

## Fusogenics™: A novel approach to antibody affinity maturation

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### ABSTRACT

Viventia's technology platforms have been designed to produce a high-throughput pipeline of antibodies for development as targeted cancer therapeutics. Our technology isolates antibodies, produced by cancer patients in response to their disease. We in turn use these antibodies to isolate and identify the cognate antigens. In addition, these antibodies are used to engineer fusion proteins to deliver a cytotoxic payload to cancer cells. VB1-011 and VB1-008 are fully-human, antibody-secreting hybridomas generated from peripheral blood lymphocytes (PBLs) of patients diagnosed with a grade II astrocytoma and breast cancer, respectively. Both antibodies demonstrated specific tumor tissue reactivity and limited binding to normal tissue. CD44E and a variant of scratch protein present on the tumor cell surface were identified as the antigens for VB1-008 and VB1-011, respectively.

Immunotoxins created from the Fab fragments of both antibodies genetically-linked to a de-immunized variant of the plant toxin bouganin (de-bouganin) had binding affinities of  $5 \times 10^{-6}$  M that were 5 to 10-fold lower than the parental antibodies. This resulted in an  $IC_{50}$  of approximately 300 nM. To improve the affinity and hence toxicity of these constructs, libraries were created and screened using Viventia's proprietary Fusogenics™ technology. This approach utilizes small libraries of soluble Fab-toxin fragments that contain hot-spot mutations in the CDR loops and uses *Pseudomonas* exotoxin (ETA) as an indicator of cytotoxicity and hence a surrogate for binding affinity. Optimal clones for each CDR loop were selected using AnnexinV/CentriRed staining measured with the 8200 cellular detection system and used to create an L- and H-chain combinatorial library. Clones with the highest biological reactivity as measured by ELISA and flow cytometry were selected. Subsequently, modeling was performed on both the affinity matured and wild-type Fab-bouganin constructs in order to identify mutated residues that interfere with the level of expression of soluble material. Ultimately, clones that retained the improved binding affinity as well as levels of expression suitable for preclinical development were selected. Affinity-matured Fab-de-bouganin fusion constructs, VB6-011-2D3 and VB6-008-T, showed an increased affinity of at least 10-times that of the wild-type molecule with  $IC_{50}$  values falling in the nanomolar range. Competition experiments with the full-length IgG and TMA staining confirmed that the specificity was preserved during the affinity maturation process. Therefore, the Fusogenics™ platform represents a novel affinity maturation approach that has yielded two fusion constructs that are excellent candidates for pre-clinical development.