A Comparison of a DNA-Sourced Neoantigen Vaccine to an RNA-Sourced Frameshift Vaccine in the Mouse Ovarian Cancer Model.

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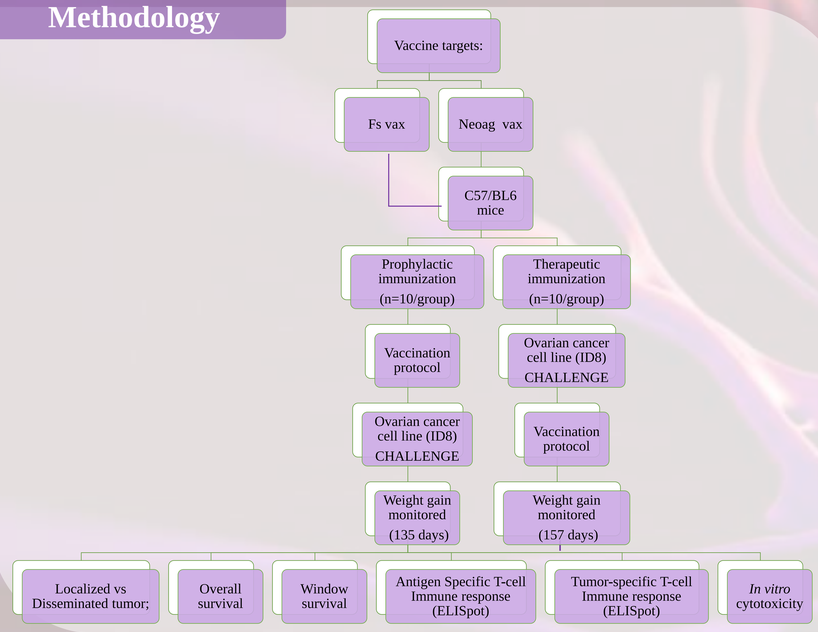
Abstract # A28

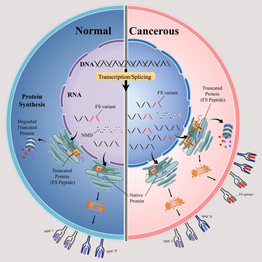
Methodology

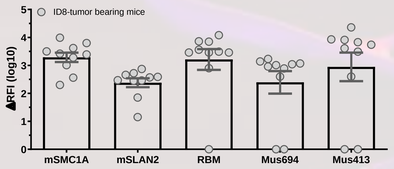


Abstract

Development of cancer vaccines is currently focused on using neoantigens arising from mutations in the DNA of tumors. We have proposed, and demonstrated, that errors in RNA processing that create frameshift (FS) neoantigens are also a good source of vaccine components, even in tumors that are DNA mutation poor. Here we directly compare the two vaccine approaches in the mouse ovarian model, ID8. Martin et al. (2016) reported that 7 DNA neoepitopes in the ovarian tumor line (ID8-G7) failed to show any protection when tested individually as peptide vaccines even though the peptides elicited an immune response. We created a pooled peptide vaccine consisting of these 7 neoantigens (Neoag vax). We compared this vaccine to one composed of 13 RNA-sourced FS neoantigens (FS vax). These FSs had conferred protection when tested in the 4T1 mammary and/or B16 melanoma models. 10 of the FSs arise from mis-transcription thru microsatellites and 3 from mis-splicing of exons. In a prophylactic vaccination protocol, both vaccines induce ~20% extended survival compared to mock controls. The FS vax elicited higher numbers of ELISpots than the ID8 control while the Neoag vax did not when splenocytes were peptide stimulated. The FS vax and NeoAg vax induced T-cells were only significantly cytotoxic to the ID8 cells if anti-PD-L1/CTLA-4 antibodies were included. In the therapeutic vaccination protocol, both the FS vax and the NeoAg vax conferred extended survival in ~20% of the mice compared to controls. Neither vaccine induced more ELISpots compared to controls using peptides. However, the FS vax did elicit more ELISpots in response to ID8 stimulation and more ID8 cytotoxicity than the NeoAg vax. From this work we first conclude that the DNA-sourced neoantigens that did not protect individually, can when pooled. Secondly, and more importantly, the RNA-sourced, FS neoantigens can perform as well as the DNA-sourced neoantigens at least in this model. This may be important as we have shown that all tumors surveyed to date, in contrast to DNA neoantigens, have abundant RNA-sourced FS neoantigens.

Results





RNA-sourced Frameshift Neoantigens (13 candidates) (FS vax).

FS type	Gene ID	Peptide Length
Microsatellite	Mus518	61
	Mus528	54
	Mus414	28
	Mus274	14
	Mus927	33
	Mus413	27
	Mus255	24
	Mus694	45
	Mus951	36
	Mus281	39
Mis-splicing	mSMC1A	27
	mSLAN2	21
	RBM	45

DNA-based Neoantigens[1] (Neoag vax).

Gene	29mer mutated peptide
B4galt3	ERPCTLALLVGSQLPVMMYLSLGGFRSLS
Cul2	VSPFLTETGEYYKQGASNLLQESNCSQYM
Dync1h1	KDRAATSPALFNRCELNWFGDWSTEALYQ
Ipo13	QAEDSPVDSQGRCLSLLELLTVLPEEFQT
Myo9a	YPSPSSPVIVRLPSVSDVPEETLSSETAM
Pkp4	SIYKKDGWNQNHFIIPVSTLERDRFKSHP
Rpl5	YLDAGLARTTTGNKFFGALKGAVDGGLSI

[1] Martin SD, Brown SD, Wick DA, Nielsen JS, Kroeger DR, et al. (2016) Low Mutation Burden in Ovarian Cancer May Limit the Utility of Neoantigen-Targeted Vaccines. PLOS ONE 11(5): e0155189. https://doi.org/10.1371/journal.pone.0155189

Figure 1. ID8-bearing mice sera react with the frameshift vaccine peptides by Fs microarray. (A) Diagram of the RNA-based frameshift peptide production in tumor cells versus normal cells. **(B)** Fs microarray reactivity with mice sera. Female C57/BL6 mice (n= 10) were intraperitonially challenged with ID8-ovarian cancer cell line and serum collected pre-injection, 30-days and 157-days post-injection. Serum reactivity of the ID8 challenged mice on the Fs array. Pre- and post-challenge (30-days) sera were assayed on FSP microarrays and (A) raw fluorescence intensity (RFI) was plotted for the 5/13 Fs peptides present on our 200-Fs array version slides. **Lower left:** Table of the frameshift antigens selected for our vaccine (Fs vax). **Lower right:** DNA-mutated neoantigens identified by Martin et al., 2016 and used as source for the DNA Neoag vax.

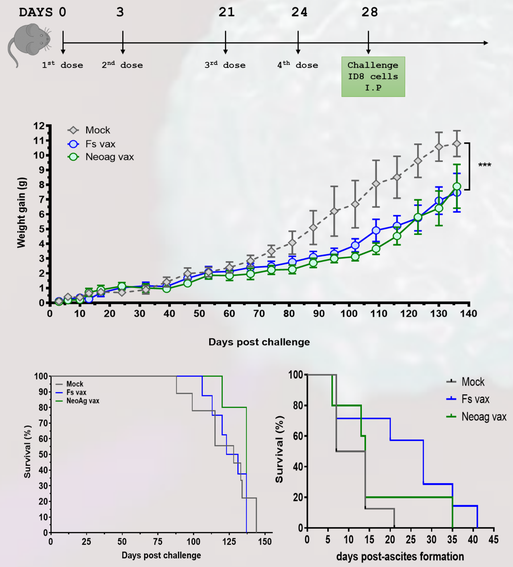


Figure 2. Prophylactic vaccination with frameshift neoantigens increased survival window in ovarian cancer mouse model. (A) Schematic diagram of the prophylactic immunization regimen. Female C57BL/6 mice (n=10/group) were immunized subcutaneously with four doses of the vaccine (Fs vax or Neoag vax) (5 µg of each antigen/dose) and Poly (I:C) (10 µg/dose), as adjuvant. On day 28, mice were challenge intraperitoneally with 1x10^6 ID8 cells and then, monitored twice per week for weight gain and illness signs for 137 days. (B) Kinetics of the change in body weight for the vaccinated and control groups. Weight gain was obtained by subtracting initial body weight at day of challenge from the weight at that day. (C) Survival curve for the prophylactic immunization. (D) Survival curve from the onset of the ascites until euthanasia. Data were compared using long-rank test (Mantel-Cox) and showed statistical significance (*, p = 0.0214). **Median survival:** Mock group= 128 days; Fs vax group= 127 days; Neoag vax= 137 days. **Median window survival:** Mock group= 10 days; Fs vax group= 28 days; Neoag vax= 14 days.

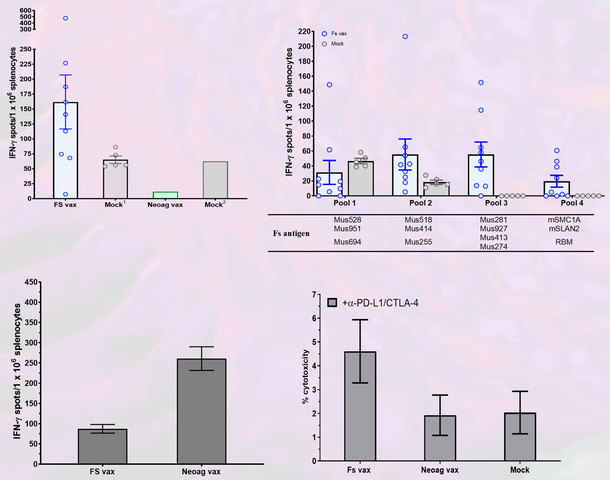


Figure 3. Fs neoantigens reactive T cells and *in vitro* cytotoxicity after prophylactic immunization. IFN-γ ELISPOT assay against neoantigens peptides (A) or ID8 tumor cell (C). Splenocytes (1 x10^6 cells) were incubated with the vaccine peptides (pooled) (20 µg/ml per peptide) or ID8 tumor cell line (1x10^5 cells/well) for 20-24 h or 72 h, respectively, at 37 °C and 5 % CO_2. As control, we used splenocytes from Mock group. For the Fs vax group the ELISPOT assay were performed individually with 4 peptide pools (B). Peptides from the Neoag vax were tested using pool of splenocytes (n= 10/group). Mock group were evaluated in pool of two mice for Fs neoantigens peptides and ten mice for the neoantigens vaccine peptides. Assay were performed in triplicate. Dots represent individual mice response or pool (2 mice) for the control group (Mock group). (D) *In vitro* CTL response against ID8 tumor cells in presence of anti-PD-L1/CTLA-4. Pooled splenocytes (1 x 10^6) from vaccinated mice were incubated with 2 x 10^4 ID8 cells for four hours, in presence or absence of mAb anti-PD-L1 and anti-CTLA-4 ((5 µg/well each), and CTL activity was measured by CytoTox 96 Non-radioactive (Promega™) cytotoxicity assay. Data represent mean ± SD of triplicate wells and are representative of one experiment. Mock[1]: splenocytes from mock group tested against Fs vax peptide pools; Mock[2]: splenocytes from mock group tested against Neoag vax peptide pool;

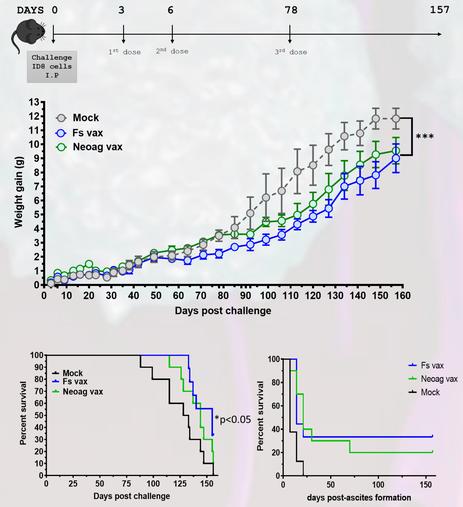
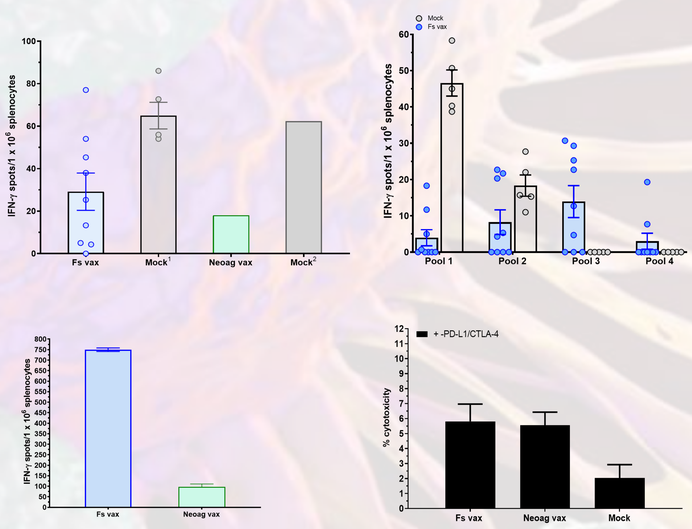


Figure 4. Therapeutic immunization with frameshift neoantigens improved overall and window survival in ovarian cancer mouse model. (A) Schematic diagram of the therapeutic immunization regimen. Female C57BL/6 mice (n=10/group) were challenge intraperitoneally with 1x10^6 ID8 cells and then immunized subcutaneously with three doses of the vaccine (Fs vax or Neoag vax) and Poly (I:C) (10 µg/dose), as adjuvant. Then, mice were monitored twice per week for weight gain and illness signs for 157 days. (B) Change in body weight for the vaccinated and control group. Weight gain was obtained by subtracting initial body weight at day of challenge from the weight at the day. (C) Survival curve for the therapeutic immunization. (D) Survival curve from the onset of the ascites until euthanasia. Data were compared using long-rank test (Mantel-Cox) and showed statistical significance (*, p < 0.05). **Median survival:** Mock group= 130 days; Fs vax group= 155 days; Neoag vax= 144 days. **Median window survival:** Mock group= 7 days; Fs vax group= 14 days; Neoag vax= 21 days.



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Conclusions

- ✓ Tumor bearing mice produce **antibodies anti-Fs specific** that can be detected by **peptide microarray**;
- ✓ **Prophylactic** immunization with **either Fs or DNA-neoag** resulted in **similar tumor control**, but **no protection**;
- ✓ **Fs vax** administrated **prophylactically** resulted in **extended survival** of the mice;
- ✓ **Prophylactic** immunization elicited a **robust peptide-specific T cell immune response**, but small tumor specific;
- ✓ Fs vax T cells-induced showed an **improved cytotoxic effect**;
- ✓ **Therapeutic** immunization with either **Fs or DNA-neoag** resulted in similar **tumor control**;
- ✓ 30% of mice were **protected** against the tumor after therapeutic immunization with Fs vax;
- ✓ Both **overall and window survival** was significatively **extended** after Fs vax immunization;
- ✓ Fs vax induced a **pronounced anti-tumor T cell** immune response;
- ✓ T cell **cytotoxicity** was **improved** in both vaccine formulations in comparison to mock group;

References

[1] Martin SD, Brown SD, Wick DA, Nielsen JS, Kroeger DR, et al. (2016) Low Mutation Burden in Ovarian Cancer May Limit the Utility of Neoantigen-Targeted Vaccines. PLOS ONE 11(5): e0155189. https://doi.org/10.1371/journal.pone.0155189

[2]Zhang J, Shen L, Johnston SA. Using Frameshift Peptide Arrays for Cancer Neo-Antigens Screening. Scientific Reports 8, 17366 (2018).

[3]Luhui Shen JZ, Hojoon Lee, Milene Tavares Batista, Stephen Albert Johnston. RNA Transcription and Splicing Errors as a Source of Cancer Frameshift Neoantigens for Vaccines. Scientific report accepted, (2019).