Immunomodulation by radiotherapy (RT) – mechanistic rationales for combination of RT with immune checkpoint inhibitors

Udo S. Gaipl
Head of Radiation Immunobiology
University Hospital Erlangen
Department of Radiation Oncology

Universitätsklinikum Erlangen

Bad Homburg, 16.11.2015
Temporary decrease of T cells during localized radiotherapy of patients with seminoma testes

Lymphocyte subsets as determined by two or triple color FACS analysis before, during and after radiotherapy

<table>
<thead>
<tr>
<th>Population</th>
<th>0 Gy</th>
<th>11 days (14 Gy)</th>
<th>21 days (26 Gy)</th>
<th>6 weeks post LRT</th>
<th>4 months post LRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+/CD19−</td>
<td>952 ± 187.27</td>
<td>482 ± 83.5 (P &lt; 0.01)</td>
<td>323 ± 109.46 (P &lt; 0.01)</td>
<td>426 ± 87.39 (P &lt; 0.01)</td>
<td>593 ± 118.09 (P &lt; 0.01)</td>
</tr>
<tr>
<td>CD3+/CD4+</td>
<td>562 ± 169.12</td>
<td>293 ± 95.79 (P &lt; 0.01)</td>
<td>202 ± 83.2 (P &lt; 0.01)</td>
<td>202 ± 83.2 (P &lt; 0.01)</td>
<td>333 ± 92.75 (P &lt; 0.01)</td>
</tr>
<tr>
<td>CD3+/CD8+</td>
<td>421 ± 85.84</td>
<td>191 ± 38.02 (P &lt; 0.01)</td>
<td>130 ± 62.74 (P &lt; 0.01)</td>
<td>221 ± 57.35 (P &lt; 0.01)</td>
<td>260 ± 108.06 (P &lt; 0.01)</td>
</tr>
<tr>
<td>CD3+/CD4+/CD45RA+</td>
<td>214 ± 71.03</td>
<td>86 ± 42.34 (P &lt; 0.01)</td>
<td>45 ± 21.79 (P &lt; 0.01)</td>
<td>51 ± 32.67 (P &lt; 0.01)</td>
<td>64 ± 36.73 (P &lt; 0.01)</td>
</tr>
<tr>
<td>CD3+/CD4+/CD45RO+</td>
<td>294 ± 60.28</td>
<td>152 ± 93.03 (P &lt; 0.01)</td>
<td>122 ± 41.61 (P &lt; 0.01)</td>
<td>165 ± 57.15 (P &lt; 0.01)</td>
<td>178 ± 35.79 (P &lt; 0.01)</td>
</tr>
<tr>
<td>CD3+/CD8+/CD45RA+</td>
<td>265 ± 65.61</td>
<td>109 ± 24 (P &lt; 0.01)</td>
<td>77 ± 28.44 (P &lt; 0.01)</td>
<td>105 ± 51.25 (P &lt; 0.01)</td>
<td>147 ± 50.8 (P &lt; 0.01)</td>
</tr>
<tr>
<td>CD3+/CD8+/CD45RO+</td>
<td>102 ± 49.22</td>
<td>84 ± 73.6 (P &lt; 0.01)</td>
<td>33 ± 13.3 (P &lt; 0.01)</td>
<td>42 ± 15.86 (P &lt; 0.01)</td>
<td>68 ± 40.57 n.s.</td>
</tr>
<tr>
<td>CD3−/CD19+</td>
<td>153 ± 57.4</td>
<td>27 ± 11.07 (P &lt; 0.01)</td>
<td>14 ± 6.72 (P &lt; 0.01)</td>
<td>32 ± 23.19 (P &lt; 0.01)</td>
<td>64 ± 22.23 (P &lt; 0.01)</td>
</tr>
<tr>
<td>CD3−/CD16+/CD56+</td>
<td>116 ± 70.62</td>
<td>68 ± 55.89 n.s.</td>
<td>29 ± 32.34 (P &lt; 0.01)</td>
<td>69 ± 30.3 n.s.</td>
<td>102 ± 57.48 n.s.</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1405 ± 258.84</td>
<td>650 ± 113.06 (P &lt; 0.01)</td>
<td>462 ± 115.56 (P &lt; 0.01)</td>
<td>710 ± 220.2 (P &lt; 0.01)</td>
<td>858 ± 192.03 (P &lt; 0.01)</td>
</tr>
</tbody>
</table>

*Mean counts of lymphocyte subsets levels ± standard deviation. The P-values (ANOVA analysis) for differences between pretreatment levels and the level at the respective timepoint were determined. All lymphocyte subsets are significantly reduced after 26 Gy. Only natural killer cells (CD3+/CD16+ /CD56−) and cytotoxic memory cells T-cells (CD3+/CD8+ /CD45RO+) recovered to normal values 4 months after treatment. The most pronounced decrease was detectable for B lymphocytes (CD3−/CD19+) and naive helper T-cells (CD3+/CD4+ /CD45RA+).

**T cell function slightly reduced, but within normal range.**

Universitätsklinikum Erlangen
**X-ray induced immune suppression – a matter of the view**

**Total body** irradiation of mice was performed using Cs-137 with a dose rate of 65 cGy/min. **Tregs** (CD4+CD25+Foxp3+) preferentially survive sub-lethal irradiation.
Analyses of about 40 immune cell subtypes and stem cells, morphology and additional activation markers in only 2.5 ml of whole blood.
Detailed immunophenotyping of patients with GBM during RCT

**Increase of:**
- CD8$^+$ (cytotoxic) T cells

**Decrease of:**
- CD4$^+$ T(helper) cells
Detailed immunophenotyping of patients with GBM during RCT

Decrease of:
- Natural Killer Cells
Tumor infiltrating lymphocytes (CD3+, CD8+) predict response to RCT in head and neck cancer
Characteristics of tumors

Modified from Hanahan D, Weinberg RA, Cell, 2011

- Sustained proliferation
- Avoiding growth regulation
- Immune suppression
- Immortalization
- Induction of chronic inflammation
- Invasion and metastases
- Angiogenesis
- Genomic instability and mutations
- Modified energy supply
- Cell death resistance

local

systemic
Cancer immunotherapy as fourth pillar of cancer treatment
Immune editing concept of tumors

Dunn et al., Immunity, 2004
Immune-activating properties of radiotherapy

Frey et al., Cancer Immunol Immunother, 2014
The central role of dendritic cells in initiating innate and adaptive immune responses

DAMP = danger signals

Modified according Torigoe et al., Int J Hyperthermia, 2009
Anti-tumor immune responses– role of tumor and its microenvironment including LNs

- Distinct tumor microenvironment
- Release of danger signals
- Release of attraction signals for DC
- Uptake of tumor antigens and cross-presentation (HMGB1-TLR2)
- CTL response against tumor cells
- T cell activation in LNs
Hypofractionated irradiation of syngeneic CT-26 tumors results in good local control

1,2x10^6 CT-26 cells s.c.

RT 5Gy

RT 5Gy

analysis of 3 mice of each group DAILY
Hypofractionated RT recruits APC and CD8+ T cells into the tumor (narrow time window!)

- Macrophages (CD11b high/ F4-80 pos)
  - Mock: Green line
  - RT 2x5Gy: Red line

- APCs (MHCII pos)
  - Mock: Green line
  - RT 2x5Gy: Red line

- CD8+ T-cells
  - Mock: Green line
  - RT 2x5Gy: Red line
Local RT with 2Gy improves adaptive T cell immune therapy for melanoma

Klug et al., Cancer Cell, 2013: “2Gy of X-ray recruits macrophages, which possess an iNOS+/M1 Phenotype, into the tumor that orchestrates effective T cell immunotherapy.”
Cancer Immunotherapy – Breakthrough of the year 2013

Science 20 December 2013:
Vol. 342 no. 6165 pp. 1432-1433
DOI: 10.1126/science.342.6165.1432

News

Cancer Immunotherapy
Jennifer Couzin-Frankel

Targeting the immune system, not the tumor itself!
Abscopal effect of RT – Reduction of tumor growth outside the field of irradiation

Abscopal effects:

- “primary” tumor
- “secondary” tumor (no RT)

RT: Single dose of 2 Gy
Flt3-L: DC growth factor

Demaria et al., Int J Radiation Oncology Biol Phys, 2004

Graph showing tumor weight (mg) over time (Days) with different treatment conditions: w/o, RT, Flt3-L, RT plus Flt3-L.
Cells of the adaptive immune system contribute to the induction of abscopal effects by RT

- w/o
- Flt3-L
- RT
- RT plus Flt3-L

Nude mice:
Radiotherapy before vaccination amplifies antigen specific local and systemic immune responses

Witek et al., Int J Radiat Oncol Biol Phys., 2014
Immunogenic tumor cell death forms are inducible by multimodal treatments

- **Dacarbazine (DTIC)**
- **B16 melanoma**
- **Chemo-therapeutics**
- **Hyperthermia**
- **Ionizing irradiation**
- **e.g. HMGB1 or HSP70 or ATP**
- **DAMP receptor**

1h 41,5° C 2Gy

+/- zVAD-fmk
Apoptosis and necrosis are inducible in melanoma cells – zVAD-fmk fosters necrosis

→ HT in combination with DTIC and/or RT increases apoptosis

→ HT in combination with RT increases necrosis
Necroptosis is inducible in B16 melanoma cells by zVAD-fmk
Multimodal treatments in combination with cell death modulation by z-VAD-fmk activates DCs
Activation of DCs is dependent on nucleotides and mediated via MyD88
In vivo effects of z-VAD-fmk on tumor growth – applications resembling the human situation
Combinatory treatment with zVAD-fmk significantly decreases tumor growth
Combinatory treatment with zVAD-fmk increases CD8+ T cells and DCs in the tumor
Tumor growth reduction by zVAD-fmk is dependent on the adaptive immune system
RT and/or CT may induce non-immunogenic or immunogenic cancer cell death
Dendritic cells are antigen presenting cells (APCs)
APCs deliver signals to T cells
Activated cells are inhibited via binding of CD80 or CD86 to CTLA-4

CTLA-4:
- Discovered in late 1980s by French researchers
- Avoids overreaction of the immune system
- Cytotoxic T-lymphocyte antigen 4 (CD152)
- Regulates early stage of T cell activation
- Medarex acquired rights to anti-CTLA-4 Ab in 1999
- Bristol-Myers Squibb (BMS) bought Medarex and introduced Ipilimumab (FDA approved in 2010)
Multiple co-stimulatory and inhibitory signals regulate T cells: immune checkpoints

Physiologically: maintenance of self tolerance
PD-1 Ligand is expressed on APCS, but also upregulated on tumor cells

**PD-1:**
- Discovered in early 1990s by Japanese biologist
- Molecule expressed on dying T cells
- Named therefore Programmed cell death protein 1 (CD279)
- Limits activity of T cells in peripheral tissues at time of inflammatory response (later than CTLA-4): major immune resistance mechanism in tumor microenvironment
- Oncologist D. Pardoll urged Medarex to test anti-PD1 in people
- Lambrolizumab (anti-PD-1) for advanced or non-resected melanomas (FDA approved in 2013)
Adaptive immune resistance mechanisms as basis for design of multimodal therapies

1 Inducer of antitumour immunity

Weak endogenous antitumour immune response

Increased endogenous antitumour immune response

Increased PDL1 expression on tumour cells or TAMs

Response

2 Anti-PD1

Single-agent anti-PD1

Strong endogenous antitumour immune response

PDL1 upregulation on tumour cells or TAMs

Response

Single-agent anti-PD1

Weak endogenous antitumour immune response

No PDL1 upregulation on tumour cells or TAMs

No response
Increased PD-L1 expression in tumor tissue after RT – rationale for combination with anti-PD-L1 Ab

Deng et al., J Clin Invest, 2014
Increased PD-L1 expression in tumor tissue after RT – rationale for combination with anti-PD-L1 Ab

Figure 2
IR and PD-L1 blockade synergistically amplify the antitumor effect. (A) Combination of anti–PD-L1 (αPD-L1) and IR significantly enhanced the inhibition of TUBO tumor growth. BALB/c mice were inoculated s.c. on day 0 with $1 \times 10^6$ TUBO cells. Tumors locally received one 12-Gy dose on day 14 and/or 200 μg anti–PD-L1 (clone 10F.9G2) or isotype control i.p. every three days for a total of four times. **$P < 0.01$; ***$P < 0.001$. 
Dosing schedule is critical for outcome of combined radioimmunotherapy – concurrently is beneficial

A. αPD-L1 mAb starting on day 1 of RT
B. αPD-L1 mAb starting on day 5 of RT
C. αPD-L1 mAb starting 7 days after the last dose of RT

Monitor tumor growth and overall survival + rechallenge of LTS mice

B. Percent survival

Universitätsklinikum Erlangen
Expression of PD-L1 is dependent on RCT and the tumor entity – B16-F10 (melanoma)
Expression of PD-L1 is dependent on RCT and the tumor entity – GL261-luc2 (glioblastoma)
Expression of PD-L1 is dependent on RCT and the tumor entity – CT26 (colorectal cancer)
Immune modulating irradiation as basis for combination of RT with ICI
Combination of radio(chemo)therapy with immune checkpoint or growth factor inhibitors

### TABLE 1 | Selected monoclonal antibodies and tyrosine kinase inhibitors against co-stimulatory and checkpoint molecules and growth factors that are in clinical phase I-II trials either alone or in combination with RT, CT or Immunotherapy.

<table>
<thead>
<tr>
<th>Target</th>
<th>Drug</th>
<th>Developer</th>
<th>Target disease (not all listed)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Co-stimulatory molecules</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD40</td>
<td>CP-870,893</td>
<td>Pfizer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dacetuzumab</td>
<td>Seattle Genetics, Inc.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lucatumumab</td>
<td>Novartis</td>
</tr>
<tr>
<td></td>
<td>CD134 (OX40)</td>
<td>MEDI6469</td>
<td>AstraZeneca</td>
</tr>
<tr>
<td></td>
<td>CD137</td>
<td>BM-663513</td>
<td>Bristol-Myers Squibb (BMS)</td>
</tr>
<tr>
<td><strong>Checkpoint inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CTLA-4</td>
<td>Tremelimunab</td>
<td>Pfizer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ipilimumab</td>
<td>BMS</td>
</tr>
<tr>
<td></td>
<td>PD-1</td>
<td>Nivolumab</td>
<td>BMS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pembrolizumab</td>
<td>Merck</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pidilizumab</td>
<td>CureTech Ltd</td>
</tr>
<tr>
<td></td>
<td>PD-L1</td>
<td>BMS-936559</td>
<td>BMS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MEDI4736</td>
<td>AstraZeneca</td>
</tr>
<tr>
<td><strong>Growth factor inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EGFR</td>
<td>Cetuximab</td>
<td>BMS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Panitumumab</td>
<td>Amgen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gefitinib</td>
<td>AstraZeneca</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Erlotinib</td>
<td>Genentech/Roche</td>
</tr>
<tr>
<td></td>
<td>HER2/neu receptor</td>
<td>Trastuzumab</td>
<td>Genentech/Roche</td>
</tr>
<tr>
<td></td>
<td>VEGFRs, PDGFRs, FLT-3, c-Kit, RET, CSF-1R</td>
<td>Sunitinib</td>
<td>Pfizer</td>
</tr>
<tr>
<td></td>
<td>VEGFRs, PDGFRs, RAP, FLT-3, c-Kit</td>
<td>Sorafenib</td>
<td>Bayer</td>
</tr>
<tr>
<td></td>
<td>VEGFRs</td>
<td>Axitinib</td>
<td>Pfizer</td>
</tr>
<tr>
<td></td>
<td>VEGFRs, PDGFRs, c-Kit</td>
<td>Pazopanib</td>
<td>GlaxoSmithKline</td>
</tr>
<tr>
<td></td>
<td>VEGFR-A</td>
<td>Bevacizumab</td>
<td>Genentech/Roche</td>
</tr>
</tbody>
</table>

*FDA-approved drugs.
Combination of radio(chemo)therapy with immune checkpoint inhibitors – systemic effects

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Systemic effects observed in pre-clinical and clinical studies after multimodal treatment of RT, CT, and immunotherapy.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Checkpoint</strong></td>
<td><strong>Tumor type</strong></td>
</tr>
<tr>
<td><strong>PRECLINICAL MOUSE-MODELS</strong></td>
<td></td>
</tr>
<tr>
<td>CTLA-4</td>
<td>Metastatic mammary carcinoma (4T1)</td>
</tr>
<tr>
<td></td>
<td>Metastatic mammary carcinoma (4T1)</td>
</tr>
<tr>
<td></td>
<td>Mammary carcinoma (TSA), colon carcinoma (MCA38)</td>
</tr>
<tr>
<td>PD-1</td>
<td>Melanoma (B16), renal cortical adenocarcinoma (RENCA)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>PD-L1</td>
<td>Mammary carcinoma (TUBC)</td>
</tr>
<tr>
<td>CD137 (4-1BB)</td>
<td>Lung carcinoma (M109)</td>
</tr>
<tr>
<td></td>
<td>Breast cancer (EMT6)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>CTLA-4 + CD137</td>
<td>Glioma (GL261)</td>
</tr>
<tr>
<td><strong>CLINICAL STUDIES</strong></td>
<td></td>
</tr>
<tr>
<td>Checkpoint inhibitors</td>
<td></td>
</tr>
<tr>
<td>CTLA-4</td>
<td>Metastatic melanoma (n = 1)</td>
</tr>
<tr>
<td></td>
<td>Metastatic melanoma (n = 1)</td>
</tr>
<tr>
<td></td>
<td>Melanoma with brain metastasis (n = 21)</td>
</tr>
<tr>
<td></td>
<td>mCRPC (n = 799) [NCT00861814]</td>
</tr>
<tr>
<td></td>
<td>Metastatic NSCLC (n = 1)</td>
</tr>
<tr>
<td>PD-1</td>
<td>Melanoma, NSCLC, mCRPC, colorectal cancer, and renal cancer (n = 238)</td>
</tr>
<tr>
<td></td>
<td>Advanced melanoma</td>
</tr>
<tr>
<td></td>
<td>Patients with DLBCL undergoing AHSC [NCT00533229]</td>
</tr>
<tr>
<td>PD-L1</td>
<td>Dose-escalation study in patients with NSCLC, melanoma, colorectal, renal-cell, ovarian, pancreatic, gastric, and breast cancer (n = 207) [NCT00726864]</td>
</tr>
</tbody>
</table>
Combination of radio(chemo)therapy with growth factor inhibitors – systemic effects

<table>
<thead>
<tr>
<th>Checkpoint</th>
<th>Tumor type</th>
<th>Treatment</th>
<th>Systemic effects + key mediator</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF-A</td>
<td>Advanced neoplastic carcinoma (n = 44) [NCT00408684]</td>
<td>IMRT (50–70 Gy) + CT + concurrent and adjuvant BEV</td>
<td>Local/regional PFS (83.7%) and distant metastasis-free interval (90.6%), PFS (74.7%), OS (90.9%) within 2 years median followup</td>
<td>(145)</td>
</tr>
<tr>
<td></td>
<td>Advanced colorectal carcinoma (n = 19)</td>
<td>RT (15x-3.4 Gy) + concurrent and adjuvant BEV + CT</td>
<td>CR (65.5%) and PR (21.1%) within 2 years median follow</td>
<td>(146)</td>
</tr>
<tr>
<td></td>
<td>Newly diagnosed GBM [NCT0094388]</td>
<td>RT (60 Gy) + concurrent and adjuvant TMZ + BEV or placebo (n = 459) or placebo (n = 483)</td>
<td>Improved PFS</td>
<td>(147)</td>
</tr>
<tr>
<td></td>
<td>Newly diagnosed GBM (n = 621) [NCT00884741]</td>
<td>RT (60 Gy) + concurrent and adjuvant TMZ + BEV or placebo</td>
<td>Improved PFS</td>
<td>(148)</td>
</tr>
<tr>
<td>EGFR</td>
<td>LA-HNC [NCT00004227]</td>
<td>RT with concurrent cetuximab (n = 211) or RT alone (n = 213)</td>
<td>Improved median OS</td>
<td>(149)</td>
</tr>
<tr>
<td></td>
<td>Unresectable LA-SCCHN (n = 60) [NCT00096174]</td>
<td>RCT with concurrent and adjuvant cetuximab</td>
<td>Improved median OS in HPV(+) tumors</td>
<td>(150)</td>
</tr>
<tr>
<td></td>
<td>Esophageal cancer [SRCTN47718479]</td>
<td>RCT with cetuximab (n = 129) or RCT alone (n = 129)</td>
<td>↓ Survival in cetuximab group</td>
<td>(151)</td>
</tr>
<tr>
<td></td>
<td>Unresectable NSCLC [SWOG 0033 ]</td>
<td>RCT with adjuvant gefitinib (n = 118) or placebo (n = 125)</td>
<td>↓ Survival in gefitinib group</td>
<td>(152)</td>
</tr>
<tr>
<td></td>
<td>LA-HNC (n = 66)</td>
<td>CRT + concurrent and adjuvant gefitinib</td>
<td>CR (90%), PFS (72%), OS (74%) within 3.5 years median followup</td>
<td>(153)</td>
</tr>
<tr>
<td></td>
<td>Metastatic NSCLC (n = 24)</td>
<td>SBRT + CT with neoadjuvant, concurrent and adjuvant erlotinib</td>
<td>Improved PFS and OS</td>
<td>(154)</td>
</tr>
<tr>
<td></td>
<td>Advanced cervical cancer (n = 36)</td>
<td>RCT with neoadjuvant, concurrent erlotinib</td>
<td>Improved PFS and OS</td>
<td>(155)</td>
</tr>
<tr>
<td></td>
<td>Lung adenocarcinoma with brain metastases (n = 23)</td>
<td>WBRT with concurrent and adjuvant erlotinib</td>
<td>Median local PFS 6.8 vs. 16.6 month (mOS: 6.8 vs. 16.6 month, response rate 54.84 vs. 95.65%) in RT vs. RT + E</td>
<td>(156)</td>
</tr>
<tr>
<td></td>
<td>Newly diagnosed GBM (n = 65)</td>
<td>RCT with concurrent and adjuvant erlotinib</td>
<td>Median PFS 8.2 vs. 4.9 month (mOS: 19.3 vs. 14.1 month) RCT + E vs. historical controls (only RCT)</td>
<td>(157)</td>
</tr>
<tr>
<td>EGFR + VEGF-A</td>
<td>LA-HNC (n = 27) [NCT00140556]</td>
<td>Neoadjuvant BEV and/or erlotinib concurrent CRT + BEV and erlotinib</td>
<td>CR (96%), local control (85%) and distant metastasis-free survival rate (63%), PFS (82%), OS (86%) within 3 years median followup</td>
<td>(158)</td>
</tr>
<tr>
<td>VEGF-A</td>
<td>Advanced hepatocellular carcinoma (n = 40)</td>
<td>RT with concurrent and adjuvant Sorafenib (S)</td>
<td>No improved efficacy of RT + S compared to RT alone</td>
<td>(159)</td>
</tr>
<tr>
<td></td>
<td>Newly diagnosed GBM (n = 47)</td>
<td>RCT with concurrent sorafenib (S)</td>
<td>No improved efficacy of RCT + S compared to RCT alone</td>
<td>(160)</td>
</tr>
<tr>
<td>RTK inhibitor</td>
<td>Patients with oligometastases (n = 25) [NCT00463090]</td>
<td>Sunitinib + IGRT (10 x 5 Gy)</td>
<td>Local (75%) and distant (52%) tumor control, PFS (65%), OS (71%) within 18-month median followup</td>
<td>(161)</td>
</tr>
<tr>
<td></td>
<td>Patients with metastases (n = 48)</td>
<td>Sunitinib + SBRT (10 x 5 Gy)</td>
<td>Local (75%) and distant (40%) tumor control, PFS (34%), OS (29%) within 4-year median followup</td>
<td>(162)</td>
</tr>
</tbody>
</table>

Co-stimulatory molecules

| CD40       | Advanced NHL (n = 74) or HL [NCT0067056] | Escalating doses of lucatumumab (once weekly for 4 weeks of an 8-week cycle) | Modest activity in relapsed/refractory patients with advanced lymphoma | (109) |

1, increase; ↓, decrease; NSCLC, non-small cell lung carcinoma; mCRPC, metastatic castration-resistant prostate cancer; GBM, glioblastoma multiforme; LA-HNC, locally advanced head and neck cancer; LS-SCHN, locally advanced squamous cell head and neck cancer; DLBCL, diffuse large B-cell lymphoma; NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma; SBRT, stereotactic body radiation therapy; SABR, stereotactic ablative RT; IMRT, intensity modulated radiation therapy; IGRT, image-guided radiotherapy; WBRT, whole brain radiotherapy; APHSC, autologous hematopoietic stem-cell transplantation; OS, overall survival; PFS, progression-free survival; CR, complete response; PR, partial response; LR, local response; BEV, bevacizumab; R-ICE, rituximab, ifosfamide, carboplatin and etoposide; MDSCs, myeloid-derived suppressor cells.
“Big challenge”: identify most beneficial combinations and chronology of R(C)T and IT

The IS needs time to act and react
“Big challenge”: which combinations of RT and IT induce anti-tumor immunity?

- Local tumor control
  - Tumor shrinkage, proliferation stop of tumor cells, cell death induction
  - Alteration of tumor cell phenotype
  - Infiltration of immune cells into the tumor
  - Release of damage associated molecular patterns

- Conversion of immune suppressive tumor microenvironment in an activating one

- Induction of systemic anti-tumor immunity

Frey et al., Cancer Immunol Immunother, 2013

Universitätsklinikum Erlangen
Abscopal anti-tumor effects after RT plus anti-CTLA-4 in a patient with malignant melanoma

Clinical reports detailing the interaction of RT and IT are limited, but on the way

- Immunological effects of **RT alone** are mostly described in patients with **melanoma** (summarized in Barker et al., 2014)
- **DC** loaded with antigens from irradiated autologous proliferating tumor cells are superior to vaccination with antigens only (Dillman et al., 2012)
- Cave: **INF-alpha** plus **RT**: toxicities
- **IL-2** (FDA 1998) **plus RT**: phase I studies; higher single doses seem to be of advantage (Seung et al., 2012)
- **Ipilimumab** (anti-CTLA4) (FDA 2011) **plus RT**: abscopal anti-tumor effects (Postow et al., 2012)
- Currently under investigation: **anti-PD1** (CD28 superfamily) **plus RT** (Verbrugge et al., 2012)
Immunotherapy – Multiple approaches

Immunotherapy

Active

Antigen-dependent

Vaccines

Antigen-independent

Modulates T cell function

Passive (adoptive)

Anti-tumor monoclonal Antibodies

Adoptive

Checkpoints inhibitors

CTLA-4 Inhibition

PD-1 Inhibition

PD-L1 Inhibition

TLR9 activation

Cytokines

GSK1572932A
TG4010 (MUC1)
Belagenpumatucel-L (NSCLC Ag + anti TGFβ)
Tergenpumatucel-L Racotumomab (ganglioside)
Stimuvax (INSPIRE)
CIMavax (EGF)
MAGE-A3
PRAME (repression of retinoic acid)
Talactoferrin (lactoferrin to stimulate circulating DC)
TLR targeting

Anti-tumor monoclonal Antibodies

Bavituximab EGFR Inhibition

Adoptive cell transfer (T cells, NK cells, DCs)

Radio(chemo)-immunotherapy: the focused beam expands

Local radiotherapy and granulocyte-macrophage colony-stimulating factor to generate abscopal responses in patients with metastatic solid tumours: a proof-of-principle trial

In Golden and colleagues' study, the combination of granulocyte-macrophage colony-stimulating factor (GM-CSF) with radiochemotherapy resulted in abscopal responses in four (22%) of 18 patients with non-small-cell lung cancer and five (36%) of 14 patients with breast cancer. These findings emphasise that systemic anti-tumour immunity can be induced by rendering the tumour cells immunogenic. Radiotherapy alone
Immune therapy works for tumors with high mutational processes

Alexandrov et al., Nature 2013
Conclusions I: Time points and fractionation

Time point of application of check-point inhibitors:

• Concurrently or at close time point after RCT

Dose and fractionation of RT:

• No conclusive preclinical data: fractionation, hypofractionation and radiosurgery may work

• From immunological point of view: hypofractionation beneficial, since less destruction of infiltrating immune cells
Conclusions II: entities and CT agents

Suitable solid tumor entities:

- Those with high mutational processes: melanoma, lung, bladder, esophagus, colorectal, cervix, head & neck

Suitable chemotherapeutic agents:

- Those which are immunogenic: anthracyclines, oxaliplatin
Conclusions III: Translational research projects

Translational research projects:

• Detailed immunophenotyping of immune cells in the peripheral blood (early markers!)

• Selected examinations in tumor biopsies: focus on CD8+T cells, dendritic cells, NK cells, PD1 and PD-1L expression

• Analyses of immune activating danger signals (HMGB1, HSP70) and cytokines (TGF-beta, IL1-beta, INFs, …) in serum
Conclusion IV: time frame of responses differ between classical and immune therapies

- Responses of CT and TKIs within weeks

- CAVE: Responses of immune checkpoint inhibitors within months (up to six). Sometimes even initial increase of metastatic lesions due to infiltrating immune cells

- Demand of re-evaluation of response criteria (e.g.: time to progression)
Malignancies of hematopoietic origin

- Also able to co-opt their local environment in order to escape immune attack
- In selected subtypes of Hodgkin (HL) (nodular sclerosing HL) and non-Hodgkin lymphoma (NHL), the PD-1 ligands are over-expressed due to a genetic amplification of the loci encoding them (Green et al., Blood 2010)
Thank you for your attention!

IMMUNE MODULATION with ICI
Future work I

• XXX
Future work II

- XXX