Human Vaccines & Immunotherapeutics 9:1, 213–218; January 2013; © 2013 Landes Bioscience

Cancer targeting vaccines

Surrogate measures of activity

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Recent FDA approval of sipuleucel-T and Ipilimumab as indicated immunologic therapy in patients with advanced prostate cancer and melanoma, respectively, has established a foothold for broader utilization of vaccine based technology in managing cancer. Despite difficulty of cell harvest and processing with sipuleucel-T and modest toxicity to Ipilimumab, when matched up with the appropriate cancer patient these immunologic approaches have provided significant benefit and have stimulated exciting forward progress in the development of new potent and less toxic (more targeted) vaccines. However, surrogate measures of activity to optimally define more sensitive subset populations and to determine length of treatment time in order to optimize management with other treatment options remain elusive. Key clinically tested vaccines under development which demonstrate correlation of patient benefit to induced immune responsiveness will be discussed. Results suggest with some vaccines correlation of patient benefit and surrogate measures of activity actually do exist. Examples will be discussed.

Keywords: cancer, vaccines, surrogate measures, immune, target

Submitted: 08/28/12

Accepted: 09/06/12

http://dx.doi.org/10.4161/hv.22091

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Introduction

So, how does it work? What are we trying to turn on or off in the immune system in order to reestablish control of our body's ability to prevent cancer from expanding? In essence, how can we prolong life, possibly with cancer, without cancer complications related to treatment and/or progressive disease? In Figure 1 the core immunologic process is demonstrated. Vaccines providing relevant tumor antigens excite the dendritic cell process to turn on afferent and efferent effector cells which create a targeted systemic attack on metastatic tumor cells.[1] Until recently systemic immune induction had been limited to therapeutic use in melanoma and renal cell cancer. Sipuleucel-T activity demonstrating statistically significant improvement in survival of advanced prostate cancer patients suggests the potential utilization of immune induction therapy (i.e., vaccines) in other solid tumors. In particular, as a proof of principle, extensive data has been demonstrated in non-small cell lung cancer (NSCLC) suggesting immune sensitivity to vaccine approaches (see **Tables 1 and 2**).

Granulocyte-macrophage Colony-stimulating Factor Gene Vaccine (GVAX). GVAX vaccine induces immune activation and exposes tumor antigens. Autologous lung cancer cells harvested from the patients are genetically modified with an adenoviral vector (Ad-GM) to secrete human GMCSF. After irradiation, they are administered intradermally over a sequential course every several weeks to months.[1]

This vaccine is well-tolerated, with the most common toxicity involving local injection-site reaction. Remarkably, 3 of 33 metastatic NSCLC patients who had failed prior standard therapy had durable complete tumor responses. The longest now more than 12 y (recent unpublished update). There appeared to be a vaccine dose-related survival advantage: longer survival was observed in patients receiving GVAX in which their vaccine secreted more than 40 ng of GMCSF per 24 h per 10^6 cells (median survival = 17 mo, 95%

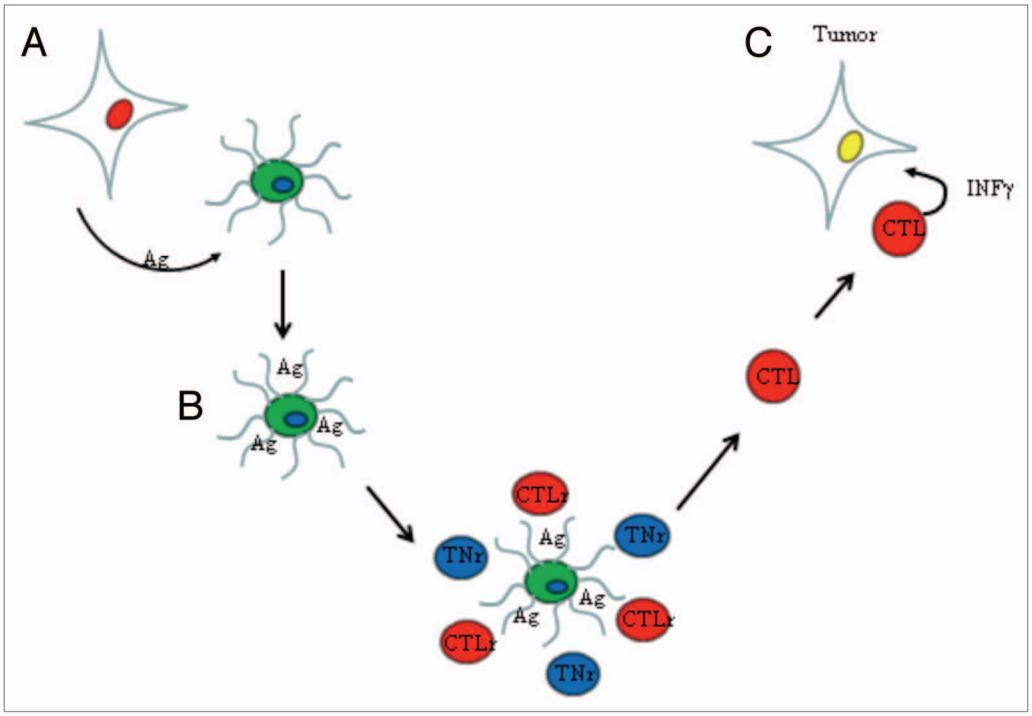

Figure 1. Vaccines (**A**) providing relevant tumor antigens (Ag) to local dendritic cells (**B**) turn on B and T effector cells, which distribute systemically, seeking metastatic tumor cells (**C**) containing the identified antigens.

confidence interval [CI], 6–23 mo) than in patients receiving GVAX secreting less GMCSF (median survival = 7 mo, 95% CI, 4–10 mo; p = 0.028).

Belagenpumatucel-L

Belagenpumatucel-L is a nonviral gene-based vaccine. This vaccine is synthesized by incorporating transforming growth factor beta2 (TGFβ2), a potent immune response inhibitor produced by some lung cancer cells, antisense gene into a pool of allogeneic tumor cells.

A randomized phase 2 trial of belagenpumatucel-L examined 3 different doses, 1.25×10^7 cells/injection, 2.5×10^7 cells/injection, and 5.0×10^7 cells/injection.[2] The dose was administered as an intradermal injection once per month for 4 mo, then once a month or every other month for a total of 12 mo. The majority of the 75 patients in the study had non-resectable stage III or IV disease. No significant side effects were observed and of 40 patients with measurable disease, 5 (13%) had a radiographic partial response. A detectable immune response occurred in a subset of patients, which correlated with lack of disease progression, and there was a dose-related effect on overall survival (**Fig. 2**). Efforts are ongoing to characterize patients who are likely to be more responsive to this vaccine, either initially or during the course of treatment, considering a panel of immune response assays, key of which involves ELISPOT assessment at baseline and at follow-up.

A phase 3 trial of belagenpumatucel-L (STOP) has just completed accrual. Results are under analysis.

Table 1. Results of Gene-Based Vaccines in IIIB/IV NSCLC

Vaccine	Stage	# Pts	Median Survival	Reference
Allogenic Ad B 7.1	IIIB/IV	19	18 mo (52% 1yr)	Raez, L.E. et al.; 2004[10]
GM-CSF gene vaccine	IV	35	Not done	Salgia, R. et al.; 2003[11]
GM-CSF gene vaccine	IIIB/IV	33	12 mo (44% 1yr)	Nemunaitis, J. et al.; 200[41]
GM-CSF gene vaccine bystander	IIIB/IV	49	7 mo (31% 1 y)	Nemunaitis, J. et al.; 2006[12]
Galactosyl-transferase	IV	7	Not done	Morris, J.C. et al.; 2005[13]
Lucanix	IIIB/IV	61	14.4 mo (56% 1 y)	Nemunaitis, J. et al.; 2006[2]
Lucanix	IIIB/IV	21	15.5 mo (72% 1 y)	Nemunaitis, J. et al.; 2009[14]
TG4010	IIIB/IV	65	14.9 mo (60% 1 y)	Ramlau, R. et al.; 2008[5]
TG4010	IIIB/IV	48	17.1 mo	Quoix E et al.; 2011[6]

TGFβ2 Antisense + rhGMCSF Tumor-associated Glycoprotein (TAG)

Experience with the results involving GVAX suggest independent benefit to

Table 2. Results of Non-Gene-Based Vaccines in IIIB/IV NSCLC

Vaccine	Stage	# Pts	Median Survival	Reference
SRL172	IIIB/IV	210	7.3 mo	O'Brien et al.; 2004[15]
Dendritic NSCLC pulsed	IA-IIIB	16	Not Applicable	Hirschowitz, E.A. et al.; 2004[16]
Dexosome MAGE load	IIIB/IV	13	Not Done	Morse, M. A. et al.; 2005[17]
CIMAvax	III, IV	40	8.2 mo	Gonzalez, G. et al.; 2003[18]
CIMAvax	IIIB, IV	43	Low dose: 6.43 mo; High does: 8.4 mo	Ramos, T.C. et al.; 2006[19]
Telomerase peptide	IIIB, IV, (I,III A)	26	8.5 mo (36% 1yr)	Brunsvig, P.F. et al.; 2006[20]
BLP 25	IIIB	88	17 mo	Butts, C. et al.; 2005[21]
BLP 25	IIIB/IV	17	5 mo (low) 15 mo (high)	Palmer, M. et al.; 2001[22]
EP2101	IIIB/IV	135	17 mo	Barve, M.; 2008[23]
1E10	IIIB/IV	71	9.9 mo	Alfonso et al.; 2007[24]
1E10	IIIB/IV	20	10.6 mo	Hernandez et al.; 2008[25]
Pulsed DC's	IIIB/IV	5	12 mo	Perroud et al.; 2011[26]
CEA pulsed DC's	IIIB/IV	14	22 mo (64% 1 y)	Zhong et al.; 2011[27]

advanced NSCLC patients based on disparate methods of enhancement (immune stimulation, inhibition of immune inhibitors, respectively) of antigen stimulation using whole cell vaccines. We thus considered combining these activities into a single vaccine. The TGFβ2 Antisense + rhGMCSF tumor-associated glycoprotein (TAG) vaccine uses an expression plasmid that coexpresses the GMCSF and TGFβ2 antisense nucleotide sequences, incorporated into autologous tumor tissue.[3,4]

During phase 1 trial, 22 advanced cancer patients were treated.[3] Patients were infused with either 1×10^7 (n = 7) or 2.5×10^7 (n = 15) cells. There was little evidence of adverse events, apart from injection site pain.

Stable disease of 3 or more months' duration was observed in 17 of 21 evaluable patients (median survival 465 d). One complete response occurred in a patient with stage IV malignant melanoma. Subsequent follow-up revealed correlation between immune response and survival, as determined by ELISPOT results which show activated T-cell expression to autologous tumor cells.

TG4010

Lessons learned from GVAX, Lucanix and TAG studies identified in **Tables 1 and 2**, as limited examples primarily involving advanced NSCLC, reveal methods of enhancing tumor antigen expression and activation of dendritic cells and other immune effectors toward

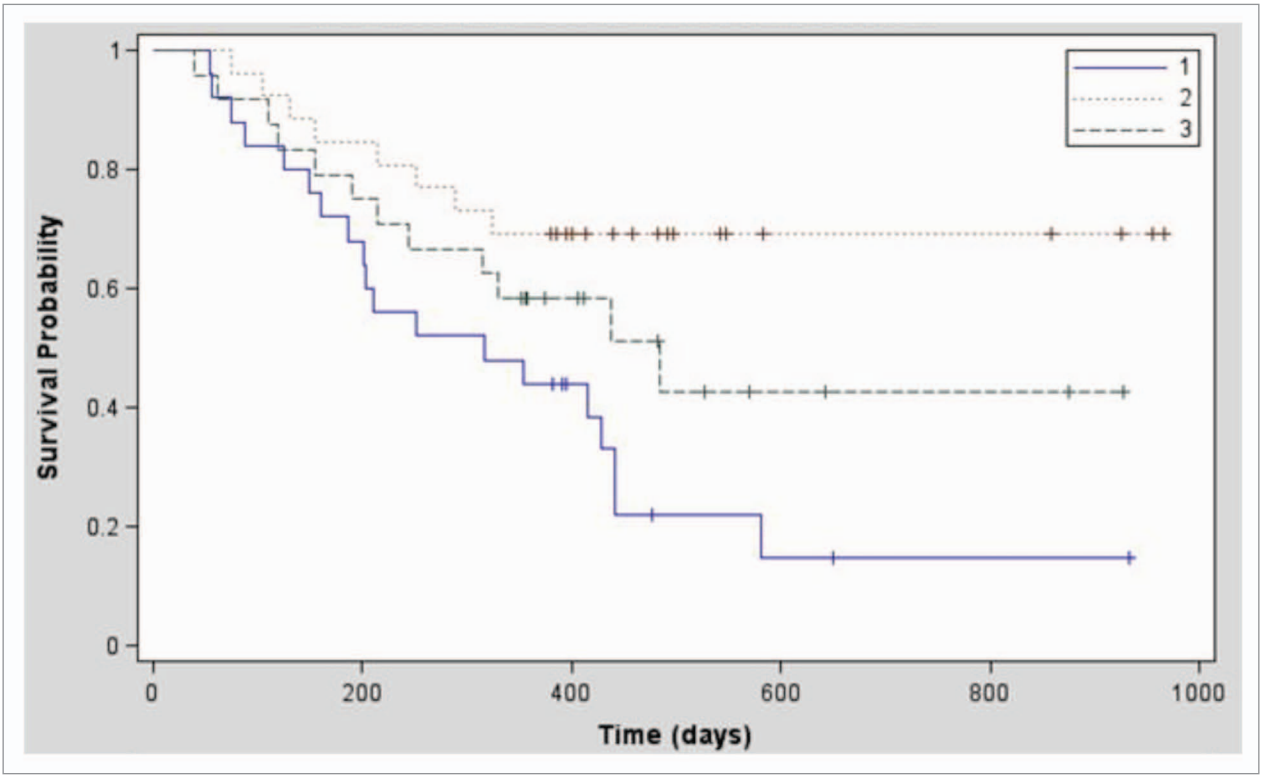

Figure 2. Dose related survival relationship is shown between cohorts of patients receiving lower and higher cell dose number (n = 75, p = 0.0155).

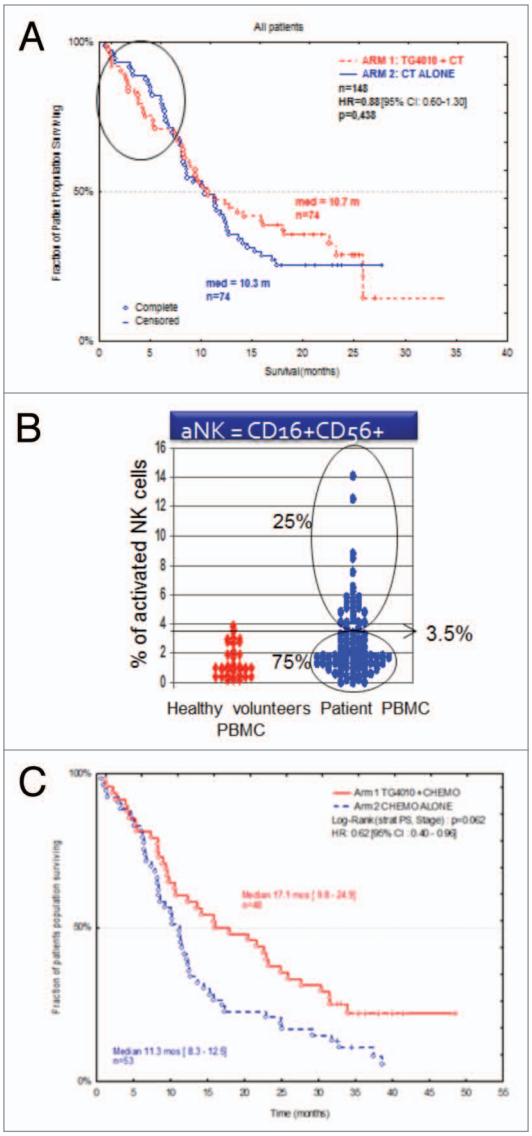

Figure 3. Survival response of patients with previously treated IIIB/IV NSCLC receiving TG4010 correlated with normal circulating activated NK (aNK) levels.[6] (**A**) Analysis of the whole population (n = 148) who received TG4010 revealed no survival difference. (**B**) However, 25% subset population was identified with abnormally elevated circulating activated NK cells (aNK). (**C**) Analysis of survival excluding patients with aNK cells revealed significant advantage in patients with normal circulating aNK cells who received TG4010.

Table 3. FANG Vaccine Transgene Expression and Knockdown Effect During Phase I Trial (n = 42).

	Pre-	Post-
(a) GMCSF (pg/106 cells/mL	7.3	1,108
(b) Furin		↓90.7%*
(c) TGFβ1		↓93.5%
d) TGFβ2		↓92.5%

* Subset population of 20.

providing targeted immunologic anticancer attack. TG4010 is another DNA based vaccine that expresses MUC1 antigen in combination with an expressive human interleukin-2 (IL-2) DNA sequence constructed into a modified vaccinia virus. A recent phase II study was conducted to evaluate the immune response induced by this vaccine in advanced stage NSCLC patients.[5] Sixty-five patients were randomized into 2 arms and treated until disease progression. Arm 1 involved 44 patients who received TG4010 combined with chemotherapy upfront, and TG4010 monotherapy was administered to 21 patients in arm 2. There were no significant toxic events observed. In the 37 evaluable patients, all experienced a MUC-1 specific cellular response. The OS for arm 1 was 12.7 mo and it was 14.9 mo for arm 2. One-year survival was 53%. In a follow-up randomized study of 148 patients, TG4010 was administered SC weekly for 6 weeks with and without chemotherapy.[6] Assessment of immunologic biomarkers revealed a 25% subset population with significantly increased circulating activated NK cells. Comparison of patients who received TG4010 with normal (low levels) circulating NK cells to similar patients with high NK cell levels receiving standard doublet chemotherapy revealed survival advantage to patients receiving TG4010 plus chemotherapy in correlation only with those patients with low activated NK levels (**Fig. 3**). These results fulfilled requirements necessary to initiate further phase III testing targeting only the low NK group.

FANG

In order to further expand upon lessons learned for prior immune stimulating approaches, we tested a dual expressive vector containing human GMCSF DNA with a novel bifunctional RNA interference technology[7] targeting furin. Furin is a proprotein convertase which upregulates both TGFβ1 and TGFβ2, potent cancer produced immune inhibitors. Activity of Lucanix and TAG are limited to TGFβ2 knockdown; however, TGFβ1 is the predominant immune inhibitor produced by most tumor cell populations. Knockdown of the direct target (furin) and the key downstream effector targets (TGFβ1, TGFβ2) was effective (Table 3).[8] Moreover, dual functions of GMCSF expression and knockdown of both TGFβ1 and TGFβ2 proteins were consistent and within predicted guidelines when expressed. No significant toxic effect was observed and suggested survival

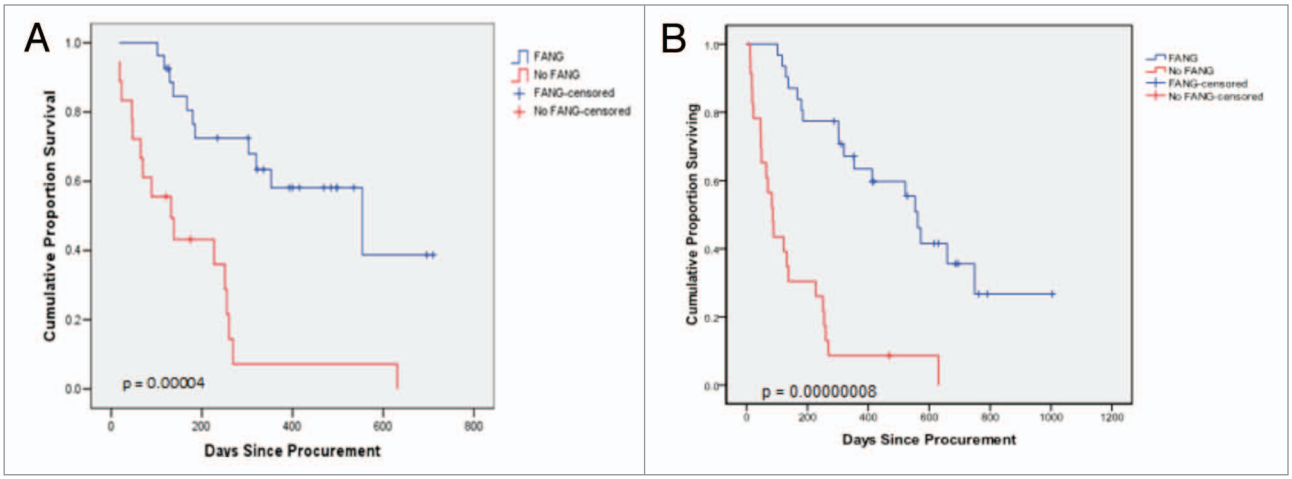

Figure 4. (**A**) Survival of advanced metastatic previously treated cancer patients receiving FANG compared with those with constructed vaccine not receiving FANG as previously published[8] based on analysis done 7/7/11. (**B**) Recent follow up on 4/26/12 revealed continued survival difference and greater than expected survival compared with historical metaanalysis of phase I trial patients.[9]

advantage was demonstrated between later stage patients receiving FANG and those electing to choose other standard of care options (**Fig. 4**).

The observed survival different between FANG and No FANG is encouraging but these are not randomized results and patients treated were not a uniform population. Nevertheless, the median survival with most recent follow-up of 522 d extending out over 1,000 d is quite impressive and compares favorably

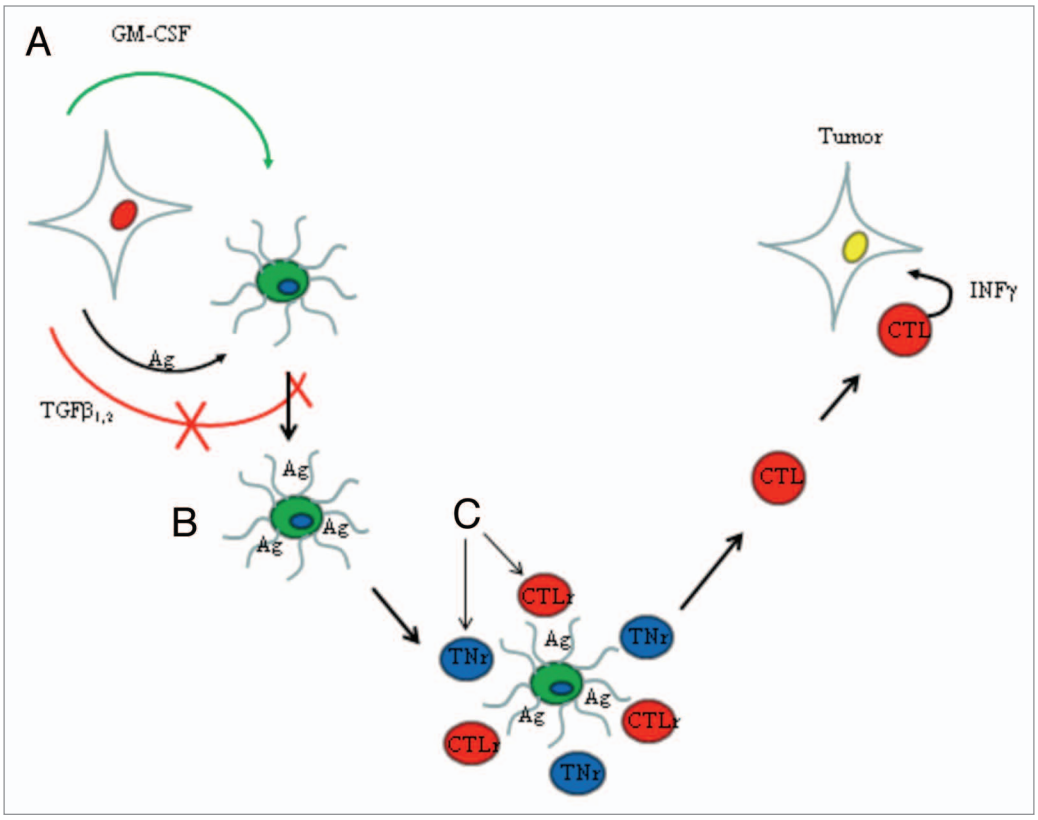

Figure 5. Methods of providing relevant tumor antigens (**A**), immune function enhancement (i.e., GMCSF) and inhibition of cancer produced immune inhibitors (i.e., TGFβ1, TGFβ2) appear to be successful in demonstrating preliminary enhancement of dendritic cell response (**B**) and enhancement of circulatory tumor targeted activated T-cells (**C**), as measured by ELISPOT assay.

to observed survival of 264 d. A recent previously published analysis at MD Anderson involving 182 phase I trial participating advanced solid tumor patients observed a median survival of only 9 mo.[9] However, more importantly, blinded comparison within the FANG treated group of patients distinguishing ELISPOT positive induced patients from ELSIPOT negative patients revealed statistically significant survival advantage of the ELISPOT positive induced group. Randomized phase II testing in frontline ovarian cancer is now ongoing.

Conclusion

Hypothesis of combining relevant cancer antigen stimulation with methods to enhance immune function and/or to reduce cancer produced immune inhibition appear on target (**Fig. 5**). Several phase III trials are either recently completed (waiting for database maturity) or are ongoing. Preliminary biomarker assessment based on key assays measuring immune function suggest clinical relevance (i.e., aNK function, ELISPOT response to autologous tumor). Over the next three years results will be known from randomized trial assessment.

Financial and Competing Interests Disclosure

The author cofounded Gradalis, Inc. and is a shareholder. Gradalis has in development a novel bifunctional RNA interference technology.

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