00476	1572	SURV
OIC R000 0332 A01	IRAK-1/4 Inhibitor I	IRAK1 (IRAK)	1.58	0.00213	2758	NEG
OIC R000 7886 A01	IRAK4 Inhibitor	IRAK4	2.54	0.00309	676	SURV
OIC R000 7904 A01	JAK compound I (VI-53)	JAK1, JAK2, JAK3	1.13	0.58735	3590	NEG
OIC R000 1147 A01	Janex-1, WHI-P131	JAK3	1.38	0.11066	2633	NEG
OIC R000 0784 B01	JNJ 28871063 hydrochloride	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU), ErbB4 (HER4)	1.44	0.12579	3892	NEG
OIC R000 0299	JNJ-10198409, RWJ-540973	PDGFRb (PDGFR, PDGFR1)	0.79	0.25954	609	SURV

A01						
OIC R000 8733 A01	JNJ-38158471	KDR (VEGFR2, VEGFR, FLK1), RET, KIT (c-KIT)	1.35	0.08461	926	SURV
OIC R000 1146 A01	JNJ-38877605	MET (c-MET, HGFR)	1.66	0.06291	2275	NEG
OIC R000 0488 A01	JNJ-7706621	AurA (AuroraA), AurB (AuroraB), AurC (AuroraC), CDC2 (CDK1, p34cdc2, CDC28p)/Cyclin B	1.53	0.16364	1642	NEG
OIC R000 0816 A01	JX-401	p38a	1.60	0.06746	3951	NEG
OIC R000 0312 A01	K-252a, K-2151	TRKA (TRK)	3.58	0.00195	335	SURV
OIC R000 0767 A01	K-252c, Staurosporine Aglycone	PKC	1.58	0.10424	3078	NEG
OIC R000 0491 A01	Kenpaullone, NSC- 664704	CDC2 (CDK1, p34cdc2, CDC28p)/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK5/p35, GSK3,	4.23	0.00237	2197	POS

		LCK (p56lck)				
OIC R000 0809 A01	Ki-8751	KDR (VEGFR2, VEGFR, FLK1), KIT (c-KIT), PDGFRa (PDGFR2), FGFR2, FLT3 (STK1, FLK2)	2.34	0.00659	1562	SURV
OIC R000 1167 A01	Ki20227 (+/-), Ki-20227	FMS (c-FMS, CSF-1R, CSF1R), KDR (VEGFR2, VEGFR, FLK1), KIT (c-KIT), PDGFRb (PDGFR, PDGFR1)	1.51	0.11390	2127	NEG
OIC R000 0489 A01	KN-62	CAMKK2 (CAMKK)	1.88	0.07871	2489	NEG
OIC R000 8710 A01	KRN 633, KRN-633	FLT1 (VEGFR1), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR2)	0.99	0.93389	3003	NEG
OIC R000 0490 A01	KU-55933, KU-0064	ATM, PI3K	2.25	0.03067	2448	POS
OIC R000 7468 A01	KU-60019	ATM	1.00	0.98770	3793	NEG
OIC R000 1180 A01	KU0063794, KU-0063794, KU-63794	FRAP (MTOR, FRAP1), mTORC1, mTORC2	2.87	0.00076	1595	SURV
OIC	KW 2449, KW-2449	FLT3 (STK1, FLK2),	1.76	0.00977	633	SURV

R000 8714 A01		ABL1 (ABL), FGFR1 (FLT2), AurA (AuroraA)				
OIC R000 0542 A01	L-779450, Raf Kinase Inhibitor IV	RAF1 (c-Raf)	4.85	0.00243	1830	POS
OIC R000 0304 H01	Lapatinib ditosylate, Tykerb, GW572016	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	2.69	0.00502	1685	NEG
OIC R000 0785 A01	Lavendustin A, RG 14355	EGFR (ERBB1, HER1), SRC (c-SRC)	1.35	0.18114	3891	NEG
OIC R000 8705 A01	LDN193189, LDN- 193189, DM-3189	ALK2, BMPR1A (ALK3)	3.79	0.00005	1002	SURV
OIC R000 0313 A01	Lestaurtinib, CEP-701, KT-5555, SPM-924	FLT3 (STK), FLK2), TRKA (TRK), JAK2	1.48	0.15762	361	SURV
OIC R000 0795 A01	LFM-A13	BTK (Bruton's tyrosine kinase)	2.59	0.00442	3772	POS
OIC R000 1121 A01	Lim2 Kinase Inhibitor	LIMK2	3.65	0.00027	1339	SURV
OIC R000	LY 333531 mesylate, Ruboxistaurin	PKC $\beta$	2.39	0.00276	1474	SURV

1117I 01							
OIC R000 8762 A01	LY-2157299	TGFbR1 (ALK5), TGFbR2	6.34	0.00000	1500	SURV	
OIC R000 0314 A01	LY-294002	PI3K	1.96	0.02257	2018	NEG	
OIC R000 0351 A01	LY-364947, HTS 466284	TGFbR1 (ALK5)	6.54	0.00041	1433	SURV	
OIC R000 8721I 01	LY2228820, LY- 2228820	p38a, p38b	1.62	0.00410	3028	POS	X
OIC R000 7881 A01	LY2784544, LY- 2784544	JAK2	0.99	0.92938	503	SURV	
OIC R000 8738 A01	LY2835219, LY- 2835219	CDK4/Cyclin D1, CDK6/Cyclin D3, PIM1	2.19	0.00034	2933	POS	X
OIC R000 0512I 01	Masitinib mesylate, AB1010	KIT (c-KIT), PDGFRb (PDGFR, PDGFR1), FGFR3	3.07	0.01221	2058	POS	X
OIC R000 3168	MAZ51, MAZ-51	FLT4 (VEGFR3)	0.91	0.53537	1469	SURV	

A02						
OIC R000 7887 A01	Merck-22-6	AKT1 (PKBa), AKT2 (PKBb)	1.89	0.00893	3841	POS
OIC R000 1170 A01	Merck-5, Mk-5, JAK Inhibitor I	JAK1, JAK2, JAK3, TYK2	1.82	0.00870	2245	NEG
OIC R000 8711 A01	MGCD-265	MET (c-MET, HGFR), RON (MST1R), TIE2 (TEK), FLT1 (VEGFR1), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3)	0.91	0.63579	2185	NEG
OIC R000 1120 A01	MK-1775, MK1775	WEE1	0.95	0.74738	574	SURV
OIC R000 1123 B01	MK-2206	AKT1 (PKBa)	1.20	0.36100	2610	NEG
OIC R000 8754 A01	MK-5108, VX-689	AurA (AuroraA)	1.31	0.02900	1887	NEG
OIC R000 7461 A01	MK-8033	MET (c-MET, HGFR)	1.10	0.62710	1775	NEG

OIC R000 0799 B01	ML 9 hydrochloride	smMLCK (MLCK, myosin light chain kinase, MYLK)	1.51	0.10264	3529	NEG
OIC R000 0289 A01	MLN-518, CT 53518, Tandutinib	FLT3 (STK1, FLK2), KIT (c-KIT), PDGFRb (PDGFR, PDGFR1)	1.65	0.08372	1597	SURV
OIC R000 1142 A01	MLN-8237	AurA (AuroraA)	1.08	0.05042	1020	SURV
OIC R000 8767 A01	MLN8054, MLN-8054	AurA (AuroraA)	1.41	0.03509	1547	SURV
OIC R000 0547 J01	Motesanib diphosphate salt, AMG-706	KIT (c-KIT), PDGFRb (PDGFR, PDGFR1), KDR (VEGFR2, VEGFR, FLK1)	1.93	0.06495	1891	NEG
OIC R000 1192 A01	MP-470, Amuvatinib	AXL (Axl), KIT (c- KIT), FLT3 (STK1, FLK2), MET (c- MET, HGFR), PDGFRa (PDGFR2), RET, RAD51 expression inhibitor	13.50	0.00002	1799	NEG
OIC R000 0545 A01	Mubritinib, TAK-165, D04025	ErbB2 (TKR1, HER2, NEU)	0.41	0.10931	99	SURV
OIC	N-(4-Pyridyl)-N'-(2,4,6-	ROCK1, ROCK2	2.84	0.00482	2378	POS

R000 0479 A01	trichlorophenyl)urea, Rho Kinase Inhibitor II	(ROCKa)					
OIC R000 0529 A01	Necrostatin-1	RIPK1 (RIP)	2.96	0.00577	2539	POS	
OIC R000 3195 A02	Neratinib, HKI-272, CPD-820	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	4.58	0.00009	1578	SURV	
OIC R000 0300 AA0 1	NH125	eEF2K	5.98	0.00048	1074	SURV	
OIC R000 3193 A02	NIK Kinase Inhibitor	NIK	0.97	0.84888	1196	SURV	
OIC R000 0496 A01	Nilotinib, Tassigna, AMN-107	BCR, ABL1 (ABL), PDGFR $\alpha$ (PDGFR, FGFR3), KIT (c- KIT)	2.56	0.03747	2005	POS	X
OIC R000 8741 A01	NQDI-1	MAP3K5 (ASK1)	1.68	0.00620	3110	POS	
OIC R000 0772 H01	NSC 109555 ditosylate, DDUG	CHK2	1.82	0.03192	3507	POS	
OIC	NSC 625987	CDK4/Cyclin D1	1.61	0.09052	1892	NEG	

R000 0290 A01						
OIC R000 0812 A01	NSC 693868	CDC2 (CDK1, p34cdc2, CDC28p)/Cyclin B, CDK5/p25, GSK3	1.27	0.28179	3524	NEG
OIC R000 0752 A01	NSC-663284	cdc25	0.81	0.45381	175	SURV
OIC R000 0753 A01	NU-2058	CDC2 (CDK1, p34cdc2, CDC28p)/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK5/p35, GSK3	1.24	0.30942	3544	NEG
OIC R000 0282 A01	NU-6102	CDC2 (CDK1, p34cdc2, CDC28p)/Cyclin B, CDK2/Cyclin A	2.01	0.01974	1448	SURV
OIC R000 0492 A01	NU-7026, LY-293646	DNAPK (DNA- PKcs)	1.73	0.11221	1923	NEG
OIC R000 8713 A01	NVP-ADW742, ADW- 742, ADW, GSK- 552602-A, NVP-ADW- 742	IGF1R (JTK13, IGFIR)	4.72	0.00001	615	SURV
OIC R000 0759 A01	NVP-AEW-541, AEW- 541	IGF1R (JTK13, IGFIR)	2.17	0.00986	1027	SURV

OIC R000 0291 A01	NVP-BEZ235, BEZ235	PI3K	2.52	0.00879	682	SURV
OIC R000 8744 A01	NVP-BHG712	RAF1 (c-Raf), SRC (c-SRC), ABL1 (ABL), EphB4	3.79	0.00001	1313	SURV
OIC R000 8732 B01	NVP-BSK805	JAK2	1.53	0.11793	2216	NEG
OIC R000 0666 A03	OICR0000666A01	PIM1	3.97	0.00001	1576	SURV
OIC R000 0857 A02	OICR0000857A	Histone Deacetylase SIRT1 inhibitor	1.40	0.05362	2757	NEG
OIC R000 0757 A01	Olomoucine	CDC2 (CDK1), p34cdc2, CDK281/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK5/p35	1.07	0.76757	3709	NEG
OIC R000 7893 R01	ON-01910, Estybon, Rigosertib	PLK1 (PLK, STPK13)	1.63	0.07731	1925	NEG
OIC R000 3207	OSI-027 hydrochloride	FRAP (MTOR, FRAP1), mTORC1, mTORC2	0.99	0.97546	1174	SURV

B01							
OIC							
R000							
7457							
A01	OSI-906, Linsitinib	IGF1R (JTK13, IGFIR)	1.31	0.16336	2901	NEG	X
		KIT (c-KIT), KDR (VEGFR2, VEGFR, FLK1), FLT1 (VEGFR1), FMS (c-FMS, CSF-1R, CSF1R), RAF1 (c-Raf), LCK (p56lck), PDGFRb (PDGFR, PDGFR1)					
OIC							
R000							
8712							
A01	OSI-930		1.48	0.03396	3167	POS	
OIC							
R000							
8734							
A01	OSI-TAK1 inhibitor	TAK1 (TGF1a, MAP3K7)	4.47	0.00001	1006	SURV	
OIC							
R000	OSU-03012						
0766	hydrochloride, AR-12,						
B01	NSC-728209	PDK1 (PDK1)	1.13	0.60374	3456	NEG	
		FLT1 (VEGFR1), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3), KIT (c-KIT), PDGFRa (PDGFR2), PDGFRb (PDGFR, PDGFR1)					
OIC	Pazopanib						
R000	hydrochloride, GW-						
1132	786034, Armala,						
B01	Votrient		1.57	0.05878	2373	NEG	
OIC							
R000		BTK (Bruton's tyrosine kinase),					
8703	PCI-32765, Ibrutinib	LCK (p56lck), LYN	1.26	0.33769	3183	NEG	

A01							
OIC							
R000							
0754	PD-0325901, PD	MAP2K1 (MEK1),					
A01	0325901	MAP2K2 (MEK2)	1.18	0.41573	1912	NEG	
OIC							
R000							
1153	PD-0332991, PD-	CDK4/Cyclin D1,					
A01	332991	CDK6/Cyclin D3	2.20	0.00239	1519	SURV	
OIC	PD-153035, AG-1517,						
R000	Compound 32, SU-						
0493	5271, ZM-252868,	EGFR (ERBB1,					
A01	WHI-P79	HER1)	2.91	0.00500	2090	POS	X
OIC		EGFR (ERBB1,					
R000		HER1), ErbB2					
0777		(TKR1, HER2,					
A01	PD-158780	NEU), ErbB4	2.84	0.00273	3376	POS	
OIC		(HER4)					
R000							
0292							
A01	PD-169316	p38 MAPK	9.46	0.00024	1542	SURV	
OIC							
R000							
0333							
A01	PD-173074	FGFR1 (FLT2),	1.36	0.00001	1386	SURV	
OIC		FGFR2, FGFR3					
R000							
0293							
A01	PD-180970	ABL1 (ABL), SRC	1.29	0.25531	185	SURV	
OIC		(c-SRC)					
R000							
0755	PD-184161	MAP2K1 (MEK1),	1.08	0.68031	3026	NEG	
		MAP2K2 (MEK2)					

A01						
OIC R000 0294 A01	PD-184352, CI-1040	MAP2K1 (MEK1), MAP2K2 (MEK2), Erk2 (ERK, p38), RAF1 (c-Raf)	1.93	0.02426	1552	SURV
OIC R000 0819 A01	PD-198306	MAP2K1 (MEK1), MAP2K2 (MEK2)	1.22	0.37059	3114	NEG
OIC R000 0530 A01	PD-407824	CHK1, WEE1	6.01	0.00139	1882	POS
OIC R000 0316 A01	PD-98059	MAP2K1 (MEK1), MAP2K2 (MEK2), Erk1	1.18	0.44340	1967	NEG
OIC R000 1136 A01	PD04217903, PF- 04217903, PF-4217903	MET (c-MET, HGFR)	1.49	0.04366	1892	NEG
OIC R000 1136 A02	PD04217903, PF- 04217903, PF-4217903	MET (c-MET, HGFR)	3.26	0.00067	1434	SURV
OIC R000 1158 A01	PD173955-Analogue 1	BCR, ABL1 (ABL)	2.13	0.01043	3780	POS
OIC R000 1157 A01	PD173955, PD-173955	BCR, ABL1 (ABL), SRC (c-SRC)	2.64	0.00080	1380	SURV

OIC R000 8720 A01	PD318088	MAP2K1 (MEK1), MAP2K2 (MEK2)	1.24	0.05303	4410	NEG
OIC R000 8708 A01	Pelitinib, EKB-569, WAY-EKB-569	EGFR (ERBB1, HER1)	3.48	0.00001	1248	SURV
OIC R000 7450 A01	PERK inhibitor	PEK (PERK)	2.30	0.00150	867	SURV
OIC R000 8695 A02	PF 3644022, PF- 3644022	MAPKAPK2 (MK2)	0.92	0.74561	3265	NEG
OIC R000 8695 A01	PF 3644022, PF- 3644022, MK2 inhibitor, compound 35	MAPKAPK2 (MK2)	0.53	0.06446	2700	NEG
OIC R000 7888 A01	PF 4708671, PF- 4708671	p70 S6K (S6K, S6K1)	1.03	0.86682	3207	NEG
OIC R000 1131 A01	PF-02341066, PF- 2341066, Crizotinib	ALK, MET (c-MET, HGFR)	2.18	0.01189	1538	SURV
OIC R000 7453 A01	PF-04691502, PF4691502, PF-502	PI3K, FRAP (MTOR, FRAP1)	2.84	0.00056	1292	SURV
OIC	PF-573228	FAK (FAK1)	2.60	0.00958	2525	POS

R000 0523 A01						
OIC R000 0516 B01	PF-670462	CK1e, CK1d	4.62	0.00282	1977	POS
OIC R000 3188 A02	PF431396, PF-431396	FAK (FAK1), PYK2 (FAK2, PTK2B, CAKB)	1.10	0.59435	2990	NEG
OIC R000 1165 A01	PF562271, PF-562271	FAK (FAK1), PYK2 (FAK2, PTK2B, CAKB)	4.64	0.00013	1023	SURV
OIC R000 0773 A01	PHA-665752	MET (c-MET, HGFR), RON (MST1R), KDR (VEGFR2, VEGFR, FLK1)	0.13	0.00000	3246	NEG
OIC R000 8719 A01	PHA-680632	AurA (AuroraA), AurB (AuroraB), AurC (AuroraC)	0.82	0.23306	1923	NEG
OIC R000 1144 A01	PHA-739358, Danusertib	ABL1 (ABL), RET, TRKA (TRK), AurA (AuroraA), FGFR1 (FLT2)	1.85	0.00709	923	SURV
OIC R000 0531 B01	PHA-767491 hydrochloride	CDC7, CDK9/Cyclin T1, MAPKAPK2 (MK2)	3.81	0.00288	1651	POS
OIC	PHA-793887	CDC2 (CDK1,	4.64	0.00009	1455	SURV

R000 7889 A01		p34cdc2, CDC28p)/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK4/Cyclin D1, CDK5/p25				
OIC R000 8761 A01	PHA-848125, PHA- 848125AC	CDK2/Cyclin A, CDK7/Cyclin H, CDK4/Cyclin D1, CDK5/p35, CDK2/Cyclin E, CDC2 (CDK1, p34cdc2, CDC28p)/Cyclin B	2.01	0.00111	504	SURV
OIC R000 1162 A01	PHA690509, PHA- 690509	CDK2/Cyclin A	0.92	0.73719	1378	SURV
OIC R000 0301 A01	PI-103	PI3K	2.12	0.01827	1402	SURV
OIC R000 0352 A01	PI-828	PI3K	2.05	0.01892	2501	POS
OIC R000 1182 A01	PI-93, PIK-93	PI3Ka	1.41	0.07688	2355	NEG
OIC R000 7884	PI3-Kinase a Inhibitor 2	PI3K	1.13	0.50308	3687	NEG

A01						
OIC						
R000						
1183						
A01	PIK-294	PI3Ka	3.38	0.00049	1598	SURV
OIC						
R000						
1183						
A02	PIK-294	PI3Ka	2.36	0.00279	1550	SURV
OIC						
R000						
0296						
B01	PIK-75 hydrochloride	PI3K	0.19	0.00000	91	SURV
OIC						
R000						
0295						
A01	PIK-90	PI3Ka	2.22	0.01259	961	SURV
OIC						
R000						
0824						
A01	PIM 1 Inhibitor 2	PIM1, PIM2	0.90	0.56714	3968	NEG
OIC						
R000						
0424	PIM1/2 Kinase					
A01	Inhibitor VI	PIM1, PIM2	3.55	0.00876	1837	POS
OIC						
R000						
0505	Pimecrolimus, Elidel,	Calcineurin (CN, PP-				
A01	SDZ-ASM-981	2B)	1.26	0.42161	364	SURV
OIC						
R000						
7451						
A01	PKC theta inhibitor	PKCt	2.41	0.00206	3621	POS

OIC R000 0317 A01	PKC-412, CGP41251, Midostaurin	FLT3 (STK1, FLK2)	1.60	0.09521	429	SURV	
OIC R000 7459 A01	PLX-4032, Vemurafenib, Zelboraf, RG-7204, RO-5185426	BRAF (B-raf)	0.96	0.81963	3040	NEG	
OIC R000 0543 A01	PLX-4720, Raf Kinase Inhibitor V	BRAF (B-raf)	4.43	0.00408	2080	POS	X
OIC R000 1126 A01	PP-1, PP1, AGL-1872, Tyrphostin-PP1	SRC (c-SRC)	2.14	0.00288	1506	NEG	
OIC R000 0483 A01	PP-2, PP2, AG-1879	LCK (p56lck), FYN (p59fyn), HCK (p56hck), SRC (c- SRC)	2.95	0.01227	1841	POS	
OIC R000 0786 A01	PP-3	EGFR (ERBB1, HER1)	1.65	0.04752	3377	POS	
OIC R000 7456 A01	Pp242, Torkinib	FRAP (MTOR, FRAP1)	3.03	0.00045	1533	SURV	
OIC R000 0353 A01	PQ401	IGF1R (JTK13, IGFIR)	2.23	0.01269	2737	POS	
OIC	PS1145 dihydrochloride	IKKb (IKK2)	1.15	0.52870	2984	NEG	

R000 0334 B01						
OIC R000 8749						
A01	PTK6 CompoundA	BRK (PTK6)	1.40	0.00219	2771	NEG
OIC R000 8750	PTK6 Inhibitor					
A01	CompoundB	BRK (PTK6)	2.62	0.00004	1494	SURV
OIC R000 0499		CDC2 (CDK1, p34cdc2, CDC28p)/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK4/Cyclin D1, CDK5/p35,				
A01	Purvalanol A, NG 60	DYRK1A (DYRK)	2.86	0.00991	2165	POS
OIC R000 0806		CDC2 (CDK1, p34cdc2, CDC28p)/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK4/Cyclin D1, CDK5/p35				
A01	Purvalanol B, NG 95		1.72	0.14727	3479	NEG
OIC R000 1196						
A01	PV-1019, NSC-744039	CHK2	2.63	0.08861	2284	NEG
OIC R000 8742	PX-866, DJM-166, DJM-2-166, Sonolisib,					
A01	NSC-722134	PI3K, PTEN inhibitor	2.14	0.00011	1496	SURV

OIC R000 0525 A01	Quercetin	PI3K	2.08	0.04862	2030	POS
OIC R000 7454 A01	Quinalizarin	CK2a1 (CK2, CKII, Casein kinase2)	1.32	0.13383	3743	NEG
OIC R000 0775 A01	R 59-022	DAGK (DGK, Diacylglycerol kinase)	1.25	0.32052	3212	NEG
OIC R000 1173 B01	R1487, R-1487	p38 MAPK	2.64	0.00247	3333	POS
OIC R000 7891 A01	R406, R-406, NSC- 742317	FLT3 (STK1, FLK2), SYK, (IL-2, IL-6 and TNFa) production inhibitor	1.54	0.04635	677	SURV
OIC R000 7906 R01	R788, R-788, Fostamatinib disodium, R935788, R-935788, NSC-745942	FLT3 (STK1, FLK2), SYK	1.03	0.87679	2180	NEG
OIC R000 7895 A01	RAF265, RAF-265, CHIR-265	KIT (c-KIT), PDGFRb (PDGFR, PDGFR1), BRAF (B- raf), RAF1 (c-Raf), KDR (VEGFR2, VEGFR, FLK1)	1.29	0.23292	1080	SURV
OIC R000 0306	Rapamycin, Sirolimus, Rapamune	FRAP (MTOR, FRAP1), CCR5 expression inhibitor	1.66	0.06618	1222	SURV

A01						
OIC						
R000	RDEA-119, AR-119,					
0749	RDEA119, BAY-	MAP2K1 (MEK1),				
A01	869766, Refametinib	MAP2K2 (MEK2)	1.03	0.87358	2256	NEG
OIC						
R000						
8751	RET Inhibitor Example					
A01	8 (Free base)	RET	1.33	0.26083	415	SURV
OIC						
R000						
0495		ROCK1, ROCK2				
A01	Rho Kinase Inhibitor V	(ROCKa)	2.29	0.01690	1670	SURV
OIC						
R000						
1186		ROCK1, ROCK2				
A01	RHO-15	(ROCKa)	2.48	0.00161	1351	SURV
OIC						
R000						
7905	Rigel JAK Compound					
A01	II, Prodrug of VI-53	JAK1, JAK2, JAK3	1.58	0.07965	1902	NEG
OIC						
R000						
0508I	Ro-31-8220 mesylate,					
01	Bisindolylmaleimide IX	PKC	1.74	0.14356	2115	NEG
OIC						
R000						
7466		MAP2K1 (MEK1),				
A01	RO-5126766	BRAF (B-raf), RAF1	1.57	0.04010	1235	SURV
OIC						
R000		CDC2 (CDK1,				
0318	Roscovitine, CYC202,	p34cdc2,				
A01	Selaciclib	CDC28p)/Cyclin B,	1.09	0.67815	2467	NEG
		CDK2/Cyclin A,				

		CDK2/Cyclin E, CDK5/p35					
OIC R000 0506 A01	Rottlerin	PKCd, TGM2 (Tissue Transglutaminase), signal transduction pathway inhibitor	4.32	0.00172	1647	POS	X
OIC R000 0818 A01	RWJ-67657	p38a, p38b	2.83	0.00252	4078	POS	
OIC R000 0763 A01	Ryuvidine	CDK4/Cyclin D1	1.30	0.26813	1310	SURV	
OIC R000 7473 A01	S-99	MAP3K5 (ASK1)	0.91	0.52652	4707	NEG	
OIC R000 8731 A01	S6K-18	p70/S6K (S6K, S6K1)	1.80	0.25115	3567	NEG	
OIC R000 0494 A01	Sal003	eIF2a	2.45	0.02160	567	SURV	
OIC R000 0354 A01	SB 203580, SB-203580	p38 MAPK	4.87	0.00088	3274	POS	X
OIC R000	SB 216763	GSK3	0.25	0.00000	3596	NEG	

0355							
A01							
OIC							
R000							
0356							
A01	SB 239063	p38 MAPK	3.06	0.00321	3575	POS	
OIC							
R000							
0358							
A01	SB 431542	TGFbR1 (ALK5)	3.04	0.00410	2434	POS	
OIC							
R000							
0750							
A01	SB-202190, FHPI	p38 MAPK	1.64	0.006406	4026	NEG	
OIC		CHK1, CDC2					
R000		(CDK1, p34cdc2,					
0788		CDC28p)/Cyclin B,					
A01	SB-218078	PKC	0.00	0.00000	0	SURV	
OIC							
R000							
0357							
A01	SB-415286	GSK3	1.43	0.14522	3190	NEG	
OIC							
R000							
0335	SB-505124						
B01	hydrochloride hydrate	TGFbR1 (ALK5)	4.15	0.00111	1563	SURV	
OIC							
R000							
8715							
A01	SB-525334	TGFbR1 (ALK5)	6.87	0.00000	1246	SURV	
OIC							
R000							
1176	SB242235, SB-242235	p38 MAPK	10.63	0.00002	3771	POS	X

A01							
OIC							
R000							
1159							
A01	SB590885, SB-590885	BRAF (B-raf)	2.65	0.00090	3856	POS	X
OIC							
R000							
0502							
A01	SC-514	IKKb (IKK2)	2.92	0.00839	2362	POS	
OIC							
R000							
8735	SCH900776, SCH-900776, MK-8776	CHK1, CDK2/Cyclin A, CDK2/Cyclin E	1.39	0.00645	582	SURV	
A01							
OIC							
R000							
0359							
A01	SD 169	p38 MAPK	1.33	0.22915	2649	NEG	
OIC							
R000							
0336							
A01	SD 208	TGFbR1 (ALK5)	4.89	0.00078	1434	SURV	
OIC							
R000							
1177							
A01	SD-06, SD-0006	p38 MAPK	3.27	0.00134	3031	POS	X
OIC							
R000							
0100							
B01	SGI-1776	PIM1, PIM2	5.85	0.00111	1631	POS	X
OIC							
R000							
1124							
A01	SGX-523	MET (c-MET, HGFR)	2.13	0.00240	3052	POS	

OIC R000 0337 B01	SIS3	TGFbR1 (ALK5)	1.50	0.16540	3068	NEG	
OIC R000 8698 A01	SJN 2511	TGFbR1 (ALK5)	4.95	0.00000	701	SURV	
OIC R000 0338 B01	SKF 86002 dihydrochloride, SKF- 86002 dihydrochloride	p38 MAPK	1.54	0.17531	298	SURV	
OIC R000 0338 A01	SKF-86002, SKF 86002	p38 MAPK	1.29	0.27917	3561	NEG	
OIC R000 0518 A01	SL327	MAP2K1 (MEK1), MAP2K2 (MEK2)	3.36	0.00337	2381	POS	
OIC R000 3167 A02	SMG-1 Compound 467	SMG1 (ATX, LIP)	1.39	0.08467	2789	NEG	
OIC R000 1161 B01	SNS-032, BMS387032, BMS-387032	CDK2/Cyclin E, CDK7, CDK9/Cyclin T1	2.87	0.00099	1448	SURV	
OIC R000 1154 A01	SNS-314	AurA (AuroraA), AurB (AuroraB), AurC (AuroraC)	1.62	0.10816	768	SURV	
OIC	Sorafenib, Bay 43-	FLT3 (STK1, FLK2),	4.52	0.00180	2643	POS	X

R000 0486 A01	9006, Nexavar	KIT (c-KIT), PDGFRb (PDGFR, PDGFR1), RET, RAF1 (c-Raf), BRAF (B-raf), FLT1 (VEGFR1), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3)					
OIC R000 0486 H01	Sorafenib,p- Toluenesulfonate Salt, Bay 43-9006, Nexavar	FLT3 (STK1, FLK2), KIT (c-KIT), PDGFRb (PDGFR, PDGFR1), RET, RAF1 (c-Raf), BRAF (B-raf), FLT1 (VEGFR1), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3)	3.19	0.01721	1800	POS	X
OIC R000 0519 A01	SP-600125	JNK1 (JNK)	2.13	0.02756	2195	POS	
OIC R000 0520 A01	Sphingosine kinase Inhibitor 2	SPK1 (SPHK, SK1, Sphingosine Kinase)	1.53	0.20254	2215	NEG	
OIC R000 1187 A01	SR3677	ROCK2 (ROCKa)	2.24	0.00350	1533	SURV	
OIC R000	Src I1	SRC (c-SRC), LCK (p56lck), KDR	1.48	0.11576	4095	NEG	

0825 A01		(VEGFR2, VEGFR, FLK1)				
OIC R000 3179 A02	ST638, ST-638	Tyrosine kinase inhibitor	0.74	0.03816	3557	NEG
OIC R000 0321 A01	Staurosporine	Pan kinase	0.63	0.09486	65	SURV
OIC R000 0339 N01	STO-609 acetic acid	CAMKK2 (CAMKK)	1.44	0.13877	3407	NEG
OIC R000 0823 A01	SU 16f	PDGFRb (PDGFR, PDGFR1), KDR (VEGFR2, VEGFR, FLK1)	0.61	0.01895	3464	NEG
OIC R000 0521 A01	SU 4312, DMBI	KDR (VEGFR2, VEGFR, FLK1)	2.53	0.01476	1646	SURV
OIC R000 0522 A01	SU 5416, SU-5416, TSU-16, NSC-696819, Semaxanib, Semaxnib, Semoxind	RET3 (STK1, FLK2), MET (c-MET, HGFR), KDR (VEGFR2, VEGFR, FLK1)	0.55	0.08507	334	SURV
OIC R000 0340 A01	SU 6656	SRC (c-SRC), YES, LYN, FYN (p59fyn)	0.12	0.00000	1219	SURV
OIC R000	SU-11274, PKI-SU11274	MET (c-MET, HGFR)	0.50	0.00376	1454	NEG

0511 A01							
OIC R000 0808 A01	SU-5402	KDR (VEGFR2, VEGFR, FLK1), FGFR1 (FLT2), PDGFRb (PDGFR, PDGFR1)	2.26	0.00880	3407	POS	
OIC R000 0538 A01	SU-6668, TSU-68, Orantinib	PDGFRb (PDGFR, PDGFR1), KDR (VEGFR2, VEGFR, FLK1), FGFR1 (FLT2), AurA (AuroraA), AurB (AuroraB), TBK1	3.21	0.02438	2105	POS	X
OIC R000 0770 A01	SU-9516	CDC2 (CDK1, p34cdc2, CDC28p)/Cyclin B, CDK2/Cyclin A, CDK4/Cyclin D1	1.61	0.07013	3786	POS	
OIC R000 0760 AB0 1	Sunitinib Malate, Sutent, SU-11248, PHA-290940AD	KIT (c-KIT), FLT3 (STK1, FLK2), FMS (c-FMS, CSF1R, CSF1R), KDR (VEGFR2, VEGFR, FLK1), PDGFRb (PDGFR, PDGFR1)	0.23	0.00000	2555	NEG	
OIC R000 0539 A01	TAE-684	ALK	2.90	0.01402	380	SURV	
OIC R000 1178	TAK-715	p38 MAPK	4.48	0.00012	2937	POS	X

A01						
OIC						
R000						
0801		CK2a1 (CK2, CKII, Casein kinase2)				
A01	TBB, NSC 231634		1.33	0.21918	3576	NEG
OIC						
R000						
7680						
A04	TBK1 Inhibitor	TBK1	2.01	0.00788	1561	SURV
OIC						
R000						
7899	TC-A 2317					
B01	hydrochloride	AurA (AuroraA)	0.86	0.32758	2477	NEG
OIC						
R000						
8748		DAPK1, DAPK3 (ZIPK, ZIP)				
A01	TC-DAPK 6		1.13	0.28203	2826	NEG
OIC						
R000						
8743						
A01	TCS 2312	CHK1	1.56	0.07107	814	SURV
OIC						
R000						
0821	TCS 2312					
B01	dihydrochloride	CHK1	1.82	0.04948	864	SURV
OIC						
R000						
0820						
A01	TCS 359	FLT3 (STK1, FLK2)	0.94	0.78275	3747	NEG
OIC						
R000						
0796		JNK1 (JNK), JNK2, JNK3, p38a				
A01	TCS JNK 5a		1.65	0.00000	299	SURV

OIC R000 0425 A01	TCS PIM-1 1	PIM1, PIM2	3.25	0.01090	2111	POS	
OIC R000 0507 A01	Temsirolimus, CCI-779, Torisel	FRAP (MTOR, FRAP1)	1.54	0.15346	737	SURV	
OIC R000 7464 A01	TG-101348	FLT3 (STK1, FLK2), JAK2	6.58	0.00003	1370	SURV	
OIC R000 3177 A02	TG003, TG-003	CLK1, CLK4	1.22	0.26278	3774	NEG	
OIC R000 8725 A01	TG100-115, TG-100-115, TG-100115	PI3K	1.95	0.00039	3137	POS	X
OIC R000 0764 A01	TGX-221	PI3K	1.16	0.49752	3404	NEG	X
OIC R000 7896 A01	Tipifarnib, NSC-702818, R-115777, Zarnestra, IND 58359	Farnesyltransferase	3.83	0.00022	3081	POS	X
OIC R000 0524 A01	TPCA-1	IKKb (IKK2)	4.37	0.00174	2590	POS	
OIC	TPL2	COT (TPL2, EST,	4.22	0.00516	1997	POS	

R000 0484 A01		MAP3K8)					
OIC R000 8746 A01	TTP 22	CK2a1 (CK2, CKII, Casein kinase2)	1.53	0.01345	3430	POS	
OIC R000 0485 A01	TWS-119	GSK3	1.92	0.08425	1316	SURV	
OIC R000 0441 A01	TX-1918	eEF2K	1.51	0.11687	1512	SURV	
OIC R000 0552I 01	Tyrphostin AG 1478 mesylate	EGFR (ERBB1, HER1)	2.96	0.00563	2301	POS	
OIC R000 0297 A01	Tyrphostin AG-1296	PDGFR $\alpha$ (PDGFR, FGFR)	1.39	0.18038	1544	SURV	
OIC R000 0787 A01	Tyrphostin B44, (-) enantiomer	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	1.68	0.05226	3478	POS	
OIC R000 0553 A01	Tyrphostin SU 1498, SU 1498	KDR (VEGFR2, VEGFR, FLK1)	2.32	0.01436	2539	POS	
OIC R000	U0126, U-0126, U 0126	MAP2K1 (MEK1), MAP2K2 (MEK2),	0.97	0.93315	2126	NEG	

0554 A01		JAK2, Erk2 (ERK, p38)					
OIC R000 0308 A01	Vandetanib, Zactima, ZD6474, AZD-6474	EGFR (ERBB1, HER1), KDR (VEGFR2, VEGFR, FLK1), RET, ABL1 (ABL), KIT (c-KIT), FLT1 (VEGFR1), FLT4 (VEGFR3), TRKA (TRK)	1.63	0.06729	1705	NEG	
OIC R000 0305 B01	Vatalanib dihydrochloride, PTK-787, ZK-222584, CGP79787D	KDR (VEGFR2, VEGFR, FLK1), PDGFRb (PDGFR, PDGFR1)	1.30	0.26230	1574	SURV	
OIC R000 8758 A01	VE-821	ATR	1.62	0.01661	1500	SURV	
OIC R000 0307 A01	VX-680, MK-0457, Tozasertib, VX6	AurA (AuroraA), AurB (AuroraB), AurC (AuroraC), ABL1 (ABL), FLT3 (FLT3, FLK2), JAK2	1.16	0.49528	644	SURV	
OIC R000 0550 A01	VX-702, VX-850, VX-954, KVK-702	p38 MAPK	3.88	0.00999	3072	POS	X
OIC R000 8723 A01	VX-745	p38 MAPK	6.85	0.00000	4077	POS	X
OIC R000	WAY-600	FRAP (MTOR, FRAP1), mTORC1,	0.38	0.07796	100	SURV	

8763 A01		mTORC2					
OIC R000 0807 A01	WHI-P 154, MASTPROM	JAK3, EGFR (ERBB1, HER1), SRC (c-SRC), ABL1 (ABL), KDR (VEGFR2, VEGFR, FLK1), p38 MAPK, PI3K	3.05	0.06334	3509	NEG	
OIC R000 0322 A01	Wortmannin, KY 12420	PI3K - Irreversible	1.15	0.51897	2408	NEG	
OIC R000 8765 A01	WYE-125132, WYE- 132	PI3K, FRAP (MTOR, FRAP1), mTORC1, mTORC2	1.21	0.50334	506	SURV	
OIC R000 7898 A01	WYE-354	FRAP (MTOR, FRAP1), mTORC1, mTORC2	1.34	0.14576	3425	NEG	
OIC R000 8709 A01	WZ3146, WZ-3146	EGFR (ERBB1, HER1)	1.71	0.00114	867	SURV	
OIC R000 7455 A01	WZ4002, WZ-4002	EGFR (ERBB1, HER1)	3.70	0.00016	3077	POS	X
OIC R000 8707 A01	WZ8040, WZ-8040	EGFR (ERBB1, HER1)	2.13	0.00140	1453	SURV	

OIC R000 1125 A01	XL-147, XL147, SAR- 245408	PI3K	1.41	0.08122	3231	NEG	
OIC R000 7469 AB0 1	XL-184, XL184, BMS- 907351, Cabozantinib malate	FLT3 (STK1, FLK2), MET (c-MET, HGFR), KIT (c-KIT), RET, TIE2 (TEK), KDR (VEGFR2, VEGFR, FLK1)	1.05	0.78872	3354	NEG	
OIC R000 1134 A01	XL-880, GSK-1363089, EXEL-2880, GSK-089, foretinib	MET (c-MET, HGFR), KDR (VEGFR2, VEGFR, FLK1), FLT3 (STK1, FLK2), TIE2 (TEK)	1.15	0.38572	665	SURV	
OIC R000 8726 A01	XL765, XL-765, SAR245409	PI3K, mTORC1, mTORC2	2.16	0.00099	3384	POS	X
OIC R000 7471 D01	XMD8-92, XMD-8-92	Erk5 (BIMK), ERK5	2.14	0.00497	3310	POS	X
OIC R000 0528 A01	XRP44X, XRP-44X, WCH-44	Ras-NET (Elk3), Tubulin polimerization	1.33	0.22979	835	SURV	
OIC R000 8704 A01	Y-27632	LRRK2, ROCK1, ROCK2 (ROCKa), Calcium sensitizer	1.96	0.00364	3718	POS	X
OIC R000	Y-27632	ROCK1, ROCK2 (ROCKa)	1.26	0.27859	3477	NEG	X

0761 A01						
OIC R000						
8701 B01	Y-39983, RKI-983, SNJ-1656	ROCK1, ROCK2 (ROCKa)	1.49	0.01804	2310	NEG
OIC R000						
1184 A01	YM201636, YM- 201636	PIKfyve (PIP5K)	3.02	0.00132	1539	SURV
OIC R000						
0810 B01	ZM 306416 hydrochloride	KDR (VEGFR2, VEGFR, FLK1)	1.49	0.12657	3440	NEG
OIC R000						
0811 B01	ZM 323881 hydrochloride	KDR (VEGFR2, VEGFR, FLK1)	1.24	0.31790	3745	NEG
OIC R000						
0527 A01	ZM 336372	RAF1 (c-Ra)	2.48	0.00802	1818	POS
OIC R000						
0341 B01	ZM 39923 hydrochloride	JAK3	1.43	0.14093	2863	NEG
OIC R000						
0551 A01	ZM-447439	AurA (AuroraA), AurB (AuroraB), AurC (AuroraC)	0.92	0.80104	779	SURV
OIC R000						
0548	ZSTK474	PI3K	2.54	0.01146	818	SURV

X

A01

Table 2. TOOL Library

Targets	Fold change (TGFb/sample)	p value	Cell number	Output	Selected for second screening
PKC activator, human embryonic stem cells (ESCs) differentiation	4.44	#####	4326	POS	X
PARP inhibitor	2.63	#####	3540	NEG	
Hsp90 inhibitor	0.00	#####	2	SURV	
Hsp90 inhibitor	0.00	#####	17	SURV	
IRE1 inhibitor	1.56	#####	3716	NEG	
Antineoplastic Drug	0.73	#####	2166	NEG	
PARP inhibitor	2.21	#####	2198	NEG	
PARP inhibitor	7.38	#####	1715	SURV	
PARP inhibitor	1.66	#####	3131	NEG	
PARP inhibitor	2.81	#####	3415	POS	
Antimitotic Drugs; Apoptosis Inducers; Pyrimidine Antagonists	1.48	#####	2081	NEG	X
Guanylate cyclase and cGMP production inhibitor	1.86	#####	2415	NEG	
Androgen Biosynthesis Inhibitors; Cytochrome P450 CYP17 (17alpha-Hydroxylase/C17-20 Lyase) Inhibitors	1.02	#####	4182	NEG	
Bcl-2, Bcl-XL, inhibitor	5.18	#####	3732	POS	
Bcl-2, Bcl-XL, Bcl-w, Mcl-1 inhibitor	6.06	#####	3424	POS	
PARP inhibitor	2.82	#####	3180	NEG	X
DNA-Directed RNA Polymerase Inhibitor	0.08	#####	100	SURV	

Sirtuin 2 inhibitor, SIRT2 inhibitor	2.24	#####	3803	NEG	
DNA Topoisomerase II Inhibitor, DNA-Intercalating Drug	1.11	#####	854	SURV	
Histone acetyltransferase (HAT) inhibitor	0.95	#####	3307	NEG	
Phosphodiesterase III Inhibitors	1.18	#####	3650	NEG	
Aromatase Inhibitors	1.46	#####	2461	NEG	
Protein synthesis inhibitor	0.75	#####	114	NEG	
Histone Deacetylase (HDAC) Inhibitors	0.65	#####	874	SURV	
Eg5 inhibitor	2.40	#####	1905	NEG	
Antimetabolite, DNA methyltransferase (DNMT) inhibitor	1.31	#####	3325	NEG	
PARP inhibitor	3.41	#####	2659	POS	X
HDAC1, HDAC2, Angiogenesis inhibitor, Apoptosis inducer	1.65	#####	1069	NEG	
Antimetabolites; Apoptosis Inducers; DNA Alkylating Drugs	1.24	#####	3361	NEG	
Retinoid X receptor agonist, RXRs agonist	4.28	#####	3100	POS	X
Androgen Receptor Antagonists	0.82	#####	3605	NEG	
DNA methyltransferase (DNMT) inhibitor, Histone-lysine N-methyltransferase (HMT2 (H3-K9-HMTase 3; G9a) inhibitor	0.82	#####	3451	NEG	
Antimitotic Drug	0.89	#####	1009	SURV	
Proteasome inhibitor	0.00	#####	9	SURV	
PARP1 inhibitor	0.96	#####	3284	NEG	
GnRH (LHRH) Agonists	1.79	#####	4196	POS	X
DNA-Damaging Drug, Antimitotic Drug, DNA alkylating agent	1.59	#####	3470	NEG	
Antifungal	1.35	#####	2855	NEG	

PARP inhibitor					
Antiinflammatory Drugs; Antioxidants; Apoptosis Inhibitors; HIV Integrase Inhibitors; NF-kappaB (NFKB) Activation Inhibitors	1.44	#####	3199	NEG	
Thymidylate synthase inhibitor	1.14	#####	3714	NEG	
Calmodulin antagonist	1.37	#####	3517	NEG	
Pyrimidine Antagonist	0.73	#####	3394	NEG	
DNA alkylating agent, NAD+ ADP- Ribosyltransferase (poly(ADP- ribose)polymerase; PARP) Inhibitors	1.41	#####	2598	NEG	
Proteasome Inhibitor	0.10	#####	43	SURV	
Apoptosis Inducers; DNA Alkylating Drugs; Glutathione Reductase (NADPH) Inhibitors; Thioredoxin Reductase Inhibitors	0.76	#####	3279	NEG	
PLD2 inhibitor, Phospholipase D2 inhibitor	1.84	#####	3297	NEG	
Histone Deacetylase 6 (HDAC6) Inhibitors	1.16	#####	1527	SURV	
Histone Deacetylase (HDAC) Inhibitors; Apoptosis Inducers	1.31	#####	3355	NEG	
Rho pathway inhibitor	0.80	#####	3369	NEG	
Phytotoxin	2.80	#####	2196	NEG	
Apoptosis inducer	0.08	#####	19	SURV	
Histone Deacetylase (HDAC) Inhibitors	0.99	#####	2329	NEG	
DNA-Damaging Drugs, DNA Alkylating Drug	1.05	#####	3149	NEG	
HDAC inhibitor	1.69	#####	2028	NEG	
DNA alkylating drug, BIRC4 expression enhancer, TNFSF6 expression inhibitor, Thioredoxin Reductase inhibitor	1.14	#####	1099	SURV	

Adenosine Deaminase (ADA) Inhibitors; Antimetabolites; Apoptosis Inducers	0.40	#####	584	SURV	
Apoptosis Inducers; BCL2 Expression Inhibitors; LGALS1 Expression Inhibitors	0.78	#####	3409	NEG	
Iron chelator	2.46	#####	3432	NEG	
Phosphodiesterase III A (PDE3A) inhibitor	1.04	#####	3010	NEG	
DNA Polymerase Inhibitors; Ribonucleoside-Diphosphate Reductase Inhibitors	1.42	#####	2719	NEG	
p53 inhibitor	1.66	#####	3410	POS	
Hedgehog inhibitor, Hh inhibitor	3.08	#####	3484	POS	X
Calcineurin inhibitor	3.19	#####	3050	POS	
Antimetabolite, DNA Polymerase Inhibitor	0.92	#####	992	SURV	
DNA Alkylating Drug	1.25	#####	3467	NEG	
Antiamyloidogenic agent, gamma-secretase inhibitor, Notch signaling inhibitor	2.05	#####	3256	POS	
Daunorubicin primary metabolite	2.69	#####	3260	POS	
DNA Topoisomerase inhibitor	0.06	#####	84	SURV	
Antimetabolite, DNA methyltransferase (DNMT) inhibitor	0.62	#####	1606	NEG	
Iron chelator	1.33	#####	2509	NEG	
Iron chelator	2.70	#####	3683	NEG	
Iron chelator	2.30	#####	2280	NEG	
Antiinflammatory drug	1.57	#####	2599	POS	X
HIF-alpha prolyl hydroxylase (HIF-PH) inhibitor	1.10	#####	3182	NEG	
Microtubule disassembly inhibitor	1.18	#####	442	SURV	
30S Ribosomal protein inhibitor, Matrix	1.91	#####	3355	POS	X

metalloproteinase inhibitor				
PARP inhibitor	2.15	#####	3641	NEG
PARP inhibitor	2.06	#####	3384	NEG
PARP inhibitor	1.50	#####	3224	NEG
Antifungal	1.14	#####	3180	NEG
DNA Topoisomerase II inhibitor	0.16	#####	438	SURV
Histone-Lysine N-Methyltransferase, H3 Lysine-79 Specific (DOT1L) Inhibitors	1.62	#####	3443	NEG
Estrogen Receptor (ER) beta agonist	1.66	#####	1096	SURV
DNA Topoisomerase II alpha Inhibitor, DNA-Intercalating Drug	0.81	#####	944	SURV
Histone Deacetylase SIRT1 inhibitor	0.85	#####	3488	NEG
Aromatase Inhibitors	1.74	#####	3755	NEG
Antifungal	3.04	#####	3062	POS
STAT1 activation inhibitor, DNA synthesis inhibitor	1.29	#####	1406	SURV
Androgen Receptor Antagonists	1.35	#####	3601	NEG
sphingosine-1-phosphate (S1P) receptors agonist	1.60	#####	3779	NEG
Histone N-Acetyltransferase (HAT) inhibitor	1.65	#####	3172	NEG
Hedgehog inhibitor, Hg inhibitor	1.66	#####	3462	NEG
DNA synthesis inhibitor	0.41	#####	441	SURV
GnRH (LHRH) Agonists	1.17	#####	3249	NEG
Estrogen-Related Receptor beta (ERRbeta, ERR2) agonist, Estrogen-Related Receptor gamma (ERRgamma) agonist	1.03	#####	3061	NEG
Bcl-2, Bcl-XL, Mcl-1 inhibitor	0.06	#####	334	SURV

Angiogenesis Inhibitors; Histone Deacetylase (HDAC) Inhibitors	1.00	#####	3491	NEG	
Glucocorticoid; Steroids	1.21	#####	2756	NEG	
Antimetabolites; Nitric Oxide Donors; Ribonucleoside-Diphosphate Reductase Inhibitors	0.73	#####	3194	NEG	
Bromodomain-Containing Protein 2 (Brd2) Inhibitors; Bromodomain-Containing Protein 3 (Brd3, RING3-like protein) Inhibitors; Bromodomain-Containing Protein 4 (Brd4, HUNK1) Inhibitors	3.44	#####	2599	POS	X
DNA Topoisomerase inhibitor	0.06	#####	74	SURV	
Stem cell differentiation	1.32	#####	3185	NEG	
Stem cell differentiation	1.54	#####	3349	POS	X
DNA Alkylating Drugs	0.90	#####	3280	NEG	
PARP inhibitor	1.92	#####	3451	NEG	
IRE1 inhibitor	1.30	#####	1782	NEG	
Topoisomerase I inhibitor	1.97	#####	2802	POS	X
	1.56	#####	711	SURV	
HDAC Inhibitor, IL-1beta production inhibitor, TNF-alpha release inhibitor	0.90	#####	564	NEG	
Wnt inhibitor	1.78	#####	3452	NEG	
Wnt inhibitor	3.21	#####	3412	POS	X
Wnt inhibitor	1.80	#####	3188	NEG	
Tankyrase inhibitor	2.30	#####	3656	NEG	
Wnt inhibitor, Tankyrase inhibitor, TNK	2.05	#####	3952	NEG	
Wnt inhibitor, Tankyrase inhibitor, TNK	1.25	#####	3579	NEG	
HDAC1 inhibitor	0.32	#####	533	SURV	

sphingosine-1-phosphate (S1P) receptors antagonist	2.91	#####	3552	POS	X
H1 histamine receptor antagonist	1.40	#####	3494	NEG	
Hsp27 inhibitor	1.60	#####	1391	SURV	
g-Secretase inhibitor	1.15	#####	2693	NEG	
Selective D2 dopamine receptor antagonist	2.84	#####	3312	POS	
HDAC inhibitor	0.26	#####	168	SURV	
Prostanoid DP (DP1) Antagonists	1.04	#####	3127	NEG	
Farnesyltransferase (geranylgeranyl pyrophosphate synthase) Inhibitors; Inhibitors of Signal Transduction Pathways	1.02	#####	2973	NEG	
HDAC inhibitor	0.48	#####	149	SURV	
TNF- $\alpha$ inhibitor	0.76	#####	3348	NEG	
Aromatase Inhibitors	1.34	#####	3297	NEG	
GnRH (LHRH) Agonists	0.93	#####	3302	NEG	
DNA Alkylating Drugs	0.87	#####	3420	NEG	
Opioid receptor agonist	1.92	#####	3456	NEG	
Protease inhibitor, Antiretroviral	3.90	#####	3287	POS	
Chemokine CCR5 antagonist	0.93	#####	3221	NEG	
HDAC2 inhibitor	1.40	#####	3008	NEG	
Androgen receptor (AR) antagonist	0.92	#####	3312	NEG	
DNA Alkylating Drug	1.02	#####	1601	NEG	
Progesterone, androgen and glucocorticoid receptor agonist	0.96	#####	3151	NEG	
Progesterone Receptor Agonists	1.10	#####	2918	NEG	
DNA Alkylating Drugs	1.33	#####	2613	NEG	

Antihyperglycemic agent	3.00	#####	3496	POS	X
Dihydrofolate Reductase (DHFR) Inhibitors; Folate Antagonists	0.83	#####	886	SURV	
HDAC 1 inhibitor	1.70	#####	1075	NEG	
Apoptosis Inducers; DNA Alkylating Drugs	0.10	#####	321	SURV	
DNA Topoisomerase II Inhibitors; DNA-Intercalating Drugs	1.39	#####	742	SURV	
HDAC inhibitor	1.88	#####	1687	NEG	
Analogue of Valrubicin	0.08	#####	339	SURV	
PKM2 activator	2.26	#####	3053	POS	
PKM2 activator	1.50	#####	2940	POS	
STAT3 inhibitor	1.12	#####	2920	NEG	
Iron chelator	1.56	#####	1887	NEG	
Androgen Receptor Antagonists	1.04	#####	3322	NEG	
DNA Alkylating Drugs	0.95	#####	3123	NEG	
PARP inhibitor	2.74	#####	3393	POS	
MDM-2 antagonist	1.60	#####	3412	NEG	
Hsp90 inhibitor	0.15	#####	103	SURV	
Cathepsin K inhibitor	3.93	#####	3309	POS	
SIRT1 inhibitor	1.02	#####	3409	NEG	
SIRT1 activator	0.96	#####	2723	NEG	
Na <sup>+</sup> , K <sup>+</sup> ATPase inhibitor	2.00	#####	3246	NEG	
DNA Alkylating Drug	1.36	#####	1045	SURV	
Histone Deacetylase (HDAC) Inhibitors	2.16	#####	1034	SURV	
Caspase activator	2.21	#####	1849	NEG	
	1.69	#####	852	NEG	

PARP inhibitor	1.06	#####	2883	NEG	X
HDAC inhibitor	1.50	#####	3337	POS	
Iron chelator	2.16	#####	3111	NEG	
Thymidylate synthase inhibitor, dihydrofolate reductase inhibitor, glycinamide ribonucleotide formyltransferase inhibitor	1.19	#####	677	SURV	X
Adenosine Deaminase (ADA) Inhibitors; Antimetabolites	1.25	#####	2049	NEG	
HDAC inhibitor	1.57	#####	3389	POS	
p53 inactivator	1.06	#####	2840	NEG	X
Iron chelator	2.43	#####	3718	NEG	
Serotonin antagonist	2.85	#####	3162	POS	
PARP inhibitor	3.17	#####	3637	POS	X
Platelet inhibitor, P2Y12 (P2T) antagonist	1.24	#####	3287	NEG	
Glucocorticoid receptors (GR) alpha and beta agonist - Irreversible	1.35	#####	2484	NEG	
glucocorticoid receptor a(GR) agonist prodrug	1.63	#####	2835	NEG	X
HGM-CoA reductase mRNA expression inhibitor	1.16	#####	3352	NEG	
DNA Alkylating Drugs	0.75	#####	3101	NEG	
Fungicide	1.01	#####	3484	NEG	X
hedgehog signaling pathway activator	1.39	#####	3629	NEG	
Promote human embryonic stem cell (hESC) survival	1.52	#####	3063	POS	
HIV integrase inhibitor	1.90	#####	3642	NEG	X
Pyrimidine Antagonists; Thymidylate Synthase Inhibitors	0.83	#####	844	SURV	

retinoic acid receptor (RAR) and the retinoid X receptor (RXR) agonist	1.59	#####	2744	POS	
DNA methyltransferase (DNMT) inhibitor	0.91	#####	3341	NEG	
MDM2 (hdm2) inhibitor, Thioredoxin reductase inhibitor	1.23	#####	2518	NEG	
NF-kB activation Inhibitor	0.93	#####	2602	NEG	
Cyclooxygenase (COX-2) inhibitor	2.21	#####	3633	NEG	
	0.94	#####	3416	NEG	
HDAC1, HDAC2 inhibitor	1.42	#####	900	SURV	
DNA Alkylating Drugs	1.32	#####	1936	NEG	
Chemokine CXCR2 (IL-8 beta Receptor) antagonist	1.05	#####	745	SURV	
OX1 receptor antagonist	3.95	#####	3422	POS	X
OX1 receptor antagonist	3.65	#####	3003	POS	X
HDAC inhibitor	1.23	#####	819	SURV	
Activator of stem cell renewal, RasGAP and ERK1 inhibitors	0.47	#####	602	SURV	
Antifungal	2.14	#####	3633	NEG	
SHH antagonist	1.90	#####	687	SURV	
Coactivator Associated Arginine Methyltransferase 1 (CARM1, PRMT <sup>1</sup> ) inhibitor	1.09	#####	3150	NEG	
Dipeptidyl-peptidase IV(DPP-4) inhibitor	2.50	#####	3238	NEG	
SMO antagonist	1.35	#####	3209	NEG	
Antioxidants	1.09	#####	1404	SURV	
HDAC inhibitor	1.05	#####	3223	NEG	
Histone Deacetylase SIRT2 Inhibitors	1.00	#####	3515	NEG	

STAT3 inhibitor	2.25	#####	2952	POS	X
Protein Kinase C (PKC) Inhibitors; Selective Estrogen Receptor Modulators (SERM)	1.00	#####	3878	NEG	
Histone Deacetylase (HDAC) Inhibitors	1.07	#####	2814	NEG	
Fungicide	1.59	#####	3519	NEG	
Antimitotic Drugs; Apoptosis Inducers; Pyrimidine Antagonists	0.97	#####	3553	NEG	
DNA Alkylating Drugs; DNA Topoisomerase II alpha Inhibitors	0.83	#####	3748	NEG	
DNA Topoisomerase II Inhibitors	1.09	#####	1001	SURV	
SIRT1 and SIRT2 inhibitors	1.20	#####	2670	NEG	
Stem cell signaling	1.31	#####	3091	NEG	
Cytochrome P450 Oxidase Inhibitors; DNA alkylating drugs	1.13	#####	2736	NEG	
Purine Antagonists	1.00	#####	1974	NEG	
PARP inhibitor	3.26	#####	3369	POS	X
CRTH2 receptor antagonist	1.31	#####	3232	NEG	
DNA Topoisomerase I Inhibitors	1.27	#####	907	SURV	
Lysine-specific demethylase 1 (LSD1) inhibitor	0.94	#####	3354	NEG	
HDAC inhibitor	0.42	#####	437	SURV	
HDAC inhibitor	2.37	#####	3337	NEG	
Histone Deacetylase 6 (HDAC6) Inhibitors	1.24	#####	1307	SURV	
Bcl-2, Bcl-XL, Mcl-1 inhibitor	3.33	#####	1798	NEG	
Histone-Lysine N-methyltransferase EHMT2 (H3-K9-HMTase 3; G9a) Inhibitors	1.08	#####	3346	NEG	

Histone-Lysine N-methyltransferase EHMT2 (H3-K9-HMTase 3; G9a) Inhibitors	1.35	#####	3445	NEG	X
Histone-lysine N-methyltransferase EHMT2 (H3-K9-HMTase 3; G9a) Inhibitor	1.04	#####	3431	NEG	
PARP inhibitor	1.07	#####	3156	NEG	
Etoposide cytotoxic Enhancer in human glioblastoma cells	1.01	#####	3592	NEG	
Antimitotic Drugs; Tubulin polymerization inhibitors	1.64	#####	539	SURV	
Tubulin polymerization inhibitors, RNA synthesis inhibitor, Cell cycle inhibitor	1.21	#####	320	SURV	
Antimitotic Drugs; Tubulin polymerization inhibitors; Cell cycle inhibitors	1.53	#####	660	SURV	
Tubulin polymerization inhibitor	1.41	#####	597	SURV	
Antifungal					
HDAC inhibitor	1.50	#####	1981	POS	
WDR5 Inhibitor	1.24	#####	3300	NEG	
Wnt inhibitor	0.98	#####	3365	NEG	
Purine Antagonists	1.42	#####	2664	NEG	
Tankyrase inhibitor	3.33	#####	3995	POS	
Survavin inhibitor	1.69	#####	2917	NEG	
Cytidine deaminase inhibitor, DNA methyltransferase (DNMT) inhibitor	1.30	#####	3472	NEG	

Table 3: Dose response hits

(A) EGFP negative FAPs were treated with TGF $\alpha$ 1 and various concentration of KIL compounds. GFP% was assessed by flow cytometry.

KIL COMPOUNDS	Concentration ( $\mu$ M)				
	TGF $\alpha$ 1 alone	0.1	0.5	1	5
SB203580	9.4	8.09	6.42	7.93	3.97
H89	9.4	11	10.3	6.53	3.21
PF670462	9.4	9.67	6.87	7.16	3.95
TPCA-1	9.4	12.3	10.8	8.49	3.99
AZD-5363	9.4	11	10.3	6.53	3.21
RafK inh	9.4	8.9	8.34	9.41	3.95
SD-06	9.4	9.99	8.15	7.28	5.55
TAK-715	9.4	5.83	4.96	6.46	3.05
Wz4002	9.4	3.09	7.08	9.53	1.84
Ly2228820	9.4	8.2	6.48	3.51	3.56
Sorafenib	9.7	6.56	6.27	5.93	3.77
Nilotinib	9.7	7.22	6.94	5.86	2.02
Rottlerin	9.4	11.2	11.3	8.01	2.7
Masitinib	9.7	8.45	8.11	9.66	3.49
PHA-767H91	9.7	9.79	10.2	10.9	4.16
SGI-1776	9.7	9.05	8.44	8.37	2.53
VX-702	9.7	7.57	7.17	5.33	3.7
SB590885	9.7	9.01	7.16	6.06	1.26
Tipifarnib	9.7	7.12	6.15	5.02	2.25
VX745	9.7	10.2	7.12	5.77	1.63
brivabinib	9.7	6.9	8.21	5.75	3.67
Y-27632	9.4	8.97	8.74	8.35	11
OSI930	9.4	9.19	10	8.29	7.47
PD153035	9.7	6.83	8.14	8.97	6.55
Oratinib	9.7	9.96	9.92	9.75	8.75
PLX-4720	9.7	9.01	8.93	8.09	9.1
IC87114	9.7	8.68	8.46	5.89	7.75
TG100-115	9.7	8.63	6.87	8.28	9.37
XL765	9.7	9.23	9.38	5.81	8.03
LY2835219	9.7	7.85	8.82	9.04	6.13

(B) EGFP negative FAPs were treated with TGF $\alpha$ 1 and various concentration of TOOL compounds. EGFP% was assessed by flow cytometry.

TOOL compounds	Concentration ( $\mu$ M)				
	TGF $\alpha$ 1 alone	0.1	0.5	1	5
Ciclopiamine	9.4	10.9	8.03	7.4	6.15

Navitodax	9.4	6.96	6.92	6.03	5.07
Metformin	9.4	7.89	4.21	4.26	4.96
ABT 737	9.4	7.89	4.21	4.26	4.96
Vorinostat	9.4	10.2	4.58	5.94	1.29
PCI-24781	9.4	9.17	2.5	0.28	
Dexamethasone	9.4	5.63	5.39	2.57	
Indolactam V	9.4	6.94	3.78	2.59	1.31
I-BET151	9.4	6.68	2.31	2.27	0.58
SB334867	9.4	11.9	8.58	11	9.44
SB408124	9.4	6.89	9.02	9.64	8.31
AZD-2281	9.4	9.66	8.23	7.91	9.32
Lopinavir	9.4	7.89	9.46	10.5	7.04
IWP-3	9.4	8.6	9.57	8.23	10.2
Bexarotene	9.4	8.7	11.1	11.5	9.65
Compostear	9.4	6.82	9.16	7.2	8.84
EL-532	9.4	6.64	10.2	8.26	7.13
ID-1	9.4	8.92	8.36	9.44	7.17
Atridox	9.4	7.56	8.75	6.79	5.92
Buserelein	9.4	9.12	10.5	9.02	6.64
STX0119	9.4	9.4	10.1	8.72	8.41
Pyrintegrin	9.4	9.4	7.01	8.79	
PJ.34	9.4	9.4	12.8	9.23	7.21
TIQ-A	9.4	9.4	11.8	9.77	6.88

Table 4. Epiprobos

Compound	Target	Fold change (sample/TGFb)	p value	Cell number	Output
A-366	G9a/GLI	1.04	0.8046 6	4511	NEG
BAZ2- ICR	BAZ2A/B bromodomains	0.78	0.1692 3	3555	NEG
C646	p300	0.80	0.3162 9	3848	NEG
CL-994	HDAC	0.82	0.4956 4	2164	NEG
GSK- LSD1	LSD1	1.30	0.1392 6	3965	NEG
GSK2801	BAZ2A/B	0.86	0.6193 1	3376	NEG
GSK343	EZH2	1.17	0.4559 5	2400	NEG
GSKJ4	JMJD3, UTX	0.31	0.0012 8	895	SURV

I-CBP112	CBP/p300	0.58	0.0642 3	3461	NEG
IOX2	PHD2	0.90	0.5448 1	3816	NEG
JQ1	pan-BRD	0.10	0.0003 2	2135	POS
LAQ824	HDAC	0.45	0.8991 8	83	SURV
LLY-507	SMYD2	0.48	0.0231 2	2305	POS
OF-1	BRPR1-3	0.33	0.0078 0	318	SURV
OICR-9429	WDR5	1.01	0.0587 9	3953	NEG
Olaparib	PARP1/2	0.69	0.0532 8	2871	NEG
PFI-1	BET	0.29	0.0052 0	1677	POS
PFI-2	SETD7	0.56	0.0186 7	3544	POS
PFI-3	SMARCA	1.53	0.0313 8	4468	NEG
SGC-CBP30	CREBBP/EP300	0.47	0.0770 0	3900	NEG
SGC0946	DOT1L	0.90	0.5785 6	3512	NEG
SGC707	PRMT3	0.77	0.4163 3	3778	NEG
UNC0638	G9a/GLP	0.88	0.4681 4	2892	NEG
UNC0642	G9a/GLP	0.20	0.0010 5	3159	POS
UNC1215	L3MBTL3	0.81	0.2720 8	3855	NEG
UNC1999	EZH1/2	0.62	0.0319 8	2730	POS

### Figure legends

**Figure 1: Collagen1a1\*3.6 EGFP transgenic mouse is a powerful tool for analyzing TGF $\beta$ 1 signaling**

**(A)** Collagen1a1\*3.6 EGFP mice were injected with notexin (NTX) in the tibialis anterior (TA)

muscle at day 0 (D0). 3 days post injury, Collagen-EGFP negative FAPs were cell sorted and plated before been treated with 1 ng/ml of TGF $\beta$ 1 for 72 hours (**B-C**). GFP expression was analyzed by flow cytometry as a measure of Collagen gene expression. n = 3 biological replicates.

**Figure 2: Drug screening targeting Collagen type I expression**

(**A-B**) Tibialis anterior (TA) muscles of Collagen1a1\*3.6 EGFP mice were injected with notexin (NTX). Three days after injury, EGFP negative FAPs were cell sorted and treated with TGF $\beta$ 1 alone or with compounds from the KIL and TOOL libraries. Collagen-GFP expression was quantified using a Cellomic Arrayscan (*Figure S1*). 4 to 9 experimental replicates were performed

(**C**) Sorted EGFP negative FAPs were treated with TGF $\beta$ 1 and selected compounds from the first round of screening of the KIL library. Percentage of EGFP positive cells was quantified by flow cytometry. Each technical replicate performed is presented (5 to 14)

(**D**) Selected positive hits from the KIL library (Sorafenib, Nilotinib and Tipifarnib) were then tested as described at concentrations ranging from 0.5 to 5  $\mu$ M. 2 biological replicates were performed.

(**E**) Sorted EGFP negative FAPs were treated with TGF $\beta$ 1 and with selected compounds from the first round of screening of the TOOL library. GFP expression was quantified using flow cytometry. Each technical replicate performed is presented (3 to 10)

(**F**) Selected compounds from the TOOL library (Indolactam V, I-BET 151 and PCI-24782) were then tested in a dose dependent manner from 0.5 to 1  $\mu$ M. 2 to 4 biological replicates were performed

(G) Compounds from the “epiprobe” library were tested at 0.25 and 1  $\mu$ M. Collagen-GFP expression was quantified using flow cytometry. 3 biological replicates were performed

Drug versus TGF $\beta$ 1: \*:  $p < 0.05$ , \*\*:  $p < 0.01$

**Figure 3: TAK1-p38 axis regulates FAP differentiation into fibroblasts**

(A-B) C3H10T1/2 cell line was stimulated for 30 min with rh-TGF $\beta$ 1 (1 ng/ml) alone or with 1  $\mu$ M of Nilotinib, Sorafenib, Masitinib, JQ-1, or (5Z)-7-Oxozeanol. Protein lysates were extracted and Western Blot for p38, phospho-p38, phospho-TAK1 and  $\beta$ -Actin performed, and the data quantified. n=4.

(C) EGFP negative FAPs were treated for 6 hours with 1 ng/ml of rh-TGF $\beta$ 1 with or without 1  $\mu$ M of (5Z)-7-Oxozeanol. Gene expression of *Col1a1*, *Acta2*, *CTGF*, *Fn1*, and *Postn* was quantified by digital droplet PCR and normalized to the expression of *hprt*. n=3.

Treatment + TGF $\beta$ 1 versus TGF $\beta$ : \*:  $p < 0.05$ ; \*\*:  $p < 0.01$

**Figure 4: Treatment of JQ1 worsens DMD pathology**

(A-F) mdx mice were fed with a control or a JQ1-medicated diet (5 mg/kg/day) for up to a year. Diaphragm collagen deposition (B-C) and myofiber size (D-F) were quantified. n=4

(G-L) mdx:utr<sup>+/-</sup> mice were implanted with an Alzet osmotic pumps containing JQ1 or its vehicle for 4 weeks. Diaphragm collagen deposition (H-I) and myofiber size (J-L) were quantified. n=10 to 12

JQ1 versus control: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$

Scale bar = 100  $\mu$ m

***Figure 5: Sorafenib and Masitinib treatments do not improve DMD histopathology***

(**A-F**) mdx:utr<sup>+/-</sup> mice were injected i.p. with 60 mg/day/kg of Masitinib from 8- to 11-weeks-old. Diaphragm collagen deposition (**B-C**) and myofiber size (**D-F**) were quantified. n=6 to 8

(**G-O**) mdx:utr<sup>+/-</sup> mice were implanted with osmotic pumps containing either Masitinib, Sorafenib or the appropriate control vehicle from 6- to 14-weeks-old. Diaphragm collagen deposition (**H, L, K, O**) and myofiber size (**I, J, M, N**) were quantified. n=4

Treated mice versus control: \*p <0.05,

Scale bar = 100 um