Title: Targeting fibrosis in the Duchenne Muscular Dystrophy mice model: an uphill battle

Marine Theret^{1#}, Marcela Low^{1#}, Lucas Rempel¹, Fang Fang Li¹, Lin Wei Tung¹, Osvaldo Contreras^{3,4}, Chih-Kai Chang¹, Andrew Wu¹, Hesham Soliman^{1,2}, Fabio M.V. Rossi¹

¹School of Biomedical Engineering and the Biomedical Research Centre, Department of Medical Genetics, 2222 Health Sciences Mall, Vancouver, BC, V6T 1Z3, Canada

²Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Minia University, Minia, Egypt

³Developmental and Stem Cell Biology Division, Victor Chang Cardiac Research Institute, Darlinghurst, NSW, 2010, Australia

⁴Departamento de Biología Celular y Molecular and Center for Aging and Regeneration (CARE-ChileUC), Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, 8331150 Santiago, Chile

Denotes Co-first authorship

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

Correspondence to:

Marine Theret

School of Biomedical Engineering and the Biomedical Research Centre

University of British Columbia

2222 Health Sciences Mall, Vancouver, British Columbia

Tel: +1(604) 822 0441 fax: +1(604) 822 7815

Email: mtheret@brc.ubc.ca

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 TGFβ1 stimulation *in vitro*. Then we treated cells with TGFβ1 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.

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 α (TNF α), and Transforming Growth Factor β (TGF β) [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 β 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 β plays an essential role in tissue modeling and remodeling, and therefore, it is considered a master molecule in the initiation and establishment of fibrosis[26]. The canonical TGF β pathway classically transmits extracellular signals via transmembrane serine/threonine kinase receptors and intracellularly via SMAD2/3/4 proteins. The non-canonical TGF β pathways include a variety of intracellular cascades activated by TGF β , independent of SMAD2/3/4. These include the molecules TGF β -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 κ B), among others[27,28]. Therapeutic manipulation of the canonical and non-canonical TGF β 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 TGFβ1

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 Collagen1a1*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 Collagen1a1-3.6*EGFP mice 3 days after NTX damage and placed in culture[15,32]. Once they reached 60% confluency, FAPs were treated with 1ng.ml⁻¹ of TGFβ1 for 72hr, and the percentage of EGFP positive cells was quantified using flow cytometry (Figure 1A). As suggested by previous studies[15], the physiological concentration of 1ng.ml⁻¹ was sufficient to increase EGFP expression by 350% (Figure 1B and C)

These results confirm that FAPs from the Collagen1a1*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⁻¹ of TGFβ1 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 TGFβ1-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 21 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 TGFβ1 (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 ($0.1\text{--}5\text{ }\mu\text{M}$) (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 β 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 β 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 κ B[37]. NF κ B pathway modulation has been associated with improvement in muscle health in mdx mice[38–40]. These results suggest that NF κ B might be modulating FAP fibrogenic differentiation and warranted further screening for non-toxic NF κ 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 $0.5\text{ }\mu\text{M}$ 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 β 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 β pathway regulates FAPs fibrogenic differentiation.

To validate that the candidate drugs act by another signaling than inhibiting the TGF β 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 β 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 β 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 α) 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 μ 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 β -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 β 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 TGFβ1-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 TGFβ1 (1 ng.ml^{-1}) with or without 5Z-7-Oxozeaenol ($1 \mu\text{M}$) 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 (*Fnl*) induced by TGFβ1 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 TGFβ 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, mdx mice (a murine model for DMD) 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. 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^{+/-}, which also lacks one allele of utrophin, a functional analog of dystrophin, have been proposed as a better alternative [57,58]. Indeed, mdx:utr^{+/-} mice mimic the human disease more closely and 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^{+/-} 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 (5 to 9-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<0.5; Figure S3E to G). However, this was not associated with changes in myofiber size (Figure S3H to J).

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, does not demonstrate toxicity when tested *in vivo* and *in vitro*[65]. 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 two weeks in mdx:utr^{+/-} 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 to the route of drug administration, we delivered Masitinib for 8 weeks 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).

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: 1) Define the intracellular pathways leading to the activation of a fibrogenic program in response to TGFβ1; 2) Identify a set of drugs capable of interfering with the fibrogenic differentiation of FAPs, to 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 TGFβ1.

Using muscle resident FAPs, we first confirmed that TGFβ1 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]. We showed that the local rise of TGFβ concentration in skeletal muscle after acute damage pushes FAPs towards fibrogenic differentiation. Similar results have been obtained by others and ourselves, where the expansion of resident FAPs is closely associated with elevated TGFβ levels and increased ECM deposition during regeneration and repair[22,69,70]. In addition, we previously showed a temporal correlation between the peak of TGFβ 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 β –TAK1 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 β 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 κ 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*.

We also tested a natural compound, Withaferin A, and two chemical derivatives, known for their inhibitory effect on the transcription factor NFkB[92,93]. Using the Col1a1-EGFP model presented here, we observed a decrease in EGFP positive cells in Withaferin A treated FAPs, suggesting that the NFkB factor might be playing a role in regulating the expression of collagen in FAPs. Similar results were found in the heart, where Withaferin A reduced type I collagen expression *in vitro* and inhibited the development of myocardial fibrosis *in vivo*[94]. Dysregulation of NFkB activity can contribute to chronic inflammatory diseases such as dystrophies. 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], and may be due to an effect on early inflammatory stages of fibrosis. However, in our hands, mice treated with Withaferin i.p (IMS-088). displayed swelling of the abdominal area and peritoneal adhesions, resulting in the interruption of its testing *in vivo* (data not shown).

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 commensurable 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]. 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 higher doses (5 μ 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 and S4). Similar results were found with the TyrK inhibitor Sorafenib. One of the 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].

In conclusion, here we show that in a model 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. These results contribute to the growing evidence that modeling fibrotic diseases *in vitro* to perform drug screening may not yield hits that will perform well in *in vivo* models[110,111].

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, *Coll1a1**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^{+/-} 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 with control diet (Research Diet, #D111112201) or JQ1 diet (control diet supplemented with JQ1 at 25 mg/kg (Cayman Chemical #11187, or Selleckchem #S7110)).

Mice were daily treated by intraperitoneal (i.p.) injection with the vehicle (DMSO/ETOH=1:1), or Masitinib (ABSCIENCE, #AB1010, 60 mg/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; Masitinib: ABSCIENCE #AB1010, 60 mg/kg/day; Sorafenib: BAYER #BXA5X4R, 6 mg/kg/day) 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. Animal were anesthetized and shaved. A 1 cm incision was performed on the skin and a pocket was created with 1 ml of sterile saline. The osmotic pump was slowly inserted, then the muscle layer was gently tented and closed with 6-0 monocril. By using an 18G needle, a hole was then poked in the muscle layer. The catheter was fed through the hole until the first retention bead passed through and into

the peritoneal space. To finish, the purse string suture was tightened using square knots. The catheter was allowed to sit naturally by adjusting the pump. Finally, the skin layer was closed using an intradermal pattern.

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.

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 × 5 min PBS washes were performed, and sections were stained with DAPI for 10 min (ThermoFisher, #D3571, 0.6 μM) 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. Hindlimbs or 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 Dickenson), 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⁻¹, Sigma) and resuspended in FACS buffer immediately before sorting. Sorting was performed on FACS Aria II (Becton Dickenson) or Influx (Becton Dickenson). Gates were defined based on fluorescence minus one (FMO) controls and EGFP negative FAPs were sorted as CD31/CD45/α7int- Sca1+ GFP-. For *in vitro* experiments, FAPs were seeded at a density of 10.000 cell/cm² in high-glucose Dulbecco's modified eagle medium (DMEM) (Invitrogen) supplemented with 10% FBS and 1.5 ng/ml β-FGF (Invitrogen).

In vitro drug screening

EGFP negative FAPs were plated at 10,000 cells/cm² in 384 well-plates (Falcon) and treated at 50-60% confluence, either with rh-TGFβ1 (1 ng.ml⁻¹) (eBioscience) or rh-TGFβ1 + 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. Viability and GFP expression were analyzed using Cellomics Array scan (Thermo Fisher Scientific).

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 μ 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 μ 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 μ M). Withaferin and its derivatives compounds (Imstar Therapeutics) were tested at a range of 0.06 to 0.5 μ 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 β 1 (1 ng.ml⁻¹) 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 µl was used in a reaction mix containing Droplet Digital PCR Supermix (Bio-Rad), TaqMan assay and RNAse free water. The Taqman probes (Suppl Table 1) used were the following: droplets were generated with a QX100 droplet generator (Bio-Rad), after mixing 20 µl of reaction mix and 70 µl 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), the cells need to be maintained at 50-70% confluence. C3H10T1/2 cells were grown at 37 °C in 5% CO₂ in growth medium; high-glucose Dulbecco's modified Eagle's medium (DMEM) (Invitrogen), supplemented with 10% fetal bovine serum (FBS) and Penicillin/streptomycin. For western blot analysis, cells were serum-starved for 1 hr before being treated with rh-TGFβ-1 (e-Bioscience) in DMEM supplemented with 2% (v/v) FBS. Specific treatments the following inhibitors were also used at 1 µM 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% CO₂.

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 9% polyacrylamide gels, transferred to PDVF membranes (Millipore, CA, USA), and probed with primary antibodies: rabbit anti-phospho-p38 (Thr180/Tyr182) (#9211S, Cell Signaling, USA), rabbit anti-p38 (#9212, Cell Signaling, USA) and mouse anti- β -actin (#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) software. Protein levels were normalized with the levels of the loading control.

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)

Figure legends

Figure 1: Collagen1a1*3.6 EGFP transgenic mouse is a powerful tool for analyzing TGF β 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 TGFβ1 for 72 hours (B-C). GFP expression was analyzed by flow cytometry as a measure of Collagen gene expression. n = 3 biological replicates. Treatment versus NT: p<0.01, ***

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 TGFβ1 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 TGFβ1 and with 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 is presented performed (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 μM. 2 biological replicates were performed.

(E) Sorted EGFP negative FAPs were treated with TGFβ1 and with selected compounds from the first round of screening of the TOOL library. GFP expression was quantified using flow cytometry. Each technical replicate is presented performed (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 μM. 2 to 4 biological replicates were performed

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

Drug versus TGF β 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 β 1 (1 ng/ml) alone or with 1 μ 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 β -Actin performed, and the data quantified. $n=4$.

(C) EGFP negative FAPs were treated for 6 hours with 1 ng/ml of rh-TGF β 1 with or without 1 μ M of (5Z)-7-Oxozeanol. Gene expression of *Colla1*, *Acta2*, *CTGF*, *Fn1*, and *Postn* was quantified by digital droplet PCR and normalized to the expression of *hprt*. $n=3$.

Treatment + rh-TGF β 1 versus rh-TGF β 1: *: $p < 0.05$; **: $p < 0.01$

Figure 4: Treatment of JQ1 worsens DMD pathology

(A-F) mdx mice were fed with a control or a JQ1-medicated diet (30 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^{+/-} 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 μ m

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

516 (A-F) mdx:utr^{+/-} mice were injected i.p. with 60 mg/day/kg of Masitinib from 8 to 11-weeks-old.
 517 Diaphragm collagen deposition (B-C) and myofiber size (D-F) were quantified. n=6 to 8
 518 (G-O) mdx:utr^{+/-} mice were implanted with osmotic pumps containing either Masitinib, Sorafenib
 519 or the appropriate control vehicle from 5 to 14-weeks-old. Diaphragm collagen deposition (H, L,
 520 K, O) and myofiber size (I, J, M, N) were quantified. n=4
 521 Treated mice versus control: *:p <0.05,
 522 Scale bar = 100 um

523 ***Figure S1: In vitro drug screening scheme.***

524 1st screening:

525 Tibialis anterior (TA) muscles of Collagen1a1*3.6 EGFP mice were injected with notexin (NTX).
 526 Three days after injury, EGFP negative FAPs were sorted, plated in 384 well-plates and treated at
 527 50-60% confluence, either with recombinant human TGFβ (1 ng.ml⁻¹) (eBioscience) or TGFβ1 +
 528 drug. After 72 hours of treatment, cells were fixed and stained for nuclei detection. Viability and
 529 GFP expression were analyzed using Cellomics Array scan (Thermo Fisher Scientific).

530 2nd screening:

531 EGFP negative FAPs were sorted from NTX-injected TAs, plated in 48 well-plates and treated
 532 when reached 50-60% confluence, either with rh-TGFβ (1 ng.ml⁻¹) (eBioscience) or rh-TGFβ1 +
 533 drug. After 72 hours, cells were detached from the plate using trypsin, resuspended in FACS buffer
 534 and stained with Hoechst 33342. Percentage of EGFP positive cells was calculated based on the
 535 total Hoechst⁺ events.

536 ***Figure S2: Masitinib inhibits Collagen-EGFP expression induced by TGFβ1***

537 Tibialis anterior (TA) muscles of Collagen1a1*3.6 EGFP mice were injected with notexin (NTX).
 538 Three days after injury, EGFP negative FAPs were cell sorted and plated before been treated with
 539 rh-TGFβ1 alone or with Masitinib as different doses (0.01 to 5μM. n= 1 to 2)

540 ***Figure S3: In vivo validation of JQ1***

541 (A-B) mdx mice were fed with control diet or JQ1 diet (30 mg/kg/day) for up to a year. Mouse
 542 mass (A) and muscle mass (B) were measured. n=4

543 (C-J) mdx:utr^{+/-} mice were implanted with pumps containing JQ1 or its vehicle for 4 weeks.
 544 Mouse body mass (C) and muscle mass (D) were measured. TA, GC, and Quad collagen deposition
 545 (E-G), as well as myofiber size (H-J) were quantified. n=10 to 12

546 End-point versus starting point: \$\$\$ p<0.001

547 JQ1 versus control: * p<0.05; ** p<0.01; *** p<0.001

548 ***Figure S4: In vivo validation of Masitinib and Sorafenib***

549 (A-C) mdx:utr^{+/-} mice were injected in i.p. with Masitinib or its vehicle from 8 to 11-weeks-old.
 550 Mouse body mass was measured (A). TA, GC, and Quad collagen deposition (B), as well as
 551 myofiber size (C) were quantified. n=6 to 8

552 (D-F) mdx:utr^{+/-} mice were implanted with minipumps containing Masitinib or its vehicle for 9
 553 weeks. Mouse body mass was measured (D). TA and GC collagen deposition (E), as well as
 554 myofiber size (F) were quantified. n=4

555 (G-I) mdx:utr^{+/-} mice were implanted with minipumps containing Sorafenib or its vehicle for 9
 556 weeks. Mouse body mass was measured (G). TA and GC collagen deposition (H), as well as
 557 myofiber size (I) were quantified. n=4

558 End-point versus starting point: \$ $p < 0.05$; \$\$\$ $p < 0.001$

559 Treatment versus control: * $p < 0.05$

560

561 **Acknowledgments**

562 The authors would like to thank Professor David W. Rowe (Center for Regenerative Medicine and
563 Skeletal Development, University of Connecticut Health Center, USA) and Doctor Lisa Hoffman
564 (Western University, London, ON, Canada) for gifting us the Col1a1*3.6-eGFP and the mdx:utr+/-
565 mouse colonies. We would like also to thank Dr. Rima Al-awar (Ontario Cancer Institute, Toronto)
566 for kindly gifting us the TOOL and KIL libraries, as well as Andrew Johnson, Justin Wong (UBC
567 Flow core), and Josh Hashimoto for their help.

568 **Author contributions**

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

573 **Competing interests**

574 The authors declare no competing or financial interests.

575 **Funding**

576 This work was supported by the Fondation pour la Recherche Médicale (FRM; 40248 to M.T.);
577 European Molecular Biology Organization (EMBO; ALTF 115-2016 to M.T.), by the Association
578 contre les myopathies (AFM; 22576 to MT), by Michael Smith Foundation for Health Research

(MSFHR; 18351 to MT), by the Agencia Nacional de Investigación y Desarrollo (ANID; POSTDOCTORADO BECAS CHILE/2011-74120052 to M.L.), by the Canadian Institutes of Health Research (CIHR-FDN-159908 to F.R.) and by the US Department of Defense (W81XWH-16-1-0327).

References

- [1] A.K. Ghosh, S.E. Quaggin, D.E. Vaughan, Molecular basis of organ fibrosis: Potential therapeutic approaches, *Exp. Biol. Med.* (2013). <https://doi.org/10.1177/1535370213489441>.
- [2] V.J. Thannickal, C.A. Henke, J.C. Horowitz, P.W. Noble, J. Roman, P.J. Sime, Y. Zhou, R.G. Wells, E.S. White, D.J. Tschumperlin, Matrix biology of idiopathic pulmonary fibrosis: A workshop report of the national heart, lung, and blood institute, *Am. J. Pathol.* 184 (2014). <https://doi.org/10.1016/j.ajpath.2014.02.003>.
- [3] J. Rosenbloom, F.A. Mendoza, S.A. Jimenez, Strategies for anti-fibrotic therapies, *Biochim. Biophys. Acta - Mol. Basis Dis.* 1832 (2013). <https://doi.org/10.1016/j.bbadis.2012.12.007>.
- [4] N.C. Henderson, F. Rieder, T.A. Wynn, Fibrosis: from mechanisms to medicines, *Nature.* (2020). <https://doi.org/10.1038/s41586-020-2938-9>.
- [5] Y. Kharraz, J. Guerra, P. Pessina, A.L. Serrano, P. Muñoz-Cánoves, Understanding the process of fibrosis in duchenne muscular dystrophy, *Biomed Res. Int.* 2014 (2014). <https://doi.org/10.1155/2014/965631>.
- [6] S. Dadgar, Z. Wang, H. Johnston, A. Kesari, K. Nagaraju, Y.W. Chen, D. Ashley Hill, T.A. Partridge, M. Giri, R.J. Freishtat, J. Nazarian, J. Xuan, Y. Wang, E.P. Hoffman, Asynchronous remodeling is a driver of failed regeneration in Duchenne muscular dystrophy, *J. Cell Biol.* 207 (2014). <https://doi.org/10.1083/jcb.201402079>.
- [7] A.L. Serrano, P. Muñoz-Cánoves, Fibrosis development in early-onset muscular dystrophies: Mechanisms and translational implications, *Semin. Cell Dev. Biol.* 64 (2017). <https://doi.org/10.1016/j.semcdb.2016.09.013>.
- [8] B. Hinz, Formation and function of the myofibroblast during tissue repair, *J. Invest. Dermatol.* 127 (2007). <https://doi.org/10.1038/sj.jid.5700613>.
- [9] A.W.B. Joe, L. Yi, A. Natarajan, F. Le Grand, L. So, J. Wang, M.A. Rudnicki, F.M.V. Rossi, Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis, *Nat. Cell Biol.* 12 (2010). <https://doi.org/10.1038/ncb2015>.
- [10] A. Uezumi, S.I. Fukada, N. Yamamoto, S. Takeda, K. Tsuchida, Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle, *Nat. Cell Biol.* 12 (2010). <https://doi.org/10.1038/ncb2014>.
- [11] N. Arrighi, C. Moratal, N. Clément, S. Giorgetti-Peraldi, P. Peraldi, A. Loubat, J.Y. Kurzenne, C. Dani, A. Chopard, C.A. Dechesne, Characterization of adipocytes derived from fibro/adipogenic progenitors resident in human skeletal muscle, *Cell Death Dis.* 6 (2015). <https://doi.org/10.1038/cddis.2015.79>.
- [12] O. Contreras, M. Cruz-Soca, M. Theret, H. Soliman, L.W. Tung, E. Groppa, F.M. Rossi, E. Brandan, Cross-talk between TGF- β and PDGFR α signaling pathways regulates the fate of stromal fibro-adipogenic progenitors, *J. Cell Sci.* 132 (2019). <https://doi.org/10.1242/jcs.232157>.
- [13] D. Burzyn, W. Kuswanto, D. Kolodin, J.L. Shadrach, M. Cerletti, Y. Jang, E. Sefik, T.G. Tan, A.J. Wagers, C. Benoist, D. Mathis, A Special Population of regulatory T Cells Potentiates muscle repair, *Cell.* 155 (2013). <https://doi.org/10.1016/j.cell.2013.10.054>.

- [14] J.E. Heredia, L. Mukundan, F.M. Chen, A.A. Mueller, R.C. Deo, R.M. Locksley, T.A. Rando, A. Chawla, Type 2 innate signals stimulate fibro/adipogenic progenitors to facilitate muscle regeneration, *Cell*. 153 (2013). <https://doi.org/10.1016/j.cell.2013.02.053>.
- [15] D.R. Lemos, F. Babaeijandaghi, M. Low, C.K. Chang, S.T. Lee, D. Fiore, R.H. Zhang, A. Natarajan, S.A. Nedospasov, F.M.V. Rossi, Nilotinib reduces muscle fibrosis in chronic muscle injury by promoting TNF-mediated apoptosis of fibro/adipogenic progenitors, *Nat. Med.* 21 (2015). <https://doi.org/10.1038/nm.3869>.
- [16] Y. Dong, K.A.S. Silva, Y. Dong, L. Zhang, Glucocorticoids increase adipocytes in muscle by affecting IL-4 regulated FAP activity, *FASEB J.* (2014). <https://doi.org/10.1096/fj.14-254011>.
- [17] X. Kang, M.Y. Yang, Y.X. Shi, M.M. Xie, M. Zhu, X.L. Zheng, C.K. Zhang, Z.L. Ge, X.T. Bian, J.T. Lv, Y.J. Wang, B.H. Zhou, K.L. Tang, Interleukin-15 facilitates muscle regeneration through modulation of fibro/adipogenic progenitors, *Cell Commun. Signal.* (2018). <https://doi.org/10.1186/s12964-018-0251-0>.
- [18] S.J. Mathew, J.M. Hansen, A.J. Merrell, M.M. Murphy, J.A. Lawson, D.A. Hutcheson, M.S. Hansen, M. Angus-Hill, G. Kardon, Connective tissue fibroblasts and Tcf4 regulate myogenesis, *Development*. 138 (2011). <https://doi.org/10.1242/dev.057463>.
- [19] A. Natarajan, D.R. Lemos, F.M.V. Rossi, Fibro/adipogenic progenitors: A double-edged sword in skeletal muscle regeneration, *Cell Cycle*. 9 (2010). <https://doi.org/10.4161/cc.9.11.11854>.
- [20] D. Fiore, R.N. Judson, M. Low, S. Lee, E. Zhang, C. Hopkins, P. Xu, A. Lenzi, F.M.V. Rossi, D.R. Lemos, Pharmacological blockage of fibro/adipogenic progenitor expansion and suppression of regenerative fibrogenesis is associated with impaired skeletal muscle regeneration, *Stem Cell Res.* 17 (2016). <https://doi.org/10.1016/j.scr.2016.06.007>.
- [21] M.N. Wosczyzna, C.T. Konishi, E.E. Perez Carbajal, T.T. Wang, R.A. Walsh, Q. Gan, M.W. Wagner, T.A. Rando, Mesenchymal Stromal Cells Are Required for Regeneration and Homeostatic Maintenance of Skeletal Muscle, *Cell Rep.* 27 (2019). <https://doi.org/10.1016/j.celrep.2019.04.074>.
- [22] A. Uezumi, T. Ito, D. Morikawa, N. Shimizu, T. Yoneda, M. Segawa, M. Yamaguchi, R. Ogawa, M.M. Matev, Y. Miyagoe-Suzuki, S. Takeda, K. Tsujikawa, K. Tsuchida, H. Yamamoto, S.I. Fukada, Fibrosis and adipogenesis originate from a common mesenchymal progenitor in skeletal muscle, *J. Cell Sci.* 124 (2011). <https://doi.org/10.1242/jcs.086629>.
- [23] G. Juban, M. Saclier, H. Yacoub-Youssef, A. Kernou, L. Arnold, C. Boisson, S. Ben Larbi, M. Magnan, S. Cuvellier, M. Théret, B.J. Petrof, I. Desguerre, J. Gondin, R. Mounier, B. Chazaud, AMPK Activation Regulates LTBP4-Dependent TGF- β 1 Secretion by Pro-inflammatory Macrophages and Controls Fibrosis in Duchenne Muscular Dystrophy, *Cell Rep.* 25 (2018). <https://doi.org/10.1016/j.celrep.2018.10.077>.
- [24] N. Ieronimakis, A. Hays, A. Prasad, K. Janebodin, J.S. Duffield, M. Reyes, PDGFR α signalling promotes fibrogenic responses in collagen-producing cells in Duchenne muscular dystrophy, *J. Pathol.* 240 (2016). <https://doi.org/10.1002/path.4801>.
- [25] T. Ito, R. Ogawa, A. Uezumi, T. Ohtani, Y. Watanabe, K. Tsujikawa, Y. Miyagoe-Suzuki, S. Takeda, H. Yamamoto, S. ichiro Fukada, Imatinib attenuates severe mouse dystrophy and inhibits proliferation and fibrosis-marker expression in muscle mesenchymal progenitors, *Neuromuscul. Disord.* (2013). <https://doi.org/10.1016/j.nmd.2012.10.025>.
- [26] G. Blobel, W. Schiemann, H. Lodish, Role of Transforming Growth Factor β in Human Disease, *N. Engl. J. Med.* 343 (2000). <https://doi.org/10.1056/nejm200007203430322>.
- [27] C. Heldin, A. Moustakas, Non-Smad TGF-beta signals, *J. Cell Sci.* (2005).
- [28] Y.E. Zhang, Non-Smad pathways in TGF- β signaling, *Cell Res.* 19 (2009). <https://doi.org/10.1038/cr.2008.328>.
- [29] R.J. Akhurst, A. Hata, Erratum: Targeting the TGF β signalling pathway in disease, *Nat. Rev. Drug Discov.* 11 (2012). <https://doi.org/10.1038/nrd3878>.
- [30] I. Kalajzic, Z. Kalajzic, M. Kaliterna, G. Gronowicz, S.H. Clark, A.C. Lichtler, D. Rowe, Use of type I collagen green fluorescent protein transgenes to identify subpopulations of cells at different stages of the osteoblast lineage, *J. Bone Miner. Res.* 17 (2002). <https://doi.org/10.1359/jbmr.2002.17.1.15>.

- [31] A.R. Gillies, R.L. Lieber, Structure and function of the skeletal muscle extracellular matrix, *Muscle and Nerve*. (2011). <https://doi.org/10.1002/mus.22094>.
- [32] M. Low, C. Eisner, F. Rossi, Fibro/adipogenic progenitors (FAPs): Isolation by FACS and culture, in: *Methods Mol. Biol.*, 2017. https://doi.org/10.1007/978-1-4939-6771-1_9.
- [33] M.A. Dawson, R.K. Prinjha, A. Dittmann, G. Giotopoulos, M. Bantscheff, W.I. Chan, S.C. Robson, C.W. Chung, C. Hopf, M.M. Savitski, C. Huthmacher, E. Gudgin, D. Lugo, S. Beinke, T.D. Chapman, E.J. Roberts, P.E. Soden, K.R. Auger, O. Mirguet, K. Doehner, R. Delwel, A.K. Burnett, P. Jeffrey, G. Drewes, K. Lee, B.J.P. Huntly, T. Kouzarides, Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia, *Nature*. (2011). <https://doi.org/10.1038/nature10509>.
- [34] M.S. Stratton, R.A. Bagchi, M.B. Felisbino, R.A. Hirsch, H.E. Smith, A.S. Riching, B.Y. Enyart, K.A. Koch, M.A. Cavaasin, M. Alexanian, K. Song, J. Qi, M.E. Lemieux, D. Srivastava, M.P.Y. Lam, S.M. Haldar, C.Y. Lin, T.A. McKinsey, Dynamic Chromatin Targeting of BRD4 Stimulates Cardiac Fibroblast Activation, *Circ. Res.* 125 (2019). <https://doi.org/10.1161/CIRCRESAHA.119.315125>.
- [35] E.M. Small, The Actin-MRTF-SRF gene regulatory axis and myofibroblast differentiation, *J. Cardiovasc. Transl. Res.* 5 (2012). <https://doi.org/10.1007/s12265-012-9397-0>.
- [36] M. Trojanowska, Ets factors and regulation of the extracellular matrix, *Oncogene*. 19 (2000). <https://doi.org/10.1038/sj.onc.1204043>.
- [37] E. Seki, S. De Minicis, C.H. Österreicher, J. Kluwe, Y. Osawa, D.A. Brenner, R.F. Schwabe, TLR4 enhances TGF- β signaling and hepatic fibrosis, *Nat. Med.* 13 (2007). <https://doi.org/10.1038/nm1663>.
- [38] G. Takaesu, R.M. Surabhi, K.-J. Park, J. Ninomiya-Tsuji, K. Matsumoto, R.B. Gaynor, TAK1 is Critical for I κ B Kinase-mediated Activation of the NF- κ B Pathway, *J. Mol. Biol.* 326 (2003). [https://doi.org/10.1016/S0022-2836\(02\)01404-3](https://doi.org/10.1016/S0022-2836(02)01404-3).
- [39] S. Messina, A. Bitto, M. Aguenouz, L. Minutoli, M.C. Monici, D. Altavilla, F. Squadrito, G. Vita, Nuclear factor kappa-B blockade reduces skeletal muscle degeneration and enhances muscle function in Mdx mice, *Exp. Neurol.* 198 (2006). <https://doi.org/10.1016/j.expneurol.2005.11.021>.
- [40] A.L. Siegel, C. Bledsoe, J. Lavin, F. Gatti, J. Berge, G. Millman, E. Turin, W.T. Winders, J. Rutter, B. Palmeiri, C.G. Carlson, Treatment with inhibitors of the NF- κ B pathway improves whole body tension development in the mdx mouse, *Neuromuscul. Disord.* (2009). <https://doi.org/10.1016/j.nmd.2008.10.006>.
- [41] R. Maitra, M.A. Porter, S. Huang, B.P. Gilmour, Inhibition of NF κ B by the natural product Withaferin A in cellular models of Cystic Fibrosis inflammation, *J. Inflamm.* 6 (2009). <https://doi.org/10.1186/1476-9255-6-15>.
- [42] J. Wang, J. Chen, H. Jin, D. Lin, Y. Chen, X. Chen, B. Wang, S. Hu, Y. Wu, Y. Wu, Y. Zhou, N. Tian, W. Gao, X. Wang, X. Zhang, BRD4 inhibition attenuates inflammatory response in microglia and facilitates recovery after spinal cord injury in rats, *J. Cell. Mol. Med.* (2019). <https://doi.org/10.1111/jcmm.14196>.
- [43] M.E. Choi, Y. Ding, S. Il Kim, TGF- β Signaling via TAK1 Pathway: Role in Kidney Fibrosis, *Semin. Nephrol.* 32 (2012). <https://doi.org/10.1016/j.semnephrol.2012.04.003>.
- [44] F. Guo, J. Hutchenreuther, D.E. Carter, A. Leask, TAK1 is required for dermal wound healing and homeostasis, *J. Invest. Dermatol.* 133 (2013). <https://doi.org/10.1038/jid.2013.28>.
- [45] S. Inokuchi, T. Aoyama, K. Miura, C.H. Österreicher, Y. Kodama, K. Miyai, S. Akira, D.A. Brenner, E. Seki, Disruption of TAK1 in hepatocytes causes hepatic injury, inflammation, fibrosis, and carcinogenesis, *Proc. Natl. Acad. Sci. U. S. A.* 107 (2010). <https://doi.org/10.1073/pnas.0909781107>.
- [46] D. Zhang, V. Gaussin, G.E. Taffet, N.S. Belaguli, M. Yamada, R.J. Schwartz, L.H. Michael, P.A. Overbeek, M.D. Schneider, TAK1 is activated in the myocardium after pressure overload and is sufficient to provoke heart failure in transgenic mice, *Nat. Med.* 6 (2000). <https://doi.org/10.1038/75037>.
- [47] N. Biesemann, L. Mendler, S. Kostin, A. Wietelmann, T. Borchardt, T. Braun, Myostatin induces interstitial fibrosis in the heart via TAK1 and p38, *Cell Tissue Res.* 361 (2015). <https://doi.org/10.1007/s00441-015-2139-2>.

- 715 [48] Y. Ogura, S.M. Hindi, S. Sato, G. Xiong, S. Akira, A. Kumar, TAK1 modulates satellite stem cell
716 homeostasis and skeletal muscle repair, *Nat. Commun.* 6 (2015). <https://doi.org/10.1038/ncomms10123>.
- 717 [49] S.M. Hindi, S. Sato, G. Xiong, K.R. Bohnert, A.A. Gibb, Y.S. Gallot, J.D. McMillan, B.G. Hill, S. Uchida,
718 A. Kumar, TAK1 regulates skeletal muscle mass and mitochondrial function, *JCI Insight.* 3 (2018).
719 <https://doi.org/10.1172/jci.insight.98441>.
- 720 [50] S. Bhatnagar, A. Kumar, D.Y. Makonchuk, H. Li, A. Kumar, Transforming growth factor- β -activated kinase
721 1 is an essential regulator of myogenic differentiation, *J. Biol. Chem.* (2010).
722 <https://doi.org/10.1074/jbc.M109.064063>.
- 723 [51] K. Yamaguchi, K. Shirakabe, H. Shibuya, K. Irie, I. Oishi, N. Ueno, T. Taniguchi, E. Nishida, K.
724 Matsumoto, Identification of a Member of the MAPKKK Family as a Potential Mediator of TGF- β 2
725 Signal Transduction, *Science* (80-.). 270 (1995). <https://doi.org/10.1126/science.270.5244.2008>.
- 726 [52] H. Hanafusa, J. Ninomiya-Tsuji, N. Masuyama, M. Nishita, J. Fujisawa, H. Shibuya, K. Matsumoto, E.
727 Nishida, Involvement of the p38 Mitogen-activated Protein Kinase Pathway in Transforming Growth
728 Factor- β -induced Gene Expression, *J. Biol. Chem.* 274 (1999). <https://doi.org/10.1074/jbc.274.38.27161>.
- 729 [53] S. Il Kim, J.H. Kwak, M. Zachariah, Y. He, L. Wang, M.E. Choi, TGF- β -activated kinase 1 and TAK1-
730 binding protein 1 cooperate to mediate TGF- β 1-induced MKK3-p38 MAPK activation and stimulation of
731 type I collagen, *Am. J. Physiol. Physiol.* 292 (2007). <https://doi.org/10.1152/ajprenal.00485.2006>.
- 732 [54] Q. Duan, S. McMahon, P. Anand, H. Shah, S. Thomas, H.T. Salunga, Y. Huang, R. Zhang, A. Sahadevan,
733 M.E. Lemieux, J.D. Brown, D. Srivastava, J.E. Bradner, T.A. McKinsey, S.M. Haldar, BET bromodomain
734 inhibition suppresses innate inflammatory and profibrotic transcriptional networks in heart failure, *Sci.*
735 *Transl. Med.* 9 (2017). <https://doi.org/10.1126/scitranslmed.aah5084>.
- 736 [55] X. Tang, R. Peng, J.E. Phillips, J. Deguzman, Y. Ren, S. Apparsundaram, Q. Luo, C.M. Bauer, M.E.
737 Fuentes, J.A. Demartino, G. Tyagi, R. Garrido, C.M. Hogaboam, C.P. Denton, A.M. Holmes, C. Kitson,
738 C.S. Stevenson, D.C. Budd, Assessment of Brd4 inhibition in idiopathic pulmonary fibrosis lung fibroblasts
739 and in vivo models of lung fibrosis, *Am. J. Pathol.* 183 (2013). <https://doi.org/10.1016/j.ajpath.2013.04.020>.
- 740 [56] E. Ardite, E. Perdiguero, B. Vidal, S. Gutarra, A.L. Serrano, P. Muñoz-Cánoves, PAI-1-regulated miR-21
741 defines a novel age-associated fibrogenic pathway in muscular dystrophy, *J. Cell Biol.* 196 (2012).
742 <https://doi.org/10.1083/jcb.201105013>.
- 743 [57] M. van Putten, M. Hulsker, V.D. Nadarajah, S.H. van Heiningen, E. van Huizen, M. van Iterson, P.
744 Admiraal, T. Messemaker, J.T. den Dunnen, P.A.C. 't Hoen, A. Aartsma-Rus, The effects of low levels of
745 dystrophin on mouse muscle function and pathology, *PLoS One.* 7 (2012).
746 <https://doi.org/10.1371/journal.pone.0031937>.
- 747 [58] L. Zhou, J.A. Rafael-Fortney, P. Huang, X.S. Zhao, G. Cheng, X. Zhou, H.J. Kaminski, L. Liu, R.M.
748 Ransohoff, Haploinsufficiency of utrophin gene worsens skeletal muscle inflammation and fibrosis in mdx
749 mice, *J. Neurol. Sci.* (2008). <https://doi.org/10.1016/j.jns.2007.08.029>.
- 750 [59] K.M. Guppel, W.T. Hrinivich, L.M. Hoffman, Skeletal muscle fibrosis in the mdx/utrn+/-mouse validates its
751 suitability as a murine model of duchenne muscular dystrophy, *PLoS One.* 10 (2015).
752 <https://doi.org/10.1371/journal.pone.0117306>.
- 753 [60] A.A. McDonald, S.L. Hebert, M.D. Kunz, S.J. Ralles, L.K. McLoon, Disease course in mdx:Utrophin+/-
754 mice: comparison of three mouse models of duchenne muscular dystrophy, *Physiol. Rep.* (2015).
755 <https://doi.org/10.14814/phy2.12391>.
- 756 [61] H. Soliman, B. Paylor, R.W. Scott, D.R. Lemos, C.K. Chang, M. Arostegui, M. Low, C. Lee, D. Fiore, P.
757 Braghetta, V. Pospichalova, C.E. Barkauskas, V. Korinek, A. Rampazzo, K. MacLeod, T.M. Underhill,
758 F.M.V. Rossi, Pathogenic Potential of Hic1-Expressing Cardiac Stromal Progenitors, *Cell Stem Cell.* 26
759 (2020). <https://doi.org/10.1016/j.stem.2019.12.008>.
- 760 [62] J.J. Moslehi, M. Deininger, Tyrosine kinase inhibitor-associated cardiovascular toxicity in chronic myeloid
761 leukemia, *J. Clin. Oncol.* 33 (2015). <https://doi.org/10.1200/JCO.2015.62.4718>.
- 762 [63] Z. White, C.H. Hakim, M. Theret, N.N. Yang, F. Rossi, D. Cox, G.A. Francis, V. Straub, K. Selby, C.

- 763 Panagiotopoulos, D. Duan, P. Bernatchez, High prevalence of plasma lipid abnormalities in human and
764 canine Duchenne and Becker muscular dystrophies depicts a new type of primary genetic dyslipidemia, J.
765 Clin. Lipidol. (2020). <https://doi.org/10.1016/j.jacl.2020.05.098>.
- 766 [64] O. Contreras, M. Villarreal, E. Brandan, Nilotinib impairs skeletal myogenesis by increasing myoblast
767 proliferation, Skelet. Muscle. 8 (2018). <https://doi.org/10.1186/s13395-018-0150-5>.
- 768 [65] P. Dubreuil, S. Letard, M. Ciufolini, L. Gros, M. Humbert, N. Castéran, L. Borge, B. Hajem, A. Lermet, W.
769 Sippl, E. Voisset, M. Arock, C. Auclair, P.S. Leventhal, C.D. Mansfield, A. Moussy, O. Hermine, Masitinib
770 (AB1010), a potent and selective tyrosine kinase inhibitor targeting KIT, PLoS One. 4 (2009).
771 <https://doi.org/10.1371/journal.pone.0007258>.
- 772 [66] A. Uezumi, S. Fukada, N. Yamamoto, M. Ikemoto-Uezumi, M. Nakatani, M. Morita, A. Yamaguchi, H.
773 Yamada, I. Nishino, Y. Hamada, K. Tsuchida, Identification and characterization of PDGFR +
774 mesenchymal progenitors in human skeletal muscle, Cell Death Dis. 5 (2014).
775 <https://doi.org/10.1038/cddis.2014.161>.
- 776 [67] H. Yokoi, M. Mukoyama, A. Sugawara, K. Mori, T. Nagae, H. Makino, T. Suganami, K. Yahata, Y.
777 Fujinaga, I. Tanaka, K. Nakao, Role of connective tissue growth factor in fibronectin expression and
778 tubulointerstitial fibrosis, Am. J. Physiol. - Ren. Physiol. 282 (2002).
779 <https://doi.org/10.1152/ajprenal.00122.2001>.
- 780 [68] K.E. Lipson, C. Wong, Y. Teng, S. Spong, CTGF is a central mediator of tissue remodeling and fibrosis and
781 its inhibition can reverse the process of fibrosis, Fibrogenesis Tissue Repair. 5 (2012).
782 <https://doi.org/10.1186/1755-1536-5-s1-s24>.
- 783 [69] O. Contreras, D.L. Rebolledo, J.E. Oyarzún, H.C. Olguín, E. Brandan, Connective tissue cells expressing
784 fibro/adipogenic progenitor markers increase under chronic damage: relevance in fibroblast-myofibroblast
785 differentiation and skeletal muscle fibrosis, Cell Tissue Res. 364 (2016). [https://doi.org/10.1007/s00441-](https://doi.org/10.1007/s00441-015-2343-0)
786 015-2343-0.
- 787 [70] D. Gonzalez, O. Contreras, D.L. Rebolledo, J.P. Espinoza, B. van Zundert, E. Brandan, ALS skeletal muscle
788 shows enhanced TGF- β signaling, fibrosis and induction of fibro/adipogenic progenitor markers, PLoS One.
789 12 (2017). <https://doi.org/10.1371/journal.pone.0177649>.
- 790 [71] C.E. Daniels, M.C. Wilkes, M. Edens, T.J. Kottom, S.J. Murphy, A.H. Limper, E.B. Leof, Imatinib mesylate
791 inhibits the profibrogenic activity of TGF- β and prevents bleomycin-mediated lung fibrosis, J. Clin. Invest.
792 114 (2004). <https://doi.org/10.1172/jci19603>.
- 793 [72] L.X. Wang, X. Yang, Y. Yue, T. Fan, J. Hou, G.X. Chen, M.Y. Liang, Z.K. Wu, Imatinib attenuates cardiac
794 fibrosis by inhibiting platelet-derived growth factor receptors activation in isoproterenol induced model,
795 PLoS One. (2017). <https://doi.org/10.1371/journal.pone.0178619>.
- 796 [73] C.K. Rhee, S.H. Lee, H.K. Yoon, S.C. Kim, S.Y. Lee, S.S. Kwon, Y.K. Kim, K.H. Kim, T.J. Kim, J.W.
797 Kim, Effect of nilotinib on bleomycin-induced acute lung injury and pulmonary fibrosis in mice,
798 Respiration. 82 (2011). <https://doi.org/10.1159/000327719>.
- 799 [74] M. Iyoda, T. Shibata, Y. Hirai, Y. Kuno, T. Akizawa, Nilotinib attenuates renal injury and prolongs survival
800 in chronic kidney disease, J. Am. Soc. Nephrol. 22 (2011). <https://doi.org/10.1681/ASN.2010111158>.
- 801 [75] P. Piñol-Jurado, X. Suárez-Calvet, E. Fernández-Simón, E. Gallardo, N. De La Oliva, A. Martínez-Muriana,
802 P. Gómez-Gálvez, L.M. Escudero, M. Pérez-Peiró, L. Wollin, N. De Luna, X. Navarro, I. Illa, J. Díaz-
803 Manera, Nintedanib decreases muscle fibrosis and improves muscle function in a murine model of
804 dystrophinopathy, Cell Death Dis. 9 (2018). <https://doi.org/10.1038/s41419-018-0792-6>.
- 805 [76] F. Hong, H. Chou, M.I. Fiel, S.L. Friedman, Antifibrotic activity of sorafenib in experimental hepatic
806 fibrosis: Refinement of inhibitory targets, dosing, and window of efficacy in vivo, Dig. Dis. Sci. (2013).
807 <https://doi.org/10.1007/s10620-012-2325-y>.
- 808 [77] T.M. Fontelonga, B. Jordan, A.M. Nunes, P. Barraza-Flores, N. Bolden, R.D. Wuebbles, L.M. Griner, X.
809 Hu, M. Ferrer, J. Marugan, N. Southall, D.J. Burkin, Sunitinib promotes myogenic regeneration and
810 mitigates disease progression in the mdx mouse model of Duchenne muscular dystrophy, Hum. Mol. Genet.
811 (2019). <https://doi.org/10.1093/hmg/ddz044>.

- [78] P. Anand, J.D. Brown, C.Y. Lin, J. Qi, R. Zhang, P.C. Artero, M.A. Alaiti, J. Bullard, K. Alazem, K.B. Margulies, T.P. Cappola, M. Lemieux, J. Plutzky, J.E. Bradner, S.M. Haldar, BET bromodomains mediate transcriptional pause release in heart failure, *Cell*. 154 (2013). <https://doi.org/10.1016/j.cell.2013.07.013>.
- [79] N. Ding, N. Hah, R.T. Yu, M.H. Sherman, C. Benner, M. Leblanc, M. He, C. Liddle, M. Downes, R.M. Evans, BRD4 is a novel therapeutic target for liver fibrosis, *Proc. Natl. Acad. Sci. U. S. A.* 112 (2015). <https://doi.org/10.1073/pnas.1522163112>.
- [80] C. Mozzetta, S. Consalvi, V. Saccone, M. Tierney, A. Diamantini, K.J. Mitchell, G. Marazzi, G. Borsellino, L. Battistini, D. Sassoon, A. Sacco, P.L. Puri, Fibroadipogenic progenitors mediate the ability of HDAC inhibitors to promote regeneration in dystrophic muscles of young, but not old Mdx mice, *EMBO Mol. Med.* 5 (2013). <https://doi.org/10.1002/emmm.201202096>.
- [81] V. Saccone, S. Consalvi, L. Giordani, C. Mozzetta, I. Barozzi, M. Sandomá, T. Ryan, A. Rojas-Muñoz, L. Madaro, P. Fasanaro, G. Borsellino, M. De Bardi, G. Frigè, A. Termanini, X. Sun, J. Rossant, B.G. Bruneau, M. Mercola, S. Minucci, P.L. Puri, HDAC-regulated myomiRs control BAF60 variant exchange and direct the functional phenotype of fibro-adipogenic progenitors in dystrophic muscles, *Genes Dev.* 28 (2014). <https://doi.org/10.1101/gad.234468.113>.
- [82] R. Müller, J. Goodrich, Sweet memories: Epigenetic control in flowering, *F1000 Biol. Rep.* (2011). <https://doi.org/10.3410/B3-13>.
- [83] K. Ono, T. Ohtomo, J. Ninomiya-Tsuji, M. Tsuchiya, A dominant negative TAK1 inhibits cellular fibrotic responses induced by TGF- β , *Biochem. Biophys. Res. Commun.* 307 (2003). [https://doi.org/10.1016/S0006-291X\(03\)01207-5](https://doi.org/10.1016/S0006-291X(03)01207-5).
- [84] C. Bao, Z. Yang, Q. Cai, Q. Li, H. Li, B. Shu, Incremental load training improves renal fibrosis by regulating the TGF- β 1/TAK1/MKK3/p38MAPK signaling pathway and inducing the activation of autophagy in aged mice, *Int. J. Mol. Med.* (2019). <https://doi.org/10.3892/ijmm.2019.4344>.
- [85] H.S. Cui, S.Y. Joo, Y.S. Cho, J.B. Kim, C.H. Seo, CPEB1 or CPEB4 knockdown suppresses the TAK1 and Smad signalings in THP-1 macrophage-like cells and dermal fibroblasts, *Arch. Biochem. Biophys.* (2020). <https://doi.org/10.1016/j.abb.2020.108322>.
- [86] K. Singh, A. Gupta, A. Sarkar, I. Gupta, S. Rana, S. Sarkar, S. Khan, Arginyltransferase knockdown attenuates cardiac hypertrophy and fibrosis through TAK1-JNK1/2 pathway, *Sci. Rep.* (2020). <https://doi.org/10.1038/s41598-019-57379-7>.
- [87] D. Xu, S. Li, L. Wang, J. Jiang, L. Zhao, X. Huang, Z. Sun, C. Li, L. Sun, X. Li, Z. Jiang, L. Zhang, TAK1 inhibition improves myoblast differentiation and alleviates fibrosis in a mouse model of Duchenne muscular dystrophy, *J. Cachexia. Sarcopenia Muscle.* (2020). <https://doi.org/10.1002/jcsm.12650>.
- [88] D. Xu, L. Zhao, J. Jiang, S. Li, Z. Sun, X. Huang, C. Li, T. Wang, L. Sun, X. Li, Z. Jiang, L. Zhang, A potential therapeutic effect of catalpol in Duchenne muscular dystrophy revealed by binding with TAK1, *J. Cachexia. Sarcopenia Muscle.* 11 (2020). <https://doi.org/10.1002/jcsm.12581>.
- [89] D. qiu Xu, L. Zhao, S. jia Li, X. fei Huang, C. jie Li, L. xin Sun, X. hua Li, L. yong Zhang, Z. zhou Jiang, Catalpol counteracts the pathology in a mouse model of Duchenne muscular dystrophy by inhibiting the TGF- β 1/TAK1 signaling pathway, *Acta Pharmacol. Sin.* (2020). <https://doi.org/10.1038/s41401-020-00515-1>.
- [90] A. Peidl, B. Perbal, A. Leask, Yin/Yang expression of CCN family members: Transforming growth factor beta 1, via ALK5/ FAK/MEK, induces CCN1 and CCN2, yet suppresses CCN3, expression in human dermal fibroblasts, *PLoS One.* (2019). <https://doi.org/10.1371/journal.pone.0218178>.
- [91] H. Sakurai, H. Miyoshi, J. Mizukami, T. Sugita, Phosphorylation-dependent activation of TAK1 mitogen-activated protein kinase kinase by TAB1, *FEBS Lett.* (2000). [https://doi.org/10.1016/S0014-5793\(00\)01588-X](https://doi.org/10.1016/S0014-5793(00)01588-X).
- [92] K. Heyninck, M. Lahtela-Kakkonen, P. Van der Veken, G. Haegeman, W. Vanden Berghe, Withaferin A inhibits NF-kappaB activation by targeting cysteine 179 in IKK β , *Biochem. Pharmacol.* 91 (2014). <https://doi.org/10.1016/j.bcp.2014.08.004>.

- [93] S. Bale, P. Venkatesh, M. Sunkoju, C. Godugu, An adaptogen: Withaferin a ameliorates in vitro and in vivo pulmonary fibrosis by modulating the interplay of fibrotic, matricellular proteins, and cytokines, *Front. Pharmacol.* 9 (2018). <https://doi.org/10.3389/fphar.2018.00248>.
- [94] A.A. Challa, M. Vukmirovic, J. Blackmon, B. Stefanovic, Withaferin-A reduces type I collagen expression in vitro and inhibits development of myocardial fibrosis in vivo, *PLoS One.* 7 (2012). <https://doi.org/10.1371/journal.pone.0042989>.
- [95] S. Dubey, H. Yoon, M.S. Cohen, P. Nagarkatti, M. Nagarkatti, D. Karan, Withaferin a associated differential regulation of inflammatory cytokines, *Front. Immunol.* 9 (2018). <https://doi.org/10.3389/fimmu.2018.00195>.
- [96] E. Ferri, C. Petosa, C.E. McKenna, Bromodomains: Structure, function and pharmacology of inhibition, *Biochem. Pharmacol.* 106 (2016). <https://doi.org/10.1016/j.bcp.2015.12.005>.
- [97] K. Kumar, B.T. DeCant, P.J. Grippo, R.F. Hwang, D.J. Bentrem, K. Ebine, H.G. Munshi, BET inhibitors block pancreatic stellate cell collagen I production and attenuate fibrosis in vivo, *JCI Insight.* 2 (2017). <https://doi.org/10.1172/jci.insight.88032>.
- [98] B. Zhou, J. Mu, Y. Gong, C. Lu, Y. Zhao, T. He, Z. Qin, Brd4 inhibition attenuates unilateral ureteral obstruction-induced fibrosis by blocking TGF- β -mediated Nox4 expression, *Redox Biol.* (2017). <https://doi.org/10.1016/j.redox.2016.12.031>.
- [99] A.C. Belkina, B.S. Nikolajczyk, G. V. Denis, BET Protein Function Is Required for Inflammation: Brd2 Genetic Disruption and BET Inhibitor JQ1 Impair Mouse Macrophage Inflammatory Responses, *J. Immunol.* 190 (2013). <https://doi.org/10.4049/jimmunol.1202838>.
- [100] H.R. Gibbons, D.J. Mi, V.M. Farley, T. Esmond, M.B. Kaood, T.M. Aune, Bromodomain inhibitor JQ1 reversibly blocks IFN- γ production, *Sci. Rep.* 9 (2019). <https://doi.org/10.1038/s41598-019-46516-x>.
- [101] C. Beyer, J.H.W. Distler, Tyrosine kinase signaling in fibrotic disorders. Translation of basic research to human disease., *Biochim. Biophys. Acta - Mol. Basis Dis.* 1832 (2013). <https://doi.org/10.1016/j.bbadis.2012.06.008>.
- [102] F. Grimminger, A. Günther, C. Vancheri, The role of tyrosine kinases in the pathogenesis of idiopathic pulmonary fibrosis, *Eur. Respir. J.* 45 (2015). <https://doi.org/10.1183/09031936.00149614>.
- [103] E. Perdiguero, P. Sousa-Victor, V. Ruiz-Bonilla, M. Jardí, C. Caelles, A.L. Serrano, P. Muñoz-Cánoves, p38/MKP-1-regulated AKT coordinates macrophage transitions and resolution of inflammation during tissue repair, *J. Cell Biol.* 195 (2011). <https://doi.org/10.1083/jcb.201104053>.
- [104] A. Reustle, M. Torzewski, Role of p38 MAPK in atherosclerosis and aortic valve sclerosis, *Int. J. Mol. Sci.* 19 (2018). <https://doi.org/10.3390/ijms19123761>.
- [105] T. Borbiev, A. Birukova, F. Liu, S. Nurmukhambetova, W.T. Gerthoffer, J.G.N. Garcia, A.D. Verin, p38 MAP kinase-dependent regulation of endothelial cell permeability, *Am. J. Physiol. - Lung Cell. Mol. Physiol.* (2004). <https://doi.org/10.1152/ajplung.00372.2003>.
- [106] S.A. Potthoff, S. Stamer, K. Grave, E. Königshausen, S.H. Sivritas, M. Thieme, Y. Mori, M. Woznowski, L.C. Rump, J. Stegbauer, Chronic p38 mitogen-activated protein kinase inhibition improves vascular function and remodeling in angiotensin II-dependent hypertension, *JRAAS - J. Renin-Angiotensin-Aldosterone Syst.* 17 (2016). <https://doi.org/10.1177/1470320316653284>.
- [107] J.D. Bernet, J.D. Doles, J.K. Hall, K. Kelly Tanaka, T.A. Carter, B.B. Olwin, P38 MAPK signaling underlies a cell-autonomous loss of stem cell self-renewal in skeletal muscle of aged mice, *Nat. Med.* (2014). <https://doi.org/10.1038/nm.3465>.
- [108] J. Segalés, E. Perdiguero, P. Muñoz-Cánoves, Regulation of muscle stem cell functions: A focus on the p38 MAPK signaling pathway, *Front. Cell Dev. Biol.* 4 (2016). <https://doi.org/10.3389/fcell.2016.00091>.
- [109] F. Girardi, F. Le Grand, Wnt Signaling in Skeletal Muscle Development and Regeneration, in: *Prog. Mol. Biol. Transl. Sci.*, 2018. <https://doi.org/10.1016/bs.pmbts.2017.11.026>.
- [110] F. Luppi, P. Spagnolo, S. Cerri, L. Richeldi, The big clinical trials in idiopathic pulmonary fibrosis, *Curr. Opin. Pulm. Med.* 18 (2012). <https://doi.org/10.1097/MCP.0b013e3283567ff9>.

- 907 [111] L. Fang, A.J. Murphy, A.M. Dart, A clinical perspective of anti-fibrotic therapies for cardiovascular disease,
908 Front. Pharmacol. 8 (2017). <https://doi.org/10.3389/fphar.2017.00186>.
- 909 [112] T. Desgeorges, S. Liot, S. Lyon, J. Bouvière, A. Kemmel, A. Trignol, D. Rousseau, B. Chapuis, J. Gondin,
910 R. Mounier, B. Chazaud, G. Juban, Open-CSAM, a new tool for semi-automated analysis of myofiber cross-
911 sectional area in regenerating adult skeletal muscle, Skelet. Muscle. (2019). [https://doi.org/10.1186/s13395-](https://doi.org/10.1186/s13395-018-0186-6)
912 018-0186-6.
- 913

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Full_Reg_Number	Synonyms	Targets	Fold change (TGFb/sample)	p value	Cell number	Output	Selected for second screen
QCR0003188801	1+P27b, P27b-00	CD32 (CD31, p34cdc2, CDC28p/Cyclin B, CDK4/Cyclin D1	1.46	0.2701	1810	NEG	
QCR000325401	Q271 (Chromomycin)	CDK1 (CDK1, MAP3K1)	2.18	0.0131	1578	NEG	
QCR0000788401	1-Naphthyl PP1, 1-NA-PP-1	SRK (c-SRC), FYN (p59lyn), ABL (ABL)	6.93	0.00023	1423	SURV	
QCR0000513801	10 DEBIC hydrochloride	AKT1 (PKB)	1.76	0.07089	1697	NEG	
QCR0000480801	2-(p-hydroxyvanilino)-4-(p-chlorophenyl)thiazole, SKI-H, ABC-29440	SPK1, SPHK, SPK3, Sphingosine Kinase	4.67	0.0024	2081	NEG	
QCR0000480401	2-(p-hydroxyvanilino)-4-(p-chlorophenyl)thiazole, SKI-H, ABC-29440	SPK1 (SPHK, SPK3, Sphingosine Kinase)	1.06	0.81000	2494	NEG	
QCR0000441401	2-methylthymine-4,5,6,7-tetrabromo-1H-benzimidazole, DNMT, Casin Kinase II	CK1a (Casin Kinase 1, CK1)	0.91	0.74226	2179	NEG	
TK1 QCR000130401	2-Thio-1-iodobenzyl-5-(1-pyrrolidin-1-yl-3-ethyl)oxazole	CK1b	1.11	0.6696	1650	NEG	
QCR0000482401	5-(3-Methoxy-4-(4-methoxybenzyl)oxybenzyl)pyrimidine-2,4-diamine	FMS (FMS, CSF-1R, CSF1R)	1.29	0.60553	2100	NEG	
QCR0000711401	1-iodotubercidin	AK (Adenosine Kinase, CK1a (Casin Kinase 1, CK1)	1.53	0.07848	2816	NEG	
QCR0000112401	6-(4-(2-Furanyl-5-(1-pyridoxylphenyl)-3-pyridin-4-ylpyrazolyl)-5-pyrimidin, Dorsomorphin, BMS-73, AMPK inhibitor, Compound C	AMPK, ALK, BMPK1 (ALK1), BMPK1B (ALK2)	2.81	0.00431	1510	SURV	
QCR0000112401	6-bromindirubin-3'-oxime, BIO	GSK3	1.27	0.31127	3355	NEG	
QCR0000509401	7-((cyclopentyl-1-(4-phenoxyphenyl)-2H-pyrazol-2-yl)-d)pyrimidin-4-ylamine	LCR (p38cdc), SRK (c-SRC)	6.30	0.00103	2208	NEG	
QCR0008716401	A-107072, A-107072-2	CK1	0.82	0.2922	2385	NEG	
QCR0008706401	A-769662, A-769662	AMPK	1.76	0.00767	3498	POS	
QCR0000752401	A-83-01	TGFBR1 (ALK5)	7.70	0.00018	1573	SURV	
QCR0000536801	A-441654, A-654	AKT1 (PKB)	0.67	0.35336	1162	SURV	
QCR0007448401	AB12134C3, PKM inhibitor	PKM (PKM)	0.92	0.48484	1648	NEG	
QCR0000739801	ABT-702 dihydrochloride	AK (Adenosine Kinase)	1.18	0.47832	3518	NEG	
QCR0001138401	ABT-869, Imlenib, Ab-39324, RG-3635	KDR (VEGFR2, VEGFR, FLK1), PDGFRb (PDGFR, PDGFR1), FMS (c-FMS, CSF-1R, CSF1R), FLT3 (FLT3, FLT2)	1.83	0.00072	1933	SURV	
QCR0001166401	AC-220, AC-220, Quizartinib, AC01020	FLT3 (FLT3, FLT2), FMS (c-FMS, CSF-1R, CSF1R), KIT (c-KIT), PDGFRb (PDGFR, PDGFR1), RET	3.55	0.00034	1289	SURV	
QCR0008728401	ABE-788, ABE-788, NVP-ABE-788	EGFR (ERBB1, HER1), ERBB2 (TKR1, HER2, NEU), KDR (VEGFR2, VEGFR, FLK1)	3.42	0.00003	1453	SURV	
QCR0000434401	ABG-3482	PKC-1 (PKC)	1.77	0.04674	2458	POS	
QCR0000778401	AG-18, AG-50810, TyrosinotinA23	EGFR (ERBB1, HER1), PDGFRb (PDGFR, PDGFR1)	2.27	0.00734	3522	POS	
QCR0000779401	AG-213, Tyrosinotin AG-213	EGFR (ERBB1, HER1), PDGFRb (PDGFR, PDGFR1)	1.15	0.50776	3632	NEG	
QCR0000774401	AG-490, Tyrosinotin AG-490	EGFR (ERBB1, HER1), JAK2, JAK2/J351, JAK3/J3P-3, JAK3/MAPK	1.46	0.10701	2528	NEG	
QCR0000780401	AG-494	EGFR (ERBB1, HER1)	1.56	0.07194	3631	NEG	
QCR0000781401	AG-825, Tyrosinotin AG-825	ERBB2 (TKR1, HER2, NEU)	1.34	0.20138	3180	NEG	
QCR0001180401	AG13958, AG-013958	PDGFRb (PDGFR, PDGFR1), KDR (VEGFR2, VEGFR, FLK1)	2.10	0.00268	1850	NEG	
QCR000044002	AG-132 Kinase inhibitor	AKT1 (PKB), AKT2 (PKB)	1.48	0.10256	2989	NEG	
QCR0001150401	AG-14	AKT1 (PKB)	1.61	0.00524	2989	NEG	
QCR0001151801	AG-1+1.2	AKT1 (PKB), AKT2 (PKB)	2.25	0.00364	3055	POS	
QCR0000751401	Alteplase/alteplase	CD32 (CD31, p34cdc2, CDC28p/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK5/p35, GSK3)	3.19	0.00180	1576	NEG	
QCR0001138401	Alteplase, HMR-1275, 1-868273, MDL-107826A, NSC-649890, 1-688276, Flapostinidol	CD32 (CD31, p34cdc2, CDC28p/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK4/Cyclin D1, CDK6/Cyclin D3, CDK7, CDK9/Cyclin T3, Bcl-2, Mcl-1, Survivin, XIAP	0.13	0.00000	89	SURV	
QCR0001172401	AMG-475	CK1 (p38cdc), SRK (c-SRC), KDR (VEGFR2, VEGFR, FLK1), p38	1.55	0.12178	1302	SURV	
QCR0007447401	AMG-900	AurA (AuroraA), AurB (AuroraB), AurC (AuroraC)	0.93	0.62919	2820	NEG	
QCR0001189401	AMG-704-1	CD2 (TIGR)	1.32	0.00681	1577	SURV	
QCR0008751401	AMK458	MET (c-MET, HGF)	1.64	0.00924	2988	POS	
QCR0001141401	AP-24534, Ponatinib	BCL, ABL1 (ABL), SRC (c-SRC), FLT3 (FLT3, FLT2), KIT (c-KIT), RET, PDGFRb (PDGFR, PDGFR1), FGFR1 (FLT2), FGFR2, FGFR3	1.27	0.25139	328	SURV	
QCR000874601	AP-24534, Ponatinib	KIT, KDR (VEGFR2, VEGFR, FLK1), FLT3 (VEGFR3), SRC (c-SRC), KIT (c-KIT)	1.75	0.00626	2893	NEG	
QCR0000513401	AP-3, Triciribine, NSC14020, TCN, Triciribine, VQD-002	AKT1 (PKB)	0.18	0.00165	222	SURV	
QCR0001340401	AR-A01418	GSK3	1.13	0.57700	2974	NEG	
QCR0000113401	Arctigenin, 1-Arctigenin	MAP3K1 (MEK1), DNA TopoisomeraseII, HIV Integrase, NFkB, AP-1	1.62	0.00606	2981	NEG	
QCR0000778401	Arctiflavon A	CDK4/Cyclin D1, CaMK2b (c-mk2)	0.91	0.68006	3067	NEG	
QCR000790401	ARG-197, Thapsigargin	MET (c-MET, HGF)	1.38	0.14162	655	SURV	
QCR0000713401	AS-152424	PI3K	1.60	0.00573	1427	NEG	
QCR0000081401	AS-601245	IKK1 (IKK)	1.37	0.20541	1227	SURV	
QCR0003201401	AS-70326, MCL1933698	MAP3K1 (MEK1), MAP2K2 (MEK2)	0.74	0.04494	1509	SURV	
QCR0000208401	AS604810, AS-604810, AS-604810	PI3K	1.38	0.22665	1403	NEG	
QCR0008722401	AS605240, AS-605240	PI3K	0.92	0.72046	2733	NEG	
QCR0004079401	ASC-033, APY-33	RET (RET3, ERN1)	1.32	0.16899	3220	NEG	
QCR0004080401	ASC-081, APY-81	RET (RET3, ERN1)	1.29	0.00000	1825	NEG	
QCR0004081401	ASC-081, APY-81	RET (RET3, ERN1)	1.42	0.12687	141	SURV	
QCR0004082401	ASC-082, APY-82	RET (RET3, ERN1)	0.38	0.00395	2843	NEG	
QCR0004083401	ASC-086, APY-86	RET (RET3, ERN1)	1.75	0.01600	362	SURV	
QCR0008697401	ASP-3026	ALK, EGFR (ERBB1, HER1)	1.24	0.08435	2768	NEG	
QCR0001160401	AT-719 derivative, AT-71919M derivative	Unknown	2.05	0.07255	3282	NEG	
QCR0001160801	AT-719 hydrochloride, AT-71919hydrochloride	CD32 (CD31, p34cdc2, CDC28p/Cyclin B, CDK2/Cyclin A, CDK3/Cyclin E, CDK4/Cyclin D1, CDK5/p35, CDK6/Cyclin D3	3.84	0.00001	1521	SURV	
QCR0008727401	AT7867, AT-7867	AKT1 (PKB), AKT2 (PKB), AKT3 (PKB), p70S6K (S6K, S6K1)	1.28	0.00003	1315	SURV	
QCR0001155401	AT9283, AT-9283	AurA (AuroraA), AurB (AuroraB), JAK2, JAK3, ABL1 mutant (ABL mutant), FLT3 (FLT3, FLT2)	0.68	0.00391	450	SURV	
QCR0008717401	Aurora A inhibitor 1	AurA (AuroraA)	1.06	0.76626	2398	SURV	
QCR0001161401	AV-412, MP-412	EGFR (ERBB1, HER1), ERBB2 (TKR1, HER2, NEU)	1.46	0.08671	1054	SURV	
QCR0001151401	AVP51, NN951, Tivozanib	FLT3 (VEGFR3), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3), KIT (c-KIT), PDGFRb (PDGFR, PDGFR1), p38 MAPK	1.47	0.03034	2708	NEG	
QCR0000902401	Avrimib, AG-13736	PDGFRb (PDGFR, PDGFR1), FLT3 (VEGFR3), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3), FMS (c-FMS, CSF-1R, CSF1R)	0.73	0.00181	139	SURV	
QCR0007882401	AZ-1146, AZ-3146	TK (ESK, MPS1), Mitogen-activated protein kinase-1 (Mps1) kinase	1.41	0.09691	2384	NEG	
QCR0008729401	AZ-1343 inhibitor	AK1 (Gp14, MAP3K7)	0.91	0.48623	564	SURV	
QCR0001184401	AZ-260	JAK2	1.71	0.01864	1247	SURV	
QCR0007472401	AZ628, AZ-628	RAF (B-raf), RAF1 (c-Raf)	1.60	0.04586	4107	POS	
QCR0001149801	AZD-7762, AZD-7762 hydrochloride	CHK1, CHK2	0.90	0.44862	521	SURV	
QCR0001143801	AZD-7762, AZD-7762 hydrochloride	CHK1, CHK2	1.76	0.01324	298	SURV	
QCR0001194401	AZD-1152-HQPA, Saracatinib	AurB (AuroraB)	1.30	0.18920	923	SURV	
QCR0001139401	AZD-1152, Saracatinib	AurB (AuroraB)	1.38	0.20552	1155	SURV	
QCR0007932401	AZD-1480, AZD1480	AK1, JAK2, JAK3	0.81	0.26910	1587	SURV	
QCR0007901401	AZD-4547	FGFR1 (FLT2), FGFR2, FGFR3, FGFR4	0.67	0.04845	1008	SURV	
QCR0008729401	AZD-5363, AZD5363	AKT1 (PKB), AKT2 (PKB), AKT3 (PKB), p70S6K (S6K, S6K1)	1.87	0.00658	1688	NEG	
QCR0003258401	AZD-8055, AZD-8055, AZD8055	PRAP RATOR, PRAP1, mTORC1, mTORC2	3.23	0.00035	1408	SURV	
QCR0000340401	AZD9130, Saracatinib, NSC-735464	ABL1 (ABL), SRC (c-SRC), KIT (c-KIT)	1.20	0.38620	501	SURV	
QCR000790401	AZD-6141, AZD-5418	CD32 (CD31, p34cdc2, CDC28p/Cyclin B, CDK2/Cyclin A, CDK9/Cyclin T3	0.91	0.50964	1313	SURV	
QCR000047401	AZD-6244, ARRY-142886, AZD-6244, Selumetinib	MAP3K1 (MEK1), MAP2K2 (MEK2), ERK2 (ERK, p38)	2.06	0.04377	2501	POS	
QCR0008724401	AZD-6482, AZD-6482	PI3K	1.44	0.01050	2848	NEG	
QCR0007894401	AZD-8330, AZD-8310, ARRY-42470A, ARRY-704	MAP3K1 (MEK1), MAP2K2 (MEK2)	0.97	0.84613	523	SURV	
QCR0008755401	Bay 57-0352, Telatinib	KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3), KIT (c-KIT), PDGFRb (PDGFR, PDGFR1)	0.96	0.73054	2852	NEG	
QCR0000315801	Bay 61-4606 hydrochloride, ac-202351	SYK	2.58	0.00686	1682	NEG	
QCR0007903401	Bay 73-4506, Regorafenib	KIT (c-KIT), PDGFRb (PDGFR, PDGFR1), RET, RAF2 (c-Raf), BRAF (B-raf), TRK2 (TRK), KDR (VEGFR2, VEGFR, FLK1), p38 MAPK	1.39	0.10227	1844	NEG	
QCR0008699401	BGI-398, NVP-BGI-398, NVP-BGI398	FGFR1 (FLT2), FGFR2, FGFR3, FGFR4	0.80	0.18353	1788	NEG	
QCR0007463401	BGT-226, NVP-BGT-226, NVP-BGT226	PI3K, PRAP RATOR, PRAP1	0.63	0.00518	861	SURV	
QCR0000544401	B1-5336	PLK1 (PLK, STPK13)	0.81	0.50603	295	SURV	
QCR0000579401	B1-7803	IKK1 (IKK)	1.69	0.05152	2581	NEG	
QCR0001149401	B1-6727, volastertib	PLK1 (PLK, STPK13)	4.47	0.00010	1715	SURV	
QCR0001188401	B1-6727	PLK1 (PLK, STPK13)	2.80	0.00261	2196	NEG	
QCR0001143401	BBI-1120, Intendatin, Vargatef	KDR (VEGFR2, VEGFR, FLK1), PDGFRb (PDGFR, PDGFR1), MET (c-MET, HGF)	1.49	0.02881	2239	NEG	
QCR0000346801	BB10-1330 dihydrochloride	EGFR (ERBB1, HER1)	0.36	0.00001	2290	NEG	
QCR0000484401	BB10-2892, Tyrosinotin, dactinib	EGFR (ERBB1, HER1)	0.43	0.00641	1620	SURV	
QCR0000346801	BBX-1382 dihydrochloride, Faldindol	EGFR (ERBB1, HER1)	1.84	0.00718	2827	POS	
QCR0001312401	BHR-796, Doramipidol	p38 MAPK	1.47	0.01682	3099	NEG	
QCR0000419801	Bisindolylmaleimide hydrochloride, FG-10203X HCl, G6-6850 HCl	Bisindolylmaleimide X, HCl salt	2.96	0.00451	1608	NEG	
QCR0001185801	Bisindolylmaleimide X, HCl salt	PKC	2.11	0.00655	3350	POS	
QCR0007885401	BIX-0189, BIX-02189	MAP2K3 (MEK3), ERK5 (BMK1, PRKMY)	2.03	0.00896	2489	SURV	
QCR0008713401	BIX-0547 inhibitor	PI3K, PI3K, PI3K, MAPKAPK1 (CK1), PI3K-43, p38-6SK2	10.17	0.00006	140	SURV	
QCR0007448401	BKM120, BKM-120, NVP-BKM-120	PI3K	1.57	0.05329	755	SURV	
QCR0008709401	BMS-777607, BMS-777607	MET (c-MET, HGF), ALK (ALK) (RON), MIST1R (TRKB), TRKS (SKY, TIF, BTK)	1.91	0.04025	2788	NEG	
QCR0007883401	BMS-794833, BMS-794833	MET (c-MET, HGF), KDR (VEGFR2, VEGFR, FLK1)	1.77	0.00696	2713	NEG	
QCR0001179401	BMS-2	MET (c-MET, HGF)	2.48	0.00147	1342	SURV	
QCR0001174401	BMS-3	LMNK1 (LMNK)	0.93	0.63912	553	SURV	
QCR0000336801	BMS-45541	AK5 (AK2)	1.43	0.15871	2341	NEG	
QCR0001175401	BMS-5	LMNK1 (LMNK)	2.30	0.00144	1419	SURV	
QCR0000778401	BMS-536224	GF13 (G13, GFR)	1.43	0.15517	2606	NEG	
QCR0008730401	BMS-581664, Brinavat aminate	FGFR1 (FLT2), FGFR2, FGFR3, FLT3 (VEGFR3), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3)	2.01	0.00266	1165	SURV	
QCR0000546401	BMS-599626, AC-480	EGFR (ERBB1, HER1), ERBB2 (TKR1, HER2, NEU), ERBB3 (HER3), ERBB4 (HER4)	1.77	0.06487	2432	NEG	
QCR0007460401	BMS-734807	GF13 (G13, GFR)	1.43	0.15517	2606	NEG	
QCR0000604401	Bosutinib, B6-606	BCR, ABL1 (ABL), SRC (c-SRC), FGR (SRC), LYN	0.53	0.10338	248	SURV	
QCR0001119401	Brinavat, BMS-540215	KDR (VEGFR2, VEGFR, FLK1), FGFR1 (FLT2), FGFR3, FLT4 (VEGFR3), FGFR2	1.34	0.11377	3094	NEG	
QCR0007467801	BR-181 hydrochloride	CDK7	1.53	0.04049	2753	SURV	
QCR0000318401	BR-795	CDK1 (CDK1, STPK13), TRK1, IKK	1.24	0.43337	415	POS	
QCR0001181401	BX112, BX-912	PDK1 (PDPR1)	2.59	0.00149	3162	POS	
QCR0008740401	BT-719, NVP-BT-719	PI3K	1.48	0.02497	2003	NEG	
QCR0001129401	C-1	PKC	1.48	0.01757	2869	NEG	
QCR0007897401	CAL-101, GS-1101	PI3K	1.73	0.03685	2637	POS	
QCR0001172401	CAL-101, INK-401	PI3K (PI3K, INK2)					

DCR000113A01	Cediranib, AZD-2171, Recetin	FLT3 (VEGFR3), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3), KIT (c-KIT), PDGFRb (PDGFR, PDGFR1)	2.95	0.00045	1544	SURV
DCR000079A01	CRK 134	ATK, ATK	1.29	0.02008	3194	NEG
DCR000047A02	CGP 57380, CGP 57380	PKA1	7.01	0.00262	2414	POS
DCR000034A01	CGP 57380, CGP 57380	MNK1	1.29	0.27700	3162	NEG
DCR000037B01	CGP 57380, CGP 57380	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B	1.29	0.00681	1124	SURV
DCR000871A01	CH-424802, AP-802, CH-5424802	ALK	0.36903	0.34903	1868	SURV
DCR000055B01	Chenerythrine chloride	PKC	0.30	0.01899	85	SURV
DCR000078A01	Chk1 124	CHK1	2.81	0.00060	1434	SURV
DCR000021A01	Chk1-98014 isomer, CT-98014	CHK1	0.05	0.03981	946	SURV
DCR000034A01	Chk2 inhibitor II, 139253	CHK2	1.41	0.16500	3273	NEG
DCR000051A01	CI 1033, Cimetidine, PD-181805, SN-26608	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU), Irreversible	1.44	0.13002	441	SURV
DCR000051A01	CI 755673	PKD1 (PKD)	3.81	0.00365	2209	POS
DCR0000771A01	Compound 401	DNAPK (DNA-PKcs), FRAP (mTOR, FRAP1)	1.81	0.03360	3336	POS
DCR000034A01	Compound 52, No-52	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B	2.07	0.02010	2420	NEG
DCR000050A01	CP-690550, CP-690550, Tacotimib, Tofacitinib	JAK3	1.18	0.49961	485	SURV
DCR000116A01	CP-724714	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	2.37	0.00318	3056	POS
DCR000747A01	CP-666722, CP-466722	PKA	0.91	0.65696	2903	NEG
DCR000876A01	Crenolamb, ARD-002, CP-868596	PDGFRa (PDGFR2), PDGFRb (PDGFR, PDGFR1)	3.10	0.00002	835	SURV
DCR000028A01	CT-49021, CHR-99021	GSK3	0.89	0.98092	1345	SURV
DCR000031A01	CS-9045	CDK3 (CK2, CK1, Caten kinase)	0.87	0.00564	809	SURV
DCR000115A01	CYC-116	AurA (AuroraA), AurB (AuroraB), KDR (VEGFR2, VEGFR, FLK1)	1.38	0.16595	997	NEG
DCR000112B01	Cyclopiolin B, Cyclopiolin B	PLK1 (PLK, STPK13)	1.38	0.11891	2941	NEG
DCR000112B01	Cyclopiolin B, Cyclopiolin B	PLK1 (PLK, STPK13)	1.33	0.88954	3140	NEG
DCR000116B01	CYT11387, CYT387, CYT-387, Mometanib	JAK2	1.83	0.01044	1435	SURV
DCR000051A01	D4476	CK1a (Caten kinase 1, CK1)	3.53	0.00356	2043	POS
DCR000871A01	Dacatinib hydroate, PF-00298804, PF-00298804-03, PF-299, PF-298804	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU), ErbB4 (HER4)	4.75	0.00001	1518	SURV
DCR000050A01	Daxatinib, BM5-354825, Spynel, NSC-732517	BLK, ABL1 (ABL), SRC (c-SRC), EphA1 (EPH), FYN (p59lyn), LCK (p56lck), PDGFRb (PDGFR, PDGFR1), YES, Etk2 (ERK, p38)	1.65	0.06302	723	SURV
DCR000111A02	Diaminoglutimidine, D1	PDK1 (PDPR1)	2.46	0.00526	3171	NEG
DCR000876B01	Dinacitib, MK-7965, NSC-727135, SCH-727965	Pin kinase	0.94	0.63635	1446	NEG
DCR000774A01	Dioxanetol, MK-7965, NSC-727135, SCH-727965	CDK2/Cyclin A, CDK5/p25, CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK9/Cyclin T1	0.28	0.00001	119	NEG
DCR000774A01	Dioxanetol, MK-7965, NSC-727135, SCH-727965	DNAPK (DNA-PKcs, FRAP (mTOR, FRAP1)	1.00	0.98617	3385	NEG
DCR000115B01	DMG-2, dicyclanetol	PDGFRb (PDGFR, PDGFR1)	1.37	0.00581	1468	NEG
DCR000034A01	Doromorphin hydrochloride, BML-275, AMPK inhibitor, Compound C	AMPK, ALK2, BMPR1A (ALK3), BMPR1B (ALK6)	1.46	0.03790	2589	NEG
DCR000765A01	Doximib, CHR-258, TKI-252, GPR-258	KDR (VEGFR2, VEGFR, FLK1), FLT3 (STK1, FLK2), KIT (c-KIT), PDGFRb (PDGFR, PDGFR1)	2.54	0.04341	682	SURV
DCR000115B01	D7080, D-7080, DR-203492-00, Levamisole	EGFR (ERBB1, HER1), Irreversible	0.00125	0.1558	1558	SURV
DCR000765A01	EM-785, CL-387785	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	0.98	0.92561	3189	NEG
DCR000808A01	Elagac acid	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.27	0.00875	3322	NEG
DCR000319A02	EMD-1214063	KIT (c-KIT), MET, HGFR	0.22	0.2280	2328	NEG
DCR000319B01	EMD-2076, EMD-981693	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.27	0.00875	3322	NEG
DCR000116A01	Enstatinib, LY-317615	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.20	0.32800	1560	SURV
DCR000081A01	EO-1428	AurA (AuroraA)	1.57	0.00625	3285	NEG
DCR000051A01	ER 77119 malate	AurA (AuroraA)	1.87	0.00204	4193	POS
DCR000115A01	ERK2 inhibitor	ERK	0.20	0.00000	209	SURV
DCR000031B01	Erlotinib HCl, CP-358774, OSI-774, Tarceva, NSC-718781, RG-1415, RG-50-8231	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	2.25	0.00550	294	SURV
DCR000031B01	Erlotinib, OSI-774, Tarceva, NSC-718781, RG-1415, RG-50-8231	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	1.10	0.00744	2795	NEG
DCR000031B01	Erlotinib, OSI-774, Tarceva, NSC-718781, RG-1415, RG-50-8231	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	1.28	0.28647	1632	NEG
DCR000031B01	Erlotinib, OSI-774, Tarceva, NSC-718781, RG-1415, RG-50-8231	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	1.64	0.27689	1663	NEG
DCR000031B01	Erlotinib, OSI-774, Tarceva, NSC-718781, RG-1415, RG-50-8231	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	1.10	0.00744	2795	NEG
DCR000031B01	Erlotinib, OSI-774, Tarceva, NSC-718781, RG-1415, RG-50-8231	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	1.22	0.38975	2880	NEG
DCR000031B01	Erlotinib, OSI-774, Tarceva, NSC-718781, RG-1415, RG-50-8231	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	1.60	0.15495	1633	NEG
DCR000117A01	EGC-0079, AKI-00341677	BRAF (B-Raf)	1.36	0.13560	3074	NEG
DCR000028B01	EGC-0941	PI3K	2.00	0.00034	1462	SURV
DCR000028B01	EGC-0941 isomerase, NG-7321, Piclitinib	PI3K	3.58	0.00002	762	SURV
DCR000031B01	Egfrinib, EMD-2076, EMD-981693	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	3.63	0.00265	1515	SURV
DCR000078A01	Emertinib	EGFR (ERBB1, HER1)	1.74	0.03944	3541	POS
DCR000065A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.50	0.00044	1206	SURV
DCR000031B01	Emertinib, EMD-2076, EMD-981693	EGFR (ERBB1, HER1), ErbB2 (TKR1, HER2, NEU)	1.15	0.58992	751	SURV
DCR000876A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.44	0.00054	2524	NEG
DCR000876A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.48	0.00226	1085	NEG
DCR000876A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.52	0.00564	1194	SURV
DCR000747A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	0.84	0.04568	3425	NEG
DCR000112A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.27	0.28603	2628	NEG
DCR000876A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.60	0.03796	294	SURV
DCR000028A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.61	0.07002	472	SURV
DCR000116A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.45	0.00673	1208	SURV
DCR000808A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.74	0.03799	3254	POS
DCR000115A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	2.52	0.00147	1536	SURV
DCR000031B01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.31	0.28065	1952	NEG
DCR000876A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	6.11	0.00000	1572	SURV
DCR000031B01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	2.09	0.05986	2084	NEG
DCR000028A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.12	0.61564	1603	SURV
DCR000031B01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.28	0.30885	3072	NEG
DCR000031B01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.19	0.43473	3111	NEG
DCR000031B01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	2.16	0.01363	1293	POS
DCR000031B01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.29	0.27768	3135	NEG
DCR000028A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.76	0.00676	2626	NEG
DCR000031B01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	4.00	0.00324	2778	POS
DCR000117B01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	2.62	0.00169	1366	SURV
DCR000774A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.22	0.34900	3667	NEG
DCR000876A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	0.86	0.55590	156	SURV
DCR000774A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.87	0.00900	869	SURV
DCR000047A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	0.52	0.05470	298	SURV
DCR000117A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	0.79	0.19401	1408	NEG
DCR0000651A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	2.41	0.00650	3192	POS
DCR000028A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	2.26	0.05734	1213	SURV
DCR000747A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.09	0.57580	4037	NEG
DCR000031B01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.52	0.13862	3354	NEG
DCR000031B01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.99	0.02411	1523	NEG
DCR000028A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	2.71	0.00365	1524	SURV
DCR000774A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.07	0.78009	1122	SURV
DCR000747A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.27	0.24037	3296	NEG
DCR000876A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.35	0.00363	1515	NEG
DCR000031B01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	2.65	0.00476	1572	SURV
DCR000031B01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.58	0.00573	2758	NEG
DCR000774A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	2.54	0.00309	876	SURV
DCR000774A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.13	0.58738	3590	NEG
DCR000117A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	0.11966	0.11966	2913	NEG
DCR000077A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.44	0.12579	892	SURV
DCR000028A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	0.79	0.25564	609	SURV
DCR000876A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.35	0.00461	926	SURV
DCR000116A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.66	0.06291	2975	NEG
DCR000048A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.53	0.16364	1642	NEG
DCR000065A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.60	0.06746	3351	NEG
DCR000031B01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	3.58	0.00195	335	SURV
DCR000077A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.58	0.10424	3078	NEG
DCR000048A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	4.23	0.00037	1697	POS
DCR000065A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	2.34	0.00655	1562	SURV
DCR000116A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.51	0.11590	2127	NEG
DCR000048A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.86	0.01971	1405	NEG
DCR000077A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	0.99	0.93389	3003	NEG
DCR000048A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	2.25	0.03067	2448	NEG
DCR000747A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A, CDK5/p25	1.00	0.89770	1753	NEG
DCR000118A01	EMD-1214063	CDK2 (CDK1, p34cdc2, CDC2b)/Cyclin B, CDK2/Cyclin A				

DKR000872101	LY2228220, LY2228820	p38a, p38b	1.62	0.00410	3028	POS	x
DKR000781A01	LY278444, LY2784544	JAK2	0.99	0.00268	503	SURV	
DKR000784A01	LY283119, LY283119	CDK4/Cyclin D3, CDK6/Cyclin D3, PIM1	2.16	0.00304	293312	POS	x
DKR000051201	Maxitinib mesylate, AB1010	KIT (c-KIT), PDGFRb (PDGFR, PDGFR1), FGFR3	3.07	0.01221	2058	POS	x
DKR001148A01	MA251, MA251	AKT1 (AKG1)	0.55351	0.00000	469	SURV	
DKR000781A01	Meca-25.6	AKT1 (PKA), AKT2 (PKB)	1.80	0.00893	3841	POS	
DKR0001170A01	Merck-5, MK-5, JAK inhibitor 1	JAK1, JAK2, JAK3, TYK2	1.82	0.00870	2245	NEG	
DKR000871A01	MK20-265	MET (c-MET, HGFb), RON (MST1R), TRK2 (TRK), FLT1 (VEGFR1), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3)	0.91	0.06370	2185	NEG	
DKR00011130A01	MK-1775, MK1775	MET (c-MET, HGFb), RON (MST1R), TRK2 (TRK), FLT1 (VEGFR1), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3)	0.16	0.74738	374	SURV	
DKR0001121801	MK-2206	AKT1 (PKA)	1.20	0.38100	2610	NEG	
DKR000871A01	MK-1508, VU-689	AurA (Aurora)	1.31	0.02900	1187	NEG	
DKR0007451A01	MK-6031	MET (c-MET, HGFb)	1.10	0.62710	1775	NEG	
DKR0007980A01	ML-119, ML-119	mlnMLCK (MLCK, myosin light chain kinase, MLC)	1.51	0.10264	3529	NEG	
DKR0000239A01	MLN-518, CT-55518, Tanduimib	FLT3 (FLT3, FLT3), KIT (c-KIT), PDGFRb (PDGFR, PDGFR1)	1.65	0.08312	1597	SURV	
DKR0001142A01	MLN-8237	AurA (Aurora)	1.08	0.65042	1020	SURV	
DKR000876A01	MLN-8054, MLN-8054	AurA (Aurora)	1.41	0.03509	1547	SURV	
DKR00005470A01	Momotinib (dihydrochloride), AMG-706	KIT (c-KIT), PDGFRb (PDGFR, PDGFR1), KDR (VEGFR2, VEGFR, FLK1)	1.93	0.04695	1901	NEG	
DKR0001152A01	MP-470, Amavivatinib	ALK (ALK), KIT (c-KIT), FLT3 (FLT3, FLT3), MET (c-MET, HGFb), PDGFRa (PDGFR2), RET, RAD51 expression inhibitor	13.50	0.00002	1799	NEG	
DKR0000545A01	Mubritinib, TAK-165, DO4025	EGFR (TKR, HER2, NEU)	0.41	0.10931	99	SURV	
DKR0000539A01	N-Propyl-6-(N-(2,4,6-trichlorophenyl)urea), Rho Kinase inhibitor II	ROCK1, ROCK2 (ROCK)	0.98	0.00463	1074	SURV	
DKR0003195A01	Necrostatin-1	RIPK1 (RIP)	2.96	0.00577	2538	POS	
DKR0003195A01	Neratinib, HKI-272, CPD-820	EGFR (ERBB1, HER1), ERBB2 (TKR1, HER2, NEU)	4.58	0.00009	1578	SURV	
DKR0003300A01	NES125	EGFR	0.98	0.00401	1074	SURV	
DKR0003193A01	Nex Kinase Inhibitor	NKX	0.97	0.84888	1198	SURV	
DKR0004396A01	Nilotinib, Tasigna, AMN-107	BCK, ABL1 (ABL), PDGFRb (PDGFR, PDGFR1), KIT (c-KIT)	2.56	0.03747	2005	POS	x
DKR000871A01	NOD9-1	MAP3K5 (AKK)	1.68	0.00200	3110	NEG	
DKR0000772H01	NOC-109555 dioxolane, DDUG	CHK2	1.82	0.03192	3507	POS	
DKR0000239A01	NOC-625987	CDK4/Cyclin D1	1.61	0.00502	1892	NEG	
DKR0000512A01	NOC-693848	CDK2 (CDK1, p34cdc2), CDK2b/Cyclin B, CDK5/p25, GSK3	1.27	0.21175	1524	NEG	
DKR0000751A01	NOC-663284	cdk25	0.81	0.45881	175	SURV	
DKR0000751A01	NO-2058	CDK2 (CDK1, p34cdc2), CDK2b/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK5/p35, GSK3	1.24	0.30942	3544	NEG	
DKR0000239A01	NO-10101	CDK2 (CDK1, p34cdc2), CDK2b/Cyclin B, CDK2/Cyclin A	2.01	0.1974	1448	SURV	
DKR0000492A01	NU-7026, LY-293646	DNAPK (DNA-PKcs)	1.73	0.11221	1923	NEG	
DKR000871A01	NVP-ADW742, ADW-742, ADW, GSK-552602 A, NVP-ADW-742	IGF1R (IGF1R), IGF1R	4.72	0.00001	815	SURV	
DKR0000751A01	NVP-459-541, ADW-541	IGF1R (IGF1R), IGF1R	2.17	0.00886	1027	SURV	
DKR0000239A01	NVP-862235, BEZ235	PI3K	2.52	0.00879	982	SURV	
DKR000874A01	NVP-BHG712	RAF1 (c-Raf), SRC (SRC), ABL1 (ABL), EphB4	3.79	0.00001	1513	SURV	
DKR000871A01	NVP-85829	AKT2	0.153	0.17793	2718	NEG	
DKR000666A01	DKR000666A01	PIM1	3.97	0.00001	1578	SURV	
DKR000871A01	DKR000871A01	Histone Deacetylase (HDAC) Inhibitor	1.40	0.05362	2757	NEG	
DKR0000751A01	Oestrone	CDK2 (CDK1, p34cdc2), CDK2b/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK5/p35	1.07	0.70751	1708	NEG	
DKR0007931A01	On-019, On-019, Etoposide, Etoposide	FLT3 (FLT3, FLT3), KIT (c-KIT), PDGFRb (PDGFR, PDGFR1)	0.93	0.07731	1925	NEG	
DKR0003207A01	OS-027, Pyrenichloride	RAF1 (RAF1), MAP3K7	1.63	0.09540	1174	SURV	
DKR0007451A01	OS-106, Lintetide	RAF1 (RAF1), MAP3K7	1.31	0.16330	2001	POS	x
DKR000871A01	OS-930	KIT (c-KIT), KDR (VEGFR2, VEGFR, FLK1), FLT1 (VEGFR1), FMS (c-FMS, CSF-1R, CSF1R), RAF1 (c-Raf), LCK (p56lck), PDGFRb (PDGFR, PDGFR1)	1.48	0.03396	3167	POS	
DKR000874A01	OT-1841, inhibitor	AKT1 (PKA), MAP3K7	4.47	0.00001	1006	SURV	
DKR0000751A01	OT-1841, inhibitor	AKT1 (PKA), MAP3K7	1.13	0.60374	1408	NEG	
DKR00011230A01	Pargamib hydrochloride, GSK-786034, Amalix, Vortioxetine	FLT1 (VEGFR1), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3), KIT (c-KIT), PDGFRa (PDGFR2), PDGFRb (PDGFR, PDGFR1)	1.57	0.09878	2373	NEG	
DKR000871A01	PD-12745, Ibrutinib	BTK (Bruton's Tyrosine Kinase), LCK (p56lck), LYN	0.26	0.33769	1183	NEG	
DKR0000751A01	PD-323901, PD-323901	MAP2K1 (MEK1), MAP2K2 (MEK2)	1.18	0.41573	1912	NEG	
DKR0001153A01	PD-323991, PD-323991	CDK4/Cyclin D1, CDK5/p35	2.20	0.00238	1519	SURV	
DKR000494A01	PD-13605, AG-1517, Compound 32, SU-6711, ZM-252868, WHI-P79	EGFR (ERBB1, HER1), ERBB2 (TKR1, HER2, NEU), ERBB4 (HER4)	0.91	0.00500	2099	POS	x
DKR0000772A01	PD-158180	EGFR (ERBB1, HER1), ERBB2 (TKR1, HER2, NEU), ERBB4 (HER4)	2.84	0.00273	3376	POS	
DKR0000239A01	PD-169316	p38 MAPK	8.46	0.00024	1542	SURV	
DKR0000333A01	PD-173074	RAF1 (RAF1), MAP3K7	0.98	0.00001	1386	SURV	
DKR0000239A01	PD-180970	ABL1 (ABL), SRC (c-SRC)	1.29	0.25531	185	SURV	
DKR0000751A01	PD-184161	MAP2K1 (MEK1), MAP2K2 (MEK2)	1.08	0.88031	3028	NEG	
DKR0000239A01	PD-184553, D-1040	MAP2K1 (MEK1), MAP2K2 (MEK2), ERK2 (ERK, p38), RAF1 (c-Raf)	1.93	0.03426	1552	SURV	
DKR0000815A01	PD-195306	MAP2K1 (MEK1), MAP2K2 (MEK2)	1.22	0.37059	3414	NEG	
DKR0000530A01	PD-407824	CHK1, WEE1	0.01	0.00138	1882	POS	
DKR0000333A01	PD-408059	MAP2K1 (MEK1), MAP2K2 (MEK2), ERK2	1.18	0.44340	1987	NEG	
DKR0001136A01	PD04217903, PF-04217903, PF-4217903	MET (c-MET, HGFb)	1.49	0.04366	1892	NEG	
DKR0001136A01	PD04217903, PF-04217903, PF-4217903	MET (c-MET, HGFb)	3.26	0.00067	1434	SURV	
DKR0001136A01	PD173955, PD-173955	BCK, ABL1 (ABL)	2.13	0.01543	1790	POS	
DKR0001157A01	PD173955, PD-173955	BCK, ABL1 (ABL), SRC (c-SRC)	2.64	0.00080	1380	SURV	
DKR000870A01	PD180808	MAP2K1 (MEK1), MAP2K2 (MEK2)	1.24	0.05303	4410	NEG	
DKR000870A01	PD180808, ERK-565, WAY-685-569	EGFR (ERBB1, HER1)	1.48	0.00001	1548	SURV	
DKR0007450A01	PERK inhibitor	PERK (PERK)	2.30	0.00160	867	SURV	
DKR000895A01	PF-364022, PF-364022	MAPKAP2 (MK2)	0.92	0.74991	3285	NEG	
DKR000895A01	PF-364022, PF-364022, MK2 inhibitor, compound 35	MAPKAP2 (MK2)	0.93	0.00440	2700	SURV	
DKR0007888A01	PF-4708671, PF-4708671	PTGSR (GSK, SGL1)	1.03	0.86882	3207	NEG	
DKR0001131A01	PF-0234066, PF-234066, Crizotinib	ALK, MET (c-MET, HGFb)	2.18	0.01189	1538	SURV	
DKR0007451A01	PF-04691501, PF-04691501, PF-502	ERK, ERAP (MTOX, FRAP1)	2.84	0.00556	252	SURV	
DKR0000512A01	PF-573228	FAK (FAK)	2.60	0.00658	2525	POS	x
DKR0000512A01	PF-670462	CK1e, CK1d	4.62	0.00262	1977	NEG	
DKR0001188A01	PF611396, PF-611396	FAK (FAK), PKC2 (FAK2, PKC2B, CASK)	1.10	0.50435	2990	NEG	
DKR0001188A01	PF162271, PF-162271	FAK (FAK), PKC2 (FAK2, PKC2B, CASK)	4.00	0.00013	1023	SURV	
DKR0000772A01	PHA-665752	MET (c-MET, HGFb), RON (MST1R), KDR (VEGFR2, VEGFR, FLK1)	0.13	0.00000	3246	NEG	
DKR000871A01	PHA-680812	AurA (Aurora), AurB (Aurora), AurC (Aurora)	0.62	0.23300	1923	NEG	
DKR0001144A01	PHA-739358, Danusertib	ABL1 (ABL), RET, TRKA (TRK), AurA (Aurora), FGFR1 (FLT2)	1.85	0.00708	923	SURV	
DKR0000333A01	PHA-767491, hydrochloride	CDK2 (CDK1, p34cdc2), CDK2b/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK4/Cyclin D1, CDK5/p35	3.81	0.00288	1651	POS	x
DKR000788A01	PHA-793881	CDK2 (CDK1, p34cdc2), CDK2b/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK4/Cyclin D1, CDK5/p35	2.44	0.00009	1455	SURV	
DKR0008761A01	PHA-848125, PHA-848125AC	CDK2/Cyclin A, CDK2/Cyclin B, CDK4/Cyclin D1, CDK5/p35, CDK2/Cyclin E, CDK2 (CDK1, p34cdc2), CDK2b/Cyclin B	2.01	0.00111	504	SURV	
DKR0001144A01	PHA-909059, PHA-909059	CDK2/Cyclin A	0.92	0.73719	1378	SURV	
DKR0000333A01	PI-103	PI3K	2.12	0.01821	1402	SURV	
DKR0000333A01	PI-828	PI3K	2.05	0.01882	2501	POS	
DKR0001182A01	PI-83, PI-83	PI3K	1.41	0.07688	2385	NEG	
DKR000788A01	PI3 Kinase inhibitor 2	PI3K	1.13	0.50308	1987	NEG	
DKR0001182A01	PIK-294	PI3K	3.38	0.00049	1588	SURV	
DKR0001182A01	PIK-294	PI3K	2.36	0.00076	1590	SURV	
DKR0000333A01	PIK-75, hydrochloride	PI3K	0.19	0.00000	81	SURV	
DKR0000239A01	PIK-90	PI3K	2.22	0.01259	961	SURV	
DKR0000815A01	PIM1 Inhibitor 2	PIM1, PIM2	0.90	0.59174	3568	NEG	
DKR0000434A01	PIM1/2 Kinase Inhibitor VI	PIM1, PIM2	3.55	0.00076	1837	POS	
DKR0000530A01	Pimocricum, Elide, SDZ-ASM-981	Calcineurin (CN, PP-2B)	1.26	0.42761	364	SURV	
DKR0007451A01	PLC-11, GPR41253, Midostaurin	PLC	0.41	0.00206	3601	NEG	
DKR0000333A01	PLX-4032, GPR41253, Midostaurin	FLT3 (FLT3, FLT3)	1.60	0.09501	429	SURV	
DKR0007451A01	PLX-4032, Vemurafenib, Zelboraf, RG-7204, RO-5185426	BRAF (B-Raf)	0.96	0.81963	3040	NEG	
DKR0000530A01	PLX-4720, Raf Kinase Inhibitor V-1	BRAF (B-Raf)	0.96	0.00408	1065	POS	x
DKR0001126A01	PP-1, PP1, AGL-1872, Typhostin-PP1	SRC (c-SRC)	2.14	0.00288	1508	NEG	
DKR000843A01	PP-2, PP2, AG-1879	LCK (p56lck), FYN (p59lyn), HCK (p58hck), SRC (c-SRC)	2.95	0.01227	1841	POS	
DKR0000333A01	Pp242, Torkinib	EGFR (ERBB1, HER1)	1.85	0.00753	1337	SURV	
DKR0000333A01	Pp242, Torkinib	FRAP (MTOX, FRAP1)	3.03	0.00045	1533	SURV	
DKR0000333A01	Pp242, Torkinib	IGF1R (IGF1R)	2.23	0.01289	2737	POS	
DKR0000333A01	Pp242, Torkinib	IGF1R (IGF1R)	1.15	0.52870	2984	NEG	
DKR000874A01	PTK6 CompoundB	BRK (PTK6)	1.40	0.00218	2771	NEG	
DKR000870A01	PTK6 Inhibitor CompoundB	BRK (PTK6)	2.82	0.00004	1484	SURV	
DKR0000492A01	Purvalanil A, NG-95	CDK2 (CDK1, p34cdc2), CDK2b/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK4/Cyclin D1, CDK5/p35, DYRK1A (DYRK)	0.98	0.00981	278	POS	
DKR0000806A01	Purvalanil B, NG-95	CDK2 (CDK1, p34cdc2), CDK2b/Cyclin B, CDK2/Cyclin A, CDK2/Cyclin E, CDK4/Cyclin D1, CDK5/p35	1.72	0.14727	3479	NEG	
DKR0001186A01	PV-1015, NSC-744039	CHK2	2.63	0.00881	2284	NEG	
DKR000871A01	PX-866, UIMA-166, DMN-2-186, Somatib, NSC-722134	FAK, FAK, FAK Inhibitor	2.14	0.00011	1486	SURV	
DKR0000512A01	Quercetin	PI3K	2.08	0.04882	2030	POS	
DKR0007451A01	Quercetin	CK1a1 (CK2, CK1, Casein Kinase)	1.32	0.13383	3743	NEG	
DKR0000772A01	Quercetin	CK1a1 (CK2, CK1, Casein Kinase)	1.29	0.30622	3172	NEG	
DKR000117801	Q1487, B-1487	PI3K	2.64	0.00247	3333	POS	
DKR000781A01	R446, R-406, NSC-742317	FLT3 (FLT3, FLT3), IGF1 (IGF1, IGF1), IGF1R (IGF1R)	1.54	0.04635	677	SURV	
DKR000750A01	R61, R-788, Etoposide, Etoposide	FLT3 (FLT3, FLT3), IGF1 (IGF1, IGF1), IGF1R (IGF1R)	1.03	0.87075	1180	NEG	
DKR000789A01	RAF-265, RAF-265, CHIR-265	KIT (c-KIT), PDGFRb (PDGFR, PDGFR1), BRAF (B-Raf), RAF1 (c-Raf), KDR (VEGFR2, VEGFR, FLK1)	1.29	0.23292	1800	SURV	
DKR0000333A01	Rapamycin, Sirtinib, Rapamycin	PI3K	1.66	0.09618	1222	SURV	
DKR0000772A01	Rap-133, AG-133, ROR1A13, BAY-609766, Redemitein	MAP2K1 (MEK1), MAP2K2 (MEK2)	0.87	0.265	258	NEG	
DKR000871A01	RET						

DKC000050801	Ro-31-4220 mesylate, Bisindolylmaleimide IX	PKC		1.74	0.14356	2115	NEG	
DKC000746A01	RD-512676	MAP2K1 (MEK1), BRAF (B-raf), RAF1 (c-Raf)		1.57	0.04010	1235	SURV	
DKC0000318A01	Penciclovir, Cyt202, Selecticb	CDK2 (CDK1, p34cdc2), CDK23(p3/cyclin B, CDK2/cyclin A, CDK2/cyclin E, CDK5/p35		1.06	0.07615	4267	NEG	
DKC0000506A01	Bortezomib	PKC-δ, TGM-δ (Tissue Transglutaminase), signal transduction pathway inhibitor		4.32	0.00172	1647	POS	x
DKC0000818A01	PW-67657	p38α, p38β		2.83	0.00292	4078	POS	
DKC0000713A01	Pseudirine	CDK4/Cyclin D1		1.30	0.26813	3150	SURV	
DKC0007473A01	S-99	MAP3K5 (AKK1)		0.91	0.52652	4707	NEG	
DKC0000711A01	SK-18	PTGSR (DEK, SEK1)		0.80	0.24115	3567	NEG	
DKC0000484A01	S4003	IKK-α		2.45	0.02160	567	POS	x
DKC0000314A01	SB-203580, SB-203580	p38 MAPK		4.87	0.00088	3274	POS	x
DKC0000315A01	SB-216749	IKK-β		0.75	0.00005	1598	POS	
DKC0000316A01	SB-219063	p38 MAPK		3.06	0.00321	3575	POS	
DKC0000318A01	SB-415142	TGFβR1 (ALK5)		3.04	0.00419	2434	POS	
DKC0000710A01	SB-520190, NPII	p38 MAPK		1.64	0.04048	4028	NEG	
DKC0000788A01	SB-218078	CHK1, CDK2 (CDK1, p34cdc2), CDK23(p3/cyclin B, PKC		0.00	0.00000	0	SURV	
DKC0000337A01	SB-415286	GSK3		1.43	0.14822	3190	NEG	
DKC0000315A01	SB-460134 hydrochloride hydrate	TGFβR1 (ALK5)		4.15	0.00111	1563	SURV	
DKC0000715A01	SB-525334	TGFβR1 (ALK5)		6.87	0.00000	1248	SURV	
DKC0001176A01	SB242235, SB-242235	p38 MAPK		10.83	0.00002	3771	POS	x
DKC0001139A01	SB505085, SB-505085	BRAF (B-raf)		2.85	0.00065	3958	POS	x
DKC0000503A01	SC-514	IKKα (IKK2)		2.92	0.00838	2362	POS	
DKC0000735A01	SC4900776, SCH-900776, MK-8776	CHK1, CDK2/Cyclin A, CDK2/Cyclin E		1.39	0.00645	582	SURV	
DKC0000319A01	SD-168	p38 MAPK		1.33	0.22015	2040	NEG	
DKC0000316A01	SD-208	TGFβR1 (ALK5)		4.89	0.00078	1434	SURV	
DKC0001177A01	SD-06, SD-0006	p38 MAPK		3.27	0.00134	3031	POS	x
DKC0001100A01	SD-1776	PI3K, PI3K2		5.85	0.00111	1631	POS	x
DKC0001124A01	SDX-523	MET (c-MET, HGFR)		2.13	0.00240	3052	NEG	
DKC0000317801	SD3	TGFβR1 (ALK5)		1.50	0.16540	3068	NEG	
DKC0000636A01	SD-1531	TGFβR1 (ALK5)		4.95	0.00000	701	SURV	
DKC0000318801	SF1-86002 dihydrochloride, SF1-86002 dihydrochloride	p38 MAPK		1.54	0.17331	298	SURV	
DKC0000318A01	SF1-86002, SF1-86002	p38 MAPK		1.29	0.27917	3581	NEG	
DKC0000513A01	SF-600125	MAP2K1 (MEK1), MAP2K2 (MEK2)		3.36	0.00331	2181	POS	
DKC00013167A01	SMG-1 Compound 467	SMG1 (ATX, LIP)		1.39	0.08467	2789	NEG	
DKC0001161801	SMG-032, BMS37032, BMS-37032	CDK2/Cyclin E, CDK7, CDK9/Cyclin T1		2.87	0.00399	1448	SURV	
DKC0001154A01	SMO-314	AurA (AuroraA), AurB (AuroraB), AurC (AuroraC)		1.82	0.18016	768	SURV	
DKC0000486A01	Sorafenib, Bay 43-9006, Nexavar	FLT3 (STK1, FLK2), KIT (c-KIT), PDGFRβ (PDGFR, PDGFR1), RET, RAF1 (c-Raf), BRAF (B-raf), FLT1 (VEGFR1), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3)		4.52	0.00180	2843	POS	x
DKC0000486A01	Sorafenib,p-Toluenesulfonate Saf, Bay-43-9006, Nexavar	FLT3 (STK1, FLK2), KIT (c-KIT), PDGFRβ (PDGFR, PDGFR1), RET, RAF1 (c-Raf), BRAF (B-raf), FLT1 (VEGFR1), KDR (VEGFR2, VEGFR, FLK1), FLT4 (VEGFR3)		3.19	0.01721	1800	POS	x
DKC0000513A01	SP-600125	IKK-1 (IKKα)		2.13	0.02756	2195	POS	
DKC0000510A01	Sphingosine kinase inhibitor 2	SPK1 (DPHK, SK-1, Sphingosine Kinase)		1.53	0.20254	2215	NEG	
DKC0001187A01	SK-3677	ROCK2 (ROCKA)		2.24	0.00350	1533	SURV	
DKC0000815A01	SK-11	SKC (c-SRC), LCK (p56lck), KDR (VEGFR2, VEGFR, FLK1)		0.14	0.11676	698	NEG	
DKC00031178A01	SK-18, ST-638	Tyrosine kinase inhibitor		0.74	0.03816	3557	NEG	
DKC0000311A01	Sevopropine	PKA Kinase		0.63	0.05486	67	SURV	
DKC000031390A1	SH-609 acetic acid	MAPK1/2 (ERK1/2)		1.44	0.13077	3407	NEG	
DKC0000813A01	SU-101	PDGFRβ (PDGFR, PDGFR1), KDR (VEGFR2, VEGFR, FLK1)		0.61	0.01895	3464	NEG	
DKC0000512A01	SU-6112, UMBI	KDR (VEGFR2, VEGFR, FLK1)		2.53	0.01476	1646	SURV	
DKC0000512A01	SU-1416, SU-1416, TSU-16, NCS-996819, Semaxanib, Semaxinib, Semomand	FLT3 (STK1, FLK2), MET (c-MET, HGFR), KDR (VEGFR2, VEGFR, FLK1)		0.9507	0.00507	134	SURV	
DKC0000340A01	SU-6656	SKC (c-SRC), YES, LYN, FYN (p59lyn)		0.12	0.00000	1219	SURV	
DKC0000511A01	SU-11774, PU-SU11774	MET (c-MET, HGFR)		0.56	0.00370	1454	NEG	
DKC0000838A01	SU-1403	KDR (VEGFR2, VEGFR, FLK1), FGFR1 (FLT3), PDGFRβ (PDGFR, PDGFR1)		2.26	0.00880	3407	POS	
DKC0000318A01	SU-6668, TSU-68, Oranitinib	PDGFRβ (PDGFR, PDGFR1), KDR (VEGFR2, VEGFR, FLK1), FGFR1 (FLT3), AurA (AuroraA), AurB (AuroraB), TK1		3.21	0.02438	2105	POS	x
DKC0000710A01	SU-6736	CHK2 (CHK1, p34cdc2), CDK43(p3/cyclin B, CDK2/cyclin A, CDK4/cyclin D1)		1.61	0.07010	2198	POS	
DKC0000740A01	Sunitinib Maleate, Sutent, SU-11248, PHA-309040AD	CHK1 (c-KIT), FLT3 (STK1, FLK2), JAK2 (c-FMS, CSF-1R, CSF1R), KDR (VEGFR2, VEGFR, FLK1), PDGFRβ (PDGFR, PDGFR1)		0.23	0.00000	2555	POS	
DKC0000319A01	TAE-684	ALK		2.90	0.01402	380	SURV	
DKC0001178A01	TAE-775	p38 MAPK		0.85	0.00572	2597	POS	x
DKC0000901A01	TBB, NCS-231634	CK2α1 (CK2, CK1, Casein kinase2)		1.33	0.21918	3578	NEG	
DKC0007850A01	TBK1 inhibitor	TBK1		2.01	0.03788	1581	SURV	
DKC0007850A01	TC-A3331 Thiochloride	CDK2 (CDK1, p34cdc2)		0.86	0.32758	1417	NEG	
DKC0008748A01	TC-DAPK-6	DAPK1, DAPK3 (DIPK, ZIP)		1.13	0.28203	2626	NEG	
DKC0008743A01	TC21312	CHK1		1.56	0.07107	814	SURV	
DKC0000813801	TC21312 dihydrochloride	CHK1		1.82	0.04844	864	SURV	
DKC0000810A01	TC3139	FLT3 (STK1, FLK2)		0.94	0.78275	3747	NEG	
DKC0000706A01	TC31NK-5a	JNK1 (JNK), JNK2, JNK3, p38α		1.65	0.00000	299	SURV	
DKC0000415A01	TC31PKA-1	PI3K, PI3K2		2.25	0.01095	2111	POS	
DKC0000507A01	Temazepam, CC-779, Torisel	FRAP (M TOR, FRAP1)		1.54	0.15346	737	SURV	
DKC0007464A01	TE-101348	FLT3 (STK1, FLK2), JAK2		6.58	0.00003	1370	SURV	
DKC0003117A01	TE083, TE-083	CHK1, CLK		1.22	0.26274	3174	NEG	
DKC0008725A01	TE100-115, TG-100-115, TG-100115	PI3K		1.95	0.00039	3137	POS	x
DKC0000704A01	TEG-221	PI3K		1.16	0.49752	3404	NEG	x
DKC0007856A01	Tegfatinib, NCS-702818, R-115777, Zanentra, IND 58359	Exonectin/tyrosinase		3.83	0.00022	3981	POS	x
DKC0000514A01	TPC-A1	IKKα (IKK2)		4.37	0.00174	2580	POS	
DKC0000484A01	TP12	CD7 (TIP12, EST, MAP3K3)		4.22	0.00516	1997	POS	
DKC0008746A01	TP122	CDK21 (CK2, CK1, Casein kinase2)		1.53	0.01345	3430	POS	
DKC0000485A01	TPW-119	GSK3		1.92	0.08426	1316	SURV	
DKC0000411A01	TR-1918	4EBP2		1.56	0.11687	1512	SURV	
DKC000051301	Tyrphostin AG-1478 mesylate	EGFR (ERBB1, HER1)		2.96	0.00661	2301	POS	
DKC0000319A01	Tyrphostin AG-1296	PDGFRβ (PDGFR, PDGFR1)		1.39	0.18038	1544	SURV	
DKC0000707A01	Tyrphostin B44, (1 enantiomer	EGFR (ERBB1, HER1), ERK2 (ERK1, ERK2, MEK)		1.68	0.05226	3478	POS	
DKC0000513A01	Tyrphostin SU-1498, SU-1498	KDR (VEGFR2, VEGFR, FLK1)		2.32	0.01436	2139	POS	
DKC0000514A01	U0126, U-0126, U-0126	MAP2K1 (MEK1), MAP2K2 (MEK2), JAK2, ERK1 (ERK, p38)		0.97	0.93315	2128	NEG	
DKC0000310A01	Vandetanib, Zactima, ZD6474, AZD-6474	EGFR (ERBB1, HER1), KDR (VEGFR2, VEGFR, FLK1), RET, ABL1 (ABL), KIT (c-KIT), FLT1 (VEGFR1), FLT4 (VEGFR3), TRKA (TRK)		1.83	0.06720	1705	NEG	
DKC0000318A01	Vandetanib dihydrochloride, PTK-787, Z-222584, CGP797870	KDR (VEGFR2, VEGFR, FLK1), PDGFRβ (PDGFR, PDGFR1)		1.30	0.29230	1574	SURV	
DKC0008738A01	VE-821	ATR		1.62	0.01861	1500	SURV	
DKC0000307A01	VE-880, MK-0457, Tositinib, V86	AurA (AuroraA), AurB (AuroraB), AurC (AuroraC), ABL1 (ABL), FLT3 (STK1, FLK2), JAK2		1.16	0.49528	644	SURV	
DKC0000510A01	VE-702, VE-800, VE-900A, VE-702	p38 MAPK		3.88	0.00991	3072	POS	x
DKC0008723A01	VE-745	p38 MAPK		6.85	0.00000	4077	POS	x
DKC000783A01	WAF-600	FRAP (M TOR, FRAP1), mTORC1, mTORC2		0.38	0.07796	100	SURV	
DKC0000807A01	Wip-P-124, MASTPROM	IKK-α, EGFR (ERBB1, HER1), SKC (c-SRC), ABL1 (ABL), KDR (VEGFR2, VEGFR, FLK1), p38 MAPK, PI3K		0.95	0.05334	3369	NEG	
DKC0000312A01	Wortmannin, WY-12420	PI3K - irreversibile		1.15	0.51897	2408	NEG	
DKC0008762A01	WYE-125132, WYE-132	PI3K, FRAP (M TOR, FRAP1), mTORC1, mTORC2		1.21	0.55334	356	SURV	
DKC0007838A01	WYE-354	FRAP (M TOR, FRAP1), mTORC1, mTORC2		1.34	0.14976	345	NEG	
DKC0008708A01	WZ1146, WZ-3146	EGFR (ERBB1, HER1)		1.71	0.00114	867	SURV	
DKC0007451A01	WZ1002, WZ-1002	EGFR (ERBB1, HER1)		3.70	0.00016	3077	POS	x
DKC0008707A01	WZ8040, WZ-8040	EGFR (ERBB1, HER1)		2.13	0.00140	1453	POS	
DKC000115A01	XL-147, XL147, SAR-245408	PI3K		1.41	0.08122	3231	NEG	
DKC0007460A01	XL-186, XL186, SAR-2607315, Cabozantinib maleate	FLT3 (STK1, FLK2), MET (c-MET, HGFR), KIT (c-KIT), RET, TIE2 (TIE), KDR (VEGFR2, VEGFR, FLK1)		0.78	0.28872	3154	NEG	
DKC0001134A01	XL-880, CSK-1363085, EXL-2885, GSK-035, Ictenibin	MET (c-MET, HGFR), KDR (VEGFR2, VEGFR, FLK1), FLT3 (STK1, FLK2), TIE2 (TIE)		1.16	0.38572	665	SURV	
DKC0008736A01	XL-765, XL-765, SAR245409	PI3K, mTORC1, mTORC2		2.16	0.00099	3384	POS	x
DKC00074710A1	XL828-R1, XL828-R2	CHK1 (BML1, PRK47)		2.14	0.04801	2119	POS	x
DKC0000518A01	XPB44X, XRP-44X, WCH-44	Ret-Net (Btk3), Tubulin polymerization		1.33	0.22979	835	SURV	
DKC0008704A01	Y-27632	LRK2, ROCK1, ROCK2 (ROCKA), Calcium sensitizer		1.96	0.00364	3718	POS	x
DKC0000313A01	Y-27633	ROCK1, ROCK2 (ROCKA)		1.26	0.27555	2477	NEG	x
DKC0008701801	Y-59983, R01-883, SNI-1656	ROCK1, ROCK2 (ROCKA)		1.49	0.01904	2310	NEG	
DKC000118A01	YM201636, YM-201636	PI3K/Akt (PI3K)		3.02	0.00133	1539	SURV	
DKC0000810801	YM-206416 hydrochloride	KDR (VEGFR2, VEGFR, FLK1)		0.48	0.02657	1440	NEG	
DKC0000811801	YM-313841 hydrochloride	KDR (VEGFR2, VEGFR, FLK1)		1.24	0.31790	3745	NEG	
DKC0000517A01	YM-336372	RAF1 (c-Raf)		2.48	0.00802	1818	POS	x
DKC0000313801	YM-39923 hydrochloride	IKK-α		1.63	0.14051	2863	NEG	
DKC0000511A01	YM-447439	AurA (AuroraA), AurB (AuroraB), AurC (AuroraC)		0.92	0.80104	779	SURV	
DKC0000548A01	YTK474	PI3K		2.54	0.01146	818	SURV	

[illegible]

[illegible]

% GFP+ KIL COMPOUNDS	0	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	8.09	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

% GFP+ TOOL compounds	TGFb1 alone 0	0.1	0.5	1	5 Concentration μM
Ciclopamine	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	8.77	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

Compound	Target	Fold change (sample/TGFb)	p value	Cell number	Output
A-366	G9a/GLP	1.04	0.80466	4511	NEG
BAZ2-ICR	BAZ2A/B bromodomains	0.78	0.16923	3555	NEG
C646	p300	0.80	0.31629	3848	NEG
CL-994	HDAC	0.82	0.49564	2164	NEG
GSK-LSD1	LSD1	1.30	0.13926	3965	NEG
GSK2801	BAZ2A/B	0.86	0.61931	3376	NEG
GSK343	EZH2	1.17	0.45595	2400	NEG
GSKJ4	JMJD3, UTX	0.31	0.00128	895	SURV
I-CBP112	CBP/p300	0.58	0.06423	3461	NEG
IOX2	PHD2	0.90	0.54481	3816	NEG
JQ1	pan-BRD	0.10	0.00032	2135	POS
LAQ824	HDAC	0.45	0.89918	83	SURV
LLY-507	SMYD2	0.48	0.02312	2305	POS
OF-1	BRPR1-3	0.33	0.00780	318	SURV
OICR-9429	WDR5	1.01	0.95879	3953	NEG
Olaparib	PARP1/2	0.69	0.05328	2871	NEG
PFI-1	BET	0.29	0.00520	1677	POS
PFI-2	SETD7	0.56	0.01867	3544	POS
PFI-3	SMARCA	1.38	0.03138	4468	NEG
SGC-CBP30	CREBBP/EP300	0.47	0.07700	3900	NEG
SGC0946	DOT1L	0.90	0.57856	3512	NEG
SGC707	PRMT3	0.77	0.41633	3778	NEG
UNC0638	G9a/GLP	0.88	0.46814	2892	NEG
UNC0642	G9a/GLP	0.20	0.00105	3159	POS
UNC1215	L3MBTL3	0.81	0.27208	3855	NEG
UNC1999	EZH1/2	0.62	0.03198	2730	POS