Targeted Nanoparticle Delivery of Doxorubicin Into Placental Tissues to Treat Ectopic Pregnancies

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Abnormal trophoblast growth can cause life-threatening disorders such as ectopic pregnancy, choriocarcinoma, and placenta accreta. EnGeneIC Delivery Vehicles (EDVs) are nanocells that can promote tissue-specific delivery of drugs and may be useful to medically treat such disorders. The objective of this study was to determine whether EDVs loaded with the chemotherapeutic doxorubicin and targeting the epidermal growth factor receptor (EGFR, very highly expressed on the placental surface) can regress placental cells in vitro, ex vivo, and in vivo. In female SCID mice, EGFR-targeted EDVs induced greater inhibition of JEG-3 (choriocarcinoma cells) tumor xenografts, compared with EDVs targeting an irrelevant antigen (nontargeted EDVs) or naked doxorubicin. EGFR-targeted EDVs were more readily taken up by human placental explants ex vivo and induced increased apoptosis (M30 antibody) compared with nontargeted EDVs. In vitro, EGFR-targeted EDVs administered to JEG-3 cells resulted in a dose-dependent inhibition of cell viability, proliferation, and increased apoptosis, a finding confirmed by continuous monitoring by xCELLigence. In conclusion, EGFR-targeted EDVs loaded with doxorubicin significantly inhibited trophoblastic tumor cell growth in vivo and in vitro and induced significant cell death ex vivo, potentially mediated by increasing apoptosis and decreasing proliferation. EDVs may be a novel nanoparticle treatment for ectopic pregnancy and other disorders of trophoblast growth. (Endocrinology 154: 911–919, 2013)

Disorders of abnormal trophoblast growth collectively represent some of the most life-threatening reproductive conditions (1–3). Ectopic pregnancies occur when the conceptus implants outside the uterus and are common, accounting for 1–2% of all pregnancies (1, 2). They can rupture, resulting in fatal intra-abdominal bleeding. Molar pregnancies and choriocarcinoma are placental tumors that can erode local tissues. Placenta accreta is an uncommon but dangerous obstetric complication in which the placenta is pathologically adherent to the myometrium.

Treatment of these disorders of trophoblast growth often requires surgery. Medical management with methotrexate is an alternative treatment option in some situations, given that the trophoblast is sensitive to this antifolate chemotherapeutic agent. However, the efficacy of methotrexate to treat these gynecological conditions is limited. For instance, methotrexate is only efficacious in resolving ectopic pregnancies that are small (4), and malignant choriocarcinoma often cannot be cured with this drug alone but requires a multiagent chemotherapeutic regimen (4). Conceivably, an efficacious treatment that is able to potently regress placental tissue could be used clinically for these conditions, decreasing the need for surgery (or multidose chemotherapy) and their attendant risks.

EnGeneIC Delivery Vehicles (EDVs) are bacterially derived nanospheres of 400-nm diameter. They can be pro-

Abbreviations: EDV, EnGeneIC Delivery Vehicle; EGFR, epidermal growth factor receptor.
pressed in the target tissue of interest (and the other targets a cell-surface antigen highly recognizes the O-antigen component of the EDV surface are coated with bispecific antibodies in which one arm tissue-specific targeting mechanism, the surface of EDVs and internalization of the packaged drug. To achieve the previously reported, despite repeat dosing (5).

Importantly, toxicity studies in mice indicate that EDVs are well tolerated, with no adverse side effects or deaths by 24 hours after administration compared with only 1/1000 to 1/8000th the amount of drug (5). EDVs were also able to initiate potent tumor inhibition and regression in case studies of lymphoma in dogs (5). Importantly, they cause little or no inflammatory response in vivo, the likely reason being the antigenic bacterial cell wall is coated with the bispecific antibodies.

The EGFR is a potent cell survival molecule. In silico analysis of EGFR expression suggests more than 3000-fold higher than average expression in placenta compared with other human tissues (Biogps.org). Other sites of modest EGFR expression in humans include the liver and lungs, which is reflected by previous findings of off-target localization after administration of EGFR-EDVs (5). This suggests that EGFR may be an appropriate placental specific epitope at which EDVs could be targeted.

We hypothesized that EDVs, loaded with a chemotherapeutic agent and targeted against EGFR, may be a novel approach to medically treat disorders of abnormal trophoblast growth. Such EDVs may facilitate tissue-specific delivery of drugs, potentially inducing potent disease regression with minimal drug toxicity. The aim of this preclinical study was to assess whether EDVs packaged with doxorubicin targeted against EGFR can induce placental cell inhibition in vivo, to investigate placental uptake and apoptosis ex vivo, and to further explore mechanisms in vitro.

Materials and Methods

EGFR and M30 cyto death immunohistochemistry

For EGFR immunohistochemistry, tissues used were paraffin sections (4 μm) of formalin-fixed tissue from ectopic pregnancy sites, term placental and placental explants, and JEG-3 choriocarcinoma cells grown on coverslips. M30 immunohistochemistry was conducted on PFA-fixed placental explant tissue collected after EDV treatment ex vivo. Antigen retrieval was carried out by either pressure-cooking for 10 minutes in TRIS EDTA or microwaving for 20 min in citrate buffer before endogenous peroxidases were blocked by treatment with 3% H2O2 for 10 min. Nonspecific binding sites were blocked before primary antibody was added for 1 hour at RT. Primary antibodies used were monoclonal mouse anti-EGFR (Clone EGFR.25: Novocastra, Leica Microsystems, Milton Keynes, United Kingdom) for ectopic sites, mouse anti-EGFR (Clone EGFR.13, Novocastra) for placental explants or JEG-3 choriocarcinoma cells, or anti-human Xenograft models, targeted EDVs were able to induce equivalent tumor regression as systemic (or naked) chemotherapeutics with only 1/1000 to 1/8000th the amount of drug (5).

Figure 1. Schematic depiction of an EDV. A, The EDVs are 400-nM sterile nanospheres coated with bispecific antibodies to promote a tissue-specific targeting mechanism. Inward facing antibody (purple) recognizes the O-antigen on lipopolysaccharide present on the EDV surface LPS, and the outward facing antibody (blue) recognizes a receptor specific for the cell to be targeted (EGFR for our study). B, Further schematic of an EDV, demonstrating the interior packaged with the chemotherapeutic doxorubicin.
M30 cyto-death antibody (Roche Diagnostics GmbH, Sandhoferstrasse, Mannheim, Germany) in 1% bovine serum albumin/phosphate-buffered saline. Control sections were stained with isotype matched controls. Ectopic pregnancy tissue was then washed, ImmPRESS Universal Antibody (antimouse IgG peroxidase) Polymer Detection Kit (Vector Laboratories, Peterborough, United Kingdom) followed by TSA Plus Cy3 (PerkinElmer, Seer Green, United Kingdom) applied before being counterstained with 1 μM SYTOX Green (Invitrogen, Paisley, United Kingdom) in TBS-T20. For JEG-3 cells, Alexafluor 488 (Life Technologies, Mulgrave, Victoria, Australia) was applied before sections were counterstained with DAPI. For placental tissue, positive staining was revealed with the use of the Invitrogen SuperPicTURe kit (Life Technologies).

Administration of EDVs to mice with JEG-3 xenograft

Female NOD/SCID mice 6–10 weeks of age were housed in standard conditions with food and water provided ad libitum and a constant light cycle of 12 hours (lights on from 08:00–20:00 hours). JEG-3 cells (106) were injected subcutaneously into the right flank. Mice were randomly allocated into one of four treatment groups: EDVs targeted to EGFR containing doxorubicin (EGFREDVDOX; 1 × 109 = 100 ng doxorubicin), EDVs targeted to GP120 (irrelevant antibody control) containing doxorubicin (GP120EDVDOX; 1 × 109 = 100 ng doxorubicin), naked doxorubicin at 100 ng/mg of doxorubicin, or saline alone. Treatments were administered intravenously from day 2 after xenograft and repeated every second day, at which time tumor volumes were measured and recorded up until 18 days after xenograft. Note, as per animal ethics requirements, animals were humanely killed once tumors reached 1000 mm3. Ethics approval for this project was granted by the Austin Animal Ethics Committee.

Treatment of placental explants with EDVs

Term placental explants were obtained from patients undergoing elective caesarean section. Cubed explants (1 mm3) were dissected from placentas and allowed to equilibrate in DMEM (Life Technologies) for 1 hour at 37°C in 10% CO2 before treatment. Three placental explants were included for treatment per placenta, with n = 5 placentas used in total. We obtained ethics approval (Mercy Hospital for Women HREC) before we undertook this study, and all patients provided written informed consent. Treatments were 1 × 109 EGFREDVDOX or GP120EDVDOX, 10 μg of doxorubicin, or saline control. Explants were incubated for 24 hours, shaking at 150 rpm at 37°C in 10% CO2 for 24 hours before explants were fixed in PFA for immunohistochemistry.

Treatment of JEG-3 cells with EDVs

JEG-3 cells were grown in Dulbecco’s Modified Eagle Medium: Nutrient Mixture F12 (DMEM/F12; Life Technologies) containing 10% fetal calf serum (FCS-Sigma, St Louis, Missouri). Cells were serum-starved to 0% FCS for 2 hours before treatments were added for 2 hours. For all in vitro studies, cells were treated with either one of four doses of EDVs targeted to EGFR containing doxorubicin (EGFREDVDOX; 2 × 108, 1 × 108, 0.5 × 108, 0.25 × 108) or one of three doses of EDVs targeted to GP120 (irrelevant antibody control) containing doxorubicin (GP120EDVDOX, 1 × 109). Control cells were treated with vehicle alone (saline). After 2 hours of treatment, cells were washed three times with sterile phosphate-buffered saline before media (containing 10% FCS) was replaced. Thereafter, cells were allowed to grow for 72 hours at 37°C in 10% CO2 before endpoint assays were carried out. A minimum of three replicates were included in each experiment, with experiments repeated three times.

Assessment of intracellular doxorubicin

Doxorubicin autofluoresces red and thus can be identified through the use of fluorescent microscopy. Xenograft or explant sections (5 μm) were wet-mounted with fluorescent mounting medium containing DAPI (Vector Laboratories, Burlingame, California) to label cell nuclei. Imaging of sections was carried out with the use of a Leica DMR upright fluorescence microscope with images acquired with the use of a ×40 objective and a color camera. Fluorescence filters for DAPI (ex560/20, em425LP), or doxorubicin (ex560/40, em645/75) were used. Images were acquired with the use of a ×1.2NA objective.

MTS assay

To assess cellular viability MTS assays (Promega, Madison, Wisconsin) were used. MTS reagent was diluted 1:6 into DMEM/F12 containing 10% FCS and added to cellular monolayers at the cessation of treatment. At 2 hours, 100 μL of conditioned media was removed and optical density was determined with the use of a BioRad X-Mark microplate spectrophotometer (BioRad, Hercules, California). Cellular viability is proportional to the optical density at 490 nm. Data are expressed as mean ± standard deviation.

Flow cytometry to assess proliferation, necrosis, and apoptosis of JEG-3 cells

To assess proliferation, cells were labeled with CFSE (carboxyfluorescein diacetate, succinimidyl ester; Life Technologies, Victoria, Australia) before being treated with EDVs as detailed above. At each analysis time point, cells were labeled with APC-annexin V (BD Pharmingen, Franklin Lakes, New Jersey) and Sytox Blue (Life Technologies) for assessment of apoptosis and cell viability. Flow cytometry was performed with the use of a FACSCanto II flow cytometer (BD Pharmingen), with the use of the following excitation lasers band pass detection filters: CFSE, 488 nm 530/30, APC-annexin V, 635 nm 660/20, Sytox Blue, 405 nm 470/40. Compensation controls consisted of 1) unstained cells, 2) CFSE-labeled cells, 3) APC-annexin V-labeled cells, 4) Sytox Blue-labeled cells, and 5) cells labeled with all markers. Flow cytometry data were analyzed with the use of FlowJo software (v7.6.4, Tree Star, Inc., Ashland, Oregon) as follows. Plots were displayed with side scatter vs. forward scatter and gated to remove debris. Plots were then displayed with Sytox Blue vs. APC-annexin V and separated into quadrants. From this plot, the percentage of live (APC-annexin Vlow / Sytox Bluelow), percentage of apoptotic (APC-annexin Vhigh / Sytox Bluelow), and percentage of dead (Sytox Bluehigh) cells were determined. To calculate the level of proliferation, the live cell gate was displayed with cell number vs. CFSE intensity, and the CFSE median value was determined.

xCELLigence

The Real-time Cell Analyzer MP instrument (Roche Diagnostics GmbH) was placed in a humidified incubator maintained...
at 37ºC with 5% CO₂. Cells were plated at either 2500 or 5000 cells/well into 96-well E Plates (Roche Diagnostics, GmbH) for 24 hours before treatment with EDVs as described above. Continuous impedance measurements were taken every 1 hour for 144 hours from the time of cell plating.

Results

EGFR is highly expressed in trophoblast from ectopic pregnancies, term placentas, and choriocarcinoma cells

Given that we propose the EGFR as the appropriate antigen to promote placental targeting of EDVs, we first set out to confirm that placental tissue from disorders of trophoblast growth has high surface EGFR expression. Immunohistochemistry revealed strong EGFR staining in the syncytiotrophoblast layer in placenta from ectopic pregnancy implantation sites (Figure 2A), term placenta (represents likely EGFR expression in third-trimester placenta accreta; Figure 2B), and JEG-3 cells, a choriocarcinoma-derived cell line (Figure 2D). Of relevance to our ex vivo studies that we describe later, strong EGFR expression was maintained in placental explants cultured for 24 hours (Figure 2C). The data suggest that placental tissue from disorders of trophoblast growth exhibit high surface EGFR expression, making them potentially amenable to EGFR-targeted EDV therapy.

EGFR-targeted EDVs decrease JEG3 tumor volume xenografts in vivo

To determine whether EDVs may be effective in targeting placental tissue in vivo, we administered EDVs targeting EGFR and loaded with doxorubicin intravenously to mice bearing subcutaneous JEG-3 tumors (Figure 2E). (Note, from here on, will use the following shorthand to describe EDVs: EGFREDVDOX, in which the superscript denotes the antigen at which the targeting antibody is directed against, the subscript denoting the drug packaged within the EDVs).

We compared the ability of EGFREDVDOX administered intravenously to inhibit subcutaneous tumor growth versus three controls: 1) normal saline, 2) systemic doxorubicin administered at /H11003 100 the total amount contained in the EDVs, and 3) GP120EDVDOX, which are EDVs loaded with doxorubicin but coated with bispecific targeting antibodies directed against an irrelevant antigen (GP120).

From day 12 onward after xenograft, tumor volumes of mice administered EGFREDVDOX remained significantly (P < .05) smaller than those administered GP120EDVDOX, saline, or naked doxorubicin (we culled mice in the saline and naked doxorubicin treatment groups at day 16 because tumor volumes for many of the mice exceeded the size permitted by our animal ethics committee of 1000 mm³). At day 18 after xenograft, the tumors of mice treated with EGFREDVDOX remained significantly (P < .05) smaller than those in mice treated with GP120EDVDOX. These findings demonstrate that EGFR-targeted EDVs loaded with doxorubicin can inhibit placental cell tumor growth in an in vivo model.

EGFREDVDOX are internalized into ex vivo human placental explants

We next assessed whether EGFREDVDOX were spontaneously internalized into ex vivo human placental ex-
plants compared with \( \text{GP120EDVDOX} \). Samples were obtained from term caesarean deliveries, and we were able to visualize the likely location of EDVs because the packaged drug doxorubicin fluoresces red.

By 7 hours after treatment, doxorubicin was present within the syncytiotrophoblast layer of explants treated with EGFREDVDOX (Figure 3, A and B). In contrast, there was no doxorubicin present in explants treated with \( \text{GP120EDVDOX} \) (Figure 3, C and D) or saline (Figure 3, E and F). At 24 hours after treatment, doxorubicin remained detectable in EGFREDVDOX-treated placental explants (Figure 3, G and H). Whereas some doxorubicin was detectable in \( \text{GP120EDVDOX} \)-treated explants at 24 hours (Figure 3, I and J), it was present to a lesser extent than in EGFREDVDOX-treated explants. At 24 hours, there was still no doxorubicin detected in placental explants treated with media alone (Figure 3, K and L) or naked doxorubicin (Figure 3, M and N).

**EGFREDVDOX induce cell death in human placental explants ex vivo**

To assess cell death within the EDV-treated explant, we carried out immunohistochemistry with the use of the M30 antibody. This detects caspase-cleaved cytokeratin products and is considered a marker of late apoptosis. EGFREDVDOX treatment for 24 h induced significant cell death, with strong M30 staining in the syncytiotrophoblast within a majority of villous tips examined (Figure 4, A and B). Whereas M30 staining was also present in the syncytiotrophoblast of placental explants treated with \( \text{GP120EDVDOX} \) (Figure 4, C and D), naked doxorubicin (Figure 4, E and F), and saline (Figure 4, G and H), the staining was much sparser than that observed in the EGFREDVDOX-treated explants. Potentially, the M30 staining observed in the normal saline group reflects a basal level of cell death known to occur within the syncytiotrophoblast as it sloughs off and regularly turns over (7). The data suggest that EGFREDVDOX can induce greater apoptosis within placental cells ex vivo compared with nontargeted EDVs (\( \text{GP120EDVDOX} \)), naked doxorubicin, or saline treatment.

**EGFREDVDOX and GP120EDVDOX decrease JEG-3 viability and proliferation and increase apoptosis in vitro**

To further assess potential mechanisms, we performed end-point assays with the use of JEG-3 cells in vitro. MTS assays revealed significant (\( P < .05 \)) dose-dependent decreases in cell viability after treatment with EGFREDVDOX. We also observed a similar dose-dependent decrease in cell viability with nontargeting EDVs (\( \text{GP120EDVDOX} \); Figure 5A). Similarly, a significant (\( P < .05 \)) dose-dependent decrease in the percentage of live cells on FACS was observed after either EGFREDVDOX or \( \text{GP120EDVDOX} \) treatment (Figure 5B).

We then performed studies on cell proliferation by FACS. Before adding our treatments, we stained the JEG-3
cells with CFSE. CFSE stains the cell membrane, meaning the degree of proliferation will be inversely proportional to CFSE retention. We observed a significant ($P < .05$) dose-dependent decrease in cell proliferation after EGFREDVDOX or GP120EDVDOX treatment (Figure 5C). Finally, we examined apoptosis by FACS, determining the percentage of cells that stained positive for annexin V. Again, we observed increased ($P < .05$) apoptosis in a dose-dependent manner after both EGFREDVDOX or GP120EDVDOX treatment (Figure 5D) compared with control.

These assays indicate that EDVs cause decreased cell viability and proliferation and they increase apoptosis when administered to JEG-3 cells in vitro. We note that in this set of experiments in JEG3 cells, similar findings were observed with both targeting and nontargeting EDVs. The probable explanation is that there is likely to be passive uptake of nontargeting EDVs when plated onto JEG-3 cells in vitro. Of note, we have already established that the targeting mechanism is likely to be functional when applied to the in vivo and ex vivo (placental explant) settings. However, we believe that these results from JEG-3 cells still provide insights into potential mechanisms.

EDVs induce decreased JEG3 proliferation and cell death by continuous monitoring

To further investigate the effects of EDVs on cell inhibition, we treated JEG-3 cells with EDVs and used the xCELLigence system to...
continuously monitor cell growth/inhibition for 96 hours. The xCELLigence measures electrical impedance across wells in which the presence of more cells increases impedance (measured as the “cell index”).

When EGFREDVDOX were administered to JEG-3 cells at 2500 cells/well (Figure 6A), all groups initially continued to proliferate for 48 hours after treatment. A divergence in cell index of all groups from 48 hours was apparent that was dose dependent. When EGFREDVDOX were added to 5000 cells/well (Figure 6B), all groups continued to proliferate for ~36 hours. From 36 hours, only JEG-3 cells that were left untreated or administered the lowest EGFREDVDOX dose (0.25 \( \times 10^9 \)) continued to proliferate. All remaining groups treated with EGFREDVDOX had significant declines in the cell index that was dose dependent. Such growth trajectories in the xCELLigence system are consistent with cell death, not cell inhibition only. Again, we noted the same dose-dependent decrease in cell growth when GP120EDVDOX were added to JEG-3 cells (data not shown).

Therefore, our results are consistent with our findings in the endpoint assays, showing that EDVs loaded with doxorubicin can promote significant JEG-3 cell inhibition/death. Furthermore, we have observed an interesting paradox in which EDVs appeared to be more effective in inducing cell inhibition/death in the presence of more JEG-3 cells.

Discussion

Disorders of trophoblast overgrowth, such as ectopic pregnancy, placental accreta, and molar pregnancies/choriocarcinoma, are the most life-threatening conditions in modern gynecology (1–3). In this report, we describe a nanoparticle drug delivery system that can promote tro-

![Figure 6](https://academic.oup.com/endo/article-abstract/154/2/911/2423708)
phoblast-specific delivery of a chemotherapeutic agent and may be a novel therapeutic approach for these disorders.

In the present study, we demonstrate for the first time the capacity of EGFREDVDOX to specifically target trophoblast tissue, where they can induce apoptosis. We have shown through the use of an in vivo xenograft model that administration of EGF-targeting EDVs yields a significant decrease in placental cell tumor volume. Compared with GP120EDVDOX or naked doxorubicin (given in the same amount contained in the EDVs), EGFREDVDOX administered to placental explants ex vivo were more readily taken up and induced greater apoptosis. Moreover, we have obtained evidence in in vitro assays that the likely mechanism of EDV action on trophoblast tissue may be a combination of decreased proliferation and increased cell death/apoptosis. We noted a lack of uptake of naked doxorubicin by placental explants but significant uptake when an equivalent amount was delivered via EDVs. The likely explanation is that a three-dimensional architecture is endocytosis occurring when EDVs are added to homogenous cells in a monolayer in vitro, and we would speculate, this interpretation is correct, the experiment highlights the potency of the EDV targeting system. Together, these findings demonstrate the utility of targeted EDVs as a novel nanoparticle vehicle that can deliver drug specifically to trophoblast.

Compared with nontargeted EDVs, EGFREDVDOX promoted greater inhibition of JEG-3 tumor cells in vivo and were more avidly taken up by placental explants and induced greater apoptosis ex vivo. These studies provide evidence that EGFREDVDOX promote placental-specific delivery of drug compared with nontargeted EDVs. However, we noted that when administered to JEG-3 cells in vitro, nontargeted EDVs elicited a similar response to the EGF-targeted EDVs. This probably is due to nonspecific endocytosis occurring when EDVs are added to homogenous cells in a monolayer in vitro, and we would speculate the explanation is that a three-dimensional architecture is required for successful targeting. Nevertheless, we believe that our in vitro studies provide important information to indicate the mechanisms via which doxorubicin exerts its actions in vivo to decrease placental cell volume.

The concept for developing targeted drug delivery systems is not new (8–10). However, there have been significant barriers to their development, including drug leakage in vivo, difficulties in production scale-up, and lack of versatility with regard to what drugs can be packaged (11). EDVs represent a new class of nanotechnology capable of stably delivering drugs to target tissues in vivo. We have previously shown that they can stably hold packaged drug, they can be produced in large volumes, and that a range of drugs of differing electrochemical properties can be successfully packaged (including shRNAs [12]). Furthermore, they appear to provoke little or no immune response and may be potentially administered in high concentrations with minimal toxicity (5).

Thus far, the literature examining the nanoparticles in pregnancy has been sparse. The work that has been done appears to have focused more on the potential risks to the fetus, should nanoparticles be administered to women to treat medical conditions who are coincidentally pregnant (13–17). Recently, Yamashita et al. (17) concluded that 70-nm silica and 35-nm titanium dioxide nanoparticles administered to pregnant mice caused fetal growth restriction and accumulated in fetal brain, raising the possibility of neurotoxicity. Thus, as far as we are aware, ours is the first report to propose the use of nanotechnology to specifically treat pregnancy complications.

Unlike inorganic silica or titanium, EDVs are organic particles in which excess EDVs that have not entered the target tissue can be degraded in the liver or immune system. This is likely to decrease the risk of permanent accumulation in vital organs, notably the fetal brain. Furthermore, given that Yamashita et al found nanoparticles of 300-nm diameter or larger did not appear to readily penetrate the placental barrier, 400-nm EDVs may also be less likely to reach the fetal circulation. Of further support of this contention, we found that EDVs are likely to be degraded in the syncytiotrophoblast, the outermost layer of the placenta abutting the maternal compartment. Therefore, it is possible that targeted EDVs might be able to effect placental specific delivery of drugs or siRNAs to the placenta but not reach fetus (or at least less drug will reach the fetal compartment compared with the placenta). If true, they may be useful to deliver drugs to treat other major complications of pregnancy.

Clinically, EDVs appear to be good candidates for the medical treatment of ectopic pregnancies and trophoblast disorders. Our previous data (5, 12) indicate that high blood flow facilitates entry of EDVs into target tissue. High blood flow is a feature of the maternal-placental interface. Moreover, it is feasible that patients could attend the clinic to receive intermittent injections of EDVs intravenously until the serum biomarker (hCG) drops to the nonpregnant range to indicate resolution. Although expense is a consideration when introducing nanoparticle therapy, we anticipate that large-scale production of EDVs could be relatively inexpensive, given that they are derived from bacteria. It is therefore possible that medical therapy with the use of EDVs may be less expensive than that required for surgical excision.

In conclusion, we have shown EDVs targeting the EGF receptor packaged with doxorubicin are able to
promote trophoblast-specific delivery of drug. EDVs may be a novel nanoparticle that can deliver high amounts of drug specifically to placental tissues and could be a novel approach to treat major disorders of trophoblast growth. Of note, a phase I clinical trial examining the toxicity of EDVs in (nonpregnant) humans is in progress (ACTRN12609000672257). Depending on those results, it may be possible that such a drug could be translated to treat women with ectopic pregnancy, placenta accreta, or choriocarcinoma.

Acknowledgments

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