Cytokine Production by Human Dendritic Cells when Administered Intratumorally Correlates with Clinical Outcome in Subjects with Advanced Cancers

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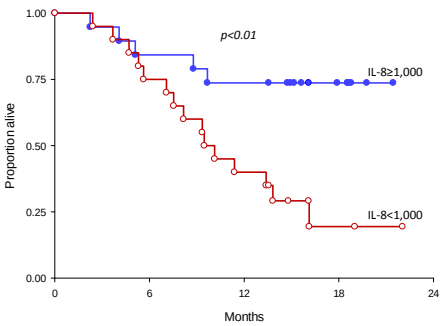
Background Dendritic cells (DC) are proficient in initiating adaptive immune responses, through the uptake and subsequent presentation to the immune system of antigenic compounds. In preclinical studies, activated DC (aDC; DCVax®-Direct) were shown to be superior to immature DC in clearing tumors from mice, upon intratumoral injection.

Methods Forty patients were enrolled in a Phase I dose escalation trial to test the safety and feasibility of intratumoral injection of aDC in solid tumors. aDC were administered intratumorally under image guidance, at a dose of 2 million, 6 million, or 15 million live, activated, autologous DC per injection. At each injection visit (days 0, 7, 14, then weeks 8, 16 and 32), a single lesion was injected. To prepare the aDC for intratumoral injection, they were activated through exposure to BCG and IFNγ. Supernatants from activated DC were collected to measure cytokine production. Tumor biopsies were assessed for tumor necrosis and for infiltrating lymphocytes. Tumor size was monitored through standard imaging procedures, and blood was collected for immune monitoring

Results Intratumoral injection under image guidance was generally well tolerated and feasible. In total, 149 i.t. injections were performed, in 17 patients at the 2 million, 20 at the 6 million, and 3 at the 15 million dose level, with fevers as the most frequently observed adverse events. Biopsies of the injected tumors showed appearance of tumor necrosis in 62%, and T cell infiltrates in 54%. Stabilization of disease was observed in more subjects treated with aDC that produced high levels of TNF ($p<0.01$). Survival is likewise associated with high production levels of TNFα, IL-6 and IL-8.

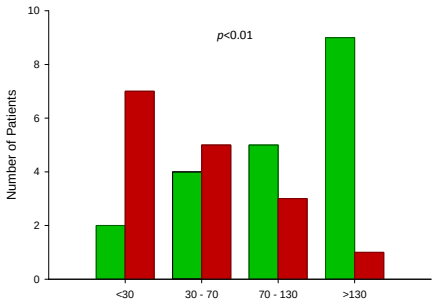
Conclusions Intratumoral injection of autologous, activated DC is feasible without significant toxicity in multiple solid tumors, and can elicit local and systemic immune responses. Clinical outcomes such as stabilization of disease and survival are significantly associated with DC potency measures such as cytokine production *in vitro*.

Interleukin 8 Production and Survival



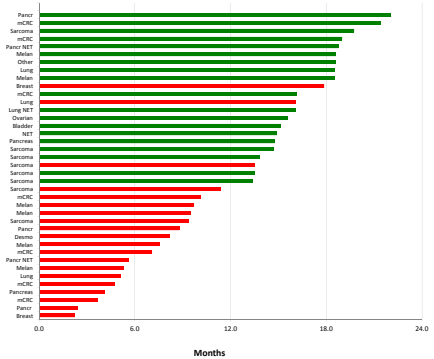
Correlation between IL-8 production, in ng/10^6 DC/24 hours, and overall survival.
In univariate analyses, after exclusion of 3 outliers, both IL-8 (p=0.013) and IL-6 (p=0.048) production are significantly correlated with OS

TNFα Production and Disease Status at Week 8



Correlation between TNFα production by the DC, and disease status at week 8:
Stable Disease (SD) vs. **Progressive Disease (PD)**
In a multivariate analysis, TNFα production correlates with survival (p = 0.02)

Survival of Study Subjects, with Indication



Patient Status as of September 1, 2015. **Green** bars denote patients that are alive, and **red** bars denote patients who have passed away

Phenotype of Activated DC

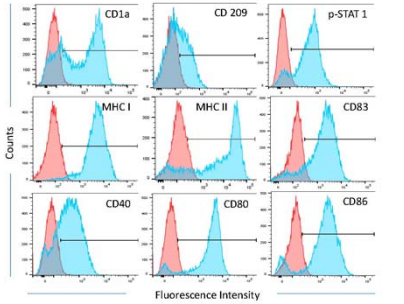
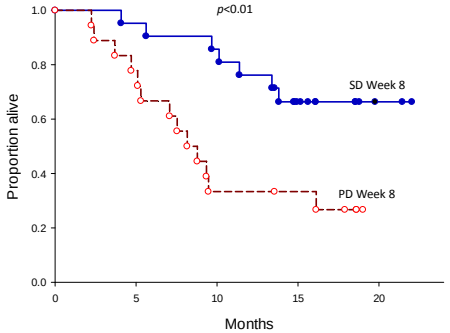


Figure 1. Representative Histograms showing different phenotypic markers of DCVax-Direct product

Expression of **cell surface markers** by the activated dendritic cells

Disease Status at Week 8 and Overall Survival

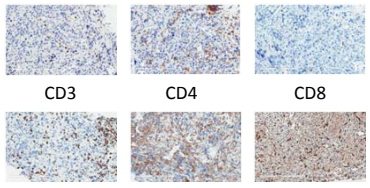


Correlation between disease status at week 8 and overall survival

T Cell Infiltration Following Treatment

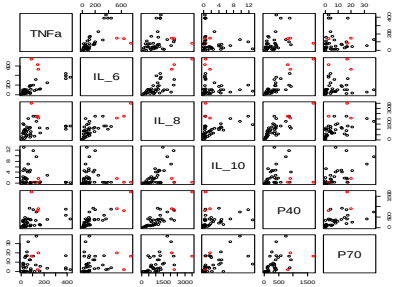
TILs, including both CD4+ helper cells and CD8+ killer cells increased from baseline, either immediately or later, in 15 of 27 assessed patients

Example: clear cell sarcoma



NORTHWEST BIOTHERAPEUTICS

Relationships Among DC-Produced Cytokine Levels



Relationships between levels of cytokines produced by the DC
Suspected outlier subjects are indicated in **red**

Conclusions

- Intratumoral (i.t.) injection of activated dendritic cells is safe and well tolerated
- Clinical outcomes following i.t. injection of activated DC are correlated with DC potency, measured by cytokine production
- Individual cytokines show different associations with clinical outcome parameters, suggesting complex correlations between DC function and possible therapeutic benefit.