

REVIEW ARTICLE

Effects of anti-beta 2-glycoprotein 1 antibodies and its association with pregnancy-related morbidity in antiphospholipid syndrome

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Abstract

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by venous, arterial, or small-vessel thrombosis and/or pregnancy-related morbidity, associated with persistent positivity of antiphospholipid antibodies (aPL). Pregnancy-related morbidity in APS patients is characterized by unexplained fetal deaths, premature birth of morphologically normal newborns, and/or consecutive pregnancy losses before the 10th week of gestation. Beta 2-glycoprotein 1 (β 2GP1) is the main antigen recognized by aPL and plays an essential role in the pathogenesis of APS. Antibodies against β 2GP1 (a β 2GP1) are involved in damage-generating mechanisms in APS due to their interaction with trophoblasts, decidua, and endothelial cells. a β 2GP1 might be used as a prognostic tool for obstetric risk stratification and β 2GP1 could be a target for molecular-targeted treatment to prevent pregnancy morbidity in APS. This review describes these aspects of a β 2GP1, including effects on different cellular targets, its association with the severity of obstetric manifestations and the potential of β 2GP1-targeted therapies for APS.

KEYWORDS

antiphospholipid antibodies, antiphospholipid syndrome, beta 2-glycoprotein 1, pregnancy outcome

1 | INTRODUCTION

Antiphospholipid syndrome (APS) is a chronic autoimmune disease characterized by persistent positivity (≥ 12 weeks) of moderate or high titers of antiphospholipid antibodies (aPL) against beta 2-glycoprotein 1 (a β 2GP1) or cardiolipin (aCL), and/or a positive lupus anticoagulant test (LA).¹ In turn, there are subtypes of antibodies against different domains of beta 2-glycoprotein 1 (β 2GP1) and aCL that are dependent on β 2GP1 binding.^{2,3} APS is defined as either primary APS⁴ or secondary to other diseases like systemic lupus erythematosus (SLE), rheumatoid arthritis or cancer.^{5–8}

APS clinical manifestations include venous, arterial, or small-vessel thrombosis (vascular APS), and pregnancy-related morbidity (obstetric APS).^{1,9} Moreover, obstetric APS has different clinical patterns: only with pregnancy-related morbidity (purely obstetric form) or combined with other vascular clinical manifestations.^{9,10} Deep vein thrombosis and early fetal loss (< 10 weeks of pregnancy) are the most common vascular and obstetric manifestations, respectively.⁷ Pregnancy-related morbidity in APS patients is furthermore characterized by one or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, one or more premature births of a morphologically normal newborn before the

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34th week of gestation because of eclampsia or severe preeclampsia, or three or more unexplained consecutive spontaneous fetal losses before the 10th week of gestation.¹ Another clinical variant of APS is the catastrophic APS (CAPS) which is characterized by rapid multiorgan failure (≥ 3 organs) due to small-vessel microthrombosis.¹¹

β 2GP1 is a five-domain plasma protein and considered the main antigen recognized by a β 2GP1 and some aCL.^{12,13} The interaction between a β 2GP1 and β 2GP1 could help understand the etiology of APS, specifically APS-related pregnancy morbidity.^{14,15} Why a β 2GP1 are formed is not known precisely; there seems to be a genetic predisposition as significant associations between the formation of a β 2GP1 and the apolipoprotein H (APOH) gene on chromosome 17, and between formation of a β 2GP1 directed against domain I and the mono-ADP ribosylhydrolase 2 gene (MACROD2) on chromosome 20 which was found in a genome-wide association study (GWAS).¹⁶ In addition, a polymorphism that leads to an exchange valine for leucine in 247 position of domain 5 of the protein is related to formation and reactivity of IgG a β 2GP1.^{17,18}

Molecular mimicry of β 2GP1 with microorganisms has also been proposed as a mechanism of formation of a β 2GP1. In a murine model, mice immunized with *Haemophilus influenzae*, *Neisseria gonorrhoeae*, or tetanus toxoid developed antibodies against the hexapeptide TLRVYK.¹⁹ This amino acid sequence is in the third domain of β 2GP1, and it is homologous with peptide domains of viruses such as Epstein-Barr and bacteria such as *Streptococcus pneumoniae*. Moreover, these specific antibodies against the hexapeptide were associated with adverse obstetric effects.¹⁹

The role of β 2GP1 itself in the pathogenesis of APS has been widely described, regardless of clinical manifestations.^{20–23} aPL promotes antiangiogenic effects, complement activation, inflammatory response and inhibition of proliferation and migration of trophoblasts in the placenta.^{9,24} Furthermore, it has been demonstrated that the aPL profile – defined as which types of aPL are present, their titers and the persistence of their positivity – is a prognostic tool in APS patients.²⁵ Patients with aPL triple positivity – meaning positive for a β 2GP1, aCL, and LA – have the highest risk of fetal loss and thromboembolic events.^{26–28} High titers of a β 2GP1 have also been identified as an additional risk factor to have adverse pregnancy outcomes even using conventional treatment with low dose aspirin (LDA) and low molecular weight heparin (LMWH).^{25,28} Moreover, next to a β 2GP1, IgM aCL has been associated with placenta-mediated complications in APS. However, it is not specified whether these aCL were β 2GP1-dependent antibodies, as it is known that some aCL are dependent on binding of β 2GP1.^{10,29}

In conclusion, a β 2GP1 have many cellular effects, positivity to a β 2GP1 might be a useful tool for obstetric risk stratification and focusing on β 2GP1 might reveal new molecular-targeted treatments in APS patients.^{22,30} In this review these aspects of a β 2GP1 are described, and the need for additional studies to determine the clinical importance of these antibodies in APS patients will be discussed.

2 | BETA 2-GLYCOPROTEIN 1: THE MAIN ANTIGEN

β 2GP1 is an apolipoprotein family phospholipid-binding protein, with a molecular weight between 43 kDa and 50 kDa.³¹ This range is due to different results obtained by techniques like sedimentation equilibrium (43 kDa) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in the presence of reducing agents (50 kDa).^{31,32} It has a plasma concentration of 0.2 mg/ml, and it is constituted by 326 amino acids arranged in five protein domains (I–V).³³ Each protein domain has ~ 60 amino acids, except domain V which has 82.^{34,35} These domains belong to the complement control protein (CCP) family, which is involved in complement regulation during protein-protein interactions.^{36,37}

β 2GP1 is mainly synthesized in hepatocytes but can be found in human endothelial cells, astrocytes, neurons, placenta and immune cells like monocytes, neutrophils, lymphocytes and macrophages in which the binding with the phosphatidylserine- β 2GP1 complex have been observed.^{36,38–44} In a murine model, β 2GP1 was detected in trophoblasts and uterine vessels endothelial cells, β 2GP1 was exhibited as well in the gut and brain in mice treated with lipopolysaccharide (LPS).⁴⁵ Furthermore, β 2GP1 is expressed in several pathological conditions, which was demonstrated by detecting the protein in myocardial cells after acute myocardial infarction.^{15,46} β 2GP1 expression is not dependent on aPL presence, and it was detected in placenta tissue from complicated pregnancies, but also from normal pregnancies.^{43,44} However, there is an increased expression of β 2GP1 on trophoblast surfaces and placentas in patients with elevated aPL titers.^{43,44}

β 2GP1 molecular structure allows binding with negative charge molecules and surfaces like lipoproteins, heparin, membrane of endothelial cells, cardiolipin or anionic phospholipids.^{12,13,47–49} These anionic phospholipids are in the inner surface of the cell membrane and could be externalized during cell apoptosis or immune cell senescence and then interact with β 2GP1. Then, β 2GP1-anionic phospholipids complex acts as an antigen for aPL.³⁴

Like other apolipoproteins, β 2GP1 has high conformational flexibility and responds to environmental variations.³² Conformational changes of β 2GP1 have been described, and there are two structure organization types: closed or circular and open or linear.⁵⁰ The closed-form is established by an interaction between the protein domain I and V and corresponds to 91% of plasma circulating β 2GP1.⁵⁰ In vitro, aCL binding to β 2GP1 immobilized on oxidized irradiated polystyrene enzyme-linked immunosorbent assay (ELISA) plates even without phospholipids was shown. This demonstrated that β 2GP1 binding to negative charge surfaces is important to induce the subsequent aCL-binding.^{3,51}

In vivo, LPS or anionic phospholipids like cardiolipin on the surface of the cell membrane bind β 2GP1.^{48,50,52,53} Then, β 2GP1 binding to negatively charged surfaces induces a conformational change to the open form that expresses an epitope in the protein that allows the binding with aPL and encourages the subsequent formation of the aPL- β 2GP1 complex that is essential in the pathophysiology of APS.^{3,37,50}

It is unknown how β 2GP1 conformational changes are induced. Some authors proposed that this is a consequence of electrostatic interactions of the molecule.⁵⁴ At high pH, the change from closed to open conformation is induced, while at low pH, the opposite-way conformation change occurs.⁵⁰ Moreover, protein reduction by thiol-oxidoreductases and disulfide isomerase has been proposed as mechanisms to induce conformational changes of β 2GP1. In turn, β 2GP1 oxidation increase its immunogenicity inducing human dendritic cell maturation and a lymphocyte T helper 1 response.⁵⁵⁻⁵⁷

Furthermore, β 2GP1 interacts with different molecules involved in coagulation and immunological pathways.¹⁵ β 2GP1 has an anticoagulant or procoagulant effect according to environmental characteristics to which it is exposed; however, these specific determinants have not been identified yet.¹⁵ β 2GP1 procoagulant effect participates in the pathophysiology of APS, and it is characterized by inhibiting protein C and anticoagulant function of annexin V, because the binding of annexin V to cardiolipin and phosphatidylserine on procoagulant surfaces of trophoblast cell, platelets and the endothelial cells are impeded.⁵⁸⁻⁶⁰

β 2GP1 could also have a direct immunological function as it is a mediator of the innate immune system.⁵² β 2GP1 inhibition of complement activation has been documented. In an assay with non-pathologic human serum, the binding of C3 to β 2GP1 creates binding sites for factor H, a mediator in the process of degradation of C3 by factor I. The inhibition of this mechanism by a β 2GP1 could be an underlying cause for accumulation of C3 activation products in the placenta of APS patients.²³

3 | PATHOGENESIS: RELEVANCE OF ANTIBODIES AGAINST β 2GP1 IN APS-RELATED PREGNANCY MORBIDITY

It has been demonstrated that aPL recognized phospholipid-binding plasma proteins and not phospholipids directly, which was an advance to understand the pathophysiology of APS.^{13,61-63} a β 2GP1 are the main antibodies related to pregnancy morbidity in APS patients due to their interaction with the trophoblasts, the decidua and the endothelium of uterine vessels,^{24,45,64} in which β 2GP1 functions like an intermediary between these aPL and target cells starting the damaging processes in APS.⁶⁵

Although placental vessel thrombosis was previously considered the most important pathological process of adverse pregnancy outcomes in APS patients, recently it has been proposed that purely vascular form and obstetric APS are two syndromes with different pathophysiology, which is explained by a predominant pro-inflammatory state related to obstetric APS.^{9,65-70} The greater expression of β 2GP1 in decidual endothelial cells in contrast to other vascular endothelium has been associated with a pro-inflammatory response mediated by a β 2GP1 that encourages defects in placenta growth, and it has been proposed that this is the leading cause of pregnancy morbidity in APS patients.^{9,45,66}

IgG polyclonal antibodies with confirmed aCL and a β 2GP1 activity purified from serum samples from patients with only pregnancy morbidity induced a reduction in the HTR8 cell (first-trimester human trophoblast) migration, conversely IgG polyclonal antibodies purified from patients with vascular thrombosis, did not have this effect.⁷¹

Furthermore, a β 2GP1 have a wide range of effects in different cellular targets that are associated with pregnancy morbidity in APS patients. These main cellular targets and their downstream effects are summarized in Figure 1 and are described in the following paragraphs.

3.1 | a β 2GP1-mediated placental dysfunction

In a systematic review by Viall and Chamley, placental dysfunction was described as a cause of pregnancy morbidity in APS.²⁴ Decidual inflammation, placental infarction, alteration in the remodeling of the uterine spiral arteries, an increase in syncytial knots, a decrease in the vasculo-syncytial membranes and the activation of the complement system (C4d) were the leading processes involved in this placental dysfunction.²⁴ Nevertheless, this review only included six studies with a β 2GP1-positive patients and did not differentiate between β 2GP1-dependent and independent aCL effect. Future research should be performed to analyze the histopathological changes produced only by a β 2GP1.²⁴

In vitro and in vivo extravillous trophoblast invasion was reduced by a β 2GP1, this altered the remodeling of the uterine spiral arteries and caused a decreased blood flow to the placenta, increased production of pro-inflammatory cytokines and angiogenic factors like the placental soluble fms like tyrosine kinase 1 (sFlt-1).⁷⁰ In an in vitro study with a toll-like receptor type 4/myeloid differentiation primary response 88 (TLR4/MyD88) dependent model, monoclonal a β 2GP1 caused an inflammatory response in trophoblastic cells, which leads to an increase in interleukin (IL) 8, IL1 β and monocyte chemoattractant protein 1 (MCP-1) production.⁷² Another in vitro TLR4/MyD88 independent model demonstrated that trophoblastic cells treated with monoclonal a β 2GP1 had lower mRNA IL6 levels, which was correlated with low levels of phosphorylated signal transducer and activator of transcription 3 (STAT3). These changes in pro-inflammatory molecules generated a decrease in early trophoblast migration.⁷³

Furthermore, aPL are internalized into the syncytiotrophoblast via a low-density lipoprotein receptor (LDLR).⁷⁴ After internalization into the syncytiotrophoblast, aPL affected the inner mitochondrial membrane, increasing the Cytochrome c release to the cytosol and the ROS production by trophoblast cells.^{74,75} Mitochondrial dysfunction altered cell death processes of the syncytiotrophoblast, leading to an increase in the release of necrotic syncytiotrophoblast debris that encourages maternal endothelial cell activation.^{74,76} In vitro, syncytiotrophoblast debris increased the endothelial cell surface expression of Intercellular Adhesion Molecule 1 (ICAM-1), and it could be avoided with RAP addition, an inhibitor of LDLR receptors.^{74,76} Binding of a β 2GP1- β 2GP1 complex to the apolipoprotein E receptor 2 (ApoER2) in the trophoblast also induced a reduction in cell migration in a murine model.⁷⁷ Moreover, β human chorionic gonadotropin hormone

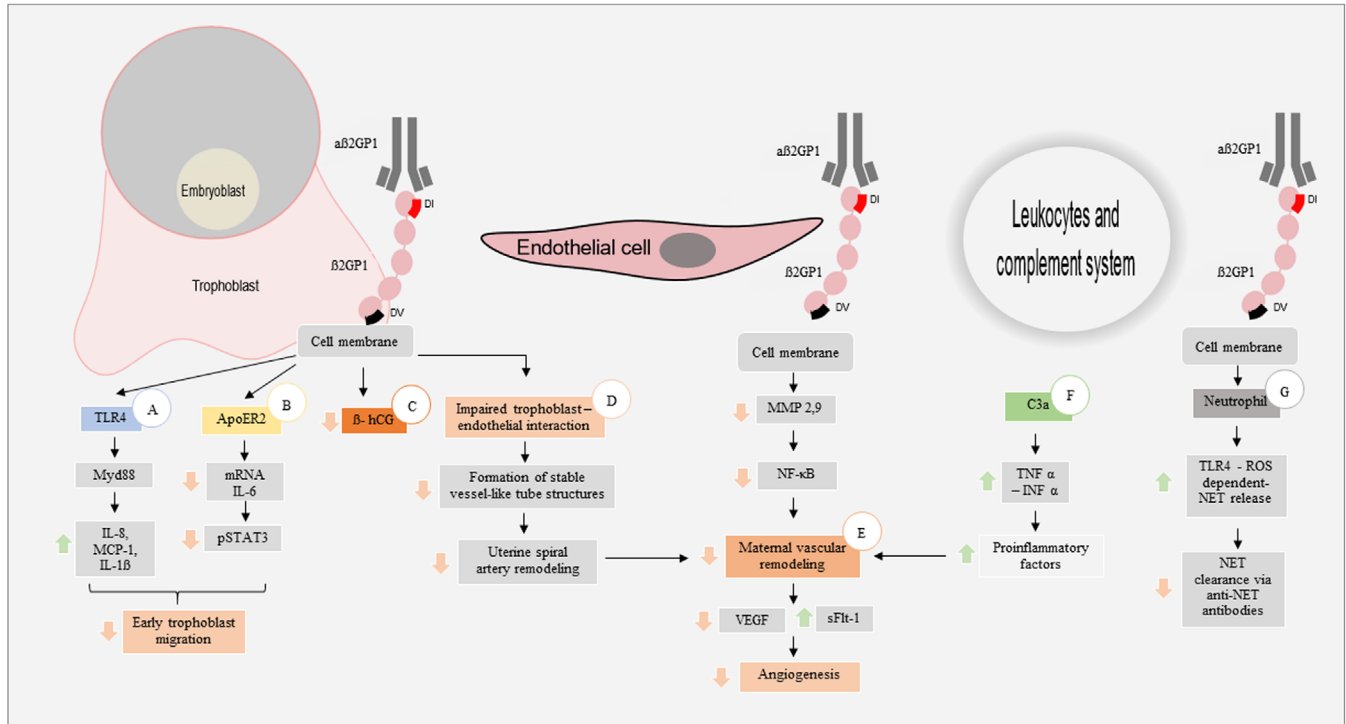


FIGURE 1 Effects of beta 2-glycoprotein 1 antibodies on different cellular targets and their damage-generating pathways (A) aβ2GP1 pro-inflammatory effect via TLR4/Myd88. (B) aβ2GP1 induced a reduction in the promigratory effect of IL6 and STAT3. (C) aβ2GP1-mediated β-hCG decrease (D) Altered trophoblast-endothelium interaction. (E) Dysfunction in maternal vascular remodeling. (F) Complement system early activation reduces maternal vascular remodeling. (G) aβ2GP1 increases the release of NETs. Arrow up. Upregulation. Arrow down. Downregulation. Abbreviations. aβ2GP1, anti-beta 2-glycoprotein-1 antibodies; β2GP1, beta 2- glycoprotein-1; TLR4, Toll-like receptor type 4; MyD88, Myeloid differentiation primary response 88; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; ApoER2, apolipoprotein E receptor 2; pSTAT3, phosphorylated signal transducer and activator of transcription 3; β-hCG, β human chorionic gonadotropin hormone; MMP, matrix metalloproteinases; NF-κB, nuclear factor kappa of activated B cells; VEGF, vascular endothelial growth factor; sFlt-1 placental soluble fms like tyrosine kinase 1, TNF-α, tumor necrosis factor alpha; INF-α, interferon alpha; ROS, reactive oxygen species; NET, neutrophil extracellular trap

(β-hCG) trophoblast production decline is another aβ2GP1 mediated mechanism that results in placental dysfunction in APS patients.^{78,79}

Thus, placental dysfunction is a leading cause of APS-related pregnancy morbidity, and aβ2GP1 play an important role in the dysfunction by promoting different pathophysiological pathways that lead to a placental pro-inflammatory state, decidual endothelial cell dysfunction and altered trophoblast cell migration (Figure 1).

3.2 | aβ2GP1-mediated endothelial dysfunction

Endothelial dysfunction has been demonstrated in APS patients with different clinical manifestations.^{24,80} This dysfunction and imbalance between proangiogenic factors, like the vascular endothelial growth factor (VEGF), and antiangiogenic factors, like sFlt-1, promote obstetric complications like preeclampsia.⁸¹ In vitro serum from women with APS-related pregnancy morbidity (with or without previous thrombosis) decreased trophoblast-endothelium interaction and disturbed formation of stable vessel-like tube structures.^{82,83} Furthermore, in vivo and in vitro aPL decreased angiogenesis, reduced VEGF production and interfered with matrix metalloproteinases (MMPs) activity which is necessary for placental angiogenesis.⁸⁴

In a human microvascular endothelial cell line (HMEC-1), trophoblastic debris derived from healthy first trimester placentas induced proteomic and transcriptomic changes characterized by an upregulated release of pro-inflammatory cytokines like IL8 and negative gene regulation of apoptosis.⁸⁵ This interaction has been considered a maternal adaptive mechanism and a part of feto-maternal communication during healthy pregnancy.⁸⁵ aβ2GP1 produced mitochondrial changes that lead to cellular death with the consequent release of syncytial nuclear aggregates that could interact with maternal endothelial cells and set off vascular pathologic changes.⁷⁰ Moreover, high aβ2GP1 concentration induced trophoblastic cell apoptosis via caspases 3, 8 and 9.⁷² Despite this, there is a lack of evidence about aβ2GP1-mediated endothelial dysfunction in purely obstetric APS form in clinical studies, considering the lack of a clear description of the APS patients clinical classification in previous studies.⁸⁰

3.3 | Role of complement and neutrophil interaction in pregnancy-related morbidity

During pregnancy, the complement helps in extensive tissue remodeling caused by trophoblast invasion, especially in remodeling of spiral

uterine arteries.⁸⁶ Early complement activation (especially the alternative pathway) and hypocomplementemia during gestation have been identified as predictors of adverse pregnancy outcomes in aPL-positive women.⁸⁷⁻⁸⁹ Besides, the Bb and sC5b-9 serum levels were higher in aPL positive patients with adverse pregnancy outcomes.⁸⁷

Complement activation allowed leucocyte recruitment, and these produced pro-inflammatory molecules like tumor necrosis factor alpha (TNF- α) and interferon alpha (INF- α) that hindered the vascular remodeling.⁹⁰ Complement activation was associated with fetal resorption and fetal growth restriction in a murine model; however, there was no demonstration of specific a β 2GP1-mediated damage.⁹¹ Furthermore, these adverse pregnancy outcomes were avoided using the C3 convertase inhibitor complement receptor 1-related gene/protein γ (Crry)-Ig, and C3 deficient mice were resistant to aPL-mediated pregnancy morbidity.⁹¹

The C5a key role in the aPL-related fetal injury was described by Girardi, *et al.*⁹² C5a activity encourages the recruitment of tissue factor-expressing neutrophils to trophoblast tissue, which leads to respiratory burst and subsequent trophoblast injury.^{93,94} Nevertheless, these studies used human IgG-containing aPL antibodies, and there was no demonstration of specific a β 2GP1-mediated damage. To our knowledge, there still are no studies investigating specific a β 2GP1-mediated effects in complement function and its role in pregnancy-related morbidity in APS patients.

Also, neutrophils have been associated with fetal damage in mice treated with aPL.⁹² Yalavarthi *et al.* demonstrated that a β 2GP1 binds to the neutrophil surface and increased the neutrophil extracellular trap (NET) release, a form of neutrophil death recognized as a leading mediator in arterial and venous thrombosis.^{42,95} β 2GP1-mediated upregulation of the beta-2 integrin Mac-1 led to a TLR-4 dependent NET release due to increased neutrophil interaction with the endothelium.⁹⁶ Moreover, a decrease in NET clearance by circulating deoxyribonucleases was shown in APS patients.^{97,98}

In vitro the IgG serum from APS pregnant women without previous thrombosis induced increase NET release via reactive oxygen species (ROS) production.⁹⁹ Furthermore, these APS-IgG stimulated NETs reduced the invasion and migration of HTR8 cells and migration and tube formation of Human Umbilical Vein Endothelial Cells (HUVEC). Interestingly, all included patients were positive for IgG a β 2GP1.⁹⁹

4 | ANTIBODIES AGAINST β 2GP1 ARE IMPORTANT DETERMINANTS OF ADVERSE PREGNANCY OUTCOMES IN APS

It has been proposed that a β 2GP1 positivity is a useful tool for obstetric risk stratification in aPL-positive patients.²² Adverse pregnancy outcomes have been more strongly associated with a β 2GP1 than with aCL and LA.^{9,22,100} All a β 2GP1 are associated with adverse pregnancy outcomes across all of gestation; however, differences have been reported in pathophysiology mechanisms between APS patients with early and late pregnancy morbidity.^{22,101,102} a β 2GP1 against domain I

are related to an increased risk of thrombotic events and, to a lesser extent, APS-related pregnancy morbidity.¹⁰³⁻¹⁰⁵ It has been proposed that the presence of this a β 2GP1 subtype is a leading predictor of late-onset pregnancy morbidity due to the role of placental thrombotic infarction in its pathogenesis.^{22,106,107}

a β 2GP1 are determinants of pregnancy outcomes in APS patients using conventional treatment.¹⁵ Specifically, high levels of a β 2GP1 are a risk factor to fetal loss and pregnancy morbidity in this patient's group.^{28,108} The PREGNANTS study including pregnant women with APS using conventional treatment demonstrated that patients only positive for a β 2GP1 had a higher incidence of pregnancy morbidities like preeclampsia, fetal growth restriction, preterm delivery and stillbirth, and a lower rate of live births than patients positive only for aCL or LA.²⁶ As mentioned above, the study did not specify whether these aCL were β 2GP1-dependent antibodies, which could affect the results.

Furthermore, by using the EUREKA algorithm, a tool to define the magnitude of the obstetric risk including any titer of aPL (diagnostic or not), it was observed that patients only positive for a β 2GP1 IgG had a higher risk of adverse pregnancy outcomes than those only positive for aCL. Moreover, patients positive for LA and a β 2GP1 IgG had the highest risk for these adverse outcomes.³⁰

Most of the available studies concerning obstetric risk stratification are retrospective and did not include different clinical manifestations, representing a barrier to extrapolating these promising results directly to the clinical practice and to possibly changing the treatment in women with the highest risk. Thereby, there is a need for prospective studies that evaluate aPL profile (and especially a β 2GP1) in relation to the severity of adverse pregnancy outcomes and used treatment in pregnant APS patients.

5 | β 2GP1 AS NOVEL MOLECULAR-THERAPEUTIC TARGET

European League Against Rheumatism (EULAR) APS treatment recommendations were published in 2019. Different treatment regimens depending on previous pregnancy morbidity, aPL profile, and response to conventional treatment were proposed.²⁵ LDA and prophylactic or therapeutic doses (according to the clinical scenario) of LMWH are the conventional treatment for APS-related pregnancy morbidity, and with this treatment a live birth is achieved in more than 70% of pregnancies.¹⁰⁹ To improve outcome, β 2GP1 have been proposed as a molecular-therapeutic target due to their role in APS pathogenesis, and a β 2GP1 association with more severe clinical manifestations despite conventional treatment. Below we describe some of the proposed strategies, that are summarized in Table 1.

5.1 | TIFI synthetic peptide

TIFI is a 20 amino acid synthetic peptide that shares similarity with the Vth domain of β 2GP1. It is a competitive inhibitor of this domain and prevents β 2GP1 binding to cell surfaces and phospholipids. It was

TABLE 1 Beta 2-glycoprotein 1 as a molecular-therapeutic target in APS-related pregnancy morbidity

Therapy	β 2GP1 relation	Mechanism of action	Effect	Model	References
TIFI peptide	Mimics Vth domain of β 2GP1.	Competitive inhibition: prevents binding of β 2GP1 to target cells.	Dose - dependent inhibition of β 2GP1 binding to trophoblast. Reduce growth retardation and fetal loss rate induced by aPL.	In vitro. Cytotrophoblast cell culture. In vivo. Murine model, pregnant C57BL/6 mice.	[95–97]
1N11 monoclonal antibody	Monoclonal antibody to β 2GP1.	Decreases antibody binding to β 2GP1 and impede interaction between β 2GP1 and apoER2.	Prevents alterations of early trophoblast migration and proliferation. Reduce increase in fetal resorption induced by aPL.	In vitro. Trophoblast cell line HTR-8SV neo. In vivo. Murine model, female Balb/c.	[98]
MBB2 Δ CH2	Non-complement fixing antibody to β 2GP1.	Competitive inhibition: prevents binding of a β 2GP1 to β 2GP1 domain I.	Reduce fetal resorption frequency and increase fetal weight.	In vitro. BeWo and HUVECs In vivo. Murine model-female BALB/c mice.	[99]

Abbreviations: aPL, antiphospholipid antibodies; apoER2, apolipoprotein E receptor 2; a β 2GP1, antibodies anti beta 2-glycoprotein-1; HTR-8SV. First-trimester human trophoblast cells; HUVEC, human umbilical cord vein endothelial cells.; β 2GP1, beta 2- glycoprotein-1.

shown to prevent aPL-mediated thrombosis and binding of β 2GP1 to endothelial cells and macrophages in a murine model.¹¹⁰ Another study in mice demonstrated TIFI-mediated inhibition of binding of the protein to trophoblast cells in a dose-dependent scheme, resulting in less fetal growth restriction and fetal losses.¹¹¹ Moreover, in vitro TIFI prevented antiangiogenic effect of a β 2GP1 in human endometrial endothelial cells.¹¹²

Despite these promising results, the mechanism of action of TIFI peptide could interfere with the physiological functions of β 2GP1 in different tissues. β 2GP1 belongs to the LPS-neutralizing proteins family, and it has a direct role in the innate immune system.^{52,113} Avoiding the binding of LPS- β 2GP1 complex to the cell surface of monocytes could be deleterious during infectious diseases in APS patients. Even though LPS binds to domain V of β 2GP1, and TIFI peptide shares similarities, there are still no studies investigating TIFI LPS-neutralizing function. Besides, β 2GP1 facilitate phagocytosis of apoptotic cells and platelets microvesicles.^{114,115} TIFI peptide could avoid the binding of these protein complexes to macrophages and leading to dysfunction in coagulation pathways.

5.2 | Monoclonal antibodies

1N11 monoclonal antibody against β 2GP1 was shown to decrease aPL binding to the protein, inhibiting its interaction with ApoER2, prevents the adverse effects of aPL on endothelial cell migration and proliferation, and reduce the prothrombotic function of aPL.¹¹⁶ Furthermore, a decrease in thrombotic and pregnancy adverse effects of aPL was shown using the non-complement fixing antibody (MBB2 Δ CH2) against β 2GP1 that prevents protein domain I binding to a β 2GP1.¹¹⁷

5.3 | Recombinant domain I molecule

This recombinant domain I molecule is able to bind to aPL and thus prevents their adverse effects.¹¹⁸ It had had promising results in murine models. However, the studies did not evaluate adverse pregnancy outcomes.^{119,120} Although a β 2GP1 directed against the domain I was associated with thrombosis and adverse pregnancy outcomes in APS patients, their prevalence in this population is around 45%.¹⁰⁵ Therefore, the contribution of antibodies with another specificity must be considering, and the new therapeutics focused only on a β 2GP1 directed against domain I could be insufficient to prevent all aPL effects.^{103–106}

Research is going concerning these promising therapeutic strategies, but still in preliminary phases. Therefore, the Rheumatology and Obstetrics associations do not recommend these drugs yet as current treatment of APS patients.^{25,121}

6 | CLINICALLY USED ALTERNATIVE TREATMENT OPTIONS NOT SPECIFICALLY TARGETING β 2GP1

6.1 | Statins

Statins have been widely used to prevent and treat cardiovascular diseases, and new evidence is emerging about their use to treat placental insufficiency.²² Pravastatin preliminary safety was shown in high-risk of preeclampsia pregnant women.¹²² Furthermore, no major congenital abnormalities have been identified in pregnant women treated with statins during the first trimester.¹²³

Previous murine models demonstrated that pravastatin improves vascular reactivity, decreasing sFlt-1 levels and its antiangiogenic

effects.¹²⁴ A reduction in adverse pregnancy outcomes in APS patients under conventional treatment plus pravastatin compared to conventional treatment alone was described.¹²⁵ Besides, the pravastatin plus therapy increases endothelial nitric oxide synthase (eNOS) synthesis leading to an increase in nitric oxide (NO) generation and improving placental vascular function.¹²⁶ Despite these promising results, statins safety and effectiveness in preventing adverse pregnancy outcomes need to be proved in large patients cohorts.

6.2 | Hydroxychloroquine

Hydroxychloroquine (HCQ) disintegrates a β 2GP1-phospholipids complexes, diminishes complement activation, and has anticoagulation effects maintaining the annexin V shield in endothelial and trophoblast cell surfaces.^{25,127,128} HCQ prevents platelet activation induced by aPL, and chloroquine could inhibit the internalization of aPL into the syncytiotrophoblast, reducing their mitochondrial deleterious effects.^{74,75,129} HCQ treatment is approved for use in APS patients with adverse pregnancy outcomes despite conventional treatment.²⁵

6.3 | Intravenous immunoglobulin

Intravenous immunoglobulin (IVIG) has been proposed for APS patients with adverse pregnancy outcomes despite conventional treatment.^{130,131} IVIG treatment decreases LA activity and interferes in the aCL binding to cardiolipin.^{132,133} Besides, anti-idiotypic antibodies to aPL in IVIG, which reduces aPL effects, have been documented. Nevertheless, effectiveness rates are controversial, IVIG therapy carries high costs, and there is a lack of evidence about clinical outcomes in APS-related pregnancy morbidity.^{130,134}

7 | CONCLUSION

β 2GP1 has a key role in the pathogenesis of APS-related pregnancy morbidity, which is different compared to the pathogenesis of vascular APS manifestations. Pro-inflammatory state and an imbalance between proangiogenic and antiangiogenic factors disturb uterine spiral artery remodeling and trophoblast proliferation and migration, resulting in adverse pregnancy outcomes like preeclampsia in APS patients. The effects of β 2GP1-dependent aCL are not determined sufficient in previous studies, therefore is poorly understood the role of these specific aCL in the pathogenesis of APS.

Furthermore, a β 2GP1 are more often associated with severe adverse clinical outcomes despite conventional treatment compared to other aPL in patients with APS-related pregnancy morbidity. Therefore, a β 2GP1 positivity is becoming a useful tool for obstetric risk stratification. However, prospective studies including cohorts of APS patients are required to determine the usefulness of a β 2GP1 based risk stratification. At last, β 2GP1 might be a possible target of

molecular-targeted treatment in the future to prevent obstetric complications in APS.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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