FAST vaccines based on frameshift neoantigens may have advantages over personal vaccines

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ABSTRACT

It is widely hoped that personal cancer vaccines will extend the number of patients benefiting from checkpoint and other immunotherapeutics. However, it is clear creating such vaccines will be challenging. It requires obtaining and sequencing tumor DNA/RNA, predicting potentially immunogenic neoepitopes and manufacturing a one-use vaccine. This process takes time and considerable cost. Importantly, most mutations will not produce an immunogenic peptide and many patient's tumors do not contain enough DNA mutations to make a vaccine. We have discovered that frameshift peptides (FSP) produced in tumors though errors in RNA production are a rich source of neoantigens. There are ~220K bioinformatically predictable possible FSP allowing us to make arrays representing them as 15aa peptides. These arrays can then be used to screen cancer patient blood antibodies for reactivity to the arrays. In screening many cancer patients blood on these array, we found both personal and cancer-type specific peptides. This suggests a new type of vaccine consisting of pre-made FSP components for a specific type of cancer. We term these FAST vaccines. Here we use the mouse 4T1 breast cancer model to test the relative effectiveness of a FAST and a PERSONAL vaccine. To create the vaccines, we initially challenged mice subcutaneously with 4T1 tumor cells and, seven days later, sera were collected. Pre-challenge and 7-days sera were assayed on peptide microarrays containing 200 FS neoantigens. For the PERSONAL vax, the top 10 candidates (higher median intensity fluorescence) were select and personal vaccines constructed and administrated to respective mice (n=10). For the FAST vax, we selected the top 10 candidates with higher prevalence among all the mice challenged (n=24), a common Breast cancer FAST vax was constructed (mBC FAST-vax). Mice were challenged with 4T1 cells subcutaneously. Vaccines were then, administrated twice with one-week interval, combined or not with checkpoint inhibitor (CPI) (anti- PD-L1/ CTLA-4). Our results demonstrated that both vaccine approaches, FAST and PERSONAL vax, alone reduced tumor growth as well as increased animal survival. Nonetheless, the FAST vax protected 70 % of mice (7/10 - tumor free) even after re-challenge, 29 days after vaccine regimen. For the Personal vax group, co-administration with CPI resulted in enhancement of tumor control with 57 % of the mice strongly controlling the tumor. The FAST vax performance was not improved by CPI. Both vaccine approaches elicited a robust and homogenous B- and T- cell immune response against both vaccine peptides and tumor cells. Additionally, use of Non-reactive FSPs and a Non-Breast cancer FAST vax were not able to control tumor development. We conclude that the FAST technology may open new opportunities to develop a low cost, feasible and efficacious vaccines against cancer.

METHODS

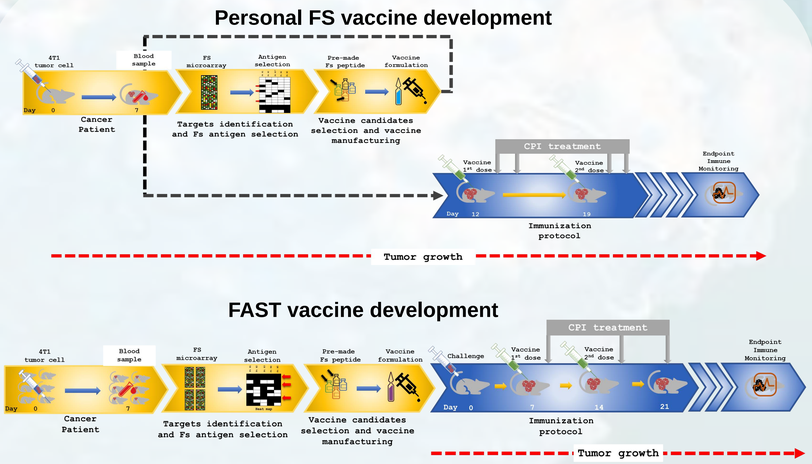
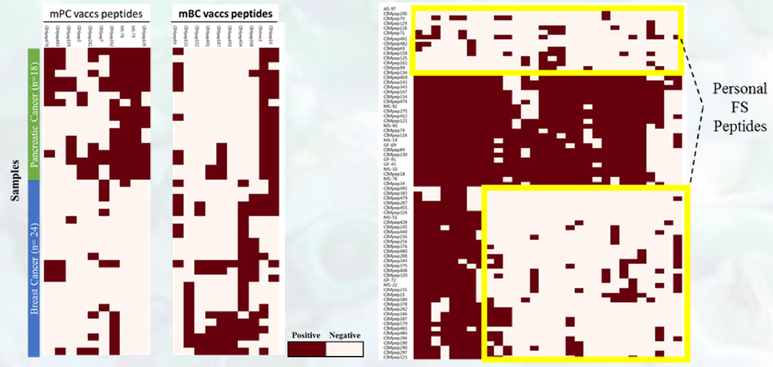


Figure 1. Frameshift (Fs) Personal and "Public" (FAST) vaccine design. Schematic diagram of Personal and FAST Fs vaccine approaches. We challenged mice subcutaneously with 4T1 tumor cells and, seven days later, sera were collected. Pre-challenge and 7-days sera were assayed on peptide microarrays containing 200 FS neoantigens. For the PERSONAL vaccs, the top 10 candidates (higher median intensity fluorescence) are selected and personal vaccines constructed and administrated to respective mice. For the FAST vaccs, we select the top 10 candidates with higher prevalence among all the mice challenged (n=24), a common Breast cancer FAST vaccs was constructed (mBC FAST vaccs). Then, mice are vaccinated . For the immunization group with the combined immunotherapy, the antibody treatment, anti-PD-L1 (200 µg/dose) and CTLA-4 (100 µg/dose) are administrated intraperitoneally.

RESULTS



Vaccine	Peptides	Positive rates (%) Cancer Samples: Breast	Pancreatic
mBC	CIMpep404	83.3	0
	CIMpep168	54.2	6
	CIMpep131	37.5	0
	CIMpep187	33.3	28
	CIMpep1	33.3	100
	CIMpep332	29.2	0
	CIMpep450	29.2	0
	CIMpep491	29.2	11
	CIMpep64	29.2	44
	CIMpep14	29.2	89
mPC	CIMpep145	0	78
	MS-75	0	100
	CIMpep105	4.2	33
	CIMpep3	4.2	28
	MS-76	4.2	94
	CIMpep479	12.5	50
	CIMpep282	16.7	44
	CIMpep493	16.7	33
	CIMpep7	25	28
	CIMpep362	29.2	89

Figure 2. Frameshift (Fs) Personal and "Public" (FAST) target candidates selection. (left) Heat map of the Fs candidates selected for the mBC FAST vaccs and mPC (Pancreatic cancer) FAST vaccs, a vaccine control. (Right) Heat map of the personal Fs selected. (lower table) FAST vaccines peptides selected and positive rates per cancer-type studied in mice model.

Table 1. Number of tumor-free mice after vaccine regimen with FAST vacc or Personal vacc.

Group	Treatment: PBS	FAST vacc	Personal vacc
Control (mock)	0 / 8	-	-
mBC	-	5 / 8	3/8
mBC + CPI	-	2 / 8	1/8
mPC	-	1 / 8	-
mPC + CPI	-	3 / 8	-
NR	-	-	0/8
NR + CPI	-	-	0/8

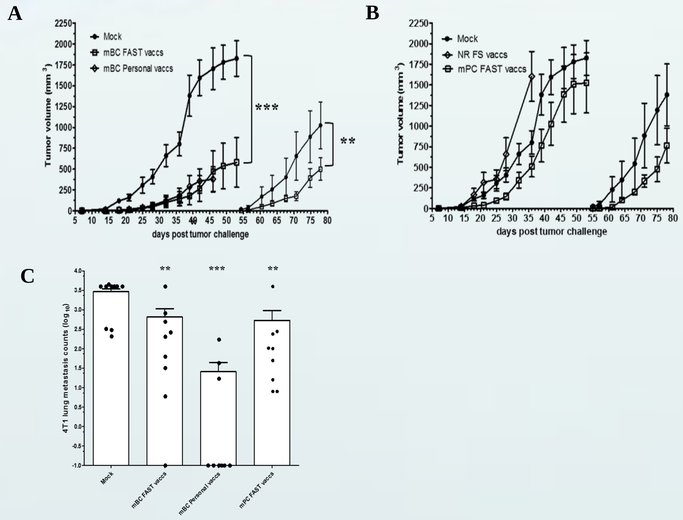


Figure 3. Protection of 4T1 breast tumor growth and lung metastasis by "shared" and personalized pool of FS neoantigens in BALB/c mice. (A-B) Average 4T1 tumor growth curves. Female Balb/c mice were challenged with 4T1 tumor cells subcutaneously and tumor size were measured twice per week. Mice were immunized as indicated in figure 1. As negative control (mock group), mice were immunized subcutaneously with PBS. As controls, we vaccinated mice with non-reactive personalized Fs peptides (NR Fs vaccs) and a pancreatic cancer vaccine (mPC FAST vaccs), composed by "public" neoantigens selected for this type of cancer. In the FAST vaccs and mock groups, the long-term tumor free mice were rechallenged s.c. with 1 x 10^3 4T1 tumor cells on day 42 and they received an additional vaccine boost on day 64. * p<0.05; **p<0.01; ***p<0.001, analyzed by two-way ANOVA with *Bonferroni* multiple comparisons post-test. (C) Quantification of pulmonary metastasis after Fs vaccs immunization protocol. Fixed clonogenic 4T1 metastatic colonies were stained with methylene blue and then counted. Bar graphs represent mean ± S.E and dots represent counts for each mice studied.* p<0.01; **, p<0.001; *** p<0.0001, analyzed by two-tailed Mann-Whitney test.

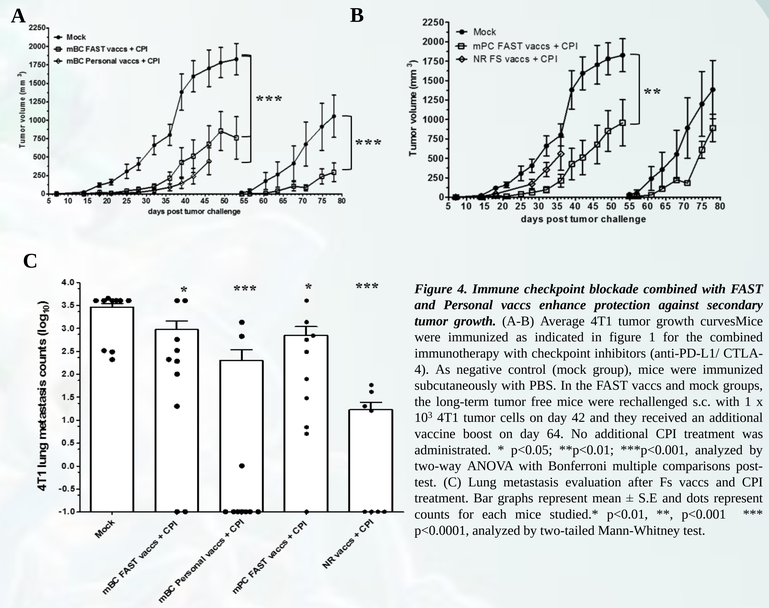


Figure 4. Immune checkpoint blockade combined with FAST and Personal vaccs enhance protection against secondary tumor growth. (A-B) Average 4T1 tumor growth curvesMice were immunized as indicated in figure 1 for the combined immunotherapy with checkpoint inhibitors (anti-PD-L1/ CTLA-4). As negative control (mock group), mice were immunized subcutaneously with PBS. In the FAST vaccs and mock groups, the long-term tumor free mice were rechallenged s.c. with 1 x 10^3 4T1 tumor cells on day 42 and they received an additional vaccine boost on day 64. No additional CPI treatment was administrated. * p<0.05; **p<0.01; ***p<0.001, analyzed by two-way ANOVA with Bonferroni multiple comparisons post-test. (C) Lung metastasis evaluation after Fs vaccs and CPI treatment. Bar graphs represent mean ± S.E and dots represent counts for each mice studied.* p<0.01, **, p<0.001 *** p<0.0001, analyzed by two-tailed Mann-Whitney test.

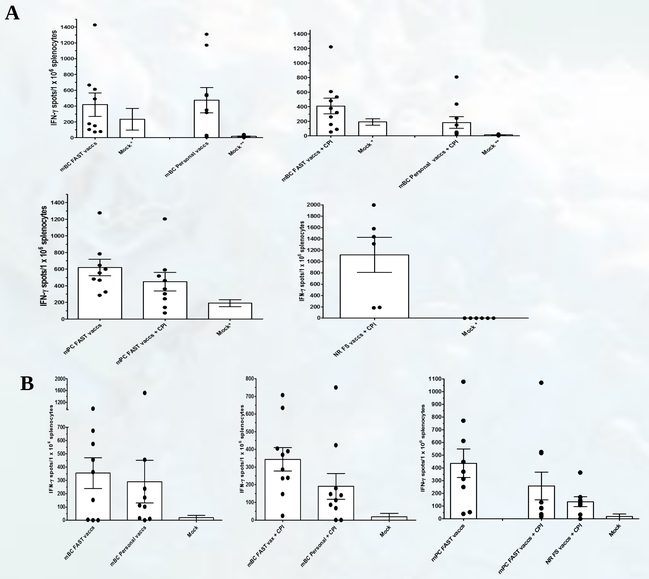


Figure 5. FAST and Personal Fs vaccs improved T cell immune response against both vaccine candidates and 4T1 tumor cells in vitro. At the endpoint, mice splenocytes were harvested and stimulated in triplicate with pooled vaccine peptides (1 mg/ml) (A) or 4T1 cells (1x10^5 cells/well) (B) and assessed by IFN-γ ELISPOT. Mice splenocytes from mock group was pooled and evaluated against all vaccine peptides (pooled) and tumor cell line. Bars represent mean ± SD of triplicate wells and each dot represents mouse individual immune response. * p<0.05; **p<0.01; analyzed by two-way ANOVA with Bonferroni multiple comparisons post-test.

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CONCLUSIONS

In conclusion, we showed that both **personalized and tumor-specific FS vaccines** selected by our Fs peptide **microarray technology** could **protect** against **primary and metastatic lesions** in a preclinical mouse model of breast cancer, inducing a **potent T cell immune response**. And, the **FAST vaccine (tumor-specific vaccine**) showed a superior ability to **eradicate initial tumor**, then, being a better choice than the personalized. Therefore, the **FAST technology** may open new opportunities to **develop a low cost, feasible and efficacious vaccine against cancer.**

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