

# Tumor Size Matters – Understanding Concomitant Tumor Immunity in the Context of Hypofractionated Radiotherapy with Immunotherapy

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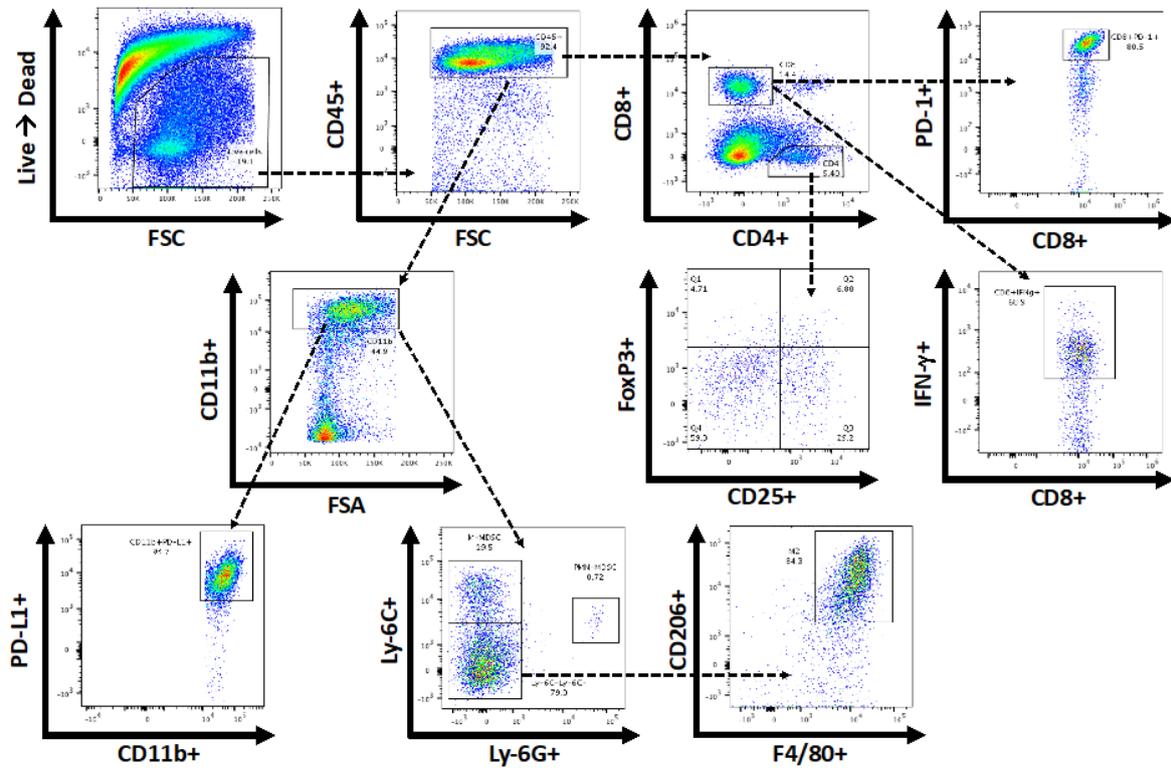
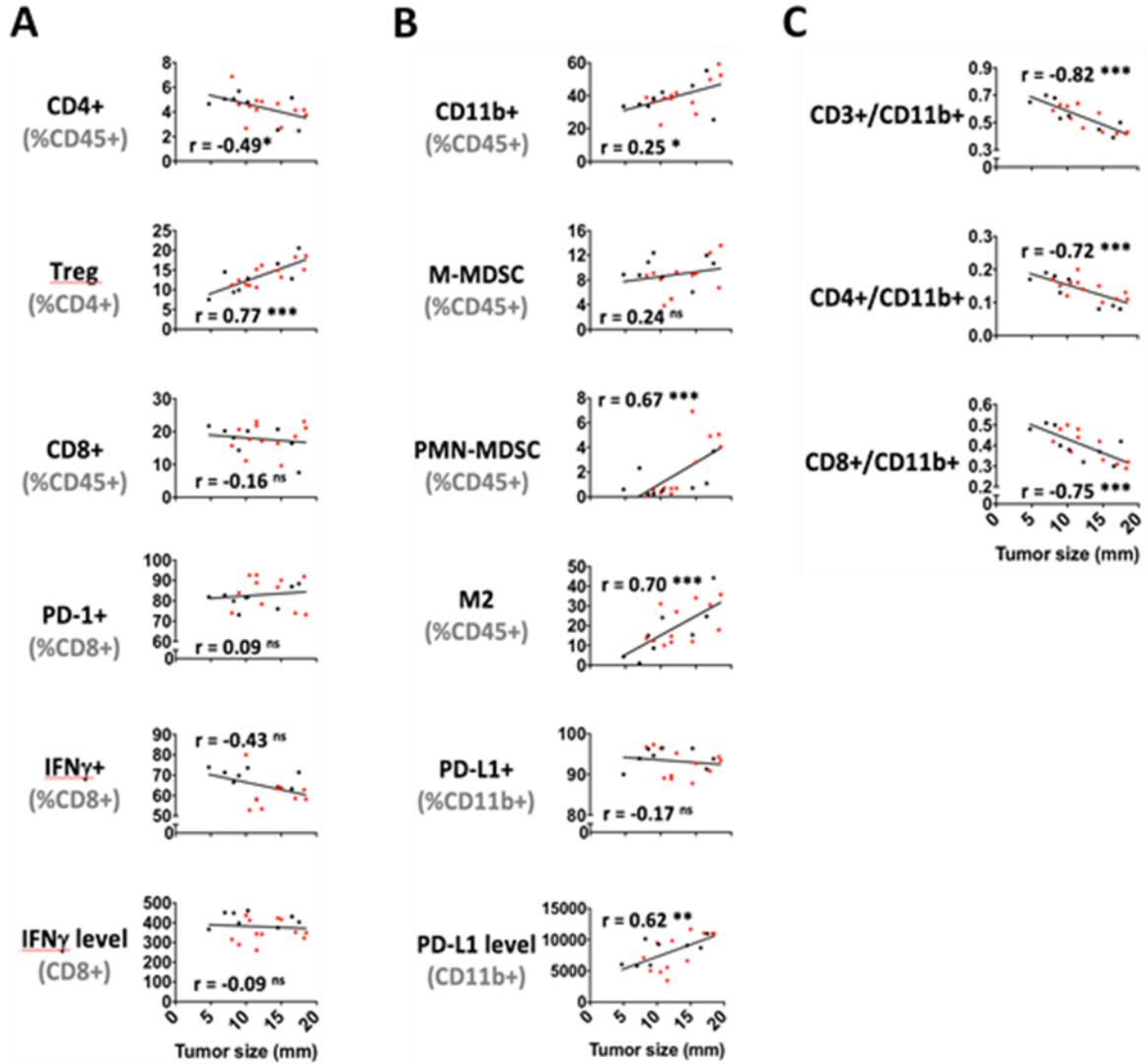
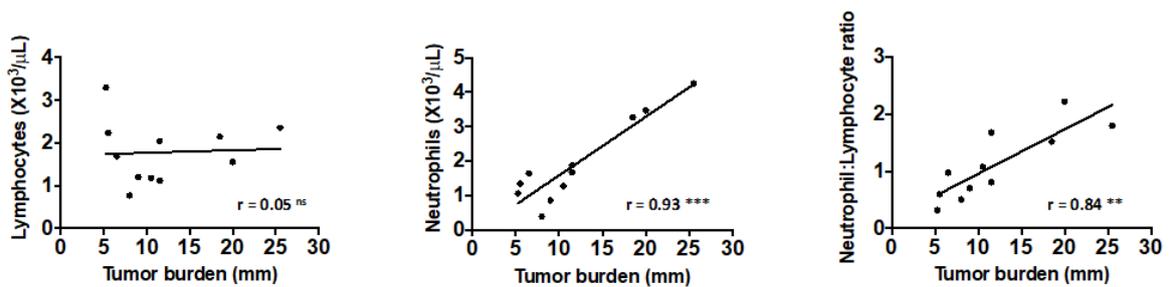


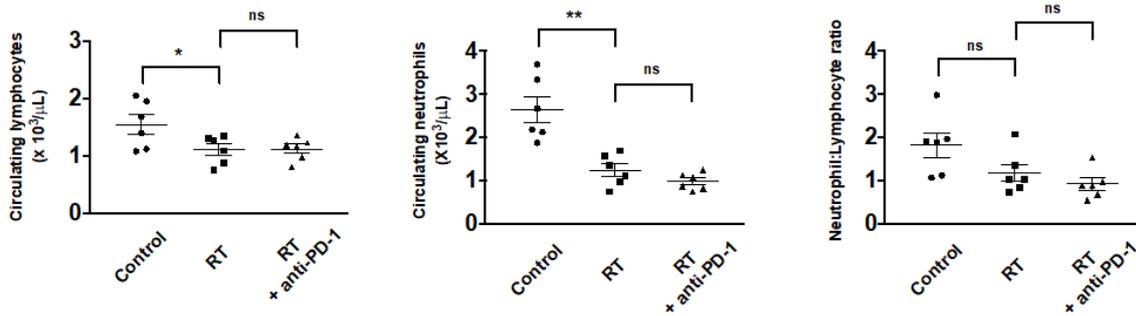
Figure S1. Multi-parametric flow cytometry gating strategies for lymphoid and myeloid cell populations.



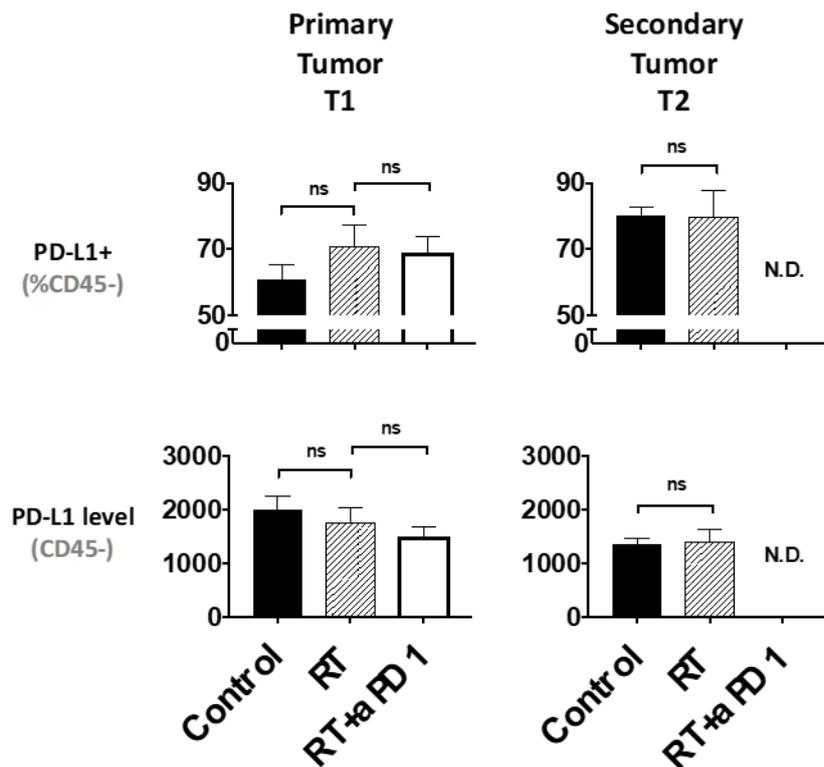
**Figure S2.** The immune cell profile in primary tumors (T1) depends on their tumor size. There is no obvious relation with the development of a secondary tumor T2. (A) Lymphoid subsets; (B) Myeloid subsets; (C) Ratio of lymphoid-to-myeloid subsets. ( $n = 20$ ). Black dots are data from T1 tumors in mice with palpable T2 tumors, red dots represent mice in which T2 did not grow.



**Figure S3.** Linear regression analysis for circulating lymphocyte counts (left), neutrophil counts (middle), and the neutrophil/lymphocyte ratio (right) versus tumor burden.  $** p < 0.01$ ;  $*** p < 0.001$ ;  $ns =$  not significant.



**Figure S4.** Impact of radiation treatment alone or in combination with PD-1 blockade on circulating lymphocyte (left) and neutrophil counts (middle), and on the ratio of neutrophils/lymphocytes (right) at d18 ( $n = 6$  per treatment group). Data are mean  $\pm$  SEM. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; ns = not significant.



**Figure S5.** PD-L1 expression on tumor cells defined as CD45<sup>-</sup> in the primary tumor T1 and the secondary tumor T2 in response to radiation therapy (RT) with or without anti-PD-1 (RT + aPD1) ( $n = 6$  per group). Data are mean  $\pm$  S.E.M. with ns not significant; ND: No Data.

**Table S1.** Phenotypic markers to define immune cells.

Cell Type	Markers
CD8 T cell	CD45+ CD8+ CD4-
CD4 T cell	CD45+ CD8- CD4+
Regulatory T cell	CD45+ CD8- CD4+ CD25+ FoxP3+
M-MDSC	CD45+ CD11b+ Ly6G- Ly6C+
PMN-MDSC	CD45+ CD11b+ Ly6G+ Ly6C(int)
M2 macrophages	CD45+ CD11b+ Ly6G- Ly6C- F4/80+ CD206+

