**Supplementary Data**

**Table S1.** Summary of radiation and chemotherapy characteristics. All radiation except in the intraoperative cases was given in the adjuvant setting.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Recurrent** | **Non-Recurrent** | **ALC < 1.3** | **ALC > 1.3** |
| **No. Patients** | 15 | 68 | 37 | 46 |
| **Radiation** | **%** | **%** | **%** | **%** |
| Unknown\* | 20 | 5.9 | 8.1 | 8.7 |
| Intraopertative Radiotherapy |  |  |  |  |
| 17 Gy, single fraction | 0 | 2.9 | 0 | 4.3 |
| Post-mastectomy Radiotherapy |  |  |  |  |
| 50.4 Gy in 28 fractions | 33.3 | 23.5 | 27 | 23.9 |
| Post-lumpectomy Radiotherapy |  |  |  |  |
| Whole Breast (45-50 Gy Gy + 10-16 Gy boost) | 40 | 61.8 | 62.2 | 54.4 |
| Partial Breast (38.5 Gy in 10 fractions) | 6.7 | 5.9 | 2.7 | 8.7 |
| **Chemotherapy** | **%** | **%** | **%** | **%** |
| None | 13.3 | 7.3 | 8.1 | 8.7 |
| Adjuvant | 46.7 | 64.7 | 54.1 | 67.4 |
| Neoadjuvant | 40 | 22.1 | 29.7 | 21.7 |
| Neoadjuvant+Adjuvant | 0 | 5.9 | 8.1 | 2.2 |
| **Chemotherapy Type** | **%** | **%** | **%** | **%** |
| Anthracycline-based regimen | 80 | 72.1 | 83.8 | 65.2 |
| Taxane | 86.7 | 85.3 | 89.2 | 82.6 |
| Gemcitabine and Carboplatin | 0 | 19.1 | 13.5 | 17.4 |
| PARP-inhibitor\*\* | 0 | 10.3 | 10.8 | 6.5 |
| Cyclophosphamide, Methotrexate, and Fluorouracil | 0 | 1.5 | 2.7 | 0 |

\*The radiotherapy regimen of patients who were not treated at Stanford hospital is unknown.

\*\*Patients who were given a PARP-inhibitor as part of a clinical trial were included.

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**Figure S1.** Viable tumor cells are present in the MFP following radiation. Enhanced *ex vivo* BLI signal from MFPs resected from Nu/Nu mice (n = 5) 10 days after RT at 20 Gy indicates viable, proliferating tumor cells.



**Figure S2.** Tumor cell recruitment is dependent on radiation dose. Dose dependence of tumor cell migration in the MFP (left), peritoneum (middle), and muscle (right) (n =12, 0 Gy; n = 5, 5 and 10 Gy; n = 10, 20 Gy). Statistical significance (\*\*\*p<0.001) was determined based on regression analysis using a GLM fit. Error bars show the 95% confidence limit.



**Figure** **S3.** Tumor growth curves and lung metastasis in mice with control (0 Gy) or irradiated (20 Gy) MFPs. 4T1 (**A**) and MDA-MB-231 (**B**) tumor growth curves in Nu/Nu mice. 4T1 tumor growth curves in BALB/c mice with antibody-depleted T cells (**C**) and treated with maraviroc (**D**). Arrow indicates time of irradiation (day 11 after inoculation for the 4T1 model and day 24 after inoculation for the MDA-MB-231 model), and error bars show the standard deviation. Lung metastasis in Nu/Nu mice (**E**) and BALB/c mice (**F**) following radiation, maraviroc treatment, and CD8+ T cell depletion (CD8-, in BALB/c mice only). Changes in lung metastases based on irradiation or treatment were not found to be significant, and the error bars show the 95% confidence limit.



**Figure S4.** Confirmation of CD4+ and CD8+ depletion in BALB/c mice using flow cytometry. **A,** Untreated spleen (n = 3). **B,** Spleen of mice treated with antibodies for CD4+ and CD8+ T cell depletion (n = 3). **C,** T-cell depletion quantification (n = 3). Error bars show the 95% confidence limit with \*\*\*p < 0.0001 as determined by ANOVA analysis.

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**Figure S5.** Baseline response to MFP radiation in BALB/c mice. **A,** Absolute lymphocyte and monocyte count in control unirradiated and irradiated mice (20 Gy) after 10 days. **B,** Mouse weight was monitored after irradiation and compared to control unirradiated mice.



**Figure S6.** Irradiation of normal cells promotes cancer cell invasion *in vitro*. MEF supernatant incubated for 2 or 7 days after irradiation promotes 4T1 (**A**) and MDA-MB-231 (**B**) cell invasion (n = 3). Error bars shown are standard deviation with \*p<0.05 and \*\*p<0.01. **C,** A Luminex assay was performed to determine the secreted cytokine profile of irradiated MEFs (n = 3). Data presented is mean fluorescence intensity (MFI) fold change compared to cytokine secretion from unirradiated MEFs. Only factors with a 1.5-fold or greater increase in MFI are shown. Black bars highlight the largest evaluated fold change. Concentrations (pg/mL) of CCL3, CCL4, and CCL5 secreted from unirradiated MEFs were 5.6, 4.3, and 103.9, respectively. The normalized MFI values are presented to account for the inherent variability in the concentration data for this assay. Error bars are reported as standard error. **D,** A Luminex assay was also performed on MFPs from Nu/Nu and BALB/c mice that were irradiated *ex vivo*. Concentrations (pg/mL) of CCL3, CCL4, and CCL5 secreted from unirradiated MFPs were 9.2, 10.6, and 427.1 for Nu/Nu and 1.5, 2.1, and 25.7 for BALB/c mice, respectively.



**Figure S7.** CD8+ T cell infiltration in irradiated normal tissues of BALB/c mice. Immunohistochemistry (IHC) was performed to detect infiltrating CD8+ T cells in BALB/c mice with (n = 10) and without (n = 8) CD8+ T cell depletion in irradiated (20 Gy) and control (n = 9; 0 Gy) MFP (**A**) and lymph nodes (**B**, LN) 10 days after RT. Scale bar is 100m. The corresponding quantification is shown in the MFP (**C**) and LN (**D**). Statistical significance determined in comparison to unirradiated control tissues. Error bars show 95% confidence limit with \*\*\*p<0.0001 as determined by a two-tailed unpaired t-test.

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**Figure S8.** Immune cell infiltration in normal tissues of Nu/Nu mice. **A,** Immunohistochemistry (IHC) was performed to detect F4/80+ macrophages in irradiated (20 Gy) and control (0 Gy) MFP and lymph nodes (n = 5). Scale bar is 100m. **B,** Quantification of infiltrating F4/80+ macrophages. Error bars show 95% confidence limit with \*\*\*p<0.001. Flow cytometry gating example for CD11b+F4/80+ macrophages in non-irradiated(**C**) and irradiated (**D**) MFPs. The box indicates the gating used for CD11b+ F4/80+ cells. **E,** Quantification of macrophage infiltration into the MFP 10 days post irradiation using flow cytometry (n = 6). Error bars show standard deviation with \*p<0.05. **F,** BLI was used to show that tumor cell migration is mitigated following irradiation by maraviroc treatment to block the CCR5 receptor (n = 9, control; n = 10, irradiated). Error bars show the 95% confidence limit. **G,** Tumor growth curves in maraviroc-treated mice with control (0 Gy) or irradiated (20 Gy) MFPs. Arrow indicates time of irradiation (day 11 after inoculation). Error bars show 95% confidence limit.



**Figure S9.** Immune cell infiltration kinetics in BALB/c mice. Time course of CD8+ T cell infiltration in the MFP (**A**) and lymph nodes (**B**, LN) of mice with (n = 10, 10 days post-RT) or without (n = 8, 10 days post-RT) CD8+ T cell depletion (n = 6, 0-5 days post-RT). Statistical significance determined in comparison to baseline infiltration 0 days post-RT. Error bars show 95% confidence limit with \*p<0.05, \*\*p< 0.01, and \*\*\*p<0.0001 as determined by a two-tailed unpaired t-test.



**Figure S10.** Secreted factors from macrophages, including CCL4, enhance 4T1 invasion and chemotaxis *in vitro*. Macrophage CM from Nu/Nu (Nu) or BALB/c mice promotes 4T1 cell invasion (**A**) and chemotaxis (**B**) when compared to complete media as determined by a transwell assay (n = 3). CCL4 neutralization (CCL4) of the CM diminished both invasion and chemotaxis. Adding recombinant CCL4 to complete medium enhanced 4T1 cell invasion. Error bars shown are standard deviation with \*p<0.05, \*\*p< 0.01, and \*\*\*p<0.0001 as determined by a two-tailed unpaired t-test. **C,** A Luminex assay was performed to determine the secreted cytokine profile of conditioned media (CM) isolated from macrophages of Nu/Nu and BALB/c mice (n = 3). The data presented is fold mean fluorescence intensity (MFI) change compared to complete media. Only the top three factors with respect to increase in MFI are shown. In terms of concentration (pg/mL), the media had low levels of MCP1 (10.74), MCP3 (0.12), and CCL4 (1.14), but macrophages secreted these factors significantly (MCP1 (3005.3, 3798), MCP3 (1093.7, 1095), and CCL4 (205.6, 547.7) for cells isolated from Nu/Nu and BALB/c mice, respectively). The normalized MFI values are presented to account for the inherent variability in the concentration data for this assay. Error bars are reported as standard error. **D,** Tumor cell migration following local CCL4 (n = 5) and systemic clodronate (n = 5) administration 10 days after RT. Statistical significance was found between the irradiated isotype control treated mice (n = 5) and all other conditions (\*p<0.05). Control unirradiated mice (n = 5) were also treated with an isotype control. Statistical significance was determined by ANOVA analysis, and error bars show the 95% confidence limit.

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**Figure S11.** Systemic factors secreted in the blood following radiation in the absence of CD8+ T cells contribute to the enhancement of macrophage migration, proliferation, and survival. A Luminex assay was performed on blood plasma in immunocompetent (CD8+), immunocompromised (CD8-), and CD8- maraviroc-treated (Maraviroc) mice (n = 5) 10 days after 20 Gy RT of normal tissues to determine the secreted cytokine profile. The data presented is fold mean fluorescence intensity (MFI) change compared to plasma in control, unirradiated mice. Only factors that have increased greater than 1.5-fold with respect to MFI in the CD8- condition and subsequently return to CD8+ levels after Maraviroc treatment are shown. Unirradiated control concentrations (pg/mL) for IL-1, MCSF, and MIP2 were 24.9, 1.42, and 13.3 for immunocompetent mice (CD8+), 53.6, 2.3, and 29.1 for immunocompromised mice (CD8-), and 34.4, 1, and 18.4 for maraviroc treated/CD8- mice. The normalized MFI values are presented to account for the inherent variability in the concentration data for this assay. Error bars are reported as standard error.