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TITLE: Dual Benefit of TGFB Inhibition on Tumor Control in the Context of Radiotherapy for Breast Cancer Brain Metastases

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CONTRACTING ORGANIZATION: Regents of University of California
San Francisco, CA 94143

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14. ABSTRACT

This project evaluates whether transforming growth factor beta (TGFβ) inhibition during radiation therapy (RT) to breast cancer brain metastases (BCBM) provides greater therapeutic benefit than RT alone using a robust proof-of-concept therapeutic protocol in combination with innovative functional imaging. Successful demonstration that TGFβ inhibition increases durable RT response that may augment immunotherapy in preclinical BCBM models would provide a strong rationale for trials of clinically viable drugs that block TGFβ signaling with gamma-knife stereotactic radiosurgery (GKSRS) for women with metastatic disease. We incorporate molecular imaging of active TGFβ to assess target levels, drug delivery, therapeutic response via tumor metabolism, and identify potential immune – mediated responses that will enable rapid clinical translation of combined RT and TGFβ inhibitory drug regimens.

15. SUBJECT TERMS-

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1. **INTRODUCTION:** This project evaluates whether transforming growth factor beta (TGFβ) inhibition during radiation therapy (RT) to breast cancer brain metastases (BCBM) provides greater therapeutic benefit than RT alone using a robust proof-of-concept therapeutic protocol in combination with innovative functional imaging. Successful demonstration that TGFβ inhibition increases durable RT response that may augment immunotherapy in preclinical BCBM models would provide a strong rationale for trials of clinically viable drugs that block TGFβ signaling with gamma-knife stereotactic radiosurgery (GKSRS) for women with metastatic disease. We incorporate molecular imaging of active TGFβ to assess target levels, drug delivery, therapeutic response via tumor metabolism, and identify potential immune – mediated responses that will enable rapid clinical translation of combined RT and TGFβ inhibitory drug regimens.

2. **KEYWORDS:** breast cancer brain metastases, transforming growth factor beta (TGFβ), immunotherapy, radiation therapy (RT), gamma-knife stereotactic radiosurgery (GKSRS), molecular imaging, positron emission computed tomography

3. **ACCOMPLISHMENTS:**
   a. **What were the major goals of the project?**

	Proposed Timeline (Months)	**Revised Timeline (Months)**	**% Complete to Date**
Specific Aim 1: Ascertain the benefit of TGFβ inhibition in preclinical immunocompetent BCBM models using targeted radiation in a small animal radiation research platform that emulates GKSRS targeted delivery and determine whether this endorses response to immunotherapy.			
Major Task 1: Evaluate TRI-Modal Therapy			
Subtask 1: Establish brain tumor metastasis models • Establish and characterize the brain metastasis models	1-3	1-6	100
Subtask 2: Establish 2 cohorts of 80 mice • Image mice using bioluminescence and ascertain tumor burden • Randomize to treatment arms • Design single fraction treatment plan for each mouse • Irradiate and monitor mice • Transfer mice for functional imaging studies • Complete imaging-based tumor response and immune modulation	3-6	6-24	80

assessments in all cohorts and collect tissue at morbidity			
Major Task 2: Correlation of biological processes with outcome			
Subtask 1: Preparation of tissues for immunoscore and tumor analysis (e.g. embedding, sectioning)	6-7	12-24	80
Subtask 2: Analyze splenic immune repertoire and circulating cells by FACS	4-6	12-24	50
Major Task 3: Replicate experiment using 2 fractionated radiation protocols			
Subtask 4: • Establish second brain metastasis model • Use optimized protocol in second brain metastasis model	12-15		25

Specific Aim 2: Characterize functional imaging methods to assess drug distribution, tumor burden and immunological response to RT			
Major Task 1: Collect imaging information as a function of time post treatment for experiment 1			
Subtask 1: Synthesize PET radiolabeled drug and optimize yields of immune-probing imaging agents	1-3	1-9	100
Subtask 2: Correlate imaging and biological responses at 7 days post treatment • Complete imaging-based tumor response and immune modulation assessments in all cohorts and collect tissue at morbidity	4-12	12-24	60
Milestone #1: *Prepare manuscript on RT responses mediated by TGF β*	12-15	24-30	50
Subtask 3: Evaluate immunological responses mediated by RT • Assess systemic and localized processes that associate with decreased tumor burden following various arms of therapy • Re-evaluate experimental design and optimize protocol	12-15	12-18	80

• Subtask 4: Assess best evidence and predictors for biological efficacy of combination.	18-24	24-30	50
Milestone #2: *Prepare manuscript on use of the TRI-MODAL therapy in pre-clinical studies*	18-24	24-36	50

b. **What was accomplished under these goals?** For this reporting period describe:
   i. Major activities

Specific Aim 1: Ascertain the benefit of TGFβ inhibition in preclinical immunocompetent BCBM models using targeted radiation in a small animal radiation research platform that emulates GKSRS targeted delivery and determine whether this endorses response to immunotherapy.

Major Activities:

- Generate and characterize two syngeneic mouse models of triple negative breast cancer (TNBC) brain metastasis.
- Image-guided radiotherapy (IGRT) of murine BCBM using the small animal radiation research platform (SARRP).
- Assessing the benefit of TGFβ inhibition in the context of IGRT
- Tumor microenvironment (TME) and immune system characterization as a function of radiotherapy and in combination with TGFβ blockade (1D11).

   ii. Specific objectives

Assessing the benefit of TGFβ inhibition in the context of IGRT. Intracranial murine models of breast cancer metastasis, 4T1-BrA, genetically modified to constitutively expressed luciferase were stereotactically inoculated into the right striatum nucleus of female syngeneic mice. Tumor growth was quantified by measuring bioluminescence (BLI) using IVIS-Xenogen. Image-guided radiation therapy (IGRT) using an Xstrahl small animal radiation research platform (SARRP) and Muriplan planning software was used to deliver a single dose of 10 Gy (sRT). Murine TGFβ neutralizing monoclonal antibody, 1D11, was administered i.p. (20 mg/kg) 24 hr before RT, and repeated every 3 days for 4 weeks. Mice were monitored by BLI and physical symptoms. A subset of mice (5 per group) were selected for brain, spleen and blood collection 6 days post-RT to characterize tumor microenvironment (TME) and immune response as a function of RT and combination treatment. Kaplan-Meier survival analysis was calculated for the remaining mice in each group. Murine brains from different treatment groups were FFPE and the immune system populations were characterized by immunofluorescence. Mice that showed complete rejection of tumor by IVIS were re-challenged with subcutaneous injections of the same tumor cells, 4T1-BrA.

TSA-BrA characterization. As previously described, we generated a second model of brain-adapted murine breast cancer cell line by inoculating TSA murine breast cancer cells into the brain of syngeneic Balb/c mice. We monitored tumor progression by BLI using IVIS and performed histology of the resulting brains at the moment of termination.

We initiated an experiment to determine latency of tumor growth with the second brain metastasis model, TSA-BrA. TSA-BrA cells were injected into the brain of Balb/c 6-7 weeks old female mice with three groups (5 mice per group) $1x10^4$, $1x10^5$, and$1x10^6$ cells. Tumor growth was monitored

with BLI and Kaplan-Meier survival curves were generated. Murine brains from different groups were collected for FFPE.

iii. Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative)

4T1-BrA intracranial tumor model During year 1 of funding we reported that combined treatment of RT and 1D11 provided benefit to fractionated RT (5 x 6 Gy) in the 4T1-BrA intracranial tumor model. In year 2, we repeated the experiments using a sub-optimal radiation dose of 10 Gy to assess whether the addition of TGFβ blockade provides long term benefit. Consistent with the results obtained in the fractionated protocol, double-treated mice bearing 4T1-BrA intracranial tumors showed significant reduction of tumor burden as measured by BLI (**Figure 1**).

Kaplan-Meier survival plots demonstrate that the double treatment was superior to RT alone as demonstrated by an increase in median survival. BLI graphs showed a decrease in tumor growth from a detectable signal to a complete regression in the RT alone and double treated group. Median survival was 17 days for control treated mice, 19 days for mice treated with 1D11, 33 days for mice treated with RT, and 41 days for double treated mice. However, 5/13 (38%) mice in the double treated group survived greater than 50 days whereas 2/12 (17%) irradiated mice did so.

Since 4T1 are highly metastatic to lung, we imaged whole lungs ex vivo by BLI to assess metastases. We found that RT alone decreased metastasis in lungs, which was decreased even more in the double-treated mice, which exhibited less lung burden (**Figure 2**).

To test for immune memory, long-term survivors were challenged with subcutaneous tumor injection. These tumors were rejected in 2/2 from RT and 3/4 mice treated with RT+1D11 (**Figure 3**). These results are contrary to the results obtained with 5 fractions of 6 Gy in which none of the long-term survivors succeeded in rejecting subcutaneous tumor re-challenge.

Notably, long term survival and demonstrable anti-tumor immunity demonstrates that blocking TGFβ is itself immunomodulatory and precludes the need for addition of an IO agent.

Our original plan was to use flow cytometry (FACS) for immune profiling, as reported in the first year. mass cytometry by time of flight (mass cytometry; CyTOF) to enable single cell resolution of up to 40 parameters in millions of cells. CyTOF combines flow cytometry with elemental mass spectrometry by using isotopes of different atomic weights to report antibody binding on single cells, rather than using fluorescence, which has been done for decades. Fluorochrome reporters can be used to identify about 15 targets, whereas CyTOF triples the content, and is more quantitative. In brief, specimens are dissociated, the single cells are incubated with a cocktail of labeled antibodies specific to selected proteins and the cytometer separates each cell from the suspension into a single droplet that is vaporized. The ion cloud is passed through a quadrupole to enrich for heavy-metal reporter ions that are separated by their charge-to-mass ratio in a time-of-flight mass spectrometer. These signals are integrated into single-cell events for analysis, in which 100,000 or more such events are compiled for each specimen.

The combination of multiplexed parameterization of many events at single cell resolution across hundreds of thousands cells provides previously unattainable capacity to study multicellular biological processes. The depth and detail as applied to the immune system, which is complex and highly heterogeneous, provides an innovative tool to analyze specimens generated in our studies.

Aw preliminary CyTOF analysis was conducted in collaboration with Dr. M. Spitzer (UCSF) using spleen samples collected five days post RT (**Figure 4**). Double-treated mice exhibited an increase in effector T cell populations, as shown by low CD27 T cells. Moreover, programmed cell death

ligand 1 (PD-L1) positive macrophages, which are known to suppress cytotoxic T lymphocyte function against tumor cells, were increased in the mice treated with RT, but this population was diminished in mice treated with RT in combination with 1D11. The scaffold maps show that there was a decrease in PDL1+ population in the combined RT + 1D11 group (red, left panel), whereas there was an increase in PD1 (blue, right panel) compared to RT alone.

TS/1-BrA intracranial tumor model

We next initiated an experiment to establish the baseline tumor growth with the second brain metastasis model, TS/A-BrA. Unexpectedly most mice injected with 1x104 and 1x105 cells did not exhibit bioluminescence and/or tumor growth, which is usually detected at 1 week post inoculation (Figure 5). However, the mice injected with 1x106 had a median survival of 27 days, with all mice dying of tumor burden. The lack of BLI suggests that either the cells have lost the reporter, that the cells were not viable and thus did not establish i.c. tumors, and/or other technical difficulties. In vitro luciferase assays demonstrated that in fact TSA-BrA cells had lost significant expression of luciferase. Currently, the TSA-BrA cells re-selected with puromycin and plan to perform additional studies with the cell line.

iv. other achievements. (Include a discussion of stated goals not met)

Completion of the objectives for second year of funding were delayed by 3 major events. The SARRP was down for approximately 12 weeks due to technical problems that resulted in loss of experimental time. In addition, Dr. Alba Gonzalez-Junca, the postdoctoral fellow who led this project on year one and half of year two, accepted a permanent research position at biotech company. I hired a new postdoctoral fellow, Dr. Luis Borrero-Garcia, who received a doctorate in Cancer Biology at the University of Puerto Rico in 2018 for his thesis research in breast cancer. He joined the lab in August and was trained by Dr. Gonzalez-Junca and lab manager Mr. William Chou. A third challenge was the erratic behavior of TSA-BrA experimental model, which required troubleshooting that identified loss of the luciferase reporter gene, also delayed experiments in the phase.

c. **What opportunities for training and professional development has the project provided?**

The project has provided the postdoctoral fellow Dr. Gonzalez-Junca the opportunity to share her work at symposiums and conferences, which included presenting at the UCSF radiology symposium, imaging conference, and breast oncology program symposium, as well as attending the AACR immunobiology of CNS meeting in February 2018. In addition, Dr. Gonzalez-Junca trained Dr. Borrero-Garcia, who is new to the field. He has now completed 2 experiments and presented the research at the UCSF Breast Oncology Program Scientific retreat. Notably he was selected for an oral presentation of the work and received an award for the abstract.

d. **How were the results disseminated to communities of interest?** The early phase of these studies were disseminated within our institution as follows:

Functional imaging platform to monitor progression and response to therapy in a pre-clinical model of BCBM. Alba Gonzalez-Junca, Denis Beckford-Vera, Niecholle Roco, Tony Hyunh, Dave Korenchan, Robert Flavell, Henry F VanBrocklin, Benjamin Franc, Mary Helen Barcellos-Hoff, UCSF Radiology Imaging Scientific Retreat 2018

TGFβ inhibition sensitizes breast cancer brain metastasis tumors to radiation treatment. Alba Gonzalez-Junca, Luis D. Borrero-Garcia, Denis Beckford Vera, Henry Van Brocklin, Benjamin Franc and Mary Helen Barcellos-Hoff. UCSF Breast Oncology Program Scientific Retreat 2019. Poster

TGFβ activation by radiation opposes immune rejection of intracranial GL261 Alba Gonzalez-Junca, Denis Beckford Vera, Henry Van Brocklin, Benjamin Franc, Renate Parry and Mary Helen Barcellos-Hoff Society of Neuological Oncology (SNO) (November 2018)- Poster presentation Mary Helen Barcellos-Hoff *This presentation reported on the use of imaging agent developed by partnering PI Dr. Benjamin Franc (#BC160513P1) and his team.*

TGFβ inhibition sensitizes breast cancer brain metastasis tumors to radiation treatment. Alba Gonzalez-Junca, Luis D. Borrero-Garcia, Denis Beckford Vera, Henry Van Brocklin, Benjamin Franc and Mary Helen Barcellos-Hoff. UCSF Breast Oncology Retreat (BOP Retreat – February 2019) – Oral presentation Luis D. Borrero-Garcia

e. **What do you plan to do during the next reporting period to accomplish the goals?**

In Year 2, we showed that effective intracranial control was achieved by a single fraction of RT of 10 Gy and TGFβ inhibition. Moreover, subsequent rejection of tumor re-challenge indicates effective intracranial tumor control can elicit immune memory. Our focus in the next term will be on repeat studies using the TSA-BrA model to evaluate the therapeutic benefit of combine treatment of TGFβ inhibition and RT. The unexpected difference between the effect of TGFβ inhibition in combination with single dose vs fractionated RT in the 4T1-BrA model warrants further exploration. TGFβ inhibition increased tumor control by both RT regimens as evidenced a median survival. However, in those mice in which tumors were eliminated, only the mice treated with a single dose were able to reject tumor rechallenge, supporting immunity as a mode of action. If confirmed upon repetition, this could suggest that GKSRS would be more effective than conventional radiation treatment plans as a means to promote immune response. Hence in NCE year 3, we plan to repeat experiments with 4T1-BrA explicitly comparing single 10 Gy versus 5 fraction of 6 Gy, as well as complete experiments with TS/A-BrA.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

a. **What was the impact on the development of the principal discipline(s) of the project?**

The development of the ^{89}Zr-DFO-fresolimumab is potentially relevant to human studies of response to therapy and can be readily moved to preliminary assessment in humans.

The demonstrable benefit of TGFβ inhibition in the context of RT for breast cancer brain metastases is clinically important.

Notably, long term survival and demonstrable anti-tumor immunity in mice bearing intracranial 4T1-BrA tumors demonstrates that blocking TGFβ is itself immunomodulatory in the context of RT, which precludes the need for addition of an IO agent as originally proposed.

b. **What was the impact on other disciplines?**

Nothing to report

c. **What was the impact on technology transfer?**

Nothing to Report.

d. **What was the impact on society beyond science and technology?**

Nothing to Report.

5. **CHANGES/PROBLEMS:**
   a. Technical problems
      i. Malfunction of SARRP needed for mouse irradiation
      ii. Cell line needed to be re-transfected with reporter
      iii. Installation of Sofie PET/CT in February but not commissioned for radioactivity by EH&S until November
      iv. These issues delayed execution of experiments and prompted the request for a no-cost extension on 10/23/18. NOTE Partnering PI, Dr. Benjamin Franc (#BC160513P1) did not request a NCE.
   b. Personnel changes
      i. Dr. Alba Gonzalez-Junca left for an industry position in July, 2018
         1. Dr. Gonzalez-Junca assisted in training new postdoc
         2. She continues to contribute to data analysis
      ii. Dr. Luis Borrero-Garcia joined August, 2018
         1. Dr. Borrero-Garcia, who received a doctorate in Cancer Biology at the University of Puerto Rico in 2018 for his thesis research in breast cancer.
         2. Dr. Borrero-Garcia completed training in September and has learned the brain metastasis model, radiation protocol and laboratory procedures
      iii. Dr. Benjamin Franc closed out the partnering grant (#BC160513P1) in imaging upon moving to a faculty position at Stanford in December

6. **PRODUCTS:** (PLEASE ALSO SEE APPENDIX A)

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
   f. What individuals have worked on the project?

Name	Role	Person Months Worked	Contribution	Funding Support
Mary Helen Barcellos-Hoff, Ph.D	PI	1.2	Designed expts and analyzed experimental data	
Alba Gonzalez-Junca, Ph.D.	Postdoctoral Fellow	5.16	Generation of pre-clinical BCBM models. Treatment and characterization.	
Luis Borrero-Garcia, Ph.D.	Postdoctoral Fellow	7	Execution of experiments and analysis of results	

William Chou	Specialist	1.8	Assistance on in vivo experiments and technical support	
Trevor Jones	Assoc Specialist	6	Assistance on in vivo experiments and technical support	

g. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

CHANGES IN OTHER SUPPORT:

PI: BARCELLOS-HOFF, MARY HELEN

Completed: Varian Medical Systems 12/01/2017 - 1/31/2019 1.2 calendar

TRI-Modal Therapy Phase 2

Pilot project focused on TGFβ inhibition during radiotherapy to define the specific conditions under which TGFβ inhibition acts in concert with immunotherapy in glioblastoma.

Role: P.I.

Added:
Varian Medical Systems 02/02/2019 - 02/01/2020 1.2 calendar
CyTOF Analytics of Systemic Immune Responses to Radiation Therapy $131,755
Role: P.I.
We propose to use mass cytometry by time of flight (mass cytometry; CyTOF) for state of the art single cell analysis to document the systemic immune response to RT in cancer patients to enable single cell resolution of up to 40 parameters in millions of cells.

R01NS109911 02/01/2019-01/31/2024 1.80 calendar
NIH/NCI $372,639
Role: MPI (Contact PI)
Reorienting the Glioblastoma Microenvironment to Respond to Immunotherapy
Goal: We hypothesize that clinically viable TGFβ blockade could both eliminate local immunosuppression by reducing MDSC viability and promote T cell infiltration by breaking down HA and TNC-driven stiff ECM.

h. What other organizations were involved as partners?
Nothing to Report.

8. **SPECIAL REPORTING REQUIREMENTS**

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to https://ers.amedd.army.mil for each unique award. QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

Dr. Benjamin Franc (#BC160513P1) did not request a NCE, and moved to Stanford University in December 2018.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report. DO NOT RENUMBER PAGES IN THE APPENDICES.

APPENDIX A: RELATED ABSTRACTS

UCSF Breast Oncology Retreat San Francisco February 19, 2019

TGFβ inhibition sensitizes breast cancer brain metastasis tumors to radiation treatment

Alba Gonzalez-Junca, Luis D. Borrero-Garcia, Denis Beckford Vera, Henry Van Brocklin, Benjamin Franc and Mary Helen Barcellos-Hoff

Department of Radiation Oncology and Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA, USA

Breast cancer brain metastases (BCBM) are associated with poor prognosis and limited therapeutic options. Current efforts focus on developing approaches to improve response to radiation therapy (RT) to test whether inhibition of transforming growth factor beta (TGFβ) improves response of brain adapted (BrA) breast cancer to radiation therapy. The rationale for this comes from previous studies that showed that TGFβ is activated in irradiated tissue affecting the composition of the tumor microenvironment and enhancing the ability of tumor cells to survive DNA damage. We first image TGFβ activity in situ using fresolimumab (GC1008), the humanized 1D11 TGFβ neutralizing antibody, radiolabeled with ^{89}Zr for PET-CT imaging (^{89}Zr-fresolimumab). Mice harboring irradiated (15 Gy) 4T1-BrA flank tumors displayed a heightened PET/CT signal compared to un-radiated tumors. We collected irradiated and non-irradiated tumors and perform dual immunofluorescence staining for active TGFβ and phospho-SMAD2. We found TGFβ intensity correlated with the radioactivity of each tumor, which shows specificity of ^{89}Zr-fresolimumab to detect TGFβ activity in vivo. Next, we tested if inhibition of TGFβ improves response of 4T1-BrA intracranial tumor models to RT. Tumor growth was quantified by measuring bioluminescence (BLI) using IVIS-Xenogen. Image-guided radiation therapy using an Xstrahl small animal radiation research platform and Muriplan planning software was used to deliver a single dose of 10 Gy (sRT) or 5 daily fractions of 6 Gy (fRT). Murine TGFβ neutralizing monoclonal antibody, 1D11, was administered i.p. and mice were monitored by BLI and physical symptoms. Combine treatment with 1D11 and RT led to an increase in median survival compared to RT alone using fRT (49 vs 31 days) or sRT (41 vs 33 days). fRT eliminated tumors in 4/9 mice whereas sRT eliminated 2/12. Double treated mice had similar response by fRT (3/8), but increased with sRT (5/13). Mice that showed complete rejection of tumor were re-challenged with subcutaneous injections of the same tumor cells. Re-challenge showed that only sRT double-treated 4T1-BrA rejected newly tumors. Effective intracranial control of BCBM was achieved by

RT and TGFβ inhibition of intracranial tumors and subsequent rejection of tumor re-challenge indicates effective intracranial tumor control can elicit immune memory.

APPENDIX B: FIGURES CITED IN TEXT

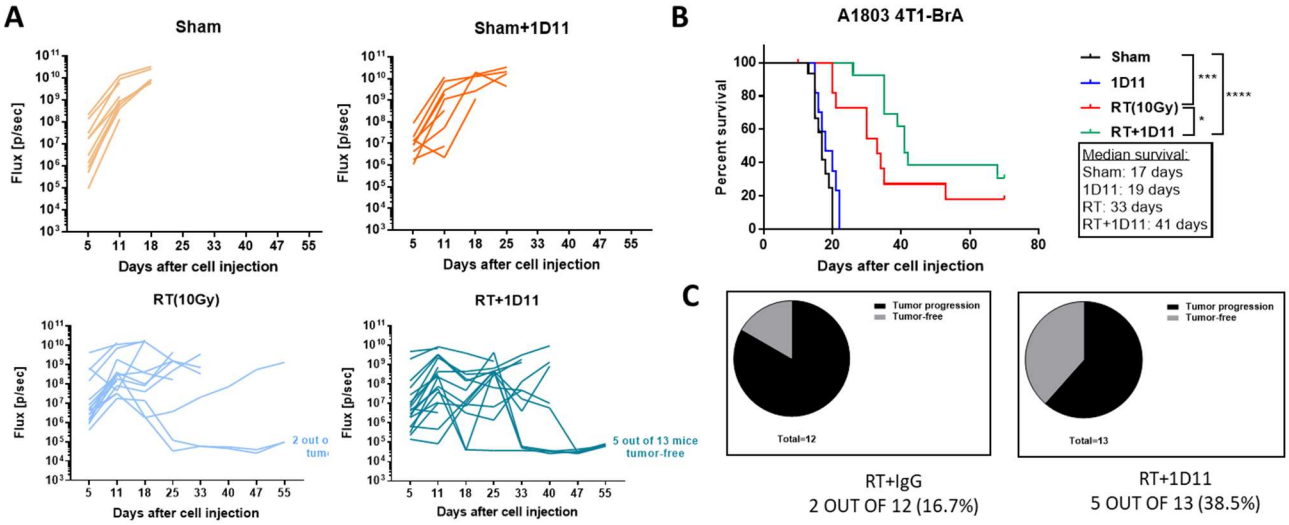

Figure 1. Treatment with RT (10 Gy) and/or IgG/1D11 in the 4T1-BrA murine model. A. Individual tumor growth curves of all mice according to treatment. B. Kaplan Meier survival plots of survival following IGRT and/or ID11. B. Tumor burden measure by BLI at 20 days. C. Frequency of tumor-free mice as a function of RT or RT and 1D11.

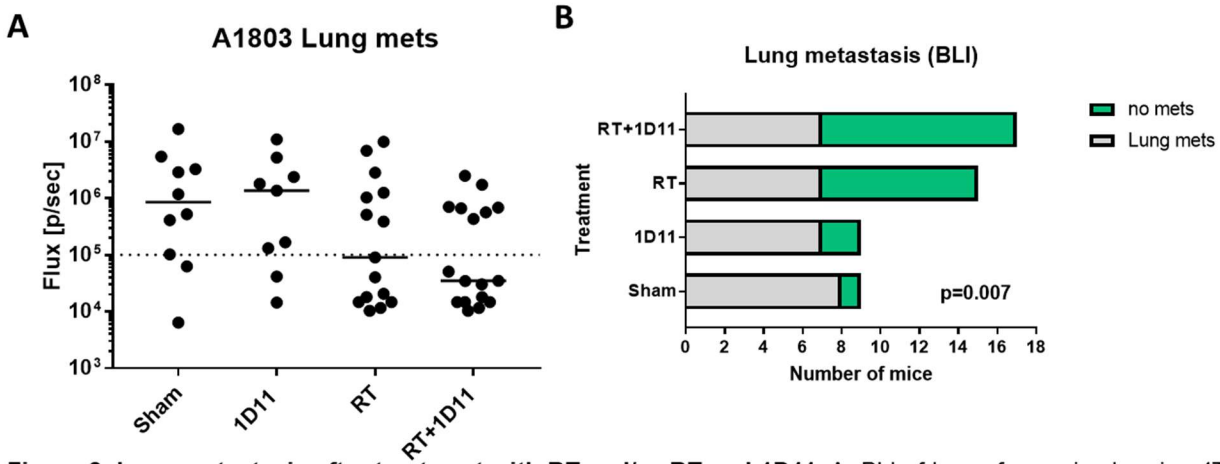

Figure 2. Lung metastasis after treatment with RT and/or RT and 1D11. A. BLI of lungs from mice bearing 4T1 tumors treated with 10 Gy RT with and without TGFβ inhibition. B. Frequency of metastasis-free mice as a function of RT or RT and 1D11.

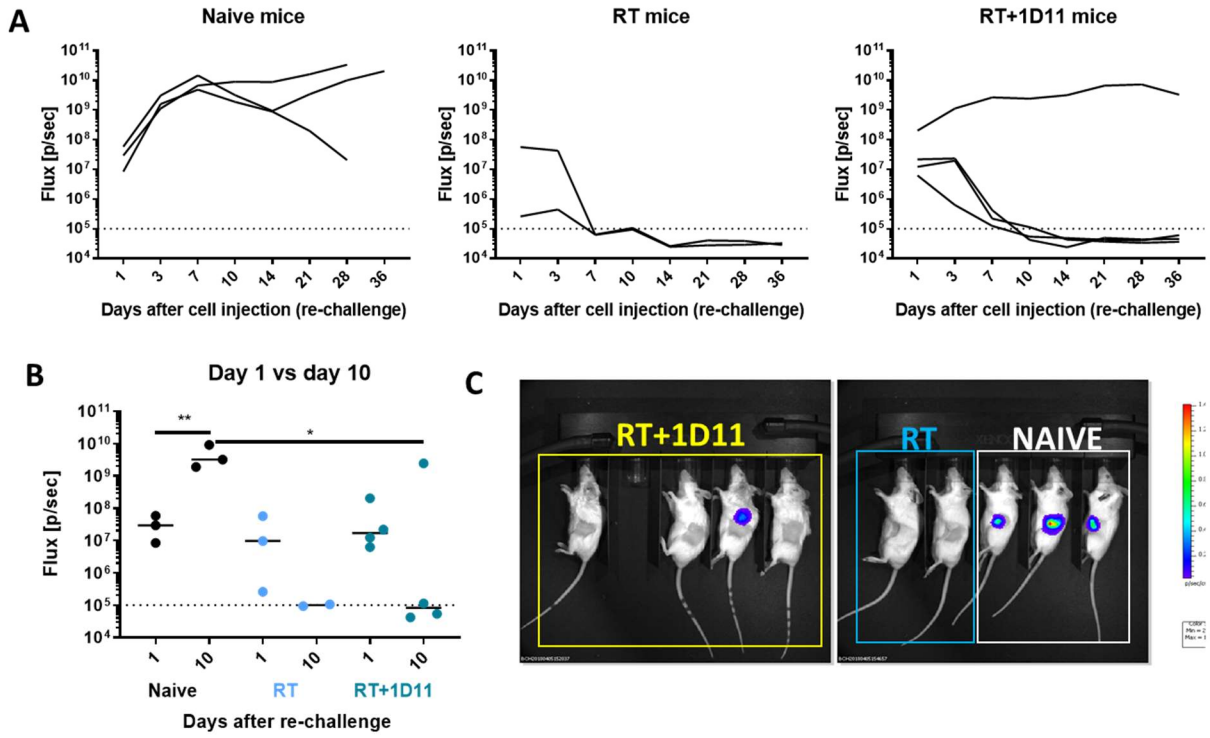

Figure 3. Rechallenge of tumor-free mice with subcutaneous tumors. A. BLI of subcutaneous tumors injection of naïve and mice who exhibited long term survival after indicated treatment B. BLI comparison of day 1 vs day 10 after rechallenge with flank tumors. C. Representative images of BLI of subcutaneous tumors.

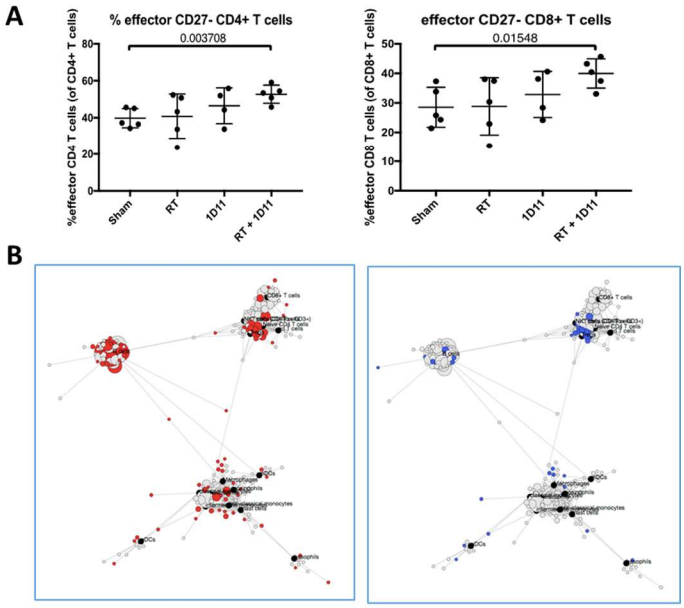

Figure 4. Preliminary CyTOF analysis of 4T1-BrA cells treated with RT (10 Gy) and /or IgG/1D11. Percentage of CD27-CD4+ T-cells and CD27-CD8+ T-cells in all treated groups. B. Scaffold maps of PD1 and PDL1 using semi-unbiased analysis where immune cells are binned into different clusters represented by the circles. The size of the circles represent number of cells in that cluster. The color (red vs. blue) represents the increase (red) vs. decrease (blue) of cells in condition 2 when compared to condition 1.