

## **A056 Highly Efficient Single-Domain Antibody Neutralization of Interleukin-6, the Factor at the Epicenter of Cytokine Storm in Acutely Ill COVID-19 Patients**

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**Introduction:** COVID-19 is a worldwide medical emergency with high mortality. Cytokine storm (CS) has been attributed as the major cause of mortality in COVID-19 patients; in CS, the uncontrolled increase in pro-inflammatory cytokines especially IL6 results in an influx of immune cells leading to progressive tissue destruction and multi-organ failure. Use of clinically available IL6 receptor (IL6R) inhibitors have shown only modest benefit in acutely ill COVID-19 cases due to need for high doses & associated side effects. More efficacious and safer agents to targets IL6 are thus urgently needed. We report generation of a potent camelid single domain antibody (sdAb) that robustly neutralizes human IL6 (hIL6). **Methods:** Two alpacas were immunized with purified hIL6 & a sdAb display phage library was prepared. The library was enriched by panning for sdAbs recognizing hIL6. One group of closely related sdAbs was identified, expressed as soluble proteins, that displayed sub-nM affinity for hIL6. These sdAbs were found to neutralize hIL6 with low pM potencies *in vitro* assays. The most potent sdAb was produced using CHO cells as a homodimer. The homodimer was also encoded in an alphavirus-based replicon RNA (repRNA) & formulated with a Lipid InOrganic Nanoparticle (LION). The homodimer protein & repRNA were tested *in vitro* and *in vivo*. **Results:** HEK293 cells transfected with STAT3-luciferase were treated for 6h with 50ng of hIL6 alone or hIL6 co-incubated 1h with 100ng of sdAb. A complete abrogation of reporter activity was observed in the sdAb group. To mimic CS, adult mice were challenged intraperitoneally (IP) for 30 min with 1mg of hIL6 alone or with sdAb, ranging from 0.25-4mg. hIL6-induced Y705-STAT3 phosphorylation in liver was completely abolished by sdAb dimer at concentration  $\geq 0.5$ mg, an effect not seen even with 500mg of IL6R Ab. Mice were intramuscularly injected with PBS or 40mg LION-formulated hIL6 sdAb dimer repRNA and given 100ng of hIL6 IP for 30m at 5 days post injection. LION-injected group showed complete absence of hepatic Y705-STAT3 in response to hIL6. **Conclusions:** The novel humanized IL6 sdAB efficiently inhibited hIL6-induced hepatic STAT3 activation and may have potential as a therapeutic for acutely ill COVID-19 patients exhibiting CS and other IL6-driven pathologies like subsets of liver cancer. Additionally, using a similar pipeline, we are developing inhibitors of ACE2 and IL-1b, which can be combined for efficacious COVID-19 treatment.