CONTROLLED FORMATION OF CPG OLIGONUCLEOTIDE MULTIMERS

Technology Summary

Unmethylated cytosine-phosphodiester-guanine (CpG) oligonucleotides (ODNs) act as immunostimulants to cells that express Toll-like receptor 9 (TLR9), such as B cells, macrophages, dendritic cells, and monocytes. CpG ODNs can be used as an adjuvant to improve a vaccine's humoral and cellular immune responses in a host. CpG ODNs tend to form troublesome G-tetrads in solution during manufacture and storage, leading to polymorphisms, aggregation, and precipitation that limits clinical applications.

FDA researchers developed a platform technology to functionalize a CpG ODN with a thermolytic substituent that prevents undesirable CpG-tetrad aggregation in solution during manufacture or storage, **while alternatively enabling CpG-tetrad formation inside the cell which enhances activity**. Use of FDA's thermolytic substituent technology on an CpG ODN can delay the formation of G-tetrads until they are internalized by their target immune cells where multimerization is required. The effect of modifying an CpG ODN with thermolytic substituents was tested on D35 (Pro-D35 modified with thermolabile substituents), a well-characterized ODN that activates the innate immune system and enhances the T-cell effector mechanism. The thermolytic substituents reduced G-tetrad formation in potassium free solutions, while allowing tetrad formation when potassium salt concentrations mimicked those of an intracellular environment. Pro-D35 did not differ in cellular uptake or biological activity compared to unmodified D35, as shown by increased cytokine production and DC maturation. Pro-D35 demonstrated clinical potential in rhesus macaques challenge studies with *Leishmania major*. The FDA thermolytic CpG ODN technology can be used to develop immunotherapeutic oligonucleotides with improved storage stability and therapeutic efficacy.

Potential Commercial Applications

- Adjuvant
- Vaccine development

Competitive Advantages

- Stabilizes CpG ODNs for manufacture/storage (reduced G-tetrad formation)
- Effective cellular uptake and controlled multimerization
- Demonstrated clinical potential in primate studies

Development Stage: *in vitro* data; *in vivo* data; preclinical primate studies

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Publications:

"Design and development of thermolytic DNA oligonucleotide prodrugs." *Ann N Y Acad Sci*. 2005 Nov;1058:26-38. PMID: 16394123

"Thermolytic CpG-containing DNA oligonucleotides as potential immunotherapeutic prodrugs." *Nucleic Acids Res.* 2005 Jun 21;33(11):3550-60. Print 2005. PMID: 15972797

"Use of thermolytic protective groups to prevent G-tetrad formation in CpG ODN type D: structural studies and immunomodulatory activity in primates." *Nucleic Acids Res*. 2006;34(22):6488-95. Epub 2006 Nov 27. PMID: 17130156

Intellectual Property: US Pat: US 9,809,824 B2, issued 11/7/2017

Product Area: adjuvant; vaccine development

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