Scgb1a1+ club cells increase the efficacy of immune checkpoint blockade in lung cancer.

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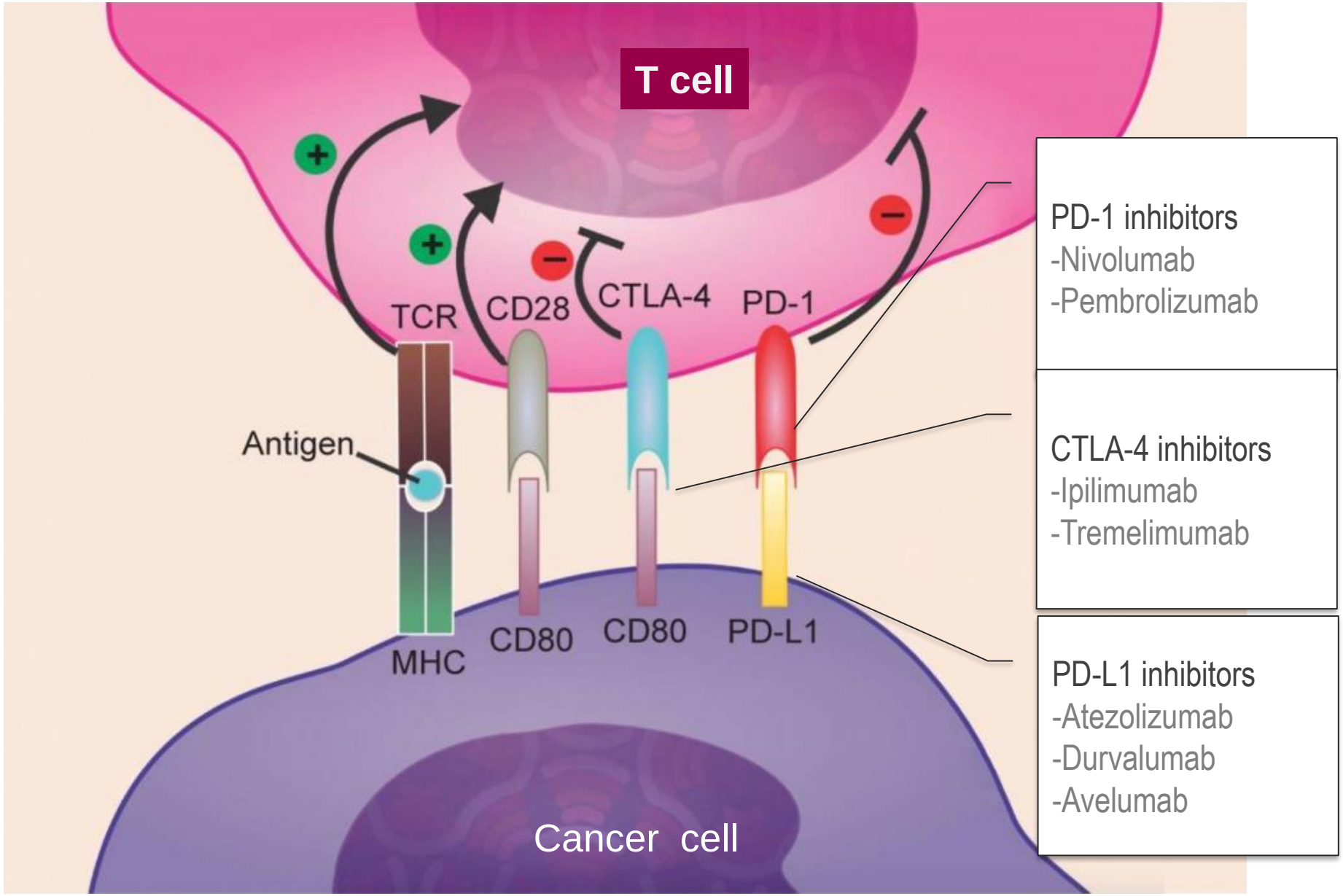
Weill Cornell Medical Center, New York

Associate Affiliate Member, Houston Methodist Research Institute Texas

Weill Cornell Medicine

Immune checkpoint blockade in advanced metastatic NSCLC



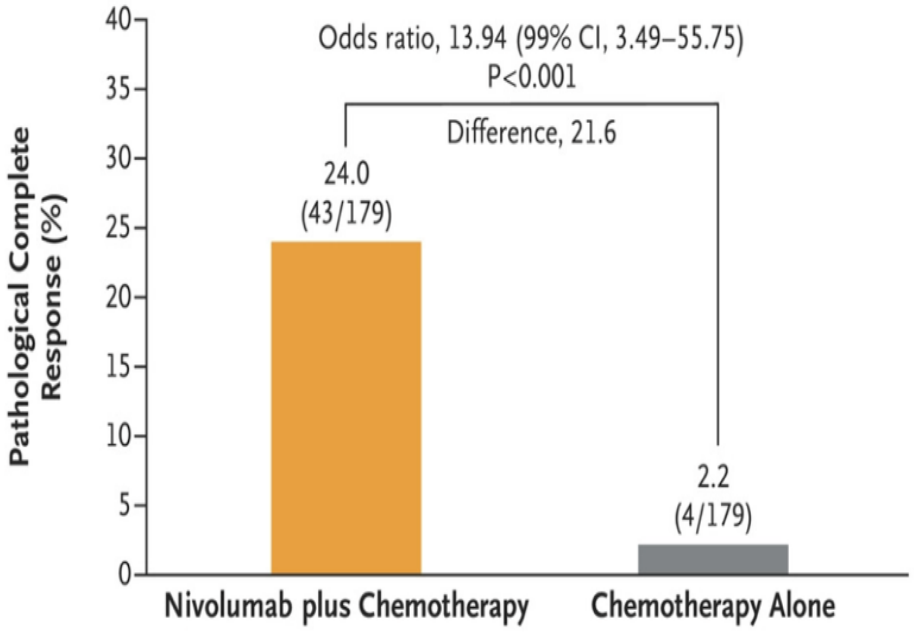
Checkmate 816 trial

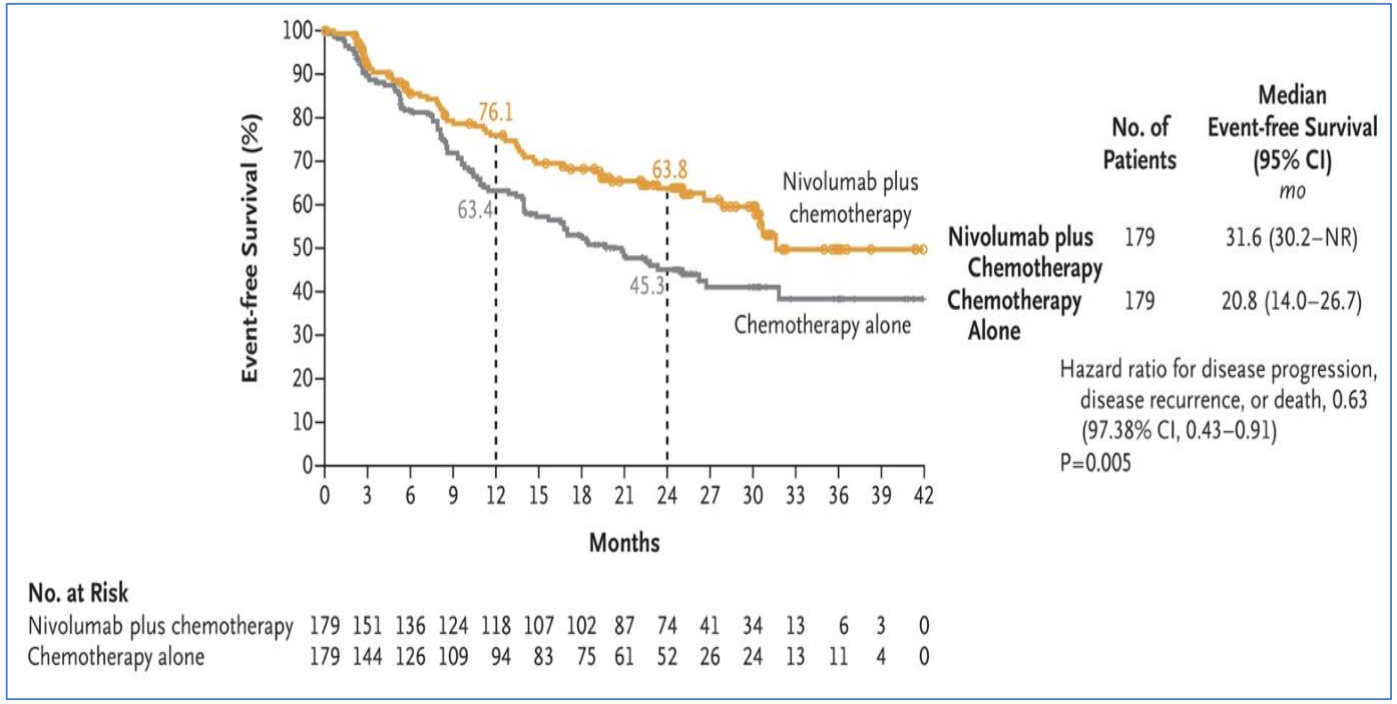
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

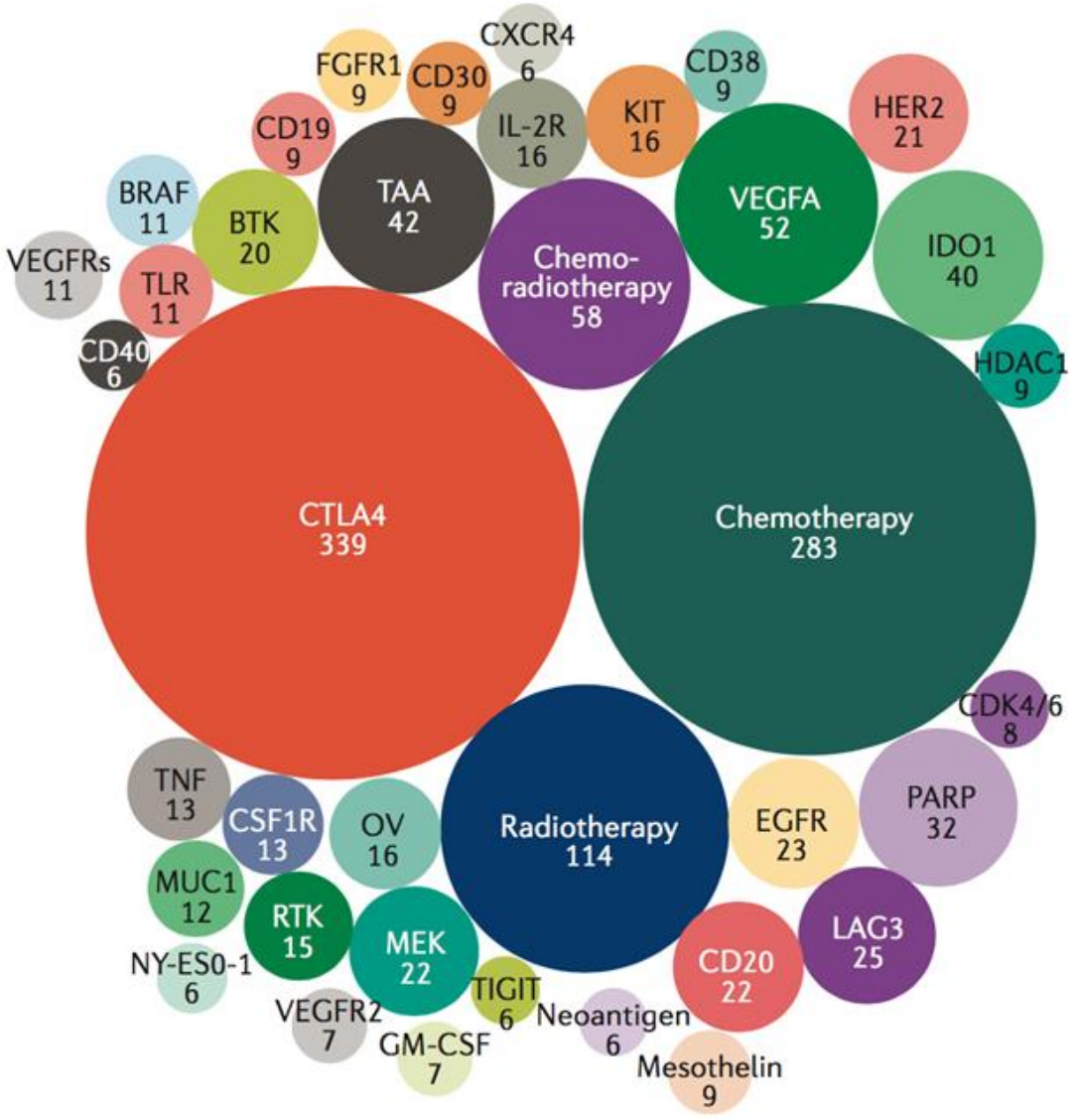
Neoadjuvant Nivolumab plus Chemotherapy in Resectable Lung Cancer

Patrick M. Forde, M.B., B.Ch., Jonathan Spicer, M.D., Ph.D., Shun Lu, M.D., Ph.D., Mariano Provencio, M.D., Ph.D., Tetsuya Mitsudomi, M.D., Ph.D., Mark M. Awad, M.D., Ph.D., Enriqueta Felip, M.D., Ph.D., Stephen R. Broderick, M.D., M.P.H.S., Julie R. Brahmer, M.D., Scott J. Swanson, M.D., Keith Kerr, M.B., Ch.B., Changli Wang, M.D., Ph.D., et al., for the CheckMate 816 Investigators*



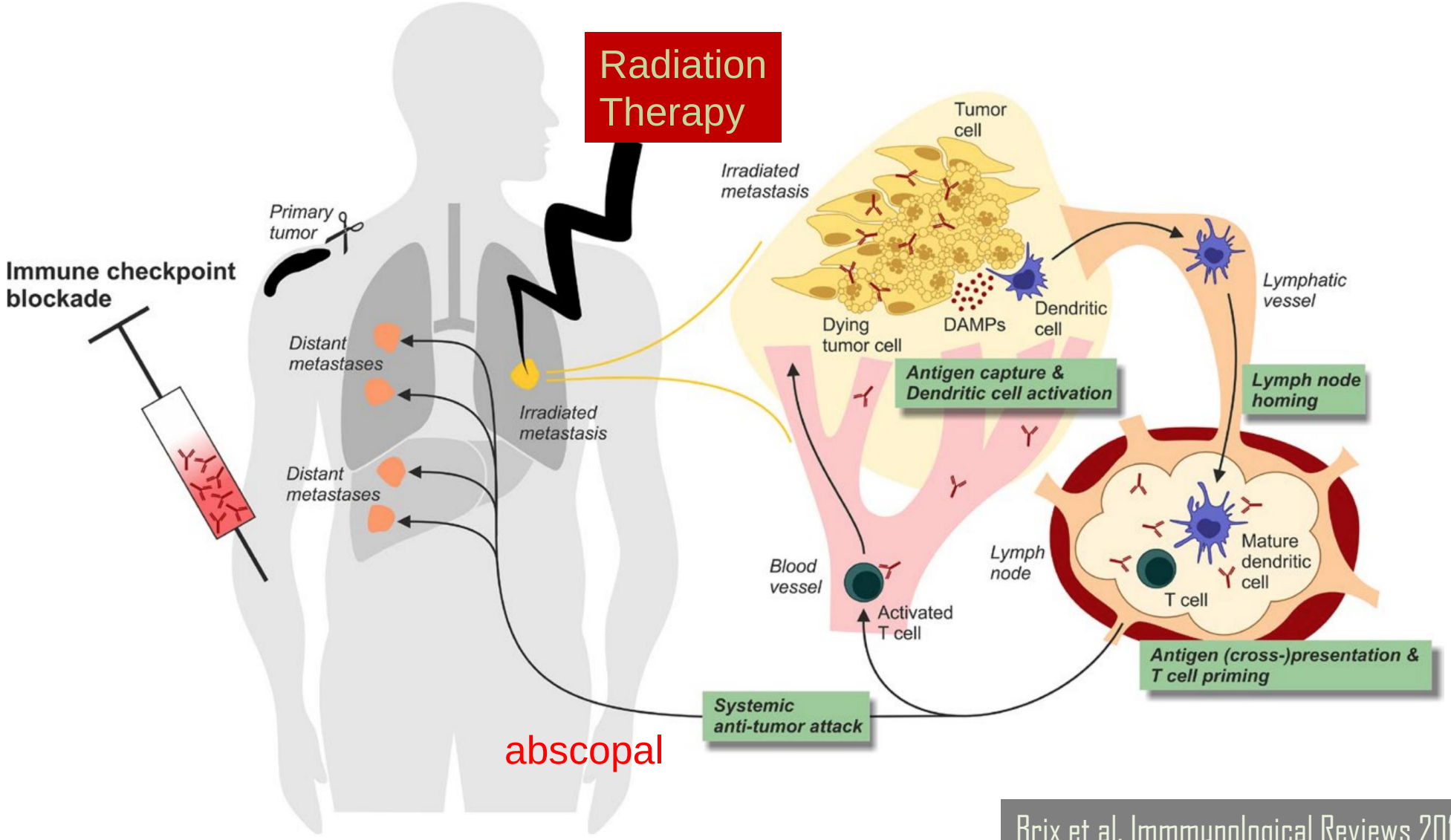


Agents being tested in combination with PD1/PD-L1 inhibition



Source: CRI

Integration of radiation therapy and immunotherapy

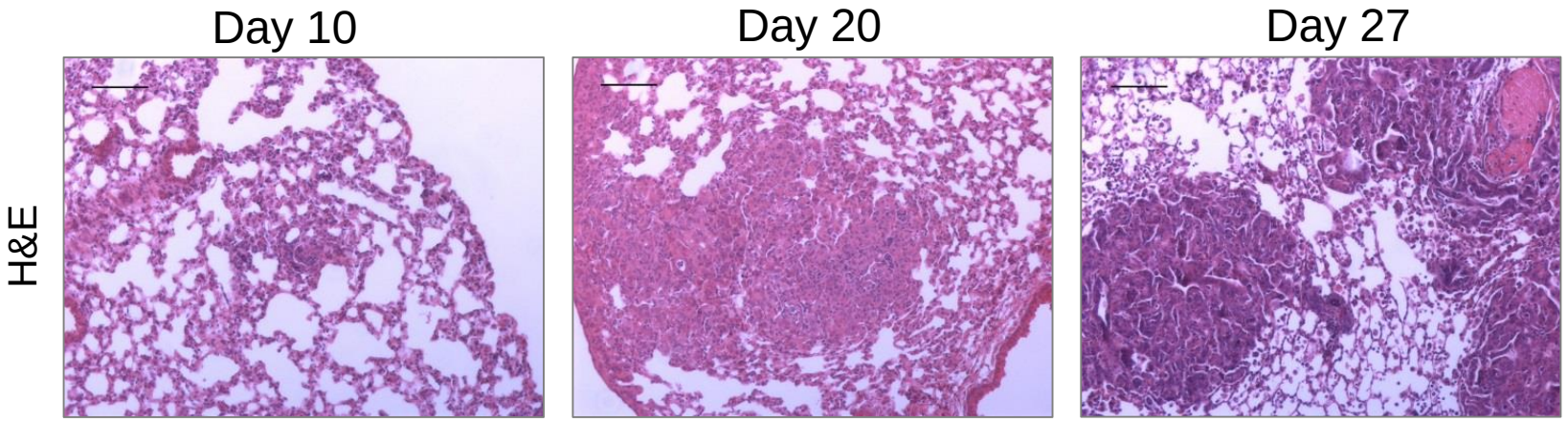


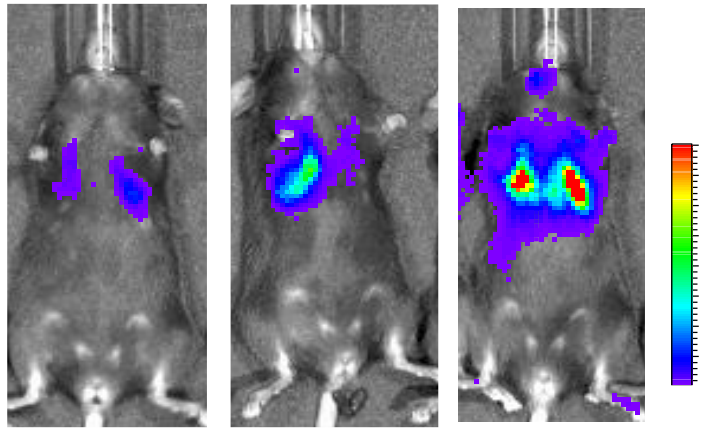
Brix et al. Immunological Reviews 2017
Ko, Raben and Formenti. CCR, 2018

Challenges in using radiation therapy as an immunomodulating agent

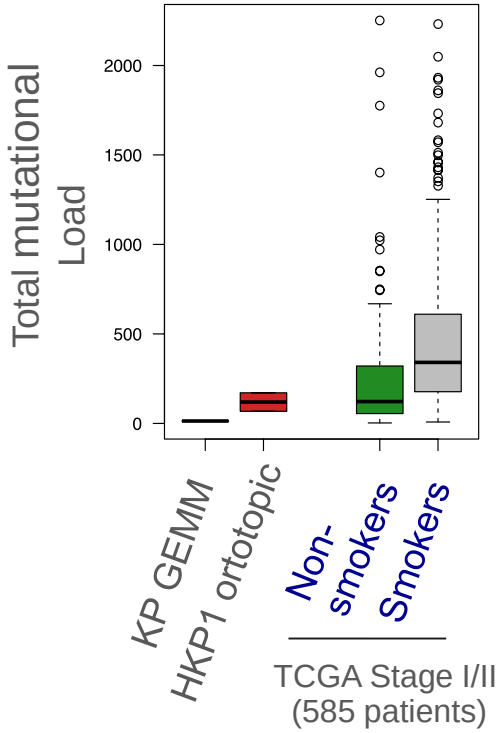
- The optimal dose of subablative RT for enhancing the immune response in cancer is under investigation.
- Optimal sequencing of therapeutic agents is unclear.
- The mechanism/s by which RT enhances the efficacy of immune checkpoint blockade are context dependent and incompletely understood.
- Need for biomarkers that predict which patients will respond to the therapy.

Orthotopic *Kras^{G12D};p53$^{-/-}$* (HKP1) lung adenocarcinoma





mCherry-Luc reporters



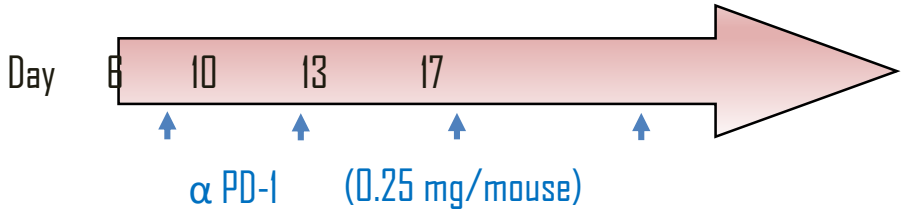
Mutation Call Stringency

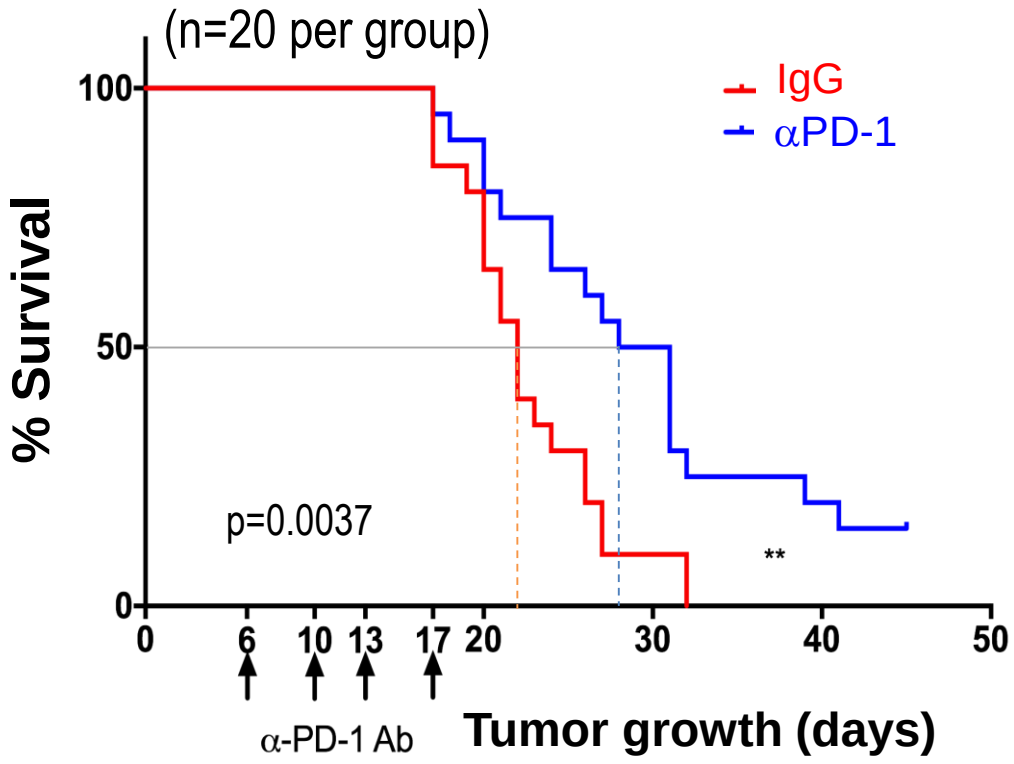
- Read depth ≥ 30 reads
- Variant allele frequency in tumor samples ≥5%, in normals is ≤1%,
- ≥5 reads supporting the mutation in the tumor.

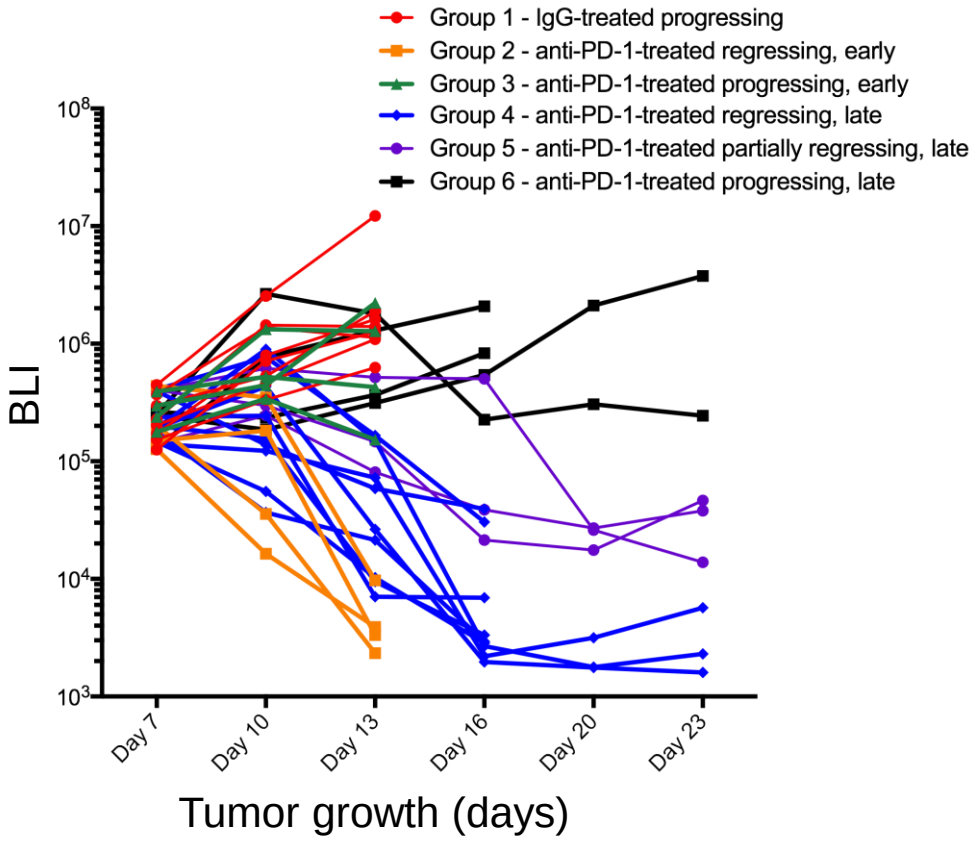
Choi et al. Cell Reports 2015
Markowitz et al. JCI Insight 2018

Treatment with anti-PD1 antibody increased OS in HKP1 mice

HKP1 mice

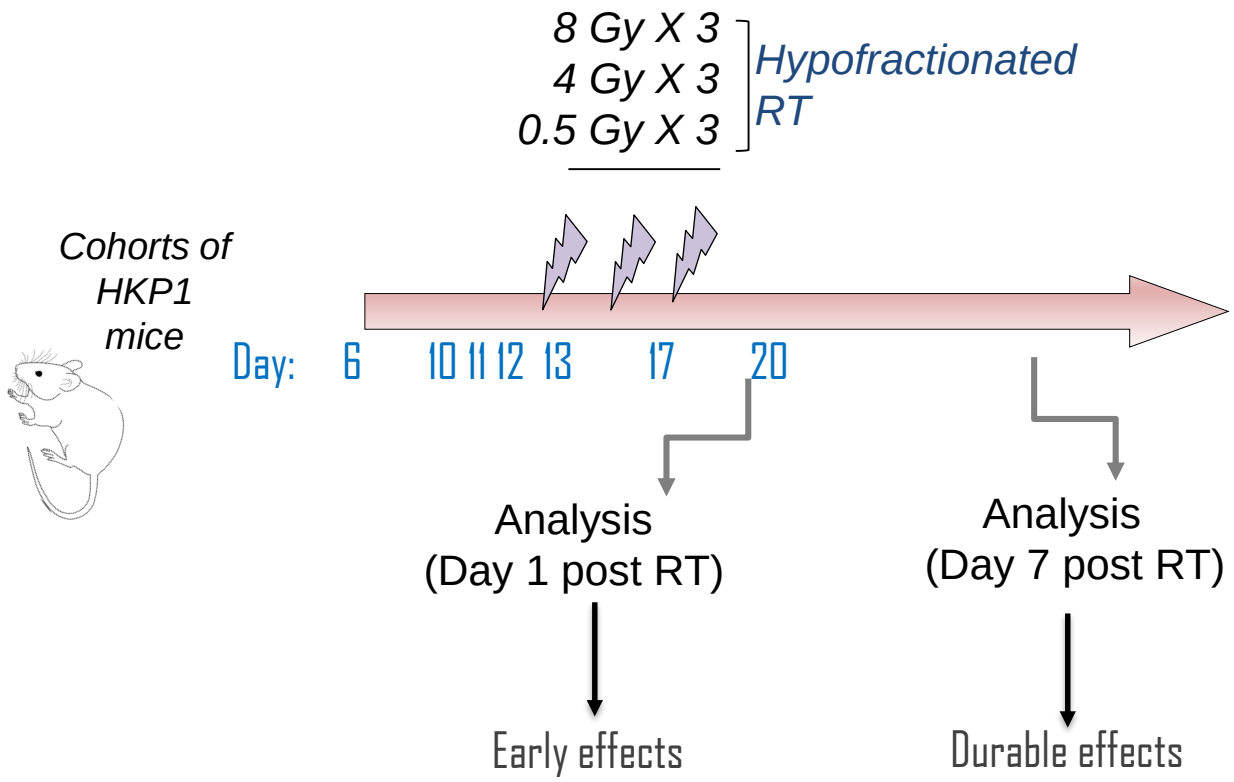




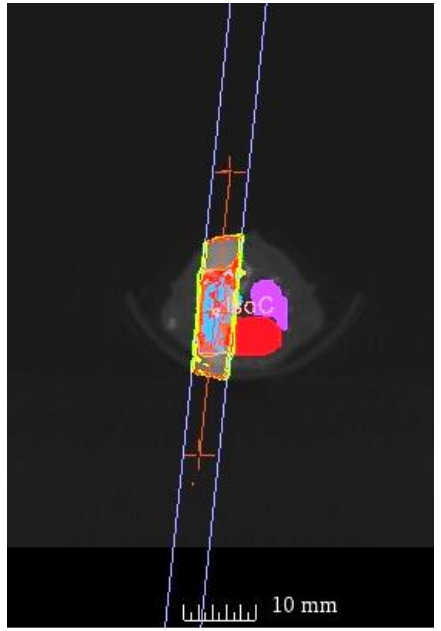


Markowitz, et al. JCI Insight 2018

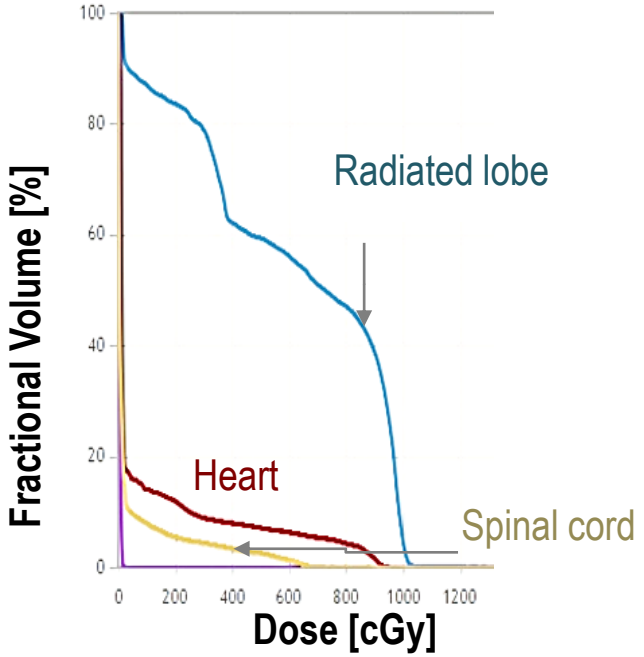
μCT guided delivery of radiation to HKP1 lung.



Lung scan

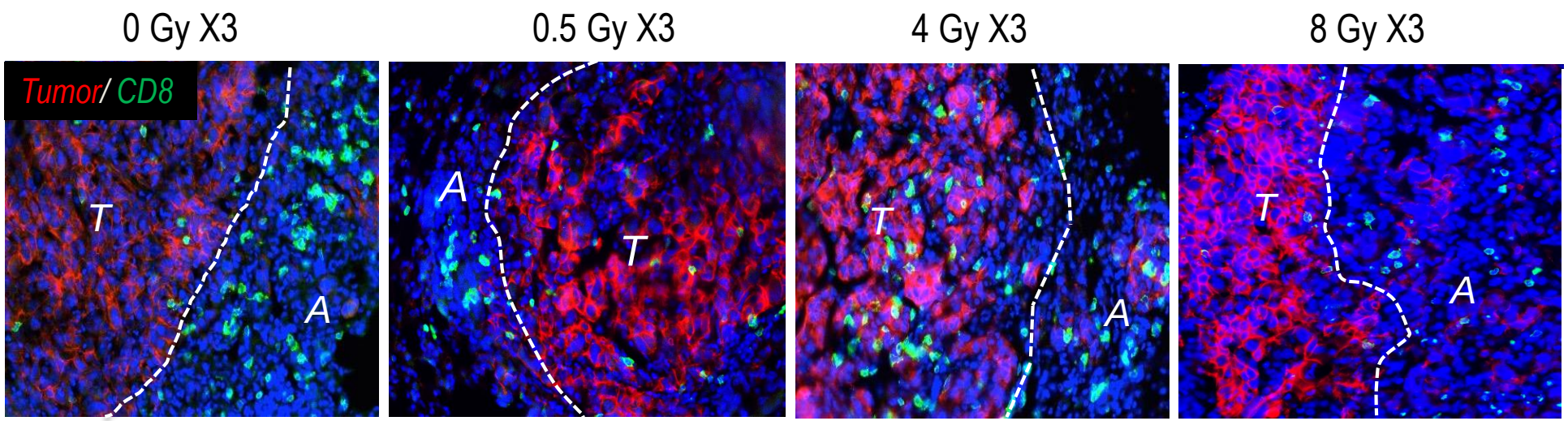


Dose volume histogram



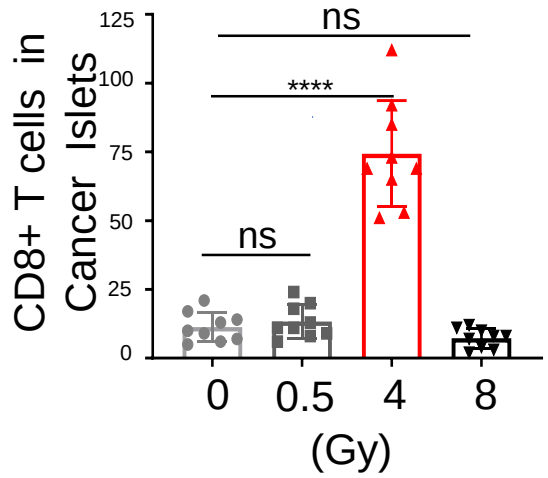
4 Gy- RT is associated with activated T cell phenotypes

(Day 1 post RT)

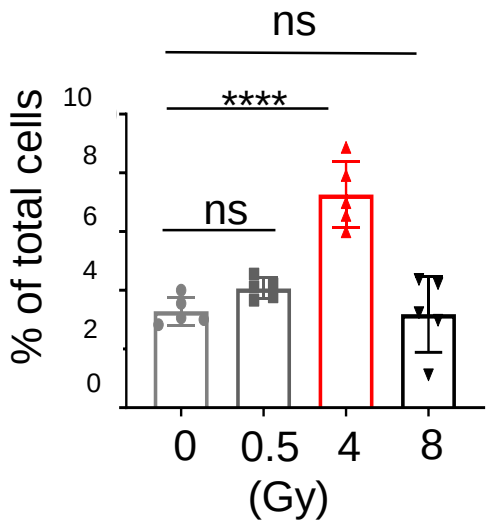


T, Tumor
A, Adjacent lung

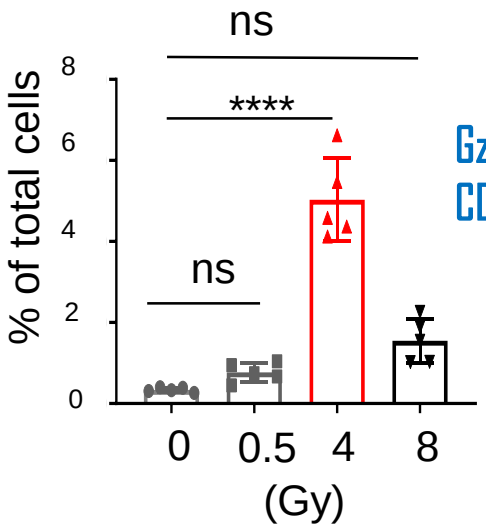
CD8 T cell infiltration



IFNγ⁺ TNFα⁺ CD4 T cells



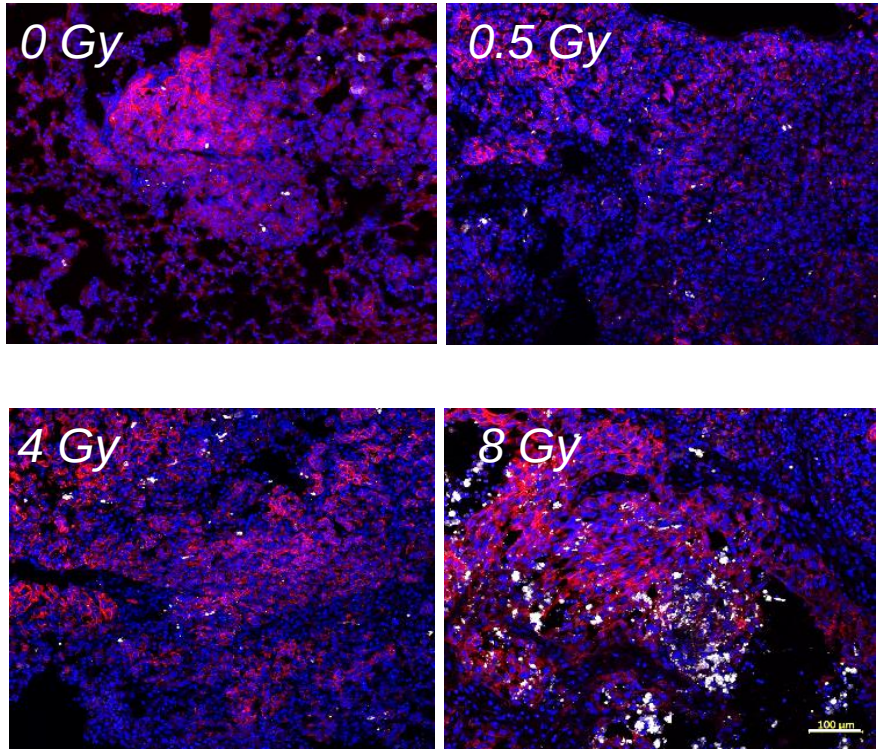
GzmB⁺ CD8 T Cells

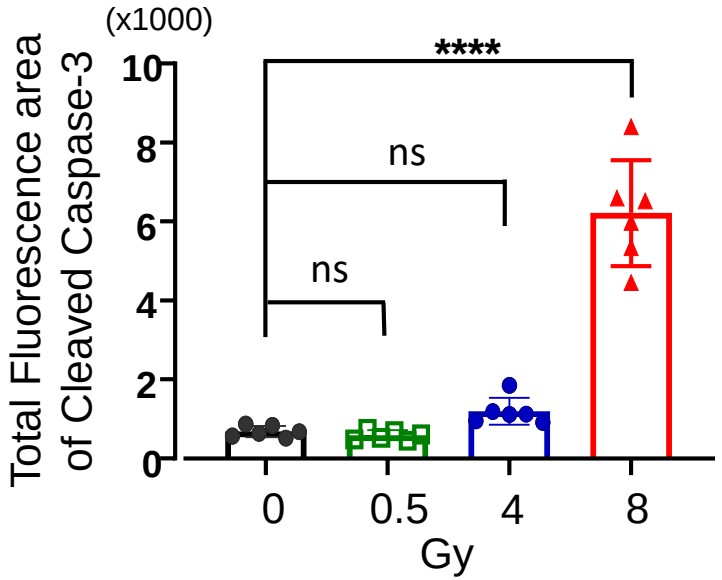


Ban et al. Nature Cancer 2021

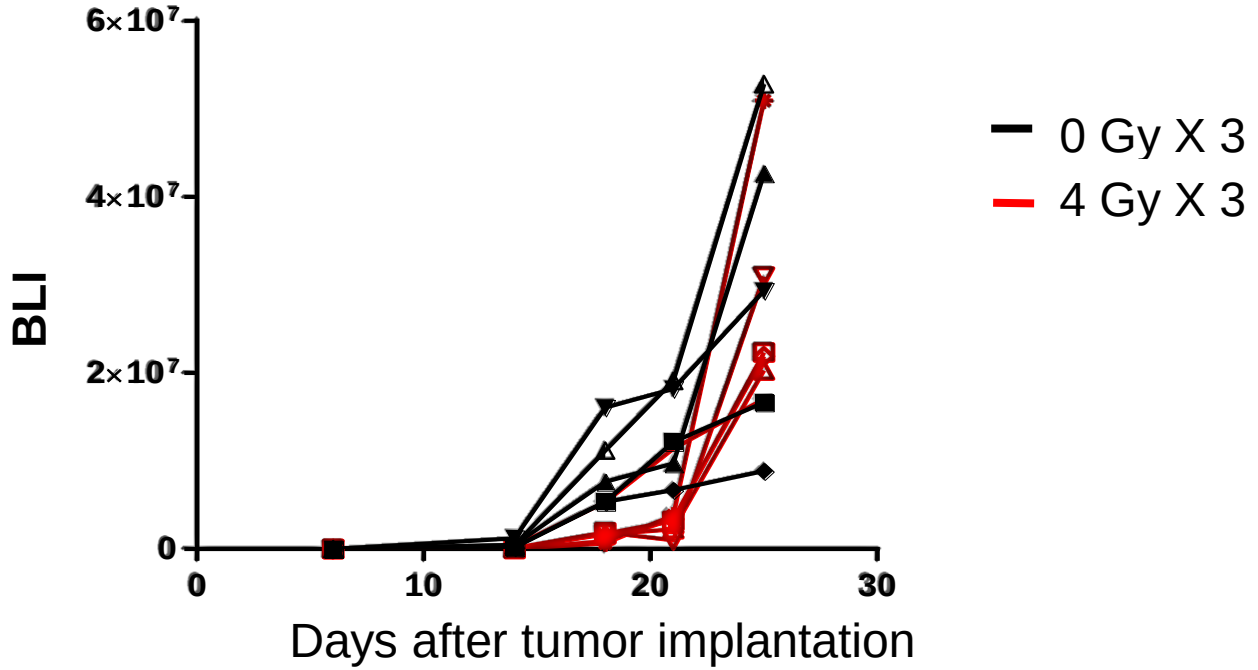
8Gy RT is associated with increased apoptosis

Tumor/Cleaved caspase-3

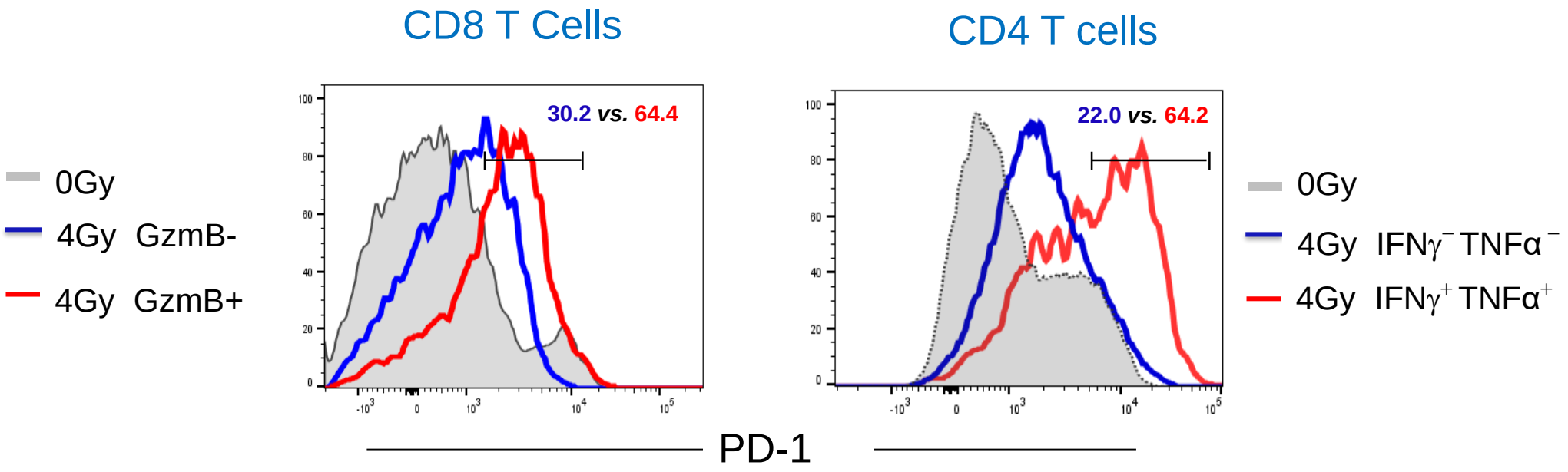




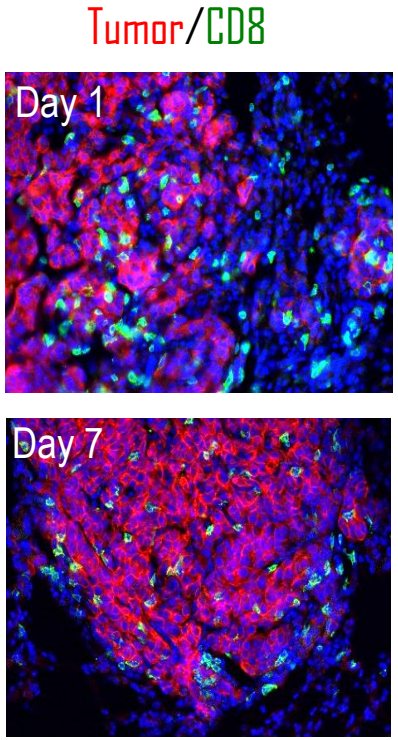
RT fails to effectively control tumor growth

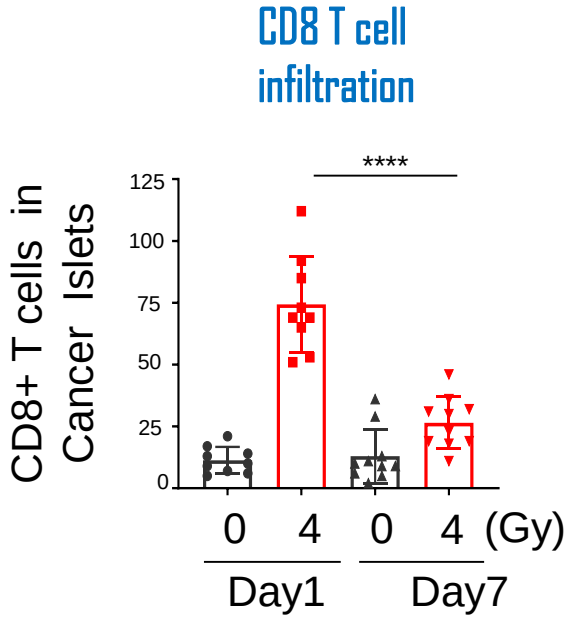


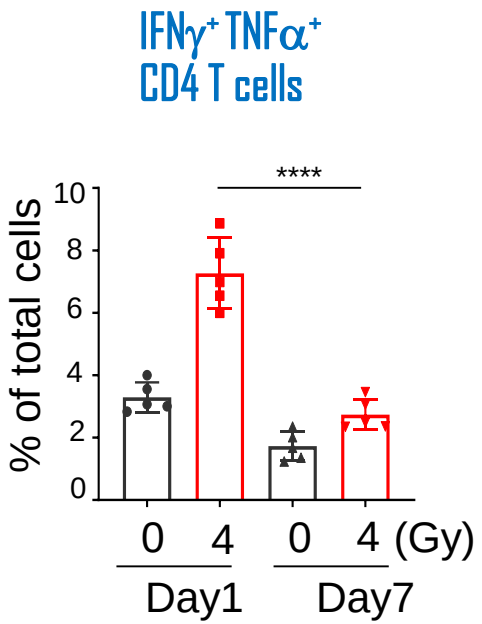
4Gy RT increased PD-1 in GzmB^{+} CD8 T cells and IFNγ^{+}/TNFα^{+} in CD4 T cells.

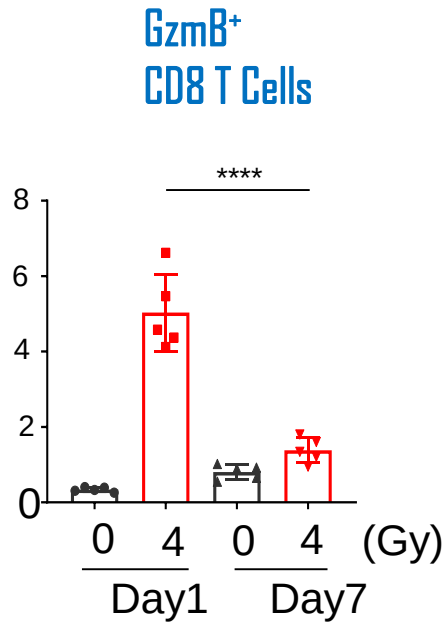


RT-mediated immune activation is not durable.

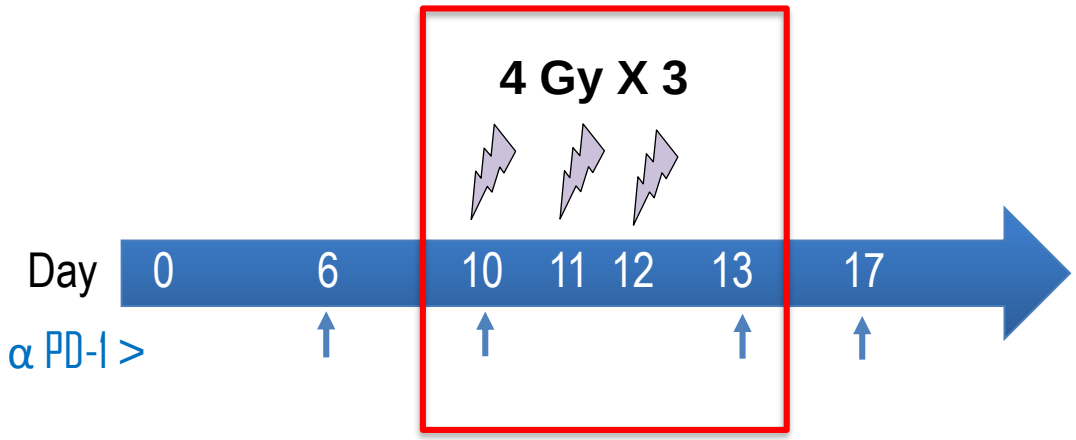




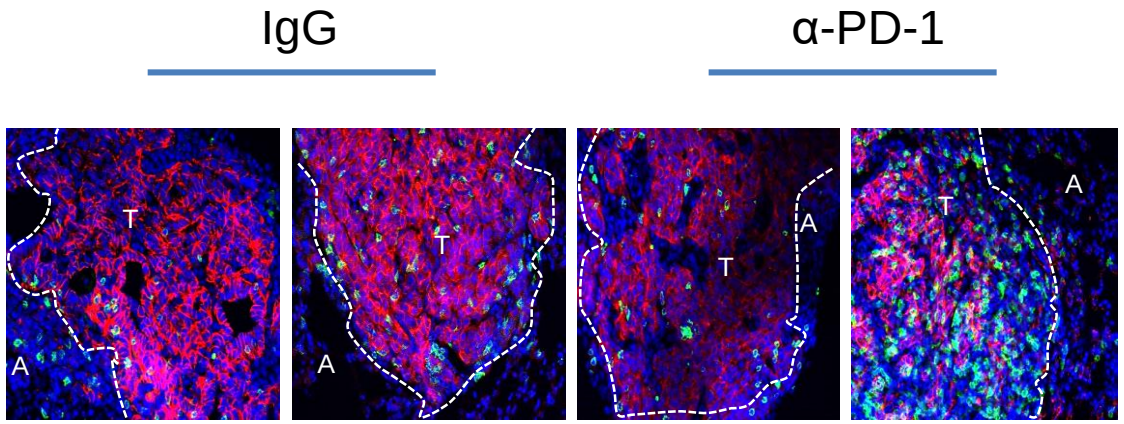


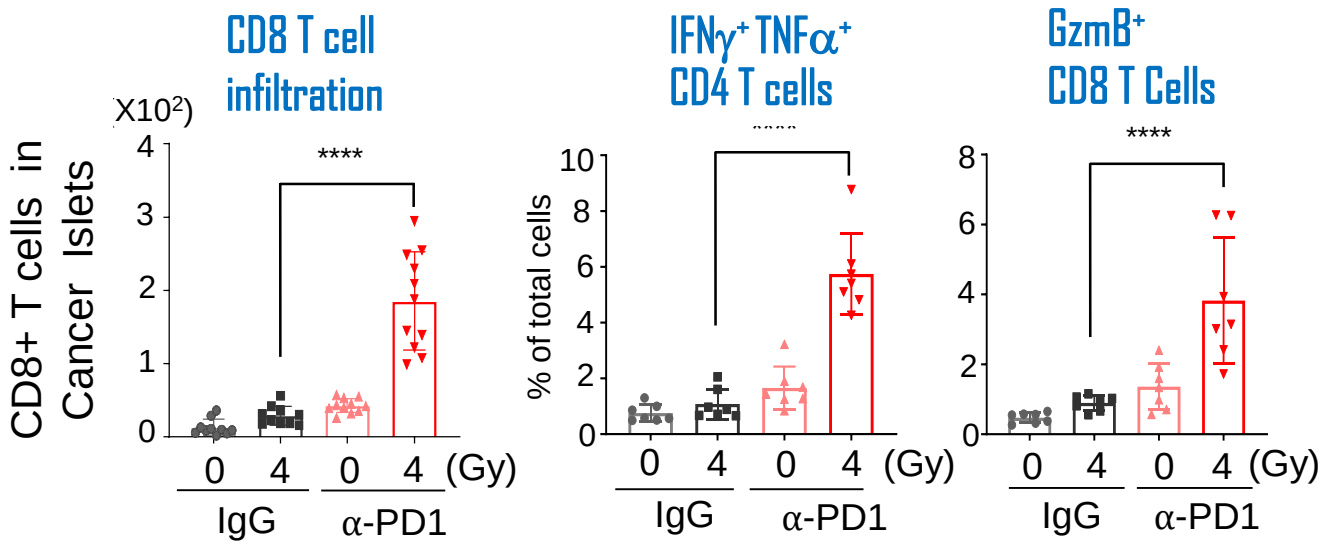


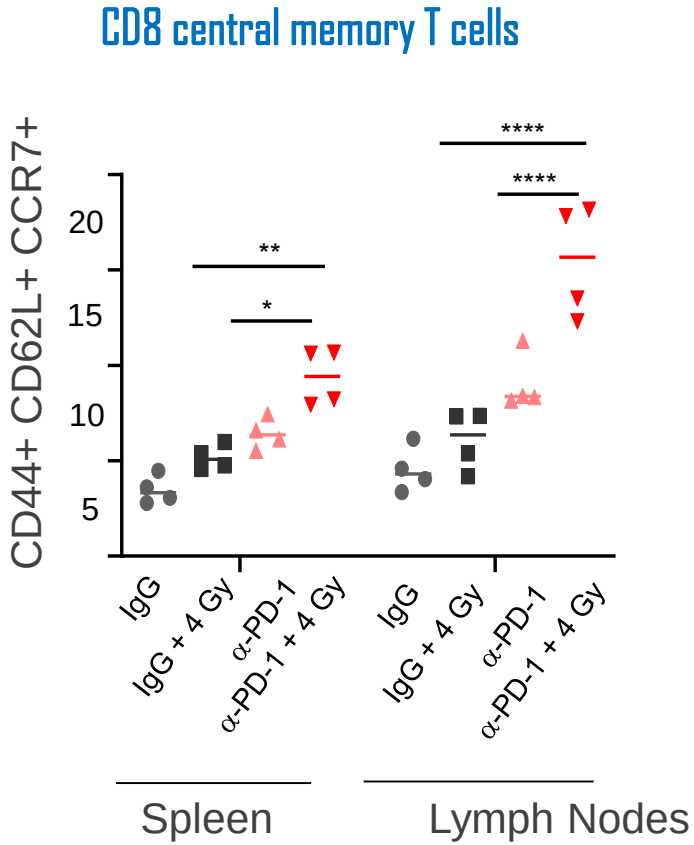
Treatment window for combination treatment



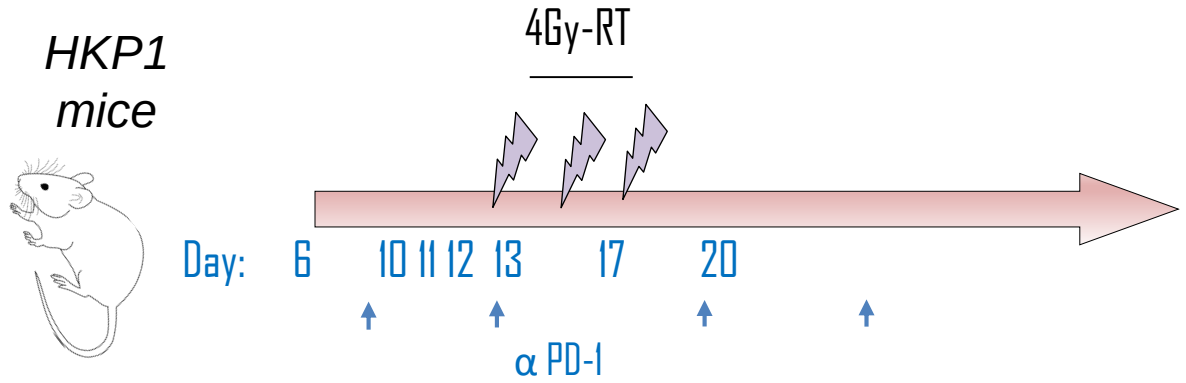
Combination treatment sustained T cell infiltration/activation and generated central memory T cell phenotypes (Day 7 post RT)



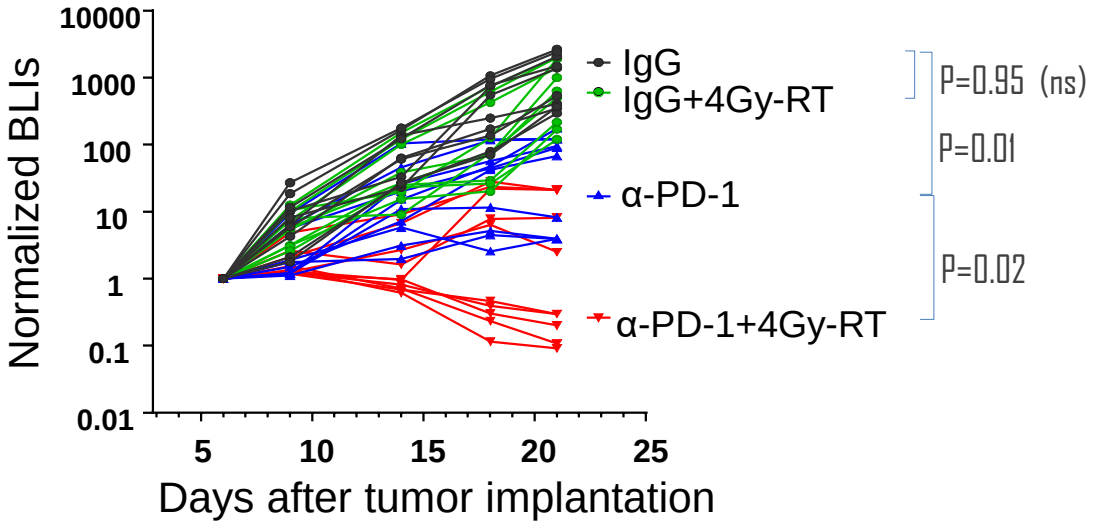




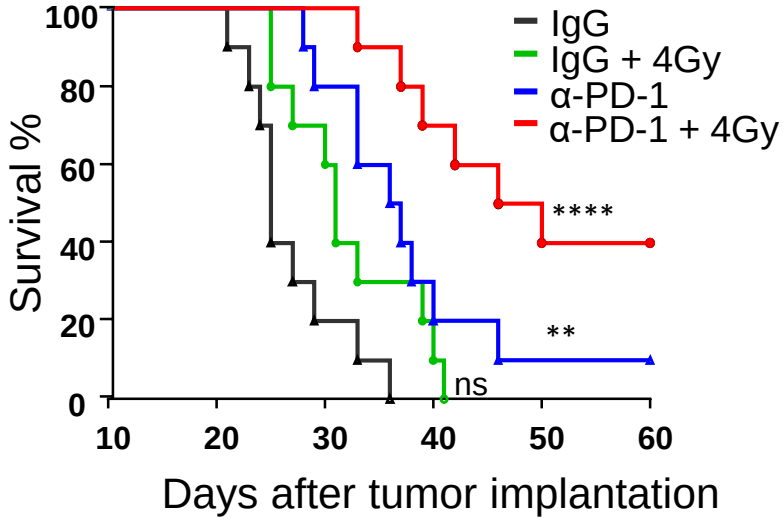
4Gy RT in combination with PD1 inhibition delays tumor growth and improves survival



Tumor Growth

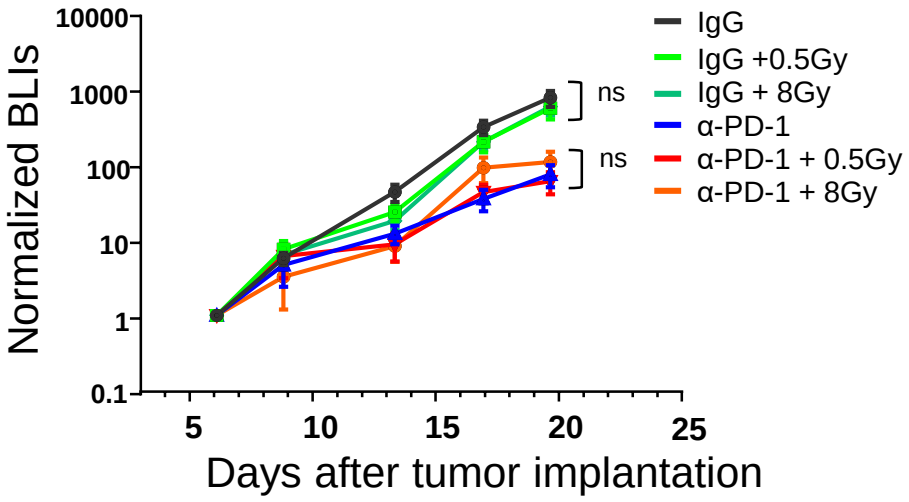


Survival

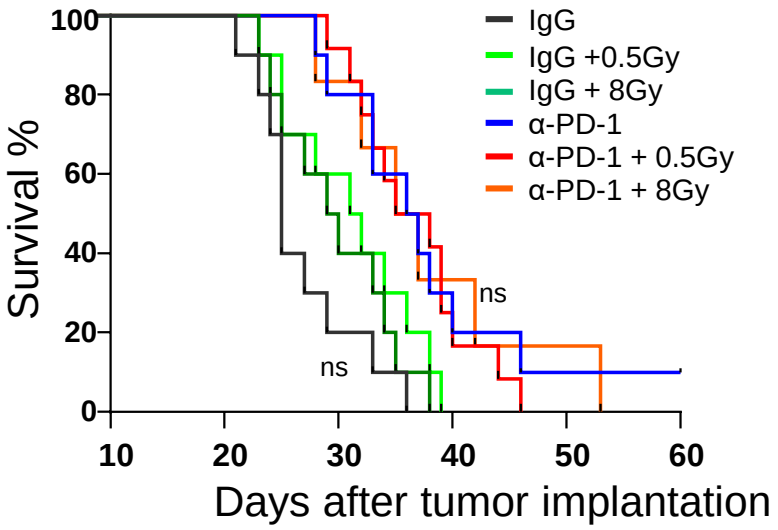


0.5 or 8 Gy RT fails to delay tumor growth or improve survival

Tumor Growth

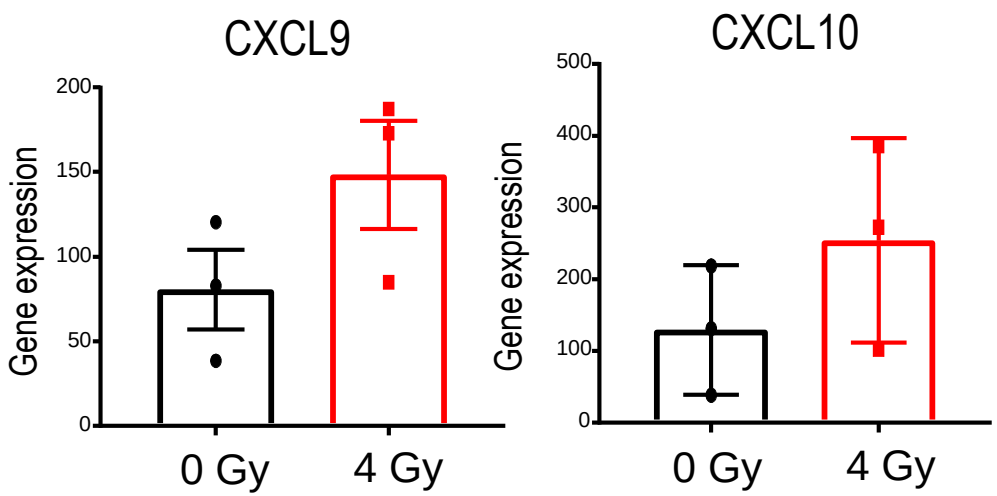


Survival



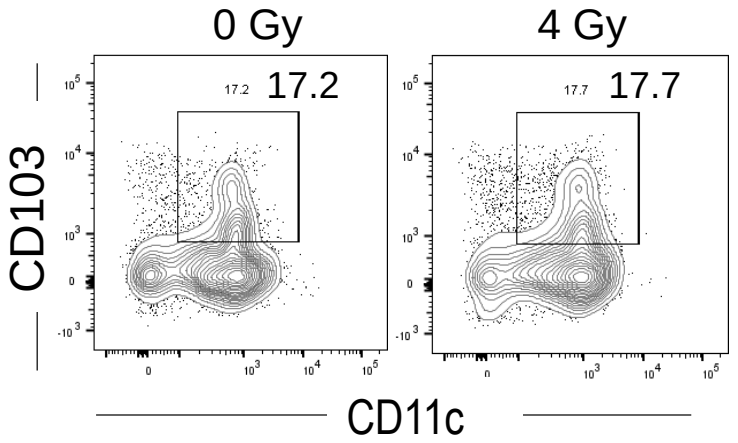
What are the mechanisms by which 4Gy RT improves efficacy of PD-1 blockade.

Chemoattractants

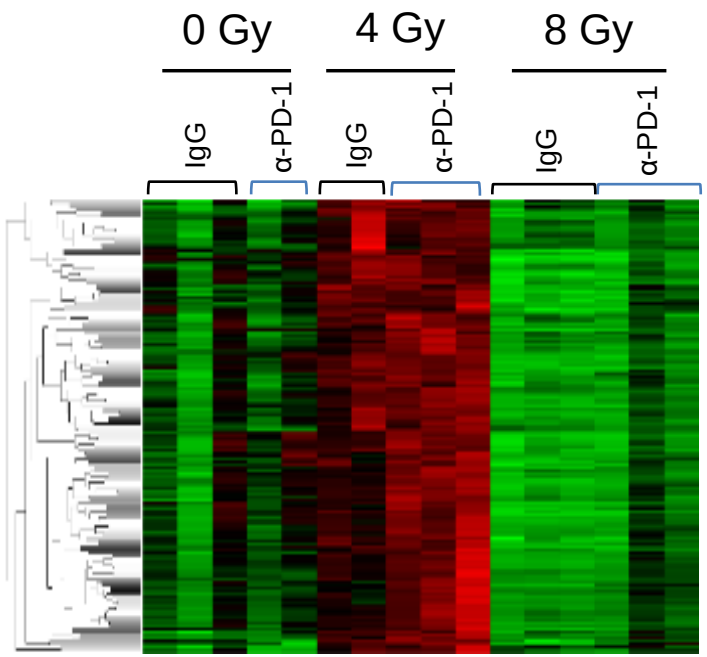


CD103+ DCs in dLNs.

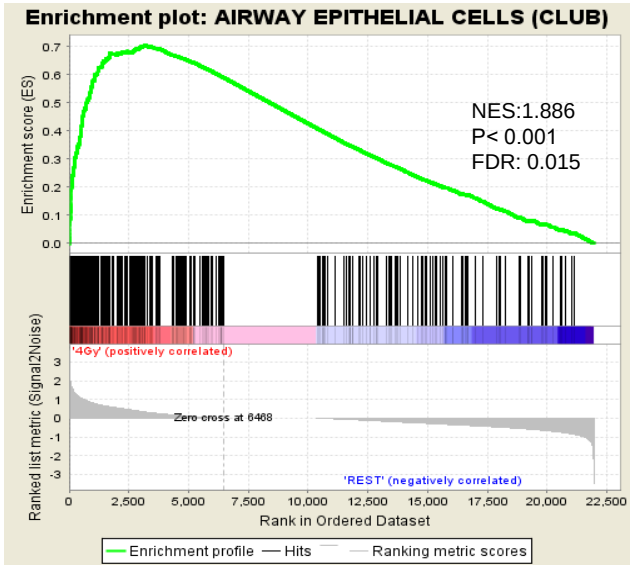
Gated on mCherry^{-} CD3^{-} CD8a^{+} MHCII^{+}

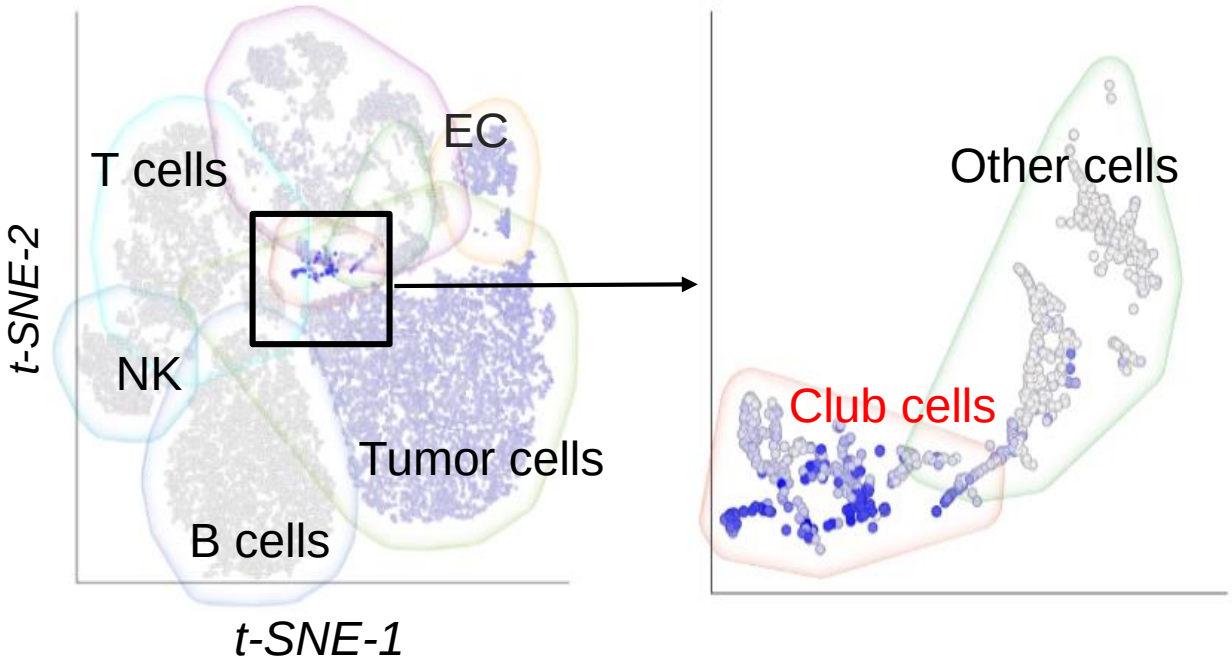


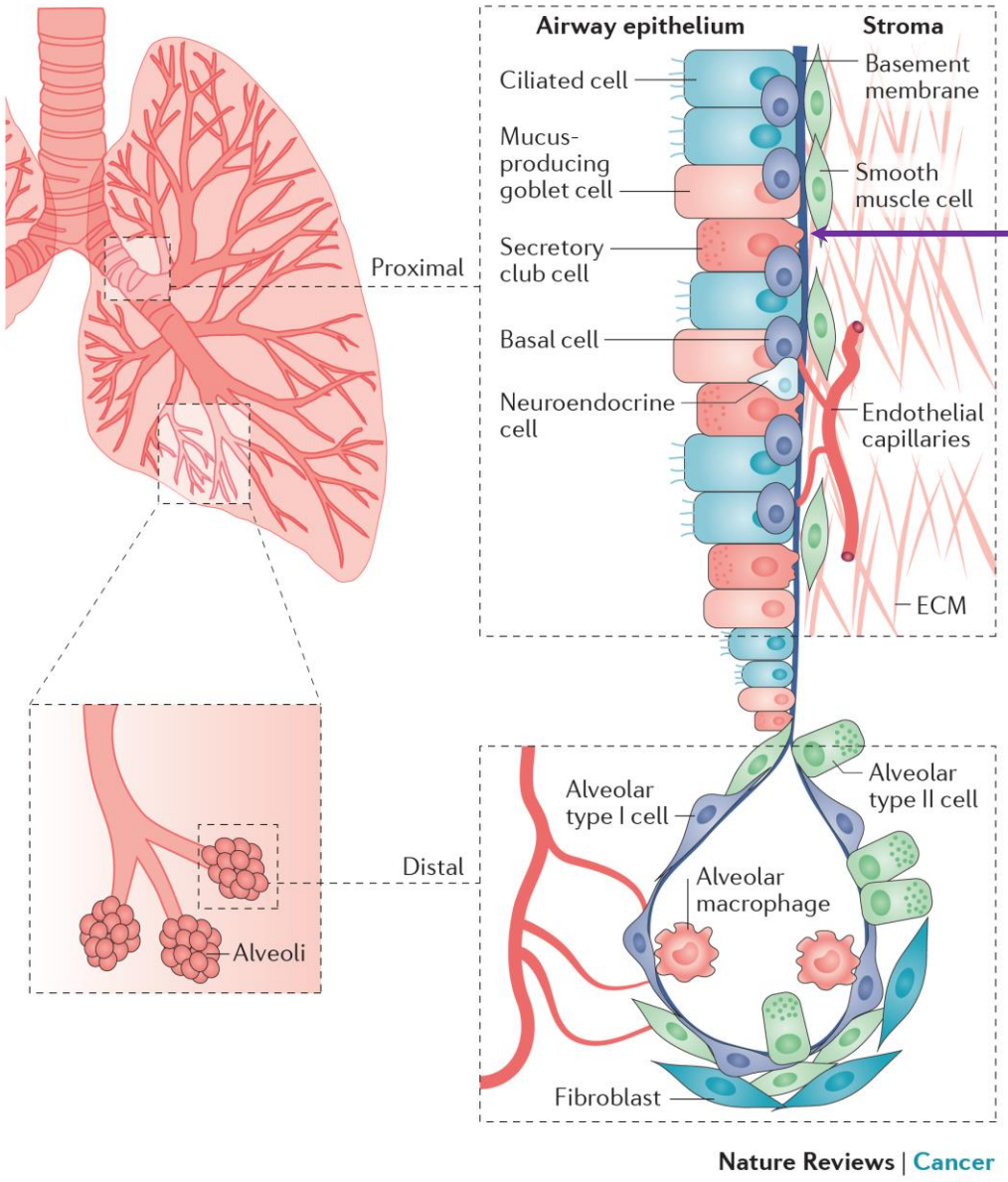
Identification of lung resident club cell gene signature in RT treated HKP1 lungs



144 Signature genes







Club cells (Clara cells)

- Non ciliated epithelial progenitor cells localized in the upper airways (bronchioles).

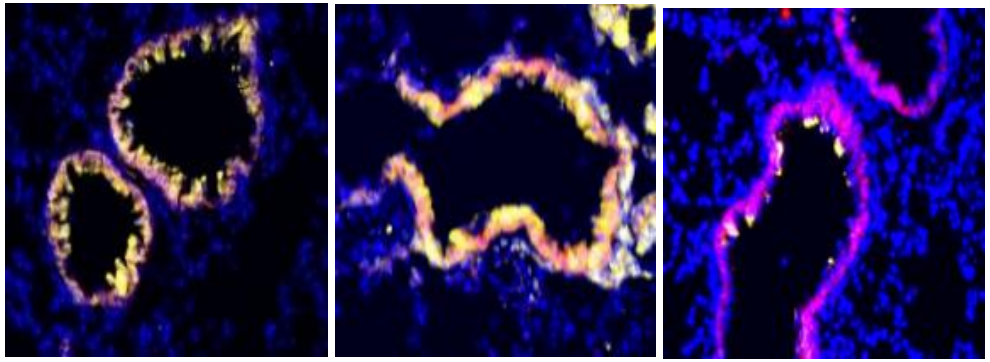
Known functions

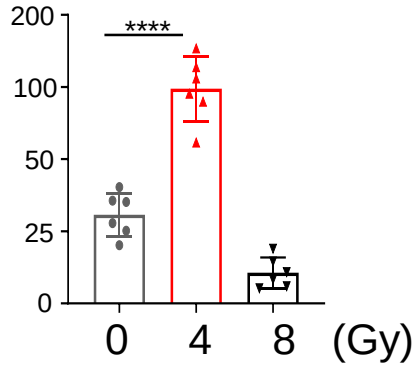
- Differentiate into epithelial cells to regenerate injured bronchiolar epithelium
- Major secretory cell: produce secretoglobins (SCGB1A1/ CC10) and pulmonary surfactants.
- Regulates airway inflammation.

Altorki et al. Nat. Rev. Cancer 2019

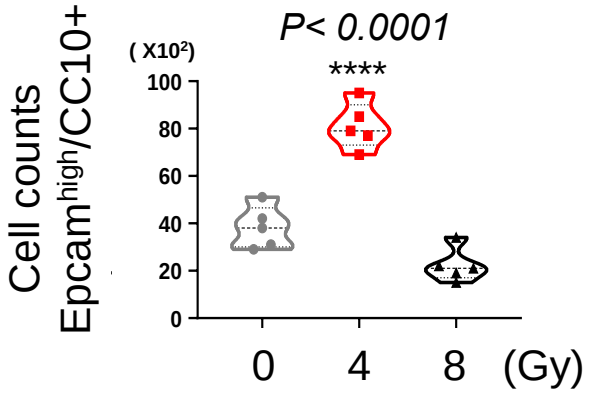
Sub-ablative RT activates club cells in the lung microenvironment.

DAPI /Ecad/CC10

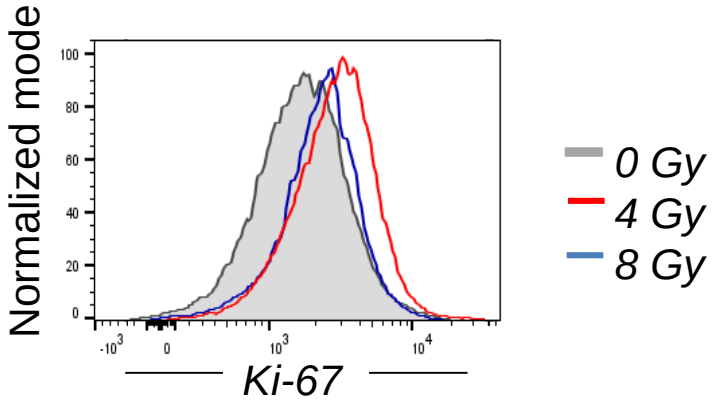




Club cell expansion



Club cell proliferation



Conditional genetic ablation of Club cells

Scgb1a1- CreER x *lox*-stop-*lox* DTR
(CC10)

+ Tamoxifen

Scgb1a1- CreER^{TM} DTR
(CC10)

Diphtheria Toxin

Scgb1a1^{cre} iDTR

HKP1 mice

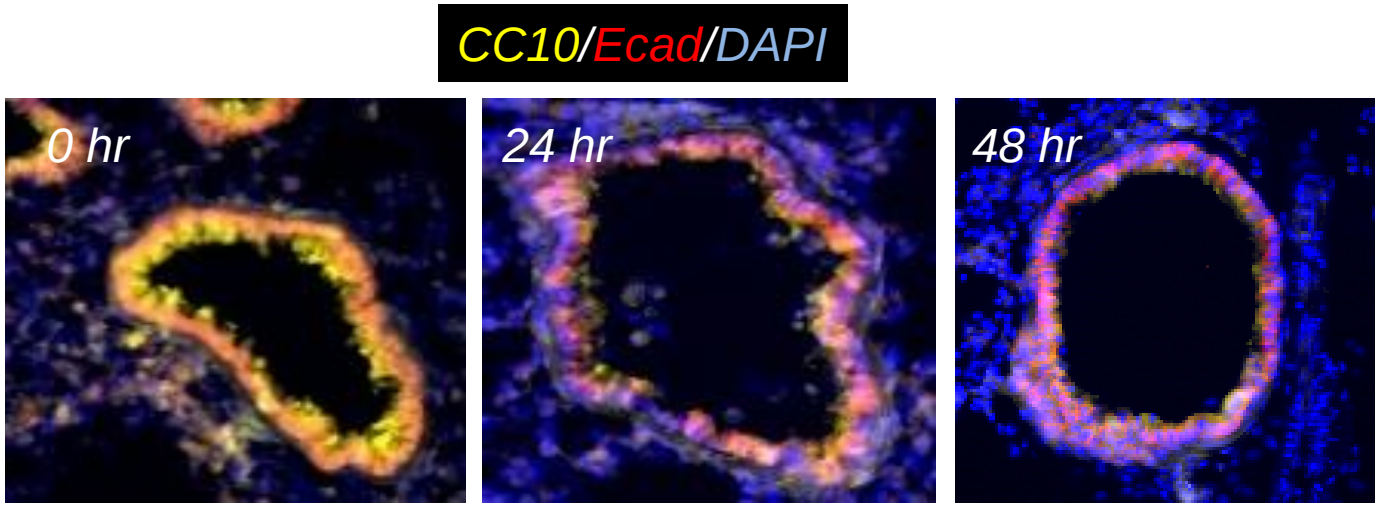
4Gy-RT

Tamoxifen (Day 3-7) *DT* (Day 8-9)

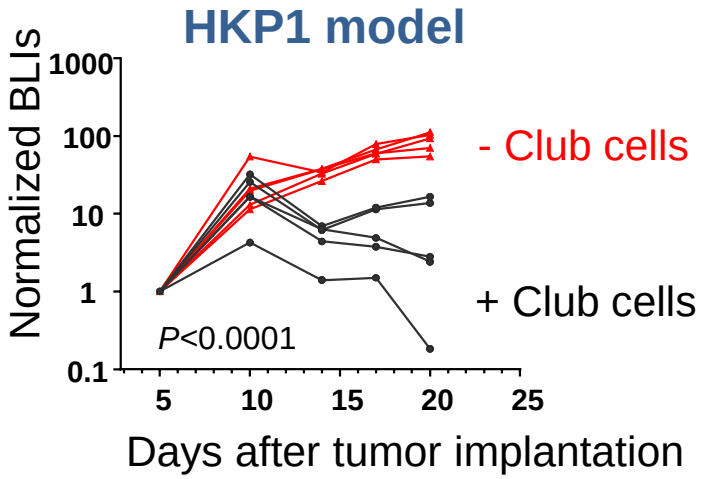
Day: 6 10 11 12 13 17

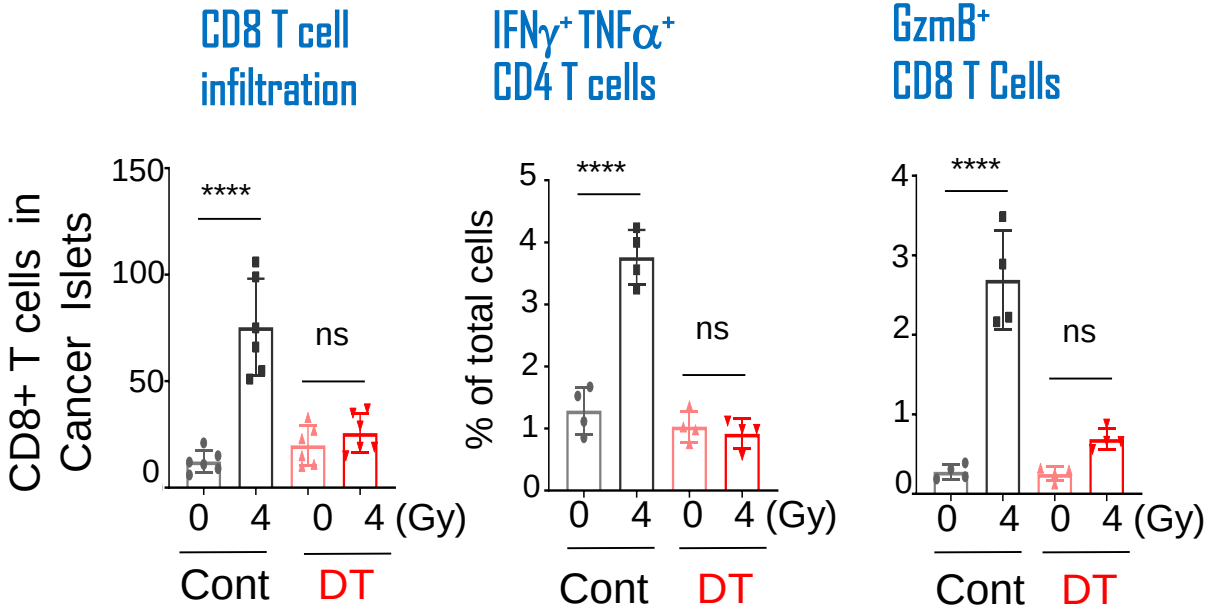
α PD-1
(0.25ug/mouse)

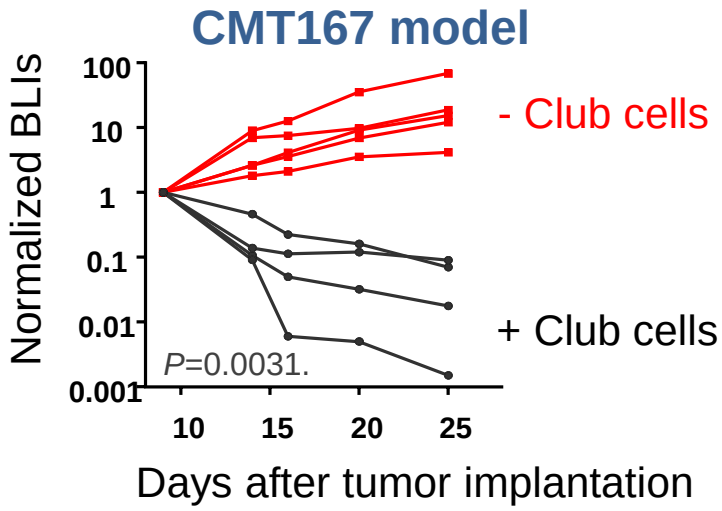
Club cell deficiency blunts immune responses and abrogates therapeutic efficacy



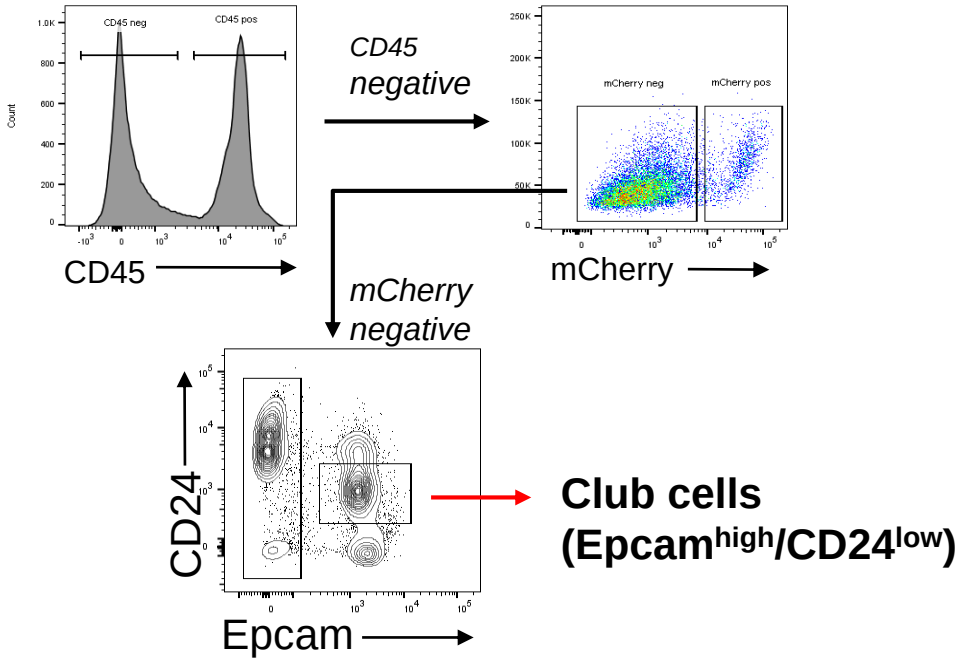
4 Gy-RT + α-PD-1

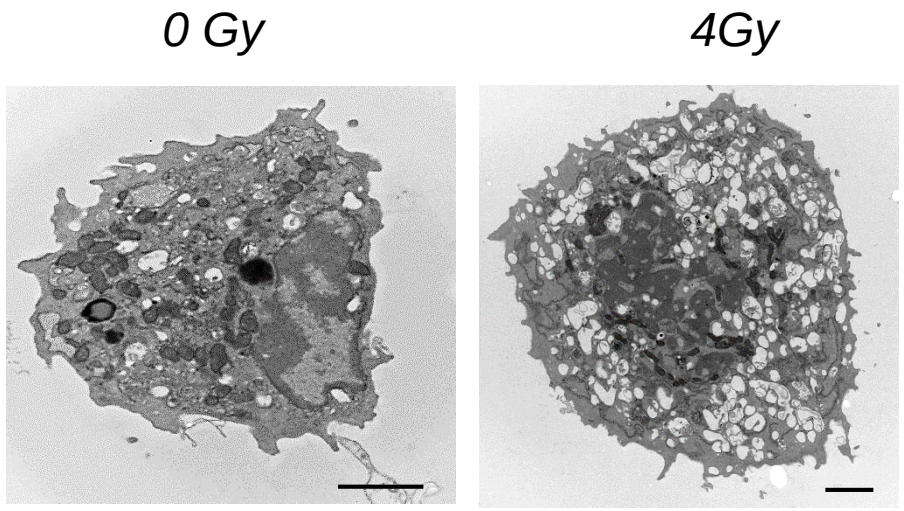






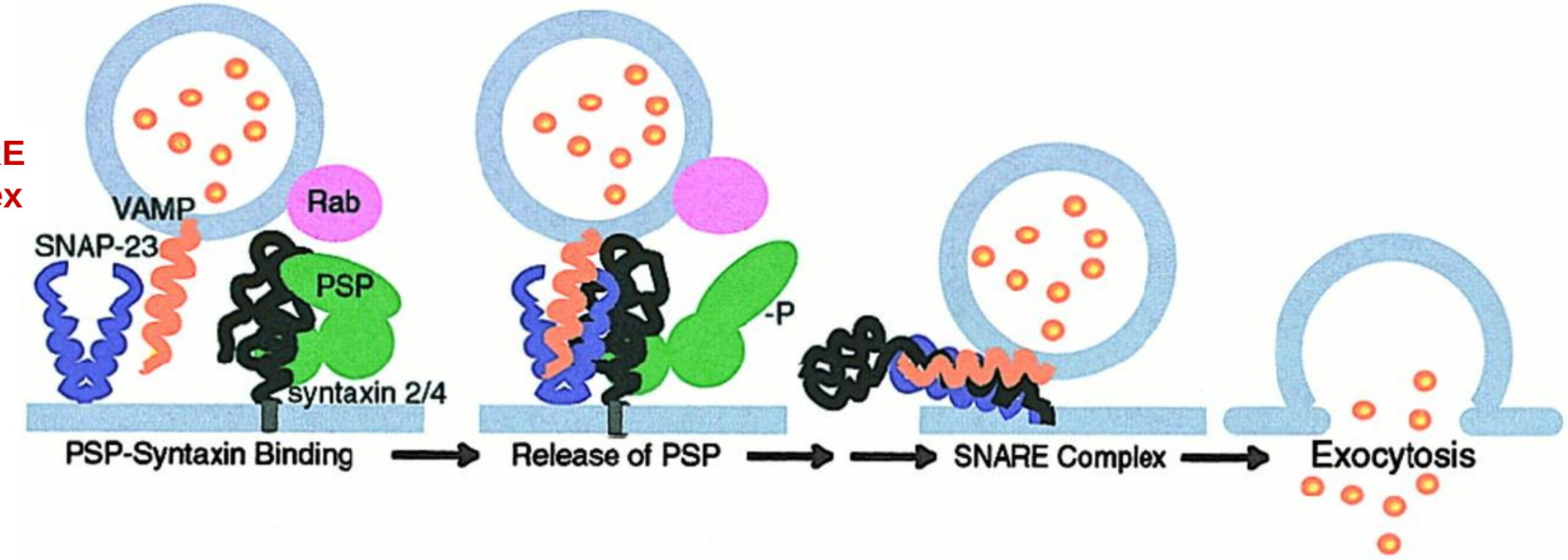
Electron microscopy of club cells sorted from RT treated HKP1 lungs show increased secretory vesicles.





Synaptsome associated protein 23 (SNAP23) regulates intracellular vesicle fusion to membranes

t-SNARE complex

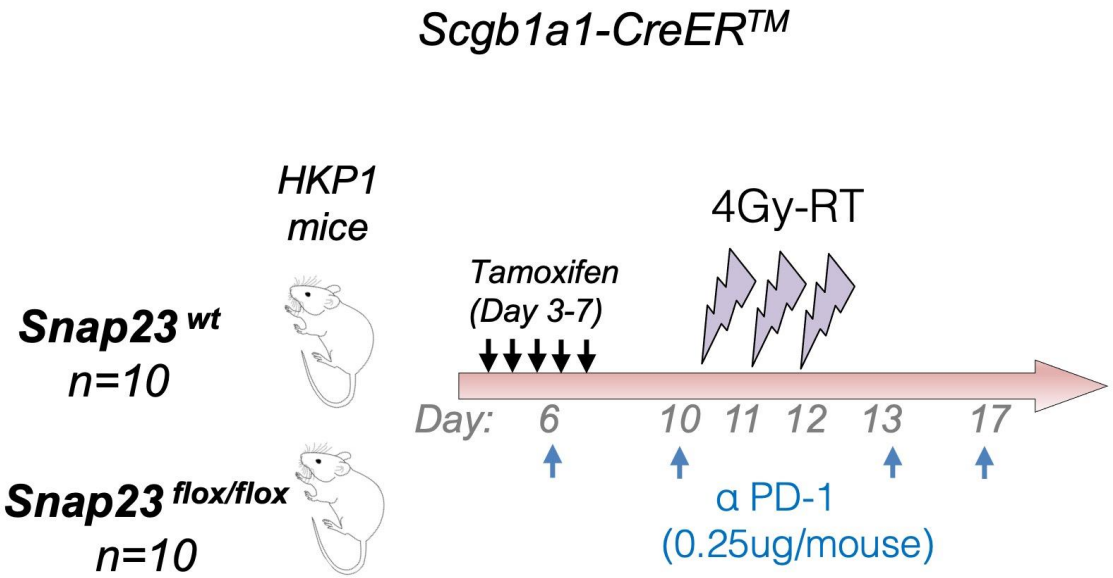


Club cell-specific conditional KO of synaptsome associated protein 23 (SNAP23)

Scgb1a1- CreER (CC10) x *SNAP23 flox/flox*

+ Tamoxifen

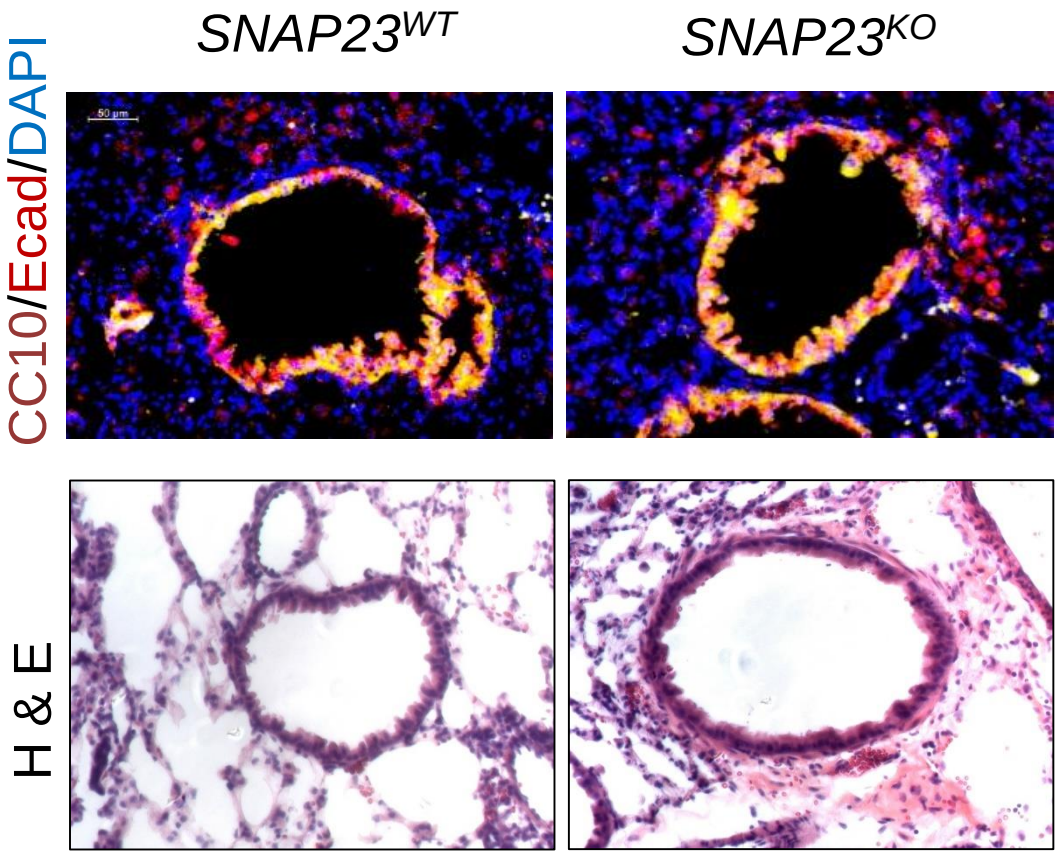
Club cell specific SNAP23^{KO}



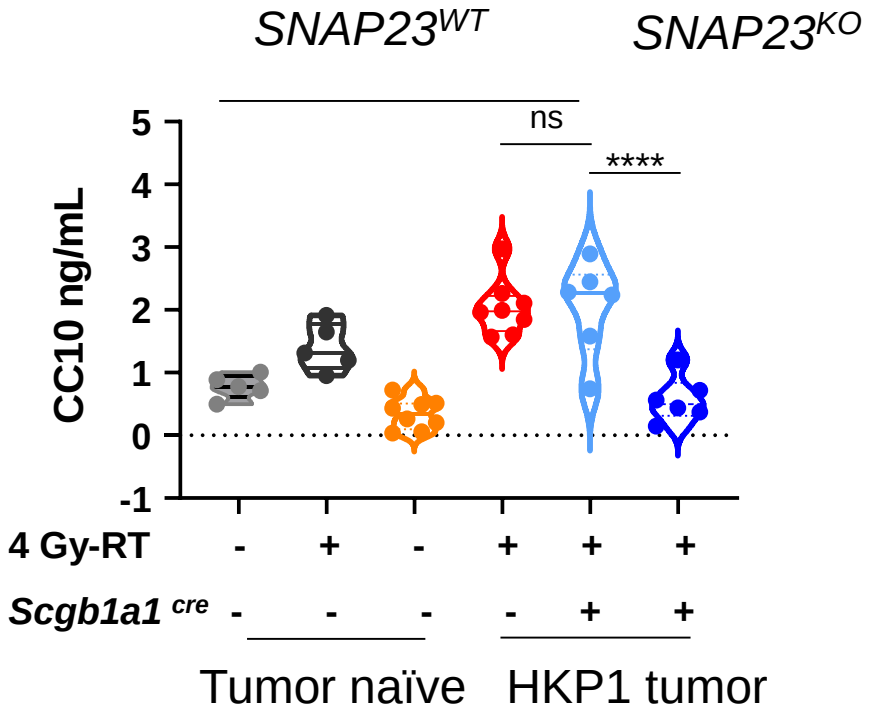
Jeff Pessin, Albert Einstein

Club cell specific SNAP23^{KO} lungs maintain bronchiole integrity

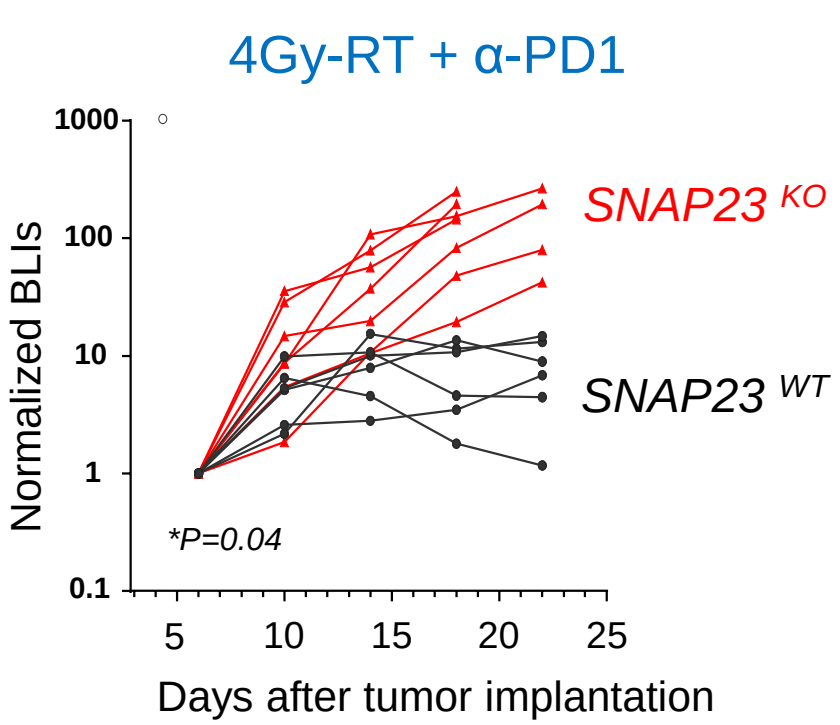
Bronchiole architecture

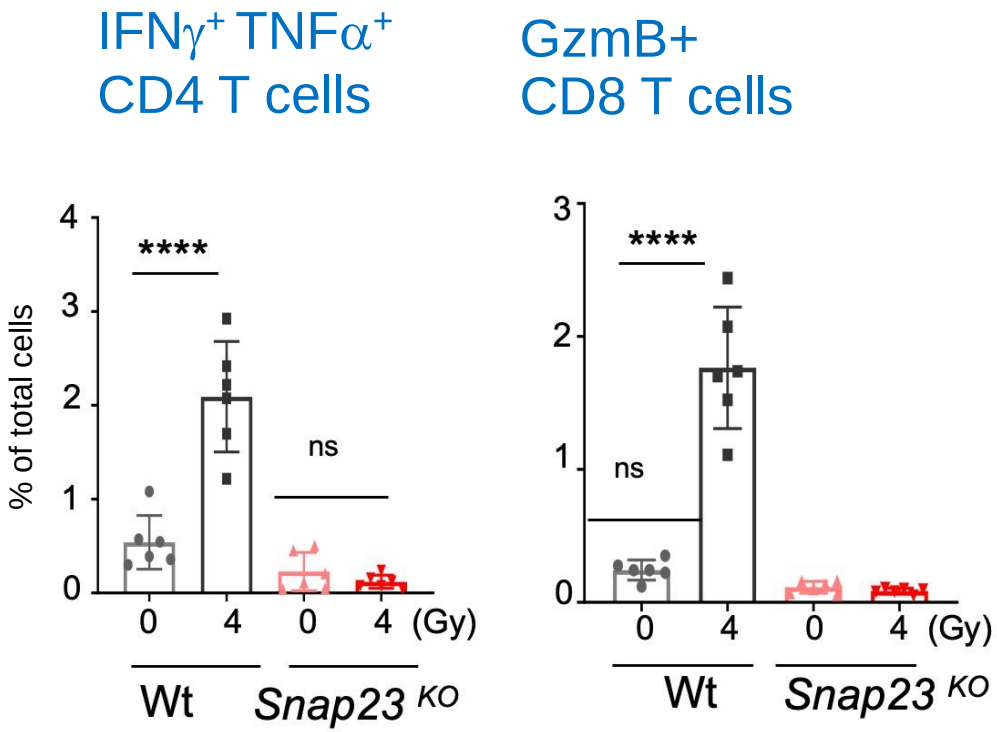


BALF analysis

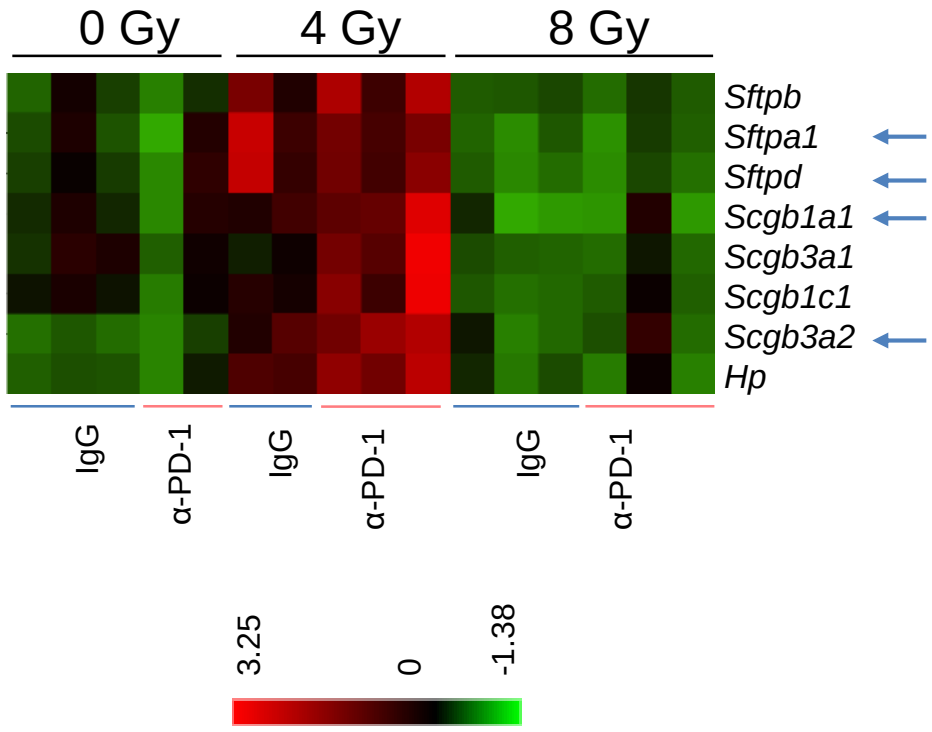


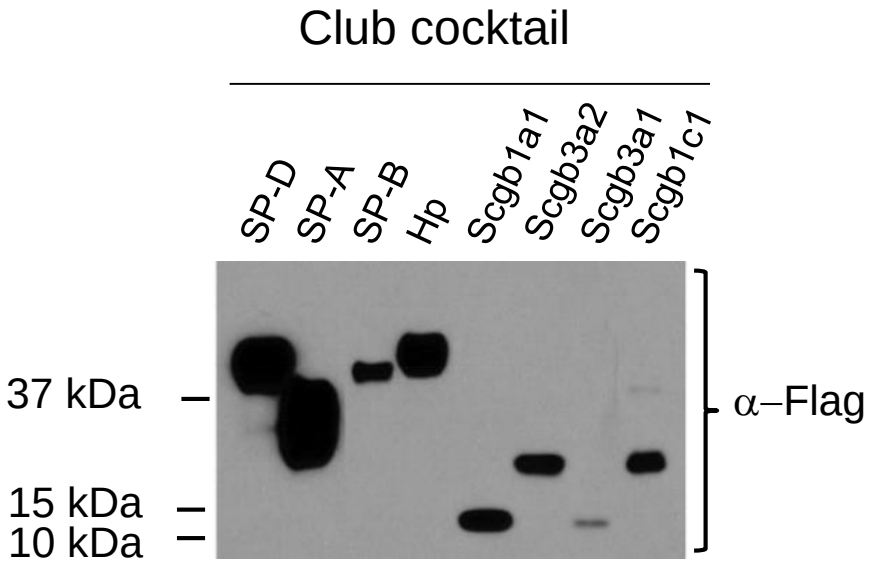
Club cell secretome: a major determinant of therapeutic efficacy



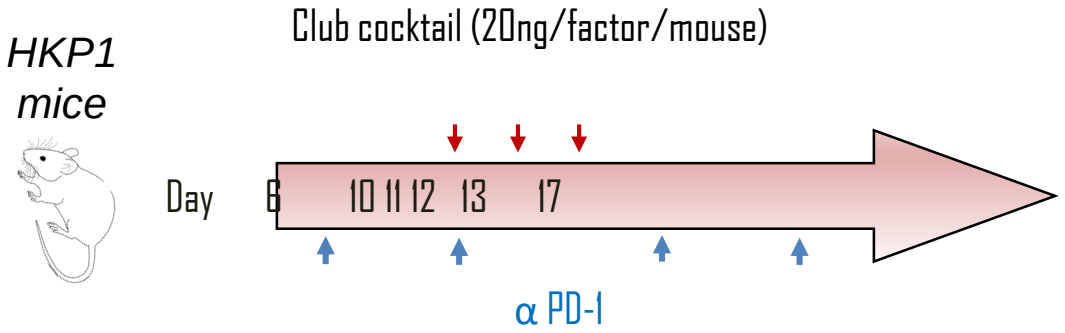


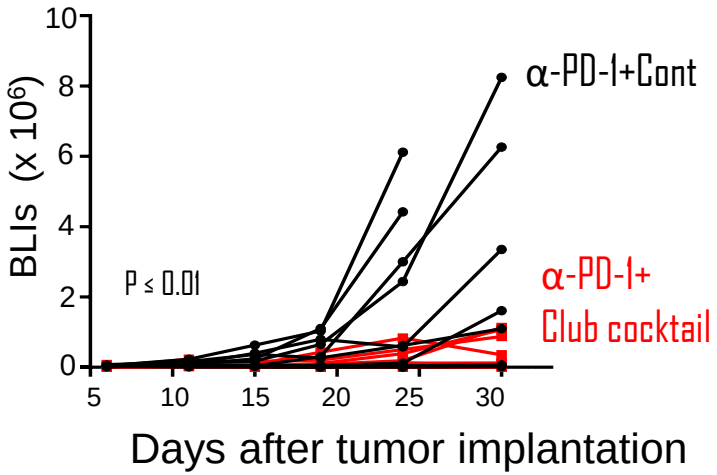
Expression of club secretory proteins.

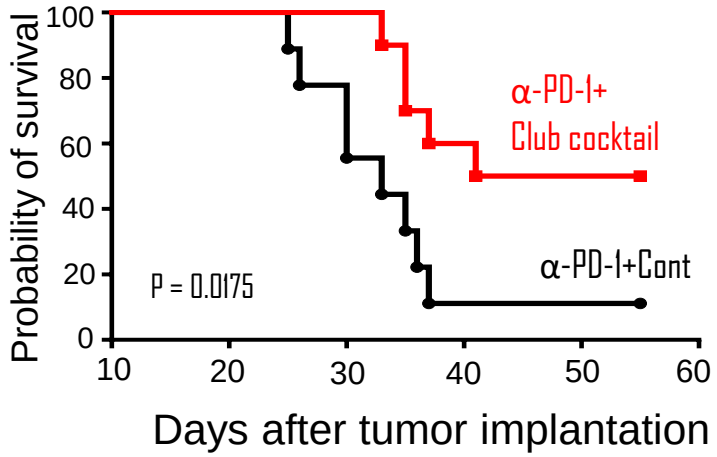


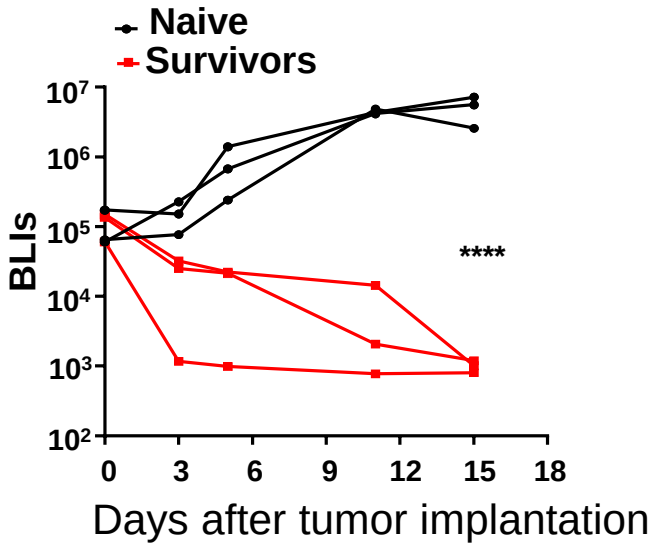


Club cell secretome: a major determinant of therapeutic efficacy.





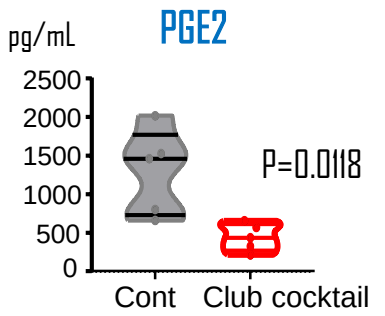


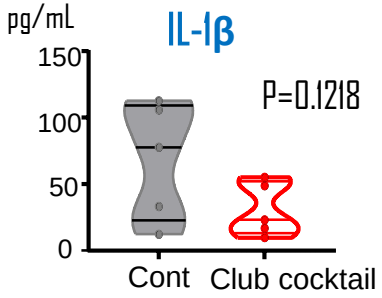


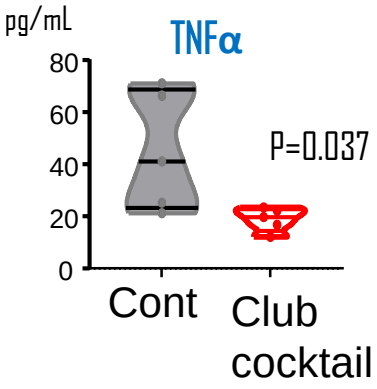
Ban et al. Nature Cancer 2021

Club cells and inflammatory mediators

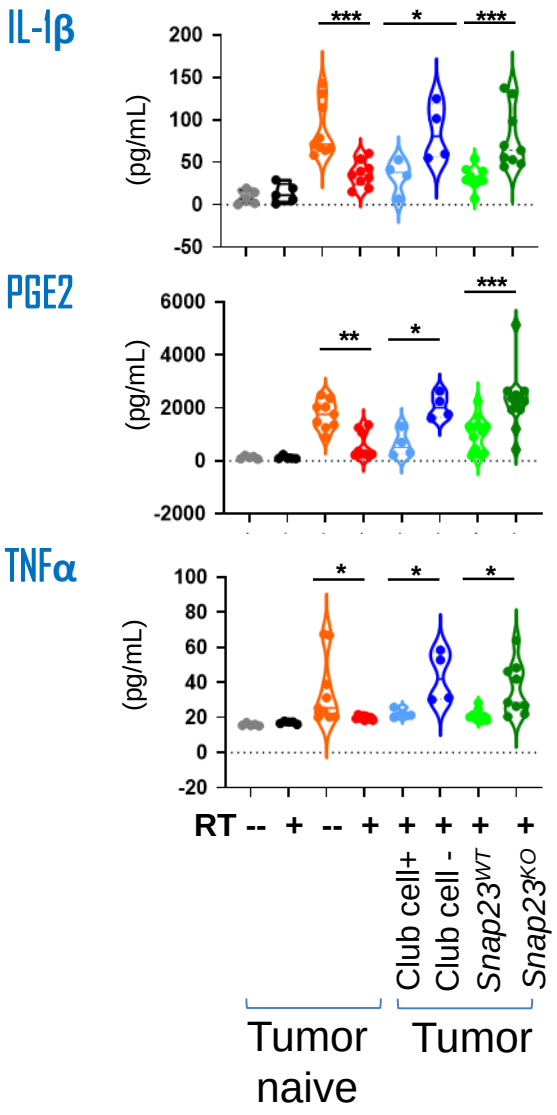
Club cell cocktail





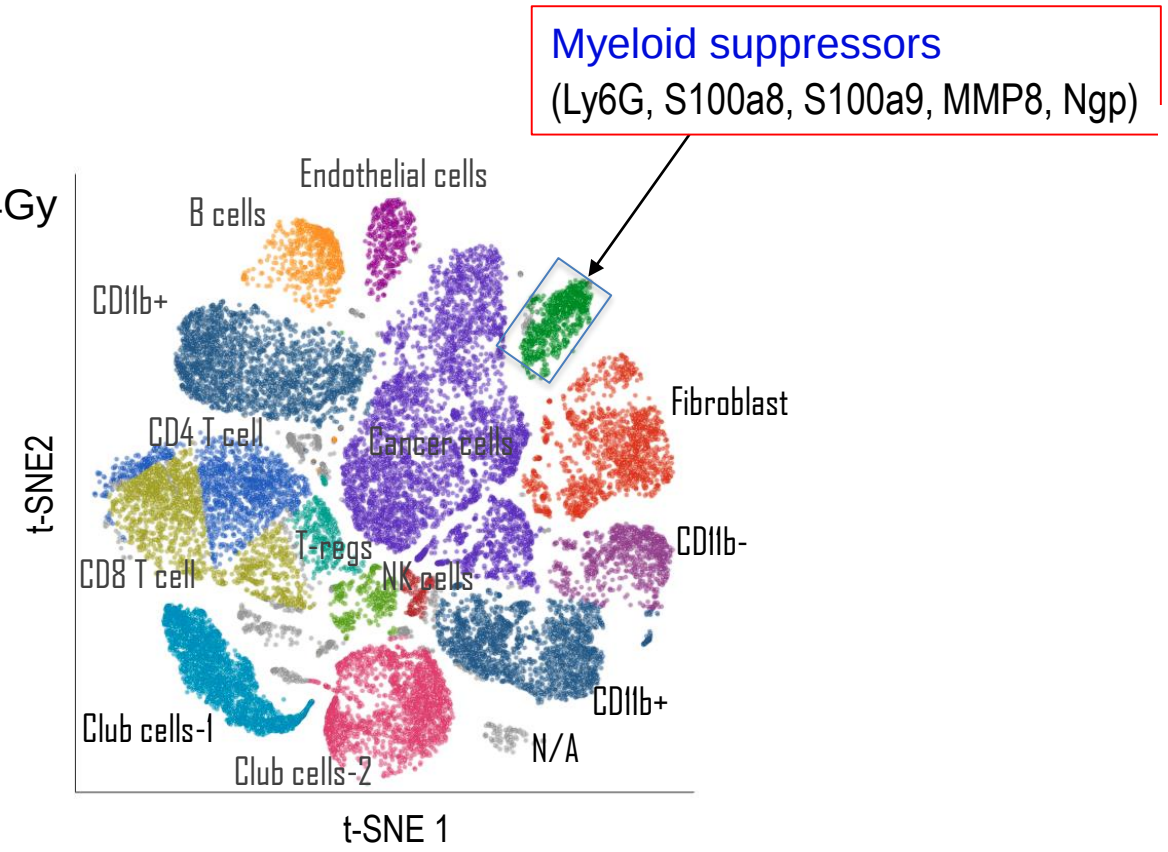


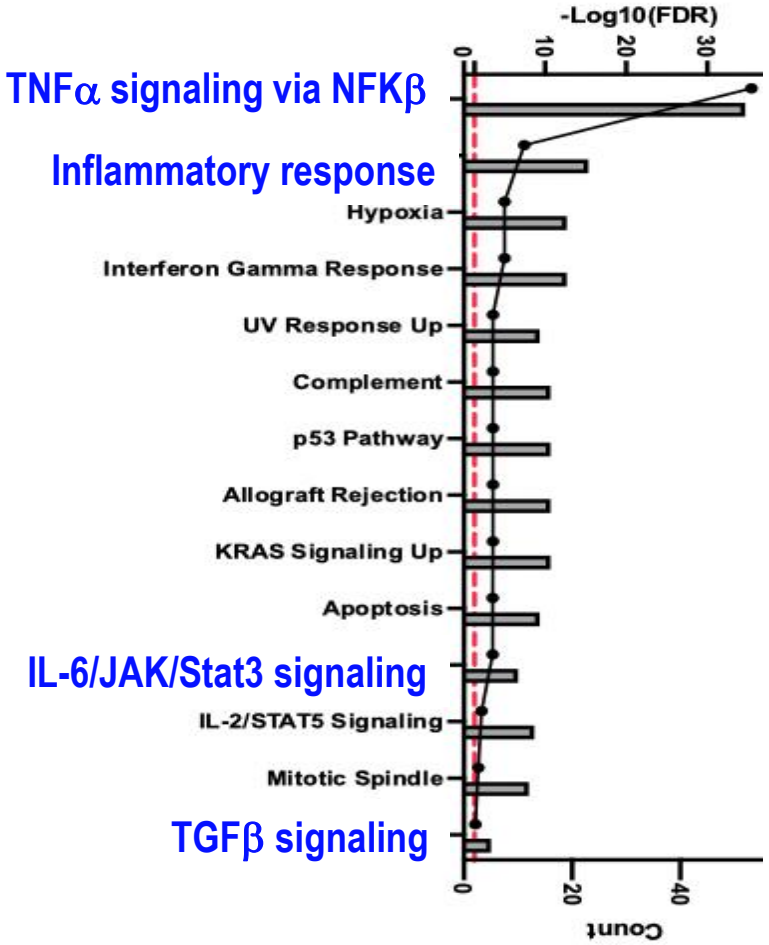
Club cell deficiency



scRNA-seq uncover activation of myeloid suppressor cells

Cohort 1: IgG
Cohort 2: IgG + 4Gy
Cohort 3: α-PD-1
Cohort 4: α-PD-1 + 4Gy

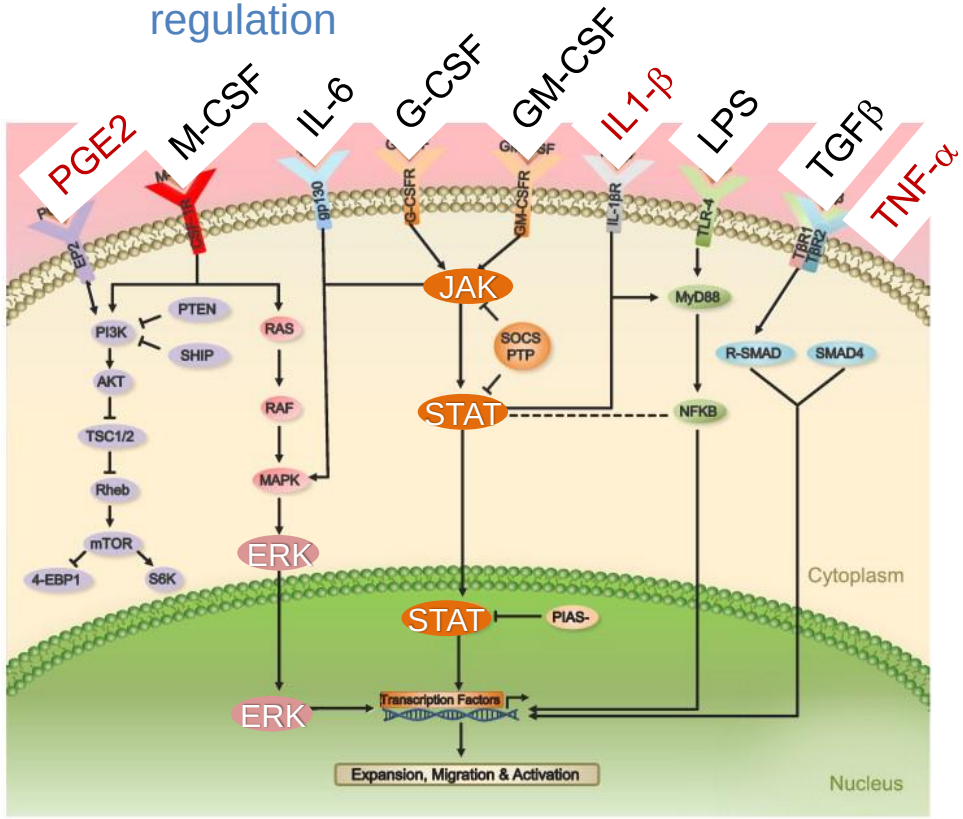




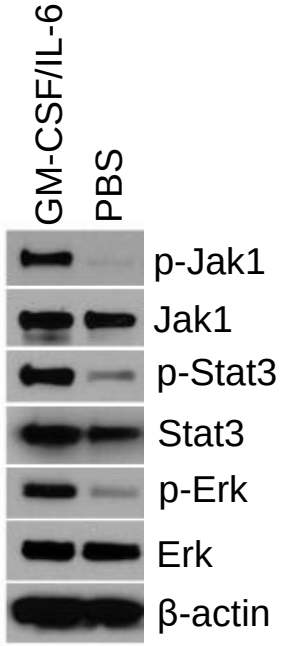
Blue: Classical myeloid suppressive pathways.

Myeloid cells and their immunosuppressive activity.

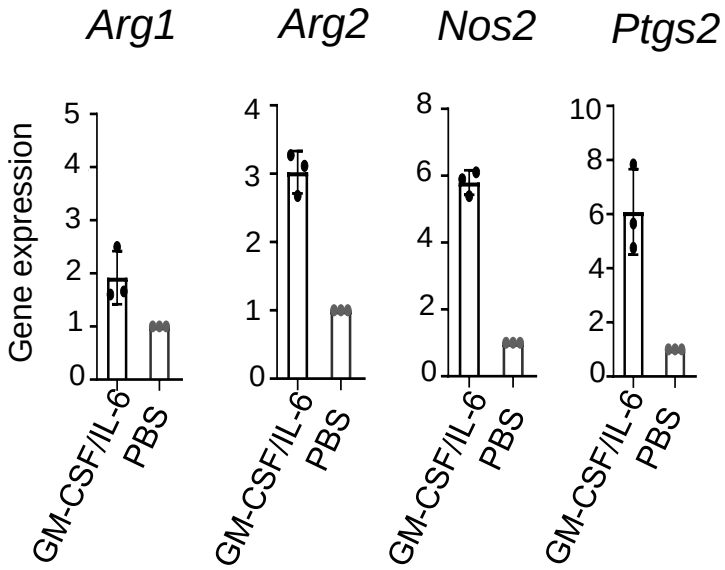
Signaling pathways in MDSC regulation



Arginase 1
Arginase 2
NOS
ROS
IL-10

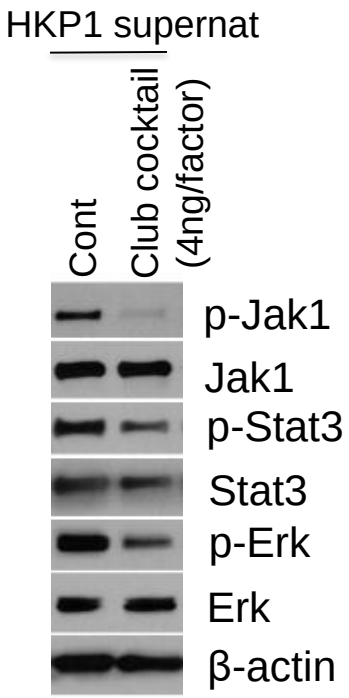


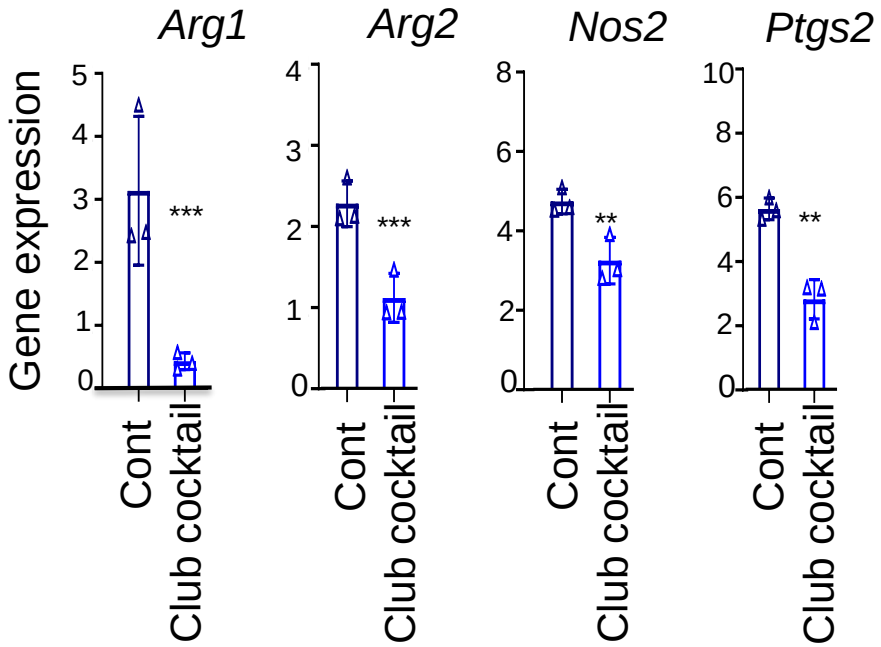
Controls

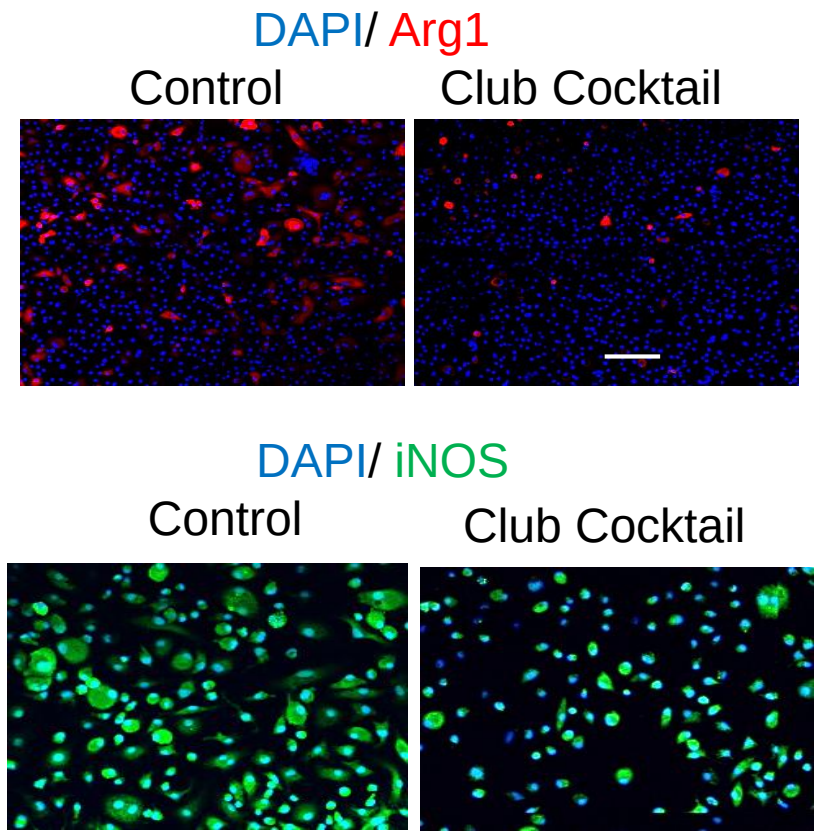


Trikha and Carlson (2014)
Biochimica et Biophysica Acta

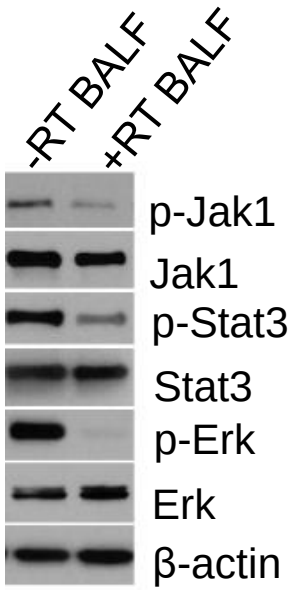
Club secretory proteins reduced immunosuppressive activity of Myeloid cells

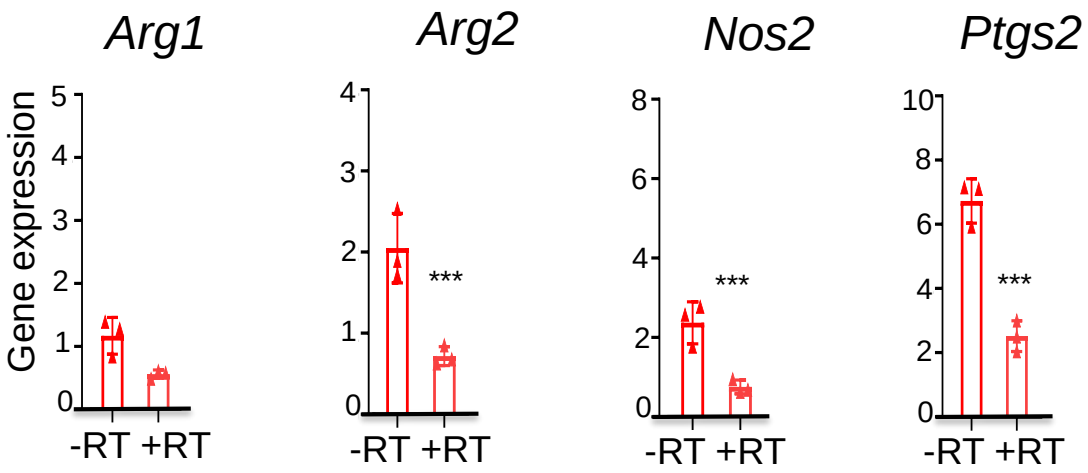




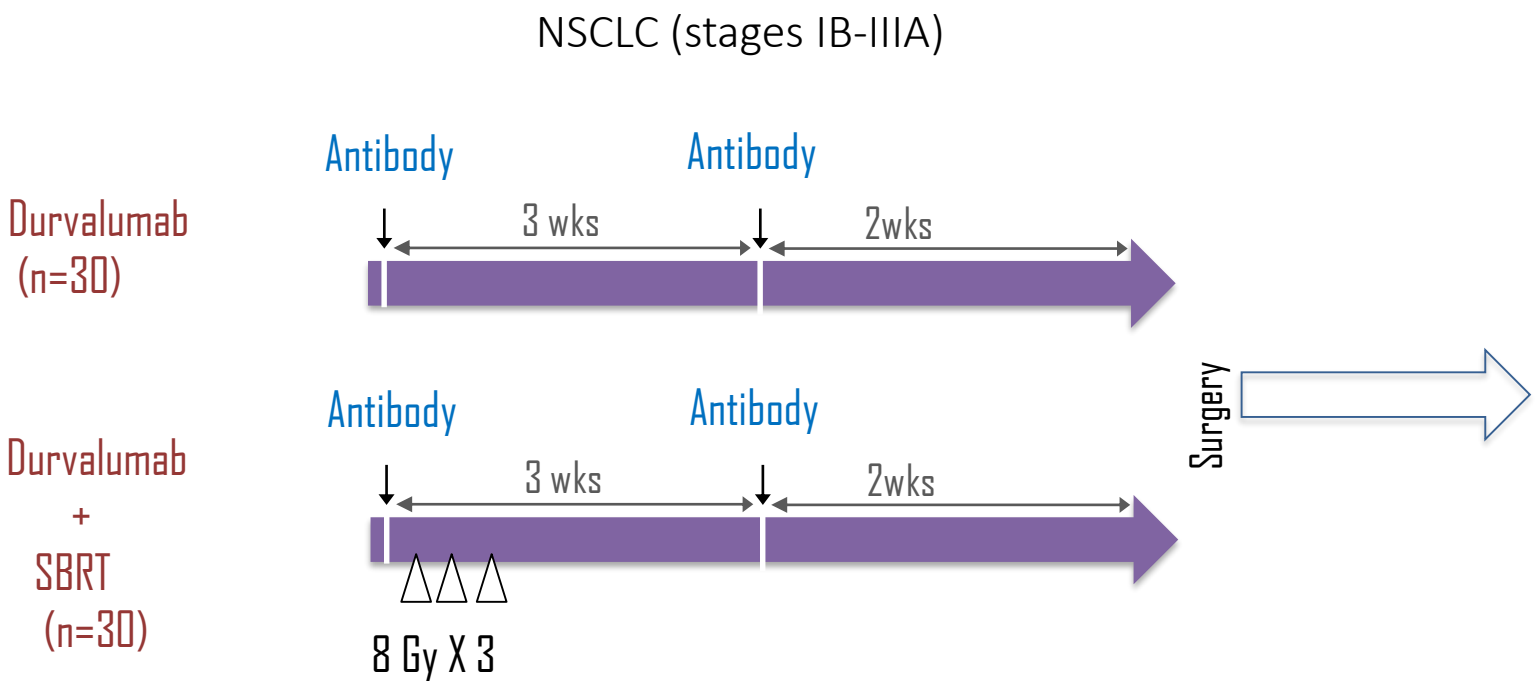


BALF from RT treated mice reduces immunosuppressive activity of Myeloid cells

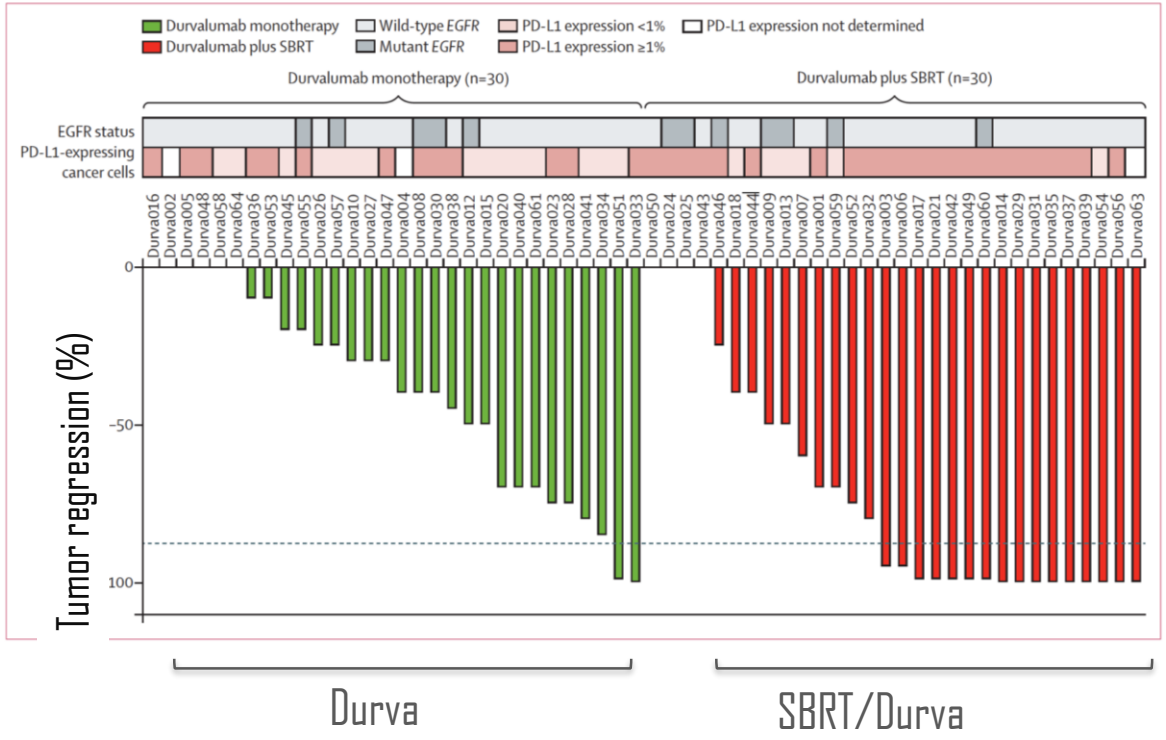




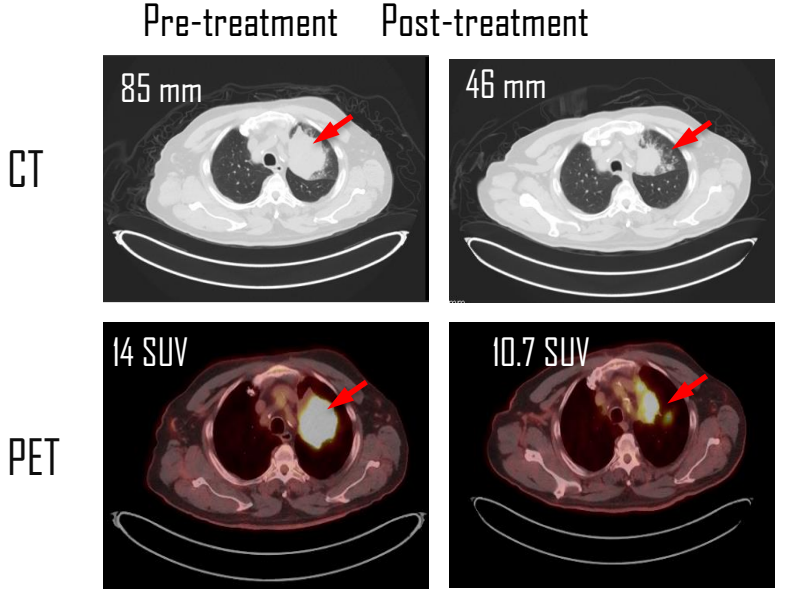
Randomized phase II neoadjuvant trial of durvalumab with SBRT in patients with resectable NSCLC (NCT02904954).



Major and complete pathological responses

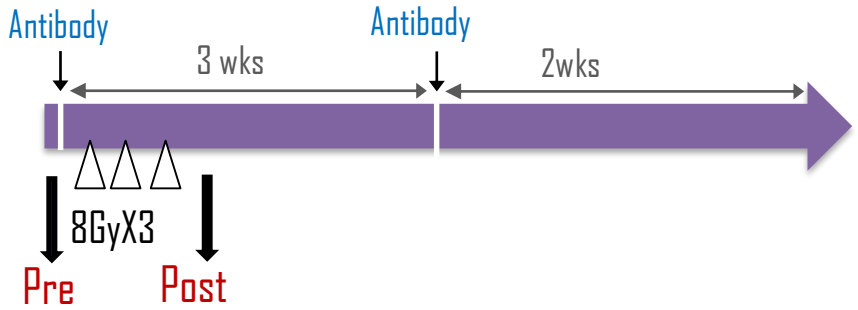


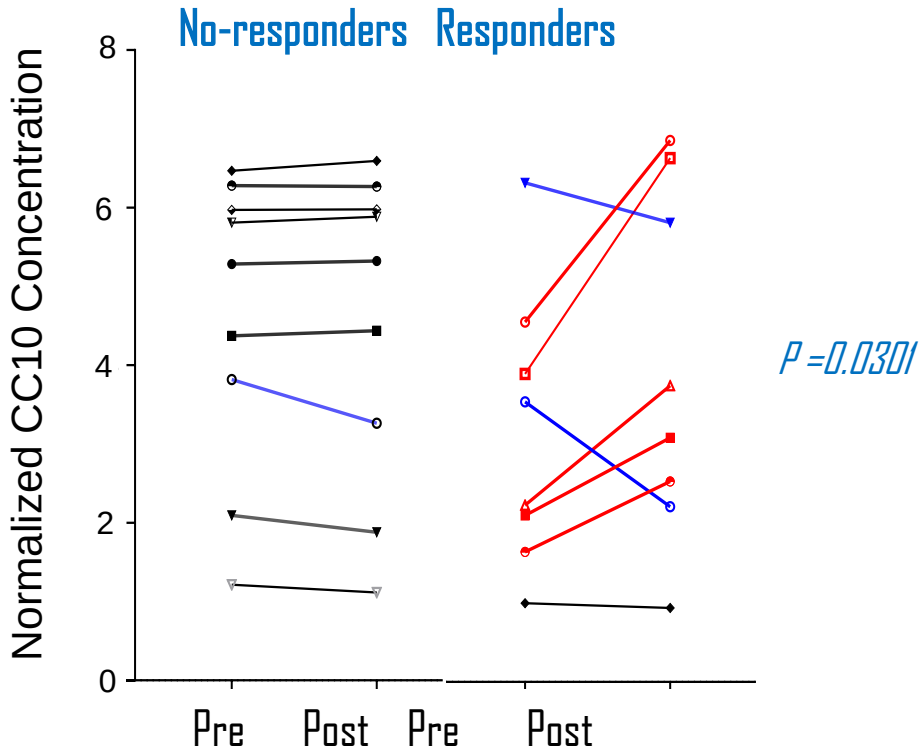
SBRT+ Durva (003) Responder



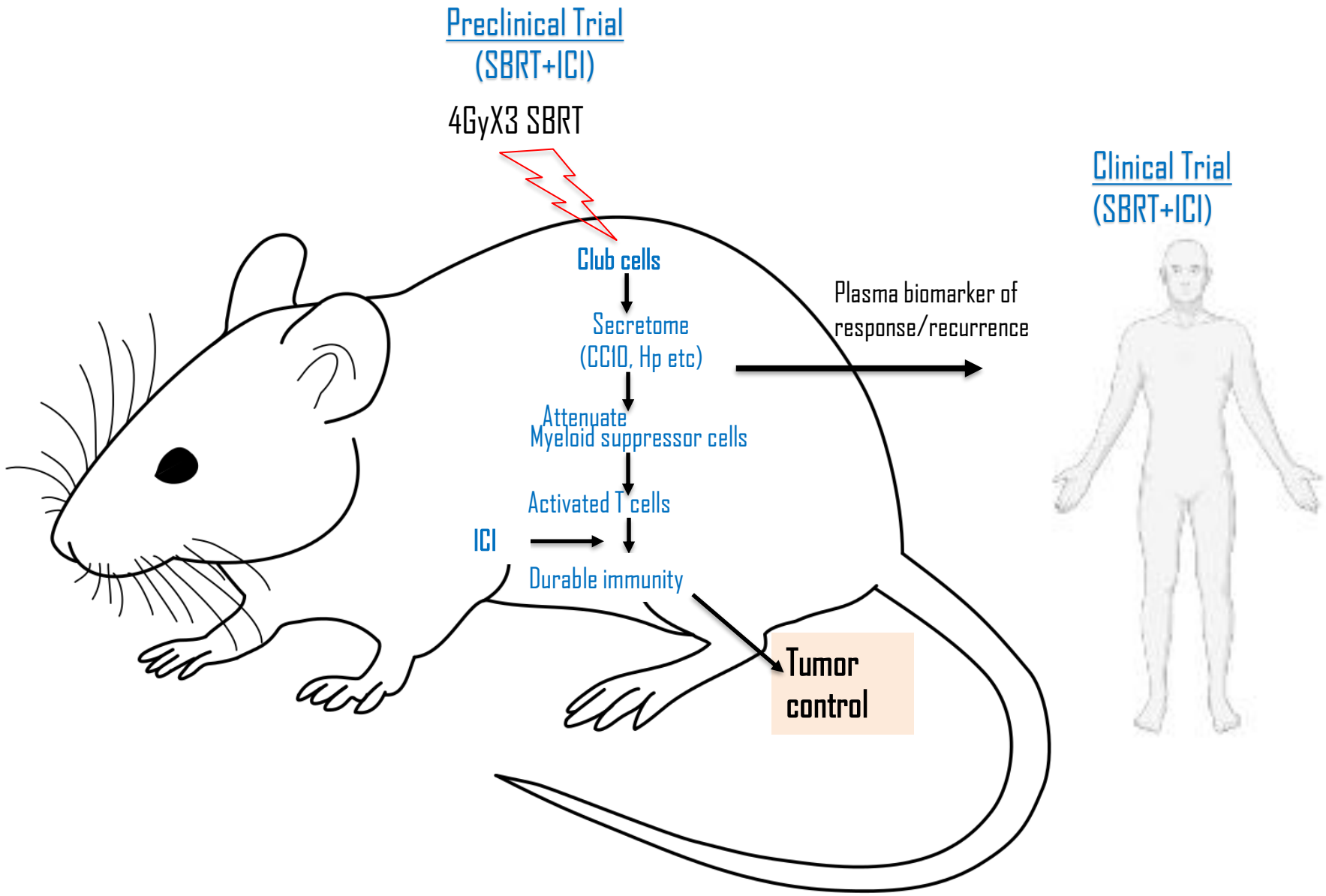
Altorki ….Formenti et al. Lancet Oncol, 2021

Increased plasma CC10 levels post SBRT in responders to therapy





P =0.0301


Preclinical Trial
(SBRT+ICI)
4GyX3 SBRT
Club cells
Secretome
(CC10, Hp etc)
Attenuate
Myeloid suppressor cells
Activated T cells
ICI
Durable immunity
Tumor
control
Plasma biomarker of
response/recurrence
Clinical Trial
(SBRT+ICI)

Yi Ban
Geoff Markowitz
Yang Bai
Michael Crowley
Divya Ramchandini
Sharrell Lee
Mitchell Martin
Aakanksha Rajeev Kapoor
Diamile Tavarez
Shira Yomtoubian
Arshdeep Singh`
Murtaza Malbari

Gao Lab
Dingcheng Gao
Yue Zhou

Houston Methodist
Stephen Wong
Jianting Shen

Thoracic Division
Nasser K. Altorki
Jeff Port
Jonathan Villena-Vargas
Cathy Spinalli
Joyce Gakuria
Abu Nasar
Murtaza Malbari

Radiation oncology/ Small animal radiator
Silvia Formenti
Sandra Demaria
Jeffery Karynak

Computational
Olivier Elemento
Yang Hu

Genomics Core
Jenny Xiang
Jason McCormick

Jay and Vicky Furman Fund
NCI T32 CA203702
CTSC KL2-TR-002385
PhRMA Foundation 2020

Summary

- A specific dose of subablative RT was identified that increased T cell infiltration and effector phenotypes.
- RT as a single agent did not generate durable anti-tumor immunity.
- RT in combination with PD1 blockade conferred tumor regression and improved survival in mouse NSCLC models
- Uncovered immune-modulating functions of RT-activated lung resident *Scgb1a1*$^{+}$ club cells.
- Identified importance of RT-activated club cell seretome in improving the efficacy of ICI.
- A set of 8 club cells secretory proteins inhibited immunosuppressive myeloid cells, reduced pro-tumor inflammatory mediators, and enhanced anti-tumor immunity to improve the efficacy of ICI.
- Notably, CC10, a member of the club cell secretome was increased in the plasma of NSCLC patients who responded to RT in combination with ICI.
- Our data suggest that future neoadjuvant radioimmunotherapy clinical trial in NSCLC may consider RT doses that can effectively mediate activation of lung resident club cells to achieve maximal therapeutic efficacy.
- Club cell factors may be developed as inhibitors of myeloid suppressor cells.

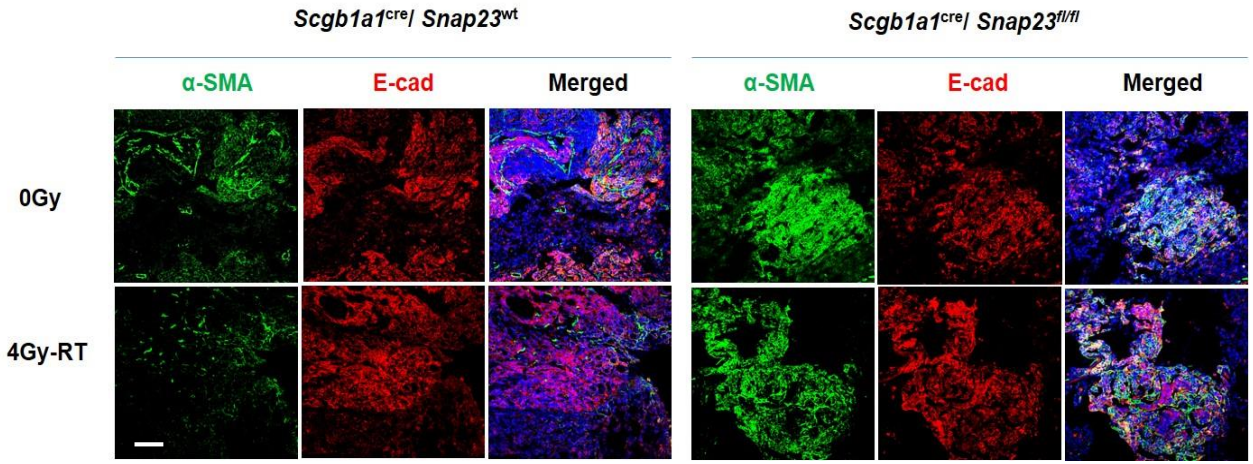
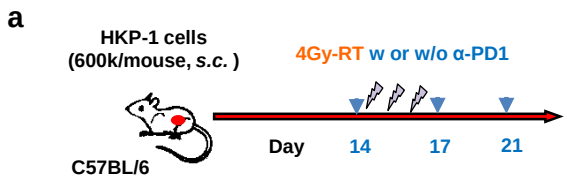
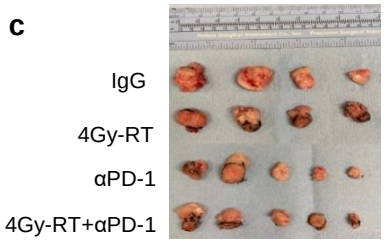


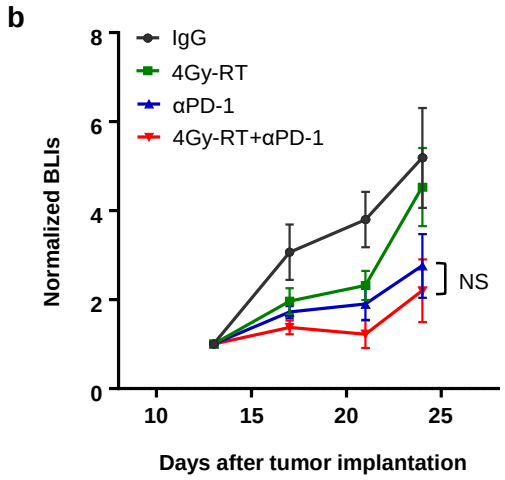
Figure 1. The secretome of club cells reduced accumulations of CAFs co-localizing with HKP1 tumor.

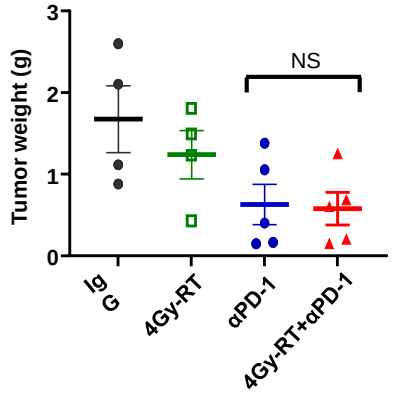
Representative stitched immunofluorescent images of 0Gy (mock) and 4Gy-RT-radiated HKP1 lobes from *wt* control mice (*Scgb1a1*^{cre}/ *Snap23*^{wt}) and mice harboring club cell-specific *Snap23* knock-out (*Scgb1a1*^{cre}/ *Snap23*$^{fl/fl}$). Sections were stained with α-SMA (a fibroblast marker, green,) and E-cadherin (tumor islets, red). Scale bar:100μM

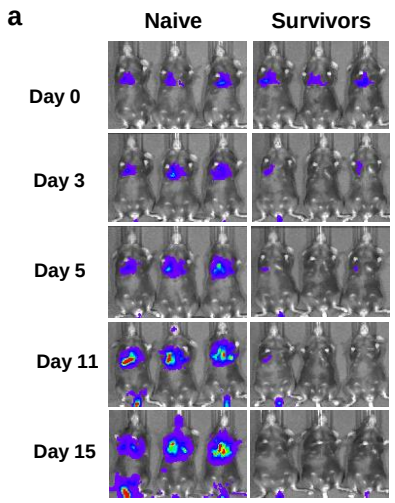
4Gy-RT did not enhance the efficacy of PD-1 inhibition in HKP-1 subcutaneous tumors.

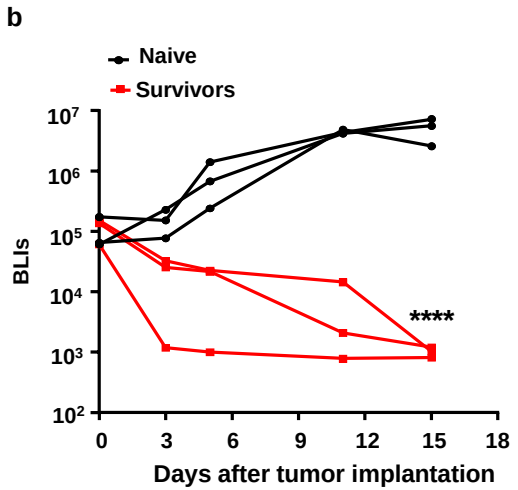


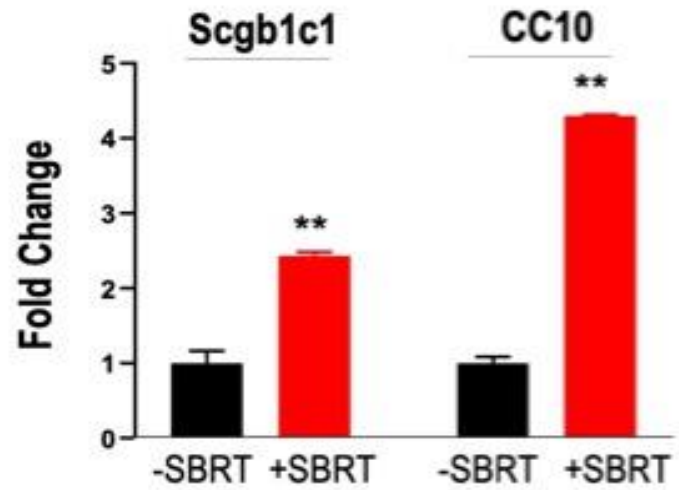







a
Naive
Survivors
Day 0
Day 3
Day 5
Day 11
Day 15


b
Naive
Survivors
BLIs
10^7
10^6
10^5
10^4
10^3
10^2
****
0
3
6
9
12
15
18
Days after tumor implantation


Scgb1c1
CC10
Fold Change
0
1
2
3
4
5
**
**
-SBRT
+SBRT
-SBRT
+SBRT