AG-120 (ivosidenib), a first-in-class mutant IDH1 inhibitor, promotes morphologic changes and upregulates liver-specific genes in IDH1 mutant cholangiocarcinoma

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BACKGROUND

- Somatic mutations in the isocitrate dehydrogenase (IDH) 1 gene are detected in 13–15% of cholangiocarcinoma (CC) cases overall and in up to 25% of intrahepatic CC (ICC) cases.[1-3]
- The mutant IDH1 (mIDH1) enzyme has a gain-of-function activity, catalyzing the reduction of alpha-ketoglutarate to produce the oncometabolite D-2-hydroxyglutarate (2-HG).[4,5]
- Accumulation of 2-HG leads to aberrant DNA and histone methylation, epigenetic dysregulation of gene expression, and a block in cellular differentiation, thereby promoting oncogenesis.[6-9]
- AG-120 (ivosidenib) is a first-in-class, oral, potent, reversible, targeted inhibitor of the mIDH1 protein that has been shown to lower 2-HG levels in plasma and tumor tissue in preclinical and clinical studies, and to induce differentiation of leukemic blasts in patients with acute myeloid leukemia.[10-12]
- AG120-C-002 (ClinicalTrials.gov NCT02073994), a first-in-human phase 1 study, assessed AG-120 in patients with mIDH1 advanced solid tumors, including 73 patients with mIDH1 CC.
  - AG-120 was well tolerated and associated with a favorable safety profile in this mIDH1 CC cohort; no dose-limiting toxicities or treatment-related deaths were reported.[13,14]
  - AG-120 demonstrated encouraging clinical activity in this heavily pretreated mIDH1 CC population (5% response rate; 56% of patients achieved stable disease; 6- and 12-month progression-free survival rates of 38.5% and 20.7%, respectively).[13,14]
- Untreated mIDH ICCs often show heterogeneous histoarchitecture, with 61% of tumors lacking a dominant pattern. The cholangiolar pattern is commonly present, but often to a limited extent (median 10% cholangiolar histology)[15] (also C. Sigel, unpublished data, 2017).
  - Cholangiolar histology is associated with better clinical outcomes and survival rates in patients with ICC[15] (also V. Deshpande, unpublished data, 2017).
- Tumor phenotype and morphologic differentiation in CC patients treated with AG-120 have not previously been examined.
- Please see poster A072 (Saha et al. Presentation date Oct 28) for details of a preclinical study of mIDH inhibition in a mouse model of ICC.

OBJECTIVES

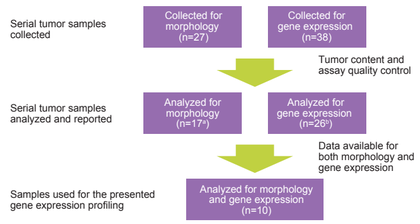
- Identify changes in morphology and gene expression in serial tumor biopsy samples obtained from patients with mIDH1 CC treated with AG-120 in the AG120-C-002 study.
- Correlate findings with the clinical outcomes of response rate and progression-free survival.

METHODS

- The 73 patients with mIDH1 CC enrolled in the trial were treated with AG-120 at doses ranging from 100 mg twice daily (BID) to 1200 mg once daily (QD).
- Tumor biopsies were collected, where feasible, from patients with CC at screening, ~2 months after treatment (Cycle 3 Day 1; C3D1), and, in some cases, 6 months after AG-120 treatment (C7D1), at disease progression, and at end of treatment (EOT).
  - 17 of 27 serial predose and postdose formalin-fixed, paraffin-embedded (FFPE) tumor samples were evaluable for morphology assessment.
  - 26 of 38 serial predose and postdose fresh frozen tumor samples underwent gene expression profiling.
  - Both morphology and gene expression data were assessed in 10 patients to elucidate their relationship (**Figure 1**).
- Biopsies with inadequate tumor content were excluded from the analyses.
- The clinical data in this poster were based on a cutoff date of May 12, 2017.

References **1.** Goyal L et al. *Oncologist* 2015;20:1019-27. **2.** Borger DR et al. *Oncologist* 2012;17:72-9. **3.** Kipp BR et al. *Hum Pathol* 2012;43:1552-8. **4.** Ward PS et al. *Cancer Cell* 2010;17:225-34. **5.** Dang L et al. *Nature* 2009;462:739-44. **6.** Saha SK et al. *Cell Cycle* 2014;13:3176-82. **7.** Saha SK et al. *Nature* 2014;513:110-4. **8.** Lu C et al. *Nature* 2012;483:474-8. **9.** Xu W et al. *Cancer Cell* 2011;19:17-30. **10.** de Botton S et al. *Haematologica* 2015;100(s1):214;P563. **11.** Fan B et al. *Haematologica* 2015;100(s1):218;P572. **12.** Fan B et al. *Blood* 2015;126(23):A1310. **13.** Lowery MA et al. *J Clin Oncol* 2017;35(Suppl):Abstr 4015. **14.** Lowery MA et al. *ASCO Annual Meeting* 2017; Poster 4015. **15.** Liau JY et al. *Mod Pathol* 2014;27:1163-73. **16.** Komuta M et al. *Hepatology* 2012;55:1876-88. **17.** Komuta M et al. *Hepatology* 2008;47:1544-56. **18.** Fan B et al. *J Clin Oncol* 2017;35(Suppl):Abstr 4082.

Figure 1. Serial tumor sample collection, process, and analyses



ªIncludes 16 patients dosed at 500 mg QD and one patient dosed at 100 mg BID
ᵇIncludes 22 patients dosed at 500 mg QD, two patients dosed at 1200 mg QD, and two patients dosed at 300 mg QD

Morphologic assessment

- Hematoxylin and eosin (H&E) stained slides from FFPE tissue were evaluated by two blinded gastrointestinal pathologists from independent institutions (Massachusetts General Hospital and Memorial Sloan Kettering Cancer Center).
- Architectural patterns, cytologic features, and stromal alterations were evaluated.
- Upon AG-120 treatment, changes in cholangiolar histology and cytoplasmic size were observed in some patients.
  - A cholangiolar pattern was defined as being composed of glands with an antler-horn configuration and angulated shapes, and lined with low cuboidal epithelium.[15-17] The percentage of tumor with a cholangiolar pattern was recorded. A predose to postdose increase was defined as a ≥20% increase in cholangiolar histology.
  - The volume of cytoplasm in tumor cells was semiquantitatively assessed and compared between pretreatment and posttreatment biopsies.

Gene expression profiling

- Gene expression profiles in serial fresh frozen biopsies from screening, C3D1, C7D1, and/or EOT were generated by RNA sequencing to relate to morphologic changes.
- Proprietary Personalis® Accuracy and Content Enhanced (ACE) Transcriptome technology was used to analyze all frozen or FFPE biopsy tissue.
- Supervised hierarchical clustering analyses were performed to identify postdose gene expression changes associated with best treatment response, increased cholangiolar histology, and cytoplasmic shrinkage.
- Unsupervised hierarchical clustering analyses were performed to assess reported marker genes for ICC, hepatocellular carcinoma, cholangiocytes, mature hepatocytes, hepatoblasts, and immune cells.

RESULTS

Morphologic changes were observed upon AG-120 treatment

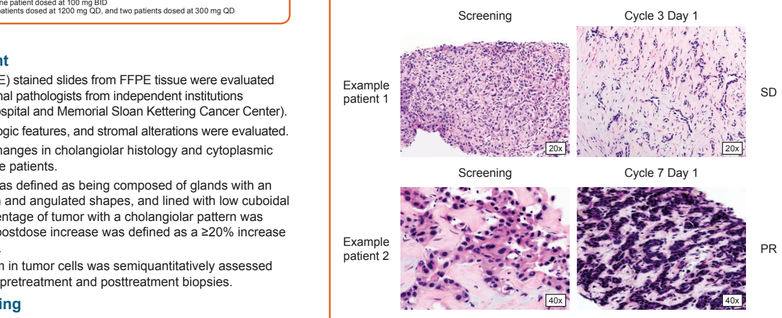
- At screening:
  - 13 of 17 (76%) patients had moderate to abundant cytoplasm.
  - 2 of 17 (12%) patients had 100% cholangiolar histology.
- Upon 8–24 weeks of AG-120 treatment, five of the 17 evaluable CCs (29%) exhibited an increase in cholangiolar histology and nine (53%) a reduction in cytoplasmic volume (**Table 1**; **Figure 2**).
  - Changes in both these phenotypes were seen in four cases.
- The two patients with a 100% cholangiolar pattern at screening also had 100% cholangiolar histology post dose.
- These morphologic changes were not associated with AG-120 dose. All patients had plasma 2-HG reduction upon AG-120 treatment, regardless of postdose morphology.[18]

Table 1. Predose to postdose morphologic changes in AG-120-treated mIDH1 CCs

	Morphology pairs available	Increase in cholangiolar histology	Cytoplasmic shrinkage	Cholangiolar and cytoplasmic changes
Number of patients	**17**	**5**	**9**	**4**
By treatment response, n				
PR	3	1	3	1
SD	12	4	6	3
PD	2	–	–	–

Treatment response measured according to Response Evaluation Criteria In Solid Tumors (RECIST) v1.1 (PR = partial response; SD = stable disease; PD = progressive disease)

Figure 2. Representative tumor section pairs showing increased cholangiolar histology and decreased cytoplasm upon AG-120 treatment

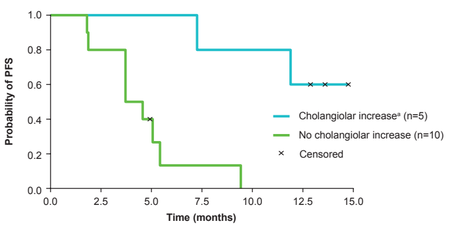


The screening biopsies lack a cholangiolar pattern, while those performed at C3D1 or C7D1 show extensive cholangiolar differentiation

Progression-free survival seems to be associated with increased cholangiolar histology (Figure 3)

- This observation should be interpreted with caution due to the small sample size and single-arm setting.

Figure 3. Kaplan-Meier curves for progression-free survival

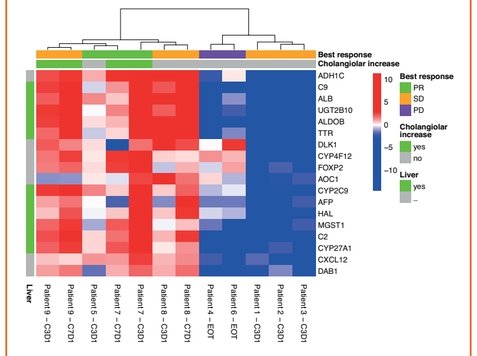


Two patients with a 100% cholangiolar pattern at screening and post dose were excluded from this analysis as they are expected to have better clinical outcomes and survival[17] (also V. Deshpande, unpublished data, 2017)
ªDefined as a ≥20% increase from screening to C3D1
PFS = progression free survival

Patients with increased cholangiolar histology show increased expression of liver-specific genes

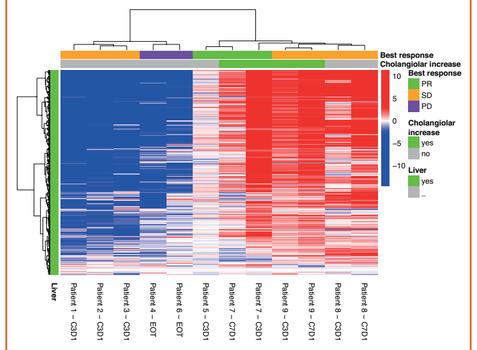
- Cluster analysis identified that the liver-specific genes *C9*, *ALB*, *UGT2B10*, *ALDOB*, *TTR*, *CYP2C9*, *AFP*, *HAL*, *MGST1*, *C2*, and *CYP27A1* were upregulated in mIDH1 CCs exhibiting an increase in cholangiolar histology upon AG-120 treatment (**Figure 4**).
- This association was subsequently reproduced in a larger set of ~500 adult liver-specific genes (**Figure 5**).

Figure 4. Cholangiolar increase is associated with upregulation of liver-specific genes



Heatmap depicts log2 fold change relative to screening
Among those with available RNA sequencing data, one patient with a 100% cholangiolar pattern at screening and post dose was excluded from this analysis
Patient 8 showed clear upregulation of liver-specific genes but no significant increase in cholangiolar histology upon AG-120 treatment (predose to postdose change in cholangiolar pattern = 10%). This patient had received another mIDH1 inhibitor prior to AG-120 treatment

Figure 5. mIDH1 CCs with cholangiolar increase show upregulation of a broad set of adult liver-specific genes

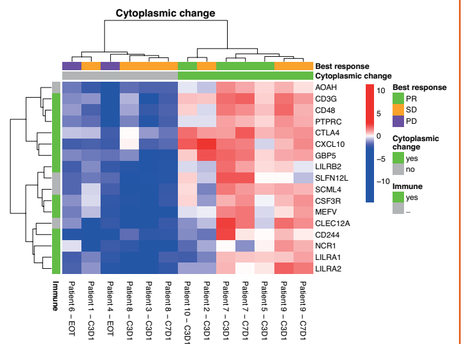


Heatmap depicts log2 fold change relative to screening
Among those with available RNA sequencing data, one patient with a 100% cholangiolar pattern at screening and post dose was excluded from this analysis

Patients with cytoplasmic decrease show increased expression of immune response-related genes

- mIDH1 CCs with a postdose cytoplasmic decrease showed upregulation of immune response-related genes such as *CTLA4*, *CXCL10*, and *CD3G* (**Figure 6**).

Figure 6. Cytoplasmic decrease is associated with upregulation of immune response-related genes



Heatmap depicts log2 fold change relative to screening

CONCLUSIONS

- This is the first demonstration that AG-120 treatment may induce morphologic and molecular changes in a subset of mIDH1 CCs.
- Increased cholangiolar histology seems to be associated with increased progression-free survival; however, this result should be interpreted with caution due to the small sample size and single-arm setting.
- Tumors with increased cholangiolar histology showed upregulation of genes associated with mature liver cells.
- The increased expression of immune response-related genes in some tumors suggests a potential rationale for AG-120 in combination with immunotherapies.
- Given the limited sample size of this dataset, additional studies are warranted to explore the biological and clinical significance of these observations.
- AG-120 is under further evaluation in an ongoing, global, phase 3, randomized, placebo-controlled study in previously treated mIDH1 CC (ClarIDHy, ClinicalTrials.gov NCT02989857).

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