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Rational Design of Nanomedicine for Placental Disorders: Birthing a New Era in Women's Reproductive Health

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The placenta is a transient organ that forms during pregnancy and acts as a biological barrier, mediating exchange between maternal and fetal circulation. Placental disorders, such as preeclampsia, fetal growth restriction, placenta accreta spectrum, and gestational trophoblastic disease, originate in dysfunctional placental development during pregnancy and can lead to severe complications for both the mother and fetus. Unfortunately, treatment options for these disorders are severely lacking. Challenges in designing therapeutics for use during pregnancy involve selectively delivering payloads to the placenta while protecting the fetus from potential toxic side effects. Nanomedicine holds great promise in overcoming these barriers; the versatile and modular nature of nanocarriers, including prolonged circulation times, intracellular delivery, and organ-specific targeting, can control how therapeutics interact with the placenta. In this review, nanomedicine strategies are discussed to treat and diagnose placental disorders with an emphasis on understanding the unique pathophysiology behind each of these diseases. Finally, prior study of the pathophysiologic mechanisms underlying these placental disorders has revealed novel disease targets. These targets are highlighted here to motivate the rational design of precision nanocarriers to improve therapeutic options for placental disorders.

1. Introduction

Obstetric complications are a leading cause of maternal and fetal morbidity and mortality.[1–3] In the United States, the rate of maternal mortality has risen from 17.4 deaths per 100,000 live births in 2018 to 23.8 deaths per 100,000 live births in 2020.[4] Many of the dangerous complications that can arise during pregnancy stem from dysfunctional placental development.[5,6] Despite the prevalence of these disorders, current treatment options are severely lacking, and drug discovery and development for maternal and fetal health are significantly understudied compared to other areas of disease.[1,7–9] Insufficient understanding of mechanisms garnering placental transfer and subsequent ethical concerns regarding the potential toxicity of therapeutic agents to the unborn fetus have resulted in a shortage of novel therapies and a general exclusion of pregnant patients from clinical trials for existing therapies.[1,10–12]

The need to design therapeutics specifically for use in pregnant patients has been evident since the thalidomide crisis of the 1960s, when the use of thalidomide to treat morning sickness resulted in increased incidence of birth defects.[13,14] Since then, this need has grown into three separate concepts: designing therapies specifically for maternal sequestration, placental accumulation, or fetal delivery. The use of engineered nanomedicine platforms has the potential to accomplish these feats. Nanomedicine has been widely explored in applications of cancer, gene therapy, diagnostics, and vaccines;[15–18] however, the use of nanomedicine for pregnancy is

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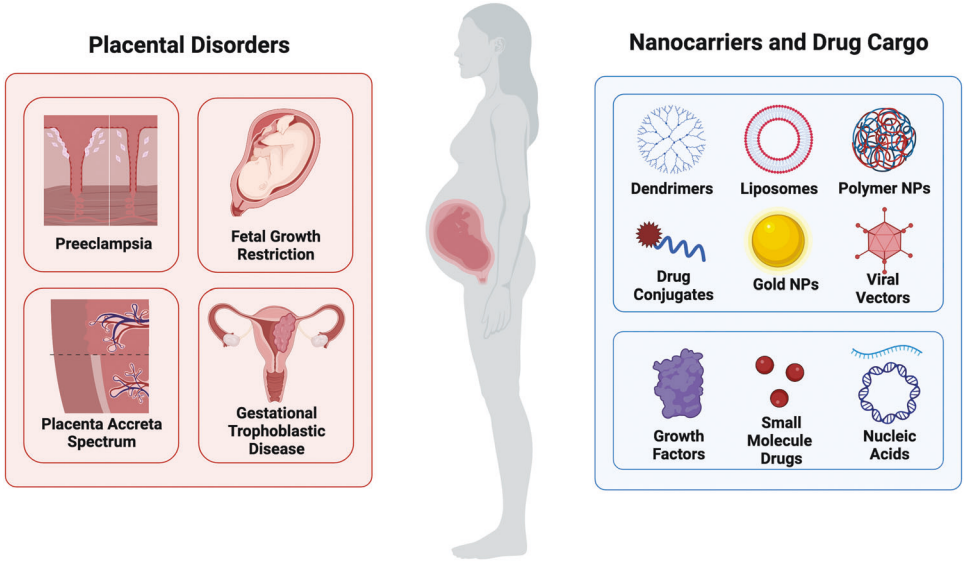

Figure 1. Overview of placental disorders and potential of nanomedicine platforms to treat placental disorders occurring during pregnancy. The four main placental disorders include preeclampsia, fetal growth restriction, placenta accreta spectrum, and gestational trophoblastic disease. Nanocarriers studied during pregnancy have ranged from polymeric nanoparticles (NPs) and gold NPs to drug conjugates and viral vectors. Nanocarriers have encapsulated growth factors, small molecules, and nucleic acids for the treatment of placental disorders during pregnancy.

in its infancy. Nanocarriers are beneficial because they are highly modular: they can encapsulate poorly soluble drugs, prolong circulation time compared to free cargo, penetrate cellular barriers, and achieve organ-specific delivery via targeting moieties.[15,16] Given this level of tunability, nanocarriers can be formulated specifically to deliver therapeutics to maternal organs during pregnancy.[6,19,20] In particular, the placenta is a target of interest, as many pregnancy complications originate during placental development.[5,19,21] Unfortunately, the pathophysiologic mechanisms underlying many placental disorders remain poorly understood, further hindering the design of successful nanomedicine therapies.

To inspire a new era of engineered platforms, this review discusses current knowledge of pathophysiology behind the following placental disorders: preeclampsia, fetal growth restriction, placenta accreta spectrum, and gestational trophoblastic disease. We then highlight nanomedicine platforms that have been established thus far to diagnose and treat these placental conditions (**Figure 1**). Last, we propose new targets for the rational design of novel nanomedicine strategies, with the ultimate goal of improving healthcare outcomes during pregnancy.

2. Placental Physiology

The placenta is a transient, yet exceedingly complex organ that develops during pregnancy to support fetal development.[22–26] Throughout gestation, the placenta not only acts as a physiological barrier, protecting the fetus from potentially harmful agents present in maternal circulation, but also as a biochemical liaison, continuously mediating nutrient, oxygen, and waste product exchange between the mother and the developing fetus (**Figure 2**A).[22,23,27,28] As one of the most functionally diverse biological systems, the placenta is solely responsible for actions usually performed in adults by separate, major organ systems, including the renal, respiratory, and endocrine systems.[23,29] For example, the placenta serves as an endocrine organ during pregnancy, secreting hormones into both maternal and fetal circulation to promote the maintenance of pregnancy, and to confer fetal immunotolerance, respectively.[5,22]

During the menstrual cycle, the uterus prepares for pregnancy by transforming the endometrium into the decidua via a process called decidualization wherein: i) tissue becomes highly vascularized, ii) endometrial stromal cells differentiate into decidualized stromal cells, transitioning from fibroblast-like phenotype to secretory cells, and iii) maternal immune cells infiltrate the endometrial environment.[30–33] This process is essential for proper blastocyst attachment and normal placental development, which begins around day seven post-fertilization.[30,33,34] Once the blastocyst has attached to the uterus, the predominant cell type of the placenta—the trophoblast—proliferates from the outer trophectoderm layer of the blastocyst.[22,35]

Together, the decidua and placenta form highly specialized tissue, often referred to as the maternal-fetal interface, which serves to promote embryo development, regulate adaption to the allogenic fetus, and protect the developing fetus from infection.[36,37] As early as the peri-implantation period, immune cells are recruited to the maternal-fetal interface to exert T helper (Th)-2/antibody-mediated immunotolerant effects over the fetus, while maintaining host immune defense again potential pathogens. Failure to develop maternal tolerance or pro-inflammatory-inducing insults at the maternal-fetal interface has been associated with miscarriage, preterm labor, spontaneous abortion, preeclampsia, and congenital infection.[36–39]

As the placenta develops post-implantation, placental cells mature from a primitive lineage of trophoblasts into cytotrophoblasts (CTBs) through two major pathways.[5,22,40] First, mononucleated CTBs fuse to form the specialized, multinucleated syncytiotrophoblast (STB) layer, also known as the

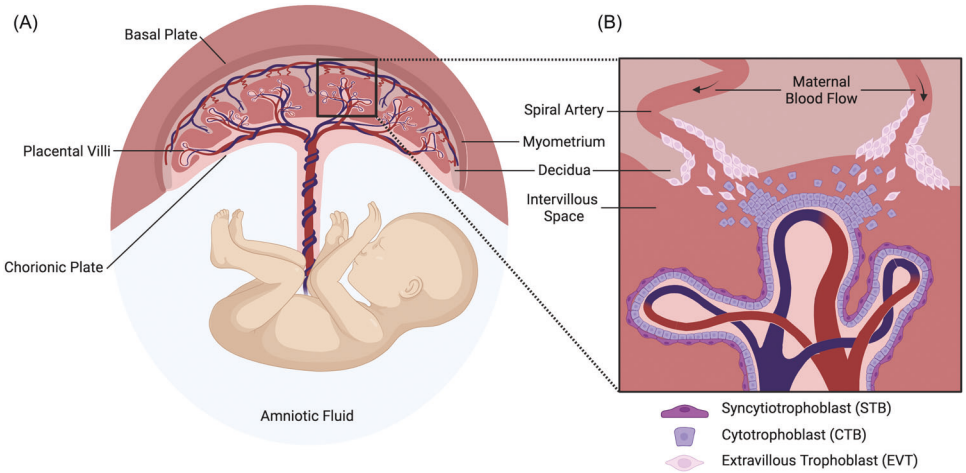

Figure 2. The biology of the placenta. A) The placenta mediates the transfer between maternal and fetal circulation during pregnancy. B) During placental development, cytotrophoblasts (CTBs) differentiate into the syncytiotrophoblast (STB) layer, forming the exterior of placental villi functional units and facilitating biochemical processes to support fetal development. CTBs can burst open placental villi and migrate towards vasculature. Here, CTBs differentiate into extravillous trophoblasts (EVTs) and invade maternal spiral arteries to create high-flow vasculature for enhanced blood supply to the placenta and fetus.

syncytium, which is responsible for initially invading the decidua to embed the embryo in the uterus.[22,34,40,41] Around eight days post-conception, fluid fills the spaces between the STB layer, creating lacunae or intervillous spaces.[5,41] At this stage, the three main zones of the placenta can be defined: the chorionic plate facing the embryo, the STB system developing into placental villi, and the basal plate in contact with the maternal endometrium.[34] The STB layer is bathed in maternal blood and shrouds the exterior of placental villi, creating functional units with extensive surface area to facilitate complex biochemical interactions between the mother and fetus.[5,22,27,35] A niche of CTBs remains beneath the STB layer and serve as a stem cell reservoir for the syncytium throughout villous development.[22,34,35] In the alternative pathway, CTBs acquire invasive abilities and differentiate into extravillous trophoblasts (EVTs), capable of invading the decidua and maternal uterine spiral arteries. EVTs replace vascular smooth muscle present in maternal arteries, with the purpose of remodeling vasculature to create large, low-resistance vessels to meet high blood-flow demands necessary to support placental perfusion and fetal growth (Figure 2B).[5,22,35,42] By the third trimester, blood flow to the uterus and placenta utilizes approximately 25% of maternal cardiac output.[27,43] Not surprisingly, EVT-mediated vascularization has been identified as a critical step during placental development, as deficiencies in artery remodeling result in reduced perfusion to the fetus and, thus, can lead to developmental abnormalities.[1,27,42]

Due to the exhaustive and versatile functionality of the placenta, the developing fetus remains reliant on the placenta to survive for nine months in utero, and abnormal placentation or dysfunction at the maternal-fetal interface can have direct consequences on the long-term health of both the mother and child.[1,5,23,27,29] Placental pathologies underlie most obstetric complications, including preeclampsia, fetal growth restriction, miscarriage, preterm labor, and fetal death.[5,27,42] Maternal consequences from placental pathologies can include kidney and/or cardiac injury, seizures, preterm labor, and premature death.[1,42] In addition, children born from pregnancies complicated by such pathologies are at a higher risk for developing cardiovascular disease, type 2 diabetes, adiposity, and psychiatric conditions later in life.[1,5,42,44] Thus, healthy placental development during pregnancy is critical for maintaining short- and long-term health of both the mother and child.

3. Nanomedicine Strategies for Placental Delivery during Pregnancy

The field of nanomedicine strives to engineer materials on the nanoscale range (1–100 nm), termed nanocarriers, to diagnose and treat disease.[45] Examples of nanocarriers include dendrimers, viral vectors, peptide-drug conjugates, and nanoparticles (NPs), including liposomes, lipid nanoparticles (LNPs), polymeric nanoparticles, and gold nanoparticles (Au NPs).[6,15] These nanocarriers can be used to encapsulate a wide range of drug cargos including small molecule drugs, nucleic acids, and proteins, making them an incredibly versatile platform for the diagnosis and treatment of disease.[15] Nanomedicine has had considerable clinical success, from the initial approval of Doxil (liposomal doxorubicin) in 1995, to the first ever RNA interference therapy ONPATTRO (small interfering RNA (siRNA) delivered via LNP), and most recently, the development of Moderna and Pfizer/BioNTech's LNP COVID-19 mRNA vaccines.[46,47]

The use of nanomedicine to systemically deliver a therapeutic agent, as opposed to administration of free drug, has many advantages, including the ability to encapsulate insoluble cargo, prolong circulation time, facilitate intracellular delivery, prevent degradation, and increase efficacy while reducing off-target side effects.[1,10,11,48] However, several challenges are associated with systemic administration of nanocarriers including rapid hepatic clearance, instability, and the potential for off-target effects.[15,48–50] Fortunately, nanocarriers are highly modular systems and, as such, their physicochemical properties can be tuned to overcome these barriers and achieve enhanced delivery to an

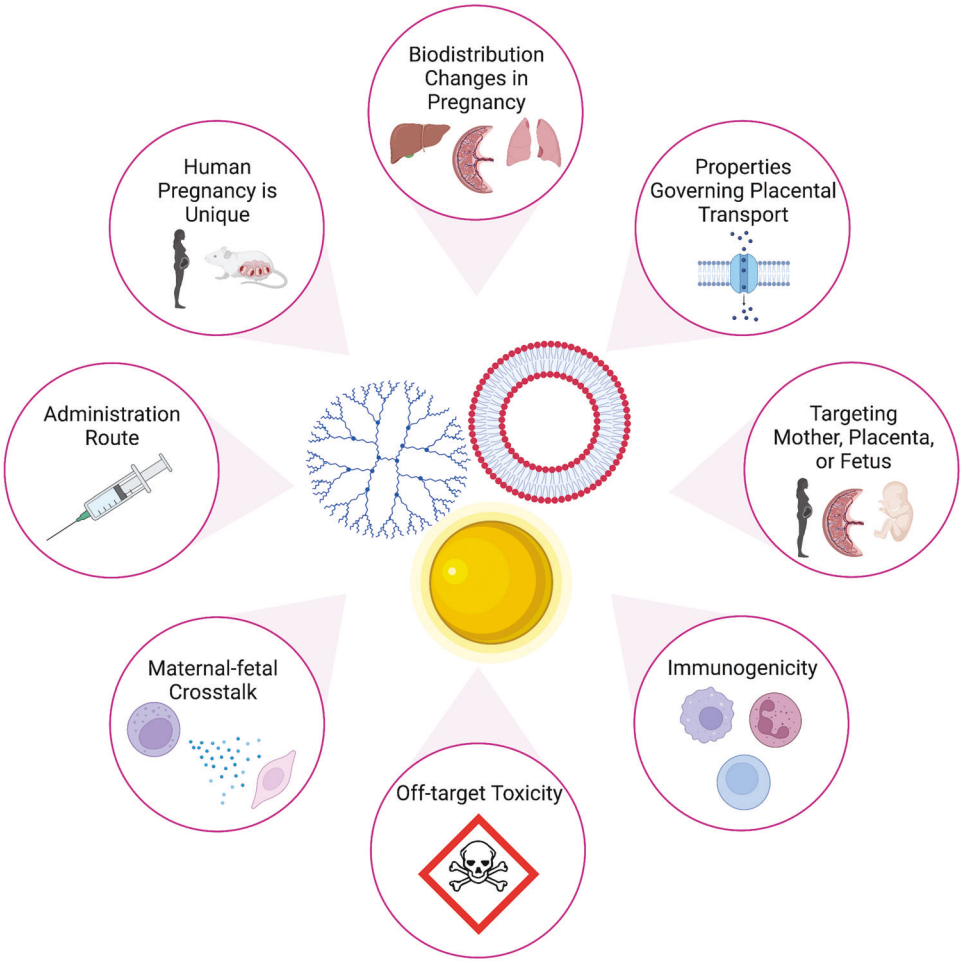

Figure 3. Design considerations of nanomedicine platforms for use during pregnancy. Physiological changes in pregnancy may alter pharmacokinetics of nanocarriers, and limited knowledge regarding how nanocarrier physicochemical properties impact placental transcytosis may present as a challenge when designing nanocarrier platforms for use during pregnancy. Nanocarriers should be designed to preferentially target maternal circulation, fetal circulation, or the placenta based on the desired application. Further, nanocarriers should not exacerbate the pro-inflammatory conditions present in many obstetric complications, and off-target effects must be minimized, as they may be detrimental to not only maternal health, but also fetal development. Cellular crosstalk at the maternal-fetal interface is complex and remains only partially understood; interactions between nanocarriers and the local immune environment must be considered. Nanomedicine platforms should be designed for use with intravenous (IV) administration, as intraplacental injections are not common in the clinic. Finally, human pregnancy is unique and proper recapitulation in in vitro, in vivo, or ex vivo models will be important in assessing nanocarrier efficacy and safety in pregnancy.

organ of interest. One of the most widely used techniques to enhance nanocarrier circulation time and reduce hepatic clearance is nanocarrier surface modification with poly(ethylene) glycol (PEG).[15,49] PEG acts to prolong circulation time by creating a hydrophilic layer around the nanocarrier surface, which reduces protein adsorption and subsequent clearance by the mononuclear phagocytic system.[48,49] Additionally, the size, shape, and charge of the nanocarrier can influence biodistribution.[15,48] Nanocarriers less than 5 nm in size have demonstrated rapid renal clearance upon injection while nanoparticles larger than 200 nm undergo splenic filtration.[15,48] Cationic nanocarriers are rapidly cleared from the bloodstream, followed by anionic nanocarriers, while neutral nanocarriers typically have the longest circulation times.[10,15] Furthermore, cationic nanocarriers can induce toxicity upon systemic administration.[10,15] To enhance the delivery of nanocarriers to the organ of interest and reduce off-target effects, targeting moieties, such as antibodies and peptides, can be conjugated to the surface of the nanocarrier.[15,51] When targeted nanocarriers reach their tissue of interest, they are taken up by cells via endocytosis, which can enable intracellular release of cargo.[15]

The use of nanomedicine platforms for drug delivery during pregnancy is in its infancy. Pregnancy drastically changes the anatomy and physiology of the body; however, due to concerns surrounding maternal and fetal safety, knowledge regarding the effects of these bodily changes remains poorly understood. To this end, several design considerations must be made when investigating nanomedicine platforms for use during pregnancy (**Figure 3**). For example, it is widely known that nanocarriers largely accumulate in the liver. However, approximately 25% of cardiac output gets shunted to the placenta during pregnancy,[43] which may alter the biodistribution of nanocarriers. In

addition, physicochemical properties that affect transplacental crossing have not been fully elucidated, and uptake into or across the placenta may be further influenced by gestational age and placental maturation.[52,53] Most small (<600 Da), lipophilic molecules cross the placenta through passive diffusion while larger proteins undergo active transport.[1,11,19,54,55] For example, IgG antibodies are actively transported across the placenta via the major histocompatibility complex class I-related (MHC) Fc receptor (FcRn) present on the STB layer.[53,55,56] Further work is required to understand how nanocarriers can be tuned to remain in maternal circulation, accumulate in the placenta, or cross into fetal circulation based on the desired application. Specifically, selective delivery of nanocarriers is important in the context of pregnancy, as off-target effects can have detrimental impacts on both the mother and the fetus.[6,11] In order to effectively study the biodistribution, therapeutic efficacy, and potential off-target effects of nanocarriers during pregnancy and, more specifically, during pregnancy complications, sophisticated animal models are required, as human pregnancy is unique and can be difficult to recapitulate in preclinical studies. While information regarding in vitro, in vivo, and ex vivo pregnancy models are beyond the scope of this review, other review articles have provided an extensive discussion on this topic.[10,20,57–60]

While the development of nanomedicine platforms for women's reproductive health remains underexplored, foundational studies over the last few decades have investigated how nanocarrier properties affect nanocarrier behavior during pregnancy. Work by Dodd et al. demonstrated that conjugation of the toxic drug, haloperidol, to PEG resulted in reduced placental uptake, which could have important implications for the use of anti-anxiety and depression medications during pregnancy.[61] A study by Kuna et al. highlighted a potential size-dependent relationship, during which increasingly large elastin-like polypeptides (ELPs) led to increased placental uptake.[62] Various types of Au NPs have also demonstrated trophoblast uptake with minimal fetal transfer and, thus, may serve as potential nanocarrier vehicles for a variety of pregnancy applications.[63–66] A study by Muoth et al. demonstrated higher uptake and deeper placental penetration for smaller Au NPs and sodium carboxylate-modified Au NPs compared to larger Au NPs or PEGylated NPs, respectively.[66] Irvin-Choy et al. reported that uptake of Au NPs varies with gestation age; higher NP accumulation was observed in earlier gestation in mice.[65] On the contrary, silica, polystyrene, and cationic NPs have been reported to cross the placenta and enter fetal circulation within hours of administration, limiting their potential use as therapeutic platforms.[19,67,68]

Liposomes have also been evaluated for delivery to the placenta, with minimal transfer to the fetus. Valero et al. reported that 100 nm PEGylated liposomes were retained in the STB layer of the placenta in both a placental perfusion model and in placental explants.[69] A subsequent study by Alfaifi et al. evaluated the effect of a targeting ligand on the surface of liposomes; gentamicin-modified liposomes, targeting the megalin protein expressed on the STB layer, demonstrated significantly greater accumulation in the placentas of mice compared to an untargeted liposome control.[70] Polymeric NPs have also been utilized for placental delivery. In a pregnant rat model, poly(glycidyl methacrylate) (PGMA) NPs or PGMA NPs functionalized with poly(ethylenimine) (PEI) were injected on gestational day 10 or 20. Ho et al. reported similar levels of NP accumulation on day 10 but increased accumulation of the cationic PGMA-PEI NPs over the anionic PGMA NPs on day 20, suggesting the impact of gestational age and surface charge on NP accumulation in the placenta.[71] Dendrimers have also been used to study the effects of surface charge and particle size on placental accumulation. Juch et al. demonstrated that positively charged dendritic polyglycerol NPs had deeper penetration in placental explants compared to their negatively charged counterparts, while Menjoge et al. showed that 5.6 nm poly(amidoamine) (PAMAM) dendrimers had minimal penetration into fetal circulation in an ex vivo placental perfusion model.[72,73]

While these studies have been pivotal in establishing a field dedicated to engineering nanomedicine for pregnancy applications, there is much work to be done. Funding focused on women's health is lacking compared to other fields.[74,75] As such, many of the cellular and molecular pathways underlying obstetric disorders, specifically placental disorders, remain only partially understood. Therefore, a thorough understanding of key pathological pathways is critical for the design of nanomedicine for placental disorders. Motivated by this gap in the field, the following section highlights what is currently known regarding the pathophysiology of preeclampsia, fetal growth restriction, placenta accreta spectrum, and gestational trophoblastic disease and discusses nanocarrier platforms that have been explored for each disease. In addition, future potential targets are proposed with the hopes of inspiring the rational design of novel nanomedicine platforms for use during pregnancy.

4. Nanomedicine Strategies to Diagnose and Treat Placental Disorders

4.1. Preeclampsia

Preeclampsia is a serious hypertensive disorder unique to human pregnancy that affects around 5–7% of all pregnancies and is a major cause of maternal morbidity and mortality worldwide.[25,76–78] Preeclampsia is characterized by maternal hypertension with endothelial and renal dysfunction after the 20th week of gestation, stemming from an abnormal vascular response to placentation. If not treated, preeclampsia can lead to stroke, kidney failure, pulmonary edema, eclampsia, and death.[25,76] In fact, preeclampsia remains the leading cause of death in pregnant people in the United States.[25] Because preeclampsia is a multisystem disease, related morbidities usually accompany preeclampsia, with fetal growth restriction often affecting the fetus, and liver and hematological disorders (HELLP syndrome) often affecting the mother.[77,79,80] Preeclampsia is also associated with a greater risk of future cardiovascular and cerebrovascular diseases in both mothers and children born to preeclamptic mothers.[25,79,80] The exact etiology of preeclampsia remains elusive; its pathogenesis is not currently attributed to a single factor.[25] Risk factors, such as diabetes mellitus, SARS-CoV-2, and previous vascular or hypertensive disorders, have been documented to increase the likelihood of preeclampsia, but are not necessarily causal of disease pathology.[78,79,81,82] The widespread lack of understanding surrounding preeclampsia may be due, in part, to disease heterogeneity; pathogenesis can vary between patients, and pathogenesis and severity of

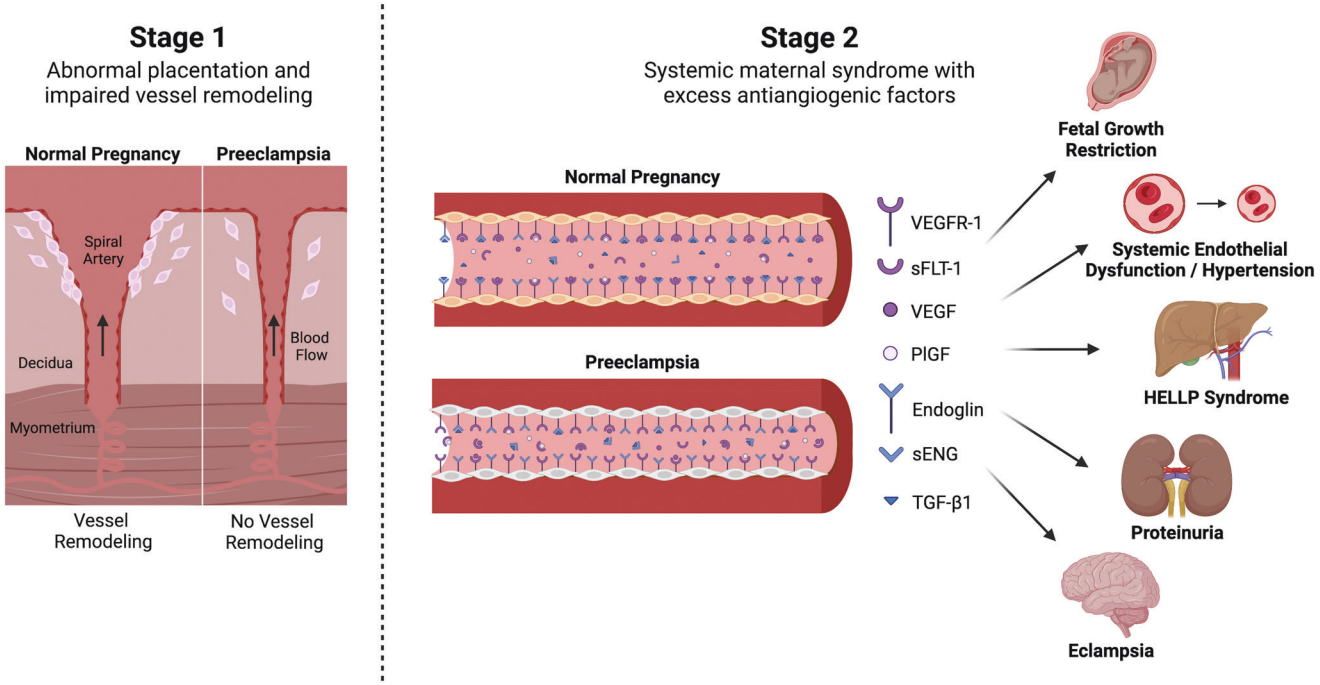

Figure 4. Schematic of preeclampsia. In stage 1, abnormal placentation is characterized by impaired spiral artery remodeling. In stage 2, deficient vascular remodeling manifests in maternal syndrome characterized by widespread endothelial activation and multi-organ disease. VEGFR-1, vascular endothelial growth factor receptor 1; sFLT-1, soluble fms-like tyrosine kinase 1; VEGF, vascular endothelial growth factor; PlGF, placental growth factor; sENG, soluble Endoglin; TGF-β1, transforming growth factor beta 1.

the disease can differ based on gestational age at the onset of disease.[25,78,80]

4.1.1. Pathophysiology of Preeclampsia

While the cause of preeclampsia remains unknown, it is widely recognized that the placenta plays a central role in preeclampsia pathogenesis. Defective decidualization, impaired CTB invasion, endothelial dysfunction, and inappropriate maternal immune response to the allogenic fetus are all thought to be major contributing factors to the disease.[25,79,82] These pathologies may separately or synergistically lead to the clinically recognizable, downstream pathway of defective spiral artery remodeling and subsequent release of proinflammatory cytokines into the maternal bloodstream.[25,78,79,83,84]

Building evidence suggests that preeclampsia can be broken down into two stages: 1) abnormal placentation and defective spiral artery remodeling occur during the first trimester followed by 2) a systemic maternal syndrome in the second or third trimester characterized by an excess of antiangiogenic factors (**Figure 4**).[78,79,84–87] During normal placentation, EVTs differentiate into an invasive phenotype and invade and remodel maternal uterine spiral arteries.[5,22,35,42,78,88] Failure of CTBs to acquire this invasive phenotype and/or inadequate spiral artery remodeling leads to shallow placentation and placental ischemia seen in preeclamptic patients, as confirmed by Doppler studies.[78,88,89] The resulting structurally abnormal placenta contributes toward the maternal syndrome observed in stage two of preeclampsia. Placental ischemia creates an imbalance between angiogenic and anti-angiogenic factors, leading to systemic maternal vascular endothelial dysfunction. During endothelial dysfunction, levels of vasodilators prostaglandin I2 (PGI2) and nitric oxide (NO) decrease, vasoconstriction occurs, and blood flow to all organs is reduced.[78,79,84,88] Serum from patients with preeclampsia display increased levels of factors associated with endothelial injury, including fibronectin, factor VIII antigen, and thrombomodulin. Increased serum levels of vascular cell adhesion molecule 1 (VCAM-1) and E-selectin, markers of endothelial cell activation, have also been observed.[88,90]

The maternal syndrome of preeclampsia is characterized by excess secretion of the hallmark inflammatory molecules soluble fms-like tyrosine kinase-1 (sFLT-1) and soluble endoglin (sENG), which contributes towards a generalized inflammatory response and systemic endothelial dysfunction, further perpetuating preeclampsia symptoms.[78,79,84] Studies have demonstrated that sFLT-1 and sENG are released directly from trophoblast cells in placental villi in response to ischemic placental conditions. In fact, increased levels of circulating sFLT-1 and sENG are present in maternal serum not only during clinical preeclampsia, but also predate clinical symptoms on the order of weeks.[88,89] The magnitude by which levels of sFLT-1 and sENG are increased in preeclamptic patients has been shown to correlate with disease severity.[77,80,83,84]

sFLT1 is the soluble version of vascular endothelial growth factor receptor 1 (VEGFR1) and acts as a decoy receptor for vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), decreasing the bioavailability of VEGF and PlGF in maternal circulation.[78] sFLT-1 also directly inhibits endothelial NO synthesis, which induces hypertension and oxidative stress. NO

is required for vascular remodeling, and NO deficiency has been shown to impair vasorelaxation in human and animal models of preeclampsia.[79,91] Another prominent antiangiogenic molecule upregulated in preeclampsia is sENG, the soluble version of Endoglin or CD105. sENG interferes with transforming growth factor β1 (TGF-β1) pathways, inhibiting endothelial NO synthase activation, and consequently promoting vasoconstriction.[84,92] sENG has been shown to display a synergistic relationship with sFLT-1, capable of potentiating the vascular effects of sFLT-1. Together, sFLT-1 and sENG have been shown to induce severe preeclampsia symptoms in rats, including severe hypertension, fetal growth restriction, and cerebral edema.[78,88]

The dysfunctional vascular pathways seen in preeclampsia are carefully intertwined with dysfunctional immune pathways at the maternal-fetal interface as well. During a healthy pregnancy, levels of T helper (Th)-1 and Th2 immune cells are carefully regulated, with an overall shift toward Th2 populations to promote fetal tolerance.[37,79] However, during preeclampsia, an increase in levels of Th1 cells and their respective immune response is observed, including increased levels of the pro-inflammatory cytokines tumor necrosis factor α (TNF-α), interleukin (IL)-6, and IL-8 and an overrepresentation of M1 macrophage phenotypes.[37,93] This shifted Th1 immune response leads to chronic inflammation similar to that seen in autoimmune diseases and results in further increased sFLT-1 expression and oxidative stress.[39,78,79] An imbalance between regulatory T cells (Treg) and Th17 cells at the maternal-fetal interface is also observed. During healthy pregnancy, naïve T cells differentiate into Treg cells to create a tolerant microenvironment and ultimately prevent rejection of the allogenic fetus. Th17 cells, differentiated from CD4+ T cells, mediate inflammatory responses against infections and may play a role in cases of fetal loss. During preeclampsia, an imbalance between Treg and Th17 cell differentiation occurs, leading to the downregulation of Treg-mediated protective functioning and upregulation of Th17-mediated inflammation.[36,39,94] There is building evidence that the crosstalk between immune cells at the maternal-fetal interface may be supported by extracellular vesicles (EVs) carrying proteins, microRNAs, and small molecules capable of inducing changes in the local immune environment. Studies have suggested that alterations in EV contents may contribute to, and potentially further exacerbate, the inflammatory maternal immune response present in preeclampsia.[10,38,95,96] Further, it's been shown that EVs from preeclamptic patients contain higher levels of sFLT-1 and sENG compared to EVs from healthy pregnancies, and the levels of EVs in maternal circulation may correlate with disease severity.[10,96]

4.1.2. Gold Standard Treatment for Preeclampsia

There are currently no curative treatment options for preeclampsia. Instead, medication can be used to manage preeclampsia symptoms. Maternal hypertension can be managed via low doses of aspirin.[80] For acute hypertension emergencies, β blocker Labetalol and/or vasodilator Hydralazine can be administered via intravenous (IV) bolus injection.[79,80] In the case that IV injection is not feasible, oral administration of Nifedipine, a calcium channel blocker, is often used to manage blood pressure.[79,80,97,98] In some cases, anticonvulsants may also be used to manage symptoms. Previous studies have demonstrated that magnesium sulfate is associated with a reduced rate of recurrent seizures and maternal death in preeclamptic patients when compared to other anticonvulsants.[80] If disease severity progresses and symptoms may no longer be managed via medication, such as the development of HELLP syndrome or multisystem organ failure, delivery of the fetus and placenta is the only available treatment option.[25,77,80] Following delivery of the placenta, maternal symptoms usually subside within a few days.[77,99]

4.1.3. Nanomedicine Strategies for Preeclampsia

A variety of nanocarriers have been explored for the treatment of preeclampsia (**Table 1**). Inspired by previous work targeting chemotherapeutics to the tumor microenvironment, Beards et al. investigated the use of tumor-homing peptide CCGKRK to selectively deliver microRNA (miRNA) inhibitors to the placenta—a method to block endogenous RNA interference.[100] Given its ability to undergo rapid proliferation and evade immune surveillance, the placenta is often referred to as a nonmalignant relative of the solid tumor, motivating the use of tumor-homing peptides as a delivery tool.[101,102] Previously, miR-675 and miR-145 have been identified as negative regulators of placental growth.[100,103,104] In this proof-of-concept study, researchers showed that intravenously injected CCGKRK-conjugated miR-675 and miR-145 inhibitor sequences significantly enhanced placental and fetal weight in healthy pregnant mice compared to controls. However, only the miR-675 inhibitor conjugate was able to reduce placental miR-675 expression in the mouse model, and neither inhibitor increased the rate of proliferation in the mouse placenta. Interestingly, when applied to human first trimester explants, both conjugates enhanced cytotrophoblast proliferation,[100] which could have implications during the early stages of abnormal placentation occurring in the first trimester in preeclampsia patients. Of note, the authors observed widespread maternal accumulation of miR inhibitors in pregnant mice during biodistribution studies, but information regarding potential off-target effects was not discussed. Further work is required to demonstrate the safety profile of miR inhibitor therapeutics during pregnancy and potential therapeutic efficacy in treating preeclampsia.

Utilizing this same targeting approach, Li et al. formulated CGKRK-targeted liposomes loaded with PFKFB3 DNA plasmids.[105] PFKFB3 is a gene involved in glycolysis regulation in endothelial cells and has been shown to promote vessel sprouting in various organs.[106] PFKFB3 expression has also been identified in placental tissues and is speculated to promote angiogenesis, making it a potential target for PE treatment.[106,107] However, PFKFB3 function during pregnancy has not yet been fully elucidated.[105] Following the administration of CGKRK-targeted liposomes loaded with PFKFB3 plasmids in healthy mice, the authors demonstrated enhanced PFKFB3 expression in placental tissues compared to the untargeted liposome control. Overexpression of PFKFB3 led to increased placental and fetal weight, as well as increased micro-vessel density in the placental tissue. Further, serum levels of blood urea nitrogen (BUN) and liver enzymes aspartate transaminase (AST) and alanine

Table 1. Preclinical Nanomedicine Strategies to Treat and Diagnose Placental Disorders.

	Delivery Vehicle	Cargo	Targeting Moiety	Treatment Outcomes	References
Preeclampsia					
	Peptide-drug conjugate	miRNA inhibitor	CCGKRK	Reduced placental miR-675 expression, enhanced placental/fetal weight	[100]
	Liposome	pDNA (PFKFB3)	CGKRK	Increased placental/fetal weight and micro-vessel density	[105]
	Peptide-drug conjugate	NF-κB inhibitor peptide	N/A	Partially reduced MAP, reduced placental TNF-α	[108]
	Lipid-drug conjugate	siRNA (sFLT-1)	N/A	>50% sFLT-1 serum knockdown, attenuation of hypertension and proteinuria	[99]
	Dendrimer	siRNA (sFLT-1)	N/A	Reduced serum sFLT-1, enhanced placental/fetal weights	[114]
	Lipid-polymer NP	siRNA (sFLT-1)	CSA-BP	Reduced serum and placental sFLT-1	[115]
	Polymer NP	siRNA (sFLT-1, Nrf2)	CSA-BP	Decreased MAP, increased angiogenic placental factors	[117]
	Lipid NP	mRNA (VEGF)	N/A	Increased blood vessel area	[119]
	Lipid NP	mRNA (PlGF)	N/A	Increased serum and placental PlGF	[120]
Fetal Growth Restriction					
	Adenoviral vector	dsDNA (IGF-1)	N/A	Improved placental glucose transport and amino acid transporter expression	[157,159]
	Polymer NP	pDNA (IGF-1)	N/A	Increased fetal weight and placental labyrinth depth	[160]
	Polymer NP	pDNA (IGF-1)	N/A	Increase glucose and amino acid transporters and fetal capillary volume density	[161,162]
	Liposome	GF (IGF-2)	CGKRK, iRGD	Increased placental/fetal weight	[102]
	Liposome	GF (EGF)	GPS	Activated downstream EGFR pathways	[142]
	Adenoviral vector	dsDNA (VEGF)	N/A	Enhanced fetal growth velocity	[167,168]
	Adenoviral vector	dsDNA (VEGF)	N/A	Increased fetal weight and placental depth	[166]
	Liposome	Small molecule (SE175)	CNKG	Increased fetal weight and mean spiral artery diameter, decreased placental oxidative stress	[172]
Placenta Accreta Spectrum					
	Liposome	Gadolinium	N/A	Clear visualization of retroplacental clear space via MRI	[216–219]
Gestational Trophoblastic Disease					
	Lipid-Polymer NP	Chemotherapeutic (methotrexate, doxorubicin)	CSA-BP	Impaired placental/fetal development, inhibited tumor growth and metastasis	[116,245]
	Polymer NP	Small molecule (prodigiosin)	CSA-BP	Decreased tumor volume and growth	[249]
	EnGenIC NP	Chemotherapeutic (doxorubicin)	Anti-EGFR Ab	Reduced tumor size	[244]
	Liposome	siRNA (SATB1)	Anti-EGFR aptamer	Reduced tumor size	[246]

(*Continued*)

Table 1. (Continued).

Delivery Vehicle	Cargo	Targeting Moiety	Treatment Outcomes	References
Gestational Trophoblastic Disease				
Liposome	Chemotherapeutic (doxorubicin, β-carotene)	N/A	Increased choriocarcinoma apoptosis	[260]
Micelle	Chemotherapeutic (methotrexate)	N/A	Inhibited tumor growth and metastasis	[264]
Iron Oxide NP	ASODN (anti-heparanase)	Anti-β-hCG	Inhibited tumor growth	[266]
Polymer NP	Chemotherapeutic (methotrexate)	Anti-hCG polypeptide	Reduced choriocarcinoma proliferation	[238]
Liposome	Chemotherapeutic (methotrexate)	Cytarabine	Inhibited tumor growth	[271]

miRNA, microRNA; pDNA, plasmid DNA; NF-κB, nuclear factor kappa B; MAP, mean arterial pressure; siRNA, small interfering RNA; sFLT-1, soluble fms-like tyrosine kinase-1; NP, nanoparticle; CSA-BP, chondroitin sulfate A-binding peptide; Nrf2, nuclear factor-erythroid 2-like 2; mRNA, messenger RNA; VEGF, vascular endothelial growth factor; PlGF, placental growth factor; dsDNA, double stranded DNA; IGF-1, insulin like growth factor-1; GF, growth factor; IGF-2, insulin like growth factor-2; EGF, epidermal growth factor; SE175, nitric oxide donor SE175; MRI, magnetic resonance imaging; EGFR, epidermal growth factor receptor; Ab, antibody; SATB1, special AT-rich sequence-binding protein-1; ASODN, antisense oligodeoxynucleotides; hCG, human chorionic gonadotropin.

transaminase (ALT) did not differ between treatment groups, suggesting no apparent toxicity from PFKFB3 loaded liposomes. While the results of this study suggest that PFKFB3 overexpression leads to enhanced placental angiogenesis, future animal studies in a model of preeclampsia are required to demonstrate the therapeutic potential of this nanomedicine strategy in treating preeclampsia.[105]

Taking an immunomodulatory approach, Eddy and team opted to target the maternal inflammatory response through a key inflammatory mediator: the transcription factor NF-κB.[108] NF-κB controls the transcription of many pro-inflammatory cytokines, including IL-6, IL-8, and TNF-α, all of which are present in the maternal syndrome phase of preeclampsia.[108–110] The activity of NF-κB is increased in both the placenta and systemic vasculature in patients with preeclampsia, and this increased activity promotes further endothelial activation and dysfunction. To overcome pharmacokinetic limitations of small peptides and ensure maternal sequestration, researchers conjugated an NF-κB inhibitor peptide (p50i) to the amenable elastin-like polypeptide (ELP) drug carrier. It has previously been shown that ELP does not cross the placental barrier.[111] Indeed, following IV injection in pregnant rats, ELP-p50i conjugates were undetectable in fetal blood or amniotic fluid, while levels of free p50i peptide were identical in maternal and fetal blood. The authors then tested the therapeutic efficacy of ELP-p50i conjugates compared to free p50i peptide in a reduced uterine perfusion pressure (RUPP) rat model of preeclampsia using continuous intraperitoneal infusion to avoid rapid clearance of p50i in the free peptide treatment group. Rats treated with both free p50i and ELP-p50i displayed a smaller rise in mean arterial pressure (MAP) compared to saline-treated groups, however neither group reached statistical significance. The similar results achieved by these treatment groups suggest that the use of continuous infusion negated pharmacokinetic advantages achieved by the fusion of p50i to the ELP carrier. Unfortunately, increased doses of the ELP-p50i conjugates did not further reduce hypertension in rats, inciting the need for additional studies to refine this therapeutic strategy. Importantly, however, the authors demonstrated no inherent toxicity of ELP-p50i conjugates following administration in healthy pregnant rats, as reflected by no significant changes in maternal weight or fetal resorption. Flow cytometry of placental T-cell populations demonstrated that ELP-p50i administration did not affect immune cell recruitment to the placenta, further confirming the safety profile of ELP-p50i conjugates. Although ELP-p50i conjugates only partially ameliorated hypertension in preeclamptic mice, ELP-p50i administration did significantly reduce placental levels of TNF-α,[108] suggesting that ELP-p50i conjugates may still hold promise as a therapeutic strategy. Additional work exploring administration routes and dosing schemes are necessary for the clinical translation of ELP-p50i conjugates for the treatment of preeclampsia.

Perhaps the most popular nanomedicine approach for the treatment of preeclampsia involves the use of siRNA to modify concentrations of placental-derived pathologic proteins in maternal circulation. Specifically, many researchers have focused on modulating sFLT-1 to disrupt its role in perpetuating widespread vascular dysfunction in preeclampsia. In a pivotal study by Turanov et al., sFLT-1 siRNA-cholesterol conjugates were examined in non-human primates using ligation of a single uterine artery to induce a uteroplacental ischemia (UPI) model of preeclampsia.[99] Chemically stabilized sFLT-1 siRNA was conjugated to cholesterol to enable nonselective siRNA distribution, favoring tissues with high blood flow and fenestrated endothelium—characteristics of the placenta.[112,113] Excitingly, a single dose of siRNA-cholesterol conjugates demonstrated >50% sFLT-1 knockdown in serum after two weeks, with sFLT-1 levels approaching those measured in control animals. Further, treatment with siRNA-cholesterol conjugates resulted in attenuation

of hypertension and proteinuria, consistent with potent sFLT-1 knockdown.[99] While these results surely demonstrate the potential of siRNA-cholesterol conjugates in treating preeclampsia, the study utilized high siRNA doses, and widespread siRNA accumulation was noted in many non-placental tissues. In addition, a trend toward lower birth weight was observed in siRNA-treated animals. Further work is required to assess dosing ranges to balance therapeutic efficacy with potential negative side effects of sFLT-1 modulation.[99]

Building upon the promising results using sFLT-1 siRNA, Yu et al. paired sFLT-1 siRNA with PAMAM dendrimers, as PAMAM dendrimers have shown to be effective siRNA carriers with minimal toxicity and low maternal-fetal transfer.[73,114] Following administration of siRNA-loaded PAMAM dendrimers in a TNF-α induced rat model of preeclampsia, circulating levels of sFLT-1 were reduced and both placental and fetal weights were enhanced compared to the untreated preeclamptic group. No major tissue abnormalities were observed in the histological analysis of major maternal organs, however more detailed safety profiling, such as analysis of inflammatory markers in the blood or placenta, was not demonstrated.[114]

In another study, Li and team employed a novel PEG-PLA NP system conjugated with the synthetic placental chondroitin sulfate A (CSA) binding peptide (P-CSA-BP) for NP-mediated sFLT-1 siRNA delivery to the placenta.[115] CSA exists on the surface of the placental STB layer, and previous work has highlighted the ability of P-CSA-BP to target NPs to the placenta.[116] In this work, researchers formulated lipid-polymer NPs and conjugated P-CSA-BP onto the NP surface to create placenta-targeted RNA delivery systems. In pregnant CD1 mice, P-CSA-BP targeted NPs demonstrated a significant increase in NP accumulation in the placenta compared to nontargeted NPs. Further, targeted NPs resulted in lower levels of sFLT-1 mRNA in placental tissue and reduced sFLT-1 protein levels in systemic circulation. Of note, the targeted lipid-polymer NP system was tested only in a healthy pregnancy model and, thus, further work is required to investigate the potential of P-CSA-BP NP-mediated delivery during preeclampsia pathogenesis. In a parallel study, Li et al. constructed P-CSA-BP targeted polymeric NPs encapsulating siRNA for modification of placental-derived pathologic molecules. In this work, NPs were formulated with siRNA against both sFLT-1 and nuclear factor-erythroid 2-like 2 (Nrf2).[117] While sFLT-1 is widely known to participate in the pathogenesis of preeclampsia and, thus, a popular target for nucleic acid therapy, the potential role of Nrf2 in preeclampsia is not as ubiquitously acknowledged. In healthy cells, Nrf2 is a transcription factor that regulates oxidative stress responses. A previous study by Nezu et al. elucidated the role of Nrf2 in preeclampsia specifically, demonstrating that Nrf2 deficiency in a preeclampsia mouse model increased angiogenesis, while Nrf2 activation resulted in decreased angiogenesis and worsened maternal and fetal outcomes.[118] As such, Li et al. probed the consequences of simultaneous downregulation of these two key pathogenic molecules. Individual NP-mediated silencing of sFLT-1 and Nrf2 both resulted in decreased blood pressure in the pregnancy-associated hypertension preeclampsia mouse model, however, only simultaneous NP-mediated silencing of sFLT-1 and Nrf2 resulted in decreased proteinuria. Excitingly, simultaneous silencing of sFLT-1 and Nrf2 increased expression of angiogenic factors chemokine ligand (CCL)-2, CCL5, chemokine ligand (CXCL)-9, and CXCL10 in the preeclamptic mouse placenta when compared to individual silencing of sFLT-1 or Nrf2 alone, suggesting that simultaneous downregulation could potentially impact preeclampsia progression.[117] While additional toxicity studies are needed to evaluate potential clinical safety, NP-mediated simultaneous downregulation of sFLT-1 and Nrf2 remains an exciting prospect for the treatment of preeclampsia.

Given that preeclampsia is characterized by an imbalance of angiogenic and anti-angiogenetic factors, much of the research in the field thus far has focused on targeting sFLT-1 (or generally silencing anti-angiogenic factors) as a therapeutic strategy. However, research exploring the supplementation of angiogenic factors, such as VEGF, has not been as widely reported. In a recent study published by our lab, Swingle et al. investigated placental delivery of VEGF mRNA using LNPs.[119] The authors first screened a library of LNPs and identified a lead LNP candidate, A4, that demonstrated significantly increased luciferase mRNA delivery in JEG-3 placental cells compared to the industry standard LNP controls MC3 and C12-200. Following IV administration in healthy pregnant mice, luciferase A4 LNPs achieved potent placental delivery and reduced liver delivery compared to C12-200. Cellular characterization following mCherry A4 LNP delivery in pregnant mice revealed approximately ≈3–5% mCherry positivity in trophoblast cells, endothelial cells, and immune cells in the placenta. Given that previous works have not delivered mRNA therapeutics to the placenta, the authors note that it is difficult to comment on the clinical significance of 3–5% positivity in placental cell populations. However, when VEGF mRNA-loaded LNPs were administered to healthy pregnant mice, authors observed not only a significant increase in fetal blood vessel area, but also an enhanced safety profile compared to C12-200. To evaluate the safety profile of A4, authors examined AST/ALT levels in mothers and cytokine expression of the following seven inflammatory markers in placentas: leptin, TNF-α, insulin-like growth factor 1 (IGF-1), IL-6, IL-1a, IL-1B, and granulocyte colony-stimulating factor (GCSF). After 48 h, C12-200 LNPs demonstrated increased AST levels compared to A4. After 6 h, cytokine levels were elevated for LNP treatment groups, but returned to baseline after 48 h, confirming the safety of LNP platforms.[119] While additional studies are necessary to investigate clinically relevant positivity rates for successful therapeutic translation, the results described here demonstrate the potential of A4 LNP-mediated local VEGF modulation in the placenta.

In a parallel study, Young et al. utilized the industry standard MC3 and C12-200 lipids to formulate LNPs with optimized lipid compositions for mRNA delivery to the placenta.[120] In vitro studies in the human trophoblast BeWo cell line demonstrated enhanced luciferase mRNA delivery with LNP formulations made with C12-200 compared to MC3. Subsequent biodistribution studies following IV administration of the top three optimized C12-200 LNP formulations encapsulating luciferase mRNA identified an LNP formulation, A14, as the top-performing LNP for luciferase mRNA delivery to the placenta. Interestingly, when the authors intravenously administered PlGF mRNA-loaded LNPs in healthy pregnant mice, LNP formulation A10 outperformed A14, with A10 demonstrating increased PlGF levels in both maternal serum and the placenta compared to

free mRNA and saline-treated groups. Additionally, the authors demonstrated significantly higher maternal serum PlGF levels than what is usually seen during pregnancy, further validating the potential of A10 in improving PlGF levels throughout pregnancy. To evaluate the safety profile of A10, authors examined AST/ALT levels in mothers and fetuses, fetal and placental weight, and concentration of the inflammatory marker IL-6 in placentas and maternal serum. No changes were observed in AST/ALT levels following A10 treatment, and no changes in overall fetal and placental health were observed among any treatment groups. IL-6 levels were slightly elevated in maternal serum following A10 administration, but no changes were observed in IL-6 levels in the placenta, suggesting that systemic IL-6 changes did not originate from immune activation in the placenta.[120] While both of these works demonstrate great promise in LNP-mediated mRNA delivery to the placenta, neither study tested LNP formulations in a preeclampsia disease model. Further work is needed to demonstrate the success of VEGF and PlGF mRNA therapies in treating preeclampsia.

The works discussed in this section demonstrate exciting prospects for nanocarrier-mediated treatment of preeclampsia. However, additional work is required for the successful clinical translation of these platforms. Specifically, extensive safety profiling of nanomedicine platforms is necessary. Given that a viable fetus is present during preeclampsia, potential off-target effects of nanocarriers could jeopardize not only maternal health, but also fetal development. Safety concerns are a major challenge for the translation of nanomedicine platforms during pregnancy, as there remains limited knowledge regarding nanocarrier properties that garner placental sequestration versus placental transport. Many of the works presented in this section utilize fetal and placental weight as markers of fetal health in rodent models, however, future studies should include more in-depth safety profiling, such as examination of liver enzymes and inflammatory markers in the placenta as reported by Swingle et al.[119] and Young et al.,[120] or immune cell profiling in the placenta as reported by Eddy et al.[108] in order to confirm the safety of both mother and fetus throughout pregnancy. Further, it is widely accepted that nanocarriers inherently exhibit immunogenic effects. Additional data is needed to evaluate the effects of drug delivery vehicles on placental function. A recent, pivotal study by Chaudhary et al. highlighted trends between LNP formulation and immunogenicity during healthy pregnancy. This work revealed that LNP transfection efficacy may be governed by the lipid polyamine headgroup, while overarching toxicity may be governed by the lipid acrylate tail. By varying lipid structure, the authors showed that inflammatory immune responses provoked immune cell infiltration in the placenta and restricted pup growth after birth.[121] Importantly, this work followed pup growth for three weeks after birth—an examination not completed by any of the studies presented in this section. It has been shown that children born from pregnancies complicated by preeclampsia have a greater risk of future cardiovascular and cerebrovascular diseases.[25,79,80] Thus, it is imperative that future studies investigate long-term effects of nanocarrier exposure in pups, both with regard to long-term safety, but also potential long-term therapeutic effects. Nanomedicine platforms that could ameliorate symptoms of preeclampsia in the mother, while demonstrating enhanced health in offspring long-term could revolutionize the treatment of preeclampsia, as well as other obstetric disorders. Finally, better animal models of preeclampsia will be critical in translating nanomedicine platforms to the clinic. Many of the animal models utilized in these works induce hypertension in rodents through mechanical means or through the delivery of an inflammatory agent. However, as discussed in this review, the pathophysiology of preeclampsia is incredibly complex, and much remains unknown. Thus, while nanomedicine platforms may demonstrate reductions in hypertension using in vivo models, additional work is needed to understand the effects that nanocarriers may have on specific cellular and molecular pathways in preeclamptic placentas. In all, the nanomedicine platforms highlighted in this section remain exciting prospects for the treatment of preeclampsia, but future work including robust, long-term safety profiling is required to bring these platforms to the clinic.

4.1.4. Potential Nanomedicine Targets for Preeclampsia

The overarching lack of treatment options currently available for preeclamptic patients has driven researchers to develop a broad range of novel drug delivery therapies. The primary focus of the field has utilized RNA interference, mainly in relation to the pathogenic sFLT-1 protein. Over the last decade, new molecular players in preeclampsia have been identified and could potentially be applied to the development of novel drug delivery strategies (**Table 2**).

Recent work by the Hu Group has elucidated the role of a new key molecule in preeclampsia–CD81–which could be harnessed in drug delivery platforms.[87,94] CD81 is a versatile molecule in normal physiological conditions, acting as an orchestrator of signaling cascades, a tumor suppressor, and a key component in endogenous exosomes. Shen et al. first identified the role of CD81 in pregnancy, demonstrating that CD81 is present in first trimester explants and slowly downregulated as pregnancy progresses. However, CD81 upregulation occurs in the STB layer, CTBs, and maternal serum in patients with preeclampsia. Exposing human umbilical vein endothelial cells (HUVECs) to high doses of exogenous CD81 induced endothelial cell activation and pathogenic angiogenesis, characteristic of preeclampsia vascular dysfunction.[87] Ding et al. followed up this study by investigating the relationship between CD81 upregulation and the maternal immune imbalance present in preeclampsia. In this work, researchers showed that CD81 overexpression in preeclamptic patients was accompanied by a decrease in Treg cells and an increase in Th17 cells in both the placenta and maternal circulation, with T cell differentiation pathways showing dependency on CD81-overexpressing trophoblast paracrine signaling of IL-6. This phenomenon was confirmed in a CD81-induced preeclampsia rat model, where the administration of IL-6 antibody mitigated both the preeclampsia phenotype and the imbalance of Treg/Th17 cells.[94] Together, these results highlight the importance of CD81 and IL-6 in preeclampsia pathogenesis and motivate their use as therapeutic targets for the treatment of preeclampsia.

The importance of vascular endothelial cadherin (VE-cadherin) in decidua invasion and spiral artery remodeling during pregnancy has also been highlighted in recent years.[122–124]

Table 2. Emerging Nanomedicine Targets for Placental Disorders.

Therapeutic Target	Motivation	References
Preeclampsia		
CD81	Upregulated in the placenta and serum of preeclamptic patients; affects T cell differentiation in the placenta; can induce endothelial cell activation	[87,94]
VE-Cadherin	Plays a role in spiral artery remodeling; deficiency results in decreased placental perfusion and fetal growth restriction	[122–124]
CD105	Promotes vasoconstriction in preeclamptic patients and may correlate with disease severity; previous success for solid tumors	[84,125]
Extracellular vesicles	Increased levels in circulation in preeclamptic patients; transports pathologic proteins systemically; can perpetuate inflammation and endothelial activation	[38,95,96]
Fetal Growth Restriction		
EGFR	Involved in regulating normal placental growth; reduced expression observed during fetal growth restriction	[142,163,174–178]
ESRRG	Regulates trophoblast differentiation, proliferation, and invasion; decreased expression in placentas with fetal growth restriction	[184–186]
sFLT-1	Antiangiogenic factor; overexpressed in fetal growth restriction	[143,155]
PAPP-A	Involved in placental function and fetal growth; decreased levels observed in fetal growth restriction	[187,188,190]
miRNAs	Unique miRNAs specific to placentas from fetal growth restriction pregnancies; general placental-derived miRNAs downregulated in fetal growth restriction	[191–194]
Exosomes	Reduced concentrations of placental-derived exosomes in fetal growth restriction	[195]
Placenta Accreta Spectrum		
Circulating trophoblast cells	Shed during placental implantation; shed at higher levels during abnormal placental invasion	[222,223]
Serum biomarkers	Include hCG, PAPP-A, cell-free fetal DNA, and cell-free placental mRNA; irregular expression during placenta accreta spectrum; can be used as diagnostic markers	[24,199,202,224]
TRAIL and TRAIL-R2	Decreased levels of TRAIL-R2 observed in placentas with placenta accreta spectrum; contribute to abnormal placental invasion	[227,228]
Uterotonics	Used to prevent postpartum hemorrhage	[212,230,232]
Gestational Trophoblastic Disease		
c-MYC	Enhanced expression in choriocarcinoma and complete hydatidiform mole; may suppress trophoblast differentiation	[26]
EGFR	Enhanced expression in gestational trophoblastic neoplasia; previous success with EGFR therapies in other cancer types	[246,252,276]
MMPs	Involved in cancer invasion and metastasis; upregulated in choriocarcinoma	[26,281]
HLA-G	Highest expression in gestational trophoblastic neoplasia compared to other cancers; may correlate with tumor progression; previously explored in renal and prostate cancer	[26,282]

VE-Cadherin, vascular endothelial cadherin; EGFR, epidermal growth factor receptor; ESRRG, estrogen-related receptor gamma; sFLT-1, soluble fms-like tyrosine kinase-1; PAPP-A, pregnancy-associated plasma protein-A; miRNA, microRNA; hCG, human chorionic gonadotropin; mRNA, messenger RNA; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TRAIL-R2, tumor necrosis factor-related apoptosis-inducing ligand receptor 2; MMPs, matrix metalloproteinases; HLA-G; human leukocyte antigen G.

VE-cadherin is expressed almost exclusively by endothelial cells, functioning to enable the formation of tight blood vessels and regulation of cellular junctions. Interestingly, it has been shown that VE-cadherin expression is acquired when CTBs differentiate into invasive EVTs, and VE-cadherin is necessary for successful placentation. VE-cadherin deficiency in mice results in failed spiral artery remodeling, decreased perfusion of the placenta, fetal growth restriction, and death.[122] As the first stage of preeclampsia is characterized by abnormal spiral artery remodeling, nanomedicine platforms aimed at stimulation of VE-cadherin expression in CTBs could offer a novel method to correct dysfunctional placentation during the early stages of pregnancy.

The role of the soluble form of CD105, or sENG, has been well described in the pathogenesis of preeclampsia, with sENG capable of acting individually to promote vasoconstriction and in

synergy with sFLT-1 to induce severe preeclampsia symptoms.[84,125] Many studies have shown that sFLT-1 treated animals develop common preeclampsia symptoms including hypertension and proteinuria; however, sFLT-1 treatment alone is not enough to induce severe preeclampsia characterized by hemolysis and thrombocytopenia seen in HELLP syndrome. Only the administration of sFLT-1 and sENG together can lead to severe preeclampsia in animals, suggesting a potential correlation between sENG levels and disease severity.[125] Despite this, many of the nanomedicine approaches explored thus far have focused on targeted silencing of sFLT-1 alone.[99,114,115,117] In the case of patients presenting with severe preeclampsia, therapeutics targeted against sENG may offer enhanced therapeutic efficacy compared to approaches targeting sFLT-1 alone. While sENG-targeted therapeutics have not yet been explored to our knowledge during pregnancy, membrane-bound CD105-targeted therapies have been previously developed in the context of solid tumors. Given its native role in angiogenic processes, CD105 is also overexpressed in cancer and thus, anti-CD105 monoclonal antibody therapies have shown success in tumor reduction.[92,126] This body of work motivates the use of endoglin as a target for vascular disorders, such as preeclampsia, and may serve as a foundation for the future development of sENG-targeted platforms. As both sFLT-1 and sENG can be found in maternal circulation months prior to clinical diagnosis,[88,89] there is great potential for nanomedicine platforms aimed at advancing diagnostic strategies and diagnostic timelines for preeclampsia and preeclampsia prevention. Further, as sENG levels may correlate with preeclampsia severity,[77,80,83,84] nanomedicine strategies for the simultaneous downregulation of sENG and sFLT-1 would be of great value as a potential treatment strategy against severe preeclampsia.

Finally, recent work has identified EVs as key players in cellular communication during pregnancy, and more specifically, during preeclampsia. EVs play a role in immunomodulation that results in a balance between Th1/Th2 immune responses during healthy pregnancy and, thus, there is evidence that EVs may be responsible for inducing the local immune shift toward Th1 overrepresentation and inflammation during preeclampsia. It has also been shown that placental ischemia present during preeclampsia stimulates the release of EVs from the placenta that may bind to monocytes in the bloodstream, stimulating the production of inflammatory cytokines and further perpetuating the inflammatory maternal syndrome.[38,95] Increased levels of EVs have been detected in the maternal bloodstream in patients with preeclampsia, making EVs a potentially attractive target for diagnostics. More specifically, these EVs have been shown to contain increased levels of sFLT-1 and sENG, and protein levels may correlate with disease severity.[38,96] Finally, Chang et al. demonstrated that placental-derived EVs transport sFLT-1 systemically and can cause widespread endothelial activation.[96] These results suggest that therapeutics targeted against sFLT-1-containing EVs could potentially inhibit endothelial activation in distal organs and ameliorate maternal symptoms of preeclampsia. While EVs have not yet been explored as nanocarriers during pregnancy, previous work has investigated their use as drug delivery vehicles for Parkinson's disease, cancer, and more.[127–129] Given their innate functionality as protein carriers for maternal-fetal communication and immunomodulation during a healthy pregnancy, EVs hold promise as nanocarrier platforms that may be explored for diagnostics or therapeutic strategies in the treatment of preeclampsia.

4.2. Fetal Growth Restriction

Fetal growth restriction is broadly defined as the failure of the fetus to reach its genetically determined growth potential and is characterized by an estimated fetal weight or abdominal circumference less than the 10th percentile for gestational age.[130–135] Fetal growth restriction occurs in 5–10% of all pregnancies, with its incidence more severe in resource-limited countries.[130,133,136] Fetal growth restriction is attributed with high fetal mortality and morbidity and is associated with an increased risk of stillbirth.[130,131,134,137] Beyond the risks of being born underweight, children born with fetal growth restriction are at higher risk of developing hypertension, obesity, coronary artery disease, stroke, and metabolic syndrome in adulthood.[130,131,133,136,138] Maternal factors—such as preeclampsia, autoimmune disorders, hypertension, age, interpregnancy interval—and fetal factors—including chromosomal abnormalities, infection, and multiple gestations—can increase the risk of fetal growth restriction during pregnancy.[130,131,136,139]

Fetal growth restriction can be split into two main categories depending on fetal ultrasound measurements: asymmetrical fetal growth restriction and symmetrical fetal growth restriction.[130,132,133] Asymmetrical fetal growth restriction is seen in 70–80% of cases and generally occurs later in gestation (second and third trimester).[130,132] Asymmetrical fetal growth restriction is caused by uteroplacental insufficiency which redistributes blood flow preferentially to supply blood to vital fetal organs, such as the brain, over more peripheral tissues.[130,132,136] This results in disproportionate growth restriction where fetal abdominal circumference measurements decrease while head circumference, biparietal diameter, and femur length remain normal.[130,132] Symmetrical fetal growth restriction occurs in 20–30% of cases and occurs during the first trimester.[130,132,136] It is hypothesized that symmetrical fetal growth restriction is caused by genetic factors or fetal infection. During symmetrical fetal growth restriction, all growth measurements of the fetus are proportionally reduced.[130,132] Symmetrical fetal growth restriction has been reported to have a poorer prognosis compared to asymmetrical fetal growth restriction.[130,132,133] While the exact etiology of fetal growth restriction can vary, and can originate from both maternal and fetal factors, placental insufficiency is the leading cause of fetal growth restriction,[134,136,139,140] and, as such, will be the focus of discussion in the subsequent sections.

4.2.1. Pathophysiology of Fetal Growth Restriction

Fetal growth restriction is a multifactorial disease[130,141,142] and, as such, the exact underlying pathogenesis varies, but placental dysfunction lies at the core of disease progression.[134,136,139,140,143] Similar to the pathophysiology of preeclampsia, fetal growth restriction progresses in two stages. The first stage occurs in the first trimester when abnormal trophoblast development and poor spiral artery remodeling lead to inadequate

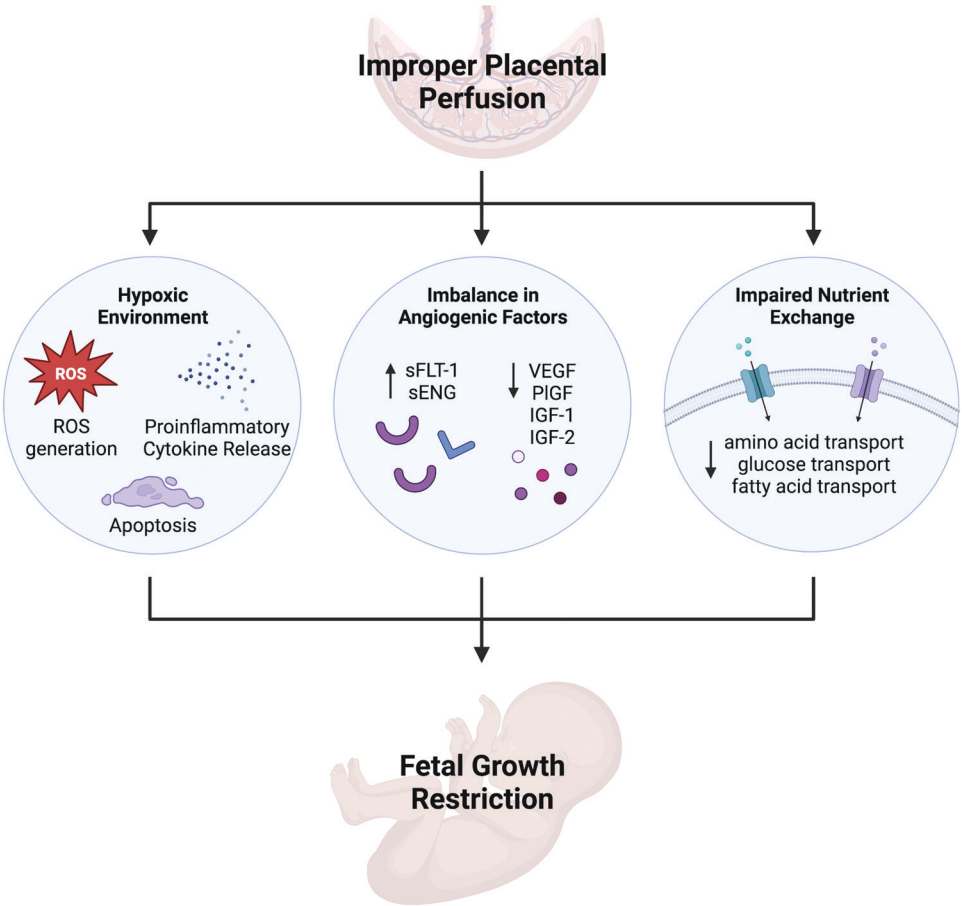

Figure 5. Schematic of placental dysfunction that occurs in FGR. Following improper remodeling of the uterine spiral arteries, impaired placental perfusion leads to a hypoxic environment, imbalance in angiogenic and anti-angiogenic growth factors, and impairment of nutrient exchange, ultimately leading to a decrease in fetal growth. ROS, reactive oxygen species; sFLT-1, soluble fms-like tyrosine kinase-1; sENG, soluble Endoglin; VEGF, vascular endothelial growth factor; PlGF, placental growth factor; IGF-1, insulin-like growth factor-1; IGF-2, insulin-like growth factor-2.

placental perfusion.[132,135,140,143,144] This improper placental development causes downstream complications in the second and third trimesters including placental ischemia, reperfusion injuries, imbalance of angiogenic and antiangiogenic factors, oxidative stress, and impaired placental transport and nutrient exchange (**Figure 5**).[132,135,139,140,145] There are many similarities between the pathologies of preeclampsia and fetal growth restriction; researchers have characterized fetal growth restriction as the fetal presentation of the disease while preeclampsia is the maternal presentation.[146]

During normal gestation, significant placental remodeling occurs during the first trimester to allow for efficient gas and nutrient exchange between the mother and fetus.[132,135,140] This process begins when CTBs differentiate into EVTs and invade the maternal decidual tissue and uterine spiral arteries.[132,133] In fetal growth restriction, abnormal trophoblast invasion results in incomplete remodeling of the spiral arteries and persistence of high resistance, low-flow blood circulation in the placenta.[132,133,135,140,143,147] Placental biopsies from fetal growth restriction pregnancies have evidence of major defects in spiral artery remodeling[140] and placental ultrasound measurements show smaller placental volume and vessel diameter.[132,135] Additionally, extensive thickening of the intima layer of spiral arteries has been identified in fetal growth restriction placentas, potentially contributing to the observed increase in vascular resistance.[132] It has been hypothesized that the dysregulation of EVT invasion seen in fetal growth restriction can be attributed to decreased expression of matrix metalloproteinases (MMPs) and increased expression of tissue inhibitors of matrix metalloproteinases (TIMPs), in particular MMP-2, -8, -9, -11, and TIMP-1 and -3,[132,148] as MMPs have been shown to facilitate EVT invasion.[149]

The poor spiral artery remodeling that occurs in fetal growth restriction impacts the velocity at which blood enters the intervillous space, and preservation of the smooth muscle layer in spiral arteries leads to intermittent perfusion of the placenta.[132,135,143] These high-resistance vessels experience increased shear forces and velocity of blood flow, which ultimately damages the placenta and causes inefficient exchange at the maternal-fetal interface.[132,135,143] Improper perfusion of the placenta can also lead to oxidative stress, which can impair cellular function and cause cell death.[135] Furthermore, reactive oxygen species (ROS) generation is increased in hypoxic and ischemic environments, and placental oxidative stress has been linked to fetal growth

restriction.[135] Additionally, the hypoxic environment observed in fetal growth restriction causes the release of proinflammatory cytokines, cellular apoptosis, and damage to the placenta vasculature, further impairing oxygen supply and transport to the fetus.[132,135] Other stress response pathways have been observed in fetal growth restriction, including endoplasmic reticulum stress in the STB layer, as well as chronic mitochondrial stress.[132,135]

The improper placental development observed in fetal growth restriction is also impacted by dysregulation of the immune microenvironment at the maternal-fetal interface.[150] During healthy pregnancy, innate immune cells, such as natural killer and dendritic cells, interact with trophoblasts to promote proper placental development and fetal growth through the promotion of spiral artery remodeling and maintenance of the immune cell response.[36,135,150–152] However, decreased numbers of dendritic cells have been found in patients affected by fetal growth restriction, and these reduced cell populations are hypothesized to negatively impact vascular development during early pregnancy.[151] Additionally, reduced levels of growth-promoting factors secreted by natural killer cells have been shown to impair fetal development, ultimately resulting in fetal growth restriction.[153]

Similar to disease pathology in preeclampsia, fetal growth restriction is characterized by an imbalance of angiogenic, antiangiogenic factors, and other growth factors in the placental environment.[134,135,140,145] PlGF and VEGF originate from placental villi and are involved in placental angiogenesis and vascular development.[134,143,154] Both PlGF and VEGF levels are decreased in fetal growth restriction in part due to suppression of protein transcription and translation by the placental villi in a hypoxic environment.[134,140,143,145,147] Additionally, reduced PlGF concentrations have been linked to fetal growth restriction severity.[134] Levels of antiangiogenic factors sFLT-1 and sENG are increased in fetal growth restriction[132,134,140,143,145,147] which also contributes to the decreased levels of PlGF and VEGF as sFLT-1 binds and inhibits both growth factors.[143,155] The insulin-like growth factor (IGF) family, which includes IGF-1 and IGF-2, influences placental growth and is important for placental cell proliferation.[135,137] Both IGF-1 and IGF-2 are downregulated in fetal growth restriction[135,137] and decreased levels of IGF-1 have been attributed with reduced rates of fetal growth and poor placental transfer.[130,139]

Healthy fetal growth is dependent on proper nutrient availability, which is impacted by maternal diet, uteroplacental blood flow, placental villous development, and placental nutrient transport.[135] In fetal growth restriction, the irregular placental vascular environment impairs nutrient exchange and transport which, in turn, leads to improper fetal growth.[132,135,139] More specifically, the STB layer expresses transporters for amino acids, fatty acids, and glucose that are essential for proper fetal growth,[132,135,136] but many of these transporters are downregulated in fetal growth restriction.[132,135,139] Glucose is particularly important in healthy development, as it is the main energy supply for both the placenta and fetus.[132,156] The fetus is dependent on the transplacental delivery of glucose from maternal circulation, but in fetal growth restriction, fetal glucose concentration is decreased due to reduced placental transport.[132,156]

4.2.2. Gold Standard Treatment for Fetal Growth Restriction

There is currently no curative treatment for fetal growth restriction and, as such, proper diagnosis and management play a large role in reducing the risks associated with fetal growth restriction. Ultrasound is the primary technique used to diagnose fetal growth restriction, but magnetic resonance imaging (MRI) is becoming increasingly preferred in high-risk pregnancy settings.[131,134] Additionally, doppler velocimetry ultrasound, particularly of the umbilical artery (UA), is the primary surveillance tool used in the management of fetal growth restriction, as it can be used to assess maternal, placental, or fetal circulation.[130,131,134,139] The UA doppler velocimetry abnormalities seen in fetal growth restriction, which reflect placental vascular resistance, include absent or reversed-end diastolic flow and are correlated with an increased risk of stillbirth, as well as neonatal and long-term morbidity.[130,132,133,139] Last, precise initial dating of gestation is essential for confirming fetal growth restriction, as the estimated fetal weight is based on gestational age.[133,139]

Several different therapeutic approaches have been investigated to prevent or treat the onset of fetal growth restriction, including nutritional and dietary supplements, amino acids, and low-dose aspirin. However, these prevention and treatment methods have all been met with variable success.[130,131,133,139,140] More specifically, the administration of low-dose aspirin has been analyzed as a therapy to prevent fetal growth restriction in high-risk patients, but there has been insufficient evidence of its success to justify its routine use in fetal growth restriction management.[131,133,139,140] Because therapeutic options for fetal growth restriction are lacking, management of the disease primarily focuses on determining the best time to deliver the fetus.[130,133,139,140] The decision of when to deliver the fetus depends on the severity of fetal growth restriction and an assessment of both the mother and fetus, taking into account gestational age, maternal health, and fetal well-being.[133,139] When determining the ideal delivery date, a balance exists between delivering the baby preterm, which increases the risk of neonatal morbidity and mortality, and waiting until fetal distress is evident, which is hypothesized to be associated with an increased risk of stillbirth.[139,140] Delivery between 38–39 weeks is recommended in cases of isolated fetal growth restriction without additional risk factors. When fetal growth restriction is diagnosed with additional risk factors for adverse outcomes, delivery is recommended between 34–38 weeks.[131] When delivery of the fetus is expected before 34 weeks, corticosteroids are administered to prevent adverse neonatal outcomes.[130,139,140]

4.2.3. Nanomedicine Strategies for Fetal Growth Restriction

The lack of treatment options for fetal growth restriction has driven the development of new therapies with a primary focus on delivering growth factors that are downregulated in fetal growth restriction (Table 1). For example, increasing IGF-1 expression in the placenta is a popular strategy employed by researchers to improve fetal growth restriction outcomes. As previously mentioned, IGF-1 is downregulated in fetal growth restriction, impacting proper fetal growth.[135,137] Jones et al. delivered human

IGF-1 (hIGF-1) to the placenta through adenoviral constructs with the goal of rescuing fetal growth restriction progression.[157] In the uterine artery branch ligation (UABL) mouse model of fetal growth restriction, administration of the adenovirus treatment (Ad-hIGF-1) via intra-placental injection led to improved placental glucose transport and, subsequently, increased fetal weight.[157,158] In a subsequent study, Ad-hIGF-1 was administered via intra-placental injection in the UABL mouse model of fetal growth restriction to examine amino acid transporter expression as a possible mechanism behind IGF-1 treatment.[159] After administration, increased levels of SNAT1, an isoform of system A amino acid transporter, were observed in the Ad-hIGF-1 treatment group, suggesting that overexpression of IGF-1 rescues amino acid transport in the placenta, helping to restore fetal weight.[159]

After the demonstrated success of IGF-1 gene therapy as a fetal growth restriction treatment, researchers investigated achieving the same therapeutic outcome through non-viral delivery of IGF-1.[160–162] Abd Ellah et al. complexed a diblock copolymer (pHPMA-b-pDMAEMA) with the hIGF-1 DNA plasmid.[160] To reduce off-target gene expression, the trophoblast-specific promoters CyP19a or PLAC1 were incorporated into the plasmids, and these polyplexes were injected via an intra-placental injection in the UABL mouse model of fetal growth restriction. In mice treated with the PLAC1-hIGF-1 polyplex, both fetal weight and placental labyrinth (the region of nutrient exchange) depth increased compared to untreated mice, indicating an improvement in placental development and fetal growth after IGF-1 administration.[160]

To determine if these IGF-1 polyplexes could be taken up by human placental trophoblasts, Wilson et al. evaluated PLAC1-hIGF-1 polyplexes in the BeWo cell line, villous fragments from term placentas, and an ex vivo human placenta perfusion model.[161] In both the BeWo cells and term placental fragments, IGF-1 expression was increased compared to both untreated and plasmid-only controls. The authors demonstrated that treatment of BeWo cells with IGF-1 polyplexes protected the cells from increased apoptosis while under oxidative stress. While they were unable to confirm IGF-1 expression in the placenta perfusion model, the authors were able to demonstrate the uptake of polyplexes in the STB layer of the placenta.[161] Wilson et al. continued this work by evaluating the hIGF-1 polyplex system in a guinea pig model of fetal growth restriction, as guinea pigs have similar placental architecture and gestational milestones to humans.[57,162] In this study, the Cyp19a1 promoter was used, as it had far better delivery to guinea pig placental cells than the PLAC1 promoter.[162] While fetal weight was not increased with intra-placental IGF-1 treatment, IGF-1 expression was confirmed in the placenta, and the treatment group was able to increase fetal glucose concentrations, placental expression of glucose and amino acid transporters, and fetal capillary volume density.[162]

Both studies by Abd Ellah et al. and Wilson et al. investigated the safety profile of IGF-1 therapy in their respective animal models. Generally, the hIGF-1 polyplex therapy was shown to be safe for both the mother and fetus, as both the polyplex and plasmid did not cross the placenta, cause any maternal morbidities, or lead to increased fetal loss.[160–162] Abd Ellah et al. further probed the safety profile of their therapy by evaluating placental morphology where the authors saw no evidence of inflammation or immune cell infiltration in mice treated with PLAC1-hIGF-1 polyplexes. Additionally, the authors confirmed specificity of the trophoblast-specific promoters in their DNA plasmids by transfecting human embryonic kidney cells in vitro with GFP plasmids and verifying negative GFP expression in the cells.[160] Wilson et al. also highlighted the importance of the trophoblast-specific promoter in reducing off-target effects of this therapy. In their study, the authors observed uptake of the polyplexes in the maternal ovary and lung after treatment but saw no transgene expression by the plasmid, confirming the trophoblast specificity of the Cyp19a1 promoter.[162] Of note, a major drawback of the described studies is the administration of the IGF-1 therapy through an intra-placental injection, which has less relevance in a clinical setting. Future work should focus on developing an IGF-1 therapy that can be administered via IV injection, potentially with the use of placental targeting techniques. Taken together, these studies demonstrate the potential for IGF-1 treatment to restore fetal and placental complications seen in fetal growth restriction and highlight the importance of including trophoblast-specific promoters to reduce off-target effects of DNA plasmid therapy.

King et al. investigated the use of tumor-homing peptides to selectively guide liposomes to the placenta to deliver IGF-2, another mediator of placental growth.[102] Biodistribution data showed that CGKRK and iRGD decorated liposomes selectively accumulated in the placental labyrinth and spiral arteries, respectively, in pregnant mice when compared to nontargeted liposomes following IV administration. The authors were able to identify calreticulin as the receptor for CGKRK on the STB layer and confirmed α_v integrin as the receptor for iRGD. IV administration of IGF-2 loaded iRGD-decorated liposomes increased placental weight in a healthy mouse model and increased fetal weights and fetal weight distribution compared to untreated and free IGF-2 controls in an IGF-2 knockout mouse model of fetal growth restriction. To assess the safety profile of this therapy, the authors monitored for abnormal pregnancy outcomes and saw no change in litter size or resorption rate for mice treated with the targeted liposomes, confirming that this therapy is well-tolerated during pregnancy. Additionally, the authors demonstrated that the targeting peptides did not affect trophoblast proliferation in vitro, the targeted liposomes remained in maternal circulation, and minimal off-target effects of IGF-2 treatment were observed in maternal clearance organs, as evident by a lack of increased weight in the maternal spleen and kidney. Taken together, King et al. demonstrated that IGF-2 loaded iRGD-decorated liposomes are safe and have potential for the treatment of fetal growth restriction.[102]

Using a similar strategy to King et al., Renshall et al. utilized phage screening to identify a new peptide sequence for the targeted delivery of epidermal growth factor (EGF) to the placenta.[142] The authors were interested in delivering EGF to the placenta as it is important for proper fetal and placental growth, and decreased epidermal growth factor receptor (EGFR) signaling has been reported in fetal growth restriction placentas.[142,163] Additionally, EGF has been shown to protect against cytokine- and ROS-induced apoptosis ex vivo in human placental explants and trophoblast cultures.[142,164,165] Through phage screening, Renshall et al. identified the peptide CGPSARAPC (GPS) that bound to the STB layer of human placental explants and localized in the junctional zone of mouse placentas.[142]

GPS-decorated liposomes loaded with EGF were able to significantly increase system A amino acid transporter activity in healthy human placental explants but not in explants from patients with fetal growth restriction. While the EGF-loaded GPS-decorated liposomes did not increase cell growth, the authors did confirm that downstream protein kinase pathways were activated upon EGF delivery with the targeted liposomes, indicating activation of EGFR following liposomal treatment. Additionally, the authors confirmed that the liposomal formulations themselves did not negatively impact normal placental function. In healthy third trimester explants, the authors observed no changes in secretion of the trophoblast-derived pregnancy hormone human chorionic gonadotropin (hCG), the percentage of apoptotic cells, or the system A amino acid transporter activity between untreated and liposome-treated samples.[142] While additional work is required to demonstrate the potential of this therapy in treating fetal growth restriction, this targeted drug delivery system may have applications in the delivery of other growth factors relevant to proper fetal and placental growth.

Overexpression of VEGF is another strategy that has been employed for the treatment of fetal growth restriction, primarily through the use of adenoviral vectors.[155,166–168] VEGF has angiogenic and vasodilatory properties, but decreased circulating VEGF levels are observed in fetal growth restriction, in part due to inhibition by sFLT-1.[143,155,169] All VEGF gene therapy treatments for fetal growth restriction deliver the VEGF isoform VEGF-A165, as it is the principal and most potent form of VEGF in humans and binds to VEGF receptors 1 and 2.[155,169] Carr et al. injected adenovirus vectors encoding either the VEGF-A165 (Ad.VEGF-A165) isoform or a non-vasoactive control vector encoding bacterial β-galactosidase (Ad.LacZ) into the uterine artery of an ovine model of fetal growth restriction.[167] In this study, the authors selected the over-nourished adolescent sheep model of fetal growth restriction as it exhibits reduced uterine blood flow and recapitulates key features observed in human fetal growth restriction, such as reduced uterine blood flow and placental weight.[155,170] Treatment with Ad.VEGF-A165 enhanced fetal growth velocity as measured by ultrasound, and fewer fetuses exhibited growth restriction at term compared to the Ad.LacZ and untreated controls.[167] In a subsequent study by Carr et al., researchers monitored the growth of Ad.VEGF-A165 treated lambs in their first 12 weeks of life and observed a higher absolute neonatal growth rate when compared to the untreated control group.[168] These results indicate that Ad.VEGF-A165 treatment has clear effects on recovering placental function and enhancing neonatal growth without adverse events in the mother or fetus.[167,168]

In order to evaluate the therapeutic effects of the Ad.VEGF-A165 treatment in vivo in a model more similar to humans, Swanson et al. evaluated Ad.VEGF-A165 treatment in a guinea pig model of fetal growth restriction.[166] In this study, the authors used the periconceptual nutrient deprivation model of fetal growth restriction which demonstrates a 40% reduction in fetal weight due to improper uteroplacental perfusion.[155,171] A laparotomy was performed on the guinea pigs with fetal growth restriction and both Ad.VEGF-A165 and Ad.LacZ vectors were administered externally to the uterine and radial arteries via a thermosensitive Pluronic gel to achieve high levels of gene transfer without the need for direct injection.[155,166] After administration, fetal weight and placental depth were increased at term for the Ad.VEGF-A165 treated group compared to the Ad.LacZ treated guinea pigs.[166] Additionally, VEGF levels were increased in the fetal blood serum for the Ad.VEGF-A165 treatment group 3–8 days following vector administration, indicating transduction of the vector after delivery. The authors also confirmed via reverse transcription (RT-) PCR that the Ad.VEGF-A165 therapy only acted in a local manner, as there was no transgenic protein expression in any fetal tissues or maternal tissues, except for one ovary which they attributed to improper positioning of the Pluronic gel during surgery.[166] These results again suggest therapeutic potential for VEGF gene therapy for fetal growth restriction treatment. However, future work should investigate systemic VEGF delivery via IV injection, potentially through other non-viral delivery platforms, to enhance the translational potential of VEGF therapy.

Another approach that has been used in the treatment of fetal growth restriction is the delivery of the vasodilator and NO donor SE175 to improve placental perfusion. Cureton et al. loaded liposomes with SE175 and attached the peptide CNKGLRNK (CNKG) to the surface of liposomes to create placental-targeted NPs.[172] Through phage screening, CNKGLRNK was shown to bind to spiral arteries and the vasculature in the labyrinth zone of the placenta.[172] Additionally, SE175 was selected, as it can relax uterine arteries in pregnant mice and enhance fetal growth in vivo.[172] Four doses of CNKG-decorated liposomes loaded with SE175 were intravenously administered to healthy or endothelial NO synthase knockout (eNOS-/-) mice, which have impaired uteroplacental blood flow and fetal growth restriction, and compared against untreated or free SE175 controls.[172] The SE175-loaded CNKG-decorated liposomes did not improve fetal weight in healthy mice but significantly increased fetal weight and mean spiral artery diameter while decreasing placental oxidative stress in eNOS-/- mice.[172] The authors confirmed that this therapy was safe throughout pregnancy, as they observed no accumulation of CNKG-decorated liposomes in fetal tissues, no significant differences between litter size and resorption rate between treatment groups, and no apparent morphological abnormalities in the maternal organs. Additionally, the authors observed that SE175 does not negatively impact normal placental function, and placental explants treated with SE175 had no changes in system A amino acid transport, hCG secretion, and trophoblast proliferation or apoptosis. However, these tests were only conducted with free SE175. Additional studies evaluating the peptide-targeted liposomes and liposomes loaded with SE175 should be completed to confirm the safety profile of this therapy.[172]

The nanocarrier strategies described above hold significant promise for treating fetal growth restriction, however there are still large safety concerns over the use of nanomedicine therapies during pregnancy. One major safety concern is the risk of off-target effects, which many of the discussed nanomedicine strategies combat through utilization of targeted delivery systems. Both external targeting moieties, such as peptides, and the inclusion of trophoblast-specific promoters in the design of DNA plasmids were shown to sequester delivery of the nanocarrier primarily to the placenta. Additionally, a complete evaluation of the safety profile of these nanomedicine strategies will be essential in progressing these therapies into the clinic. While many of the discussed studies confirmed that their

nanocarriers did not enter fetal circulation or cause fetal toxicity, more in-depth studies are needed to evaluate the impact of these nanocarriers on normal placental function. Following the work done by Abd Ellah et al., researchers should investigate inflammation in the placenta and maternal organs following nanocarrier treatment, either through examination of inflammatory markers in the blood or immune cell infiltration into the placenta.[160] Studies evaluating trophoblast proliferation and hormone secretion in the placenta following nanocarrier treatment, as done by King et al., Renshall et al., and Cureton et al., can be used to confirm that normal placental function is retained even after nanocarrier treatment.[102,142,172] Additionally, there is a need for long-term safety data when evaluating the outcome of these therapies on the progression of fetal growth restriction. Since fetal growth restriction can cause complications into adulthood, following offspring health will be important in understanding both the long-term curative consequences of these treatments, as well as ensuring that there are no long-term toxic effects on offspring development. Last, researchers should consider the clinical relevance of their nanocarrier therapy, regarding both the type of nanocarrier used and the route of administration. Generally, non-viral nanocarriers, such as liposomes or polymer NPs, are advantageous over viral vectors due to safety concerns of immunogenicity and broad tropisms associated with viral delivery systems.[173] Additionally, nanocarriers that can be administered via IV injections rather than local intra-placental injections are preferred, as IV injections are more common in clinical settings. Taken together, the nanocarrier therapies described above hold promise for treating fetal growth restriction, but future preclinical work should include more extensive nanocarrier safety profiling.

4.2.4. Potential Nanomedicine Targets for Fetal Growth Restriction

Many of the strategies employed in the development of new therapies to treat fetal growth restriction involve delivering growth factors that are downregulated in fetal growth restriction, including EGF, IGF-1, and IGF-2.[102,142,157,159–162,166–168] An alternative approach could aim to upregulate the expression of receptors to these growth factors (Table 2). EGFR is a promising target for fetal growth restriction treatment, as reduced expression of placental EGFR has been observed in placentas from patients with fetal growth restriction[163,174,175] and EGFR knockout mice exhibit reduced fetal growth with the possibility of mid-gestational embryonic lethality.[176] EGFR is mainly expressed on the STB layer and binding of EGF to EGFR causes receptor dimerization, stimulation of tyrosine kinase activity, and initiation of a signaling cascade that leads to cell proliferation and division, ultimately resulting in placental growth.[142,163,174,177,178] Thus, increasing the expression of EGFR on the STB layer may offer an alternative approach for fetal growth restriction therapy.

Recent work in the field has elucidated the potential role of estrogen-related receptor gamma (ESRRG) in fetal growth restriction.[179,180] ESRRG is a member of the estrogen-related receptor family of orphan nuclear receptors and, interestingly, an endogenous ligand for ESRRG has yet to be identified.[179–181] ESRRG is highly expressed in the placenta and has greater expression in villous trophoblasts, especially in the STB layer, compared to EVTs.[182,183] ESRRG is involved in the regulation of trophoblast differentiation, proliferation, and invasion; decreased levels of ESRRG have been reported at both the mRNA and protein levels in placentas from patients with fetal growth restriction.[184,185] Its role in regulating trophoblast function suggests that decreased levels of ESRRG may contribute to the placental dysfunction observed in fetal growth restriction.[185,186] Additionally, reduced levels of ESRRG have been observed in hypoxic environments, further supporting its potential role in fetal growth restriction.[179,184] While a better understanding of ESRRG's role in both normal trophoblast development and placental dysregulation is needed, ESRRG represents another possible target for the treatment of fetal growth restriction, either through upregulation of the receptor or through the delivery of a synthetic ligand.[179,185]

As outlined in the previous section, the silencing of sFLT-1 through siRNA is a widely used strategy in the treatment of preeclampsia. Similar to the pathophysiology of preeclampsia, sFLT-1 is overexpressed in fetal growth restriction and its circulation is partly responsible for reduced levels of PlGF and VEGF.[143,155] As such, many of the NP-mediated sFLT-1 siRNA strategies employed for preeclampsia could potentially be applied for the treatment of fetal growth restriction.

The role of pregnancy-associated plasma protein-A (PAPP-A) has been well characterized in the context of pregnancy. PAPP-A is a large glycoprotein that is mainly secreted by the STB layer and is directly involved in placental function and proper fetal growth.[187,188] The primary role of PAPP-A is to cleave IGF from IGF binding protein 4 (IGFBP-4), an inhibitor of IGF action in the placenta.[130,188,189] Furthermore, decreased levels of circulating PAPP-A have been identified in fetal growth restriction,[190] leading to its use as a potential biomarker of the disorder.[130,187] The important role of PAPP-A during healthy pregnancy and its decreased levels in fetal growth restriction make it a promising target for the development of new nanomedicine-based therapies and diagnostic tools for fetal growth restriction.

Last, since diagnosis of fetal growth restriction remains a challenge, circulating miRNAs and exosomes, a type of EV, have been investigated as alternative approaches for the diagnosis of fetal growth restriction.[191–195] Several different placental-derived circulating miRNAs have been implicated in fetal growth restriction. Some miRNAs, including hsa-miR-518b and hsa-miR-1323, have been directly identified as miRNAs specific to the placentas of fetal growth restriction pregnancies, whereas many placental-derived miRNAs, such as miR-26a-5p and miR-103a-3p, have been shown to be downregulated in pregnant patients with fetal growth restriction.[191–194] Nanocarriers can be used to improve isolation methods for these miRNAs, but more work is needed to validate these miRNAs as biomarkers of fetal growth restriction and to determine their role in the progression of fetal growth restriction.[196] Placental-derived exosomes have also been evaluated as diagnostic markers of fetal growth restriction. In a study by Miranda et al., patients with fetal growth restriction were shown to have a significantly reduced ratio of placental-derived exosomes to total plasma exosomes in comparison to healthy patients.[195] As such, concentrations of placental-derived exosomes may also be used as a biomarker for fetal growth restriction. While exosomes have primarily been investigated for diagnostic applications in pregnancy, they have been used as

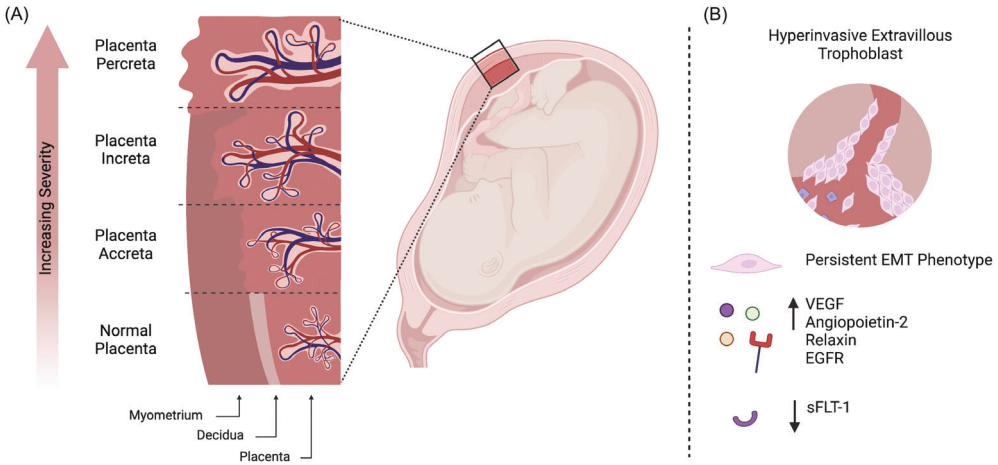

Figure 6. Schematic of placenta accreta spectrum. A) Placenta acrreta spectrum can be divided into three subtypes, placenta accreta, placenta increta, and placenta percreta, depending on the degree of placental invasion into the maternal myometrium. B) Factors impacting aberrant trophoblast invasion that occurs during placenta accreta spectrum. VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor; sFLT-1, soluble fms-like tyrosine kinase-1.

drug delivery vehicles in other disease applications.[197,198] Future studies should investigate exosome use for novel placental therapies and the treatment of fetal growth restriction.

4.3. Placenta Accreta Spectrum

Placenta accreta spectrum is a disorder characterized by abnormal placental attachment and trophoblast invasion into the maternal myometrium.[24,199,200] This abnormal attachment results in a failure of the placenta to spontaneously detach from the uterine wall following delivery of the fetus.[24,201,202] Placenta accreta spectrum includes the spectrum of disorders known as placenta accreta, placenta increta, and placenta percreta, each of which has varying degrees of abnormal placental invasion.[24,200,202,203] Placenta accreta spectrum is associated with high rates of maternal morbidity and mortality due to the risk of severe postpartum hemorrhage. Incidence of placenta accreta spectrum has risen in recent years due to the rising global rates of cesarean delivery.[24,199–204] Additional risk factors for placenta accreta spectrum include advanced maternal age, multiparity, Asherman's syndrome, placenta previa, and prior uterine surgeries, but the predominant risk for placenta accreta spectrum is the number of previous cesarean deliveries.[24,199–202]

The leading hypothesis for what causes placenta accreta spectrum is that a defect, such as a uterine scar, at the endometrial-myometrial interface leads to a failure of normal decidualization which allows for deep anchoring of the placental villi and trophoblast infiltration.[24,199,201–203] The degree to which this abnormal anchoring and infiltration occur can vary from a mild adhesion of myometrial fibers to the basal plate to severe placental invasion through the uterus and into the peritoneal cavity.[24,205] The most common form of placenta accreta spectrum is placenta accreta during which the chorionic villi attach directly to the myometrium in the absence of the decidua. The more severe forms of placenta accreta spectrum include placenta increta, during which the chorionic villi invade the myometrium as far as the external layer, and placenta percreta, where the chorionic villi invade through the myometrium and potentially into surrounding structures such as pelvic tissue and the bladder (**Figure 6**A).[24,200,202]

4.3.1. Pathophysiology of Placenta Accreta Spectrum

Proper decidualization is vital for normal placental development.[30,203] It is hypothesized that the decidua can regulate trophoblast invasion[30,203] and in normal placentation, the EVTs do not invade past the inner third of the myometrium due to spatial and temporal regulations in the decidual environment.[24,203] However, the disruption of the decidua that is seen in placenta accreta spectrum may contribute to loss of this regulation and abnormal EVT invasion.[202,203] The degree to which EVTs penetrate the myometrium is hypothesized to be impacted by the degree of damage at the endometrial-myometrial interface during placenta accreta spectrum.[202] These defects and scars have been shown to increase local fibrous tissue, inflammatory cell infiltration, and reepithelization and vascular remodeling of the scar area, which all may contribute to abnormal placental invasion and trophoblast infiltration.[202,203] Additionally, migration of EVTs is believed to be regulated, in part, by matrix metalloproteinases (MMPs) through degradation of the extracellular matrix, although there is conflicting information regarding the role of MMPs in placenta accreta spectrum.[24,32,203]

Immune imbalance at the maternal-fetal interface is also thought to play a role in the progression of placenta accreta spectrum. CD4+ T cells and natural killer cells have reduced populations during placenta accreta spectrum compared to healthy pregnancies. T cells typically have important immunosuppressive functions during pregnancy, but in placenta accreta spectrum, increased trophoblast invasion is hypothesized to be impacted by reduced T cell populations and a suppressed T cell response.[24,206] During healthy pregnancy, natural killer cells are

believed to regulate trophoblast invasion through the secretion of soluble factors, such as cytokines.[203,207] The diminished natural killer cell population observed during placenta accreta spectrum also coincides with the downregulation of several cytokines, including IL-10 and interferon-γ, all of which are believed to contribute to enhanced trophoblast invasion and the onset of placenta accreta spectrum.[206]

Angiogenesis in the placental microenvironment plays an important role during placenta accreta spectrum, and abnormal uteroplacental neovascularization is present in the majority of patients with placenta accreta spectrum.[24,202] Angiogenesis during placenta accreta spectrum has been characterized through the analysis of local protein expression in the placental microenvironment. Upregulation of VEGF, angiopoietin-2 (ang-2), and EGFR on the STB layer during placenta accreta spectrum has been demonstrated along with reduced expression of sFLT-1 in EVTs, suggesting that abnormal EVT invasion and placental adherence may occur in part due to irregular expression of different growth and angiogenic factors (Figure 6B).[24,203] Reduction of sFLT-1 levels is particularly evident in placenta increta and percreta patients.[24] Increased expression of the placental relaxin (RLN) protein in the basal plate and its receptor (RXFPI) on villous trophoblasts and the basal plate has also been observed during placenta accreta spectrum.[24] RLN and its receptor play an important role in angiogenesis in the endometrium by stimulating VEGF secretion.[24,208] The overexpression of RLN during placenta accreta spectrum may suggest that placenta accreta spectrum causes the production of various autocrine and paracrine factors to encourage the upregulation of angiogenic factors while suppressing antiangiogenic factors, facilitating neovascularization.[24] Despite the cellular changes observed during placenta accreta spectrum, they likely occur secondary to the abnormal myometrial-endometrial interface where the invasive placenta develops.[203]

Last, while the proliferative capacity of the placenta has long been compared to tumor pathogenesis, several similarities have also been documented between the placental microenvironment during placenta accreta spectrum and the tumor microenvironment, namely the proliferative and invasive trophoblast phenotype observed in placentas during placenta accreta spectrum.[24] Cells in both of these microenvironments are required to overcome local immune systems, sustain proliferative signaling, and induce angiogenesis.[24] In particular, the epithelial-to-mesenchymal transition (EMT), which has long been associated with cancer progression,[209] has been documented to play a role in placenta accreta spectrum progression. After implantation in a normal pregnancy, CTBs proliferate at the surface of anchoring villi. These cells make contact with the decidua and undergo EMT, after which they become EVTs and invade the decidua.[24,203] The EMT of EVTs is important to ensure proper invasion and attachment of the placenta during pregnancy, however this transition should not occur throughout pregnancy.[24] In placenta accreta spectrum, it has been shown that there is abnormal EMT persistence throughout the pregnancy and EVTs can have EMT features through the third trimester, which may play an important role in the migration of EVTs during placenta accreta spectrum (Figure 6B).[24]

4.3.2. Gold Standard Treatment for Placenta Accreta Spectrum

Proper care of patients with placenta accreta spectrum is heavily impacted by antenatal diagnosis, as early diagnosis has been shown to improve maternal and neonatal outcomes through accurate risk assessment and well-planned deliveries.[24,199,201,202,210,211] The primary method of diagnosis for placenta accreta spectrum is ultrasound imaging, which can be supplemented with magnetic resonance imaging (MRI).[24,199,201,202,210,211] Most patients are diagnosed with placenta accreta spectrum during the second and third trimesters, but signs can be observed as early as the first trimester on ultrasound.[199,200,211] Ultrasound is the preferred technique for placenta accreta spectrum diagnosis, due to its high sensitivity and selectivity, but ultrasound technology lacks the ability to determine the depth of invasion or the type of placenta accreta spectrum.[199,202,211] MRI has also been reported to have high sensitivity and specificity for diagnosing placenta accreta spectrum, and additionally has the ability to assess the depth of myometrial invasion.[199,202,210,211] However, MRI has not been shown to improve placenta accreta spectrum diagnosis over ultrasound, and its high cost and limited accessibility prevent its use as the primary technique to diagnose placenta accreta spectrum.[199,202] While current diagnostic methods for placenta accreta spectrum remain quite accurate, several population studies[212–214] have shown that over half of placenta accreta spectrum cases go undiagnosed prior to delivery, greatly increasing the risk of massive obstetric hemorrhage due to the surgical team attempting to manually remove the placenta from the uterine wall.[24,202,210]

There are two main treatment strategies for patients diagnosed with placenta accreta spectrum, either conservative management or surgical management. Additionally, the management of placenta accreta spectrum is greatly impacted by proper antenatal diagnosis, the use of a multidisciplinary care team with experience in treating placenta accreta spectrum patients, and delivery at an experienced maternity center.[199,202,211] The predominant management strategy used in patients diagnosed with placenta accreta spectrum is surgical management, where the placenta is left in situ after delivery and a total hysterectomy is performed.[199,210,211] This approach can lead to high rates of maternal morbidity due to the risk of hemorrhage or damage to surrounding organs.[199,202,215] Another approach that is employed, especially for patients with placenta percreta or who are diagnosed with placenta accreta spectrum at birth, is a delayed hysterectomy.[199,202,210,211,215] When a patient wishes to preserve their fertility or is at high risk of hemorrhage with a hysterectomy, conservative management strategies can be employed during which the placenta or uteroplacental tissue is removed without removal of the uterus.[199,202,211,215] With this approach, the umbilical cord is ligated and the entire placenta or only the parts of the placenta that do not spontaneously separate after delivery are left in situ.[202,211] Long-term monitoring is required for these patients until the placenta is either expelled or resorbed, which can occur on the order of weeks to months.[202,211,215] Defining the best time for delivery is also essential for managing patients with placenta accreta spectrum. Delivering the fetus early can help reduce bleeding for the mother, but delivering too early can result in increased risks for the fetus.[202,211] As such, delivery for patients

with placenta accreta spectrum is usually scheduled between 34–36 weeks.[199,210,211]

4.3.3. Nanomedicine Strategies for Placenta Accreta Spectrum

To our knowledge, nanomedicine strategies aimed at treating placenta accreta spectrum have not yet been explored, and thus nanomedicine platforms covered in this section will only discuss diagnostic strategies for placenta accreta spectrum, in particular nanomedicine techniques for improving MRI diagnosis of placenta accreta spectrum (Table 1). As previously mentioned, diagnosis in placenta accreta spectrum remains a challenge with over half of placenta accreta spectrum cases going undiagnosed until delivery.[24,202,210,216] New methods to clearly identify abnormalities at the placental-endometrial interface are highly desired in order to improve the rates of antenatal placenta accreta spectrum diagnosis.[216–218] In particular, proper visualization of the retroplacental clear space, a hypoechoic region at the placental-endometrial interface that is a marker of normal placentation, will aid in placenta accreta spectrum diagnosis.[217,218]

Many of the groups working in this space have focused on developing a liposomal-gadolinium contrast agent to enhance MRI imaging of placental margins, the retroplacental clear space, and the placental-endometrial border to improve placenta accreta spectrum diagnosis.[216–219] In each of these studies, gadolinium (Gd) was selected as a contrast agent as it has been demonstrated to improve the diagnostic capabilities of MRI for placenta accreta spectrum through improved visualization of the placental-endometrial interface.[216,218,220] However, concerns about fetal exposure to Gd have prevented its mainstream use in MRI imaging for placenta accreta spectrum diagnosis.[216,218] In a study by Ghaghada et al., liposomes loaded with Gd were injected into pregnant rats and MRI images of the placental interface were collected.[216] The authors were able to acquire MRI images that demonstrated clear visualization of the placental margins and placental-uterine interface.[216] In a follow-up study, Badachhape et al. confirmed that MRI imaging of mice following the administration of liposomal-Gd led to visualization of the retroplacental clear space with a high signal-to-noise ratio.[217] Most recently, Badachhape et al. injected liposomal-Gd into the Gab3 knockout mouse model (Gab3-/-), which has higher rates of placental invasion compared to wildtype mice.[217,221] After injection, MRI imaging was performed on the mice, and disruption of the retroplacental clear space was clearly observed, supporting the translational potential of this liposomal-Gd agent in the diagnosis of placenta accreta spectrum via MRI.[217] In these works, authors confirmed that the 125 nm size of the liposomal-Gd agents prevented transplacental transport as documented by inductively coupled plasma mass spectrometry (ICP-MS) analysis of fetal and placental tissue.[216,219] Additionally, Ghaghada et al. evaluated the liposomal-Gd agent in a perfused human placental model, where authors used healthy placentas as well as placentas from gestational diabetes and fetal growth restriction pregnancies. In all three models, there was no placental permeability of the liposomal-Gd agent as evidenced by constant lipid concentrations in both the maternal and fetal compartments, further confirming that the liposomal-Gd agent remains in maternal circulation.[216] These results demonstrate the potential of a liposomal-Gd agent for contrast-enhanced MRI to improve placenta accreta spectrum diagnosis and outcomes, but additional studies are needed to demonstrate whether the preclinical success of this strategy in rodents can be applied to humans.

The above studies clearly demonstrate the potential of liposomal-Gd in improving the diagnosis of placenta accreta spectrum with MRI. However, all experiments thus far have been performed in rodent models of placenta accreta spectrum. In order to progress this liposomal-Gd diagnostic platform into the clinic, studies in larger animal models are needed to confirm its translatability into humans and to evaluate its diagnostic capabilities in species with placental structures more similar to humans. Additionally, given the initial concern of fetal exposure to gadolinium, a better understanding of the safety profile of liposomal-Gd is needed. While the authors confirmed that liposomal-Gd did not cross the placenta and enter fetal circulation, experiments evaluating potential toxicity or inflammation in the placenta or other maternal organs following liposomal-Gd administration should be conducted. Finally, the nanomedicine field thus far has only focused on developing nanocarriers to improve the diagnosis of placenta accreta spectrum, and future work should investigate new nanocarrier-based therapeutic strategies to increase treatment options for patients with placenta accreta spectrum.

4.3.4. Potential Nanomedicine Targets for Placenta Accreta Spectrum

Improving outcomes associated with placenta accreta spectrum is primarily focused on intensive delivery planning, which is dependent on accurate diagnosis prior to giving birth. While ultrasound and MRI are important tools in diagnosing placenta accreta spectrum, over half of placenta accreta spectrum cases go undiagnosed prior to delivery, highlighting a need for new diagnostic techniques (Table 2).[24,202,210] Taking an alternative approach from the liposomal-Gd agents described above, recent work in the field has focused on capturing circulating trophoblast cell clusters or microparticle proteins from maternal blood to aid in the early detection of placenta accreta spectrum.[222,223] Circulating trophoblast cells are of particular interest as they are shed during placental implantation and development and are hypothesized to shed at higher levels in cases of abnormal placental invasion, such as placenta accreta spectrum.[222] Additionally, several potential diagnostic serum markers have been identified for placenta accreta spectrum, including alpha-fetoprotein, hCG, PAPP-A, VEGF, PlGF, cell-free fetal DNA, and cell-free placental mRNA.[24,199,202,224] While more research is needed to elucidate their roles in placenta accreta spectrum progression, new nanomedicine strategies can be developed to isolate these biomarkers from maternal blood as diagnostic tools to be used in addition to ultrasound. Design of these NPs could take inspiration from the cancer nanodiagnostics field, where NP probes, such as Au NPs and quantum dots, have been extensively used to identify tumor biomarkers.[225,226]

New nanomedicine therapies are needed to help treat placenta accreta spectrum (Table 2). One potential approach is to design nanocarriers that can reduce deep trophoblast invasion into the maternal myometrium. To this end, a potential target to regulate trophoblast function is tumor necrosis factor-related

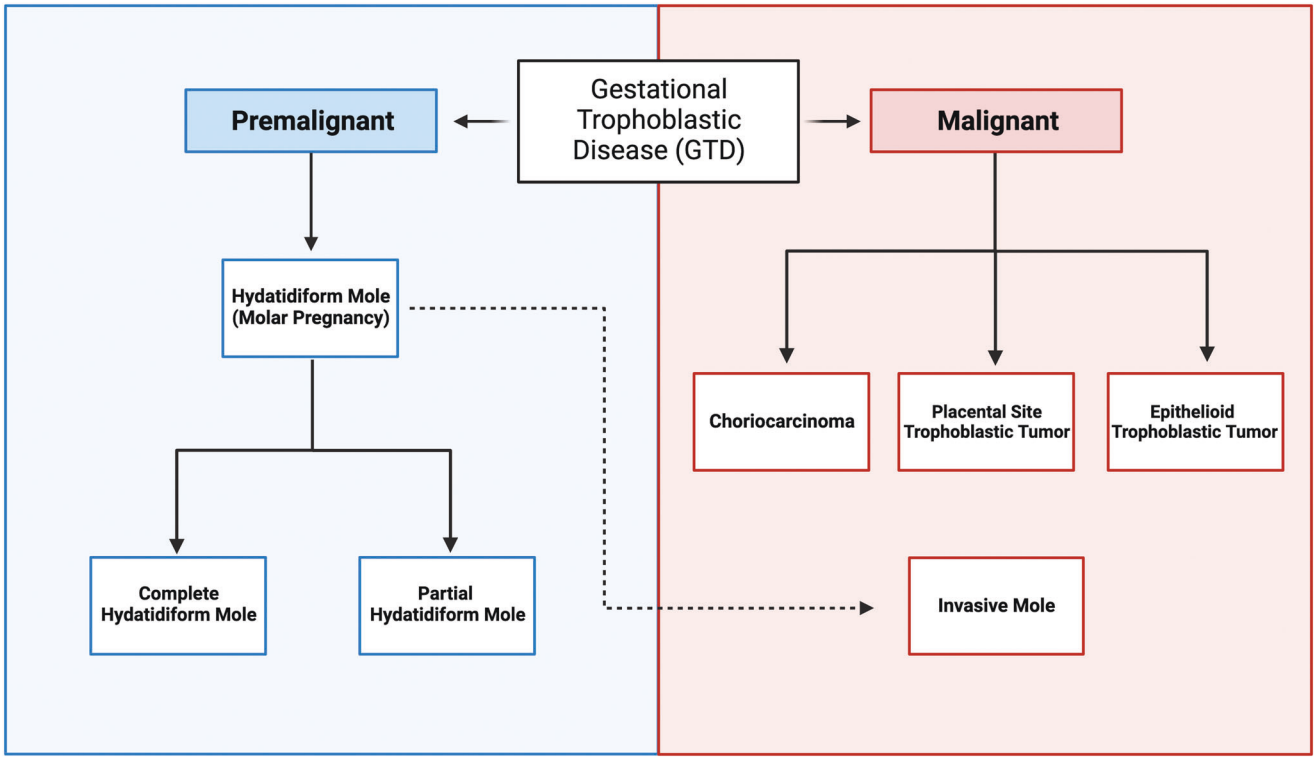

Figure 7. Flow chart describing benign and malignant forms of gestational trophoblastic disease. Complete and partial hydatidiform moles lead to benign clumps of trophoblast cells in place of a fetus; these moles can be evacuated surgically. Invasive moles are biologically benign, but deep invasion into the myometrium can occur, leading to metastasis and a malignant classification. Choriocarcinoma, placental site trophoblastic tumor, and epithelioid trophoblastic tumor are all classified as malignant, as tumor metastasis can occur in various distal organ systems.

apoptosis-inducing ligand (TRAIL) and its receptor TRAIL receptor 2 (TRAIL-R2).[227,228] Binding of TRAIL to its receptor leads to the formation of a signaling complex that initiates caspase activation and apoptosis.[227] TRAIL-R2 has been detected throughout pregnancy, and EVTs have been shown to express TRAIL and its receptor.[229] In patients with placenta accreta spectrum, placental and serum levels of TRAIL-R2 were decreased compared to healthy controls, suggesting that decreases in TRAIL receptor levels may lead to decreased apoptosis in the placenta, contributing to the abnormal placental invasion observed in placenta accreta spectrum.[228] Thus, using nanomedicine to overexpress the TRAIL receptor in trophoblasts or delivery of TRAIL itself to the placenta, presents a potential approach to the treatment of placenta accreta spectrum.

Last, massive obstetric hemorrhage is one of the major causes of maternal mortality associated with placenta accreta spectrum, with burden of hemorrhage higher in resource-limited countries due to limited access to advanced maternity centers.[199,202,215,230] The development of new nanomedicines to prevent or treat hemorrhage could aid in reducing mortality rates associated with placenta accreta spectrum. Uterotonics are the recommended postpartum hemorrhage prevention strategy by the World Health Organization and include the drugs oxytocin, carbetocin, and misoprostol.[230,231] Uterotonics cause the myometrium to contract, constricting the spiral arteries and reducing blood flow.[231] One potential approach would be to load a uterotonic agent into an NP targeted to the placenta to help stop bleeding, a technique that has been previously evaluated in myometrial samples in vitro.[232] Localized delivery of these drugs to the placenta could help reduce off-target effects of the drug and prevent administration of the drug into the fetal compartment.[232,233] Alternatively, nanomedicine strategies to reduce other forms of hemorrhage could be applied to patients with placenta accreta spectrum. NPs loaded with coagulation proteins, clotting activators, or peptides that interact with activated platelets have demonstrated success in reducing blood loss and could be applied to the treatment of postpartum hemorrhage.[234]

4.4. Gestational Trophoblastic Disease

Gestational trophoblastic disease is an umbrella term encompassing benign and malignant forms of pregnancy-associated tumors. Benign gestational trophoblastic diseases, also known as hydatidiform moles or molar pregnancy, include complete hydatidiform moles, partial hydatidiform moles, and invasive moles. Malignant gestational trophoblastic diseases, also termed gestational trophoblastic neoplasia, include choriocarcinoma, placental site trophoblastic tumors, and epithelioid trophoblastic tumors (**Figure 7**).[235–237] Although invasive moles are biologically characterized as benign gestational trophoblastic diseases, invasive moles can result in metastasis to the lungs or vagina—which has led to their dual classification as a benign disease that can transform into a malignant disease.[26,235] All forms of gestational

trophoblastic disease arise from the abnormal proliferation of trophoblast cells following fertilization. Gestational trophoblastic diseases are characterized by an overexpression of hCG, and are often diagnosed clinically using ultrasound in conjugation with monitoring of hCG levels in maternal circulation during pregnancy.[235,238]

Incidence rates and etiology for gestational trophoblastic diseases have not yet been well characterized for a variety of reasons, including inconsistent clinical definitions and diagnostic criteria, lack of global centralized databases, rarity of disease, and more.[235,237] For example, morphological distinction of non-molar miscarriage from hydatidiform moles can be difficult since placental villous dysmorphism can be seen in both conditions. Molecular-level testing is often required to differentiate between conditions.[236] Unfortunately, this detailed level of testing may not always be performed due to time constraints, cost, and/or equipment accessibility, which ultimately contributes to the widespread lack of reported cases and available data for gestational trophoblastic diseases.[26,236] Based on available data, established risk factors for hydatidiform moles include extremes in maternal age, prior molar pregnancy, and history of spontaneous abortion.[237] Risk factors for choriocarcinoma include prior complete hydatidiform mole and advanced maternal age.[237] Placental site trophoblastic tumors mostly follow non-molar gestation and originate specifically from the placental implantation site. Epithelioid trophoblastic tumors usually present many years following gestation and often stimulate choriocarcinoma. Epithelioid trophoblastic tumors demonstrate moderate increases in hCG levels but are aggressive in nature, with a mortality rate of around 13%.[235] In addition, risk of gestational trophoblastic neoplasia following hydatidiform mole has been associated with long-term oral contraceptive use, although this connection remains controversial.[239,240]

While gestational trophoblastic diseases still result in significant morbidity and mortality, treatment options have improved drastically over the past few decades with increased efficacy and accessibility of uterine evacuation procedures and chemotherapeutic agents.[237] Despite this, gestational trophoblastic diseases still can pose serious health threats for women. Molar pregnancies do not support a viable fetus and, thus, surgical evacuation is required.[235,237] Evacuation procedures are generally safe when performed in clinical settings, but have some risks associated with them, including uterine perforation, vaginal laceration, and hemorrhage.[241] In addition, because histological examination is not mandated after all termination procedures, women are at risk for misdiagnosis, which can lead to substantial future morbidity with a need for chemotherapy and complex surgical procedures.[235] Invasive mole and gestational trophoblastic neoplasia pose many of the threats commonly associated with cancer, including weakened immune system, metastasis, and death. Choriocarcinoma can result in cancer metastasis in both the mother and infant, with choriocarcinoma metastasis hotspots including the cervix, vagina, lungs, brain, kidney, intestine, spleen, and liver. Although placental site trophoblastic tumors and epithelioid trophoblastic tumors are much rarer, these conditions can be equally as dangerous. Placental site trophoblastic tumors have been shown to metastasize to the uterus and lungs, and epithelioid trophoblastic tumors have been shown to metastasize to the liver, lungs, vagina, brain, spine, and gallbladder. In addition, both placental site trophoblastic tumors and epithelioid trophoblastic tumors often demonstrate chemoresistance, making treatment without loss of fertility nearly impossible.[235]

4.4.1. Pathophysiology of Gestational Trophoblastic Disease

All gestational trophoblastic diseases are derived from the placenta, however the molecular mechanisms underlying different gestational trophoblastic diseases, especially gestational trophoblastic neoplasia, are largely unknown.[26] Hydatidiform moles refer to pregnancies characterized by abnormal CTB and STB proliferation and swelling of placental villi, usually with an absent or nonviable fetus. The cellular events leading to the formation of complete hydatidiform mole and partial hydatidiform mole vary but involve abnormal fertilization. Complete hydatidiform mole occurs when an anucleated ovum (no maternal chromosomes) fuses with a single sperm or a diploid sperm, with both cases resulting in a diploid androgenetic karyotype where all chromosomes are paternally derived.[235,236] Complete hydatidiform mole development is indicated by trophoblast hyperplasia, the presence of few or no blood vessels and/or collapsed vessels, stromal debris, and nuclear pleomorphisms.

In a distinctly different pathway from complete hydatidiform mole, partial hydatidiform mole occurs from fertilization of a seemingly healthy ovum by two sperm simultaneously, resulting in a triploid karyotype.[235,236] Similar to complete hydatidiform mole, partial hydatidiform mole development is also characterized by trophoblastic hyperplasia, but appears patchy and less extensive than that seen in complete hydatidiform mole. Additional features of partial hydatidiform mole include fibrosis of villi and the presence of blood vessels with few nucleated fetal red cells.[235] Invasive moles occur when hydatidiform moles, typically complete hydatidiform moles, progress with deep villous invasion of trophoblasts into the myometrium, either directly into the tissue or through vasculature, during which uterine perforation can occur.[235–237] Despite the well-established cytogenetic mechanisms associated with hydatidiform moles, the molecular etiology behind molar pregnancy remains poorly understood. Upregulation of several oncogenes has been associated with hydatidiform moles, including c-MYC, c-ERB-2, c-FMS, and BCL-2 (**Figure 8**). Frequent promoter hypermethylation and decreased expression of phosphatase and tensin homolog (PTEN), chromodomain helicase DNA binding protein 1 (CHD1), hypermethylated in cancer 1 (HIC-1), and cyclin-dependent kinase inhibitor 2A (CDKN2A) proteins have also been observed in hydatidiform moles compared to healthy placentas.[26]

Gestational trophoblastic neoplasia specifically occurs following the neoplastic transformation of CTB stem cells, after which differentiation programs dictate the type of tumor that develops (choriocarcinoma vs. placental site trophoblastic tumor vs. epithelioid trophoblastic tumor). Choriocarcinoma is derived from neoplastic CTBs, STB cells, and a group of cells possessing markers of both CTB and STB cells, deemed intermediate trophoblasts (ITBs). Placental site trophoblastic tumor is mainly composed of ITBs, and epithelioid trophoblastic tumor is a subtype of placental site trophoblastic tumors, which differentiates specifically into chorionic-type ITBs.[26,237,242] Gestational trophoblastic neoplasia is characterized by abnormal

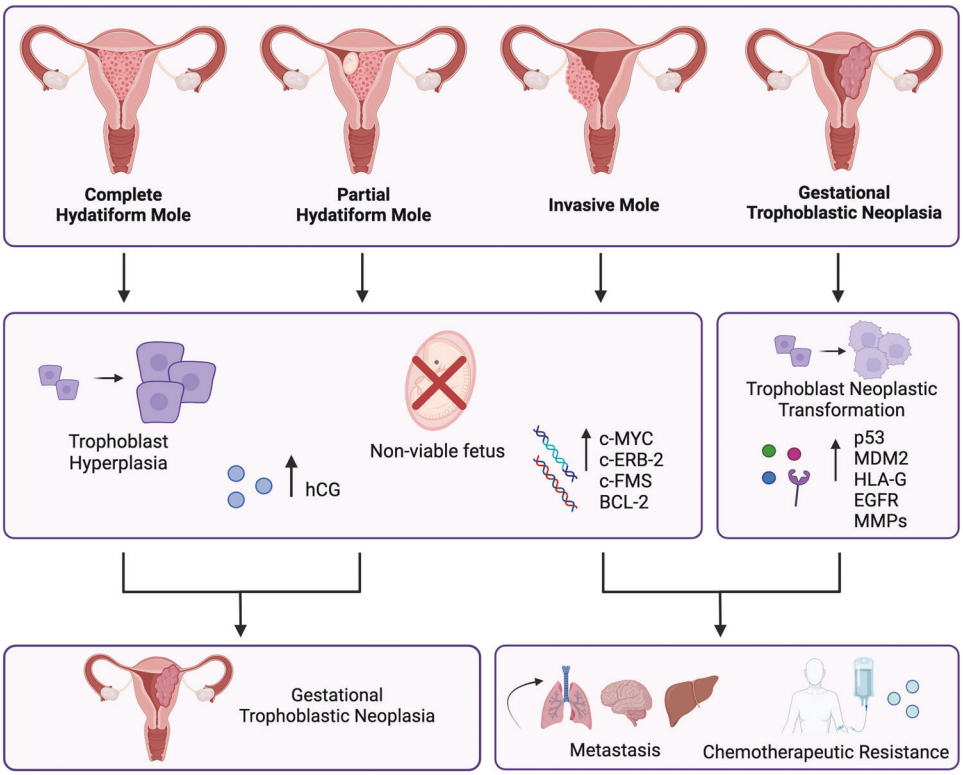

Figure 8. Schematic of Gestational Trophoblastic Diseases. In molar pregnancy, abnormal trophoblast differentiation and proliferation result in an absent or nonviable fetus. The pregnancy hormone hCG and oncogenes c-MYC, c-ERB-2, c-FMS, and BCL-2 are upregulated during molar pregnancy. Invasive moles form when trophoblasts invade deep into the myometrium during molar pregnancy. Molar pregnancy often leads to the development of malignant gestational trophoblastic neoplasia. Risks of invasive mole and gestational trophoblastic neoplasia include cancer metastasis and chemotherapeutic resistance. hCG, human chorionic gonadotropin; p53, p53 tumor suppressor protein; MDM2, mouse double minute 2; HLA-G, human leukocyte antigen G; EGFR, epidermal growth factor receptor; MMPs, matrix metalloproteinases.

trophoblastic hyperplasia and anaplasia of both CTB and STB cells, extensive hemorrhage, absence of placental villi, and tissue necrosis.[235] It's also been demonstrated that hydatidiform moles are often precursor lesions of gestational trophoblastic neoplastic transformation.

Preliminary molecular-level studies have demonstrated overexpression of the p53 tumor suppressor protein in choriocarcinoma, but further analysis failed to show somatic mutations. The p53-associated protein, mouse double minute 2 (MDM2), however, is overexpressed in many cancers, including choriocarcinoma, and may overcome wild-type p53-mediated growth suppression, contributing to the growth of choriocarcinoma.[26] Other important genes that may play a role in choriocarcinoma development and growth include EGFR, tumor suppressor gene DOC-2, and the gene for ras GTPase activating protein. Synergistic regulation of common oncoproteins has also been suggested to play a role in choriocarcinoma pathogenesis.[26,235] Beyond genetic abnormalities, immune regulation within the tumor microenvironment may promote tumor growth. Both healthy and neoplastic trophoblasts alike have been shown to upregulate the non-classic MHC class I molecule, human leukocyte antigen G (HLA-G), which normally plays a role in escaping immune surveillance. Choriocarcinomas contain the highest levels of HLA-G expression among other cancers, suggesting a key role in HLA-G mediated tumor growth. MMPs, which play a role in tumor invasion, have also been suggested to orchestrate choriocarcinoma development, as choriocarcinomas have been shown to display increased levels of MMPs. This overexpression may contribute toward the highly metastatic nature of choriocarcinoma if left untreated (Figure 8).[26,235] Due to the rarity of placental site trophoblastic tumors and epithelioid trophoblastic tumors, molecular mechanisms underlying these tumor pathogeneses have been studied even less than choriocarcinoma. Genetic analysis has shown that both placental site trophoblastic tumor and epithelioid trophoblastic tumor contain paternal alleles not present in adjacent healthy uterine tissue, confirming fetal (trophoblastic) origin.[26,243] Contrary to many other gestational trophoblastic diseases, placental site trophoblastic tumors are not accompanied by a rise in hCG levels, but rather, an elevation in levels of human placental lactogen (hPL). Placental site trophoblastic tumors have been associated with abnormal expression of cell-cycle regulatory genes, including cyclins, cyclin-dependent kinases, and p53. Epithelioid trophoblastic tumors express markers of epithelial cells, including cytokeratin, E-cadherin, and EGFR. In addition, epithelioid trophoblastic tumors, but not placental site trophoblastic tumors, express the p63 gene, a transcription factor belonging to the p53 family, although its functional role has not been identified.[26,235]

4.4.2. Gold Standard Treatment for Gestational Trophoblastic Disease

Hydatidiform moles are diagnosed almost exclusively via ultrasound. Complete hydatidiform moles can be observed readily due to the characteristic swollen chorionic villi. Partial hydatidiform moles may be observed via ultrasound by identifying focal cystic spaces within the placenta and an increase in the transverse diameter of the gestational sac. Given that abnormal hCG levels are present in most gestational trophoblastic diseases, urine and blood tests are also often used for diagnosis and to determine the severity of disease.[237]

Following the diagnosis of a hydatidiform mole, uterine evacuation is the gold standard treatment, as the fetus is non-viable. For patients wishing to maintain fertility, suction evacuation and curettage (D&C, also known as medical abortion) is the recommended method.[236,237] For patients not wishing to preserve fertility, a hysterectomy is recommended. In addition to evacuating the molar pregnancy, hysterectomy results in permanent sterilization and eliminates the risk of myometrial invasion. However, hysterectomy does not eliminate the potential for metastatic disease, and thus additional follow-up is required to monitor for post-molar gestational trophoblastic neoplasia. Medical induction of labor is not recommended for molar pregnancy, as this method leads to increased maternal morbidity, including blood loss, incomplete evacuation, requirement for cesarean delivery in subsequent pregnancy, and potential development of post-molar gestational trophoblastic neoplasia.[236,237]

The standard of care for choriocarcinoma is chemotherapy. Fortunately, choriocarcinoma is one of the few cancers that are responsive to chemotherapy, and metastatic choriocarcinoma is potentially curable using combined chemotherapy and adjuvant surgical procedures.[26,237] Conventional chemotherapy includes methotrexate (MTX) for low-risk disease and EMACO (contains etoposide, methotrexate, actinomycin D, cyclophosphamide, and vincristine) for intermediate or high-risk disease. Unfortunately, some choriocarcinomas do not respond to single-agent chemotherapy and require multiagent chemotherapy regiments.[244–247] In addition, a significant portion of choriocarcinoma patients develop recurrent diseases. Placental site trophoblastic tumors and epithelioid trophoblastic tumors usually demonstrate chemotherapeutic resistance and, thus, do not currently have curative treatments. For some patients, especially those with epithelioid trophoblastic tumor, the diagnosis is terminal.[26] While the use of chemotherapy has demonstrated some success for many choriocarcinoma patients, chemotherapeutic drugs for all cancer applications are widely known to be toxic and present dangerous systemic side effects.[245,246,248] Improved chemotherapeutic regimens with less toxicity and enhanced placental targeting are needed. Further, novel treatment strategies are needed for placental site trophoblastic tumors and epithelioid trophoblastic tumors, as there are currently no treatment strategies for these diseases.

4.4.3. Nanomedicine Strategies for Gestational Trophoblastic Disease

To our knowledge, nanomedicine strategies specifically aimed at treating hydatidiform moles, placental site trophoblastic tumors and epithelioid trophoblastic tumors have not been explored and, thus, nanomedicine platforms covered in this section will only discuss strategies for choriocarcinoma treatment (Table 1). Because many molecular mechanisms remain unknown surrounding gestational trophoblastic diseases, the nanomedicine platforms described for choriocarcinoma may have value for future investigations into nanocarrier development for hydatidiform moles, placental site trophoblastic tumors, and epithelioid trophoblastic tumors as well.

One common strategy that has been explored to target chemotherapeutic drugs to the placenta has been the attachment of P-CSA-BP to NP formulations. Zhang et al. have previously demonstrated the ability of P-CSA-BP lipid-polymer NPs to deliver MTX effectively and specifically to the placenta to treat placental disorders.[116] The delivery of MTX via NP nanocarrier could offer several benefits including reduced systemic toxicity and increased drug delivery to the site of disease and, thus, allow for lower chemotherapeutic doses and maternal sequestration in the case of pregnancy. In this work, IV-injected P-CSA-BP NPs specifically accumulated in mouse placentas. Interestingly, researchers administered P-CSA-BP NPs loaded with MTX in healthy pregnant mice to assess the efficacy of the platform as a drug delivery vehicle. Of note, the clinical use of such a platform would be used to treat ectopic pregnancy or choriocarcinoma, during which a viable fetus most likely would not be present. In this study, administration of MTX-loaded P-CSA-BP NPs resulted in dramatic impairment of placental and fetal development, confirming drug delivery capacity. In addition, MTX was detected in fetuses following treatment with free MTX or nontargeted NPs, but no MTX was detected in fetuses following P-CSA-BP NP treatment, suggesting that P-CSA-BP targeting restricts drug delivery to the placenta and may sequester drug there. The authors evaluated the safety of P-CSA-BP NPs by performing histological analysis of liver and kidney tissues; free MTX groups and nontargeted NP groups resulted in tissue abnormalities in the liver and kidney whereas tissue architecture from targeted P-CSA-BP NPs groups resembled that of saline-treated groups, demonstrating that P-CSA-BP NPs did not cause off-target toxicity. Of note, the successful delivery of MTX during healthy pregnancy in this work resulted in drastic placental and fetal toxicity, which may have masked potential placental inflammation and/or toxicity that may emerge when a viable pregnancy is not present. Toxicity and safety profiling beyond histological analysis, and within a relevant cancer model, is necessary to validate the potential of this NP platform for treating choriocarcinoma.[116]

In a parallel study, Zhang et al. evaluated their CSA-targeted lipid-polymer NP platform in a mouse cancer model. The authors investigated the efficacy of targeted NPs loaded with the chemotherapeutic drug doxorubicin in alleviating tumor burden in nude mice bearing Fluc-GFP-JEG-3 xenografts, derived from the choriocarcinoma cell line JEG-3.[245] Following IV injection, CSA-NPs rapidly localized to the tumor whereas nontargeted NPs did not demonstrate any signal in the tumor. CSA-NPs loaded with doxorubicin inhibited primary tumor growth and suppressed metastasis compared to nontargeted NPs and free doxorubicin. Impressively, luciferase activity in the Fluc-GFP-JEG3 cells could not be detected in two of five mice in the CSA-NP treated group 18 days after treatment. All mice in the CSA-NP group survived beyond 30 days, whereas all control PBS mice died within 18 days. While this work demonstrated the efficacy

of CSA-NPs in reducing tumor burden in mice, the safety profiling was not extensive. The only metric recorded in addition to survival length was body weight following treatment. Histological analysis of organs, immune cell profiling, and cytokine levels in the blood and tumor environment were not discussed in this work. In all, CSA-NPs hold promise for the targeted delivery of doxorubicin to treat choriocarcinoma, however additional studies are required to investigate potential safety and toxicity concerns of this platform.[245]

Zhao et al. utilized the targeting capabilities of P-CSA-BP to explore the efficacy of a novel drug carrier material and simultaneously investigate the anticancer potential of the drug prodigiosin in choriocarcinoma.[249] Prodigiosin is an antimalarial and immunosuppressive drug of bacterial origin that has recently been shown to have anticancer and antimetastatic properties.[250] The novel platform investigated in this work employed P-CSA-BP conjugated to dendrigraft poly-L-lysines (DGLs). DGLs are synthetic, biodegradable polymers rich in external amino acid groups that can stably encapsulate drugs. DGLs are tunable and can be easily modified with PEG to improve circulation time and targeting moieties for tissue-specific drug delivery.[251] In luminescent JEG-3 tumors in mice, targeted DGLs demonstrated significantly decreased luminescence compared to free prodigiosin and nontargeted DGLs. Size and growth rate of tumors in the P-CSA-BP DGL treated group were significantly reduced compared to controls. Histological analysis revealed no major changes in liver, lung, and kidney tissues 30 days post-treatment, suggesting that DGLs do not cause off-target effects. In addition, survival rate was around 70% for the targeted DGL group, but less than 25% in all other treatment groups after 18 days. Molecular analysis revealed that P-CSA-BP DGLs may induce choriocarcinoma apoptosis specifically through p53 and caspase-3 pathways.[249] Interestingly, the authors noted that biodistribution data had previously been reported with P-CSA-BP lipid polymer NPs, and thus, biodistribution data of DGLs was not discussed. Given the inherent differences between lipid polymer NPs and DGLs, the inclusion of biodistribution data is suggested to confirm that DGLs do not elicit off-target effects. While histological analysis of lung, liver, and kidney tissue was reported, additional safety profiling, such as analysis of AST/ALT levels and inflammatory cytokine markers, is needed for the clinical translation of DGLs as therapeutic platforms.

Previous work has documented high levels of EGFR expression in the placenta, with potential additional increases in expression occurring during placental pathologies, including choriocarcinoma and preeclampsia.[252–254] Kaitu'u-Lino et al. developed EGFR-targeted EnGenIC Delivery Vehicles (EVDs) to deliver doxorubicin to the placenta for placental diseases that may benefit from chemotherapeutic agents. The authors list potential therapeutic applications including ectopic pregnancy, molar pregnancy, and choriocarcinoma.[244] EVDs are bacterially derived nanospheres that can be sterilized and can stably encapsulate chemotherapeutic drugs. Previous biodistribution work by this group demonstrated that potentially 30% of EGFR-targeted EVDs can reach the placental within 2 hours of administration with minimal toxicity.[255] In their latest study, researchers observed potent decreases in the viability and proliferation of JEG-3 cells following treatment with doxorubicin-loaded EVDs. The authors then demonstrated that EGFR-EVDs loaded with the chemotherapeutic drug doxorubicin resulted in reduced JEG-3 xenograft tumor size in mice compared to nontargeted EVDs and systemic doxorubicin. Beyond tumor volume and survival data, the safety and toxicity of EVDs as a therapeutic platform were not evaluated.[244] Additional toxicity data are needed to evaluate the potential of EVD technology for chemotherapeutic drug delivery to treat placental disorders.

Also focusing on EGFR expression in the placenta, Dong et al. investigated choriocarcinoma delivery of EGFR aptamer-conjugated liposome-polycation-DNA (LPD) complexes.[246] This same group has previously demonstrated potent siRNA delivery to hepatocellular carcinoma and breast cancer via LPD complexes.[256,257] In this work, researchers aimed to investigate the consequences of using siRNA to silence SATB1, a regulator of gene expression. SATB1 has been shown to promote metastasis of various cancers, including gastric, liver, and breast cancer, and SATB1 knockdown has resulted in decreased cancer growth and metastasis.[258,259] The potential role of SATB1 suppression in choriocarcinoma has not previously been investigated. To investigate SATB1 silencing as a potential therapeutic strategy against choriocarcinoma, researchers conjugated EGFR aptamers, or oligonucleotide sequences with affinity against EGFR, to LPDs and encapsulated SATB1 siRNA to form EGFR-LPDS. Researchers first investigated SATB1 expression in choriocarcinoma cell lines and observed a 19x increase in SATB1 expression in choriocarcinoma cells, motivating the use of SATB1 as a therapeutic target. In vitro data showed that SATB1 knockdown in choriocarcinoma cell lines could inhibit choriocarcinoma proliferation. In JEG-3 xenografts in mice, EGFR-LPDS could inhibit expression of SATB1 and, excitingly, exhibited a tumor weight inhibitory rate of around 81%. These results suggest that EGFR-targeted LPDs may be an effective strategy for treating EGFR-expressing cancers, such as choriocarcinoma. However, safety profiling beyond monitoring mouse body weight was not discussed.[246] Further work investigating potential toxicity and off-target side effects is necessary to demonstrate the clinical potential of EGFR-LPDS in treating choriocarcinoma.

In a proof of concept study, Erol et al. investigated a new chemotherapeutic regimen in vitro using JEG-3 and JAR choriocarcinoma cell lines.[260] Cell lines were dosed with PEGylated liposomal doxorubicin (PLD), β-carotene, or a combination of PLD and β-carotene, and apoptosis was quantified after 72 hours. PLD has previously been approved for the treatment of other cancers, and doxorubicin encapsulation using PEGylated liposomes has been reported to affect biodistribution and decrease toxicity, motivating the use of liposomal doxorubicin for choriocarcinoma treatment.[261,262] β-carotene is a naturally occurring vitamin A precursor possessing chemotherapeutic properties. A previous study by Parazinni et al. has suggested that low β-carotene may be related to gestational trophoblastic disease development.[263] In this preliminary study, researchers showed that increasing amounts of PLD in combination with a fixed amount of β-carotene resulted in increased apoptosis in choriocarcinoma cells. Further work is required to investigate the potential synergistic effects of this chemotherapeutic regimen in vivo.[260]

Wei et al. developed self-assembling ascorbic acid derived (PEG-ss-aAPP) micelles to deliver MTX as a potential treatment strategy for choriocarcinoma.[264] To decrease systemic side

effects of MTX and increase drug action at the site of disease, researchers loaded MTX in self-assembling micelles, where hydrophilic PEG segments minimize rapid removal by the reticuloendothelial system (RES). The ascorbic acid derivative, APP, was utilized given its ability to form micelles in aqueous solution and its inherent anti-cancer properties.[265] Micelles specifically were selected as a drug carrier vehicle given their tunability; micelles can be engineered to release drugs in response to specific stimuli. In this work, redox and pH-sensitive micelles were formulated such that the combination of an intracellular reducing environment and the acidity of the endosome or tumoral microenvironment will synergistically lead to the release of drug cargo. Using Cy 5.5 dye-loaded micelles and the organelle tracker Lysotracker Green, researchers identified specific localization of Cy5.5 dye, confirming that drug cargo had escaped the endosome and leaked into the cytoplasm. Through in vitro studies with choriocarcinoma cell lines and a wound healing assay, researchers showed that PEG-ss-aAPP micelles loaded with MTX (PEG-ss-aAPP/MTX) were able to slow cancer cell proliferation and reduce cancer cell migration compared to free MTX and PEG-free micelles loaded with MTX (APP/MTX micelles). Specifically, PEG-ss-aAPP/MTX micelles demonstrated potent anticancer effects via APP- and MTX-mediated ROS production, which has inhibitory effects on NF-κB translocation to the nucleus. This inhibition results in the suppression of gene expression pathways known to drive inflammation, invasion, and metastasis.[109] Finally, in an orthotopic mouse model of choriocarcinoma, PEG-ss-aAPP/MTX micelles strongly inhibited primary tumor growth and suppressed metastasis compared to controls. These results were supported by reduced levels of the choriocarcinoma tumor marker hCG in PEG-ss-aAPP/MTX treated mice. Safety profiling revealed no changes in body weight, AST/BUN levels, or IL-6 serum concentration among treatment groups.[264] Together, these results frame PEG-ss-aAPP/MTX micelles as a potentially exciting treatment option for choriocarcinoma.

Another strategy that has been employed to specifically shunt chemotherapeutics to the placenta and reduce systemic toxicity during choriocarcinoma is targeting the overexpressed hCG receptor. Huining et al. have previously shown that dextran-coated iron oxide NPs modified with anti-β-hCG antibodies can be used to target choriocarcinoma cells.[266,267] In this work, researchers used this hCG-targeted platform to deliver heparanase (Hpa) antisense oligodeoxynucleotide (ASODN) in JEG-3 cells. Hpa has been reported to play a role in cancer metastasis, motivating the use of antisense therapy (translation inhibition) as a potential treatment strategy.[268] Western blot analysis confirmed inhibition of Hpa in JEG-3 cells following treatment with Fe3O4-dextran-anti-βhCG-Hpa ASODN. Following treatment to hypodermal transplant tumors in nude mice, Fe3O4-dextran-anti-βhCG-Hpa ASODN resulted in significant inhibition of tumor growth compared to empty Fe3O4-dextran-anti-βhCG NPs and Fe3O4-dextran-anti-βhCG nonspecific oligodeoxynucleotide NPs.[266] While NP-mediated reduction in tumor growth suggests potential antitumor effects, additional in vivo data, such as toxicity and/or survival study data, were not recorded. In a more recent study, Cong et al. developed hCG polypeptide-conjugated polymeric NPs to enhance the delivery of MTX for applications in both choriocarcinoma and ectopic pregnancy. In vitro data demonstrated significantly reduced cell proliferation in both JAR and JEG-3 cells compared to free MTX and nontargeted NPs.[238] In vivo studies were not conducted and, thus, additional work is required to probe the effects of hCG polypeptide-conjugated NPs for the treatment of choriocarcinoma.

In recent years, new findings have demonstrated that nanodrugs conjugated with specific membrane transporter substrates can not only enhance the specificity of drug delivery but can physically enter cancer cells through mediation by the transporter.[269,270] Thus, a new class of promising targets may be highly expressed transporter proteins on tumor cells. A recent study by Fei et al. utilized this concept to develop liposomes targeted against human equilibrative nucleoside transporter 1 (ENT1), which is overexpressed in choriocarcinoma cells.[271] To target liposomes to ENT1, liposomes were conjugated with cytarabine (Cy, an analog of the nucleoside cytosine, serves as substrate for ENT1) to create Cy-Lipo. Further, Cy has been shown to interfere with the cell cycle and have therapeutic effects on choriocarcinoma.[272] In vitro studies confirmed that Cy-Lipo formulations have a high affinity for ENT1, and ENT1 can mediate endocytosis of the drug carrier vehicles in JEG-3 cells. When administered via IV injection to choriocarcinoma xenograft nude mice, Cy-Lipos loaded with MTX demonstrated powerful antitumor effects, with a tumor growth inhibition index of around 93%. In addition, Cy-Lipo-treated mice displayed an enhanced safety profile compared to controls as demonstrated by body weight, AST/ALT/BUN levels, and histological analysis.[271] Together, these data highlight Cy-Lipo as an exciting therapeutic platform for choriocarcinoma.

The works presented here demonstrate promising therapeutic platforms for the treatment of choriocarcinoma. Like the nanomedicine platforms developed for other placental disorders, investigations into the safety and toxicity of such platforms are required before nanocarriers for gestational trophoblastic disease can progress to the clinic. Robust safety profiling is particularly important in the context of cancer, as it can be difficult to distinguish between inherent nanocarrier toxicity, cancer cell-mediated toxicity, and delivered chemotherapeutic-mediated toxicity. As bodily immune defenses are already weakened during cancer, it is of the utmost importance to ensure that drug delivery platforms don't further elicit harsh inflammatory responses. Many of the works discussed here reported days of survival and mouse weight as metrics of health. Future studies should include more detailed safety profiling, such as the profiling performed by Wei et al.[264] and Fei et al.[271] including histological analysis and examination of body weight, AST/BUN levels, and IL-6 serum concentrations post-treatment. Further, long-term studies, such as the metastatic tumor analysis by Wei et al., are necessary to evaluate the timeline of nanocarrier therapeutic effects on tumor burden and long-term cancer metastasis.[264]

As mentioned prior, there are no works to our knowledge that have investigated the use of nanomedicine to treat hydatidiform moles, placental site trophoblastic tumors, and epithelioid trophoblastic tumors. Given that treatment for hydatidiform moles often utilizes evacuation procedures, and abortion rights have recently been restricted across much of the United States, it is critical that novel nanomedicine platforms be developed as safe and effective treatment strategies for pregnancy complications, including molar pregnancies. While placental site

trophoblastic tumors and epithelioid trophoblastic tumors are less common than choriocarcinoma, these tumor types can exhibit drug resistance and often demonstrate an increased risk of metastasis.[26,243] Thus, it is critical to utilize nanoengineering tools to develop therapeutic platforms for these tumors for which few treatment options currently exist.

4.4.4. Potential Nanomedicine Targets for Gestational Trophoblastic Disease

While the use of nanocarriers to selectively deliver gold-standard chemotherapeutics and reduce systemic toxicity remains an important and exciting prospect for the treatment of gestational trophoblastic neoplasia, novel treatment strategies are needed to advance the field beyond toxic MTX delivery, develop options for chemotherapeutic-resistant gestational trophoblastic neoplasia, and decrease gestational trophoblastic neoplasia recurrence. Target-based strategies may hold the most potential for the treatment of gestational trophoblastic neoplasia, as these nanoplatforms can target specific molecular pathways and selectively eliminate tumor cells whereas standard chemotherapy affects almost all proliferating cells (Table 2).

As mentioned previously, many proto-oncogenes are upregulated in choriocarcinoma. Specifically, abnormal enhanced expression of c-MYC is present in many cancer types, including choriocarcinoma, and, also, the nonmalignant complete hydatidiform mole.[26] Upregulated c-MYC proteins may suppress trophoblast differentiation while enhancing proliferation, leading to neoplasia. The idea of targeting MYC pathways as a cancer treatment strategy is not new; however, nanomedicine platforms for c-MYC modulation in gestational trophoblastic neoplasia have not been reported.[273–275] Drug delivery carriers could be used to selectively modulate c-MYC overexpression in choriocarcinoma and other gestational trophoblastic neoplasia via small molecule inhibitors, peptide inhibitors, siRNA, or antisense oligonucleotides.

EGFR is normally highly expressed in placental cells; however, EGFR is further upregulated in choriocarcinoma, placental site trophoblastic tumors, and epithelioid trophoblastic tumors.[246,252] Previous work has also shown that high EGFR immunoreactivity in trophoblasts in complete hydatidiform mole may significantly correlate with the subsequent development of gestational trophoblastic neoplasia.[26,276] Like MYC, EGFR has been previously explored as an anticancer target, with EGFR antibody medications showing some success in lung, colorectal, and head and neck cancer.[26,277,278] EGFR-targeted therapies remain relatively underexplored for gestational trophoblastic neoplasia applications. Given the compounded significant EGFR overexpression present in many gestational trophoblastic diseases, EGFR represents an exciting target for the development of novel targeted nanomedicine platforms for complete hydatidiform mole and gestational trophoblastic neoplasia treatment.

MMPs play a role in the invasion and metastasis of many cancers and, thus, have been a target of interest for anticancer therapies.[279,280] MMP inhibitors may be used to reduce tumor growth, inhibit metastasis, and block tumor angiogenesis. MMPs are upregulated in choriocarcinoma and may contribute to the highly metastatic and chemo-resistant nature seen in many choriocarcinomas.[26,281] Broad-spectrum MMP inhibitors have previously gone to clinical trials as anticancer agents, but results demonstrated poor bioavailability and toxicity.[280] Thus, small molecule or nucleic acid derived MMP inhibitors may be ideal candidates for inclusion in a targeted nanomedicine platform for the treatment of gestational trophoblastic neoplasia. Nanocarrier-mediated MMP inhibition could increase availability at the site of disease and reduce systemic toxicity, potentially demonstrating anti-metastatic properties in gestational trophoblastic neoplasia.

Gestational trophoblastic neoplasia contains some of the highest levels of HLA-G compared to other cancers, making HLA-G an attractive target for placental cancers specifically.[26,282] Further, HLA-G levels may also correlate with tumor progression, metastasis, and poor clinical outcome, suggesting future potential functionality of HLA-G as a target for diagnostic measures.[282,283] HLA-G immunotherapy has been preliminarily explored using HLA-G derived peptides in renal cell carcinoma and prostate cancer.[284,285] HLA-G may hold potential as an exciting target for both diagnostic measures and treatment strategies for gestational trophoblastic neoplasia, but, to date, remains unexplored.

5. Conclusions and Future Perspectives

In this review, we discuss nanomedicine strategies to diagnose and treat preeclampsia, fetal growth restriction, placenta accreta spectrum, and gestational trophoblastic disease. Through exploration of the underlying pathophysiologic mechanisms behind these conditions, we highlight several novel disease targets. We hope that researchers in the field of maternal and fetal health can apply the pathophysiology reviewed in this article to engineer new rationally designed nanocarriers to improve currently available treatment strategies for placental disorders. We believe nanomedicine can be a powerful tool to improve outcomes for both mother and fetus during pregnancy—especially now in a post-Roe era of women's reproductive rights in the United States.

In light of the recent overturning of Roe v. Wade which restricts abortion access across much of the United States, we feel it is vital to direct attention to issues of women's reproductive health and the rights of pregnant patients. With the revocation of this constitutional right, millions of women across the U.S. will be forced to carry pregnancies to term.[286,287] Naturally, increased rates of pregnancy will result in increased incidence of pregnancy-related complications and maternal mortality.[288,289] Further, restricted access to safe abortions will disproportionately affect women of color and low-income individuals, who already experience an increased incidence of pregnancy complications and healthcare disparities.[290–292] Ramifications of this policy change correlate directly with the pregnancy complications discussed in this review. As previously mentioned, the gold standard treatment for molar pregnancy is medical abortion.[237,293] In addition, pregnant people experiencing ectopic pregnancy and miscarriage similarly may require medical abortion procedures.[286,294–296] During instances of these conditions, the fetus is non-viable, and thus, a medical abortion is required to evacuate harmful contents from the uterus of the mother.[236,237,297–299] In states with abortion bans, women have lost access to safe medical abortions, which puts them at greater risk for adverse events and death.[286,296,300] The use of new nanocarrier platforms may be

critical in ensuring safe treatment options for these women moving forward.

Now, more than ever, there is a need to develop safe and effective treatment strategies for pregnancy complications. The nanomedicine platforms described in this review highlight the potential successes of nanocarriers in treating placental disorders. These works may serve as a foundation for future development and clinical translation of novel drug delivery technology for applications in pregnancy. While this review solely focused on nanomedicine to diagnose and treat placental disorders, nanomedicine has broader applications in women's health. For example, nanocarriers could be used to treat other pregnancy complications, such as preterm birth and gestational diabetes, and has applications beyond pregnancy to treat ovarian cancer, breast cancer, and endometriosis. Thus, it is vital that research in these fields is promoted through the creation of grants and funding pathways to advance our mechanistic understanding of these diseases and subsequently develop therapeutic strategies. Together, these efforts will unlock new avenues for advancing diagnostic and therapeutic strategies specifically aimed at treating disorders that disproportionately affect women, ultimately fulfilling the goal of nanomedicine—improving human health.

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Conflict of Interest

The authors declare no conflict of interest.

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