Anti-Ovarian Cancer Activity of a Lectibody Targeting Tumor-associated High-Mannose Glycans

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INTRODUCTION

- **Ovarian cancer** is the most lethal gynecological cancer [1]
  - Current treatment includes surgery and platinum-based chemotherapy, but most patients experience chemoresistant disease recurrence [1,2]
  - Few targeted therapies are available
- Ovarian cancers present excessive **high-mannose glycans** on the cellular surface in contrast to healthy tissues [3]
- **Avaren-Fc (AvFc)** is a lectibody, an antibody-like molecule consisting of a high-mannose-glycan-binding lectin fused to the Fc region of human IgG1 [4]
- AvFc selectively binds to high-mannose glycans on cancer cells, inducing an antibody-dependent cell-mediated cytotoxicity (ADCC).
- **Aim: Test the efficacy of AvFc as an anti-ovarian cancer drug**

References
1. Lheureux, S., et al., *Epithelial ovarian cancer.* The Lancet, 2019. **393**(10177): p. 1240-1253.
2. Tewari, K.S., et al., *Final Overall Survival of a Randomized Trial of Bevacizumab for Primary Treatment of Ovarian Cancer.* Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 2019. **37**(26): p. 2317-2328.
3. Chen, H., et al., *Mass spectrometric profiling reveals association of N-glycan patterns with epithelial ovarian cancer progression.* Tumour Biol, 2017. **39**(7): p. 1010428317716249.
4. Hamorsky, K.T., et al., *Engineering of a Lectibody Targeting High-Mannose-Type Glycans of the HIV Envelope.* Molecular Therapy, 2019. **27**(11): p. 2038-2052.

AVAREN-FC (AvFc)

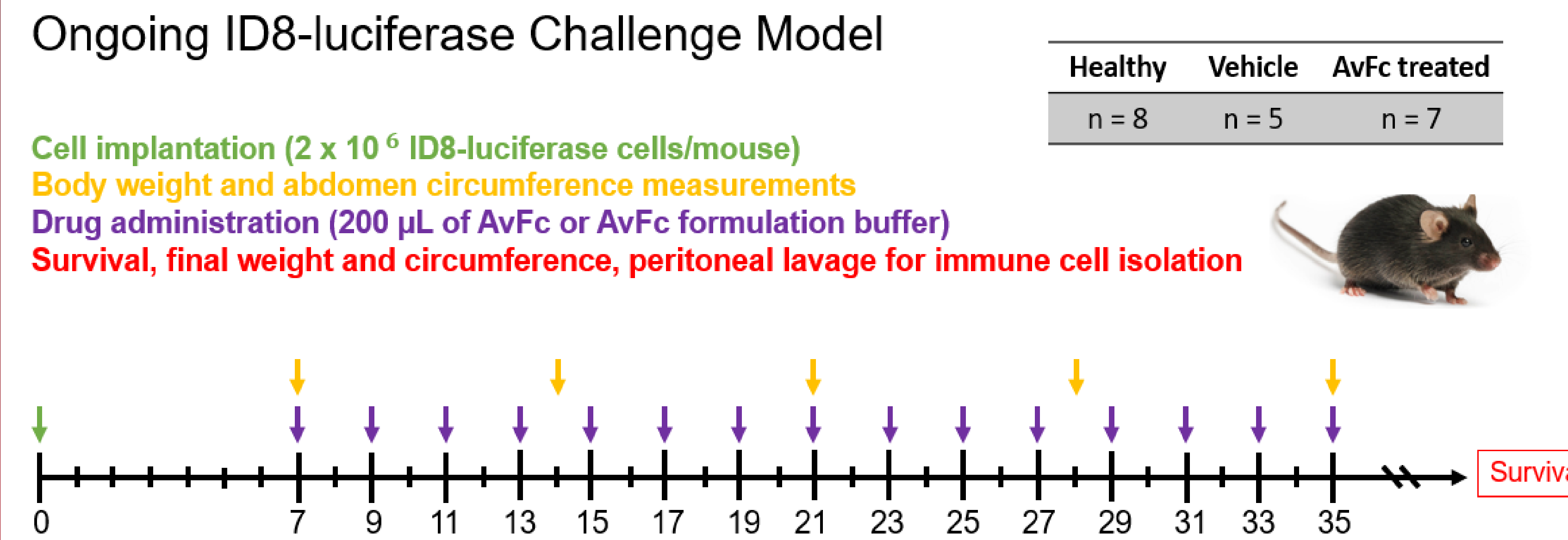### 1. AvFc differentiates malignant from normal adjacent tissues
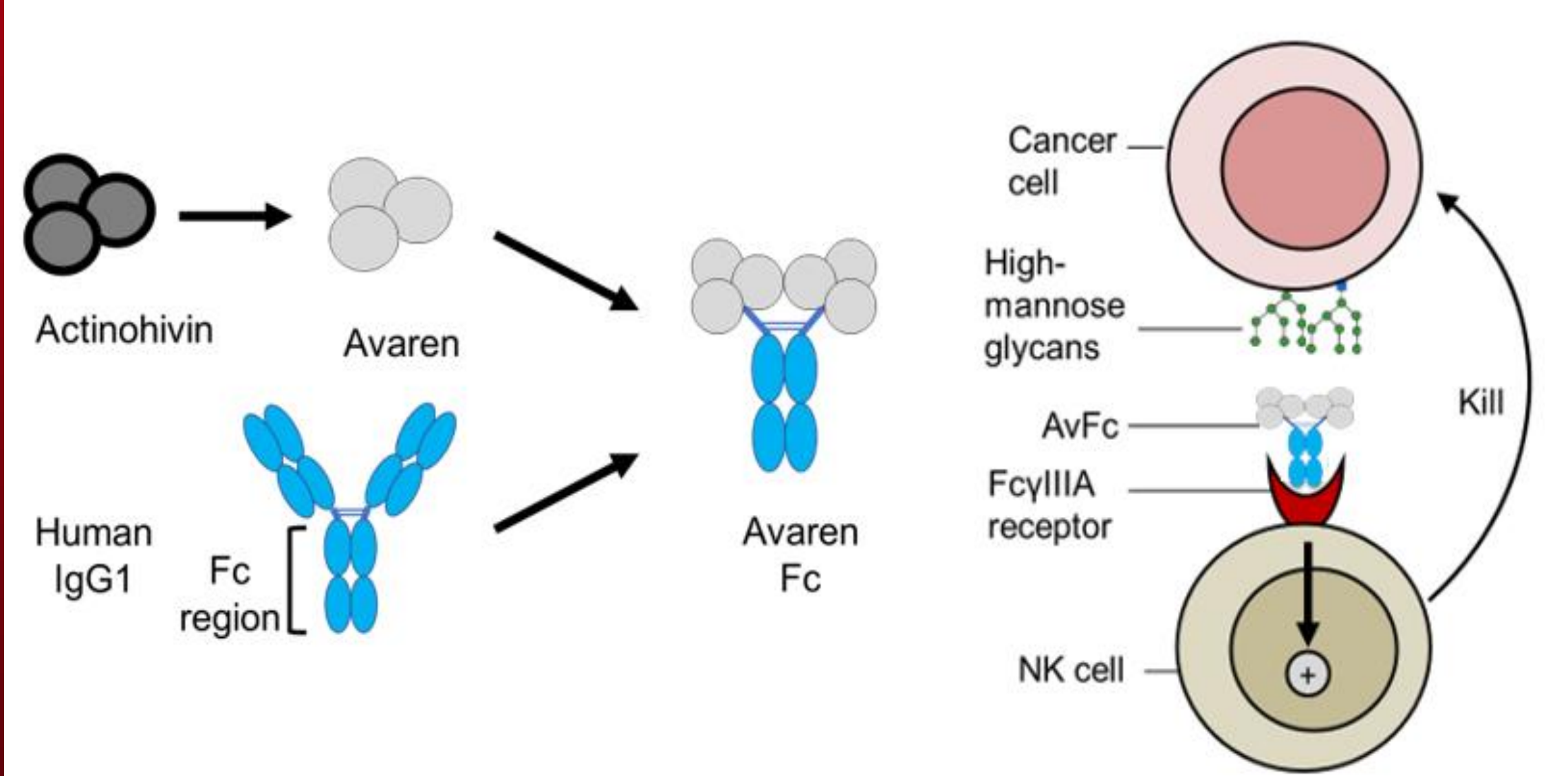
AvFc is an engineered high-mannose-binding bacterial lectin fused to the human IgG1 Fc, allowing the induction of antibody-dependent cell-mediated cytotoxicity (ADCC). Variants of AvFc have been made to improve ADCC activity, including the afucosylated **GnGn** glycovariant.
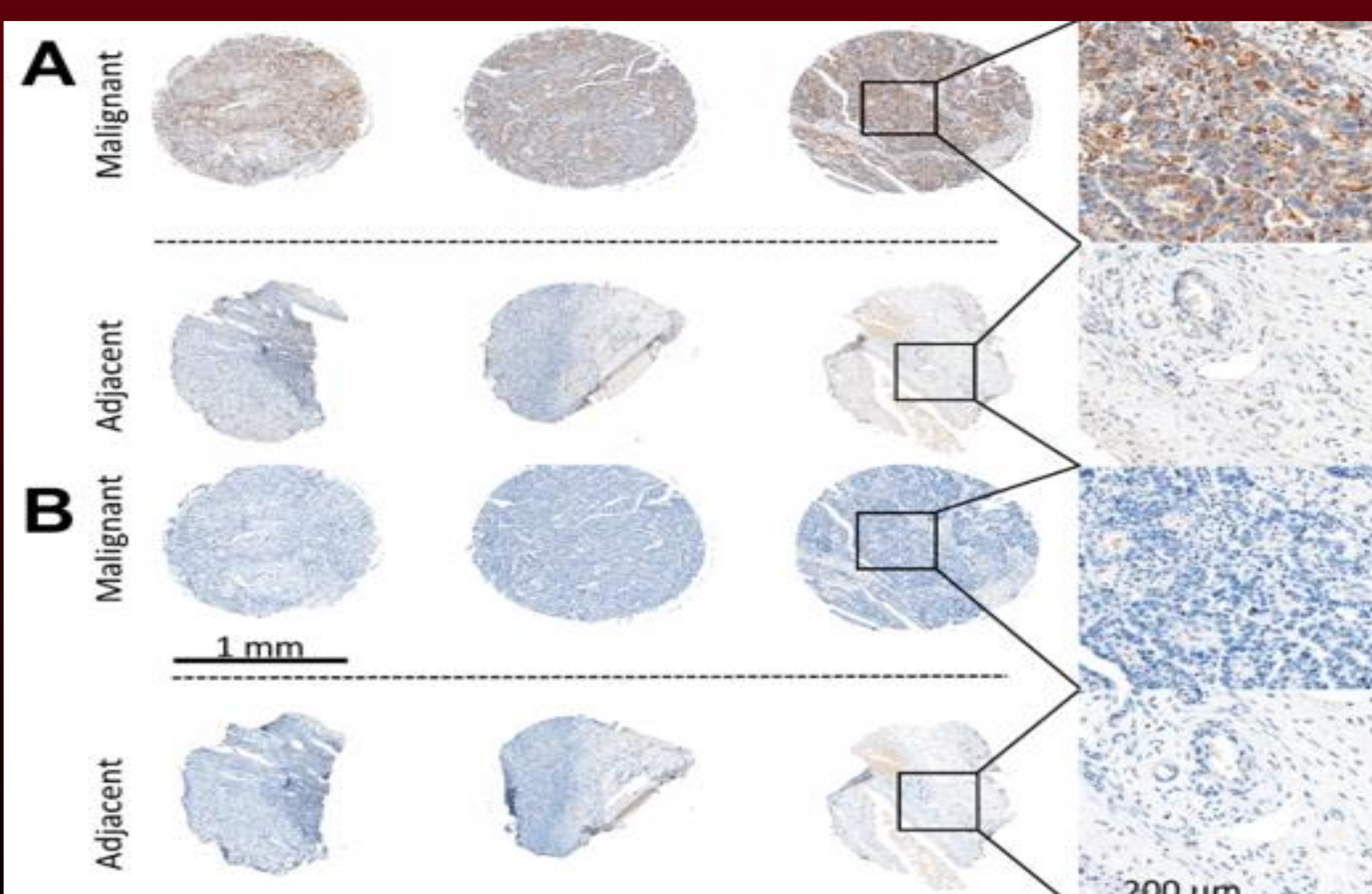
Figure 1 – Immunohistochemistry (IHC) of ovarian cancer patient tissue. IHC was performed on a commercial tissue array, which contained 3 Stage I high grade serous ovarian cancer tissues and 3 adjacent normal ovarian tissues. **A.** AvFc. **B.** A non-sugar-binding AvFc mutant (AvFc[lec-]).

RESULTS

2. Preliminary results in an ongoing *in vivo* experiment show signs of AvFc's efficacy against ovarian cancer

Ongoing ID8-luciferase Challenge Model

	Healthy	Vehicle	AvFc treated
	n = 8	n = 5	n = 7

Cell implantation (2 x 10^{6} ID8-luciferase cells/mouse)
Body weight and abdomen circumference measurements
Drug administration (200 µL of AvFc or AvFc formulation buffer)
Survival, final weight and circumference, peritoneal lavage for immune cell isolation

Figure 2 – Mouse ID8-luciferase ovarian cancer challenge model. The high-ADCC variant of AvFc (AvFc GnGn) was used. Animal weights and abdomen circumferences were measured weekly until animals reach a weight of 35 g or a circumference of 10 cm.
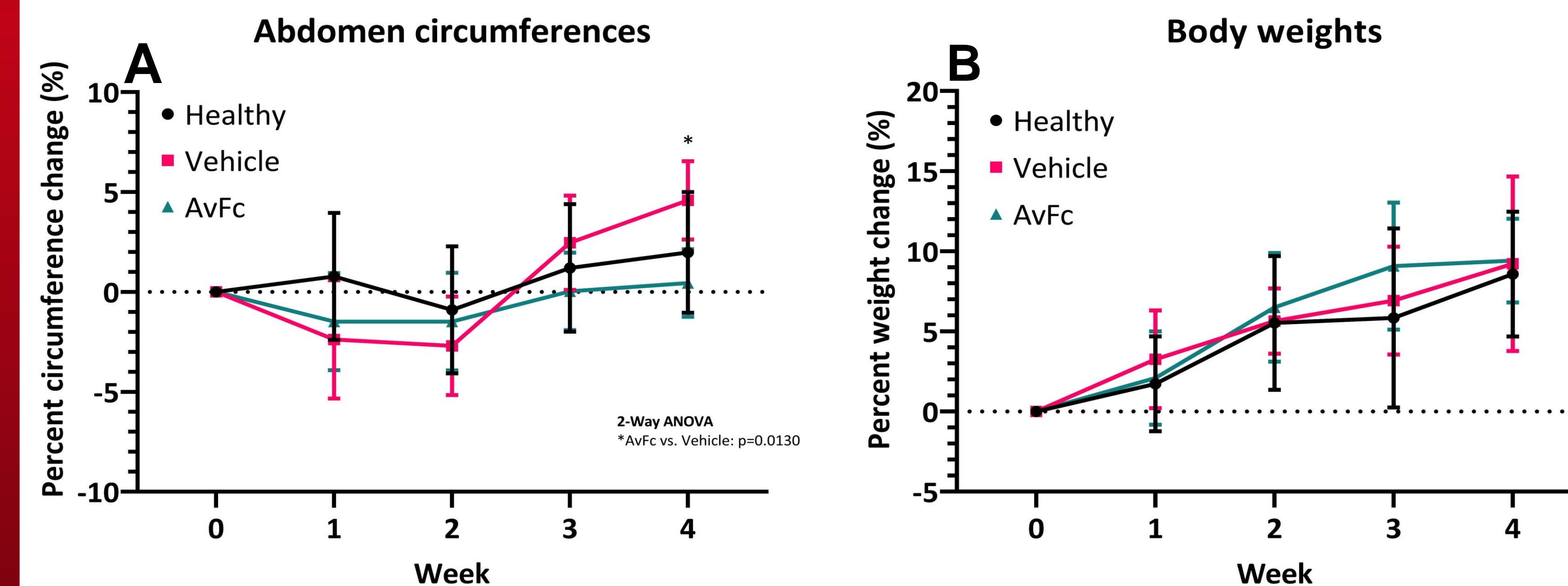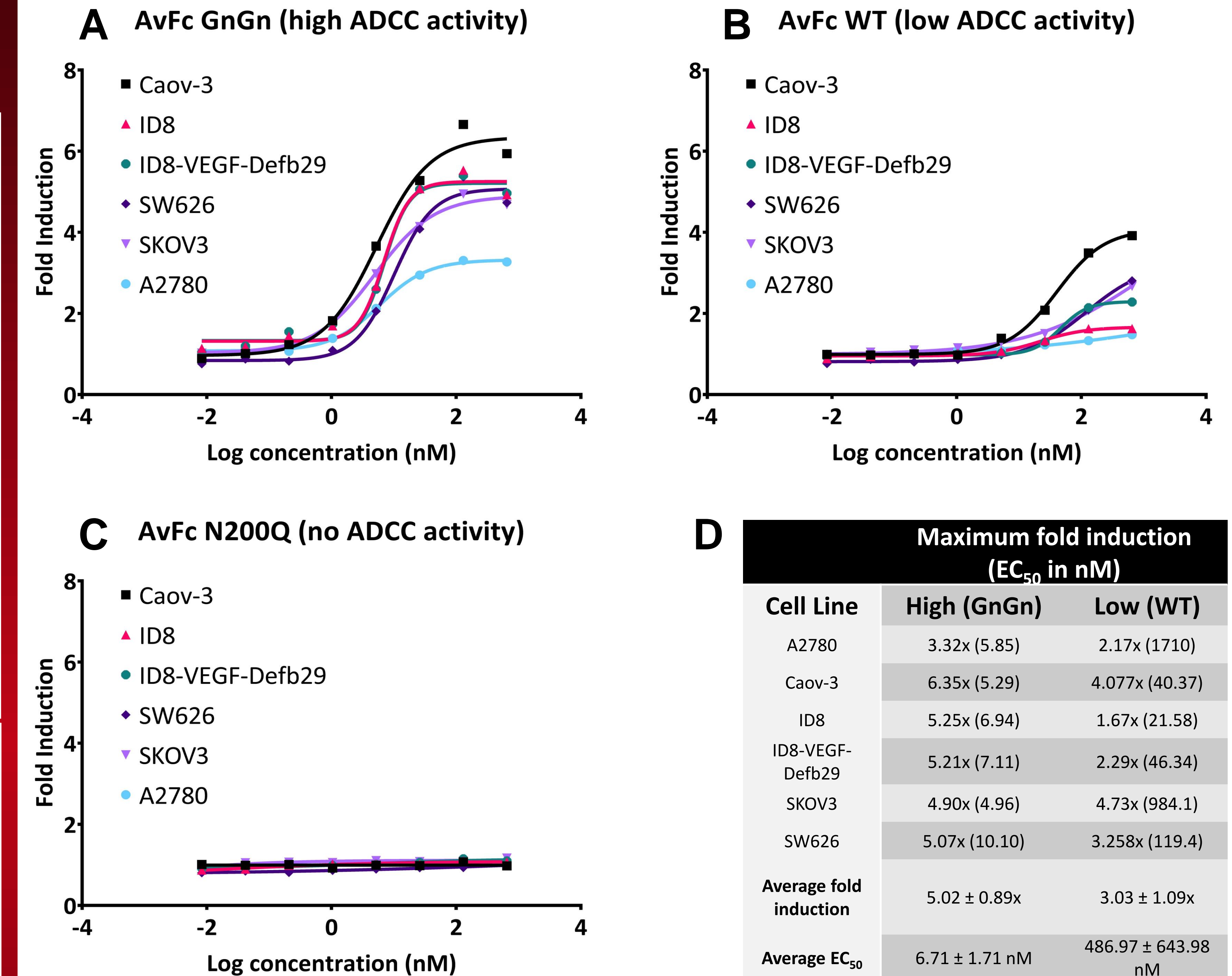
Figure 3 – Ongoing measurements of abdomen circumferences (A) and body weights (B). *P* < 0.05; 2-Way ANOVA with Bonferroni posttests. Unlike abdomen circumferences, body weights have remained similar between the three groups with no statistically significant difference thus far (as of week 4 post-implantation).

3. AvFc exhibits dose-dependent binding to human ovarian cancer cell lines

	1.5 nM	15 nM	150 nM
ID8-VEGF-Defb29	38.0	89.9	98.9
ID8	19.4	91.7	95.3
SKOV3	62.3	98.6	99.7
A2780	94.0	97.8	98.7
CAOV3	4.6	38.7	56.5
SW626	19.4	50.0	60.5

Figure 4 – Binding of AvFc to ovarian cancer cells by flow cytometry. The binding of AvFc to a panel of ovarian cancer cell lines was evaluated by single-color flow cytometry.

4. AvFc induces ADCC against ovarian cancer cell lines

A AvFc GnGn (high ADCC activity)
B AvFc WT (low ADCC activity)
C AvFc N200Q (no ADCC activity)

Legend: Caov-3, ID8, ID8-VEGF-Defb29, SW626, SKOV3, A2780

D

Cell Line	Maximum fold induction (EC$_{50}$ in nM)	
	High (GnGn)	Low (WT)
A2780	3.32x (5.85)	2.17x (1710)
Caov-3	6.35x (5.29)	4.077x (40.37)
ID8	5.25x (6.94)	1.67x (21.58)
ID8-VEGF-Defb29	5.21x (7.11)	2.29x (46.34)
SKOV3	4.90x (4.96)	4.73x (984.1)
SW626	5.07x (10.10)	3.258x (119.4)
Average fold induction	5.02 ± 0.89x	3.03 ± 1.09x
Average EC$_{50}$	6.71 ± 1.71 nM	486.97 ± 643.98 nM

Figure 5 – ADCC activity of AvFc variants. The ability of AvFc to induce ADCC against ovarian cancer cell lines was evaluated using a reporter-cell-based luciferase assay. **ADCC induction varied with each cell line, with AvFc GnGn on average producing a much higher response (A, D) than the wild type (B, D).**

SUMMARY

- AvFc selectively recognized ovarian cancer cells and induced ADCC
- Ongoing *in vivo* experiment suggests AvFc's efficacy against ovarian cancer

FUTURE DIRECTIONS

- Completion of the murine ID8 EOC challenge model followed by additional endpoint analyses (e.g. bioluminescent imaging, ascites immunoprofiling, ELISA & qRT-PCR)
- Perform co-immunoprecipitation followed by mass spectrometry to identify glycoproteins targeted by AvFc on ovarian cancer cells

ACKNOWLEDGEMENTS