Phase II CALM study: Changes in the tumor microenvironment induced by the immunotherapeutic agent Coxsackievirus A21 delivered intratumorally in patients with advanced melanoma

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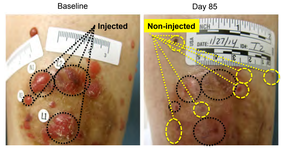
Introduction

CAVATAK, an oncolytic immunotherapy, is a bio-selected oncolytic strain of Coxsackievirus A21 (CVA21). Following intratumoral (IT) injection, CVA21 preferentially infects ICAM-1 expressing tumor cells, resulting in viral replication, cell lysis, and a systemic anti-tumor immune response. Preclinical studies have confirmed enhanced antitumor activity using a combination of intratumoral CVA21 and PD-1 or CTLA-4 blockade. The Phase II CALM study investigated the efficacy and safety of IT CVA21 in pts with advanced melanoma. In a preliminary analysis of response, the primary endpoint of the study was achieved with 22 of 57 (38.6%) evaluable pts displaying immune-related PFS (irPFS) at 6 months. Preliminary analysis of secondary endpoints showed: median irPFS of 4.2 months (95% CI 2.8, 8.3), 1-year survival 75.0% (36 of 48 pts), on-going best objective response rate of 28.1% (16 of 57 pts), median time to response 2.8 months. Responses were observed in both injected and uninjected melanoma metastases (Figure 1). In further support of the generation of CVA21-mediated immune anti-tumor activity was the identification a possible novel serum cytokine signature of elevated levels of IL-8 and γ-IFN in treated patients linked to systemic tumor response (Figure 2). IL-8 is known to be secreted by activated macrophages, while γ-IFN is produced by stimulated cytotoxic T-cells / NK cells. Here we report on a continuation study aimed at further understanding the immune-mediated effects of CVA21 replication within the tumor micro-environment.

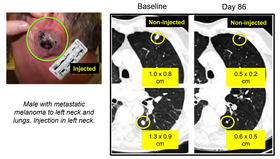
Figure 1: Examples of injected and uninjected responses in advanced melanoma patients receiving multi-intralesional doses of CVA21

Pt 12-002: Local injected and non-injected lesion responses



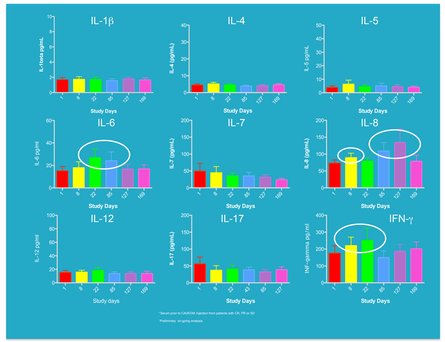
Male with metastatic melanoma to the leg. Injection in leg lesions .

Pt 03-032: Non-injected distant visceral lesion response



Male with metastatic melanoma to left neck and lungs. Injection in left neck.

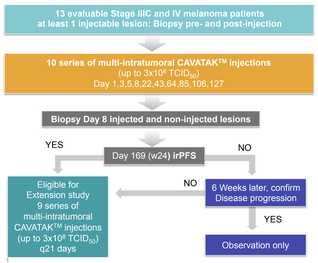
Figure 2: Preliminary analysis of serum cytokine levels following multi-intralesional CVA21 dosing in patients with advanced melanoma



Study Design

The primary objective is to elucidate the nature of the systemic anti-tumor responses, a CALM study extension cohort of 13 pts will receive up to 3 x 10^8 $TCID_{50}$ CVA21 IT on study days 1,3,5 and 8 and then every three weeks for a further 6 injections. Sequential tumor biopsies of both injected and non-injected lesions will be monitored for levels of viral replication and evidence of viral-induced immune activation within the tumor micro-environment. Serial serum samples are being monitored for viral loads, anti-CVA21 neutralizing antibody (nAb) and levels of immune-inflammatory cytokines

Phase 2: CALM study extension cohort design
(CAVATAK in **L**ate **S**tage **M**elanoma)



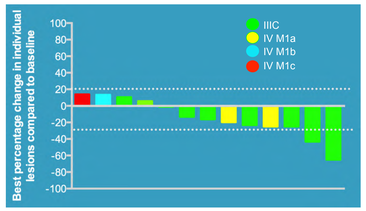
Biopsy Analysis

- Multi-spectral IHC, CD3,CD8,CD163, PD-L1, Melanoma antigen
- Nanostring gene expression using immuno-profiling panel
- CVA21 viral protein IHC

Preliminary Data

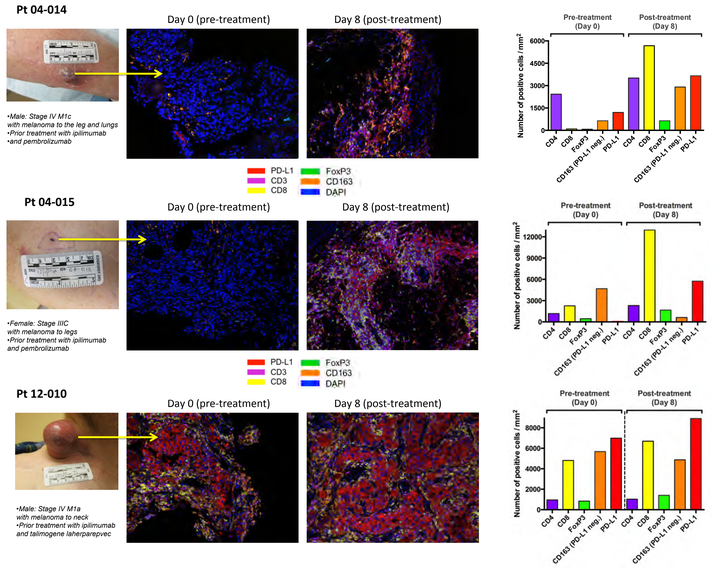
Active tumor-specific cytolytic viral replication is postulated to contribute to the generation of systemic anti-tumor immune responses. Normal kinetics of CVA21 decay would lead to complete viral clearance from the circulation around 24-30 hrs post-viral administration. Preliminary serum testing of CALM study extension patients (n=13) for CVA21 load by viral-RNA RT-PCR serum CVA21 revealed that at 48 hrs post-IT injection on treatment days 1 and 3, 46.2% of pts respectively possessed circulating CVA21, indicating possible tumor-specific cytolytic viral replication and detection of progeny virus. Detection of persistent serum CVA21 levels reduced to approx. 8% of pts at study day 8, a time where significant levels of anti-CVA21 neutralising antibodies started to develop.

Figure 3: Current analysis: Best percentage change in target lesions (Investigator assessed)

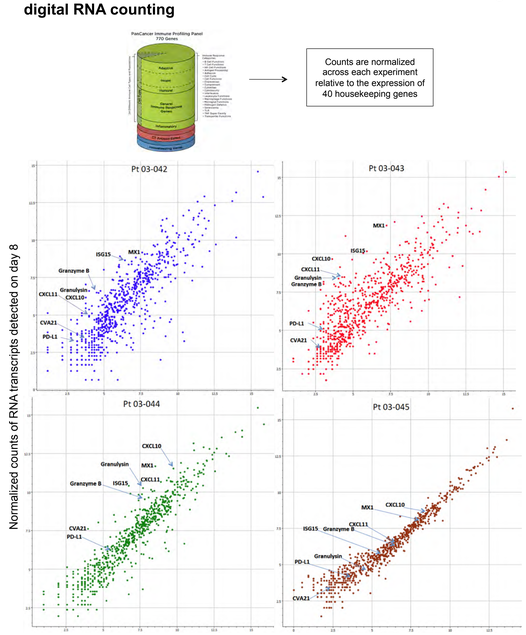


Preliminary Data

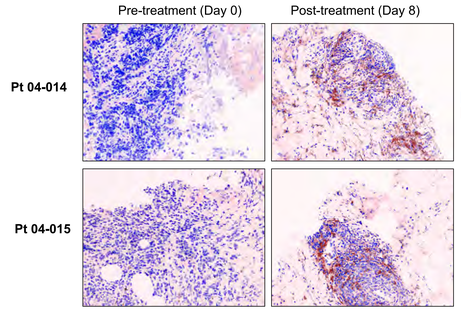
Coxsackievirus A21-induces immune cell infiltration in the micro-environment of melanoma lesions: Multi-spectral analysis



Coxsackievirus A21-induces up-regulation of immune response genes in the micro-environment of melanoma lesions: NanoString digital RNA counting



Coxsackievirus A21 increases the number of PD-L1 expressing cells in the micro-environment of melanoma lesions: Immuno-histochemistry



Conclusions

- Intratumoral administration of CVA21 induces multi-cycle viral replication releasing detectable levels of systemic progeny virus in approximately 50% of patients
- CVA21 replication within the tumor microenvironment induced increases in immune cell infiltrates and expression of PD-L1 as assessed by multi-spectral imaging
- Analysis of a number of pre- and post-treatment biopsy samples by NanoString digital RNA counting identified sizable up-regulation of a number of immune modulation elements, including Interferon-induced 17 kDa protein, Interferon-induced GTP-binding protein Mx1, Granulysin, Granzyme B, Perforin, CXCL10 and CXCL11
- Oncolytic and immunotherapeutic activities of CVA21 warrant further clinical evaluation of intratumoral delivery of CVA21 in combination with immune checkpoint inhibitor strategies (anti-CTLA-4, anti-PD-1 or anti-PD-L1).

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