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POSTER ABSTRACTS

614.ACUTE LYMPHOBLASTIC LEUKEMIAS: THERAPIES, EXCLUDING TRANSPLANTATION AND CELLULAR IMMUNOTHERAPIES

The AKR1C3-Activated Prodrug, Achm-025, Eradicates Disease in Preclinical Models of Aggressive T-Cell Acute Lymphoblastic Leukemia

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Introduction: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy that is exceptionally difficult to cure after relapse. T-ALL expresses significantly higher levels of the enzyme aldo-keto reductase family 1 member C3 (AKR1C3) compared with B-cell ALL. To exploit this finding, we developed a novel prodrug, ACHM-025, which is selectively activated by AKR1C3 to form a potent cell-entrapped DNA alkylating agent. ACHM-025 was designed to improve drug specificity and minimize toxicity observed with currently used DNA alkylating agents, such as cyclophosphamide (CPM), a prodrug which is activated systemically via liver enzymes. We evaluated the *in vivo* efficacy and AKR1C3 selectivity of ACHM-025 against a panel of 25 pediatric T-ALL patient-derived xenografts (PDXs) alongside standard-of-care therapy.
Methods: AKR1C3 expression in T-ALL PDXs was determined by RNA-seq, immunoblotting and intracellular flow cytometry. For *in vivo* efficacy studies, PDXs were established as orthotopic models in immune-deficient NSG mice. Engraftment was assessed by enumerating the proportion of human versus mouse $CD45^+$ cells (%$huCD45^+$) in the peripheral blood. Treatment commenced when the %$huCD45^+$ reached ≥1% (Day 0), and events were defined as %$huCD45^+$ ≥25% or leukemia-related morbidity. Drug efficacy was assessed by mouse event-free survival (EFS) and stringent objective response measures.
For the single agent study, ACHM-025 (IP weekly, Days 0, 7, 14) was assessed against 25 T-ALL PDXs using a single mouse trial (SMT) format (one vehicle treated mouse, one drug treated mouse). For the consolidation therapy comparison, ACHM-025 (IP Days 0, 7) or CPM (IP Days 0, 7) combined with cytarabine (Ara-C; IP Days 0-4, 7-11) and 6-mercaptopurine (6MP; IP Days 0-4, 7-11) were assessed against a T-ALL PDX derived from a patient at relapse. For the relapsed/refractory (R/R) therapy study, ACHM-025 (IP Days 0, 7, 14) and nelarabine (IP Days 0-4, 14-18) were assessed against a T-ALL PDX derived from a patient at relapse.
Results: ACHM-025 dose-limiting toxicity in cynomolgus monkeys was neutropenia, where the pharmacokinetic equivalent dose in NSG mice was well tolerated. We first evaluated the *in vivo* efficacy of ACHM-025 as a single agent using the SMT format across an extended panel of 25 T-ALL PDXs, to address the impact of genetic heterogeneity in pediatric ALL on drug response. Remarkably, 7/25 PDXs treated with ACHM-025 did not relapse over 250 days after the last treatment and a total of 22/25 T-ALL PDXs scored an objective response. In comparison, vehicle treated EFS ranged from 3-26 days for the 25 T-ALL PDXs. Importantly, AKR1C3 expression (mRNA, protein or intracellular) provides a predictive biomarker of efficacy, as ACHM-025 was significantly more effective against T-ALL PDXs with high AKR1C3 expression versus those with low AKR1C3 expression ($p<0.0001$).
CPM is included in standard-of-care consolidation therapy for ALL in combination with Ara-C and 6MP. Comparing single agents, ACHM-025 was significantly more effective than CPM (T-C 25 vs. 57 days, $p=0.0005$, Table 1). The combination of ACHM-025, Ara-C and 6MP was significantly more effective than standard-of-care consolidation therapy (CPM, Ara-C and 6MP), more than doubling survival (T-C 36 vs. 75 days, $p=0.0005$, Table 1). Finally, we compared ACHM-025 with nelarabine, which is the only FDA approved agent for R/R T-ALL. Comparing single agents, ACHM-025 was substantially more effective than nelarabine (T-C 19 vs. 198 days, $p=0.0005$, Table 1). Importantly, relapsed disease remained sensitive to ACHM-025

re-treatment *in vivo*, with no evidence of acquired resistance. Remarkably, no mice treated with the ACHM-025/nelarabine combination relapsed over 250 days after the last treatment (Table 1).
Conclusions: ACHM-025 exerted profound *in vivo* efficacy against T-ALL PDXs and eradicated the disease in 7 aggressive T-ALL PDXs. ACHM-025 was significantly more effective than CPM both as a single agent and when used in combination with Ara-C/6MP. Notably, ACHM-025 in combination with nelarabine was curative when used to treat a chemoresistant T-ALL PDX *in vivo*. The *in vivo* efficacy of ACHM-025 directly correlated with AKR1C3 expression levels, providing a predictive biomarker for response. These data provide strong preclinical evidence highlighting the potential of ACHM-025 as a targeted and effective therapy for aggressive forms of T-ALL.

Disclosures Ashoorzadeh: *Lixin Pharmaceuticals:* Patents & Royalties: Inventor on patent CN110809576B; *Achilles Medical Co. Ltd.:* Patents & Royalties: Inventor on patent US11661404B2; *Health Innovation Ventures:* Patents & Royalties: Inventor on patents DK2888227T3, EP2888227B1, US10202408B2, CA2886574C, US9873710B2, AU2013/306514B2, US9505791B2; *Rain Oncology:* Patents & Royalties: Inventor on patents CN107427515B, AU2015/358384B2, JP6769962B2, US10507210B2, CA2754808C, MX336332B, EP2406262B1, JP5925680B2, AU2010/290199B2, RU2568639C2, JP5793428B2, US9073916B2, US9101632B2, CN102574846B. **Patterson:** *Achilles Medical Co. Ltd.:* Patents & Royalties: Inventor of patent US11661404B2; *Health Innovation Ventures:* Patents & Royalties: Inventor of multiple patents (DK2888227T3, EP2888227B1, US10202408B2, CA2886574C, US9873710B2, AU2013/306514B2, US9505791B2); *Rain Oncology:* Current holder of *stock options* in a privately-held company, Patents & Royalties: Inventor of multiple patents (CN107427515B, AU2015/358384B2, JP6769962B2, US10507210B2, CA2754808C, MX336332B, EP2406262B1, JP5925680B2, AU2010/290199B2, RU2568639C2, JP5793428B2, US9073916B2, US9101632B2, CN102574846B); *Lixin Pharmaceuticals:* Patents & Royalties: Inventor on patent CN110809576B. **Smaill:** *Achilles Medical Co. Ltd.:* Patents & Royalties: Inventor on patent US11661404B2; *Rain Oncology:* Current holder of *stock options* in a privately-held company, Patents & Royalties: Inventor on multiple patents (CN107427515B, AU2015/358384B2, JP6769962B2, US10507210B2, CA2754808C, MX336332B, EP2406262B1, JP5925680B2, AU2010/290199B2, RU2568639C2, JP5793428B2, US9073916B2, US9101632B2, CN102574846B); *Lixin Pharmaceuticals:* Patents & Royalties: Inventor on patent CN110809576B; *Health Innovation Ventures:* Patents & Royalties: Inventor on multiple patents (DK2888227T3, EP2888227B1, US10202408B2, CA2886574C, US9873710B2, AU2013/306514B2, US9505791B2).

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Table 1. *In vivo* activity of ACHM-025 in combination against a relapsed/refractory T-ALL PDX.

Treatment	Dose (mg/kg)	EFS (days)	T-C (days)	T/C (days)	p-value (vs vehicle)	p-value (vs treatment)	ORM
Vehicle	0	9.2	-	-	-	-	-
Ara-C	12.5	31.7	22.5	3.4	0.0005	-	CR
6MP	12.5	7.4	-1.8	0.8	0.0053	-	PD1
CPM	75	34.5	25.3	3.7	0.0005	0.0005	CR
ACHM-025	5	66.6	57.4	7.2	0.0005		MCR
CPM + Ara-C + 6MP	-	45.5	36.3	4.9	0.0005	0.0005	MCR
ACHM-025 + Ara-C + 6MP	-	83.9	74.7	9.1	0.0005		MCR
ACMH-025	10	207	198	22.5	0.0005	0.0005	MCR
Nelarabine	125	28.4	19.1	3.1	0.0005		SD
ACHM-025 + Nelarabine	-	>293	>284	>31.8	0.0005	-	MCR

6MP, 6-mercaptopurine; CPM, cyclophosphamide; CR, complete response; EFS, median event free survival; MCR, maintained complete response; ORM, median objective response measure; PD1, progressive disease 1; p-value, log-rank (Mantel-Cox) test (conservative); SD, stable disease; T-C, treated EFS – control EFS; T/C, treated EFS / control EFS.

Figure 1