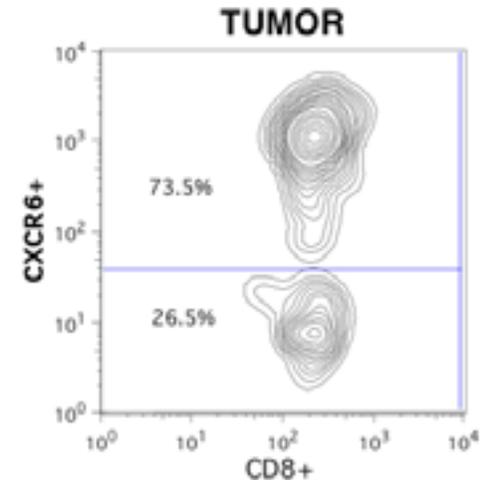
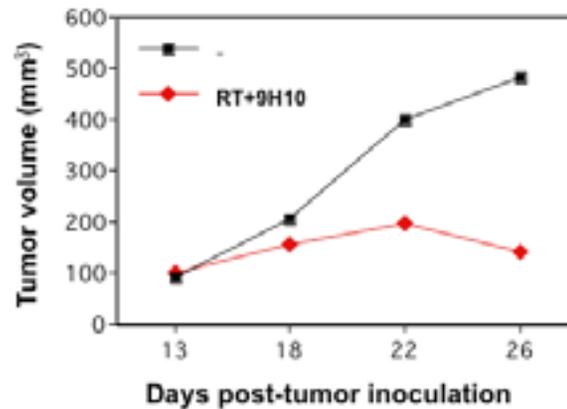
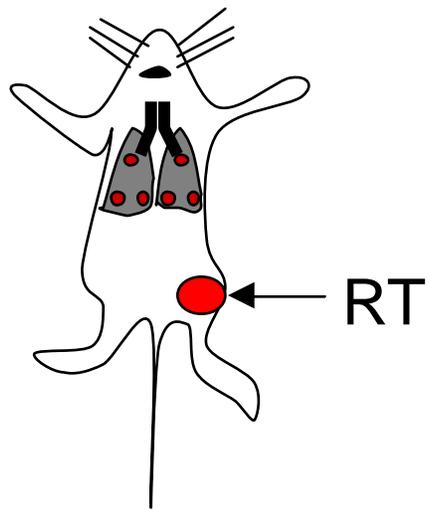


**Stress-induced signals promote
T cell-tumor cell interactions
following anti-CTLA4 therapy**

**Maria Grazia Ruocco
NYU School of Medicine**

4T1 mouse breast cancer model



Anti-CTLA4 mAb 9H10 in combination with local RT but NOT alone induced anti-tumor CD8 T cell responses that inhibited the primary tumor and its metastases

CD8 T cells infiltrating the regressing tumors were CXCR6+

Demaria et al., Clin. Cancer Res. 11:728-34, 2005

Matsumura et al., J Immunol 181:3099-3107 2008

Hypothesis

Ionizing radiation alters the expression of cell surface molecules on tumor cells, resulting in changes in the interactions between tumor cells and T cells.

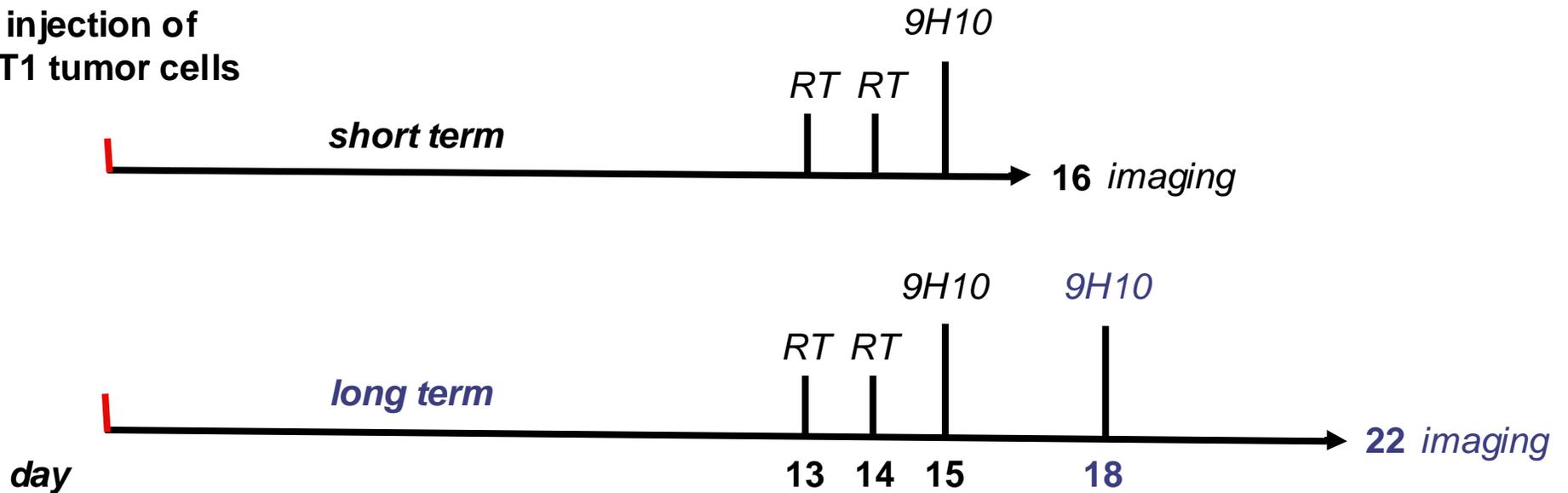
Aim

A better understanding of tumor cell-T cell interactions will contribute to improve clinical approaches.

Experimental model

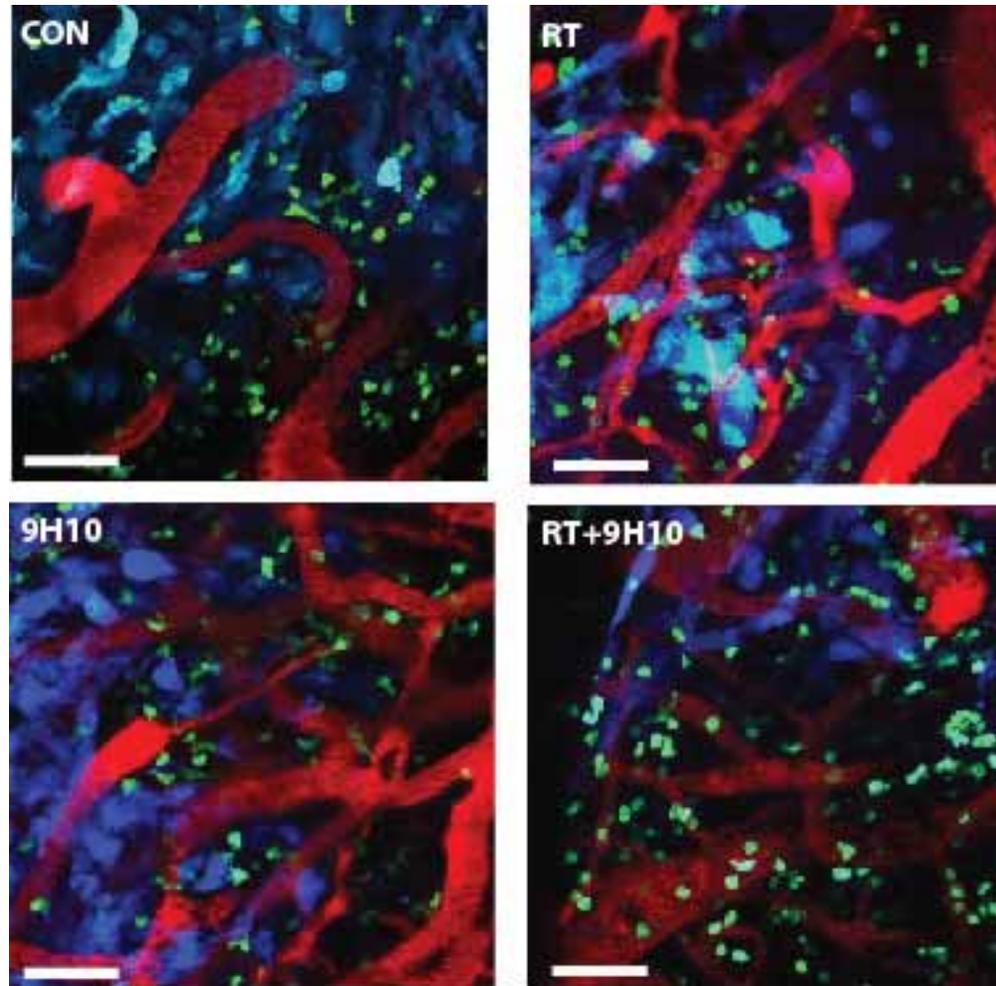


injection of
4T1 tumor cells



Blood vessels
T cells
2nd harmonic

T cells infiltrate the tumor microenvironment



Control

Blood vessels
T cells
4T1 tumor cells

RT

Blood vessels
T cells
4T1 tumor cells

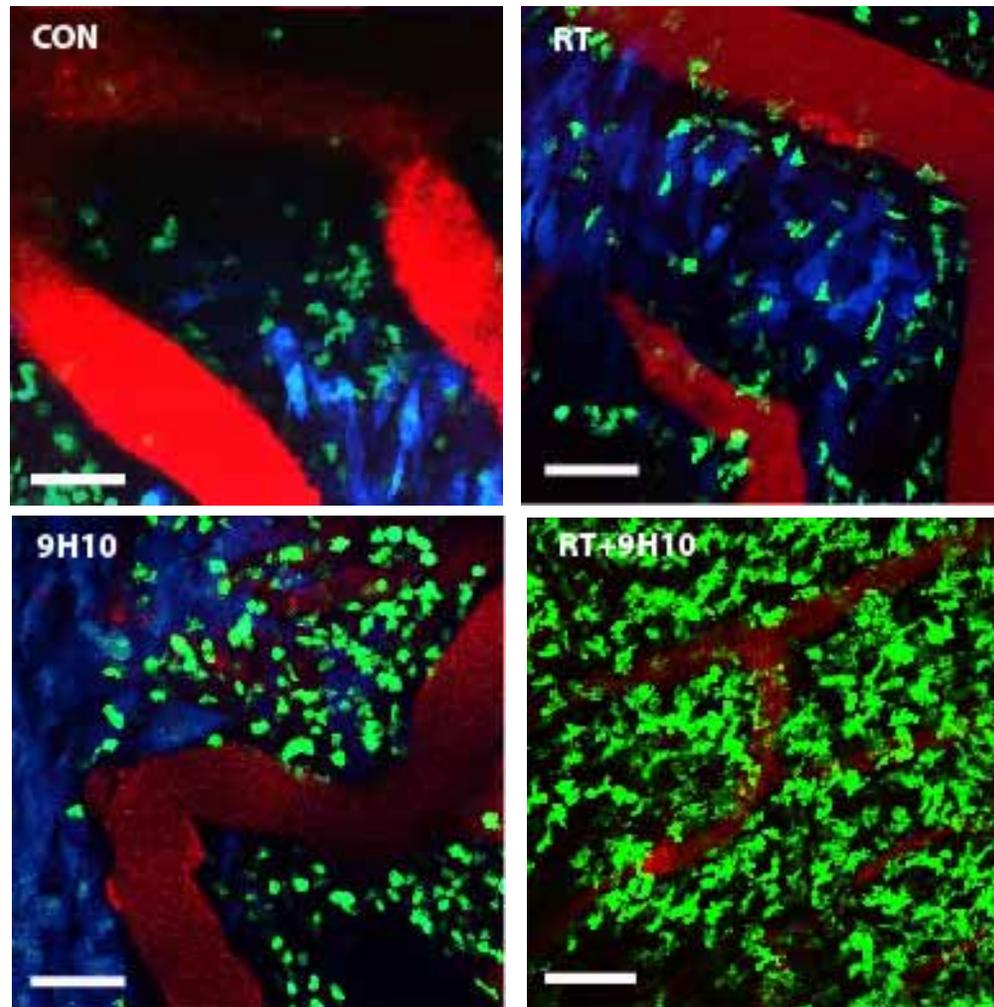
9H10

Blood vessels
T cells
4T1 tumor cells

RT+9H10

Blood vessels
T cells
4T1 tumor cells

RT+9H10 leads to tumor eradication



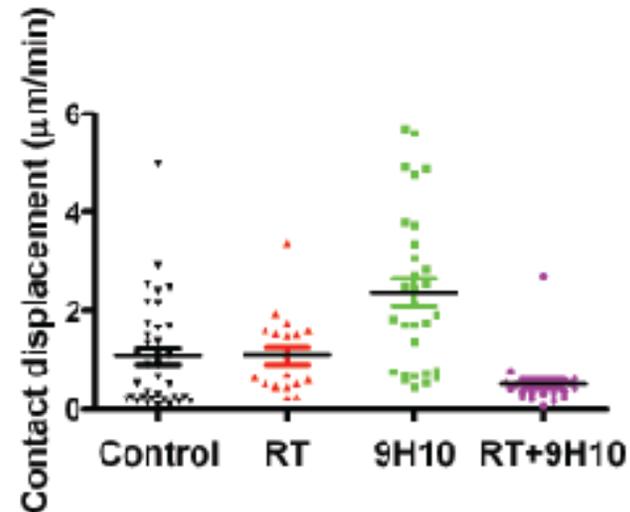
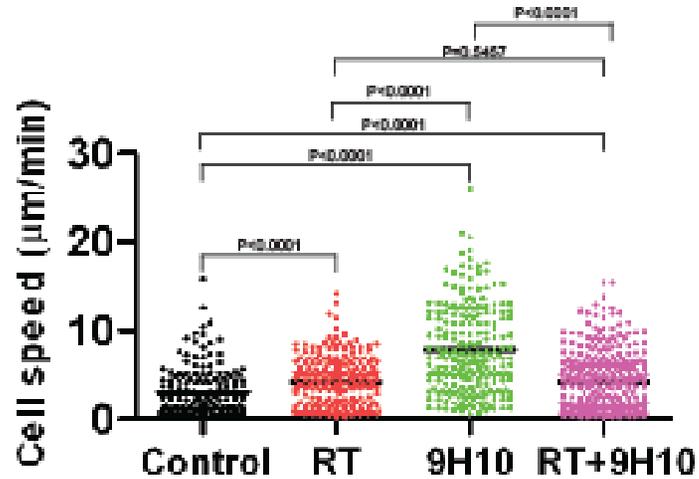
RT

Blood vessels
T cells
4T1 tumor cells

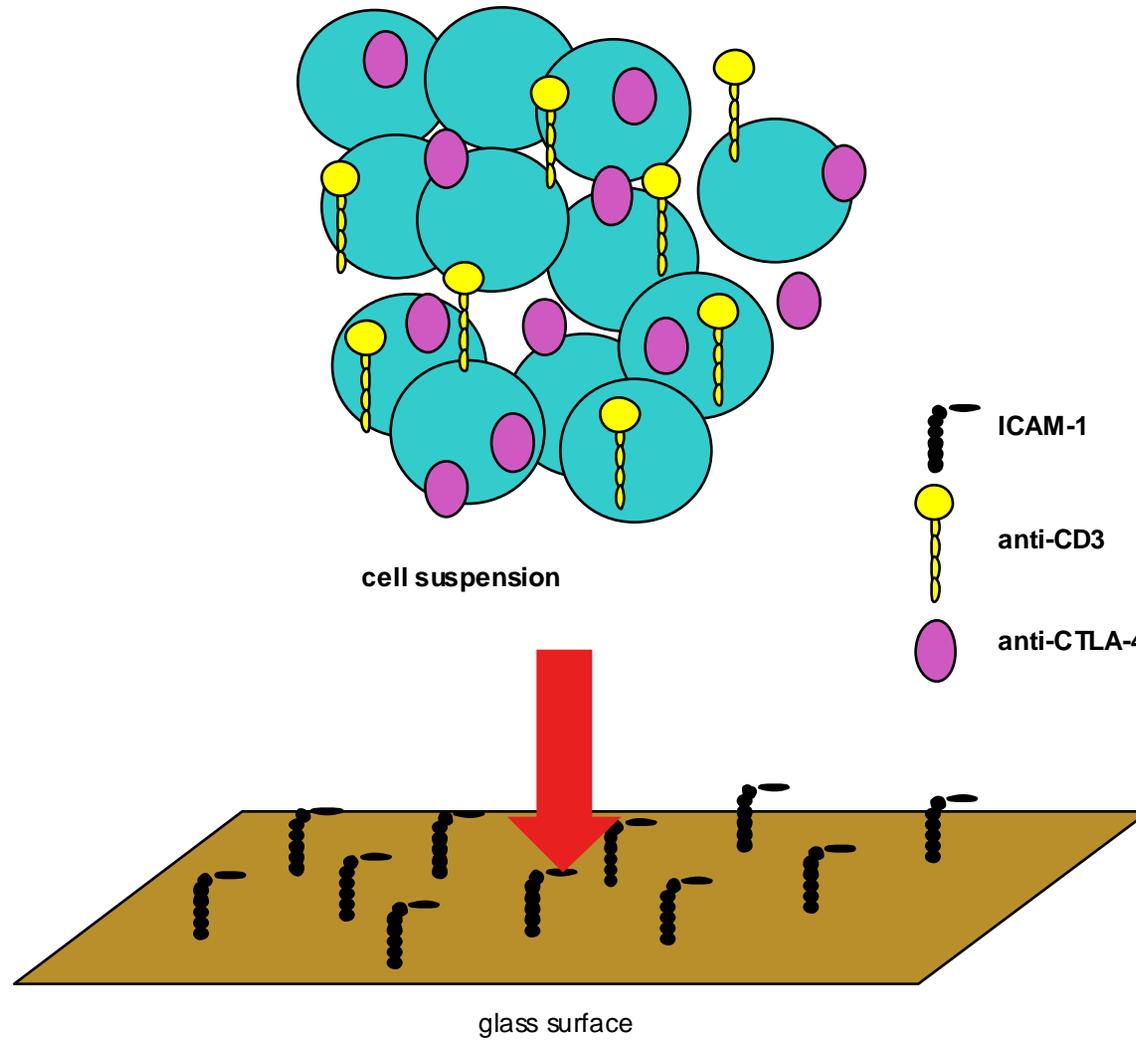
RT+9H10

Blood vessels
T cells
4T1 tumor cells

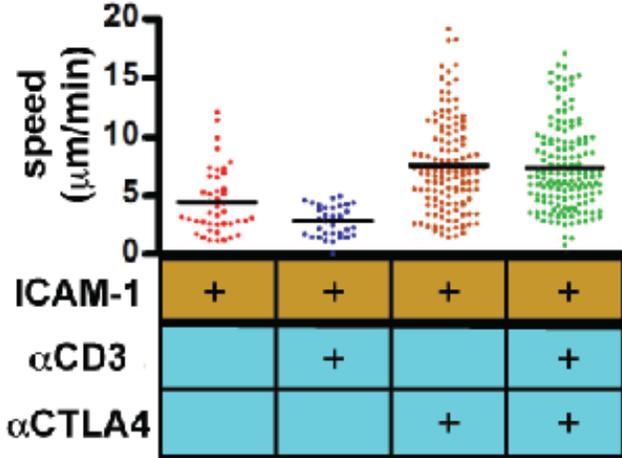
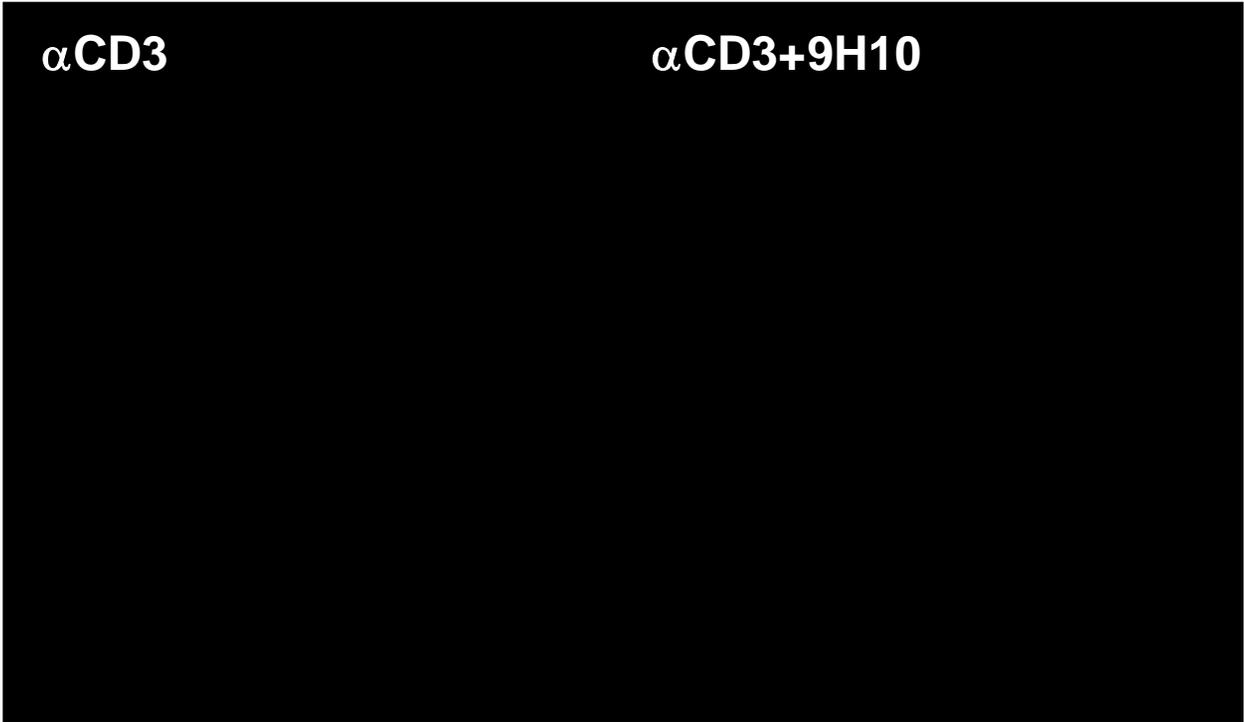
RT and 9H10 combined treatment increases the duration of T cells-tumor cell interactions



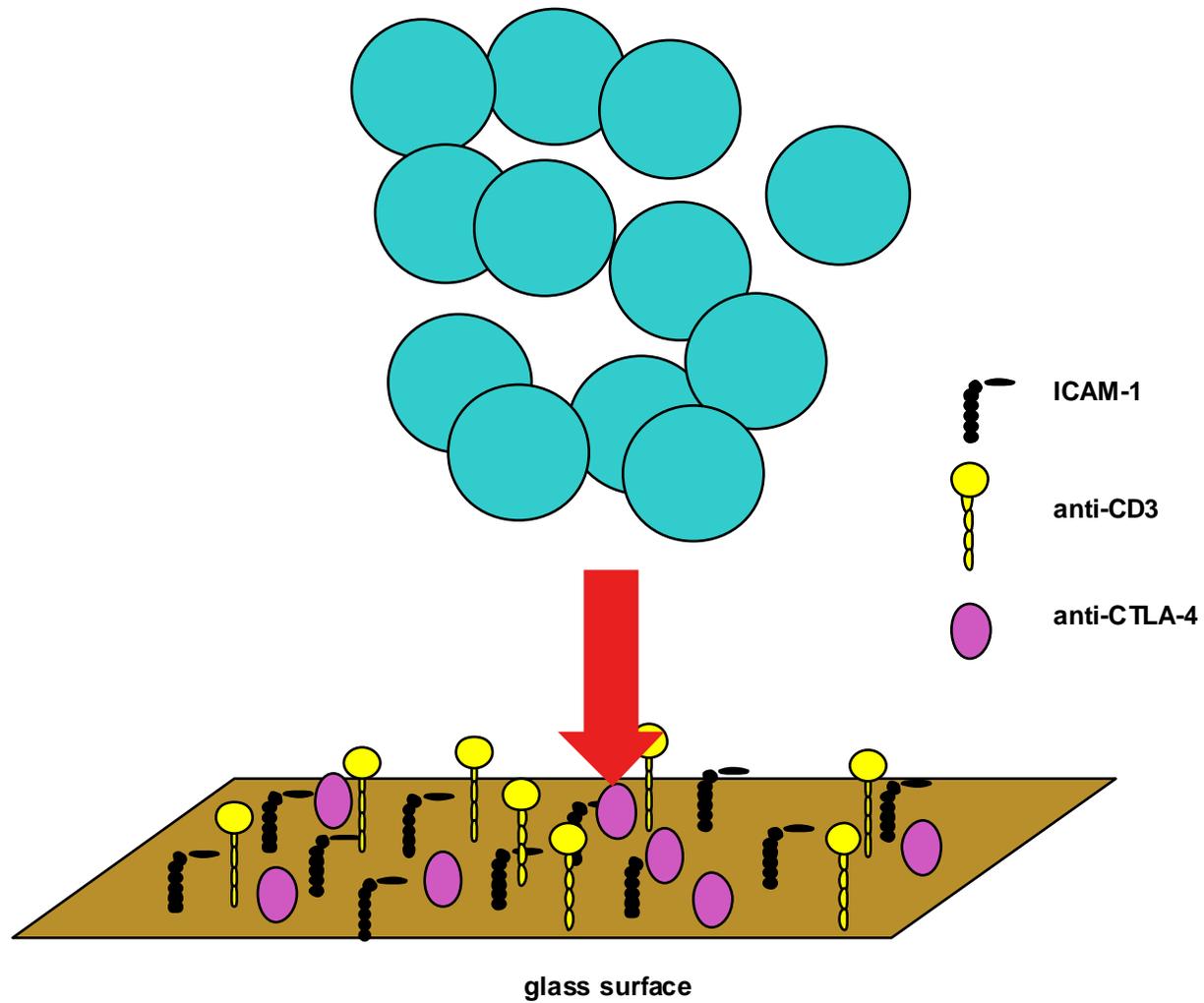
In vitro T cell migration assay



Anti-CTLA4 induces high T cell motility and prevents stable T cell-tumor cell interactions

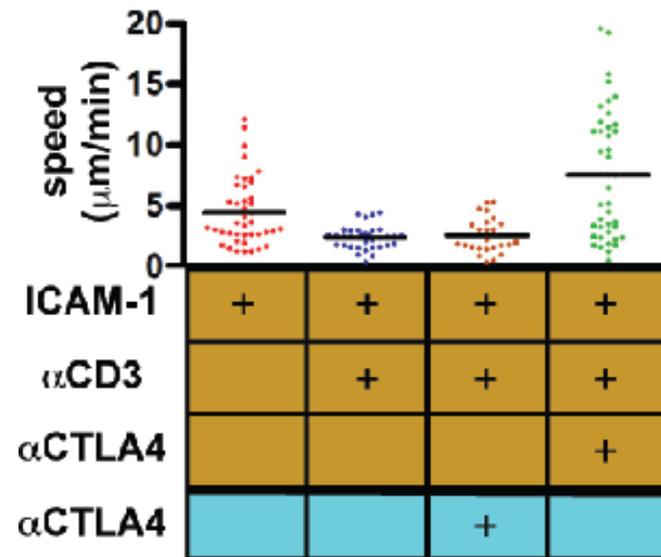


In vitro T cell migration assay

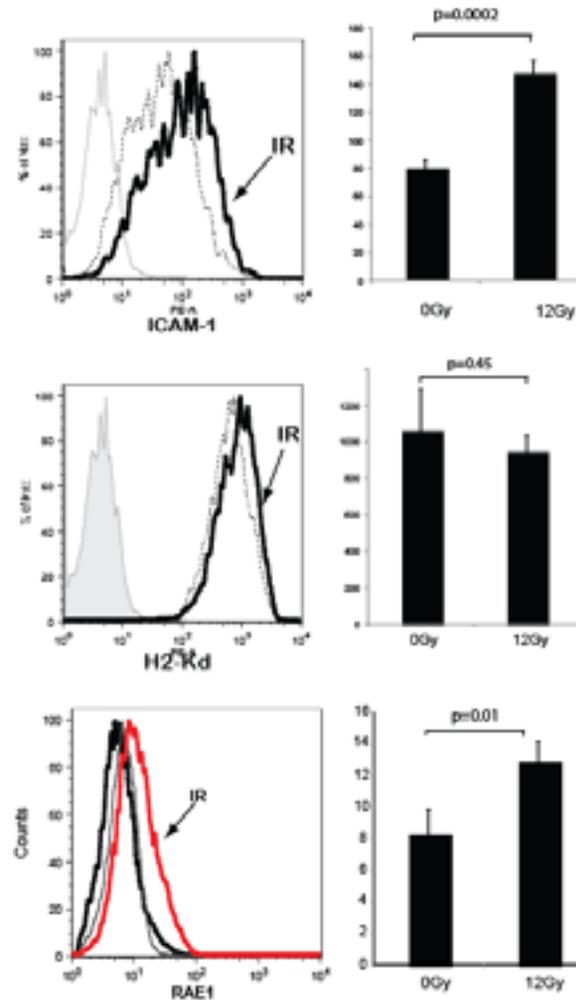


Soluble anti-CTLA4 fails to override a bound anti-CD3 stop signal

bound α CD3+sol9H10



ICAM-1 and Rae1 expression is increased in 4T1 tumor cells following *in vivo* RT



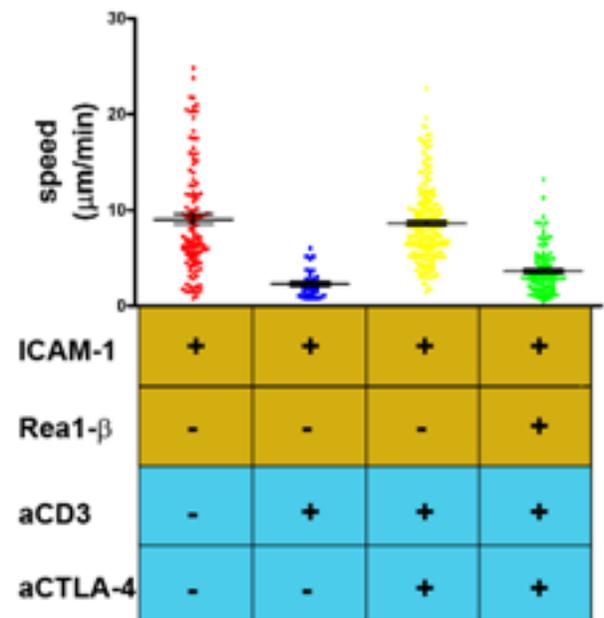
Rae1- β

- A stress-activated molecule, expressed on transformed cells.
- Binds to NKG2D, expressed on effector cells including activated cytolytic CD8 T cells.
- Interaction of Rae1 with NKG2D expressed on anti-tumor CD8+ T cells is important for immune-mediated tumor inhibition in the 4T1 tumor model (Nam JS et al. Cancer Res., 2008).

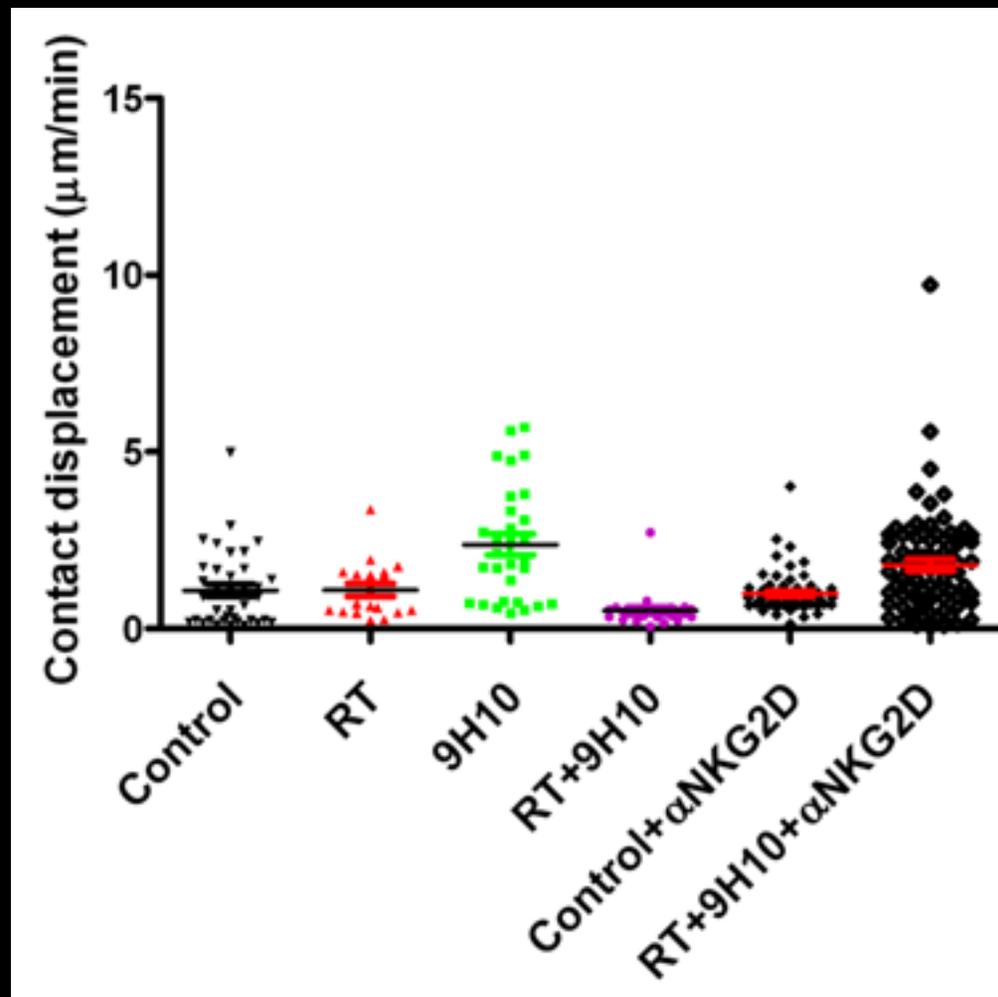
Rae1- β converts the anti-CTLA4 'go' signal into a 'stop' signal

α CD3+9H10

α CD3+9H10+ α NKG2D



In vivo anti-NKG2D antibody treatment:
reverses the effects of RT+9H10



Conclusions

- RT+9H10 increases the duration of T cell-tumor cell interactions *in vivo*.
- 9H10 treatment alone increases T cell migration decreasing the duration of T cell- tumor cell interactions.
- RT upregulates ICAM1 and Rae1 on tumor cells
- Blocking the interactions between Rae1 and NKG2D prevents stable interactions suggesting that these molecules are important for formation of stable IS and T cell activation.

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