

**Enhancement of expression of a Fab format of a cytotoxic therapeutic protein produced in an *Escherichia coli* expression system; scale-up of process from 15 L development scale to 1200 L production scale for phase I trials.**

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**ABSTRACT**

VB6-845 is a recombinant Fab expressed in *E. coli* using a dicistronic expression unit. The first unit is comprised of the V<sub>H</sub>-C domain containing a H<sub>2</sub>H histidine affinity tag placed at the N-terminus. The second unit is comprised of a cytotoxic bouganin protein linked C-terminally to the V<sub>H</sub>-C domain via a L<sub>2</sub>L furin-sensitive linker. The dicistronic unit is cloned into the pING3302 expression vector under the control of the arabinose-inducible *araBAD* promoter. Both the V<sub>H</sub>-C and V<sub>L</sub>-C -cytotoxin expression units are preceded H<sub>2</sub>H L H by a PelB leader sequence that targets both polypeptide chains into the periplasmic space, and allows the recovery of fully formed and active VB6-845 in the culture supernatant. An initial process was designed at the 15 L development scale to generate material using high cell density cultivation at expression titers of 10 mg/L in the supernatant. Supernatant was harvested using centrifugation and microfiltration, and purified using a six-step purification process. Subsequently, the process was scaled-up to the 1,200 L fermentation level and material generated was used to undertake pre-clinical trials. In preparation of clinical trials, process optimization steps, including modifications of the coding region of VB6-845, led to a 10-fold increase in titers in the supernatant. The process was scaled up to the 1,200 L fermentation scale, including all associated harvesting and downstream process parameters, to produce material for Phase I clinical trials.