| RA-200 Research Activity Plan | |
|---|---|
| Sidra Research Activity ID (SDR) Title: | **In Vitro Characterization of *R. bromii*–Tumor–Immune Interactions in Colorectal Cancer** |
| SDR Lead Scientist: | **Christophe Raynaud** |
| Project ID (PRJ): | PRJ12002 |
| PRJ Budget Holder/Line Manager: | **Wouter Hendrickx** |
| Budget Source: | **13 000 qar** |
| Date: | 17-Jul-25 |

## Instructions

This form - **RA-200 Research Activity Plan** – is a planning guide before a research study begins. If you have questions, please contact researchpmo@sidra.org:

- *Research Activity (SDR): Research activity to be performed.*
- *Research Project (PRJ): Research topic / research question to investigate.*
  *Research Program: Disease-oriented program under which this research falls.*

## Research Activity Details

An SDR is a research activity a scientific team is working on as part of a project. Research activities are focused on operational aspects of the components that make up a Project. *Each SDR needs a separate **abstract** of the planned research activities (5000 characters including space)*


**ABSTRACT**

*Ruminococcus bromii* is a gut commensal bacterium known for its ability to degrade resistant starch and produce short-chain fatty acids such as acetate. Recent studies have highlighted its emerging role in modulating immune responses, attenuating fibrosis, and influencing the tumor microenvironment. This project explores the in vitro effects of *R. bromii*-derived metabolites on colorectal cancer cells, fibroblasts, and T cells using advanced 3D tumor spheroid models, transcriptomic analyses, and ECM-based assays. By integrating cancer biology and microbiome science, we aim to characterize the immunomodulatory and anti-tumorigenic potential of *R. bromii* and identify the metabolites responsible for these effects.

**Background & Rationale**

*Ruminococcus bromii* is an anaerobic, Gram-positive bacterium, and a prominent member of the human gut microbiota. It is especially known for its efficient degradation of resistant starch (RS), enabling the production of beneficial short-chain fatty acids (SCFAs), primarily acetate. Its keystone function facilitates the activity of other microbial species in the colon, contributing to colonic health and metabolic balance.

Recent studies have highlighted the multifaceted role of *Ruminococcus bromii* in cancer and tissue homeostasis. Depending on the tumor context and host factors, *R. bromii* has been associated with both protective and potentially detrimental effects. While some studies suggest its involvement in dysbiosis under specific conditions, emerging data increasingly point toward its beneficial roles in modulating immune responses, shaping the tumor microenvironment, and enhancing treatment efficacy. In 2022, Messaoudene et al. (Cell Reports Medicine) found *R. bromii* enriched following treatment with castalagin, correlating with improved immune infiltration and tumor control. Our own findings in the AC-ICAM study (Roelands et al., *Nature Medicine*, 2023) identified *R. bromii* as a strong predictor of survival in cancer patients, linked to increased $CD103^{+}$ dendritic cell infiltration. Most recently, Liu et al. (2025, *Frontiers in Immunology*) showed acetate from *R. bromii* reduced liver fibrosis by dampening fibroblast activation and collagen deposition. These findings suggest *R. bromii*

plays a broader role in shaping the tumor stroma and immune landscape, warranting mechanistic dissection.

**Objectives and Preliminary Work**

We aim to:

- Assess the impact of *R. bromii* conditioned media (CM) on colorectal cancer cell proliferation, migration, gene expression, and cytokine production.
- Investigate how CM influences T cell cytotoxicity, activation, and infiltration within 3D spheroid models.
- Evaluate ECM deposition and decellularization outcomes from tumor cells and fibroblasts ± CM, and assess subsequent T cell behavior on these matrices.
- Perform metabolomic profiling to identify active bacterial metabolites and validate their effects in vitro.

Preliminary achievements include the generation of GFP-labeled DLD-1 colon cancer cells and establishment of stable 3D tumor spheroids. Optimization of *R. bromii* anaerobic culture and CM harvesting is underway.

**Approach (Brief Summary of Methods)**

Human colorectal cancer cell lines will be transduced with GFP-expressing lentivirus and used to generate 3D tumor spheroids. Conditioned media from *R. bromii* cultures will be collected and applied to spheroids. Tumor proliferation and migration will be assessed, and RNAseq will be performed to identify transcriptomic changes. T cells labeled with RFP will be co-cultured with tumor spheroids ± CM to assess cytotoxicity and infiltration using confocal microscopy and flow cytometry.

Fibroblasts will be incorporated into spheroid models to evaluate stromal interactions, fibroblast activation, and ECM modulation. Decellularized ECM matrices will be used in functional assays to test T cell activation, migration, and proliferation. Finally, metabolomic analysis of *R. bromii* CM will guide functional validation of candidate bacterial metabolites.

**Discussion / Conclusion**

This project proposes a novel, integrative approach to investigate how the gut commensal *Ruminococcus bromii* modulates the colorectal tumor microenvironment. By combining microbiology, cancer immunology, and systems biology, we aim to unravel the mechanisms by which microbial-derived metabolites influence tumor and immune cell behavior, including effects on extracellular matrix composition and immune infiltration. Using 3D tumor spheroids, T cell co-cultures, fibroblast-based ECM assays, and multi-omics technologies, this study will identify new pathways linking microbial signals to immune function and stromal remodeling. With strong relevance to precision oncology and microbiome-based therapeutics, the project aligns with institutional and national priorities and holds significant translational potential.

## Research Activity Requirements

| Yes | No | Topic |
|---|---|---|
| **Ethics**[1] | | |
| ☐ | ☒ | **Work with human-subject samples/data** |
| ☐ | ☒ | **IRB needed** |
| ☐ | ☒ | **Work with animal samples** |
| ☐ | ☒ | **IACUC needed** |
| ☐ | ☒ | **This is an interventional clinical trial** |
| **Collaborations** | | |
| ☐ | ☒ | **Work with collaborators outside Sidra** |
| ☐ | ☒ | **Data will be shared outside Sidra** |
| **Budget** | | |
| ☐ | ☒ | **No Cost at all** |
| ☐ | ☒ | **External party outside Sidra is covering costs** |
| ☒ | ☐ | **Have a budget at Sidra for this research activity *and* I confirm that all related costs (testing/screening/etc.) will be covered by this budget** |
| **Sample/Data processing** | | |
| ☐ | ☒ | **Lab work will be done in collaboration with a Sidra PI** |
| ☒ | ☐ | **Lab work will be done by Sidra Cores** |

## Duration of the Research Activity

Duration in Months: 10

## Core Labs/Service Providers

In Table.2 below, please check the areas where you require support:

*Table.2 Core labs/service providers*

| | |
|---|---|
| ☒ Genomics Core | ☐ Pathology |
| ☒ Omics Core | ☐ HR (e.g. temp staffing) |
| ☒ Microscopy Core | ☒ Flow Core |
| ☐ Mass Spec Core | ☐ Research Contracts Office |
| ☐ Zebrafish Facility Core | ☐ Research Scientific Data Management |
| ☐ Advanced Cell Therapy Core | ☐ Grants Office |
| ☐ Applied Bioinformatics Core | ☐ Other *specify* |
| ☐ Computational & Informatics Core | ☐ Not Applicable |

**APPENDIX.A**

[1] N.B.: We ask that your answers accurately reflect your research study's activities.

**Remove all the instructions in red and replace with your content.**

## 1) What are you going to do (Study Design, Materials, Methods)

MATERIAL AND METHODS

*Ruminococcus bromii* will be cultured under strict anaerobic conditions. Once optimal growth is achieved, the cultures will be centrifuged and filtered to obtain cell-free conditioned media (CM).

To model the tumor environment, human colon cancer cell lines (including DLD-1, HT-29, HCT116…) will be stably transduced with GFP-expressing lentiviruses (puromycin-resistant) and used to generate 3D tumor spheroids under low-attachment conditions. Experimental conditions will include four key setups: tumor cells alone, tumor cells with *R. bromii* CM, tumor cells with T cells, and tumor cells with both T cells and *R. bromii* CM.

Tumor cell proliferation will be assessed using cell counting. Migration capacity will be measured using wound healing (scratch) assays. Gene expression changes induced by *R. bromii* CM will be evaluated *via* Lexogen RNA sequencing. The impact on extracellular matrix (ECM) components will be analyzed by western blot and immunofluorescence. Markers of epithelial-mesenchymal transition (EMT), such as E-cadherin, N-cadherin, and vimentin, will also be assessed. Additionally, we will monitor genotoxic stress by analyzing γH2AX and p53 levels, and cytokine production will be evaluated using human cytokine arrays.

For functional T cell assays, primary human T cells will be labeled with RFP and co-cultured with GFP+ tumor spheroids. Tumor cell killing will be assessed through live imaging and quantification of GFP signal loss. T cell infiltration and migration will be examined using confocal microscopy, flow cytometry, and transwell assays. Gene expression analyses of both T cells and tumor cells will be performed by Lexogen RNAseq under each experimental condition.

To explore ECM-mediated effects, tumor cells or fibroblasts will be cultured for seven days in the presence or absence of *R. bromii* CM to allow ECM deposition. After decellularization, T cells will be seeded onto the resulting matrices, and their proliferation (via Ki67 staining), activation (CD69, CD25), and migration (live-cell imaging) will be evaluated.

As a complementary approach, human fibroblasts will be incorporated into the spheroid models. We will assess fibroblast activation using α-SMA and FAP markers, and collagen production will be measured via western blot and immunofluorescence. T cell cytotoxicity in the presence of fibroblasts and *R. bromii* CM will also be examined. ECM produced by fibroblasts under different conditions will undergo decellularization, followed by T cell functional assays as described.

Finally, metabolomic analyses (both untargeted and targeted) will be performed on *R. bromii* CM to identify active microbial metabolites, including short-chain fatty acids, amino acids, and other bioactive compounds. Selected candidate molecules will be used to validate their individual contributions in functional assays.

**PROPOSAL, OBJECTIVES AND PRELIMINARY DATA**

**Achieved Goals:**

- Successfully generated GFP-labeled colon cancer cells using lentiviral transduction.
- Established robust 3D tumor spheroids with colon cancer cells.

**Current and Future Goals:**

- **Bacterial conditioned medium**:

    - Optimize *R. bromii* growth and CM harvesting under anaerobic conditions.
- **Functional assays**:
    - Assess impact of *R. bromii* CM on tumor cell proliferation, migration, and transcriptome.
    - Study CM-mediated enhancement or inhibition of T cell killing of tumor cells.
    - Perform Lexogen RNAseq on tumor cells and T cells.
- **ECM essays**:
    - Induce ECM production by tumor cells/fibroblasts ± CM.
    - Decellularize and test T cell functional responses on matrix.
- **Fibroblast models**:
    - Incorporate fibroblasts into spheroids to study stromal interactions.
    - Analyze fibroblast-mediated ECM changes ± CM and their impact on T cells.
- **Metabolite identification**:
    - Perform metabolomic profiling of *R. bromii* CM.
    - Test synthetic metabolites to identify active compounds.
- **Validate and extend findings across additional human colon cancer cell lines**

**Study Population**

The study will utilize in vitro models simulating the human colorectal tumor microenvironment. These include:

- Human colon cancer cell lines: *DLD-1*, *HT-29*, *HCT116*, Lovo, LS174T, SW620 available from our lab or provided by collaborators within Qatar.
- Primary human T cells, isolated from peripheral blood of healthy donors.
- Primary human fibroblasts, either commercially sourced or isolated from healthy colon tissue.

**Sample Size**

- A minimum of 3 biological replicates (n=3) per experimental condition will be performed, with **3–5 technical replicates** each to ensure robustness and reproducibility.

**Source of Data and Experimental Setting**

- All experiments will be conducted at Sidra Medicine in dedicated cell culture, imaging, and sequencing facilities.

**Statistical Power and Sample Size Calculation**

G*Power:

- For detecting a 25% change, assuming SD = 15%, $\alpha = 0.05$, and power = 0.8, a minimum of 5–6 biological replicates per group is required.

## 2) What are the deliverables:

- By **September 2025**, we aim to complete stable GFP lentiviral transduction of colorectal cancer cell lines, enabling robust visualization of tumor spheroids and real-time monitoring of cell viability in functional assays. In **October 2025**, we will optimize the anaerobic culture of *Ruminococcus bromii* and standardize the production of high-quality, cell-free conditioned media (CM). This will allow consistent experimental conditions for downstream analyses.
- By **December 2025**, we plan to perform a series of functional assays on colorectal cancer cell lines to assess the impact of *R. bromii* CM on tumor cell proliferation, migration, and global transcriptomic changes. Following this, by **January 2026**, we will conduct T cell functional assays in 3D tumor spheroid models, evaluating killing efficiency, infiltration capacity, and activation markers in the presence or absence of *R. bromii* CM.
- In **February 2026**, we expect to have established and validated ECM-deposition and decellularization protocols using both tumor cells and fibroblasts, with and without CM exposure. This will provide insight into how microbial signals influence the biophysical and biochemical landscape of the tumor microenvironment.

- By **April 2026**, we will carry out comprehensive metabolomic profiling (untargeted and targeted) of *R. bromii* CM to identify candidate bioactive metabolites. Selected metabolites will be tested individually in functional assays to validate their effects on tumor and immune cell behavior.
- Finally, by **May 2026**, we will integrate transcriptomic, metabolomic, and functional data to identify key pathways and molecular targets involved in the immunomodulatory and anti-tumor effects of *R. bromii*, providing a foundation for future translational studies or therapeutic exploration.

**7) Budget Projection (max. 300 words):**

This project requires a budget that supports the key experimental components essential for investigating the immunomodulatory and anti-tumor potential of *Ruminococcus bromii* in colorectal cancer. The budget is structured to cover the following areas:

1. Anaerobic Bacterial Culture and Conditioned Media (CM) Preparation
    - Cultivating *R. bromii* under strict anaerobic conditions requires specialized consumables, growth media, and access to anaerobic chambers. Reagents for CM collection, filtration, and quality control are also included.
2. 3D Tumor Spheroid and Co-culture Assays
    - The establishment of 3D models (tumor cells ± fibroblasts ± T cells) requires low-attachment culture plates, cell culture reagents, lentiviral vectors (e.g., for GFP/RFP labeling), and consumables for imaging and flow cytometry. Live-cell imaging reagents and antibodies for phenotypic analysis are included.
3. Transcriptomic and Metabolomic Analyses
    - Budget covers RNA extraction kits, Lexogen RNAseq services, library prep, and computational analysis. Untargeted and targeted metabolomics require sample processing kits and LC-MS service fees. Synthetic versions of candidate metabolites may also be purchased for validation.
4. Extracellular Matrix and Decellularization Assays
    - Includes reagents for matrix deposition, decellularization kits, and staining reagents (e.g., for Ki67, α-SMA, FAP, and EMT markers). Confocal imaging and data analysis resources are also budgeted.
5. Bioinformatics and Data Integration
    - Multi-omics analysis (transcriptomics, metabolomics, functional data) requires computational infrastructure and may involve external support for pathway analysis and data visualization. Licenses for specialized software (e.g., MetaboAnalyst, Ingenuity Pathway Analysis) are included.
6. Contingency
    - A contingency allocation is included to cover unforeseen experimental repetitions (e.g., batch variations in CM), technical troubleshooting, or additional sample processing.

This carefully planned budget ensures that each phase of the study—from bacterial growth to molecular and functional characterization—is well supported. By enabling a comprehensive, mechanistic dissection of *R. bromii*'s role in modulating the tumor-immune microenvironment, this investment aligns with the project's innovative goals.

Christophe Raynaud_____

**Name of SDR - Lead Scientist**

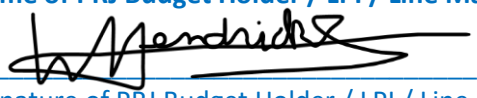
_____

07/09/2025

Signature of SDR - Lead Scientist Date

Wouter Henndrickx_____

**Name of PRJ Budget Holder / LPI / Line Manager Name:**



_____ 07/09/2025

Signature of PRJ Budget Holder / LPI / Line Manager Name: Date