



Scan QRCode
to receive the poster!

¹OncoDesign, 25-27 rue du Quebec, F-91140 Villebon-sur-Yvette, France
² DNDi, 15 chemin Louis-Dunan, 1202 Geneva, Switzerland

Development of an immunogenicity assay for Anti-Drug Antibody (ADA) detection directed against an oligonucleotide CpG-D35 using ECL technology (MSD)

Karine Mouchain¹ | Jerome Nugier¹ | Nina Gonzalez¹ | Michael Jegat¹ |
Beatrice Bonnet² | Mouhssin Oufir¹ | Fabrice Viviani¹



1

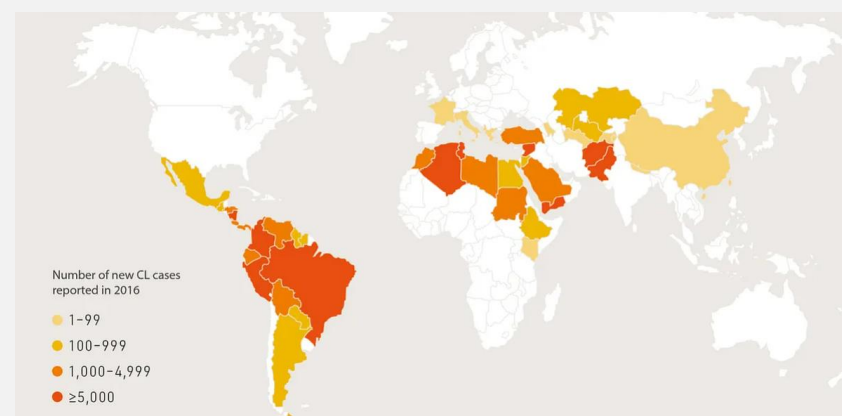


CONTEXT & OBJECTIVES

CpG OligoDeoxyNucleotides (CpG ODN) contain **CpG motifs** that mimic immune stimulation induced by bacterial DNA and stimulate the immune system through **Toll-like receptor 9 (TLR9)**. Thus, they promote activation and/or maturation of antigen presenting cells (APC) and strongly induce the synthesis of IFN- α by activating plasmacytoid dendritic cells.

CpG-D35 is developed by **Drugs for Neglected Diseases initiative (DNDi)** as a combination therapy for the treatment of complicated **cutaneous leishmaniasis (CL)** and **post kala-azar dermal leishmaniasis (PKDL)** impacting 0.6-1.2 M people each year. CpG-D35 has shown encouraging results in the rhesus CL model and DNDi wishes to determine its suitability for progression to phase I clinical trials.

As a part of an IND-enabling preclinical safety package, this study aims to develop an immunogenicity assay for the detection of ADA directed against CpG-D35 using ECL technology (MSD) in Cynomolgus macaque serum samples.



- 600,000-1.2 million people infected every year
- Endemic in 87 countries
- Effectiveness of current treatments can be as low as 50%

2



RESULTS

Production, purification and characterization of the positive control

- Immunization of 4 rabbits with CpG-D35 coupled to BSA / titration of serum by ELISA to follow immune response
- Pooling of bleedings
- Purification: Caprylic acid precipitation / Protein A (Total IgG) / Immuno-purification using a streptavidin agarose resin coupled with CpG-D35
- SDS-PAGE : Integrity and purity calculation of purified antibody

→ Different tests (ELISA and ECLA) have shown that immuno-purified anti-CpG antibodies preferentially recognize BSA (carrier protein directly linked to CpG without linker) which was present both on the plate (used traditionally for saturation) and in the assay buffer. In solution, non-fat dry milk was used as replacement for BSA and gave a better S/N ratio.

Tested assay formats:

1/Bridging ECLA using CpG-D35 conjugated to BSA: The presence of free SulfoTag (ST) induces a non-specific signal increase and consequently a method sensitivity reduction. Moreover, the MW of ST (1.15 kDa) is too close to CpG MW (6.7 kDa) to be removed after conjugation. Thus CpG was firstly coupled to BSA (at 5'-end of CpG) and then BSA-CpG was conjugated to Biotin or ST in a second step.

→ Due to high CpG-D35 aggregation in the coupling media, it was impossible to couple CpG to BSA.

→ As rabbit polyclonal antibodies recognize BSA, this format was no longer possible.

2/Bridging ECLA using CpG-D35 directly conjugated to Biotin and ST: First, biotin was directly conjugated to CpG-D35 during synthesis. In parallel, an NH₂ functionality was added at 5'-end of CpG (= short end of CpG) in order to promote both CpG recognition of the 3'-end and the loop by anti-CpG antibodies (better exposure sites for recognition by ADA and the same structure as the immunogen used to produce anti-CpG antibodies).

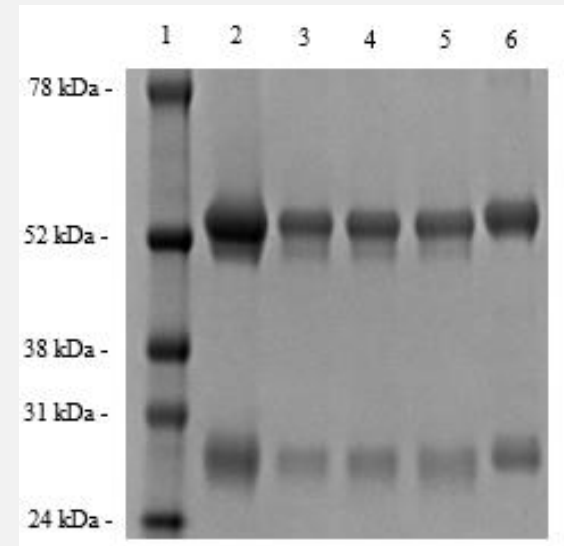
→ Despite optimization of experimental conditions, this format failed to meet regulatory bioanalytical expectations (high S/N ratio) and has been discontinued.

3/Direct binding: One labeled secondary Ab specific to rabbit IgG present in the positive control / one labeled secondary antibody specific to monkey IgG present in serum samples.

Definition of experimental conditions using rabbit positive control:

→ High non-specific interaction of positive control on the MSD streptavidin plate in the absence of CpG-biotin: use of polypropylene plates (ELISA) coated with avidin instead of streptavidin and modification of buffer compositions.

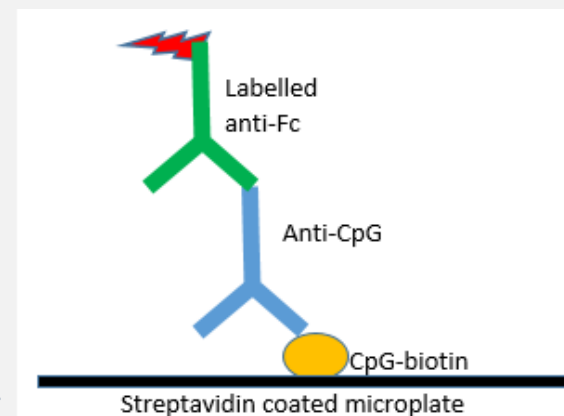
→ Evaluation of experimental conditions previously defined for rabbit positive control on macaque serum samples, but high non-specific binding.



SDS-PAGE in reducing conditions
Lane 1: MW Marker
Lane 2 to 6: different batches of immuno-purified antibodies



Bridging format (www.mesoscale.com)



Direct binding format

3



CONCLUSION

Due to the nature and the MW of this oligonucleotide, conjugation techniques traditionally used for proteins cannot be implemented and free biotin / ST could not be removed easily, which was challenging in the development of the bridging format. The direct binding format does not involve the conjugation of the oligonucleotide but involves the use of species-specific secondary antibodies and is more prone to background noise.

The solutions proposed to overcome these analytical challenges are as follows:

- Use of the same CpG-NH₂ molecule for conjugation to biotin and ST in order to avoid recognition of either of the two entities in the bridging format.
- Evaluation of specific oligonucleotide purification spin columns to remove free biotin and ST, or testing a coupling protocol with an inverted ratio
- Use of another technology (Surface Plasmon Resonance SPR or Single Molecule Counting SMC).