A Long-Acting Exendin-4 Analog Conjugate to the Human Fc Fragment Reveals Low Immunogenic Potential

1009-P

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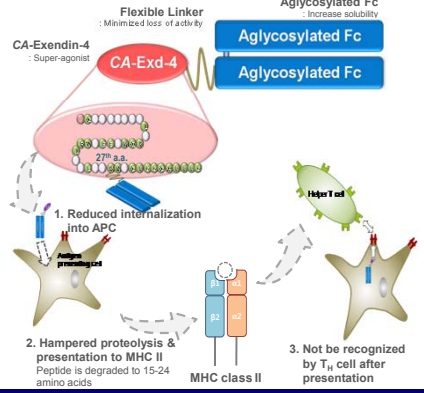
ABSTRACT

Exendin-4 (Exd-4) is a glucagon-like peptide-1 receptor agonist used for the treatment of type 2 diabetes, but it elicits an immune response in 37 to 57% of patients. We have developed an *CA*-Exendin-4 with a single amino acid modification. A long-acting form, named LAPS*CA*-Exendin-4, was developed by conjugation of the human Fc fragment to the 27th amino acid residue of the *CA*-Exendin-4. The Fc carrier conjugation to the middle of the peptide might lower immunogenicity by impeding proteolytic fragmentation of the *CA*-Exendin-4 prior to antigen presentation via MHC class II. In order to predict immunogenicity of LAPS*CA*-Exendin-4 in humans, *ex vivo* CD4+ T cell activation and proliferation assays were performed. LAPS*CA*-Exendin-4 showed positive responses in 6% of the study cohort, which suggests that it may have relatively lower levels of immunogenicity than exendin-4. Further, antibodies against LAPS*CA*-Exendin-4 were assayed in SD rats and monkeys after once weekly administration over 26 weeks. Binding antibodies developed only in 1% of rats and not at all in monkeys. In a phase 2 clinical trial with exposure for at least 3 months (8 weekly or 3 monthly doses), no immune response was detected in patients with type 2 diabetes (n=51). In fact 7.8% of the subjects were identified as antibody positive prior to the first exposure but no increased titers were observed after the treatments. All antibodies were non-neutralizing. These observations suggest that LAPS*CA*-Exendin-4 has a very low potential for immunogenicity in humans.

BACKGROUND

- **Exendin-4 is a well characterized GLP-1 receptor agonist.**
- **Due to the differences in the molecular structure of Exendin-4 compared to GLP-1, the molecule is immunocgenic in humans: 40-60% of patients develop antibodies [1)]**
- **Immunogenicity of LAPS*CA*-Exendin-4 may be lowered by site-specific conjugation of Fc carrier, thereby interfering with proteolysis and MHC II presentation [2)]**



METHODS

- ***In silico* prediction of T cell epitopes**

To identify potential T-cell epitopes of *CA*-Exendin-4, its amino acid sequence was analyzed *in silico* using Immune Epitope Database (IEDB). 39 amino acids were parsed into 15-mer frames overlapping by fourteen amino acids. Percentile ranks for the binding of overlapping 15-mer peptides to the six major HLA alleles were determined.

- ***Ex vivo* T cell activation assays (Antitope limited, UK)**

A cohort of 50 donors was selected to best represent the number and frequency of HLA-DR allotypes expressed in the general population. Test articles were added to the PBMC and incubated for a total of 8 days at 37°C. On days 5, 6, 7 and 8, the cultures were pulsed with [^{3}H]-Thymidine for further 18 hours and counts per minute (cpm) were determined. To identify T cell activation, IL-2 secretion was measured by Elispot assay. Test articles were tested in sextuplicate cultures and for each donor. After an 8-day incubation period, spots per well (spw) were determined.

- **Pre-clinical studies in SD rats and cynomolgus monkeys for 26 weeks**

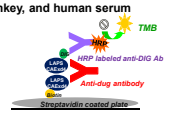
Three doses of LAPS*CA*-Exendin-4 and vehicle administered weekly by subcutaneous injection into SD rats (n=200) and cynomolgus monkey (n=40) for 26 weeks, respectively. These animals were treated for 26 weeks and then allowed a 4-week treatment-free recovery period. For immunogenicity evaluation, serum samples were collected during acclimatization, in week 13 (week 12 for monkey), 19, 26 of treatment, and week 4 of recovery.

- **Phase II repeated dosing clinical trial**

In a double-blind, randomized, placebo-controlled, multiple ascending-dose Phase II study in 6 groups of 12 patients with T2DM each receiving subcutaneous injections of LAPS*CA*-Exendin-4 or placebo in a 3:1 ratio. Eight doses of the weekly regimens 1, 2, and 4 mg, and three doses of the monthly regimens 8, 12 and 16 mg of LAPS*CA*-Exendin-4 were administered. For immunogenicity evaluation, serum samples were collected on day –1 (baseline) and at follow-up for all cohorts and an additional sample was taken on day 57 for weekly cohorts, day 64 for monthly cohorts.

- **Determination of anti-drug antibody in rat, monkey, and human serum**

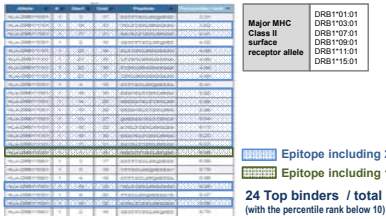
For determination of anti-drug antibodies (ADA), the bridging ELISA method was used. The sensitivity of the rat, monkey, and human is 31, 125, and 34.1 ng/mL, respectively. For determination of neutralizing antibody, the cell based neutralizing antibody assay measuring cAMP level in hGLP-1R/CHO cells was used.



RESULTS

Non-clinical Studies in Low Immunogenicity

Figure 1. *In silico* prediction of T cell epitopes depends on conjugation site



Major MHC Class II surface receptor allele
DRB1*01:01
DRB1*03:01
DRB1*07:01
DRB1*09:01
DRB1*11:01
DRB1*15:01

Epitope including 27th a.a.
Epitope including 1st a.a.

24 Top binders / total 125
(with the percentile rank below 10)

- **Among the top potential T-cell epitopes of *CA*-Exendin-4, 62.5% includes the 27th amino acid. PEG conjugation on internal site might be more effective to lower antigenic potential of *CA*-Exendin-4 compared with N-terminal conjugation.**

Figure 2. *Ex vivo* T cell activation depends on conjugation site

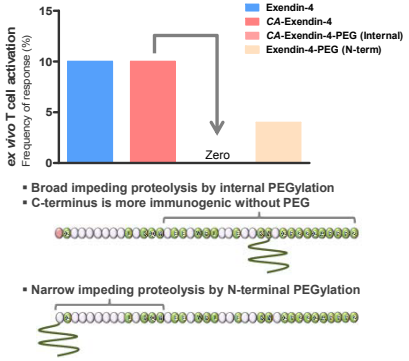


Table 1. Pre-clinical immunogenicity

Antibody status in SD rats at 26 weeks

Group	Dose	n /group	Positive	Time point	% positive	Neutralizing Ab
1	Vehicle	60	0	-	0	0
2	Low dose	40	1	Week 13	2.5	0
3	Mid dose	40	0	-	0	0
4	High dose	60	1	recovery	1.6	0
	Total	200	2		1.0	0

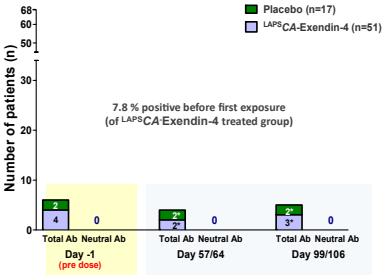
Antibody status in cynomolgus monkeys at 26 weeks

Group	Dose	n /group	Positive	% positive
1	Vehicle	12	0	0
2	Low dose	8	0	0
3	Mid dose	8	0	0
4	High dose	12	0	0
	Total	40	0	0

- **Binding antibodies against LAPS*CA*-Exendin-4 developed only in 1% of rats, and was absent in cynomolgus monkeys at 26 weeks.**

Clinical Studies

Figure 3. Clinical immunogenicity (Phase II repeated dosing study)



* Case that anti drug antibody was confirmed at pre-dose

- **No immune response was detected in patients (n=51) for 3 months. 7.8% of subjects were identified as antibody positive before the first drug exposure, but all are no treatment-emergent antibodies and not neutralizing for LAPS*CA*-Exendin-4.**
- **The reasons for the pre-existing antibodies are unclear.**

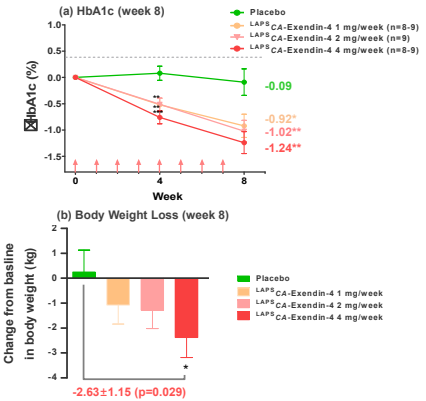
Figure 4. Comparison of GLP-1R agonists in immunogenicity

Immunogenicity of GLP-1R Agonist		
GLP-1 receptor agonist subclass	Exendin-4 backbone	GLP-1 backbone
Immunogenicity (% of patients with antibodies)	Exenatide / Exenatide QW [1] (37% / 57%)	Liraglutide [5] (8%)
	Lixisenatide [3] (60-71%)	Albiglutide [6] (3%)
	LAPS*CA*-Exendin-4[4] (8%)†	

† **All are no treatment-emergent antibodies**

- **LAPS*CA*-Exendin-4 displays very low immunogenicity in humans despite of non-human GLP-1 backbone.**

Figure 5. Efficacy in glucose lowering & body weight loss (phase II repeated dosing study)



- **LAPS*CA*-Exendin-4 showed potent HbA1c reduction with BWL in addition to no immune response.**

CONCLUSIONS

- **Conjugation on internal site of *CA*-Exendin-4 was identified as a key factor lowering immunogenicity of LAPS*CA*-Exendin-4.**
- **Binding antibodies against LAPS*CA*-Exendin-4 developed only in 1% of rats (n=140) and not at all in cynomolgus monkeys (n=28) after once weekly administration over 26 weeks.**
- **In a phase 2 clinical trial with exposure for 3 months no immune response was detected in patients with type 2 diabetes (n=51). 7.8% of subjects were identified as antibody positive prior to the first exposure but no increased titers were observed after the treatments.**
- **These observations suggest that LAPS*CA*-Exendin-4 has a very low potential for immunogenicity in humans.**

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American Diabetes Association's (ADA) 74th Scientific Sessions, San Francisco, CA, USA; June 13-17, 2014

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