Data Supplement

First-in-Human Phase 1 Study of iadademstat (ORY-1001): a First-in-Class Lysine-Specific Histone Demethylase 1A Inhibitor, in relapsed or refractory Acute Myeloid Leukemia

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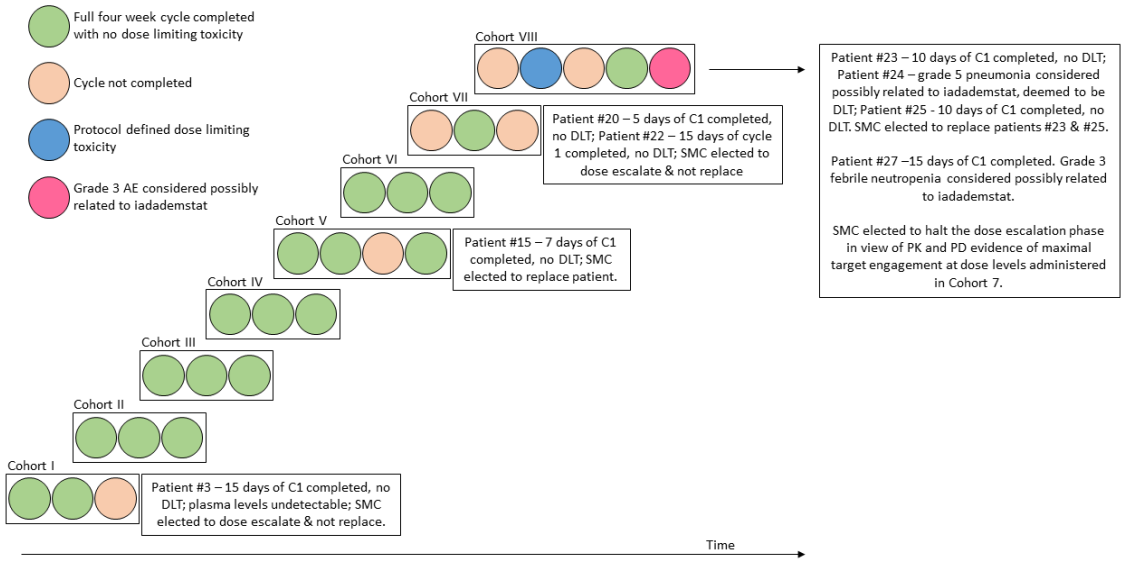
Supplemental Figures

a



b

Cohort	Dose ($\mu g/m^2/d$)	Log_{10} dose ($\mu g/m^2/d$)	N
I	5	0.70	3
II	15	1.18	3
III	30	1.48	3
IV	45	1.65	3
V	60	1.78	4
VI	80	1.90	3
VII	140	2.15	3
VIII*	220	2.34	5
E	140	2.15	14

Figure S1. Phase 1 trial 3+3 DE scheme

a) Image shows study DE scheme. b) DE and additional dose (E:EC) (* indicates MTD identified by the Safety Monitoring Committee).

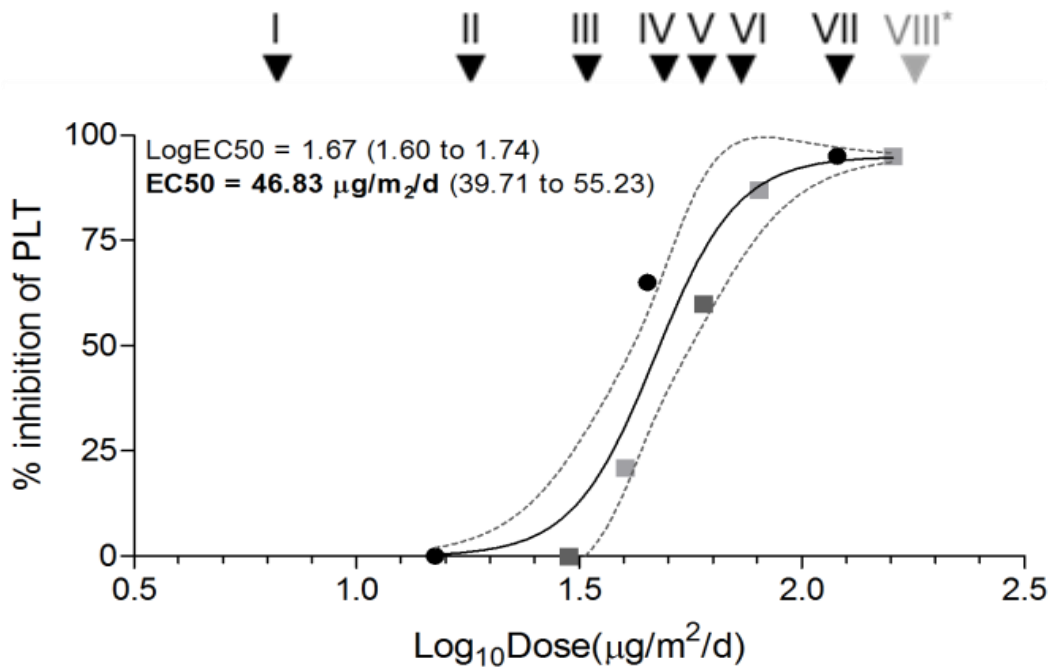


Figure S2. Impact of iadademstat on platelet levels (PLT) in non-clinical species in GLP toxicity studies

Impact of iadademstat on platelet levels represented as % inhibition compared with control animals. Black dots: 28 day GLP toxicity in Sprague Dawley rats. Light and dark grey squares: 14 and 28 day toxicity in Beagle dogs. Black arrows represent equivalent clinical doses ranging from cohorts I (5µg/m2/d) to VIII (220µg/m2/d). * indicates MTD identified by the Safety Monitoring Committee. See also Supplemental Methods.

FAB		M4		M6a		M6a		M6a	
Patient ID		29		32		35		40	
Morphological differentiation		YES		NO		NO		NO	
% PB blasts D0		95		0		1		20	
% PB blasts D29		92		0		0		14	
% BM blasts D0		97		17		51		20	
% BM blasts D29		70		8		23		54	
		-ΔΔCp, 600-768	Log_2(RSEMD29/D0)	-ΔΔCp, 98-168	Log_2(RSEMD29/D0)	-ΔΔCp, 600-768	Log_2(RSEMD29/D0)	-ΔΔCp, 600-768	Log_2(RSEMD29/D0)
PB qRT-PCR biomarker panel	LY96	5.9	∞	1.5	-1.1	-2.0	1.9	3.4	2.5
	GPR65	4.8	2.3	1.5	0.6	-2.6	-0.3	2.3	0.7
	PI16	4.4	3.0	1.1	2.9	3.9	-0.8	-3.3	-3.2
	CD86	3.9	0.5	-1.3	0.9	-0.9		2.8	0.6
	ITGAM	3.7	3.7	-1.8	-0.2	-1.6	-1.1	1.3	0.0
	ANXA2	3.2	1.8	1.4	1.1	2.3	0.6	2.5	0.8
	CAMSAP2	3.0	1.5	1.4	-0.2	2.6	0.5	4.8	1.3
	S100A12	2.8	6.3	2.1	-0.2	-3.2	-1.8	-3.6	
	VCAN	2.2	∞	-1.2	-0.4	3.7	0.2	2.6	1.5
	LYZ	0.9	3.7	-1.2	0.7	-3.1		2.5	0.9
	VIM	0.7	-0.1	0.6	0.5	2.7		3.5	-0.3
	CTSG	2.3	3.2	0.7	-0.4	-3.9	-1.8	-9.1	-0.2
BM RNAseq Erythro	PROCR		1.3		-0.4		1.7		1.4
	HBB		-4.4		-1.2		-1.5		-3.9
	HBA2		-2.7		-0.9		-1.9		-3.7
	HBA1		-2.7		-0.8		-1.7		-4.2
	GYPA		∞		-1.2		-0.2		-5.3
	GYPB		∞		-0.7		-0.5		-6.7

Figure S3. Molecular response to treatment with iadademstat.

Relative gene expression levels in selected AML cases from the EC. Expression analysis was performed in PB cells by qRT-PCR (represented as –ΔΔCp) or in BM by RNA sequencing (represented as Log_2(Treatment/Pre-dose)). Magenta values show gene up regulation and pink values down regulation. Information on the occurrence of blast morphological differentiation and percentage variation (D0: baseline; D29: end of Cycle 1) is also shown. RSEM: RNA-Seq by Expectation Maximization.

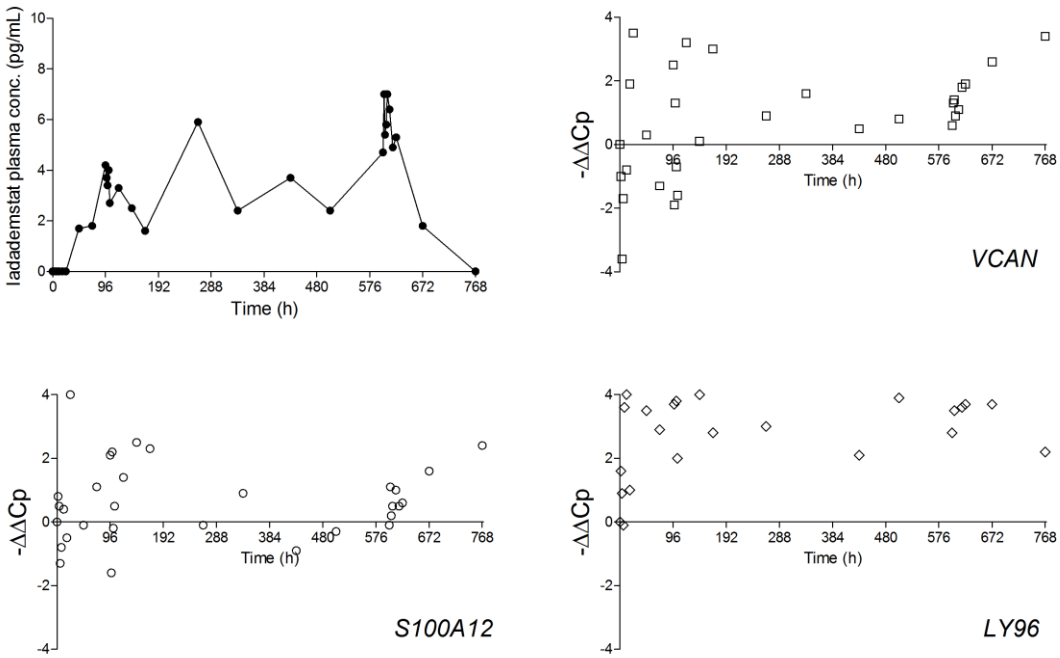


Figure S4: PK and PD in patient #13 (cohort V)

a) PK profile of iadademstat. Plasma levels were assessed by HPLC-MS/MS in serial samples (D1, D5, D26), trough and washout samples. (b-d) PD profiles of iadademstat. Time course of changes in expression of the indicated differentiation biomarkers in blood cells analyzed by qRT-PCR: b) *VCAN*, c) *S100A12* and d) *LY96*.

Supplemental Tables

Table S1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
1. Patients aged 16 and above	1. Cancer history that according to the investigator might confound the assessment of the study endpoints
2. Patients must have relapsed or refractory AL (excluding promyelocytic leukaemia) considered by the investigator ineligible for intensive chemotherapy regimen at that time	2. Patients with uncontrolled hypertension or diabetes, hepatitis or HIV.
3. Patients must have ECOG Performance Status (PS) of 0-2	3. Inter-current illness or social situation that will limit compliance with study requirements
4. Women of child-bearing potential must have negative serum or urine pregnancy test within two weeks prior treatment start	4. Pregnancy or lactating / breast feeding
5. Fertile male or female patients must use highly efficient contraception for the duration of the study and 6 months after the last iadademstat dose	5. Any medical condition which in the opinion of the investigator places the patient at an unacceptably high risk for toxicities if entered into the clinical study
6. Male patients must use condoms to avoid drug exposure of their partner	6. Acute myeloid leukaemia treatment within the previous 14 days. Hydroxyurea or 6-mercaptopurine are allowed until 12 hours prior study treatment start and after the first treatment block (day 1-5) in case of hyperleucocytosis
7. Patients must be capable of understanding and complying with protocol requirements, and they must be able and willing to sign a written informed consent	7. Patients medicated with anti-depressants reported to have KDM1A/LSD1 inhibitory activity: Tranylcypromine or Phenelzine
8. Life expectancy of at least 2 months	8. Radiotherapy less than 2 weeks prior to the start of the study

Table S2. Flowchart of planned interventions in Cycle 1

Trial period	Screening	Dose assignment	Treatment																											
Cycle			1																											
Visit	Screening																													
Days	14-0		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Informed consent	X																													
Demographics and patient characteristics including detailed documentation of comorbidities/concomitant diagnosis	X																													
Medical history	X																													
Review excl./incl. criteria	X	X																												
Physical examination	X		X							X							X							X						
Vital Signs	X		X							X							X							X						
ECOG score	X		X							X							X							X						
Weight	X		X							X							X							X						
Height	X																													
Concomitant therapy	X		X							X							X							X						
12-lead ECG	X		X																											
Safety laboratory (Clinical biochemistry)	X		X							X							X							X						
Hematology	X		X		X		X			X		X		X			X		X		X			X		X		X		
Coagulation	X		X							X																				
Urinalysis (dipstick)	X																													
Administration of ORY-1001			X	X	X	X	X			X	X	X	X	X			X	X	X	X	X			X	X	X	X	X		
Blood for Pharmacokinetics			X	X	X	X	X	X	X	X				X			X				X			X				X	X	
Blood for Pharmacodynamics			X	X	X	X	X	X	X	X				X			X				X			X				X	X	
Bone marrow aspirate																														
- Cytology																														
- Flow cytometry																														
- Genetics																														
- Molecular biology (MLL, FLT3, NPM1, CEBPA)	X		X																											
Adverse Events			X							X							X							X						
QoL assessment	X		X																											
Disease assessment	X																													
Eligibility for next cycle																														

Days	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
Informed consent																													
Demographics and patient characteristics including detailed documentation of comorbidities/concomitant diagnosis																													
Medical history																													
Review excl./incl. criteria																													
Physical examination	X							X							X							X							X
Vital Signs	X							X							X							X							X
ECOG score	X							X							X							X							X
Weight	X							X							X							X							X
Height																													
Concomitant therapy	X							X							X							X							X
12-lead ECG	X																												X
Safety laboratory (Clinical biochemistry)	X							X							X							X							X
Hematology	X		X		X			X							X							X							X
Coagulation																													
Urinalysis (dipstick)																													
Administration of ORY-1001																													
Blood for Pharmacokinetics	X				X																								
Blood for Pharmacodynamics	X				X																								
Bone marrow aspirate																													
- Cytology																													
- Flow cytometry	X																							X					
- Genetics																													
- Molecular biology (MLL, FLT3, NPM1, CEBPA)																													
Adverse Events	X							X								X						X							X
QoL assessment	X																												
Disease assessment	X																												
Eligibility for next cycle																													X

Table S3. Patient population characteristics

COHORT		PATIENT	TYPE	FAB	WHO 2008[a]	MLL[b]	WT1	FLT3-ITD	NPM1	MUTATIONS AND CYTOGENETICS	CYTOGENETIC RISK[c]	CYCLE 1[d]	CYCLE 2[d]	CYCLE 3[d]
DOSE ESCALATION	1	1	AML	M5b	2					45, X, -Y [6]/46, X, -Y, +8 [2]/47, +8 [2]/46, XY [10]	2			
		2	ALL	NA	5					46, XY, dup(1)(?q23q43), add(9)(p22), add(13)(q32), inc [cp18]/46, XY [4]	3			
		3	AML	NA	2					NA	NA			
	2	4	AML	M7a	1					46, XY, t(3;3)(q21;q26) [4]/45, X, -Y, t(3;3)(q21;q26) [15]/46, XY [1]	3			
		5	AML	NA	2					44, XY, -7, -12, del(13)(q22q34), add(21q22), +mar [cp10]/46, XY[4].	3			
		6	AML	NA	2					45, XY, add(3)(q26), der(3)add(3)(p21), del(5)(q22q33), -6, der(7)t(3;7)(p21;q22), [cp20]	3			
	3	7	AML	NA	3					47, XX, +11 [20]	2			
		8	AML	M7a	1					NA	NA			
		9	AML	NA	4					47, XY,+8 [13]/46, XY [7]	2			
	4	10	AML	NA	4					Normal	2			
		11	AML	M2	2					46, XX, del(5)(q11.2q31) [14]/46, idem, +8 [3]/46, idem, -16, t(16;21)(p11;p13) [3]	3			
		12	AML	NA	4					44, X, -Y, -21, der(21)t(1;21;22)(q31;q11.2;p11.2) [20]	2			
	5	13	AML	NA	2					43-49, XY, r(4), -5, del(?10)(q22), -13, der(16)t(13;16)(q21;q24), -17, -20, +mar1, +mar2, +mar3, +mar4, +mar5, +mar6 [cp18]/46, XY[2]; TP53 mutated	3			
		14	AML	M2	4					NA	NA			
		15	AML	NA	NA					Normal	2			
		16	AML	M2	3					46, XY, add(3)(q27), -5, +4 dmin, inc [cp10]	3			
	6	17	AML	M0	4					92, XXXX[6]/46, XX[14]	2			
		18	AML	NA	2					45, XY, -7 [4]/46,XY[7]	3			
		19	AML	NA	4					52, XY, +X, +8, +13, +14, +17, +19 [17]/46, XY [3]	3			
	7	20	AML	M0	NA					NA	NA			
		21	AML	NA	2					47, XY, -5, -7, -8, +4 mar [12]/48, XY, idem, +mar [4]/46, XY [1]	3			
		22	AML	M2	1					Normal	2			
	8	23	AML	NA	1					45, X, -Y, t(8;21)(q22;q22) [20]	1			
		24	AML	NA	4					47, XY, +8 [20]	2			
		25	AML	M0	4					46, XY, t(2;12) (q31;p13) [1] / 47, XY, idem, +8 [19]	2			
		26	AML	NA	2					Normal	2			
		27	AML	NA	4					45, X, -Y [1]/46, XY [19]	2			
EXTENSION		28	AML	M4	1					46, XY, t(10;11)(p12;q23) [20]	3			
		29	AML	M4	1					46, XX, t(9;11)(p21;q23) [20]	2			
		30	AML	M5b	3					46, XY, t(1;8), der(1)t(1;11)(q43q23), der(8)t(1;8)(p22q21) [20]	2			
		31	AML	M4	1					46, XX, t(9;11)(p21;q23) [20]	2			
		32	AML	M6a	4					46, XY, del(20)(q11q13), +mar [20]	2			
		33	AML	M4	3					46, XX, t(9;11)(p21;q23) [20]	2			
		34	AML	M6a	4					43~46, XY,-4, del(5)(q31?q35), -7, -12, -16, add(17)(p1), -20, ?add(21)(q22), +2~4mar [cp20]; TP53	3			
		35	AML	M6a	4					47, XY, +8 [6]/47, sl, +1, der(1;15)(q10;q10) [7]/48, sdl, +19 [3]/47, sl, +1 der(1;21)(q10;q10) [4]	3			
		36	AML	M5a	1					47, XX, +21, t(11;17)(q23;q21) [20]	3			
		37	AML	M5a	4					47, XX, +8, t(6;17)(p21;p13) [20]	2			
		38	AML	M2	2					NA	NA			
		39	AML	M2	2					Normal	2			
		40	AML	M6a	2					46, XY, del(7)(q22q22) [9]/47, XY, +8, del(7)(q22q22) [11]	3			
		41	AML	M2	1					46, XY, t(11;19) (q23;q13)[20]	2			

[a] *WHO 2008 subtypes: AML with recurrent genetic abnormalities, 1; AML with multilineage dysplasia, 2; AML therapy-related, 3; AML not otherwise categorized, 4; B-acute lymphoblastic leukemia, 5*

[b] *Grey background indicates chromosome alterations involving MLL; dark grey MLL fusion*

[c] *Cytogenetic risk (Ref.26) : Favourable, 1; Intermediate, 2; Adverse, 3*

[d] *Treatment cycles: black completed; grey incomplete*

NA: Not available

Table S4. Summary of all adverse events (AEs) and serious adverse events (SAEs).

Description of event	Total AEs		Grade 3&4 AEs		Total SAEs		Grade 5 AEs	
	DE	EC	DE	EC	DE	EC	DE	EC
General								
- Asthenia	21	9	2	4	-	1	-	-
- Anorexia	5	6	-	2	-	-	-	-
- Mucositis	9	5	1	-	-	-	-	-
- Xerostomia	3	2	-	-	-	-	-	-
- Oedema	14	4	1	1	-	-	-	-
Infection								
- Fever of unknown origin or neutropenic fever	23	17	10	11	7	9	1	-
- Septic shock or sepsis	3	3	2	3	3	2	1	-
- Intravenous device infection (line infection)	-	1	-	-	-	-	-	-
- Cutaneous/sub-cutaneous	6	6	3	2	1	2	-	1
- Respiratory infection or pneumonia	13	3	5	2	10	3	8	1
- Colitis	1	-	-	-	-	-	-	-
- Fungal mucosal infection	5	-	-	-	-	-	-	-
- Herpetic stomatitis	4	1	-	-	-	-	-	-
- Herpes zoster	1	-	-	-	-	-	-	-
- Conjuctivitis	1	-	-	-	-	-	-	-
- Sinusitis	1	-	-	-	1	-	1	-
- Tonsilitis	-	1	-	1	-	-	-	-
- Nasopharyngitis	1	1	-	-	-	-	-	-
- Urinary tract infection	1	-	-	-	-	-	-	-
Hemorrhage								
- Cutaneous: catheter related (2), petechiae (11), spontaneous hematoma (6), ear hemorrhage (1)	17	3	2	-	-	-	-	-
- Mucosal: hemorrhagic diathesis (4), hematemesis (1), melaena (1), epistaxis (5), haemoptysis (2), gastrointestinal hemorrhage (1)	13	1	1	-	-	-	-	-
- Retinal hemorrhage	1	-	-	-	-	-	-	-
- Intracranial hemorrhage	4	-	1	-	3	-	1	-
- Coagulopathy	1	-	-	-	-	-	-	-
Respiratory (other than mentioned)								
- Respiratory failure or distress	5	-	1	-	2	-	2	-
- Pleural effusion	3	-	2	-	-	-	-	-
- Cough	7	2	-	-	-	-	-	-
- Rhinorrea & bronchorrhea	4	-	-	-	-	-	-	-
- Dyspnoea	2	2	-	-	-	-	-	-
- Tachypnoea	2	-	-	-	-	-	-	-

Table S4. Summary of all adverse events (AEs) and serious adverse events (SAEs) (continued)

Description of event	Total AEs		Grade 3&4 AEs		Total SAEs		Grade 5 AEs	
	DE	EC	DE	EC	DE	EC	DE	EC
Gastrointestinal (other than mentioned)								
- Dysgeusia	1	5	-	-	-	-	-	-
- Gastro-esophageal reflux disease	-	1	-	-	-	-	-	-
- Diarrhoea	11	11	-	-	-	1	-	-
- Constipation	12	2	-	-	-	-	-	-
- Nausea	7	4	-	-	-	-	-	-
- Vomiting	4	3	-	-	-	-	-	-
- Abdominal pain	6	2	1	-	-	-	-	-
- Hemorrhoids	2	1	-	-	-	-	-	-
- Abdominal distension, enlarged liver or spleen	6	-	-	-	-	-	-	-
Neurological								
- Headache	10	-	-	-	-	-	-	-
- Visual hallucination	-	1	-	-	-	-	-	-
- Seizure	1	-	1	-	-	-	-	-
- Depressed level of conciousness	1	-	-	-	1	-	1	-
- Presyncope	1	-	1	-	-	-	-	-
- Ataxia (1), Dysarthria (1) , Aphasia (1), lower limb parasthesia (1)	4	-	-	-	-	-	-	-
Skin								
- Skin rash (8) or dry skin (2)	8	2	-	-	-	-	-	-
- Skin ulcer	2	-	-	-	-	-	-	-
- Hyperhydrosis	2	-	-	-	-	-	-	-
- Basal cell carcinoma	-	1	-	-	-	-	-	-
- Pruritus (4), ear wax (1)	3	2	-	-	-	-	-	-
Hematological								
- Thrombocytopenia	8	3	8	3	-	1	-	-
- Leukocytosis	5	3	3	1	-	3	-	-
- Leucopenia	1	-	1	-	-	-	-	-
- Neutropenia	3	2	3	1	-	-	-	-
- Anemia	2	1	1	1	-	-	-	-
- Differentiation syndrome	-	2	-	-	-	2	-	2
- Disease progression	1	5	-	-	1	5	1	5
Musculoskeletal disorders								
- Musculoskeletal pain or discomfort	21	7	-	1	-	1	-	-
- Joint pain or swelling	4	2	-	-	-	-	-	-
- Fall	3	-	-	-	-	-	-	-
- Steroid myopathy	1	-	-	-	-	-	-	-
Metabolism disorders								
- Hypokalemia	4	5	1	-	-	-	-	-
- Hypocalcemia	1	2	-	-	-	-	-	-
- Dehydration	-	2	-	-	-	-	-	-
- Hypophosphatemia	1	1	-	-	-	-	-	-
- Hyperglycemia (same patient)	8	-	7	-	-	-	-	-
- Hypoglycemia	1	-	-	-	-	-	-	-
- Hypomagnesemia	1	-	-	-	-	-	-	-

Table S4. Summary of all adverse events (AEs) and serious adverse events (SAEs) (continued)

Description of event	Total AEs		Grade 3&4 AEs		Total SAEs		Grade 5 AEs	
	DE	EC	DE	EC	DE	EC	DE	EC
Cardiac								
- Angina pectoris	1	-	-	-	-		-	-
- Pericarditis	-	1	-	-	-	1	-	1
- Bradycardia (2) or tachycardia (1)	3	-	-	-	-	-	-	-
- Cardiac failure	1	-	-	-	1	-	1	-
- Supraventricular tachycardia	-	3	-	2	-	2	-	-
- Hypotension	4	1	2	1	-	1	-	-
Urinary tract and kidney								
- Renal impairment	2	1	-	1	-	-	-	-
- Urinary retention (1), cystitis (1), urinary frequency (1)	1	2	-	-	-	-	-	-
Psychiatric								
- Insomnia	2	1	-	-	-	-	-	-
- Disorientation	2	-	-	-	-	-	-	-
- Agitation	-	1	-	-	-	-	-	-
- Depression	2	-	-	-	-	-	-	-
Other								
- Abnormal liver function tests	4	2	-	-	-	-	-	-
- Vitreous detachment	1	-	-	-	-	-	-	-
- Eye pain	1	-	-	-	-	-	-	-
- Ptosis	1	-	-	-	-	-	-	-
- Catheter site pain	1	-	-	-	-	-	-	-
- Cell death	-	1	-	-	-	-	-	-
- Infusion reaction	1	-	1	-	1	-	-	-
- Graft vs host disease skin (1), liver (1), mouth (1) (same patient)	3	-	1	-	1	-	-	-
TOTAL	**347**	**150**	**62**	**37**	**32**	**34**	**17**	**10**

** AEs which changed from a lower to a higher grade or vice versa (n=21) are only counted once, at the highest grade.

Table S5. Summary of all AEs considered possibly, probably or definitely related to study drug

Description of event	Total AEs		Grade 3&4 AEs		Total SAEs		Grade 5 AEs	
	DE	EC	DE	EC	DE	EC	DE	EC
General								
- Asthenia	3	5	-	2	-	1	-	-
- Anorexia	1	5	-	2	-	-	-	-
- Xerostomia	-	1	-	-	-	-	-	-
Infection								
- Fever of unknown origin or neutropenic fever	2	5	1	3	1	3	-	-
- Cutaneous/sub-cutaneous	-	1	-	-	-	1	-	1
- Respiratory infection or pneumonia	1	1	-	1	1	1	1	-
Hemorrhage								
- Cutaneous: catheter related (1), petechiae (2), spontaneous haematoma (1)	3	1	-	-	-	-	-	-
- Mucosal: hemorrhagic diathesis (1), epistaxis (1)	2	-	-	-	-	-	-	-
Respiratory (other than mentioned)								
- Cough	-	1	-	-	-	-	-	-
- Dyspnoea	-	1	-	-	-	-	-	-
Gastrointestinal (other than mentioned)								
- Dysgeusia	1	4	-	-	-	-	-	-
- Nausea	-	1	-	-	-	-	-	-
- Diarrhoea	1	4	-	-	-	-	-	-
Skin								
- Rash	2	1	-	-	-	-	-	-
- Pruritus	1	-	-	-	-	-	-	-
- Basal cell carcinoma	-	1	-	-	-	-	-	-
Hematological								
- Thrombocytopenia	3	2	3	2	-	1	-	-
- Neutropenia	2	-	2	-	-	-	-	-
- Anemia	1	-	-	-	-	-	-	-
- Differentiation syndrome	-	2	-	-	-	2	-	2
Musculoskeletal disorders								
- Joint pain or swelling	-	1	-	-	-	-	-	-
Other								
- Abnormal liver function tests	3	1	-	-	-	-	-	-
- Graft versus host disease	1	-	-	-	-	-	-	-
- Cell death	-	1	-	-	-	-	-	-
TOTAL	**27**	**39**	**6**	**10**	**2**	**9**	**1**	**3**

** AEs which changed from a lower to a higher grade or vice versa (n=21) are only counted once, at the highest grade.

Table S6. Taqman assays (PD biomarker panel)

Gen	Taqman Assay	Source
LY96	Hs01026734_m1	Life Technologies
S100A12	Hs00942835_g1	Life Technologies
ANXA2	Hs01561520_m1	Life Technologies
CAMSAP2	Hs01115863_m1	Life Technologies
CD86	Hs01567026_m1	Life Technologies
CTSG	Hs01113415_g1	Life Technologies
GPR65	Hs01097741_s1	Life Technologies
ITGAM	Hs00355885_m1	Life Technologies
LYZ	Hs00426232_m1	Life Technologies
VCAN	Hs00171642_m1	Life Technologies
CRISP9/PI16	Hs00542137_m1	Life Technologies
VIM	Hs00185584_m1	Life Technologies
HPRT1	Hs02800695_m1	Life Technologies

Supplemental Methods

Selection of starting dose for dose escalation

Based on preclinical toxicology studies and in accordance with ICH S9 on non-clinical evaluation for anticancer pharmaceuticals, CHMP/ICH/646107/08, the starting dose for iadademstat was established at 1/10th of the Severely Toxic Dose in 10% of rats, corresponding to 5µg/m^2/d. No off-target activity was observed in 28-day rat or dog toxicology studies. The impact of iadademstat on platelet levels tested in preclinical toxicology studies in rats and dogs, as well as the doses chosen for the Phase I trial, are shown in Figures S1B and S2.

Determination of maximum tolerated dose (MTD)

The study protocol defined the MTD as the highest dose at which no more than 33% of patients experience a dose limiting toxicity (DLT). DLT was defined as: (i) any grade 4 or 5 drug-related toxicity adverse event in the first cycle of treatment; (ii) in terms of myelotoxicity, as persistent grade 4 neutropenia (ANC <500/L), anemia (Hb <80g/L) and/or thrombocytopenia (platelet count <10x109/L) with hypocellular bone marrow (<5%) after Day 35 of treatment if not related to acute leukemia (patients with a baseline ANC of 0.5x10^9/L and/or platelet count of 25x10^9/L were excluded); (iii) any grade 3 or higher adverse event related to study drug or intercurrent illness (not underlying disease) which did not resolve despite appropriate medical intervention (e.g. grade 3 nausea, diarrhoea or vomiting); (iv) any treatment-related effect resulting in missing three ORY-1001 doses in the 28-day treatment period or, any treatment-related non-hematologic toxicity delaying initiation of the second oral ORY-1001 cycle by longer than 14 days.

Study monitor

The implementation, conduct and closure of this clinical trial was performed by a contract research organization (CRO), including regulatory activities, project management, site monitoring, source data verification, data management and medical writing.

Safety

Safety and tolerability were evaluated by summary drug-related DLT, AE reports, physical examinations and laboratory safety evaluations. National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.03 (1) was used for grading AEs and investigators assessed causality as either unrelated, possibly, probably or definitely related. The safety of this study was overseen by a safety monitoring committee (SMC). All patients who received iadademstat were included in the data summaries for safety.

Sampling

PB and BM samples were obtained at different time points according to the protocol. Core PK/PD sampling was performed from Day 1 to 8; sampling continued at selected time points throughout cycle 1 (C1) and during wash-out (Table S2).

PK analysis

Iadademstat concentrations were determined in human plasma using a highly sensitive GLP-validated HPLC-MS/MS method (LLOQ: 1pg/mL) with ESI in positive ion mode and deuterated iadademstat as internal standard, developed at Pharm-Analyt Labor GmbH. Sample analysis was GLP compliant and pharmacokinetic parameters were calculated by means of non-compartmental analysis by using WinNonlin® 6.4 software. Patients treated for at least one week were evaluable for PK.

PD analysis

PD studies were performed on total RNA isolated from stabilized blood cells using the PAX Blood RNA kit (Pre-Analytix, Cat# 762174) at Oryzon Genomics S.A. RNA was quantified using a Nanodrop™ 1000 spectrophotometer (Thermo Scientific) and quality was verified using the Agilent 2100 Bioanalyzer™. First strand synthesis was performed from 0.5µg of total RNA with the iScript™ Reverse Transcription Supermix for RT-qPCR (BioRad, Cat# 1708841). Gene expression analyses were performed in triplicate using validated off-the-shelf Taqman qRT-PCR assays (Table S6) in a Roche Lightcycler II 480 according to

manufacturer recommendations. ΔΔCp values were calculated relative to the pre-dose sample (T0) and relative to the endogenous control gene HPRT1. Variations in expression were presented as -ΔΔCp values.

BM biomarker analysis

RNAseq analysis was performed at Q2 Solutions (a Quintiles joint-venture), on pre-dose (D0) and post-treatment (D29) samples from five patients of the EC. BM samples were processed and nucleic acid libraries prepared and sequenced to a depth of 50M (D0) or 30M (D29) using Illumina HiSeq RNA sequencing by Q2 Lab Solutions according to standard protocols, followed by bioinformatics analysis in order to allow for differential gene expression analysis. Biomarker data were represented as Log_2(Treated/Pre-treatment) values.

Response assessment

Stained blood or BM aspirate smears were examined by light microscopy and manual differentials performed for morphological assessment together with blood cell counts. Responses were assessed according to the described revised recommendations of the International Working Group (IWG) for Diagnosis, Standardization of Response Criteria, Treatment Outcomes and Reporting Standards for Therapeutic Trials in AML (2). Patients who completed at least one cycle were evaluable for response.

Statistical analysis

No formal sample size estimation was performed. The assumptions concerning reasonable sample size were based on the dose-escalating scheme applied for determination of DLTs and the MTD.

All patients who received any amount of iadademstat were included in the summaries of baseline characteristics and safety. One-way analysis of variance (ANOVA) was used to compare PK parameters at different doses in a subset of DE patients who underwent PK evaluation. The Student T test was used to analyze time-dependence and dose-dependence of biomarker expression and changes in peripheral blood (PB) and BM blast percentage.

Data access

Access to clinical trial data is available through a data sharing agreement upon reasonable request and in compliance with applicable laws. Enquiries should be directed to the study sponsor (info@oryzon.com).

References

1. Common Terminology Criteria for Adverse Events (CTCAE): https://www.eortc.be/services/doc/ctc/ctcae_4.03_2010-06-579 14_quickreference_5x7.pdf

2. Cheson BD, Bennett JM, Kopecky KJ, et al; International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol. 2003; 21(24):4642-9, Correction: J Clin Oncol. 2004; 22(3):576.