PHASE 1 STUDY: SAFETY AND TOLERABILITY OF INCREASING DOSES OF CB-839, AN ORALLY-ADMINISTERED SMALL MOLECULE INHIBITOR OF GLUTAMINASE, IN ACUTE LEUKEMIA

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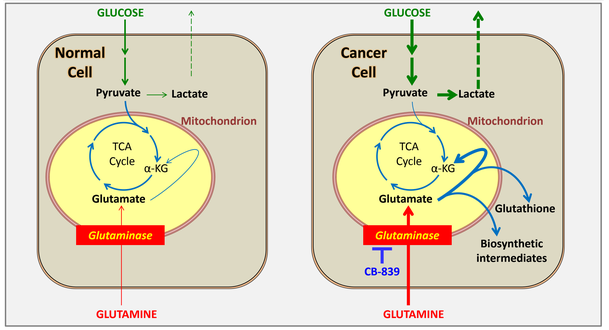
BACKGROUND AND RATIONALE

- Glutamine is required for the growth and survival of many tumor types[1,2]
- Glutaminase (GLS) controls the formation of glutamate (Figure 1), which is used to:
  - Generate TCA cycle intermediates
  - Synthesize glutathione
  - Generate NADPH and maintain redox balance
  - Synthesize anabolic building blocks, including nucleotides and fatty acids
- CB-839 is a highly selective, reversible, allosteric inhibitor of glutaminase[3]
- CB-839 has broad preclinical *in vitro* and *in vivo* anti-tumor activity in solid and hematologic malignancies[3,4,5]
- Herein we describe the initial results from CX-839-003 (ClinicalTrials.gov Identifier: NCT02071927)

Figure 1: Altered Glucose and Glutamine Metabolism of Cancer Cells



STUDY OBJECTIVES

Primary Objective

- To evaluate the safety and tolerability and determine the Recommended Phase 2 Dose (RP2D) of CB-839 for relapsed and/or treatment-refractory leukemia and IDH-mutated myelodysplastic syndrome (MDS)

Secondary Objectives

- To explore the pharmacokinetics (PK) of CB-839
- To describe evidence of anti-tumor responses in patients with acute leukemia
- To describe evidence of responses or hematological improvements in patients with MDS

Exploratory Objective

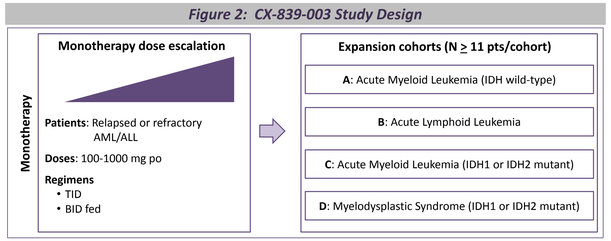
- To explore the pharmacodynamics (PDn) of CB-839

METHODS

Study Design

- Phase 1 multicenter, open-label, dose-escalation of CB-839 as a single-agent in AML/ALL
- Accelerated titration dose escalation design with 3 week cycle length
  - Pharmacodynamic assessments include assessment of GLS inhibition
  - Bone marrow evaluations after first and second cycle, then every three (3) cycles
- Expansion Cohorts are planned in defined patient populations (see Figure 2)

Figure 2: CX-839-003 Study Design



Key Inclusion Criteria

- Eastern Cooperative Oncology Group (ECOG) Performance Status of 0-2
- Adequate hepatic, renal and hematological function
- All patients must have bone marrow involvement of their tumor, with documented blast percentage of > 5%

Key Exclusion Criteria

- Patients with acute promyelocytic leukemia (APL)
- Newly diagnosed patients with favorable cytogenetic abnormalities
- Allogeneic hematopoietic stem cell transplant (HSCT) or Donor Lymphocyte Infusion (DLI) within 90 days prior to Day 1
- Any conditions that may preclude adequate absorption of study drug

Study Treatment

- Oral CB-839 administered in 21-day cycles using one of two regimens:
  - **TID**: Three times daily (upon waking, at ~3 pm and at bedtime)
  - **BID fed**: Two times daily with meals

Pharmacokinetics (PK), Pharmacodynamics (PDn) and Efficacy Assessments

- PK: Serum CB-839 measured on Cycle 1/Day 1 (C1D1), C1D15 and D1 of each subsequent cycle
- PDn: Blood draws for collection of platelets/PBMCs on C1D1 predose and at 4 hr
  - PDn data were analyzed together with PDn data from parallel Phase 1 study in solid tumors (Study CX-839-001[6])
- Efficacy: Bone marrow evaluations were performed at baseline and on D1 of Cycle 2, Cycle 3, and after every third cycle thereafter

STUDY STATUS

- As of April 15, 2015, 18 patients had been treated and had data in the clinical database
- Summaries of patient enrollment and baseline characteristics are provided in Tables 1 and 2, respectively
- An MTD has not been established (no DLTs on study)
- 600 mg po BID fed was selected for expansion cohorts based on results from this study and a concurrent larger Ph1 solid tumor study (N=59)[6]:
  - PK consistently above target threshold
  - Clear PK/PDn relationship in peripheral blood (platelets and PBMCs)
  - Evidence of pharmacodynamic activity in solid tumor biopsies
  - Reduced incidence of liver function abnormalities with BID dosing (see Table 4 below)

Table 1: Enrollment Summary

Dose cohorts (N=18)		
Dose level	**Schedule**	**N**
100 mg	TID	1
200 mg	TID	1
400 mg	TID	1
600 mg	TID	8
800 mg	TID	3
1000 mg	TID	2
600 mg	BID with food	2
Tumor types: AML (16), B-ALL (1), Mixed (1)		

Table 2: Baseline Characteristics

Characteristic		N=18
Age: median (range)		75 (35 — 84)
Female/Male: N (%)		9 (50%)/9 (50%)
Number of lines of prior systemic therapy	Median (range)	2.5 (0 — 7)
	0-1 regimens: N (%)	6 (33%)
	2-3 regimens: N (%)	10 (56%)
	≥4 regimens: N (%)	2 (11%)
Prior transplant: N (%)	Allogeneic	4 (22%)
	Autologous	0
ECOG Score: N (%)	0	3 (17%)
	1	12 (67%)
	2	3 (17%)

PHARMACOKINETICS*

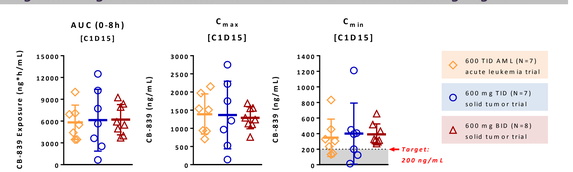
- CB-839 has acceptable PK properties in cancer patients
  - Half-life approximately 4 hr
  - Exposure generally increases with dose
- PK parameters were highly variable with the TID regimen and less variable with the BID fed regimen (Table 3 and Figure 3)
  - AUC, C_{max} and C_{min} on Cycle 1/Day 15 have lower variability (%CV) with BID fed regimen
  - C_{min} dropped below target concentration of 200 ng/mL in 5/14 (36%) patients on 600 mg TID regimens
  - C_{min} remained above the target concentration of 200 ng/mL for all patients that received 600 mg BID fed in the dose escalation cohort on study CX-839-001

**due to the small number of patients enrolled in most dose cohorts on this study, PK data were combined with PK data obtained in a companion solid tumor study, CX-839-001[6]*

Table 3: More Consistent Exposure with BID Fed Dosing Regimen

Trial: CX-839-		001	001	001	001	003	001	001	001
Dose (mg):		100	150	250	400	600	600	800	600
Schedule:		TID	TID	TID	TID	TID	TID	TID	BID
N:		3	4	10	3	7	7	3	8
AUC (0-8h) (ng*hr/mL)	Average	1980	7167	3852	3959	5833	6125	5575	6201
	Variation (%CV)	33%	98%	101%	46%	40%	70%	72%	34%
C_{max} (ng/mL)	Average	432	1467	778	846	1391	1366	1019	1291
	Variation (%CV)	46%	87%	89%	38%	40%	68%	35%	23%
C_{min} (ng/mL)	Average	134	585	241	306	347	400	457	366
	Variation (%CV)	32%	108%	105%	80%	69%	98%	92%	35%

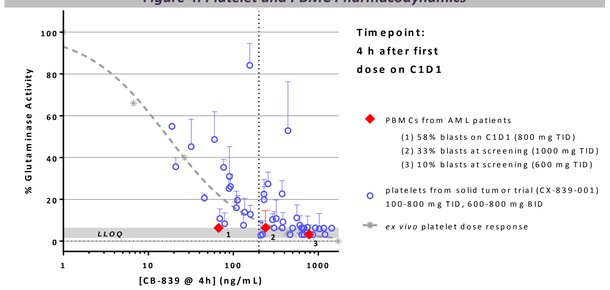
Figure 3: Target CB-839 Levels are Maintained with BID Fed Dosing Regimen



PHARMACODYNAMICS

- Strong inhibition of GLS was demonstrated in PBMCs and platelets in data from Phase 1 studies in AML/ALL patients (CX-839-003) and solid tumor patents (CX-839-001[6]), respectively (Figure 4)
  - GLS inhibition was measured in platelets/PBMCs 4 hr after dosing on C1D1
  - A clear exposure-response relationship is evident in platelets
  - C_{min} concentrations with 600 mg BID fed regimen maintain exposures that should provide ≥90% inhibition of GLS (≥ 200 ng/mL)

Figure 4: Platelet and PBMC Pharmacodynamics



SAFETY AND TOLERABILITY

- 16.7% (3/18) of patients experienced a Gr3 Adverse Event (AE) suspected to be related to CB-839 (Table 4)
- No patients discontinued due to an AE
- No DLTs occurred on study
  - One DLT of reversible Gr3 creatinine elevations occurred in the parallel solid tumor Phase 1 study (CX-839-001[6]); this patient had Type 2 diabetes mellitus with significant end-organ damage (retinopathy, Gr3 proteinuria at baseline)
- Reversible, asymptomatic elevations in liver function tests (LFTs) have been the primary toxicity signal to date
  - One patient experienced Gr3 elevations in ALT and total bilirubin
  - All LFTs rapidly returned to ≤Gr1 within ~1 week of drug discontinuation
- Based on combined data from CX-839-001 and CX-839-003, the frequency of ALT elevations is reduced when CB-839 is dosed BID with food (Table 5)

*Table 4: Drug-related Adverse Events in ≥ 2 Patients**

Total patients: 18	Number (%) of patients			
MedDRA Preferred Term	**All AEs**	**Grade 1**	**Grade 2**	**Grade 3^**
Patients with any AE	11 (61.1)	10 (55.6)	5 (27.8)	3 (16.7)
Alanine aminotransferase increased	6 (33.3)	3 (16.7)	2 (11.1)	1 (5.6)
Aspartate aminotransferase increased	6 (33.3)	4 (22.2)	1 (5.6)	1 (5.6)
Gamma-glutamyltransferase increased	5 (27.8)	2 (11.1)	2 (11.1)	1 (5.6)
Blood alkaline phosphatase increased	2 (11.1)	2 (11.1)		
Blood bilirubin increased	2 (11.1)	1 (5.6)		1 (5.6)
Fatigue	2 (11.1)	1 (5.6)	1 (5.6)	
Oral pain	2 (11.1)	2 (11.1)		
Vomiting	2 (11.1)	2 (11.1)		
Anaemia	1 (5.6)			1 (5.6)

**All Gr3 drug-related events are included* *^ There were no Grade 4 drug related AEs reported*

Table 5: Reduced Frequency of LFT Elevations in BID Cohorts

Dose (mg) and Schedule		100-250 TID	400-800 TID	600 & 800 BID
Total Patients = 77*		19	29	29
ALT Increase (Number of Patients)	All AEs	1	12	2
	Gr 1	0	3	0
	Gr 2	1	3	1
	Gr 3	0	6	1
Bilirubin Increase (Number of Patients)	All AEs	1	4	0
	Gr 1	0	1	0
	Gr 2	1	1	0
	Gr 3	0	2	0
Days on Study: Median (Range)		44 (11 - 105)	56 (14 - 286)	43 (9 - 112)

**combined data from CX-839-001 and CX-839-003 trials*

CLINICAL OUTCOMES

- This Phase 1 study has enrolled a limited number of patients across a range of doses on two different dosing regimens [TID (n=16) and BID fed (n=2)]
  - The patients enrolled included AML (n=16), ALL (n=1) and mixed leukemia (n=1)
  - AML patients included IDH1-mutant (n=1), IDH2-mutant (n=2) and IDH-wild-type (n=13)
- 5 of 18 patients remained on study for at least 4 cycles (Figure 5)
- One IDH2-mutant AML patient achieved a Complete Response with incomplete recovery of peripheral counts (CRi)
  - Bone marrow blast count steadily dropped from a baseline count of 16% to <5% over the course of 8 cycles (~6 months); subject remains on study (10+ months)
- One recently enrolled patient achieved a rapid reduction in peripheral blasts from 30% at baseline to 3% on Day 20 (Figure 6)
  - Upon discontinuation of study drug (due to CNS progression), peripheral blasts rose to 13% over two days

Figure 5: Treatment Duration

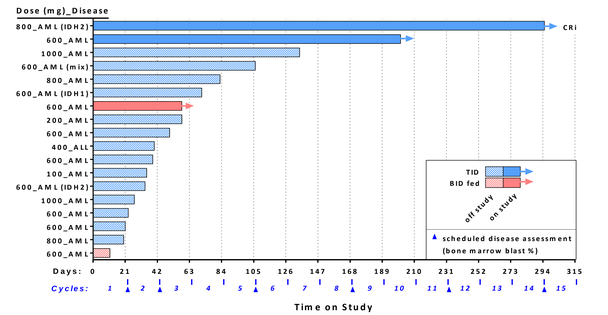
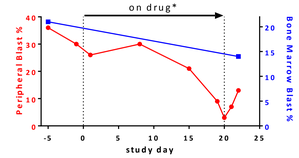


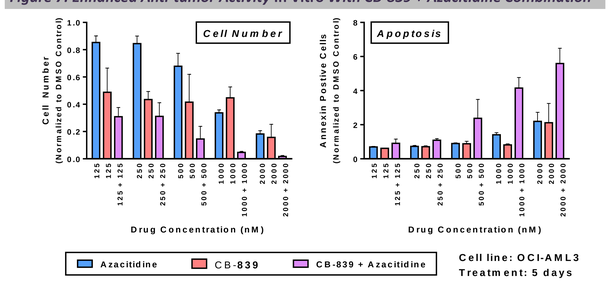
Figure 6: Peripheral Blast Response



SUMMARY AND CONCLUSIONS

- **CB-839 is well tolerated in advanced leukemia patients**
- **Robust inhibition of GLS demonstrated in platelets and PBMCs**
- **One patient achieved a CRi and has been on study for >10 months; 4 additional patients remained on study for at least 12 weeks**
- **BID dosing with meals provides optimal PK and safety profile**
- **Evaluation of clinical efficacy as monotherapy and in combination using BID dosing schedule is ongoing**
- **Future development of CB-839 in AML will include the combination with azacitidine (Vidaza)**
  - Supported by preclinical combination activity in AML cell line (Figure 7)

Figure 7: Enhanced Anti-tumor Activity In Vitro With CB-839 + Azacitidine Combination



References

1. Wise and Thompson (2010) *Trends Biochem Sci* **35**:427-433
2. DeBerardinis and Cheng (2010) *Oncogene* **29**:313-324
3. Gross et al. (2014) *Mol Cancer Ther* **13**:890-901
4. Parlati et al. (2013) *Blood* **122**:4226
5. Matre et al. (2014) *Blood* **124**:3763
6. Harding et al. (2015) *JCO* **33 (suppl)**:abstr 2512