Early Detection of Ovarian Cancer Using Uterine Lavage and Duplex Sequencing

GCI G Spring Meeting TRL Research Committee JUNE 2, 2017
Paul Speiser
Medical University Vienna, Dept. Gynecologic Oncology
Molecular Oncology Group
Screening Recommendations
US Preventative Services Task Force

Grade A
There is **high certainty** that the net **benefit is substantial** - recommended

Grade: **A Recommendation**
Screening **cervical cancer** ages 21 to 65 years with Pap smear every 3 years.
Screening **colorectal cancer**: fecal occult blood testing, sigmoidoscopy, or colonoscopy, in adults, beginning at age 50

**Innovative Concepts for Screening**

Detection of TP53 Mutations in Tampons of HGSC advanced stage patients 3 pos. out of 5  (60%)

DNA from Liquid Pap to detect Ovarian Cancer
22 Patients (4 early stage) 9 pos. (2 early stage)  41%

Uterine Lavage to detect Müllерian Duct Carcinomas
30 Patients (3 early stage) 24 pos. (3 early stage) 80%

[www.uspreventiveservicestaskforce.org](http://www.uspreventiveservicestaskforce.org)
Lavage of the Uterine Cavity for Molecular Detection of Müllerian Duct Carcinomas: A Proof-of-Concept Study

LUSTIC – Lavage of the Uterine cavity for diagnosis of Serous Tubal Intraepithelial Carcinoma

Study aim:

• Are exfoliated cells from STICs/occult carcinomas transported into the uterine cavity?
• Is it possible to collect those cells via uterine lavage and detect them in the lavage fluid?

→ Earlier diagnosis of OC, or even its precursor lesions
→ Monitoring of HROC patients
Study protocol:

Uterine lavage → Histopath. examination
Study protocol:

Uterine lavage → Histopath examination

STIC microdissection

TP53 Sequencing

Design a specific ddPCR assay

TP53 mutation analysis of lavage sample
<table>
<thead>
<tr>
<th>Institution</th>
<th>LUSTIC</th>
<th># Patients</th>
<th>Positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klinikum Essen Mitte</td>
<td>✓</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Medical University of Graz</td>
<td>✓</td>
<td>53</td>
<td>1 ✓ Tissue analysis</td>
</tr>
<tr>
<td>Charles University Prag</td>
<td>✓</td>
<td>59</td>
<td>1 × Foll. phase</td>
</tr>
<tr>
<td>Hamburg-Eppendorf Universitätsklinikum</td>
<td>✓</td>
<td>5</td>
<td>1 ✓ Lavage analysis</td>
</tr>
<tr>
<td>Catholic University Leuven</td>
<td>✓</td>
<td>27</td>
<td>4 × Shipment ✓✓✓ Tissue analysis</td>
</tr>
<tr>
<td>University College London</td>
<td>✓</td>
<td>50</td>
<td>1 ✓ Tissue analysis</td>
</tr>
<tr>
<td>Trinity College Dublin</td>
<td>✓</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Radboud University Medical Center</td>
<td>✓</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Kopenhagen Rigshospitalet</td>
<td>✓</td>
<td>11</td>
<td>1 ✓ Tissue analysis</td>
</tr>
<tr>
<td>Kepler Universitätsklinikum Linz</td>
<td>✓</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Brno Masaryk Memorial Cancer Institute</td>
<td>✓</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Medical University of Vienna</td>
<td>✓</td>
<td>36</td>
<td>2 ✓ Lavage positive ✓ Tissue analysis</td>
</tr>
</tbody>
</table>

308 = 3.6%
### Applicability of the Lavage Concept for Screening Purposes

#### TRL Research Committee June 2, 2017 / Paul Speiser

<table>
<thead>
<tr>
<th>Test/Condition</th>
<th>Lavage (n = 93)</th>
<th>IUD (n = 92)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>93</td>
<td>92</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>Mean 51 (19 – 80)</td>
<td></td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>OC</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Other cancers</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Borderline</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>High-risk</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td><strong>Menopause status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-menopausal</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Post-menopausal</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td><strong>Center</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vienna</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>Essen</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Hamburg</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Pilsen</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><strong>Lavage volume out</strong></td>
<td>Mean 8.3 (2.5-10)</td>
<td></td>
</tr>
<tr>
<td><strong>Catheter insertion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Easy</strong></td>
<td>77</td>
<td>77</td>
</tr>
<tr>
<td><strong>Difficult</strong></td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td><strong>Dilation applied</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>No</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td><strong>Lavage sample collected</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

| **Skin Test**                           | 93              | 92           |
| **Lavage**                              | 93              | 92           |
| **IUD**                                 | 92              | 92           |

| **Local anesth.**                        | 0               | 0            |
| **VAS**                                  | 3               | 4            |
| **minutes**                              | 8               | 7            |
| **VAS interval**                         | 0               | 0            |
| **Local anesth.**                        | 0               | 0            |
| **VAS**                                  | 4               | 3.5          |
| **minutes**                              | 7               | 8            |

**Median**

- **Lavage**: 1.8 VAS, 6.7 minutes
- **IUD**: 1.7 VAS, 6.04 minutes
Detecting ultralow-frequency mutations by Duplex Sequencing

Scott R Kennedy, Michael W Schmitt, Edward J Fox, Brendan F Kohn, Jesse J Salk, Eun Hyun Ahn, Marc J Prindel, Kawai J Kuong, Jiang-Cheng Shen, Rosa Ana Risques & Lawrence A Loeb

Affiliations | Contributions | Corresponding author

Published online 09 October 2014 | Corrected online 22 October 2014

Ultra-deep sequencing detects ovarian cancer cells in peritoneal fluid and reveals somatic TP53 mutations in noncancerous tissues


*Department of Pathology, University of Washington, Seattle, WA 98195; **Division of Hematology and Medical Oncology, Department of Medicine, University of Washington, Seattle, WA 98195; †Department of Obstetrics and Gynecology, University of Washington, Seattle, WA 98195; and ‡Department of Statistics, University of Washington, Seattle, WA 98195

Edited by Philip C. Hanawalt, Stanford University, Stanford, CA, and approved March 31, 2016 (received for review January 25, 2016)
Duplex Sequencing removes technological background

Starting DNA molecule

Top and bottom strands amplified and sequenced. PCR copies are grouped by unique tag sequence and strand

Errors removed by comparing the sequences of PCR duplicates

Individual strand consensus sequences are compared to eliminate remaining errors

Increase signal
Decrease noise

> 10,000-fold error reduction
Tumor TP53 mutation is identified in 80% of ovarian cancer patients, >10-fold over biological background mutation

Low-frequency TP53 mutations are prevalent in women with and without ovarian cancer: “biological background”
Biological background *TP53* mutations in uterine lavage from cases and controls increase with age.

![Graph showing the increase in TP53 mutations with age in cancers and controls](image)

<table>
<thead>
<tr>
<th>CANCERS - ALL MUTATIONS</th>
<th>CONTROLS - ALL MUTATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of TP53 Biological Background Mutations</td>
<td>Frequency of TP53 Biological Background Mutations</td>
</tr>
<tr>
<td>age</td>
<td>p=0.003</td>
</tr>
</tbody>
</table>

30 40 50 60 70 80 90

1.0E-07 2.0E-06 3.0E-06 4.0E-06 5.0E-06 6.0E-06

**Medical University of Vienna**

GCIG Spring Meeting TRL Research Committee JUNE 2, 2017 / Paul Speiser

Department Gynecologic Oncology, Molecular Oncology Group
Background mutations are not random.

Exonic background mutations by class

- Frameshift: 0.1
- Nonsynonymous: 0.9
- Splicing: 0.0
- Stopgain: 0.0
- Synonymous: 0.0

Cancer cases: Dark blue, Control cases: Light green

Proportion of mutations

Mutations are disproportionately non-synonymous

Background mutations by pathogenicity

- Inactive: 0.0
- Partial activity: 0.0
- No loss of activity: 0.0

Cancer cases: Dark blue, Control cases: Light green

Mutations are disproportionately pathogenic
Background mutations are not random

Distribution of uterine lavage TP53
"Biological Background" mutations

Distribution of TP53 mutations among all cancers in COSMIC
TP53 mutations in normal GU tissues

Mutation load by tissue type

- Healthy 54 year old

- Endometrium
- Cervix
- Fallopian tube
- Ovary
- Myometrium
- Leukocytes
- Uterine lavage

Mutations per basepair
TP53 mutations in normal GU tissues

- Healthy 54 year old
- Average of 52-57 year olds

Mutations per basepair

Endometrium, Cervix, Fallopian tube, Ovary, Myometrium, Leukocytes, Uterine lavage
Next steps...

- Analysis of „healthy tissue“

- SBIR Grant:
  Phase I
    Ideal sample preparation
    Validate sensitivity and reproducibility of high-throughput DS

  Phase II
    *TP53* Sequencing of uterine lavage samples of
    100 average risk women with HGSC
    100 average risk cancer-free controls
    15 high-risk *BRCA* 1/2 mutation carriers with microscopic HGSC
    100 high-risk *BRCA* 1/2 mutation carriers controls
The Early Detection Research Network (EDRN) June 7, 2017
Presentation to the Breast/GYN Collaborative Group

LOVE Trial - Lavage of the uterine cavity and fallopian tubes for OVarian cancer Early detection

Sensitivity & Specificity lavage concept in detecting STIC/occult EOC

2160 HBOC women, 6-monthly screen, 5%, 108 occult HGSC or STIC

FDA Presubmission Meeting, Washington
May 2016

FDA has determined that your proposed clinical investigation is a nonsignificant risk (NSR) device study because it does not meet the definition of a significant risk (SR) device under § 812.3(m) of the investigational device exemptions (IDE) regulation (21 CFR 812).
Acknowledgements

University of Washington
Larry Loeb
Rosana Risques
Katy Loubet-Senear
Loeb lab
Risques lab
Kennedy lab

TwinStrand Biosciences Inc.
Jesse Salk

Medical University of Vienna
Molecular Onvology Group
Robert Zeillinger
Elisabeth Maritschnegg

Isaac Kinde, Yuxuan Wang,
Lius Diaz, Kenneth Kinzler, Nickolas Papadopoulos, Bert Vogelstein

MUG, Universitätsklinik f. Frauenheilkunde u. Geburtshilfe, Gunda Pristauz, Karl Tamussiono – Graz, Austria
Klinikum Essen Mitte, Department Gynecology, Gynecologic Oncology, Florian Heitz, Andreas du Bois – Essen, Germany
Charité University - Campus Virchow Clinic, Department of Gynecology, Jalid Sehouli – Berlin, Germany
Charles University, Department of Obst.&Gyn., Gyn. Oncology Center, David Cibula – Prague, Czech Republic
Charles University, Department of Obst.&Gyn., Dr. Jiri Bouda, Dr. Alena Bartakova – Pilsen, Czech Republic – 16 Samples
University Medical Center Hamburg-Eppendorf, Department of Gynecology, Sven Mahner – Hamburg, Germany
Catholic University Leuven, Division Gynaecological Oncology, Ignace Vergote – Leuven, Belgium
The UK Familial Ovarian Cancer Screening Study (UK FOCSS), UCL, Martin Widschwendter – London, UK
Radboud Universiteit/Research Inst. Oncology, Univ. of Nijmegen Leon Massuger, – Nijmegen, Netherlands
Trinity College, St James’s Hospital, Department Gynaecologic Oncology, Noreen Gleeson – Dublin, Ireland
University of Copenhagen, Rigshospitalet, Department of Gynaecology, Mikkel Rosendahl – Kopenhagen, Denmark