Removal of clinically relevant contaminants from cGMP grade preparations of plasmid DNA: implications for non-viral gene therapy and DNA-based vaccines

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Investigators have observed toxicity leading to the death of animals following intravenous (IV) injections of plasmid DNA-liposome complexes at high doses. However, administration of liposomes alone at high doses without plasmid DNA does not cause toxic effects. Thus, toxic effects are related to the combination of plasmid DNA and liposomes. We have identified previously undiscovered polysaccharide contaminants in plasmid DNA, including cGMP clinical preparations that contribute significantly to *in vivo* toxicity. Thus, we developed six different assays for detection and novel methods for removal of the toxic polysaccharides. This further proprietary purification process is termed the Super-Clean DNA process. Our animal testing validates that these contaminants in standard kit and GMP plasmid DNA preparations are responsible for the toxicity observed. Moreover, we have shown that these contaminants adversely affect transgene function and efficacy. In particular, colanic acid contributes to the marked toxic effects and is highly viscous. Colanic acid is tightly intertwined with DNA and must be degraded in order to be effectively removed. We have produced a recombinant, truncated colanic acid degrading enzyme (CAE) that successfully accomplishes this task as demonstrated by reduced viscosity of plasmid DNA after CAE digestion, for example. Standard plasmid DNA preparations can be digested with CAE and further purified. We also showed *in vivo* that Super-Clean DNA-liposome complexes (at least 100 ug of plasmid DNA) can alter mortality from 100% (n= 90) to 0% (n= 120) in a series of five separate studies.

In Conclusion: Super-Clean DNA processing provides a critical advance to the success of clinical development for non-viral gene delivery approaches.