Clinical relevance of DNA-damage response in metastatic breast and lung cancer under irradiation

Yvonne Goy, Kai Rothkamm, Cordula Petersen, Andreas Krüll, Klaus Pantel, Harriet Wikman, Kerstin Borgmann
**Circulating tumor cells**

**Number of CTCs:**
- Independent prognostic and predictive marker in several different cancer types (e.g. breast and lung cancer).
- Cut-off level: 5 cells/7,5ml blood (CellSearch®)
  - Significant shorter survival

Persistently increased number of CTCs under therapy:
- indicates particularly effective DNA repair mechanisms.
• Locoregional radiotherapy can reduce the risk of distant metastasis (e.g. in early breast cancer)

  **vs.**

• Radiotherapy can mobilize viable tumor cells into the circulation

• In early stage of fractionated radiotherapy (such as 2,0-6,0 Gy in 1-3 fractions): tumor cells are much more likely to survive if they escape into the circulation

• Irradiated tumor cells: increased genomic instability and plasticity -> can become more radioresistant

Martin et al, Nature reviews/ clinical oncology, January 2017
Working hypothesis: worse prognosis of patients with metastatic cancer is the result of a more effective DNA damage response in CTCs and in primary lymphocytes.

- **01/2017- today:** inclusion of **43 brain metastatic patients**
  - 19x breast cancer, 24x lung cancer

- **83 blood samples** *(76x CellSearch®)*
  - at least 5 patients died shortly after irradiation

- CTC status:
  - DNA repair and DNA fiber assay
    - Of CTCs as well as primary lymphocytes
  - **Increase** 2x, **Decrease** 8x, **No change** 16x
  - Only single CTCs, no clusters
Pat-ID 1007

- Brain metastatic NSCLC (female, 58y, + LYM)
- 03/2017: Stereotactic radiosurgery of four brain metastases, 1x 23,0 Gy

**CTC status:**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>1. bs</td>
<td>56 CTCs (few CTCs M30 +)</td>
<td></td>
</tr>
<tr>
<td>2. bs</td>
<td>70 CTCs (30 CTCs M30+)</td>
<td></td>
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<tr>
<td>3. bs</td>
<td>0 CTCs</td>
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</tbody>
</table>

- Systemic therapy with Pembrolizumab one week after irradiation
- 09/2017: last FU, complete remission, very good general condition
Pat-ID 1020

- Brain metastatic breast cancer (female, 56y, HER2+)
- 05/2017 Intensity modulated irradiation of two brain metastases, 7x 5.0 Gy

CTC status:

<table>
<thead>
<tr>
<th>1. bs</th>
<th>1 CTCs</th>
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<tbody>
<tr>
<td>2. bs</td>
<td>1 CTCs (weakly M30 +)</td>
</tr>
<tr>
<td>3. bs</td>
<td>0 CTCs</td>
</tr>
</tbody>
</table>

- Systemic therapy with TDM-1 inhibitor one week after irradiation
- 08/2017: 1.FU, partial remission, good general condition
Pat-ID 995

- Multiple brain metastases of breast cancer (female, 70y, also LYM, HEP)
- 02/2017 Stereotactic radiosurgery of the biggest brain metastases, 1x 23,0 Gy

<table>
<thead>
<tr>
<th>CTC status</th>
<th>Count</th>
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<tbody>
<tr>
<td>1. bs</td>
<td>19 CTCs</td>
</tr>
<tr>
<td>2. bs</td>
<td>32 CTCs (30x apoptotic)</td>
</tr>
<tr>
<td>3. bs</td>
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</tbody>
</table>

03/2017 abdominal progression, patient died in a hospice
DNA damage repair processes and replication stress in patients primary lymphocytes

**Replication processes**

<table>
<thead>
<tr>
<th>untreated</th>
<th>Irradiation 6Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>replication fork speed, kb/min</td>
<td></td>
</tr>
</tbody>
</table>

- 995: 0.8 ± 0.2
- 1007: 0.8 ± 0.1
- 1020: 0.8 ± 0.1

**DNA damage response**

- Foci positive cells (%)
  - untreated: 5%
  - Irradiation 6Gy: 50%

<table>
<thead>
<tr>
<th>untreated</th>
<th>Irradiation 6Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foci/cell</td>
<td></td>
</tr>
</tbody>
</table>

- 995: 5 (untreated), 15 (irradiated)
- 1007: 5 (untreated), 10 (irradiated)
- 1020: 5 (untreated), 10 (irradiated)

**Stalled forks**

- 995: 10.2%
- 1007: 6.8%
- 1020: 6.6%

**New oris**

- 995: 2.8%
- 1007: 0.0%
- 1020: 0.8%

(untreated)
Conclusion and Outlook

- Everything presented is ongoing work:

  DNA repair after ex vivo irradiation differs in the analyzed patient cohort, indicating that individual DNA repair capacity correlates with poor prognosis.

  Also an increase in apoptosis was observed in some patients indicating that irradiation seem to be more effective in a subgroup of patients.

- Next steps:
  - At present data collection is ongoing reaching a minimum of 50 patients at the end of the year.
  - Further we will elucidate the responsible DNA damage response pathways in CTCs after irradiation.
  - (When possible) analysis of tumor and metastasis tissue will follow.
  - Identification of patients with a more effective DNA damage response to intensify radiotherapy via PARPi/CHKi.
Thanks for your attention!

Any questions?

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