Several previous reports indicated that cetuximab (Cmab) rechallenge may be efficacious in patients for whom Cmab was previously effective. On the other hand, some reports did not support this. Considering the plasticity of a single arm, we believe that the results presented here are valuable with benefit from the field of liquid biopsy.

**Study Design**

multicenter phase II study
main eligibility criteria:
- mCRC patients who have become refractory to fluoropyrimidines, L-OHP, CPT-11, Cmab and bevacizumab, and in whom previous treatment with Cmab was effective
- in any earlier line (achieving CR, PR, or SD that persisted for ≥6 months)
- RAS wild-type
- measurable disease
- aEFI ≥ 2:6 weeks between the last dose of Cmab during previous treatment and the start of Cmab rechallenge


Primary endpoint: response rate (RR)

Secondary endpoints: progression-free survival (PFS), overall survival (OS), association between the aEFI and efficacy, and safety

**Statistical considerations:** Using a single-stage binomial design, 45 patients were required; a RR of 20% was considered promising, and a RR of ≤5% unacceptable (one-sided α = 2.5%, β = 10%).

**Results**

- **Between Dec 2014 and Oct 2015, 33 patients** enrolled. The registration of this trial was closed in Oct 2017 due to insufficient accrual.
- **The primary endpoint:** the rates of PR/SD/PD (95%CI) were PR 15.2% (5.3-31.9%)/30.9% (22.9-47.9%)/PD 42.4% (25.5-59.8%).
- **Secondary endpoints:** median PFS and OS (95%CI) were 88 days (62-113days) and 252 days (195-307days).
- **There were no statistical significant difference of RR, PFS and OS stratified by median aEFI (≥12days).**
- **New safety information were not identified.**
- **Twenty four patients were enrolled** the additional liquid biopsy research which was conducted optionally.

**Methods**

**Liquid biopsy research**

Additional research of ctDNA was conducted optionally. Blood samples at baseline collected in STRECK BCT® tubes. DNA was extracted from plasma using the QIAamp Circulating Nucleic Acid Kit (Qagen).

We performed ddPCR assays on a QX200 digital PCR system (BioRad laboratories). The PCR data were quantified as copies/ml using QuantSnip™ software (BioRad laboratories).

A mutation was considered positive with more than 0.1% fractional abundance of KRAS 12/13/61G52/526Q, BRAF (V600E) and EGFR 542R mutant droplets. The uniplex ddPCR method had been used for the present study, because of the computational analysis of a dilution series of synthetic copies of each indicated mutation allele.

Lib® Probe of KRAS G12/G13 Screen (Riken Genes)
Lib® Probe of KRAS 52/526 Screen (Riken Genes)
Lib® Probe BRAF V600 Screen (Riken Genes)

Additionally, we used ddPCR® probe EGFR 542R as the detection probe for EGFR 542R (c.1647A>C) and 542Q (c.1647C>C) (BioRad laboratories).

**Results of liquid biopsy research** (Table 4 and 5, Figure 4)

- 8 samples were positive
- 5 samples were negative
- 9 samples were negative

**Conclusion**

Cmab rechallenge showed some activity in the salvage setting, in patients for whom Cmab was previously effective. KRAS and BRAF screening by liquid biopsy may contribute to identify the patients with benefit from Cmab rechallenge.