heterogeneity. Moreover, HER2 status is dynamic during the clinical course due to genetic differentiation accompanied by neoplastic progression and clonal selection via various factors including chemo- and radiotherapies. Thus, the assessment of HER2 gene copy number in liquid biopsy recently gained a lot of interest for its non-invasiveness, suitability for repeat testing and homogeneity compared to tissue biopsy; however, the limited signal-to-noise ratio (cirdulating tumor DNA (ctDNA) represents a very small fraction in cell-free DNA, which may be less than 0.1%) poses a great challenge for the accuracy and robustness of the tests (either targeted sequencing or droplet digital PCR).

Methods: Targeted bisulfite sequencing using an enriched panel with pre-selected GC-associated CpG sites was performed on 74 FFPE tissue samples (44 IHC0/1+ and 30 IHC3+) to identify HER2-overexpression-specific methylation markers. Then we verified the performance of these markers for HER2 status determination using methylation-specific quantitative PCR (qMSP) in 71 independent tissue samples, as well as three GC cell lines (N-87 and MKN-7 (Her2+), and MKN-28 (Her2-)). We further validated the performance of the markers on 110 GC plasma samples collected before surgery. A HER2-status diagnostic model was built and the performance was evaluated.

Results: We first discovered 105 statistically significant methylation markers for inferring HER2 status in tissue based on the results from targeted sequencing. 69 out of the 105 markers (66%) are located in chromosome 17. qMSP assays were designed for these candidate markers and validated on 110 GC plasma samples. A 3-marker diagnostic model was built and demonstrated sensitivity of 86.7% and specificity of 96.9%, which discriminates HER2-positive from HER2-negative GC patients. The overall plasma-tissue concordance of this liquid biopsy test was 95.5%. Furthermore, the HER2-status test can stratify HER2 IHC2+ patients into either HER2-negative or HER2-positive status, which was confirmed by conventional FISH test.

Conclusions: We have developed a novel, accurate and noninvasive qMSP test for determining HER2 status in GC patients. The high concordance with IHC/FISH results of this blood test holds great promise as an auxiliary method to guide HER2-targeted therapy in GC patients.

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P-52 FOLFIRI or FOLFOX in second line of advanced biliary tract cancer: A retrospective analysis

M. Balarine, T. Felismino, M. Camandaroba

A.C.Camargo Cancer Center, São Paulo, Brazil

Background: Cholangiocarcinoma (CCA) are rare malignancies, globally accounting for 3% of upper gastrointestinal cancer. Despite recent advances in first-line treatment leading to gains in progression-free survival (PFS) and overall survival (OS) with the association of immunotherapy, further lines of treatment are yet underrepresented in large randomized clinical trials. Most robust data in second line treatment is a phase 3 randomized trial ABC06, which has established 5-Fluorouracil and Oxaliplatin (FOLFOX) versus active symptom control, as the best treatment option.

Methods: Single center retrospective study of metastatic or irresectable CCA treated with Cisplatin and Gemcitabine in first-line setting, further exposed to second line treatment with 5-Fluorouracil and Irinotecan (FOLFIRI) or FOLFOX. Primary endpoint was OS and secondary endpoints included PFS and toxicity analysis. OS time was analyzed using the Kaplan-Meier method and differences in survival outcomes were assessed using the log-rank test. Prognostic factors were assessed using univariate and multivariate Cox analysis. A p value < 0.05 was considered significant.

Results: From November 2020 to December 2021 103 patients in first-line setting with Gemcitabine and Cisplatin were included at the study database. Among these, 67 (65%) patients received a second line treatment after disease progression, of which 25 (29.9%) received FOLFIRI and 26 (38.8%) received FOLFOX. Median of treatment cycles was 5 (Interquartile Range [IQR] 2 — 8) in FOLFOX group and 4 (IQR 2 — 9) in FOLFIRI group. Grades 3 and 4 adverse events were no difference between the group FOLFOX (n= 16; 61.5%) vs FOLFIRI (n=14; 56%, p= 0.688). In a median follow up time of 45.5 months, the unadjusted median OS was 8 months (95% confidence interval [CI] 3.31 — 12.68) in FOLFIRI group versus (vs) 5 months (95% CI 0.68 — 9.32; p= 0.259) in FOLFOX group. In Cox's analysis for OS, platinum resistant/refractory chemotherapy had a worse outcome with Hazard Ratio 2.58 (IC 95% 1.35 — 4.92) p= 0.004.

Conclusions: Despite the limitations of retrospective single center study, analysis shows that FOLFIRI may be a safe second-line treatment for metastatic cholangiocarcinoma.

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P-53 A phase 2, multi-center, open-label study of cinrebafusp alfa (PRS-343) in patients with HER2-high and HER2-low gastric or gastroesophageal junction (GEJ) adenocarcinoma

G. Ku[1], S. Piha-Paul[2], M. Gupta[3], D. Oh[4], Y. Kim[5], J. Lee[6], S. Rha[7], Y. Kang[8], M. Díez García[9], T. Fleitas Kanonnikoff[10], V. Arrazubi[11], K. Aviano[12], T. Demuth[12]

[1]Memorial Sloan Kettering Cancer Center, New York, United States; [2]The University of Texas MD Anderson Cancer Center, Houston, United States; [3]Sansum Clinic, Santa Barbara, United States; [4]Seoul National University College of Medicine, Seoul, South Korea; [5]Korea University Anam Hospital, Seoul, South Korea; [6]Samsung Medical Center, Sungkyunkwan University School of Medicine, Gangnam-gu, Souel, South Korea; [7]Yonsei Cancer Center, Yonsei University College of Medicine, Seodaemun-gu, Seoul, South Korea; [8]University of Ulsan, Seoul, South Korea; [9]Vall d'Hebrón University Hospital, Vall d'Hebrón Institute of Oncology, Barcelona, Spain; [10]Valencia University Clinic Hospital, Valencia, Spain; [11]Complejo Hospitalario de Navarra, Pamplona, Spain; [12]Pieris Pharmaceuticals, Boston, United States

Background: For patients (pts) with HER2-overexpressing metastatic gastric cancer, trastuzumab + chemotherapy +/- pembrolizumab is a standard first-line option but only provides an incremental overall survival (OS) benefit vs chemotherapy. Anticalin® proteins are recombinant human proteins based on lipocalins. Cinrebafusp alfa, a first-in-class bispecific antibody-Anticalin fusion protein, targets HER2 and the co-stimulatory immune receptor 4-1BB on T cells. In a previous phase 1 study cinrebafusp alfa monotherapy was generally well tolerated and showed deep and durable responses in patients with HER2-positive gastrointestinal malignancies at doses of 8mg/kg Q2W and 18mg/kg Q2W. Significant induction of plasma 4-1BB as well as increase of CD8+ cells was observed in on-treatment tumor biopsies at active dose levels (Piha-Paul, SITC 2020). Based on pharmacokinetics (PK), pharmacodynamics (PD) and clinical efficacy data, a phase 2 dose of 18mg/kg Q2W in C1 followed by 8mg/kg Q2W maintenance was chosen.

Trial design: This is a global, open-label, multicenter, two-arm phase 2 trial of cinrebafusp alfa in patients with metastatic gastric or gastroesophageal junction cancer. Arm 1 is enrolling patients with HER2 high (Immunohistochemistry (ICH) 3+ or IHC 2+ with HER2/neu gene amplification) disease. Pts who have received one prior treatment regimen for metastatic disease, including HER2-directed therapy such as trastuzumab are eligible. Pts will receive cinrebafusp alfa in combination with ramucirumab and paclitaxel. Arm2 is enrolling patients with HER2 low (IHC 1+ or 2+ without HER2/neu gene amplification) disease. Pts who have received at least one prior treatment regimen for metastatic disease are eligible. Pts will receive cinrebafusp alfa in combination with tucatinib. After a run-in consisting of 3 pts in each arm, an additional 17 patients will be enrolled in each arm. For Arm 1, an additional 40 patients may be enrolled after a futility analysis has been conducted. Treatment will continue until disease progression, unacceptable toxicity, or consent withdrawal. Primary endpoint is confirmed overall response rate per RECIST 1.1 and key secondary endpoints are duration of response, progression free survival, overall survival, safety, PK, and immunogenicity. Recruitment is ongoing. Approximately 10 sites in 3 countries in US, Asia and Europe are expected to participate.

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