Background
Recurrence is the leading cause of increased morbidity and mortality after colorectal cancer surgery, with up to one-third of patients undergoing curatively intended surgery having a recurrence. The degree of tumor-infiltrating immune cells is crucial for the risk of recurrence, which is why interventions targeting the tumor and the local microenvironment are in increased focus. Intratumoral injection of the vaccine has in experimental studies shown to increase the proportion of infiltrating immune cells and lead to tumor shrinkage in both treated and untreated tumors. The purpose of this combined phase 1 and 2 study was to determine the safety of intratumoral influenza vaccine injection and whether it induces local and systemic elicitation of anti-tumor immune response.

Methods
All patients with non-metastatic sigmoid and rectal cancer were eligible for inclusion. The intratumoral influenza vaccine was administered by an additional sigmoidoscopy 7-14 days before the scheduled surgery. The primary outcome was safety. The clinical outcome was evaluated as Mandard tumor regression grade (TRG) assessed by two independent pathologists. Translational outcomes included local and systemic immunological changes, analyzed via immunohistochemistry, and local mRNA gene expression.

Results
Ten patients were included in the study, four with sigmoid cancer and six with rectum cancer. No serious adverse reactions or events occurred. TRG was rated as five in all patients, except one rated as four by a single pathologist. A significant increase of CD8+ but not CD3+ T cell count was noted based upon immunohistochemical staining. mRNA gene expression showed several differentially expressed genes when comparing pre vs. post vaccination specimens. Functional enrichment analysis showed significant suppression of pro-tumor inflammatory related pathways. Spatial analysis of protein expression revealed an increased expression of PD-1 in areas of CD8+ T cell infiltrated regions of the post-vaccination specimens compared with similar regions in pre-vaccination specimens.

Conclusions
Intratumoral influenza vaccine is a safe intervention. The intervention did not lead to tumor regression, while immunohistochemistry and mRNA gene expression analyses revealed a significant increase in CD8+ T cell count and suppression of pro-tumor inflammation, suggesting that intratumoral influenza vaccine induces an anti-tumor response in the tumor microenvironment.

Trial Registration
Trial registration on clinicaltrials.gov: NCT04591379

Ethics Approval
The study was approved by the regional ethics committee: SJ-834