Background: The role of inflammatory immune responses in colorectal cancer (CRC) development and response to therapy is a matter of intense debate. While inflammation is a known driver of CRC, inflammatory immune infiltrates are a positive prognostic factor in CRC and predispose to response to immune checkpoint blockade (ICB) therapy. Unfortunately, over 85% of CRC cases are primarily unresponsive to ICB due to the absence of an immune infiltrate and even the cases that show an initial immune infiltration can become refractory to ICB. The identification of therapy supportive immune responses in the field has been partially hindered by the sparsity of suitable mouse models to recapitulate the human disease. In this study, we aimed to understand how the dysregulation of the complement anaphylatoxin C3aR, observed in subsets of CRC patients, affects the immune responses, the development of CRC and response to ICB therapy.

Methods: We used a comprehensive approach encompassing analysis of publicly available human CRC datasets, inflammation-driven and newly generated spontaneous mouse models of CRC, and multi-platform high dimensional analysis of immune responses using microbiota sequencing, RNASeq, and mass cytometry.

Results: We found that patients’ regulation of the complement C3aR is associated with epigenetic modifications. Specifically, down-regulation of C3ar1 in human CRC promotes a tumor microenvironment characterized by the accumulation of innate and adaptive immune cells that support anti-tumor immunity. Using our novel spontaneous mouse model of CRC (APCMin+/C3aR−/−) we showed that loss of C3aR resulted in enhanced immune infiltration in typically “cold” tumors and modified the intestinal microbiota. We also identified a species of E. faecalis that increased over time in the tumor microenvironment of APCMin+/C3aR−/− mice. Notably, treatment with a-PD1 in APCMin+/C3aR−/− but not APCMin+ mice resulted in significant tumor reduction. Therefore, the lack of C3a in the colon activates a microbiota-mediated pro-inflammatory program, which promotes the development of tumors with an immune signature that renders them responsive to the ICB therapy.

Conclusions: The complement system in the gastrointestinal tract is essential to avoid overt inflammation in health conditions. However, this regulatory mechanism may restrain the activation of immune responses during tumor development. Our findings reveal that C3aR may act as a previously unrecognized checkpoint to enhance anti-tumor immunity in CRC. C3aR can thus be exploited to overcome ICB resistance in a larger group of CRC patients.

Acknowledgements: We would like to acknowledge the Italian Association of Cancer Research, the Umberto Veronesi foundation, the American Cancer Society and NIH P20GM130457 for supporting this study.

Ethics Approval: All samples used in this study were obtained following individual informed consent and ethical approval by the National Research Ethics Service in the United Kingdom (ref 15/EE/0241).