EARLY VARIATIONS OF KRAS MUTATIONS IN CIRCULATING CELL-FREE TUMOR DNA: A PROMISING BIOMARKER TO MONITOR CHEMOTHERAPY RESISTANCE IN PANCREATIC CANCER

1)Rofi E. 2)Vivaldi C. 3)Del Re M. 4)Fornaro L. 5)Vasile E. 6)Falcone A. 7)Danesi R.

Dept. of Clinical and Experimental Medicine

Background. Despite recent improvements with chemotherapy treatments (FOLFIRINOX or gemcitabine + nab-paclitaxel), a significant number of patients with pancreatic ductal adenocarcinoma (PDAC) do not have benefit from these regimes, developing early resistance to treatment (Chand et al. 2016). In support to imaging approaches, carbohydrate antigen 19.9 (CA 19-9) is the only approved biomarker to monitor tumor response, but it has several limitations (Ballehaninna et al., 2012). Consequently, increasing efforts are focused to identify both molecular and clinical predictors of benefit. Mutant KRAS is a driving oncogene occurring in 75-95% of PDAC (Bailey et al. 2016). Therefore, its detection in circulating cell-free tumor DNA (cftDNA) released in plasma could represent a valid non-invasive method to monitor treatment response in PDAC patients undergoing chemotherapy. **Aim.** To prospectively evaluate the changes in plasma levels of KRAS mutations (MUTKRAS) in PDAC patients undergoing first-line chemotherapy and correlate the early variations of cftDNA MUTKRAS after 15 days of treatment with therapeutic outcome. **Methods.** Twenty-seven patients affected by advanced PDAC were enrolled in this study. Six ml of plasma were prospectively collected at baseline, after 15 days of chemotherapy and at each clinical follow-up. CftDNA was extracted from plasma with the QIAmp Circulating nucleic acid Kit (Qiagen®, Valencia, CA, USA) and analyzed by the Droplet Digital™ PCR (ddPCR, BioRad®, Hercules, CA, USA) for KRAS mutations in codon 12 (G12X) and G13D. **Results.** Preliminary data obtained from ddPCR analysis of cftDNA revealed that at baseline 8 out of 27 patients were KRAS wild type, while 19 were carriers of one of the KRAS mutations. Of note, 14 patients harbored the KRAS p. G12D mutation, 2 patients had the p. G12V, 2 the p. G12R and another one the p. G13D. One out of 8 KRAS wild type patients became positive at day 15 and another one at the first radiological evaluation (2 months after treatment start). Patients with baseline positive cftDNA MUTKRAS or wild type had no significant statistically differences in terms of median PFS (7.4 vs not reached months; p=0.24, respectively) and OS (11.5 vs not reached months; p=0.16, respectively). During treatment, MUTKRAS monitoring was able for 25 out of 27 patients, of which 17 were KRAS positive and 8 were KRAS wild type. There was a statistically significant difference in PFS between patients characterized by an increasing in cftDNA MUTKRAS and those with a stability or a reduction in cftDNA MUTKRAS at the 15th day of treatment (median PFS 2.5 vs 7.5 months, p=0.03, figure 1). Interestingly, all patients with an increase in cftDNA MUTKRAS at the 15th day displayed a progression of disease, that was confirmed by the radiological evaluation 2 months after the beginning of treatment. Moreover, patients with an increase in cftDNA MUTKRAS appeared to have a shorter median OS than patients with a cftDNA MUTKRAS reduction (6.5 vs. 11.5 months, respectively; p=0.009). Even if the early cftDNA MUTKRAS variations did not correlate with tumor response (Fisher's exact test p=0.09; Mann-Whitney test p=0.156), a trend toward better disease control in patients with early cftDNA MUTKRAS decrease was found (Fisher's exact test p=0.08; Mann-Whitney test p=0.059). Furthermore, as reported in figure 2 in a

case of partial response, the variations in cftDNA MUTKRAS levels were found to be better correlated to tumor dynamics than changes in CA 19-9 levels. Finally, our preliminary results showed that: 1) cftDNA analysis of MUTKRAS could be a reliable non-invasive approach to early predict the tumor progression and responsiveness to chemotherapy and 2) cftDNA measurements seem to have a higher potential than conventional monitoring approaches. However, in order to assess these preliminary data, further analyzes are needed, while also increasing the cohort of KRAS mutant PDAC patients.

Chand et al. (2016). Int J Biol Sci. 12(3):273-82.

Ballehaninna et al. (2012). J Gastrointest Oncol. 3(2):105-19.

Bailey et al. (2016). Nature. 531(7592):47-52.

Figure 1. PFS according to cftDNA MUTKRAS variation at day 15.

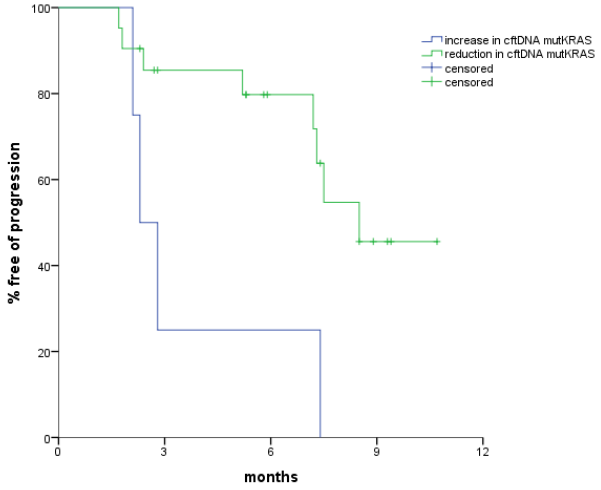

Figure 2. cftDNA MUTKRAS compared to CA19-9 monitoring in a case of partial response.