## Supplementary Online Content

Cercek A, Boerner T, Tan BR, et al. Assessment of hepatic arterial infusion of floxuridine in combination with systemic gemcitabine and oxaliplatin in patients with unresectable intrahepatic cholangiocarcinoma: a phase 2 clinical trial. *JAMA Oncol*. Published online October 31, 2019. doi:10.1001/jamaoncol.2019.3718

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This supplementary material has been provided by the authors to give readers additional information about their work.

 

## eMethods. Detailed Methodology

***Inclusion/exclusion criteria***

Inclusion criteria:

- Age ≥21 years
- Histologically confirmed intrahepatic cholangiocarcinoma (also variously reported as peripheral cholangiocarcinoma, cholangiolar carcinoma, or cholangiocellular carcinoma)
- Clinical or radiographic evidence of metastatic disease to regional lymph nodes will be allowed, provided it is amenable to resection
- Radiographically measurable disease; measurable disease is defined as disease that can be assessed with 2-dimensional measurements on cross-sectional imaging; minimum lesion size is 2 cm in greatest diameter as per RECIST criteria
- Disease must be considered unresectable at the time of preoperative evaluation
- Presence of less than 70% liver involvement by cancer
- Patients may have failed ablative therapy
- Patients previously treated with systemic chemotherapy will be eligible
- Karnofsky performance status (KPS) ≥60% and be considered candidates for general anesthesia, abdominal exploration, and hepatic artery pump placement
- Patients with chronic hepatitis and/or cirrhosis are eligible, but must be Child-Pugh class A
- Patients must be able to read, understand, and sign informed consent
- White blood cell (WBC) count ≥2,000 cells/$mm^3$
- Platelet count ≥75,000/$mm^3$
- Creatinine ≤1.8 mg/dl
- Total bilirubin <1.5 mg/dl

Exclusion criteria:

- Presence of distant metastatic disease; patients will undergo radiographic evaluation to exclude the possibility of distant metastatic disease; clinical or radiographic evidence of metastatic disease to regional lymph nodes will be allowed, provided it is amenable to resection
- Prior treatment with floxuridine
- Prior external beam radiation therapy to the liver
- Diagnosis of sclerosing cholangitis
- Clinical evidence of portal hypertension (ascites, gastroesophageal varices, or portal vein thrombosis; surgically related ascites does not exclude the patient)
- Active infection
- Pregnant or lactating women
- History of other malignancy within the past 3 years (except non-melanoma skin cancer)
- Life expectancy less than 12 weeks
- Inability to comply with study and/or follow-up procedures

*Pretreatment evaluation*

Pretreatment evaluation included a complete history and physical examination, routine laboratory studies, and analysis of tumor markers (CEA and CA 19-9). Disease extent was assessed with cross-sectional imaging (CT scan of chest, abdomen, and pelvis or MRI of abdomen and pelvis). Findings concerning for extrahepatic disease were evaluated with a PET scan. All patients underwent endoscopy, colonoscopy, and mammogram (females) to exclude other primary tumor sites.

*Surgical HAI pump placement*

Preoperative hepatic CT angiogram, including visualization of the celiac and superior mesenteric arteries, was used to evaluate hepatic arterial blood supply. Intraoperative injection of dye (either fluorescein or methylene blue) was used to check the flow immediately after pump placement. Postoperatively, a perfusion study utilizing technetium-99m macroaggregated albumin via the pump's sideport was compared with a standard sulfur colloid liver-spleen

 

scan to confirm that distribution of the pump effluent was confined to the liver and that adequate perfusion of the liver remnant was achieved (eFigure 1). Regional lymph nodes were removed at the time of pump placement.

*Treatment schema*
Hepatic arterial infusion (HAI) chemotherapy consisted of continuous infusion of floxuridine into the liver circulation via a surgically implanted hepatic pump. The study design at both institutions consisted of monthly cycles, with treatment beginning approximately 14 days post-HAI pump placement (Cycle 1 Day 1). The treatment schemes at the 2 institutions differed slightly; Memorial Sloan Kettering Cancer Center patients received only HAI floxuridine over 14 days starting on Cycle 1 Day 1 and initiated systemic chemotherapy on Day 15 of Cycle 1, while the Washington University in St. Louis patients received HAI floxuridine over 14 days and systemic gemcitabine/oxaliplatin starting on Cycle 1 Day 1 (eTable 1).

*Chemotherapy administration*
HAI chemotherapy was initiated two weeks after pump placement on a 4-week cycle. All patients received an infusion of HAI floxuridine ([0.12 mg/kg x kg x 30]/pump flow rate) and dexamethasone (30 mg/pump flow rate; either 23 or 25 mg) with heparin sulfate (30,000 units) and saline to a volume of 30 mL on Day 1 of a 14-day infusion. After the 2-week infusion, the residual volume was removed, and heparinized saline (30 mL) was instilled. The patients began systemic gemcitabine (800 mg/m$^2$) with oxaliplatin (85 mg/m$^2$) on Day 1 or 15 (as described above) and received gemcitabine/oxaliplatin with each subsequent pump access, which occurred every 2 weeks.

Patients were seen biweekly, and history, physical examination, and routine bloodwork, including liver function tests, were obtained at each visit. A CT scan of the chest, abdomen, and pelvis was obtained at baseline and then at 1, 3, 5, 8, 12, and 15 months after starting treatment. Patients also underwent MRI with Eovist contrast for research purposes at baseline, 4 weeks after starting treatment, and 3, 6, and 9 months post-treatment. RECIST 1.1 response was calculated based on CT scans. Treatment with HAI floxuridine and systemic gemcitabine/oxaliplatin chemotherapy continued until the patients developed one of the following: disease progression, extrahepatic progression, treatment-related complications or toxicity, or surgical resection of disease.

*Toxicity grading*
Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria version 4.0.[1] The floxuridine dose adjustment schedule has been previously described.[2]

*Tumor genomic analyses (continued)*
A tumor biopsy was collected either at the time of diagnosis or at the time of HAI pump placement. A pathologist reviewed all tumor samples and resected lymph nodes. Microdissection was performed as needed to ensure adequate cellularity. The MSK-IMPACT deep sequencing assay was used for genomic analysis.[3] Custom DNA probes were designed for targeted sequencing of all exons and selected introns of 341 to 468 genes, including known oncogenes, tumor suppressor genes, and members of pathways deemed potentially actionable by targeted therapies. Genomic DNA from tumor and patient-matched normal samples (blood) were used for library preparation (Kapa Biosystems, Wilmington, MA) and exon capture (Roche NimbleGen, Madison, WI). Barcoded sequence libraries were pooled at equimolar concentrations and subjected to a single exon capture reaction, as previously described.[3, 4] FASTQ files were aligned to the human reference assembly GRCh37 by using BWA-MEM (Burrows-Wheeler Aligner version 0.7.5a), and PCR duplicates were identified through the MarkDuplicates tool in Picard Tools version 1.96. Genomic regions with greater than 20x coverage were subjected to indel realignment by Assembly-Based ReAligner version 0.92. Variant calling was performed in paired tumor/normal mode using MuTect software version 1.1.4 for single nucleotide variants and SomaticIndelDetector and Pindel software version 0.2.5a7 for small insertions and deletions. Variants were subsequently annotated using Annovar relative to UCSC-derived canonical transcripts and then subjected to a series of filtering steps to ensure only high-confidence calls were admitted to a final step of manual review.[5, 6] Somatic copy number alterations were identified by comparing sequence coverage of targeted regions in a tumor sample relative to a standard diploid normal as previously described.[5] Somatic structural aberrations were identified using DELLY software from tumor and matched normal paired read data.

*Statistical analyses (continued)*
Progression-free survival (PFS) was calculated from the date of pump placement until the date of progression or death, whichever occurred first. If at any time patients came off of the study because of toxicities or due to

 

symptoms that made further treatment impossible, these patients were treated as events at the off-study date. Patients with disease that was converted to resectability during treatment, those unable to comply with study requirements, or those that requested alternative treatment were censored at the off-study date.

 

## eReferences

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2. Kemeny N, Capanu M, D'Angelica M, Jarnagin W, Haviland D, Dematteo R, et al. Phase I trial of adjuvant hepatic arterial infusion (HAI) with floxuridine (FUDR) and dexamethasone plus systemic oxaliplatin, 5-fluorouracil and leucovorin in patients with resected liver metastases from colorectal cancer. Ann Oncol. 2009;20(7):1236-41.
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## eTable 1: Treatment schema.

| **Memorial Sloan Kettering Cancer Center** | |
|---|---|
| Cycle schema for Cycle 1, repeated every 4 weeks | |
| Cycle 1 Day 1 | HAI floxuridine/dexamethasone (14-day infusion) |
| Cycle 1 Day 15 | Systemic gemcitabine + oxaliplatin<br>HAI heparin/saline (14-day infusion) |
| **Washington University in St. Louis** | |
| Cycle schema for Cycle 1, repeated every 4 weeks | |
| Cycle 1 Day 1 | Systemic gemcitabine + oxaliplatin<br>HAI floxuridine/ dexamethasone (14-day infusion) |
| Cycle 1 Day 15 | Systemic gemcitabine + oxaliplatin<br>HAI heparin/saline (14-day infusion) |

HAI: hepatic arterial infusion

**eTable 2: Chemotherapy and hepatic arterial infusion floxuridine dosing.**

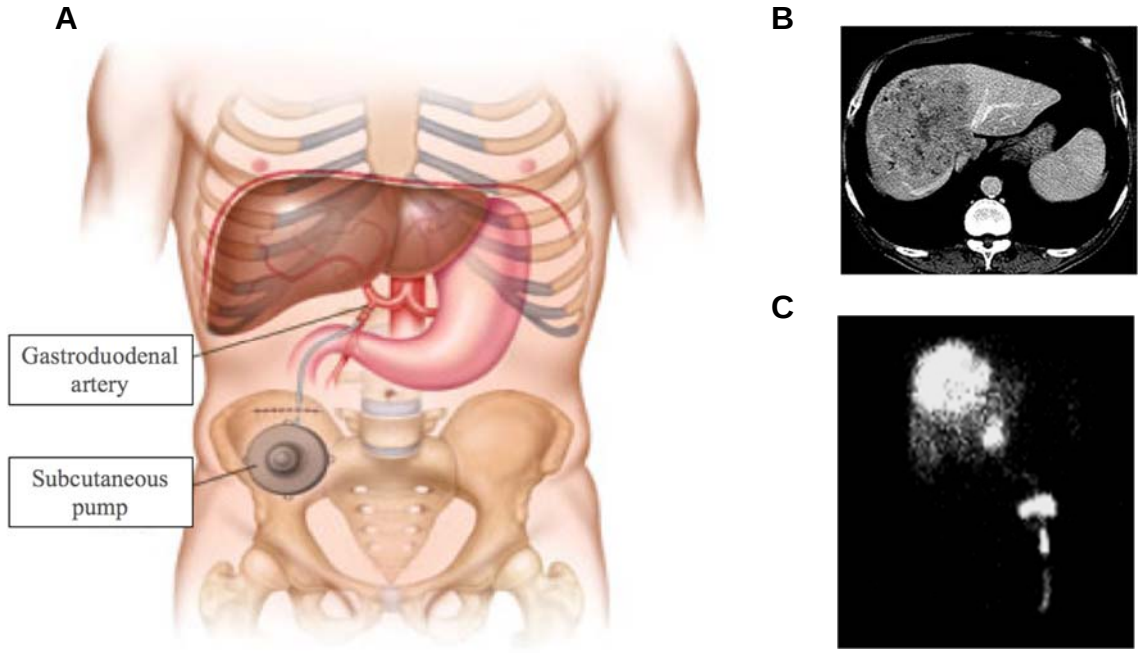
| | **n=38** |
|---|---|
| **Floxuridine** | |
| Number of doses | Average % of total floxuridine dose received |
| First 3 doses | 70 |
| First 6 doses | 47 |
| **Gemcitabine** | |
| **Duration** | % of pts who received gemcitabine |
| < 2 months | 0 |
| 2-4 months | 16 |
| 4-6 months | 10 |
| > 6 months | 74 |
| **Oxaliplatin** | |
| **Duration** | % of pts who received oxaliplatin |
| < 2 months | 8 |
| 2-4 months | 42 |
| 4-6 months | 29 |
| > 6 months | 21 |

**eTable 3: Clinical response Memorial Sloan Kettering Cancer Center cohort (n=38).**

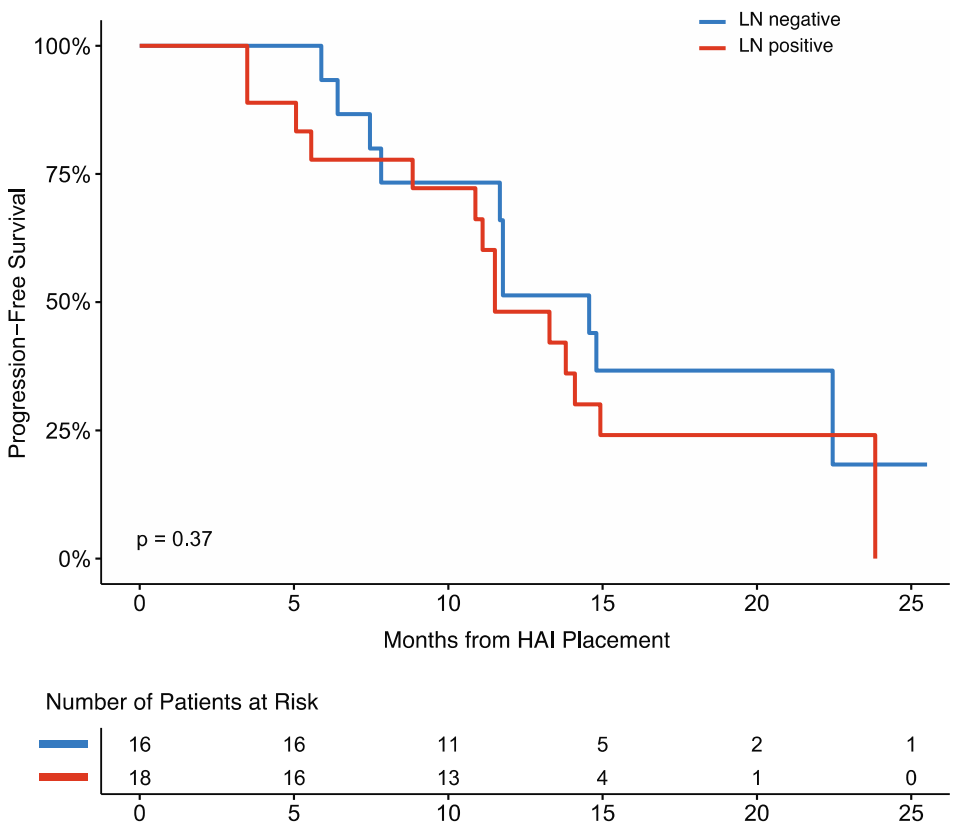
| **Response** | |
|---|---|
| Partial radiographic response | 57.8% |
| Stable disease | 42.1% |
| Progressive disease | 0% |
| Six-month progression-free survival | 84.1% |
| One-year overall survival | 89.5% |
| Median progression-free survival | 11.8 months |
| Median overall survival | 25.0 months |



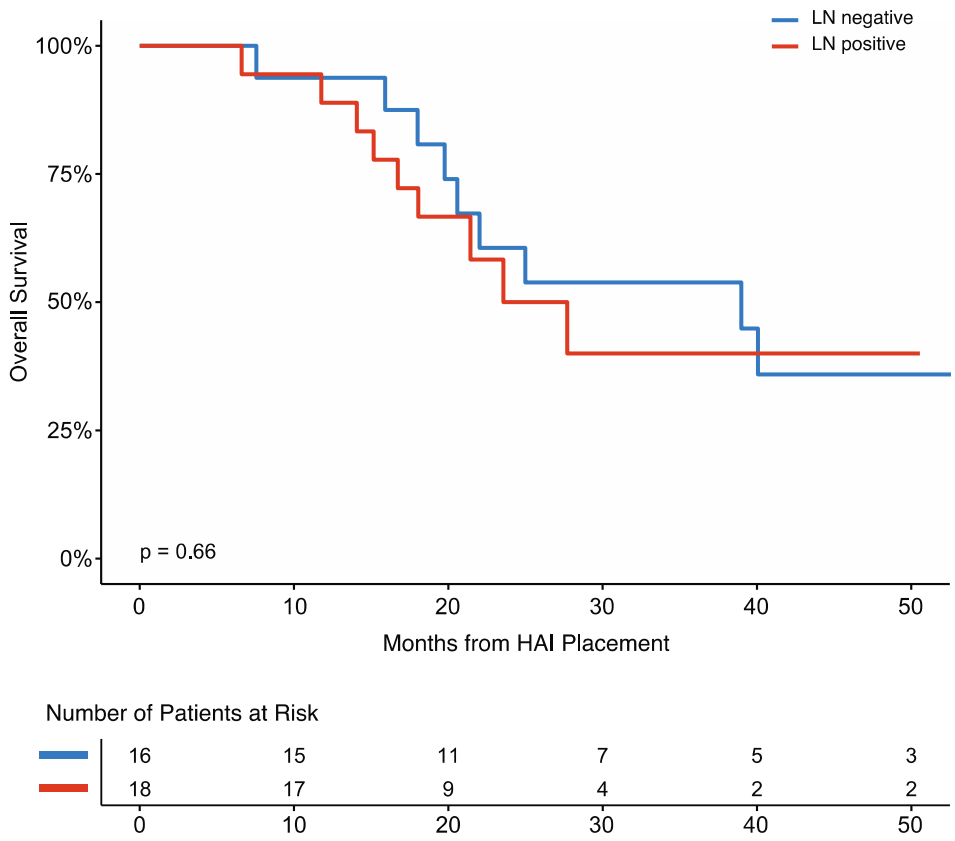

**eFigure 1. Hepatic arterial infusion pump chemotherapy for intrahepatic cholangiocarcinoma.** (A) Diagram depicting hepatic arterial infusion pump placement. (B) CT scan of an intrahepatic cholangiocarcinoma prior to pump placement. (C) Nuclear medicine scan from perfusion study after pump placement depicting that the perfusion of radiolabeled albumin is confined to the liver.



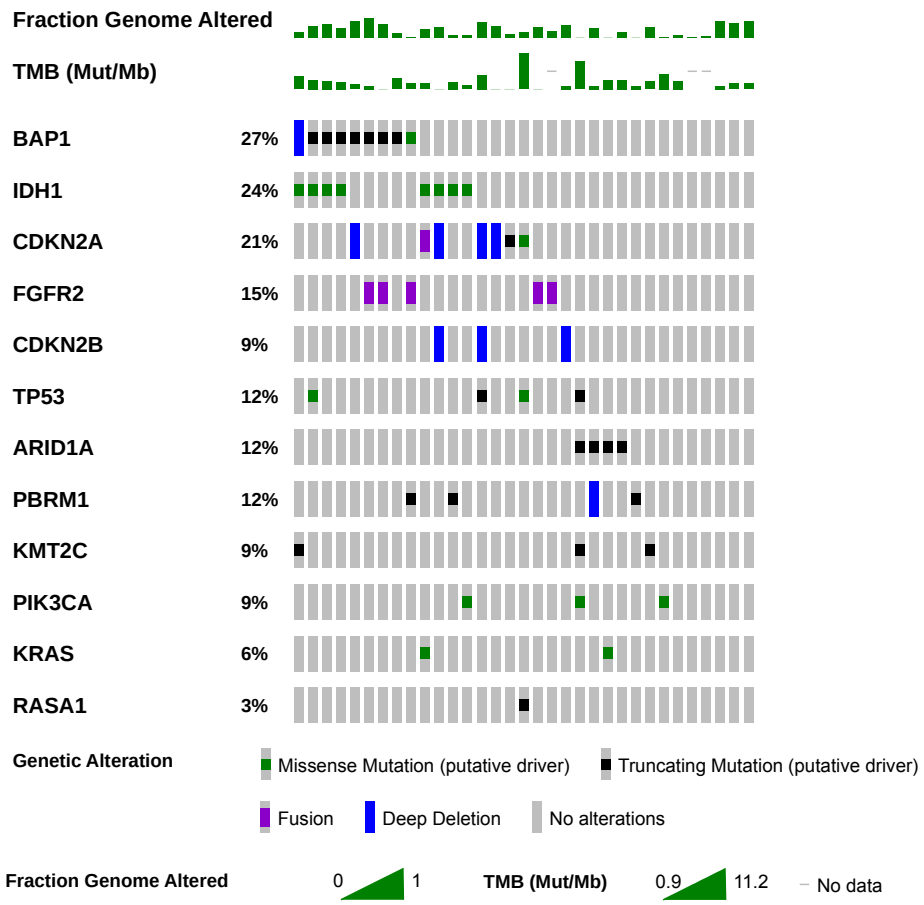

**eFigure 2: Progression-free survival in the Memorial Sloan Kettering Cancer Center cohort by lymph node status.** Patients were grouped as lymph node (LN) negative or LN positive. LN status was unknown for four patients, and they were excluded from the analysis.




**eFigure 3: Overall survival in the Memorial Sloan Kettering Cancer Center cohort by lymph node status.** Patients were grouped as lymph node (LN) negative or LN positive. LN status was unknown for four patients, and they were excluded from the analysis.

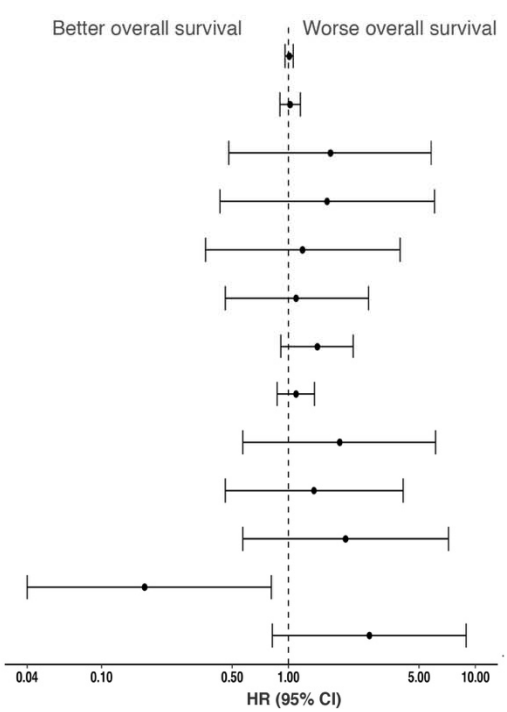
 




**eFigure 4: The oncoprint of mutations found in 33 patients enrolled in the study.**
Only the most common mutations, as defined as those found in ≥4 patients, are shown.

| Characteristic | n | HR (95% CI) | p-value |
|---|---|---|---|
| **Age at diagnosis** (per 1-year increase) | 33 | 1.00 (0.95-1.04) | 0.831 |
| **Tumor size** (per unit increase) | 27 | 1.02 (0.9-1.16) | 0.758 |
| **Baseline AST** (per unit increase) | 27 | 1.78 (0.59-5.42) | 0.305 |
| **Baseline ALT** (per unit increase) | 27 | 1.46 (0.43-4.96) | 0.544 |
| **Baseline bilirubin** (per unit increase) | 27 | 1.51 (0.49-4.69) | 0.473 |
| **Baseline ALK** (per unit increase) | 27 | 1.01 (0.45-2.28) | 0.973 |
| **Baseline CEA** (per unit increase) | 26 | 1.41 (0.9-2.2) | 0.123 |
| **Baseline CA19–9** (per unit increase) | 33 | 1.14 (0.92-1.4) | 0.23 |
| **Tumor Grade** (well/moderate vs. poor) | 14/11 | 1.62 (0.54-4.83) | 0.386 |
| **LN status** (positive vs. negative) | 16/14 | 1.02 (0.39-2.66) | 0.976 |
| **Multifocal disease** (yes vs. no) | 19/8 | 2.17 (0.61-7.64) | 0.217 |
| **IDH1/2** (MUT vs. WT) | 10/23 | 0.21 (0.06-0.75) | 0.01 |
| **TP53** (MUT vs. WT) | 4/29 | 2.12 (0.68-6.56) | 0.183 |




**eFigure 5: Forest plot showing the relative hazard ratio (HR) for overall survival according to patient characteristics at baseline.** Univariable analysis of prognostic features in unresectable intrahepatic cholangiocarcinoma was used to assess correlation with overall survival. No clinical or tumor genomic features were associated with survival except for sequence alterations in *IDH1* or *IDH2* that were significantly associated with a survival benefit (p=0.01).