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Clinical Trial Results

Phase II and Pharmacodynamic Study of Autophagy Inhibition Using Hydroxychloroquine in Patients With Metastatic Pancreatic Adenocarcinoma

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Author Summary

Abstract

Background. Autophagy is a catabolic pathway that permits cells to recycle intracellular macromolecules, and its inhibition reduces pancreatic cancer growth in model systems. We evaluated hydoxychloroquine (HCQ), an inhibitor of autophagy, in patients with pancreatic cancer and analyzed pharmacodynamic markers in treated patients and mice.

Methods. Patients with previously treated metastatic pancreatic cancer were administered HCQ at 400 mg ($n = 10$) or 600 mg ($n = 10$) twice daily. The primary endpoint was 2-month progression-free survival (PFS). We analyzed peripheral lymphocytes from treated mice to identify pharmacodynamic markers of autophagy inhibition that were then assessed in peripheral lymphocytes from patients.

Results. Among 20 patients enrolled, 2 (10%) were without progressive disease at 2 months. Median PFS and overall survival were 46.5 and 69.0 days, respectively. Treatment-related grade 3/4 adverse events were lymphopenia ($n = 1$) and elevated alanine aminotransferase ($n = 1$). Tolerability and efficacy were similar at the two dose levels. Analysis of treated murine lymphocytes suggested that LC3-II expression by Western blot is a reliable marker for autophagy inhibition. Analysis of LC3-II in patient lymphocytes demonstrated inconsistent autophagy inhibition.

Conclusion. Mouse studies identified LC3-II levels in peripheral lymphocytes as a potential pharmacodynamic marker of autophagy inhibition. In patients with previously treated metastatic pancreatic cancer, HCQ monotherapy achieved inconsistent autophagy inhibition and demonstrated negligible therapeutic efficacy. ***The Oncologist*** 2014;19:637–638

Discussion

Autophagy is a catabolic pathway that permits cells to recycle intracellular macromolecules and organelles [1, 2]. The role of autophagy in cancer is complex and likely is dependent on tumor type, genetic landscape, and phase of tumorigenesis [2–4]. Nevertheless, a subset of malignancies require autophagy for growth and survival [1]. Pancreatic cancers have high basal levels of autophagy, and inhibition of autophagy impeded their growth in vitro and in mouse models [5]. Chloroquine (CQ) and hydroxychloroquine (HCQ) inhibit autophagy in vitro [5–7]. We conducted a phase II clinical trial and translational study of HCQ in patients with previously treated metastatic pancreatic cancer. Concurrently, we examined peripheral lymphocytes from CQ-treated mice to identify pharmacodynamic markers of autophagy inhibition. With more than 35 trials assessing HCQ as cancer therapy, it is paramount to identify reliable pharmacodynamic markers in humans.

In mice receiving CQ at doses sufficient to inhibit autophagy in tumors and to cause tumor regression, we noted increased levels of LC3-II, but not p62, in peripheral lymphocytes and hepatocytes (Fig. 1). This suggests that monitoring LC3-II levels in human peripheral lymphocytes may provide a useful pharmacodynamic marker for monitoring autophagy inhibition. In our patients, HCQ at 800 mg or 1,200 mg daily resulted in inconsistent autophagy inhibition, as measured by LC3-II in peripheral lymphocytes. Furthermore, the 2-month PFS rate of 10% was inadequate to justify further studies of single-agent HCQ in this patient population.

Several mechanisms may explain the lack of efficacy for HCQ. First, autophagy inhibition alone in metastatic human pancreatic

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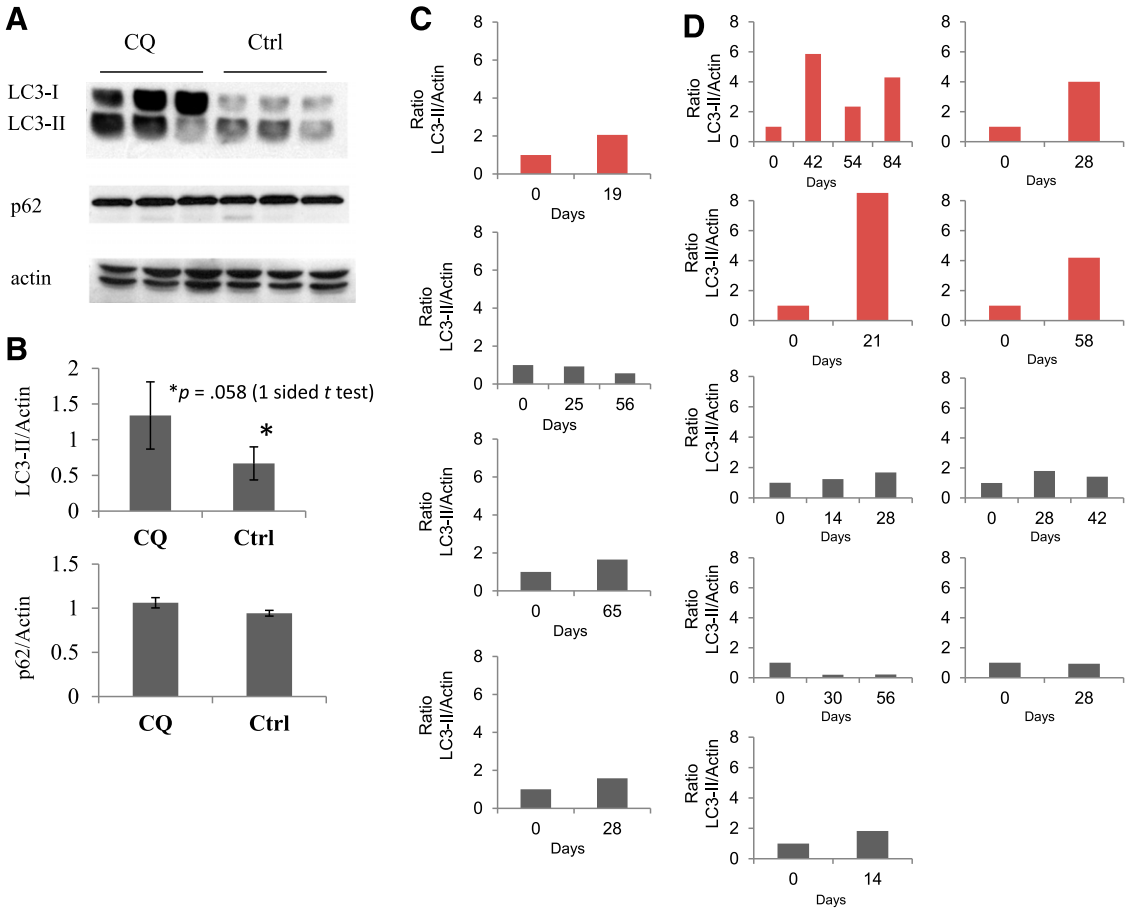

Figure 1. Ratio of LC3-II to actin as a biomarker for autophagy inhibition. **(A)**: Autophagy inhibition in mouse lymphocytes. A Western blot probed for LC3, p62, and β-actin in the presence (first three lanes) or absence (second three lanes) of CQ treatment. Each lane comprised pooled lymphocyte samples from two to three individual mice treated with drug or control. **(B)**: A bar graph displays the relative quantity of LC3-II (upper graph) and p62 (lower graph) as a ratio to β-actin as assessed by densitometry. Autophagy inhibition in circulating lymphocytes from patients receiving hydroxychloroquine (HCQ) at either 400 mg b.i.d. **(C)** or 600 mg b.i.d. **(D)**. Each bar graph reflects results from a single patient prior to treatment (day 0), and then at one time point or more while receiving HCQ. For each patient, a baseline ratio of LC3-II to actin was determined based on assessment by densitometry of Western blot prior to starting (day 0) and then at one time point or more following initiation of HCQ. Graphs in red depict patients with a more than twofold increase in relative LC3-II levels on serial blood draws.

Abbreviations: CQ, chloroquine; Ctrl, control.

cancer may not be sufficient to affect tumor growth. Indeed, studies have suggested that autophagy inhibition can act synergistically with cytotoxic chemotherapy [6, 8]. Second, autophagy inhibition at the HCQ doses tested appeared inconsistent when assessed in circulating lymphocytes. Consequently, the doses tested may not adequately inhibit autophagy within tumors. The use of HCQ with concurrent chemotherapy may obviate the need for complete autophagy inhibition in tumors, and such trials are ongoing. Optimization of HCQ dosing or administration of more potent inhibitors may also be necessary in future studies. Third, this study was conducted in patients who received multiple lines of prior chemotherapy. Given the short survival of patients with previously treated pancreatic cancer, patients may not have received sufficient HCQ to manifest a tumor response. Alternatively, chemotherapy may promote the upregulation of autophagy as a survival mechanism [9], making autophagy inhibition in future lines of therapy more difficult.

Despite our negative efficacy results for HCQ in patients with previously treated metastatic pancreatic cancer, inhibition of autophagy remains an intriguing therapeutic strategy for pancreatic cancer and other tumor types. Successful implementation of this therapeutic approach will require reliable markers of autophagy inhibition, and our data suggest LC3-II as a candidate pharmacodynamic marker for use in clinical trials.

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