Cytokine production by intratumorally administered activated dendritic cells correlates with survival in a Phase I clinical trial

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ABSTRACT

Background Activated, autologous dendritic cells (aaDC) can be used to induce anti-tumor immune responses. A unique method of applying aaDC is through intratumoral injection, where the tumor cells serve as the source of antigen required for an adaptive anti-tumor response. A local effect may also occur as a result of cytokine production by the injected DC which makes the tumor more susceptible to a pre-existing or an induced immune attack.

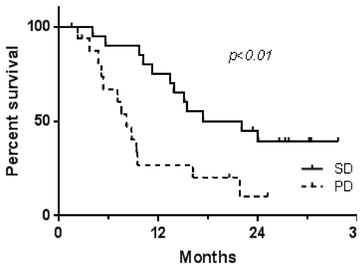
Methods Forty patients with locally advanced or metastatic solid tissue cancers were treated in a dose escalation trial in which aaDC were injected percutaneously under image guidance into a single tumor. Subjects had a median of 3 tumors (range 1 – 5) and had received an average of 3.1 prior treatment regimens. To generate the aaDC, autologous monocytes were converted *ex vivo* into DCs which were then activated. All batches of DCs were released based on pre-specified criteria which included immunophenotyping and a T cell-stimulation assay, as well as sterility and endotoxin levels. Cytokine levels produced by the activated DCs during manufacturing were measured and patient outcomes were correlated to these expression levels.

Results All three doses levels were well tolerated. The main adverse events related to treatment were grade 1 and 2 fevers. Twenty-one patients achieved stable disease (SD) 8 weeks after initiating treatment, and this was found to correlate with survival. Levels of certain cytokines, such as such IL-8 and IL-12 p40, and TNFα were substantially elevated *in vitro* and IL-8 and IL-12 p40 production were predictive of survival). TNFα levels also correlated with SD at week 8. More than 70% of patients tested were found to have significant T cell responses, and/or *de novo* or significantly enhanced PD-L1 expression in the tumor post treatment, with a trend towards improved survival.

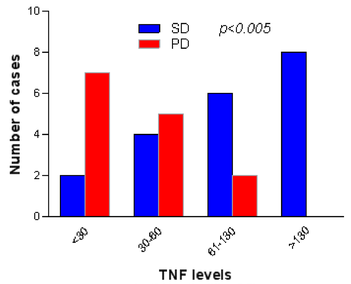
Conclusions Study outcomes such as stabilization of disease and survival correlated with high DC cytokine levels, in the absence of meaningful toxicity. The DCVax treatment may be mediated through direct cytotoxic effects, as well as modulation of the tumor microenvironment to increase tumor infiltration by T cells, and attraction of inflammatory cells to the tumor. The development of PD-L1 expression likely reflects an induced immune response.

Stable Disease and Survival

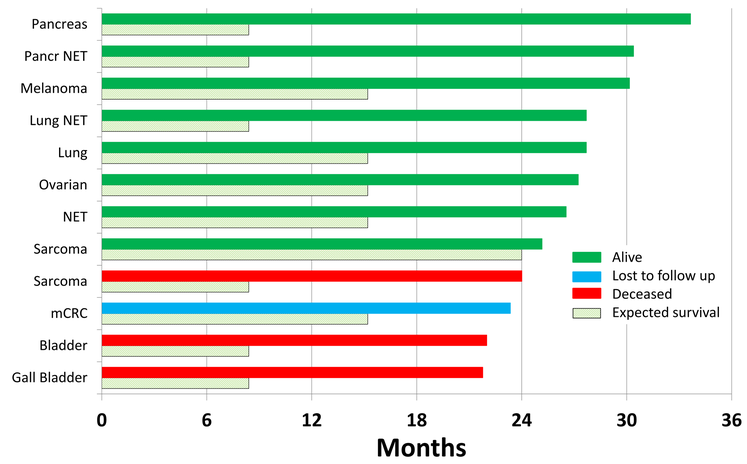
SD at week 8 correlates with survival



TNFα production by the DCs correlates with SD at week 8



Top 30% of patients
Actual vs. Expected Individual Survival*



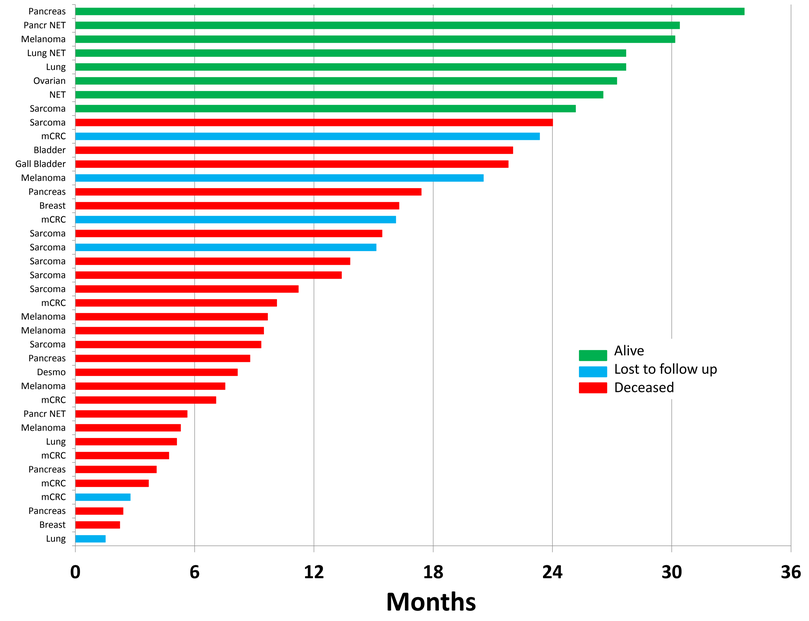
Wheler et al. (2012)* provides a basis for determining individual life expectancies for each individual cancer patient, based upon clinical experience with 1,181 patients with diverse cancers at the MD Anderson Cancer Center Phase I clinic. These individual patient assessments provide more specific information and comparisons than median survival measures based on groups of patients, and can be especially useful in exploratory early stage trials.

Using the Wheler et al. system, individual life expectancies were determined for the top 30% of the patients in the DCVax-Direct Phase I Trial, who have lived ≥23 months to date. Under the Wheeler system, 1 – 5 risk factors were assigned to each patient. The 5 risk factors are measures of serum albumin, serum LDH, number of metastases, GI tumor, and ECOG performance status. Individual patient life expectancy is driven by the number of risk factors for that particular patient, as follows: the median expected survival is 24.0, 15.2, 8.4, 6.2, and 4.1 months for patients with 0, 1, 2, 3, and 4 or 5 of the above risk factors, respectively. The individual life expectancies of these DCVax-Direct patients are plotted in the graph as the shaded horizontal bars, whereas the actual survival is plotted as solid horizontal bars.

Average expected survival for these 12 patients is 12.3 months, and average actual survival time to date is 26.7 months, for a difference of 14.4 months.

**Wheler et al. 2012: Survival of 1,181 Patients in a Phase I Clinic: The MD Anderson Clinical Center for Targeted Therapy Experience. Clin. Cancer Res. 2012 May 15; 18(10): 2922–2929.*

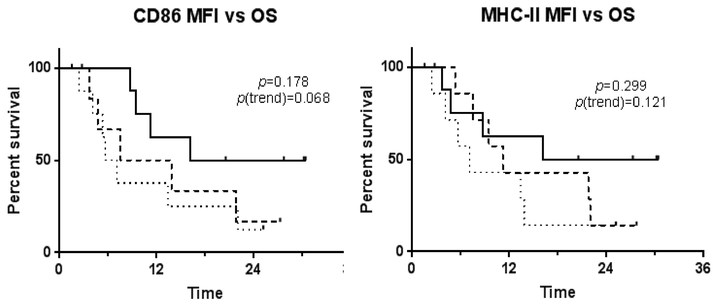
Overall Patient Survival



Conclusions

- Activated DCs can be safely administered into the tumor, in patients with multiple unresectable tumors and different cancer types
- A meaningful proportion of patients show long term survival >24 months
- Early T cell infiltration demonstrates modulation of the tumor microenvironment by the injected DCs to allow influx of pre-existing anti-tumor T cells
- Later T cell infiltration, coupled with the emergence of shared TCR sequences between tumor and peripheral blood, demonstrates induction of a systemic anti-tumor immune response
- Functional staining of infiltrating T cells for IFNγ reveals cytokine production by these cells, which is indicative of cytotoxic T cell activity
- Induction of PD-L1 in tumor tissue in response to DCVax-Direct indicates the potential for combination therapy with immune checkpoint inhibitors
- DC quality, defined either phenotypically or by the production of soluble factors, is predictive for survival
- The noted correlations between cytokine/chemokine production and survival supports the hypothesized mechanisms of action of DCVax-Direct:
  - Direct killing of tumor cells
  - Changing the tumor micro-environment to become more conducive to immune activities
  - Inducing anti-tumor T cells to initiate tumor cell killing
- DC-produced cytokines such as TNFα may be directly responsible for mediating tumor control in patients treated with DCVax-Direct

DC Phenotype and Survival



DC-Produced Cytokines and Survival