CD28 shedding is a novel immune-regulatory mechanism found in cancer patients which directly inhibits anti-PD-1 effect

**Background**

The interaction between the CD28 receptor and its B7 ligands has a crucial role in the induction of a patient and persistently tumour T cell response towards effective immune surveillance and control of tumour growth. Although PD-1 and CTLA-4, antibodies that directly downregulate CD28 activity, are the mainstays of current anti-immunosuppressive therapy, CD28 shedding has been recently shown to be a novel mechanism in the regulation of CD28 activity. Understanding the mechanisms behind CD28 shedding will lead to a better understanding of the regulation of CD28.

**A.** CD28 shedding is uniquely sensitive to H&N patient PBMC γ

**B.** Urothelial carcinoma patient IL-6 subjects. NR- healthy subjects. 40 plasma samples with low to high soluble CD28 levels were assayed for CRP and IL-6 levels with specialized ELISA.

**C.** This regulatory mechanism occurs in the setting of cancer as aberrant levels of soluble CD28 were found in the plasma of cancer patients, where its decreased plasma levels of soluble CD28.

**D.** Elevated levels of soluble CD28 were found in 15% of plasma samples from cancer patients across various tumor types. A variety of T cell stimulations in the need for better understanding of the regulation of CD28.

**E.** Indeed, the most effective immune oncology treatments target CTLA-4 and PD-1, proteins that directly downregulate CD28 activity, further emphasizing that CD28 shedding is a novel immune-regulatory mechanism found in cancer patients which directly inhibits anti-PD-1 activity.

**Model describing the immuno-suppressive effect of CD28 shedding**

**Soluble CD28 attenuates anti-PD-1 immunostimulatory effect**

**Soluble CD28 dynamics relates with response to PD-1 pathway therapy**

**BION-206 - An efficient VHH antibody that blocks the shedding of CD28 receptor**

**Conclusions**

- We describe a novel mechanism in the CD28 pathway involving the proteolytic shedding of CD28.
- CD28 shedding occurs upon T cell activation and causes the release of a dimeric soluble CD28 decoy receptor.
- Soluble CD28 inhibits T cell activity and counters anti-PD1 activity.
- High levels of sCD28 were found in cancer patients’ blood, which were associated with response to PD-1 blockade.
- A lead VHH antibody has been characterized and shown to bind specifically to CD28’s proteolytic site, blocking CD28 cleavage with no agonistic or agonistic activity.