Experimental Hematology / Oncology

Primary and Acquired Resistance to Targeted Treatment in BRAF V600E-mutated Metastatic Colorectal Cancer - PARTACER-Suisse (SAKK 41-23, Trial in Progress)

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Summary

BRAF V600E–mutated, microsatellite-stable (MSS) metastatic colorectal cancer (mCRC) represents the most aggressive molecular subtype of mCRC, characterized by a poor prognosis. Targeted therapy with cetuximab (anti-EGFR) plus encorafenib (a class I BRAF inhibitor), alone or in combination with chemotherapy, is the current standard of care for patients with BRAF V600E-mutated mCRC. However, **the development of secondary resistance to targeted therapy in BRAF V600E mCRC remains a major clinical challenge, and effective strategies to prevent, delay, or overcome acquired resistance are still lacking.** We hypothesize that a more comprehensive understanding of the genomic and non-genomic landscape of acquired resistance to cetuximab and encorafenib (CE) will enable the identification of novel therapeutic targets. To address this, **PARTACER-Suisse (SAKK/SCI 41-23), a prospective, multicenter, non-interventional study (HRO) with an extensive translational research program**, is currently enrolling patients across 12 Swiss study sites. Here, we present preliminary *in vitro* data establishing 2D and organoid models of BRAF V600E mCRC derived from patient samples.

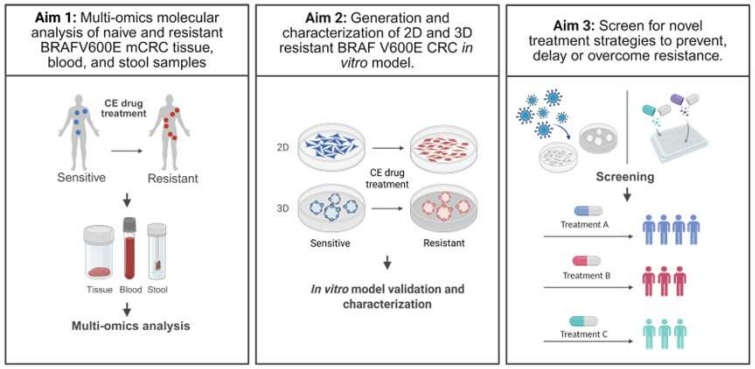

Figure 1. Aims and methods. Paired tissue, blood, and stool samples, will be prospectively collected from patients with BRAF V600E-mutated mCRC undergoing molecularly targeted treatment with encorafenib/cetuximab, and will undergo comprehensive molecular analyses to uncover the full molecular landscape of acquired resistance to targeted treatment in this setting **(Aim 1)**. BRAFV600E colorectal cancer cell lines together with patient-derived organoid cultures (PDO) will be employed for modeling acquired resistance *in vitro*. PDO will be established from tissue biopsies prior to and after treatment with CE **(Aim 2)**. PDO and cell lines will be employed to establish novel strategies to prevent, delay, modify, or overcome acquired resistance. **(Aim 3)**. Figure generated with BioRender.

In vitro modelling of acquired resistance in MSS vs MSI-H BRAF V600E mCRC

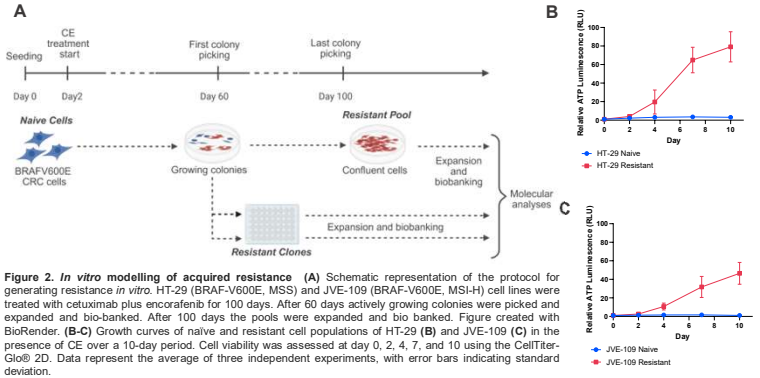

Figure 2. *In vitro* modelling of acquired resistance **(A)** Schematic representation of the protocol for generating resistance *in vitro*. HT-29 (BRAF-V600E, MSS) and JVE-109 (BRAF-V600E, MSI-H) cell lines were treated with cetuximab plus encorafenib for 100 days. After 60 days actively growing colonies were picked and expanded and bio-banked. After 100 days the pools were expanded and bio banked. Figure created with BioRender. **(B-C)** Growth curves of naïve and resistant cell populations of HT-29 **(B)** and JVE-109 **(C)** in the presence of CE over a 10-day period. Cell viability was assessed at day 0, 2, 4, 7, and 10 using the CellTiter-Glo® 2D. Data represent the average of three independent experiments, with error bars indicating standard deviation.

Genomic and non-genomic resistance alterations

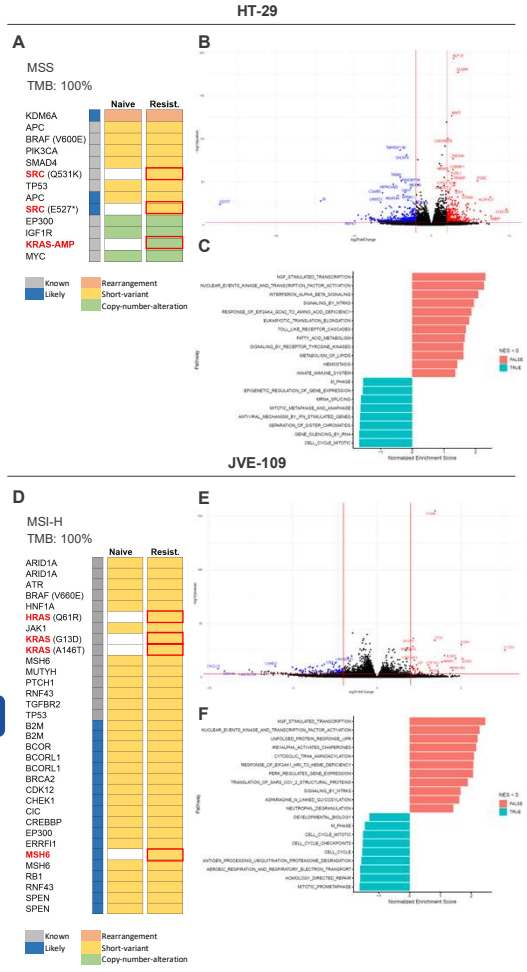

Figure 3. BRAF V600E CRC cells acquire genomic and non-genomic resistance alterations in vitro. (A, C) *FoundationOne*® comprehensive genomic profiling of naïve and resistant **(A)** HT-29 and **(C)** JVE-109 colorectal cancer cell lines. **(B, E)** Volcano plots representing differential gene expression analysis between naïve and resistant **(B)** HT-29 and **(E)** JVE-109 cell lines, performed using DESeq2. Horizontal and vertical dashed lines denote *padj* < 0.05 and the fold change cutoff, respectively. **(D, F)** Gene Set Enrichment Analysis (GSEA) comparing naïve and resistant **(D)** HT-29 and **(F)** JVE-109 cell lines, highlighting the top significantly enriched Hallmark pathways (*FDR* < 0.25). Normalized enrichment scores (NES) indicate activation or repression of biological programs associated with acquired resistance.

Patient-derived BRAFV600E CRC organoid models

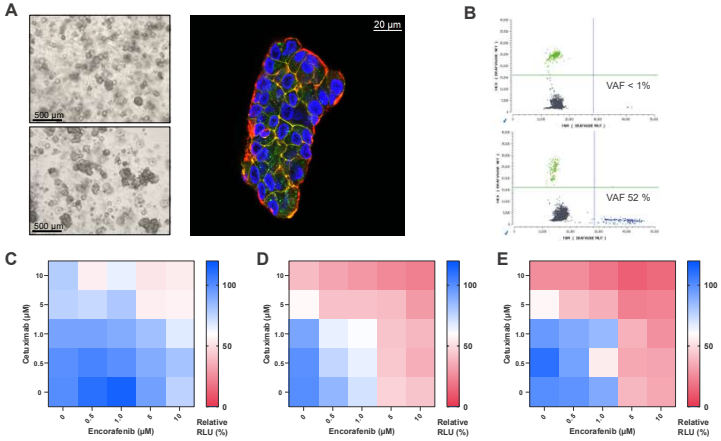

Figure 4. Patient-derived colorectal cancer organoids enable molecular validation and functional drug testing.(A) Representative patient-derived colon cancer organoids generated in our laboratory, shown under phase contrast (left) and stained for DAPI (blue), actin (red), and E-cadherin (green). **(B)** Digital droplet PCR (ddPCR) analysis of tumor tissue (top) and corresponding organoid DNA (bottom) used to estimate tumor cell content based on variant allele frequency (VAF) and to monitor potential culture drift. **(C–E)** Combination drug testing to evaluate therapeutic synergy between cetuximab and encorafenib in 3D organoid models exhibiting differential treatment sensitivity: **(C)** low, **(D)** intermediate, and **(E)** high sensitivity. Organoids were treated with increasing concentrations of the cetuximab/encorafenib combination, and cell viability was measured using CellTiter-Glo® 3D after 5 days.

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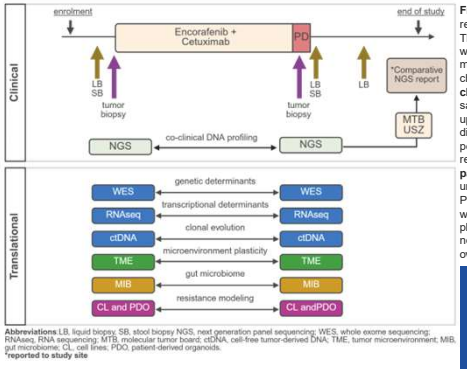

Abbreviations LB, liquid biopsy; SB, stool biopsy NGS, next generation panel sequencing; WES, whole exome sequencing; RNAseq, RNA sequencing; MTB, molecular tumor board; ctDNA, cell-free tumor-derived DNA; TME, tumor microenvironment; MIB, gut microbiome; CL, cell lines; PDO, patient-derived organoids.
*reported to study site

Figure 5. The PARTACER-Suisse study. Schematic representation of the PARTACER-Suisse study workflow. The study will prospectively enroll 30 evaluable patients with confirmed BRAF V600E mutated mCRC prior to molecularly target treatment with CE, with or without chemotherapy, independent of treatment line. During the **clinical part of the study**, paired tissue and liquid samples will be collected prior to treatment initiation and upon disease progression. Comparative NGS panel diagnostics from tissue and liquid biopsies will be performed, and a comparative research report will be reported back to the recruiting site. For the **translational part of the project**, tissues, liquid and stool samples will undergo comprehensive comparative molecular analyses. PDOs established from pre- and post-treatment biopsies will be exploited for functional analyses *in vitro* including pharmacological and genetic screens, aiming to identify novel targets and treatment strategies to prevent, delay or overcome acquired resistance in BRAF V600E mCRC.

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