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# The PD-1/PD-L1 Checkpoint, Patterns in Preeclampsia Pathogenesis

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## Abstract

Programmed cell death protein PD-1 and its ligand PD-L1 perform an inhibitory checkpoint function in the adaptive immune response. Their function as a gatekeeper between tolerance and resistance makes them appropriate drug targets for a class of cancer drugs known as immune checkpoint blockers (ICB). (Borgers et al., 2021) (Sun et al.,2023) The balance between tolerance and resistance is particularly relevant in pregnancy. The maternal immune system needs to tolerate the foetal allograft while simultaneously providing resistance to pathogens and neoplasms. The PD1/PD-L1 axis is considered to be an essential mechanism in the maintenance of this maternal-foetal tolerance. (Zhang, Tian, Tang, Liu, & Liao, 2015) During normal pregnancy PD1 is increased in the uteroplacental interphase. (Teodora Maria Toadere et al., 2024) This is thought to drive the formation of Tregs specific to paternal antigens on the foetal allograft resulting in increased tolerance. (Alijotas-Reig, Llurba, and Gris, 2014) Preeclampsia is a pregnancy complication involving an overactive immune response to the foetal allograft. The pathogenesis of preeclampsia is to date not fully understood. (Mittelberger et al., 2022) Recent studies have found alterations in PD-1 and PD-L1 signalling in pregnant patients with preeclampsia, pointing to a possible role of this mechanism in the development of the disease. In this review, we will discuss these new findings in the context of the PD1/PD-L1 mechanism in healthy pregnancy and the maternal-foetal tolerance.


## Introduction

### The PD-1/PD-L1 checkpoint

The PD-1/PD-L1 axis is best characterized for its role in peripheral tolerance induction. In the normal immune response this inhibitory pathway counteracts the costimulatory signals required for T cell activation. Figure 1 depicts the signaling cascades in the activation of a T helper(Th) cell by an Antigen Presenting Cell (APC). (Wang et al., 2021)

Activation of a naïve T cell requires recognition of an antigen presented on an MHC-II molecule. The T cell receptor (TCR) has a variable alpha and beta domain that specifically recognizes this conjugate antigen. The TCR coreceptor CD3 contains Immunoreceptor Tyrosine based Activation Motifs (ITAMs) at its cytosolic tails. Upon antigen recognition, the tyrosine residues in these ITAM motifs become phosphorylated by Lymphocyte-specific protein tyrosine Kinase (LCK). Coreceptor CD4 facilitates this process by bringing LCK physically closer to the ITAM motifs.

Phosphorylated ITAM motifs provide docking sites for downstream signaling proteins. Such as the protein kinase Zap-70 which is activated by LCK and further propagates the stimulatory signaling cascade. (Bhattacharyya et al 2020) (Hwang et al 2020) In addition to TCR signaling (signal 1) a second costimulatory signal (signal 2) is required. CD28 has two ligands, CD80 and CD86. Engagement between CD28 and either of its ligands promotes phosphorylation by PI3K. Phosphorylation of the YMNM motif of the CD28 cytoplasmic tail creates a PI3K binding site. PI3K generates PIP3 which acts a 2$^{nd}$ messenger in T cell activation as well as cell proliferation and survival. (Sharpe., 2017)

The membrane protein PD-1 is expressed on activated Th cells. Its cytosolic tail contains an Immunoreceptor Tyrosine based Inhibition Motif (ITIM) and Immunoreceptor Tyrosine based Switch Motif (ITSM). Upon interaction between PD-1 and either PD-L1 or PD-L2 the ITIM and ITSM are phosphorylated. This provides a docking site for SH2 domain containing signaling molecules. SH2 domain-containing inositol phosphatase SHIP1,

counteracts PI3K function. Thereby it inhibits the stimulatory cascade that PI3K sets in motion. SH2 domain-containing phosphoserine phosphatase SHP2 counteracts LCK function, which deactivates the ITAMs and Zap70. In summary PD-1/PD-L1 engagement on and activated T cell, leads to the recruitment of cytosolic inositol lipid and protein tyrosine phosphatases that counteract signal 1 and 2, resulting in reduced T cell activation and proliferation and alterations in cytokine production and cell metabolism. (Crute et al 2020) (Wang et al., 2021) (Ghosh et al.,2021) (Sharpe et al., 2017)

PD-1 is expressed on many other cell types besides activated T cells, including B cells natural killer cells, monocytes, dendritic cells and myeloid progenitor cells. PD-L1 is expressed on APCs, T cells and B cells and can be induced by pro-inflammatory signals cytokines. (Sharpe., 2017) This makes it possible for PD-L1 expressed on T cells to react with PD-1 on other T cells or macrophages (reverse signaling mode), or with PD-1 on the same cell (cis interaction). Ligand PD-L1 can also bind to CD-80, and this might interfere with CD28/CD80 binding and thus disrupt signal 2 in T cell activation. (Sharpe., 2017) (Wang et al., 2021) PD-L2 interactions with repulsive guidance molecule b (RGMb) have been linked to respiratory tolerance. (Xiao et al., 2014) In addition to membrane bound mPD-1 and mPD-L1 mentioned above soluble sPD-L1 and sPD-1 are characterized. Alterations in sPD-1 and sPD-L1 expression are associated with (auto)immune disease and inflammatory conditions as in cancer and pregnancy. (Bailley., 2021)

In the acute inflammatory response, the PD-1/PD-L1 checkpoint provides protection from tissue damage as well as helps to return to homeostasis after the treat has cleared. Increased antigen exposure will induce PD-1 expression resulting in peripheral tolerance. (Sharpe., 2017) Several cancers have coopted the checkpoint to avoid the immune system. Immune checkpoint blockers (ICB) are antibodies that are used in clinic to block Immune checkpoint molecules such as PD-1 and PD-L1. This allows the immune system to overcome cancer induced tolerance. However, diminished PD-1/PD-L1 signaling is associated with (auto)immune disease. Controlled regulation of this checkpoint is thus essential to avoid pathology. (Bailley et al., 2021)

**PD-1/PD-L1 in the maternal-foetal tolerance**

Pregnancy provides a significant challenge for the maternal immune system. It involves continued exposure to foreign antigens on the semi-allogeneic foetus. Tolerance to these foreign peptides is essential to prevent serious tissue damage. There are several mechanisms involved in the continued tolerance throughout pregnancy. Distinct subtypes of Th cells affect and respond to their environment through cytokine signalling. Throughout pregnancy Th1/Th2/Th17 T cell subset proportions change. In the initial phase of pregnancy Th1 is dominant, whereas this shifts to Th2 for most of the second and third trimester. In addition, the amount of regulatory T cells increases. Increased progesterone further drives Th2 differentiation and increased PD-1 activity. (Teodora Maria Toadere et al., 2024)

PD-1/PD-L1 induced tolerance is believed to be an essential mechanism in maintaining the maternal-foetal tolerance. (Teodora Maria Toadere et al., 2024) (Borgers et al., 2021) Inhibiting or blocking of the PD1 or PD-L1 receptors with ICB is associated with foetal reabsorption, increased risk of growth restriction, premature delivery and foetal and neonatal death in animal models. (Borgers et al., 2021) Data from ICB clinical trials in humans is very limited. Borgers et all reviewed 7 case studies of (combination) ICB treatment in pregnant women with melanoma (n=6) and Placental site trophoblastic tumour (n=1). Of those cases, 5 women conceived

while they were being treated. Given the unfavourable results in animal studies, treatment was halted upon discovery of the pregnancy in the first trimester in 4 of those pregnancies. This leaves us with limited data on the continued use of ICB throughout pregnancy. In one of the 1st trimester exposure pregnancies the child was born with hyperthyroidism. However, this condition was spontaneously resolved at 6 months of age. Other side effects included intra-uterine growth restriction (n=2) and placental insufficiency (n=1). Interestingly, at their last follow up all 7 pregnancies resulted in healthy children. Suggesting that 1st trimester exposure (n=4) as well as 2nd trimester exposure (n=1) and exposure throughout pregnancy (n=2) did not result in lasting immunological effects in the offspring. In leu of randomized control trials on the use of checkpoint inhibitors in pregnant patients remains controversial. (Borgers et al., 2021) (Menzer et al., 2018) (Mehta et al., 2018) (Burroto et al., 2018) (Xu et al., 2019)(Bucheit et al., 2020) (Haiduk and Zimmer, 2021) (Polnaszek et al., 2021)

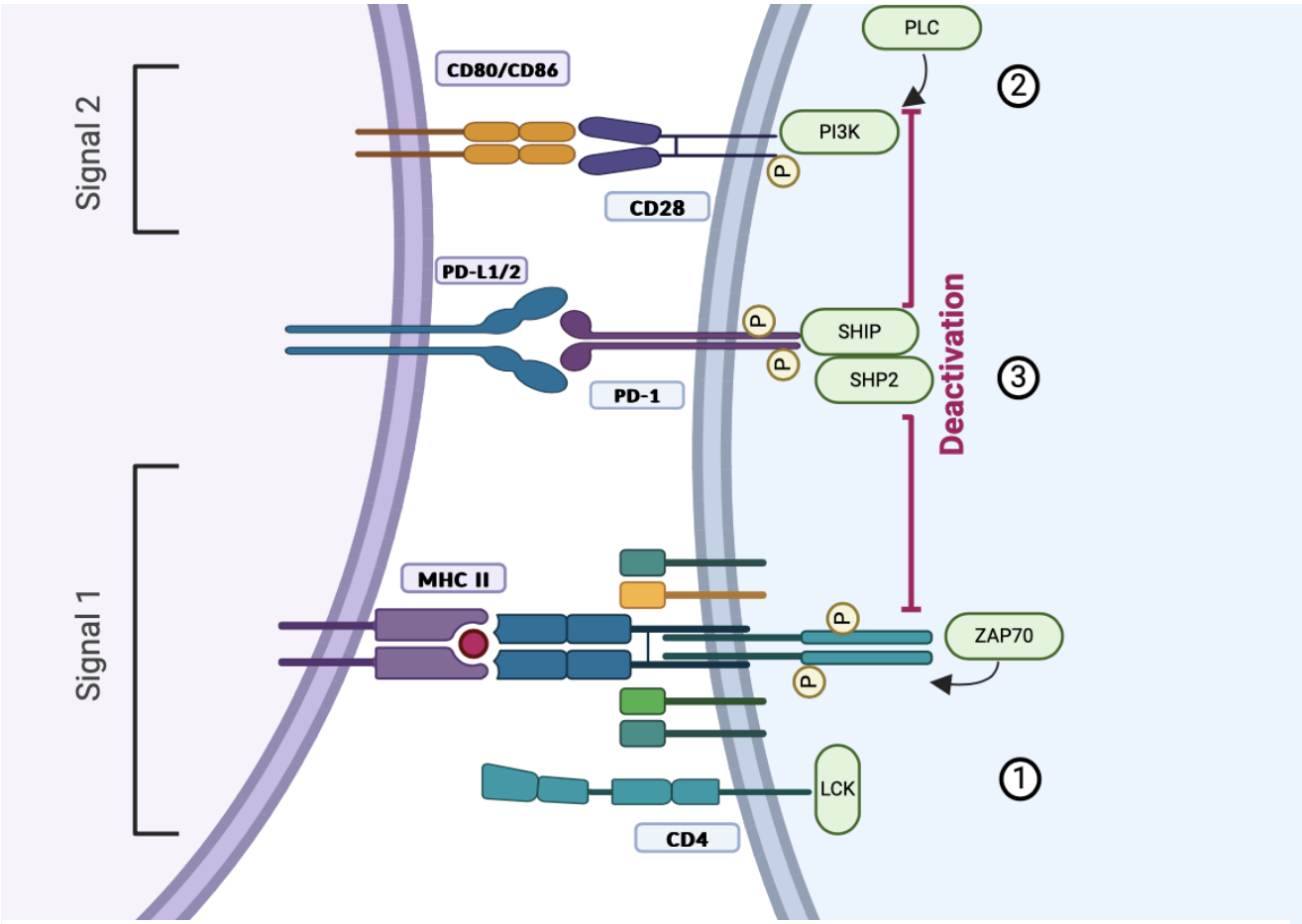


**Figure 1** The PD-1/PD-L1 interaction between an APC (purple) and T helper cell (blue) **(1)** Interactions between the T cell Receptor (TCR) and antigen presented on MHC-II (signal 1) leads to the recruitment of co-stimulatory receptor CD4 and kinase LCK. LCK phosphorylates tyrosine residues in the ITAM on the cytosolic TCR complex subunits. ZAP70 is recruited to p-ITAM and once activated by LCK further propagates the signal. **(2)** Engagement of CD28 by CD80 or CD86 leads to phosphorylation of the YMNM motif on its cytosolic tail. This results in recruitment of PI3 kinase which activates downstream signaling components. (signal 2) **(3)** Interaction between PD-1 and PD-L1/2 results in phosphorylation of the ITAM and ITSM motifs. This recruits SH2 domain bind phosphatases SHP2 and SHIP1. SHP2 counteracts tyrosine phosphorylation. SHIP1 counteracts inositol lipid phosphorylation. This image was made with Biorender.

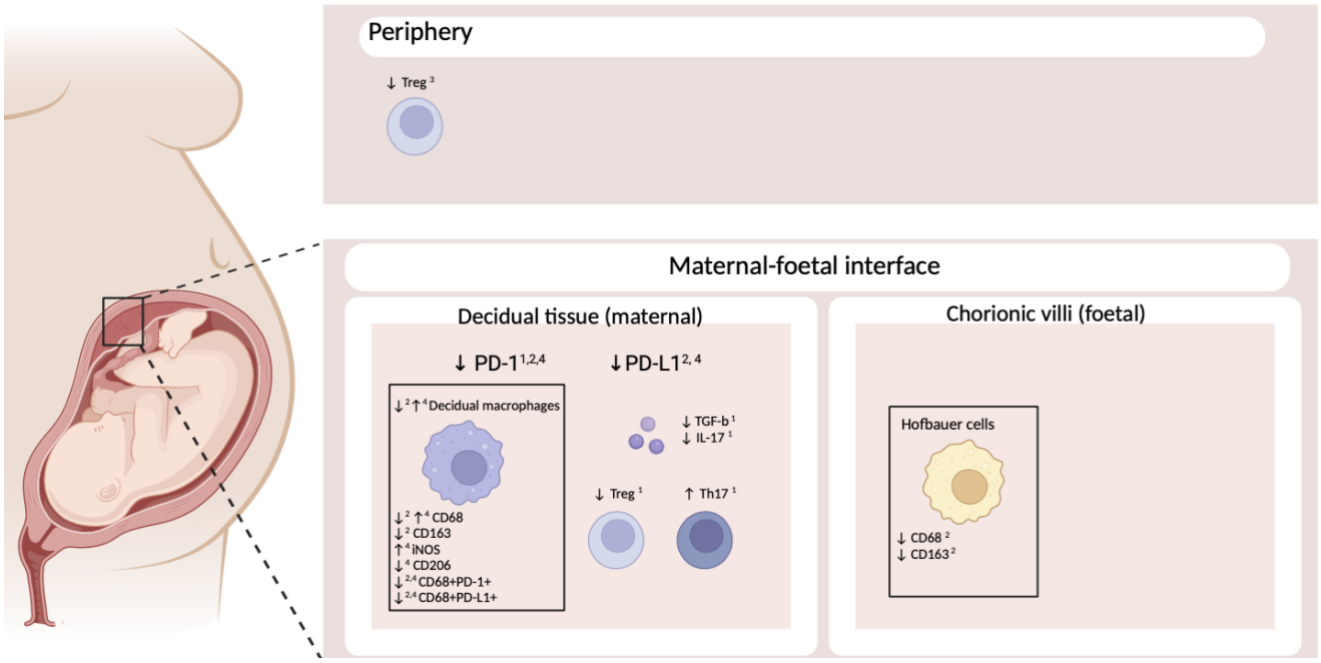


**Figure 2** Summary of alterations in PD-1/ PD-L1 signaling at the maternal-foetal interface in PE pregnancies compared to healthy pregnancies. In addition alterations in T cell proportions polarization markers and cytokine expression are indicated. The symbol↑ means upregulation ↓ means downregulation. (1) Madidi et a., 2022 (2) Mittelberger et a., 2023 (3) Chen et al., 2024 (4) Du et al., 2024 This image was made with Biorender.

**Multiple pregnancies and paternal factors**

Women who previously developed preeclampsia (PE) have a higher occurrence in subsequent pregnancies, however the symptoms appear to be less severe. (Mittelberger et al., 2023)
A 2019 review by Hernandez et al points at several parental factors influencing PE onset. In subsequent pregnancies with new partners, there is a higher incidence of PE compared to consecutive pregnancies with the same partner. Multiple pregnancies with the same partner might as a result of prolonged exposure to parental antigens upregulate PD-1 on Th cells, thus compensating for the lower PD-1 expression associated with this disease. High intervals between pregnancies as well as lower exposure to parental antigens and donor-conceived pregnancies have been associated with increased risk too. Hernandez at all argues that reduced sexual contact might give the maternal immune system less opportunity to create tolerance to paternal antigens. In addition, seminal fluid is known to contain high amounts of TGF-β, which is involved in Treg induction. The risk of developing preeclampsia is also increased by fathers born out of preeclamptic pregnancies themselves. (Galaviz-Hernandez et al., 2019) There might be heritable deviations in seminal fluid composition. It might be interesting to study TGF-β levels in the seminal fluid of fathers of PE children.

## Preeclampsia

Preeclampsia (PE) is an immune disease in pregnant women, where the maternal immune system is unable to tolerate the allogeneic antigens on the foetal tissues. It is a leading cause in foetal and maternal mortality. The disease onset is around 20 weeks gestational and characterized by hypertension and failure of the liver and kidneys. Placental dysfunction is closely related to the symptoms associated with PE. Its exact pathogenesis is unclear and treatment options are limited to delivery of the foetus and placenta. (Dimitriadis et al., 2023) (Mittelberger et al., 2022)

## PD-1/PD-L1 (dys)function in preeclampsia

A 2022 review by Mittelberger et al discussed the PD-1/PD-L1 checkpoint in relation to preeclampsia. The authors reason that aberrant PD-1/PD-L1 expression at the maternal-foetal interface as well as in the periphery leads to the Treg and T helper imbalances associated with PE. Their analysis of previous work comparing PE to healthy pregnancies yielded a downregulation of PD-1 and PD-L1 at the placenta. Conversely, they reported an upregulation of PD-1 on Tregs, CD8+ and CD4+ T cells in the peripheral blood. The soluble sPD-1 and sPD-L1 proteins were also upregulated in peripheral blood samples. They also highlighted a significant increase in Th17 cells, which led to a lower Treg/Th17 balance (Mittelberger et al., 2022). Decreased PD-1 and PD-L1 expression can be expected to result in more activated T effector cells and inflammatory conditions consistent with PE symptoms. The review of Mittelberger et all sheds light on several key players surrounding the role of PD-1 and its ligand in PE. However, its exact mechanism remains unclear. Here, we summarize and discuss recent literature to add to our understanding of the checkpoint's role in PE.

## Methods

A literature search was performed in the PubMed data base with key-words (preeclampsia) AND (PD1) on Friday the 7th of June 2024. This yielded 22 results. The search results were further limited to work published between January 2022 and June 2024 as there was a related comprehensive review published in January of 2022. (Mittelberger et al., 2022) This yielded 10 results. Eligible for inclusion were original articles involving the PD1/ PDL-1 checkpoint in pregnant women with preeclampsia. This resulted in 4 papers summarized below.

## Results

Figure 2 depicts a summary of notable alterations in PD-1/PD-L1 signalling at the maternal-foetal interface in PE pregnancies compared to healthy pregnancies. The decidua is a part of the maternal uterine tissue that forms the point of contact between the placenta and maternal immune system. (Mori et al., 2016)

Madidi et al collected the decidual tissue of 25 PE patients and 25 healthy pregnant women during c-section. PD-1 gene expression was assessed using Real-time PCR. Protein expression was measured with western blots. They found a significant ($p < 0.0001$) reduction for both values. (effect sizes; 1.76 and 3.02 respectively) Flow cytometry on the peripheral blood of PE patients yielded a significant increase in Th17 cell frequency ($p = 0.0091$ effect size 0.679) and a decrease in Tregs frequency ($p = 0.0016$ effect size 1.05) compared to healthy test subjects, in concordance with previous literature. (Zhang et al., 2018) Several inflammatory and anti-inflammatory cytokines in the serum were measured using ELISA. As expected, markers associated with tolerance such as Il-10 (effect size 1.27) and TGFβ (effect size 0.717) were significantly downregulated in PE while pro-inflammatory markers IL-1β, IL-17 and TNF-α were elevated (effect sizes; 0.716, 0.673 and

0.950 respectively) in agreement with loss of tolerance observed in PE (Madidi et al 2022)

Mittelberger et al followed up on their 2022 review with a research paper that specifically looked into PD-1 and PD-L1 expression on maternal macrophages in the decidua and foetal macrophages, or Hofbauer cells, in the chorionic villi. The placental and decidual tissue samples were obtained during c-sections over a period of several years. This resulted in tissue samples from a study population of 40 PE and 40 healthy pregnancies. A distinction was made between early onset and late onset of PE and the foetus's gender was considered. (Mittelberger et al 2023)

The decidua and chorionic villi were examined using immune-histochemistry and quadrupole immune-fluorescence. They used the CD68 protein as a general macrophage marker and CD163 as a marker for M2 subtype macrophages. The authors report a reduction in overall CD68 and CD163 expression in both the decidua and chorionic villi in PE patients. In both tissues. The majority of maternal macrophages were CD163 positive. These findings indicate that the total amount of macrophages is reduced, and the majority of macrophages is M2 polarized. (Mittelberger et al 2023)

Regarding PD-1 expression, they found a significant decrease of PD-1+ macrophages in the decidual tissue as well as the chorionic villi of PE pregnancies. There was additionally a significant downregulation of PD-L1+ macrophages in both tissue types in concordance with previous studies. (Mittelberger et al 2023)

According to this study, there appear to be some foetal sex-specific effects on the expression of PD-1 and PD-L1 as well as the expression of macrophages. For instance, downregulation of CD163 positive maternal macrophages in PE (p=0.043) was only significant with female offspring. (p= 0.02) while there was no significant change for male offspring (p= 0.364). Downregulation PD-L1 on maternal macrophages in the decidua (p=0.043) on the other hand was only significant in male offspring (p=0.004). There was no significant difference in female offspring (p=0.841). Many other studies do not include foetal sex which makes it difficult to compare these findings to previous studies. The authors report significant variations in some of the clinical details of the study population such as BMI, which increase the risk of confounding, as high BMI is a known risk factor for PE development. (Mittelberger et al 2023)

Chen et al measured the proportions of CD4+ T cell and Treg subsets in the peripheral blood of healthy pregnant women, post-partum women, non-pregnant women and PE patients. Each group consisted of 20 women. They distinguished between 1$^{st}$, 2$^{nd}$ and 3$^{rd}$-trimester pregnancies. PD-1 expression on the T cell subsets were determined using flow cytometry. They used CD3+ CD4+ CD25+ CD127(low) as a Treg marker. The authors reported a reduction in the Treg proportion in PE pregnancies. They did not find significant differences in PD-1 expression in either of the cell types between PE patients and healthy 3$^{rd}$ trimester pregnancies. (Chen et al 2024)

Du et al speculate that aberrant PD-1/PD-L1 signalling influences macrophage polarization which may be an important factor in PE pathogenesis. Macrophage polarization is determined by their environment. The classically activated or M1 macrophage is mostly associated with inflammatory conditions, while the replacement activated or M2 type is associated with tolerance, in a similar manner to Th1 and Th2 as discussed above. Macrophage and lymphocyte polarization are strongly connected through excretion of cytokines that affect both cell types. (Yao, 2019)
In this study, the decidual tissue of 20 healthy pregnant women and 18 PE pregnancies, sampled during c-section, was analysed using

immunohistochemistry and western blot analysis. They found a significant increase in general macrophage marker CD68 and M1 polarization marker iNOS, and a decrease in M2 polarization marker CD206 in PE patients compared to healthy pregnancies, indicating that the overall macrophage count increased in the direction of M1 polarization. Contradictory to findings by Mittelberger et al. (Mittelberger et al., 2023)
Both PD-1 and PD-L1 were downregulated in the PE decidual tissues. Immunofluorescence double staining was used to study the co-expression of PD-1 and PD-L1 with general macrophage marker CD68. In line with the expression of the checkpoint proteins in decidual tissues, CD68+PD-1+ and CD68+PD-L1+ cells were significantly reduced in PE samples. (Du et al 2024)

To further understand the connection between PD-1/PD-L1 signalling and macrophage polarization they blocked the pathway during M1 polarization. THP-1 cells were exposed to M1-inducing conditions in two groups ICB were added. The expression of M1 markers increased in both the pembrolizumab (PD-1 blocker) and durvalumab (PD-L1 blocker) groups. These findings indicate increased M1 polarization upon blockage of PD1 or PD-L1 in macrophages. ICB pre-treated M1 macrophages were additionally co-cultured with a trophoblast cell line. Trophoblasts are embryonal cells from the outer blastocyst in early pregnancy. They are essential in placentation of the embryo and later placental development. Trophoblast invasion is the process of trophoblast cells invading the maternal uterine tissue/decidua. (Anin et al., 2004) Transwell assays were used to investigate their invasion ability. They found a significantly reduced number of migrated cells. (Du et al 2024)

## Discussion and Conclusions

Preeclampsia is a potentially life-threatening pregnancy complication characterized by high blood pressure and proteinuria (Mittelberger et al., 2022). Previous efforts to understand its pathogenesis have pointed in the direction of immune imbalances. As discussed above, the PD-1/PD-L1 axis has been well characterized for its role in foetal-maternal tolerance. Our analysis of recent literature identified local aberrations in PD1, PD-L1, sPD1 and sPD-L1 expression in PE pregnancies, as well as a change in the lymphocyte and myeloid polarization, consistent with prolonged inflammation. Below these patterns are discussed and related to previous works.

### *PD-1 and PD-L1 expression is reduced at the foetal-maternal interface of pregnant women with PE*

The decidual tissue forms an interface between the foetal and maternal immune systems. General downregulation of PD-1 and PD-L1 has been described in the placentas of PE patients. And on CD8+ effector memory cells specifically. (Mittelberger et al 2022).
In their 2022 paper, Madidi et al showed reduced PD-1 expression and protein levels in PE decidual tissues. (Madidi et al., 2022) A recent study by Du et al confirmed a significant reduction in the expression of PD-1 as well as its ligand PD-L1 in PE decidual tissue. In addition, they characterized PD-1 and PD-L1 expression on decidual macrophages. This yielded a significant reduction in CD68+PD-1+ and CD68+PD-L1+ co-expressing cells. (Du et al., 2024) Another recent paper found reduced PD-1 and PD-L1 expression in decidual tissue, as well as in the chorionic villi. In addition to a reduction of PD-1+ maternal macrophages and foetal macrophages. (Mittelberger 2023). These findings indicate that the foetal immune system displays a similar decrease of PD-1/PD-L1 signalling as observed on the maternal tissues.

Paternal effects have previously been considered to contribute to the development of PE to a smaller extent as maternal factors. (Galaviz-Hernandez et al., 2019) The reduction of PD-1/PD-L1 expression at the

maternal-foetal interface has now been well established. Specifically, the expression of PD-1+ maternal and foetal macrophages adds to the canonical understanding of PD-1/PD-L1 regulation. In the textbook mechanism, PD-1 expression is mostly associated with T cells. However, PD-1 expression on macrophages is now understood to, upon binding of its ligand, result in dysfunctional macrophages which in turn inhibit the T cell response. (Lei et al., 2024)

*Aberrant PD-1/PD-L1 signalling affects macrophage polarization*

Du et al reported a general increase in decidual macrophages in PE patients. As well as an increase in M1 over M2 polarization. (Du et al., 2024) This is contrary to previous work by Mittelberger et al where they found a reduction of overall macrophage counts of which a majority were M2 polarized. (Mittelberger et al., 2023) Both papers used the same general macrophage marker, but a different M2 marker. Mittelberger et al used CD163 whereas Du et al opted for CD206. CD163 expression on macrophages is understood to be induced by interleukin IL-10. Madidi et al found a significant reduction of IL-10 in the decidua of PE pregnancies. This might result in reduced CD163 expression and give a wrongful M2 polarized macrophages count. Additionally macrophages are highly dynamic structures and it may be difficult to conclusively determine their polarization state.

Du et al further explored the effects of disrupted PD-1/PD-L1 signalling on M1 polarization. They found that blocking these checkpoints resulted in higher M1 over M2 proportions. In the decidual tissue healthy pregnancy the proportion of M2 over M1 is known to increase and partly attributed to local tolerance induction. (Yao, 2019)

*Aberrant PD-1/PD-L1 signalling affects lymphocyte polarization and the Treg/Th balance.*

Previous work has made the connection between PD-1/PD-L1 dysfunction and the Treg/Th17 balance. For instance, Zhang et al hypothesized that decreased PD-1 expression promotes Th17 proliferation and reduces Treg differentiation. (Zhang et al., 2017) Chen et al confirmed a reduction of Treg cells in the peripheral blood of PE pregnancies compared to healthy 3rd trimester pregnancies. (Chen et al, 2024). In the decidua, Madidi et al also found a reduction of Tregs and an increase of Th17. They also measured several cytokines involved in lymphocyte polarization, for instance a reduction in TGF-β and an increase in IL-17. (Madidi et al., 2022) TGF-β is known to induce Treg and Th17 differentiation. IL-17 is produced by Th17 cells. (Zhu, 2018)

*Peripheral PD-1/PD-L1 aberrations are dissimilar from those observed at the maternal-foetal interface*

Mittelberger et al described an upregulation of PD-1 on Tregs, CD8+ and CD4+ in the peripheral blood of PE patients. The increased peripheral PD-1 expression seemingly contradicts the inflammatory conditions associated with PE. However, the exact mechanisms of PD-1/PD-L1 dysregulation may very well be differently organized locally or governed by multiple mechanisms. A 2019 paper by Gu et al found upregulation of soluble PD-1 in the peripheral blood of PE patients. It follows that the increased sPD-1 in peripheral blood may prevent PD-1/PD-L1 signalling through competitive binding, essentially acting through the same mechanism as checkpoint inhibitors used in cancer treatment. This might counter the effect of increased PD-1. This might be studied by administering sPD-1 in mouse models and comparing the response with ICB.

*Limitations*

PE is usually detected around 20 weeks of gestation when symptoms reveal themselves. Treatment options are limited to removal of the foetus and placenta through premature

delivery or abortion. (Mittelberger et al., 2023). Alterations in the PD-1/PD-L1 in PE pregnancies are different between the foetal-maternal interface and the peripheral blood. It is possible to monitor the peripheral blood throughout the pregnancy. At the foetal-maternal interface this becomes more complex. The studies described in this text harvested decidual tissue and or placental tissue during c-section. This limits the data to 3rd trimester values and excludes vaginal births. Non-human primate animal models are preferred for studying preeclampsia because their placentation structure is very similar to that of humans. However, gestation is long and they mostly have singleton pregnancies. Mice or rat models have shorter gestation and large litter sizes. Placental development in rodents is however very different from that in humans and abnormalities associated with pathology in humans, such as abnormal spiral artery remodelling, do not have the same effect. Additionally, tail-cuff blood pressure methods used in mice are unreliable and a more dependable direct blood pressure method in rats requires anaesthesia which might affect results. (Chau et al., 2021)

*Preeclampsia pathogenesis*

Preeclampsia pathogenesis is not yet fully understood. Previous works have divided its development into two stages. Stage 1 is characterized by abnormal placentation in early pregnancy. Stage 2 or the maternal syndrome is when the typical symptoms such as hypertension and kidney or liver dysfunction arise. (Chau et al., 2021)

The alterations of the PD-1/PD-L1 inhibitory checkpoint in preeclamptic pregnancies described above, contribute to an inflammatory environment at the maternal-foetal interface and periphery. Downregulation of PD-1 and PD-L1 at the maternal-foetal interface reduces inhibition of T cell activation. This creates a chronic inflammatory environment through secretion of pro-inflammatory cytokines and lymphocyte differentiation towards pro-inflammatory T cell subsets Th1 and Th17. PD-1 and PD-L1 expression is likewise downregulated on decidual and foetal macrophages. Resulting in more activated macrophages that can interact and secrete inflammatory cytokines.

Du et al reported a decrease of macrophage counts and increased M1/M2 proportions in PE patients compared to healthy pregnancy. Macrophage polarization towards the pro-inflammatory M1 subtype further increases inflammation at the maternal-foetal interface. (Du et al., 2024) At the periphery a reduction of Tregs decreases peripheral tolerance. In addition, increased sPD-1 and sPD-L1 in the peripheral blood might induce inflammation through competitive binding.

Trophoblast invasion is a critical step in placentation in early pregnancy. (Abbas et al., 2020) (Anin et al., 2004) Reduced invasion of foetal trophoblast cells into the decidua has been associated with PE pregnancies. (Moffett and Loke, 2006) (Pijnenborg et al., 2006) Du et used anti-PD-1 and anti-PD-L1 checkpoint inhibitors to study the effect of checkpoint inhibition on macrophage polarization. They found an increase in M1 polarization in both groups. Interestingly, trophoblast invasion was decreased in both groups as well. (Du et al., 2024) The inflammatory environment created by loss of peripheral tolerance might ultimately limit trophoblast invasion, leading to abnormal placentation and placental development observed in PE pregnancies.

*Future research and treatment opportunities*

Prolonged antigen exposure is understood to result in the upregulation of PD-1 and PD-L1. Pregnancy involves increasing exposure to foetal antigens at the maternal-foetal interface. The work discussed above indicated uncharacteristically low PD-1/PD-L1 levels in PE pregnancies. sPD-1 and sPD-L1 levels in the peripheral blood might be a good biomarker for early detection of PE. More studies need to

be done measuring their values in the different stages of pregnancy with larger sample sizes and comparing it to uncomplicated pregnancies.

The exact mechanism of how this deficiency arises remains unclear. These alterations are found in the context of chronic inflammation and increased proportions of inflammatory cytokines and lymphocyte and monocyte subsets. The order of events in cascade of altered cytokine expression, differentiation and PD-1/PD-L1 downregulation remains uncertain. In vitro, blocking of the checkpoint molecules has been shown to induce M1 polarization. (Du et al., 2024) However, as a result of differentiation cytokine expression will be affected which in turn affects macrophage polarization. Given the inflammatory conditions associated with PE, chronic inflammation models could be used to further elucidate these mechanisms.

A 2024 study by Lei et al found that TGF-β1 directly induces PD-1 expression on macrophages in vivo and in vitro. Macrophage specific knockdown of the TGF-β1 receptor TGF-βRI significantly reduced PD-1 expression on macrophages in M38 colon cancer tissue and *S. japonicum*–infected mouse livers. In macrophage specific TGF-βRI knockdown mice models drastically reduced PD-1 expression. They hypothesize that macrophages in response to chronic inflammatory conditions upregulate PD-1 expression in a TGF-β1 dependent manner. Interestingly, further transcription factor analysis showed that this process is independent of NFATc1. The transcription factor that is understood to govern PD-1 expression upon T cell activation. (Oestreich et al., 2008) Instead S*MAD3/STAT3* signaling induces PD-1 upregulation under these chronic inflammatory conditions. (Lei et al., 2024)

TGF-β plays a key role in lymphocyte and macrophage differentiation and the establishment of peripheral tolerance. Based on Lei et al. findings there may be some therapeutic advantage to administering it to PE patients. However, increasing tolerance this way could present the risk of cancer development and other immunological pathology. Further research is in PE animal models is needed of this method to increase PD-1 expression at the foetal-maternal interface.

## References


Abbas, Y., Turco, M. Y., Burton, G. J., & Moffett, A. (2020). Investigation of human trophoblast invasion in vitro. *Human Reproduction Update*, *26*(4), 501–513. https://doi.org/10.1093/humupd/dmaa017

Alijotas-Reig, J., Llurba, E., & Gris, J. Ma. (2014). Potentiating maternal immune tolerance in pregnancy: A new challenging role for regulatory T cells. *Placenta*, *35*(4), 241–248. https://doi.org/10.1016/j.placenta.2014.02.004

Anin, S. A., Vince, G., & Quenby, S. (2004). Trophoblast invasion. *Human Fertility (Cambridge, England)*, *7*(3), 169–174. https://doi.org/10.1080/14647270400006911

Bailly, C., Thuru, X., & Quesnel, B. (2021). Soluble Programmed Death Ligand-1 (sPD-L1): A Pool of Circulating Proteins Implicated in Health and

Diseases. *Cancers*, *13*(12), 3034. https://doi.org/10.3390/cancers13123034

Bhattacharyya, N. D., & Feng, C. G. (2020). Regulation of T Helper Cell Fate by TCR Signal Strength. *Frontiers in Immunology*, *11*. https://doi.org/10.3389/fimmu.2020.00624

Borgers, J. S. W., Heimovaara, J. H., Cardonick, E., Dierickx, D., Lambertini, M., Haanen, J. B. A. G., & Amant, F. (2021). Immunotherapy for cancer treatment during pregnancy. *The Lancet. Oncology*, *22*(12), e550–e561. https://doi.org/10.1016/S1470-2045(21)00525-8

Bucheit, A. D., Hardy, J. T., Szender, J. B., & Glitza Oliva, I. C. (2020). Conception and viable twin pregnancy in a patient with metastatic melanoma while treated with CTLA-4 and PD-1 checkpoint inhibition. *Melanoma Research*, *30*(4), 423. https://doi.org/10.1097/CMR.0000000000000657

Burotto, M., Gormaz, J. G., Samtani, S., Valls, N., Silva, R., Rojas, C., Portiño, S., & de la Jara, C. (2018). Viable Pregnancy in a patient with metastatic melanoma treated with double checkpoint immunotherapy. *Seminars in Oncology*, *45*(3), 164–169. https://doi.org/10.1053/j.seminoncol.2018.03.003

Chau, K., Welsh, M., Makris, A., & Hennessy, A. (2021). Progress in preeclampsia: the contribution of animal models. *Journal of Human Hypertension*, *36*(8), 705–710. https://doi.org/10.1038/s41371-021-00637-x

Chen, Z., Zhou, X., Qu, H., Zhang, X., Kwak-Kim, J., & Wang, W. (2024). Characteristics and functions of memory regulatory T cells in normal pregnancy cycle and pregnancy complications. *Journal of Reproductive Immunology*, *163*, 104235. https://doi.org/10.1016/j.jri.2024.104235

Crute, B. W., Sheraden, R., Ott, V. L., Isaac, Getahun, A., & Cambier, J. C. (2020). Inhibitory Receptor Trap: A Platform for Discovery of Inhibitory Receptors That Utilize Inositol Lipid and Phosphotyrosine Phosphatase Effectors. *Frontiers in Immunology*, *11*.

https://doi.org/10.3389/fimmu.2020.592329

Dimitriadis, E., Rolnik, D. L., Zhou, W., Estrada-Gutierrez, G., Koga, K., Francisco, R. P. V., Whitehead, C., Hyett, J., da Silva Costa, F., Nicolaides, K., & Menkhorst, E. (2023). Pre-eclampsia. *Nature Reviews Disease Primers*, *9*(1), 1–22. https://doi.org/10.1038/s41572-023-00417-6

Du, X., Liu, H., Shi, J., Yang, P., Gu, Y., & Meng, J. (2024). The PD-1 /PD-L1 signaling pathway regulates decidual macrophage polarization and may participate in preeclampsia. *Journal of Reproductive Immunology*, *164*, 104258. https://doi.org/10.1016/j.jri.2024.104258

Galaviz-Hernandez, C., Sosa-Macias, M., Teran, E., Garcia-Ortiz, J. E., & Lazalde-Ramos, B. P. (2019). Paternal Determinants in Preeclampsia. *Frontiers in Physiology*, *9*. https://doi.org/10.3389/fphys.2018.01870

Haiduk, J., & Ziemer, M. (2021). Pregnancy in a patient with metastatic uveal melanoma treated with nivolumab. *Journal of the German Society of Dermatology*, *19*(5), 762–765. https://doi.org/10.1111/ddg.14463

Hwang, J.-R., Byeon, Y., Kim, D., & Park, S.-G. (2020). Recent insights of T cell receptor-mediated signaling pathways for T cell activation and development. *Experimental & Molecular Medicine*, *52*(5), 750–761. https://doi.org/10.1038/s12276-020-0435-8

Lei, Z., Tang, R., Wu, Y., Mao, C., Xue, W., Shen, J., Yu, J., Wang, X., Qi, X., Wei, C., Xu, L., Zhu, J., Li, Y., Zhang, X., Ye, C., Chen, X., Yang, X., Zhou, S., & Su, C. (2024). TGF-β1 induces PD-1 expression in macrophages through SMAD3/STAT3 cooperative signaling in chronic inflammation. *JCI Insight*, *9*(7). https://doi.org/10.1172/jci.insight.165544

Madadi, S., Mohammadinejad, S., Alizadegan, A., Hojjat-Farsangi, M., Dolati, S., Samadi Kafil, H., Jadidi-Niaragh, F., Soltani-Zangbar, M. S., Motavalli, R., Etemadi, J., Eghbal-Fard, S., Aghebati-Maleki, L., Danaii, S., Taghavi, S., &

Yousefi, M. (2022). Expression level of immune checkpoint inhibitory factors in preeclampsia. *Human Immunology*, *83*(8-9), 628–636. https://doi.org/10.1016/j.humimm.2022.07.004

Mehta, A., Kim, K. B., & Minor, D. R. (2018). Case Report of a Pregnancy During Ipilimumab Therapy. *Journal of Global Oncology*, *4*, 1–3. https://doi.org/10.1200/jgo.17.00019

Menzer, C., Bernd Beedgen, Rom, J., Christin Maria Duffert, Anna-Lena Volckmar, Sedlaczek, O., Richtig, E., Enk, A., Jäger, D., & Hassel, J. C. (2018). Immunotherapy with ipilimumab plus nivolumab in a stage IV melanoma patient during pregnancy. *European Journal of Cancer*, *104*, 239–242. https://doi.org/10.1016/j.ejca.2018.09.008

Mittelberger, J., Seefried, M., Franitza, M., Garrido, F., Ditsch, N., Jeschke, U., & Dannecker, C. (2022). The Role of the Immune Checkpoint Molecules PD-1/PD-L1 and TIM-3/Gal-9 in the Pathogenesis of Preeclampsia—A Narrative Review. *Medicina-Lithuania*, *58*(2), 157–157. https://doi.org/10.3390/medicina58020157

Mittelberger, J., Seefried, M., Sanja Löb, Kuhn, C., Franitza, M., Garrido, F., Carl Mathis Wild, Ditsch, N., Jeschke, U., & Dannecker, C. (2023). The programmed cell death protein 1 (PD1) and the programmed cell death ligand 1 (PD-L1) are significantly downregulated on macrophages and Hofbauer cells in the placenta of preeclampsia patients. *Journal of Reproductive Immunology*, *157*, 103949–103949. https://doi.org/10.1016/j.jri.2023.103949

Moffett, A., & Loke, C. (2006). Immunology of placentation in eutherian mammals. *Nature Reviews Immunology*, *6*(8), 584–594. https://doi.org/10.1038/nri1897

Mori, M., Bogdan, A., Balassa, T., Csabai, T., & Szekeres-Bartho, J. (2016). The decidua—the maternal bed embracing the embryo—maintains the pregnancy. *Seminars in Immunopathology*, *38*(6), 635–649. https://doi.org/10.1007/s00281-016-0574-0

Nielsen, M. C., Hvidbjerg Gantzel, R., Clària, J., Trebicka, J., Møller, H. J., & Grønbæk, H. (2020). Macrophage Activation Markers, CD163 and CD206, in Acute-on-Chronic Liver Failure. *Cells*, *9*(5), 1175. https://doi.org/10.3390/cells9051175

Oestreich, K. J., Yoon, H., Ahmed, R., & Boss, J. M. (2008). NFATc1 Regulates PD-1 Expression upon T Cell Activation. *The Journal of Immunology*, *181*(7), 4832–4839. https://doi.org/10.4049/jimmunol.181.7.4832

Pijnenborg, R., Vercruysse, L., & Hanssens, M. (2006). The Uterine Spiral Arteries In Human Pregnancy: Facts and Controversies. *Placenta*, *27*(9-10), 939–958. https://doi.org/10.1016/j.placenta.2005.12.006

Polnaszek, B., Mullen, M., Bligard, K., Raghuraman, N., & Massad, L. S. (2021). Term Pregnancy After Complete Response of Placental Site Trophoblastic Tumor to Immunotherapy. *Obstetrics and Gynecology*, *138*(1), 115–118. https://doi.org/10.1097/AOG.0000000000004434

Sharpe, A. H., & Pauken, K. E. (2017). The diverse functions of the PD1 inhibitory pathway. *Nature Reviews Immunology*, *18*(3), 153–167. https://doi.org/10.1038/nri.2017.108

Sun, Q., Hong, Z., Zhang, C., Wang, L., Han, Z., & Ma, D. (2023). Immune checkpoint therapy for solid tumours: clinical dilemmas and future trends. *Signal Transduction and Targeted Therapy*, *8*(1). https://doi.org/10.1038/s41392-023-01522-4

Teodora Maria Toadere, Ţichindeleanu, A., Daniela Andreea Bondor, Topor, I., Şerban Ellias Trella, & Iuliana Nenu. (2024). Bridging the divide: unveiling mutual immunological pathways of cancer and pregnancy. *Inflammation Research*. https://doi.org/10.1007/s00011-024-01866-9

Wang, Q., Bardhan, K., Boussiotis, V. A., & Patsoukis, N. (2021). The PD-1 Interactome. *Advanced Biology*, *5*(9), 2100758.

https://doi.org/10.1002/adbi.202100758

Xiao, Y., Yu, S., Zhu, B., Bedoret, D., Bu, X., Francisco, L. M., Hua, P., Duke-Cohan, J. S., Umetsu, D. T., Sharpe, A. H., DeKruyff, R. H., & Freeman, G. J. (2014). RGMb is a novel binding partner for PD-L2 and its engagement with PD-L2 promotes respiratory tolerance. *Journal of Experimental Medicine*, *211*(5), 943–959. https://doi.org/10.1084/jem.20130790

Xu, W., Moor, R. J., Walpole, E. T., & Atkinson, V. G. (2019). Pregnancy with successful foetal and maternal outcome in a melanoma patient treated with nivolumab in the first trimester: case report and review of the literature. *Melanoma Research*, *29*(3), 333–337. https://doi.org/10.1097/cmr.0000000000000586

Yao, Y., Xu, X.-H., & Jin, L. (2019). Macrophage Polarization in Physiological and Pathological Pregnancy. *Frontiers in Immunology*, *10*(792). https://doi.org/10.3389/fimmu.2019.00792

Zhang, Y., Liu, Z., Tian, M., Hu, X., Wang, L., Ji, J., & Liao, A. (2017). The altered PD-1/PD-L1 pathway delivers the "one-two punch" effects to promote the Treg/Th17 imbalance in pre-eclampsia. *Cellular & Molecular Immunology*, *15*(7), 710–723. https://doi.org/10.1038/cmi.2017.70

Zhang, Y.-H., Tian, M., Tang, M.-X., Liu, Z.-Z., & Liao, A.-H. (2015). Recent Insight into the Role of the PD-1/PD-L1 Pathway in Feto-Maternal Tolerance and Pregnancy. *American Journal of Reproductive Immunology*, *74*(3), 201–208. https://doi.org/10.1111/aji.12365

Zhu, J. (2018). T Helper Cell Differentiation, Heterogeneity, and Plasticity. *Cold Spring Harbor Perspectives in Biology*, *10*(10), a030338. https://doi.org/10.1101/cshperspect.a030338