Supplementary Appendix

Inflammasome-targeted therapy to prevent adverse perinatal outcomes of recurrent chronic intervillositis of unknown etiology

Short title: Inflammasome-targeted therapy in recurrent chronic intervillositis

Table of Contents

1. Supplementary Methods 2

1.1. Selection of the placenta samples with CHI 2

1.2. Treatment protocol 3

1.3. Immunohistochemistry acquisition 4

1.4. RNA quantification and preparation for NanoString nCounter mRNA analysis 5

2. Supplementary FIGURES 6

Figure 1S : TOP-Scoring regulatory cytokines 6

3. Supplementary TABLES 7

Table S1: List of genes used to calculate zScores for each gene signature 7

Table S2: Patient characteristics for transcriptomic analysis of inflammasome pathway 8

Table S3: Baseline maternal characteristics of recurrent CHI patients receiving inflammasome targeted therapy 9

4. REFERENCES 10

1. SUPPLEMENTARY METHODS

1.1. Selection of the placenta samples with CHI

Cases were identified as previously described **(1)**. Briefly, cases were identified by searching the Department of Histopathology placenta database for the term 'intervillositis.' Serial 2-µm-thick sections of formalin-fixed paraffin-embedded potential CHI tissues, stained with hematoxylin–eosin–saffron (HES) or immunostained for CD68, were re-analyzed independently by three pathologists specialized in fetal pathology. Discordant cases were included or excluded according to consensus among the three pathologists. Kappa values for interobserver variability of the diagnosis of CHI was 0.70. Diagnosis of CHI was confirmed according to the criteria of Bos et al **(2)**: (1) presence of cellular infiltrate in the intervillous space; (2) ~ 80% of the mononuclear cells in the intervillous space positive for CD68; (3) infiltration occupying at least 5% of the intervillous space; and (4) no clinical or histopathological sign of infection

1.2. Treatment protocol

The four following drugs were administrated as soon as the intra-uterine pregnancy was confirmed according to this protocol:

- Anakinra 100 mg once daily subcutaneously
- Hydroxychloroquine (HCQ) 400 mg orally per day
- Colchicine 1 mg orally per day,
- Low dose of aspirin (LDA) (100 mg) orally per day (in the evening).

If possible, HCQ and colchicine were started periconceptional. LDA was prescribed until 34 weeks of gestation in accordance to French guidelines in case of IUGR **(3).** The three other molecules were maintained until the delivery.

N.B: low-molecular-weight heparin was prescribed at preventive dose for patient 3 because of obstetrical antiphospholipid syndrom.

1.3. Immunohistochemistry acquisition

The paraffin-embedded placental tissues were cut in serial 3-µm-thick sections. First, the tissue sections were dewaxed at 56°C for 2 hours and then antigen retrieval was performed using the PT Link pH 9 buffer (Agilent Dako, Santa Clara, CA) for 40 minutes at 95°C then cooled down to 65°C for 20 minutes. Next, sections were incubated with an endogenous peroxidase inhibitor for 10 minutes at room temperature and after being washed in TBS-tween buffer (tris-buffered saline and polysorbate 20, Dako) were incubated with the primary antibodies for one hour at room temperature (anti-NLRP3, Enzolife, ALX804819 ; anti-PYCARD, Sigma-Aldrich, HPA-049074). Sections were washed again, and immunoreactive signals were visualized using the Dako EnVision FLEX peroxidase detection system, as per the manufacturer's instructions. Finally, the sections were counterstained with hematoxylin and mounted in Eukitt® mounting medium. Sections were visualized with a Nikon DS-Fi2 microscope (Leica) and images were acquired using the NIS Elements imaging software. Negative controls were performed by repeating the experiment in absence of the primary antibody.

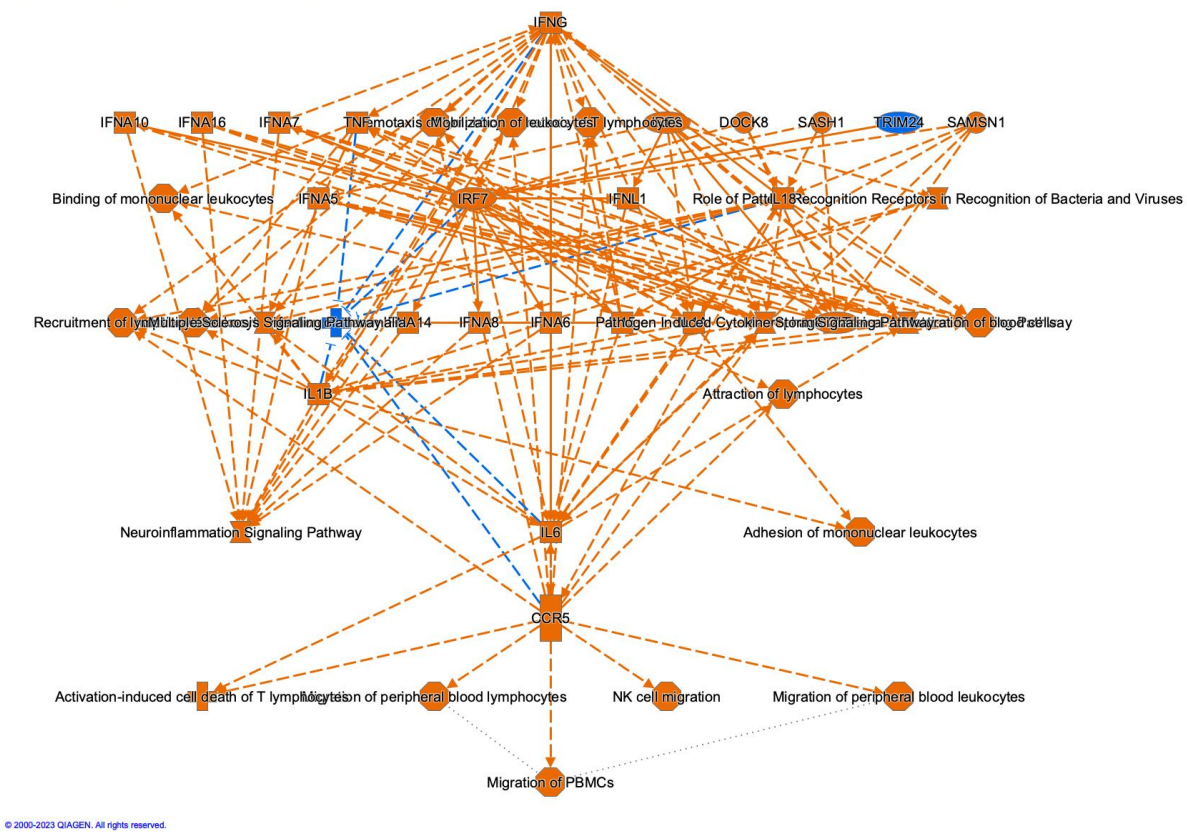
1.4. RNA quantification and preparation for NanoString nCounter mRNA analysis

Total RNA from serial 20-μm-thick sections of formalin-fixed paraffin-embedded (FFPE) was extracted using a RNeasy FFPE kit (QIAGEN) according to the manufacturer's protocol. RNA quantification was performed using the Thermo Scientific™ NanoDrop 2000. For each sample, 185 ng of purified RNA was added to 8 μL of Master Mix Reporter CodeSet and 2 μL Capture ProbeSet using an nCounter master kit as recommended (NanoString Technologies).

2. SUPPLEMENTARY FIGURES

Figure 1S : TOP-Scoring regulatory cytokines

The Top-Scoring regulatory cytokines identified with the IPA software corresponded to interferon type I (IFN type I), tumor necrosis factor (TNF), interleukin-6 (IL-6), interleukin-1B (IL-1B), and interleukin-18 (IL-18).



3. SUPPLEMENTARY TABLES

Table S1: List of genes used to calculate zScores for each gene signature

	Signature Targets
IL-1 β	*CCL2, CCL20, CXCL2, IL1A, IL1B, IL6, LILRB1, NFKB1, NFKBIZ, POU2F2*
IL-18	*ARG1, B2M, BAX, BCL2, BID, CASP3, CASP8, CCL18, CCL19, CCL2, CCL20, CCL3, CCL4, CCL5, CD36, CD81, CD83, CEBPB, CHUK, CTNNB1, CXCL2, FADD, FAS, FN1, ICAM1, IFNG, IKBKB, IL10, IL12B, IL13, IL18, IL18R1, IL18RAP, IL1B, IL2RA, IL6, IL9, IRAK1, IRF1, ITGA2B, LCK, MAPK1, MYD88, NFKB1, NFKB2, NFKBIA, NFKBIZ, NOS2, PRKCD, PTGS2 RELA, SOCS3, SPP1, TBX21, TNF, TNFAIP3, TNFSF11, TP53, TRAF1, TRAF6*
Inflammasome	*IL1B, IL18, MEFV, casp1, NLRP3, PYCARD*

Table S2: Patient characteristics for transcriptomic analysis of inflammasome pathway

	CHI	Healthy donors
Number of subjects	18	6
Age, years (median, range)	30.9 [22-43]	30.0 [21-39]
Race, n (%)		
Caucasian	6 (33)	5 (83)
Asian	0 (0)	0 (0)
Black	7 (39)	0 (0)
Missing data	5 (28)	1 (16)
BMI, kg/m^2 (median, range)	21.6 [17.1-34.0]	19.9 [17.2-28.0]
Active smoking, n (%)	8 (44)	2 (33)
Hypertension disorder history, n (%)	0 (0)	1 (17)
CHI history, n (%)	2 (11)	-
Ongoing Treatment (when analysis was performed), n (%)	None: 15 (83) LDA: 2 (11) LDA + LMWH + Ig IV + HCQ: 1 (6)	None: 5 (28) LDA: 1 (6)
Obstetrical outcomes, n		
Preeclampsia	1	0
SGA	16	0
Stillbirth	1	0
Cesarean section	7	3
Gestational age at birth (median, range)	37.7 [35.0-41.3]	37.8 [36.3-39]
Birth weight, g (median, range)	2247 [940-3160]	3018 [2590-3540]

Abbreviations: BMI, body mass index; CHI, chronic histiocytic intervillositis; SGA, small for gestational age; LDA, low dose aspirin; LMWH, low molecular weight heparin; IV Ig, intravenous immunoglobulins; HCQ, hydroxychloroquine.

Table S3: **Baseline maternal characteristics of recurrent CHI patients receiving inflammasome targeted therapy**

	Patient 1	**Patient 2**	**Patient 3**
Age, race	34 years, Caucasian	41 years, Caucasian	28 years, Caucasian
Body Mass Index (kg/m^2)	22	27	24
Active smoking	No	Yes	Yes
Hypertension disorder history	No	No	No
Diabetes mellitus	No	No	No
History of pregnancy loss			
Preeclampsia	0	0	0
< 14 WG only—no	6	6	0
- Abortion	0	1	
- Miscarriage—no./total no.	6	5	0
- Ectopic or molar pregnancy—no./total no.	0	0	0
≥ 14 WG only—no	0	3	2
Number of live births	1/7 pregnancies	0/9 pregnancies	0
Confirmed CHI	3/6 fetal losses	5/8 fetal losses	2/2 fetal losses
Previous treatment received	LDA, LMWH, PRED, HCQ	LDA, LMWH, PRED, HCQ, IV Ig, AZA, ADA	LDA, LMWH

Abbreviations: CHI, chronic histiocytic intervillositis; IUGR, intra-uterine growth restriction; IV Ig, intravenous immunoglobulins; LDA, low dose aspirin; LMWH, low molecular weight heparin; PRED, prednisone; TOP, termination of pregnancy; AZA, azathioprine; ADA adalimumab

4. REFERENCES

1. Mattuizzi A, Sauvestre F, André G, et al. Adverse perinatal outcomes of chronic intervillositis of unknown etiology: an observational retrospective study of 122 cases. Sci Rep 2020;10(1):12611.

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3. Vayssière C, Sentilhes L, Ego A, et al. Fetal growth restriction and intra-uterine growth restriction: guidelines for clinical practice from the French College of Gynaecologists and Obstetricians. Eur J Obstet Gynecol Reprod Biol 2015;193:10–8.