Background Virotherapeutics leverage oncolytic and pro-inflammatory properties to ultimately engage multiple mechanisms of action that lead to holistic modulation of the tumor microenvironment (TME) and induction of anti-tumor immunity. As such, they serve as off-the-shelf in situ cancer vaccines to generate a personalized immune response. We have previously introduced CodaLytic, a codon-modified influenza virus, as an efficient kick-starter of the cancer immunity cycle in several murine models with different baseline immune contexture and in human tumor explant cultures. Here, we are presenting a deeper dive into the immune cell composition infiltrating syngeneic mouse tumors after treatment with CodaLytic.

Methods Effects on the TME were measured after intratumoral administration of 10^8 PFU CodaLytic as monotherapy and in combination with immune checkpoint blockade (ICB) and/or chemotherapy in orthotopic EMT6 and 4T1 triple-negative breast cancer, subcutaneous B16-F10 melanoma and MC38 colon cancer mouse models. Tumors were harvested and dissociated for characterization of immune infiltrate by flow cytometry after ≥2 doses of virus and after onset of efficacy based on tumor volume in at least one experimental group.

Results Across all models, virus treatment increased tumor infiltration with T and antigen-presenting cells, which could be further boosted by addition of ICB. Both total CD3+ T and importantly CD8+ T cells were recruited to tumors in response to treatment and their frequency within the immune infiltrate correlated directly with tumor volumes (CD3+: MC38 R²=0.58, p<0.02; EMT6 R²=0.31, p<0.02; 4T1 R²=0.50, p<0.0001; CD8+: MC38 R²=0.60, p<0.02; EMT6 R²=0.20, p<0.05; 4T1 R²=0.15, p<0.05). Interestingly, addition of ICB further supported the increase of T cell infiltration in alignment with improved efficacy, while Granzyme B expression was primarily driven by CodaLytic, in particular in B16 and MC38 models, independently of combination with PD-1 blockade. Importantly, these increases were not offset by parallel recruitment of regulatory T cells in B16 and EMT6 models. Furthermore, the frequency of the cross-presenting subset of dendritic cells increased after CodaLytic treatment and directly associated with tumor volume (4T1: R²=0.30, p<0.01). A TME conducive to anti-tumor immunity was further supported by recruitment of B, NK and CD4+ T cells.

Conclusions CodaLytic treatment induced changes in the murine tumor immune infiltrate suggesting anti-tumor immune activity independently of the baseline immune contexture associated with the tumor model. This supports the utility of CodaLytic, a codon-modified virus being developed for breast cancer immunovirotherapy, as a valuable component of novel therapeutic regimens.

Ethics Approval The animal work in this study was approved after MisPro Biotech Services IACUC review, protocols 2019–01-17-COD-1, 2022-COD-02 and LC 2022-COD-03. MC38 data was generated according to the IACUC guidelines of Champions Oncology.