CAVATAK, an oncolytic immunotherapy, is a bio-selected oncolytic strain of Coxsackievirus A21 (CVA21). Following intratumoral (I.T.) injection, CVA21 preferentially infects ICAM-1 expressing tumor cells, resulting in viral replication, cell lysis, and a systemic anti-tumor immune response. The Phase II CALM study investigated the efficacy and safety of I.T. CVA21 in patients with advanced melanoma with durable responses being observed in both injected and non-injected melanoma metastases, suggesting the generation of significant host anti-tumor responses. Pre-clinical studies in an immune-compotent mouse model of melanoma revealed that combinations of intratumoral CAVATAK and anti-PD-1 or anti-CTLA-4 mAbs mediated significantly greater antitumor activity and compared to use of either agent alone. Here we report on an extension study aimed at understanding the impact of CVA21 on immune infiltrates and immune checkpoint molecules within treated lesions of advanced melanoma patients.

Coxsackievirus A21 injection up-regulates in interferon-induced genes and immune checkpoint molecules within the micro-environment of melanoma lesions

**Study Design**

- **Progression**
- **Disease control**

Levels of lesion T-cell infiltrates: Multispectral images obtained and enumerated with PerkinElmer Vectra imaging system and InForm software.

**Conclusions**

- CVA21 treatment facilitated notable changes within the tumor microenvironment by inducing increases in immune cell infiltrates (CD3+CD8+) and expression of PD-L1, in particular within lesions displaying stable disease or response.
- CVA21 treatment induces a Th1 gene shift, with increases in interferon-induced genes.
- CVA21 treatment notably up-regulates many immune checkpoint inhibitory molecules in injected melanoma lesions, including CTLA-4, PD-L1, LAG-3, TIM-3 and IDO.
- CVA21 induced up-regulation of CD122 may potentially increase the clinical activity of anti-CTLA-4 blockade in advanced melanoma patients.
- In general, CVA21 injection appears to facilitate more widespread up-regulation of immune checkpoint inhibitory molecules than immune checkpoint stimulatory molecules.
- Up-regulation in immune cell infiltrates and/or immune checkpoint inhibitory molecules in CVA21-treated lesions as early as 7 days post initial viral administration may be predictive of future tumor response.

**Future Directions**

- Clinical evaluation of the activity of intratumoral injection of CVA21 in combination with systemic administration of ipilimumab in patients with unresectable melanoma is currently underway (Phase Ib MITCl study: ClinicalTrials.gov Identifier:NCT02307149).
- Clinical evaluation of the activity of intratumoral injection of CVA21 in combination with systemic administration of pembrolizumab in patients with unresectable melanoma is currently underway (Phase Ib CAPRA study: ClinicalTrials.gov Identifier:NCT02565992).
- CVA21 treatment may be used to reconstitute the immune cells within the tumor microenvironment of cancers that currently respond poorly to immune checkpoint blockade (ie, melanoma liver metastases, colorectal and prostate cancers).