Experience from TransPORTEC

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Management of endometrial cancer – past 20 yrs

- Move from ‘same for all’ to selected treatment approach
- Randomised trials for adjuvant therapy
- Increasingly risk-based treatment selection
- Current focus on
  - effective adjuvant therapy for high-risk disease
  - molecular basis of endometrial cancer development and new targets for therapy
  - individual risk assessment and treatment directed by molecular characteristics
Stage I intermediate risk (n=714):

- grade 1 or 2 with $\geq 50\%$ invasion
- grade 2 or 3 with $< 50\%$ invasion
- TAH-BSO without lymphadenectomy

\[
\begin{align*}
\text{pelvic radiotherapy (46 Gy)} & \\
\text{R} & \\
\text{no further treatment} &
\end{align*}
\]
Stage I-IIA endometrial carcinoma, $N = 427$

- **High**-intermediate risk factors
- Hysterectomy and BSO

pelvic radiotherapy

vaginal brachytherapy

PORTEC-2 trial (2000-2006)

Nout et al, Lancet 2010
In total **947 (85%)** endometrioid endometrial tumour tissues from PORTEC-1 and -2 trials obtained for biobank.

Strong clinical and pathology collaboration in NL

After central pathology review:

- **283 Low-/low-intermediate risk**
- **588 High-intermediate-risk**
- **76 High-risk**
Quantification of LVSI in PORTEC-1 and 2 (n=954)

Risk of distant metastases by LVSI

Substantial LVSI: HR 4.6
Mild LVSI: HR 2.2
Quantification of LVSI in PORTEC-1 and 2

Risk of pelvic recurrence

All 954 patients

Substantial LVSI (5%)

Substantial: HR 6.1 (2.3-15.9)

p<0.001

p=0.08

Nout et al, ASTRO 2014; Bosse et al, EJC 2015
L1-CAM

L1-CAM strong negative prognostic factor
- About 7-10% overall L1CAM+
- More often L1CAM+ in grade 3, p53+, NEEC
- Confirmed in large ENITEC series (1200)

Zeimet et al 2013
Bosse et al 2014
Molecular characteristics of endometrial cancer

TGCA, Kandoth et al, Nature 2013
The Cancer Genome Atlas

Limitations:

- Heterogenous cohort (stage I: 70%, stage II-IV: 30%)
- Variable adjuvant treatment (RT 20%, CT 10%, RT+CT 10%, unknown 60%)
- Small numbers
- Not a practical approach
- Validation needed
PORTEC-1 and -2 translational studies

• Improved risk assessment of endometrial cancer by comprehensive analysis of molecular factors
• High concordance of molecular tumor alterations between pre-operative curettage and hysterectomy specimens
• Substantial lymph-vascular space invasion (LVSI) is a significant risk factor for recurrence in endometrial cancer
• L1 cell adhesion molecule is a strong predictor for distant recurrence and overall survival in early stage endometrial cancer
• Prognostic significance of POLE proofreading mutations

Molecular analysis (FFPE) - methods

• Identification of the 4 molecular subgroups by surrogate markers
  1. *POLE*: Sanger sequencing exon 9 and 13 (coverage >85% of proofreading mutations)
  2. MSI: promega MSI assay and MMR protein expression
  3. p53: IHC and *TP53* Sanger sequencing exon 5-8 in uncertain cases
  4. NSMP: -

• Analysis of potential other classifiers
  - Hotspot mutation analysis in 13 genes: Sequenom Massarray
    *BRAF, CDKNA2, CTNNB1, FBXW7, FGFR2, FGFR3, HRAS, KRAS, NRAS, PIK3CA, PPP2R1A, PTEN*
  - IHC: ARID1a, β-catenin, ER, PR, L1CAM, PTEN

• Association with outcome
  - Log rank and univariable analysis, Multivariable Cox models, AUC
Four molecular subgroups in PORTEC-1 and 2

- 861 tumours classified

- 59.0% NSMP
- 26.3% MSI
- 8.9% p53
- 5.9% POLE
- 3% Multiple classifying alterations
  - 1.5% MSI & p53
  - <1% p53 & POLE
  - <1% MSI & POLE
  - <1% MSI, p53 & POLE

Stelloo et al, Clinical Cancer Research 2016
Molecular analysis PORTEC-1 and 2 cohort (N=834)

The 4 TCGA subgroups by surrogate markers

Locoregional recurrence

Distant metastasis

Overall survival

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P-value < 0.001

Stelloo et al, Clinical Cancer Research 2016
A clinically applicable molecular-based classification for endometrial cancers

- 152 -> 143 patients evaluable
- 17% serous/mixed
- 39% low risk, 16% intermediate risk, 45% high risk
Molecular analysis PORTEC-1 and 2 cohort (N=834)

- Clinical and pathological characteristics:
  Age, grade, myometrial invasion, LVSI, treatment

- Four molecular subgroups:
  **POLE, MSI, p53** remaining

- Hotspot mutations:
  BRAF, CDKNA2, **CTNNB1** FBXW7, FGFR2, FGFR3, HRAS, KRAS, NRAS, PIK3CA, PPP2R1A, PTEN

- Protein expression:
  ARID1a, β-catenin, ER, PR, **L1CAM** PTEN
• 55% of high-intermediate risk patients reclassified to favourable
• 15% of high-intermediate risk patients reclassified to unfavourable
New PORTEC-4a trial design

- Molecular integrated vs standard indications for adjuvant treatment:

  Endometrial carcinoma
  
  Surgery and pathology diagnosis
  
  FIGO 2009 – high intermediate risk
  Stage IA (with invasion), any age and grade 3 (with or without LVSI)
  Stage IB, grade 1-2 and age > 60
  Stage IB, grade 1-2 and LVSI+
  Stage IB, grade 3 without LVSI
  Stage II (microscopic), grade 1

  Randomisation
New PORTEC-4a trial design

- Molecular integrated vs standard indications for adjuvant treatment:

  **Randomisation**

  1. **Standard treatment recommendation based on clinicopathological factors**
     - Vaginal brachytherapy
  2. **Individual treatment recommendation based on molecular pathology analysis**
     - Favourable
       - Observation (~55%)
     - Intermediate
       - Vaginal brachytherapy (~40%)
     - Unfavourable
       - External beam radiation therapy (~5%)

  **Follow-up and Quality of Life**
PORTEC-3 trial for high-risk endometrial cancer

- Stage I high risk, stage II-III, NEEC

Recruitment completed December 2013
Pooled randomised NSGO/EORTC/Iliade trials
Radiotherapy +/- Chemotherapy

Progression free survival

Overall survival

Hogberg et al, EJC 2010

Serous and clear cell cancers in NSGO/EORTC trial (33%)

PFS 69 vs 78%, p=0.009
OS 75 vs 82%, p=0.07
PORTEC-3 trial – toxicity and quality of life

de Boer et al, Lancet Oncology 2016
TransPORTEC Consortium

• International collaboration
• PORTEC-3 participating groups
• Clinical PI, pathologists, scientists, stats
• Central PORTEC-3 biobank
• Joint projects with different work packages
• Ultimate aims
  - Molecular prognostic factors
  - Predictors for efficacy of chemotherapy
  - Immune response
  - New targets for therapy
  - In depth understanding of driver mutations, mechanisms of cancer development and spread
TransPORTEC Consortium

- Central biobank, well defined processes and QA

TransPORTEC participants

Collect tumorblocks
~500 cases

Distribute TMA and DNA

 Courtesy of Tjalling Bosse
TransPORTEC Biobank - workflow

1 tumorbloclk per case with PORTEC-3 number

REGISTRATION
PORTEC-3 DATABASE

- Database with PORTEC-3 numbers
- Unique ID ensures confidentiality
- Patient ID is maintained in a locked area

Processing - Step 1
HE & 15-20 blancs

QUALITY CONTROL
GYN-PATHOLOGIST

- Quality score in database
- Indicate tumor, tumor/stroma and normal for TMA and DNA isolation

Processing – Step 2
TMA en DNA

PORTEC-3 Biobank (approx. 450 tumor samples)

<table>
<thead>
<tr>
<th>I. PORTEC-3 TMA</th>
<th>II. PORTEC-3 DNA</th>
<th>III. PORTEC-3 slides</th>
<th>IV. PORTEC-3 stock</th>
</tr>
</thead>
<tbody>
<tr>
<td>3x Tumor</td>
<td>10ng/ul tumor+normal</td>
<td>15-20 blanc slide</td>
<td>3x tumorcores in tubes</td>
</tr>
<tr>
<td>3x Tumorstroma</td>
<td>Storage -20°C matrix</td>
<td>Storage at -4°C</td>
<td>Storage at -4°C</td>
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</table>
• Meetings twice yearly
• Started with collection of TransPORTEC-pilot series (n=100) test of biobanking and distributing of material to groups
• Collection and shipping of PORTEC-3 tissue samples
• Problems and issues
  - Consent for donation of tissues sample at the time of patient consent -> in principle this was clear from the start
  - Some groups had to check again for patient consent, and have a MTA with each center despite this being in the PORTEC-3 CTA
  - Some pathology labs refused to participate despite patient consent
  - Range of 0 to 97% completeness of biobanking, overall ~63%
  - Delays with identification, collection, MTA, shipping .....
TransPORTEC pilot studies

- 3 joint papers published

**Prediction model for regional or distant recurrence in...**

Prognostic significance of L1CAM expression and its association with mutant p53 expression in high-risk endometrial cancer

POLE in high grade / high risk EC

Church et al, JNCI 2015

Stelloo et al, Mod Path 2014

Meng et al, Gyn Oncol 2014

TransPORTEC pilot study

Multivariable HR=0.11 95%CI 0.001–0.84, \( P=0.028 \)

POLE-wildtype

POLE-mutant

POLE-wildtype

POLE-mutant

Meng et al, Gyn Onc. 2014
Immune response in *POLE*-mutant EC

Proposed mechanism how ultramutation results in a favourable prognosis

Van Gool et al, *OncoImmunology* 2015
TransPORTEC pilot studies

- 3 papers published
- 4 in press / in preparation

Topics:
- Markers of the p53 pathway further refine molecular profiling in high risk endometrial cancer
- Immunological profiling of molecularly classified high-risk endometrial cancers identifies POLE-mutant and MSI cancers as candidates for checkpoint inhibition
- DNA repair pathways and genomic instability score
- Combined molecular and immunological stratification for immunotherapeutic treatment of high risk endometrial cancer
TransPORTEC Consortium

- PORTEC-3 biobank nearly completed
- Processing started
- Plans for funding applications to be submitted at time of PORTEC-3 trial analysis
- Combined umbrella project with work packages
- Preliminary work and independent validation
Recent progress and future developments

- Towards selective use of adjuvant treatment
- Clinical trials standard with translational component
  - Standard consenting for tissue collection
  - Immediate transfer of block to central biobank with rigorous QA and expert pathology review
  - Clear collaboration rules within the group
- Clinical trials with molecular factor-directed therapy
- Individualised treatment based on integrated clinico-pathological and molecular characteristics
- Integration of molecular and immunological factors and treatment approaches
- New targets for therapy