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Initial Testing of the Aurora Kinase A Inhibitor MLN8237 by the Pediatric Preclinical Testing Program (PPTP)

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Abstract

Background—MLN8237 is a small molecule inhibitor of Aurora Kinase A (AURKA) that is currently in early phase clinical testing. AURKA plays a pivotal role in centrosome maturation and spindle formation during mitosis.

Procedures—MLN8237 was tested against the Pediatric Preclinical Testing Program (PPTP) *in vitro* panel at concentrations ranging from 1.0 nM to 10 μ M and was tested against the PPTP *in vivo* panels at a dose of 20 mg/kg administered orally twice daily $\times$ 5 days. Treatment duration was 6 weeks for solid tumor xenografts and 3 weeks for ALL xenografts.

Results—MLN8237 had a median IC₅₀ of 61 nM against the PPTP *in vitro* panel. The ALL cell lines were more sensitive and the rhabdomyosarcoma cell lines less sensitive than the remaining PPTP cell lines. *In vivo*, MLN8237 induced significant differences in event-free survival (EFS) distributions compared to controls in 32/40 (80%) solid tumor models and all (6/6) ALL models. Maintained complete responses (CRs) were observed in 3 of 7 neuroblastoma xenografts, and all 6 evaluable ALL xenografts achieved CR (n=4) or maintained CR (n=2) status. Maintained CRs were observed among single xenografts in other panels, including the Wilms tumor, rhabdoid tumor, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, and medulloblastoma.

Conclusions—The *in vivo* activity observed against the neuroblastoma panel far exceeds that observed for standard agents evaluated against the panel by the PPTP. High levels of *in vivo* activity were also observed against the ALL xenograft panel. These data support expedited clinical development of MLN8237 in childhood cancer.

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Keywords

Preclinical Testing; Developmental Therapeutics; MLN8237

INTRODUCTION

The process of mitosis has been exploited for treatment of cancer, and antimetabolic agents form an important group of chemotherapeutics for both adult and childhood malignancies. For many childhood solid tumors and leukemias, microtubule destabilizing drugs such as vincristine and vinblastine remain important components of curative regimens. In addition to microtubule interacting agents (taxanes, epothilones, *Vinca* alkaloids) other components of the mitotic apparatus have been identified as potential therapeutic targets. These include the mitotic kinesins [1,2], including centromere components such as the CENP family of proteins required for correct chromosome alignment and complete formation of the spindle assembly complex [3], as well as tubulin-associated kinases, Polo-like kinases and the Aurora kinases [4]. Aurora and Polo-like kinases also play essential roles in centrosome separation, chromosome alignment, segregation, and cytokinesis. Inhibition of any of these kinases results in abnormal mitotic events and potentially apoptosis [5]. For example, AURKA inhibition by MLN8054 leads to p73-dependent apoptosis in p53-deficient cells [6].

The Aurora serine/threonine protein kinases are a family encoded by three genes (*AURKA*, *AURKB* and *AURKC*) that play key roles in mitosis. Aurora A is an essential mammalian protein, as genetically engineered null mice die at the blastocyst stage with pronounced cell proliferation failure, mitotic arrest, and monopolar spindle formation [7]. AURKA, in complex with a cofactor, Bora, is required for phosphorylation of Polo-like kinase 1 (PLK1) and G2/M transition [8]. In addition, Aurora A activity is involved in centrosome duplication and separation, microtubule-kinetochore attachment, spindle checkpoint, and cytokinesis [9,10].

The Aurora kinases have been shown to act as oncogenic drivers in a variety of human cancers. High level expression of AURKA is often seen in cancer cells and is associated with amplification of the *AURKA* gene locus on chromosome 20 in a significant subset of adult tumors [11], but this has not been reported in pediatric cancers. In contrast, overexpression of Aurora B kinase is rarely associated with gene amplification. Overexpression of Aurora A overrides the spindle assembly checkpoint induced by microtubule destabilizing agent nocodazole [12], confirming the role of this kinase in the spindle activated checkpoint and providing a potential explanation of how deregulation of Aurora A can contribute to genetic instability and tumorigenesis. Finally, Aurora A has recently been shown to directly interact with the *MYCN* protein to sequester this transcription factor and prevents its ubiquitination and proteasomal degradation in neuroblastoma cell lines in a kinase independent manner [13,14].

Several small molecule inhibitors of Aurora kinases have entered clinical trials [11]. Most of these drugs primarily target Aurora B. The primary focus of the PPTP is to identify novel agents that have significant antitumor activity against models of childhood solid tumors and acute lymphoblastic leukemia (ALL) as one approach to prioritize future clinical development in the pediatric setting. Here we report the *in vitro* and *in vivo* activity of MLN8237, a second generation AURKA inhibitor.

MATERIALS AND METHODS

In vitro testing

In vitro testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system that quantifies viable (using fluorescein diacetate [FDA]) cell numbers in tissue culture multiwell plates [15]. Cells were incubated in the presence of MLN8237 for 96 hours at concentrations from 1 nM to 10 μ M and analyzed as previously described [16]. Two measures of sensitivity were used; the IC₅₀, defined as the drug concentration inhibiting growth by 50% compared to controls, and the EC₅₀, defined as the drug concentration yielding 50% of the maximum inhibitory effect.

In vivo tumor growth inhibition studies

CB17SC-M *scid*^{-/-} female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted kidney/rhabdoid tumors, sarcomas (Ewing, osteosarcoma, rhabdomyosarcoma), neuroblastoma, and non-glioblastoma brain tumors, while BALB/c nu/nu mice were used for glioma models, as previously described [17–19]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*^{-/-} mice as described previously [20]. Details of these tumor panels can be obtained at <http://pptp.nchresearch.org/documents.html>. Female mice were used irrespective of the patient gender from which the original tumor was derived. All mice were maintained under barrier conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the appropriate consortium member. Ten mice (solid tumor models) and 8 mice (ALL models) were used in each control or treatment group. Tumor volumes (cm³) [solid tumor xenografts] or percentages of human CD45-positive [hCD45] cells [ALL xenografts] were determined as previously described [21].

Determination of response

Responses were determined using three activity measures as previously described [21]. For individual mice, progressive disease (PD) was defined as < 50% regression from initial volume during the study period and > 25% increase in initial volume at the end of study period. Stable disease (SD) was defined as < 50% regression from initial volume during the study period and 25% increase in initial volume at the end of the study. Partial response (PR) was defined as a tumor volume regression 50% for at least one time point but with measurable tumor (≥ 0.10 cm³). Complete response (CR) was defined as a disappearance of measurable tumor mass (< 0.10 cm³) for at least one time point. A complete response was considered maintained (MCR) if the tumor volume was < 0.10 cm³ at the end of the study period. For treatment groups only, if the tumor response was PD, then PD was further classified into PD1 or PD2 based on the tumor growth delay (TGD) value. TGD values were calculated based on the numbers of days to event. For each individual mouse that had PD and had an event in the treatment groups, a TGD value was calculated by dividing the time to event for that mouse by the median time to event in the respective control group. Median times to event were estimated based on the Kaplan-Meier event-free survival distribution. If a mouse had a TGD value ≤ 1.5 , that mouse was considered PD1. If the TGD value was > 1.5, the mouse was considered PD2. Mice that had PD but did not have an event at the end of the study were coded as PD2. An in-depth description of the analysis methods is included in the Supplemental Response Definitions section.

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SAS®, was used to compare event-free survival (EFS) distributions between treatment and control groups. P-

values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies.

Drug Information and Formulation

Millennium Pharmaceuticals, Inc., through the Cancer Therapy Evaluation Program (NCI), provided MLN8237 to the Pediatric Preclinical Testing Program. MLN8237 was dissolved in 10% 2-hydroxypropyl- β -cyclodextrin, 1% sodium bicarbonate in water and administered via oral gavage twice daily for 5 days repeated each week for a total of 6 weeks for the solid tumor xenografts, and 3 weeks for the leukemia xenografts, at 20 mg/kg/dose. MLN8237 was provided to each consortium investigator in coded vials for blinded testing against a placebo control consisting of drug vehicle only.

RESULTS

MLN8237 *in vitro* testing

MLN8237 uniformly inhibited growth of a majority of the cell lines from the PPTP *in vitro* panel (Table I) with only one cell line (Rh18) having an IC₅₀ value greater than 10 μ M. The median EC₅₀ and IC₅₀ values for all of the cell lines in the panel were 49 nM and 61 nM, respectively (Table I). The only significant differences in median EC₅₀ or IC₅₀ values between tumor panels (ALL, Ewing, neuroblastoma, and rhabdomyosarcoma) were for the rhabdomyosarcoma panel, which had significantly higher median EC₅₀ and IC₅₀ values than the remaining PPTP cell lines tested. (Figure 1, panel A). MLN8237's cytotoxic activity (as assessed by minimum T/C values approaching 0) was most notable for the ALL panel, with 4 of 5 cell lines showing minimum T/C values < 1%. Minimum T/C values for the other panels were higher, with the median value for the entire panel being 8.0% and with the rhabdomyosarcoma panel having the highest median minimum T/C value (22.7%). Examples of concentration response curves for the neuroblastoma line CHLA-90 and the leukemia cell line MOLT-4 illustrate the plateau effects observed for some cell lines and the near complete cytotoxic effect observed for others (Figure 1, panel B).

MLN8237 *in vivo* testing

MLN8237 was evaluated in 49 xenograft models. Thirty-three of 935 mice died during the study (3.5%), with 3 of 462 in the control arms (0.6%) and 30 of 473 in the MLN8237 treatment arms (6.3%). Two lines (CHLA-79 and ALL-8) were excluded from analysis due to toxicity greater than 25 percent. ALL-7 was excluded due to all animals succumbing to lymphomas. A complete summary of results is provided in Supplemental Table I, including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice with events and average times to event, tumor growth delay, as well as numbers of responses and T/C values.

Antitumor effects were evaluated using the PPTP activity measures for time to event (EFS T/C), tumor growth delay (tumor volume T/C), and objective response. MLN8237 induced significant differences in EFS distributions compared to controls in 32/40 (80%) solid tumor models and each of the six evaluable ALL models (Table II). Eleven out of 38 evaluable lines met the criteria for high activity with EFS T/C values greater than 2 and with final tumor volumes less than the initial volumes (Table II). An additional 15 models met criteria for intermediate activity for the EFS T/C activity measure by having EFS T/C values exceeding 2.0 and significant differences in EFS distribution between treated and control groups.

The *in vivo* testing results for the objective response measure of activity are presented in Figure 2 in a 'heat-map' format as well as a 'COMPARE'-like format, based on the scoring

criteria described in the Material and Methods and the Supplemental Response Definitions section. The latter analysis demonstrates relative tumor sensitivities around the midpoint score of 5 (stable disease). Objective responses were seen in 10 of 40 solid tumor models with examples of typical solid tumor response shown in Figure 3 (SK-NEP-1, NB-1643 and NB-EBc1). Complete responses were seen in all 6 ALL models with two models maintaining the response throughout the observation period. Examples of typical ALL tumor response are shown in Figure 4 (ALL-2, ALL-17 and ALL-19).

DISCUSSION

The Aurora family of serine/threonine protein kinases play a critical role in the regulation of chromosomal segregation and cytokinesis during mammalian cell division. There are several lines of evidence supporting Aurora kinase A as a cancer therapeutic target [11]. First, *AURKA* is amplified or overexpressed in many tumors [22–24]. Second, the overexpression of *AURKA* results in the transformation of normal cells, supporting the hypothesis that Aurora A is an oncogene [25]. Third, knockdown of *AURKA* using RNA interference technology results in mitotic spindle defects, mitotic delay, and apoptosis in human cells [26]. Finally, two common polymorphisms in *AURKA* (Phe31Ile and Val57Ile) have been shown to alter the enzyme's kinase function, are associated with breast cancer susceptibility, [27] and are preferentially amplified in some human cancers [28,29]. To date, aurora kinase inhibitors have shown only limited clinical activity infrequently inducing objective responses in solid tumors, although showing more activity in leukemia patients [11]. However, there are no data available to support *AURKA* as a therapeutic target in pediatric cancers.

MLN8237 is a small molecule reversible inhibitor of *AURKA* via competition with ATP binding that is being developed for the treatment of cancer. It is approximately 200-fold more selective for Aurora kinase A compared to Aurora kinase B, and is also relatively selective compared to other receptor and non-receptor kinases. Such an inhibitor would be expected to have potential application across a broad range of human tumors, given the central role of mitosis in the progression of virtually all malignancies, but perhaps with an increased therapeutic index when the gene is amplified and/or overexpressed as an acquired somatic event in cancer cells. MLN8237 has demonstrated activity against a broad range of adult preclinical tumor models, and is also expected to be toxic to proliferating normal tissues (such as bone marrow and GI epithelium) due to *AURKA*'s central role in mammalian cell division.

The PPTP is designed to prioritize agents being developed for cancer in general to the field of pediatric oncology. The results here show the potential power of this approach, as there remain no *a priori* explanation for the broad and potent activity seen against both the ALL (including a CR in ALL-7 when tested at $0.5 \times \text{MTD}$) and neuroblastoma panels, as well as potent activity against some cell models in other histotypes. *AURKA* was not identified to date in any of the genomics efforts focused on leukemia or neuroblastoma as a potential therapeutic target. In addition, there appears to be no correlation between *AURKA* copy number or expression with activity level in the xenografts studied here (data not shown, see [30]). This suggests that oncogenicity of this kinase is determined by post-translational events and/or is cell-type specific. For example, Otto and colleagues discovered that *AURKA* binds MYCN and sequesters it from proteasomal degradation in a kinase independent manner [13,14]. However, the neuroblastoma cell lines sensitive in this experiment were not only those with *MYCN* amplification, and MLN8237 acts via inhibition of kinase function, suggesting that the MYCN (and perhaps MYC) binding function of *AURKA* potentially contributes to, but does not determine, cytotoxicity to MLN8237.

Development of MLN8237 for use in the pediatric cancer setting will require further work to identify MLN8237-based drug combinations with high-level activity. Preclinical testing of combinations that include an AURKA inhibitor have to date focused on adult cancer models. This early work suggests promising levels of activity when combining AURKA inhibition with microtubule-targeted agents such as taxanes and *Vinca* alkaloids [31–33].

The robust anti-tumor activity observed in this screen has been validated in a second stage of testing (manuscript under preparation) and led to the fast-tracking of this agent to the clinic. A pediatric phase 1/2 trial was designed and opened in the Children's Oncology Group Phase 1 Consortium during the past year. This trial is exploring both once a day or twice daily dosing for 7 days, followed by 14 days of rest to recover from the anticipated myelosuppression. This is a substantially different dosing schedule than explored in the PPTP and was driven largely by the adult Phase 1 experience [34]. As results from the clinical trial emerge, it will be important to correlate any observed anti-tumor activity with pharmacokinetic and pharmacodynamic measurements from both the human and murine experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. MLN8237 *in vitro* activity, figure 1A is a dot plot chart that illustrates the relative sensitivity of the cell lines using the IC₅₀ values displayed by histology. The black line indicates the median IC₅₀ (61 nM) for the panel. Figure 1B illustrates typical cytotoxicity curves for CHLA-90 (multidrug resistant *TP53*-mutated neuroblastoma) and Molt-4 (T cell ALL). Error bars represent standard deviations for each concentration tested.

Figure 2. MLN8237 *in vivo* objective response activity. Left: A high level of activity is indicated by a score of 6 or more, intermediate activity by a score of ≥ 2 but < 6 , and low activity by a score of < 2 . The colored heat map depicts group response scores: MCR (red), CR (orange), PR (yellow), SD (gray), PD2 (light green), PD1 (dark green). Right: representation of tumor sensitivity based on the difference of individual tumor lines from the midpoint response (stable disease). Bars to the right of the median represent lines that are more sensitive, and to the left are tumor models that are less sensitive. Red bars indicate lines with a significant difference in EFS distribution between treatment and control groups, while blue bars indicate lines for which the EFS distributions were not significantly different.

Figure 3. MLN8237 activity against individual solid tumor xenografts, Kaplan-Meier curves for EFS, median relative tumor volume graphs, and individual tumor volume graphs (controls, gray and treated, black lines) are shown for selected lines: (A) SK-NEP-1 (B) NB-1643, and (C) NB-EBc1.

Figure 4. MLN8237 activity against individual ALL xenografts, Kaplan-Meier curves for EFS and graphs of median and individual percentages of hCD45 cells (controls, gray and treated, black lines), are shown for selected lines: (A) ALL-2, (B) ALL-17, and (C) ALL-19.

Table I

Activity of MLN8237 against Cell Lines in the PPTP *in Vitro* Panel

Cell Line	Status	Histology	Minimum T/C(%)	EC ₅₀ (nM)	IC ₅₀ (nM)
RD		Rhabdomyosarcoma	20.5	150	228
Rh41	Post-Therapy	Rhabdomyosarcoma	22.7	57	90
Rh18	Diagnosis	Rhabdomyosarcoma	60.5	>10,000	>10,000
Rh30	Diagnosis	Rhabdomyosarcoma	22.6	89	230
BT-12	Diagnosis	Rhabdoid	29.3	9	60
CHLA-266	Diagnosis	Rhabdoid	28.4	27	72
TC-71	Post-Therapy	Ewing	10.0	100	102
CHLA-9	Diagnosis	Ewing	4.2	16	18
CHLA-10	Post-Therapy	Ewing	4.7	56	60
CHLA-258	Post-Bone Marrow Transplant	Ewing	18.8	82	132
SJ-GBM2	Post-Therapy	Glioblastoma	8.0	45	50
NB-1643	Diagnosis	Neuroblastoma	10.2	32	37
NB-EBcl	Post-Therapy	Neuroblastoma	3.6	49	50
CHLA-90	Post-Bone Marrow Transplant	Neuroblastoma	16.3	48	61
CHLA-136	Post-Bone Marrow Transplant	Neuroblastoma	10.4	36	39
NALM-6	Post-Therapy	ALL B-precursor	0.5	58	62
COG-LL-317	Post-Therapy	ALL T-cell	0.0	47	47
RS4;11^l	Post-Therapy	ALL B-precursor	0.3	18	18
MOLT-4	Post-Therapy	ALL T-cell	0.2	26	26
CCRF-CEM		ALL T-cell	4.1	85	94
Kasumi-1	Post-Bone Marrow Transplant	AML	3.4	98	103
Karpas-299	Post-Therapy	ALCL	2.5	40	38
Ramos-RA1		NHL	2.5	120	127
Median			8.0	49	61
Minimum			60.5	9	18
Maximum			0.0	>10,000	>10,000

T/C, Treated/Control; EC₅₀, concentration inducing 50% maximal effect; IC₅₀, drug concentration causing 50% growth inhibition relative to control.

Table II

Activity for MLN8237 against the PPTP *in Vivo* Panel

Xenograft Line	Histology	KM Estimate of Median Time to Event	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-Value	T/C Activity	EFS Activity	Response Activity
BT-29	Rhabdoid	> EP	<0.001	> 3.6	0.8	0.47	0.002	Low	High	Int
KT-14	Rhabdoid	> EP	<0.001	> 2.6	1.3	0.39	<0.001	Int	Int	Int
KT-12	Rhabdoid	> EP	<0.001	> 3.6	0.0	0.15	<0.001	High	High	High
KT-10	Wlms	> EP	<0.001	> 4.7	0.0	0.18	<0.001	Int	High	High
KT-11	Wlms	40.0	<0.001	3.5	>4	0.32	<0.001	Int	Int	Int
KT-13	Wlms	> EP	<0.001	> 5.7	0.9	0.41	<0.001	Int	High	Int
SK-NEP-1	Ewing	> EP	<0.001	> 5.3	0.0	0.14	<0.001	High	High	High
EW5	Ewing	14.9	<0.001	1.3	>4	0.77	<0.001	Low	Low	Low
EW8	Ewing	16.3	<0.001	1.2	>4	0.78	0.001	Low	Low	Low
TC-71	Ewing	13.4	0.013	1.7	>4	0.63	0.105	Low	Low	Int
CHLA258	Ewing	11.7	0.351	1.3	>4	0.93	0.393	Low	Low	Low
Rh28	ALV RMS	> EP	<0.001	> 2.2	2.3	0.32	<0.001	Int	Int	Int
Rh30	ALV RMS	18.1	0.553	0.6	>4	1.28	0.280	Low	Low	Low
Rh30R	ALV RMS	27.8	0.006	1.6	>4	0.54	<0.001	Low	Low	Low
Rh41	ALV RMS	19.6	<0.001	1.4	>4	0.73	<0.001	Low	Low	Low
Rh65	ALV RMS	> EP	<0.001	> 2.4	0.0	0.06	<0.001	High	High	High
Rh18	EMB RMS	37.2	<0.001	3.1	>4	0.24	<0.001	Int	Int	Int
BT-28	Medulloblastoma	8.4	0.175	1.1	>4	0.88	0.912	Low	Low	Low
BT-45	Medulloblastoma	33.9	<0.001	2.5	>4	0.57	0.013	Low	Int	Int
BT-46	Medulloblastoma	> EP	0.087	.	0.0	0.10	<0.001	High	NE	High
BT-50	Medulloblastoma	> EP	0.474	.	1.0	0.94	0.912	Low	NE	Int
BT-41	Ependymoma	> EP	0.531	.	1.9	0.93	0.243	Low	NE	Int
BT-44	Ependymoma	37.7	0.002	1.8	>4	0.79	0.481	Low	Low	Int
GBM2	Glioblastoma	30.9	<0.001	1.6	>4	0.69	0.001	Low	Low	Int
BT-39	Glioblastoma	> EP	<0.001	> 3.3	1.9	0.39	<0.001	Int	Int	Int
D645	Glioblastoma	30.3	<0.001	4.7	>4	0.32	<0.001	Int	Int	Int

Xenograft Line	Histology	KM Estimate of Median Time to Event	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-Value	T/C Activity	EFS Activity	Response Activity
D456	Glioblastoma	> EP	<0.001	> 6.2	0.7	0.18	<0.001	Int	High	High
NB-SD	Neuroblastoma	> EP	<0.001	>4.4	0.9	0.47	<0.001	Low	High	Int
NB-1771	Neuroblastoma	> EP	<0.001	> 1.5	0.0	0.13	<0.001	High	NE	High
NB-1691	Neuroblastoma	29.4	<0.001	3.4	>4	0.60	0.019	Low	Int	Int
NB-EBc1	Neuroblastoma	> EP	<0.001	> 2.1	0.0	0.06	<0.001	High	High	High
NB-1643	Neuroblastoma	> EP	<0.001	> 1.7	0.0	0.02	<0.001	High	NE	High
NB-1382	Neuroblastoma	> EP	<0.001	> 1.7	2.1	0.31	<0.001	Int	NE	Int
SK-N-AS	Neuroblastoma	7.6	0.077	1.4	>4	0.71	0.139	Low	Low	Low
OS-1	Osteosarcoma	> EP	<0.001	> 1.6	0.1	0.23	<0.001	Int	NE	High
OS-2	Osteosarcoma	34.9	<0.001	1.9	>4	0.38	<0.001	Int	Low	Int
OS-17	Osteosarcoma	39.6	<0.001	2.2	>4	0.40	<0.001	Int	Int	Int
OS-9	Osteosarcoma	> EP	0.206	.	0.9	0.58	<0.001	Low	NE	Int
OS-33	Osteosarcoma	38.8	<0.001	3.2	>4	0.24	<0.001	Int	Int	Int
OS-31	Osteosarcoma	28.4	<0.001	2.0	>4	0.51	<0.001	Low	Int	Int
ALL-2	ALL B-precursor	> EP	<0.001	> 2.7	2.3	.			Int	High
ALL-3	ALL B-precursor	> EP	<0.001	>11.8	11.2	.			Int	High
ALL-4	ALL B-precursor	36.2	0.005	36.2	>25	.			Int	High
ALL-16	ALL T-cell	> EP	0.002	> 4.1	0.4	.			High	High
ALL-17	ALL B-precursor	> EP	<0.001	> 8.0	4.2	.			Int	High
ALL-19	ALL B-precursor	> EP	<0.001	> 4.2	0.5	.			High	High

KM, Kaplan-Meier estimate; EP, median time to event exceeded the observation period (6 weeks)