FIRST-IN-MAN STUDY OF TK-POSITIVE ONCOLYTIC VACCINIA VIRUS DELIVERED BY ADIPOSE STROMAL VASCULAR FRACTION CELLS

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Abstract

Background. Recent oncolytic virus clinical studies have shown safety and implied anti-tumor activity. However, a major obstacle to this approach has been the rapid oncolytic virus elimination by patient's immune system. We hypothesized that oncolytic viruses would be protected and delivered efficiently to tumor sites by autologous adipose stromal vascular fraction (SVF) cells. Effective virus protection by adipose derived cells has been confirmed in preclinical studies. Here, we report the results of a first-in-man trial to determine the safety and feasibility of this approach in patients with advanced solid tumors and AML.

Methods. In this single-arm, open-label safety study, 24 patients with advanced solid tumors and 2 patients with AML were treated with a single administration of the oncolytic virus ACAM2000 (vaccinia) delivered by SVF cells. Patients received ACAM2000/SVF by intravenous application, or by a combination of intravenous and intratumoral or intra-peritoneal injections. The dose for ACAM2000 was between 1.4 x 10^6 pfu to 1.8 x 10^7 pfu incubated with same number of SVF cells. The primary endpoint was safety/tolerability by incidence of dose-limiting toxicity. Secondary endpoints included evaluations of overall survival and induction of anti-tumor and anti-vaccinia immune responses. Blood samples were collected at multiple time points for quantifying vaccinia virus DNA in peripheral blood by qPCR. In addition, levels of 30 plasma cytokines and the effects on activated T cells, Tregs, memory T cells, NK cells, and MDSC were analyzed.

Results. No serious toxicities (> grade 2) were reported. Eight of the 26 subjects reported an AE: self-limiting skin lesions, lasting 7 to 18 days – an expected reaction to ACAM2000. No infusion related AEs were reported. No AEs leading to study discontinuation were reported. Viral DNA was detected in all patients immediately following treatment. Interestingly, in 8 patients viral DNA disappeared 1 day and re-appeared 1 week post treatment, suggesting active viral replication, possibly at tumor sites. This viral DNA reappearance correlated with longer survival of these patients. No major increase in cytokine levels was observed in any of the patients. No correlation between cytokine levels and pox lesions was noted. Flow cytometry showed gradual changes suggesting improved immune cell activation status. Tumor size reduction was documented in several patients.

Conclusions. Treatment with ACAM2000/SVF in patients with advanced solid tumors and AML is safe and well tolerated, with clear antitumor effects in several patients. These promising initial clinical results merit further investigation of therapeutic utility.

Methods

- Open-label, non-randomized dose-escalation trial
- Mini-liposuction procedure was performed to isolate up to 100 milliliters of adipose tissue
- The SVF cells were prepared in a closed system according to an established protocol (*Berman et al. The American Journal of Cosmetic Surgery. 2017:1 – 14*)
- The lyophilized ACAM2000 vaccine was reconstituted, added to a syringe containing SVF cells and incubated at 37°C for 15 minutes – 1 hour on a rotator
- Patients received ACAM2000/SVF by intravenous application, or by a combination of intravenous and intratumoral or intra-peritoneal injections
- The dose for ACAM2000 was between 1.4 x 10^6 pfu to 1.8 x 10^7 pfu incubated with same number of SVF cells
- Blood samples were collected at multiple time points for quantifying vaccinia virus DNA in peripheral blood by qPCR. In addition, levels of 30 plasma cytokines and the effects on activated T cells, Tregs, memory T cells, NK cells, and MDSC were analyzed

Results

TABLE 1. Demographics

Twenty-six patients were enrolled in this study:
- 15 male and 11 female patients
- 24 patients with different solid tumors and 2 patients with AML were enrolled

Age (years)	
Median	60.4
Range	19-92

Gender	N	%
Male	15	58
Female	11	42

Cancer Type	N	%
Lung cancer	1	4
Breast cancer	2	8
Prostate cancer	3	12
AML	2	8
Pancreatic cancer	2	8
Colorectal cancer	2	8
Head and Neck cancer	4	15
Adrenal cancer	1	4
Liver cancer	1	4
Astrocytoma	1	4
Bronchial carcinoid	1	4
Ovarian Cancer	1	4
Uterine cancer	1	4
Sarcomatoid	1	4
Squamous cell carcinoma neck	1	4
Esophageal cancer	1	4
Thyroid cancer	1	4

Results

TABLE 2. Adverse Events

Adverse Events	Number	%	Related	Resolved
Skin rashes	8	40	Yes	Yes
Lipo- puncture bleeding	1	5	Yes	Yes
Fever (100.5 F)	2	10	No (10 d and 16 d a/t)	Yes
Pain	4	20	No (7 d, 10 d and 1month a/t)	Yes
Hemoptysis	1	5	No (2mths a/t)	Yes
Pleural effusion	1	5	No (3mths a/t)	Yes
Headache and weakness of one site	1	5	No (3wks a/t)	Yes
Blood Transfusion	1	5	No (10d a/t)	Yes
Pneumonia	1	5	No (16d a/t)	Yes

FIGURE 1. Representative analysis of 9 cytokines and the relationship with the appearance of skin rashes in patients.

Patients' plasma samples were analyzed using The Cytokine Human Magnetic 30-Plex Panel for the Luminex™ platform (Thermo Fisher) at different time-points after treatment: 1W – 1 week post treatment; 1M – 1 month post treatment.

Data is presented as Log2 of fold change after treatment.

Patient ID	IL-8 1W	IL-8 1M	IL-6 1W	IL-6 1M	IL-1β 1W	IL-1β 1M	IL-12 1W	IL-12 1M	IFN-g 1W	IFN-g 1M	TNF-α 1W	TNF-α 1M	IL-10 1W	IL-10 1M	IL-2 1W	IL-2 1M	IL-4 1W	IL-4 1M	Pox lesions
21																			Yes
14																			No
26																			No
15																			No
3																			No
5																			No
29																			No
10																			Yes
18																			Yes
24																			No
27																			No
28																			No
30																			Yes
8																			No

-6 0 4 (Log2 of fold change after reatment)

FIGURE 2. Viral DNA in patients' peripheral blood.

DNA was extracted using the Quick-gDNA™ Blood MidiPrep (Zymo Research, CA). The copy number amount of the ACAM2000 gene A56R was quantified by qPCR. Viral DNA was analyzed by qPCR before treatment (bt), and 1 minute (1 min), 60 minutes (60 min), 1 day (1d), 1 week (1w), 1 month (1mo), 3 months (3mo) and 6 months (6mo) after treatment.

Patient ID	Treatment Dose: Total PFU	Skin Rashes	Copies of Viral DNA in the peripheral blood: bt	1 min	60 min	1 d	1 w	1 mo	3 mo	6 mo
30	1.40E+06	Yes	0.0	57054.4	0.0	0.0	27.6	0.0	0.0	0.0
31	1.40E+06	No	0.0	36095.0	1245.0	399.7	2312.4	*	*	*
32	1.40E+06	Yes	0.0	84858.0	423.7	0.0	59.7	0.0	0.0	0.0
33	1.40E+06	No	0.0	2737.8	0.0	0.0	0.69	*	*	*
34	1.40E+06	No	0.0	10403.2	106.0	0.0	0.0	*	*	*
35	1.40E+06	No	0.0	58336.1	225.3	0.0	201.7	*	*	*
36	1.40E+06	Yes	0.0	7615.1	64.5	0.0	279.5	na	na	na
1	2.50E+06	Yes	0.0	na	na	0.0	0.0	0.0	0.0	na
3	3.00E+06	No	0.0	na	na	0.0	0.0	0.0	0.0	0.0
4	3.00E+06	No	0.0	349.6	997.2	0.0	na	0.0	0.0	0.0
8	3.00E+06	No	0.0	1383.3	0.0	0.0	48.1	0.0	0.0	0.0
14	3.00E+06	No	0.0	14099.0	78.3	0.0	0.0	0.0	0.0	0.0
18	3.00E+06	Yes	0.0	26214.8	269.7	0.0	98.8	73.5	0.0	0.0
21	3.00E+06	Yes	0.0	59483.1	0.0	0.0	190.7	0.0	0.0	0.0
22	3.00E+06	Yes	0.0	11398.7	43.64#	0.0	0.0	0.0	*	*
25	3.00E+06	No	0.0	475.0	0.0	0.0	0.0	0.0	*	*
26	3.00E+06	No	0.0	na	0.0	154.3	0.0	2.3	193.8	0.0
27	3.00E+06	No	0.0	2345.2	271.9	0.0	0.0	0.0	0.0	na
28	3.00E+06	No	0.0	3850.6	4010.3	0.0	0.0	0.0	0.0	0.0
29	3.00E+06	No	0.0	889.6	360.6	0.0	0.0	0.0	0.0	na
15	3.60E+06	No	0.0	135241.9	622.1	0.0	0.0	0.0	0.0	0.0
47	5.00E+06	No	0.0	327.0	0.0	0.0	0.0	0.0	0.0	na
5	6.00E+06	No	0.0	572.7	na	0.0	0.0	0.0	0.0	*
23	6.60E+06	No	0.0	2357.3	0.0	0.0	0.0	0.0	0.0	0.0
24	1.32E+07	No	0.0	na	na	1796.1	11.5	101.1	0.0	na
10	1.80E+07	Yes	0.0	na	na	0.0	0.0	0.0	*	*

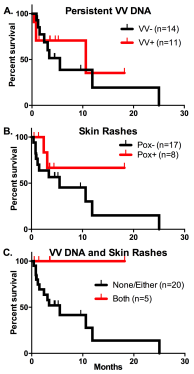
na - sample not available

* - Patient deceased

Copies of Viral DNA/ml blood: > 1001 | 501-1000 | 101-500 | < 100

Results

FIGURE 3. Kaplan-Meier plots showing the relative survival of a total of 25 patients (23 patients with solid tumors and 2 patients with AML).



(A) Patient survival relative to the presence of persistent vaccinia virus DNA in the blood for 1 week to 3 months post treatment. Median survival: without persistent VV, 5 months, (n=14) vs. with persistent VV, 10 months (n=11)

(B) Patient survival relative to reported presence of skin rashes. Median survival: without skin rashes, 5 months (n=17) vs. with skin rashes undefined (n=8)

(C) Patient survival relative to the presence of both skin rashes and persistent vaccinia virus DNA in the blood. Median survival: with either or none, 5 months, (n=20) vs. with both, undefined (n=5)

Vertical tick marks indicate patients who dropped from the study or were censored from analysis after switching to alternative cancer treatments.

FIGURE 4. Patient #21: tumor regression of patient's poorly differentiated squamous cell carcinoma (Stage: IV_B) (Synergistic effect with inhibition of PD-1).

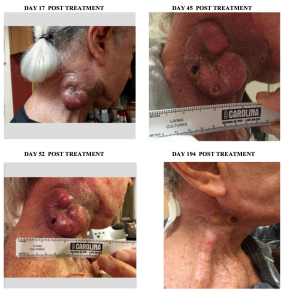
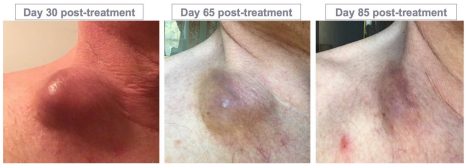


FIGURE 5. Patient #47: tumor regression of patient's metastatic papillary thyroid carcinoma. Treatment effects in the treated right supraclavicular lymph node (Synergistic effect with inhibition of CTLA-4).



Conclusions

1. **The combined application of SVF and ACAM2000 was well tolerated in all patients. The MTD was not reached**
2. **The plasma cytokine assays suggested mild inflammatory reaction starting approximately one week after treatment, not associated with any clinical symptoms**
3. **There is a trend towards improved survival associated with vaccinia virus activity *in vivo* including the persistence of viral DNA in the blood, skin rashes or both**
4. **Some patients experienced significant tumor size reduction, especially when the ACAM2000/SVF treatment was combined with checkpoint inhibition**
5. **These promising initial clinical results merit further investigation of therapeutic utility**