Tumor associated antigen uptake tracking following immuno-radiotherapy

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Background

Radiation therapy (RT) leads to immunologic cell death of cancer cells where tumor-associated antigens (TAA) is released together with immune stimulatory factors leading to priming of adaptive anti-cancer responses. Immuno-radiotherapy is a promising strategy that combines the strong anti-cancer properties of RT with synergistic immunotherapies like Toll-Like-Receptor agonists (TLRa) that further facilitates immunologic cancer recognition, rejection, and induction of anti-cancer memory.
Although presentation of tumor-associated antigen (TAA) is essential for mounting efficient anti-cancer responses, it is often inefficient due to suppressive factors present in the TME, e.g. immuno-suppressive subsets such as monocytic myeloid-derived suppressor cells (Mo-MDSCs). Overcoming the suppressive milieu is possible by treatment with TLR7 agonists to repolarize the tumor micro environment (TME) and facilitate antigen presentation leading to an anti-tumor response.

In order to evaluate the effects of immuno-radiotherapy, we tracked TAA-uptake in response to treatment using a B16.F10 tumor cell line stably expressing mCherry (a pH-stable fluorochrome). This method allows tracking of TAA in immune-subsets in both the tumor and tumor-draining lymph node (tdLN) and evaluation of the subsets responsible for antigen presentation.

Tumor-Associated Antigen Tracking

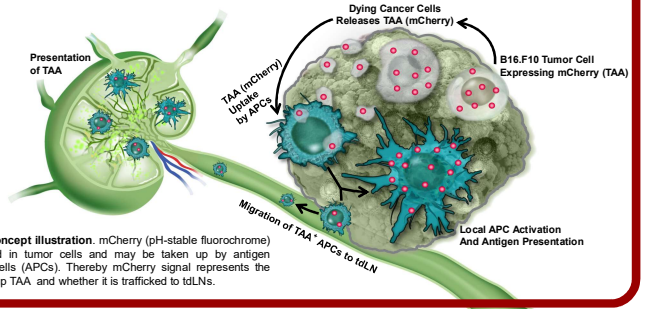

Figure 1. Concept illustration. mCherry (pH-stable fluorochrome) is expressed in tumor cells and may be taken up by antigen presenting cells (APCs). Thereby mCherry signal represents the cells taking up TAA and whether it is trafficked to tdLNs.

Immuno-Radiotherapy Induces Potent Tumor Control

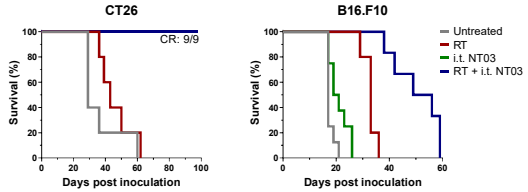

Figure 4. Immuno-radiotherapy cures tumors in the immunogenic cancer model CT26 and increases survival in the poorly immunogenic B16.F10 cancer model.
Left: Survival of CT26-bearing mice treated with 5x2 Gy local RT given as q1d combined with 4x NT03 containing TLR7a given in q7d. Immuno-radiotherapy was initiated on day 14 (mean tumor volume = 110 mm^3). n=5-9 per group. CR = complete responders.
Right: Survival of B16.F10-wildtype-bearing mice with 15 Gy local RT combined with 4x NT03 containing TLR7a given as q7d. Immuno-radiotherapy was initiated on day 9 (mean tumor volume = 110 mm^3). n=5-8 per group.

Methods

The potency of immuno-radiotherapy was determined in efficacy on CT26- and B16.F10-bearing mice (BALB/cJRj or C57BL/6JRj, respectively). Mice were inoculated with 2-3x10^5 cancer cells and treatment was initiated on day 9 and 14, respectively (mean tumor size = 110 mm^3).
Mice were treated with RT (5x2 Gy given q1d for CT26 and 1x15 Gy for B16.F10) combined with 4 treatments with a intratumoral sustained release depot containing a TLR7 agonist (RT + i.t. NT03).

TAA-tracking was performed using C57BL/6JRj mice inoculated with 5x10^5 B16.F10-mCherry (obtained from Mikala Egeblad, Cold Spring Harbor Laboratory) subcutaneously. Established tumors (mean of 100-130 mm3) mice were treated with 1x15 Gy RT and the TLR7 agonist (TLR7a) either i.v. or intratumorally in NT03 gel.

Tumors and tdLNs were excised and processed for flow cytometry.
Tumors were weighted and total cells in tumors were counted on a Muse Cell Analyzer.
Both organs were subject to 12-color flow cytometry on a 4-laser BD LSRFortessa X-20.
All populations were gated as singlet, scatter, viable, and CD45$^+$ and further characterized as:

- Macrophages (MΦ) and tumor-associated MΦ (TAMs): CD11b$^+$CD11c$^+$CD64$^+$
- Patrolling monocytes (pMo): CD11b$^+$CX3CR1$^+$CD11c$^-$Ly6g$^-$Ly6c$^-$
- Conventional dendritic cell 1 (cDC1): CD64^{Low}CD11c^{High}XCR1$^+$CD11b$^-$
- Conventional DC2 (cDC2): CD64^{Low}CD11c^{High}CD11b$^+$XCR1$^-$
- Plasmacytoid DC (pDC): CD64^{Low}Siglec H$^+$
- B cells: I-A/I-E$^+$CD11b$^-$CD11c$^-$CD64$^-$XCR1$^-$CD8$^-$
- Mo-MDSCs: CD11b$^+$Ly6c^{High}Ly6g$^-$CD11c$^-$
- Polymorphnuclear-MDSCs (PMN-MDSCs): CD11b$^+$Ly6g$^+$Ly6c^{Int}CD11c$^-$
- CD8α$^+$ T cells: CD8a$^+$XCR1$^-$
- Tumor cells: CD45$^-$mCherry$^+$

mCherry uptake was based on Fluorescence Minus One (FMO) controls using B16.F10-wildtype and reported as %mCherry$^+$ or median fluorescent intensity (MFI) for each population.

Data on all bar-plots are mean±SEM

Tumor panel	
Fluorophore	Target
BV421	I-A/I-E
BV480	CD11b
BV650	XCR1
BV711	Ly6c
BV786	CD11c
AF488	CD86
BB700	Ly6g
mCherry	TAA
PE-Cy7	CD64
APC/AF647	CX3CR1
AF700	CD45
eFluor780	Live/Dead

tdLN panel	
Fluorophore	Target
BV421	CD11c
BV480	I-A/I-E
BV650	XCR1
BV711	CD11b
BV786	CD8α
AF488	CD86
BB700	Siglec H
mCherry	TAA
PE-Cy7	CD64
APC	CD103
AF700	CD45
eFluor780	Live/Dead

Tumor-Associated Antigen Uptake in Cellular Subsets

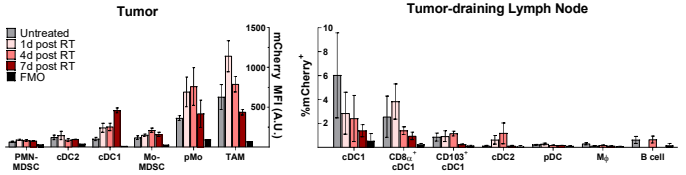

Figure 2. TAA-uptake (mCherry) is highest in pMos and TAMs in the TME and resident cDC1s in tdLNs. TAA-uptake was investigated 1, 4, and 7 days after RT.
Left: mCherry MFI in myeloid populations in tumors.
Right: Percentage mCherry positive in myeloid populations in tumor-draining lymph nodes.

Antigen Uptake is Associated with CD86 and MHC II

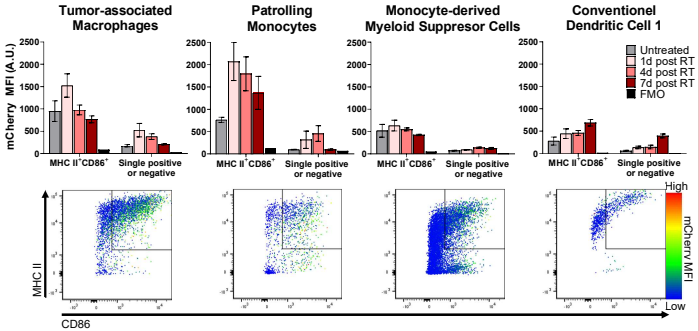

Figure 3. TAA (mCherry)-uptake is strongly associated with a MHC II$^+$ CD86$^+$ phenotype of relevant myeloid populations in the TME. TAA-uptake was investigated 1, 4, and 7 days after RT.
Top row: mCherry MFI in subsets of myeloid populations based on 15 Gy local radiotherapy. Single positive or negative refers to MHC II$^+$, CD86$^-$, and MHC II$^-$ CD86$^-$ cells.
Bottom row: Representative sample of the population plotted based on CD86 and MHC II expression with mCherry overlay

Modulation of the TME and tdLN Activation

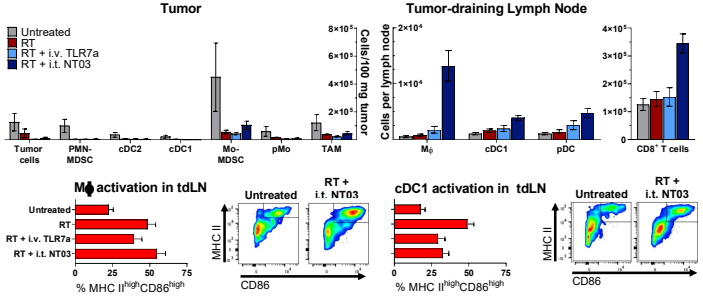

Figure 5. Immuno-radiotherapy eradicates all subsets in the tumor microenvironment and induces recruitment of myeloid and CD8α$^+$ T cells to the tumor-draining lymph node. The TME and tdLN were investigated 7 days after immuno-radiotherapy.
Top left: Cells per 100 mg tumor of relevant phenotypes after immuno-radiotherapy
Top right: Total cells of relevant phenotypes per tdLN after immuno-radiotherapy.
Bottom left: Macrophage activation in tdLNs after immuno-radiotherapy. Representative sample of MΦs from untreated and RT + i.t. NT03 tdLNs to the right of graph.
Bottom right: cDC1 activation in tdLNs after immuno-radiotherapy. Representative sample of cDC1s from untreated and RT + i.t. NT03 tdLNs to the right of graph.

Conclusion

Immuno-radiotherapy cures immunogenic cancer models and significantly increases survival in a poorly immunogenic cancer model

Immuno-radiotherapy strongly increases recruitment and activation of central myeloid subsets to tumor-draining lymph nodes.

mCherry-transduced cancer cell lines are useful models of TAA-uptake for evaluating kinetics in relation to both trafficking and therapies.

TAA-uptake is strongly associated with a CD86$^+$ MHC II$^+$ phenotypes.

TAA-uptake is strongly associated with TAMs and pMos in the tumor and primarily resident (CD8α$^+$) cDC1s in tdLNs.