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Selective Accumulation Of Attenuated Salmonella In Solid Tumors - A New Anti-Cancer Vector. Mario Sznol, John Mao, Susan MacDonald, and Ivan King. Vion Pharmaceuticals, New Haven, CT, 06511.

VNP20009 is an attenuated *Salmonella typhimurium* with partial deletions of the genes for *msbB* and *purI*. The *msbB* gene deletion results in an altered lipopolysaccharide (LPS) that has reduced capacity to stimulate TNF production in monocytes. The *purI* gene deletion creates a requirement for external sources of purines. In murine models, the LD50 for intravenously (IV) administered VNP20009 compared to wild-type is increased > 10,000 fold. VNP20009 administered IV to tumor-bearing mice effectively colonizes tumors, reaching tumor-to-normal tissue ratios that exceed 300-1000:1. Among normal tissues, spleen and liver contain the highest number of organisms. High tumor to normal tissue ratios are maintained for > 30 days. Furthermore, VNP20009 inhibits the growth of murine syngeneic, murine spontaneous, and human xenograft tumors. Second generation vectors carrying various gene constructs have demonstrated improved anti-tumor activity compared to VNP20009. Based on these data, phase 1 trials of the base vector VNP20009 have been initiated in patients with advanced cancer. To date, intra-tumoral administration has been well-tolerated, a maximum tolerated dose has not been reached, and colonization of tumors for at least 2 weeks has been documented in most patients. The IV phase 1 trials are ongoing to determine an optimal dose and schedule for colonization of systemic tumors. VNP20009 has not been detected in stool or urine of patients treated with either the intra-tumoral or IV route, and clearance from blood in humans is rapid. In conclusion, attenuated *Salmonella* represent a new vector with innate anti-tumor activity in animal models and the potential to deliver gene-based products to the tumor micro-environment.

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Preclinical and Clinical Studies with GM-CSF Transduced Tumor Vaccines. Bernard A Fox. Earle A. Chiles Research Institute, Robert W. Franz Cancer Research Center, Oregon Health Sciences University and Providence Cancer Center, Portland, OR USA 77213

Recently we reported that the failure of vaccination to induce antitumor immunity was not due to the absence of an immune response but due to the development of an inappropriate (IL-4 / type 2) immune response. This led us to hypothesize that a tumor-specific type 1 cytokine response (IFN-γ / TNF-α) is critical for T cell-mediated tumor regression. We have monitored patients from a phase I/II autologous tumor vaccine/immunotherapy trial and identified the development of tumor-specific immune responses that are weak or mixed (both type 1 and 2). No patient exhibited a highly polarized IFN-γ or TNF-α response (type 1) to specific tumor. Since our animal models predict that a polarized type 1 response is critical for therapeutic efficacy, the lack of a strong type 1 response may explain why this strategy failed to mediate objective clinical responses. Our preclinical studies suggest that to augment priming and polarization of a type 1 antitumor response, a GM-CSF secreting autologous tumor cell vaccine is optimal. We have shown that this strategy is effective in animals with significant tumor burden and eliminates a requirement for CD4 help in the priming of therapeutic immunity. Since tumor-specific CD4 help may be reduced in patients with cancer, this represents another advantage of GM-CSF-modified tumor vaccines. Based on these studies we participated in a multicenter phase I/II trial of autologous GM-CSF gene-modified cancer vaccines (GVAX) in subjects with non-small cell lung cancer (NSCLC). Three of 23 stage III/IV NSCLC patients experienced a CR. Current studies are examining the antitumor immune response of these patients.

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MESENCHYMAL STEM CELLS (MSC) AS CARRIERS OF BIOLOGICALLY ACTIVE MOLECULES IN CANCER AND LEUKEMIA THERAPY.

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MSCs are non-hematopoietic cells that reside in the bone marrow. They can be isolated, expanded ex vivo, and retransplanted. We here investigate the ability of MSC to expand and home and to deliver therapeutic molecules for the therapy of chronic myeloid leukemia (CML). MSC from normal and CML donors were expanded ex vivo, and infected with an adeno-associated virus expressing either CMV-driven INFα or progesterone regulated INFα. MSC infected w/10^5 genome equivalents of AAV-CMV INF expressed over 10,000u/ml of INFα. MSC infected with the regulated system (but not induced) expressed < 150u/ml; 24hrs after induction w/10^{-7}, 10^{-8}, or 10^{-9}M mifepristone (MFP), 5000u, 6500u, 4800u/ml of INFα was measured. IFNα produced by MSC resulted in upregulation of MHC Class 1 antigens on CML patient samples, and in inhibition of growth of K562 and BV173-CML cell lines. One 18-hr exposure of MSC to MFP resulted in a 12-day expression of high levels of IFNα, before levels decreased, and could be reactivated to the same level with another exposure. Hence, this concept has promise as a novel therapy for CML, with IFNα being delivered by MSC into the bone marrow. In a second approach, we demonstrate that MSC conbribute to stroma formation of solid tumors and that IFNβ produced locally by MSC can arrest tumor growth.