Orthopedic Toxicities Among Adolescents and Young Adults Treated on DFCI ALL Consortium Trials

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Abstract:
Adolescent and young adult patients with acute lymphoblastic leukemia (ALL) have superior outcomes when treated on pediatric regimens. Pediatric ALL regimens rely heavily on corticosteroids and asparaginase and are known to increase the risk of osteonecrosis (ON) and fractures in children, particularly adolescents. Orthopedic toxicity among young adults treated on pediatric-inspired regimens is not well described. Here, we report the symptomatic orthopedic toxicities of patients aged 15-50 years treated on sequential Dana-Farber Cancer Institute (DFCI) ALL Consortium protocols. Among 367 patients with a median age of 23 years (range 15-50, 68% < 30 years), 60 patients were diagnosed with ON (5-year cumulative incidence (CI) 17%; [95% confidence interval 13-22]) and 40 patients experienced fracture (5-year CI 12% [95% CI 8-15]). Patients < 30 years were significantly more likely to be diagnosed with ON (5-year CI 21% vs 8%, p=0.004). Patients treated more recently on pegaspargase-based protocols were significantly more likely to be diagnosed with ON compared to those treated on earlier trials with native E.coli asparaginase (5-year CI 24% vs 5%, p=0.001). Of the 54 ON events for which adequate information was available, surgery was performed in 25 (46%). Patients with ON had superior overall survival (OS) compared to those without (multivariable OS HR 0.15 [95% CI: 0.05-0.46], p=0.001; ON included as a time-varying exposure). Increased rates of orthopedic toxicity in late generation protocols may be driven by the pharmacokinetic drug interaction between pegaspargase and dexamethasone, leading to higher dexamethasone exposure.

Conflict of interest:
COI declared - see note

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Regular article

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Short title: Orthopedic toxicity in AYAs treated on DFCI ALL trials

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Abstract

Adolescent and young adult patients with acute lymphoblastic leukemia (ALL) have superior outcomes when treated on pediatric regimens. Pediatric ALL regimens rely heavily on corticosteroids and asparaginase and are known to increase the risk of osteonecrosis (ON) and fractures in children, particularly adolescents. Orthopedic toxicity among young adults treated on pediatric-inspired regimens is not well described. Here, we report the symptomatic orthopedic toxicities of patients aged 15-50 years treated on sequential Dana-Farber Cancer Institute (DFCI) ALL Consortium protocols. Among 367 patients with a median age of 23 years (range 15-50, 68% < 30 years), 60 patients were diagnosed with ON (5-year cumulative incidence (CI) 17%; [95% confidence interval 13-22]) and 40 patients experienced fracture (5-year CI 12% [95% CI 8-15]). Patients < 30 years were significantly more likely to be diagnosed with ON (5-year CI 21% vs. 8%, p=0.004). Patients treated more recently on pegaspargase-based protocols were significantly more likely to be diagnosed with ON compared to those treated on earlier trials with native *E.coli* asparaginase (5-year CI 24% vs 5%, p<0.001). Of the 54 ON events for which adequate information was available, surgery was performed in 25 (46%). Patients with ON had superior overall survival (OS) compared to those without (multivariable OS HR 0.15 [95% CI: 0.05-0.46], p=0.001; ON included as a time-varying exposure). Increased rates of orthopedic toxicity in late generation protocols may be driven by the pharmacokinetic drug interaction between pegaspargase and dexamethasone, leading to higher dexamethasone exposure.
Key points (140 character limit for each bullet)

- Orthopedic toxicity was common in adolescent and young adult patients treated on DFCI Consortium pediatric ALL protocols.
- Younger age and exposure to pegaspargase were associated with higher risk of osteonecrosis (ON). Patients with ON had superior OS.

Introduction

Adolescents and young adults (AYAs) diagnosed with acute lymphoblastic leukemia (ALL) are less likely to be cured of their disease than younger children. While adverse disease biology is more commonly present in this population, it is now recognized that the therapeutic approach also has a major impact on outcome. A seminal 2008 analysis observed that patients aged 16 to 20 years treated on Children’s Cancer Group (CCG) trials from 1998 to 2001 had superior outcomes compared to same-aged patients enrolled in adult Cancer and Leukemia Group B (CALBG) trials. Subsequently, several retrospective studies were conducted worldwide that confirmed the observation that AYAs treated on pediatric protocols had improved outcomes compared to those treated on adult protocols. This prompted multiple groups of investigators in the United States and Europe to prospectively study pediatric or pediatric-inspired protocols in adults up age 50 years demonstrating safety and favorable outcomes compared to historical cohorts. These efforts have established pediatric-inspired regimens as a standard of care for AYA patients with ALL making it essential that the unique toxicities of pediatric regimens – which rely heavily on corticosteroids and asparaginase – also be carefully studied in this population.

Orthopedic toxicity, including osteonecrosis (ON) and fracture, is a known complication of pediatric ALL treatment regimens and has been attributed primarily to corticosteroid exposure with many, though not all, studies showing that dexamethasone, compared to prednisone, increases risk of ON. The incidence of symptomatic ON has been reported to range
between 6% and 9.3% in pediatric cohorts with adolescents at more risk than younger children.\textsuperscript{10,11,12} Orthopedic toxicity in adults treated for ALL has been studied less but younger adults have been reported to be more at risk than older adults whether treated on traditional adult\textsuperscript{13} or pediatric-inspired protocols.\textsuperscript{14} Some, but not all studies have shown a higher incidence of ON among female patients,\textsuperscript{11,12} while recent investigations have shown that African ancestry might be protective against ON and fracture.\textsuperscript{15} Given the long-term impact of orthopedic toxicity on ALL survivors, it is important to understand the frequency of, risk factors for, and impact of orthopedic events among AYAs treated on pediatric-inspired regimens.

Here, we present our experience with orthopedic toxicities in a large cohort of AYAs treated on sequential DFCI ALL Consortium protocols or off-study according to these protocol regimens. We aimed to characterize the incidence and risk factors for developing orthopedic toxicity in this treatment context.

\textbf{Methods}

\textit{Patients:} Patients aged 1-50 years were treated on four sequential multi-center DFCI ALL Consortium protocols between 2000 and 2018. We identified all trial participants 15 years and older at time of diagnosis for the current analysis. We also identified patients 15 years and older not enrolled on these studies but treated per the same protocols at DFCI/Brigham and Women’s Hospital (BWH), Massachusetts General Hospital (MGH), and Boston Children’s Hospital (BCH) using chart review. Earlier patients were enrolled on parallel pediatric 00-001 (2000-2004) and adult 01-175 (2002-2008) trials while later patients were enrolled on parallel pediatric 05-001 (2005-2011) and adult 06-254 (2007-2011) trials. This research was approved by the Dana Farber Cancer Institute Institutional Review Board and conducted in accordance with the Declaration of Helsinki.

\textit{Treatment:} The treatment protocols of 00-001, 05-001, and 01-175 have been previously published.\textsuperscript{7,10,16} The 06-254 trial was based on the very high risk arm of 05-001.\textsuperscript{17} All trials used
prednisone during induction. Protocol 00-001 randomized patients between dexamethasone (18mg/m²/day for days 1-5 of each 3-week cycle) and prednisone (120mg/m²/day for days 1-5 of each 3-week cycle) during post-induction treatment phases, while the other three trials used dexamethasone (18mg/m²/day for days 1-5 of each 3-week cycle). Both 00-001 and 01-175 used native *E.coli* asparaginase. Protocol 05-001 randomized patients between pegaspargase and native *E.coli* asparaginase, while 06-254 used pegaspargase for all patients. All patients were intended to receive 30 consecutive weeks of asparaginase during post-induction therapy (Figure 1).

**Orthopedic toxicities:** Cases of symptomatic ON and fracture were extracted from case report forms (CRFs) for trial patients and by chart review for those not on trial. Toxicity events were submitted in CRFs by treating providers and the selection of imaging modality for verification was per provider discretion. Events identified through chart review were verified either by magnetic resonance imaging, X-ray, or computed tomography. Patients were not classified as having a symptomatic fracture if the fracture resulted specifically from a procedure for treatment of osteonecrosis. Individual patients may have had more than one ON and/or more than one fracture during the treatment period. Additional chart review was performed on all patients treated at DFCI/BWH, MGH, and BCH (Boston cohort) to identify surgical events.

**Statistical analysis:** All patients were combined in the modeling based on the intended treatment protocol. The 5-year cumulative incidences (CIs) of ON and fracture were estimated and compared using the Gray test. Univariate and multivariable competing risk regression models were constructed with death as a competing risk. Leukemic relapse and second malignant neoplasm were not included as competing risk, as we hypothesized that all surviving patients were at risk of developing ON from the initial exposure to the ALL regimen. Multivariable models included age (<30 vs. ≥30 years), sex, body mass index (BMI, underweight or normal vs. overweight vs. obese/morbidly obese), and treatment regimen backbone. Covariates were chosen based on previously published literature. In subset analysis excluding...
patients on protocol 00-001 (due to lack of available data elements), the association of treatment-related dyslipidemia (grade 4 toxicity including hypercholesterolemia and hypertriglyceridemia) with ON and fracture was explored. Additionally, similar analyses were performed for patients who either received pegaspargase or native \textit{E.coli} asparaginase post-induction. Median follow-up was calculated using the reverse indicator for the Kaplan Meier method. Disease-free survival (DFS) was calculated from the time of remission to the time of relapse or death and censored at the last time known alive without relapse. Overall survival (OS) was calculated from the time of study registration to the time of death censored at the time last known alive. A time-varying covariate was included for ON and fracture was included in a Cox regression model to assess impact on DFS and OS. P-values are two-sided and considered significant if <0.05.

\textbf{Results}

\textbf{Patient Characteristics}

A total of 367 patients were identified and included in the analysis (Table 1, Figure 1b). Early generation protocols (00-001 and 01-175) accounted for 32\% (n=117) of patients with the remaining patients (68\%, n=260) treated on or as per late generation protocols (05-001 and 06-254). Fifty-nine (16\%) patients were treated as per these protocols, most commonly because protocol enrollment was closed at the time of presentation. The majority (61\%, n=225) of patients were male. The median age at time of diagnosis was 23 years, with 68\% (n=249) younger than 30 years (Table 1). The median BMI at diagnosis was 24.5 kg/m$^2$ with 46\% classified as overweight or obese. Seventy-seven percent (n=281) of patients had B-lineage ALL with 77\% (n=281) classified as CNS 1 status (negative). The median follow-up time for patients remaining alive on early generation protocols was 4.9 years (range: 0.08-14.1) and on late generation protocols was 5.1 years (range: 0.01-11.8) and did not significantly differ between protocol generations (Wilcoxon rank-sum test; p=0.15).
Orthopedic Events

In total, there were 100 ON events in 60 patients (5-year CI 17% [95% CI: 13-22]), and 51 fractures in 40 patients (5-year CI 12% [95% CI: 8-15]). The median time from diagnosis to ON event was 1.6 years (range: 0.5-7.7). The median time from diagnosis to fracture event was 1.4 years (range: 0.2-5.2) (Figure 2). Nine out of 40 first fractures (22.5%) and 22 out of 60 first ON events (36.7%) occurred 2 years or more from diagnosis. Table 2 shows the 5-year CI of orthopedic events for patient subgroups. The majority of the 100 ON events documented occurred in the hip (48) with the remainder occurring in the knee (20) and other locations (32). Of the 51 fractures, 13 were vertebral, 10 affected the foot, and the rest were in other locations (Supplementary Table 3). Among 54 ON events for which adequate follow-up information was available (Boston cohort), surgery was performed in 25 (46%), among whom 18 (33%) patients received total joint replacements. Of the 9 fracture events with available follow-up, 3 (33%) required surgery.

Risk Factors for Orthopedic Toxicity

To understand factors associated with increased risk for development of orthopedic toxicities, we performed univariate and multivariate competing risk regression analyses for ON and fracture. Patients younger than 30 years were significantly more likely to be diagnosed with ON (5-year CI 21% [95% CI: 16-27]) compared to those aged 30-50 years (5-year CI 8% [95% CI: 4-14], univariate HR 2.77 [95% CI: 1.35-5.65], p=0.005) (Figure 3a). Of note, patients 30-50 years were just as likely to have received 26 or more weeks of asparaginase than those younger than 30 years (41 vs 47% p=0.39), a threshold previously identified as being associated with improved outcomes.18 Patients treated on late generation pegaspargase-based protocols were significantly more likely to be diagnosed with ON (5-year CI 24% [95% CI: 18-30]) compared to those treated on early generation native *E.coli* asparaginase-based protocols.
(5-year CI 5% [95% CI: 2-10], HR 5.28 [95% CI: 2.24-12.48], p<0.001) (Figure 3b). Patients younger than 30 years on late generation protocols had a 5-year CI of 29% [95% CI: 22-37]. The association between age younger than 30 years and treatment on late generation protocols and risk for ON remained statistically significant in multivariate analysis including treatment backbone, age, BMI, and sex. BMI and sex were not associated with ON on univariate analysis. There were no significant associations of age, protocol generation, or any other variable and risk for fracture although there were fewer fractures in male patients, which was of marginal statistical significance (HR 0.55 [95% CI: 0.29-1.02], p=0.057) (Table 3). Only 20 patients in this cohort treated on a early generation protocol received post-induction prednisone and thus the impact of steroid formulation could not reliably be investigated.

To further investigate the role of asparaginase formulation, we performed a subset analysis among the 247 patients (67%) who received post-induction asparaginase (defined as receiving one or more doses of asparaginase after completion of induction) and classified patients by their intention-to-treat type of asparaginase (E.coli versus pegaspargase). Reasons for not proceeding to post-induction asparaginase included early death, severe early asparaginase toxicity, or treatment decision to be consolidated with allogeneic stem cell transplant. In this analysis, patients who were assigned to receive post-induction pegaspargase were more likely to develop ON than those assigned to received native E.coli-asparaginase (univariate HR 2.53 [95% CI: 1.42-4.49], p=0.002) (Supplementary Table 1). This result remained statistically significant in a multivariable analysis. Of note, in this group, duration of asparaginase was not statistically associated with ON, whether considered as a continuous variable (p=0.36) or as a categorical variable (< or ≥ 26 weeks, p=0.89).

To assess the contribution of treatment-emergent dyslipidemia (defined as grade 4 hypercholesterolemia or hypertriglyceridemia) to orthopedic toxicity, we performed a separate analysis excluding patients from protocol 00-001, as dyslipidemia was not reported consistently for those patients (Supplementary Table 2). In this analysis, dyslipidemia was associated with a
higher ON incidence in a univariate analysis (univariate HR 2.04 [95% CI: 1.15-3.64], p=0.016). This association was not statistically significant in a multivariable analysis. Of note, no patient in this analysis received post-induction prednisone and the associations between late generation protocols and ON as well as between age and ON remained statistically significant.

**Association of Orthopedic Toxicity and Survival**

The association of orthopedic toxicity and survival was explored by constructing multivariable models of OS with ON or fracture as the time-varying exposure of interest. After adjusting for covariates including age, presenting leukocyte count, immunophenotype, BMI, treatment regimen, Philadelphia chromosome status, and CNS status, patients experiencing an ON or fracture event were significantly less likely to die than those who did not experience an event (multivariable HR for ON 0.15 [95% CI: 0.05-0.46], p=0.001 and HR for fracture 0.40 [95% CI: 0.16-0.99], p = 0.048) (Table 4). Of note, when post-induction steroid formulation (dexamethasone vs. prednisone) was included in the model, it was not associated with OS in a statistically significant fashion (data not shown).

**Discussion**

Our study represents the largest investigation to date of orthopedic toxicity associated with ALL treatment in AYA patients treated on pediatric-inspired regimens. We found that orthopedic toxicities are common and that age between 15 and 30 years at treatment initiation, as compared to age greater than 30 years, is associated with a higher risk of developing ON. Our cumulative incidence was higher than in other recently published studies, which might be related to longer follow up, higher treatment completion rates, or differences in the doses and schedule of asparaginase and steroid exposure between DFCI and other protocols. The finding that younger adults are more at risk for orthopedic toxicity compared to older adults is consistent with reports of the recent adult Eastern Cooperative Oncology Group 2993 trial and the NOPHO
ALL2008 trial (which applied a pediatric regimen to patients aged 1-45). As it has been previously established that adolescents older than 10 years old are at higher risk of ON than younger children, our study helps confirm a “vulnerable window” of late adolescence and young adulthood during which ON risk is highest for patients receiving ALL therapy. A definitive mechanistic explanation for why adolescents and young adults are particularly vulnerable to ON has not been established. The procoagulant effects of increasing sex hormone concentrations, the timing of epiphyseal closure, and the peak of growth hormone/IGF1 during puberty leading to increased bone metabolic activity and increased vulnerability to hypoxia have all been identified as possible contributors. Our study did not confirm previous associations between ON and female sex, high BMI, and race/ethnicity. With regard to sex, this may be due to the fact that the increased risk for ON among females is more prominent in younger adolescents (aged 10-15 years). With regard to race/ethnicity, our cohort was predominantly white and did not reliably capture ethnicity data.

A key finding of our study is the demonstration that asparaginase formulation influences risk for ON with the cumulative incidence of ON being 24% in those on pegaspargase-based protocols compared to 5% in those treated on E.coli based protocols. One of the contributing studies to our analysis did not demonstrate this difference: DFCI 05-001, in which 7% of patients were older than 15 years at time of diagnosis, showed statistically similar ON rates between patients receiving E.Coli vs. pegaspargase. Since AYAs are at significantly higher risk for ON than younger children, we hypothesize that DFCI 05-001 was not adequately powered to detect differences between these formulations.

We hypothesize that pegaspargase increases the risk of ON through more sustained asparaginase exposure with the pegylated formulation. ON may result due to more toxicity from asparaginase itself and/or the interaction of asparaginase with steroid clearance. Asparaginase is thought to directly influence risk for orthopedic toxicity via its influence on coagulation parameters and lipid metabolism. Asparaginase induces a hypercoagulable state through
suppression of antithrombin and elevation of vWF/Factor VIII complex which may contribute to ON.\textsuperscript{23} It also causes alterations in lipid metabolism which could lead to lipid droplet formation in bony vascular beds and subsequent damage to the vascular endothelium.\textsuperscript{24} Recent experiments in mice have showed that reduction of serum triglycerides with fenofibrate can reduce ON incidence.\textsuperscript{25} Our study also demonstrated an association (although not statistically significant on multivariable analysis) between dyslipidemia and orthopedic toxicity which was previously reported in patients treated on the NOPHO ALL2008 protocol.\textsuperscript{26} Asparaginase may also indirectly affect risk for orthopedic toxicity via its pharmacokinetic interaction with steroids. One prior analysis showed that patients who were exposed to lower amounts of asparaginase as a result of developing an asparaginase allergy had higher dexamethasone clearance, leading to lower overall dexamethasone exposure.\textsuperscript{27} Hence, asparaginase appears to reduce steroid clearance when administered concurrently, which may lead to increased corticosteroid exposure and thus increase the risk for ON. This hypothesis can be tested in the future with formal monitoring of both asparaginase activity and corticosteroid levels in prospective studies.

Since pegaspargase has a longer half life than native \textit{E.coli} asparaginase,\textsuperscript{28} we propose that pegaspargase may lead to longer, more sustained exposure to both asparaginase and dexamethasone, thus leading to higher ON risk. It is important to note that pediatric and young adult regimens are now predominantly based on pegylated asparaginase formulations, including pegaspargase and calaspargase, a formulation with an even longer half life.\textsuperscript{29} While these formulations may offer important oncologic benefits, they may also increase orthopedic toxicity especially in vulnerable adolescents and young adults.

Another major finding of our study is the association of an orthopedic event and improved oncologic outcomes in our cohort. We found that patients with ON had an overall survival hazard ratio at 5 years of 0.15 compared to those without ON, after adjusting for relevant covariates. A similar finding was reported in the CCG-1961 trial where an improved EFS (HR of 0.32) was reported for patients older than 10 years who had an ON event compared
to those who did not.\textsuperscript{12} We hypothesize that patients who develop ON are likely to have had higher and/or more sustained steroid and asparaginase exposure due to individual differences in drug metabolism. Thus, ON may be a proxy for chemotherapeutic exposure and may provide reassurance for oncologists choosing to omit corticosteroids in patients who have experienced an orthopedic event while still on treatment as these patients have likely already had more intense exposure than patients who have not had an orthopedic event. For patients who have already completed treatment at the time of ON detection, focus will be on addressing the orthopedic issue and ensuring appropriate orthopedic care and bone health.

The retrospective nature of our analysis is a limitation as there may have been differences in adverse event ascertainment in earlier (\textit{E. coli} based) versus later (pegaspargase based) treatment regimens. However, our findings are consistent with those from CCG-1961, in which a subset of patients exposed to pegaspargase had higher ON rates than patients exposed to \textit{E. coli} asparaginase.\textsuperscript{12} It is important to note that our study only investigated clinical (symptomatic) ONs and fractures. While prospective radiographic screening in asymptomatic patients identifies more events,\textsuperscript{30} the clinical significance of asymptomatic lesions remains unclear and would benefit from further prospective study in patients followed during survivorship.

We hope our study will inspire several areas of future research. First, the generalizability of our findings will need to be confirmed in other regimens. DFCI AYA protocols are unique in that consolidation is based on 30 weeks of continuous asparaginase with corticosteroid pulses. Deliberate prospective characterization of orthopedic toxicities is required in all ALL protocols treating children and young adults. This is especially important as pediatric-inspired protocols are being used more frequently in the AYA population and incorporated in national guidelines.\textsuperscript{31} Second, careful documentation of surgical interventions, quality of life measures, and functional outcomes should be incorporated in future AYA protocol design to capture the full clinical impact of orthopedic toxicity beyond what is captured in routine CTCAE grading. The fact that over a
third of the first ON events in our study occurred 2 years or more from diagnosis highlights the fact that ON events may be underascertained in clinical trials. This should be a focus of future survivorship studies recognizing that “loss to follow up” can be a particular challenge in transitory AYA patients. Third, additional studies should focus on identifying reliable individual predictors and risk scores for clinically significant orthopedic complications. Candidate predictors include serum biomarkers of bone metabolism, specific radiographic indicators, additional asparaginase toxicities (i.e. pancreatitis, thromboembolism), and combinations of clinical factors (age, BMI, lipid profile). Of note, germline polymorphisms have been identified as risk factors for ON: GWAS studies have identified genes involved in adipogenesis and the glutamate receptor pathways to be associated with ON, while polymorphisms in the pro-apoptotic \textit{BCL2L11} gene have been implicated in ON risk through both retrospective clinical studies and \textit{in vitro} assays. With better understanding of toxicity burden and at-risk populations, prospective interventions to mitigate incidence and morbidity of ON in patients who are at high risk of ON based on demographics, genetics, and treatment regimen could be developed.

In summary, our study defines a vulnerable window of adolescence and young adulthood where orthopedic toxicity is a particular risk when being treated on pediatric-inspired ALL regimens associated with prolonged steroid and asparaginase exposure. Pegylated formulations of asparaginase now in common use may potentiate risk for this toxicity. As survival improves in patients treated with intensive pediatric-inspired regimens, focus on toxicity and survivorship will be increasingly important. Progress in this realm will require cooperation between pediatricians, adult medical oncologists, endocrinologists, orthopedic surgeons, and survivorship experts to achieve the best outcomes for adolescent and young adult patients with ALL.

**Data sharing statement**

Data is available by e-mailing the corresponding author: marlise_luskin@dfci.harvard.edu.
Authorship contributions

YV extracted the data from the charts, designed the data analysis, and drafted the manuscript. KS designed and performed the data analysis. AP, LS, LV, GG, and AB designed the analysis, and identified patients for inclusion in the cohort. MN extracted pharmacy data. DD conceptualized the study and helped design the analysis. ML oversaw the creation of the cohort, the design of the analysis, and the drafting of the manuscript. All authors revised the final manuscript.

Disclosure of Conflicts of Interest

LS was on the advisory board of Jazz Pharma, Takeda, Servier, and Syndax. AB received consultancy funding from Acceleron Pharma, Biogen, Celgene/BMS, Forty Seven, Jazz Pharma, Novartis, Takeda, and Xcenda, and research funding from Celgene/BMS, Novartis, Takeda, GSK, Janssen, and Astra Zeneca. DD received consultancy funding from Amgen, AutoLos, Agios, Bluepring Medicines Corporation, Forty Seven, Incyte Corporation, Jazz Pharma, Novartis, Pfizer, Shire, and Takeda, and research funding from Bluperint Medicines Corporation, Novartis, Abbvie, and Glycomimetics.
Figure 1: Overview of DFCI AYA ALL Treatment protocols. 1a: Earlier patients were enrolled on parallel Pediatric 00-001 (2000-2004) and Adult 01-175 (2002-2008) trials while later patients were enrolled on parallel Pediatric 05-001 (2005-2011) and Adult 06-254 (2007-2011) trials. 1b: Breakdown of patients by treatment protocol, age, and asparaginase formulation. 1: Intention-to-treat post-induction asparaginase formulation. Patients that did not receive post-induction asparaginase are excluded from this table. 2: Cumulative incidence (death included as competing risk). ON: Osteonecrosis. Fx: Fracture.

Figure 2: Incidence over time of ON and fracture in 367 AYA ALL patients. Median time to event was 1.6 years for ON and 1.4 years for fracture.

Figure 3: Probability of ON by risk group. (A) shows the probability of ON by age group and (B) shows the probability of ON by treatment protocol. Early generation protocols 00-001 and 01-175 used E.coli asparaginase while late generation protocols 05-001 and 06-254 mostly used pegaspargase.

Table 1: Patient demographics and disease characteristics

|                                | N (%) |
|--------------------------------|-------|
| Total, Eligible Pts.           | 367   |
| Protocol/Treatment Regimen     |       |
| 00001 Pediatric Early          | 35 (10)|
| 05001 (n=11 treated as per) Pediatric Late | 82 (22) |
| 01175 Adult Early              | 92 (25)|
| 06254 (n=48 treated as per) Adult Late | 158 (43)|
| Age (yrs), median (range)      | 23 (15, 50)|
| 15-19                          | 138 (38)|
| 20-29                          | 110 (30)|
| 30-39                          | 62 (17)|
| 40-50                          | 57 (16)|
| WBC (x10^-3), median (range)   | 12.2 (0.1, 708.8)|
| < 30                           | 249 (68)|
| ≥ 30                           | 117 (32)|
| Unknown                        | 1 (<1)|
| Blast %, median (range)        | 23 (0, 98)|
| Sex                            |       |
| Female                         | 142 (39)|
| Male                           | 225 (61)|
| Immunophenotype†               |       |
| B-cell                         | 281 (77)|
| T-cell                         | 86 (23)|
| CNS Status                     |       |
| CNS 1                          | 281 (77)|
| CNS 2                          | 38 (10)|
| CNS 3                          | 11 (3)|
| Traumatic Tap with Blasts      | 7 (2)|
| Traumatic Tap without Blasts   | 18 (5)|
| Not performed                  | 12 (3)|
| Mediastinal Mass               |       |
| Category                                                | N (%)     |
|---------------------------------------------------------|-----------|
| Yes                                                     | 62 (17)   |
| No                                                      | 299 (82)  |
| Not Evaluated                                          | 6 (1)     |
| Philadelphia chromosome positive                        | 44 (12)   |
| KMT2A rearrangement (n=330)                             | 20 (6)    |
| High hyperdiploid (51-67 chromosomes) (n=325)           | 42 (13)   |
| Complex Karyotype >=3 Abnormalities (n=228)              | 45 (20)   |
| Body Mass Index at Diagnosis*                           | 24.5 (13.9, 57.0) |
| underweight < 18.5                                      | 16 (4)    |
| normal 18.5-24.9                                       | 182 (50)  |
| overweight 25-29.9                                      | 94 (26)   |
| obese 30-39.9                                           | 59 (16)   |
| morbid obese ≥40                                       | 16 (4)    |

*BMI=weight(kg) / [height(m)]²
†2 B cell and 1 T cell patient had myeloid co-expression
WBC = white blood cell, CNS = central nervous system
Table 2: Cumulative incidence of ON and fracture

|                          | 5-year Cumulative Incidence ON % [95% CI] | p-value† | 5-year Cumulative Incidence Fracture % [95% CI] | p-value† |
|--------------------------|------------------------------------------|----------|-----------------------------------------------|----------|
| Overall                  | 17 [13-22]                               |          | 12 [8-15]                                    |          |
| Treatment Regimens       |                                          |          |                                               |          |
| Early generation         | 5 [2-10]                                 | <0.001   | 9 [5-16]                                      | 0.74     |
| Late generation          | 24 [18-30]                               |          | 13 [9-18]                                    |          |
| Age                      |                                          |          |                                               |          |
| <20 yrs.                 | 18 [12-25]                               | 0.003    | 14 [9-21]                                    | 0.44     |
| 20-29 yrs.               | 26 [17-36]                               |          | 10 [5-18]                                    |          |
| 30-39 yrs.               | 12 [5-23]                                |          | 14 [6-26]                                    |          |
| ≥40 yrs.                 | 4 [1-11]                                 |          | 7 [2-17]                                     |          |
| Age                      |                                          |          |                                               |          |
| <30 yrs.                 | 21 [16-27]                               | 0.004    | 12 [8-17]                                    | 0.54     |
| ≥30 yrs.                 | 8 [4-14]                                 |          | 11 [6-18]                                    |          |
| BMI at Diagnosis         |                                          |          |                                               |          |
| Underweight/Normal       | 19 [14-26]                               | 0.53     | 14 [9-20]                                    | 0.39     |
| Overweight               | 14 [8-23]                                |          | 9 [4-16]                                     |          |
| Morbidly Obese/Obese     | 15 [8-26]                                |          | 10 [4-18]                                    |          |
| Sex                      |                                          |          |                                               |          |
| Male                     | 16 [11-22]                               | 0.50     | 9 [6-13]                                     | 0.051    |
| Female                   | 19 [13-27]                               |          | 17 [10-24]                                   |          |
| Lipidemia (grade 4) (N=332)* |                                      |          |                                               |          |
| Yes                      | 32 [18-46]                               | 0.019    | 5 [1-16]                                     | 0.11     |
| No                       | 16 [12-21]                               |          | 13 [9-17]                                    |          |

†the Gray test *excludes 00001 protocol no grade 5 events observed includes hypertriglycerides and hypercholesterolemia
|                      | Osteonecrosis          | Fracture                  |
|----------------------|------------------------|---------------------------|
|                      | ON Univariate HR [95% CI] | ON Multivariable HR [95% CI] | Fracture Univariate HR [95% CI] | Fracture Multivariable HR [95% CI] |
|                      | p-value                | p-value                   | p-value            | p-value                         |
| **Treatment Regimen**| **ON**                 | **ON**                    | **Fracture**       | **Fracture**                    |
| Late vs. early       | 5.28 [2.24-12.48]      | <0.001                    | 1.12 [0.57-2.17]   | 0.75                            |
| **Age**              |                        |                           |                   |                                 |
| <30 vs. 30-50 years  | 2.77 [1.35-5.65]       | 0.005                     | 1.25 [0.63-2.50]   | 0.52                            |
| **BMI**              |                        |                           |                   |                                 |
| Morbidly Obese/Obese | 0.81 [0.41-1.60]       | 0.55                      | 0.72 [0.31-1.67]   | 0.45                            |
| vs. Underweight/Normal |                      |                           |                   |                                 |
| Overweight vs.       | 0.70 [0.36-1.34]       | 0.28                      | 0.58 [0.25-1.32]   | 0.19                            |
| Underweight/Normal   |                        |                           |                   |                                 |
| Sex                  |                        |                           |                   |                                 |
| Male vs. Female      | 0.85 [0.51-1.41]       | 0.52                      | 0.85 [0.51-1.41]   | 0.52                            |

**Table 3: Competing risk regression models of ON and fracture**
Table 4. Univariate and Multivariable Cox Modeling for OS

|                | OS Univariate HR [95% CI] | OS Multivariable HR [95% CI] | P       |
|----------------|---------------------------|-------------------------------|---------|
| **ON‡**        | 0.11 [0.04-0.35]          | 0.15 [0.05-0.46]              | 0.001   |
| Age 30-50 vs. <30 years | 1.88 [1.27-2.78]          | 1.42 [0.93-2.16]              | 0.11    |
| Sex M vs. F    | 1.08 [0.72-1.61]          | 0.72                          |         |
| WBC >=50 vs. <50 | 2.10 [1.42-3.10]          | 2.17 [1.45-3.23]              | 0.0002  |
| B-cell vs. T-cell | 2.31 [1.29-4.14]          | 2.31 [1.27-4.18]              | 0.006   |
| CNS Blasts vs. No Blasts | 1.41 [0.84-2.35]     | 1.90                          |         |
|                | 0.89 [0.45-1.79]          | 0.75                          |         |
| Ph+ Yes vs. No | 1.89 [1.12-3.19]          | 1.03 [0.59-1.79]              | 0.92    |
|                | 1.81 [0.25-12.98]         | 2.25 [0.30-17.01]             | 0.43    |
| BMI            |                           |                               |         |
| Morbidly Obese/Obese vs. Underweight/Normal | 2.25 [1.39-3.64]          | 1.91 [1.16-3.15]              | 0.011   |
|                |                           |                               |         |
|                |                           |                               |         |
|                |                           |                               |         |
| Fracture‡      | 0.31 [0.13-0.77]          | 0.40 [0.16-0.99]              | 0.048   |
| Age 30-50 vs. <30 years | 1.88 [1.27-2.78]          | 1.62 [1.06-2.48]              | 0.026   |
| Sex M vs. F    | 1.08 [0.72-1.61]          | 0.72                          |         |
| WBC >=50 vs. <50 | 2.10 [1.42-3.10]          | 2.30 [1.54-3.44]              | <0.001  |
| B-cell vs. T-cell | 2.31 [1.29-4.14]          | 2.31 [1.27-4.18]              | 0.006   |
| CNS Blasts vs. No Blasts | 1.41 [0.84-2.35]     | 0.19                          |         |
|                | 0.89 [0.45-1.79]          | 0.75                          |         |
| Ph+ Yes vs. No | 1.89 [1.12-3.19]          | 1.06 [0.61-1.85]              | 0.84    |
|                | 1.81 [0.25-12.98]         | 1.68 [0.22-12.70]             | 0.61    |
| BMI            |                           |                               |         |
| Morbidly Obese/Obese vs. Underweight/Normal | 2.25 [1.39-3.64]          | 1.89 [1.14-3.13]              | 0.013   |
|                |                           |                               |         |
|                |                           |                               |         |
| Fracture‡      | 0.31 [0.13-0.77]          | 0.40 [0.16-0.99]              | 0.048   |

‡Included as a time-varying covariate
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Figure 1: Overview of DFCI AYA ALL treatment protocols and patients

1a:

Early generation: Pediatric (DFCI 00-001) and Adult (DFCI 01-175)
- Induction: CNS Prophylaxis
  - Pediatric: IT Chemo + XRT
  - Adult: IT Chemo + XRT
- Consolidation: E. Coli Asparaginase (30 wk)
- Maintenance: Until 2 years from CR

Late generation: Pediatric (DFCI 05-001) and Adult (DFCI 06-254)
- Induction: CNS Prophylaxis
  - Pediatric: IT Chemo + XRT
  - Adult: IT Chemo + XRT
- Consolidation I: E. Coli Asparaginase (30 wk)
  - Pediatric: E. Coli Asparaginase (30 wk)
  - Adult: PEG Asparaginase (3 wk)
- Consolidation II: PEG Asparaginase (30 wk)
- Maintenance: Until 2 years from CR

1b:

Met inclusion criteria (367)

Early generation protocols: 127

- Pediatric: 00-001: 35
  - On trial: 35
  - As per: 0
- Adult: 01-175: 92
  - On trial: 92
  - As per: 0

Late generation protocols: 240

- Pediatric: 05-001: 82
  - On trial: 71
  - As per: 11
- Adult: 06-254: 158
  - On trial: 110
  - As per: 48

| Age at dx Post-ind. asp.¹ | < 30y E.Coli | 30-50y E.Coli |
|---------------------------|-------------|-------------|
| n                         | 51          | 28          |
| 5y ON Cl ²                | 10 [4-20]   | 4 [1-16]    |
| 5y Fx Cl ²                | 10 [4-20]   | 11 [3-25]   |

| Age at dx Post-ind. asp.¹ | < 30y E.Coli | 30-50y E.Coli | < 30y PEG | 30-50y PEG |
|---------------------------|-------------|-------------|----------|----------|
| n                         | 42          | 1           | 89       | 36       |
| 5y ON Cl ²                | 25 [13-40]  | NA          | 37 [26-48] | 21 [9-36] |
| 5y Fx Cl ²                | 20 [9-34]   | NA          | 13 [7-22] | 20 [8-37] |

Figure 1: Overview of DFCI AYA ALL Treatment protocols. 1a: Earlier patients were enrolled on parallel Pediatric 00-001 (2000-2004) and Adult 01-175 (2002-2008) trials while later patients were enrolled on parallel Pediatric 05-001 (2005-2011) and Adult 06-254 (2010-2011) trials. 1b: Breakdown of patients by treatment protocol, age, and asparaginase formulation. 1: Intention-to-treat post-induction asparaginase formulation. Patients that did not receive post-induction asparaginase are excluded from this table. 2: Cumulative incidence (death included as competing risk). ON: Osteonecrosis. Fx: Fracture.
**Figure 2: Probability of ON and fracture**

A) Osteonecrosis (ON)

- 100 events in 60 patients
- 5-year CI: 17% (95% CI, 13-22%)
- Median time to event: 1.6 years

B) Fracture

- 51 events in 40 patients
- 5-year CI: 12% (95% CI, 9-16%)
- Median time to event: 1.4 years

**Figure 2:** Incidence over time of ON and fracture in 367 AYA ALL patients. Median time to event was 1.6 years for ON and 1.4 years for fracture.
Figure 3: Probability of ON by risk group. (A) shows the probability of ON by age group and (B) shows the probability of ON by treatment protocol. Early generation protocols 00-001 and 01-175 used *E. coli* asparaginase while late generation protocols 05-001 and 06-254 mostly used pegaspargase.