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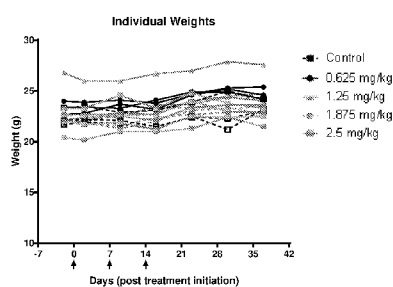
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(54) Title: METHOD FOR TREATING LEUKEMIA

(A)



(B)

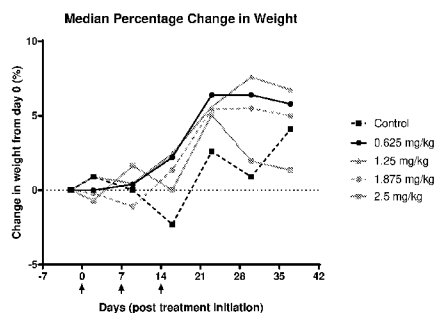


Figure 1

(57) Abstract: The present disclosure is related to a method and composition for treating leukemia. The method comprises administering subject in need with an effective amount of a compound named OBI-3424 or OBI-3423. It shows particular efficacy in treating acute lymphoblastic leukemia (ALL), including T-ALL and B-ALL.

MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

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Method for Treating Leukemia

Related Applications

【0001】 The present disclosure claims priority of United States provisional applications having serial number 62/565,986 filed September 29, 2017 and 62/690,786, filed June 27, 2018, which are hereby incorporated by reference herein for all purposes.

Technical Field

【0002】 The present disclosure relates a method for treating Leukemia; particularly for treating Acute Lymphoblastic Leukemia.

Description of Related Art

【0003】 Aldo-keto reductases (AKRs) are a superfamily of NADPH-dependent oxidoreductases that primarily catalyze the reduction of aldehydes and ketones to their corresponding alcohols (Penning TM and Drury JE. *Arch Biochem Biophys.* 2007; 464(2): 241-250). AKR family I member C3 (AKR1C3), is a hydroxysteroid dehydrogenase involved in the synthesis of steroid hormones and prostaglandins (Penning TM. *Chem Biol Interact.* 2015; 234: 236-246) and is widely expressed in normal human tissues (Penning TM et al. *Biochem J.* 2000; 351(Pt 1): 67-77). AKR1C3 is also expressed in pediatric and adult ALL, with significantly higher mRNA levels detected in T-ALL than B-ALL (Jamieson SM *et al. Biochem Pharmacol.* 2014; 88(1): 36-45). Recently, elevated expression and activity of AKR1C3 and other subfamily members were shown in diagnostic samples from pediatric T-ALL patients who went on to respond poorly to treatment when compared to treatment responders, suggesting a relationship between AKR1C3 expression and sensitivity of T-ALL cells to conventional drugs including daunorubicin, vincristine, and L-asparaginase. Moreover, the activity of AKR1C3 and its subfamily members affects the *in vitro* sensitivity of primary T-ALL cells and cell lines to vincristine (Bortolozzi R *et al. Br J Cancer.* 2018; 118(7): 985-994). Therefore, AKR1C3 overexpression in pediatric T-ALL is a potential therapeutic target as well as a possible biomarker for treatment resistance.

【0004】 Leukemia is a kind of cancer resulting from carcinogenesis in bone marrow. Patients bearing leukemia have abnormal white blood cells that are immaturely developed and lack normal functions. Common symptoms of leukemia include bleeding and bruising problems, feeling tired, fever, and an increased risk of infections. Clinically available treatments include chemotherapy,

radiation therapy, targeted therapy, and bone marrow transplant. The average five-year survival rate is about 57% in the United States.

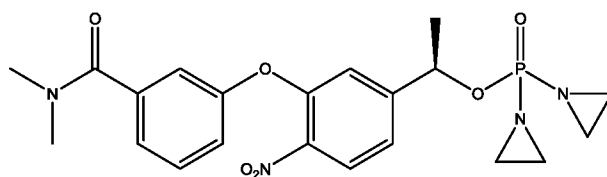
【0005】 Acute Lymphoblastic Leukemia (ALL), also known as Acute Lymphocytic Leukemia, is a rare blood cancer affecting the maturation of B-cell and T-cell lymphoblasts from progenitor cells. Acute Lymphoblastic Leukemia arises from the malignant transformation of B cell progenitors and T cell progenitors are named B-ALL and T-ALL respectively. Subtypes of B-ALL include early pre-B-ALL, common B-ALL (constitutes about 50% of the population), pre-B-ALL, and mature B-ALL. Subtypes of T-ALL include pre-T-ALL and mature T-ALL. Generally, T-ALL has a better prognosis compared to mature B-ALL. Other types of B-ALL have prognosis that falls in between.

【0006】 The current prevalence of ALL in the US is around 86,462 cases in 2018. The disease affects primarily children, with 60% of cases occurring at age <20 years. The remission rate for pediatric ALL has been high at around 90%, with overall survival at around 60-70% in recent years. However, current treatments for ALL have been less successful for patients with recurrent disease as well as infant and adult patients, leading to an unmet medical need for new treatments.

SUMMARY

【0007】 In one aspect, the present disclosure provides a method for treating leukemia. Said method comprises administering a subject in need with an effective amount of a compound having the following Formula I or Formula II:

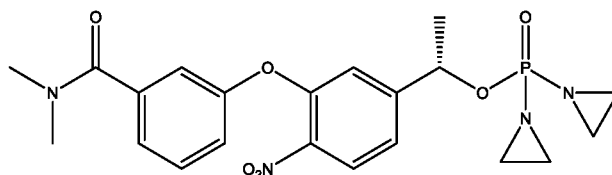
(R)-1-(3-(3-N,N-dimethylaminocarbonyl)phenoxy)-4-nitrophenyl)-1-ethyl-N,N'-bis(ethylene)phosphoramidate:



I

or

(S)-1-(3-(3-N,N-dimethylaminocarbonyl)phenoxy)-4-nitrophenyl)-1-ethyl-N,N'-bis(ethylene)phosphoramidate:



II

or a salt, an isotopic variant, a solvate, or a hydrate of each thereof.

【0008】 In another aspect, the present disclosure provides pharmaceutical composition for treating leukemia. Said pharmaceutical composition comprises a compound of Formula I, Formula II, or a salt, an isotopic variant, a solvate, or a hydrate of each thereof, and at least one pharmaceutically acceptable carrier or excipient.

Brief Description of the Drawings

【0009】 Figure 1 shows the results of the tolerability study. (A) The curve of weight of the mice during experiment period. (B) The median percentage change in weight during experiment period. The arrows indicate OBI-3424 administration.

【0010】 Figure 2 shows the results of the efficacy study of ALL-8 PDX. (A) The change of the percentage of human CD45⁺ during experiment period. The arrows indicate OBI-3424 administration. (B) Percentage of survival during experiment period.

【0011】 Figure 3 shows the results of the efficacy study of ALL-27 PDX. (A) The change of the percentage of human CD45⁺ during experiment period. The arrows indicate OBI-3424 administration. (B) Percentage of survival during experiment period.

【0012】 Figure 4 shows the results of the efficacy study of ALL-29 PDX. (A) The change of the percentage of human CD45⁺ during experiment period. The arrows indicate OBI-3424 administration. (B) Percentage of survival during experiment period.

【0013】 Figure 5 shows the results of the efficacy study of ALL-30 PDX. (A) The change of the percentage of human CD45⁺ during experiment period. The arrows indicate OBI-3424 administration. (B) Percentage of survival during experiment period.

【0014】 Figure 6 shows the results of the efficacy study of ALL-31 PDX. (A) The change of the percentage of human CD45⁺ during experiment period. The arrows indicate OBI-3424 administration. (B) Percentage of survival during experiment period.

【0015】 Figure 7 shows the results of the efficacy study of ALL-32 PDX. (A) The change of the percentage of human CD45⁺ during experiment period. The arrows indicate OBI-3424 administration. (B) Percentage of survival during experiment period.

【0016】 Figure 8 shows the results of the efficacy study of ALL-11/EV PDX. (A) The change of the percentage of human CD45⁺ during experiment period. The arrows indicate OBI-3424 administration. (B) Percentage of survival during experiment period.

【0017】 Figure 9 shows the results of the efficacy study of ALL-11/AKR1C3 PDX. (A) The change of the percentage of human CD45⁺ during experiment period. The arrows indicate OBI-3424 administration. (B) Percentage of survival during experiment period.

【0018】 Figure 10 shows the results of the efficacy study of ALL-28 PDX. (A) The change of the percentage of human CD45⁺ during experiment period. The arrows indicate OBI-3424 administration. (B) Percentage of survival during experiment period.

【0019】 Figure 11 shows the results of the organ infiltration study. EV=Empty vector; AKR1C3= aldo-keto reductase family 1 member C3; ALL = acute lymphoblastic leukemia. Note: Left graphs are samples taken from peripheral blood (PB), spleen (SPL). Right graphs are samples taken from central (C) and endosteal (E) regions of bone marrow (BM) taken from left (L) and right (R) sides. Red triangles= OBI-3424 treated group; Black squares= control group; Gray circles= Day 0 time point group. *P<0.05, **P<0.01, ****P<0.001 comparing OBI-3424 treated mice to control mice. (A) ALL-8; (B) ALL-27; (C) ALL-29.

【0020】 Figure 12 shows the results of the organ infiltration study. EV=Empty vector; AKR1C3= aldo-keto reductase family 1 member C3; ALL = acute lymphoblastic leukemia. Note: Left graphs are samples taken from peripheral blood (PB), spleen (SPL). Right graphs are from samples taken from central (C) and endosteal (E) regions of bone marrow (BM) taken from left (L) and right (R) sides. Red triangles= OBI-3424 treated group; Black squares= control group; Gray circles= Day 0 time point group. ****P<0.0001 comparing OBI-3424 treated mice to control mice. (A) ALL-30; (B) ALL-31; (C) ALL-32.

【0021】 Figure 13 shows the results of the organ infiltration study. EV=Empty vector; AKR1C3= aldo-keto reductase family 1 member C3; ALL = acute lymphoblastic leukemia. Note: Left graphs are samples taken from peripheral blood (PB), spleen (SPL). Right graphs are from samples taken from central (C) and endosteal (E) regions of bone marrow (BM) taken from left (L) and right (R) sides. Red triangles= OBI-3424 treated group; Black squares= control group; Gray circles= Day 0 time point group. *P<0.05, **P<0.01, ****P<0.0001 comparing OBI-3424 treated mice to control mice. (A) ALL-28; (B) ALL-11/EV; (C) ALL-11/AKR1C3.

【0022】 Figure 14 shows the tumor burden growth curve. AST-106: OBI-3424. mpk: mg/kg. Arrows represent the time a dose is administered. “^” indicates only dosing for Group 1 and Group 2.

【0023】 Figure 15 shows the anti-tumor efficacy in blood, spleen and bone marrow samples. AST-106: OBI-3424. mpk: mg/kg. *P<0.05, ***P<0.001.

【0024】 Figure 16 shows the *in vivo* results of the dosage evaluation. (A) ALL-8; (B) ALL-31. Left panels show engraftment of each PDX as the %huCD45⁺ over time. Middle panels show the mouse EFS and right panels show the %huCD45⁺ of murine bone marrow. Right panels, % infiltration of murine bone marrow pre-treatment (black circles), control mice at event (open circles), OBI-3424 at 0.5 mg/kg (grey squares), 1.0 mg/kg (yellow triangles), 2.5 mg/kg (red inverted triangles), at Day 28 post treatment initiation. BM L, left bone marrow; BM R, right bone marrow. ****P<0.0001 comparing OBI-3424 treated and control mice.

【0025】 Figure 17 shows the *in vivo* results in combination with nelarabine treatment. (A) ALL-8; (B) ALL-31. **P<0.01, ***P<0.001, ****P<0.0001 comparing OBI-3424 treated and control mice. Left panels show engraftment of each PDX as the %huCD45⁺ over time. Middle panels show the mouse EFS and right panels show the %huCD45⁺ of murine bone marrow. Right panels, % infiltration of murine bone marrow pre-treatment (black circles), control mice at event (open circles), OBI-3424 at 0.5 mg/kg (grey squares), 1.0 mg/kg (yellow triangles), 2.5 mg/kg (red triangles), nelarabine treated (blue triangles), and OBI-3424 in combination with nelarabine treated (green inverted triangles) at Day 28 post treatment initiation. BM L, left bone marrow; BM R, right bone marrow.

DETAILED DESCRIPTION

【0026】 AKR family I member C3 (AKR1C3) is a hydroxysteroid dehydrogenase involved in the synthesis of steroid hormones and prostaglandins⁸ and is over expressed in pediatric and adult ALL. A potential novel therapeutic approach is to exploit the enzymatic activity of AKR1C3 to activate novel prodrugs. Without being bound by theory, OBI-3424 (the Formula I and Formula II compound of the present disclosure), is a prodrug that is selectively activated by AKR1C3 to a potent DNA alkylating agent.

【0027】 *Definitions*

【0028】 The following definitions are provided to assist the reader. Unless otherwise defined, all terms of art, notations, and other scientific or medical terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the chemical and medical arts. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not be construed as representing a substantial difference over the definition of the term as generally understood in the

art.

【0029】 All numerical designations, *e.g.*, pH, temperature, time, concentration, and weight, including ranges of each thereof, are approximations that typically may be varied (+) or (-) by increments of 0.1, 1.0, or 10.0, as appropriate. All numerical designations may be understood as preceded by the term “about”. Reagents described herein are exemplary and equivalents of such may be known in the art.

【0030】 “A,” “an,” and, “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a compound refers to one or more compounds or at least one compound. As such, the terms “a” (or “an”), “one or more”, and “at least one” are used interchangeably herein.

【0031】 As used herein, the term “comprising” is intended to mean that the compositions and methods include the recited elements, but not excluding others. “Consisting essentially of” when used to define compositions and methods, shall mean excluding other elements of any essential significance to the composition or method. “Consisting of” shall mean excluding more than trace elements of other ingredients for claimed compositions and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this invention. Accordingly, it is intended that the methods and compositions can include additional steps and components (comprising) or alternatively including steps and compositions of no significance (consisting essentially of) or alternatively, intending only the stated method steps or compositions (consisting of).

【0032】 The terms "optically active" and "enantiomerically active" refer to a collection of molecules, which has an enantiomeric excess of no less than about 10%, no less than about 20%, no less than about 30%, no less than about 40%, no less than about 50%, no less than about 60%, no less than about 70%, no less than about 80%, no less than about 90%, no less than about 91%, no less than about 92%, no less than about 93%, no less than about 94%, no less than about 95%, no less than about 96%, no less than about 97%, no less than about 98%, no less than about 99%, no less than about 99.5%, no less than about 99.8%, or no less than about 99.9%. In certain embodiments, the enantiomeric excess for an optically or enantiomerically active compound is no less than about 90%, no less than about 95%, no less than about 98%, or no less than about 99%.

【0033】 In describing an optically active compound, the prefixes R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The (+) and (-) are used to denote the optical rotation of the compound, that is, the direction in which a plane of polarized light is rotated by the optically active compound. The (-) prefix indicates that the compound is

levorotatory, that is, the compound rotates the plane of polarized light to the left or counterclockwise. The (+) prefix indicates that the compound is dextrorotatory, that is, the compound rotates the plane of polarized light to the right or clockwise. However, the sign of optical rotation, (+) and (-), is not related to the absolute configuration of the molecule, R and S.

【0034】 The terms "optically pure" and "enantiomerically pure" refer to a collection of molecules, which has an enantiomeric excess (ee) of no less than about 80%, no less than about 90%, no less than about 91%, no less than about 92%, no less than about 93%, no less than about 94%, no less than about 95%, no less than about 96%, no less than about 97%, no less than about 98%, no less than about 99%, no less than about 99.5%, no less than about 99.8%, or no less than about 99.9%. In certain embodiments, the enantiomeric excess for an optically or enantiomerically pure compound is no less than about 90%, no less than about 95%, no less than about 98%, or no less than about 99%. An enantiomeric excess of a compound can be determined by any standard methods used by one of ordinary skill in the art, including, but not limited to, chiroptical chromatography (gas chromatography, high-performance liquid chromatography, and thin-layer chromatography) using an optically active stationary phase, isotopic dilution, electrophoresis, calorimetry, polarimetry, NMR resolution methods with chiral derivatization, and NMR methods with a chiral solvating agent or chiral shift reagent.

【0035】 The terms "substantially pure" and "substantially homogeneous" mean sufficiently homogeneous to appear free of readily detectable impurities as determined by standard analytical methods used by one of ordinary skill in the art, including, but not limited to, thin layer chromatography (TLC), gel electrophoresis, high performance liquid chromatography (HPLC), gas chromatography (GC), nuclear magnetic resonance (NMR), and mass spectrometry (MS); or sufficiently pure such that further purification would not detectably alter the physical, chemical, biological, and/or pharmacological properties, such as enzymatic and biological activities, of the substance. In certain embodiments, "substantially pure" or "substantially homogeneous" refers to a collection of molecules, wherein at least about 50%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or at least about 99.5% by weight of the molecules are a single stereoisomer of a compound, as determined by standard analytical methods.

【0036】 The term "isotopic variant" refers to a compound that contains an unnatural proportion of an isotope at one or more of the atoms that constitute such compounds. In certain embodiments, an "isotopic variant" of a compound contains unnatural proportions of one or more isotopes, including, but not limited to, hydrogen (^1H), deuterium (^2H), tritium (^3H), carbon-11 (^{11}C), carbon-12 (^{12}C), carbon-13 (^{13}C), carbon-14 (^{14}C), nitrogen-13 (^{13}N), nitrogen-14 (^{14}N), nitrogen-15

(¹⁵N), oxygen-14 (¹⁴O), oxygen-15 (¹⁵O), oxygen-16 (¹⁶O), oxygen-17 (¹⁷O), oxygen-18 (¹⁸O), fluorine-17 (¹⁷F), fluorine-18 (¹⁸F), phosphorus-31 (³¹P), phosphorus-32 (³²P), phosphorus-33 (³³P), sulfur-32 (³²S), sulfur-33 (³³S), sulfur-34 (³⁴S), sulfur-35 (³⁵S), sulfur-36 (³⁶S), chlorine-35 (³⁵Cl), chlorine-36 (³⁶Cl), chlorine-37 (³⁷Cl), bromine-79 (⁷⁹Br), bromine-81 (⁸¹Br), iodine-123 (¹²³I), iodine-125 (¹²⁵I), iodine-127 (¹²⁷I), iodine-129 (¹²⁹I), and iodine-131 (¹³¹I). In certain embodiments, an "isotopic variant" of a compound is in a stable form, that is, non-radioactive. In certain embodiments, an "isotopic variant" of a compound contains unnatural proportions of one or more isotopes, including, but not limited to, hydrogen (¹H), deuterium (²H), carbon-12 (¹²C), carbon-13 (¹³C), nitrogen-14 (¹⁴N), nitrogen-15 (¹⁵N), oxygen-16 (¹⁶O), oxygen-17 (¹⁷O), oxygen-18 (¹⁸O), fluorine-17 (¹⁷F), phosphorus-31 (³¹P), sulfur-32 (³²S), sulfur-33 (³³S), sulfur-34 (³⁴S), sulfur-36 (³⁶S), chlorine-35 (³⁵Cl), chlorine-37 (³⁷Cl), bromine-79 (⁷⁹Br), bromine-81 (⁸¹Br), and iodine-127 (¹²⁷I). In certain embodiments, an "isotopic variant" of a compound is in an unstable form, that is, radioactive. In certain embodiments, an "isotopic variant" of a compound contains unnatural proportions of one or more isotopes, including, but not limited to, tritium (³H), carbon-11 (¹¹C), carbon-14 (¹⁴C), nitrogen-13 (¹³N), oxygen-14 (¹⁴O), oxygen-15 (¹⁵O), fluorine-18 (¹⁸F), phosphorus-32 (³²P), phosphorus-33 (³³P), sulfur-35 (³⁵S), chlorine-36 (³⁶Cl), iodine-123 (¹²³I), iodine-125 (¹²⁵I), iodine-129 (¹²⁹I), and iodine-131 (¹³¹I). It will be understood that, in a compound as provided herein, any hydrogen can be ²H, for example, or any carbon can be ¹³C, as example, or any nitrogen can be ¹⁵N, as example, and any oxygen can be ¹⁸O, where feasible according to the judgment of one of skill. In certain embodiments, an "isotopic variant" of a compound contains unnatural proportions of deuterium.

【0037】 The phrase “or a salt, an isotopic variant, a solvate, or a hydrate of each thereof” has the same meaning as the phrase “or a salt of the compound referenced therein, an isotopic variant of the compound referenced therein, a solvate of the compound referenced therein, or a hydrate of the compound referenced therein”.

【0038】 “Administering” or “administration of” a drug to a patient (and grammatical equivalents of this phrase) refers to direct administration, which may be administration to a patient by a medical professional or may be self-administration, and/or indirect administration, which may be the act of prescribing a drug. For example, a physician who instructs a patient to self-administer a drug and/or provides a patient with a prescription for a drug is administering the drug to the patient.

【0039】 “Patient,” “subject,” and “subject in need” are used interchangeably to refer to a mammal in need of treatment for cancer, particularly leukemia, more particularly T-cell acute lymphoblastic leukemia. Generally, the patient is a human. Generally, the patient is a human

diagnosed with cancer. In certain embodiments a “patient,” “subject,” or “subject in need” may refer to a non-human mammal used in screening, characterizing, and evaluating drugs and therapies, such as a non-human primate, a dog, cat, rabbit, pig, mouse, or rat.

【0040】 “Effective amount” used herein is referred to the amount of each active agent required to confer the desired effect on the subject, either alone or in combination with one or more other active agents. An effective amount varies, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size, gender and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons. In a preferable embodiment, the effective amount is about 0.10 µg/kg to about 100 mg/kg.

【0041】 “Treating,” “treatment of,” or “therapy of” a condition or patient refers to taking steps to obtain beneficial or desired results, including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation or amelioration of one or more symptoms of cancer; diminishment of extent of disease; delay or slowing of disease progression; amelioration, palliation, or stabilization of the disease state; or other beneficial results. Treatment of cancer may, in some cases, results in partial response or stable disease.

【0042】 “Pharmaceutically acceptable” component (such as a carrier or excipient) means that the compound or composition is suitable for administration to a subject to achieve the treatments described herein, without unduly deleterious side effects in light of the severity of the disease and necessity of the treatment. “Carrier” means a material that does not cause significant stimulation to an organism and does not eliminate the biological activity and characteristics of a given compound. “Excipient” means an inert substance added into a pharmaceutical composition to facilitate administration of a compound.

【0043】 “Purine nucleoside analog (PNA)” used herein is referred to an important group of cytotoxic drugs active in the treatment of neoplastic and autoimmune diseases. Three of them, fludarabine (FA), cladribine (2-chlorodeoxyadenosine, 2-CdA) and pentostatin (2'-deoxycoformycin, DCF) have established clinical activity in hematological malignancies and have been approved by FDA. These drugs are also investigated in some autoimmune disorders.

Recently four novel PNA: clofarabine (CAFdA), nelarabine, immucillin H (BCX-1777, forodesine) and 8-chloroadenosine (8-Cl-Ado) have been synthesized and introduced into clinical trials (Robak T *et al*, Curr Med Chem. 2006; 13(26): 3165-89).

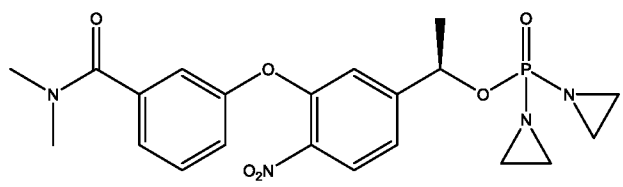
【0044】 A “combination” refers to combination therapy having an amount of OBI-3424 and/or an amount of other medicines administered together (either as co-administration and/or co-formulation), either sequentially or simultaneously, on the same or different days during a treatment cycle, has a synergistic effect that is therapeutically effective and more than therapeutically additive.

【0045】 A usable pharmaceutically acceptable carrier, or excipient are disclosed in various references including *Handbook of Pharmaceuticals Excipients* edited by Raymond C Rowe, Paul J Sheskey, and Marian E Quinn. In a unlimited embodiment, said pharmaceutically acceptable carrier, or excipient can be selected from the group consisting of inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Said compositions optionally further comprises at least one additional biologically active compound or agent.

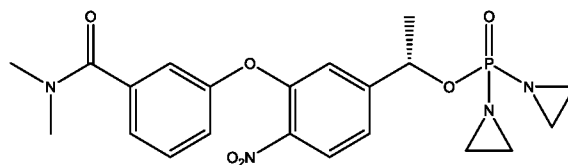
【0046】 The pharmaceutical composition might comprise but not limited to a single unit dose of the active ingredient (for instance, the compound of the present disclosure). For purposes of treatment, a dose unit can be in the form of a discrete article such as but not limited to a tablet or capsule, or can be a measurable volume of a solution, suspension or the like containing a unit dose of the active ingredient. The term “unit dose” herein refers to an amount of active ingredient intended for a single but not limited to oral, intravenous, intramuscular, cutaneous, subcutaneous, intrathecal, transdermal, implantation, sublingual, buccal, rectal, vaginal, ocular, otic, nasal, inhalation, or nebulization administration to a subject for treatment such as but not limited to alcohol dependence, opioid dependence, pain relief, or other diseases. The treatment may require periodic administration of unit doses of the compound of the present disclosure, for example one unit dose two or more times a day, one unit dose with each meal, one unit dose every four hours or other interval, or only one unit dose per day.

【0047】 The First Aspect of the present disclosure.

【0048】 The first aspect of the present disclosure provides a method for treating leukemia comprising administering a subject in need with an effective amount of a compound having the following Formula I or Formula II:



I



II

or a salt, an isotopic variant, a solvate, or a hydrate of each thereof.

【0049】 The manufacture method and exemplary structures of Formula I and Formula II compounds are recited in **WO2017087428A1** filed on November 11, 2016, incorporated herein by reference.

【0050】 In a specific embodiment, said compound has an enantiomeric excess of no less than 80%, no less than 90%, or no less than 95%.

【0051】 In a preferable embodiment, said compound is substantially pure.

【0052】 In a specific embodiment, said compound has a purity of at least 50% or at least 90%.

【0053】 In a preferable embodiment, said leukemia is acute lymphoblastic leukemia.

【0054】 In a preferable embodiment, said acute lymphoblastic leukemia is T-cell acute lymphoblastic leukemia.

【0055】 In a preferable embodiment, the method further comprises administering the subject an anti-cancer medicine. The anti-cancer medicine includes but not limited to nelarabine, clofarabine, fludarabine, cladribine, pentostatin, forodesine, 8-chloroadenosine, azathioprine or a combination thereof.

【0056】 In specific embodiment, administration of OBI-3424 and administration of the anti-cancer medicine can be conducted at any time interval. In an alternative embodiment, the time interval is 1 minute, 3 minutes, 5 minutes, 10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 5 hours, 10 hours, 1 day, 3 days, 5 days, 1 week, 2 weeks, 3 weeks, or 1 month.

【0057】 In a preferable embodiment, said acute lymphoblastic leukemia is B-cell acute lymphoblastic leukemia.

【0058】 Second Aspect of the Present Disclosure.

【0059】 The second aspect of the present disclosure provides a pharmaceutical composition for treating leukemia. Said pharmaceutical composition comprises a compound of above Formula I or Formula II and at least one pharmaceutically acceptable carrier or excipient.

【0060】 In a preferable embodiment, said leukemia is acute lymphoblastic leukemia.

【0061】 In a preferable embodiment, said acute lymphoblastic leukemia is T-cell acute lymphoblastic leukemia.

【0062】 In a preferable embodiment, said acute lymphoblastic leukemia is B-cell acute lymphoblastic leukemia.

【0063】 In an alternative embodiment, said pharmaceutical composition is formulated to be administered through oral, intravenous, intramuscular, cutaneous, subcutaneous, intrathecal, transdermal, implantation, sublingual, buccal, rectal, vaginal, ocular, otic, nasal, inhalation, or nebulization administration route.

【0064】 In a preferable embodiment, the pharmaceutical composition further comprises an anti-cancer medicine. The anti-cancer medicine includes but not limited to nelarabine, clofarabine, fludarabine, cladribine, pentostatin, forodesine, 8-chloroadenosine, azathioprine or a combination thereof.

【0065】 Another aspect provides a pharmaceutical combination comprising a Formula I or II compound and a second compound having anti-cancer properties or other therapeutic effects.

EXAMPLES

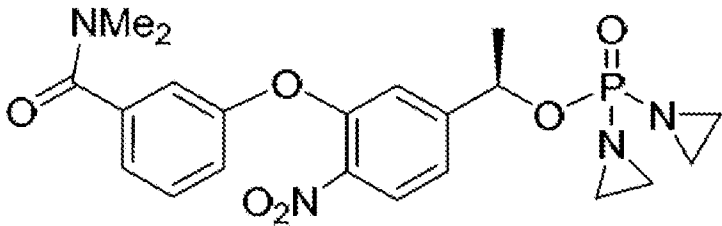
【0066】 Various features and embodiments of the disclosure are illustrated in the following representative examples, which are intended to be illustrative, and not limiting. Those skilled in the art will readily appreciate that the specific examples are only illustrative of the invention as described more fully in the claims which follow thereafter. Every embodiment and feature described in the application should be understood to be interchangeable and combinable with every embodiment contained within.

【0067】 Example 1: Cytotoxicity study.

【0068】 In-vitro cytotoxicity of AKR1C3-associated **OBI-3424** was investigated in eleven T-ALL cell lines, one B-ALL cell line transfected with granulocyte-colony stimulating factor, and one BCP-ALL cell line. Expression levels of AKR1C3 protein were determined using western blot

analysis. The *in vitro* cytotoxicity, calculated as 50% maximal inhibitory concentration (IC₅₀) of OBI-3424, was determined using a CellTiter-Glo assay. The properties of OBI-3424 were listed as **Table 1**.

【0069】 Table 1: The properties of OBI-3424.

 OBI-3424	
Full Chemical Name:	3-[5-[(1R)-1-[bis(aziridin-1-yl)phosphoryloxy]ethyl]-2-nitrophenoxy]-N,N-dimethylbenzamide
Formula:	C ₂₁ H ₂₅ N ₄ O ₆ P
Molecular Weight:	460.43 g/mol
CAS number	2097713-68-1

【0070】 OBI-3424 displayed *in vitro* cytotoxicity with an IC₅₀ range of around 3.0–30.0 nM in six cell lines expressing high (strong) levels of AKR1C3. For the cell lines with moderate AKR1C3 expression levels, the IC₅₀ range was 3.0–84.0 nM (**Table 2**).

【0071】 Table 2: AKR1C3-dependent *in vitro* Cytotoxicity of OBI-3424 in ALL Cell Lines.

Leukemia	Cell Line	AKR1C3 Expression	IC ₅₀ (nM)
T-ALL (child)	PF-382	Strong	15.0
T-ALL (child)	SUP-T1	Strong	3.0
T-ALL (child)	TALL-1	Strong	30.0
T-ALL (19 yo)	MOLT-4	Strong	10.0
T-ALL (child)	CCRF-CEM	Strong	3.7
T-ALL (child)	Jurkat	Moderate	40.0
T-ALL (child)	Jurkat, Clone E6-1	Moderate	24.0
T-ALL (adult)	NOMO-1	Moderate	11.0
T-ALL (Jurkat mutant)	P116	Moderate	84.0
B-ALL (transfected with G-CSF)	GR-ST	Strong	9.9
BCP-ALL	Reh	Moderate	3.0

AKR1C3 = aldo-keto reductase family 1 member C3; ALL = acute lymphoblastic leukemia; B-ALL= B-cell acute lymphoblastic leukemia; IC₅₀ = fifty percent inhibitory concentration; T-ALL = T-cell acute lymphoblastic leukemia; G-CSF= Granulocyte-colony stimulating factor; BCP-ALL= B-Cell Precursor ALL

【0072】 Another *in-vitro* cytotoxicity using ALL patient-derived xenografts (PDX) cell lines was conducted. The experiment included four B-ALL PDX cell lines, seven T-ALL cell lines, and six ETP-ALL (Early T-cell Precursor Acute Lymphoblastic Leukemia) cell lines. ALL PDX cells were cultured in QBSF medium (Quality Biological Inc., Gaithersburg MD, USA) supplemented with Flt-3 ligand (Bionovus Life Sciences, Cherrybrook, NSW, Australia) or IL-7 (Jomar Life Research, Melbourne, VIC Australia). Cells were plated according to optimal cell density and incubated for 3 h or overnight (37 °C, 5% CO₂). PDX cells were treated with OBI-3424 or vehicle control for 48 h or 72 h, respectively. Viability was determined using Alamar Blue reduction assay, or Cell Titer-Glo Luminescent Cell Viability Assay (Promega, Madison, WI). The half-maximal inhibitory concentration (IC₅₀) was calculated by interpolation of non-linear regression curves calculated by Graphpad Prism 7 software.

【0073】 The results were shown in **Table 3**. OBI-3424 exerted potent cell killing efficacy against ALL PDXs. The median IC₅₀ values were 36.9 nM for B-ALL, 112.4 nM for T-ALL and 42.0 nM for ETP-ALL.

【0074】 Table 3. Patient demographics of PDXs tested *in vivo* and *in vitro* with OBI-3424.

PDX	Age (y)	Sex	Diagnosis	Disease Status at Biopsy	OBI-3424 IC ₅₀ (nM)
ALL-2	5.5	F	B-ALL	Relapse	92.3
ALL-7	7.4	M	B-ALL	Diagnosis	3.2
ALL-11	3.1	F	B-ALL	Diagnosis	28.3
ALL-28	1.7	M	B-ALL	Diagnosis	23.6
ALL-8	12.8	M	T-ALL	Relapse 1	4.1
ALL-27	8.5	M	T-ALL	Diagnosis	10.3
ALL-29	4.9	M	T-ALL	Diagnosis	1.1
ALL-30	7.5	M	T-ALL	Diagnosis	5.5
ALL-31	10.2	M	T-ALL	Diagnosis	9.7
ALL-32	-	-	T-ALL	Relapse 1	11.3
ALL-42	2.6	M	T-ALL	Diagnosis	744.7
ETP-1	16	M	ETP-ALL	Diagnosis	130.3
ETP-2	3	M	ETP-ALL	Diagnosis	15.7
ETP-3	14	M	ETP-ALL	Diagnosis	47.2
ETP-4	8.9	M	ETP-ALL	Diagnosis	6.8
ETP-5	15.9	M	ETP-ALL	Diagnosis	48.1
ETP-6	19.5	F	ETP-ALL	Diagnosis	4

【0075】 Furthermore, the results of *in vitro* cytotoxicity between several leukemia cell lines were in the following **Table 4**. It demonstrated that OBI-3424 could be treated in T-ALL, B-ALL, Acute myeloid leukemia (AML), Acute promyelocytic leukemia (APL) and Erythroleukemia.

【0076】 Table 4. *In vitro* cytotoxicity of OBI-3424 following 72 hour exposure in leukemia cell lines.

Cell Lines	AKR1C3 Expression	IC ₅₀ (nM)	Disease Characteristics
CCRF-CEM	Strong	3.7	T-ALL
MOLT-4	Strong	10	T-ALL (19 yrs)
PF-382	Strong	15	T-ALL (pediatric)
SUP-T1	Strong	3	T-ALL (pediatric)
TALL-1	Strong	30	T-ALL (pediatric)
Jurkat	Medium	40	T-ALL (pediatric)
Jurkat, Clone E6-1	Medium	24	T-ALL
NOMO-1	Medium	11	T-ALL (adult)
P116	Medium	84	Jurkat mutant
GR-ST	Strong	9.9	B-ALL (transfected with G-CSF)
KG-1	Strong	153	Erythroleukemia (59 yrs)
TF-1	Strong	2.8	Erythroleukemia (35 yrs)
HEL	Medium	224	Erythroleukemia (30 yrs)
Reh	Medium	3	ALL (non-T, Non-B)
HL-60	Medium	52.6	APL (36 yrs)
HL-60 Clone 15	Medium	87	APL (36 yrs)
Mono-Mac-6	Medium	29.8	AML-M5 (64yrs)

【0077】 **Example 2: Tolerability study.**

【0078】 Naïve NSG mice were weighed and bled (for biochemistry & hematology markers) weekly to monitor drug toxicity. The mice were treated intraperitoneally once weekly with **OBI-3424** at various dosage (0.625, 1.25, 1.875, and 2.5 mg/kg) for 3 weeks. The weight of mice was measured and recorded; the changes in weight were calculated. The results (**Figure 1**) showed that the treatment has minimal impact on mouse weight. OBI-3424 was well tolerated, with a toxicity rate of only 2.8% in the drug-treated groups.

【0079】 Example 3: Efficacy study.

【0080】 ALL patient-derived xenografts (PDXs) grew in an orthotopic manner following intravenous inoculation into NSG mice. *See Table 5.* Mice were bled (IV) weekly to determine %human CD45⁺ (%huCD45⁺) in the peripheral blood (PB). Treatment of OBI-3424 (2.5 mg/kg by the intraperitoneal route once weekly for 3 weeks) or vehicle commenced when the %huCD45⁺ exceeded a median of 1%. An event was defined as the %huCD45⁺ exceeding 25% or leukemia-related morbidity. Leukemia growth delay (LGD) was calculated as the median EFS of treated mice minus that of control.

【0081】 Mice were divided into 9 groups of 27 mice (**Table 6**). Each group was subdivided into treatment groups receiving either vehicle (n=12), OBI-3424 (n=12), or no treatment with collection at Day 0 time point/baseline (n=3). The vehicle and treatment subgroups were further divided into two study cohorts measuring treatment efficacy and Day 28/event time point. The Day 0 time point/baseline mice and Day 28/event time point mice were used to calculate event free survival (EFS). Mice were weighed and bled weekly throughout the study. Mice with greater than 10% weight loss were closely monitored. Any mouse that had $\geq 20\%$ weight loss was euthanized and autopsied to identify the cause of weight loss.

【0082】 The Kaplan-Meier method was used to compare event-free survival (EFS) between treatment (T) and control (C) groups. Stringent objective response measures (ORM) were assigned to each mouse and reported as group medians (Houghton *et al*, *Pediatr Blood Cancer*, 2007; 49:928-40). The Objective Response categories were progressive disease (PD, which was subdivided into progressive disease without and with growth delay, PD1 and PD2 respectively, defined only for mice in treatment groups), stable disease (SD), partial response (PR), complete response (CR), and maintained complete response (MCR). Detailed description thereof was set forth in **Table 7**.

【0083】 Table 5: ALL patient-derived xenografts (PDXs) mouse model.

PDX	Diagnosis	Stage of Disease	Comments
ALL-8	T-ALL	Relapse 1	Patient died from disease. Normal cytogenetics
ALL-27	T-ALL	Diagnosis	Patient died in CR2. Normal cytogenetics
ALL-29	T-ALL	Diagnosis	Patient in CR1. Normal cytogenetics
ALL-30	T-ALL	Diagnosis	Patient dies from disease
ALL-31	T-ALL	Diagnosis	Patient died from disease. Cytogenetics: del(6)(q21), del(11)(q23)

ALL-32	T-ALL	Relapse 2	Patient in CR2. Cytogenetics: del(9)(q34)
ALL-28	BCP-ALL	Diagnosis	Patient in CR1. Hyperdiploid
ALL-11/EV	BCP-ALL	Diagnosis	Empty vector (EV) control
ALL-11/AKR1C3	BCP-ALL	Diagnosis	AKR1C3 transduced

PDX= patient derived xenograft; ALL= Acute lymphoblastic leukemia; T-ALL= T-Cell Acute Lymphoblastic Leukemia; CR2= Second complete remission; CR1= First complete remission; BCP-ALL= B-Cell Precursor Acute Lymphoblastic Leukemia; EV= empty vector; AKR1C3= Aldo-keto reductase family 1 member C3

【0084】 Table 6: Efficacy result of Nine PDXs mice.

PDX (Diagnosis)	Treatment Group	Animal Counts		
		Efficacy (n)	Day 28/Event (n)	Total (n)
ALL-8 (T-ALL)	Vehicle (2.5 mg/kg equivalent)	8	4	12
	OBI-3424 (2.5 mg/kg)	8	4	12
	Day 0 Time Point/Baseline	-	-	3
ALL-11/AKR1C3 (BCP-ALL)	Vehicle (2.5 mg/kg equivalent)	8	4	12
	OBI-3424 (2.5 mg/kg)	8	4	12
	Day 0 Time Point/Baseline	-	-	3
ALL-11/EV (BCP-ALL)	Vehicle (2.5 mg/kg equivalent)	8	4	12
	OBI-3424 (2.5 mg/kg)	8	4	12
	Day 0 Time Point/Baseline	-	-	3
ALL-27 (T-ALL)	Vehicle (2.5 mg/kg equivalent)	8	4	12
	OBI-3424 (2.5 mg/kg)	8	4	12
	Day 0 Time Point/Baseline	-	-	3
ALL-28 (T-ALL)	Vehicle (2.5 mg/kg equivalent)	8	4	12
	OBI-3424 (2.5 mg/kg)	8	4	12
	Day 0 Time Point/Baseline	-	-	3
ALL-29 (T-ALL)	Vehicle (2.5 mg/kg equivalent)	8	4	12
	OBI-3424 (2.5 mg/kg)	8	4	12
	Day 0 Time Point/Baseline	-	-	3
ALL-30 (T-ALL)	Vehicle (2.5 mg/kg equivalent)	8	4	12
	OBI-3424 (2.5 mg/kg)	8	4	12
	Day 0 Time Point/Baseline	-	-	3
ALL-31 (T-ALL)	Vehicle (2.5 mg/kg equivalent)	8	4	12
	OBI-3424 (2.5 mg/kg)	8	4	12
	Day 0 Time Point/Baseline	-	-	3
ALL-32	Vehicle (2.5 mg/kg equivalent)	8	4	12

(T-ALL)	OBI-3424 (2.5 mg/kg)	8	4	12
	Day 0 Time Point/Baseline	-	-	3

PDX= patient-derived xenograft; ALL= acute lymphoblastic leukemia; T-ALL= T-cell acute lymphoblastic leukemia; BCP-ALL= B-cell precursor acute lymphoblastic leukemia; EV= empty vector; AKR1C3= Aldo-keto reductase family 1 member C3; N= total number of mice

【0085】 Table 7: Detailed description of Objective Response.

ORM	Description	Score
Progressive Disease 1 (PD1)	%HuCD45 ⁺ never drops below 1% and reaches event before end of study, with EFS $\leq$ 200% of median control EFS	0
Progressive Disease 2 (PD2)	%HuCD45 ⁺ never drops below 1% and reaches event before end of study, with EFS >200% of median control EFS	2
Stable Disease (SD)	%HuCD45 ⁺ in PB never < 1% and mouse never reaches event during study period	4
Partial Response/ Remission (PR)	%HuCD45 ⁺ in PB <1% once during study period	6
Complete Response/ Remission (CR)	%HuCD45 ⁺ in PB <1% for at least two consecutive weekly readings during study period	8
Maintained Complete Response/Remission (MCR)	%HuCD45 ⁺ in PB <1% for at least 3 consecutive weekly readings at any time after treatment has been completed	10

ALL = acute lymphoblastic leukemia; CR = complete response; EFS = event-free survival; LGD = leukemia growth delay; MCR= maintained complete response; ORM = objective response measure; PB = peripheral blood; PD1 = progressive disease 1; PD2 = progressive disease 2; PDX = patient-derived xenograft; PR = partial response; SD = stable disease; %HuCD45⁺ = proportion of human CD45⁺ cells.

【0086】 For ALL, an event was defined as %HuCD45 cells exceeding 25% in PB. The exact time-to-event was established by interpolating between the measurements directly preceding and following the event, assuming log-linear growth. Differences in EFS between treated and control groups were tested using the Gp test of Harrington & Fleming (Biometrika 69:553-566, 1983; $\alpha=0.05$, two-sided alternative) with $\rho=1$, which is equivalent to the Peto & Peto modification of Gehan-Wilcoxon. This test is more efficient than the Mantel-Cox log-rank test under shift (i.e. translation) alternatives (Tarone and Ware, Biometrika 64: 166-80, 1977), which is a pattern we have described in previous testing data with similar xenograft mouse models.

【0087】 Overall group response was determined by the median response among evaluable mice as follows: each individual mouse was assigned a score from 0 to 10 based on their response, PD1=0, PD2=2, SD=4, PR=6, CR=8, and MCR=10, and the median for the group determines the overall response. If the median score is half-way between an objective response number category, the objective response is assigned to the lower response category (e.g. an objective response score

of 9 is scored CR). Studies in which toxicity was greater than 25%, or in which the control group was not SD or worse, were considered in-evaluable and were excluded from analysis.

【0088】 Treatment groups with PR, CR, or MCR were considered to have an objective response. Agents inducing objective responses were considered highly active against the tested line, while agents inducing SD or PD2 were considered to have intermediate activity, and agents producing PD1 were considered to have low level of activity against the tested line.

【0089】 Data is shown as both %huCD45⁺ engraftment in the PB and Kaplan Meier curves (EFS). *See Figures 2-10 and Table 8.* Leukemia progression delay (T-C) was observed in OBI-3424 treatment group for all six T-ALL PDX models, with valued ranging from 17.1 days (p <0.001) to 77.8 days (p < 0.001) and EFS tumor growth inhibition rate (EFS T/C) valued ranging from 3.92% (p < 0.001) to 14.0% (p < 0.001; **Table 8**).

【0090】 The BCP-ALL PDX panel, which had a lower expression of AKR1C3, had T-C values ranging from 21.2 days to 58.2 days (p < 0.001), and EFS T/C values of around 2.5% (p < 0.001; **Table 6**). The BCP-ALL PDX model ALL/AKR1C3, which expresses AKR1C3 at levels equivalent to those seen in T-ALL models, had a leukemia progression delay of 47.2 days (p < 0.001), falling within the range observed in the T-ALL PDX panel and EFS T/C value of 3.53% (p < 0.001). Each mouse was assigned an ORM as per the PPTC criteria (**Tables 7-8**), from which the median was calculated.

【0091】 Of the T-ALL PDXs, 5/6 showed objective responses, with 1/6 showing a CR, 4/6 showing a MCR, and 1/6 showing SD. The ALL-27 T-ALL PDX model was contaminated with a mouse-origin lymphoma, so only four mice in this group were used for EFS analyses (**Table 8**). Of the BCP-ALL PDXs, 1/3 showed CR and 2/3 showed MCR. The response rate of each PDX to the transduction of AKR1C3 in BCP-ALL PDX model ALL-11/AKR1C3 improved the median EFS from 34.9 days (ALL-11/EV, p < 0.001) to 65.9 days (ALL-11/AKR1C3, p < 0.001) when treated with OBI-3424 (**Table 8**). Efficacy of OBI-3424 treatment was also measured using the maximum decrease in %HuCD45⁺ in PB from baseline value (Day 0 PB value from Day 0 mice).

【0092】 Table 8: The Objective Response of Nine PDXs mice.

PDX	N	Na	EFS T-C (Days)	EFS T/C	p- value	Min CD45	Median Response	Mean BM %huCD45+ (day*)		
								Control	OBI-3424	p-value
ALL-8	8	8	67.3	8.7	<0.001	0.0	MCR	97.9 (15)	0 (28)	<0.0001
ALL-27	8	4	70.6	11.4	0.008	0.0	MCR	49.8 (8,14)	0 (28)	NS
ALL-29	8	8	38.1	14	<0.001	0.013	CR	92.3 (8)	10.7 (28)	<0.0001
ALL-30	8	7	42.1	7.5	<0.001	0.0	MCR	95.7 (7)	0.25 (28)	<0.0001

ALL-31	8	8	77.8	7.5	<0.001	0.0	MCR	98.7 (14,21)	0 (28)	<0.0001
ALL-32	8	8	17.1	3.9	<0.001	1.1	SD	94.4 (8,15)	96.3 (22,28)	NS
ALL-28	8	8	58.2	2.5	<0.001	0.0	MCR	69.5 (28)	0.69 (28)	0.05
ALL-11/EV	8	8	21.1	2.5	<0.001	0.13	CR	66.2 (19)	61.7 (28)	NS
ALL-11/1C3	8	8	47.2	3.5	<0.001	0.0	MCR	65.8 (20,25)	11.2 (28)	<0.0001

N, total number of mice entering experiment; Na, number of mice in analysis; EFS T – C, difference in median time-to-event (days) between T and C groups; EFS T/ C, ratio of median time-to-event (days) between T and C groups; *P*-value, between C and T EFS by Gehan-Wilcoxon test; Min CD45, average minimum huCD45% for treated group; BM, bone marrow; *, days post treatment initiation on which BM samples were harvested; NS=not significant.

【0093】 **Example 4: Organ Infiltration.**

【0094】 Organ infiltration was determined by flow cytometry, and dead cells were excluded using a fixable viability dye. Data were analysed by a Two-way ANOVA coupled with a Tukey's multiple comparison test. Data are expressed as individual values, with a line indicating the median. For the T-ALL PDX models, 4/6 showed significantly decreased leukemic infiltration of PB, CP, SPL, and BM samples taken from central and endosteal regions of both femurs in mice treated with OBI-3424 compared to the mice treated with the vehicle control. ALL-27 showed significant decreases in leukemic infiltration in PB, CP, and SPL, but no decrease in bone regions. ALL-32 showed no significant decrease in leukemic infiltration. Of the BCP-ALL PDXs, 2/3 showed significant decreases in infiltration across all sampled organs (**Figures 11-13**). ALL-11/AKR1C3 showed infiltration levels similar to those seen in the T-ALL PDX models. ALL-11/EV only showed significant decreases in infiltration in PB and CP.

【0095】 **Example 5: *In vivo* Antitumor Efficacy in ALL PDX Mouse Models.**

【0096】 Forty female NOD/SCID mice, impaired T-cell, B-cell, and natural killer cell lymphocyte development, were inoculated via IV injection with 100 μ L saline containing 2×10^6 prepared HuKemia[®] ALL model AL7473 cells. Mice were divided into 4 treatment groups of 10 mice each one week after tumor inoculation. Groups were treated intravenously with either dextrose 5% in water (D5W) solution as vehicle control (Group 1), OBI-3424 (coded as AST-106) at 0.5 mg/kg (Group 2), 1.5 mg/kg (Group 3), or 4.5 mg/kg (Group 4) weekly for 4 weeks. All mice were sacrificed 6 days after the fourth treatment dose. PB, SPL, and BM were collected and analyzed using HuCD45⁺ fluorescent-activated cell sorting (FACS) detection.

【0097】 All OBI-3424 treatment groups had significantly lower HuCD45⁺ levels in PB than

the placebo group, with a p -value <0.01 (**Figure 14**). In addition, all treatment groups had significantly lower tumor burdens than the control group in PB, SPL, and BM at the end of the study (**Figure 15**).

【0098】 Experiment 6: Dosage evaluation and Combination Treatment.

【0099】 In this experiment, two T-ALL PDXs derived from patients who experienced aggressive and fatal disease (ALL-8 and -31) were tested over a five-fold reduction in OBI-3424 dose. OBI-3424 (or vehicle control) was administered at 2.5 mg/kg, 1 mg/kg or 0.5 mg/kg via intraperitoneal (IP) injection once weekly for three weeks.

【00100】 OBI-3424 at all three doses significantly delayed the progression of both PDXs (**Figure 16A and B** left and middle panels) and induced objective responses in ALL-31, while objective responses in ALL-8 were elicited at the two highest doses (1.0 and 2.5 mg/kg). Moreover, significant reductions in blood (cardiac puncture), spleen and bone marrow infiltration were observed on Day 28 (14 days after the last of three weekly treatments) for ALL-31 at all three dose levels (**Figure 16B** right panel), and in ALL-8 at the two highest doses (**Figure 16A** right panel).

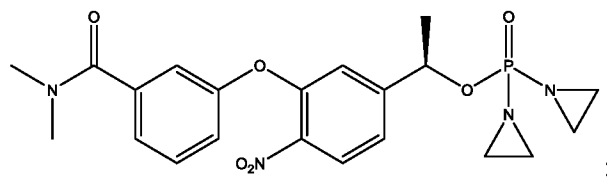
【00101】 Then, the *in vivo* efficacy of OBI-3424 in combination with the nucleoside analogue nelarabine, which is FDA approved for the treatment of relapsed T-ALL and T-cell lymphoblastic lymphoma was conducted. Mice were treated with OBI-3424 administered at 2.5 mg/kg via IP injection once weekly for 3 weeks, nelarabine administered at 150 mg/kg via IP injection once daily for 5 days and repeated on Day 14, or both drugs at their respective treatment schedules. It indicated that nelarabine significantly delayed the progression of ALL-8 and -31 (**Figure 17A and B** left and middle panels), but did not significantly decrease organ infiltration at event (prior to Day 28) (**Figure 17A and B** right panels). The single-agent efficacy of OBI-3424 was consistent with that reported above, and OBI-3424 combined with nelarabine significantly enhanced the effects of each single agent alone (**Figure 17A and B** left and middle panels). The criterion for Therapeutic Enhancement ($P<0.01$) of the OBI-3424/nelarabine combination was achieved in ALL-8. The OBI-3424/nelarabine combination also profoundly decreased organ infiltration at Day 28 compared to nelarabine alone or vehicle control (**Figure 17A and B**, right panels).

【00102】 Unless defined otherwise, all technical and scientific terms and any acronyms used herein have the same meanings as commonly understood by one of ordinary skill in the art in the field of this invention. Although any compositions, methods, kits, and means for communicating information similar or equivalent to those described herein can be used to practice this invention, the preferred compositions, methods, kits, and means for communicating information are described herein.

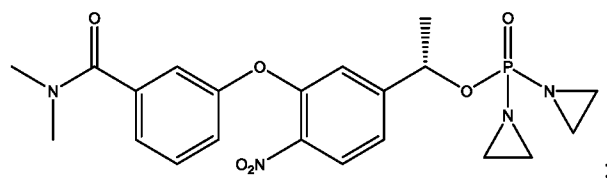
【00103】 All references cited herein are incorporated herein by reference to the full extent allowed by law. The discussion of those references is intended merely to summarize the assertions made by their authors. No admission is made that any reference (or a portion of any reference) is relevant prior art. Applicants reserve the right to challenge the accuracy and pertinence of any cited reference

What is claimed is

1. A method for treating acute lymphoblastic leukemia, comprising administering a subject in need with an effective amount of a compound having the following Formula I or Formula II:



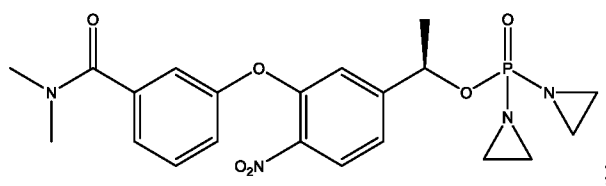
I



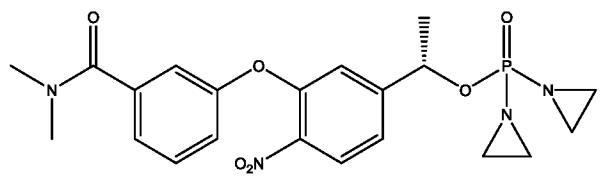
II

- or a salt, an isotopic variant, a solvate, or a hydrate of each thereof.
2. The method of claim 1, wherein the compound is (R)-1-(3-(3-N,N-dimethylaminocarbonyl)phenoxy)-4-nitrophenyl)-1-ethyl-N,N'-bis(ethylene)phosphoramidate or (S)-1-(3-(3-N,N-dimethylaminocarbonyl)phenoxy)-4-nitrophenyl)-1-ethyl-N,N'-bis(ethylene)phosphoramidate.
 3. The method of claim 1, wherein the compound is the Formula (I) compound.
 4. The method of claim 1, wherein said compound has an enantiomeric excess of no less than 80%.
 5. The method of claim 4, wherein said compound has an enantiomeric excess of no less than 90%.
 6. The method of claim 5, wherein said compound has an enantiomeric excess of no less than 95%.
 7. The method of claim 1, wherein said compound is substantially pure.
 8. The method of claim 7, wherein said compound has a purity of at least 50%.
 9. The method of claim 8, wherein said compound has a purity of at least 90%.

10. The method of claim 1, wherein said compound is formulated as a pharmaceutical composition; wherein said pharmaceutical composition further comprises at least one pharmaceutically acceptable carrier or excipient.
11. The method of claim 10, wherein said pharmaceutically acceptable carrier or excipient is selected from inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and oils.
12. The method of claim 1, wherein said acute lymphoblastic leukemia is T-cell acute lymphoblastic leukemia.
13. The method of claim 1, wherein said acute lymphoblastic leukemia is B-cell acute lymphoblastic leukemia.
14. The method of claim 3, wherein therapeutically effective amount of OBI-3424 in the composition comprises from about 0.10 $\mu\text{g/kg}$ to about 100 mg/kg.
15. The method of claim 1, wherein the method comprising administering to the subject in need thereof an effective amount of a combination of an additional purine nucleoside analog (PNA).
16. The method of claim 15, wherein the purine nucleoside analog comprises nelarabine, clofarabine, fludarabine, cladribine, pentostatin, forodesine, 8-chloroadenosine, azathioprine or a combination thereof.
17. The method of claim 15, wherein the combination provides a synergistic effect in cancer treatment and enhanced therapeutic efficacy.
18. The method of claim 1, the subject is human.
19. A pharmaceutical composition for treating acute lymphoblastic leukemia comprising a compound having the following Formula I or Formula II:



I



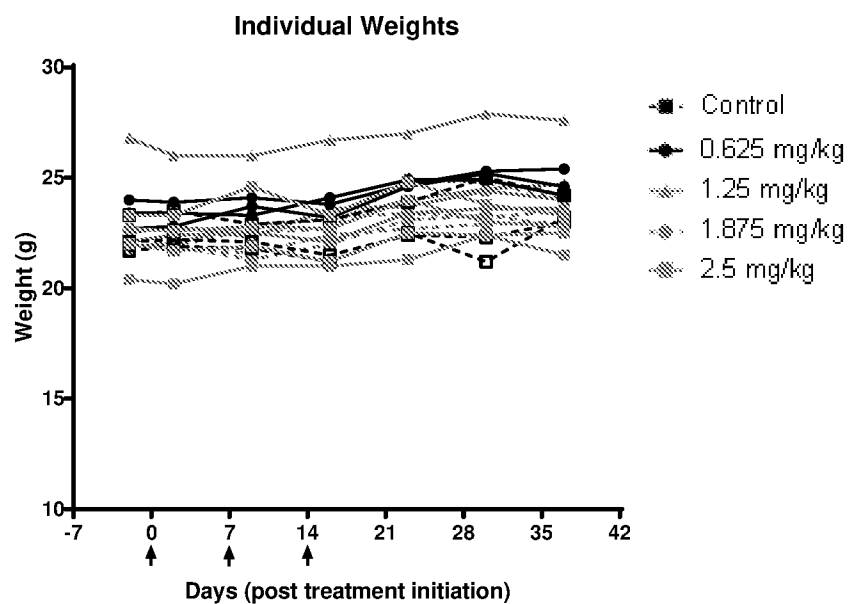
II

or a salt, an isotopic variant, a solvate, or a hydrate of each thereof;

and at least one pharmaceutically acceptable carrier or excipient.

20. The pharmaceutical composition of claim 19; wherein said acute lymphoblastic leukemia is T-cell acute lymphoblastic leukemia or B-cell acute lymphoblastic leukemia.
21. The pharmaceutical composition of claim 19; wherein said pharmaceutically acceptable carrier or excipient is selected from inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and oils.
22. The pharmaceutical composition of claim 19, further comprising a combination of an additional purine nucleoside analog (PNA).
23. The pharmaceutical composition of claim 22, wherein the purine nucleoside analog comprises nelarabine, clofarabine, fludarabine, cladribine, pentostatin, forodesine, 8-chloroadenosine, azathioprine or a combination thereof.
24. The pharmaceutical composition of claim 13, wherein the combination provides a synergistic effect in cancer treatment and enhanced therapeutic efficacy.
25. The pharmaceutical composition of claim 19, wherein a therapeutically effective amount of the Formula I compound or the Formula II compound is from about 0.10 $\mu\text{g/kg}$ to about 100 mg/kg .

(A)



(B)

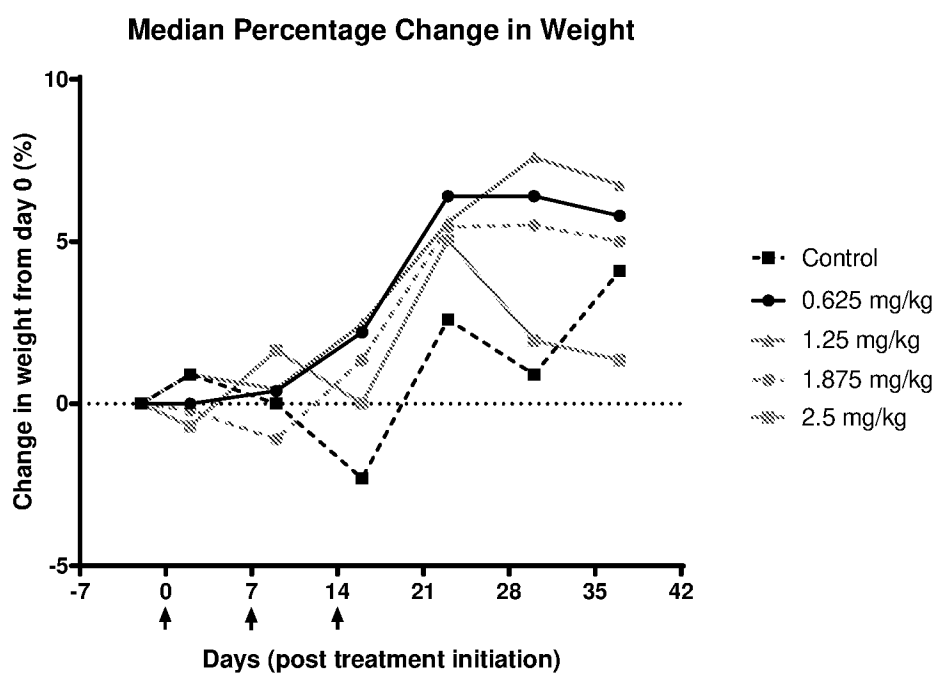
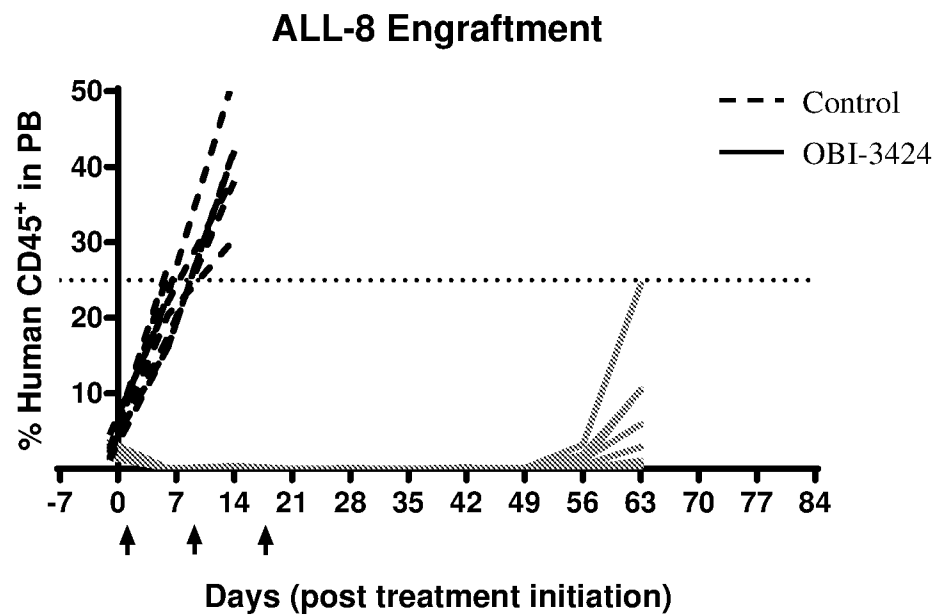


Figure 1

(A)



(B)

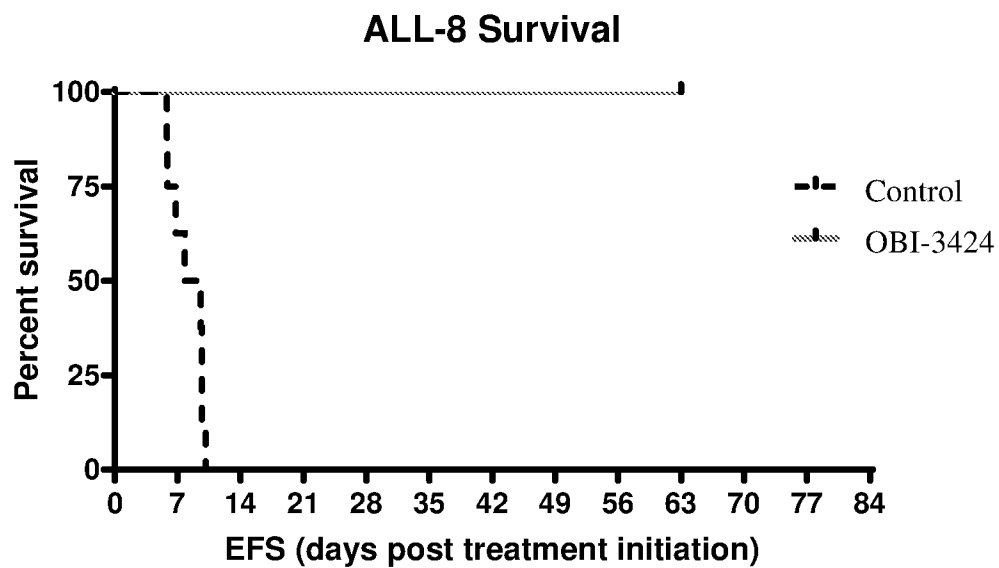
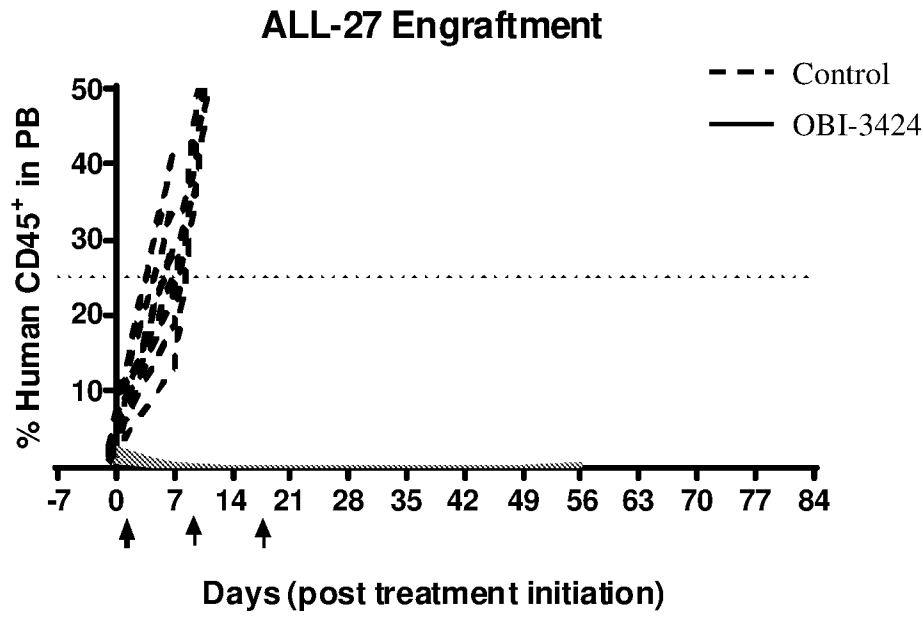


Figure 2

(A)



(B)

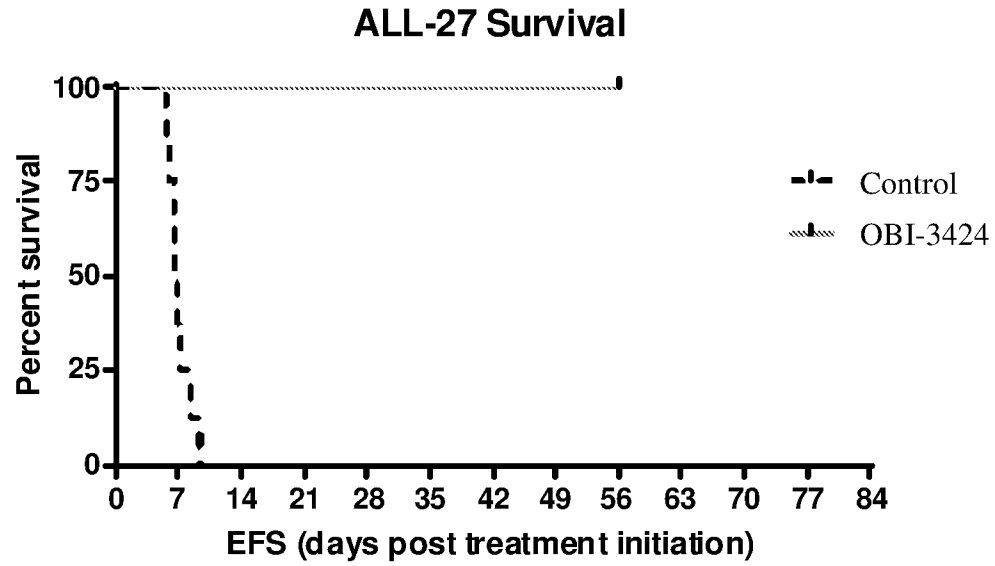
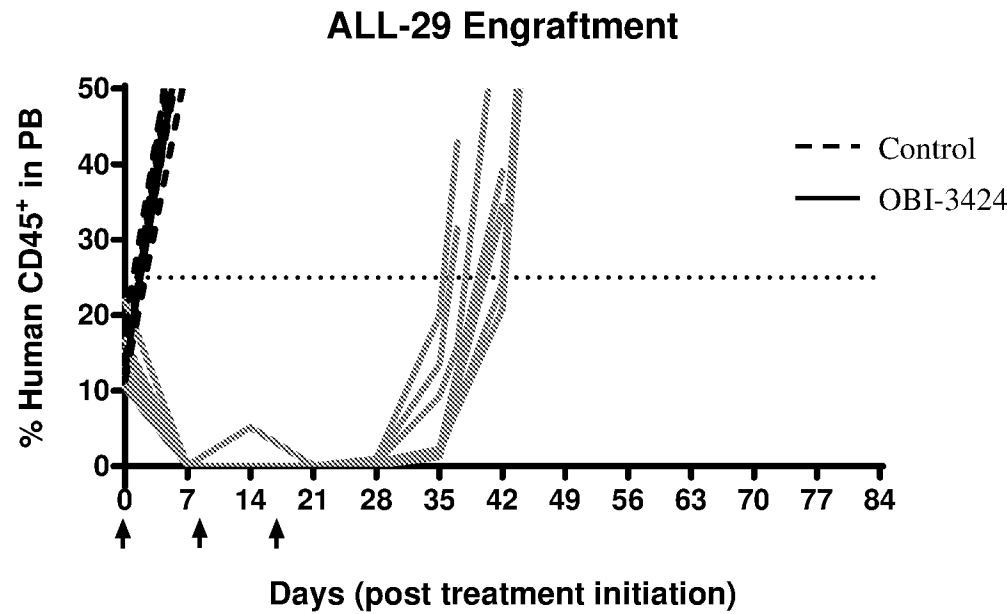


Figure 3

(A)



(B)

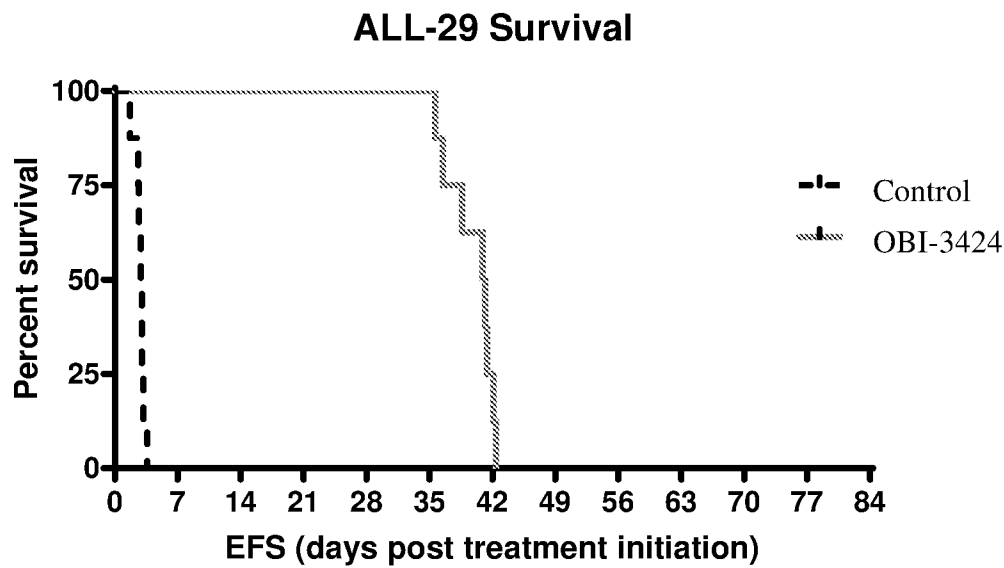
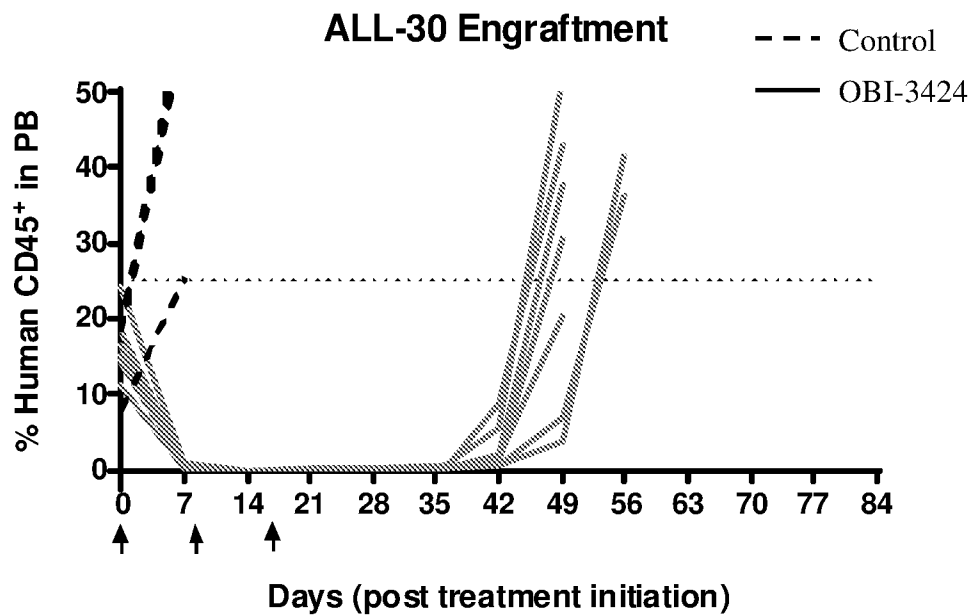


Figure 4

(A)



(B)

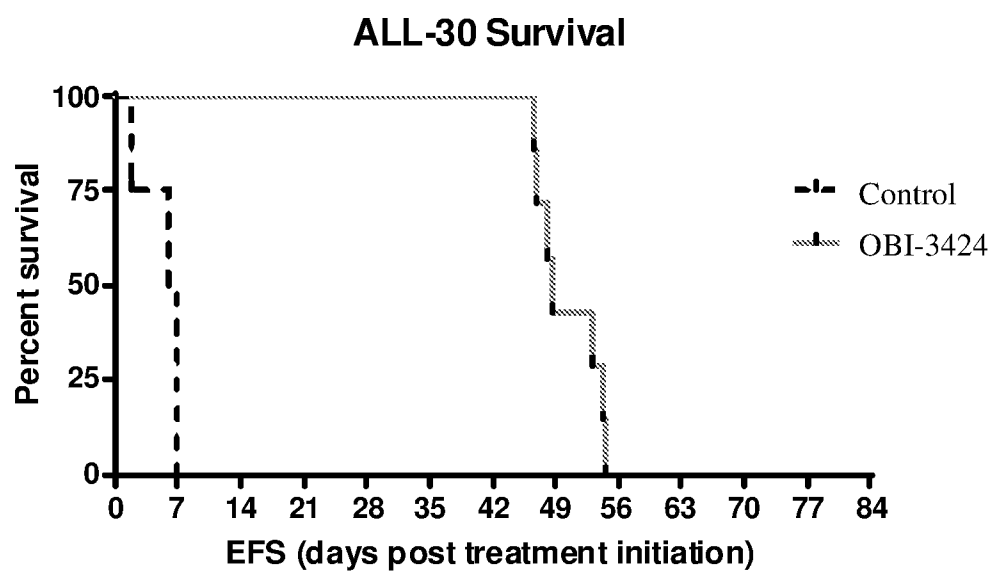
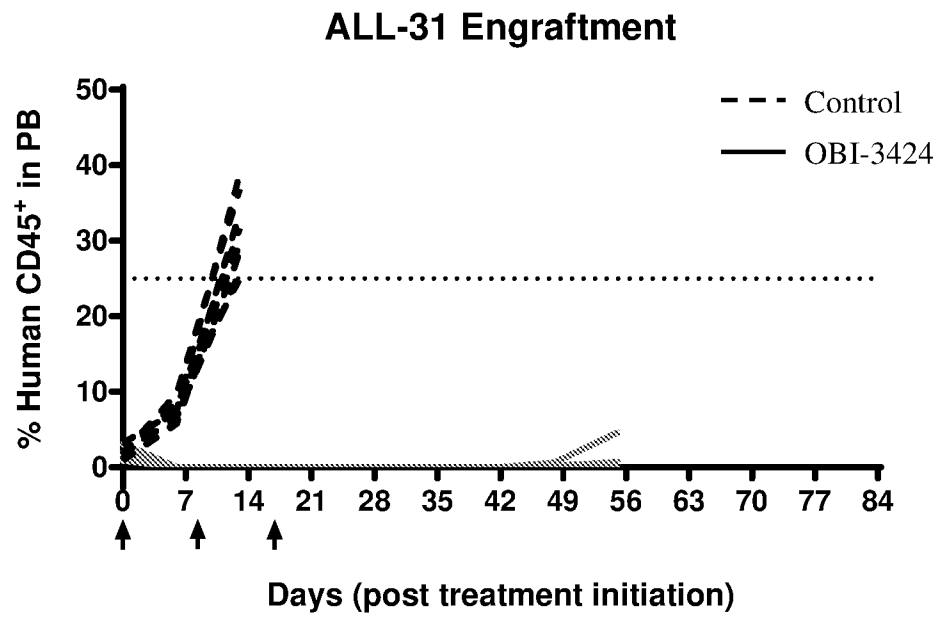


Figure 5

(A)



(B)

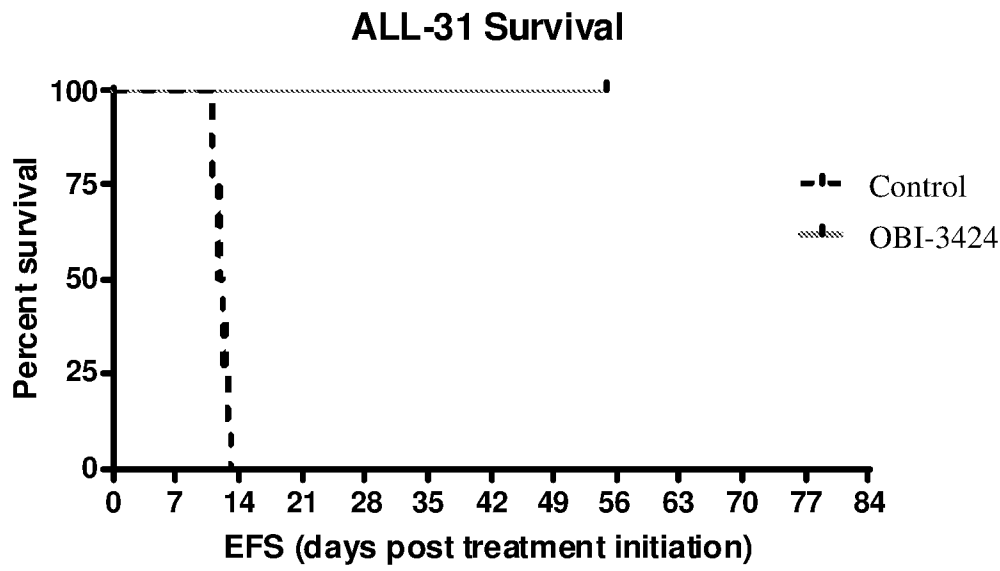
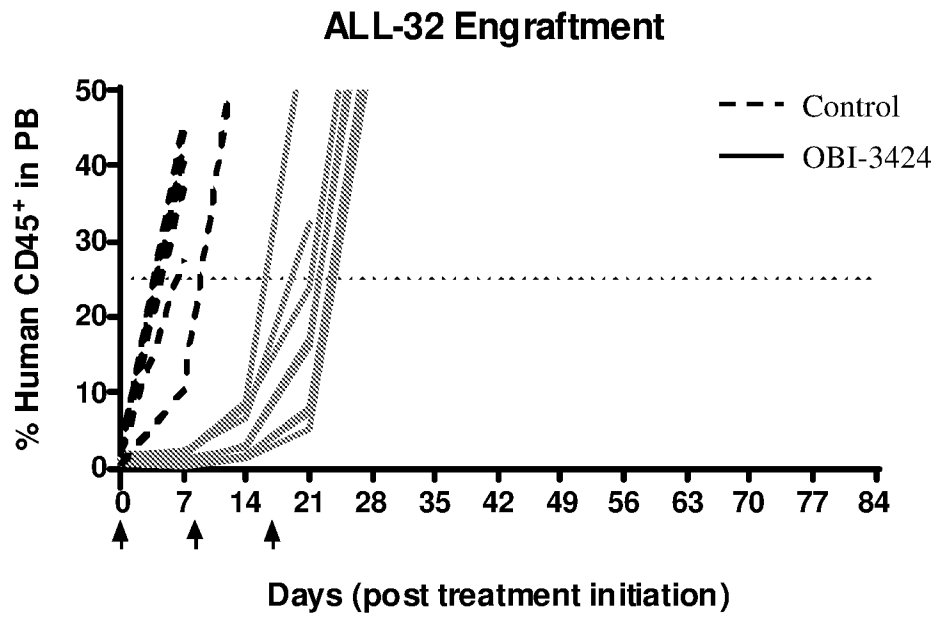


Figure 6

(A)



(B)

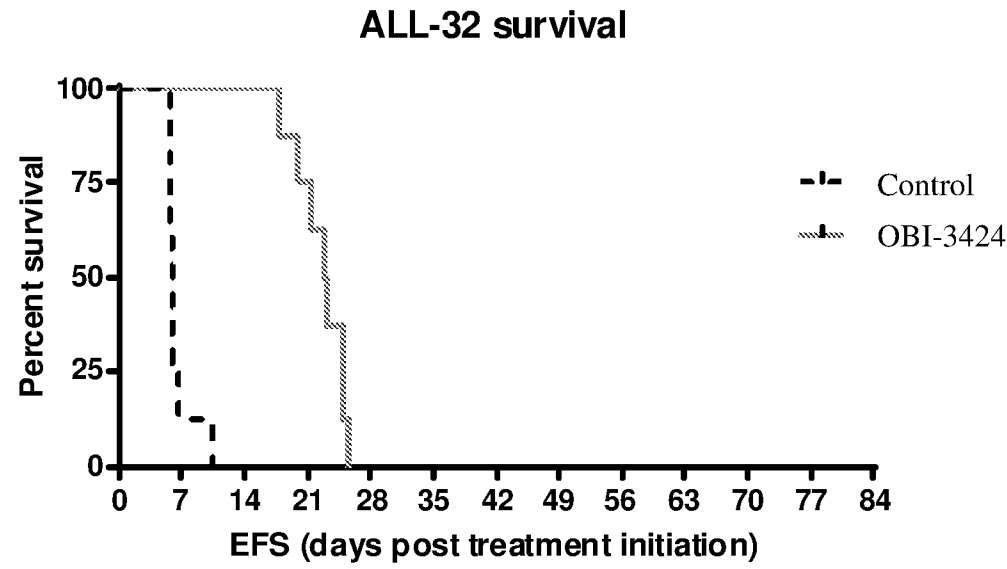
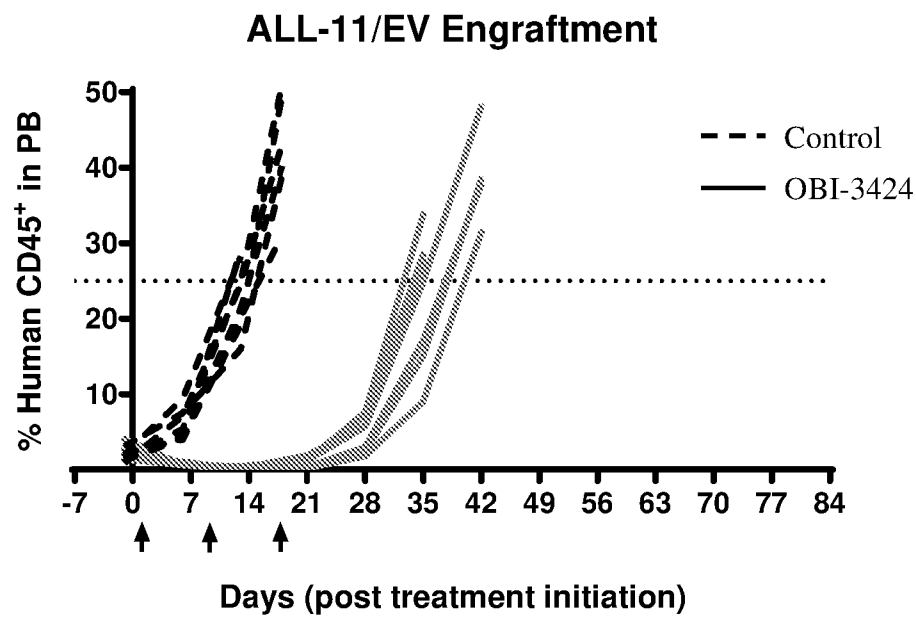


Figure 7

(A)



(B)

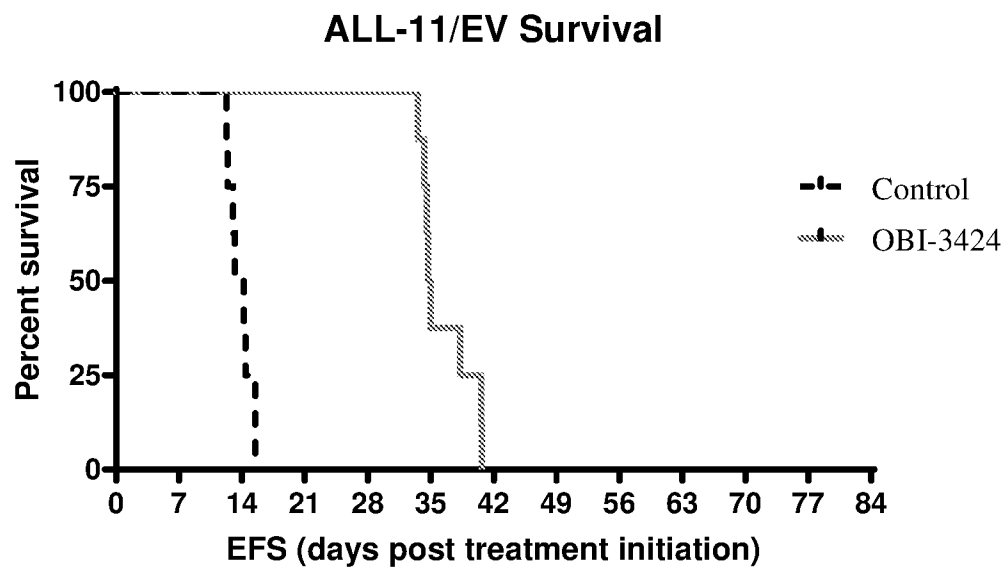
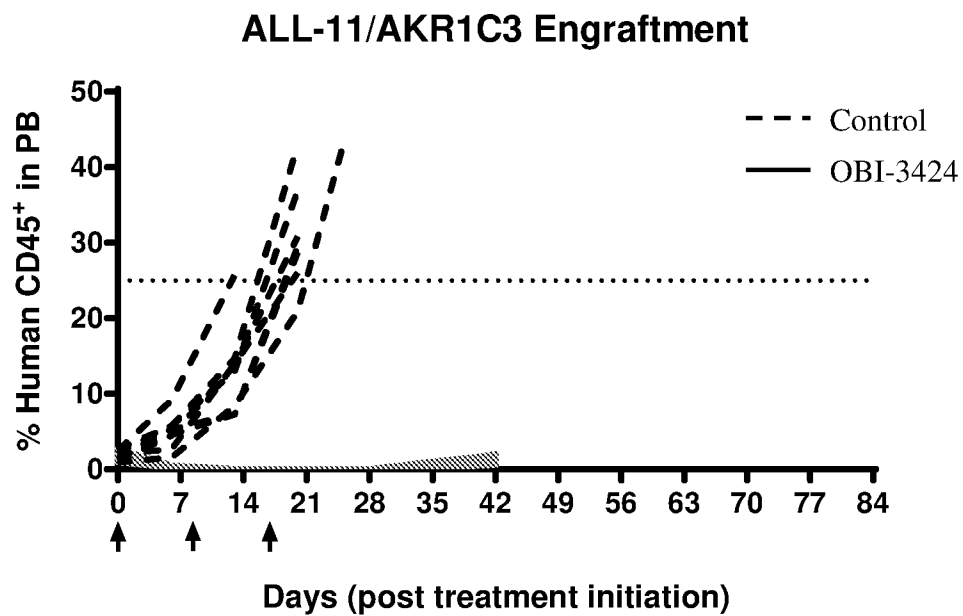


Figure 8

(A)



(B)

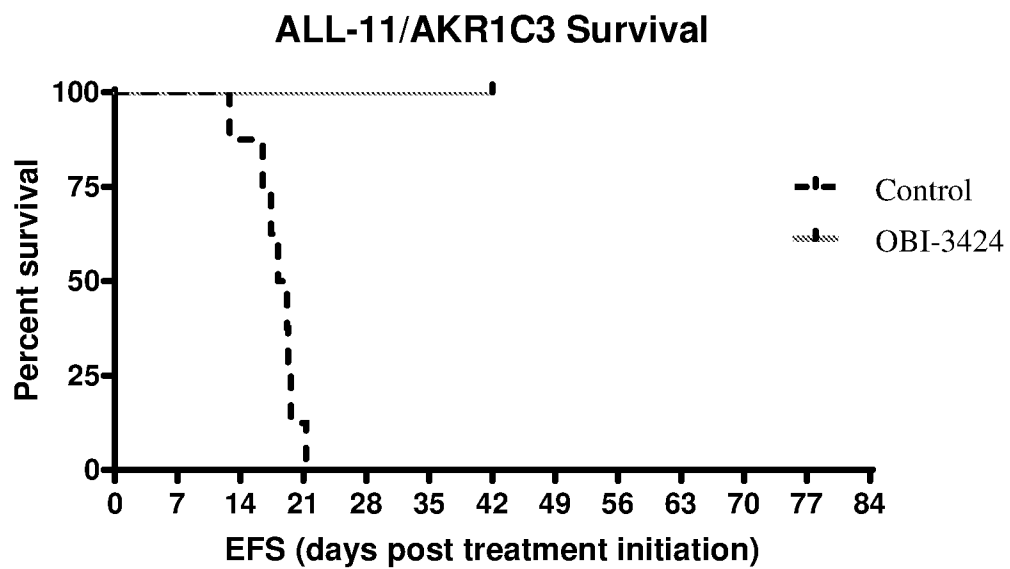
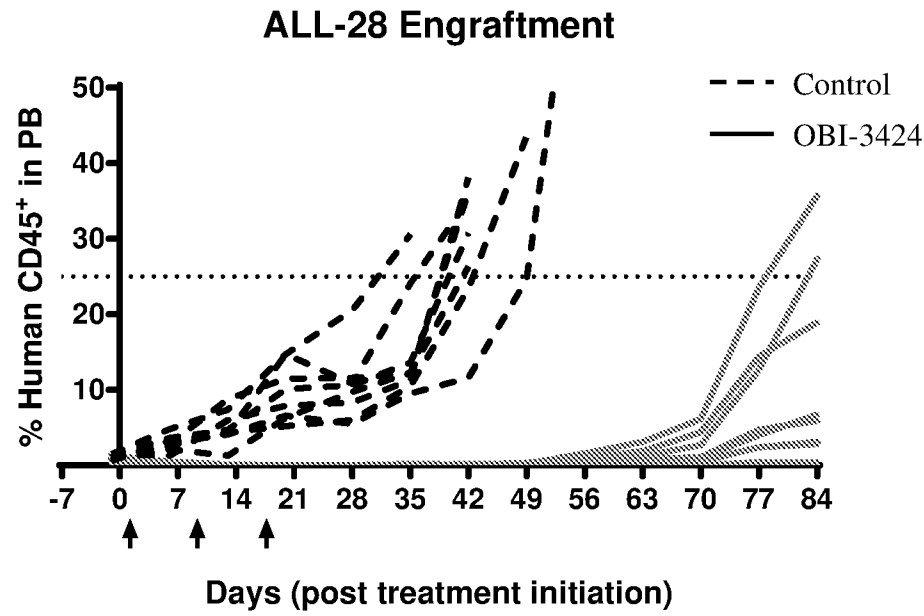


Figure 9

(A)



(B)

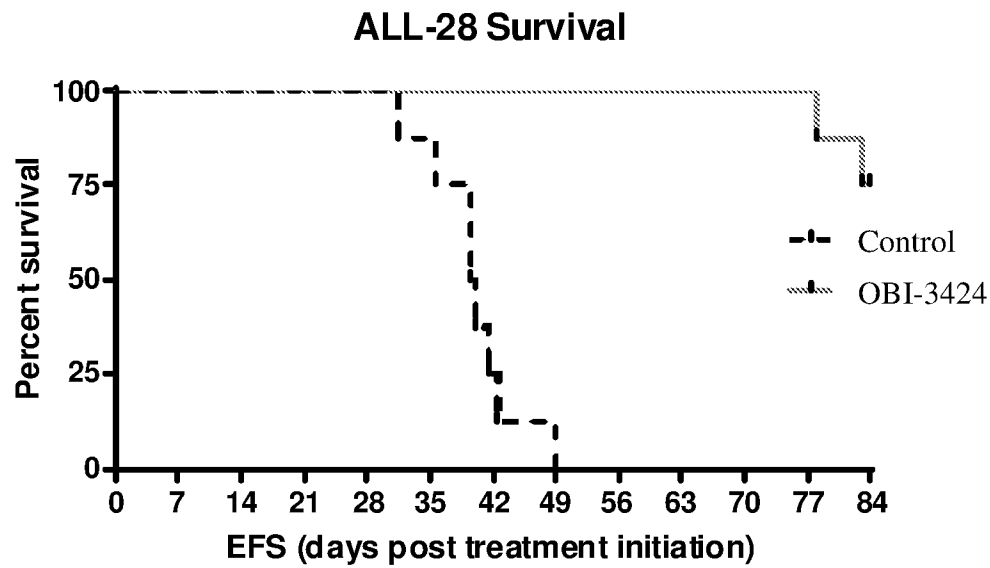


Figure 10

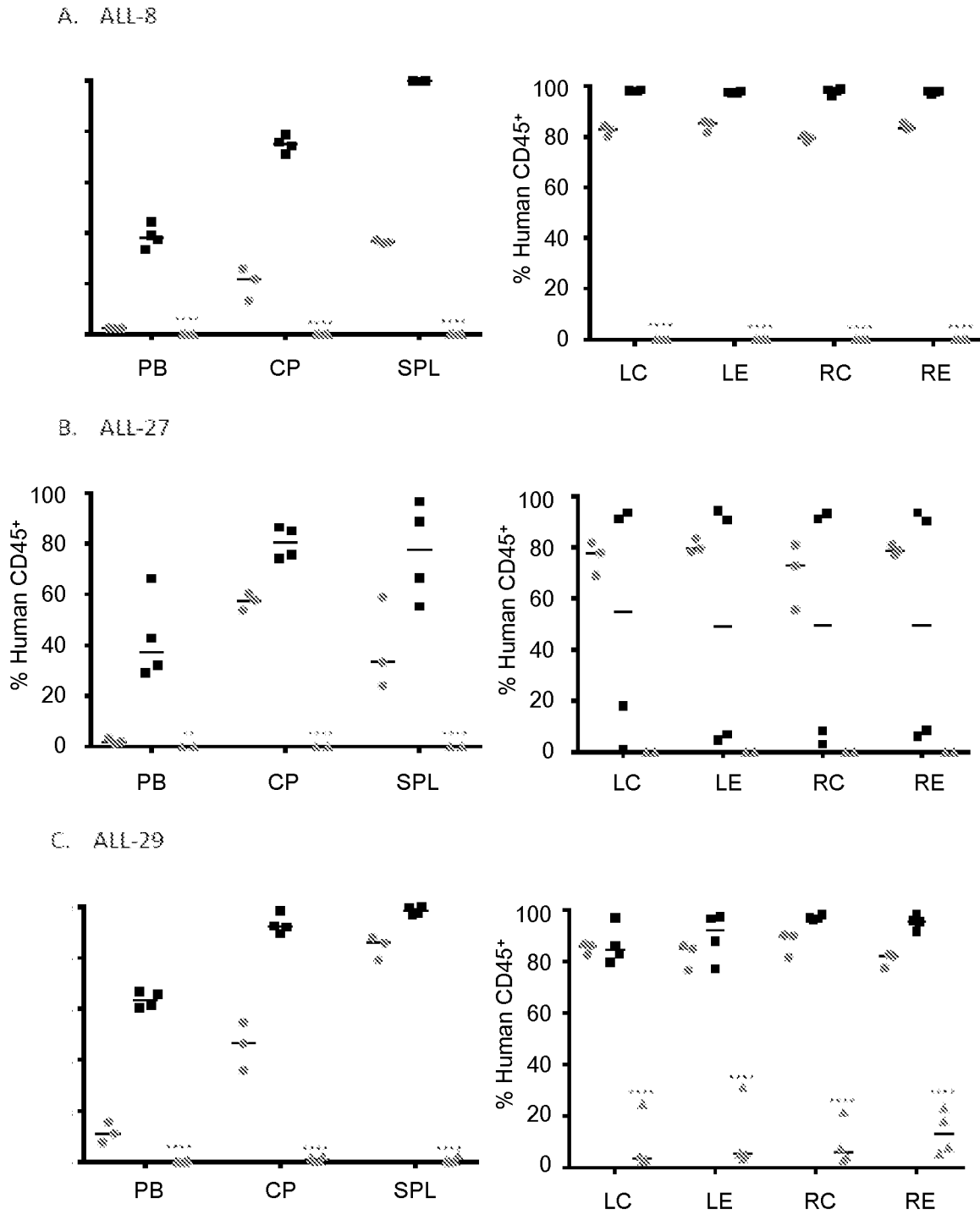


Figure 11

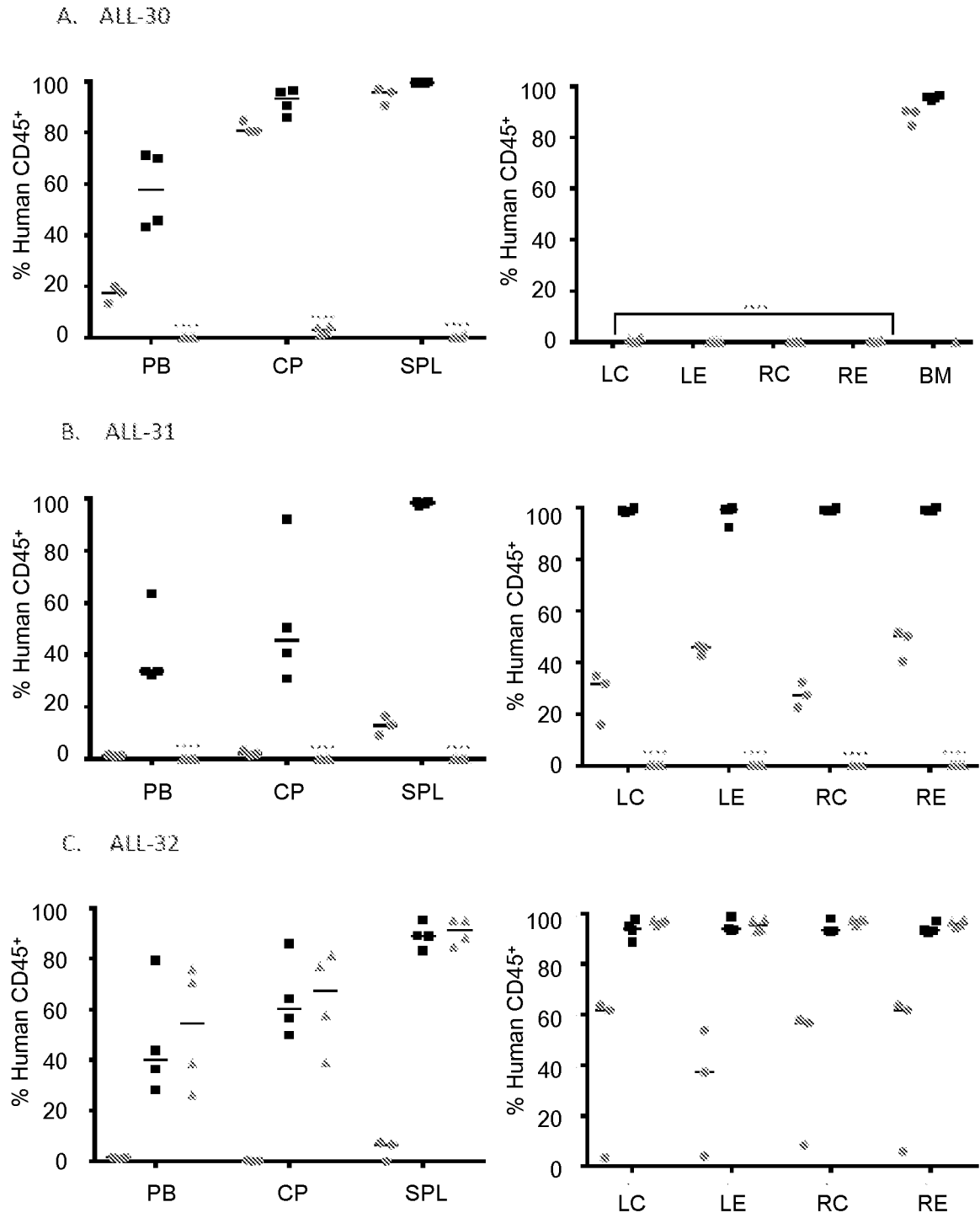


Figure 12

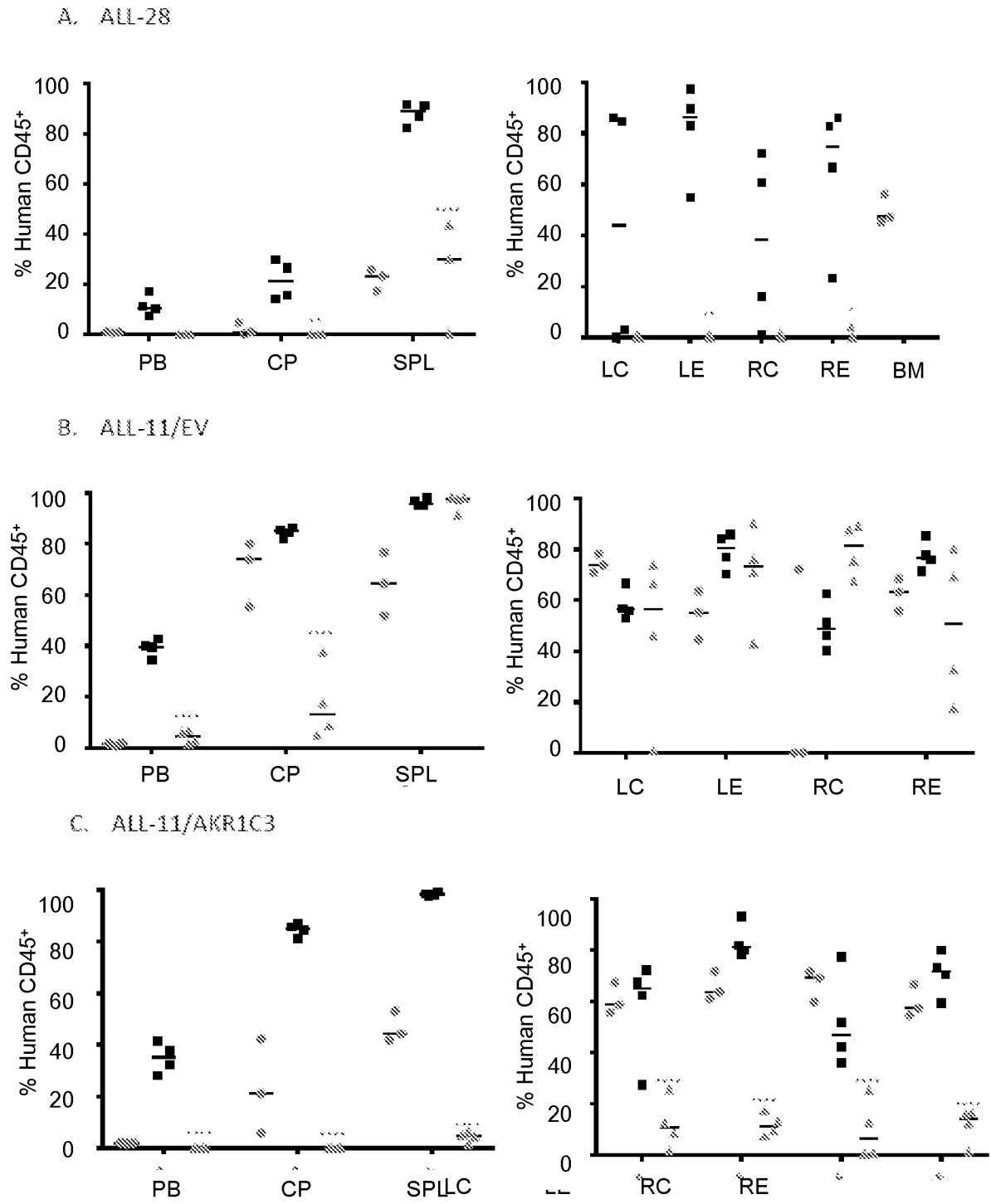


Figure 13

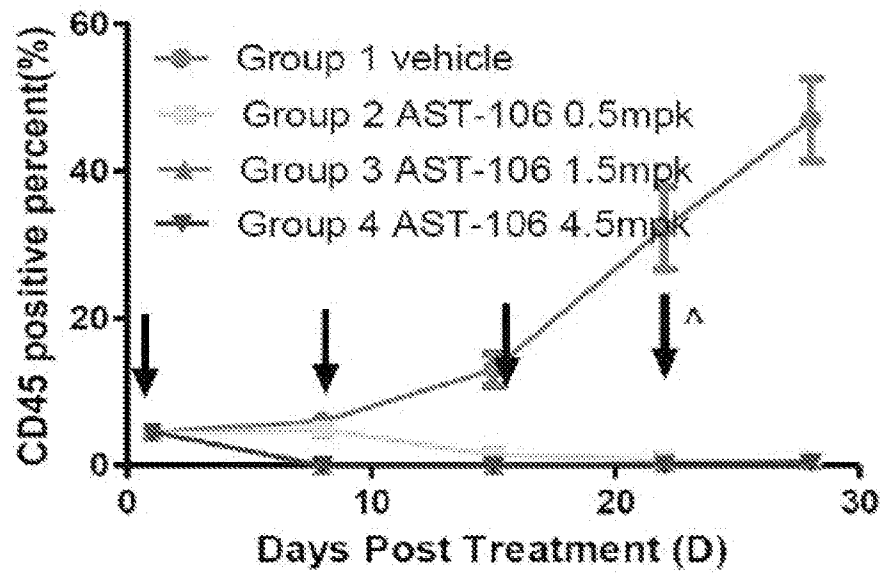


Figure 14

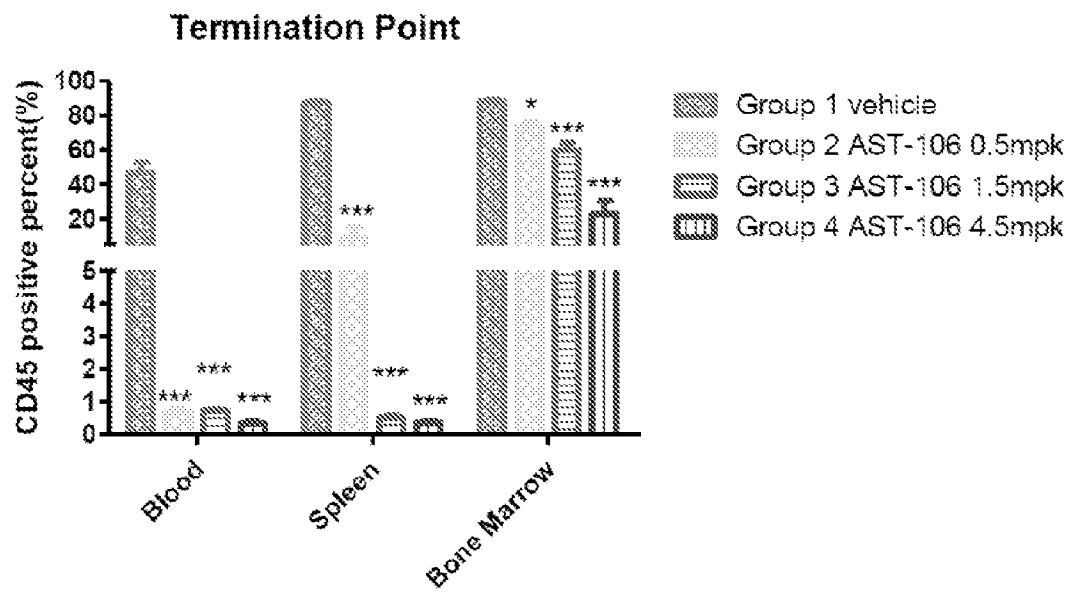


Figure 15

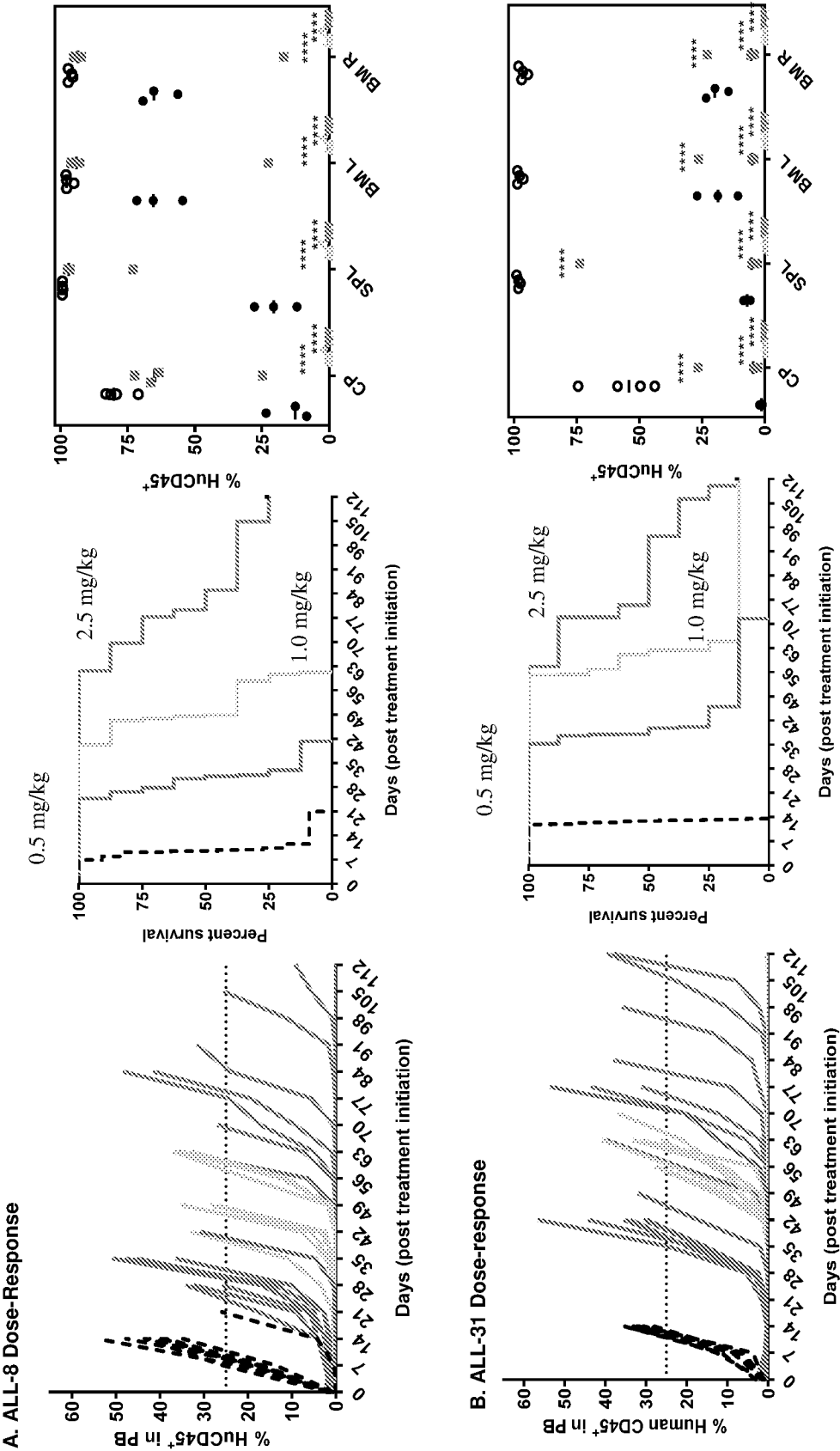


Figure 16

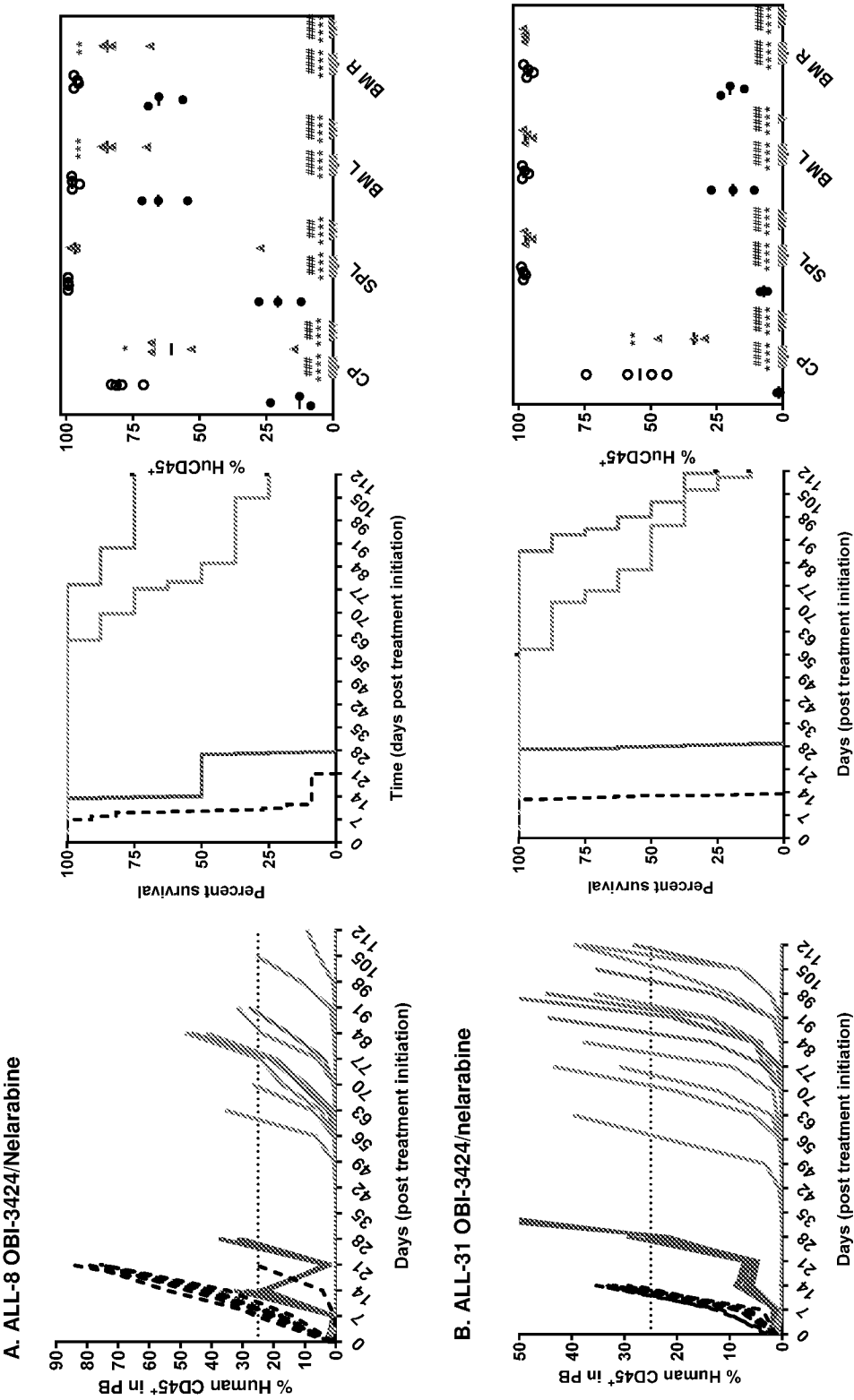


Figure 17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2018/108649

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/04(2006.01)i; A61K 31/06(2006.01)i; A61P 35/02(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

VEN, CNKI, NCBI PubMed, GOOGLE Scholar, ISI Web of Knowledge, STN, ASCENTA PHARM, OBI PHARMA INC, SHENZHEN AIHENGHAO MEDICAL TECHNOLOGY, 2097713-68-1, 2097713-69-2, 2006311-00-6, AST-3424, OBI-3424, JurKat, acute lymphoblastic leukemia, purine nucleoside analog, PNA, leukemia, T-cell, B-cell

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017087428 A1 (THRESHOLD PHARMACEUTICALS, INC.) 26 May 2017 (2017-05-26) the abstract, claims 1-11, paragraph 114, example 6	1-25
X	WO 2016145092 A1 (THRESHOLD PHARMACEUTICALS, INC.) 15 September 2016 (2016-09-15) paragraph 72	19-25
A	CN 101998964 A (PACIFIC ARROW LTD.) 30 March 2011 (2011-03-30)	1-25
A	CN 106687471 A (BAYER PHARM. CO., LTD.) 17 May 2017 (2017-05-17) claims 1-2	1-25
A	WO 2016161342 A2 (THRESHOLD PHARMACEUTICALS, INC.) 06 October 2016 (2016-10-06) claims 1-2	1-25

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

12 December 2018

Date of mailing of the international search report

28 December 2018

Name and mailing address of the ISA/CN

National Intellectual Property Administration, PRC
6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing
100088
China

Authorized officer

TU, Haihua

Facsimile No. (86-10)62019451

Telephone No. 53962084

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2018/108649**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **1-18**
because they relate to subject matter not required to be searched by this Authority, namely:
 - [1] Claims 1-18 direct to a method of treating acute lymphoblastic leukemia, They do not meet the criteria set out in PCT Rules 39.1(iv). The search report has been carried out and based on the use of a compound in the manufacture of a pharmaceutical composition for treating acute lymphoblastic leukemia.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2018/108649

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2017087428	A1	26 May 2017	BR	112017025778	A2	14 August 2018
				TW	201726695	A	01 August 2017
				CA	2990696	A1	26 May 2017
				IL	256569	D0	28 February 2018
				EP	3390415	A1	24 October 2018
				KR	20170130615	A	28 November 2017
				JP	2018517710	A	05 July 2018
				AU	2016357728	A1	30 November 2017
				CN	108290911	A	17 July 2018
WO	2016145092	A1	15 September 2016	EP	3277380	A4	01 August 2018
				SG	11201707293V	A	30 October 2017
				AU	2016229136	B2	09 August 2018
				KR	20170128280	A	22 November 2017
				CN	107530556	A	02 January 2018
				IL	254377	D0	30 November 2017
				BR	112017019287	A2	02 May 2018
				JP	2018513876	A	31 May 2018
				AU	2016229136	A1	05 October 2017
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				EP	3277380	A1	07 February 2018
				CA	2979251	A1	15 September 2016
				EP	2254899	A4	30 May 2012
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				WO	2009117196	A1	24 September 2009
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				AU	2009226063	A1	24 September 2009
				EP	2254899	A1	01 December 2010
				WO	2009117196	A8	26 November 2009
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				JP	2017526721	A	14 September 2017
				EP	3194419	A1	26 July 2017
				US	2017260226	A1	14 September 2017
				WO	2016037956	A1	17 March 2016
				CN	106687471	B	30 October 2018
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				US	2018086693	A1	29 March 2018
				WO	2016161342	A2	06 October 2016
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				SG	11201707375U	A	30 October 2017
				CN	108136214	A	08 June 2018
				CA	2981494	A1	06 October 2016
				TW	201706267	A	16 February 2017
				KR	20170127463	A	21 November 2017
				BR	112017021167	A2	03 July 2018
				JP	2018511612	A	26 April 2018
				EP	3277381	A2	07 February 2018