The following protocol information is provided solely to describe how the authors conducted the research underlying the published report associated with the following article:

Outcome of Children with BCR-ABL1-like Acute Lymphoblastic Leukemia Treated with Risk-directed Therapy Based on the Levels of Minimal Residual Disease

Roberts, et al

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TOTAL THERAPY STUDY XV FOR NEWLY DIAGNOSED PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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TOTAL XV
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1.0 STUDY OBJECTIVES

The overall objective of this protocol is to improve the cure rate of children with non-B-cell acute lymphoblastic leukemia (ALL). Specific aims are as follows (primary aims indicated by bold type).

1.1 Therapeutic Aims

1.1.1 To estimate the overall event-free survival of children at least one year of age at diagnosis who are treated with risk-directed therapy and to monitor the molecular remission induction rate.

1.1.2 To determine if CNS irradiation can be safely omitted in the context of the systemic therapy used in the protocol.

1.1.3 To assess the prognostic value of biologic markers in childhood ALL.

1.2 Pharmacologic Aims

1.2.1 To identify whether prolonged (24 hour) intravenous infusions of HDMTX produce greater methotrexate polyglutamate (MTXPG) accumulation than short (4 hour) infusions 42 hours after 1 gm/m^2 of HDMTX, stratified for lineage (T- vs B-lineage) and ploidy (hyperdiploid vs non-hyperdiploid B-lineage).

1.2.2 To determine whether prolonged (24 hour) intravenous infusions of HDMTX produce greater antileukemic effects than short (4 hour) infusions, based on the inhibition of *de novo* purine synthesis in bone marrow blasts and the decrease in circulating blasts during the 4 day "window" prior to initiation of conventional remission induction therapy.

1.2.3 To assess the mechanisms underlying lineage- (B- vs T) and ploidy- (hyperdiploid vs nonhyperdiploid B-lineage) related differences in MTXPG accumulation, e.g., expression or activity of the reduced folate carrier (RFC), gamma glutamyl hydrolase (GGH), efflux pumps (e.g. MRPs), or folylpolyglutamate synthetase (FPGS). To explore the molecular basis of such differences, including the contribution of genetic polymorphisms to interpatient variability in MTXPG accumulation.

Amend 1.0 (deleted infectious disease aims)

1.3 Biologic Aims

1.3.1 To determine whether levels of minimal residual disease in peripheral blood reflect those measured in the bone marrow by immunologic or molecular techniques.

1.3.2 To explore whether genetic polymorphisms of enzymes important in the disposition of antileukemic agents (e.g. CYP3A4, methylene tetrahydrofolate reductase, thiopurine methyltransferase, glutathione

transferases, glucuronosyltranferases) are correlated with antileukemic drug pharmacodynamics in lymphoblasts, clinical toxicities, and long-term outcome.

1.3.3 To determine whether auto-induction of dexamethasone pharmacokinetics occurs during reinduction, and to assess whether apparent clearances, inducibility or pharmacodynamics of dexamethasone are related to genetic polymorphisms in CYP3A4, PXR, or other relevant determinants of dexamethasone disposition and effect.

1.3.4 To explore whether the development of anti-asparaginase antibodies or CSF depletion of asparagine is correlated with acute toxicities and long-term outcome.

1.3.5 To assess the degree of DNA alteration in somatic cells (leukocytes) during and after treatment.

1.3.6 To assess whether somatic cell genetic polymorphisms correlate with *in vitro* drug sensitivity of blasts.

1.4 Cancer Control Aims

Amend 2.0, Amend 4.0

1.4.1 To assess the relationship among MRI changes of brain (especially white matter abnormalities) after HDMTX (5 gm/m^2 versus 2.5 gm/m^2 dosage and one dose versus 5 doses) and intrathecal treatment, CSF or plasma levels of homocysteine and excitatory amino acids, **folates,** cognitive deficits, and diminished quality of life.

Amend 2.0

1.4.2 To investigate the hypothesis that early MRI changes are predictive of late MRI abnormalities, cognitive deficits, and diminished quality of life.

1.4.3 To investigate the incidence and risk of osteopenia/osteoporosis in patients receiving intensive antimetabolites and dexamethasone therapy and to explore whether dexamethasone pharmacokinetics, pharmacogenetics or pharmacodynamics are related to its risk.

1.4.4 **To estimate the incidence and timing of hypercalciuria during treatment for ALL.**

Amend 4.0 (deleted text)

1.4.5 To relate non-invasive cardiac evaluation (fractional shortening and afterload), clinical cardiovascular status (New York Heart Association Class), and health- related quality of life in children with acute lymphoblastic leukemia treated with cardiotoxic cancer therapy.

2.0 BACKGROUND AND RATIONALE

This is a comprehensive **TOTAL** therapy study of ALL, including multiple primary and secondary objectives. Many primary and secondary objectives are related. Background and rationale are organized according to the scientific nature of the aims.

2.1 Therapy

2.1.1 Risk Assignment

Despite the high cure rates (70% to 80%) in childhood ALL,[1] resistant forms of this disease still represent a leading cause of cancer-related deaths in children[2] and pose formidable challenges for the future. While efforts are being made to identify new effective antileukemic agents or new approaches to therapy, current treatment emphasizes early and vigorous assessment of the risk of relapse in individual patients, so that patients are not under- or over-treated. In this regard, the Total XV protocol applies the most comprehensive risk classification to date, combining blast cell immunophenotype and genotype, with presenting clinical features and early treatment response. Accordingly, cases are divided into three risk groups -- low-, standard-, and high-risk (corresponding to standard-, high-, and very high-risk categories in other protocols).

B-cell precursor ALL cases with age between 1 and 10 years and presenting leukocyte count $<50 \times 10^9$/L, leukemic cell DNA index ≥ 1.16 or *TEL-AML1* fusion have an overall excellent prognosis and are generally considered to have low-risk leukemia.[1] However, as many as 15% of these cases still relapse on antimetabolite-based therapy, for unknown reasons. Recent studies showed that response to early treatment can identify patients who have good presenting features but nonetheless at high risk of relapse.[3-6] In this regard, we have shown that despite favorable presenting features, patients with ≥5% bone marrow blasts at day 19 or 26 of remission induction and those with minimal residual leukemia ≥0.01% on remission date (~ day 46) are at high risk of relapse.[7-9] Hence, these patients will be treated as having <u>standard-risk</u> ALL.

Because of their generally poor outcome, cases with CNS 3 status (≥5 WBC/:L of cerebrospinal fluid with identifiable blasts or cranial nerve palsy), testicular leukemia, or hypodiploidy (<45 chromosomes),[1] will also be treated on the <u>standard-risk</u> arm. Patients with T-cell ALL, pre-B-cell ALL with the t(1;19) and *E2A-PBX1* fusion or *MLL* rearrangement fare well provided that they receive intensive therapy.[1] Hence they are also assigned to the standard-risk group in Study XV.

The remaining patients with favorable presenting features and good response to remission induction therapy will be treated on low-risk arm.

We and others have shown that patients with Philadelphia-chromosome (*BCR-ABL)*-positive ALL have very poor prognosis.[10,11] Recent studies showed that their prognosis can be improved with allogeneic hematopoietic stem cell transplantation.[11] It is well recognized that patients with induction failure (i.e., ≥5% bone marrow blasts on remission date) have dismal prognosis.[12,13] Our recent studies showed that patients with ≥1% blasts on remission date and those with ≥0.1% at 16 weeks post remission induction also have very poor prognosis.[8,14] Therefore, these 4 groups of patients are considered to have high-risk leukemia and are candidates for allogeneic hematopoietic stem cell transplantation during their first remission.

2.1.2 Remission Induction Therapy

Since 1984, all patients had received 6-drug remission induction with prednisone, vincristine, L-asparaginase, daunorubicin, and etoposide (or teniposide) plus cytarabine. While the remission rates have been excellent (98% to 99%),[15] our recent analysis indicated that even 3 to 4 doses of etoposide plus cytarabine during remission induction and reinduction can result in a 2% to 3% cumulative risk of therapy-related acute myeloid leukemia (Pui et al, unpublished observation). To decrease this risk, we will limit the use of epipodophyllotoxins in Total XV to only high-risk cases during reintensification therapy (see section 6.4). For remission induction, we will continue to use the first 3 ½ weeks of remission induction of Total XIII studies, consisting of methotrexate, prednisone, vincristine, daunorubicin and L-asparaginase. In the second half of remission induction, instead of etoposide plus cytarabine, a combination of high-dose cyclophosphamide, 6-mercaptopurine and cytarabine is used. This combination has been widely used in the BFM or modified BFM regimens with only 20% frequency of grade 1 to 3 infection (Schrappe M, personal communication). This rate is lower than the 50% to 60% observed in Studies XIII and XIV.

Since 1991, all patients received "upfront window" therapy with methotrexate, 6-mercaptopurine or both, before the more conventional remission induction treatment. In Study XIII, upfront HDMTX was well tolerated with less than a 10% incidence of grade 3 or 4 mucositis in patients receiving HDMTX at 1 gm/m^2 with or without 6-mercaptopurine.[16] In addition, the use of upfront HDMTX at 1 g/m^2 did not compromise the delivery of remission induction therapy, as the number of days required to complete induction plus consolidation was similar (p=0.379) between patients treated in different arms of Total XIIIB (6MP alone, low or high-dose MTX, or no window therapy) and

between the 49 children who were randomized to receive low, medium, or high steady state concentrations of HDMTX on Total XIV (p=0.35). This treatment component also did not compromise treatment outcome in St. Jude studies XIIIA and XIIIB, which have had an excellent 5-year event-free survival of 81% ± 8%.[1] In fact, preliminary results of Total XIIIA suggest that among patients with B-lineage ALL, those who received upfront HDMTX (1 gm/m^2) may have a better outcome than those who received lower-dose MTX (180 mg/m^2)(Pui C-H, unpublished observation).

In the DFCI 81-01 protocol, Sallan et al reported improved treatment outcome among children randomized to receive a single dose of HDMTX (4 gm/m^2), compared to those who received low-dose MTX (40 mg/m^2) as "upfront window" therapy prior to conventional remission induction therapy.[17] To this end, in the most recent DFCI Protocol, HDMTX (4 gm/m^2) is given during the first week of remission induction treatment, immediately after 2 doses of daunorubicin (Sallan SE, personal communication). Therefore, in addition to providing a unique opportunity to study the *in vivo* lymphoblast pharmacology of methotrexate, we now consider high-dose methotrexate (1 gm/m^2) to be an integral component of remission induction treatment.

Excessive toxicities were observed during the early phase of remission induction in Study XIV, in which upfront methotrexate was given at dosages ranging from 250 to 8000 mg/m^2 (Pui, unpublished observation). Although the observed toxicities did not have a statistical association with the level of exposure to the upfront HDMTX, we believe it is prudent to limit the dosage of methotrexate to 1 gm/m^2 at this time. To further reduce the risk of potential mucositis, leucovorin dosage is increased in this study as compared to those used in studies XIII and XIV, the trimethoprim/sulfamethoxazole has been delayed to day 19 (its early start at day 5 may have contributed to early antifolate toxicity in Total XIV), and triple intrathecal treatment is given on day 19 instead of day 4 (as scheduled in Total XIII and XIV studies). However, an additional triple intrathecal treatment will be given on day 8 to patients who have CNS2, CNS3 or contaminated CSF with blasts at diagnosis to prevent subsequent CNS relapse.

The leucovorin dosage that we have chosen for the up-front MTX therapy on Total XV (a total of 155 mg/m^2) is higher than we gave on Total XIIIA (25 mg/m^2) and Total XIIIB (50 mg/m^2) but lower than that given by the DFCI[17] (~296 mg/m^2) to newly diagnosed ALL patients who receive a higher dose than we plan (4 g/m^2) but a shorter infusion (1 hour). We believe that this dose is a reasonable compromise to maximize patient safety while maintaining a beneficial antileukemic effect of the MTX. In fact, given that the decrease in peripheral white blood cell count and blast count over the first 4 days of therapy can be

assumed to be due to MTX effect, this decrease was observed on all of the last five patients on Total XIV who received doses of MTX <1500 mg/m^2 (range 184 to 1432 mg/m^2), even after the third amendment to Total XIV which greatly increased the dose and early start of leucovorin. For these five patients, the ratio of leucovorin to MTX ranged from 0.07 to 0.27, none had delayed MTX excretion, and all exhibited decreases in their peripheral blood counts, despite their only exposure to anticancer agent being the up-front MTX with leucovorin rescue. The ratio of leucovorin to MTX that we propose on Total XV is 0.16. We acknowledge that this is a higher ratio than that used by DFCI (which is not directly comparable due to their shorter infusion length) and is much more leucovorin per MTX exposure than will be used during consolidation (see section 6.3), but we prefer to err on the side of aggressive rescue to prevent toxicity in these newly diagnosed patients.

Amend 4.0

Recombinant urate oxidase (**rasburicase, Elitek)** may be used to treat hyperuricemia or to prevent this complication in patients at high risk. In this regard, SJCRH clinicians have vast experience in using this drug, which has proved to be very effective.[18]

Amend 8.0

2.1.2.1 Rationale for Additional Asparaginase Doses During Induction (Amendment 8.0)

Patients with 1% or more blasts on day 19 had poor treatment outcomes in total 13B with a 5-year cumulative risk of relapse of 36% +-9%. Although the overall result of Total XV has improved substantially, our interim analysis showed that this group of patients still has a high 5-year cumulative risk of relapse of 34%+-22%. Therefore, early therapy will be intensified in these patients.

2.1.3 Consolidation Therapy

Consolidation therapy consists of HDMTX (5 gm/m^2 or 2.5 gm/m^2 over 24 hours in standard-/high-risk or low-risk cases, respectively) given every other week for 4 doses with leucovorin rescue, together with three weekly doses of intrathecal treatment plus concomitant daily 6-mercaptopurine (50 mg/m^2 in standard-/high-risk cases and 75 mg/m^2 in low-risk cases). The feasibility and effectiveness of HDMTX given at 5 gm/m^2 have been documented in large numbers of patients treated in the Berlin-Frankfurt-Mηnster (BFM)[3] and AIEOP studies.[18b] In fact, the improved treatment outcome of T-cell ALL cases in the BFM studies and of intermediate-risk B-lineage ALL cases in the AIEOP study[18b] was attributed in part to the use of HDMTX at 5 gm/m^2. Preliminary results of Total XIII A & B pharmacologic studies suggest that while HDMTX at 2.5 gm/m^2 is sufficient for most B-lineage ALL cases,[19,20] non-hyperdiploid B-lineage blasts accumulate more methotrexate polyglutamates with high-dose than with low-dose methotrexate (Relling MV, unpublished observation). In a DFCI study with vast majority of

cases of B-lineage phenotype, children received a single up-front dose of 4 gm/m^2 had better overall leukemia-free survival than those receiving 40 mg/m^2.[17] In addition, in our study X, higher exposure to methotrexate was most important among the higher-risk B-lineage cases (p=0.007).[20a] Hence, in Study XV, HDMTX will be given at 5 gm/m^2 for T-cell and standard-/high-risk B-lineage ALL and at 2.5 gm/m^2 for low-risk B-lineage ALL. In Total XV study, the number of HDMTX (including up-front dose) is limited to 5 doses (as opposed to 11 doses at 2 gm/m2 used in Total XIII) to avoid excessive neurotoxicities. As targeted systemic exposure has been shown to be particularly important for high-dose methotrexate treatment,[1] the dosage will be adjusted accordingly for individual patients (see Section 6.3.3.1).

It should be noted that approximately 10% of provisional low-risk ALL will be changed to standard-/high-risk ALL because of positive minimal residual leukemia at the end of induction. In these cases, HDMTX dosage after the first dose will be changed to 5 gm/m^2.

2.1.4 Continuation Treatment

After consolidation therapy, patients will receive risk-directed continuation treatment. Low-risk patients will receive daily 6-mercaptopurine and weekly methotrexate with pulses of dexamethasone and vincristine every 3 to 5 weeks (for the first two years). In a randomized trial of low-risk ALL, dexamethasone treatment was superior to prednisone in reducing CNS relapse and improving event-free survival,[21] possibly due to its superior CNS penetration.[22] Unlike continuation treatment in the Total XIII studies, 6-mercaptopurine will be continued during the week of dexamethasone and vincristine treatment in this study to intensify systemic therapy. To this end, the dose intensity of 6-mercaptopurine is the most important pharmacologic variable in determining treatment outcome in Total XII study.[23] Methotrexate will not be given during the week of dexamethasone plus vincristine to reduce the risk of typhlitis.

Continuation treatment for standard- and high-risk cases incorporates effective treatment components of the most successful clinical trials to date. L-asparaginase will be used intensively during the early phase of treatment based on improved results of DFCI studies, which used 30 weekly doses of E. coli preparation at 25,000 U/m^2 for all patients (93% of whom had B-lineage ALL);[24] CCG augmented therapy for poor early responders (90% of whom had B-lineage ALL) which included 55 doses at 6,000 to 15,000 U/m^2 during the first 44 weeks of postremission therapy;[25] and the POG8704 study for T-cell ALL which incorporated 20 weekly doses at 25,000 U/m^2.[26] Hence, intensive L-asparaginase therapy improved outcome in patients with T-cell ALL as well as those with high-risk B-lineage ALL. In study XV, 25 to 28 doses of L-

asparaginase at 10,000-25,000 U/m^2 will be given to patients with standard- or high-risk ALL. (Note: intensive L-asparaginase treatment failed to improve outcomes in a study of low-risk B-lineage ALL;[27] hence, we have not incorporated an intensive asparaginase regimen for the low-risk arm of Study XV).

Improved treatment outcome of a recent DFCI study[24] and of a Dutch study[28] was attributed to the use of dexamethasone, which is also used in Total XV. In fact, in the DFCI 91-01 study, improved treatment outcome in standard/high-risk patients was partly attributed to the use of very high dose of dexamethasone (18 mg/m^2/day) (Sallan SE, personal communication). Use of epipodophyllotoxins will be limited to high-risk ALL in Total XV to decrease the risk of therapy-related AML.[15] We will use cyclophosphamide and anthracyclines to intensify treatment in standard-/high-risk cases. However, total cumulative dose of cyclophosphamide is limited to 4.6 gm/m^2 to reduce the problem of sterility (especially in boys) and anthracycline cumulative dosage is limited to 230 mg/m^2. (In low-risk cases, the cumulative dosage of anthracycline is 110 mg/m^2). These dosages of anthracyclines are associated with a very low risk of cardiomyopathy.[29]

Reinduction treatment is an integral component of most contemporary trials, with benefits extending to low-risk cases. A recent CCG study showed that an augmented BFM study (equivalent to the use of double reinduction) can even abolish the adverse prognostic impact of poor early response.[25] Hence, in study XV, all patients (regardless of risk status) will receive two courses of reinduction, albeit at different intensity as determined by risk group assignment. In standard-risk and high-risk groups, high-dose cytarabine (2 gm/m^2) is incorporated because our Study XII suggested that T-cell cases benefit from this treatment [30] and an adult BFM study showed that high-dose cytarabine can markedly improve outcome, even in patients with the t(4;11) ALL.[31]

Gender has long been recognized as a significant prognostic factor in childhood ALL. Boys have consistently fared worse than girls, given equivalent therapy. Recent improvements in ALL therapy have not eliminated the gender difference in prognosis, according to St. Jude[32] and POG studies[33]. CCG and BFM groups have treated their male patients with a longer duration of treatment (i.e. 3 years), based upon their data. Hence, in Study XV, the duration of continuation treatment in boys will be extended to 3 years, as opposed to 2 ½ years in girls. Continuation treatment beyond 2 years in Study XV consists of 6-mercaptopurine and methotrexate only (no dexamethasone and vincristine pulses).

2.1.5 Subclinical CNS Treatment

Isolated CNS relapse has been virtually eliminated in recent Total Therapy studies with a 5-year cumulative risk of only 1.2% (95% confidence interval, 0 to 2.9%) in Study XIIIA;[34] only three of 247 patients treated in Study XIIIB with a median follow-up of 3 years have thus far had an isolated CNS relapse. We attribute this success to early intensification of intrathecal treatment, especially in patients at high risk of relapse, and effective systemic treatment including the use of dexamethasone. In Studies XIIIA & B, intrathecal treatment was intensified during induction treatment (i.e., weekly for at least 4 doses) for patients with any amount of leukemic blasts identifiable in the cerebrospinal fluid at diagnosis. Patients with cerebrospinal fluid contaminated with leukemic-blast cells due to a traumatic lumbar puncture at diagnosis also require intensive intrathecal treatment because they are at higher risk of CNS relapse and have a poorer event-free survival if treatment is not intensified.[35] These patients then received additional intrathecal doses every 4 weeks during the first 56 weeks of continuation therapy, as did patients with a CNS-1 status (i.e., no identifiable blasts in CSF) who were judged to have an increased risk of CNS relapse based on other features (i.e., those with WBC $\geq$100 x 10^9/L, T-cell cases with WBC $\geq$50 x 10^9/L and Philadelphia-chromosome positive cases). CNS irradiation was reserved for patients in the high-risk category (except those with only CNS-2 status; that is, <5 WBC/:L in cerebrospinal fluid with identifiable leukemic blasts), which comprised about 23% of the overall patient population.

Since CNS irradiation can induce serious long-term sequelae, including growth retardation, intellectual impairment and brain tumors,[36] most contemporary treatment protocols specify cranial irradiation for only a small fraction of patients at a very high-risk of CNS relapse. In view of the outstanding CNS control in Studies XIIIA & B, CNS irradiation is eliminated altogether in this study, except in patients who clear their CSF blasts very slowly during remission induction (i.e., persistent blasts after 3 intrathecal treatments) and those who during continuation treatment have morphologic evidence of lymphoblasts in the CSF, confirmed by immunological testing (i.e. TdT) on two separate occasions. We expect that these events should not occur in more than 5% of the patients. Moreover, based on our experience in Total XIII and XIV studies, CNS relapse very rarely occurs in the first year of treatment during which intensive intrathecal treatment is given. Reinduction treatment with weekly intrathecal treatment followed by CNS irradiation plus 5 triple intrathecal treatments will be given to patients who are found to have low levels of lymphoblasts in the CSF during treatment. To prevent excessive CNS relapse due to the elimination of "prophylactic" CNS irradiation, stopping rules have been developed and

will be monitored closely during the trial period (see section 19.2.2 of Statistical Considerations).

2.2 Pharmacologic Studies

2.2.1 Relation Between Infusion Length and Intracellular Accumulation of MTX PG

Prior to work conducted in Total XIIIA & B studies,[16,20] relatively little was known about the *in vivo* intracellular disposition of MTX in human leukemic lymphoblasts. One reason for the paucity of such data is the lack of blast cells after the initial 2 to 4 weeks of remission induction treatment, at which time the majority of patients have achieved a complete remission. In the absence of cellular pharmacokinetic and pharmacodynamic studies in patients, selection of the optimal dosage, route and schedule of antileukemic drugs are based on empiric clinical observations and suboptimal preclinical models. In this regard, the dosage of MTX in the treatment of ALL has evolved largely on an empirical basis, such that there is more than a 100-fold difference in the dosages used between various contemporary treatment protocols worldwide, and the method of dosage administration in ALL encompasses oral and intramuscular dosing and IV infusions which range in length from 5 minutes to 36 hours.

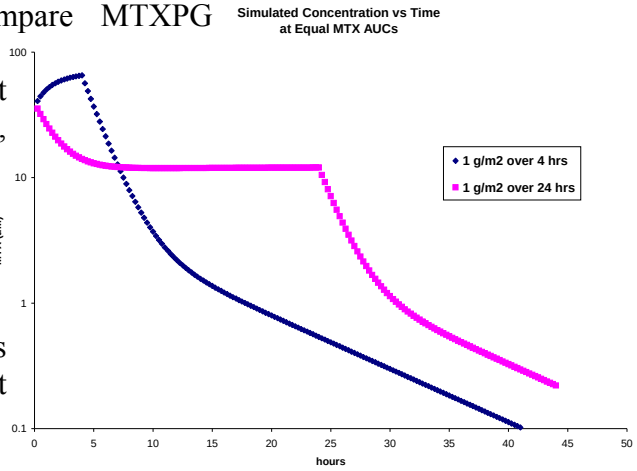
The length of exposure to MTX is an important determinant of MTXPG formation and antitumor effect in preclinical *in vitro* and *in vivo* models, with longer exposures to equal extracellular concentrations resulting in greater polyglutamylation and effect.[37-44] Preclinical data suggested equivalent bone marrow suppression at an AUC of 120,000 nM•hrs (12 hr exposure) and at 24,000 nM•hrs (24 hr exposure),[43] suggesting that duration of exposure was a more important determinant of MTX effect—as long as a putative minimum threshold value has been exceeded.[38-41,44] We compared MTXPG accumulation following different MTX concentrations and durations of exposure *in vitro*, and found that with similar "AUCs" of exposure (e.g. 1 ΦM for 24 hours vs 5 ΦM MTX for 4 hours), greater MTXPG accumulation occurred with the former than with the latter schedule.[19] Moreover, the "advantage" of prolonged exposure was more pronounced with a T-lineage than with a B-lineage ALL cell line. Increasing the duration of exposure has recently been hypothesized to circumvent MTX resistance on the basis of overexpression of MRPs, which are involved in effluxing MTX from leukemic cells.[45] However, caution must be exercised in extrapolating findings from cell lines to patients, as *in vitro* data had suggested that low-dose MTX might be as effective as high-dose MTX, which *in vivo* pharmacologic studies[16,20] and clinical trials have shown not to be the case.[30,46-48] Thus, it is not clear whether prolonged infusions encompassing the dosage range relevant for ALL treatment are more

effective than short infusions of MTX, and if they are, whether increasing the dose could compensate for the shorter duration of exposure.

There have been no randomized trials comparing short versus prolonged infusions of MTX in ALL. In Total XI and during consolidation and continuation therapy in Total XIII, HDMTX was given at 2 gm/m^2 IV as short infusions over 2 hours (Table 1). The rationale was that 2 gm/m^2 over 2 hours maintains plasma concentrations > 0.1 μmol/L (a putatively cytotoxic concentration) for the same time period (~ 42 hours) as had 1 gm/m^2 over 24 hours (as had been effectively used on Total XS[48]). After 42-48 hours of MTX exposure, leucovorin rescue must be started. However, the pharmacologic studies from the upfront window of Total XIII proved that not only duration of concentrations exceeding 0.1 μmol/L, but also the extent by which it is exceeded, is important for MTXPG accumulation.[16,20] Thus, we and others are concerned that short infusions may be less efficacious than longer infusions. However, the need for hospitalization for 24 hour infusions, with their attendant increased workload for clinical staff, disruption to patients' families, and possible increased toxicity, dictate that the question of optimal infusion length be rigorously addressed. The clinical relevance of addressing the issue of infusion length is illustrated by the fact that major treatment groups (the POG, as piloted in 9201 and 9705 protocols for ALL, and the BFM, in mature B-lineage leukemia and lymphoma) are prospectively comparing outcome in patients randomized to long vs short infusions of MTX. Thus, as we did in Total XIIIA, we herein propose to address the pharmacologic effects of two different schedules of MTX in the up-front setting. Our findings should provide biochemical data with direct relevance to the manner in which MTX is being tested in randomized ALL therapeutic trials conducted by other treatment groups.

HDMTX in SJCRH ALL Protocols

Protocol (years of accrual)	Dose of HDMTX (gm/m^2)	Infusion Length (hrs)	Place in Therapy
XS-IVIT (1979-1984)	1	24	15 doses over first 72 weeks of continuation
XI (1984-1988)	2	2	2 doses as consolidation
XII (1988-1991)	1.5 vs individualized based on clearance	24	5 doses during continuation
R15 (1991-1996)	5 (targeted to Cpss of 65 μmol/L)	24	once or twice as consolidation
XIII (1991-1998)	1 (vs 30 mg/m^2 po q 6 hrs x 6)	24	as up-front pre-induction therapy
	2	2	2 doses as consolidation and 8 doses during continuation
XIV (1998-1999)	Individualized to Cpss of 2.5 to 90 μmol/L (~ 0.25 to 7 gm/m^2)	24	as up-front pre-induction therapy
	2.5 (low-risk arm) or 5 (high-risk arm)	24	2 doses as consolidation and 4 doses during continuation



In Total XV, we will compare MTXPG accumulation and MTX pharmaco-dynamics at equivalent MTX plasma AUCs, giving 1 gm/m^2 over 24 vs over 4 hours. We recognize that this comparison of comparable AUCs will result in lower plasma MTX concentrations at 42-44 hours with the 4-hr infusion, and that we and others have attempted to circumvent this by giving a somewhat higher dose over 4 hours (e.g. 2 gm/m^2) than that given over 24 hours (e.g. 1 gm/m^2). However, it is most appropriate to **start** by comparing equivalent doses, because (a) if we compared 2 gm/m^2 over 4 hrs vs. 1 gm/m^2 over 24 hrs and observed higher MTXPGs with the former schedule, we would not know whether doubling of the dose or infusion length affects MTXPG accumulation and (b) the data we generate by comparing equivalent doses will be an informative starting point for future comparisons, in which we might alter dosage in an attempt to produce better MTXPG accumulation with a short infusion.

It is important to establish the optimal infusion length of MTX for children with different lineage and genetic subtypes of ALL, for the following reasons. Clinical data indicate that the level of systemic exposure following 1 to ~1.5 g/m^2 over 24 hours significantly affected the event-free survival of children with B-lineage ALL[30,48] but not of those with T-lineage ALL.[30] Moreover, in Study XIIIA, we demonstrated that given the same dosages of MTX, total accumulation of MTXPG and long-chain MTXPG (more active metabolites) were significantly lower in T-lineage blasts than B-lineage blasts, and in non-hyperdiploid B-lineage blasts than hyperdiploid B-lineage blasts.[20] Such differences have also been observed in vitro.[49,50] It is not known whether infusion length is a more important determinant of MTXPG accumulation in hyperdiploid B-lineage, nonhyperdiploid B-lineage, or T-cell ALL. Our own data from in vitro incubations with B-lineage vs T-ALL cell lines suggest a greater impact of 24 hour compared to 4 hour MTX exposures in T compared to B-lineage cell lines.[19] Moreover, our preliminary data from Total XIV, in which the peripheral blast MTXPGs were measured serially at 20 minutes, 3 hours, and 24 hours during constant infusion of MTX, demonstrate substantial interpatient variability in the kinetics of accumulation during MTX exposure, suggesting that lineage- and ploidy-specific differences in the determinants of MTXPG accumulation (such as RFC-mediated uptake, hydrolase-mediated cleavage, MRP-mediated efflux, and FGPS-

mediated polyglutamylation)[20,39,45,50-55] may result in a different optimal duration of MTX exposure among the major ALL subtypes. Thus, we propose to define whether there are infusion-length related differences in MTXPG accumulation between hyperdiploid B-lineage, nonhyperdiploid B-lineage, and T-cell ALL in a randomized, stratified comparison. Because of the inconvenience, increased hospitalization, and possible increase in toxicity of long compared to short infusions, identifying lineage- and ploidy-related differences in infusion length could translate into individualizing MTX infusion length for different ALL subtypes in the future, potentially allowing for less toxic schedules in some subsets of patients.

2.2.2 To determine whether prolonged (24 hour) intravenous infusions of HDMTX produce greater antileukemic effects than short (4 hour) infusions, based on the inhibition of de novo purine synthesis in bone marrow blasts, and the decrease in circulating blasts during the 4 day "window" prior to initiation of conventional remission induction therapy.

In Study XIIIA, a significant correlation was found between the degree of MTXPG accumulation in lymphoblasts and the extent of decrease in *de novo* purine synthesis (DNPS) (from pretreatment to 42 hours following the start of MTX) and the clearance of circulating blasts in a small group of 11 patients with non-hyperdiploid B-lineage ALL.[16] Our preliminary data also indicate that the degree of inhibition of thymidylate synthesis and DNPS, as well as the MTX exposure, correlate with the extent of clearance of circulating blast cells.[56] That the inhibition of DNPS and thymidylate synthesis and the decrease in circulating blasts differ among patients and correlate with MTXPG accumulation suggests that these pharmacologic endpoints and early clinical responses can be used to characterize the pharmacodynamics of HDMTX. In Study XIIIB, we found that the pre-treatment rate of DNPS was significantly higher in T-lineage blasts as compared to hyperdiploid or non-hyperdiploid B-lineage blasts.[56] Different MTXPG concentrations are likely to be required to produce comparable cytotoxicity in these subtypes of ALL, due to the different baseline levels of DNPS, dihydrofolate reductase,[57] or other target enzymes. The immediate decrease in circulating lymphoblasts and the *ex vivo* change in purine synthesis are reasonable indices of antileukemic response. Conceivably, short infusions may produce adequate antileukemic response in one ALL subtype but not in another. In Total XV, we will study a larger number of patients to definitively address whether antileukemic effects differ by infusion duration among hyperdiploid B-lineage, nonhyperdiploid B-lineage, and T-cell ALL subgroups.

To circumvent the DNPS inhibition effect of allopurinol,[58] we will use recombinant urate oxidase (Uricozyme or Rasburicase) to prevent or

treat hyperuricemia,[18] if needed prior to the initiation of conventional induction therapy.

2.2.3 To assess whether differences in expression or activity of the reduced folate carrier (RFC), gamma glutamyl hydrolase (GGH), efflux pumps (e.g. MRPs), or folylpolyglutamate synthetase (FPGS) account for lineage- (B- vs T) and ploidy- (hyperdiploid vs nonhyperdiploid B-lineage) related differences in MTXPG accumulation, and to identify the molecular basis of such differences, including the contribution of genetic polymorphisms to interpatient variability in MTXPG accumulation.

Amend 4.0

A number of factors could influence differences in MTXPG accumulation between ALL subtypes. In Study XIIIA, a trend toward higher FPGS activity was found in B-lineage blasts as compared to T-lineage blasts,[20] a finding confirmed by others[52] but this difference could not fully account for the lineage differences in intracellular MTXPG accumulation. Moreover, we did not find a significant difference in FPGS activity between hyperdiploid and non-hyperdiploid blasts.[55] Preliminary data indicate that hyperdiploid ALL blasts have higher RFC expression than non-hyperdiploid B-lineage blasts and that the number of copies of chromosome 21 (to which the RFC is localized) is correlated with MTXPG accumulation.[59] The role of GGH as a determinant of lineage differences has been questioned,[52] but was evaluated using only MTXPG-2 (rather than longer chain MTXPGs) as the substrate and without accounting for the known lysosomal localization[60] of GGH. The contribution of MRPs to MTXPG accumulation is just beginning to be evaluated,[45] and in collaboration with Dr. Schuetz,[61] we will assess their role in differential MTXPG accumulation with short vs long infusions of MTX among various ALL subtypes. For any targets or enzymes that are important to determining MTXPG accumulation, we are exploring whether somatic-cell genetic polymorphisms (e.g. in RFC) contribute to blast accumulation, which of course, may have implications for interpatient variability in susceptibility to MTX-induced toxicity, as well. Initially, we will use currently available limited gene arrays to assess expression levels of genes potentially involved in methotrexate metabolism and effects. As more dense gene arrays become available and as we establish the ability to develop customs arrays in the Hartwell Center at SJCRH, we will use them to assess more global changes in gene expression and their relationship with methotrexate's antileukemic effects. **As reliable proteomic tools become available, we will use them to assess more global changes in protein expression and their relationship with methotrexate's antileukemic effects.**

Amend 1.0 (deleted infectious disease section)

2.3 Biologic Studies

2.3.1 Minimal residual disease

We and others have shown that detection of submicroscopic ("minimal") disease strongly correlates with leukemia relapse.[8,72,73] Minimal residual disease (MRD) can now be studied in all patients with ALL. Using both PCR and immunologic methods, we were able to study minimal residual leukemia in all 62 samples recently obtained with highly concordant results.[24] However, these studies entail sequential bone marrow aspirations. It would be more practical if bone marrow aspirates could be replaced by peripheral blood, which would also allow more frequent sampling, hence closer monitoring of submicroscopic levels of leukemia. Most of our patients will have a central venous device in place, obviating a need for venipuncture. However, the relative distribution of MRD in bone marrow and peripheral blood in children treated for ALL is not well established.

To investigate the potential value of examining peripheral blood for residual disease, we have compared percentages of leukemic lymphoblasts in peripheral blood and bone marrow in 58 children with ALL, using immunologic techniques. In 43, MRD was undetectable in both tissues, while 7 were MRD positive in both marrow and blood. The eight remaining patients (all B-lineage ALL) were MRD positive in marrow but MRD negative in the blood.[75] A recent study of MRD by PCR amplification of IgH genes in 35 paired peripheral blood and bone marrow samples (from 15 children) during remission induction chemotherapy found that the presence of detectable minimal residual disease in peripheral blood correlated well with that in bone marrow.[76] Thus, studies of peripheral blood could yield potentially useful clinical information.

In Total XV, we will determine the degree of correlation between levels of residual leukemia in blood versus bone marrow by studying paired samples from patients enrolled in the study using immunologic and/or molecular techniques. The information gained could reveal differences in tissue distribution and recirculation capacity among different leukemic subtypes. Ultimately, it could help establish a fundamentally new approach to monitor remission status, one that would be much more practical and potentially allow early therapeutic intervention.

2.3.2 Somatic cell DNA alteration from chemotherapy

Long-term adverse effects of ALL therapy include secondary acute myeloid leukemias and secondary brain tumors.[1,77] DNA damage in peripheral leukocytes may presage the development of tumors[78] and can

be induced by exposure to environmental mutagens and chemotherapy.[79-82] In Total XIIIB, we have used a large V(D)J-recombinase mediated deletion of the *HPRT* gene in lymphocytes as a biomarker,[83] with particular interest in the recombinogenesis induced by etoposide,[84] as a relevant mechanism involved in the genesis of secondary AML. For study XV, we have eliminated the use of etoposide to decrease the risk of secondary AML. The increased emphasis on asparaginase and anthracyclines should result in much less leukemogenesis. However, because there is always the possibility for unanticipated effects, we propose to continue to serially collect somatic cells for analysis of possible early indicators of leukemogenesis or DNA alterations in Total XV. Anthracycline use has been associated with secondary AML exhibiting characteristics of either topoisomerase II-inhibitor and alkylator-induced AML,[84a] and thus having somatic cells available for possible future study is a prudent action to take. Examples of DNA rearrangements to assess include *MLL* itself as an indicator of DNA alteration, as this gene has been shown to rearrange both *in vitro* and *in vivo*, immediately following etoposide and anthracycline exposure.[85] Moreover, as additional indicators of DNA alterations become available, we will use them as in vivo markers of chemotherapy effects.

Telomeres are specialized structures at the ends of chromosomes that consist of several kilobases of tandem TTAGGG repeats and associated proteins. Telomeres promote karyotypic stability by preventing chromosome end-to-end associations, breaks, and loss during mitosis.[85a] Additionally, they provide a buffer of expendable DNA to accommodate the "end replication problem," which is the erosion of DNA that occurs with each round of cell division. When telomeres reach a critically short length, chromosomal instability ensues and senescence is triggered.[85b] Because hematopoietic cells undergo many cell divisions to repopulate the bone marrow after chemotherapy-induced cytopenia, it is possible that repeated cycles of chemotherapy greatly accelerate telomere shortening. To test the possibility, leukocyte telomere length from six children treated on the TOTAL XIIIB study was measured during induction, as well as during and after continuation therapy. In two patients, telomere length was stable throughout treatment. In the other four patients, telomeres shortened by 3,000-8,000 bases in a two year time period, which greatly exceeds the normal shortening rate of 40 bases per year.[85c,85d] Two of the children ended therapy with telomere length equivalent to that of a 100 year old individual. The clinical implications of this observation remain to be determined. In particular, it is unclear whether short telomeres, with their associated chromosomal instability, predispose to the genesis of secondary leukemia. It is also unclear whether hematopoiesis can be sustained for a full lifespan in children who end therapy with very short telomeres. To further evaluate

the phenomenon of treatment-induced telomere shortening, leukocyte telomere length will be measured prospectively in this study.

Amend 2.0

Somatic cell DNA alternation will be compared between standard/high risk patients (who receive far more mutagenic agents) and low risk patients (who receive largely antimetabolite-based therapy) at identical times during therapy (Pre-consolidation, **end of consolidation,** during remission reinduction I, pre-reinduction II, **at week 64** and upon completion of treatment). RNA (stored as cDNA) will be isolated to facilitate some assays. Somatic cell DNA damage will also be compared within individual patients at these time points.

2.3.3 Genetic polymorphisms in drug metabolizing enzymes

Several enzymes which are known or suspected to be important to the action of drugs used in ALL therapy are genetically regulated and polymorphically distributed in various ethnic populations. For example, methylenetetrahydrofolate reductase (MTHFR) is crucial in folate homeostasis and its relative deficiency is known to predispose to hyperhomocysteinemia[86] and has been hypothesized to predispose to MTX neurotoxicity[87] and asparaginase-induced thrombosis. Although our own preliminary data do not confirm these hypotheses, other acute toxicities may be more common among the 10% of the population who are homozygous for the thermolabile form of MTHFR and the 40% who are heterozygotes (Relling, unpublished observations). If confirmed, it is possible that MTHFR status could be used to assist in individualizing continuation therapy in the future. Because intracellular folate pools have been found to be important determinants of antifolate pharmacology,[88] it is also important to assess if MTHFR genotype is related to MTXPG accumulation in leukemic lymphoblasts.

Thiopurine methyltransferase (TPMT) also exhibits genetic polymorphism, with <1% of the population homozygous deficient and 10% heterozygous.[89] We have been using information on patients' TPMT status to assist in dosage individualization for continuation therapy since 1991 in patients enrolled on Total XIII. We will continue to assess patients for TPMT phenotype, and where helpful, TPMT genotype, and use these data (along with data on clinical tolerance of chemotherapy and measurements of active thiopurine metabolites in red blood cells) as part of our algorithm for dosage adjustment during continuation therapy on Total XV.

There are fewer data to indicate whether the genetic polymorphisms in glutathione transferases (GSTM1 and GSTT1)[90] are important in ALL therapy. GSTs are important in inactivating many alkylating anticancer agents[91] (e.g. cyclophosphamide) and the active metabolites of drugs such as etoposide,[92] *in vitro*. Importantly, they are involved in protecting DNA from endogenous and exogenous free radical damage[93]

and thus may be involved more generally in mediating toxicity and efficacy of antileukemic agents *in vivo*. Our preliminary analysis suggested a trend toward fewer CNS relapses among patients with GSTM1 null genotype, although this genotype did not affect overall treatment outcome in children with ALL.[94] In these preliminary, retrospective studies, it was not possible to determine whether GST status was associated with any acute or long term toxicities. Thus, in Total XV, we propose to study all patients, and explore whether acute toxicities are related to GST genotype. Should long-term toxicities (such as second cancers) occur, GST genotype could be examined as a possible risk factor. In addition, polymorphisms in mismatch repair enzymes[94a] (e.g. MSH2, MSH6) or HPRT may affect their response and toxicity, and polymorphisms in CYP3A4 and its regulators (e.g., PXR, p-glycoprotein) could affect response and toxicity related to vincristine, glucorticoids, and anthracyclines.

2.3.4 Asparaginase Pharmacology

Allergic reactions occur commonly with administration of asparaginase (an enzyme not present in humans) and can limit its use.[95] More importantly, development of anti-asparaginase antibodies (with or without clinical hypersensitivity) may inactivate asparaginase and thus attenuate its ability to deplete asparagine systemically.[96] However, this assertion has yet to be tested systematically in any large front-line ALL trial. Data from a recent EORTC trial which randomized patients to identical dosages of two different preparations of native asparaginase (*E. coli* vs *Erwinia*) suggested a significant difference in treatment outcome. Patients who received E. coli asparaginase, which has a longer half-life, had a better treatment outcome.[97] This finding has stimulated interest in optimizing use of this class of agents. Missing from this EORTC trial and most other large ALL trials are studies of whether anti-asparaginase antibodies play a role in efficacy of asparaginase.

It is believed to be important to deplete asparagine systemically to optimize asparaginase effect. CSF constitutes a convenient tissue for sampling, because CSF asparagine levels reflect systemic levels[98] and are not subject to the difficulties in immediate sample processing caused by asparaginase in blood.[99] In our front-line relapse study, R16, we are carefully assessing asparaginase pharmacokinetics, asparagine depletion, and antibody levels. With careful sample processing, plasma asparagine measurements are feasible. In this larger-scale study (Total XV), we propose to measure anti-asparaginase antibodies, as well as CSF and plasma asparagine depletion in all patients at identical time periods during therapy, to explore whether these variables are related to treatment outcome and whether the two variables (antibodies and asparaginase depletion) are related to each other.

2.3.5 Glucocorticoid Pharmacology

Glucocorticoids make up a large component of ALL continuation therapy in Total XV and other contemporary ALL regimens. Glucocorticoid-induced adverse effects, such as immunosuppression, osteoporosis, and avascular necrosis, may be dose-limiting for some patients. However, glucocorticoid pharmacokinetics and dynamics remain essentially undefined in this patient population. Data from transplant patients suggest that the extent of plasma cortisol suppression may be a predictor of steroid-induced bone toxicities.[129] Moreover, glucocorticoids are potent inducers of CYP3A4, *in vitro* and *ex vivo*. CYP3A4 is the most abundant P450 in human liver and is responsible for metabolism of etoposide, vincristine, oxazaphosphorines and steroid.[100] However, steroid-induced drug interactions remain largely uncharacterized *in vivo*. Thus, Total XV provides a unique platform to characterize pharmacokinetics of dexamethasone in children with ALL, to determine whether autoinduction occurs during the periods of reinduction therapy with dexamethasone, and to explore whether there are relationships between dexamethasone pharmacokinetics and pharmacodynamics (e.g., cortisol suppression) and between either kinetics/dynamics and genetic polymorphisms of CYP3A4, its regulatory proteins (e.g. PXR) or other relevant somatic cell pharmacogenetic polymorphisms, (e.g. glucocorticoid receptors,[100a] p-glycoprotein).

2.3.6 Determination of ALL blast sensitivity to antileukemic agents, in vitro

We have previously used the stroma-supported immunocytometric assay (SIA) to determine the relative sensitivity and resistance to antileukemic agents of primary leukemia cells isolated from newly diagnosed patients with ALL.[101] This SIA method permitted a comparison of the relative action of dexamethasone and prednisone[102] and the screening of new agents for antileukemic activity.[101] We acknowledge that IC50 values, particularly those for thiopurines, may not correlate with in vivo values as to absolute concentrations, but such in vitro tests may be useful in identifying outlyers in a relative fashion. We propose to use this SIA method to assess the relative sensitivity of ALL blasts for newly diagnosed patients entered on the Total XV protocol, with the goal of identifying patients whose leukemia cells exhibit a high level of resistance or sensitivity to mercaptopurine, and dexamethasone, data that will be used to assess the relation between plasmacogenetic polymorphisms and blast sensitivity to these antileukemic agents. We previously used this SIA method to determine sensitivity of primary ALL blasts to mercaptopurine, in 23 patients entered on the Total XII protocol, revealing substantial heterogeneity in blast sensitivity to 6MP. The IC50 values, after 96 hour exposure to 6MP, ranged from 0.45 :M to

120 :M (median 47.9 :M, n=16); thus there was 270-fold range in sensitivity. Studies for prednisone and dexamethasone revealed >3,000-fold range for prednisone (n=20) and 514-fold range for dexamethasone (n=22), with the IC50 of prednisone and dexamethasone being highly correlated.[102] However, in these previous studies (conducted on samples from Total XII), data were not available on germline polymorphisms that might affect the leukemic cells and host susceptibility to these agents. Our goal in studies conducted as part of the Total XV protocol, is to elucidate mechanisms underlying this large range of sensitivity to these agents, thereby providing insights that may improve the efficacy of these agents for treating childhood ALL. We will initially focus on specific genes and proteins that are involved in the metabolism and cytotoxicity of these agents, including thiopurine methyltransferase, HPRT, mismatch repair proteins (e.g., MSH2/MSH6, etc), and enzymes involved in the disposition of glucocorticoids (e.g., CYP3A4, PXR). We will explore whether germline polymorphisms in these genes that may affect host susceptibility are associated with differences in ex vivo blast sensitivity, as assessed by the SIA method.

Amend 4.0

2.4 Cancer Control Studies

While neuropsychologic deficits are well-recognized side effects of cranial irradiation, intrathecal and systemic chemotherapy (especially MTX) can also cause brain atrophy and spinal cord dysfunction.[103-106] In fact, methotrexate given orally in several divided doses can also cause neurotoxicities when leucovorin rescue is not given.[107] It has been postulated that CSF or plasma concentrations of homocysteine (or homocysteic acid) may be related to MTX treatment and/or neurotoxicities.[108] **Whether CSF levels of folate may predispose to or presage neurotoxicity has not been previously studied**. In studies XIII A and B, patients received intensive triple intrathecal treatment and 10 courses of HDMTX (2 gm/m^2 over 2 hours). Although transient encephalopathy was observed in up to 3% of cases, long-term neurotoxicities are rare.[109] We are now assessing the neuropsychological function of long-term survivors from Study XIIIA. In this investigation, 41% (18/44) of the patients treated without RT had Full-Scale IQ scores at least 1 standard deviation below normal but only 10% (4/44) had sufficient academic and attentional deficits to qualify for an interventional feasibility study (MEMFIX). Nineteen of the 44 patients had MRI examinations suitable for segmentation analysis. Three of these 19 long-term survivors (16%) had leukoencephalopathy on these examinations. A relationship between white matter volume and neurocognitive performance (Full-Scale IQ [r=0.33, p=0.165] and CPT Hits Reaction Time [r=0.44, p=0.059]) was also demonstrated in this pilot study. Cross-sectional studies of neurocognitive performance in long-term survivors are designed to detect severe impairments compared to the normal population and are relatively insensitive to more subtle localized treatment-induced deficits in individual patients.

Amend 4.0

In Study XIV, we used intensive triple intrathecal treatment and HDMTX, with the latter given at a higher dosage over a longer period of time (2.5 gm/m^2 or 5 gm/m^2 over 24 hours) than HDMTX on Total XIII. MRI of the brain was prospectively studied in all patients after 1, 4, and 7 courses of HDMTX. Preliminary results showed that focal white matter changes were found in 2 of 47 patients in the first study period, 25 of 43 in the second period, and 17 of 24 in the third period (Reddick WE, unpublished observation). Because MRI studies have seldom been performed on asymptomatic patients, the clinical significance of these findings is currently unknown. We will continue to monitor prospectively the neuropsychologic function, quality of life, CSF and plasma levels of homocysteine, **folates**, and related components (in collaboration with J. Greiner, Dallas Southwestern **and with U. Jaehde at the University of Bonn**) and changes in the brain MRI of patients treated in Total XV. Results will be used to establish whether imaging changes or biochemical indices have clinical predictive importance that may be useful in future studies of ALL.

While several chemotherapeutic agents used to treat childhood ALL, including methotrexate[110] and glucocorticoids,[111] cause bone demineralization, two previous studies reported that severely diminished bone mineral density (more than 2 SD below the mean for age-and sex-matched control) in survivors of childhood cancers was limited to patients who received cranial irradiation. Our recent preliminary study showed that this abnormality is not limited to patients who received cranial irradiation, and in fact is more prevalent than that suggested by previous studies. Using quantitative computed tomography densitometry,[112] we studied bone density in 145 patients who have completed treatment on Total Therapy XI. Of these patients, 21% had bone density more than 1.645 standard deviations below the normal mean (Kaste S, unpublished observation). This distribution is significantly different from the expected normal distribution ($p<0.0001$ by goodness-of-fit test). Three factors were associated with increased risk of osteopenia/osteoporosis: male gender, Caucasian race, and 24 Gy of cranial irradiation. Since peak bone mass is usually achieved during puberty, with little if any subsequent accumulation,[113] it is essential to identify this abnormality early for therapeutic intervention. We plan to determine bone density in patients at diagnosis and at week 120 of therapy for ALL. The goal is to determine the prevalence and the risk factors of this complication in the absence of cranial irradiation. In this regard, level of physical activity, nutritional status, family history, and age of onset of puberty may each influence the development of this complication. Patients with abnormal bone density at the end of therapy will be referred to the endocrine clinic for evaluation and treatment.

Amend 4.0 (deleted text)
Amend 3.0

Asparaginase therapy is well known to cause decreased protein synthesis and is the primary explanation for the commonly seen changes in circulating levels of coagulation proteins. Increased circulating levels of unusually large vWF multimers has been reported in patients who experience thrombosis while receiving multiagent chemotherapy which

includes asparaginase[114a]. Under normal conditions, these unusually large multimers are rapidly removed from the blood by a vWF cleaving protease [114b]. In patients who develop thrombosis, asparaginase may also reduce the synthesis of vWF-cleaving protease, thus leaving the large multimers in the circulation and initiating thrombosis. To explore this idea, we will obtain one 5 ml blood sample (red top tube) at the time of the diagnosis of thrombosis, and measure vWF multimers and cleaving protease concentrations, along with anti-asparaginase antibodies. One 3 ml tube of blood will also be obtained for plasma asparagine. Serum and plasma from patients already being collected for asparaginase antibodies will serve as an initial control.

Acute cardiotoxicity from anthracycline chemotherapy is reasonably well understood and characterized. Late toxicity, on the other hand, has only recently been recognized as a potential cause of morbidity and mortality in childhood cancer survivors. Late-onset congestive heart failure, arrhythmias, and sudden death have been observed in childhood cancer survivors and have occurred independently of congestive heart failure during therapy.[114c-114g] Recent investigations have indicated that significant numbers of survivors demonstrate asymptomatic echocardiographic abnormalities (elevated afterload) after completion of anthracycline therapy. Clinical factors correlated with these changes have varied by study and include young age at treatment, female gender, high cumulative anthracycline dose, mediastinal irradiation, and time interval since completion of therapy. Of concern, the prevalence and severity of cardiac abnormalities appears to be increasing in these patients with prolonged follow-up. As a result, longitudinal data of clinically well-defined cohorts are needed to accurately assess the relationship between echocardiographic abnormalities and risk of clinically significant cardiovascular disease.

Amend 4.0

Children undergoing therapy for ALL are predisposed to urolithiasis, which can cause pain, hospitalization, delays in chemotherapy, and poor renal clearance of chemotherapy with increased toxicity.[114h, 114i] Furthermore, a third of the patients would suffer recurrent stone formation.[114h] Urolithiasis is caused by increased urine calcium excretion in response to glucocorticoid therapy. The incidence and timing of hypercalciuria (a urine calcium:creatinine ratio > 0.21[114j]) in childhood ALL are not known. Finally, children who develop osteoporosis during ALL therapy could benefit from calcium supplementation, but we have avoided this treatment since it may increase the risk of urolithiasis. Identifying children with hypercalciuria, who are at risk for urolithiasis, and determining the times during ALL therapy when children are at highest and lowest risk for calciuria would allow optimal treatment of osteoporosis and implementation of preventive measure for urolithiasis.

Amend 5.0

2.5 Philadelphia Chromosome (Ph)-positive ALL

As of June 2004, five patients with Ph+ ALL have been enrolled on this trial. One patient has relapsed after hematopoietic stem cell transplantation and another had a very poor early response, illustrating the need for more effective therapeutic management of this small subgroup of ALL patients. In view of recent promising results with single agent imatinib mesylate in patients with Ph+ ALL in the refractory/relapsed setting[114n, 114o, 114p], in preparation for allogeneic hematopoietic stem cell transplant[114q, 114r, 114s], and most recently in the setting of combination chemotherapy for relapsed/refractory disease[114t], this drug will be added to the treatment regimen for Ph+ ALL patients only.

Thomas, et al[114t], reported the results of a trial in adult Ph+ ALL in which twenty patients were treated with hyper-CVAD and imatinib (400 mg/day x 14 days in each treatment course). The treatment was well tolerated even in combination with high-dose methotrexate and high-dose cytarabine. Imatinib at 600 mg per day was given continuously during continuation treatment with mercaptopurine and vincristine plus prednisone pulses. No excessive toxicities were noted as compared with historical controls treated with hyper-CVAD without imatinib.

In the ongoing COG study, imatinib is given at 340 mg/m^2 for 21 days with various courses of post-remission chemotherapy (Schutz K, personal communication). Four cohorts of patients have been treated. For the last cohort of patients, imatinib will be given continuously during continuation treatment. In the current European (BFM) study, imatinib is given at 300 mg/m^2 at protocol Ib of induction with mercaptopurine, cytarabine and cyclophosphamide, and between various pulses of therapy (Schrappe M, personal communication). In both COG and BFM studies, the combination has been well tolerated with no unusual toxicities observed.

In this trial, imatinib will be started at day 26 of induction (at 340 mg/m^2 per day, maximum 600 mg per day) until the end of therapy. For patients who undergo HSCT, imatinib will be held during the transplant period, and then resumed for 6 months after recovery of marrow function. The reasons for starting imatinib on day 26 of induction are (1) European study showed that it is well tolerated when used in combination with cyclophosphamide, mercaptopurine and cytarabine; (2) since imatinib is both an inhibitor and substrate for cytochrome P450 3A4, it is best to avoid the use of this agent during prolonged treatment with prednisone and vincristine in the first three weeks of induction; and (3) we can still assess early responses to chemotherapy, which is an important factor in determining whether transplant is indicated or not for certain Ph$^+$ cases.

3.0 DRUG INFORMATION

3.1 PREDNISONE

Source and Pharmacology: Prednisone is a synthetic congener of hydrocortisone, the natural adrenal hormone. Prednisone is a white or yellowish crystalline powder. It binds with steroid receptors on nuclear membranes, impairs cellular mitosis and inhibits protein synthesis. Prednisone also has potent anti-inflammatory effects and suppresses the immune system. Prednisone is well absorbed orally. It is converted to prednisolone, the pharmacologically active metabolite, in the liver. Prednisolone is further metabolized to inactive compounds in the liver. The metabolites are excreted mainly in the urine.

Formulation and Stability: Prednisone is available as 2.5, 5, 10, 20, 25 and 50 mg tablets. In addition, it is available as a 1 mg/ml solution. All dosage forms can be stored at room temperature. At St. Jude Childrens Research Hospital, prednisolone 1mg/ml oral solution may be substituted for prednisone liquid due to its superior palatability.

Supplier: commercially available

Toxicity: Side effects of prednisone vary depending on the duration of its use. Side effects that can occur with short term use include sodium and water retention with associated hypertension, peptic ulcer with possible perforation and hemorrhage, impaired wound healing, increased susceptibility to infections, emotional instability, insomnia, increased appetite, weight gain, nausea, acne and hyperglycemia. Less common side effects include pancreatitis, hypersensitivity reactions and aseptic necrosis. Side effects more commonly associated with prolonged use include cataracts, increased intraocular pressure and associated glaucoma, development of a “cushingoid” state, compression fractures, menstrual irregularities, suppression of growth in children, secondary adrenocortical and pituitary unresponsiveness particularly in times of stress as in trauma, surgery or illness, osteoporosis and muscle wasting.

3.2 DEXAMETHASONE (Decadron®)

Source and Pharmacology: Dexamethasone is a synthetic congener of hydrocortisone, the natural adrenal hormone. Dexamethasone is a white or yellowish crystalline powder. It binds with steroid receptors on nuclear membranes, impairs cellular mitosis and inhibits protein synthesis. Dexamethasone also has potent anti-inflammatory effects and suppresses the immune system. Dexamethasone is well absorbed orally. It is metabolized in the liver and the metabolites are excreted mainly in the urine.

Formulation and Stability: Dexamethasone is available as 0.25 mg, 0.5 mg, 0.75 mg, 1 mg, 1.5 mg, 2 mg, 4 mg and 6 mg tablets and a 0.5 mg/5 ml elixir. It is available as a 4 mg/ml, 10 mg/ml and 20 mg/ml solution for parenteral use. All formulations of the drug can be stored at room temperature. The injectable form may be further diluted in

5% dextrose or 0.9% NaCl containing solutions and is stable for at least 24 hours at room temperature.

Supplier: Commercially available

Toxicity: Side effects of dexamethasone vary depending on the duration of its use. Side effects that can occur with short term use include sodium and water retention with associated hypertension, peptic ulcer with possible perforation and hemorrhage, impaired wound healing, increased susceptibility to infections, emotional instability, insomnia, increased appetite, weight gain, nausea, acne and hyperglycemia. Less common side effects include pancreatitis, hypersensitivity reactions and aseptic necrosis. Side effects more commonly associated with prolonged use include cataracts, increased intraocular pressure and associated glaucoma, development of a "cushingoid" state, compression fractures, menstrual irregularities, suppression of growth in children, secondary adrenocortical and pituitary unresponsiveness particularly in times of stress as in trauma, surgery or illness, osteoporosis and muscle wasting.

3.3 VINCRISTINE (Oncovin®)

Source and Pharmacology: Vincristine is an alkaloid obtained from the periwinkle *(Vinca rosea)* plant. It reversibly binds to microtubule and spindle proteins causing metaphase arrest. Vincristine has poor penetration into the CSF. It is approximately 75% protein bound. Extensive metabolism occurs in the liver. Excretion is primarily in the bile and feces. A dosage decrease is recommended in patients with a bilirubin > 3 mg/dl.

Formulation and Stability: Vincristine is supplied in multiple-dose vials containing 1 ml, 2 ml and 5 ml. Each ml of solution contains 1mg of vincristine and 100 mg of mannitol. In addition, acetic acid and sodium acetate are added for pH control. The intact vials should be stored under refrigeration and protected from light. The drug can be further diluted with 5% dextrose or 0.9% NaCl containing solutions.

Supplier: Commercially available

Toxicity: Dose limiting toxicity is neurotoxicity. This can be characterized by constipation and/or paralytic ileus, ptosis, vocal chord paralysis, weakness, jaw pain, abdominal pain, peripheral neuropathies, loss of deep tendon reflexes and "foot drop". Peripheral neuropathy is often the first sign of neurotoxicity and is initially reversible. Other toxicities reported commonly include alopecia and mild nausea and vomiting. Vincristine is a vesicant and may cause severe tissue damage if extravasation occurs. Other toxicities reported less commonly include SIADH, myelosuppression, stomatitis, seizures, altered mental status, anaphylaxis, headache and optic atrophy. NOTE: dose reduction may be necessary in patients < 1 year of age. Dosing on a per kg (rather than per m^2) basis has been advocated for infants in order to decrease toxicity.

3.4 DAUNORUBICIN (Daunomycin, Cerubidine®)

Source and Pharmacology: Daunorubicin is an anthracycline antibiotic derived from *Streptomyces peucetius*. Daunorubicin intercalates between base pairs of DNA causing steric obstruction, disruption of DNA function and inhibition of RNA synthesis. In addition, daunorubicin inhibits topoisomerase II, an enzyme responsible for allowing strands of DNA to pass through one another as they unwind. Even though daunorubicin exerts its major effects in the S phase, it is considered to be cell cycle phase-nonspecific. Daunorubicin is widely distributed in tissues but does not cross the blood brain barrier. It is metabolized to daunorubicinol which is the major active metabolite and aglycones (inactive). The major route of elimination is through the bile (40%) with additional elimination through the urine. Dosages should be reduced in patients with liver dysfunction or renal dysfunction (creatinine > 3 mg/dl).

Formulation and Stability: Daunorubicin is supplied in vials containing 20 mg of reddish colored lyophilized powder and 100 mg of mannitol. The intact vials should be stored at room temperature. Each vial can be reconstituted with 4 ml of sterile water for injection to give a final concentration of 5 mg/ml. Reconstituted solutions are stable for 24 hours at room temperature and 48 hours if refrigerated.

Supplier: Commercially available

Toxicity: Dose-limiting toxicities of daunorubicin include myelosuppression and cardiotoxicity. Two forms of cardiac toxicity can occur. Acute toxicity may take the form of arrhythmias, heart block or pericarditis and may be fatal. The chronic form of cardiotoxicity is related to total cumulative dose and is characterized by heart failure. Mediastinal radiotherapy and/or other cardiotoxic drugs may increase the risk of cardiotoxicity. In general, total lifetime dosages of 450-550 mg/m^2 should not be exceeded. Other toxicities include nausea and vomiting, mucositis, alopecia, diarrhea and red discoloration of the urine and other body fluids. Severe tissue damage and necrosis can occur upon extravasation. Radiation recall reactions can occur and can be severe. Rarely, allergic reactions have occurred.

3.5 DOXORUBICIN (Adriamycin®)

Source and Pharmacology: Doxorubicin is an anthracycline antibiotic produced by *Streptomyces peucetius*. Doxorubicin exerts its anti-tumor effects in several different ways. Doxorubicin intercalates between base pairs of DNA causing steric obstruction, disruption of DNA function and inhibition of RNA synthesis. In addition, doxorubicin inhibits topoisomerase II, an enzyme responsible for allowing strands of DNA to pass through one another as they unwind. Lastly, doxorubicin undergoes enzymatic electron reduction to generate highly reactive species, including the hydroxyl free radical, which is thought to be responsible for the drug's cardiac toxicity, but may play a role in its anti-tumor activity as well. Doxorubicin is cell-cycle, phase non-specific. Doxorubicin is widely distributed in the tissues and plasma, but does not cross the blood brain barrier to an appreciable extent. It is metabolized to doxorubicinol, which is thought to be the major active metabolite, and aglycones. Doxorubicin and its

metabolites are excreted mainly in the bile and feces (≈80%). The remainder is excreted in the urine. Dosage should be reduced in patients with liver dysfunction or renal dysfunction (creatinine > 3 mg/dl).

Formulation and Stability: Doxorubicin is available in vials containing 10 mg, 20 mg, 50 mg and 200 mg as a 2 mg/ml red-orange solution. It is also available in vials containing 10 mg, 20 mg, 50 mg, 100 mg and 150 mg of doxorubicin as a red-orange lyophilized powder. Intact vials of doxorubicin solution should be stored under refrigeration while the lyophilized product should be stored at room temperature. Both products should be protected from light. Lyophilized doxorubicin can be reconstituted by adding 5, 10, 25, 50 or 75 ml of 0.9% NaCl respectively to the 10, 20, 50, 100 and 150 mg vials to produce a final concentration of 2 mg/ml. Bacteriostatic diluents are not recommended. After reconstitution, the resultant solution should be protected from light and is stable for 7 days at room temperature and 15 days if refrigerated.

Supplier: Commercially available

Toxicity: Dose-limiting toxicities include myelosuppression and cardiotoxicity. Two forms of cardiac toxicity can occur. Acute toxicity may take the form of arrhythmias, heart block or pericarditis and may be fatal. The chronic form of cardiotoxicity is related to total cumulative dose and is characterized by heart failure. Mediastinal radiotherapy and/or other cardiotoxic drugs may increase the risk of cardiotoxicity. In general, total lifetime dosages of 450-550 mg/m^2 should not be exceeded. Other toxicities include nausea and vomiting, mucositis, alopecia, diarrhea and red discoloration of the urine and other body fluids. Severe tissue damage and necrosis can occur upon extravasation. Radiation recall reactions can occur and can be severe. Rarely, allergic reactions have occurred.

3.6 L-ASPARAGINASE (Elspar®)

Source and Pharmacology: Asparaginase is an enzyme. It is derived from *Escherichia coli*. Asparaginase hydrolyzes serum asparagine (an amino acid required to synthesize proteins) to aspartic acid and ammonia, and is therefore lethal to cells that cannot synthesize asparagine. Asparaginase is active during all phases of the cell cycle. Asparaginase is not absorbed from the GI tract must be given parenterally. Although asparaginase does not cross into the CSF well. The plasma half life of L-asparaginase when given IV is ≈ 8-30 hours. When given IM the half life is ≈30 hours. Only minimal urinary and biliary excretion occurs. Clearance is unaffected by age, renal function or hepatic function.

Formulation and Stability: E. Coli asparaginase is available in vials containing 10,000 IU of lyophilized drug and 80 mg mannitol. Unused vials should be refrigerated. The contents of each vial should be diluted with 0.5 cc of preservative-free normal saline or sterile water, giving a resultant solution of 20,000 units/ml. Once in solution, it is recommended that it be used within 8 hours as no preservative is added. Occasionally a small number of gelatinous-like fibers may develop upon

standing. If this occurs, the solution can be filtered through a 5 micron filter to remove the particles with no change in potency.

Supplier: Commercially available

Toxicity: Acute toxicity includes anaphylactic reactions that occur most commonly when the drug is given IV. These can be characterized by laryngeal constriction, hypotension, diaphoresis, fever, chills, edema and loss of consciousness. Allergic reactions at the site of IM injection include pain, swelling and erythema. Other adverse effects include neutropenia and associated immunosuppression, mild nausea and vomiting, malaise, anorexia, elevated LFT's, pancreatitis and hyperglycemia. A decrease in protein synthesis including albumin, fibrinogen and other coagulation factors may occur which can result in hemorrhage. Thrombosis and/or pulmonary embolism can also occur. Less common side effects include renal dysfunction and CNS complications including somnolence, weakness, lethargy, coma and seizures.

Amend 8.0

3.7 PEG-L-ASPARAGINASE (Pegaspargase, Oncaspar®)

Source and Pharmacology: PEG-asparaginase is a modified version of the enzyme, L-asparaginase. L-asparaginase is modified by covalently conjugating units of polyethylene glycol (PEG) to the enzyme. The asparaginase used in the manufacturing of PEG-asparaginase is derived from *Escherichia coli.* Asparaginase hydrolyzes serum asparagine (an amino acid required to synthesize proteins and DNA) to aspartic acid and ammonia, and is therefore lethal to cells that cannot synthesize asparagine. Asparaginase is active during all phases of the cell cycle. Asparaginase is not absorbed from the GI tract and must be given parenterally. PEG-asparaginase has a plasma half-life of approximately 6 days, but is measurable for at least 15 days following the initial treatment. It cannot be detected in the urine.

Formulation and Stability: PEG-asparaginase is available in single-use vials containing 5 ml of PEG-asparaginase as a clear solution. Each vial contains 3750 units of drug at a concentration of 750 units/ml. The intact vials should be stored under refrigeration. Freezing destroys its activity, which cannot be detected visually. It should not be used if it is cloudy or a precipitate is present.

Supplier: Commercially available

Toxicity: Acute toxicity includes anaphylactic reactions which occur most commonly when the drug is given IV. These can be characterized by laryngeal constriction, hypotension, diaphoresis, fever, chills, edema and loss of consciousness. Allergic reactions at the site of IM injection include pain, swelling and erythema. The incidence of hypersensitivity reactions to PEG-asparaginase may be less than with conventional E. Coli derived asparaginase although cross-sensitivity can occur. Other adverse effects include neutropenia and associated immunosuppression, mild nausea and vomiting, malaise, anorexia, elevated LFT's, pancreatits and hyperglycemia. A decrease in protein synthesis including albumin, fibrinogen and other coagulation factors may occur which can result in thrombosis or pulmonary embolism. Less

common side effects include renal dysfunction and CNS complications including somnolence, weakness, lethargy, coma and seizures.

3.8 METHOTREXATE

Source and Pharmacology: Methotrexate is a folate analogue that acts by inhibiting dihydrofolate reductase. Dihydrofolate reductase is an enzyme important in the conversion of folic acid to tetrahydrofolic acid, which is necessary in the synthesis of purine nucleotides and thymidylate. By inhibiting the production of tetrahydrofolic acid, methotrexate interferes with DNA, RNA and protein synthesis. Methotrexate is poorly and variably absorbed orally, with an average of ≈ 40% for doses of ≤ 30 mg/m^2. At higher dosages, the extent of absorption decreases. Methotrexate is approximately 50% protein bound. It distributes widely into body tissues and fluids with sustained concentrations in the kidney and the liver. Methotrexate undergoes metabolism by cytosolic aldehyde oxidase to hydroxy methotrexate. It is excreted mainly in the urine as unchanged drug with small amounts being excreted in the bile and feces. The percent recovered as unchanged drug in the urine is higher with short infusions than with prolonged infusions. Methotrexate has a biphasic elimination with an initial half-life of ≈ 2-3 hours and a terminal half-life of 10-12 hours. Methotrexate may be "sequestered" in body fluid collections and eliminated slowly from these areas. Patients with effusions or GI obstruction should have plasma levels monitored closely for delayed excretion following high-dose methotrexate.

Formulation and Stability: Methotrexate is supplied in single-dose vials containing 50mg, 100mg, 200mg, and 250mg of methotrexate as a 25mg/ml preservative-free solution and in vials containing 20mg, 50mg, 100mg, 250mg and 1000mg of lyophilized drug. It is also available in 2.5mg tablets. Methotrexate preservative-free solution and lyophilized drug should be stored at room temperature and protected from light. Methotrexate tablets can also be stored at room temperature. The vials containing 20, 50, 100 and 250mg of lyophilized product can be reconstituted by adding sterile water, 0.9% NaCl or D5W to a final concentration not exceeding 25mg/ml. The 1000mg vials containing lyophilized product are reconstituted to a final concentration of 50mg/ml.

Supplier: Commercially available

Toxicity: The dose limiting toxicities of methotrexate are generally bone marrow suppression, ulcerative stomatitis, severe diarrhea or acute nephrotoxicity. Toxicities reported frequently include nausea and vomiting, diarrhea, anorexia, alopecia, hepatic toxicity and alopecia. Less common side effects include blurred vision, photosensitivity, anaphylaxis, headache, pneumonitis, skin depigmentation or hyperpigmentation, rash, vasculitis and encephalopathy. During high-dose methotrexate therapy, most patients experience a transient decrease in GFR, but renal failure can occur, particularly if the patient does not receive urinary alkalinization and aggressive hydration before, during and after receiving high dose methotrexate. Leucovorin rescue should be initiated within 48 hours of starting high-dose methotrexate and adjusted based on MTX levels to prevent bone marrow toxicity and

mucositis. Leucovorin may also be necessary after IT administration, especially if IT methotrexate therapy is given to patients with renal dysfunction. Patients with Down Syndrome have a tendency to have delayed methotrexate clearance and a greater risk of toxicity, despite increased leucovorin rescue.

3.9 CYCLOPHOSPHAMIDE (Cytoxan®)

Source and Pharmacology: Cyclophosphamide is a nitrogen mustard derivative. It acts as an alkylating agent that causes cross-linking of DNA strands by binding with nucleic acids and other intracellular structures, thus interfering with the normal function of DNA. Cyclophosphamide is cell-cycle, phase non-specific. Cyclophosphamide is well absorbed from the GI tract with a bioavailability of >75%. Cyclophosphamide is a prodrug that requires activation. It is metabolized by mixed-function oxidases in the liver to 4-hydroxycyclophosphamide, which is in equilibrium with aldofosfamide. Aldofosfamide spontaneously splits into cyclophosphamide mustard, which is considered to be the major active metabolite, and acrolein. In addition, 4-hydroxycyclophosphamide may be enzymatically metabolized to 4-ketocyclophosphamide and aldofosfamide may be enzymatically metabolized to carboxyphosphamide which are generally considered to be inactive. Cyclophosphamide and its metabolites are excreted mainly in the urine. Dosage adjustments should be made in patients with a creatinine clearance of < 50 ml/min.

Formulation and Stability: Cyclophosphamide is available in 25 and 50 mg tablets. Cyclophosphamide is also available in vials containing 100, 200, 500, 1000 and 2000 mg of lyophilized drug and 75 mg mannitol per 100 mg of cyclophosphamide. Both forms of the drug can be stored at room temperature. The vials are reconstituted with 5, 10, 25, 50 or 100 ml of sterile water for injection respectively to yield a final concentration of 20 mg/ml. Reconstituted solutions may be further diluted in either 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically stable for 24 hours at room temperature and 6 days if refrigerated, but contain no preservative, so it is recommended that they be used within 24 hours of preparation.

Supplier: Commercially available

Toxicity: Dose limiting toxicities of cyclophosphamide are bone marrow suppression and cardiac toxicity. Cardiac toxicity is typically manifested as congestive heart failure, cardiac necrosis or hemorrhagic myocarditis and can be fatal. Hemorrhagic cystitis may occur and necessitates withholding therapy. The incidence of hemorrhagic cystitis is related to cyclophosphamide dose and duration of therapy. Forced fluid intake and/or the administration of mesna decreases the incidence and severity of hemorrhagic cystitis. Other toxicities reported commonly include nausea and vomiting (may be mild to severe depending on dosage), diarrhea, anorexia, alopecia, immunosuppression and sterility. Pulmonary fibrosis, SIADH, anaphylaxis and secondary neoplasms have been reported rarely.

3.10 CYTARABINE (Ara-C) (Cytosar-U®)

Source and Pharmacology: Cytarabine is a deoxycytidine analogue. It must be tri-phosphorylated to its active form, ARA-CTP, by deoxycytidine kinase and other nucleotide kinases. Ara-CTP inhibits DNA polymerase. In addition, ara-CTP is incorporated into DNA as a false base, causing inhibition of DNA synthesis.. It is cell cycle, S phase specific. Cytarabine does penetrate the blood brain barrier. It is converted to its inactive form, uracil arabinoside, by pyrimidine nucleoside deaminase. Approximately 80% of the dose is recovered in the urine, mostly as uracil arabinoside (ara-U).

Formulation and Stability: Cytarabine is available in multi-dose vials containing 100, 500, 1000 and 2000 mg of lyophilized drug. Intact vials can be stored at room temperature. For IV use, either sterile water for injection or bacteriostatic water for injection can be used to reconstitute the lyophilized drug. For intrathecal use, only sterile water for injection should be used for reconstitution. The 100 and 500 mg vials are reconstituted with 5 and 10 ml respectively resulting in a final concentration of 20 mg/ml or 50 mg/ml respectively. The 1000 and 2000 mg vials are reconstituted with 10 ml and 20 ml respectively resulting in a final concentration of 100 mg/ml. After reconstitution, the drug is stable for 8 days at room temperature.

Supplier: Commercially available

Toxicity: Myelosuppression is the dose limiting adverse effect, with leukopenia and thrombocytopenia being predominant. Other adverse effects reported commonly include nausea and vomiting (may be severe at high doses), diarrhea, mucositis, anorexia, alopecia, skin rash and liver dysfunction. A flu-like syndrome characterized by fever, muscle and bone aches is common. Less common side effects include allergic reactions and cellulitis at the injection site. High doses of cytarabine can cause conjunctivitis, hepatitis, and a group of CNS symptoms including somnolence, peripheral neuropathy, ataxia and personality changes. CNS symptoms are usually reversible and are more common in patients who have received previous cranial irradiation. In addition, a syndrome of sudden respiratory distress progressing to pulmonary edema has occurred.

3.11 ETOPOSIDE (Vepesid, VP16)

Source and Pharmacology: Semi-synthetic epipodophyllotoxin derived from Podophyllum peltatum. It causes inhibition of thymidine incorporation into DNA. It is phase specific killing cells during late G_1/Early S. It is extensively bound to serum proteins with excretion by liver, kidneys, and small intestines. Plasma disposition is biphasic with a terminal half-life 3.5-7 hours in children. Dosage decrease may be necessary in patients with low serum albumin and/or hyperbilirubinemia.

Formulation and stability: Available as a clear, yellow solution containing 30% ethanol in 50 mg ampules. The intact vials are stable for 4 years when stored at room temperature and protected from light. Reconstitute: with equivalent volumes of diluent (sodium

chloride or D_5W). The solution is stable for 4 hours at room temperature. Diluted to 0.2 mg/ml and 0.4 mg/ml in D5W or NS, results in stable solutions at room temperature for 96 and 48 hours respectively.

Supplier: Commercially available.

Toxicity: Acute dose-limiting toxicity is marrow suppression especially leukopenia with the nadir at 3-14 days, generally occurring at one week. Hypotension has been a problem, but only after rapid IV push or with high doses. Other adverse reactions include immunosuppression, fever, chills, allergic reaction and alopecia. Premedication with diphenhydramine ? steroids can reduce or eliminate allergic reactions. Chemical phlebitis at the injection site can occur. Secondary leukemia has been reported in patients treated with VP16.

3.12 6-MERCAPTOPURINE (6-MP) (Purinethol®)

Source and Pharmacology: Mercaptopurine is a purine antimetabolite. It must be converted intracellularly to 6-thioguanine nucleotides (6-TGNs), the active forms of the drug. The 6-TGNs are then incorporated into DNA and RNA and cause inhibition of DNA and RNA synthesis. Mercaptopurine is cell cycle, S phase specific. Absorption is variable and incomplete (5-37%) and is decreased by the presence of food in the gut. Mercaptopurine does distribute into the CSF, with CSF concentrations of ≈ 27% of plasma concentrations when given by continuous infusion. Mercaptopurine undergoes first pass metabolism in the GI mucosa and the liver. It is metabolized in hematopoietic tissues by HPRT to the active nucleotide forms. It is inactivated to methylated metabolites by TPMT (thiopurine methyl transferase) and to 6-thiouric acid by xanthine oxidase. TPMT is a genetically regulated, polymorphically distributed enzyme and is deficient in about 1 in 300 persons who cannot tolerate usual doses of 6-MP. Mercaptopurine is eliminated through the urine as both unchanged drug and metabolites.

Formulation and Stability: Mercaptopurine is available as a 50 mg tablet. In addition, a 500 mg vial of lyophilized product is available through the NCI. Both the tablets and the parenteral product should be stored at room temperature and protected from light. The 500 mg vials can be reconstituted with 49.8 ml of sterile water for injection resulting in a final solution of 10 mg/ml. This solution is chemically stable for 21 days at room temperature or refrigerated. Further dilution to a concentration of 1-2 mg/ml in D5W prior to administration is recommended to reduce the incidence of vein irritation. Diluted solutions are chemically stable for at least 3 days at room temperature or refrigerated.

Supplier: Tablets are commercially available. The parenteral form is available investigationally through the NCI.

Toxicity: The dose-limiting toxicity of mercaptopurine is bone marrow suppression. Mercaptopurine can cause intrahepatic cholestasis and focal centrolobular necrosis and is usually manifested by hyperbilirubinemia and increased liver function tests. Other

toxicities include mild nausea and vomiting, anorexia, mucositis, stomatitis, immunosuppression and skin rash or hyperpigmentation

Amend 1.0 (deleted atovaquone information)

3.13 COTRIMOXAZOLE (SMX/TMP, Bactrim®, Septra®)

Source and Pharmacology: Co-trimoxazole is a combination of two antibacterial drugs, trimethoprim and sulfamethoxazole. Co-trimoxazole acts by sequentially inhibiting enzymes of the folic acid pathway. Sulfamethoxazole interferes with bacterial folic acid synthesis by inhibiting dihydrofolic acid formation from para-aminobenzoic acid. Trimethoprim inhibits dihydrofolic acid reduction to tetrahydrofolate. Co-trimoxazole is well absorbed from the GI tract (90-100%). Both trimethoprim and sulfamethoxazole are metabolized in the liver and excreted mainly in the urine as unchanged drug and metabolites. Dosage adjustments should be made for patients with impaired renal function.

Formulation and Stability: Cotrimoxazole is available in tablets containing a 1:5 ratio of trimethoprim to sulfamethoxazole as follows: 80 mg trimethoprim/400 mg sulfamethoxazole and 160 mg of trimethoprim/800 mg sulfamethoxazole. (NOTE: St. Jude pharmacy carries only the 80mg/400mg tablet, also known as the single strength tablet) It is also available in a suspension for oral use containing 40 mg trimethoprim/200 mg sulfamethoxazole per 5 ml. An IV formulation is available that contains 16 mg trimethoprim and 80 mg sulfamethoxazole per ml.

Supplier: Commercially available

Toxicity: Common toxicities include nausea, vomiting, anorexia, skin rashes and photosensitivity. Toxicities reported less commonly include confusion, depression, hallucinations, seizures, fever, ataxia, diarrhea, stomatitis, erythema multiforme, pseudomembranous colitis, hemolysis (in patients with G-6PD deficiency), serum sickness, and interstitial nephritis. Toxicities that are rare but severe when they occur include agranulocytosis, aplastic anemia, megaloblastic anemia, hepatitis, toxic epidermal necrolysis and Stevens-Johnson syndrome.

Dosage and Route of Administration: 5-10 mg TMP-SMZ/kg/day or 150 mg TMP-SMZ/m^2/day in divided doses every 12 hours, 3 days/week.

3.14 HYDROCORTISONE (Cortef, Solu-Cortef)

Source and Pharmacology: Hydrocortisone is a synthetic steroid akin to the natural adrenal hormone cortisol. It is phase-specific, killing lymphoblasts primarily during S phase. It has catabolic effect on proteins and alters the kinetics of peripheral blood leukocytes. It is excreted in the urine and catabolized in the liver.

Formulation and Stability: Solu-Cortef sterile powder is supplied in the following packages: 100 mg plain, and 100 mg, 250 mg, 500 mg, and 1000 mg ACT-O-VIAL (MIX-O-VIAL). Store unreconstituted product at controlled room temperature 15-30°C (59-86°F). Store reconstituted solution in the refrigerator and protect from light.

Unused solution should be discarded after 3 days. Use Solu-Cortef (plain vial) for intrathecal use, and reconstitute with 0.9% sodium chloride, USP for injection.

Supplier: Commercially available

Toxicity: Hyperphagia, obesity, striae, acne, immunosuppression, hypertension, osteoporosis, avascular necrosis of bone, personality changes, diabetes and Cushingoid Syndrome. Peptic ulcer and/or GI bleeding has also been noted. If given intrathecally, sterile arachnoiditis may occur.

Dosage and Route of Administration: See Section 6.2.2 for IT dosing

Amend 3.0,
Amend 4.0

3.15 Rasburicase (Urate oxidase, Elitek)

Source and Pharmacology: Rasburicase is a recombinant urate oxidase enzyme demonstrated in vitro and in vivo to be a highly potent uricolytic agent. Urate oxidase is active at the end of the purine catabolic pathway, where it acts as a catalyst in the enzymatic oxidation of uric acid into allantoin, a readily excretable substance about five times more soluble than uric acid. The mean elimination half-life of rasburicase from plasma is approximately 18 hours. Pharmacology studies have not demonstrated any modifying effects of rasburicase on cardiovascular, respiratory, or central nervous system functions. Hydrogen peroxide is a by-product of the conversion of uric acid to allantoin by rasburicase. In patients with a specific enzyme deficiency such as glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxidase or methemoglobin reductase, increased levels of hydrogen peroxide may cause oxidative damage leading to intravascular homolysis or methemoglobinemia.

Formulation and Stability: Rasburicase is supplied as sterile, lyophilized powder containing 1.5 mg rasburicase protein per vial in addition to mannitol, alanine, and disodium phosphate. It is to be reconstituted with 1 ml sterile diluent (water for injection with Poloxamer 188) from supplied ampoules. The lyophilized vials must be stored in the refrigerator. After reconstitution, the 1.5 mg/ml solution should be further diluted to a total of 50 ml in normal saline and infused intravenously over 30 minutes. Do not filter the solution. The final rasburicase solution should be refrigerated and infused within 24 hours.

Supplier: Commercially available

Toxicity: Rasburicase is administered as supportive care during chemotherapy for advanced malignancies, and most adverse events observed are related to the underlying disease state and its treatment. The following toxicities may be partially related to the urate oxidase: any allergic reaction with bronchospasm or rash, fever, headache, gastrointestinal pain, and nausea or vomiting. .

Dosage and Route of Administration: 0.1-0.2 mg/kg/dose in 50 ml normal saline IV over 30 minutes. Two doses per day may be given the first two days if clinically indicated.

3.16 LEUCOVORIN (Folinic Acid, Wellcovorin®)

Source and Pharmacology: Leucovorin is a racemic mixture of tetrahydrofolic acid, which is involved as a cofactor for 1-carbon transfer reactions in the synthesis of purine and pyrimidines. Leucovorin is a potent antidote for both the hematopoietic and reticuloendothelial toxic effects of folic acid antagonists by replenishing reduced folate pools. It is postulated that in some cancers, leucovorin enters and "rescues" normal cells from the toxic effects of folic acid antagonists, in preference to tumor cells, because of differences in membrane transport and affinity for polyglutamylation. Leucovorin is converted in the intestinal mucosa and the liver to 5-methyl-tetrahydrofolate, which is also active as a reduced folate. It is excreted primarily in the urine with minor excretion occurring in the feces.

Formulation and Stability: Leucovorin is supplied in 5, 15 and 25 mg tablets and vials containing 50, 100 or 350 mg of leucovorin as a lyophilized powder. The tablets and the lyophilized powder can be stored at room temperature. The 50 mg and 100 vials can be reconstituted by adding 5 or 10 ml of sterile water or bacteriostatic water for injection respectively to yield a final concentration of 10 mg/ml. The 350 mg vials can be reconstituted with 17 ml of sterile water or bacteriostatic water for injection to yield a final concentration of 20 mg/ml. The reconstituted solution is stable for at least 7 days at room temperature. Leucovorin may be further diluted in 5% dextrose or 0.9% NaCl containing solutions.

Supplier: Commercially available

Toxicity: Leucovorin is generally well tolerated. Toxicities that have been reported uncommonly include rash, mild nausea, headache, thrombocytosis and wheezing (possible allergic reaction).

Amend 5.0

3.17 Gleevec (Imatinib Mesylate, formerly known as STI571)

For Ph+ ALL patients only. See package insert for additional information

Source and Pharmacology: imatinib mesylate is a phenylaminopyrimidine derivative and is a 4-[(4-Methyl-1-piperazinyl)methyl-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimid-inyl]amino]-phenyl]benzamidemethanesulfonate. It is a protein-tyrosine kinase inhibitor that inhibits the Bcr-Abl tyrosine kinase, the constitutive abnormal tyrosine kinase created by the Philadelphia chromosome abnormality.

Formulation and Stability: Each film-coated tablet contains 100 mg or 400 mg of imatinib free base. The drug should be stored at 25°C (77°F); excursions permitted to 15°C-30°C (59°F - 86°F). The tablets should be dispensed in a tight container, USP, and protected from moisture.

The prescribed dose should be administered orally, with a meal and a large glass of water. In children, the daily dose may be split into two – once in the morning and once in the evening. For patients unable to swallow the film-coated tablets, the tablets may be dispersed in a glass of water or apple juice. The required number of tablets should be placed in the appropriate volume of beverage (approximately 50 mL for a 100-mg tablet, and 200 mL for a 400-mg tablet) and stirred with a spoon. The suspension should be administered immediately after complete disintegration of the tablet.

Supplier: Commercially available

Amend 8.0

Toxicity: Common toxicities include dyspepsia/heartburn, nausea/vomiting, headache, myelosuppression, and fatigue. Occasional toxicities include fever, edema in limbs, face, periorbital area, weight gain, increased SGOT/SGPT, alkaline phosphatase, bilirubin, abdominal pain and cramping, myalgia, arthralgia, decreased bone marrow cellularity, lymphopenia, eczema dermatitis, rash, muscle pain and cramping, anorexia, and pigmentation changes (hypo-vitiligo). Rare toxicities include cerebral edema, melena/GI bleeding, anemia, diarrhea, dysphagia, esophagitis, odynophagia, hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, pneumonitis/pulmonary infiltrates, late hepatotoxicity and **decrease in the heart's ability to pump blood.**

Amend 8.0

3.18 ERWINIA ASPARAGINASE (Erwinase®)

Source and Pharmacology: Erwinia asparaginase is an enzyme. It is derived from *Erwinia caratovora* and may be useful in patients with an allergy to the *E. coli* derived product. Asparaginase hydrolyzes serum asparagine (an amino acid required to synthesize proteins) to aspartic acid and ammonia, and is therefore lethal to cells that cannot synthesize asparagine. Asparaginase is active during all phases of the cell cycle. Asparaginase is not absorbed from the GI tract and must be given parenterally. Asparaginase does not cross into the CSF. The plasma half life of erwinia asparaginase when given IM is ≈16 hours. Only minimal urinary and biliary excretion occurs. Clearance is unaffected by age, renal function or hepatic function.

Formulation and Stability: Erwinia asparaginase is available in vials containing 10,000 units of lyophilized drug and 80 mg mannitol. Unused vials should be refrigerated. The contents of each vial should be diluted with 1 cc of preservative-free normal saline or sterile water, giving a resultant solution of 10,000 units/ml. Once in solution, it is recommended that it be used within 8 hours as no preservative is added. Occasionally a small number of gelatinous-like fibers may develop upon standing. If this occurs, the solution can be filtered through a 5 micron filter to remove the particles with no change in potency.

Supplier: Available through OPi Pharmaceuticals. Patients/LARs will be required to sign separate consent and enroll on Compassionate Use protocol.

Toxicity: Acute toxicity includes anaphylactic reactions that occur most commonly when the drug is given IV. These can be characterized by laryngeal constriction, hypotension, diaphoresis, fever, chills, edema and loss of consciousness. Allergic reactions at the site of IM injection include pain, swelling and erythema. Other adverse effects include neutropenia and associated immunosuppression, mild nausea and vomiting, malaise, anorexia, elevated LFT's, pancreatitis and hyperglycemia. A decrease in protein synthesis including albumin, fibrinogen and other coagulation factors may occur which can result in hemorrhage. Thrombosis and/or pulmonary embolism can also occur. Less common side effects include renal dysfunction and CNS complications including somnolence, weakness, lethargy, coma and seizures.

4.0 ELIGIBILITY CRITERIA

4.1 Diagnosis of non-B-cell ALL by immunophenotyping, as determined by the reactivity pattern to a panel of monoclonal antibodies with flow cytometry (e.g. T-cell by the presence of cytoplasmic or surface CD3, B-cell precursor by the presence of cytoplasmic CD79a), as well as morphology and cytochemical staining properties (i.e., no Auer rods and negative for myeloperoxidase, Sudan black B [myeloid pattern], α naphthyl butyrate esterase [myeloid pattern]), or acute undifferentiated leukemia (after excluding MO leukemia).

4.2 Age 1 to 18 years (inclusive).

4.3 One week or less of prior therapy, limited to glucocorticoids, vinca alkaloids, emergency radiation therapy to the mediastinum and one dose of intrathecal chemotherapy.

4.4 Written, informed consent following Institutional Review Board, NCI, FDA, and OPRR Guidelines.

5.0 RISK CLASSIFICATION

Patients are classified into one of three categories (low-, standard-, or high-risk) based on the presenting age, leukocyte count, presence or absence of CNS 3 status or testicular leukemia, immunophenotype, cytogenetic and molecular diagnosis, DNA index, and early response to therapy. Hence, definitive risk assignment (for provisional low-risk cases based on presenting features) will be made after completion of remission induction therapy. The criteria and the estimated proportion of patients in each category (based on data from Total XIII studied) are provided below.

Amend 8.0

5.1 Criteria for Low-Risk ALL (approximately 40% of patients)

- B-cell precursor ALL with DNA index ≥1.16, *TEL-AML*1 fusion, or age 1 to 9.9 years and presenting WBC <50 x 10^9/L.

- **Must not have** (i) CNS 3 status (≥5 WBC/:L of cerebrospinal fluid with morphologically identifiable blasts or cranial nerve palsy), (ii) overt testicular leukemia (evidenced by

ultrasonogram), (iii) adverse genetic features [t(9;22) or *BCR-ABL* fusion; t(1;19) with *E2A-PBX1* fusion; rearranged *MLL;* or hypodiploidy (<45 chromosomes)], or (iv) poor early response (≥ **1**% lymphoblasts on day 19 or 26 of remission induction, **≥ 0.01% lymphoblasts by immunologic or molecular methods on remission date).**

5.2 Criteria for Standard-Risk ALL (approximately 50 of patients)

- All cases of T-cell ALL and those of B-cell precursor ALL that do not meet the criteria for low-risk or high-risk ALL

Amend 9.0

5.3 Criteria for High-Risk ALL (approximately 10% of patients)

- t(9;22) or *BCR-ABL* fusion
- Induction failure or ≥1% leukemic lymphoblasts in the bone marrow on remission date.
- ≥ 0.1% leukemic lymphoblasts in the bone marrow in week 7 of continuation treatment (i.e. before reinduction I, ~14 weeks post remission induction).
- **Re-emergence of leukemic lymphoblasts by MRD (at any level) in patients previously MRD Negative.**
- **Persistently detectable MRD at lower levels.**

6.0 TREATMENT PLAN

Treatment will consist of three main phases, Remission Induction, Consolidation, and Continuation. Treatment with an Upfront HDMTX Window for research purposes will be optional (see Section 6.1.5).

6.1 Stratification and Randomization in Upfront HDMTX Treatment

6.1.1 CNS Intrathecal (IT) Treatment

Diagnostic lumbar puncture is performed on Day 1 before upfront HDMTX treatment. As a traumatic tap at diagnosis may result in a poorer outcome and the need for extra intrathecal therapy subsequently, all diagnostic lumbar punctures will be performed by experienced personnel, preferably under general anesthesia or deep sedation. Intrathecal cytarabine (ara-C) will be administered immediately after cerebrospinal fluid is collected for diagnosis, and its dosage is age-dependent as following:

Age (months)	IT ara-C Dosage (mg)	Volume (ml)
12-23	40	8
24-35	50	10
≥36	60	12

This dose of intrathecal cytarabine is consistent with the concept of early intensification of intrathecal treatment.[34] Cytarabine is used because intrathecal treatment results in high levels in CSF (with little metabolism to the inactive compound uracil arabinoside) but has little, if any, systemic effect (due to rapid inactivation in plasma).[115] Thus, intrathecal cytarabine treatment will not affect the systemic MTX studies.

6.1.2 Upfront High-Dose Methotrexate Window

Patients with pre-existing renal failure (i.e. creatinine level >1.4 mg/dL in children and >2 mg/dL in adolescents ≥13 years or creatinine clearance <50 ml/min/1.73 m^2) or Down syndrome are ineligible for the up-front HDMTX treatment (see Sections 6.1.5 and 7.1). All the other consenting patients will be stratified by leukemic cell lineage, DNA index and presenting WBC, and randomized to receive HDMTX (1 g/m^2) as a 4 hour infusion versus as a 24 hour infusion. In the rare instances that results of lineage or DNA index cannot be obtained, they will be assumed to be B-lineage and 1.0, respectively. Standard orders must be used for upfront HDMTX treatment.

Hydration and Alkalinization: Prehydration IV fluid (with 40 mEq $NaHCO_3$/L) should be administered at the rate of 200 ml/m^2/hr for at least 2 hours before the start of HDMTX. At the start of prehydration, one IV dose of $NaHCO_3$ 12 to 25 mEq/m^2 diluted in 50 ml D5W may be given over 15 minutes. HDMTX treatment will follow, provided that urinary pH is ≥ 6.5 and uric acid level has been corrected to 8 mg/dL or less; exceptions must be cleared with the Pharmacokinetics Service and the attending physician. Hydration should continue at a rate of at least 100 ml/m^2/hr for 24 hours from the start of HDMTX and until the plasma MTX concentration is < 1.0 ΦM.

Urine pH will be monitored with each void during infusion. An IV bolus of 12.5 mEq/m^2 $NaHCO_3$ will be given if urine pH is 6.0; and 25 mEq/m^2 will be given if urine pH is < 6.0. Acetazolamide 500 mg/m^2 orally every 6 to 8 hours may be used if systemic alkalosis limits the administration of bicarbonate for urinary alkalinization.

6.1.3 HDMTX administration

The concentration of MTX will be determined according to pharmacy guidelines, as indicated on the standard physician orders, to be compatible with current IV administration devices and supplies at SJCRH. Those randomized to receive short infusions will receive 1 g/m^2 in 100 ml D5W (to piggy-back with hydration) over 4 hours. Those randomized to receive long infusions will receive 200 mg/m^2 IV push, followed by 800 mg/m^2 in 250 ml of D5W (to piggy-back with hydration).

The MTX should start at 2:00 p.m.; exceptions should be limited to patients in whom it is deemed clinically necessary to begin treatment without any delay.

An outline of pharmacokinetic testing for the Upfront HDMTX window is found in Section 10.0.

6.1.4 Leucovorin administration

Leucovorin rescue should be started after both the 42 hr peripheral blood and bone marrow samples have been obtained and must not be delayed beyond 48 hours. The initial dosage of leucovorin for both infusion durations will be 50 mg/m^2 IV at 44 hours followed by 15 mg/m^2 IV q 6 hrs for 7 doses (hrs 50, 56, 62, 68, 74, 80, and 86 hrs).

Amend 1.0

The leucovorin will be increased in those with 42 hour plasma MTX levels > 1.0 μM. **Additional measures, such as hydration, hemoperfusion, or carboxypeptidase will be considered in patients with 42-hour MTX levels > 10 μM.**

6.1.5 Contingency plan for patients ineligible or who decline upfront HDMTX

Patients who do not receive upfront HDMTX should receive intrathecal cytarabine (as per section 6.1.1) and proceed immediately to induction therapy that would otherwise begin on Day 5 (see Section 6.2).

6.2 Remission Induction (6 to 7 weeks)

Ninety-six hours after the start of HDMTX, the remaining induction treatment will begin with prednisone, vincristine, daunorubicin, asparaginase and triple intrathecal treatment, followed by cyclophosphamide plus cytarabine plus 6-mercaptopurine. This phase of induction treatment may be started early should progressive disease occur after HDMTX or if other clinical conditions (e.g., mediastinal mass severely compromising airway, epidural compression) warrant more rapid reduction of leukemic mass. However, we have not yet encountered a case with progressive disease after treating over 400 patients with upfront antimetabolites in studies XIII and XIV. The first dose of daunorubicin on day 5 may be delayed in patients with evidence of mucositis or increased hyperbilirubinemia (i.e., total bilirubin ≥2.0 mg/dl and direct bilirubin >1.4 mg/dl). Omit this dose of daunorubicin if total bilirubin is still ≥2 mg/dl and direct bilirubin >1.4 mg/dl on day 12. A dose of daunorubicin may be given as soon as hyperbilirubinemia has resolved. Patients with mucositis should be evaluated for herpes simplex infection and treated with acyclovir if work-up is positive.

Amend 4.0
Amend 5.0

6.2.1 Drug Dosages

Agents	Dosages and Routes	# Dose	Schedules
Prednisone§	40 mg/m^2/day PO (tid)	84	Days 5 – 32
Vincristine	1.5 mg/m^2/week IV	4	Days 5, 12, 19, 26
Daunorubicin	25 mg/m^2/week IV	2	Days 5, 12
L-asparaginase*	10,000 **Unit**/m^2/dose IM (thrice weekly)	6 to 9	Days 6, 8, 10, 12, 14, 16 (19, 21, 23)
Cyclophosphamide†	1000 mg/m^2/dose IV over **15-45 minutes**	1	Day 26
Cytarabine	75 mg/m^2/dose IV	8	Days 27-30, 34-37
6-Mercaptopurine†	60 mg/m^2/dose PO	14	Days 26-39

*See 6.2.3.1 for modifications and the criteria to receive three additional doses
†See 6.2.3.2 for the need of bone marrow exam and the criteria of starting cyclophosphamide plus 6-mercaptopurine on day 26.
§ Oral prednisone can be substituted with methylprednisolone at 20 mg/m2/day IV (t.i.d.) for patients who cannot tolerate the oral medication.

Please see Section 7.12 regarding Imatinib Mesylate dosing.

Amend 1.0

6.2.1.1 Remission Induction Dose Modifications

See Section 7.0 for treatment modifications for vincristine, daunorubicin, and asparaginase. Following Day 26 treatment, cytarabine and 6-mercaptopurine may be held if patient develops febrile neutropenia or grade 3 or 4 mucositis. If c-reactive protein is normal, fever subsides, cultures are not significant and mucositis resolves, cytarabine and 6-mercaptopurine may be resumed. Doses may be completely omitted if the patient is beyond day 34 of remission induction (i.e., half or more doses of 6-mercaptopurine and cytarabine have been given), allowing early bone marrow recovery and early initiation of consolidation therapy.

Rev 4.1

6.2.2 IT Chemotherapy Doses

Triple intrathecal chemotherapy (MHA) is used for the remaining treatment with dosages based on age as follows:

Age (months)	Methotrexate (mg)	Hydrocortisone (mg)	Ara-C (mg)	Volume (ml)
12-23	8	16	24	8
24-35	10	20	30	10
≥36	12	24	36	12

Frequency and total number of triple intrathecal treatments for Remission Induction are based on the patient's risk of CNS relapse, as follows:

- All patients will receive intrathecal ara-C on day 1 (Section 6.1.1) and triple intrathecal treatment on days 19.
- Patients with the following features will received additional triple intrathecal treatment on days 8 and 26:
  - CNS-3 status (i.e., ≥5 WBC/μL of CSF with blasts or cranial nerve palsy)
  - CNS-2 status (<5 WBC/μL of CSF with blasts)
  - Traumatic status (>10 RBC/μL of CSF with blasts)
  - T-cell with WBC >50 x 10^9/L at presentation
  - WBC >100 x 10^9/L at presentation
  - Presence of Philadelphia chromosome, *MLL* rearrangement or hypodiploidy (< 45)
- Leucovorin rescue (5 mg/m^2/dose) PO will be given at 24 and 30 hours after each triple intrathecal treatment during induction and consolidation. (IT with HDMTX is rescued by leucovorin for HDMTX only.)

6.2.3 Bone Marrow Evaluations

6.2.3.1 Day 19

Amend 3.0, 8.0

A bone marrow aspirate will be done on day 19 of remission induction to assess antileukemic response. The presence of **approximately 1% or more** leukemic blasts in the bone marrow **by morphologic exam or by MRD study** is an indication for three additional doses of L-asparaginase to be administered on days 19, 21, and 23. (for modifications due to L-asparaginase allergic reactions, see Section 7.9)

Amend 5.0, 8.0

6.2.3.2 Day 26

Bone marrow aspirate on day 26 will be performed in patients who had received extra doses of asparaginase due to the presence of 1% or more leukemic blasts in the bone marrow on day 19. Patients with residual leukemia identified on day 26 should receive cyclophosphamide, 6-mercaptopurine, and cytarabine as scheduled if their clinical condition permits, regardless of their ANC. For other patients, the treatment can be delayed for 3 to 7 days to allow adequate hematopoietic recovery if ANC <300/mm^3.

6.2.3.3 End of Induction-MRD response

A bone marrow aspirate will be performed around day 43 to day 46 of remission induction when ANC has recovered to ≥300/mm^3 and platelet count to ≥50 x 10^9/L. If the date falls on Saturday or Sunday, the procedure may be performed on Friday or Monday, respectively. MRD level will be determined in this bone marrow sample. Poor response will be defined as MRD level ≥0.01% (one or more lymphoblasts among 10^4

mononuclear cells in bone marrow) by either immunologic or molecular assay. If both assays are unsuccessful or the sample is inadequate, the MRD will be assumed to be negative. If the result of MRD is positive, the provisional low-risk case will then be classified as standard-risk (MRD $\geq$0.01% but less than 1%) or high-risk (MRD $\geq$1%) accordingly, and will receive subsequent 3 doses of HDMTX at a higher dosage (i.e., 5 gm/m^2). (These cases would have received the first HDMTX of consolidation therapy at 2.5 gm/m^2.)

6.3 Consolidation Treatment (8 weeks)

Amend 1.0

6.3.1 Drug Dosages

Agent	Dosage and Route	# Doses	Schedule
HDMTX	Targeted to 33 :M (low-risk) or 65 :M (standard-/high-risk)	4	days 1, 15, 29 and 43
6-Mercaptopurine	**50** mg/m^2/day	56	Days 1 to 56

When ANC $\geq$300/mm^3, WBC $\geq$ 1000/mm^3, and platelet count $\geq$ 50 x 10^9/L, consolidation treatment will be started, consisting of HDMTX (every other week for 4 doses) and daily 6-mercaptopurine. HDMTX will be held if total bilirubin >2 mg/dl and direct bilirubin >1.4 mg/dl.

6.3.2 6-MP administration

Amend 1.0

6-Mercaptopurine should be taken on an empty stomach (i.e., >2 hours after meal) at bedtime;[116] patients should not drink milk or take dairy products together with 6-mercaptopurine. **In patients for whom HDMTX treatment is delayed, 6-mercaptopurine may be continued until 14 days after the last course of HDMTX, even if this result is more than 56 days of 6-mercaptopurine treatment. However, 6-mercaptopurine may be held in the presence of ANC < 300/mm^3, platelet count < 50,000/mm^3 or grade 3 or 4 mucositis. Dosage of 6-mercaptopurine in subsequent courses may be reduced to 25 mg/m^2/day in patients who have prolonged neutropenia after HDMTX and 6-mercaptopurine treatment.**

Amend 4.0

6.3.3 HDMTX administration

The dosage of HDMTX depends on the risk classification of individual patients and on each patient's estimated systemic clearance based on their initial MTX clearance with the window dose. Patients with standard- or high-risk ALL who did not receive window MTX will receive 5 gm/m^2, and those with low-risk ALL 2.5 gm/m^2, administered over 24 hr intravenously. (Standard orders must be used for HDMTX, which should be administered at SJCRH **or at Cook Children's Medical Center**.) The subsequent dose of HDMTX, 6-mercaptopurine and intrathecal treatment will be delayed if ANC <300/mm^3, WBC < 1000/mm^3, platelet count < 50 x 10^9/L, SGPT >500 U/L, total bilirubin >2 mg/dl and direct bilirubin >1.4

mg/dl, or mucositis is present. Sodium bicarbonate may be given orally at 1 gm/m^2 every 6 hours or intravenously with prehydration fluid starting the day before HD MTX. For patients with Down Syndrome, HDMTX administration will be modified (See Section 7.1.2).

Amend 2.0, 4.0

6.3.3.1 Dosage Individualization of MTX In studies Total XII and R15,[30,117] infusion rates of MTX were adjusted during a 24-hour infusion in order to achieve a target AUC or steady-state concentration (Cpss). In Total XII, wherein patients received five 24-hour infusions of MTX, intra-patient CV in clearance was less than that of inter-patient throughout the first year of continuation therapy, raising the potential for performing inter- rather than intra-course MTX dose adjustments.

Amend 6.0

On Total XIV, MTX clearance with the first course of "window" MTX predicts clearance for subsequent MTX courses (p=.035, .0025, and .0127 for consolidation #1, consolidation #2, and continuation #1, respectively). Thus, in Total XV, we propose to adjust the dose for consolidation 1 based upon clearance during the window MTX, and adjust the doses for consolidation #2, #3, and #4 based on the prior consolidation course. **Plasma MTX concentrations obtained during the infusion may be used to adjust the MTX dose, provided the intra-infusion targeting is documented.** The dose will be adjusted to achieve a Cpss of 33 μM in the low-risk patients and 65 μM in the standard/high-risk patients as follows. Based on prior experience of adjusting MTX doses in children with ALL,[30] we anticipate median (5^{th} – 95^{th} percentile) doses in the low vs standard/high-risk cases to be 2.5 g/m^2 (1.5-3.5 g/m^2, with higher doses in patients with clearance >102 ml/min/m^2 and lower doses in those with clearance <88 ml/min/m^2) and 5.0 g/m^2 (3.2-6.6 g/m^2, with higher doses in patients with clearance >102 ml/min/m^2 and lower doses in those with clearance with <88 ml/min/m^2), respectively. Clearance is estimated from the plasma concentrations up through 48 hours from the window or consolidation courses, using nonlinear curve fitting and a Bayesian estimation strategy as implemented in ADAPT. The dose (10% as a loading dose over 1 hour and the remainder over 23 hours) is targeted to achieve the indicated target Cpss's. There will be a limit of a maximum of 50% increment or decrement in total MTX dose with any adjustment between courses. If, a patient experiences renal dysfunction or received concurrent nephrotoxic therapy with a course of HDMTX, clearance for that course will not be used to determine a subsequent course in which renal function has improved, but the dose will be based on the most recent non-nephrotoxic course. **If dosage individualization is not possible for practical reasons, the dose will be 2.5 g/m^2 for low-risk cases and 5 g/m^2 for standard/high-risk cases, respectively. For each MTX course, the precise method for targeting is documented in the research database, along with the reason(s) if the dose was not targeted. Bayesian priors incorporating updated clearance estimates and patient characteristics will be updated periodically.** The pharmacist

Amend 6.0

will indicate estimated clearance and recommended dose for each course in the Cerner/Milli system.

6.3.3.2 Prehydration: At least two hours before HDMTX, prehydration IV fluid (D5W + 40 mEq $NaHCO_3$/L) will be administered at the rate of 200 ml/m^2/hr. At start of prehydration, one IV dose of $NaHCO_3$ (unless otherwise clinically indicated, 12 mEq/m^2 for low-risk patients and 25 mEq/m^2 for standard-/high-risk patients) diluted in 50 ml D5W will be given over 15 minutes. Prehydration fluid may also be given overnight at a rate of at least 100 ml/m^2/hr. HDMTX treatment will follow, provided that urinary pH is $\geq$6.5; exceptions must be cleared with the pharmacokinetics service and the attending physician.

6.3.3.3 HDMTX infusion: MTX loading dose will be given over 1 hour, followed immediately by maintenance infusion over 23 hours. During the MTX infusion, patients should receive hydration fluid with D5W + 40 mEq/L $NaHCO_3$ at 100-150 ml/m^2/hr. Urine pH will be monitored with each void during infusion. An IV bolus of 12 mEq/m^2 $NaHCO_3$ will be given if urine pH is 6.0; and 25 mEq/m^2 will be given if urine pH is <6.0. Acetazolamide 500 mg/m^2 orally every 6 to 8 hours may be used if systemic alkalosis limits the administration of bicarbonate for urinary alkalinization. Patients with evidence of renal dysfunction or delayed clearance during the MTX infusion may receive less than a 24 hour MTX infusion.

Blood samples for MTX pharmacokinetics will be drawn according to Section 10.2.

Amend 1.0

6.3.3.4 Leucovorin rescue: Leucovorin [15 mg/m^2 IV or PO for standard/high-risk or 10 mg/m^2 PO (or IV) for low risk cases] will be started at 42 hours after the start of MTX and repeated every 6 hours for a total of five doses. The dosage of leucovorin will be increased in patients with high plasma MTX concentrations (>1.0 μM at 42 hours) and continued until the MTX concentration is less than 0.10 μM. **Additional measures, such as hydration, hemoperfusion, or carboxypeptidase will be considered in patients with 42-hour MTX levels > 10μM.** Patients with a history of Grade 3 or 4 gastrointestinal toxicity with prior MTX or a history of typhlitis with any chemotherapy should have leucovorin begin at 36 hours with subsequent MTX; if toxicity recurs, the baseline leucovorin dosage should also be increased. Conversely, patients with minimal toxicity (grade 1 or less GI toxicity and no delays in therapy) following full leucovorin rescue (15 or 10 mg/m^2 for five doses) should receive only 3 doses of leucovorin with subsequent course of HDMTX (i.e. at 42, 48, and 54 hours as has been utilized by the BFM)[3], **provided that the MTX dose and steady state level are not > 110% of those associated with the prior MTX course.** Patients who tolerate HDMTX

well without delayed MTX excretion are eligible to receive oral leucovorin with subsequent courses.

Amend 1.0

6.3.4 IT Chemotherapy

All patients will receive triple intrathecal therapy **every other week for four** doses on Days 1, 15, **29, and 43** (dosages are based upon age, according to Section 6.2.2.). The intrathecal treatment will be given on the same day of the HDMTX administration.

Amend 2.0

6.3.5 Interim Continuation Treatment

Interim continuation treatment will be given to the occasional patients who, upon attaining complete remission, are deemed unable to tolerate HDMTX. Specific criteria to use interim continuation therapy include disseminated fungal infection requiring systemic antifungal therapy, recent development of cerebral thrombosis, or grade 3 or 4 renal or hepatic dysfunction. There may be other unforeseen reasons that warrant temporary withholding of HDMTX.

Interim treatment will consist of 6-mercaptopurine 50 mg/m^2 per day and MTX 40 mg/m^2 per week; intrathecal therapy may be given every other week during this period of time and continued during the subsequent HDMTX treatment for a total of 4 doses. HDMTX will be started when the patient's physical condition allows. In the event that the interim therapy is longer than 4 weeks, an extra intrathecal therapy may be given with the last course of HDMTX.

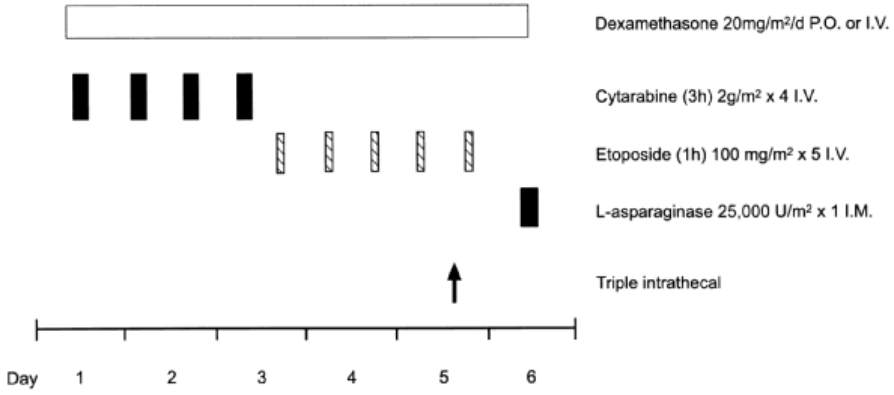
6.4 Reintensification Treatment (for High-Risk ALL)

Rev 3.2, Amend 4.0

Patients with high-risk leukemia **may** receive reintensification therapy and then will be offered the option of transplant. This treatment will attempt to maximize leukemic cell kill before allogeneic hematopoietic stem cell transplantation. For patients with Philadelphia chromosome positive ALL and those with induction failure or >1% leukemic lymphoblasts (determined by MRD study) in bone marrow on remission date, this treatment will be given after consolidation therapy**. However, consolidation therapy may be shortened, depending on patient's response to therapy and on the timing of transplantation.** For patients with >0.1% leukemic lymphoblasts (determined by MRD study) in bone marrow in week 7 of continuation treatment, this treatment will be given after the reinduction I. Upon marrow recovery (i.e., ANC ≥ 300/mm^3, WBC ≥ 1000/mm^3 and platelet count ≥ 50 x 10^9/L) after a course of reintensification, bone marrow examination with MRD study will be repeated.

This treatment course may be repeated only once if the patient still has persistently positive MRD (i.e. >0.01% blasts). Allogeneic hematopoietic stem cell transplantation may proceed after 1 course of the treatment if MRD becomes negative with the first course of treatment; otherwise, transplant will be performed

after two courses of treatment. Patients deemed unsuitable for the transplant or who decline the procedure or whose donor has yet to be identified, will remain on study and receive subsequent chemotherapy as scheduled. The treatment scheme and dosage of chemotherapy are summarized below.



6.5 Continuation treatment (120 weeks for girls and 146 weeks for boys)

Postremission continuation treatment begins 7 days after the fourth course of HDMTX of the consolidation treatment, provided that the ANC ≥ 300/mm^3, WBC ≥ 1000/mm^3 and platelet count ≥ 50 x 10^9/L as well as no evidence of mucositis. Continuation treatment (120 weeks for girls and 146 weeks for boys) differs according to the risk classification, as follows (abbreviations as defined below).

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Revision 6.1

6.5.1 Treatment (Weeks 1 to 20)

Week	Standard/High Risk	Low Risk
1	DEX + DOX + VCR + 6MP + ASP	6MP + DEX + VCR
2	6MP + ASP	6MP + MTX
3	¥6MP + ASP	6MP + MTX
4	DEX + DOX + VCR + 6MP + ASP	6MP + DEX + VCR
5	6MP + ASP	6MP + MTX
6	6MP + ASP	6MP + MTX
7	*†Reinduction I§	*†Reinduction I
8	Reinduction I	Reinduction I
9	Reinduction I	Reinduction I
10	6MP + ASP	6MP + MTX
11	DOX + VCR + 6MP + ASP	6MP + MTX
12	*6MP + ASP	*6MP + MTX
13	6MP + ASP	6MP + MTX
14	DEX + DOX + VCR + 6MP + ASP	6MP + DEX + VCR
15	6MP + ASP	6MP + MTX
16	6MP + ASP	6MP + MTX
17	*†Reinduction II	*†Reinduction II
18	Reinduction II	Reinduction II
19	Reinduction **********II	Reinduction II
20	No chemotherapy	6MP + MTX

¥Triple intrathecal treatment will be given to other standard/high-risk cases with WBC ≥100 x 10^9/L, T-cell ALL with WBC ≥ 50 x 10^9/L, presence of Philadelphia chromosome, *MLL* rearrangement, hypodiploidy <45, or CNS-3 status
*IT MHA (methotrexate + hydrocortisone + cytarabine; see section 6.2.2 for dosage)
*† IT MHA and bone marrow examination
§Patients with MRD >0.1% at week 7 receive reintensification treatment after Reinduction I (see Section 6.4)
See Section 6.5.2 for details on Reinduction treatment.

During weeks 1 to 20, dexamethasone, vincristine and L-asparaginase can be given regardless of blood counts, provided that the patient is not sick. Methotrexate, 6-mercaptopurine and doxorubicin will be held if ANC <300/mm^3, WBC <1000/mm^3, or platelet count <50 x 10^9/L (see section 7.0 for other dose modifications).

Amend 4.0

6.5.1.1 Drug Dosages, Schedules and Routes for Continuation Therapy Weeks 1 to 6 and 10 to 16

DEX (dexamethasone) 12 mg/m^2 (std/high risk) or 8 mg/m^2 (low risk) PO daily (tid) x 5 days, Days 1-5
DOX (doxorubicin) 30 mg/m^2 IV, Day 1

VCR (vincristine)	2.0 mg/m^2 IV push (max. 2 mg), Day 1
6MP (6-mercaptopurine)	50 mg/m^2 PO h.s. daily x 7 days (std/high risk), Days 1-7 75 mg/m^2 PO h.s. daily x 7 days (low risk), Days 1-7
ASP (L-asparaginase)	25,000 **Unit**/m^2 IM, Day 1
MTX (methotrexate)	40 mg/m^2 IV or IM, Day 1

6.5.2 Reinduction Treatment

This phase of treatment will be started at weeks 7 and 17 after bone marrow examination confirms complete remission. All patients must also have ANC ≥ 500/mm^3, WBC ≥ 1000/mm^3, and platelet count ≥ 50 x 10^9/L before the start of reinduction treatment.

Reinduction treatment will be given twice: weeks 7 to 9 and weeks 17 to 19 for all patients.

Intrathecal treatment will be followed by leucovorin rescue (5 mg/m^2) at 24 and 30 hours only in patients with prior CNS toxicities.

6.5.2.1 *Reinduction I for Standard/High-Risk ALL*

Amend 1.0, 4.0, 5.0

Agents	Dosages and routes	# Doses	Schedules
Dexamethasone	8 mg/m^2/day PO (t.i.d.)	**45**	Days 1-8, **15**-21
Vincristine	1.5 mg/m^2/week IV (max 2 mg)	3	Days 1, 8, 15
Doxorubicin	30 mg/m^2	2	Days 1, 8
L-asparaginase	25,000 **Unit**/m^2, weekly IM	3	Days 1, 8, 15
Methotrexate + hydrocortisone + ara-C	Age-dependent, IT	1	Day 1

6.5.2.2 *Reinduction II for Standard/High-Risk ALL*

Amend 1.0, 4.0**,** 5.0

Agents	Dosages and routes	# Doses	Schedules
Dexamethasone	8 mg/m^2/day PO (t.i.d.)	**45**	Days 1-8, **15**-21
Vincristine	1.5 mg/m^2/week IV (max 2 mg)	3	Days 1, 8, 15
L-asparaginase	25,000 **Unit**/m^2, weekly IM	3	Days 1, 8, 17
Methotrexate + hydrocortisone + ara-C	Age-dependent, IT	1	Days 1
High-dose cytarabine	2 gm/m^2 IV q 12 hr	4	Days 15, 16

6.5.2.3 *Reinduction I and II for Low-Risk ALL*

Amend 1.0**,** 4.0**,** 5.0

Agents	Dosages and routes	# Doses	Schedules
Dexamethasone	8 mg/m^2/day PO (t.i.d.)	**45**	Days 1-8, **15**-21
Vincristine	1.5 mg/m^2/week IV (max 2 mg)	3	Days 1, 8, 15
L-asparaginase	10,000 **Unit**/m^2/thrice weekly IM	9	Days 2, 4, 6, 8, 10, 12, 15, 17, 19
Doxorubicin	30 mg/m^2/week IV	1	Day 1
Methotrexate + hydrocortisone + ara-C	Age-dependent, IT	1	Day 1

6.5.3 IT Chemotherapy

Amend 1.0, 2.0, 6.0

- Triple intrathecal treatment will be given to low-risk cases with CNS-1 status (no identifiable blasts in CSF) on weeks 7, 12, 17, 24, 32, 40, and 48.
- Triple intrathecal treatment will be given to low-risk cases with CNS-2, traumatic CSF with blasts status, **or WBC > 100 x 10^9/L** on weeks 7, 12, 17, 24, 28, 32, 36, 40, 44 and 48.
- Triple intrathecal treatment will be given to standard/high-risk cases on weeks 7, 12, 17, 24, 28, 32, 36, 40, 44 and 48.
- Triple intrathecal treatment will be given to other standard/high-risk cases with WBC ≥100 x 10^9/L, T-cell ALL with WBC ≥ 50 x 10^9/L, presence of Philadelphia chromosome, *MLL* rearrangement, hypodiploidy <45, or CNS-3 status on weeks **3,** 7, 12, 17, 24, 28, 32, 36, 40, 44, 48, 56, 64, 72, 80, 88 and 96.

Amend 1.0, Rev 3.1, 6.0

Thus, the total numbers of intrathecal treatments for various subgroups of patients are 13 (low-risk cases with CNS1 status), 18 (low-risk cases with CNS2 status, traumatic CSF with blast status, or WBC > 100 x 10^9/L), 16 (standard-risk cases with CNS1 status), 18 (standard risk cases with CNS2 status), and 25 (standard/high-risk cases with WBC > 100 x 10^9/L, presence of Philadelphia chromosome, MLL rearrangement, hypodiploidy < 45, or CNS 3 status). The number of intrathecal treatments for high risk patients will depend on whether and when the patients will receive stem cell transplantation. Leucovorin will not be given after intrathecal treatment during continuation treatment unless the patient has an adverse reaction with previous intrathecal or methotrexate treatment, e.g., seizure or encephalopathy. While intrathecal treatments may be given at SJCRH affiliate institutions, all patients must have intrathecal treatment given at St. Jude at least every 8 weeks, because close monitoring of cerebrospinal fluid is an essential component of therapy.

6.5.4 Treatment (weeks 21 to end of therapy)

Week	Standard/High Risk	Low Risk
21	6MP + MTX	6MP + MTX
22	6MP + MTX	6MP + MTX
23	Cyclo + Ara-C	6MP + MTX
24	*DEX + VCR	*6MP + DEX + VCR
25	6MP + MTX	6MP + MTX
26	6MP + MTX	6MP + MTX
27	Cyclo + Ara-C	6MP + MTX
28	*DEX + VCR	(*)6MP + DEX + VCR

*IT MHA (methotrexate + hydrocortisone + cytarabine; see section 6.2.2 for dosage)
(*)IT MHA for patients with CNS-2 or traumatic CSF with blast status; see section 6.5.3 for details.

6.5.4.1 Drug Dosages, Schedules and Routes for Continuation Therapy from Week 21 To End of Therapy

6MP (6-mercaptopurine)	75 mg/m^2 PO h.s. daily x 7 days, Days 1-7
MTX (methotrexate)	40 mg/m^2 IV or IM, Day 1
Cyclo (Cyclophosphamide)	300 mg/m^2 IV, Day 1
Ara-C (Cytarabine)	300 mg/m^2 IV, Day 1
DEX (dexamethasone)	12 mg/m^2 (std/high risk) or 8 mg/m^2 (low risk) PO daily (tid) x 5, Day 1-5
VCR (vincristine)	2.0 mg/m^2 IV push (max. 2 mg), Day 1

The same treatment (weeks 21-28) will be repeated for a total of 6 times (until week 68). After week 68, all patients will receive daily 6MP and weekly MTX with pulses of dexamethasone and vincristine every 4 weeks until week 100, after which only 6MP and methotrexate will be given. Intrathecal treatment will be given every 8 weeks only to patients at high risk of CNS relapse after week 48 and will be discontinued after week 96. Continuation therapy will be discontinued after 120 weeks in girls and after 146 weeks in boys.

6.6 Hematopoietic Stem Cell Transplantation

Patients who meet the criteria of high-risk ALL are candidates for allogeneic hematopoietic stem cell transplantation. However, if the option is declined by the patients or guardians, or the procedure is deemed unsuitable by the attending physician and the principal investigator, the patient will remain on study and continue to receive chemotherapy.

7.0 TREATMENT MODIFICATIONS

7.1 Down Syndrome patients

Patients with Down syndrome are eligible for enrollment, with the following modifications.

7.1.1 Window Therapy: Cases with Down syndrome are ineligible for Upfront Window treatment and should proceed to Day 5 of induction therapy (as per section 6.2).

7.1.2 Consolidation and continuation HDMTX: Dosages of high-dose MTX should be modified due to their well-documented altered pharmacokinetics and enhanced tissue sensitivity to MTX's effects. Their hydration and alkalinization regimen should be the same as outlined in Section 6.3.3. However, the dose of HDMTX is 500 mg/m^2 (50 mg/m^2 over 1 hour and 450 mg/m^2 given over 23 hours).

The baseline leucovorin rescue should begin early (at hour 30 at 30 mg/m^2 IV q 6 hours x 2 doses, followed by 10 mg/m^2 IV q6 hours x 6 doses). If MTX plasma levels are elevated, increased leucovorin rescue will be recommended by the Pharmaceutical Science Department. Vigorous hydration should be assured until the 42 hour MTX level is known.

7.1.3 Continuation Low Dose MTX: The low dose weekly MTX (40 mg/m^2) dosage should be administered at full dosage if possible. If the patient has severe neutropenia (which delays subsequent therapy) or grade 4 mucositis (or mucositis which delays subsequent therapy) following the dose of 40 mg/m^2, the dosage should be decreased to 30 mg/m^2. If that dosage is similarly not tolerated, then the dosage may be further decreased to 20 mg/m^2 and finally to 10 mg/m^2, if necessary. If 10 mg/m^2 is also not tolerated, then leucovorin should be added at 5 mg/m every 6 hour for 4 doses starting 42 hours from the MTX dosage, with titration to acceptable toxicity.

7.1.4 Intrathecal Therapy: Intrathecal treatment should be administered as outlined in Section 6.2.2.

7.2 Renal dysfunction

Subclinical renal impairment (normal serum creatinine but decreased GFR) may be present in patients receiving concurrent nephrotoxic drugs. If possible, amphotericin and IV acyclovir should be held during and for 20 hours after HDMTX infusions or until adequate MTX clearance has been documented. Consideration to delaying MTX should be given if a patient's serum creatinine indicates renal impairment.

7.3 Hepatic dysfunction

Anthracyclines and vincristine dosages should be modified in patients with elevated direct bilirubin concentrations or other evidence of biliary obstruction. (More conservative criteria will be used for anthracycline treatment during initial remission induction. See Section 6.2.)

Direct bilirubin 2-4 mg/dl - 50% dosage decrease
Direct bilirubin 4-6 mg/dl - 75% dosage decrease
Direct bilirubin >6 mg/dl - withhold dose

L-asparaginase may need to be withheld in patients with elevated direct bilirubin concentrations, especially if there is evidence of mucositis. HDMTX should be withheld or given at reduced dosages if direct bilirubin if >2 mg/dl and should be withheld if there is evidence of existing mucositis.

Amend 5.0

See Section 7.12.2 for Imatinib Mesylate dose modification guidelines for Ph+ ALL patients with hepatic toxicity/dysfunction.

Subclinical hypertransaminasemia (SGPT >500 IU/L) is an indication to delay only high-dose methotrexate but not other chemotherapy.

7.4 Obesity

Actual body weight will be used to calculate body surface area in all patients and used for dosage calculations (with the exception that vincristine dosage is capped at 2.0 mg).

7.5 Testicular Leukemia at Diagnosis

Overt testicular leukemia occurs in 2% of boys at diagnosis, generally in infants or adolescents with hyperleukocytosis[118]. Ultrasonogram should be performed to differentiate testicular leukemia from hydrocele and to measure the testicular volume. Testicular size should also be measured and followed with the use of an orchiometer. Overt testicular leukemia at diagnosis per se is not an indication for testicular irradiation, as many patients can be successfully treated with chemotherapy, including high-dose methotrexate.[118] Ultrasonogram should again be performed upon completion of remission induction. If testicular size is still abnormally enlarged, the sonogram should be repeated after consolidation treatment with high-dose methotrexate and mercaptopurine. Persistently enlarged testes after consolidation treatment will be biopsied. Testicular irradiation (24 Gy) will be administered in the rare patients with positive biopsies, after consultation with a radiotherapist.

7.6 Vincristine Neurotoxicity

Amend 1.0

The maximum single dose of vincristine must not exceed 2 mg. Mild vincristine toxicities (jaw pain, constipation, decreased deep tendon reflexes) are anticipated. Loss of voice due to vocal cord paralysis may be a complication of vincristine toxicity but it must be differentiated from pharyngitis or Candida infection of the cord. If persistent, severe abdominal cramps, gait impairment, **severe pain (requiring narcotic treatment),** or SIADH develop, the dose may be reduced to 1 mg/m^2. Only motor paralysis or typhlitis warrants discontinuation of vincristine.

7.7 Cerebral thrombosis

Amend 3.0

For patients who develop cerebral thrombosis during induction, **a 5 ml blood sample (red top tube) will be obtained for analysis of vWF multimers and cleaving protease concentrations, along with anti-asparaginase antibodies. One tube of 3 ml blood will also be obtained for plasma asparagine.** Subsequent treatment will be modified. In low-risk cases, during each reinduction treatment, dexamethasone will be given only in the first week and L-asparaginase in the second and third weeks (i.e., omit dexamethasone in the third week and L-asparaginase in the first week) and low molecular weight heparin will be given throughout reinduction treatment. In standard-/high-risk cases, dexamethasone will be omitted from weeks 4 and 9 and low molecular weight heparin will be given during the first 19 weeks of continuation treatment.

7.8 Avascular Necrosis of Bone

Amend 3.0

MRI exams will be interpreted by the radiologist. If there is evidence of epiphyseal or metaphyseal hip lesions, knee epiphyseal or metaphyseal lesions, or lesions of talus consistent with avascular necrosis of the bone, the patient will be referred to the orthopedic surgeon, who will evaluate symptoms, and will assess the severity and estimated risk of progression. Physical therapy, activity modifications, and surgical procedures will be recommended as needed. Patients with hip epiphyseal lesions or talus lesions affecting > 30% of weight-bearing area will have an X-ray of the affected area, and will be assessed at higher risk for progression. Symptomatic patients with such findings will likely have their dexamethasone stopped, especially if they are past reinduction II in therapy. Asymptomatic patients with such findings will likely have their dexamethasone dose halved, especially if they are past reinduction II in therapy. Any patients with X-ray findings of AVN are candidates for dexamethasone modification, regardless of symptoms. All modifications (or lack thereof) of dosage will be recorded in the research database. Patients with progression of any lesions or with worsening symptoms will be re-evaluated by imaging and, if appropriate, by additional orthopedic follow-up. If the dexamethasone is discontinued, the, first choice will be to replace each week's dosing with one dose of methotrexate (40 mg/m^2), if tolerated. See Section 12.4 for additional guidelines.

7.9 L-asparaginase Hypersensitivity

Amend 4.0, **Rev. 10.1**

Patients with allergic reactions (e.g., severe pain and tenderness at injection site, urticaria) to *E. coli* L-asparaginase will be subsequently given *Erwinia* L-asparaginase. **Please note: Enrollment and informed consent on the ERWASE protocol is *required* in order for patients to receive Erwinia L-asparaginase**. Erwinia L-asparaginase will be given at 20,000 **Unit**/m^2/dose during remission induction and, in low-risk cases, during reinduction; it will be given at 25,000 **Unit**/m^2 twice weekly (3 to 4 days apart) from weeks 1 to 19 of continuation treatment in standard-/high-risk cases. Patients allergic to both *E. coli* and *Erwinia* preparations will receive PEG-asparaginase (at 2500 **Unit**/m^2 per week). Acute hemorrhagic pancreatitis or severe pancreatitis (abdominal pain $\geq$72 hours and increased amylase $\geq$3 x normal) is a contraindication to continue L-asparaginase treatment. In the case of mild to moderate pancreatitis, asparaginase should be held until symptoms and signs subside, and amylase levels return to normal and then resumed. Asparaginase treatment should be delayed until at least 2 hours after intrathecal treatment. If patients become allergic to all three forms of asparaginase, the week of continuation treatment with asparaginase should constitute at least 6-mercaptopurine 50 mg/m^2/day and methotrexate 40 mg/m^2/week.

Amend 2.0**,** 8.0

7.10 TPMT Status and 6MP Dosage

Thiopurine methyltransferase (TPMT) defects have been linked to the risk of therapy-related AML (t-AML),[84a,b] even in the context of ALL therapy consisting primarily of antimetabolities.[84a] Both studies reporting an association of TPMT defects with risk of t-AML incorporated 6MP at a dosage of 75 mg/m^2/day, whereas many ALL treatment groups use a starting dosage of 60 mg/m^2/day and then titrate up. Although the mechanism is not known, patients with TPMT defects have higher concentrations of thioguanine nucleotides,[84c,e] and substitution of thioguanine for guanine in DNA can affect topoisomerase II-induced cleavage of DNA in the presence and absence of a topoisomerase II inhibitor.[84d] Together, the clinical and preclinical evidence suggest that the 10% of patients who carry a mutant TPMT allele may be at higher risk for t-AML. Because such patients often require dosage decreases of 6MP,[84c,84e] and because (in a setting in which dosage was adjusted in about 1/3 of patients with TPMT defects) overall leukemia-free survival was outstanding among patients with TPMT defects,[23] we are recommending that patients with phenotype or genotype consistent with at least one mutant TPMT allele receive no more than 60 mg/m^2/day of 6MP unless it is clearly documented that compliance has been good and metabolite levels remain low (< 100 pmol/1x10^8 RBCs), or unless MTX dose has been increased and WBC or ANC remain high. Further changes in 6MP dose should be titrated based on WBC and ANC. Every effort should be made to keep other anticancer agent doses at protocol levels for these patients (see sections 8.1 and 8.2). A blood sample (5-10 ml) will be drawn

along with routine lab work at Day 5 of remission induction to allow for timely TPMT genotyping.

Amend 2.0

Assessing TPMT status: TPMT genotype will be determined by mutation-specific PCR, directed against the 3 most common inactivating mutations at positions 238, 460, and 719 of the cDNA. These 3 mutations account for > 90% of all mutant alleles.(127) The detection of mutations at any of these positions will confer TPMT heterozygous status. As specified in section 8.2, all patients also have 6TGN thiopurine metabolite measures at the start of reinduction I and TPMT activity in RBCs assessed at least by week 17. The threshold for on-therapy, non-transfused TPMT activity that is considered the upper bounds for heterozygous status (14 units/ml) (84f) is higher than that for off-therapy, non-transfused patients (10 units/ml).(127) If the ratio of methyl thiopurine metabolites to TGNs is < 15 (95^{th} %-tile for heterozygotes), the patient will be considered to have a TPMT defect. If TPMT genotype or activity or metabolite measures are consistent with heterozygous status, the patient will be considered heterozygote; using these criteria, approximately 10% of patients are expected to be heterozygote or homozygous mutants,(84f) in agreement with population studies for TPMT status. Thus, approximately 10% of patients are expected to require dose decreases of 6MP on the basis of TPMT status.

Amend 3.0

7.11 Pancreatitis

Any patients with abdominal pain suspected of pancreatitis should have serum amylase and lipase measured as well as an abdominal sonogram or CT scan done. In the case of severe pancreatitis (i.e. abdominal pain of 72 hours or more, amylase level three times or more of the upper limit of normal, and sonographic or CT scan evidence of pancreatitis), asparaginase will be discontinued permanently. Treatment with octreotide (Sandostatin®), a potent analog of somatostatin which has an inhibitory effect on the pancrease by suppression of pancreatic endocrine and exocrine function, should be considered. The suggested dose is 3 to 5 mcg/kg (max 10mcg/kg) every 12 hours, given SQ or IV over 30 minutes. The most common side effects of the treatment include hyperglycemia, hypoglycemia, nausea, and hyperbilirubinemia. In cases with mild to moderate pancreatitis (abdominal pain less than 72 hours and amylase level less than three times the upper limit of normal), asparaginase should be held and resumed once symptoms and signs subsided. Call the PI or co-PI to discuss the management if the patient is asymptomatic (without abdominal pain) and has only elevated amylase levels. Because of potential drug-drug interactions, a PharmD should be consulted for patients receiving octreotide.

Amend 5.0

7.12 Philadelphia Chromosome Positive (Ph+) ALL

Patients with Ph+ ALL will receive imatinib mesylate, starting on day 26 of induction chemotherapy. Imatinib will be given at a dose of 340 mg/m^2, rounded to the nearest 100 mg increment, once daily (maximum 600 mg per

day) until the end of all therapy. For patients undergoing transplant, imatinib will be stopped when conditioning therapy begins, and then resumed upon recovery of hematological function. Imatinib therapy will continue for 6 months post transplant.

The prescribed dose should be administered orally, with a meal and a large glass of water. In children, the daily dose may be split into two – once in the morning and once in the evening. For patients unable to swallow the film-coated tablets, the tablets may be dispersed in a glass of water or apple juice. The required number of tablets should be placed in the appropriate volume of beverage (approximately 50 mL for a 100-mg tablet, and 200 mL for a 400-mg tablet) and stirred with a spoon. The suspension should be administered immediately after complete disintegration of the tablet.

7.12.1 Concomitant Therapy with Imatinib

<u>Anticonvulsants</u>: Imatinib mesylate is metabolized by the P-450 enzyme system (CYP 2D6), with reported interaction with 3A4). Therefore, if patients require anticonvulsant medication, they should receive a medication unlikely to activate the P-450 system. Permissible medications would therefore include gabapentin, valproic acid, and medications of the benzodiazepine and succinimide classes (See Appendix V).

<u>Anticoagulants</u>: Use of coumadin is not allowed. Low molecular weight heparin may be used with caution. Its use should be avoided, if possible, in patients whose platelet counts fall below 50,000/mm^3.

Medications which may interact with the study drug are not allowed (e.g., certain anticonvulsants, see above). Medications which interfere with P-450 metabolism should be avoided. The following medications and foods can interfere with P-450 metabolism: grapefruit juice, erythromycin, azithromycin, clarithromycin, rifampin and its analogs, Voriconazole, fluconazole, ketoconazole, itraconazole, cimetidine, cannabinoids (marijuana or dronabinol) and leukotriene inhibitors used in asthma such as zafirlukast and zileuton. In addition, drug interactions in patients receiving prochlorperazine (Compazine) and coumadin are possible. Patients who require prochlorperazine during therapy should be monitored for akathisia. This medication should not be used during imatinib mesylate administration unless there is unavoidable medical need and no appropriate alternative agents are available. Also, acetaminophen should not be taken in greater than the recommended dose. See Appendix V for more extensive list of drugs with potential interaction with imatinib mesylate.

Investigators are warned to avoid coumadin, carefully monitor therapeutic anti-coagulation in patients receiving imatinib mesylate,

and more specifically, to maintain adequate platelet counts (≥ 20,000/μl) in such patients. In patients with platelets under 50,000/mm^3, anticoagulation should be used only with great caution.

Amend 5.0

7.12.2 Dose Modifications for Imatinib

All dose modifications should be based on the worst preceding toxicity.

Grade III/IV Non-Hematological Toxicity

If a patient experiences Grade III/IV non-hematological toxicity, the study drug must be withheld until the toxicity resolves. Upon recovery, study drug may be resumed at a 30% dose reduction (calculated on the basis of the dose actually administered). If the Grade III/IV toxicity recurs, further dose reductions can be performed using the above procedures (also see guidelines for hepatic toxicity below).

Hepatic Toxicity

Patients with SGOT/SGPT (ALT/AST) ≤ 1 x ULN baseline who experience Grade III hepatic toxicity should be managed using the criteria detailed above. In patients enrolling with SGOT/SGPT > 1 x - ≤ 3 x ULN at baseline who experience > 5-fold increase in the levels of the most elevated transaminase (unrelated to high-dose methotrexate), study drug will be withheld until the transaminase levels return to baseline values and then resumed at the same dose. If a similar degree of toxicity recurs, study drug must be held until the transaminase levels return to the baseline values. Study drug may then be resumed at a 30% dose reduction (calculated on the basis of the dose actually administered). If the toxicity recurs, further dose reductions can be performed using the above procedures.

Grade IV Hematological Toxicity

No dose modifications will be allowed during the first 28 days of therapy. If Grade IV neutropenia (ANC < 500/μl) is present on Day 28 or later, study drug must be held until ANC > 500/μl, at which time treatment may be resumed at full dose. If Grade IV neutropenia persists, a bone marrow and aspirate should be performed at the investigator's discretion to assess the cellularity and percentage of blasts. If Grade IV neutropenia recurs after resuming imatinib mesylate, it will be held until ANC is > 500/μl. Imatinib mesylate will then be resumed at a 30% dose reduction (calculated on the basis of the dose actually administered). If the toxicity recurs, further dose reductions can be performed using the above procedures.

If the platelet count falls below 25,000/μl, Imatinib mesylate should be held until the platelet count is > 50,000/μl. Upon recovery, Imatinib mesylate may be resumed at a 30% dose reduction (calculated on the basis of the dose actually administered). Further dose reductions may be performed, using the above procedures, if thrombocytopenia (platelets < 25,000/μl) is again encountered. If the platelet count does not recover to > 50,000/μl within 7 days of stopping Imatinib mesylate, a bone marrow aspirate should be performed to exclude progressive leukemia or bone marrow fibrosis. Progressive leukemia is an off therapy criterion. If bone marrow fibrosis leading to isolated thrombocytopenia occurs, call the principal investigator to discuss dose.

8.0 DOSE MODIFICATIONS DURING CONTINUATION THERAPY

Full doses of treatment will be administered when WBC ≥ 1000/mm^3, ANC is ≥ 300/mm^3 and platelet count ≥ 50 x 10^9/L. Dexamethasone and vincristine will be given regardless of blood counts, provided that the patient is clinically well. Adjustments of dosages should be made in the following circumstances, with re-evaluation of tolerance and toxicities every 8 to 16 weeks.

8.1 Dose modifications for inadequate myelosuppression

Amend 2.0

Patients who miss less than 25% of therapy but have persistently (>50% of time; not counting the week after dexamethasone/vincristine) high WBC (>4 x 10^9/L) and high ANC (>1000/mm^3) should be counseled on compliance, particularly if 6TGN levels are <100 pmol/8 x 10^8 RBCs. If the WBC remains high, 6-mercaptopurine and methotrexate dosages should be increased by 30% (e.g., to 100 mg/m^2 and 50 mg/m^2, respectively), using a stepwise approach if needed. **If patients have a TPMT defect, 6MP dosage should not be increased unless the TGN levels are < 100 pmol/8 x 10^8 RBCs.**

8.2 Dose decreases based on 6MP Pharmacology (low risk and standard/high risk)

Amend 2.0

All patients on both arms will have 6TGN measured at the start of reinduction I (i.e., week 7). TPMT will be measured at the start of reinduction II (week 17) **or earlier if TPMT defects are suspected. TPMT genotype[127] may be used to identify TPMT mutant alleles.** 6TGN and TPMT will be measured subsequently in patients with high 6TGN level, suspected noncompliance, problems with toxicities or high blood counts.

Patients missing ≥ 25% of therapy who have 6TGN levels >1000 pmol/8 x 10^8 RBCs will have 6-mercaptopurine dosage reduced to achieve a steady-state 6TGN level between 200 to 1000; those with 6TGN level <1000 will have both 6-mercaptopurine and methotrexate dosages reduced by 30%.

Dosages will be re-evaluated every 8 to 16 weeks. Other causes of low blood counts should also be considered (see Section 12.5).

Patients missing less than 25% of therapy and with WBC <4 x 10^9/L do not need any change in dosage, regardless of 6TGN level.

Amend 2.0 **See section 7.10 for 6MP dosage adjustments.**

Amend 5.0

See Section 7.12.1 for Imatinib dosage adjustments (Ph+ ALL patients only)

9.0 CONTINGENCY PLANS FOR REFRACTORY DISEASE OR RELAPSE

Amend 8.0 (deleted text)

9.1 Induction failures

Patients who do not attain complete remission (≥ 5% leukemic blasts in bone marrow) after remission induction, consolidation treatment and reintensification treatment **are eligible for other protocols.** Those who do not achieve a remission after induction therapy, but subsequently attain complete remission after consolidation or reintensification treatment, are candidates for allogeneic hematopoietic stem cell transplantation.

Amend 8.0 (deleted text)

9.2 Hematologic relapse

Patients with ≥ 25% lymphoblasts in marrow aspirate will become eligible for relapse protocols.

Amend 8.0 (deleted text), **9.0**

9.3 Extramedullary relapse

Patients with any form of extramedullary relapse (testes, ovarian, etc) except that of CNS will become eligible for relapse protocols. Patients with overt CNS relapse (i.e. ≥ 5 WBC/μL of CSF with blasts) will remain on study and receive **a second remission re-induction as outlined in Section 6.2. During the second remission induction, patients will receive weekly triple intrathecal for at least 4 doses (with CSF clear of leukemic blasts in at least two exams); triple intrathecal therapy will be continued every 3 to 4 weeks until time for CNS irradiation (in consultation with Radiation Oncologist). All patients with CNS relapse will also receive 1-2 cycles of re-intensification as outlined in Section 6.4 to consolidate bone marrow remission after induction (when bone marrow function is adequate) but before CNS irradiation. Whether 1-2 cycles are to be given will depend on the MRD status and individual patient tolerance. This should be discussed with the principal investigator**. **They will receive** continuation treatment for at least one year from time of relapse (at least 2½ years in girls and 3 years in boys including initial treatment). Patients who have <5 WBC/μL of CSF with identifiable blasts are not considered to have overt CNS relapse and will be treated as outlined in Section 9.4.

Amend 9.0

9.4 Refractory CNS Leukemia at Diagnosis and Lymphoblasts During Remission Requiring CNS Radiation

In contrast to previous Total Therapy studies in which cranial irradiation was given prophylactically to patients with CNS leukemia at diagnosis and to those with high-risk leukemia, only patients with refractory CNS leukemia at diagnosis

(i.e., failure to clear CSF blasts after three consecutive intrathecal treatments) and those with immunologically proven leukemic lymphoblasts in CSF (regardless of cell count) during hematologic remission on two occasions in the study will receive therapeutic CNS irradiation in consultation with radiotherapists. The former group will receive 24 Gy cranial irradiation with five triple intrathecal treatments (with leucovorin rescue) at one year of remission (weeks 48 to 50). The latter group will receive systemic **a second remission induction as outlined in Section 6.2, triple intrathecal therapy and an additional 1-2 cycles of re-intensification as outlined for patients with isolated CNS relapse in Section 9.3.** CNS irradiation **will be given** as following: cranial irradiation (24 Gy in 16 fractions) for patients with <5 WBC/:l of CSF occurring within the first 18 months of remission; cranial irradiation (18 Gy in 12 fractions) for those with any number of leukemic lymphoblasts in CSF after 18 months of initial remission; and craniospinal irradiation (24 Gy cranial irradiation in 16 fractions plus 15 Gy spinal irradiation in 10 fractions) for patients with $\geq$5 WBC/:L of CSF occurring within the first 18 months of remission. Those patients receiving cranial irradiation only should receive 4 to 5 triple intrathecal therapy during irradiation. 6MP and MTX will be withheld for at least one week prior to and during irradiation; systemic chemotherapy during irradiation will include dexamethasone and vincristine with or without L-asparaginase.

10.0 PHARMACOKINETIC/PHARMACODYNAMIC STUDIES

10.1 Upfront HDMTX Window

10.1.1 Measurement of MTXPG

Intracellular MTX and MTXPG concentrations in the bone marrow lymphoblasts at 42 hours and in the peripheral blasts at 1.0, 4.0, 24, and 42 hours after the start of MTX infusion will be determined by previously described methods.[16,20] Briefly, cell pellets (~5 x 10^6 cells) are extracted for MTXPG, and stored at -80°C until analysis. Individual polyglutamate fractions are separated by HPLC and then quantified by a competitive DHFR-binding REA method,[119] with results (pmol) normalized to 10^9 cells.

10.1.2 Determination of Antileukemic Effects

Two primary endpoints will be used to assess antileukemic effects: (1) inhibition of *de novo* purine synthesis (DNPS) and (2) the decrease in circulating white blood cells and blast cell counts. For the determination of DNPS synthesis,[58,120] 5 x 10^6 cells collected at diagnosis and at 42 hours after the start of MTX treatment are resuspended in medium (90% RPMI/10% dialyzed FBS, 2 mM glutamine) containing 0.5 mM ^{14}C formate (50 dpm/pmol) for 2 hours, washed, harvested and frozen at -80°C; then assayed by a HPLC-UV method with in-line scintillation counting. Based on our past experience, almost all patients will be

evaluable for decreasing white blood cell counts and 85% of children will have circulating blasts at diagnosis and thus be evaluable for the decrease in circulating blasts as a measure of MTX cytotoxicity.[4] (Patients who have received antileukemic systemic therapy prior to Total XV up-front MTX are eligible for window therapy but will not be assessed for antileukemic effect.) Bone marrow blasts will be available from >90% of patients for the DNPS synthesis assay. Daily complete blood counts with differential will be performed through 96 hours after the start of therapy.

10.1.3 Measurements of FPGS, GGH, MRP, and reduced folate carrier (RFC)

FPGS activity will be measured in cytosol from 5 x 10^6 bone marrow cells obtained at diagnosis and at 42 hours after the start of MTX infusion, by a modification of the ^{3}H-glutamic acid incorporation microassay we have used previously.[55] RFC function will be assayed directly by assessing ^{3}H-MTX uptake at 1.0 :M MTX over a 10 minute period. FPGS, MRPs, and RFC mRNA will be quantified by Northern-blotting and RT-PCR, using methods we have established in our laboratory.[19,59] GGH will be measured in blast lysosomes, cytosol, and total cell lysate using modifications of the methods described[60] with MTXPG(5) as the substrate. Pre-therapy peripheral blasts from the pre-MTX blood sample will be compared with diagnostic bone marrow when possible.

10.1.4 Plasma MTX

Plasma MTX concentrations will be measured pre and at 1, 4, 24, and 42 hours from the start of MTX, and every 24 – 48 hours thereafter in patients with delayed MTX clearance.

10.2 Methotrexate – Consolidation

Amend 6.0

2-3 ml of blood will be obtained in heparinized tubes (green top) pre-dose and **between 0.5 and** 6 hr, 23 hr, and 42 hr from the start of the infusion. Additional samples will be obtained in patients **in whom there is clinical suspicion of poor clearance, or in those who have** high plasma MTX concentration (e.g., >0.5 μM at 42 hours), to adjust leucovorin rescue.

10.3 Thiopurines

Blood (3 ml) will be obtained at week 7 and at week 17 for red blood cell thiopurine concentrations and TPMT, respectively. Additional samples may be obtained as indicated in Section 8.0.

Amend 5.0

10.4 Asparaginase (Not required at Cook)

Amend 3.0

Serum (3 ml blood) will be obtained on Days 5, 19, and 33 of remission induction therapy and again at Day 1 of reinductions I and II in all patients for measurement of anti-asparaginase antibodies, according to our previously established methods.

Using the serum obtained for vWF multimers and cleaving protease concentrations, those with sagittal sinus thrombosis or any deep vein thrombosis will also have measurement of anti-asparaginase antibodies at the time of thrombosis.

Amend 3.0

CSF from therapeutic lumbar punctures will be collected at SJCRH and placed into sulfosalicylic acid-containing tubes prior to induction therapy (with IT ara-C) and on Day 19 of remission induction therapy and on Day 1 of reinductions I and II in all patients. CSF asparagine levels will be measured according to our previously established methods.[121] Plasma (5 ml in green top tube) for asparagine will be obtained together with the CSF sample. **An additional blood sample (3 ml green top tube) will be obtained on the day of diagnosis of sagittal sinus thrombosis or any deep vein thrombosis for measurement of asparagine levels and asparaginase concentration.**

11.0 PATIENT EVALUATION

Amend 4.0

11.1 Pretreatment Evaluation

All patients should be invited to participate in the Tissue Banking Protocol (97BANK) at the time of study entry.

1. Complete history and physical exam with careful notation and assessment of clinical signs relevant to leukemia: size of liver, spleen and lymph nodes, testicular or skin involvement, local or systemic infection, etc. and careful history for the prior use of allopurinol, steroids or other antileukemic drugs.
2. Complete blood count with differential
3. Coagulation screen **as clinically indicated** (see Section 18.5)
4. Chemistry profile: glucose, electrolytes, BUN, creatinine, LDH, uric acid, bilirubin, SGOT, SGPT, calcium, phosphorous, magnesium, total protein and albumin.
5. Thyroid function test **(Not required at Cook's)**
6. Chest x-ray
7. Bone marrow evaluation: morphology, cytochemistry, immunophenotyping, cytogenetics, DNA index, molecular diagnosis
8. Lumbar puncture with CSF examination (cell count with differential of cytospin preparation) –performed on day 1 or day 2 (i.e., day 1 of HDMTX treatment), immediately followed by first intrathecal treatment with ara-C.
9. Other studies as clinically indicated, e.g. sickle cell prep, hemoglobin electrophoresis and G6PD screen for black children; varicella titer; Hepatitis B antigen; HIV (with informed consent), EBV, TOXO, CMV titers.

Amend 1.0, 2.0, Amend 4.0, Rev 4.1**,** 8.0

11.2 Data to be Obtained Serially While on Study

	Initial HDMTX (4 day)	Induction/ Consolidation	Continuation	Reinduction I & II
Physical Exam	Qd	q3-7 days	q4 week	q3-7 day
CBC with differential	Qd	**Qwk**	Qwk	qwk
Coagulation screen	As **clinically indicated**	As indicated	As indicated	As indicated
Uric acid, lytes, calcium phosphorous, magnesium	Qd	***Before each HDMTX and as indicated**	As indicated	Day 1 and as indicated
Serum glucose, urinalysis	As indicated	As indicated	As indicated	As indicated
Bilirubin, SGOT, SGPT, total protein, albumin, LDH, BUN, creatinine	At diagnosis	* Before each HDMTX and as indicated	As indicated	Day 1 (weeks 7 & 17) and as indicated
Bone Marrow and peripheral blood (MRD)	At diagnosis and at 42 hrs	Day 19, ± day 26 and end of induction*	Week 48, Week 120 Week 146 (boys)	Day 1 (weeks 7 & 17)
CSF studies	At diagnosis (with first intrathecal treatment)	With each intrathecal treatment	With each intrathecal treatment; then ≈q16wk **until week 96, and then at Week 120 for girls and 140 for boys.**	With each Intrathecal treatment
Chest x-ray	At diagnosis	End of induction in cases with mediastinal mass at diagnosis, then as indicated	As indicated	As indicated
MRI of hips and knees			Off therapy	After each reinduction phase (Weeks **12-14 and 22-24)**
Somatic Cell DNA	**Day 5**	Remission date (before 1st HDMTX in consolidation) **and Week 7 (before 4th consolidation MTX)**	Week **64**, 120, week 146	Day 8 of Reinduction I, Day 1 of Reinduction II
RBC TPMT and TGN				Day 1 (weeks 7 & 17)
MTX Pharmacokinetics	Days 1-3	Each HDMTX		
Asparaginase antibodies		Days 5, 19, and 33		Day 1 (week 7 and 17)
MRI of brain		**Between Days 33-46**	Weeks 48 and 120	**Week 1 of Reinduction I**
CSF **homocysteine/ folates a**nd plasma	Day 1	Weeks 1 and 3 consolidation		

homocysteine				
CSF and plasma asparagine	Day 1	Day 19		Day 1 (weeks 7 and 17)
Serum Cortisol		Week 3 of consolidation	Weeks 2 and 12	Days 1 and 8 of Reinduction I
Dex PK				Days 1 & 8 Reinduction I
Quality of Life		Week 6 induction	Weeks 48, 120, and 146 (in boys)	**Week 7 of Consolidation**
Psychology		Week 6 induction	Weeks 120 and 146 (in boys)	**Week 1 of Reinduction I**
QCT for bone density	Week 1		Weeks 120 and **146 (boys)**	
Cardiac evaluation	Between day 1 to day 4		Off therapy	**After** reinduction II
Urinalysis	As needed	As needed	As needed	As needed
Spot urine sample for calcium, creatinine, and sodium	At diagnosis	*During induction after completion of prednisone therapy	*Week 24	* Before and after reinduction I and II
24-hour urine sample for calcium, creatinine, and sodium		*During induction after completion of prednisone therapy (within one day of the spot urine measurement)		*Before reinduction I (within one day of the spot urine measurement)
Thyroid function	**At diagnosis**		As **clinically indicated**	

***Recommended for good clinical care**

11.3 Evaluation Criteria

11.3.1 Complete remission: M1 marrow status with restoration of normal hematopoiesis and normal performance status. These findings must persist for at least one month.

11.3.2 Induction failure: ≥ 5% leukemic blasts in marrow after 46 days of remission induction treatment.

11.3.3 Bone marrow relapse: ≥ 25% leukemic blasts in marrow

11.3.4 CNS relapse: ≥ 5 WBC/μL of CSF with definite blasts on cytospin preparation

11.3.5 Testicular relapse: Isolated testicular relapse must be confirmed pathologically; in the event of bone marrow relapse, combined testicular relapse can be based on testicular enlargement (documented by sonogram) without biopsy.

11.4 Off-therapy Evaluation

Amend 1.0, 5.0

It is recommended that patients will be followed every 4 months for 1 year, every 6 months for 1 year and then yearly until the patient is in remission for 10 years and is at least 18 years old. Thereafter, the patient will become alumni and will be followed according to the institution's policy. During the St. Jude visit, **the following evaluations are recommended:** CBC with differential and blood chemistries as well as other laboratory studies as needed will be obtained.

Neuropsychologic exam, quality of life evaluation, MRI of brain (in patients with abnormal study at week 120) and cardiac evaluation will be performed at 2 years off therapy. **It is recommended that QCT for bone density be performed at 5 years off-study.**

Patients will be considered off therapy 30 days after last treatment taken. Adverse events will not be reported while patient is off-therapy unless they are deemed related to therapy by the site PI.

After attaining continuous, complete remission for 6 years or more, **SJCRH** patients will be invited to participate in the long-term follow-up umbrella protocol and may be referred to the After Completion of Therapy Clinic (ACT).

When a SJCRH patient has been in remission for 10 years and is at least 18 years of age, the patient will become SJCRH alumni and will be followed according to the SJCRH institutional policy.

12.0 SUPPORTIVE CARE

12.1 Fever at diagnosis

All patients with fever at diagnosis will be admitted for broad spectrum parenteral antibiotic treatment until an infectious etiology can be excluded.

12.2 Metabolic derangements

It is important to prevent or treat hyperuricemia and hyperphosphatemia with secondary hypocalcemia resulting from spontaneous or chemotherapy-induced leukemic cell lysis, especially in T-cell ALL.

- Patients with large leukemic cell burden should receive hydration and oral phosphate binder (aluminum hydroxide in cases with relatively high calcium level and calcium carbonate in those with low calcium level or sevelamer (renagel) in patients who can't tolerate aluminum hydroxide or calcium carbonate).

Amend 3.0, Amend 4.0

- Patients with large leukemic cell burden with or without hyperuricemia (e.g., WBC $\geq$ 100 x 10^9/L, uric acid $\geq$ 7.5 mg/dl or $\geq$ 6.5 mg/dl in patients <13 years old) may be treated with **rasburicase** if they have no history of severe allergy (e.g., bronchial asthma requiring bronchodilator, atopic eczema), G6PD deficiency or ongoing pregnancy. **Rasburicase** is given at a dose of 0.1- 0.2 mg/kg/day and may be given twice for the first two days, with repeated doses as necessary. For all other patients not at high risk of hyperuricemia, hydration and judicious use of alkalinization (keeping urine pH between 6.5 and 7.4) may be sufficient. Allopurinol should be avoided until the 42-hour bone marrow sampling and preferably also the 96-hour CBC are obtained to avoid confounding effects of allopurinol on purine synthesis.

12.3 Hyperleukocytosis

For patients with extreme hyperleukocytosis (i.e., WBC >200 x 10^9/L), leukapheresis or exchange transfusion (in small children) may be considered. The Director of the Blood Bank should be consulted for this procedure.

12.4 Avascular Necrosis of Bone

Amend 3.0

Osteonecrosis of the bone, a known complication of treatment with corticosteroids, can be expected to occur in approximately 10-15% of patients, especially in those older than nine years of age.[122,123] This devastating complication may result in collapse of the articulating surface with subsequent pain and development of arthritis. Early detection of small lesions will permit intervention which may prevent pain and irreversible damage of the joints. In this study, all patients have MRI scans of the pelvis/hips and knees after each reinduction phase, at off therapy date, and as needed thereafter. Patients diagnosed with osteonecrosis (see section 7.8) will be referred to orthopedics. Any patient who develops symptoms of joint pain prior to or between scheduled MRI scans should have an MRI performed to rule out osteonecrosis or progression of this complication.

For patients who require surgical intervention, treatment will vary based on degree of progression, i.e., observation, core decompression, bone grafting and resurfacing hemiarthroplasty.[124-126]

12.5 Pancytopenias

Patients with prolonged (>3 weeks) unexplained anemia (hemoglobin <7 g/dl) or neutropenia (ANC <300/mm^3) during remission should be evaluated for B19 parvovirus infection or hemolysis or toxicity from non-chemotherapeutic agents (e.g., TMP/SMZ).

12.6 Nutritional Supplementation

Nutritional or vitamin therapies should not result in patients receiving more than the RDA for folic acid with dietary and supplement intake, to prevent interference with the effectiveness of methotrexate.

12.7 Drug Interactions

Amend 3.0

Because concurrent use of enzyme inducing anticonvulsants (e.g. phenytoin, phenobarbital, and carbamazepine) with antileukemic therapy has recently been associated with inferior EFS,[126a] every effort should be made to avoid these agents, as well as rifampin, which also induces many drug metabolizing enzymes. Gabapentin does not induce hepatic drug metabolizing enzymes and may be a suitable alternative anticonvulsant. Azole antifungals (fluconazole, itraconazole, **voriconazole,** and ketoconazole) **and the macrolide group of antibiotics (e.g.**

erythromycin, rifampin, and zithromax) may have potent inhibitory effects on drug-metabolizing enzymes, and the doses of some antileukemic drugs (e.g. vincristine, anthracyclines, etoposide) may need to be reduced in some patients on chronic azole antifungals **or antibiotics.** Consult Pharmacokinetics if long-term use of these interacting drugs is unavoidable.

13.0 TOXICITY AND COMPLICATIONS CRITERIA

Amend 1.0

13.1 Definitions of infection (See Appendix II)

Amend 1.0

13.2 Toxicity of treatment will be evaluated using the NCI guidelines (See Appendix III)

Amend 8.0 (added and deleted text)

14.0 OFFSTUDY AND OFF TREATMENT CRITERIA

14.1 Off Treatment Criteria

14.1.1 Development of unacceptable toxicity during treatment (with concurrence of the principal investigator)

14.1.2 Patients with severe congenital immunodeficiency (e.g. ataxia telangiectasia) or HIV infection will be taken off study and will receive alternate therapy (to prevent excessive toxicities) with curative intent.

14.1.3 Progressive Disease/Relapse

14.1.4 Second Malignancy

14.2 Off Study Criteria

14.2.1 Death

14.2.2 Refusal of therapy or noncompliance (with the concurrence of the principal investigator)

14.2.3 Found to be ineligible (e.g., incorrect diagnosis)

14.2.4 **Lost to Follow-up**

14.2.5 Withdrawal of Consent for Further Follow-up

15.0 DATA SAFETY MONITORING BOARD

Amend 2.0

The protocol progress will be reviewed and monitored by the St. Jude Children's Research Hospital Data Safety Monitoring Board (DSMB). Data summaries will be provided to the DSMB by the **Statistical Office** after review by the Principal Investigator. The data will include patient accrual, demographic summaries, grade 3/4 toxicities, major adverse events (i.e. deaths, relapses, second malignancies) and results of interim and final analyses of various endpoints as specified in the protocol. **The data are retrieved from the database and are reviewed at every Total XV meeting, which is generally held twice a month, and are reflected in the minutes, which are provided to the biostatistician.** When the appropriate number of patients have failed **or accrued**, triggering interim or final analysis**, the biostatistician will be informed by the PI. Should a safety stopping rule be exceeded, the protocol will be temporarily closed until the DSMB can review the situation**. The first report will be provided to the DSMB after all the patients enrolled in the first 6 months have completed remission

induction (i.e. ~8 months after enrollment of the first patient). At the DSMB meeting to review the protocol data, the PI and appropriate Co-Investigators will meet with the DSMB to discuss any relevant issues with the DSMB. The DSMB's report, summarizing their evaluation of the data, will be simultaneously forwarded to the PI, the IRB and the CMO. If the investigators disagree with the evaluation, a rebuttal will be made within 10 days to the DSMB, with a copy to the IRB and the CMO. The PI will inform the chair of the IRB and the CMO if a rebuttal will be made so that the IRB may choose to postpone review of the protocol until the rebuttal has been received. If there are no excessive, unexpected events observed during the first 6 months of the trial, subsequent reports to the DSMB will be made coincident with the continuing review report submitted to the IRB, if deemed appropriate by the DSMB and the IRB.

Amend 1.0 (deleted the PCP substudy)

16.0 BIOLOGIC STUDIES

16.1 Minimal Residual Disease

We will apply immunologic and molecular methods as previously described.[8,82,83] The leukemia markers commonly used to study minimal residual disease by 4-color flow cytometry are listed in the table below.

Immunophenotypic markers currently used to study MRD in children with ALL

ALL lineage	Phenotype	Frequency (%)†
B-lineage	CD19/CD34/CD10/TdT*	30-50
	CD19/CD34/CD10/CD22*	20-30
	CD19/CD34/CD10/CD38*	30-50
	CD19/CD34/CD10/CD45*	30-50
	CD19/CD34/CD10/CD13	10-20
	CD19/CD34/CD10/CD15	5-10
	CD19/CD34/CD10/CD33	5-10
	CD19/CD34/CD10CD65	5-10
	CD19/CD34/CD10/CD21	5-10
	CD19/CD34/CD10/CD56	5-10
	CD19/CD34/CD10/CD66c	10-20
	CD19/CD34/TdT/cytoplasmic μ*	10-20
	CD19/7.1	3-5
	CD19/p53	3-5
T-lineage	TdT/CD3	90-95
	CD34/CD3	30-50

* The use of these immunophenotypes for MRD studies relies mainly on differences in intensities of expression between leukemic lymphoblasts and normal lymphoid progenitor cells. The remaining combinations rely mainly on the aberrant expression of one of the markers.

†Proportion of childhood ALL cases in which one leukemic cell in 10^4 normal bone marrow cells can be detected with the listed immunophenotypic combination. Most cases express more than one combination suitable for MRD studies.

We will test whether proportions of detectable leukemic cells in peripheral blood samples correspond to levels of leukemia infiltration in bone marrow aspirates, a possibility suggested by our preliminary findings of quantifying residual disease in paired blood/marrow samples collected at remission date. In our initial experiments, we will study residual disease in peripheral blood and bone marrow samples collected at remission date. We expect approximately one-fourth of children to have detectable MRD at this stage of treatment. If these studies corroborate our preliminary observations, we will then investigate the correlation between levels of residual disease in paired samples collected at other time points during treatment.

Any child registered in Total XV whose leukemic cells are amenable to our assays will be eligible for testing. Peripheral blood samples (5-10 ml) will be taken at completion of induction therapy, during remission (e.g. day 1 of reinduction I and II), week 48 of continuation treatment, and before elective cessation of chemotherapy.

16.2 Somatic Cell DNA Alteration

Amend 1.0, 2.0, 4.0

Peripheral blood (10 ml) will be obtained at the start of consolidation, on day 8 of reinduction I **(pre-doxorubicin and with the 8-hour dexamethasone level)**, at the start of reinduction II, **at week 64,** at week 120, at week 146, and with each off-therapy visit up to 3 years. **White cells from samples collected for other tests (e.g., MTX blood levels at weeks 1 and 7 of consolidation and from asparagine levels at Reinduction), will be used for evaluating telomeres.** Leukocytes will be isolated, and cells will be cryopreserved or DNA, and RNA **and protein** will be isolated using standard techniques. DNA alterations will be assessed using previously published techniques,[91,92] or using other assays for DNA rearrangements or gene/**protein** expression that may be informative. In high-risk patients, an additional sample one week post etoposide therapy will be obtained.

16.3 Genetic Polymorphisms in Drug Metabolizing Enzymes

Leukocytes from blood samples during and after the HDMTX during consolidation (required for clinical plasma monitoring of MTX) will be pooled and used for DNA extraction by standard techniques. Genotyping for pharmacogenetic polymorphisms (e.g. methylene tetrahydrofolate reductase, thiopurine methyltransferase, mismatch and DNA repair enzymes, CYP3A4, glutathione transferases, glucocorticoid receptors, and p-glycoprotein)[100] will be assessed using molecular techniques in place in Pharmaceutical Department.[86,127,128] This DNA will also be used to assess Vitamin D-receptor polymorphisms. An aliquot of DNA from every patient who has signed 97BANK (or its succeeding banking protocol) will be available from the pharmacogenetics DNA sample to the Cell Bank laboratory.

16.4 Dexamethasone Pharmacodynamics

Dexamethasone pharmacokinetics will be assessed on Day 1 and Day 8 of reinduction I. Blood samples (2 ml each) will be drawn pre and at 1, 2, 4, and 8 hours from the morning dexamethasone doses on these days. In addition, *in vivo* pharmacodynamics will be assessed by measuring the serum cortisol during week 3 of consolidation (baseline), weeks 2 and 12 of continuation (3 days after last dose of dexamethasone) and days 1 and 8 of reinduction I (along with dexamethasone pharmacokinetics). In addition, heights are now recorded carefully monthly on a stadiometer (using the average of 3 replicate measures) and these data are entered in the AS400 and MILLI/Powerchart systems. Both cortisol and height change have been related to glucocorticoid plasma AUC previously.[129]

16.5 Determination of ALL Blast Sensitivity to Antileukemic Agents, *in vitro*

We will use a portion of the diagnostic bone marrow from each patient having an adequate number of cells (should be the majority of patients) and the stroma-supported immunocytometric assay (SIA) to determine the relative sensitivity and resistance to antileukemic agents, as we have done previously.[101,102] Such studies require only ~3 x 10^5 cells per well, 5 drug dilutions or 2 drugs plus a control in duplicate, 2 drugs plus control, for a total of 3.6 x 10^6 cells. Thus, samples should be available for the majority of patients (see sections 2.3, 17.3), underlying this large range of sensitivity to these agents, thereby providing insights that may improve the efficacy of these agents for treating childhood ALL. Germline polymorphisms will be determined from normal white blood cell DNA as described in 17.3.

17.0 CANCER CONTROL STUDIES

17.1 MRI, Neuropsychological, Quality of Life and Non-Invasive Cardiac Changes

Amend 1.0, 2.0

The first aim is to describe the natural history of changes in neuropsychological testing scores and quality of life associated with HDMTX treatment. Because most MRI changes are observed within the first few HDMTX administrations,[130,131] the first surveillance studies will be performed after only one dose of HDMTX **(between days 33 to 46 of Remission Induction)** as an early assessment before neurological changes should become evident. Subsequent surveillance studies will be performed after 3 doses (week **1 of reinduction** therapy) and 5 doses (week 7 of consolidation therapy) of HDMTX, at Week 48 and Week 120. Off therapy studies are conducted to define late effects, if any. This schedule has been developed for neurologically asymptomatic patients because neurologically symptomatic patients will be referred for neurological examination and imaging studies by their attending oncologist whenever the symptoms or signs should occur.

Amend 1.0, 2.0, Rev 3.1, 3.2, Amend 4.0

Study	1 Day 1 induction Post 0 HDMTX	2 Week 6 induction Post 1 HDMTX	3 Week 3 Consolidation	**Week 7 Consolidation**	4 Week **1, Reinduction I**	5 Week **22-24 after** Reinduction II	6 Week 48	7 Week 120	8 Week 146 (for boys)	9 Two year Post therapy
Psychology#		X			X			X	X	X
MRI-brain#		X**			X		X	X		X*
QOL		X		**X**			X	X	X	X
Homo-cysteine in CSF	X With IT Ara-C		X With IT MHA Weeks 1 and 3							
Cardiac Evaluation	X					X		X	X	X

*only in patients with abnormal MRI findings at week 120 of continuation therapy

**** Between days 33 and 46.**

 # Not required at Cook's

17.1.1 Psychological Testing

Amend 1.0

Testing will be performed following remission induction (Week 6), after the 5th dose of HDMTX (Week **1 of Reinduction I**) , at Week 120, at Week 146 (in boys), and two years post therapy. Psychological tests will be age-dependent, taking approximately 2 hours or less of the patient's time. For children 6 years of age or older, the testing will be comprised of a brief estimate of IQ[132,133] and academic achievement,[134] and measures of attention,[135] memory,[136] learning capacity,[137] and emotional adjustment.[138] For younger children, only a brief estimate of IQ will be possible.[139,140]

17.1.2 MRI

Amend 1.0, 2.0

MRI of the brain will occur at 4 intervals: after one HDMTX (**between days 33 and 46 of Remission Induction**), after 5 HDMTX (Week **1 of Reinduction I** therapy), Week 48, and Week 120 of continuation therapy. These studies were designed to yield longitudinal measures of white matter abnormalities during treatment and determine their relationship to neurocognitive performance. The first MRI examination at the end of induction is used as the baseline examination for the patient. The next MRI examination is **during Reinduction I** after all five courses of HDMTX. This will give an immediate measure of the occurrence and relative volume of white matter abnormalities which may be attributed to HDMTX exposure. The third MRI examination is at week 48 of continuation therapy (approximately one year after the second MRI). This time point will allow us to determine if the white matter abnormalities observed at the end of consolidation are resolving in the absence of addition HDMTX. The fourth MRI examination is at week 120 of continuation (end of therapy for female patients). By assessing all patients at this time point, we can determine if the white matter abnormalities have resolved and the relationship between the remaining volume of normal

appearing white matter and neurocognitive performance. Patients with white matter changes at week 120 will have an extra exam at 2 years off therapy to determine if the changes are permanent.

These abbreviated clinical MRI scans consist of T1 sagittal for localization followed by 3 mm thick contiguous transverse T1, T2/PD, and FLAIR images covering the entire cerebrum. These are followed by turbo-PAIR and turbo-spin echo imaging sets to evaluate quantitative T1 and T2 relaxation times of abnormal and normal-appearing white matter with two single sections at the level of the basal ganglia and the centrum semiovale. The full examination can be performed in approximately 40 minutes for children not requiring sedation and one hour for children requiring sedation. Most children six years of age and older can tolerate the procedure without sedation. All scans will be evaluated by a neuroradiologist for clinically relevant findings that will be captured in a data reporting form.

The T1, T2/PD, and FLAIR images will be analyzed with a hybrid neural network segmentation algorithm that has been developed and validated at our center specifically for quantification white matter changes.[141] Quantitative volumes (cc) of abnormal and normal appearing white matter will be determined from the segmented maps and normalized to brain volume. This method has been previously applied to investigate the impact of radiation dose, age at irradiation and time since irradiation on normal appearing white matter volumes in children surviving medulloblastoma.[142] Our subsequent studies have shown that white matter volumes in these patients are significantly related to neurocognitive performance.[142a]

Amend 2.0 (deleted neuro/EEG), Amend 4.0

17.1.3 Quality of Life Assessments

Quality of life will be measured at key points during and following the completion of treatment using a functional assessment (completed by the nurse who is most familiar with the patient), a self-report from patients 5 years of age and older, and a parent-report whose child is 5 years of age or older. The functional assessment will include ratings of vision, hearing, speech, ambulation, dexterity, emotion, cognition, and pain as measured by the McMaster University HU13 Health-Related Quality of Life Inventory (4 minutes).[143] Patients will complete the 23-item Pediatric Quality of Life Inventory[144] and the 27-item Pediatric Cancer Module (7 minutes) while parents complete the corresponding parent-report forms (7 minutes). **Patients who are 8 years of age and older will also complete two open-ended interview questions and a 5-item, recently developed subscale on meaning of being ill. [155]**

17.1.4 Homocysteine and folates

Amend 1.0, 4.0

A 2 ml aliquot of CSF, obtained with intrathecal treatment (before Day 1 of induction, Weeks 1 and 3 of consolidation treatment), and 4 ml blood will be placed in Homocysteine Collection tubes (to stabilize homocysteine and related compounds) with the pre, 23 and 42-hour plasma samples of the 1st and 2nd HDMTX during consolidation, sent to the pharmacokinetics lab, frozen and assayed subsequently for homocysteine, and homocysteic acid, **and folates**, in collaboration with Dr. J. Griener (Dallas Southwestern) **and with Dr. U. Jaehde, of University of Bonn.**

17.2 Prediction of Late Effects from Early Changes

The second Cancer Control aim is to determine if early changes on MRI or biochemical measures of excitatory amino acids are predictive of chronic MRI abnormalities, psychological, neurological, and quality of life decrements. This aim is addressed by methods of statistical correlation of the above surveillance measures. The approach to analysis is presented in the next section.

17.3 Bone Density Measurement

Amend 1.0, 4.0, 6.0

A CT bone density of the thoracolumbar spine (T11-L3) will be performed at diagnosis and at Week 120. Patients with a vertebral compression fracture at these sites will be excluded from the study. Bone density measurements will be obtained using the Mindwaves QCT Calibration phantoms and software (Mindwaves Software, Inc., South San Francisco, CA). The anticipated scan time is approximately 8 minutes, and is painless, noninvasive and the risk is **minimal**, roughly equivalent to the amount of x-ray exposure from one chest x-ray. Total effective radiation dose (28.8 μSv) compares favorably to a chest x-ray (50 μSv), round trip transcontinental flight (60 μSv), and monthly natural background exposure (200 μSv).[112]

Hepatic, renal, and thyroid function (free T4, T4, TSH) will be evaluated by blood tests. Vitamin-D-polymorphisms will be assessed as predictors for osteoporosis using somatic cell DNA collected during consolidation[145] (see Section 17.3).

Rev. 4.1, deleted text

Upon completion of all treatment, all patients will have dietary counseling and instructions of exercise. For patients whose bone density is between 0 and -2 standard deviations below the mean, annual bone density determination will be obtained for 5 years or until the density returns to normal. If during these annual evaluations, bone density remains stable or improves, no further intervention will be initiated. For those who have bone density below -2 standard deviations below the mean, they will be treated with vitamin D and calcium and referred to endocrinology clinic for further evaluation and treatment.

17.4 Detection of Osteonecrosis by MRI

Amend 2.0

Identification and monitoring of patients for the development of osteonecrosis will be accomplished by MRI of the hips and knees **after** completion of reinduction 1 and 2 and at the completion of therapy in asymptomatic patients as indicated in Section 12.4. Should patients present with symptoms suggesting development of osteonecrosis (avascular necrosis on bone), MRI will be obtained as clinically indicated (Appendix 1).

Amend 4.0, 8.0

17.5 The Calciuria Study

Putative risk factors for hypercalciuria include the dose, schedule, and choice of glucocorticoid. It is recommended, but not required, that a spot urine calcium, creatinine, and sodium be collected at diagnosis, during remission induction after completion of prednisone, before and after each reinduction phase, and at week 24 of continuation therapy. The urine measurements are timed to assess calciuria at the times it is expected to be highest (just after intensive glucocorticoid therapy), or lowest (just before the next phase of therapy that contains a glucocorticoid, and during continuation). This will allow identification of periods of highest risk, when low dietary calcium may be protective, and periods of lowest risk, when calcium supplementation may be safe.

Testing of spot second morning void urine for calcium and creatinine is inexpensive and non-invasive. Spot urine specimens have lower precision in children 4-10 years old[114k], but the more accurate 24-hour urine collection is more burdensome to families, more expensive, and not possible in children who are not toilet trained. A number of studies have compared spot to 24-hour urine collections in healthy children,[114k-114m] but none has evaluated their correlation in patients receiving glucocorticoids. Therefore, in children who are toilet trained, it is recommended, but not required that we will measure both the 24-hour and spot urine calcium at 2 time points to compare the spot urine results to the "gold standard" 24-hour results. At all other time points only spot urine samples will be evaluated as recommended. The incidence of hypercalciuria at each time point will be estimated and the 95% confidence interval calculated.

17.6 Non-Invasive Cardiac Evaluation

Amend 1.0, 2.0, 5.0, 7.0 (deleted text)

Study participants will have noninvasive cardiac evaluation periodically during and after their leukemia therapy. A baseline evaluation will be performed before initiation of anthracycline therapy. Subsequent evaluations will be performed after completion of Reinduction II (**weeks 22-24**) after administration of all anthracycline chemotherapy (110 mg/m^2 for low-risk and 230 mg/m^2 for high-/standard-risk); after completion of all antineoplastic therapy (week 120 for girls and week 146 for boys) and 2 years post-therapy. The study evaluation will comprise a **1) 2D scanning, color Doppler, and M-mode echocardiography and 2) 12-lead electrocardiography.**

18.0 STATISTICAL CONSIDERATIONS

Total XV has a number of primary objectives. Each will be addressed in the following sections as to design, sample size and statistical methodology for analysis. The refusal of participation in any of the research aims will not render the patient ineligible for Total XV; the patient will still be evaluable for other study aims including the estimation of event-free survival. The largest sample size requirement among all objectives will be the defining sample size for Total XV. No adjustment will be made for multiple questions among the primary objectives, but within an objective it may be appropriate to apply methods to control the type I error rate. Group sequential designs will be used to provide guidelines for any early stopping decisions for safety, efficacy and/or early publication of preliminary results. All primary analyses of randomized questions will be consistent with the "intent to treat" hypothesis. All eligible randomized patients will be included in analyses of randomized questions. To the extent possible, as indicated in the following sections, all eligible patients will be included in all analyses.

Methodology for analyzing data related to secondary objectives are presented, but in general no power calculations will be provided since the sample size for the trial is determined by achieving the primary objectives. Furthermore, if more appropriate statistical methods are identified or developed during the course of this trial then those methods will be used to analyze the data.

Amend 5.0
Amend 9.0 (added and deleted text)

Sample Size: The total planned accrual to this study is 300 **evaluable** patients. This sample size is dictated by therapeutic aim 1.1.2 as discussed in section 19.2.2. This sample size is further required to attain the pharmacological aim 1.2.1 as discussed in section 19.2.1. **Based on the experience with the first 400 patients, we estimate that approximately 62% will participate in the pharmacologic studies and have adequate samples for the analyses. Hence, we need to accrue approximately 480 patients for the study. Based on current accrual rate, and with the participation of Cook's Children Medical Center, we expect to complete the accrual by late August or early September 2007.**

Overall, 76% of the total enrollments have been randomized. Since Cook Children's Medical Center was added as a collaborating site, only 74% of the enrollments have been randomized. We have also been experiencing reduced randomization recently -- only 63% of the enrollment in the calendar year 2006 was randomized. In conjunction with the fact that not all samples from the randomized patients can be successfully assayed, we anticipate that at the best we can successfully assay 62% of the entire enrollment. Because 300 successful samples are required for the pharmacological aims, the entire enrollment needs to be increased to at least 480.

The estimated total enrollment of 400 in the previous amendment was based on a successful rate more optimistic than our recent experience.

The increment from 400 to 480 represents a 20% increase of the total sample size for the biomarker aims. This is certainly not a trivial increase and will substantially improve the statistical power for the tests of each individual biomarker. Consequently, the average power among the massive multiple tests for the biomarkers is then increased, which will in turn help substantially in reducing or controlling the false discovery rate. This principle was recently demonstrated in Pounds and Cheng (2005).[156]

For strata defined by immunophenotype and DNA index we will make the following assumptions: If immunophenotype is unknown at the time the randomization is to be executed then the immunophenotype will be assumed B-lineage. If DNA index is unknown at the time of randomization then the index will be assumed to be 1.0. Past experience indicate that few patients will fall into these unknown categories.

Amend 10.0

Sample Size Addendum (6/28/2007)

The sample size for this study will be increased with Amendment 10 to allow enrollment to continue until 510 participants are enrolled, or the successor study, TOTXVI is activated (whichever occurs first).

18.1 Safety Monitoring

Amend 2.0

18.1.1 During Induction

As indicated in section **19.3**, we will closely monitor toxicity during induction therapy including death and will use specific monitoring rules for seizures (grades 3 or 4), infections (grade 4), **disseminated fungal infections,** mucositis (grades 3 or 4), and delays in initiation of consolidation therapy. Due to the short induction period and, with the single exception of death, the toxic events do not represent competing events. Safety monitoring rules will be based on the binomial distribution. The acceptable baseline rates for these three toxicities are based on the experience with Total XIIIB, which has an excellent overall outcome. The monitoring rules will be interpreted as guidance for unacceptable toxicity and will be interpreted as indicating that consideration should be given to amendments in therapy if the stopping criteria are reached. **If any stopping threshold is reached, accrual will be suspended until the DSMB has had the opportunity to review the data.** Note that for safety monitoring one is less concerned with the significance level of the test and more concerned that the rule has relatively good power. All rules assume a total sample size of 300 patients and interim analyses are after 30, 60, 120, 180 and 240 patients. These times roughly correspond to six months, one year, two years, three years and four years of accrual. Note that the analyses will be conducted no earlier than two months after the last patient in a cohort is registered due to the time to complete induction therapy, collect the data and complete the analyses and write the report. The stopping boundaries are derived by the method of Xiong.152

Amend 9.0**, 10.0**

Death During Induction

Past experience by St. Jude and other groups using very intensive induction therapy suggest that a 2-3% mortality rate is to be expected. We will use modification of a group sequential plan to monitor for excessive death during induction. We will monitor against a baseline rate of 2% and will modify the study if there is evidence that the death rate exceeds 5%. The original plan had an overall significance level of **0.0542** with a power of **0.9843** to detect the unacceptably high rate. The modification reduces the stopping rule at the first two stages from 4 to 2 and from 5 to 3 deaths, respectively. This modification increases both power and significance level. This change was necessary, as no one would consider 3 deaths in the first 30 patients to be acceptable. The modified criteria are given in the following table. If the stopping criterion is reached, consideration will be given to either amending the induction therapy or stopping the trial.

Amend 9.0, 10.0

Interim Analysis	Sample Size (Patients)	*Modify if # Death ≥*
1	30	2
2	60	3
3	120	7
4	180	9
5	240	10
6	**300**	**12**
7	**360**	**13**
8	**420**	**14**
9	**480**	**16**

Amend 9.0, 10.0

Seizures

We will monitor against a baseline rate of 5% and will suggest modifying the study if there is statistical evidence that the seizure rate exceeds 10%. The overall significance level will be **0.0567** with a power of **0.9967** to detect the unacceptable higher rate.

Amend 9.0

Interim Analysis	Sample Size (Patients)	Modify if # Patients with Seizures ≥
1	30	**6**
2	60	**9**
3	120	**14**
4	180	**18**
5	240	**21**
6	**300**	**28**
7	**360**	**31**
8	**420**	**33**
9	**480**	**35**

With this sample size we would have more than 70% power to detect a difference between 5% and 8%. Thus we have good power for relatively small differences.

Amend 9.0, 10.0

Infections

We will monitor against a baseline rate of 10% and will suggest modifying the study if there is statistical evidence that the infection rate exceeds 15%. The overall significance level will be **0.0636** with a power of **0.9713** to detect the unacceptable higher rate.

Amend 2.0, 9.0, **10.0**

Interim Analysis	Sample Size (Patients)	Modify if # Patients with Grade 4 Infections ≥
1	30	**9**
2	60	**14**
3	120	**23**
4	180	**31**
5	240	**37**
6	**300**	**45**
7	**360**	**53**
8	**420**	**58**
9	**480**	**63**

Amend 9.0, 10.0

Mucositis

We will monitor against a baseline rate of 20% and will suggest modifying the study if there is statistical evidence that the mucositis rate exceeds 30%. The overall significance level will be **0.0560** with a power of .99**98** to detect the unacceptable higher rate.

Amend 9.0, 10.0

Interim Analysis	Sample Size (Patients)	Modify if # of Patients with Mucositis ≥
1	30	**14**
2	60	**23**
3	120	**39**
4	180	**53**
5	240	**65**
6	**300**	**84**
7	**360**	**96**
8	**420**	**106**
9	**480**	**117**

Delay in Initiation of Consolidation Therapy

Toxicity associated with excessive neutropenia combined with other toxicities during induction resulting in delays in therapy will be reflected in the time from the first dose of therapy (in the window) to the initiation

of consolidation therapy. The average time in Total XIIIB was 49.0 days with a standard deviation of 3.6 days (N=232). At each of the planned interim analyses for safety we will construct 95% lower bounds for the mean time from initiation of the window therapy to consolidation therapy. If any of these lower bounds exceeds 49 days, we will consider amending the induction/window therapy.

Amend 2.0. **10.0**

18.1.2 Over Entire Therapy Duration

Disseminated fungal infections

We will monitor against a baseline rate of 5% and will suggest modifying the study if there is statistical evidence that the disseminated fungal infection rate exceeds 10%. The overall significance level will be 0.0567 with a power of 0.9967 to detect the unacceptable higher level.

Amend 3.0, **9.0**

Only the first event in a given patient will be counted although we will closely monitor for repeated infections. Sample size (denominator) is the number of patients either who have recovered from the second re-induction or developed visceral fungal infection before recovery from second reinduction. The numerator is the number of the first events among all monitored patients (i.e. those in denominator). Accrual will be suspended if the number of events (numerator) is equal to or exceeds that corresponding to each sample size.

Amend 9.0, 10.0

Interim Analysis	Sample Size (Patients)	Modify if # Patients with Disseminated Fungal Infections ≥
1	30	6
2	60	9
3	120	14
4	180	18
5	240	21
6	300	28
7	360	31
8	420	33
9	480	35

18.2 Therapeutic Aims

18.2.1 To estimate the event-free survival (EFS) of children at least one year of age at diagnosis treated with Total XV risk-directed therapy and to monitor the molecular remission induction rate.

EFS will be measured from the date of complete response to the date of initial failure for patients who fail. Failure includes the traditional endpoints of failure to achieve a complete remission, relapse in any site,

secondary malignancy, and death during induction or remission. EFS will be measured to the date of last contact for patients who are failure free at the time of analysis. EFS is defined to be zero (0) for patients who die during induction therapy or fail to achieve a complete remission.

Since Total XV will have several new categories of events that are not considered failures in the traditional mode, but which result in patients being treated as if they had had a traditional failure, we will estimate a newly defined EFS_2. For this secondary EFS_2 any event that is treated as a "failure" will be considered as the similar, traditional failure. For example, patients in remission who test positive for TDT+ cells in the CSF for two consecutive taps will be classified as having had an isolated CNS failure at the date of the second tap.

Kaplan-Meier estimates of EFS will be provided and compared historically with those of Total XIIIA and XIIIB for patients who were at least one year of age at diagnosis. All eligible patients entered on Total XV will be included in these comparisons. Comparisons will be made both unstratified and stratified by Total XV defined risk groups. Some assumptions will be necessary as not all factors defining risk on Total XV will be available for all patients treated on Total XIIIA and B. These comparisons define the standards with which the final results of this study should be compared to assure continued progress towards the St. Jude goal of improving the cure rate of childhood ALL. No interim analyses for the purpose of decision making are planned for this study objective.

The following table shows the distribution of EFS for Total XIIIA and B as of December 31, 1999. Note that the classical definition of failure is used and no events such as two TDT+ CSF failures have been excluded. Only children who were at least one year of age at diagnosis are included.

Total (N)	1-year	2-year	3-year	4-year	5-year	6-year	7-year
XIII A (160)	97.5±1.2%	89.4±2.4%	86.3±2.7%	81.9±3.0%	78.4±3.5%	74.8±5.1%	74.8±9.4%
XIII B (237)	95.4±1.4%	90.0±2.2%	87.0±3.3%	81.2±5.6%			

Data for the molecular remission rate at the end of induction therapy are available for 113 children treated on Total XIIIB who were at least one year of age at diagnosis. The distribution is shown in the next table.

MRD Level	**< 0.01%**	**.01% - 0.1%**	**0.1% - 1.0%**	**≥ 1.0%**	**Total**
N (%)	84 (74.3%)	14 (12.4%)	9 (8.0%)	6 (5.3%)	113

Since the induction therapy has been modified significantly, we will monitor the accumulating molecular remission data from Total XV as a safety precaution. If there is evidence that the remission rate is statistically inferior to that of Total XIIIB, consideration will be given to amending Total XV induction therapy. Day 43-46 MRD levels are expected to be available for 90% of patients.

Amend 9.0, 10.0

Based on the above data, a 95% lower bound on the proportion of patients who would have MRD levels below .01% of day 43 is 0.675. Thus the safety monitoring rule will be derived around testing the hypothesis that the proportion of patients who have MRD levels below 0.01% on day 43-46 is at least 74% with power of **0.9699** to detect a drop to below 67%. Xiong's sequential design[152] will be used with an overall significance level of **0.0555**. The timing of the sequential analysis is approximately annually. If 60 patients are accrued annually then we expect 54 of these patients to have day 43-46 MRD levels assessed. Note that the data will not be available until the patients have completed day 43 of therapy.

Amend 9.0, 10.0

Interim Analyses	Sample Size (Patients)	Amend if # with MRD <0.01%
1	54	**29**
2	108	**65**
3	162	**103**
4	216	**142**
5	270	**187**
6	**330**	**233**
7	**390**	**276**
8	**450**	**318**
9	**486**	**337**

For example, if 65 (60.2%) or fewer of the first 108 patients evaluated at day 43-46 have MRD levels less than 0.01%, consideration will be given to amending the induction therapy.

18.2.2 To determine whether CNS irradiation can be safely omitted with early intensification of systemic and intrathecal chemotherapy.

This objective will be addressed with a historical comparison based on a Mantel-Haenszel test (unstratified) comparing patients who received prophylactic irradiation on Total XIIIA & B with comparable patients treated on Total XV. Since only a subset of patients who were in CCR at one year were irradiated on Total XIIIA & B, the comparison will be conditioned on the subset of the same group of patients who remain in CCR on Total XV therapy at "one year" of continuation therapy.

On Total XIIIA & B children with the following presenting features who were in remission at approximately week 56 of continuation therapy

would have been irradiated. This group of patients will be referenced as "HR-CNS."

- WBC ≥ 100,000
- Ph+
- CNS-3 at diagnosis
- T-Lineage and WBC ≥50,000

	HR-CNS	**Total**
XIIIA	34 (21.3%)	160
XIIIB	50 (21.1%)	237
Totals	84 (21.2%)	397

A subset of HR-CNS patients who present with CNS-3 disease or T-Lineage ALL and WBC ≥ 50,000 will be referenced as "VHR-CNS." The next table shows the distribution of VHR-CNS patients on the two studies that would have received irradiation at one year.

VHR-CNS: Total XIIIA & B				
	CNS-3	**T & WBC ≥ 50,000**	**Total (%)**	**XIIIA & B**
Enrolled	13	35	48 (12.1%)	397
Irradiated	10	26	**36 (9.1%)**	

As indicated below there were a variety of reasons why some patients, while meeting the criteria (HR-CNS), failed to receive irradiation. We expect similar findings on Total XV.

	Total Study		
Events	XIIIA (N = 34)	XIIIB (N = 50)	Totals (N = 84)
Induction Failure	1	2	3
BM Relapse	3	2**	5
CNS Relapse	0 (0%)	0 (0%)	0 (0%)
BM + CNS Relapse	1 (2.9%)	0 (0%)	1 (1.2%)
BM + Extramedullary	0	1	1
Died	0	0	0
2nd Malignancy	1	1***	2
Toxicity	0	1	1
BMT (Non-Protocol)	0	2	2
BMT	0	0	0
In Remission, not irradiated	1	12	13
Total not irradiated	7 (20.6%)	21 (42.0%)	28 (33.3%)
Too Early	0	0	0
Total Irradiated	27 (79.4%)	29 (58.0%)	56 (66.7%)

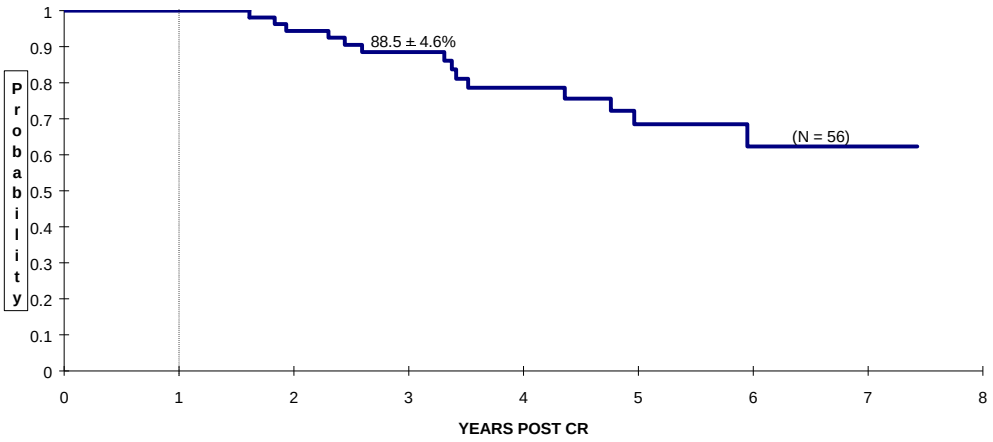
**: One Patient not irradiated as scheduled; failed on week 57

***: Not irradiated as scheduled; failed on week 68

Total XIIIB was amended later to omit irradiation for patients with B-lineage and WBC > 100,000. It was this amendment that resulted in most of the 12 patients in the "In Remission, not irradiated" category of Total XIIIB.

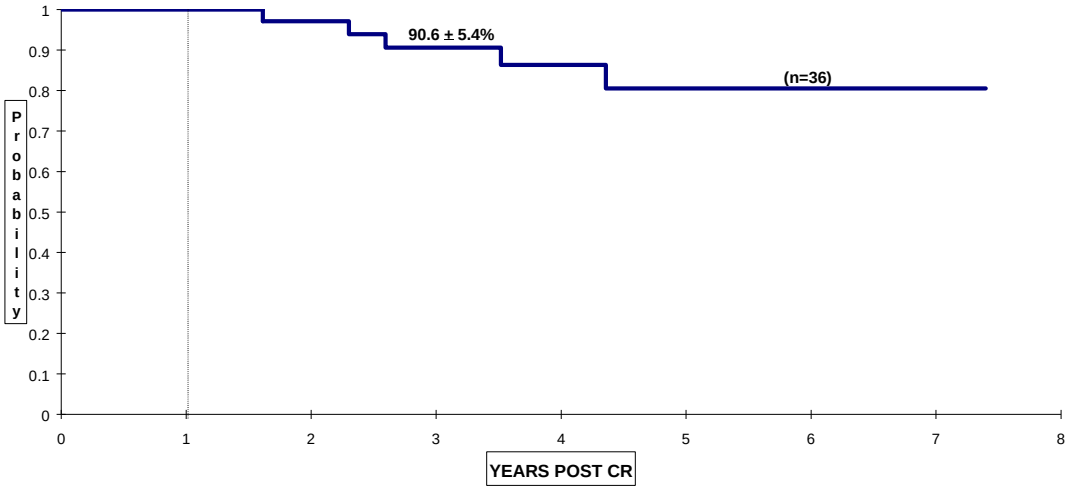
The following figure shows Kaplan-Meier estimates of duration of CCR for the 56 children in the HR-CNS group who were treated on Total XIIIA or B who were irradiated at approximately week 56 of continuation therapy. The 14 failures post irradiation are 6 hematological relapse, 1 CNS and mediastinal relapse and 7 second malignancies.

Duration of CCR for HR-CNS Patients irradiated on Total XIIIA & B



The following figure shows Kaplan-Meier estimates of duration of CCR for the 36 children in the VHR-CNS group who were treated on Total XIIIA or B who were irradiated at approximately week 56 of continuation therapy. The five failures post irradiation are 2 hematological relapse, 1 CNS and mediastinal relapse and 2 second malignancies.

Duration of CCR for VHR-CNS Patients Irradiated on Total XIIIA & B



We propose to test based on historical controls that the distributions of CCR post week 56 of continuation therapy does not differ whether or not the patients receive prophylactic irradiation. The alternative hypothesis is that the distribution of duration of CCR post week 56 of continuation therapy is superior when irradiation is given. The design will be based on Dixon and Simon.[153] The investigators of the Hematological Malignancies program discussed the two errors that could result from testing these hypotheses and concluded that both errors are equally important. The type I error would be concluding that irradiation was needed when in fact it is not required. The type II error would be concluding that irradiation was not required when in fact it was needed. The decision was made to conduct the investigation with $\alpha = \beta = 0.10$. We note that the HR-CNS patients who were irradiated prophylactically on Total XIIIA and B serve as a good control for dropping irradiation. These patients would still receive irradiation in many treatment protocols to date.[24] The VHR-CNS group of patients are still considered by some investigators at St. Jude as candidates to receive prophylactic irradiation.

We expect the following accrual of patients eligible to provide information relative to this study objective.

- Five years of accrual at 60 patients per year
- Total of 300 patients
- Experimental comparison groups
- HR-CNS: 13.6 patients per year with 8.2 per year in CCR at week 56 of continuation therapy
- VHR-CNS: 7 patients per year with 5.5 in CCR at week 56 of continuation therapy

The following table summarizes the power considerations for the two historical comparisons. In the HR-CNS group we will have 90% (80%) power to detect a decrease in the 3-year CCR rate from 88.5% to 77.0% (79%). For patients in the VHR-CNS group we will have 90% (80%) power to detect a drop in the 3-year CCR rate from 90.6% to 77.6% (80.1%). Only patients in the HR-CNS and VHR-CNS groups who are in CCR at week 56 of continuation therapy will be included in the comparisons.

1-Sided Test; α = 0.10; 5 Years Accrual; 2 Years Follow Up (post week 56)				
	3-year CCR Rate			
	RT	**No RT**	**Delta**	**Power**
HR-CNS	0.885	0.770	11.5%	**91.16%**
HR-CNS	0.885	0.775	11.0%	**89.30%**
HR-CNS	0.885	0.790	9.5%	**81.95%**
VHR-CNS	0.906	0.776	13.0%	**89.82%**
VHR-CNS	0.906	0.796	11.0%	**82.08%**
VHR-CNS	0.906	0.801	10.5%	**79.60%**

Amend 2.0 (deleted the 4 year interim analysis of HR-CNS)

A secondary endpoint of proportion of isolated CNS relapses will also be analyzed primarily for safety monitoring. CNS relapse will include the traditional definition as well as by TDT+ CSF in two consecutive taps. As of December 31, 1999 there have been no isolated CNS failures post week 56 of continuation therapy for the HR-CNS group of patients who received prophylactic irradiation. In HR-CNS (VHR-CNS) we will monitor for less than 1.79% (2.78%) versus more than 11.79% (12.78%) isolated CNS relapses during years 2 through 4 of continuation therapy on Total XV. Based on 40 (30) patients in CCR at week 56 in the HR-CNS (VHR-CNS) group and the exact binomial distribution we will have at least 86.6% (75.6%) power (α=0.0347 [0.0499]) to reject the null hypothesis of less than a 1.79% (2.78%) isolated CNS relapse rate. If more than 3 isolated CNS relapses are observed in the cohort of 40 patients, we will conclude that prophylactic irradiation is likely necessary and detailed analyses will attempt to refine the precise subgroup who require irradiation. If we see fewer than 3 isolated CNS relapses in 40 patients, we will conclude that prophylactic irradiation is not necessary. Note since the VHR-CNS patients are a sub-group of the HR-CNS patients and the decision rule is the same, the safety-monitoring rule essentially means monitoring the isolated relapse rate among the HR-CNS patients.

Amend 9.0

18.2.3 The assessment of the prognostic value of biologic markers and clinical features of the patients is an important component of analyzing and correctly interpreting outcome data for children treated with ALL. We propose to combine the outcome data for Total XV with previous Total protocols to investigate the prognostic implications of markers now known and those that will be identified in the future. Appropriate statistical methods of evaluating these factors will be utilized including overview analyses and stratified Cox regression models. **The biomarkers consist of identified polymorphisms in a large set of candidate genes, DNA microarray, and single-nucleotide-polymorphisms microarray. Thus, there will be hundreds of thousands of markers to screen and it is imperative to control or assess accurately the number of false positive findings, yet at the same time to maintain adequate statistical power. An increase in sample size by additional accrual will benefit this endeavor substantially, especially for the analyses of relatively infrequent but clinically important failures such as isolated CNS relapse.**

18.3 Pharmacological Aims

18.3.1 To identify whether prolonged (24 hour) intravenous infusions of HDMTX produce greater methotrexate polyglutamate (MTXPG) accumulation than short (4 hour) infusions 42 hours after 1 gm/m2 of HDMTX, stratified for lineage (T-vs B-lineage) and ploidy (hyperdiploid vs non-hyperdiploid B-lineage).

The following table summarizes the findings relative to MTXPGs 1-7 in the leukemic cells for patients randomized to the window therapy of Total XIIIA. A total of 167 patients were registered on study and 150 (90%) were randomized and successfully studied for MTXPGs. The window question for Total XV is to compare the HD MTX given in exactly the same way as in Total XIIIA with 1 g/m^2 HD MTX given as a four-hour infusion. The hypothesis is that the 24-hour infusion will produce MTXPGs 1-7 that are on the average twice that observed for the 4-hour infusion of 1g/m^2 of MTX. The randomization will be stratified by the four lineage-ploidy defined strata.

Total XIIIA MTXPGs 1-7 (pmol/10^9 cells)									
		HD MTX (1 g/m^2 over 24 hrs.)				**LD MTX**			
LINEAGE/PLOIDY	**N (%)**	**N**	**Mean**	**SD**	**Med.**	**N**	**Mean**	**SD**	**Med.**
B-lineage non-hyper.	101(60%)	52	1952	1656	1450	49	677	621	517
B-lineage hyper.	28 (17%)	13	3188	2215	2167	15	901	396	855
T-lineage non-hyper.	18 (11%)	8	649	312	605	10	211	120	206
T-lineage hyper.	3 (2%)	1				2			
Not Studied	17 (10%)								

The above data have been used to estimate sample sizes for the window question for Total XV. We estimated sample sizes by assuming the

analyses would be conducted using a t-test with equal variances. The variance estimates from the HD MTX data were used in the sample size calculations. Since only 90% of patients registered can be expected to be randomized and successfully studied for MTXPGs the sample size has been increased accordingly.

For children who present with B-lineage non-hyperdiploid ALL, we propose to test ($\alpha = 0.05$; two-sided test),

H_0: the mean MTXPGs 1-7 for 24-hour infusion is the **SAME** as the mean MTXPGs 1-7 for the 4-hour infusion

H_a: the mean MTXPGs 1-7 for 24-hour infusion is DIFFERENT FROM the mean MTXPGs 1-7 for the 4-hour infusion

By randomizing and successfully studying 162 patients with B-lineage non-hyperdiploid ALL, we will have 90% power for detecting a difference of 1000 pmol/10^9 cells (1950 pmol/10^9 cells for the 24-hour infusion of MTX versus 950 pmol/10^9 cells for the 4-hour infusion of MTX). Since only 60% of evaluable patients are expected to present with B-lineage non-hyperdiploid ALL and be successfully studied for MTXPGs, we propose a total accrual to Total XV of 300 patients.

By entering 300 patients on Total XV we will randomize approximately 44 patients with B-lineage hyperdiploid ALL. This sample size will provide approximate 79% power to detect 2-fold increase (difference) in mean MTXPGs 1-7 for the 24-hour infusion and 80% power to detect a mean difference of 1900 pmol/10^9 cells (3200 pmol/10^9 cells for the 24-hour infusion of MTX versus 1300 pmol/10^9 cells for the 4-hour infusion of MTX).

By entering 300 patients on Total XV we will randomize approximately 28 patients with T-lineage non-hyperdiploid ALL. This sample size will provide approximate 77% power to detect a 2-fold increase (difference) in mean MTXPGs 1-7 for the 24-hour infusion and 80% power to detect a mean difference of 330 pmol/10^9 cells (660 pmol/10^9 cells for the 24-hour infusion of MTX versus 330 pmol/10^9 cells for the 4-hour infusion of MTX).

18.3.2 To determine the relation between MTXPG accumulation in leukemic lymphoblasts and antileukemic effects, as measured by the inhibition of *de novo* purine synthesis, and by the decrease in circulating blasts during the 4 days after initiation of single-agent high-dose methotrexate treatment.

We will correlate the degree of MTXPG accumulation in leukemic lymphoblasts with antileukemic effects, as measured by the inhibition of *de novo* purine synthesis (DNPS), and the decrease in circulating blasts

during the four days after initiation of single-agent high-dose methotrexate treatment.

General linear models[147] will be used to evaluate the relationships among $MTXPG_{4-6}$, percent change in DNPS, and percent decrease in circulating blast cells. Whether evidence exist that the multivariate distributions differ between lineage and genetic defined subsets of children with ALL will be tested using Hotelling's T^2 statistic. Since not all patients present with circulating blast cells, a second set of analyses will be conducted on all patients by deleting the measure of decrease in circulating blast cells. Due to the relatively large sample size we do not anticipate statistical difficulties with this aim. We expect at least 270 patients will have all assays completed and of these 84% (based on Total XIIIA & B experience) will present with circulating blasts.

18.3.3 General linear models will be used to evaluate relationships between assessment of pharmacologic variables (e.g., RFC, FPGS, GGH) and MTXPG concentrations.

Amend 1.0 (deleted infectious disease aims)

18.4 Biologic Aims

18.4.1 To determine whether levels of minimal residual disease in peripheral blood (PB) reflect those measured in the bone marrow (BM) by immunologic or molecular techniques.

The key question here is whether PB can replace BM as the source of tissue for measuring MRD. MRD is to be measured by both immunologic (IM) and molecular (MB) techniques. At the end of induction, patients will have bone marrow and peripheral blood assessed by both techniques. Either method applies to approximately 90% of patients. All patients who have BM positive for MRD by either method will have BM and PB examined again on days one of reinduction I and II.

For each technique the generalized Kappa statistic will be used to compare agreement between BM and PB. MRD results are ordinal with two categories, no evidence, and $\geq 1x10^4$.

A second objective is to compare the two techniques for samples (BM and PB, jointly) for which both are applicable. The rate of MRD will be analyzed using a generalized linear mixed-effects model, which takes into account the correlation between locations (in peripheral blood or in bone marrow) and between techniques (IM and MRD) using all the data. This model also permits the estimation of variance components and agreement coefficients.[150,151]

The investigation of MRD measured at days one of reinduction I and II presents some difficult problems and newer statistical approaches may be

available by the time the data matures. This investigation will be conditioned upon patients who are positive at the end of induction and will represent few patients. We expect that at most 25% of patients will test positive by one of the two methods. These data are likely to include informative censoring as patients who have the higher MRD levels may fail prior to the second reinduction. Initially the mixed effect model discussed above will be used to analyze the data acknowledging limitations due to small sample sized and skewed data.

18.4.2 To assess the degree of DNA damage in somatic cells (leukocytes) during treatment.

Somatic cell DNA damage will be compared between standard risk patients (who receive more mutagenic agents) and low risk patients (who receive largely antimetabolite-based therapy) at identical times during therapy (pre and post anthracyclines during continuation therapy). Patients in the high-risk arm will be analyzed separately. The measure of somatic cell DNA damage is ordinal and in initial analyses will be treated as dichotomous. The time points will remain fixed for all patients. The longitudinal data will be analyzed to determine whether there are differences between treatment groups, time and interaction between group and time, utilizing the proportional odds model. If subsequent analyses require investigations using continuous covariates and if the missing values are missing at random, then a random effects proportional odds model will be used. The effect of non-random missing values will be examined as well.

18.4.3 To explore whether genetic polymorphisms of enzymes important in metabolism of antileukemic agents (e.g. methylenetetrahydrofolate reductase, thiopurine methyltransferase, glutathione transferases) are correlated with MTX pharmacology in lymphoblasts, clinical toxicities and long-term outcome.

This exploratory objective will be addressed with analyses utilizing failure-time models and longitudinal models of categorical data.

18.4.4 To explore whether the development of anti-asparaginase antibodies or CSF depletion of asparaginase is correlated with acute toxicities and long-term outcome.

Time dependent covariates in a Cox life table regression model will be used to explore relations between development of anti-asparaginase antibodies and CSF depletion of asparagine and event-free survival. Investigation of relationships with acute toxicities will utilize longitudinal models.

18.5 Cancer Control Aims

Because the clinical importance of the MRI changes are unknown, these measures will be blinded to the physicians except for patients where clinical evidence of neurologic problems exist. Only after Total XV will we begin to understand the relevance of these changes on the clinical outcome. However, an interim analysis will be performed after the first 100 patients are enrolled to determine if the studies should be terminated early, continued, or conducted more frequently.

Amend 2.0, 4.0

18.5.1 To assess the relation between MRI changes of brain (especially white matter abnormalities) from HDMTX (2.5 gm/m^2 versus 5 gm/m^2 dosage) and intrathecal treatment, cognitive deficits, CSF levels of homocysteine **or folates** and diminished quality of life.

Random effects models will be used to characterize the change in white matter volumes, psychological parameters and quality of life indices. Analyses of these relations will account for possible biases resulted from an early stopping of the trial. The associations between MRI changes, cognitive deficits, CSF homocysteine and quality of life indices over time will be assessed using a random-effects model with time-dependent covariates.

Amend 2.0

18.5.2 To investigate whether early MRI changes are related to late MRI abnormalities, cognitive deficits, and diminished quality of life.

We shall assess how early MRI changes (e.g., the white matter volume) are associated with the changes at the off therapy date. Both the changes will be analyzed via a semi-parametric generalized linear model with covariates such as type of lineage or genotype assessed. Robust-variance estimator will be used for hypothesis testing and constructing confidence intervals of the changes in white matter volume.

Amend 2.0

18.5.3 To correlate changes in MRI, cognitive deficits and diminished quality of life with selected pharmacokinetic variables.

Methods similar to those described above will be used to investigate the correlations.

18.5.4 To investigate the incidence and risk of osteopenia/osteoporosis in patients receiving intensive antimetabolites and dexamethasone therapy and to explore whether dexamethasone pharmacokinetics, pharmacogenetics or pharmacodynamics are related to its risk.

Amend 4.0 (deleted text)

18.5.5 To describe and model cardiac function overtime in children with ALL, descriptive statistics and unbalanced repeated measures models will be utilized to correlate clinical and treatment parameters with cardiac function assessed by non-invasive methodologies.

Amend 4.0

18**.5.6 To determine the incidence and timing of hypercalciuria during treatment of ALL,** descriptive statistics along with longitudinal and repeated measure models will be used.

Rev 4.1

19.0 ADVERSE EVENT REPORTING

19.1 Adverse Event Reporting

Adverse events will be monitored and scored according to the NCI CTC, version 2.0. A copy of the CTC 2.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov).

All serious and unexpected adverse events that occur during treatment and for 30 calendar days after treatment must be reported in writing to the St. Jude PI within 2 working days of knowledge of the event at the address below:

Ching Hon Pui, MD
Department of Hematology/Oncology
St. Jude Children's Research Hospital
332 N. Lauderdale St.
Memphis, TN 38105
Phone: 901-495-3335
FAX: 901-521-9005
E-mail: Ching-Hon.Pui @Stjude.org

A Serious Adverse Event (SAE) refers to any event in which the outcome:

- Is fatal
- Is life-threatening
- Causes permanent disability or incapacity
- Is a congenital anomaly, cancer, or overdose
- Causes or prolongs inpatient hospitalization

An unexpected adverse event refers to those not identified in their specificity or severity in the current risk documents (e.g., investigator's brochure or package insert) or through clinical practice.

For the purposes of this protocol, the following events will NOT be considered serious or unexpected adverse events:

- Hospitalization for treatment related febrile neutropenia
- Hospitalization for expected complications of treatment or expected toxicities of the commercially available agents used in this study (except for grade 4 non-hematologic toxicities).
- Hospitalization for treatment of expected signs or symptoms of disease complications or progression of disease
- Death unequivocally related to disease progression.

Amend 7.0

Typhilitis and thrombosis/embolism are expected toxicities.

Reporting Requirements

All participating investigators are responsible for submitting annual continuing review reports, and serious and unexpected adverse event information to their Institutional Review Board (IRB)/Ethics Committee according to local requirements.

Unexpected death while a patient is receiving treatment on this protocol, and for 30 days after protocol therapy is discontinued, or any death more than 30 days after protocol treatment, but which is felt to be treatment related, will be reported in writing to the St. Jude PI and IRB within 48 hours of knowledge of the death.

Serious and unexpected adverse events must be reported to the St. Jude PI and IRB within 10 calendar days of knowledge of the event. Grade 3 hematologic toxicity need not be reported.

The first occurrence of a toxicity not previously known (no matter what grade) that may be due to protocol treatment will be reported to the St. Jude PI and institutional IRB within 10 calendar days of knowledge of the event.

19.2 Reporting to the IRB

Amend 1.0

In addition to the annual progress reports to the IRB, toxic deaths on study will be reported to the IRB within 24 hours and serious and unexpected toxicities within 10 days. IRB will also be informed should the following adverse events exceed the thresholds: induction deaths, grade 3 or 4 seizure; grade 4 infections, grade 3 or 4 mucositis and delay in initiation of consolidation therapy (see Section **18.1**).

20.0 DATA MANAGEMENT SYSTEMS

Data Management will be supervised by the Hematology/Oncology Data Management Supervisor, working with Dr. Pui or his designee, in concert with the IS department. Detailed Data Collection Forms, on which all protocol-specific data and all grade 3 or 4 toxicity will be recorded by the data manages, have been developed. Each page of the forms has a physician signature line; all forms will be reviewed with the attending physician, and all questions will be directed to the PI and reviewed at regularly-scheduled working meetings. The attending physicians (or their designees) are responsible for keeping up-to-date roadmaps in the patient's primary SJCRH medical chart.

Data from the Data Collection Forms will be entered into the Progedd database (or its applicable successor as implemented by IS), and regular (at least monthly) summaries of toxicity will be generated for the PI and the department of Biostatistics to review. Biostatistics will work with the P.I. in generating the progress reports for the IRB and the DSMB.

Amend 1.0, 5.0

The CPDMO, which is separate from the Data Management Office, will conduct source document verification of eligibility of all SJCRH cases, performed within two weeks of completion of enrollment and during a monitoring visit for outside sites. This will include verification of appropriate documentation of consent. Monitoring

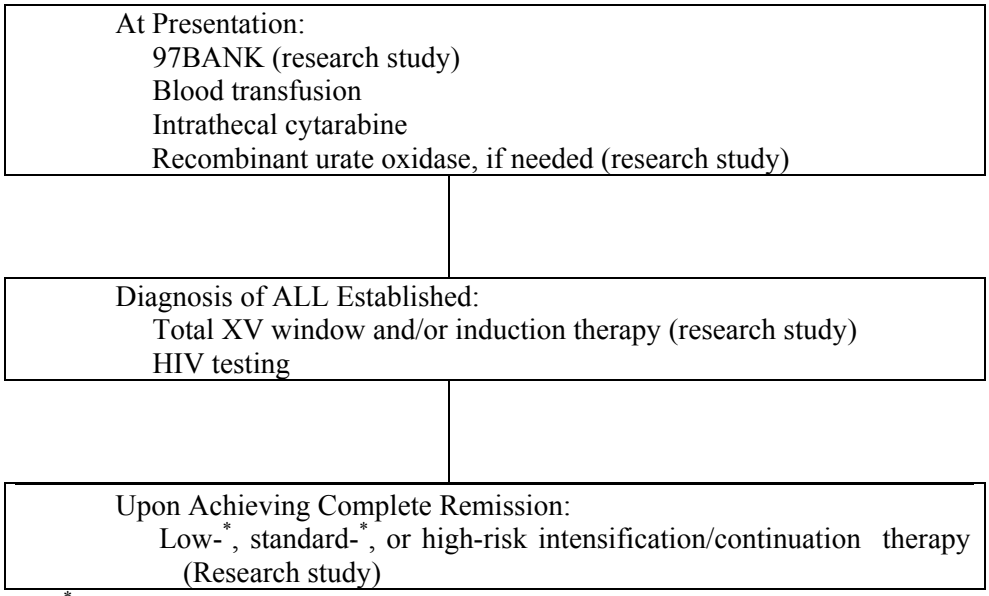
of timeliness of adverse events and serious adverse event reporting will be done as events are reported. Monitoring of CDUS elements, adverse event reporting, and compliance with the conduct of the protocol will be conducted according to the DSMP plan recommended schedule for this study.

21.0 INFORMED CONSENT PROCESS

Amend 1.0, 2.0

The process of informed consent for Total XV will follow institutional policy. The informed consent process is an ongoing one that begins at the time of diagnosis and ends after the completion of therapy. Informed consent should be obtained by the attending physician or his/her designee, in the presence of at least one non-physician witness. Initially, informed consent will be sought for the 97BANK protocol (research study), blood transfusion (if needed), the first intrathecal therapy with cytarabine (if the diagnosis of leukemia is certain either by referral bone marrow smears or blood smears at SJCRH), and treatment with recombinant urate oxidase, if needed (research study). After the diagnosis of ALL is established, we will invite the patient to participate in the Total XV window and/or induction therapy, as well as obtain standard medical consent for HIV testing. After the patient attains complete remission, we will seek informed consent for their participation **in** risk-directed intensification/continuation therapy (low-, standard- or high-risk according to presenting features, treatment response and the level of minimal residual leukemia). Rare patients with low- or standard-risk leukemia may be reassigned to high-risk therapy due to persistently high minimal residual leukemia (see Section 5.3). These patients will be asked to re-consent with the high-risk therapy consent. The timeline for various informed consents is indicated in the figure below.

Amend 2.0



*Rare patients will subsequently be treated with high-risk therapy due to the persistence of minimal residual disease, per protocol.

Throughout the entire treatment period, patients and their parents receive constant education from health professionals at SJCRH and are encouraged to ask questions regarding alternatives and therapy. All families have ready access to chaplains, psychologists, social

workers, and the St. Jude ombudsperson for support, in addition to that provided by the primary physician and other clinicians involved in their care.

We will also obtain verbal assent from children 7 to 14 years old and written assent for all patients ≥14 years of age.

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Amend 1.0 (deleted references 62 to 72 regarding infectious disease aims)

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Amend 2.0

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Remission Induction Pretreatment

Use New ALL Standard orders: enter Tissue Banking Protocol (97 Bank); Hx, PE, hx for use of allopurinol, steroids or other antileukemic drugs; CBC diff, coags, lytes, BUN, creatinine, LDH, uric acid, bilirubin, SGOT, SGPT, calcium, phosphorous, magnesium, total protein and albumin; CXR, BM for morphology, cytochemistry, immunophenotyping, cytogenetics, DNA index, molecular diagnosis; NP cx for S. pneumoniae**;** photo; others as clinically indicated, e.g. sickle cell prep, hemoglobin electrophoresis and G6PD screen for black children; varicella titer; Hepatitis B antigen; HIV (with informed consent); EBV, TOXO, CMV titers.

Amend 2.0, Rev 3.1, Amend 4.0, Amend 5.0, 8.0

1 LP, IT **AraC**; Start MTX 1400; PE, CBC diff, URA, Spot urine, lytes, Ca, P, Mg, MTX PK (8, 9, 12, 14)	2 complete MTX PE, CBC diff, URA, lytes, Ca, P, Mg, MTX PK	3 PE, CBC diff, URA, lytes, Ca, P, Mg, MTX PK †42 hr BM LV ___ mg	4 PE, CBC diff, URA, lytes, Ca, P, Mg LV ___ mg	5 PE, CBC diff, URA, lytes, Ca, P, Mg Pred, bili, SGPT VCR Dauno (**2**, 6)	6 PE, CBC diff, URA, lytes, Ca, P, Mg, Pred ASP	7 Pred, CBC, diff, URA, lytes, Ca, P, Mg
8 Pred ASP (*)ITMHA	9 Pred	10 Pred ASP	11 Pred	12 CBC diff Pred VCR ASP Dauno	13 Pred	14 Pred ASP
15 Pred	16 Pred ASP	17 Pred	18 Pred	19 †BM CBC diff Pred VCR *ITMHA (ASP) (6,8) TMP-SMZ	20 Pred TMP-SMZ	21 Pred (ASP) TMP-SMZ
22 Pred	23 Pred (ASP)	24 Pred	25 Pred	26 †BM (*)ITMHA CBC diff Pred VCR Cyclo 6MP TMP-SMZ **‡Imatinib**	27 Pred TMP-SMZ Ara-C 6MP **‡Imatinib**	28 Pred TMP-SMZ Ara-C 6MP **‡Imatinib**
29 Pred Ara-C 6MP **‡Imatinib**	30 Pred Ara-C 6MP **‡Imatinib**	31 Pred 6MP **‡Imatinib**	32 Pred 6MP **‡Imatinib**	33 Stop pred CBC diff, bili, TMP-SMZ, 24-hour urine, Spot urine, 6MP (6, **10)** **‡Imatinib**	34 TMP-SMZ Ara-C 6MP **‡Imatinib**	35 TMP-SMZ Ara-C 6MP **‡Imatinib**
36 Ara-C 6MP **‡Imatinib**	37 Ara-C 6MP **‡Imatinib**	38 6MP **‡Imatinib**	39 6MP **‡Imatinib**	40 CBC diff TMP-SMZ **(11)** **‡Imatinib**	41 TMP-SMZ **‡Imatinib**	42 TMP-SMZ **‡Imatinib**
43**‡Imatinib**	44**‡Imatinib**	45**‡Imatinib**	46 †BM (see consolidation calendar) TMP-SMZ (4) **‡Imatinib**			

Amend 5.0 (13 and 14 deleted from footnote)

*IT methotrexate + hydrocortisone + cytarabine; (*)Patients with the following features will received additional triple intrathecal treatment on days 8 and 26: CNS-3 status (i.e., ≥ 5 WBC/μL of CSF with blasts or cranial nerve palsy), CNS-2 status (<5 WBC/μL of CSF with blasts), Traumatic status (>10 RBC/μL of CSF with blasts), T-cell with WBC >50 x 10^9/L, WBC >100 x 10^9/L, Presence of Philadelphia chromosome, *MLL* rearrangement or near haploidy

†All bone marrow samples for MRD (except 42 hr on Day 3). If ≥ 5% BM blasts Day 19, 3 doses of L-asparaginase on days 19, 21, and 23. Day 26 BM in patients with any blasts on day 19.

Special Studies: (1) blood chemistries (Chem 18 profile); (2) somatic DNA; (3) 6TGN or TPMT; (4) blood for MRD; (5) MRI of hips ; (6) asparaginase studies (blood); (7) **deleted;** (8) CSF and plasma asparagines (Not required at Cook's); (9) CSF for homocysteine/folates and plasma homocysteine; (10) MRI of brain(Not required at Cook's) (to be done **between days 33 and 46** before remission procedures); (11) psychology (not required at Cook's), and quality of life; (12) QCT for bone density (to be done in the first week); (15) serum cortisol; (16)

dexamethasone pharmacokinetics; (17a) **Recommended-**24-hour for measurement of calcium, creatinine, and sodium (not required at Cook;s); (17b)**Recommended**- Spot urine for measurement of calcium, creatinine, and sodium (not required at Cook's).

CBC with differential is needed daily for the first week and then **weekly**.

‡ **Only Ph+ patients**

Amend 1.0, 2.0, Rev 3.1,3.2, Amend 4.0, Amend 5.0**,** 8.0

Consolidation

End of Induction-MRD response: BM aspirate will be performed around day 46 of remission induction when ANC has recovered to ≥300/mm^3 and platelet count to ≥50 x 10^9/L. If the date falls on Saturday or Sunday, the procedure may be performed on Friday or Monday, respectively. MRD level will be determined in this bone marrow sample. Poor response will be defined as MRD level ≥ 0.01% lymphoblasts by either immunologic or molecular assay. If both assays are unsuccessful or the sample is inadequate, the MRD will be assumed to be negative. If the result should become available later and disclose a level ≥ 0.01%, the provisional low-risk case will then be classified as standard-risk or high-risk accordingly, and will receive subsequent HDMTX at a higher dosage (i.e., 5 gm/m^2).

1 *IT CBC diff, HDMTX #2 6MP,(1, 2, 9) ‡**Imatinib**	2 6MP, ‡**Imatinib**	3 6MP LV ___ mg ‡**Imatinib**	4 6MP LV ___ mg ‡**Imatinib**	5 6MP ‡**Imatinib**	6 6MP ‡**Imatinib**	7 6MP ‡**Imatinib**
8 CBC diff 6MP ‡**Imatinib**	9 6MP ‡**Imatinib**	10 6MP ‡**Imatinib**	11 6MP ‡**Imatinib**	12 6MP ‡**Imatinib**	13 6MP ‡**Imatinib**	14 6MP ‡**Imatinib**
15 *IT CBC, diff, HDMTX #3 6MP (1, 9, 15) ‡**Imatinib**	16 6MP ‡**Imatinib**	17 6MP LV ___ mg ‡**Imatinib**	18 6MP LV ___ mg ‡**Imatinib**	19 6MP ‡**Imatinib**	20 6MP ‡**Imatinib**	21 6MP ‡**Imatinib**
22 6MP ‡**Imatinib** CBC, diff	23 6MP ‡**Imatinib**	24 6MP ‡**Imatinib**	25 6MP ‡**Imatinib**	26 6MP ‡**Imatinib**	27 6MP ‡**Imatinib**	28 6MP ‡**Imatinib**
29 *IT CBC, diff, HDMTX #4 (1) 6MP ‡**Imatinib**	30 6MP ‡**Imatinib**	31 6MP LV ___ mg ‡**Imatinib**	32 6MP LV ___ mg ‡**Imatinib**	33 6MP ‡**Imatinib**	34 6MP ‡**Imatinib**	35 6MP ‡**Imatinib**
36 CBC, diff, 6MP ‡**Imatinib**	37 6MP ‡**Imatinib**	38 6MP ‡**Imatinib**	39 6MP ‡**Imatinib**	40 6MP ‡**Imatinib**	41 6MP ‡**Imatinib**	42 6MP ‡**Imatinib**
43 *IT CBC, diff, HDMTX #5 6MP **(2,)** ‡**Imatinib**	44 6MP ‡**Imatinib**	45 6MP LV ___ mg ‡**Imatinib**	46 6MP LV ___ mg ‡**Imatinib**	47 6MP ‡**Imatinib**	48 6MP ‡**Imatinib**	49 6MP ‡**Imatinib**
50 CBC, diff, 6MP ‡**Imatinib**	51 6MP ‡**Imatinib**	52 6MP ‡**Imatinib**	53 6MP ‡**Imatinib**	54 6MP ‡**Imatinib**	55 6MP ‡**Imatinib**	56 6MP ‡**Imatinib**

Amend 5.0 (13 and 14 deleted from footnote)

*IT methotrexate + hydrocortisone + cytarabine, to be given on days 1,**15, 29, and 43**; see protocol section 6.3 for criteria for delaying 3rd IT and HDMTX/6MP.

Special Studies: (1) blood chemistries (Chem 18 profile); (2) somatic DNA; (3) 6TGN or TPMT; (4) blood for MRD; (5) MRI of hips ; (6) asparaginase studies (blood); (7) **deleted**; (8) CSF and plasma asparagines (not required at Cook's) ; (9) CSF for homocysteine/folates and plasma homocysteine; (10) MRI of brain (not required at Cook's); (11) psychology(not required at Cook's), and quality of life; (12) QCT for bone density; (15) serum cortisol; (16) dexamethasone pharmacokinetics; (17a) **Recommended-**24-hour for measurement of calcium, creatinine, and sodium (not required at Cook's); (17b) **Recommended-**Spot urine for measurement of calcium, creatinine, and sodium (not required at Cook's)

CBC with differential is needed weekly.

‡ **Only Ph+ patients**

Amend 1.0, 2.0, rev 3.1,3.2, Amend 4.0**,** Rev. 4.1, Amend 5.0, Amend 6.0, Revision 6.1, 8.0

TREATMENT SCHEMA and Special Laboratory Tests During Continuation Therapy

	Standard/High Risk		**Low Risk**	
Week		**Special Studies**		**Special Studies**
1	**DEX + DOX + VCR + 6MP + ASP +‡Imatinib**	**1**	**6MP + DEX + VCR**	
2	**6MP + ASP+‡Imatinib**	**15**	**6MP + MTX**	**15**
3	**¥6MP + ASP+‡Imatinib**		**6MP + MTX**	
4	**DEX + DOX + VCR + 6MP + ASP+‡Imatinib**	**1**	**6MP + DEX + VCR**	
5	**6MP + ASP+‡Imatinib**		**6MP + MTX**	
6	**6MP + ASP+‡Imatinib**		**6MP + MTX**	
7	***†Reinduction I (DEX + DOX + VCR + ASP+‡Imatinib)**	**1, 3, 4, 6, 8, 10,11a,15, 16, 17a,b**	***†Reinduction I (DEX + VCR + Doxo + Asp x 3)**	**1, 3, 4, 6, 8, 10,11a,15, 16, 17a, b**
8	**Reinduction I (DOX + VCR + ASP+‡Imatinib)**	**1, 2, 15, 16**	**Reinduction I (VCR + ASP x 3)**	**2, 15, 16**
9	**Reinduction I (DEX + VCR + ASP+‡Imatinib)**	**17b**	**Reinduction I (DEX + VCR + ASP x 3)**	**17b**
10	**6MP + ASP+‡Imatinib**		**6MP + MTX**	
11	**DOX + VCR + 6MP + ASP+‡Imatinib**	**1**	**6MP + MTX**	
12	***6MP + ASP+‡Imatinib**	**(5), 15**	***6MP + MTX**	**(5), 15**
13	**6MP + ASP+‡Imatinib**	**(5)**	**6MP + MTX**	**(5)**
14	**DEX + DOX + VCR + 6MP + ASP+‡Imatinib**	**1,(5)**	**6MP + DEX + VCR**	**(5)**
15	**6MP + ASP+‡Imatinib**		**6MP + MTX**	
16	**6MP + ASP+‡Imatinib**		**6MP + MTX**	
17	***†Reinduction II (DEX + VCR + ASP+‡Imatinib)**	**1, 2, 3, 4, 6, 8, 17b**	***† Reinduction II (DEX + VCR + DOXO + ASP x 3)**	**1, 2, 3, 4, 6, 8, 17b**
18	**Reinduction II (VCR + ASP+‡Imatinib)**		**Reinduction II (VCR + ASP x 3)**	
19	**Reinduction II (DEX + VCR + HD Ara-C + Asp+‡Imatinib)**	**1, 17b**	**Reinduction II (DEX + VCR + ASP x 3)**	**1, 17b**
20	**‡Imatinib**		**6MP + MTX**	
21	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
22	**6MP + MTX+‡Imatinib**	**(5)**	**6MP + MTX**	**(5)**
23	**Cyclo + Ara-C+‡Imatinib**	**(5)**	**6MP + MTX**	**(5)**
24	***DEX + VCR+‡Imatinib**	**(5), 17b**	***6MP + DEX + VCR**	**(5), 17b**
25	**6MP+ MTX+‡Imatinib**		**6MP + MTX**	
26	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
27	**Cyclo + Ara-C+‡Imatinib**		**6MP + MTX**	
28	***DEX + VCR+‡Imatinib**		**(*)6MP + DEX + VCR**	
29	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
30	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
31	**Cyclo + Ara-C+‡Imatinib**		**6MP + MTX**	
32	***DEX + VCR+‡Imatinib**	**1**	***6MP + DEX + VCR**	**1**
33	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
34	**6MP + MTX+‡Imatinib**		**6MP + MTX**	

	Standard/High Risk		Low Risk	
Week		**Special Studies**		**Special Studies**
35	**Cyclo + Ara-C+‡Imatinib**		**6MP + MTX**	
36	***DEX + VCR+‡Imatinib**		**(*)6MP + DEX + VCR**	
37	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
38	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
39	**Cyclo + Ara-C+‡Imatinib**		**6MP + MTX**	
40	***DEX + VCR+‡Imatinib**		***6MP + DEX + VCR**	
41	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
42	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
43	**Cyclo + Ara-C+‡Imatinib**		**6MP + MTX**	
44	***DEX + VCR+‡Imatinib**		**(*)6MP + DEX + VCR**	
45	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
46	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
47	**Cyclo + Ara-C+‡Imatinib**		**6MP + MTX**	
48	**†*DEX + VCR+‡Imatinib**	**1, 4, 10, 11b**	**†*6MP + DEX + VCR**	**1, 4, 10, 11b**
49	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
50	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
51	**Cyclo + Ara-C+‡Imatinib**		**6MP + MTX**	
52	**DEX + VCR+‡Imatinib**		**6MP + DEX + VCR**	
53	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
54	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
55	**Cyclo + Ara-C+‡Imatinib**		**6MP + MTX**	
56	**(*)DEX + VCR+‡Imatinib**		**6MP + DEX + VCR**	
57	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
58	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
59	**Cyclo + Ara-C+‡Imatinib**		**6MP + MTX**	
60	**DEX + VCR+‡Imatinib**		**6MP + DEX + VCR**	
61	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
62	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
63	**Cyclo + Ara-C+‡Imatinib**		**6MP + MTX**	
64	**§(*)DEX + VCR+‡Imatinib**	**1, 2**	**§6MP + DEX + VCR**	**1, 2**
65	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
66	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
67	**Cyclo + Ara-C+‡Imatinib**		**6MP + MTX**	
68	**DEX + VCR+‡Imatinib**		**6MP + DEX + VCR**	
69	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
70	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
71	**6MP + MTX+‡Imatinib**		**6MP + MT**	
72	**(*)6MP + DEX + VCR+‡Imatinib**		**6MP + DEX + VCR**	
73	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
74	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
75	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
76	**6MP + DEX + VCR+‡Imatinib**		**6MP + DEX + VCR**	
77	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
78	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
79	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
80	**§(*) 6MP + DEX + VCR+‡Imatinib**	**1**	**§6MP + DEX + VCR**	**1**
81	**6MP + MTX+‡Imatinib**		**6MP + MTX**	

	Standard/High Risk		Low Risk	
Week		**Special Studies**		**Special Studies**
82	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
83	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
84	**6MP + DEX + VCR+‡Imatinib**		**6MP + DEX + VCR**	
85	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
86	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
87	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
88	**(*)6MP + DEX + VCR+‡Imatinib**		**6MP + DEX + VCR**	
89	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
90	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
91	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
92	**6MP + DEX + VCR+‡Imatinib**		**6MP + DEX + VCR**	
93	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
94	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
95	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
96	**§(*)6MP + DEX + VCR+‡Imatinib**	**1**	**§6MP + DEX + VCR**	**1**
97	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
98	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
99	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
100	**6MP + DEX + VCR+‡Imatinib**		**6MP + DEX + VCR**	
101	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
102	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
103	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
104	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
105	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
106	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
107	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
108	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
109	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
110	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
111	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
112	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
113	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
114	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
115	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
116	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
117	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
118	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
119	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
120$^{\Omega}$	**6MP + MTX+‡Imatinib**	**1,2, 4, 5, 10, 11a,b, 12**	**6MP + MTX**	**1,2, 4, 5, 10, 11a,b, 12**

Amend 5.0 (13 and 14 deleted from footnote), Revision 6.1, 8.0, **9.0**

Rev 3.2 *****IT methotrexate + hydrocortisone + cytarabine

(*) For **low risk** patients with CNS-2 status or traumatic tap with blasts and for high risk patients with WBC $\geq$100 x 10^9/L, T-cell with WBC $\geq$50 x 10^9/L, presence of Philadelphia chromosome, MLL rearrangement, near haploidy or CNS 3 status.

†Bone marrow Examination; sample for MRD.

§Surveillance cerebrospinal fluid examination

Special Studies: (1) blood chemistries (Chem 18 profile); (2) somatic DNA; (3) 6TGN or TPMT; (4) blood for MRD; (5) MRI of hips (**any time within weeks 12-14 is acceptable for post-reinduction I, any time within weeks 22-24 is acceptable for post-reinductin II,** week 120 for girls and week 146 for boys); (6) asparaginase studies (blood); (7) **deleted** (8) CSF and plasma **asparagines (not required at Cook's)**; (9) CSF **homocysteine/folates** and plasma homocysteine**;** (10) MRI of brain **(not required at Cook's)**; (11a) psychology **(not required at Cook's)** and (11b) quality of life; (12) QCT for bone density; (15) serum cortisol; (16) dexamethasone pharmacokinetics. **(17a) Recommended-24-hour urine for measurement of calcium, creatinine and sodium (not required at Cook's); (17b) Recommended-Spot urine-for measurement of calcium, creatinine, and sodium (not required at Cook's)**

CBC with differential is needed weekly.

‡ Only Ph+ patients

[γ]Triple intrathecal treatment will be given to other standard/high-risk cases with WBC ≥100 x 10^9/L, T-cell ALL with WBC ≥ 50 x 10^9/L, presence of Philadelphia chromosome, *MLL* rearrangement, hypodiploidy <45, or CNS-3 status

[Ω]If participant has a CNS relapse, treatment may be extended beyond week 120 (girls) due to additional contingency therapy (See Sections 9.3 and 9.4)

TREATMENT SCHEMA/Special Laboratory Tests During Continuation Therapy, cont.
MALE PATIENTS ONLY

Amend 2.0, Amend 4.0, Amend 5.0**,8.0**

	Standard/High Risk		Low Risk	
Week		**Special Studies**		**Special Studies**
121	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
122	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
123	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
124	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
125	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
126	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
127	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
128	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
129	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
130	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
131	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
132	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
133	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
134	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
135	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
136	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
137	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
138	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
139	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
140	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
141	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
142	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
143	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
144	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
145	**6MP + MTX+‡Imatinib**		**6MP + MTX**	
146$^{\Omega}$	**§†6MP + MTX+‡Imatinib**	**1, 2, 4, 5, 11a, b, 12**	**§ †6MP + MTX**	**1, 2, 4, 5, 11, 12**

Amend 5.0 (13 and 14 deleted from footnote)**,** 8.0, **9.0**

†Bone marrow Examination; sample for MRD.

§Surveillance cerebrospinal fluid examination

Special Studies: (1) blood chemistries (chem 18 profile); (2) somatic DNA; (3) 6TGN or TPMT; (4) blood for MRD; (5) MRI of hips (week 120 for girls and week 146 for boys); (6) asparaginase studies (blood); (7) **deleted** (8) CSF and plasma asparagines **(not required at Cook's);** (9) CSF **homocysteine/folates** and plasma homocysteine; (10) MRI of brain (**not required at Cook's**); (11a) psychology **(not required at Cook's)** and (11b) quality of life; (12) QCT for bone density; (15) serum cortisol; (16) dexamethasone pharmacokinetics; **(17a) Recommended-24-hour urine for measurement of calcium, creatinine,and sodium (not required at Cook's); (17b) Recommended-Spot urine-for measurement of calcium, creatinine, and** sodium (not required at Cook's).

CBC with differential is needed weekly.

‡ Only Ph+ patients

$^{\Omega}$If participant has a CNS relapse, treatment may be extended beyond week 146 (boys) due to additional contingency therapy (See Sections 9.3 and 9.4)

APPENDIX I: MANAGEMENT OF OSTEONECROSIS (Deleted with amendment 3.0)

Amend 1.0 (deleted appendix relating to atovaquone sub-study)

APPENDIX II: DEFINITIONS RELATED TO INFECTIOUS DISEASES

I. ASSESSMENTS

A. Fever: A single temperature of > 38.3°C (101°F) or ≥38.0°C (100.4°F) on two occasions within 12 hours. The measurement must be oral with glass or IVAC[7] thermometers or at the tympanic membrane by infrared instruments (Thermoscan[7]). The same method should be used throughout the febrile episode for each patient.

B. Neutropenia: Neutrophil count < 500/mm^3 or < 1,000/mm^3 with predicted decline to ≤500/mm^3.

C. Duration of Fever (for episodes of fever with neutropenia): The initial temperature is the one immediately before the first dose of antibiotics and GCSF. This is designated as zero hour. The end of a febrile period is at the time of the first temperature of 38.0°C or less which is sustained at this level over a period of 24 hours or longer without antipyretic intervention. The duration of fever is the number of hours from zero hour to the end of the febrile period.

D. Antibiotic Therapy: Any systemic antibacterial drug will be considered as antibiotic therapy whether given orally or parenterally. Topical antibiotics will not be included.

E. Antifungal Therapy: Any antifungal drug administered orally or parenterally will be considered antifungal therapy. Antifungal therapy will be categorized for analysis as:

1. Treatment for oral candidiasis
   Nystatin
   Oral amphotericin B
   Clotrimazole troches
   Fluconazole
   Ketoconazole

2. Treatment for systemic mycoses (includes empirical use)
   Systemic amphotericin B (standard and liposomal)
   Fluconazole
   Flucytosine
   Itraconazole

II. BACTERIAL

1. Bacteremia only: positive blood culture of any bacterium, but no clinical evidence of infection. (Specify catheter-related or not.)
2. Bacterial sepsis: positive blood culture for any bacterium plus clinical evidence of infection (fever, chills, hypotension, etc.). (Specify catheter-related or not.)

3. Urinary tract infection: urine colony count of 100,000 or greater of a single organism plus symptoms, dysuria, flank pain, etc. Asymptomatic bactiuria is the same colony count without symptoms.
4. Pneumonia (bacterial): radiographic discernible infiltrate plus isolation of potentially causative bacteria from bronchoalveolar lavage, blood or biopsy specimen. If positive blood culture, code as bacterial sepsis with pneumonia.
5. Meningitis: positive culture of causative bacteria from CSF plus symptoms compatible with meningitis.
6. Osteomyelitis: radiographic lesions plus positive blood or bone aspirate/biopsy cultures.
7. Acute Otitis Media: physician diagnosis plus antibiotic treatment.
8. Pharyngitis: only if group A beta hemolytic streptococcus is isolated from throat culture in patient with symptoms. A positive rapid streptococcal test is acceptable in place of culture. Other types of pharyngitis will not be considered.
9. Cellulitis: erythema and induration plus isolation of bacterim from aspirate or drainage.

III. FUNGAL INFECTIONS

A. Candidiasis

1. Systemic: isolation of Candida species from an otherwise sterile body fluid or tissue (e.g., blood, CSF, aspirate or biopsy of tissue, bone marrow, joint fluid, etc.) along with clinically compatible illness; or, demonstration histologically of typical budding yeast and pseudohyphae in tissue biopsy plus isolation of Candida species in culture from the same tissue or otherwise sterile body fluid or tissue. Typical histopathology with pseudohyphae in pulmonary, hepatic, splenic, brain or renal lesions is adequate since the organism often fails to grow from cultures of organs.
2. Oral: presence of typical whitish lesions on the mucosal surface with yeast or pseudomycelia on gram stain or KOH preparation or isolation of *Candida* species in culture from the mouth.
3. Esophageal or urinary bladder: evidence of tissue involvement proven by endoscopy and biopsy plus isolation of fungus in culture.

B. Aspergillosis

1. Systemic: the isolation of Aspergillus species from otherwise sterile body fluid or tissue plus clinically compatible illness (e.g., pulmonary infiltrate, sinusitis, CNS lesion, hepatic or splenic lesion). The demonstration of typical branching septate hyphae in biopsies or aspirated tissue specimens plus isolation of the organism in culture also permits the diagnosis of systemic diagnosis.
2. Pulmonary: typical cystic lesion with fungus ball ("half moon sign") in chest radiograph and/or CT scan, and Aspergillus species isolated from sputum, tracheal aspirate or bronchoalveolar lavage fluid.

C. Histoplasmosis: isolation of *Histoplasma capsulatum* in culture from patient with compatible illness.

D. Presumptive Systemic Fungal Infection: typical lesions in liver, spleen, kidney, or brain on CT or MRI scans plus compatible clinical features and treated with systemic antifungal drugs.

E. Others: the investigator may include certain other infectious diseases of unique nature. Acceptance requires decision before decoding occurs and with the agreement of the principal investigator and an attending from the Infectious Disease Department.

IV. PROTOZOAN

A. *Pneumocystis carinii* Pneumonia: discernible radiographic lesion plus identification of *P. carinii* in bronchoalveolar lavage fluid, biopsy or induced sputum.
B. *Cryptosporidiosis*: *C. parum* identified in stool plus diarrhea.
C. *Toxoplasmosis:* see ACTG protocol 254.

V. TOPOGRAPHICAL

A. The following diagnosis are acceptable for objectively identified infections without confirmation of etiology.

1. Pneumonia: lesion on radiograph
2. Osteomyelitis: radiological diagnosis
3. Sinusitis: radiological diagnosis (x-ray, CT, MRI)
4. Cellulitis: physician diagnosis

VI. FEVER OF UNDETERMINED ETIOLOGY

B. Febrile episode with no etiological or topographical evidence of disease. Identify as:
1. neutropenic = ANC $<500/mm^3$
2. nonneutropenic = ANC $\geq 500/mm^3$

APPENDIX III: NCI TOXICITY CRITERIA ARE AVAILABLE ON THE ST. JUDE INTRANET FOR REVIEW.

APPENDIX IV: NON-INVASIVE CARDIAC EVALUATION METHODOLOGY

Amend 5.0 (deleted text) (deleted with Amendment 7.0)

Amend 5.0

APPENDIX V: DRUGS KNOWN TO BE METABOLIZED BY CYP450 ISOENZYMES 2D6 AND 3A4

CYP2D6	
Substrates	
Amitriptyline (hydroxylation)	**Methamphetamine**
Amphetamine	**Metoclopramide**
Betaxolol	**Metoprolol**
Bisoprolol	**Mexitetine**
Brofaromine	**Mianserin**
Buturolol	**Meperidine**
Bupropion	**Methadone Mirtazapine (hydroxylation)**
Captopril	**Molindone**
Carvedilol	**Morphine**
Cevimeline	**Nortriptyline (hydroxylation)**
Chlorpheniramine	**Olanzapine (minor, hydroxymethylation)**
Chlorpromazine	**Ondansetron**
Cinnarizine	**Orphenadrine**
Clomipramine (hydroxylation)	**Oxycodone**
Clozapine (minor pathway)	**Papaverine**
Codeine (hydroxylation, o-demelhylation)	**Paroxetine (minor pathway)**
Cyclobenzaprine (hydroxylation)	**Penbutolol**
Cyclophosphamide	**Pentazocine**
Debrisoquin	**Perhexiline**
Delavirdine	**Perphenazine**
Desipramine	**Phenformin**
Dexfenfluramine	**Pindolol**
Dextromethorphan (o-demethylation)	**Promethazine**
Dihydrocodeine	**Propafenone**
Diphenhydramine	**Propranolol**
Dolasetron	**Quetiapine**
Donepezil	**Remoxipride**
Doxepin	**Risperidone**
Encainide	**Ritonavir (minor)**
Fenlluramine	**Ropivacaine**
Flecainide	**Selegiline**
Fluosetine (minor pathway)	**Sertindole**
Fluphenazine	**Sertraline (minor pathway)**
Haiofantrine	**Sparteine**
Haloperidol (minor pathway)	**Tamoxifen**
Hydrocodone	**Thioridazine**
Hydrocortisone	**Tiagabine**
Hydroxyamphetamine	**Timolol**
Imipramine (hydroxylation)	**Tolterodine**
Labetalol	**Tramadol**
Loratadine	**Trazodone**

Substrates

Maprotiline
m-Chlorophenylpiperazine (m-CPP)
Trimipramine
Tropisetron
Veniafaxine (o-desmethylation)
Yohimbine

CYP2D6
Inhibitors

Amiodarone
Celecoxib
Chloroquine
Chlorpromazine
Cimetidine
Citalopram
Clomipramine
Codeine
Deiavirdine
Desipramine
Dextropropoxyphene
Diltiazem
Doxorubicin
Entacapone (high dose)
Fluoxetine
Fluphenazine
Fluvoxamine
Haloperidol
Labetalol
Lobeline
Lomustine
Methadone
Mibefradil
Moclobemide
Nortluoxeline
Paroxetine
Perphenazine
Propafenone
Quinacrine
Quinidine
Ranitidine
Risperidone (weak)
Ritonavir
Sertindole
Sertraline (weak)
Thioridazine
Valproic acid
Venlafaxine (weak)
Vinblastine
Vincristine
Vinorelbine
Yohimbine

CYP3A3/4

Substrates

Acetominophen
Aifentanil
Alosetron
Alprazolam
Amiodarone
Amitriptyline (minor)
Amlodipine
Anastrozole
Androsterone
Antipyrine
Astemizole
Atorvastatin
Benzphetamine
Bepridil
Chlorpromazine
Cimetidine
Cisapride
Citalopram
Clarithromycin
Clindamycin
Clomipramine
Clonazepam
Clozapine
Cocaine
Codeine (demethylation)
Cortisol
Cortisone
Cyclobenzaprine (demethylation)

Bexarotene	**Cyclophosphamide**
Bromazepam	**Cyclosporine**
Budesonide	**Dehydroepiandrostendione**
Bupropion (minor)	**Delavirdine**
Buspirone	**Desmethyldiazepam**
Busulfan	**Dexamethasone**
Caffeine	**Dextromethrophan (minor, N-demethylation)**
Cannabinoids	**Diazepam (minor; hydroxylation, N-demethylation**
Carbamazepine	**Nefazodone**
Cevimeline	**Nelfinavir**
Cerivastatin	**Nevirapine**
Digitoxin	**Nicardipine**
Diltiazem	**Nifedipine**
Disopyramide	**Niludipine**
Docetaxel	**Nimodipine**
Dolasetron	**Nisoldipine**
Donepezil	**Nitrendipine**
Doxorubicin	**Omeprazole (sulfonation)**
Doxycycline	**Ondansetron**
Dronabinol	**Oral contraceptives**
Enalapril	**Orphenadrine**
Erythromycin	**Paclitaxel**
Estradiol	**Pantoprazole**
Ethinyl estradiol	**Pimozide**
Ethosuximide	**Pioglitazone**
Etoposide	**Pravastatin**
Exemestene	**Prednisone**
Dofefilide (minor)	**Progesterone**
Felodipine	**Proguanil**
Fentanyl	**Propafenone**
Fexotenadine	**Quercetin**
Finaxteride	**Quetiapine**
Fluoxetine	**Quinidine**
Flutamide	**Quinine**
Glyburide	**Repaglinide**
Granisetron	**Retinoic acid**
Halofantrine	**Rifampin**
Hydrocortisone	**Risperidone**
Hydroxyarginine	**Ritonavir**
Lfosfamide	**Salmeterol**
Lmipramine	**Saquinavir**
Lndinavir	**Sertindole**
Lsradipine	**Sertraline**
Ltrazonazole	**Sibutramine**
Ketoconazole	**Sildenafil citrate**

Lasoprazole (minor)	**Simvastatin**
Letrozole	**Sirolimus**
Levobupivicaine	**Sufentanil**
Lidocaine	**Tacrolimus**
Loratadine	**Tamoxifen**
Losartan	**Temazepam**
Lovastatin	**Teniposide**
Methadone	**Terfenadine**
Mibefradil	**Testosterone**
Miconazole	**Tetrahydrocannabinol**
Midazolam	**Theophyline**
Mifepristone	**Tiagabine**
Mirtazapine (N-demethylation	**Tolterodine**
Montelukast	**Vincristine**
Navelbine	**Warfarin (R-warfarin)**
Toremifene	**Yohimbine**
Trazadone	**Zaleplon (minor pathway)**
Tretinoin	**Zatoestron**
Triazolam	**Zileuton**
Troglitazone	**Ziprasidone**
Troleandomycin	**Zolpidem**
Venlafaxine (N-demethylation)	**Zonisamide**
Verapamil	
Vinblastine	
Inducers	
Carbamazepine	**Phenytoin**
Dexamethasone	**Primidone**
Ethosuximide	**Progesterone**
Glucocorticoids	**Rifabutin**
Griseofulvin	**Rifampin**
Nafcillin	**Rofecoxib (mild)**
Nelfinavir	**St. John's Wort**
Nevirapine	**Sulfadimidine**
Oxcarbazepine	**Sulfinpyrazone**
Phenylbutazone	**Troglitazone**
CYP3A3/4	
Inhibitors	
Amiodarone	**Ketoconazole**
Anastrozole	**Metronidazole**
Azithromycin	**Mibefradil**
Cannabinoids	**Miconazole (moderate)**
Cimetidine	**Nefazodone**
Clarithromycin	**Nelfinavir**
Clotrimazole	**Nevirapine**
Cyclosporine	**Norfloxacin**

Danazol	**Norfluoxetine**
Delaviridine	**Omeprazole (weak)**
Dexamethasone	**Oxiconazole**
Diethyldithiocarbamate	**Paroxetine**
Dirithromycin	**Quinidine**
Disulfiram	**Quinine**
Entacapone (high dose)	**Quinupristine and dalfopristin**
Erythromycin	**Ranitidine**
Ethinyl estradiol	**Ritonavir**
Fluconazole (weak)	**Saquinavir**
Fluoxetine	**Sertindole**
Fluvoxamine	**Sertraline**
Gestodene	**Troglitazone**
Grapefruit juice	**Troleandomycin**
Indinavir	**Valproic acid (weak)**
Isoniazid	**Verapimil**
Itraconazole	**Zafirlukast**
	Zileuton