Title: Blood-borne infections exacerbate incidence and severity of symptomatic glucocorticoid-induced osteonecrosis

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Keywords: acute lymphoblastic leukemia, bacteremia, glucocorticoid, lipopolysaccharide, osteonecrosis

Abbreviations:

ALL	acute lymphoblastic leukemia
CI	confidence interval
i.p.	intraperitoneal
LPS	lipopolysaccharide
OR	odds ratio

Abstract:

Background: Osteonecrosis is a common toxicity associated with glucocorticoid (e.g. dexamethasone and prednisone) treatment of children with acute lymphoblastic leukemia (ALL), but risk factors are incompletely defined. Infections are also a common complication of ALL therapy. Lipopolysaccharide (LPS) is used experimentally to mimic infection-related systemic effects. To our knowledge, the contribution of systemic infections to the risk of glucocorticoid-induced osteonecrosis has not been investigated.

Procedure: Patients with ALL on St. Jude Total Therapy XV (n= 365) were assessed for documented bacteremia prior to development of osteonecrosis, which was confirmed by MRI, and graded using the National Cancer Institute's Common Terminology for Adverse Events (version 3.0). In a pre-clinical model, Balb/cJ mice treated with dexamethasone plus or minus LPS were assessed for frequency and severity of osteonecrosis and arteriopathy.

Results: We found that patients with ALL who experienced bacteremia had a higher frequency of symptomatic osteonecrosis ($\geq$ grade 2) than those who did not (OR: 1.88, 95% CI: 1.03-3.41, $p=0.038$). LPS exacerbated experimental dexamethasone-induced osteonecrosis. Mice treated with dexamethasone plus LPS had a higher incidence of osteonecrosis ($p=0.00086$) and arteriopathy ($p=0.0047$) than did those treated with dexamethasone alone, and the severity of osteonecrosis ($p=0.00045$) and arteriopathy ($p=0.0048$) were also more pronounced with the addition of LPS treatment. The increase in osteonecrosis was not explained by any alteration of dexamethasone pharmacokinetics by LPS.

Conclusions: These data identify systemic infection during ALL therapy as a novel risk factor in the development of glucocorticoid-induced osteonecrosis.

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Introduction:

The association between glucocorticoid (e.g. dexamethasone and prednisone) use and the development of osteonecrosis has been described in patients treated for various inflammatory diseases and lymphoid malignancies (1). The incidence of osteonecrosis varies by the intensity and schedule of glucocorticoid exposure, age of the patient, gender, race, concurrent drug use, and genetic factors (2, 3).

Glucocorticoid-induced osteonecrosis is a serious and common adverse event in children treated for acute lymphoblastic leukemia (ALL) (4-7), with prevalence varying across studies (3, 4, 8-11). Though the risk factors for osteonecrosis have been extensively studied, incidence of the disease varies based on anti-leukemia treatment regimen and study population. Older age and higher glucocorticoid exposure are two risk factors consistently associated with osteonecrosis in children with ALL (3).

Infection is one of the most common therapy-related causes of morbidity and mortality in pediatric patients with ALL (12-15) due to therapy related immunosuppression and myelosuppression. Infection remains the most common cause of treatment-related mortality (16-18); a retrospective analysis of the UKALL2003 cohort identified bacterial infections as the most common cause of death (12). Likewise, in our recent retrospective study, bacteremia was the most common microbiologically documented infection (13). Infections are most common during the induction phase of therapy, which includes glucocorticoids (13, 15, 19, 20). While severe infections are known to cause end-organ damage, the effect on bone has not been previously studied in patients with ALL. Based on evidence that lipopolysaccharides (LPS), or endotoxins, components of gram-negative bacterial

outer membrane that mimic the systemic effects of infection in experimental models, have been shown to predispose to prednisone-induced osteonecrosis in animal models (21), we hypothesized that infection in children with ALL would increase their risk of osteonecrosis. We therefore tested if blood-borne bacterial infections were associated with the risk of osteonecrosis in pediatric patients with ALL, and tested if LPS exacerbated osteonecrosis in our mouse model of dexamethasone-induced osteonecrosis

Methods:

Murine studies

Dexamethasone sodium phosphate solution (0.4 mg/mL sodium citrate dihydrate; 0.1 mg/mL sodium sulfite; 0.01 mg/mL benzyl alcohol) was obtained from Fresenius Kabi (Lake Zurich, IL). Sulfamethoxazole (600 mg/L) and trimethoprim (120 mg/L) oral suspension was obtained from Aurobindo Pharma USA, Inc (Dayton, NJ), and tetracycline was purchased from Sigma (St. Louis, MO). The folic-acid deficient diet was purchased from TestDiet (Richmond, IN). Lipopolysaccharide (LPS) isolated from *E. coli* 0111:B4 was purchased from EMD Millipore (Burlington, MA). Sodium chloride (0.9% saline) injection solution was obtained from APP Pharmaceuticals, LLC (Schaumburg, IL).

The treatment protocol was a variation of those used in our clinically relevant model of dexamethasone-induced osteonecrosis in Balb/cJ mice (22-25). Male Balb/cJ mice were bred in house at St. Jude Children's Research Hospital (Memphis, TN). At postnatal days 26 to 28, mice were placed into one of four treatment groups (Figure 1). The dexamethasone only group (n=50) received continuous exposure to

dexamethasone (2 mg/L) in drinking water for 42 days (23). LPS only mice (n=14) received LPS (1 mg/kg) intraperitoneal (i.p.) injections on days 0 and 14. Dexamethasone plus LPS mice (n=50) received both dexamethasone and LPS, and the control mice (n=10) received sham injections of saline and no dexamethasone. To prevent opportunistic infections, drinking water for all mice contained tetracycline (1 g/L) for 7 days per week, and sulfamethoxazole (600 mg/L) and trimethoprim (120 mg/L) oral suspension for 3.5 days per week; the antibiotics have no effect on osteonecrosis (22, 23). Mice were fed a folic-acid deficient diet (< 0.05 ppm folic acid) (22). Blood was collected at time of sacrifice and plasma was frozen at -80 C until assayed for dexamethasone concentrations by HPLC (26). 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, with free access to food and water, and a 12-hour light/dark cycle. All experiments were approved by the Institutional Animal Care and Use Committee of St. Jude Children's Research Hospital.

Previous studies showed that osteonecrosis occurred predominately in the distal femoral epiphysis, therefore only the knee joints were evaluated (22). At necropsy, both hind limbs were collected, fixed in 10% formalin, decalcified in 10% formic acid, and processed (22-25). Hind limbs were sagittally sectioned beginning at the medial aspect of the knee. A minimum of 5 sections of a 4 µm thickness were collected at 20 µm apart, and stained with hematoxylin and eosin. The sections were examined by a board-certified Veterinary Pathologist (L.J.J) to evaluate the presence and extent of distal femoral epiphyseal necrosis and arteriopathy. If the initial five sections collected did not contain an evaluable arteriole, additional sections were cut

and stained. Even with this extensive sectioning, there were cases that an evaluable arteriole was not able to be sectioned. The percent area of necrosis of the marrow and trabecular bone in the distal femoral epiphysis was estimated on the section containing the greatest area of necrosis. Osteonecrosis was defined by necrotic bone marrow adipose tissue and hematopoietic cells, ghost osteocytes, and empty lacunae within trabecular bone. Vessels were scored (arteriopathy score) from 0-4 where 0 = no abnormalities (within normal limits, WNL); 1 = loss of smooth muscle cell nuclei, endothelium intact; 2 = loss of smooth muscle cell and endothelial cell nuclei, with or without mild thickening of the vessel wall but no luminal occlusion; 3 = loss of smooth muscle cell and endothelial cell nuclei with thickening of the vessel wall to the point of causing luminal occlusion; and 4 = loss of smooth muscle cell and endothelial cell nuclei with thickening of the vessel wall, luminal occlusion, and thrombosis of the vessel proximal to/above the physis.

Patients, osteonecrosis, and bacteremia

A retrospective analysis investigating the association between osteonecrosis and bacteremia was performed on patients with ALL (n=498) treated on the Total XV study from June 2000 to October 2010 at St. Jude Children's Research Hospital (27). Risk classification and treatment regimens (27) and use of antibiotics has been described elsewhere (13). Patients (n= 365) were prospectively screened by MRI of the hips and knees (after reinduction I (weeks 7-9) & II (weeks 17-19) of continuation therapy, and at the completion of therapy); additional joints may have been evaluated by MRI on a clinical basis depending on symptoms. All cases of osteonecrosis were confirmed by MRI. At the time of each MRI, possible symptoms of osteonecrosis were assessed, and osteonecrosis was prospectively graded

according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 3.0 (CTCAE v.3: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae3.pdf): grade 0 (no osteonecrosis by imaging), grade 1 (osteonecrosis by imaging but asymptomatic), grade 2 (osteonecrosis by imaging with moderate symptoms), grade 3 (osteonecrosis by imaging with severe symptoms) or grade 4 (osteonecrosis by imaging with disabling symptoms), as has been previously reported (5). Cases of bacteremia were defined based on clinical presentation and positive blood cultures according to modified National Healthcare Safety Network criteria as previously described (13, 14). Patients without documented bacteremia were classified as negative for bacteremia. The date of MRI for the highest grade of osteonecrosis per patient was used as the cut-off date to assess whether bacteremia had or had not occurred prior to osteonecrosis. This retrospective study was approved by the institutional review board.

Statistical Analysis

The Chi-square test was used to compare differences in categorical variables between groups when the expected number of cases in each category was ≥ 5 ; the Fisher's exact test was used when the expected number of cases was < 5 . The Mann-Whitney test was used to compare continuous variables. The log-rank test was used to compare survival between treatment groups. The effect size was estimated by calculating the summary odds ratio (OR) and its 95% confidence intervals (CI). Multivariate analyses utilized generalized linear regression. All statistical analyses were conducted using the R program (version 3.3.1). A P-value of less than 0.05 was considered statistically significant.

Results:

Dexamethasone and LPS in mice

There was no difference in body weight of mice prior to placement on treatment (Supplemental Figure S1). Both control and LPS only mice gained more weight than dexamethasone-treated mice, consistent with our prior findings dexamethasone-treated mice weigh less than control-treated mice in this model (22, 23, 25).

Dexamethasone plus LPS mice showed a transient delay, but not decrease, in weight gain compared to dexamethasone-only mice (Figure 2A). There was no difference in survival among treatment groups ($p=0.92$), indicating that LPS exposure was not toxic (Figure 2B).

LPS given alone at the doses used herein (low enough to cause no effect on weight gain or survival) did not increase the frequency of osteonecrosis ($p > 0.9$) or arteriopathy ($p > 0.9$) compared to control (Figure 3). However, in dexamethasone-treated mice, the addition of LPS increased the frequency of both osteonecrosis (55% versus 20%, $p=0.00086$) and arteriopathy (65% versus 35%, $p=0.0047$) compared to those treated with dexamethasone only (Figure 3).

We have previously shown a significant association between occurrence of osteonecrosis and arteriopathy in our mouse model (24). In the current study, lesions were present in the evaluable vessels of 100% (36/36) of mice with and in 21% (13/61) of mice without osteonecrosis ($p=3.38 \times 10^{-13}$) (Supplemental Table S1).

There was no difference in arteriopathy in osteonecrosis-positive mice by treatment group: 100% (9/9) of only dexamethasone-treated mice exhibited arteriopathy versus

100% (27/27) of those treated with dexamethasone plus LPS ($p=1$) (Supplemental Table S1). These data support vascular damage as the primary event in the development of glucocorticoid-induced osteonecrosis.

In a representative subset of mice, we evaluated the severity of osteonecrosis and arteriopathy by utilization of numeric scoring schemes. To determine the degree of osteonecrosis, we calculated the percent of epiphysis that was necrotic in each distal femur. Anything greater than 0% necrosis was considered positive for osteonecrosis. The sum of necrosis in each mouse (sum of the two distal femur percentages) was recorded as the “necrosis score”: a mouse could have a score from 0 (negative for osteonecrosis in both joints) to 200 (100% necrosis in both joints) (Figure 4A, 4B). Arteriopathy was graded for the damage to the arteriolar branches of the medial genicular artery of each distal femur (Figure 4C, 4D). For the mouse overall score, the sum of the arteriolar damage to the two distal femurs was calculated: a mouse could have an arteriopathy score of 0 (negative) to 8 (thrombosis in both hind limbs). Not only was the frequency of osteonecrosis and arteriopathy greater after dexamethasone plus LPS versus dexamethasone alone (Figure 3), but the severity was also greater: the mean necrosis was 39.2% (range 0-175) versus 1.6% (range 0-25), $p=0.00045$ (Figure 4A, 4B). Likewise, the mean arteriopathy score was 2.48 (range 0-7) in mice treated with dexamethasone plus LPS versus 0.92 (range 0-4) in those treated with dexamethasone only ($p=0.0048$) (Figure 4C, 4D). Three mice in the dexamethasone plus LPS group (and none in the dexamethasone alone group) showed bilateral osteonecrosis and arteriopathy, further supporting exacerbated disease with LPS treatment.

LPS did not affect the pharmacokinetics of dexamethasone ($p = 0.38$, Supplemental Figure S2A). We have previously shown that higher plasma dexamethasone concentration is associated with osteonecrosis in mice (25) and in patients with ALL (5). However, in this cohort of mice, we did not observe a significant difference in plasma dexamethasone in mice positive versus negative for osteonecrosis ($p=0.29$, Supplemental S2B) nor in mice positive versus negative for arteriopathy ($p=0.14$, Supplemental S2C).

Bacteremia preceding osteonecrosis in St. Jude TXV cohort

Osteonecrosis outcomes were available for 365 patients. Twenty-nine percent (105 patients) of these patients developed bacteremia; in 68 cases, the episode occurred prior to osteonecrosis and these cases were considered positive for bacteremia in our analysis; in 37 cases, bacteremia occurred after the MRI for highest-grade osteonecrosis, and thus these patients were classified as negative for bacteremia in our analysis. Most patients with bacteremia had only one recorded episode of bacteremia during their 2.5-year course of treatment (Supplemental Table S2). There was a significant association between bacteremia and subsequent development of symptomatic osteonecrosis ($\geq$ grade 2 by CTCAE v.3): 29% (20/68) of patients with bacteremia developed symptomatic osteonecrosis versus 18% (54/297) of patients without bacteremia (OR: 1.88, 95% CI: 1.03-3.41, $p=0.038$) (Figure 5). This association remained significant after adjustment for race and gender ($p=0.047$), which had been associated with bacteremia (13), although not ($p=0.087$) after additional adjustment to include age, a known risk factor for osteonecrosis. Though most patients with bacteremia had only one documented episode, an increased number of episodes of bacteremia was associated with development of symptomatic

osteonecrosis ($p=0.0073$): 8.1% (6/74) of patients with symptomatic osteonecrosis ($\geq$ grade 2) had > 1 episode of bacteremia versus 1.4% (4/291) of patients without symptomatic osteonecrosis ($<$ grade 2). This association remained even after adjustment for race, gender, and age ($p=0.04$) (Supplemental Table S2).

Osteonecrosis was also evaluated as an ordinal variable (grades 0-4): prior bacteremia was significantly associated with increased grade of osteonecrosis ($p=0.034$), which was significant after adjustment for race and gender ($p=0.031$), but not after additional adjustment for age ($p=0.082$). We found that 16% (30/188) of patients with grade 1 osteonecrosis; 22% (10/45) of patients with grade 2 osteonecrosis; 30% (8/27) of patients with grade 3 osteonecrosis and 100% (2/2) of patients with grade 4 osteonecrosis had prior bacteremia (Supplemental Table S3),

Discussion:

Glucocorticoids are immunosuppressive, and when given chronically, they can cause vascular damage and osteonecrosis (1, 24, 28, 29). The same schedules of glucocorticoid that are used clinically, and are mimicked in our murine model of dexamethasone-induced osteonecrosis, place patients at high risk of infection due to chronic immunosuppression (12-14, 19). Herein, we show for the first time an association between bacteremia and a higher risk of osteonecrosis (Figure 5).

Our clinical data suggest infections complicating ALL therapy may have long-term effects on bone health in survivors. Due to the relatively small number of patients with information on both bacteremia and osteonecrosis (studied via prospective MRI), we did not have the power to further classify type of bacteremia or evaluate incidence of a specific type of infection as it related to osteonecrosis. The

association between osteonecrosis and bacteremia did not remain statistically significant after multivariate analysis including patient age (Table 5, Supplemental Table S3). Older age (≥ 10 years) is one of the most important risk factors for osteonecrosis in children with ALL (3-5, 10). Interestingly, patients with a higher number of bacteremic episodes were at higher risk for symptomatic osteonecrosis even after adjustment for age, gender, and race (Supplemental Table S2). There was no association between bacteremia and age in our cohort. Additional data in larger cohorts of adolescents and young adults will be helpful to better understand the relationship between infection and osteonecrosis.

Although the observed association between infection and osteonecrosis could be explained by an independent increased risk of both complications due to varying exposure to glucocorticoids, there are also numerous plausible mechanisms by which the systemic inflammatory response to infection could exacerbate the damage to vasculature (28) and bone (29-31) that contribute to osteonecrosis, as shown in our experimental model.

Testing whether infection predisposes to glucocorticoid-induced osteonecrosis in our murine model is a challenge. Deliberate infection during the six weeks of continuous glucocorticoid exposure that are required to induce osteonecrosis (22, 23) would likely cause immediate death, and therefore make the mice non-evaluable. Keeping mice alive long enough to develop glucocorticoid-induced osteonecrosis without succumbing to infection has been a major challenge to modelling this phenotype preclinically in mice (23). To mimic the systemic effects of infection, injections of LPS have been widely used. LPS is known to exacerbate glucocorticoid-induced

osteonecrosis in animal models, likely, at least in part, due to activation of the TLR4 signaling pathway (21, 32-38). The mouse model described herein is unique compared to prior models for several reasons, including the systemic glucocorticoid exposure, the LPS administration, the age of the mice, and the anatomic location of osteonecrosis, all of which make our model relevant for testing risk factors for osteonecrosis among children with ALL.

The experimental model of osteonecrosis used in this study has several strengths. It reflects the clinical route of oral administration of glucocorticoids, whereas most other models utilize intermittent injections. These intermittent exposures to large doses of glucocorticoids do not reflect the clinical exposure of most patients with ALL, who receive constant plasma levels of the drug over multiple consecutive days (27, 39, 40). The plasma dexamethasone concentrations we report herein (15-30 nM, Supplemental Figure S2) are analogous to those observed clinically (5, 41). Our data showed that LPS increased the frequency of osteonecrosis when continuous dexamethasone is administered to mice, and that the mechanism was not due to a pharmacokinetic effect of LPS on inhibition of dexamethasone clearance (Supplemental Figure S2).

A prior murine model of osteonecrosis that utilized both glucocorticoids and LPS used repetitive exposure to LPS to induce a chronic inflammatory condition (34, 42). However, repeated exposure to sublethal doses of LPS can lead to immune tolerance, and decreased inflammation (43). Non-lethal systemic infections are generally acute in patients with ALL: chronic exposure to LPS is not a reflective model of the effect of infection in the development of osteonecrosis. While 29% of

patients had bacteremia complicating ALL therapy, most patients had only one episode (Figure 5, Supplemental Table S2). Those with more than one episode had distinct incidents: chronic infections were not observed. Many prior models used doses of LPS so substantial that animals experienced significant weight loss or mortality from the LPS (32, 34, 38, 44) whereas our mice exhibited neither more weight loss nor mortality than that caused by dexamethasone alone (Figure 2), making our LPS trigger more relevant to the level of stress caused by non-lethal systemic infections in patients with ALL.

Our experimental model utilized mice 26-28 days of age, equating to the pediatric/adolescent age range that is relevant for children with ALL. It is known that the immune response to blood-borne bacterial infections varies with age (45), with aged mice showing a more pronounced decrease in anticoagulant pathway activation and a greater degree of endothelial damage following LPS administration (46). We report that acute LPS exposure increased glucocorticoid-induced osteonecrosis in a murine model that is representative of the pediatric population.

Other models investigated the femoral head and/or the vessels supplying the femoral head (lateral epiphyseal arteries) for diagnosis of experimental osteonecrosis (21, 32, 34, 35, 37). In our model, we observe the development of osteonecrosis in the distal femur (femoral condyles), which has limited vasculature compared to the femoral head (24). This allows for the role of vascular damage to be more sensitively assessed as it relates to development of osteonecrosis, as other vessels cannot compensate for the initial vascular damage and lack of blood supply.

We observed a nearly 3-fold increase in the incidence of osteonecrosis in mice treated with dexamethasone plus LPS (55%) versus dexamethasone only (20%), with the rate of arteriopathy increasing from 35% in dexamethasone only mice to 65% in dexamethasone plus LPS mice (Figure 3). The severity of both osteonecrosis and arteriopathy was higher in mice treated with LPS (Figure 4). These findings are consistent with our prior work, showing that proximal vasculature damage (e.g. arteriopathy) is the likely preceding and possibly causative event prior to development of osteonecrosis following dexamethasone with or without concurrent asparaginase (24, 25). LPS has been shown to cause apoptosis of vascular endothelial cells and inhibition of vascular repair (28, 47). Thus, it is possible that the higher incidence of arteriopathy and osteonecrosis we observed with LPS added to dexamethasone was a direct consequence of additional vascular insult caused by LPS, although arteriopathy was rare following LPS alone (Figure 3). LPS treatment could also be secondarily affecting the bone through activation of pro-inflammatory mediators that promote bone resorption (30, 31).

We conclude that blood-borne bacterial infection may predispose to glucocorticoid-induced osteonecrosis in children being treated for ALL and that this clinical association can be modeled experimentally in dexamethasone-treated mice via acute administration of LPS. This finding is clinically important because it provides evidence that, in addition to the acute morbidity and mortality associated with infections during treatment for ALL, they may have serious consequences that extend into survivorship (48). This provides impetus for development of methods to prevent and pre-emptively treat infection in this vulnerable population (14).

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

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Legends:

Figure 1: Dexamethasone and lipopolysaccharide treatment regimens. Top two horizontal lines indicate continuous exposure to dexamethasone (2 mg/L DEX in drinking water + prophylactic antibiotics) and the bottom two horizontal lines indicate control mice on prophylactic antibiotic water only, with or without lipopolysaccharide (LPS) injection. Vertical arrows indicate LPS (1 mg/kg body weight) or sham (saline) i.p. injections at days 0 and 14 of a 6-week experiment. The number of mice represent two independent experiments.

			DEX (water)	LPS (i.p.)
DEX only (50 mice)	↓ 14d ↓	→ 6 weeks	2 mg/L	sham
DEX + LPS (50 mice)	↓ 14d ↓	→ 6 weeks	2 mg/L	1 mg/kg BW
LPS only (14 mice)	↓ 14d ↓	→ 6 weeks	0 mg/L	1 mg/kg BW
Control (10 mice)	↓ 14d ↓	→ 6 weeks	0 mg/L	sham

Figure 2: Weight and survival in mice by treatment group. A. Weight was measured weekly. Line represents group mean with shaded area showing the limits of the 95% confidence interval. Lipopolysaccharide (LPS) and control mice weighed significantly more than dexamethasone (DEX) only and DEX + LPS mice from weeks 1-6 of treatment (# $p < 1 \times 10^{-6}$; Kruskal-Wallis test at each time point). Mann-Whitney test was used to compare relative weight of DEX only and DEX + LPS mice (** $p < 0.0001$; * $p < 0.05$). B. Survival curve. 100% survival in Control (10/10) and LPS only (14/14) groups, 98% survival in both DEX only (49/50) and DEX + LPS (49/50) groups. Log-rank test was used to calculate survival probability p-value.

Figure 3: Lipopolysaccharide treatment increased the frequency of osteonecrosis and arteriopathy in dexamethasone-treated mice. Chi-square P values were calculated between dexamethasone (DEX)-treated mice that received versus those that did not receive lipopolysaccharide (LPS). All control mice were negative for osteonecrosis and arteriopathy (data not visible on graph). One DEX-Only mouse was not evaluable for arteriopathy.

Figure 4: LPS treatment exacerbated the severity of osteonecrosis and arteriopathy in DEX treated mice. A. Representative image of H&E-stained sagittal section of the distal femoral epiphysis by treatment group. Right images show magnification of associated boxed area on left. B. Osteonecrosis scores by treatment group. Necrosis score for each mouse represents the sum of necrosis in the two distal femurs. C. Arteriopathy scores in mice by treatment group: sum of scores in both distal femurs are shown. D. Representative image and description for arteriopathy grading scheme. Arrows indicate areas that define the arteriopathy grade (text below image). Triangles on boxplots indicate group mean. P-value calculated using Mann-Whitney test. N=25/treatment group.

Figure 5: Frequency of symptomatic osteonecrosis and bacteremia in patients with leukemia. Of the 498 patients in TXV, 365 were evaluable by MRI and symptoms for osteonecrosis (ON) [assigned using CTCAE v.3 criteria: grade 0 (absent), grade 1 (MRI positive but asymptomatic), grade 2 (MRI positive with moderate symptoms), grade 3 (MRI positive with severe symptoms), or grade 4 (MRI positive with disabling symptoms)]. 29% (105/365) of evaluable patients were positive for bacteremia; 19% (68/365) were positive for bacteremia prior to highest grade of osteonecrosis event. 29% (20/68) of patients with prior bacteremia developed symptomatic osteonecrosis (grade 2-4) versus 18% (54/297) of patients without bacteremia prior to highest grade osteonecrosis event. Odds ratio (with 95% confidence interval) indicates risk of symptomatic osteonecrosis given bacteremia status. Using a linear regression model including bacteremia, genetic race and sex as covariates, bacteremia remained significantly associated with symptomatic osteonecrosis ($p=0.047$); but was not statistically significant when age was added to the model ($p=0.087$).

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