Evaluation of the potential of oncolytic vaccinia virus delivered by autologous SVF to modulate innate and adaptive immunity in patients with diverse solid and hematological malignancies

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Background

The development of oncolytic viruses for the treatment of cancer has been significantly hampered by their rapid clearance in circulation due to complement and antibody-mediated neutralization. In our recent first-in-human Phase I clinical trial, we evaluated the safety and feasibility of our approach to enhance virus delivery and improve tumor targeting by utilizing an autologous stromal vascular fraction (SVF) based cell delivery system. Patient sample analysis demonstrated that patients could be stratified based on the level of vaccinia virus amplification in vivo, as evidenced by analysis of persistent viral DNA in the blood.

Methods

In the current study, we evaluated the immunomodulatory potential of vaccinia virus delivered by autologous stromal vascular fraction (SVF)-derived cells and attempted to identify immunological correlates of successful vaccinia virus amplification in vivo. To this end, we performed an extensive time-course analysis of cytokines in patients' plasma as well as various peripheral blood immune subpopulations using Luminex multi-analyte profiling and multiparameter flow cytometry, respectively. We also analyzed the impact of this therapeutic approach on the innate and adaptive immune subpopulations, including NK cells, myeloid cells, as well as effector, regulatory and memory T cells.

Results

Therapy with SFV-delivered oncolytic vaccinia virus induced a coordinated activation of cytokine, T cell and NK responses in patients as early as 1 day after treatment, which peaked around 1-week and lasted for up to 1-month post treatment. The ability of the oncolytic virus to effectively amplify in cancer patients correlated with significant changes of multiple innate (NK) and adaptive (T cell) immunological parameters. Interestingly, patient stratification into groups with transient versus persistent viral DNA was linked to opposing and mutually exclusive patterns of robust activation of NK versus T cell responses, respectively.

Conclusions

Overall, this study establishes the timeline of treatment-related immunological changes and identifies potential immunological correlates associated with the persistence of virus amplification in vivo. Therefore, our findings provide new insights into the role of interpatient immunological variability and will contribute to the proper evaluation of the therapeutic potency of oncolytic virotherapy in future clinical trials.

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Table 1. Patient population and description of stratification approach. Blood from patients with solid tumors treated on the study was analyzed by qPCR PowerUp™ SYBR® Green Master Mix (ThermoFisher, CA) at different timepoints and patients were stratified based on the presence of persistent/re-emerging vaccinia virus DNA in the blood, VV (+) group, versus patients where viral DNA disappeared and was undetectable 24h post administration, VV (-) group.

SVF-ACAM2000 TRIAL		TREATMENT		DOSE	OUTCOME	VAX STATUS	SKIN LESION	COPIES OF VIRUS (A56R) DNA / mL BLOOD						
PATIENT STRATIFICATION	Group ID	Patient ID	IT	Total PFU	Live Dec Drop Cens (Months)	Yes / No	Yes / No	1 min	60 min	1 d	1 w	1 mo	3 mo	6 mo
BLOOD VV DNA (-) GROUP N=12	NONE	23		6.60E+06	1.0	Yes	No	2357.3	0.0	0.0	0.0	0.0	0.0	0.0
		4		3.00E+06	1.0	Yes	No	349.6	997.2	0.0	na	0.0	0.0	0.0
		15	(+)	3.60E+06	1.0	Yes	No	135241.9	622.1	0.0	0.0	0.0	0.0	0.0
		25	(+)	3.00E+06	1.5	Yes	No	475.0	0.0	0.0	0.0	0.0	*	*
		5	(+)	6.00E+06	5.0	Yes	No	572.7	na	0.0	0.0	0.0	0.0	*
		27	(+)	3.00E+06	5.0	Yes	No	2345.2	271.9	0.0	0.0	0.0	0.0	na
		14		3.00E+06	12.0	Yes	No	14099.0	78.3	0.0	0.0	0.0	0.0	0.0
		3	(+)	3.00E+06	24.0	Yes	No	na	na	0.0	0.0	0.0	0.0	0.0
		28	(+)	3.00E+06	24.0	Yes	No	3850.6	4010.3	0.0	0.0	0.0	0.0	0.0
	SKIN LESIONS	22	(+)	3.00E+06	2.5	Yes	Yes	11398.7	43.64#	0.0	0.0	0.0	*	*
		10	(+)	1.80E+07	3.0	Yes	Yes	na	na	0.0	0.0	0.0	*	*
		1		2.50E+06	3.0	No	Yes	na	na	0.0	0.0	0.0	0.0	na
BLOOD VV DNA (+) GROUP N=11	PERSISTENT VIRAL DNA	31	(+)	1.40E+06	0.5	Yes	No	30095.0	1245.0	399.7	2312.4	*	*	*
		33	(+)	1.40E+06	1.0	Yes	No	2757.8	0.0	0.0	0.69	*	*	*
		35	(+)	1.40E+06	1.0	Yes	No	38336.1	225.3	0.0	201.7	*	*	*
		26	(+)	3.00E+06	3.0	Yes	No	na	0.0	154.3	0.0	2.3	193.8	0.0
		24		1.32E+07	7.0	Yes	No	na	na	1796.1	11.5	101.1	0.0	na
		8	(+)	3.00E+06	10.0	No	No	1383.3	0.0	0.0	48.1	0.0	0.0	0.0
	PERSISTENT VIRAL DNA AND SKIN LESIONS	32	(+)	1.40E+06	0.5	Yes	Yes	44838.0	423.7	0.0	59.7	0.0	0.0	0.0
		21	(+)	3.00E+06	1.0	Yes	Yes	59483.1	0.0	0.0	190.7	0.0	0.0	0.0
		36		1.40E+06	3.0	No	Yes	7615.1	64.5	0.0	279.5	na	na	na
		30	(+)	1.40E+06	6.0	Yes	Yes	27054.4	0.0	0.0	27.6	0.0	0.0	0.0
		18		3.00E+06	18.0	Yes	Yes	26214.8	269.7	0.0	98.8	73.5	0.0	0.0

Table 2. Patterns of cytokine responsiveness in SVF-ACAM2000 treated patients. Cytokine levels were normalized to baseline for each individual patient. Table shows normalized means for all patients on the study and for each timepoint, with statistically significant changes to baseline as indicated. Normalized means were also calculated after stratification to compare potential differences between the VV (-) and (+) groups. Statistically significant differences in the responses between the two groups are shown as indicated, p values based on unpaired two-tailed Student's t-test

CYTOKINES	NORM. MEANS				NORM MEAN P-VALUES				BLOOD VV DNA (-) GROUP				BLOOD VV DNA (+) GROUP				VV DNA (+) / (-) P-VALUES			
TIMECOURSE	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3
PATIENTS (N)	23	22	16	11	23	22	16	11	12	11	10	6	11	11	6	5				
FGF-Basic	1.0	0.9	1.0	1.4	;0.037				1.0	0.9	0.9	1.4	1.0	0.9	1.0	1.4				
IL-1b	1.0	1.0	1.0	1.8					1.0	1.0	0.9	1.8	0.9	0.9	1.0	1.7				
G-CSF	1.0	1.0	1.0	1.4					1.0	1.0	1.0	1.5	1.0	1.0	1.0	1.3				
IL-10	1.2	1.1	1.1	1.8				;0.030	1.0	1.0	1.1	1.7	1.4	1.1	1.0	2.0				
IL-13	1.0	1.0	1.0	1.0					1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.0				
IL-6	1.1	2.5	2.2	4.8				;0.043	1.0	2.1	1.2	4.3	1.1	2.9	3.7	5.5				
IL-12	1.0	1.3	1.1	2.0					1.0	1.1	0.9	1.5	1.1	1.4	1.5	2.5				
RANTES	1.0	1.0	1.1	1.1					1.0	1.0	1.0	1.0	1.0	1.0	1.1	1.1				
EXOTAXIN	1.1	1.2	1.2	1.8					1.0	1.0	0.9	1.5	1.3	1.3	1.6	2.1				
IL-17	1.0	1.0	1.0	1.0					1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.1				
MIP-1a	0.8	0.9	1.3	2.0					0.9	0.9	1.5	2.6	0.8	0.9	1.0	1.3				
GM-CSF	1.0	1.0	1.0	1.0					1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0				
MIP-1b	0.8	1.2	1.7	2.6					0.9	1.4	1.9	2.7	0.7	1.0	1.2	2.6				
MCP-1	1.1	1.3	1.1	1.9	;0.023			;0.014	1.1	1.0	1.0	1.6	1.1	1.6	1.2	2.1				
IL-15	1.0	0.9	1.0	1.5	;0.042	;0.012			1.0	0.9	0.9	1.7	1.0	0.9	1.0	1.2				
EGF	1.0	1.4	1.6	2.0		;0.021	;0.019		1.0	1.6	1.6	2.3	1.0	1.2	1.7	1.7				
IL-5	1.0	1.0	1.0	1.0					1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0				
HGF	0.9	1.0	1.1	1.6	;0.011				0.9	1.0	1.2	1.8	0.9	1.0	0.8	1.5				
VEGF	1.0	1.5	1.6	3.0				;0.029	0.9	1.5	1.4	3.1	1.2	1.5	1.7	2.8				
IFN-g	1.0	1.0	1.0	1.4				;0.043	1.0	0.9	1.0	1.5	1.0	1.0	1.0	1.2				
IFN-a	1.0	1.0	1.0	1.5				;0.037	1.0	1.0	1.0	1.6	1.0	1.0	1.0	1.4				
IL-1RA	0.9	1.6	1.3	3.0		;0.032		;0.017	0.9	1.3	1.4	3.1	1.0	1.8	1.2	2.9				
TNF-a	0.9	0.9	1.0	1.1	;0.033	;0.035			0.9	0.9	1.0	1.1	0.9	0.9	0.8	1.0				
IL-2	1.0	0.9	1.0	1.1	;0.010	;0.004			1.0	0.9	1.0	1.1	1.0	0.9	1.0	1.1				
IL-7	1.0	1.0	1.0	0.9					1.0	1.0	1.0	0.8	1.0	1.0	1.0	1.0				
IP-10	1.2	1.5	1.2	2.0	;0.007	;0.008			1.1	1.5	1.2	1.5	1.3	1.6	1.3	2.7				
IL-2R	1.0	1.2	1.3	1.7				;0.013	0.97	1.1	1.4	1.8	1.05	1.4	1.1	1.7	;0.011			
MIG	1.0	1.3	1.1	2.5				;0.032	1.0	1.0	0.9	2.2	1.1	1.6	1.3	2.8				
IL-4	1.0	1.0	1.0	1.0					1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0				
IL-8	0.9	2.2	2.3	6.7				;0.044	1.0	1.9	1.8	6.5	0.9	2.6	3.2	7.0				

Table 3. Differential innate and adaptive PBMC subset responsiveness in SVF-ACAM2000 treated patients. Levels of different immune subsets were quantified as % of all PBMC and normalized to baseline for each individual patient. Table shows normalized means for all patients on the study and for each timepoint, with statistically significant changes to baseline as indicated. Normalized means were also calculated after stratification to compare potential differences between the VV (-) and (+) groups. Statistically significant differences in the responses between the two groups are shown as indicated, p values based on unpaired two-tailed Student's t-test

T CELL PANELS	NORM. MEANS				NORM MEAN P-VALUES				BLOOD VV DNA (-) GROUP				BLOOD VV DNA (+) GROUP				VV DNA (+) / (-) P-VALUES			
TIMECOURSE	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3
PATIENTS (N)	23	22	16	11	23	22	16	11	12	11	10	6	11	11	6	5				
T CELL ACTIVATION																				
CD3+	1.1	0.9	0.9	0.8					1.0	0.8	0.8	0.7	1.2	1.0	1.1	1.1				
CD3+/CD4+	1.0	0.9	0.8	0.8		;0.038			0.9	0.7	0.7	0.6	1.2	1.0	1.0	1.0		;0.029		
CD3+/CD8+	1.3	1.0	1.0	1.0					1.2	1.0	0.9	0.8	1.4	1.1	1.2	1.2				
CD3+/CD4+/CD183+	1.1	0.6	0.6	0.6	;0.001	;0.001			1.0	0.7	0.6	0.4	1.1	0.6	0.7	0.9				
CD3+/CD4+/CD278+	0.9	0.7	0.9	0.8	;0.005				0.8	0.6	0.7	0.6	1.1	1.0	1.2	1.0	;0.001	;0.010	;0.006	
CD3+/CD8+/CD183+	1.3	0.9	0.8	0.8					1.2	1.1	0.7	0.6	1.4	0.7	1.0	1.1				
CD3+/CD8+/CD278+	1.2	1.0	1.1	0.9					1.2	0.9	1.0	0.7	1.2	1.1	1.2	1.1				
CD3+/CD4+/CD152+	1.2	0.8	0.9	0.7					1.0	0.7	0.8	0.7	1.3	1.0	1.0	0.8				
CD3+/CD4+/CD279+	1.1	0.8	0.8	0.8					1.0	0.8	0.8	0.8	1.1	0.9	0.8	0.7				
CD3+/CD8+/CD152+	1.3	1.2	0.9	1.7					1.0	1.0	0.8	1.8	1.7	1.3	1.2	1.5				
CD3+/CD8+/CD279+	1.5	1.1	1.0	1.2	;0.011				1.5	1.2	0.9	1.3	1.5	1.0	1.2	0.9				
CD3+/CD4+/CD69+	1.5	1.7	3.0	5.7					0.9	1.2	1.0	0.9	1.9	2.0	7.1	12.9				
CD3+/CD8+/CD69+	1.7	1.4	1.3	1.0	;0.007	;0.025			1.6	1.3	1.2	0.6	1.8	1.5	1.6	1.7				;0.022
Tregs	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3
CD3+	1.1	0.9	0.9	1.0					1.0	0.8	0.8	1.0	1.1	1.0	1.0	1.0				
CD3+/CD4+	1.0	0.9	0.8	0.9		;0.020			0.9	0.7	0.8	0.9	1.1	1.0	0.9	0.9				
CD4+/CD25+	0.9	0.9	0.8	1.5		;0.010			0.8	0.7	0.8	2.0	1.0	1.1	0.8	0.8				
CD4+/CD25hi+	1.0	0.9	0.8	0.9					0.9	0.8	0.8	0.9	1.1	1.0	0.9	0.9				
CD4+/CD25hi+Foxp3+	1.0	0.9	1.0	0.9	;0.038				1.0	1.0	1.0	0.9	1.0	0.9	1.0	0.9				
circ. Foxp3+ .Treg cells	1.0	0.8	0.8	0.9	;0.026	;0.021			0.9	0.7	0.7	0.9	1.0	0.9	0.9	0.9				
CD4+/CD25+/CD127 low+	1.0	1.1	0.9	1.0					1.0	1.1	0.9	1.1	0.9	1.0	0.9	1.0				
CD4+/CD25hi+/CD39+	1.0	1.0	1.1	1.0					1.0	1.1	1.1	1.0	0.9	1.0	1.1	0.9				
CD4+/CD25hi+/CD152+	1.1	1.3	1.0	0.7					1.2	1.3	0.8	0.7	1.1	1.4	1.2	0.7				
T CELL MEMORY	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3
CD3+	1.1	0.9	1.0	0.9					1.1	0.8	0.9	0.8	1.1	1.0	1.2	1.1				
CD3+/CD4+	1.0	0.9	0.9	0.9					0.9	0.7	0.8	0.8	1.1	1.0	1.2	1.1				
CD3+/CD8+	1.4	1.1	1.1	0.8					1.3	1.0	1.0	0.7	1.6	1.2	1.2	0.9				
CD3+/CD4+/CD45RA+/CD197+	1.0	0.9	0.9	0.9					0.9	0.8	0.8	0.8	1.1	1.0	1.2	1.1				
CD3+/CD4+/CD45RA-/CD197+	1.0	0.8	0.8	0.6				;0.022	0.8	0.7	0.7	0.6	1.1	1.0	1.1	0.7				
CD3+/CD4+/CD45RA+/CD197-	1.2	0.9	0.9	5.4					1.0	0.8	0.7	4.5	1.3	0.9	1.4	6.5				
CD3+/CD4+/CD45RA-/CD197-	1.1	1.0	1.0	1.1					0.9	0.8	0.8	0.9	1.3	1.1	1.3	1.5				
CD3+/CD8+/CD45RA+/CD197+	1.0	1.0	1.9	0.8					1.0	0.9	1.2	0.7	1.1	1.1	3.2	1.0				
CD3+/CD8+/CD45RA-/CD197+	0.9	0.8	1.1	9.2					0.9	0.8	1.0	1.4	0.9	0.7	1.3	22.2				
CD3+/CD8+/CD45RA+/CD197-	1.5	1.2	1.1	0.7					1.5	1.0	1.0	0.7	1.5	1.4	1.2	0.7				
CD3+/CD8+/CD45RA-/CD197-	1.2	1.1	1.3	0.9					1.1	0.9	1.3	0.7	1.3	1.3	1.3	1.2				

MDSC PANEL	NORM. MEANS				NORM MEAN P-VALUES				BLOOD VV DNA (-) GROUP				BLOOD VV DNA (+) GROUP				VV DNA (+) / (-) P-VALUES			
TIMECOURSE	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3
PATIENTS (N)	23	22	16	11	23	22	16	11	12	11	10	6	11	11	6	5				
Lineage-DR-CD33+CD11b+	0.8	1.2	1.4	0.9					0.9	1.2	1.2	0.6	0.8	1.2	1.6	1.1				
HLA-DR-CD14+	1.1	1.2	1.2	1.1					1.0	1.1	1.1	1.1	1.3	1.3	1.4	1.1				
HLA-DRlow+ CD14+	1.0	1.1	1.2	0.6			;0.015		0.9	1.1	1.2	0.6	1.2	1.1	1.1	0.7				
CD33+HLA-DRlow+CD14+CD15+	1.1	1.0	1.2	0.6			;0.022		1.0	1.0	1.1	0.5	1.1	1.1	1.4	0.7				
CD33+HLA-DR-CD14-CD15+	0.8	1.6	1.7	1.6					0.8	1.3	1.6	0.8	0.9	1.8	1.9	2.1				

NK PANEL	NORM. MEANS				NORM MEAN P-VALUES				BLOOD VV DNA (-) GROUP				BLOOD VV DNA (+) GROUP				VV DNA (+) / (-) P-VALUES			
TIMECOURSE	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3	D1	W1	M1	M3
PATIENTS (N)	23	22	16	11	23	22	16	11	12	11	10	6	11	11	6	5				
CD3+	0.8	0.9	0.8	1.0					0.9	0.9	0.7	0.9	0.8	1.0	1.0	1.1				
CD3+CD56+	0.9	1.0	0.9	1.0					0.9	1.0	0.8	1.0	0.9	1.0	0.9	1.0				
CD3-CD56+	1.0	1.0	1.0	1.1					1.0	1.1	1.0	1.3	0.9	1.0	1.0	0.8				
CD3+CD314+	0.9	1.1	0.9	1.1					1.0	1.0	0.8	1.0	0.8	1.1	1.1	1.1				
CD3-CD56+CD16+CD314+	1.1	1.1	1.0	1.1					1.2	1.2	1.1	1.5	1.0	1.1	1.0	0.7				
CD3-CD56+CD16+CD314-	0.5	0.5	0.6	0.6	;0.000	;0.000	;0.001		0.4	0.6	0.5	0.6	0.5	0.4	0.7	0.7				
CD3-CD56+CD16-CD314+	1.0	1.5	1.2	1.8		;0.007			1.0	1.2	1.1	1.9	1.1	1.6	1.3	1.6				
CD3-CD56+CD16-CD314-	1.0	1.2	1.3	1.4					1.2	1.8	1.5	1.2	0.9	0.8	0.9	1.6				
CD3-CD56+CD16+CD69+	0.9	0.9	1.1	0.7					0.9	0.9	1.1	0.6	1.0	0.9	1.2	0.8				
CD3-CD56+CD16-CD69+	1.0	1.0	1.0	1.0					1.1	1.5	1.2	1.0	0.8	0.7	0.8	1.0		;0.044		
CD3-CD56+CD16+CD69-	1.0	1.0	0.9	1.0					1.1	1.1	1.0	1.3	0.9	0.9	0.9	0.7				
CD3-CD56+CD16-CD69-	1.0	1.4	1.2	1.7		;0.004	;0.025		1.0	1.5	1.2	1.9	1.0	1.4	1.2	1.6				
CD3-CD56+CD16+CD69+IFN+	0.9	1.1	2.1	0.4					1.2	1.7	2.7	0.5	0.6	0.6	1.0	0.0		;0.005		
CD3-CD56+CD16-CD69+IFN+	1.0	0.7	1.5	0.3					1.0	1.0	2.0	0.2	0.9	0.5	0.7	0.3		;0.048		
CD3-CD56+CD16+CD69-IFN+	0.9	1.3	1.8	0.3					1.1	1.7	2.5	0.5	0.7	1.1	0.8	0.2				
CD3-CD56+CD16-CD69-IFN+	0.9	2.0	3.0	0.8					1.0	2.9	4.3	1.1	0.9	1.5	1.1	0.5				

COLOR CODE: 0.5 0.7 0.9 1.0 1.2 1.5 1.8 2.0 (FOLD CHANGE TO BASELINE)