Safety of GPI-0100 as Adjuvant When Administered with a Vaccine: A Narrative Review of Clinical Studies

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Abstract

Background - Vaccination is the most effective method of preventing infectious diseases. The immunogenicity of a vaccine can be stimulated by an adjuvant. GPI-0100 adjuvant has shown great promise in mice studies. However, research of GPI-0100 in humans is limited.

Objective - Here the safety aspect of the use of GPI-0100adjuvant from human studies is summarized.

Methods- The Pubmed and Embase databases were searched with the search terms: 'GPI-0100', 'Clinical' and 'Adjuvant'. The criteria for reporting were studies that were done in humans.

Results – The most common local adverse events that were observed in the three studies were mild (grade I) to moderate (grade II) local reactions and mild (grade I) pain at the injection site. GPI-0100 administered at a dose of 3000 µg showed comparable toxicity profile in the three studies. The dose of GPI-0100 appeared to be no cause in increasing numbers of local adverse events.

Conclusion- GPI-0100 is a promising adjuvant that shows little safety concerns and a low threshold of toxicity.

Keywords GPI-0100; clinical; adjuvant

Introduction

Since the first vaccine made by Edward Jenner, vaccination has been the most effective method of preventing infectious diseases (NIAID). Vaccines are most famous for their usefulness against many pathogens and have been used extensively. Besides being used as a prophylactic vaccine, studies have been working on using effective therapeutic vaccines against cancer. However many of these vaccines contain antigens which are weak in immunogenicity. To help stimulating vaccine elicited immune response, adjuvants are being used (Walkowicz. W, 2016). Adjuvants can help vaccines in a numerous of ways. For instance, some adjuvants can function as a delivery system that delivers the antigen into the antigen-presenting cells (APCs) to stimulate the induction of antigen-specific immune responses (Saupe. P, 2006; Moser. C, 2003). Some adjuvants contain e damage-associated molecular (DAMPs) and/or pathogen-associated molecular patterns (PAMPs), such as microbial components, that activate the innate immune cells. This results in the induction of potent innate and adaptive immune responses against the vaccine antigens (Kawai. T, 2010; Takeuchi. O, 2010). Vaccine adjuvants are classified in three classes based on their modes of action: a delivery system, an immunostimulant or a combination of both.

An effective protective immune response against intracellular pathogens requires a successful induction of cell-mediated immune responses or T-cell immunity (Stenger. S, 1998; Vile. R, 1996). Although aluminum-based adjuvants have been widely used in marketed vaccines or those in clinical studies, they elicit only cellular immune responses (Kennedy, R, 2008). The use of aluminum-based adjuvants has therefore been limited to stimulate antibody responses (Kwak. L, 1996). A group of tritierpenoid saponin adjuvants, extracted from *Quillaja saponaria* Molina have been used for a long time in veterinary medicine. A purified mixture called Quil A® consists of more than 25 different saponin molecules and stimulates both the humoral and cellular responses against co-administered antigens, with a particular strength to induce Th1 cytotoxic T cell responses. This mixture however, has undesirable side effects in humans such as hemolytic activity, local reactions and occasional events of systemic toxicity and has therefore been restricted from human use (Kensil. C, 1991; O'Hagan. D, 2015).

A new version of the saponin adjuvant called GPI-0100 is a semi-synthetic analog. GPI-0100 was developed to have the adjuvanticity of quillaja saponins, but with less toxicity and greater stability in aqueous solutions. In addition, the stability of the GPI-0100 derivative allow for the development of vaccines with long shelf lives. Saponins from *Quillaja saponaria* Molina contain lipophilic acyl groups that appear to be important for stimulation of cytotoxic T cells against exogenous antigens as well as their toxicity (Marciani. D, 2000; Cox. J, 1997). These acyl groups are linked to a fucosyl residue which is attached at position C-28. This linkage however, is very unstable and hydrolyzes under conditions where the pH is higher than 6. This deacylation has a negative effect on the adjuvant itself. The result is a loss of saponins capability to stimulate a cell-mediated immune response. The GPI-0100 adjuvant combats these problems by removing the relatively toxic and unstable acyl moieties. The adjuvant also introduces a stable non-toxic lipophilic moiety in the glucuronic acid residue. GPI-0100, with immune stimulating properties similar to those of native saponins, is a dodecylamide saponin derivative that stimulates an antibody isotype profile that corresponds to a Th1 type immune response, as well as CTL production against exogenous antigens (Marciani. D, 2000). Although the

immune stimulatory activity of GPI-0100 is significantly lower than that of *Q.saponins*, such as QS-21, the toxicity is low enough to be used at high doses to induce an effective immune response without the side effects of Q. saponins (Marciani. D, 2003).

In general, adjuvants that are used in human vaccines should not: (1) cause intolerable toxicity; (2) cause pyrogenicity; (3) induce autoimmunity; (4) decrease the humoral and T-cell immunity; (5) cause immunogenicity and (6) lack stability. The first three points are dealing with the safety and tolerability of adjuvants. This review is focused on the safety aspects of the GPI-0100 use in clinical settings. The safety and tolerability data observed from the identified studies were.

Methods

Pubmed and Embase were used to search for the term ‘GPI-0100’, ‘clinical’ and ‘adjuvant’. The criteria for reporting were studies performed in humans.

Results

Study I: A bivalent conjugate vaccine in the treatment of biochemically relapsed prostate cancer: a study of glycosylated MUC-2-KLH and Globo H-KLH conjugate vaccines given with the new semi-synthetic saponin immunological adjuvant GPI-0100 or QS-21(Slovin. S, 2005)

Study set up

In this study, the safety, pharmacokinetics, and antitumor activity were assessed of a novel immunotherapeutic regimen. This prescribed course of medical treatment is known as Folate Immune that involves an EC90 vaccine administered with a GPI-0100 adjuvant that is followed by EC17. This folate-targeted hapten immunotherapy targets folate receptor expressing cancer cells and is designed to convert poorly immunogenic tumors to highly immunogenic tumors in patients with metastatic renal cell carcinoma (mRCC). First, patients are vaccinated with EC90 and GPI-0100 adjuvant to stimulate the anti-fluorescein antibody production. The next step is to administer EC17 to mark folate receptor positive tumor cells with the fluorescein hapten and to allow the surface-bound fluorescein to attract anti-fluorescein antibodies to the tumor cell surface.

This was a phase I trial study. Two preparations of GPI-0100 (unfractioned and purified) and QS-21 were tested in groups of five treated prostate cancer patients who had no evidence of disease except for rising prostate-specific antigens (PSA) levels. The adjuvant doses in each group ranged from 100 to 5000 μg (table 1). Each dose of GPI-0100 was given subcutaneously to the patient to single random sites on the upper arm and upper leg at weekly intervals for 3 weeks. A bivalent vaccine containing the glycolipid Globo against Globo H and the glycosylated mucin MUC2 conjugated to keyhole limpet hemocyanin (KLH) was mixed with the GPI-0100. In every cohort, each patient was given a 5 μg dose of MUC-2G-KLH and 10 μg of Globo H-KLH.

In the first cohort, 19 patients were recruited and assigned in groups of five to the 100, 300 and 1000 μg doses of unfractioned GPI-0100 (UF-GPI-0100). Four patients instead of the intended five were assigned to five vaccines of 3000 μg dose each due to poor compliance of one patient that received only four vaccines. The age of the participants ranged from 52 to 76 years with a median age of 67 years.

In cohort II, fifteen patients were seen as recruited and assigned in groups of five to dose levels of 1000, 3000 and 5000 μg of the purified GPI-0100 (GPI-0100-P). The age of the participants ranged from 57 to 77 years with a median age of 69 years.

In the third cohort, nine patients were recruited and all assigned to a dose of 100 μg of QS-21. The age of the participants ranged from 56 to 78 years with a median age of 69 years.

Table 1. Treatment groups for the UF-GPI-0100, GPI-0100-P and QS-21(table cited from the Slovin et al, 2005).

Cohort	No. of patients	Dose MUC-2G/Globo H-KLH (μg) per vaccination + GPI-0100
1	5	5/10 + 100 μg UF-GPI-0100
1	5	5/10 + 300 μg UF-GPI-0100
1	5	5/10 + 1000 μg UF-GPI-0100
1	4[a]	5/10 + 3000 μg UF-GPI-0100
II	5	5/10 + 1000 μg GPI-0100-P
II	5	5/10 + 3000 μg GPI-0100-P
II	5	5/10 + 5000 μg GPI-0100-P
III	9	5//10 + 100 μgQS21

[a] One patient received four vaccines instead of the intended five vaccines. This was due to poor compliance.

Local Adverse Event

There were similar grade I local reactions between UF-GPI-0100 and GPI-0100-P but fewer grade II local reactions when using GPI-0100-P (Table 2). No adjuvant dose effect was observed from the toxicity profiles for both UF-GPI-0100 and GPI-0100-P.The most common adverse events observed in both the unfractioned and purified preparations of GPI-0100 were local reaction and pain at the injection site. Furthermore, there were only two events of flu-like symptoms observed in the UF-GPI-0100, while the GPI-0100-P resulted in eight total events of flu-like symptoms.

When QS-21 was administered, four of nine patients experienced local reactivity. Seven out of the nine patients had grade I pain at the injection site, and one patient had symptoms similar to the flu. Two of the nine patients experienced grade II local reactivity. No grade II pain was observed at the injection sites in patients receiving either UF-GPI-0100 or GPI-0100-P, despite the high adjuvant doses used.

Systemic Adverse Event

No systemic adverse effects were observed in the participated patients.

Efficacy

Patients generated high titer antibodies to MUC-2G and to Globo H when receiving doses of the bivalent vaccine plus GPI-0100-P and QS-21. When the dose of GPI-0100-P increased, the patients generated higher titer antibodies to MUC-2G and Globo-H

IgM antibodies with a mean titer in patients treated with GPI-0100-P at 5000 μg were significantly higher than those developed with a dose of 100 μg of QS-21.

Table 2. Comparison of toxicities of bivalent vaccine using unfractionated (A) and purified (B) preparations of GPI-0100 (Table cited from the Slovin et al, 2005)

Toxicity	Dose (μg) (Grade I)				Dose (μg) (Grade II)			
	100	300	1000	3000	100	300	1000	3000
(A) Unfractionated preparation								
Bruising	0	0	0	0	0	0	0	0
Chills	0	0	0	0	0	0	0	0
Fatigue	0	0	1	0	0	0	0	0
Fever	0	0	0	0	0	0	0	0
Flu-like symptoms	0	1	0	1	0	0	0	0
Flushing	0	1	0	0	0	0	0	0
Headache	0	0	0	1	0	0	0	0
Local reaction	4	2	2	3	2	4	2	2
Pain at injection site	4	2	2	4	0	0	0	0
Pruritus	1	0	1	1	1	0	0	0
Abnormal ALT	0	0	0	0	0	0	0	0

Toxicity	Dose (μg) (Grade I)			Dose (μg) (Grade II)		
	1000	3000	5000	1000	3000	5000
(B) Purified preparation						
Bruising	0	1	0	0	0	0
Chills	0	1	0	0	0	0
Fatigue	1	1	1	0	1	0
Fever	0	0	0	0	0	0
Flu-like symptoms	0	1	2	1	2	2
Flushing	0	0	0	0	0	0
Headache	1	0	0	1	0	0
Local reaction	4	3	1	1	1	3
Pain at injection site	4	4	2	0	0	0
Pruritus	0	1	1	1	0	0
Abnormal ALT	0	0	3	0	0	0

Study II: A Phase I Study of Folate Immune Therapy (EC90 Vaccine Administered With GPI-0100 Adjuvant Followed by EC17) in Patients With Renal Cell Carcinoma (Amato. R, 2013)

Study Setup

In this study, the safety, pharmacokinetics, and antitumor activity were assessed of a novel immunotherapeutic regimen. This prescribed course of medical treatment is known as Folate Immune that involves an EC90 vaccine administered with a GPI-0100 adjuvant that is followed by EC17. This folate-targeted hapten immunotherapy targets folate receptor expressing cancer cells and is designed to convert poorly immunogenic tumors to highly immunogenic tumors in patients with metastatic renal cell carcinoma (mRCC). First, patients were vaccinated in the primary vaccination phase with EC90 and GPI-0100 adjuvant to stimulate the anti-fluorescein antibody production. The next step was the treatment phase where the EC17 marked folate receptor positive tumor cells with the fluorescein hapten that was administered. This allowed the surface-bound fluorescein to attract anti-fluorescein antibodies to the tumor cell surface.

This study consisted of 2 phases: a dose-escalation phase and an extension phase. During the dose-escalation phase, the safety was assessed of the EC90/GPI-0100 vaccine and the increasing doses of EC17.

This was a phase I, multicenter, open-label, baseline controlled, dose-ranging safety study of Folate Immune (EC90 vaccine administered with GPI-0100 adjuvant, followed by EC17) in adult patients with recurrent or mRCC.

Table 3. Vaccine and Treatment Scheme by Cohort (table cited from the Amato et al, 2013)

Cohort	No. Patients	EC90 Dose (mg)	No. EC90/GPI-0100 Vaccinations (Weekly)	EC17 Dose (mg/kg)	EC17 Duration (5 d/wk) (wk)	Follow-up (wk)
1	6	0.2	3	0.031	3	4
2	2	0.2	3	0.092	3	4
1R	6	0.2	4	0.031	4	2
2R	6	0.2	4	0.092	4	2
3	6	0.2	4	0.092	4	2
4	3	0.2	5	0.092	4	2
5	—	—	—	—	—	2
6	—	—	—	—	—	2
7	4	0.2	4 with booster day 43	0.276	6	2
8	—	—	—	—	—	2

In the study 41 patients were enrolled and 33 patients were evaluated. Before the primary vaccination phase, two patients failed the screening and dropped out of the study. 39 patients received one more vaccination(s) of EC90/GPI-0100, and 33 patients received one or more doses of EC17. Six patients dropped out during the study due to disease progression or symptomatic detoriation. Patients were eligible to have additional cycles of EC17 corresponding to their cohort when they exhibited signs of disease stabilization or tumor regression.

The age of the participants ranged from 34 to 74 years and the median age was 60 years. The treatment group consisted of 12 (31%) patients that were female and 27 (69%) that were male.

During the vaccination phase, patients received weekly vaccinations with 0.2 mg of EC90 mixed with a dose of 3.0 mg GPI-0100 adjuvant for 3, 4, or 5 weeks (Table 3). The patients in all the cohorts received the same dose of EC90 and GPI-0100 adjuvant. The vaccination was subcutaneously injected to the patients. The dose of 3.0 mg of GPI-0100 was chosen based on previous data showing that this dose has an acceptable toxicity profile (Slovin, 2005).

During the treatment phase, EC17 was administered as a bolus subcutaneous injection. This injection was given in each patient after the last dose of EC90/GPI-0100 vaccination was received. In cohort 1, patients had 3 weeks of treatment with EC17 and patients in cohort 1R had 4 weeks of treatment with EC17. Each treatment in cohort 1 and cohort 1R consisted of a dose of 0.031 mg/kg of EC17. Four weeks of treatment with EC17 was given to the patients in cohort 2, 2R, 3 and 4.These patients received a dose of 0.092 mg/kg of EC17 in the treatment phase. In the last group, cohort 7, patients received 6 weeks of treatment with EC17 at a dosage of 0.276 mg/kg. These patients received four vaccinations of EC90/GPI-0100 and a booster vaccination on day 43 (Table 3).

Local adverse events

In the study of Amato, a dose of 3.0 mg of GPI-0100 with 0.2 mg of EC90 was well tolerated when injected in the patients weekly for 3 or 4 weeks with or without a booster dose on day 43. 62 % (24/39) of the patients experienced at least 1 adverse event during the primary vaccination phase. The most frequently observed local adverse events during the primary vaccination phase were mild (grade I) to moderate (grade II) injection site reactions, hypotension and nausea. During the primary vaccination phase at least 1 injection site reaction was reported in 36% (14/39) of the patients. Most of the local adverse events were reported after the EC17 treatment which has no further adjuvantation from GPI-0100. Since the local adverse events reported at this stage could be resulted from the use of EC17 instead of the GPI-0100 adjuvanted study vaccine, we exclude them from the result reporting.

Systemic adverse events

Systemic adverse events were not reported during the vaccination phase. Two patients discontinued treatment due to grade 4 anaphylaxis or grade 3 pancreatitis at a later stage during the EC17 treatment. One grade 4 hypersensitivity reaction was reported throughout the whole study, which was the first patient treated with EC17 who experienced anaphylactic shock and treatment was discontinued. The EC17 vaccine that this patient received was found to be impure because of a bisfluorescein contamination which is known to produce immediate hypersensitivity reactions in immunized hosts. The following EC17 vaccines were prepared to minimize impurity with better processing techniques. Furthermore, there was a patient that died from disease progression before completing the cycle.

Study III: Phase I/Ib Study of Folate Immune (EC90 Vaccine Administered With GPI-0100 Adjuvant Followed by EC17) With Interferon-a and Interleukin-2 in Patients With Renal Cell Carcinoma (Amato. R, 2014)

Study Set up

This study followed a similar immunotherapeutic regimen in patients with recurrent or metastatic renal cell carcinoma (RCC) as the previous. In addition, interleukin-2 (IL-2) and interferon-α (IFN-α) were included to enhance the immune-mediated killing of FR-targeted tumor cells by stimulating the Fc receptor-bearing immune cells to mount an antitumor response against the anti-fluorescein antibody-opsonized tumor cells.

This was a phase I/II, multicenter, open-label, baseline controlled, dose-ranging safety study of folate immune with concurrent IL-2 and IFN-α treatment in adult patients with recurrent or metastatic RCC (Table 4). The treatment was administered subcutaneously. At day 1 of week 1, patients in all the cohorts received 1.2mg of EC90 vaccine and 3.0 mg of GPI-0100 adjuvant once per week for four weeks. At the start of the second week, patients received a dose of 0.3 mg/kg of the EC17 vaccine 5 days per week for 4 consecutive weeks. At the start of the third week, a dose of 7.0 milli-international units (MIU) of IL-2 and 3.0 MIU of IFN-α were given 3 days per week. Cohort 1 was used as a safety cohort. Cohort 2 and 3 were done to optimize the cytokine dosage regimens. In cohort 2, the initial two cycles started at day one of the third week and consisted of a cycle where IL-2 at a dose of 12.0 MIU and IFN-α at a dose of 3.0 MIU were administered three times per week. This continued for two weeks. At the start of the fifth week, the cycle started again but now IL-2 and IFN-α were given at a dose of 3.0 MIU 3 days per week.

In cohort 2, the extension cycle started with doses of IL-2 and IFN-α at 3.0 MIU each give to the patients three days per week for four consecutive weeks. In cohort 3, the first cycle (n=12) started at the first day of the third week. IL-2 at a dose of 7.0 MIU and IFN-α at a dose of 3.0 MIU were administered 3 times weekly for 3 weeks. The same doses of IL-2 and IFN-α were given in cycle 2 of cohort 3 three times weekly for four weeks. (Figure 1.)

This study consisted of a total of 24 patients. The age ranged from 39 to 73 years and the median age was 60 years. Seven (29%) patients were female and 17 (71%) patients were male.

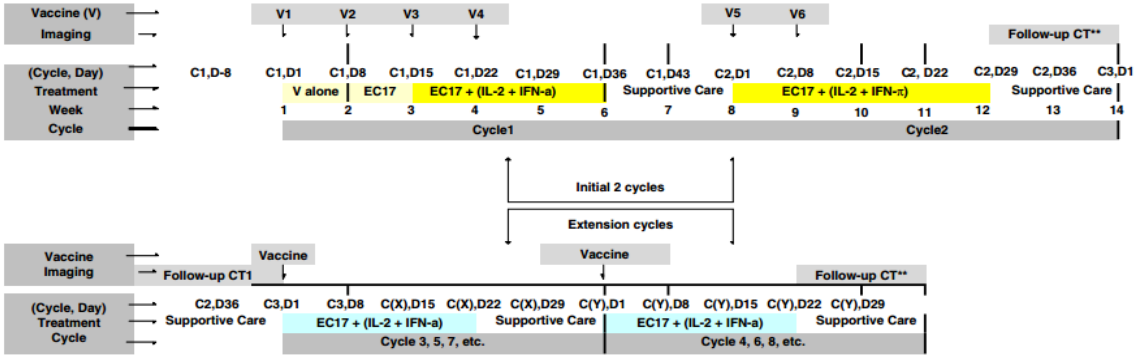

Figure 1. Treatment schedule and timeline (Figure cited from the Amato et al, 2014).

Table 4. Dosing scheme for cohorts 1-3. CT indicates computed tomography; CTL, cytotoxic T lymphocytes; IFN, interferon; IL, interleukin (Table cited from the Amato et al, 2014).

Cohorts	EC90 (mg)	GPI-0100 (mg)	EC17 (mg/kg)	IL-2 (MIU)		IFN-α (MIU)
				Initial 2 cycles	Extension cycles	
1	1.2	3	0.3	7	7	3
2	1.2	3	0.3	Cycle 1@12; Cycle 2 @ 3	3	3
3	1.2	3	0.3	7	2.5	3

Local Adverse Event

In all 24 participated patients there was at least 1 adverse event reported. The most commonly reported local adverse events were chills (87.5%), pyrexia (83.3%), vomiting (75.5%) and nausea (62.5%) (Table 5).

Systemic Adverse Event

In 12 (50%) of the participated patients, grade 3 or 4 systemic adverse events were reported. The investigator considered however only one patient (in cohort 3) with grade 3 eosinophilia to have a possible relationship to the study drug. The other grade 3 and 4 (systemic adverse events?) were regarded to be unrelated to the study drug. In 3 (12.5%) out of the 24 participated patients , grade 4 systemic adverse events were reported. These were spinal compression fracture (in cohort 1), metastases to trachea (in cohort 3), and acute pancreatitis (in cohort 3). None of these events were considered to have a relationship to the study drug.

Table 5. Adverse events (Grade I and Grade II) that were reported in the patients.

	Patients
Adverse events	
Chills	21
Pyrexia	20
Vomiting	18
Nausea	15
Fatigue	10
Injection-site reaction	9
Constipation	7
Dyspepsia	6
Headache	6
Hyperhidrosis	6

Discussion

The most common local adverse events that were observed in the three studies were mild (grade I) to moderate (grade II) local reactions and mild (grade I) pain at the injection site. GPI-0100 administered at a dose of 3000 µg showed comparable toxicity profile in the three studies. The dose of GPI-0100 appeared to be no cause in increasing numbers of local adverse events.

In one of the three studies a systemic adverse event was reported that was related to the treatment. In the patient grade 3 eosinophilia was observed and it was considered to have a possible relationship to the study drug.

In study II and study III, the focus was on designing the safety dose of EC90 and EC17 and not the dose of GPI-0100 which remained at 3.0 mg. Therefore, the efficacy in the two studies was not taken in to consideration. However, the adverse events were observed when GPI-0100 was given to the patients.

QS-21 is a saponin adjuvant which has shown great immune boosting potential for numerous vaccines. QS-21 is shown to enhance both humoral and cellular immune responses elicited by vaccines. When patients in prior studies received MUC-2G-KLH and Globo H-KLH with the QS-21, they tolerated the vaccines well but often reported redness, tenderness, swelling and itching at the injection site with occasional systemic complaints such as fever and arthralgias and myalgias attributed to QS-21 (Slovin. S, 2005). Research in Alzheimer's disease has already used QS-21 as an adjuvant for anti-Aβ vaccine therapy where it induced high sustained anti-Aβ antibody titers (Arai. H 2015). However, a variety of adverse events were reported from the patients during the studies. The most common adverse event was injection site reactions. Local and general reactions were more frequently reported by patients that were given QS-21 at a dose of 50 µg, but the toxicity profile remained considerable (Vandepapelière. P, 2005). These adverse events with QS-21 are consistent with the observations in study I elicited by QS-21 at a dose of 100 µg. In study I, GPI-0100 has shown to cause fewer adverse events in the patients and a lower threshold of toxicity. Further research on the direct comparison of the safety and tolerability aspects of GPI-0100 and QS-21 could be interesting.

Another saponin that is currently studied is a saponin fraction similar to *Q.saponaria* that has been extracted from *Quillaja brasiliensis* leaves and is called QB-90 (Kauffmann, C, 2016). This fraction was found to be comparable to Quil A ® in adjuvant efficacy. QB-90 was in addition less toxic than Quil A® and had shown similar patterns of antibody induction and stimulation of cellular immunity by generation of Th1 responses (Silveira. F, 2011). ISCOMs formulations, where the Quil A® was replaced by QB-90, has shown to be another promising alternative to classic ISCOMs as vaccine adjuvants. QB090 showed lower toxicity levels than Quil A® *in vitro* and *in vivo*. (Cibulski. S, 2016)

GPI-0100 needs more elaborated research to demonstrate their detailed mechanism of protection against various diseases. Furthermore, their specific enhancement of humoral en cellular immune response should be further confirmed in various novel vaccines for human use. So far, clinical studies are limited in using GPI-0100 as an adjuvant for immunotherapy but results show great promise.

These clinical studies show that GPI-0100 has little overall local reactivity and also little systemic side effects.

Conclusion

GPI-0100 is a promising adjuvant that shows little safety concerns and a low threshold of toxicity.

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References

Amato, R. J., Shetty, A., Lu, Y., Ellis, P. R., Mohlere, V., Carnahan, N., et al. (2014). A phase I/Ib study of folate immune (EC90 vaccine administered with GPI-0100 adjuvant followed by EC17) with interferon-alpha and interleukin-2 in patients with renal cell carcinoma. *Journal of Immunotherapy (Hagerstown, Md.: 1997), 37*(4), 237-244.

Amato, R. J., Shetty, A., Lu, Y., Ellis, R., & Low, P. S. (2013). A phase I study of folate immune therapy (EC90 vaccine administered with GPI-0100 adjuvant followed by EC17) in patients with renal cell carcinoma. *Journal of Immunotherapy (Hagerstown, Md.: 1997), 36*(4), 268-275.

Arai, H., Suzuki, H., & Yoshiyama, T. (2015). Vanutide cridificar and the QS-21 adjuvant in japanese subjects with mild to moderate alzheimer's disease: Results from two phase 2 studies. *Current Alzheimer Research, 12*(3), 242-254.

Cibulski, S. P., Mourglia-Ettlin, G., Teixeira, T. F., Quirici, L., Roehe, P. M., Ferreira, F., et al. (2016). Novel ISCOMs from quillaja brasiliensis saponins induce mucosal and systemic antibody production, T-cell responses and improved antigen uptake. *Vaccine, 34*(9), 1162-1171.

Coffman, R. L., Sher, A., & Seder, R. A. (2010). Vaccine adjuvants: Putting innate immunity to work. *Immunity, 33*(4), 492-503.

Cox, J. C., & Coulter, A. R. (1997). Adjuvants--a classification and review of their modes of action. *Vaccine, 15*(3), 248-256.

Hogenesch, H. (2013). Mechanism of immunopotentiation and safety of aluminum adjuvants. *Frontiers in Immunology, 3*, 406.

Kauffmann, C., Machado, A. M., Fleck, J. D., Provensi, G., Pires, V. S., Guillaume, D., et al. (2004). Constituents from leaves of quillaja brasiliensis. *Natural Product Research, 18*(2), 153-157.

Kawai, T., & Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: Update on toll-like receptors. *Nature Immunology, 11*(5), 373-384.

Kennedy, R., & Celis, E. (2008). Multiple roles for CD4+ T cells in anti-tumor immune responses. *Immunological Reviews, 222*, 129-144.

Kensil, C. R., Patel, U., Lennick, M., & Marciani, D. (1991). Separation and characterization of saponins with adjuvant activity from quillaja saponaria molina cortex. *Journal of Immunology (Baltimore, Md.: 1950), 146*(2), 431-437.

Kim, S. K., Ragupathi, G., Cappello, S., Kagan, E., & Livingston, P. O. (2000). Effect of immunological adjuvant combinations on the antibody and T-cell response to vaccination with MUC1-KLH and GD3-KLH conjugates. *Vaccine, 19*(4-5), 530-537.

Leroux-Roels, G. (2010). Unmet needs in modern vaccinology: Adjuvants to improve the immune response. *Vaccine, 28 Suppl 3*, C25-36.

Lovgren Bengtsson, K., Morein, B., & Osterhaus, A. D. (2011). ISCOM technology-based matrix M adjuvant: Success in future vaccines relies on formulation. *Expert Review of Vaccines, 10*(4), 401-403.

Marciani, D. J. (2015). Is fucose the answer to the immunomodulatory paradox of quillaja saponins? *International Immunopharmacology, 29*(2), 908-913.

Marciani, D. J., Press, J. B., Reynolds, R. C., Pathak, A. K., Pathak, V., Gundy, L. E., et al. (2000). Development of semisynthetic triterpenoid saponin derivatives with immune stimulating activity. *Vaccine, 18*(27), 3141-3151.

Marciani, D. J., Reynolds, R. C., Pathak, A. K., Finley-Woodman, K., & May, R. D. (2003). Fractionation, structural studies, and immunological characterization of the semi-synthetic quillaja saponins derivative GPI-0100. *Vaccine, 21*(25-26), 3961-3971.

Moser, C., Metcalfe, I. C., & Viret, J. F. (2003). Virosomal adjuvanted antigen delivery systems. *Expert Review of Vaccines, 2*(2), 189-196.

United States National Institute of Allergy and Infectious Diseases (NIAID). NIAID Biodefense Research Agenda for Category B and C Priority Pathogens. Accessed 18 june 2016. "Vaccines are the most effective method of protecting the public against infectious diseases."

O'Hagan, D. T., & Fox, C. B. (2015). New generation adjuvants--from empiricism to rational design. *Vaccine, 33 Suppl 2*, B14-20.

Ragupathi, G., Gardner, J. R., Livingston, P. O., & Gin, D. Y. (2011). Natural and synthetic saponin adjuvant QS-21 for vaccines against cancer. *Expert Review of Vaccines, 10*(4), 463-470.

Saupe, A., McBurney, W., Rades, T., & Hook, S. (2006). Immunostimulatory colloidal delivery systems for cancer vaccines. *Expert Opinion on Drug Delivery, 3*(3), 345-354.

Silveira, F., Cibulski, S. P., Varela, A. P., Marques, J. M., Chabalgoity, A., de Costa, F., et al. (2011). Quillaja brasiliensis saponins are less toxic than quil A and have similar properties when used as an adjuvant for a viral antigen preparation. *Vaccine, 29*(49), 9177-9182.

Sjolander, S., Drane, D., Davis, R., Beezum, L., Pearse, M., & Cox, J. (2001). Intranasal immunisation with influenza-ISCOM induces strong mucosal as well as systemic antibody and cytotoxic T-lymphocyte responses. *Vaccine, 19*(28-29), 4072-4080.

Slovin, S. F., Ragupathi, G., Fernandez, C., Jefferson, M. P., Diani, M., Wilton, A. S., et al. (2005). A bivalent conjugate vaccine in the treatment of biochemically relapsed prostate cancer: A study of glycosylated MUC-2-KLH and globo H-KLH conjugate vaccines given with the new semi-synthetic saponin immunological adjuvant GPI-0100 OR QS-21. *Vaccine, 23*(24), 3114-3122.

Slovin, S. F., Ragupathi, G., Musselli, C., Fernandez, C., Diani, M., Verbel, D., et al. (2005). Thomsen-friedenreich (TF) antigen as a target for prostate cancer vaccine: Clinical trial results with TF cluster (c)-KLH plus QS21 conjugate vaccine in patients with biochemically relapsed prostate cancer. Cancer Immunology, Immunotherapy : CII, 54(7), 694-702.

Stenger, S., & Modlin, R. L. (1998). Cytotoxic T cell responses to intracellular pathogens. *Current Opinion in Immunology, 10*(4), 471-477.

Takeuchi, O., & Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell, 140*(6), 805-820.

Vandepapeliere, P., Rehermann, B., Koutsoukos, M., Moris, P., Garcon, N., Wettendorff, M., et al. (2005). Potent enhancement of cellular and humoral immune responses against recombinant hepatitis B antigens using AS02A adjuvant in healthy adults. *Vaccine, 23*(20), 2591-2601.

Walkowicz, W. E., Fernandez-Tejada, A., George, C., Corzana, F., Jimenez-Barbero, J., Ragupathi, G., et al. (2016). Saponin variants with central glycosidic linkage modifications exhibit distinct conformations and adjuvant activities. *Chemical Science (Royal Society of Chemistry : 2010), 7*(3), 2371-2380.