ADVANCES IN THE USE OF TROPHOBLASTIC CELLS FOR PRENATAL NON-INVASIVE DIAGNOSTICS OF GENETIC DISORDERS

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Disclosure
Inventor/coinventor of ISET patents
Founder ans scientific advisor of Rarecells
Academic tasks:
Teaching (University Paris Descartes)
Developing and implementing new tests (Hôpital Necker)
Research activity (INSERM Unit)
Circulating Fetal Trophoblastic Cells (CFTC)

CFTC do not have to cross the placenta

Endovascular trophoblasts invade the lumen of spiral arteries coming in contact with maternal blood

Invasion follows two waves: middle of 1T and end of 1T

- Isolation by density gradient, MACS, from 20 to 50 ml of blood, followed by Y PCR or FISH: very inconsistent results (Oudejans C, 2003)
- Trophoblastic markers proven to be not specific: HLA-G, NeuroD2, placenta GF (Tioa ML, 2007)
- 30 ml blood, CD105, CD141, CK: Hatt L et al, 2013; Shlutter et al 2014; Shlutter et al, 2015:

ISET® TECHNOLOGY for CIRCULATING FETAL TROPHOBLASTIC CELLS

ISET by Rarecells: Patented combination of parameters allowing very sensitive and rapid isolation of CFC
Workflow for ISET® in vitro assay of sensitivity and reproducibility with fixed cells counted by micropipetting

1. Counting by micropipetting of single A549 fluorescent cells
2. Addition of A549 cells to whole blood one by one
3. Dilution with Rarecells® buffer and incubation for 10 minutes
4. Filtration by Rarecells® Device and Block: fixed cells workflow
5. Microscopy analysis: fluorescent cells counting

Table 2: Sensitivity and repeatability of the ISET® system (fixed cells)

<table>
<thead>
<tr>
<th>mL of blood processed</th>
<th>1 mL</th>
<th>5 mL</th>
<th>10 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of spiked tumor cells</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Number of tumor cells detected by ISET®</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total found/spiked</td>
<td>10/12</td>
<td>10/12</td>
<td>12/12</td>
</tr>
<tr>
<td>Recovery success rate</td>
<td>83.3%</td>
<td>83.3%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Results


H. MOUAWIA, ......P. PATERLINI-BRÉCHOT. Trophoblasts enriched from maternal blood provide definitive genetic diagnosis in 63 consecutive fetuses at risk for Cystic Fibrosis or Spinal Muscular Atrophy, Reproductive Biomedicine Online, 2012.

PFEIFER I, ......P. PATERLINI-BRÉCHOT. Cevical Trophoblasts for non-invasive single cell genotyping and prenatal diagnosis, Placenta, 2015.
Successful clinical validation - Mouawia et al, RBMO 2012

Effect of circulating fetal trophoblastic cell replicated tests on the overall reliability of the diagnostic procedure.

When using 10 CFTC replicates, the protocol of independent diagnostic analysis (correlation = 0), virtually never gives rise to a global (clinical) mistake at an error rate of 10%, i.e. the probability of global diagnostic error rate is 1 in 10 billion. With five CFTC replicates, this extraordinary performance can be reached if the error rate is 1% or less.

SMA + CF: total (31+32) = 63 pregnant women

Consecutive cases

1191 microdissected cells: 475 CFTC

7 + 7 affected fetuses

as compared to CVS

Sensitivity: 100%
Specificity: 100%
**Kinetics of Circulating Fetal Trophoblastic Cells in maternal blood after In Vitro Fertilization (4th to 12th WG) (collab. Prof R Frydman):**

**CFTC start to circulate at the 5th WG**

![Graph showing CFTC levels over gestation](image)

Total 473 CFTC

**Isolation of trophoblasts from the cervix**

Transcervical cells (TCC): inner part of the cervix and lower pole of the uterine cavity (Schetts 1971)

1. Decidua parietalis
2. Decidua capsularis
3. Decidua basalis
4. Uterine cavity

**TCC sampling methods:**
- Intrauterine lavage
- Endocervical lavage
- Endocervical mucus aspiration
- Endocervical sampling by a cytobrush

![Image showing TCC collection](image)

Collection of cells from external part of the cervix

**Pfeifer I et al Placenta, 2015**
Non-invasive isolation of trophoblasts from the cervix
2ml out of 10 ml analyzed - Pfeifer I 2015

<table>
<thead>
<tr>
<th>Couple</th>
<th>Term of pregnancy (WG)</th>
<th>Informative STR marker</th>
<th>Cytotrophoblast/ Syncytiotrophoblast* - NIIPD**</th>
<th>N° of microdissected cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(CF)</td>
<td>12</td>
<td>D7S460/D7S523</td>
<td>4 - carrier</td>
<td>10</td>
</tr>
<tr>
<td>2(CF)</td>
<td>12</td>
<td>D7S523</td>
<td>6 - carrier</td>
<td>12</td>
</tr>
<tr>
<td>3(CF)</td>
<td>12</td>
<td>D16S539/D7S523</td>
<td>10 - carrier</td>
<td>19</td>
</tr>
<tr>
<td>4 (SMA)</td>
<td>12</td>
<td>D5S816/D21S1437</td>
<td>6 - not affected</td>
<td>13</td>
</tr>
<tr>
<td>5 (SMA)</td>
<td>12</td>
<td>D21S1435</td>
<td>10 - not affected</td>
<td>21</td>
</tr>
<tr>
<td>6 (SMA)</td>
<td>12</td>
<td>D16S539/D7S523</td>
<td>6 - not affected</td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>D5S816/D21S1437</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>8d</td>
<td>12</td>
<td>D16S539</td>
<td>3/2</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>D16S539/D6S816</td>
<td>4/2</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>D21S1435</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>11d</td>
<td>12</td>
<td>D21S1435</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>D16S539</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>13d</td>
<td>12</td>
<td>D21S1435/D7S523</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>14d</td>
<td>12</td>
<td>D16S539/D6S816</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>15d</td>
<td>12</td>
<td>D16S539/D21S1435</td>
<td>0/2</td>
<td>16</td>
</tr>
<tr>
<td>16d</td>
<td>12</td>
<td>D16S539/D21S1435</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td>17d</td>
<td>9</td>
<td>D16S539/D6S816</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>18d</td>
<td>9</td>
<td>D5S615/D16S539</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>19d</td>
<td>8</td>
<td>D16S539/D6S816</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>20d</td>
<td>11</td>
<td>D16S539/D21S11</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>21d</td>
<td>8</td>
<td>D5S615/D5S816</td>
<td>3/1</td>
<td>8</td>
</tr>
</tbody>
</table>

A- ISET® filtration workflow for enrichment and analysis of fixed circulating rare cells

1- Blood Collection 10 ml EDTA tube
2- Dilution with Rarecells® Buffer and incubation (10 minutes)
3- Filtration by Rarecells® Block and Device
4- Fixed cancer cells on the filter
   Extract filter and air dry
   Possible storage and shipment
Cytological staining
5a- Microscopic analysis
5b- Molecular analysis (on pools or at the single cell level)

B- ISET® filtration workflow for enrichment and analysis of fixed/live circulating rare cells

1- Blood Collection 10 ml EDTA tube
2- Dilution with Specific Rarecells® Buffer and incubation (5 minutes)
3- Filtration by Rarecells® Block and Device
4- Fresh cells recovery in suspension
5a- In vitro culture and/or in vivo functional studies
5b- Optional immunomagnetic depletion of CD45 positive cells
5c- Optional micromanipulation
6- Molecular analysis (on pools or at the single cell level)
- Workflow for ISET® *in vitro* assay of sensitivity and reproducibility with fixed/live cells counted by micropipetting

Table 5. Sensitivity and repeatability of the ISET® system for live tumor cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>A549</th>
<th>LNCaP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of spiked cells</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Blood volume (mL)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Number of spiked cells</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Number of rare cells detected by ISET®</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Recovery rate (%)</td>
<td>90% (7)</td>
<td></td>
</tr>
<tr>
<td>SEM, standard error of mean</td>
<td>21/60</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity of the ISET® system for dual collection of live rare cells followed by CD45-immunomagnetic depletion

> 30

CFTC per 10 ml blood

30% = > 10

CFTC

WGA
genotyping

(single cells or pooling)

NGS

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