Purpose or Objective: The aim of this retrospective study is to evaluate the effect of neoadjuvant radiochemotherapy on the density of CD8+ tumor infiltrating lymphocytes (TILs) of rectal adenocarcinoma, by comparison of the density of CD8+ TILs in endoscopical biopsies before and resection specimens after the therapy.

Material and Methods: In total 53 patients with locally advanced rectal cancer were studied retrospectively. Neoadjuvant treatment comprised 50.4 Gy/28 fractions external radiation with continual 5-fluorouracil. Four to six weeks after the radiochemotherapy, surgical resection was performed. Immunohistochemistry was applied to assess CD8+ expression in both the pretreatment biopsies and resected specimens.

Results: During radiochemotherapy 30 patients (57%) had increased the density of CD8+ TILs, in 18 patients (34%) decreased, in 1 patient there was no change, in 4 patients it was not possible to assess the dynamics of the density of CD8+ TILs (in 2 patients due to insufficient amount of tissue for immunohistochemical analysis and in other 2 patients due to pathologic complete response after radiochemotherapy). The median of follow-up was 75 months (6.3 years). In 2 patients resection with microscopic residual tumor (R1) was performed and for 51 patients radical resection with microscopically negative margins (R0) was performed. Downstaging after preoperative radiochemotherapy was observed in 34 patients (64%). Five-year overall survival was 56% (95%CI: 43-70%). The density of CD8+ TILs was not significant in Cox regression analysis (p=0.16) or log-rank test (p=0.16). According to chi-square test (p=0.37) there was no significant impact of the increase of the density of CD8+ TILs after radiochemotherapy on downstaging. The increase of the density of CD8+ TILs after radiochemotherapy was associated with a trend of 2.5 longer overall survival in comparison with patients with the decrease of the density of CD8+ TILs after radiochemotherapy.

Conclusion: In the present study we did not observe any predictive or prognostic significance of the density of CD8+ TILs in endoscopical biopsies before radiochemotherapy, in resection specimens after the radiochemotherapy nor in changes of the density of CD8+ TILs after radiochemotherapy. The limitation of our study is the number of patients (53). It is not excluded that in a larger number of patients predictive or prognostic significance of the density of CD8+ TILs could be detected.

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Mechanisms and abscopal effects of combined mRNA-based radioimmunotherapy in a syngenic mouse model.
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Purpose or Objective: Tumor metastasis and tumor immune evasion present major challenges of cancer treatment. Radiotherapy has been demonstrated to overcome the immunosuppressive tumor microenvironment and anecdotal reports suggest that local tumor irradiation alone may also exert systemic or abscopal anti-tumor effects by immune-response stimulation with subsequent control of non-irradiated tumor metastases. This study aimed to assess abscopal effects of radiation alone and in combination with an mRNA-based tumor vaccination in a syngenic mouse model.
Material and Methods: Syngenic C57BL/6 mice were subcutaneously injected with ovalbumin-expressing murine thymoma cells (E.G7-OVA, 3x10^5) into the right hind leg on day -13 and into the left flank on day -9. On days 0, 1 and 2, the primary tumors (right hind leg) were irradiated (IR) with fractions of 2 Gy photons by the use of a linear accelerator. The secondary tumors at the left flank were shielded and received only 1.1 ± 0.3% of the IR dose applied to the primary tumor as confirmed by film dosimetry. Twice per week, tumor length and width were measured by caliper for tumor volume calculation and vaccination groups were intradermally injected with the mRNA-based vaccine RNActive® encoding Ovalbumin beginning day 0. At the end of the experiments, the secondary tumors were analyzed for cytokine abundances by protein microarray.

Results: Primary and secondary tumors of control mice developed with similar growth kinetics. IR and combined radioimmunotherapy significantly delayed tumor growth leading to primary tumor control in 15% and 53% of mice. Importantly, in secondary tumors with starting volumes below 30mm³ radioimmunotherapy induced a significant growth delay compared to vaccination alone (p=0.002) and control group (p=0.01). IR alone delayed the growth of the secondary, unirradiated tumor in an insignificant manner. Combined radioimmunotherapy for CCL5/RANTES and CXCL9/MIG expression as compared to the other groups, both suggesting increased T-cell activation. Similar but unsignificant trends were found for CCL2/MCP-1, CCL3, IL-1α, VEGF, M-CSF and other cytokines. The secondary tumors at the left flank were shielded and received only 1.1 ± 0.3% of the IR dose applied to the primary tumor as confirmed by film dosimetry. Twice per week, tumor length and width were measured by caliper for tumor volume calculation and vaccination groups were intradermally injected with the mRNA-based vaccine RNActive® encoding Ovalbumin beginning day 0. At the end of the experiments, the secondary tumors were analyzed for cytokine abundances by protein microarray.

Conclusion: Immunotherapy can enhance radiation-induced abscopal effects in small immunogenic tumors. This effect exhibits the potential of a combined radioimmunotherapy for the control of micrometastases. The characterization of the underlying immunological processes has to await further experiments.

Conclusion:

Functional imaging for ART; biological bases and potential impact on clinical outcome

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Developments in high-precision radiotherapy by means of on-board imaging, such as IMRT and stereotactic radiotherapy, have extended the possibilities for dose escalation to tumor localizations, while de-escalating doses to surrounding normal tissues. Advances in imaging technologies allow for better differentiation of tumor extension and target localization, in addition to superior anatomical imaging possibilities, functional and molecular imaging can be utilized to convey information regarding inherent tumor characteristics relevant for prognostication and prediction of therapy response. In many different tumor types, studies have investigated the potential of especially magnetic resonance imaging (MRI) and positron emission tomography (PET) / computed tomography (CT) scan to bring various tumor features to light. Repetitive imaging of malignancies before and during treatment can give rise to response adaptive treatment as has been successfully shown by integrating 18F-Fluorodeoxyglucose (18F-FDG) PET/CT imaging in chemotherapy response evaluation of Hodgkin’s Lymphoma, in order to define the eventual radiotherapy target and dose or to avoid radiotherapy altogether. For response evaluation of Hodgkin’s Lymphoma on 18F-FDG PET/CT images, application of the internationally accepted Deauville criteria reduce interobserver variability and standardize response criteria.

In many solid tumor types, numerous mostly single-center studies have described a plethora of functional or molecular imaging characteristics for description of tumor features, prognostication and prediction purposes, radiotherapy target delineation or direction of targeted therapy. This illustrates the drive towards personalized medicine in oncology, where individual therapy and therapy adaptation are based on specific patient and tumor characteristics. PET/CT studies concerning prognostic and predictive imaging properties have focused on depiction of tumor characteristics and their changes during therapy; such as metabolism (e.g. 18F-FDG PET), hypoxia (e.g. 18F-fluoromisonidazole PET, 18F-fluorooazomycin arabinosine PET, Blood Oxygen Level-dependent MRI), proliferation (e.g. 18F-fluorothymidine PET), cell membrane synthesis (e.g. 11C-choline PET), tumor cellularity (e.g. Diffusion-weighted MRI) or tumor perfusion (e.g. Dynamic Contrast-enhanced MRI). Clinical and pre-clinical PET/CT studies have illustrated the possibility to quantify presence and abundance of targets for antibody-based therapies, such as radio-labeled cetuximab in the case of the epidermal growth factor receptor. Studies on adaptive radiotherapy based on PET/CT imaging, in e.g. head-and-neck squamous cell carcinoma and non-small cell lung cancer, have mainly focused on definition of radiotherapy-resistant tumor subvolumes relevant for dose-escalation. Longer follow up results of these studies will reveal if these therapy intensifications will lead to better disease outcomes. What such imaging studies bring forward, is that different imaging modalities with different specific biological markers will define different tumor subvolumes, mostly with different spatial and temporal properties. The challenge is to define the correct individual therapy regulations for the correct tumor within the correct timeframe. Moreover, how can one reliably quantify the imaging signal, delineate radioresistant tumor subvolumes or evaluate therapy response, if most