Fetal Cells in Maternal Blood

*Game Changer in Non-Invasive Prenatal Diagnosis*

RIPUDAMAN SINGH. PhD, MBA

Chief Technology Officer

ARCEDI Biotech ApS, Denmark
Cell-based Non-Invasive Prenatal Diagnosis (cbNIPD)

“If you can do it, it changes the game. There’s no question about it.”
Disclosures
CVS/Amniocentesis

ARCEDI GOAL

- Current Prenatal Testing/Diagnosis Technology Scenario
ARCEDI GOAL

- Future Prenatal Testing/Diagnosis Technology Scenario

Cell-based
RATIONALE

- High coverage with low risk
ARCEDI TECHNOLOGY OVERVIEW
- Isolation, Extraction and Analyses of Circulating Fetal Cells

Pregnant women GA: 10 to 13 weeks
Blood Processing (30mL of whole blood)
Selection and Staining using ARCEDI markers
Scanning and Identification of Fetal Cells

CbNIPD using CMA/NGS
Whole Genome Amplification (WGA)
‘Picking’ Fetal Cells
Positively Identified Fetal Cells
Fetal Cells in Maternal Blood – A History
It’s been known for years that fetal cells do circulate in pregnant women’s blood.

Alternative to invasive prenatal diagnosis was envisaged – Focus on fetal cells. Reasons:
  - Mitigate the risk of intervention associated with invasive methods
  - Make prenatal diagnostics simple and cost-effective

Earlier attempts to isolate fetal cells from maternal circulation consistently and in good numbers were not very successful.
FETAL CELLS IN MATERNAL BLOOD

- Challenges

Fetal Cell Type  Markers

Rarity of the Fetal Cells
FETAL CELLS IN MATERNAL BLOOD
- A Love story rekindled

Figure 1. The number of publications targeting fetal cells in maternal blood. Graph generated from citations in Pubmed.

Singh et al. 2017
FETAL CELLS IN MATERNAL BLOOD
- A Love story rekindled

FETAL ERYTHROCYTES IN THE MATERNAL CIRCULATION
ALVIN ZIPURSKY

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PRACTICAL AND THEORETICAL IMPLICATIONS OF FETAL/MATERNAL LYMPHOCYTE TRANSFER
Janina Walkowska, Felix A. Conte, Melvin M. Grumbach

Fetal cells in the blood of pregnant women: Detection and enrichment by fluorescence-activated cell sorting
(Y chromatin/HLA/prenatal diagnosis/chromosome abnormalities)

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Skepticism

Intact fetal cells in maternal plasma: are they really there?

Rare fetal cells can be recovered from maternal blood, which suggests that non-invasive prenatal diagnosis is possible. However, recovery and analysis of fetal cells from blood is complex, and sensitivity is low because of the rarity of these cells in the maternal circulation. An alternative strategy, which suggested that intact fetal cells can be found in maternal plasma by use of simple enrichment methods, has been reported. We aimed to replicate this technique. However, five independent laboratories were unable to identify any intact male cells from the plasma of 38 women known to be carrying male fetuses. Although apoptotic intact fetal cells could contribute to the detection of fetal DNA in maternal plasma, we believe that recovery of these cells is difficult and not clinically practical.

Lancet 2003; 361: 139-40

Singh et al. 2017
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LEONARD A. HERZENBERG*, DIANA W. BIANCHI*, JIM SCHRÖDER*, HOWARD M. CANN*, and G. MICHAEL IVerson*

Skepticism

Intact fetal cells in maternal plasma: are they really there?
Fardad Z. Shojafar, Saeideh Hafez, Katy L. Johnson, Jose Leigh Skippon, Diana W. Bianchi, Dorothy E. Lewis, William D. Weber, Katherine Klipser, Sherman E. Eiser, Ladd G. Jackson, Mani Evans, Wolfgang Holzgreve, Fabio de la Cruz

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Arcedi Singh et al. 2017
Addressing the Challenges – The ARCEDI Way
CHECKLIST

- Technology to be approved as a cbNIPD should fulfil the following criteria

  • The technology should target specific cell type(s).

  • There should be antibodies that are both sensitive and specific for those cell types.

  • Technology should be platform and parameter independent.

  • After identifying the true fetal cells it should be possible to get access to the cells for downstream applications.

  • The fidelity of the DNA from enriched fetal cells should be high so that genetic analyses using chromosomal microarray (CMA) or next-generation sequencing (NGS) can be performed.

Singh et al. 2017
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*Singh et al. 2017*
FETAL CELL TYPE
FETAL CELL TYPE

- Establishing the Fetal Cell gene expression profile

Characterization of Fetal Cells from the Maternal Circulation by Microarray Gene Expression Analysis – Could the Extravillous Trophoblasts Be a Target for Future Cell-Based Non-Invasive Prenatal Diagnosis?

Lotte Hatt, Marie Brinch, Ripudaman Singh, Kristine Møller, Rune Hoff Lauridsen, Niels Uldbjerg, Berthold Huppertz, Britta Christensen, Steen Kølvraa
FETAL CELL TYPE

- Trophoblast mediated Uterine Vessel Remodelling

Moser et al. 2016
(Histochem Cell Biol)
FETAL CELL TYPE

- Trophoblast mediated Uterine Vessel Remodelling

Routes of extravillous trophoblast invasion (6-11 weeks)

Moser et al. 2016
(Histochem Cell Biol)
FETAL CELL TYPE

- Trophoblast mediated Uterine Vessel Remodelling

**Routes of extravillous trophoblast invasion (6-11 weeks)**

- Interstitial
- Endovascular
- Endoglandular

**Moser et al. 2016**

*(Histochem Cell Biol)*
- Trophoblast mediated Uterine Vessel Remodelling

**Routes of extravillous trophoblast invasion (6-11 weeks)**

Moser et al. 2016
(Histochem Cell Biol)
FETAL CELL TYPE

- Trophoblast mediated Uterine Vessel Remodelling

- EMT (Epithelial – Mesenchymal Transition)

- Fetal Cells that shed in the Maternal Circulation express markers for both:
  - EPITHELIAL CELLS
  - ENDOTHELIAL CELLS

- ARCEDI MARKER COMBINATION!
  - 8 Markers for Enrichment and Staining of Fetal Cells

Moser et al. 2016
(Histochem Cell Biol)
Enrichment and identification of fetal cells in maternal blood and ligands for such use

Nov 9, 2011

The present invention relates to enrichment and/or identification of fetal cells of a maternal blood sample using fetal cell specific ligands and/or fetal cell specific hybridization probes wherein the ligand or probes are directed to an endothelial/mesenchymal marker, e.g. CD105, CD146 or CD141, in a first round of enrichment and the ligand or probes, in a second round of enrichment, are directed to an epithelial marker, e.g. a cytokeratin, such as CK7, CK8, CK18 or CK19. Enriched or identified fetal cells may be subjected to steps of detection or diagnosis, wherefore the present invention enables non-invasive 5 prenatal diagnostics.

Patent History
Patent number: 9429520
Type: Grant
Filed: Nov 9, 2011
Date of Patent: Aug 30, 2016
Patent Publication Number: 20130331284
Assignee: Arcedi Biotech ApS (Vejle)
Inventors: Andreas Eckelt (Odenthal), Britta Christensen (Birkerød), Steen Kolvraa (Skødstrup), Marie Brinch (Vejle), Ripudaman Singh (Århus O), Lotte Hatt (Skanderborg)
Primary Examiner: Jeanine A Goldberg
Application Number: 13/833,455

Classifications
Current U.S. Class: Involving Fixed Or Stabilized, Nonliving Microorganism, Cell, Or Tissue (e.g., Processes Of Staining, Stabilizing, Dehydrating, Etc., Compositions Used Therefore, Etc.) (435/40.5)
International Classification: C07H 21/04 (20060101); C12Q 1/58 (20060101); G01N 21/64 (20060101); G01N 33/569 (20060101);
FETAL CELL MARKERS
ARCEDI METHOD – MARKER SENSITIVITY

- Performance - Retrieval of Fetal Cells

• 190 PREGNANT WOMEN at NT SCAN- 30ml Blood.
  • 99 SAMPLES FROM ‘LOW RISK’ GROUP
  • 91 SAMPLES FROM ‘HIGH RISK’ GROUP (offered CVS)
ARCEDI METHOD – MARKER SENSITIVITY

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<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of Fetal Cells</td>
<td>2440</td>
</tr>
<tr>
<td>Mean (per sample)</td>
<td>12.8/30ml blood</td>
</tr>
<tr>
<td>Median</td>
<td>10</td>
</tr>
<tr>
<td>Range</td>
<td>1-46</td>
</tr>
</tbody>
</table>
ARCEDI METHOD – MARKER SENSITIVITY

- Frequency Distribution (‘High Risk’ vs ‘Low Risk’)
ARCEDI METHOD – MARKER SENSITIVITY

- Frequency Distribution (‘High Risk’ vs ‘Low Risk’)

Fetal Cell from Every Sample!
ARCEDI METHOD – MARKER SPECIFICITY

- Classification of Fetal Cells

• 208 FETAL CELLS FROM MALE PREGNANCIES.
  • After identification, Fetal Cells subjected to XY FISH

Kølvraa et al. 2016
(Prenatal Diagnosis)
ARCEDI METHOD – MARKER SPECIFICITY
- Classification of Fetal Cells

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<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>XY</td>
<td>164</td>
</tr>
<tr>
<td>XX</td>
<td>0</td>
</tr>
<tr>
<td>No. FISH Signal</td>
<td>32</td>
</tr>
<tr>
<td>Lost during FISH</td>
<td>12</td>
</tr>
</tbody>
</table>

Kølvraa et al. 2016
(Prenatal Diagnosis)
FETAL CELL MORPHOLOGY
FETAL CELLS

- Gallery
FETAL CELLS

- Gallery

Fetal Cell Identification Criteria

- Cytoplasmic staining pattern (green)
- Nuclear morphology/staining pattern (blue)
- Counterstain
- Size of the cell
METHOD ROBUSTNESS
ARCEDI METHOD – ROBUSTNESS

- Turnaround time/sample

Sampling  Blood Processing  FetalCell Enrichment and staining  Mounting and scanning  Visual inspection  Picking  WGA

ARCEDI method: Processing Time per sample in Hours (continuous)
ARCEDI METHOD – AUTOMATION

- Turnaround time/sample

Sampling

Blood Processing

FetalCell Enrichment and Staining (MutiMACS & Tecan Robot)
Takes 24 samples

Collection of enriched fetal cells using FACS (BD Aria III)
Takes 2 samples per hour

WGA
2 samples

* 10

ARCEDI method: Processing Time per sample in Hours (continuous)
ARCEDI METHOD – ROBUSTNESS

- Sample Stability (Parameter/Platform Independent)

Fetal Cell Number and Distribution unaffected by:

- Blood collection **tubes** (BD vs Streck Tubes)
- **Time** before blood processed – 72 hrs
- **Transportation** – air and road and processed after 72 hrs
- Fetal cells from **every sample**!
GENETIC ANALYSES ON FETAL CELLS
DNA FIDELITY
- Deciphering Genetic Information from Fetal Cells

WGA
Picoplex

CMA
(180K)
DNA FIDELITY

- One ‘High Risk’ case

Genome-wide copy number analysis on DNA from fetal cells isolated from the blood of pregnant women

Steen Kalvraa1,2, Ripudaman Singh1,2, Elizabeth A. Normand2, Sadeem Qaderi2, Ignatia B. van den Veyver2,3, Laird Jackson4, Lotte Hatt1, Palle Schelde1, Niels Uldbjerg5, Else Marie Vestergaard6, Li Zhao2, Rui Chen2, Chad A. Shaw2, Amy M. Breman2 and Arthur L. Beaudel2

1 ARCEDI Biotech ApS, Vejle, Denmark
2 Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA
3 Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, USA
4 Department of OB-Gyn, Drexel University College of Medicine, Philadelphia, PA, USA
5 Department of Obstetrics and Gynecology, Aarhus University Hospital, Aarhus N, Denmark
6 Department of Clinical Genetics, Aarhus University Hospital, Aarhus N, Denmark

* Correspondence to: Ripudaman Singh. Email: rs@arcedi.com
1 Deceased.
DNA FIDELITY

- One ‘High Risk’ case

  • ‘High Risk’ Pregnancy offered Amniocentesis
  
  • Maternal Blood collected before Amniocentesis
  
  • 2 Fetal Cells enriched and identified using ARCEDI Method
  
  • WGA and Array CGH (Agilent 180k microarray) performed at Baylor College of Medicine, Houston TX.
DNA FIDELITY
- NIPT413: Mosaicism [45,X/46,X,r(X)] – array CGH

Cell 1

Kölvraa et al. 2016
(Prenatal Diagnosis)
DNA FIDEILITY
- NIPT413: Mosaicism [45,X/46,X,r(X)] – array CGH

Cell 1

Cell 2

Kølvraa et al. 2016
(Prenatal Diagnosis)
DNA FIDELITY

- NIPT413: Mosaicism [45,X/46,X,r(X)] – array CGH

Cell 1

Cell 2

Amnio

Kølvraa et al. 2016
(Prenatal Diagnosis)
DNA FIDELITY

- NIPT413: Mosaicism [45,X/46,X,r(X)] – NGS

45,X: cell 377

46,X,r(X): cell 450

Kølvraa et al. 2016 (Prenatal Diagnosis)
GOING BEYOND ANEUPLOIDIES

- Five ‘High Risk’ cases

**PRENATAL DIAGNOSIS**

**ORIGINAL ARTICLE**

On the road to replacing invasive testing with cell-based NIPT: five clinical cases with aneuploidies, microduplication, unbalanced structural rearrangement or mosaicism

Else Marie Vestergaard, Ripudaman Singh, Palle Schelde, Lotte Hatt, Katarina Ravn, Rikke Christensen, Dorte Launholt Lildballe, Olav Bjørn Petersen, Niels Uldbjerg, Ida Vogel

Accepted manuscript online: 7 September 2017  Full publication history
DOI: 10.1002/pd.5150  View/save citation
Cited by (CrossRef): 0 articles  Check for updates  Citation tools

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/pd.5150
GOING BEYOND COMMON ANEUPLOIDIES

- Five ‘High Risk’ cases

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<th>BMI</th>
<th>Indication</th>
<th>NT (mm)</th>
<th>Karyotype [hg19] on CVS (fetal sex)</th>
<th>cfNIPT †</th>
<th>Analyzed cells</th>
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<tr>
<td>1</td>
<td>36 (12+3)</td>
<td>28.4</td>
<td>iNT</td>
<td>5.2</td>
<td>arr (21)x3 (male)</td>
<td>na</td>
<td>7</td>
<td>Confirmed</td>
</tr>
<tr>
<td>2</td>
<td>40 (12+1)</td>
<td>21.8</td>
<td>aFTS (1:180)</td>
<td>1.5</td>
<td>arr (13)x2~3 (male)</td>
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<td>32 (12+5)</td>
<td>31.0</td>
<td>aFTS (1:37)</td>
<td>1.7</td>
<td>arr (2)x2~3 (60-70%) (male)</td>
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Table 1. Summary of patient characteristics, indications, karyotype on CVS, and cfNIPT results in a series of fetuses presenting with aneuploidies, microduplication and unbalanced structural rearrangement in first trimester pregnancies. BMI, body mass index; NT, nuchal translucency; iNT, increased nuchal translucency; aFTS, abnormal first trimester screening; na, not examined; † blood samples for cfNIPT and cbNIPT were all drawn shortly before invasive testing.

(Vestergaard et al. 2017; In Press)
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(Vestergaard et al. 2017; In Press)
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- Five ‘High Risk’ cases

(cbNIPD)

(Vestergaard et al. 2017; In Press)
GOING BEYOND COMMON ANEUPLOIDIES
- Five ‘High Risk’ cases

(Vestergaard et al. 2017; In Press)
GOING BEYOND COMMON ANEUPLOIDIES

- 4.9 Mb Deletion on Chr 3 (Male Fetus)

3 fetal cells

cbNIPD
GOING BEYOND COMMON ANEUPLOIDIES

- 4.9 Mb Deletion on Chr 3 (Male Fetus)

3 fetal cells

cbNIPD

CVS
CLINICAL VALIDATION - ONGOING
CLINICAL VALIDATION
- (CVS vs cbNIPD vs cfNIPT)

- Recruit ‘High Risk’ pregnancies (who are undergoing CVS) from 6 different hospitals in Denmark
- Inclusion criteria: Singleton pregnancies between the GA of 10-13 weeks, opting for CVS.
- Enrich Fetal Cells from the blood and perform cell based fetal DNA analysis on the cells
- Perform cell free NIPT
- Check whether the results from three tests correlate

Pregnancies (6 Hospitals in DK)

CVS  cfNIPT  cbNIPD
## CLINICAL VALIDATION (CVS vs cbNIPD vs cfNIPT)

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>cbNIPD</th>
<th>cfNIPT</th>
<th>CVS</th>
<th>FF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>12</td>
</tr>
<tr>
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<tr>
<td>3</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>T21</td>
<td>No-call</td>
<td>T21 (Mosaic 80-85%)</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>Normal</td>
<td>Normal</td>
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<td>3</td>
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<tr>
<td>8</td>
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<td>Normal</td>
<td>Normal</td>
<td>13</td>
</tr>
<tr>
<td>9</td>
<td>T13</td>
<td>T13</td>
<td>T13 (Mosaic 90%)</td>
<td>8</td>
</tr>
<tr>
<td>10*</td>
<td>T16</td>
<td>?</td>
<td>na</td>
<td>?</td>
</tr>
<tr>
<td>11</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>3</td>
</tr>
</tbody>
</table>
cbNIPD TEST LAUNCH IN DENMARK
TEST LAUNCH

HIGH RISK PREGNANCIES

2/3

CVS

1/3

NON-INVASIVE

CELL BASED

CELL FREE

If Abnormal

If Abnormal
IN BRIEF

• Identified the Circulating Fetal Cell Type

• Tested Markers which are highly sensitive and specific

• Robust method of enriching fetal cells

• Picked the cells and perform downstream analyses (WGA/array CGH/NGS)

• Detected Aneuploidies as well as CNVs using Fetal Cells
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