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Fenofibrate reduces osteonecrosis without affecting antileukemic efficacy in dexamethasone treated mice

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Author Contributions:

Conceptualization of experiments by Emily R. Finch; methodology and data were collected by Emily R. Finch, Monique A. Payton, David A. Jenkins, Xiangjun Cai, Lie Li, Seth E. Karol, Mary V. Relling, Laura J. Janke; formal analysis by Emily R. Finch, Mary V. Relling, and Laura J. Janke; the original manuscript was written by Emily R. Finch, Mary V. Relling, and Laura J. Janke; all authors reviewed and approved the manuscript.

Short running title: Fenofibrate reduces experimental osteonecrosis

Word count: 1,893

Summary Statement:

In children treated for acute lymphoblastic leukemia, hypertriglyceridemia is associated with increased risk of symptomatic osteonecrosis. Here, we demonstrate that prevention of hypertriglyceridemia with fenofibrate decreases the risk of dexamethasone-induced osteonecrosis while maintaining antileukemic efficacy, and thus may be considered for clinical trials.

Conflict of interest: We have no relevant conflicts of interest to disclose.

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National Institutes of Health. We are thankful for St. Jude's Animal Resource Center and Veterinary Pathology Core for technical assistance.

Abstract:

Recent clinical trials in children with acute lymphoblastic leukemia (ALL) indicate that severe hypertriglyceridemia (> 1000 mg/dL) during therapy is associated with increased frequency of symptomatic osteonecrosis. Interventions to lower triglycerides have been considered, but there have been no pre-clinical studies investigating impact of lowering triglycerides on osteonecrosis risk, nor whether such interventions interfere with the antileukemic efficacy of ALL treatment. We utilized our clinically relevant mouse model of dexamethasone-induced osteonecrosis to determine if fenofibrate decreased osteonecrosis. To test whether fenofibrate affected the antileukemic efficacy of dexamethasone, we utilized a BCR-ABL^{+} model of ALL. Serum triglycerides were reduced with fenofibrate throughout treatment, with the most pronounced 4.5-fold decrease at week 3 ($p<1x10^{-6}$). Both frequency (33% versus 74%, p=0.006) and severity (median necrosis score of 0 versus 75; $p=6x10^{-5}$) of osteonecrosis were reduced with fenofibrate. Fenofibrate had no impact on BCR-ABL^{+} ALL survival (p=0.65) nor on the antileukemic properties of dexamethasone (p=0.49). These data suggest that lowering triglycerides with fenofibrate reduces dexamethasone-induced osteonecrosis while maintaining antileukemic efficacy, and thus may be considered for clinical trials.

Introduction:

Hypertriglyceridemia occurs in 4-19% of children treated with glucocorticoids and/or asparaginase during therapy for acute lymphoblastic leukemia (ALL) (1-7). Though hypertriglyceridemia has been thought to be transient and relatively benign (1, 2), recent studies suggest patients with severe hypertriglyceridemia (>1000 mg/dL) during ALL therapy may be at increased risk for long-term complications, including symptomatic osteonecrosis (3, 7, 8).

Recent recommendations for pharmaceutical management of severe hypertriglyceridemia includes the use of fibrates, specifically fenofibrate (9). In addition to being associated with fewer drug-drug interactions than statins (10-12), fenofibrate specifically reduces serum triglycerides up to 50%, with minimal adverse reactions (13-15), whereas the main effect of statins is the decrease of serum low-density lipoprotein cholesterol (9, 16, 17). The active metabolite of fenofibrate, fenofibric acid, targets peroxisome proliferator activated receptor alpha, leading to activation of lipoprotein lipase which increases lipolysis and elimination of triglyceride-rich particles (18), resulting in reduction of circulating serum triglycerides (19, 20). Therefore, fenofibrate has been suggested to manage acute hypertriglyceridemia, such as that observed during ALL therapy. However, there is no evidence that reducing triglycerides with fenofibrate can reduce risk of osteonecrosis. Moreover, with very effective ALL therapy, caution must be exercised before adding any new agents to treatment regimens, to avoid any untoward drug interactions.

In these proof-of-principle experiments, we tested the hypothesis that lowering serum triglycerides with fenofibrate in dexamethasone-treated mice would result in decreased osteonecrosis and would not interfere with antileukemic efficacy.

Methods:

Treatment

Experiments were approved by the Institutional Animal Care and Use Committee of St. Jude Children's Research Hospital (SJCRH; Memphis, TN): protocol numbers 423-100428 and 465-100549.

All mice received prophylactic antibiotics in drinking water (herein as "base-water"): continuous tetracycline (1 g/L; Sigma-Aldrich, St. Louis, MO) and intermittent sulfamethoxazole/trimethoprim oral suspension (600/120 mg/L for 3.5 days/week; Aurobindo Pharma, USA, Inc., Dayton, NJ). Mice were randomized to receive continuous dexamethasone (0.4 mg/mL sodium phosphate solution; Fresenius Kabi, Lake Zurich, IL) in drinking water, herein as "dexamethasone water" (3 mg/L in osteonecrosis model and 4 mg/L in BCR-ABL$^+$ ALL model (21-23).

The folic acid deficient diet (0.2 ppm folic acid; TestDiet, Richmond, IN) was the "base-diet" (21). Fenofibrate (Sigma Aldrich, St. Louis, MO) was added to the base-diet for a final concentration of 0.2% fenofibrate (w/w) ("fenofibrate-supplemented diet"; TestDiet, Richmond, IN), a dose used in prior experimental rodent models (24-26).

Serum triglycerides were measured (ABX Penta 400 instrument; Horiba, Montpellier, France) after a 12-16 hour fast (food was withheld; mice had free access to water).

Mice were randomized to one of four treatment groups: control (base-water and base-diet); fenofibrate-only (base-water and fenofibrate-supplemented diet); dexamethasone-only (dexamethasone-water and base-diet); or dexamethasone + fenofibrate (dexamethasone-water and fenofibrate-supplemented diet).

Osteonecrosis model

The protocol was modified from that previously described (21, 22, 27-29). At postnatal days 26 to 28, male Balb/cJ mice (bred in house at SJCRH) were randomized by body weight (13.5 g, 95% CI 13.0- 14.7) to 6-weeks of treatment. Fasting serum triglycerides were measured after 1, 3, and 6 weeks of treatment. At end of treatment, white adipose tissue (WAT); and plasma dexamethasone and fenofibric acid levels were measured. Osteonecrosis and epiphyseal arteriopathy were evaluated and scored in both distal femurs, by board-certified veterinary pathologist (L.J.J.), as described (21, 22, 27-29). See Supplemental Methods for details.

BCR-ABL$^+$ ALL model

The BCR-ABL (p185$^+$, *Arf*$^{-/-}$) luciferase-positive cell line (BCR-ABL$^+$) was generated (23, 30-32). Female 8-week-old matched syngeneic mice (C57Bl/6J, *Arf*-wildtype; Jackson Laboratory, ME) received intravenous injections of 2000 BCR-ABL$^+$ cells. Bioluminescent imaging was performed weekly (23), to monitor leukemic burden. At day

3, mice were stratified by luminescent signal and body weight to treatment (n=10/treatment group). Fasting serum triglycerides were measured at day 24. Treatment ended at day 28: mice were maintained on base-diet/base-water until humane endpoint or the end of study at day 63. See Supplemental Methods for details.

Statistical Analysis

The frequency of osteonecrosis/arteriopathy was compared using chi-square test. Survival comparisons utilized log-rank testing. For continuous variables, the two-tailed Mann-Whitney test was utilized (two-groups); the Kruskal-Wallis test with Dunn's correction for multiple comparisons was utilized (three or more groups). All continuous statistics are expressed as median with 95% confidence interval (CI). Statistical analyses and visual representation utilized the R program (version 3.5.1) (33) or GraphPad Prism (34). A significance threshold of $p<0.05$ was used for all analyses.

Results:

Fenofibrate in experimental osteonecrosis

Consistent with previous observations (21, 22, 28, 29), mice that received dexamethasone (dexamethasone-only and dexamethasone + fenofibrate treatment groups) gained less weight over time versus those not treated with dexamethasone (control and fenofibrate-only treatment groups) (Figure 1B). Fenofibrate supplementation suppressed weight gain over the course of treatment. By week 6 of treatment, control mice gained 50% more weight than fenofibrate-only mice ($p=2x10^{-5}$); dexamethasone-only mice gained 10% more weight than dexamethasone + fenofibrate

mice (p=0.01; Figure 1B). There was no difference in survival between groups (p=0.55; Figure 1C).

In mice treated with dexamethasone, fenofibrate significantly reduced serum triglycerides as early as week 1, from 130.1 mg/dL (95% CI 85.2- 201.1) to 37.4 mg/dL (95% CI 17.1- 60.3), a 3.5-fold decrease (p= 0.0004). Fenofibrate supplementation continued to suppress dexamethasone-induced hypertriglyceridemia at week 3 (4.5-fold decrease, $p<1x10^{-6}$) through the end of treatment at week 6 (3.5-fold decrease, $p=5x10^{-6}$). There was no difference in triglycerides between fenofibrate-only and fenofibrate + dexamethasone mice at weeks 1 and 3 (p>0.99 at each time point). By week 6, triglycerides were increased in fenofibrate + dexamethasone versus fenofibrate-only mice (p=0.005), however, levels were comparable to control mice (p>0.99; Figure 2A).

In dexamethasone treated mice, fenofibrate reduced WAT: perigonadal fat (representative of visceral fat) was reduced from 0.30 g (95% CI 0.22- 0.54) to 0.17 g (95% CI 0.11- 0.20; p=0.01) and inguinal fat (representative of posterior subcutaneous fat) was reduced from 0.25 g (95% CI 0.19- 0.47) to 0.12 g (95% CI 0.10- 0.15; p= 0.0003; Figure 2B).

As expected, only mice that received dexamethasone developed osteonecrosis and/or arteriopathy (Figure 3). The frequency of both osteonecrosis and arteriopathy was 74% in dexamethasone-only versus 33% in dexamethasone + fenofibrate mice (p=0.006;

Figure 3A). Not only were the frequency of osteonecrosis and arteriopathy reduced over 2-fold with fenofibrate, but the severity was significantly reduced. In dexamethasone-only mice, the necrosis score was reduced from 75 to 0 with fenofibrate supplementation ($p=6x10^{-5}$; Figure 3B); arteriopathy score was reduced from 4 to 0 (p= 0.0006; Figure 3C).

In dexamethasone + fenofibrate treated mice, there was a correlation between week 3 triglycerides and osteonecrosis: mice that developed osteonecrosis had triglycerides of 67.5 mg/dL (95% CI 21.1-96.9) versus 37.2 mg/dL (95% CI 30.9-53.1) in osteonecrosis-negative mice (p=0.03; Supplemental Figure 1A). By the end of treatment, there was no difference in triglycerides by osteonecrosis status (p>0.99; Supplemental Figure 1B).

At the end of treatment, there was no difference in plasma dexamethasone in mice treated with dexamethasone-only (26.6 nM, 95% CI 20.0- 58.3nM) versus dexamethasone + fenofibrate (20.5 nM, 95% CI 16.7- 25.9 nM; p=0.1, Supplemental Figure 2A). Fenofibric acid was significantly lower in dexamethasone + fenofibrate (1.9 µg/mL, 95% CI 1.6 -2.4 µg/mL) versus fenofibrate-only mice (6.7 µg/mL, 95% CI 6.1 - 8.8 µg/mL, $p<1x10^{-}6$; Supplemental Figure 2B).

Fenofibrate in experimental BCR-ABL ALL

In a BCR-ABL dexamethasone-sensitive model of murine ALL, (Figure 4A), all mice that did not receive dexamethasone succumbed to disease by day 35 (Figure 4C), with prolonged survival in both dexamethasone-only and dexamethasone + fenofibrate arms

compared to no dexamethasone ($p<1x10^{-6}$). Mice were monitored for relapse until the end of follow-up at day 63. There was no difference in disease burden between dexamethasone-only versus dexamethasone + fenofibrate treatment groups, as indicated in survival (p=0.49; Figure 4C), spleen weight (p>0.99, Supplemental Figure 3A), and WBC count (p>0.99, Supplemental Figure 3B). In dexamethasone treated mice, fenofibrate decreased triglycerides from 89.9 mg/dL to 29.0 mg/dL (p= 0.001; Figure 4D).

Discussion:

Though the etiology of osteonecrosis is not completely understood, the involvement of lipid metabolism has been well documented (35-37). However, there have been few studies investigating whether pharmacologically lowering triglycerides prevents osteonecrosis (38).

We found that fenofibrate supplementation successfully managed dexamethasone-induced hypertriglyceridemia (Figure 2) and was associated with reduction in the frequency and severity of osteonecrosis (Figure 3). The correlation between week 3, but not week 6 triglycerides (Supplemental Figure 1), and osteonecrosis suggest that management of triglycerides is especially important early in the progression of the disease. Although there was an increase in triglycerides between fenofibrate + dexamethasone and fenofibrate-only mice by week 6 (Figure 2A), fenofibrate was able to maintain triglycerides comparable to control mice, even with prolonged continuous exposure to dexamethasone.

Any addition to ALL therapy must be thoroughly assessed to ensure it does not interfere with highly effective modern drug regimens. For our purposes, we utilized a model of BCR-ABL$^+$ ALL known to be sensitive to dexamethasone treatment. Fenofibrate supplementation did not cause observable differences in the efficacy of dexamethasone in treatment of experimental BCR-ABL$^+$ ALL (Figure 4).

In our experimental models, the dosage of both dexamethasone and fenofibrate were well tolerated (Figure 1C) and resulted in plasma levels of the drug/metabolites in the clinical range (13, 39, 40).Though the plasma concentration of fenofibric acid was reduced in mice treated with dexamethasone + fenofibrate compared to fenofibrate-only (Supplemental Figure 2B), it was still effective in reducing dexamethasone-induced hypertriglyceridemia (Figure 2A) and osteonecrosis (Figure 3), while not impacting BCR-ABL$^+$ ALL treatment (Figure 4). Statins have been suggested as possible agents to reduce osteonecrosis (41-43) but because they have potent interactions with CYP3A and with SLCO1B1 (involved in metabolism and disposition of multiple ALL drugs), their likelihood of drug interactions makes them less desirable agents to use in patients with ALL.

Dexamethasone treatment is associated with hypertriglyceridemia (1, 3, 44). It has been shown that elevated serum triglyceride-rich lipoproteins (TRLs) contribute to endothelial dysfunction and subsequent atherosclerosis (45). We hypothesized that dexamethasone-induced arteriopathy and subsequent osteonecrosis are related to

hypertension caused by endothelial dysfunction (27, 46). Therefore, we suspect that the mechanism by which fenofibrate reduced the incidence of osteonecrosis and arteriopathy was by diminishing the dexamethasone-induced elevation of TRLs and thereby mitigating the effects of endothelial dysfunction.

In children will ALL, there are no specific guidelines for managing triglyceride levels during glucocorticoid and/or asparaginase treatment. However, conservative use of fibrates has been successful in managing triglycerides in specific cases, with no reported adverse events (3, 5, 47). In general, the use of fibrates in children is limited to cases of extreme hypertriglyceridemia to prevent pancreatitis, primarily in individuals with familial lipid disorders (20, 48-50). Fibrates have been shown to effectively reduce triglycerides in children between 39-54% (49, 51). As seen in adults, data suggest fibrates are well tolerated in children, with the most frequently reported adverse reactions being gastrointestinal disturbances and muscle cramps (13, 14, 20, 49). However, transient increased liver function tests have been reported in a small number of cases (48, 51) indicating potential for hepatotoxicity when used in combination with other hepatotoxic chemotherapeutic agents.

Though fibrates have been included in a case-by-case basis to manage hypertriglyceridemia during ALL therapy (3, 5, 47), there has been no systematic analysis on the effect of lipid-lowering intervention and long-term outcomes in ALL survivors. Our proof of principle preclinical experiments shows fenofibrate as a potential intervention to reduce dexamethasone-induced osteonecrosis in ALL without substantial

drug interactions and without any untoward effect on the efficacy of ALL therapy. Our findings support the rationale for controlled clinical trial with fenofibrate in chemotherapy-related hypertriglyceridemia.

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Figure legends:

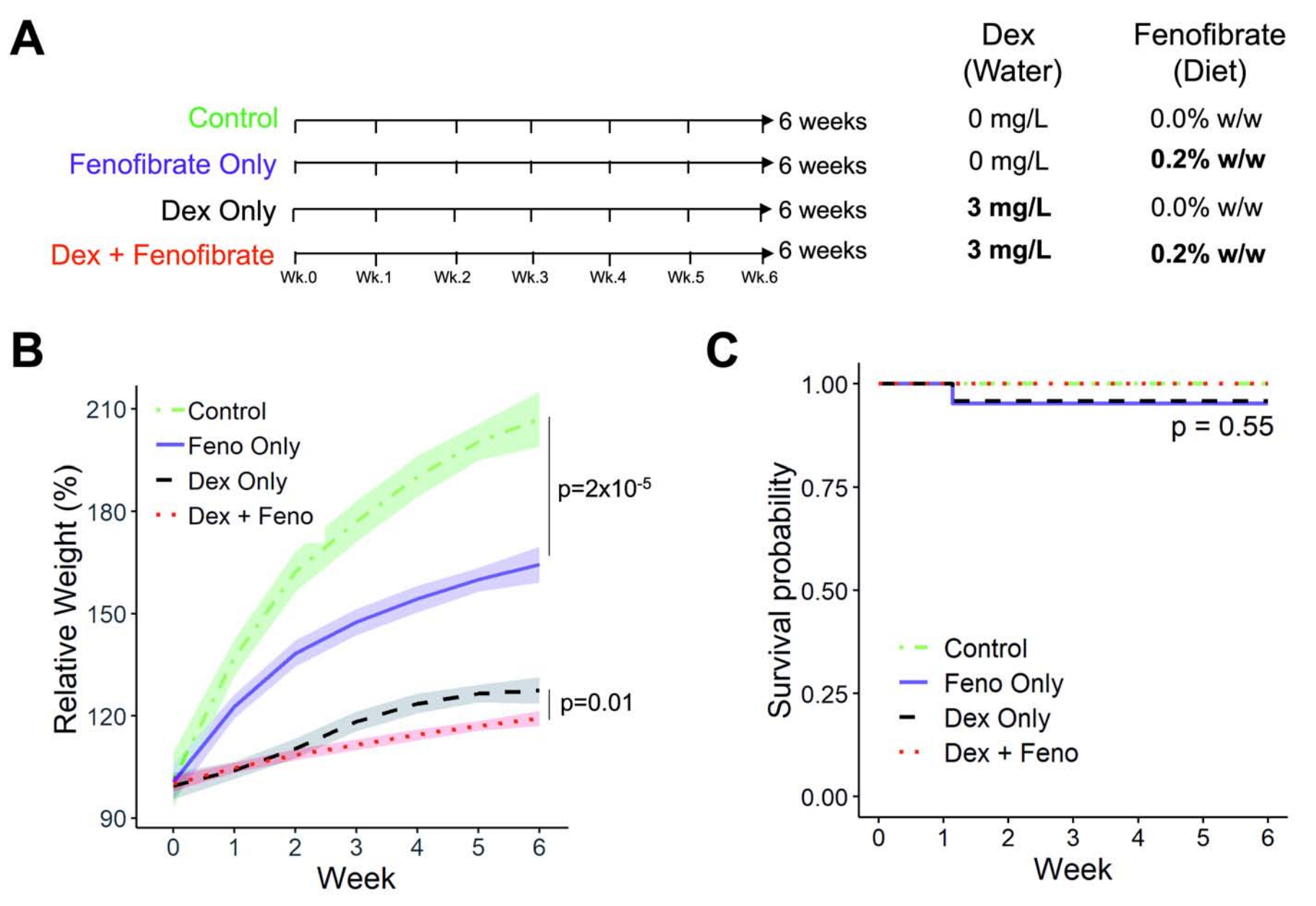
Figure 1. Experimental design, body weight, and survival in dexamethasone-induced osteonecrosis A. Experimental design: male Balb/cJ mice were placed on 6-weeks of continuous treatment with dexamethasone, fenofibrate, or both. B. Body weight relative to week 0, median and 95% CI shown. Control and Fenofibrate (Feno) only mice weighed more than Dex Only and Dex + Feno mice from weeks 1-6 of treatment ($p< 1x10^{-6}$). C. Survival did not differ by treatment group. 100% survival in Control (12/12) and Dex + Feno (33/33) groups, 98% survival in both Dex Only (23/24) and Feno Only (20/21) groups (p = 0.55).

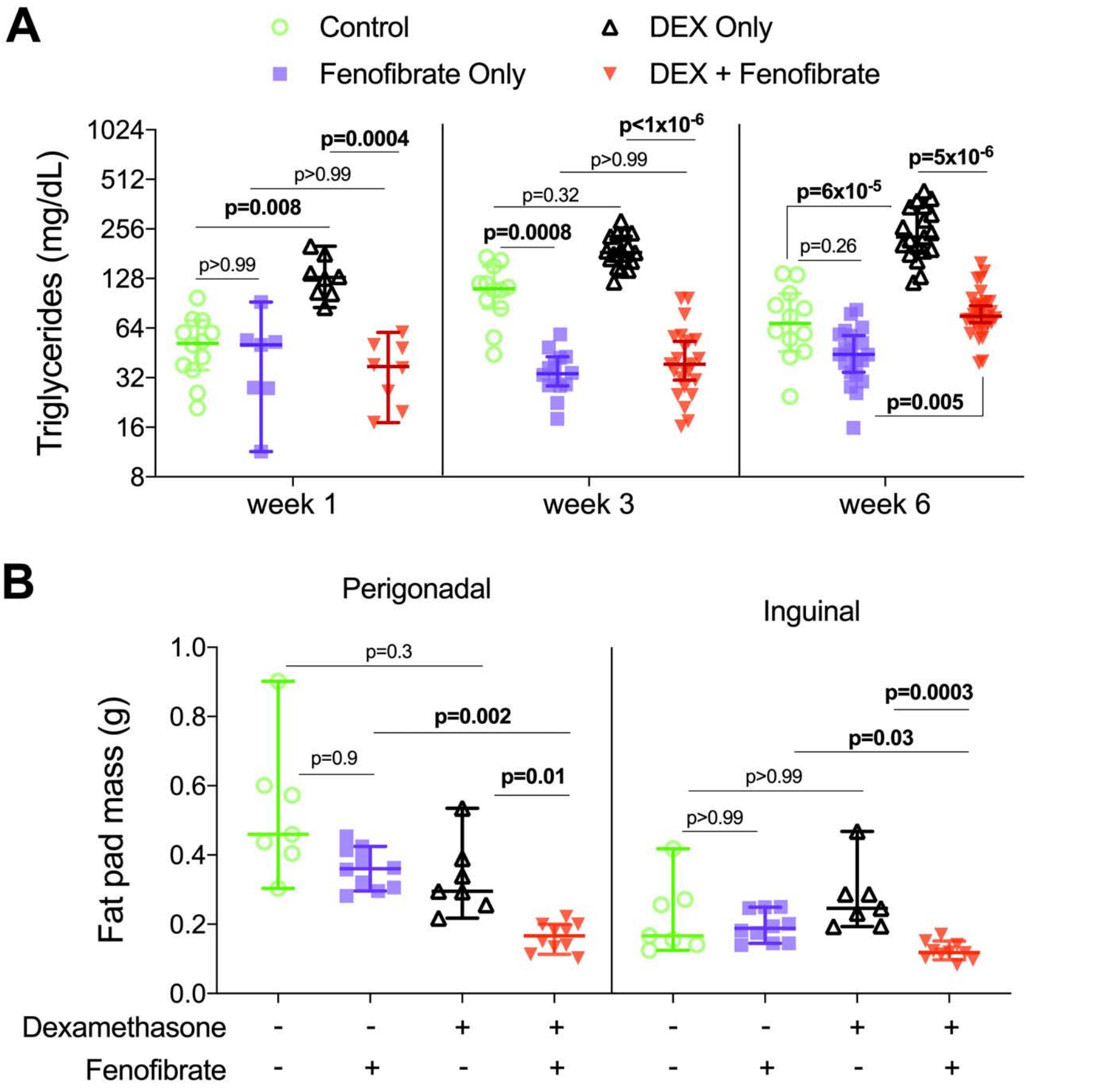
Figure 2: Fenofibrate reduces serum lipids and fat depots in dexamethasone treated mice. A. Median (95% CI) fasting serum triglycerides at weeks 1, 3, and 6 of treatment. Week 1, n=8-12/group; week 3, n= 12-27/group; week 6, n= 12-31/group**.** B. Perigonadal and inguinal fat pads at week 6. N=8-10/group. P-values shown between relevant groups: control vs. fenofibrate-only; control vs. DEX-Only; fenofibrate-only vs. DEX+ fenofibrate; DEX-Only vs. DEX + fenofibrate.

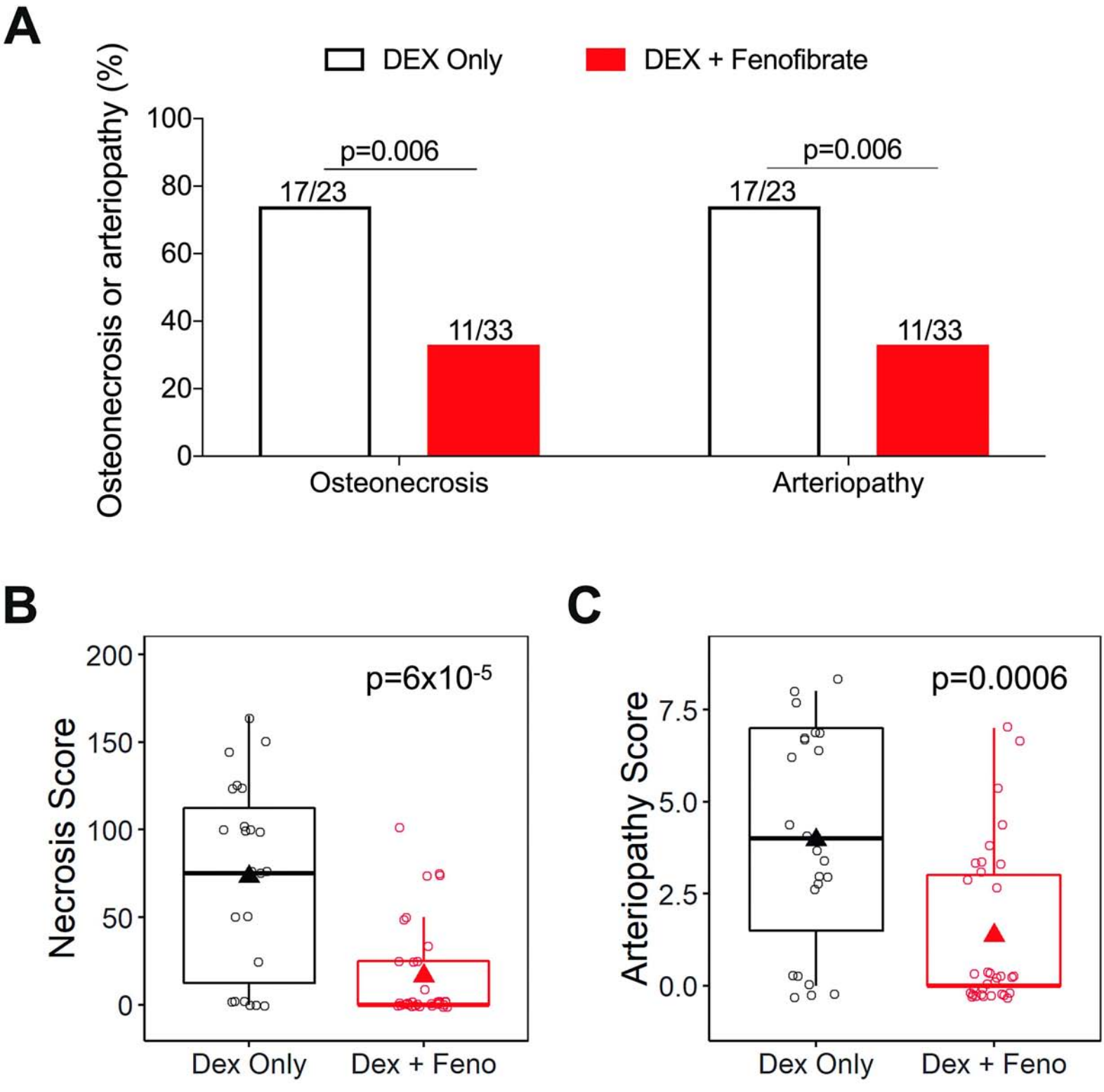
Figure 3: Fenofibrate reduces the frequency and severity of osteonecrosis and arteriopathy in dexamethasone treated mice. A. Frequency of osteonecrosis or arteriopathy, compared with chi-square test. B. Necrosis scores (possible range of 0 (negative for osteonecrosis) to 200 (equivalent to 100% necrosis in both hind limbs) and C. Arteriopathy scores (possible range of 0 (negative for arteriopathy) to 8 (thrombotic

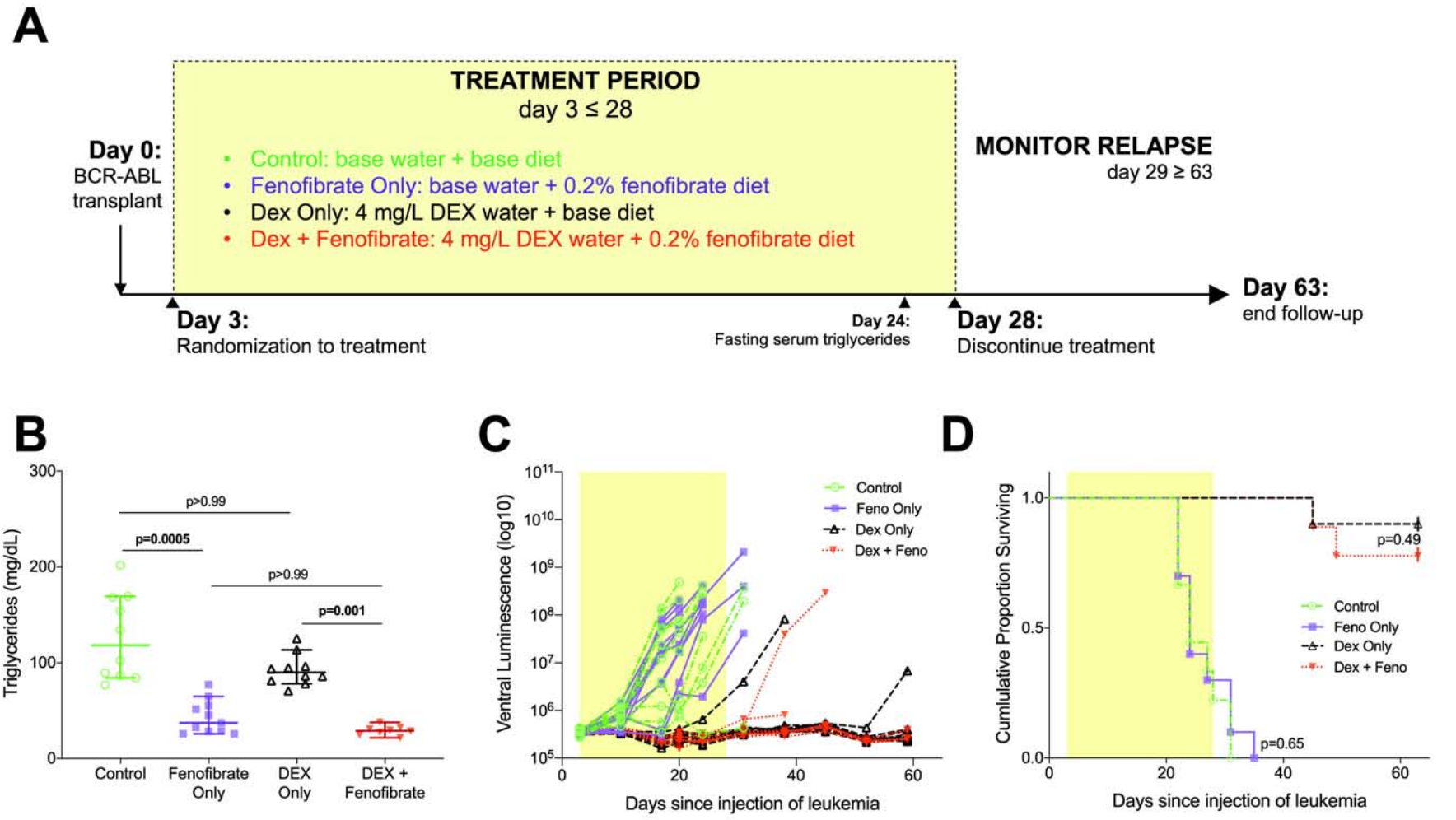
arteriopathy in both limbs) were reduced with fenofibrate. Median with 95% CI (box and whiskers) and mean (triangle). NOTE: no control or fenofibrate-only mice developed osteonecrosis or arteriopathy.

Figure 4: Fenofibrate does not affect efficacy of dexamethasone in BCR-ABL$^+$ ALL. A. Experimental design. B. Ventral luminescence was measured weekly. C. Survival log-rank test shows p-values between control vs. fenofibrate-only and dexamethasone-only vs. dexamethasone + fenofibrate. $P<1x10^6$ between dexamethasone treated groups (dexamethasone-only and dexamethasone + fenofibrate) vs. no dexamethasone treatment (control and fenofibrate-only). Shaded yellow area indicates time on therapy. D. Fasting serum triglycerides at day 24 (3-weeks post-BCR-ABL transplant). N=9-10/group.


A
Dex (Water)
Fenofibrate (Diet)
Control
6 weeks
0 mg/L
0.0% w/w
Fenofibrate Only
6 weeks
0 mg/L
0.2% w/w
Dex Only
6 weeks
3 mg/L
0.0% w/w
Dex + Fenofibrate
6 weeks
3 mg/L
0.2% w/w
Wk.0
Wk.1
Wk.2
Wk.3
Wk.4
Wk.5
Wk.6
B
Control
Feno Only
Dex Only
Dex + Feno
Relative Weight (%)
210
180
150
120
90
p=2x10-5
p=0.01
0
1
2
3
4
5
6
Week
C
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.55
Control
Feno Only
Dex Only
Dex + Feno
0
1
2
3
4
5
6
Week


A
Control
DEX Only
Fenofibrate Only
DEX + Fenofibrate
Triglycerides (mg/dL)
1024
512
256
128
64
32
16
8
p=0.0004
p>0.99
p=0.008
p>0.99
p<1x10-6
p>0.99
p=0.32
p=0.0008
p=5x10-6
p=6x10-5
p=0.26
p=0.005
week 1
week 3
week 6
B
Perigonadal
Inguinal
Fat pad mass (g)
1.0
0.8
0.6
0.4
0.2
0.0
p=0.3
p=0.002
p=0.9
p=0.01
p=0.0003
p=0.03
p>0.99
p>0.99
Dexamethasone - - + + - - + +
Fenofibrate - + - + - + - +


A
DEX Only
DEX + Fenofibrate
Osteonecrosis or arteriopathy (%)
100
80
60
40
20
0
p=0.006
p=0.006
17/23
17/23
11/33
11/33
Osteonecrosis
Arteriopathy
B
Necrosis Score
200
150
100
50
0
p=6x10-5
Dex Only
Dex + Feno
C
Arteriopathy Score
7.5
5.0
2.5
0.0
p=0.0006
Dex Only
Dex + Feno


A
TREATMENT PERIOD
day 3 ≤ 28
Day 0:
BCR-ABL
transplant
Control: base water + base diet
Fenofibrate Only: base water + 0.2% fenofibrate diet
Dex Only: 4 mg/L DEX water + base diet
Dex + Fenofibrate: 4 mg/L DEX water + 0.2% fenofibrate diet
MONITOR RELAPSE
day 29 ≥ 63
Day 63:
end follow-up
Day 3:
Randomization to treatment
Day 24:
Fasting serum triglycerides
Day 28:
Discontinue treatment
B
Triglycerides (mg/dL)
p>0.99
p=0.0005
p>0.99
p=0.001
Control
Fenofibrate Only
DEX Only
DEX + Fenofibrate
C
Ventral Luminescence (log10)
Control
Feno Only
Dex Only
Dex + Feno
Days since injection of leukemia
D
Cumulative Proportion Surviving
p=0.49
p=0.65
Control
Feno Only
Dex Only
Dex + Feno
Days since injection of leukemia

Supplement to Fenofibrate reduces osteonecrosis without affecting antileukemic efficacy in dexamethasone treated mice

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Supplemental Methods:

Mice:

Mice (up to 5/cage) were maintained in sterile microisolator cages (Micro Vent System 75 JAG, Allentown, NJ) and housed on ventilated racks in a temperature- and humidity-controlled room, a 12-hour light/dark cycle and with free access to food and water. Food was withheld prior to measurement of fasting serum triglycerides: serum triglycerides were measured after a 12-16 hour fast (food was withheld; mice had free access to water).

Evaluation of osteonecrosis and arteriopathy:

Evaluation of osteonecrosis and arteriopathy have been extensively described previously (1-5). Briefly, both femurs were collected, fixed in 10% formalin, decalcified in 10% formic acid, paraffin-embedded, sagittally sectioned beginning at the medial knee, and stained with hematoxylin and eosin. Osteonecrosis was evaluated by a board-certified veterinary pathologist (L.J.J.) for presence of empty lacunae, ghost or pyknotic nuclei in osteocytes in the bone trabeculae, and necrosis of the adjacent marrow and stromal elements (1-4); and scored from 0 (negative for osteonecrosis) to 200 (equivalent to 100% necrosis in both hind limbs) as described (5). Arteriopathy lesions were evaluated in arteriolar branches of the medial genicular artery on the surface of the distal femoral condyles (3) and scored from 0 (negative for arteriopathy) to 8 (thrombotic arteriopathy in both limbs) (5).

White adipose tissue:

White adipose tissue (WAT) was evaluated at sacrifice: perigonadal (representative of visceral fat) and inguinal (representative of posterior subcutaneous fat) fat pads were dissected and weighed at time of necropsy (6, 7).

Quantification of dexamethasone:

Plasma dexamethasone were measured at the end of treatment using HPLC-MS with a linear reportable range of (5.1–1275 nM), as previously described (8).

Quantification of fenofibrate/fenofibric acid:

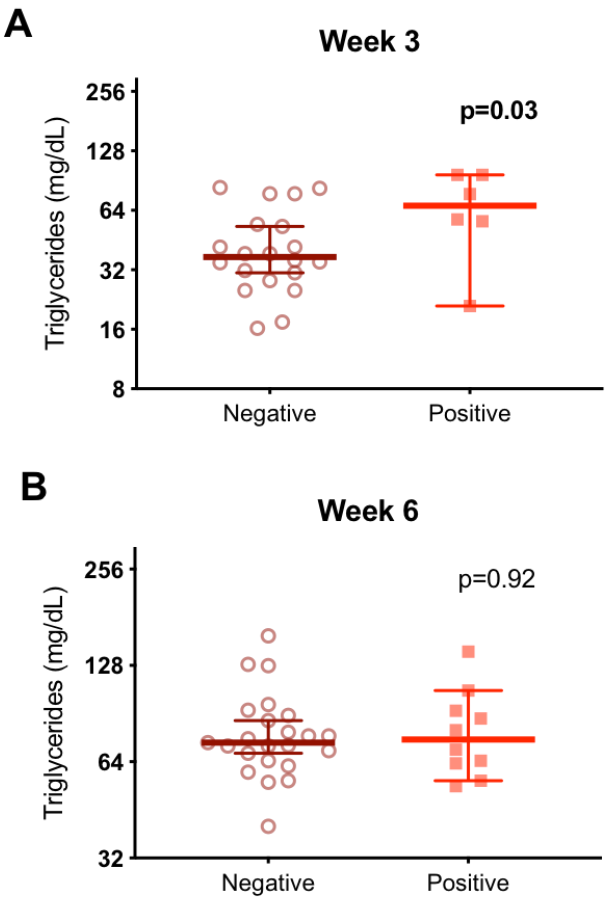
Quantitation of fenofibrate and analytes was carried out with a Waters ACQUITY separation system (Milford, MA) and Xevo TQ triple-quadrupole system (Beverly, MA). Separation was achieved on a Waters ACQUITY BEHC_{18} column (1.7 µm, 50 x 2.1 mm) using a column heater operating at 40°C with a Waters ACQUITY in-line filter. Autosampler temperature was maintained at 15° ± 5°C. The gradient mobile phase was composed of 0.1% formic acid of acetonitrile (B) and 0.1 % formic acid water (A). The flow rate was 0.6 ml/min, and the separation was completed within 5 min. The instrument was equipped with an electrospray interface, and was controlled by Masslynx 4.1 software (Waters, MA). The analysis was performed in MRM mode: *m/z* 361 > 232 for fenofibrate; *m/z* 318 > 232 for fenofibric acid; m/z 367> 234 for fenofibrate_d6; m/z 324>138 for fenofibric acid_d6. The MS/MS conditions were as follows: capillary voltage: 2.5kV; source temperature: 150°C; desolvation temperature: 500°C; cone gas flow: 0 l/h; desolvation gas flow: 1000 l/h. Note that no fenofibrate was detectable in our experimental samples.

Frozen samples were thawed at room temperature. A 10 µL aliquot of standard, QC or mouse sample was spiked into a polypropylene microcentrifuge tube and 30 µL of internal standard solution (5 ng/ml of fenofibrate and 25 ng/mL fenofibric acid in MeOH) was added. The tube was vortex-mixed for 20 sec, followed by centrifugation at 15,000 rpm for 8 min at 4°C. The supernatant was transferred to sample vial and 1µL was injected for analysis.

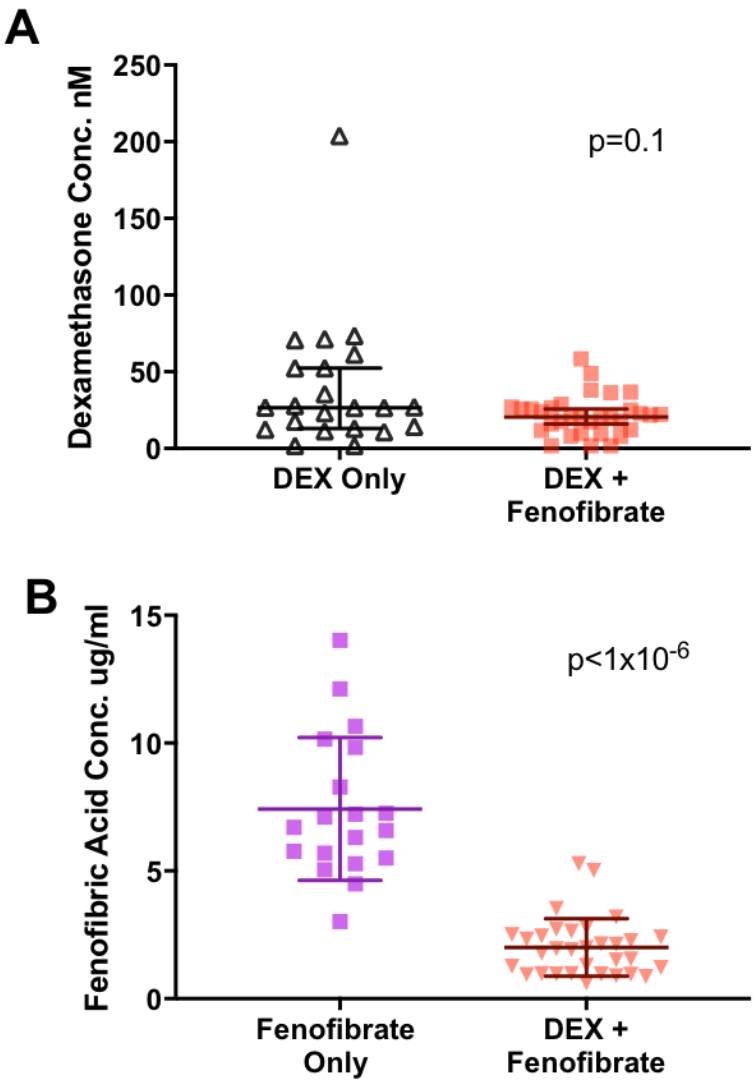
BCR-ABL model:

The BCR-ABL ($p185^{+}$, $Arf^{-/-}$) luciferase-positive cell line ($BCR\text{-}ABL^{+}$) was generated (8-11) . Female 8-week-old matched syngeneic mice (C57Bl/6J, *Arf*-wildtype; Jackson Laboratory, ME) received intravenous injections of 2000 $BCR\text{-}ABL^{+}$ cells. Bioluminescent imaging was performed weekly (8), to monitor leukemic burden. At day 3, mice were stratified by luminescent signal and body weight to treatment (n=10/treatment group). Fasting serum triglycerides were measured at day 24. Treatment ended at day 28: mice were maintained on base-diet/base-water until humane endpoint or the end of study at day 63. Mice were observed daily and sacrificed when luminescence was nearing saturation (pre-determined ventral-luminescence threshold of $1x10^{10}$) or they displayed clinical signs (hind limb paralysis, ruffled fur, respiratory distress, poor mobility) or at the end of study at day 63. Peripheral white blood cell count (WBC) and spleen weight were measured at sacrifice. In both the control and dexamethasone-only treatment groups, one mouse was censored from analysis due to early death not related to leukemia.

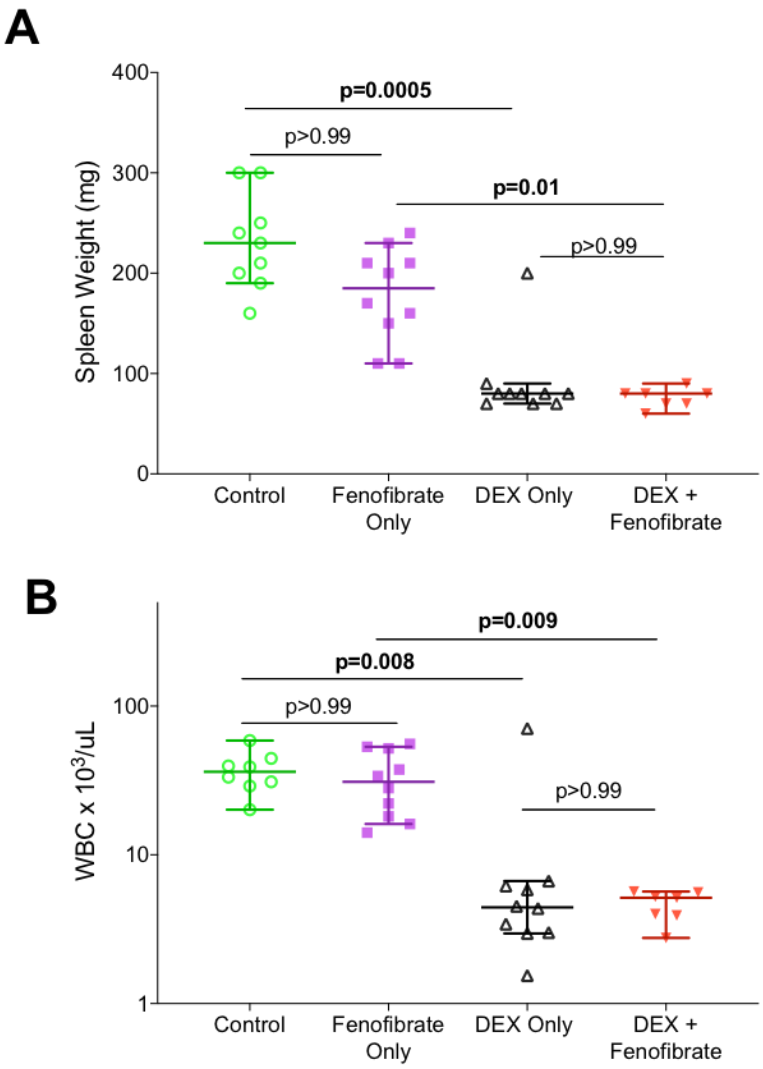
Supplemental Figures:



Supplemental Figure 1: In dexamethasone + fenofibrate mice, week 3 triglycerides differed by osteonecrosis status. Fasting serum triglycerides were measured at week 3 (A) and 6 (B). Graph plots serum triglycerides by osteonecrosis status in dexamethasone + fenofibrate mice. P-value was calculated using the two-tailed Mann-Whitney test. The graph show values from individual mice and the group median with 95% CI. At week 3, n= 20 (negative); n= 6 (positive). At week 6, n= 23 (negative); n= 10 (positive).



Supplemental Figure 2: Fenofibrate supplementation did not affect plasma dexamethasone. At end of treatment at week 6, dexamethasone (A) and fenofibric acid (B) were measured by HPLC-MS. P-value was calculated using the two-tailed Mann-Whitney test. The graph show values from individual mice and the group median with 95% CI. N=22 (DEX Only); n= 32 (DEX + fenofibrate).



Supplemental Figure 3: Fenofibrate does not affect efficacy of dexamethasone in BCR-ABL model. At time of humane sacrifice or end experiment at day 63, spleen weight (A) and WBC count (B) were measured. N=7-10/group. Kruskal-Wallis test was used to compare groups: only p-values between relevant groups: control vs. fenofibrate-only; control vs. DEX-Only; fenofibrate-only vs. DEX+ fenofibrate; DEX-Only vs. DEX + fenofibrate. Graph show values from individual mice and the group median with 95% CI.

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