# Virotherapy with CodaLytic™, a codon-modified influenza virus, induces an anti-tumor immune microenvironment across models of different immune contextures

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## INTRODUCTION

Virotherapeutics derived from multiple virus species have been demonstrated to induce beneficial changes in the tumor microenvironment, increasing immune cell infiltration and activating stimulatory immune responses, which ultimately support induction of an anti-tumor immune response. Here, we characterize the immune-stimulatory mechanism of action of a novel virotherapeutic influenza virus developed utilizing Codagenix's SAVE codon pair modification platform[1,2] in models of differing immune contexture.

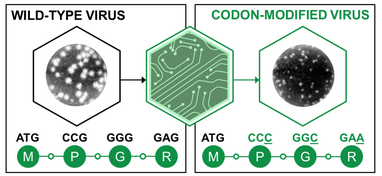



**Fig. 1: Codon pair modification concept**

**CodaLytic™**: influenza virus strain A/California/07/2009 with > 600 silent mutations in hemagglutinin and neuraminidase genes to introduce codon pairs that are less favored in human cells[3]; is attenuated in healthy cells due to slower translation but can be easily grown *in vitro*; achieves tumor specificity due to tumor hypersialylation and defects in interferon response and apoptosis pathways.

## METHODS

Monotherapy efficacy of CodaLytic, anti-tumor immune memory and immuno-modulation of the tumor microenvironment using phenotypic and transcriptomic characterization method was assessed in the moderately infiltrated, immune-active orthotopic triple-negative breast cancer model EMT6 and the well infiltrated colon cancer model CT26. Combination with a mouse-reactive PD-1 checkpoint inhibitor was tested in the immune-resistant B16-F10 melanoma models using efficacy and flow cytometry readouts. *Ex vivo* responses to CodaLytic alone or in combination with pembrolizumab in tumoroid cultures of primary human cancer tissues were determined using Nilogen Oncosystem's bioimaging technique to measure tumor cell killing, MSD multiplexed cytokine quantification and flow cytometry. Tissues were collected with approval of the Vanderbuilt University's Ethics review board, approval no. 031078.

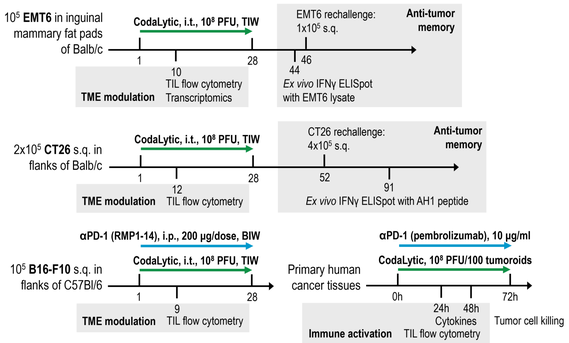



**Fig. 2: Experimental overviews**

## EFFICACY

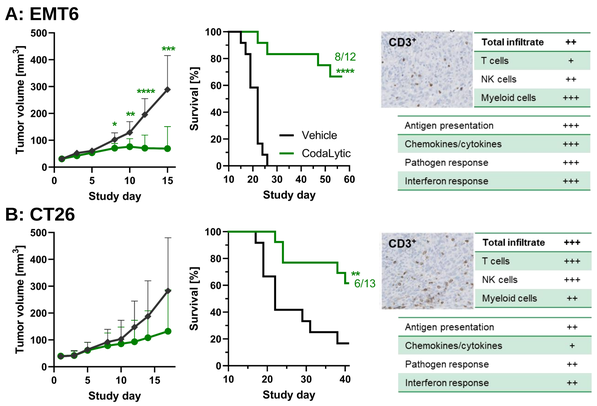
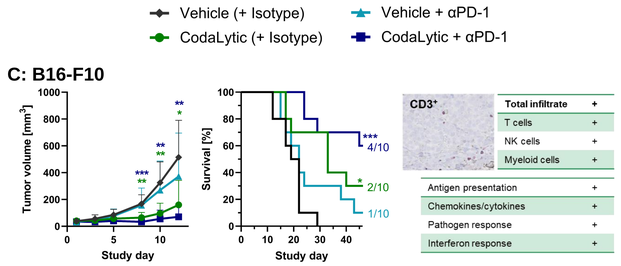



**Fig. 3: Anti-tumor efficacy and survival in 3 tumor models. A:** Orthotopic EMT6 model (n=12). **B:** S.q. CT26 model (n=13). **C:** S.q. B16-F10 model (n=10). For tumor growth (left), means and standard deviations are shown until the first study day an animal had to be euthanized. Two-way ANOVA with Geisser-Greenhouse correction and Tukey's multiple comparison's correction where applicable. Survival is shown as Kaplan-Meier curves (middle). Log-rank test with Bonferroni adjustment for multiple comparisons where applicable. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001 vs Vehicle/Vehicle + Isotype control. Immune contexture modified from Yu *et al*.[4] (right).




## TUMOR IMMUNE INFILTRATE

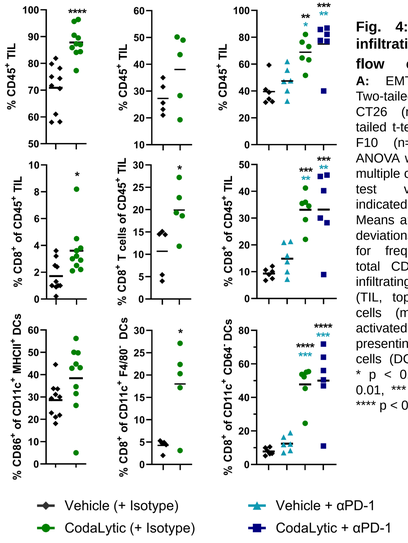



**Fig. 4: Immune infiltration by flow cytometry. A:** EMT6 (n=10). Two-tailed t-test. **B:** CT26 (n=5). Two-tailed t-test. **C:** B16-F10 (n=6). 1-way ANOVA with Tukey's multiple comparisons test vs group indicated by color. Means and standard deviations are shown for frequencies of total CD45⁺ tumor-infiltrating leukocytes (TIL, top), CD8⁺ T cells (middle) and activated / cross-presenting dendritic cells (DCs, bottom). * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

## EFFECT IN HUMAN TUMOROIDS

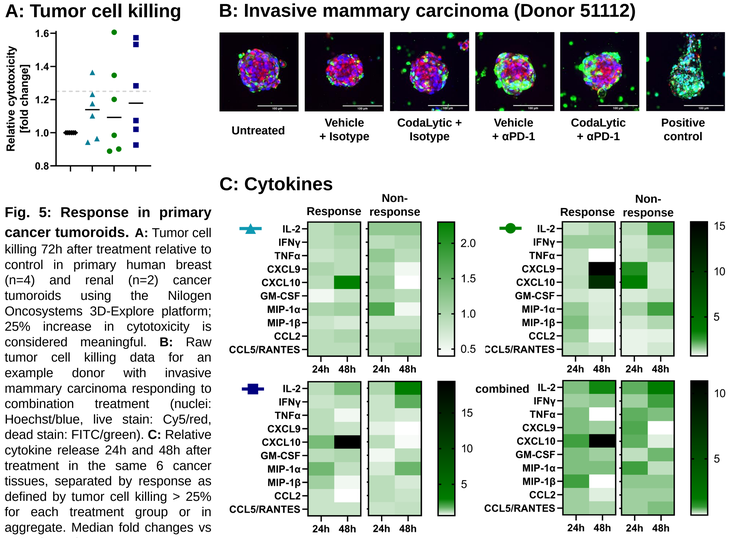



**Fig. 5: Response in primary cancer tumoroids. A:** Tumor cell killing 72h after treatment relative to control in primary human breast (n=4) and renal (n=2) cancer tumoroids using the Nilogen Oncosystems 3D-Explore platform; 25% increase in cytotoxicity is considered meaningful. **B:** Raw tumor cell killing data for an example donor with invasive mammary carcinoma responding to combination treatment (nuclei: Hoechst/blue, live stain: Cy5/red, dead stain: FITC/green). **C:** Relative cytokine release 24h and 48h after treatment in the same 6 cancer tissues, separated by response as defined by tumor cell killing > 25% for each treatment group or in aggregate. Median fold changes vs control are shown.

## TME TRANSCRIPTOME

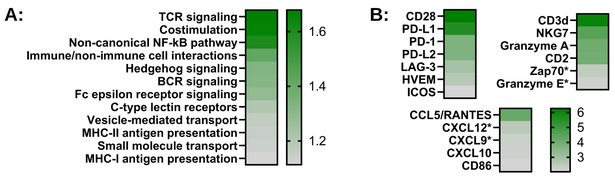



**Fig. 6: Transcriptomic changes in the EMT6 tumor microenvironment (TME) using the NanoString IO360 panel. A:** Top 12 transcriptionally upregulated pathways after CodaLytic vs Vehicle treatment. Log2-transformed directed pathway scores filtered for > 1.5x upregulation and p < 0.05 are shown. **B:** Linear fold changes of selected upregulated genes related to T cell function, dendritic cell activation and chemoattraction with > 2x fold change and p < 0.05 unless indicated by an asterisk.

## ANTI-TUMOR IMMUNE MEMORY

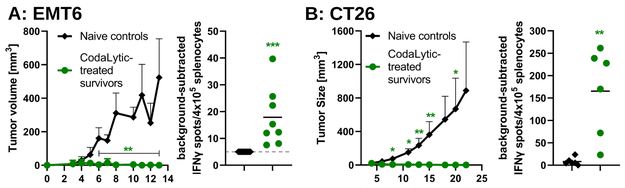
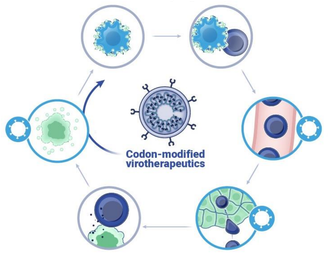



**Fig. 7: Induction of tumor antigen-specific immune memory. A:** EMT6 rechallenge (n=7), ELISpot (n=8). **B:**. CT26 rechallenge (n=7), ELISpot (n=6). For s.q. rechallenge (left), means and standard deviations are shown. 2-way ANOVA with Geisser-Greenhouse correction and Sidak's multiple comparisons test. For IFNγ recall response in splenocytes isolated from survivors and naïve control animals after 24h restimulation with EMT6 cell lysate (n=8) or AH1 peptide (n=6), individual values and means are shown. Two-tailed t-test. * p < 0.05, ** p < 0.01, *** p < 0.001.

## SUMMARY AND CONCLUSIONS

The novel immunotherapeutic influenza virus CodaLytic was able to induce broad innate and adaptive immune changes in the tumor microenvironment that translate into anti-tumor efficacy and long-term survival in several preclinical mouse models with varying responsiveness to immunotherapies and baseline immune contextures. Key components of the multi-faceted mechanism of action correlated with tumor cell killing were replicated *ex vivo* in well infiltrate primary human tumoroid cultures when used in combination with anti-PD-1 checkpoint blockade. With no signal in preclinical toxicology studies and excellent clinical tolerability of this attenuated influenza virus when given as an intranasal or injectable vaccine, CodaLytic emerges as a promising immunotherapeutic with the potential to deepen or broaden responses to checkpoint inhibitors.




REFERENCES: [1] Coleman JR *et al*. Science 2008;320(5884):1784; [2] Mueller S *et al*. Nature Biotechnology 2010;28(7):723; [3] Broadbent AJ *et al*. Vaccine 2016;34(4):563; [4] Yu JW et al. PLoS ONE 2018;13(11):e0206223.

ABBREVIATIONS: SAVE, synthetic attenuated viral engineering; s.q., subcutaneous; i.p., intraperitoneal; IFNγ, interferon-γ; TIW, 3x/week; BIW, 2x/week; PFU, plaque-forming units; TIL, tumor-infiltrating leukocytes; DCs, dendritic cells; TME, tumor microenvironment.

CODAGENIX