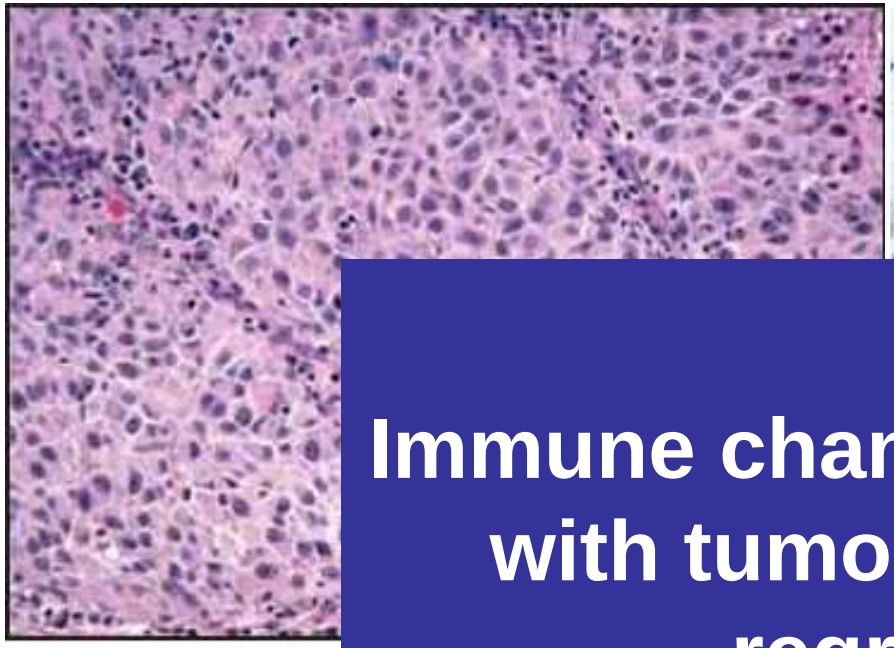
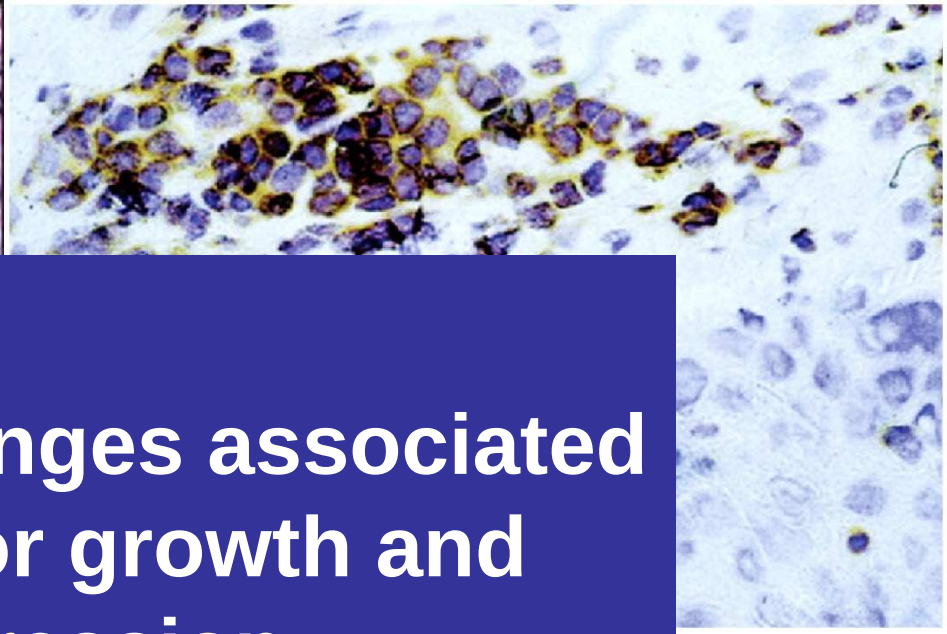
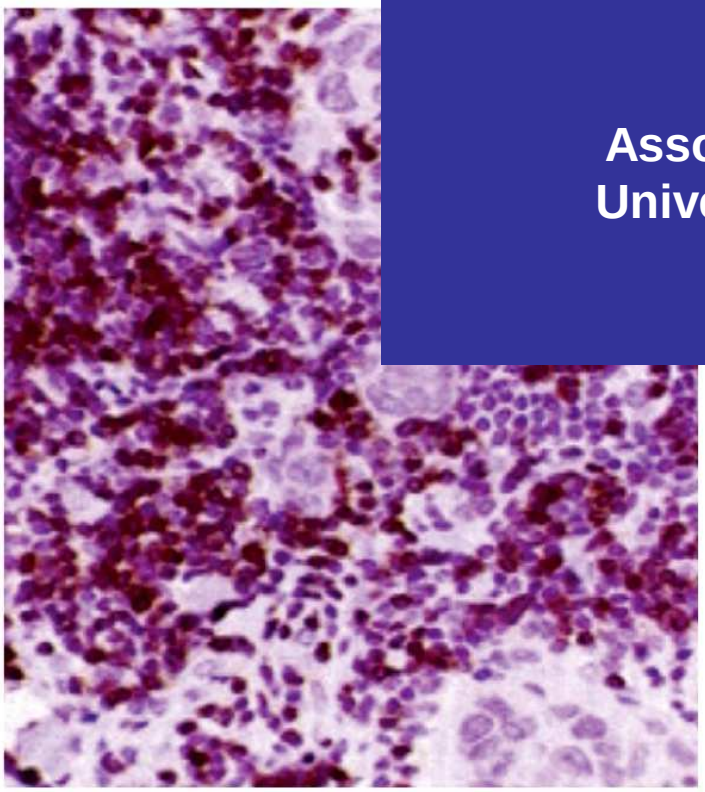
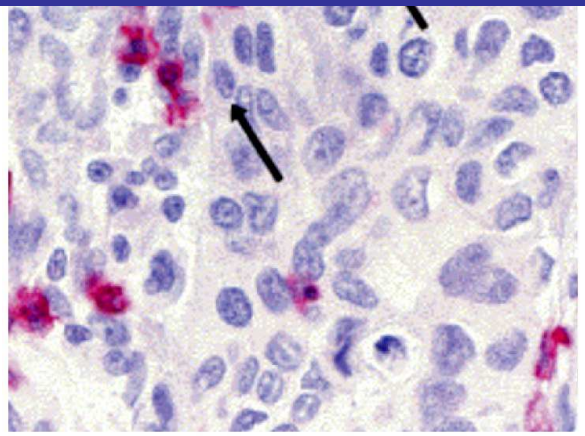
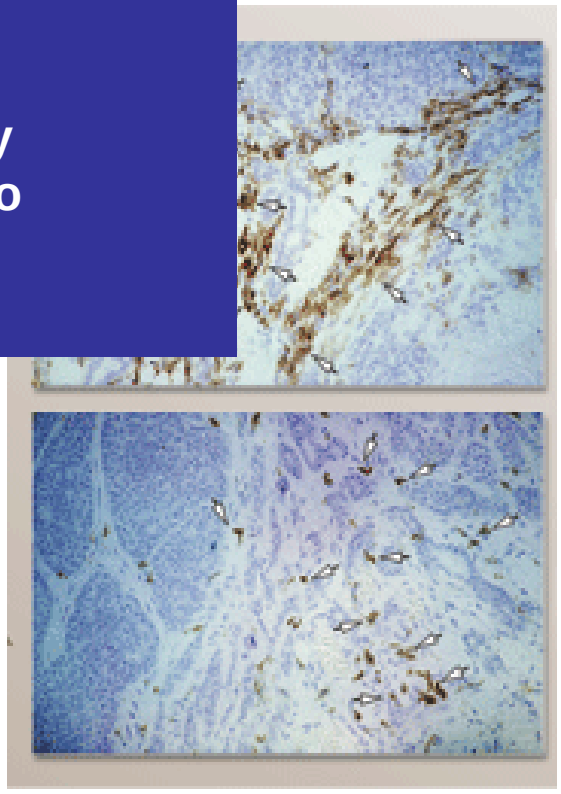



Immune changes associated with tumor growth and regression

Jack Bui, MD, PhD
Associate Professor of Pathology
University of California, San Diego

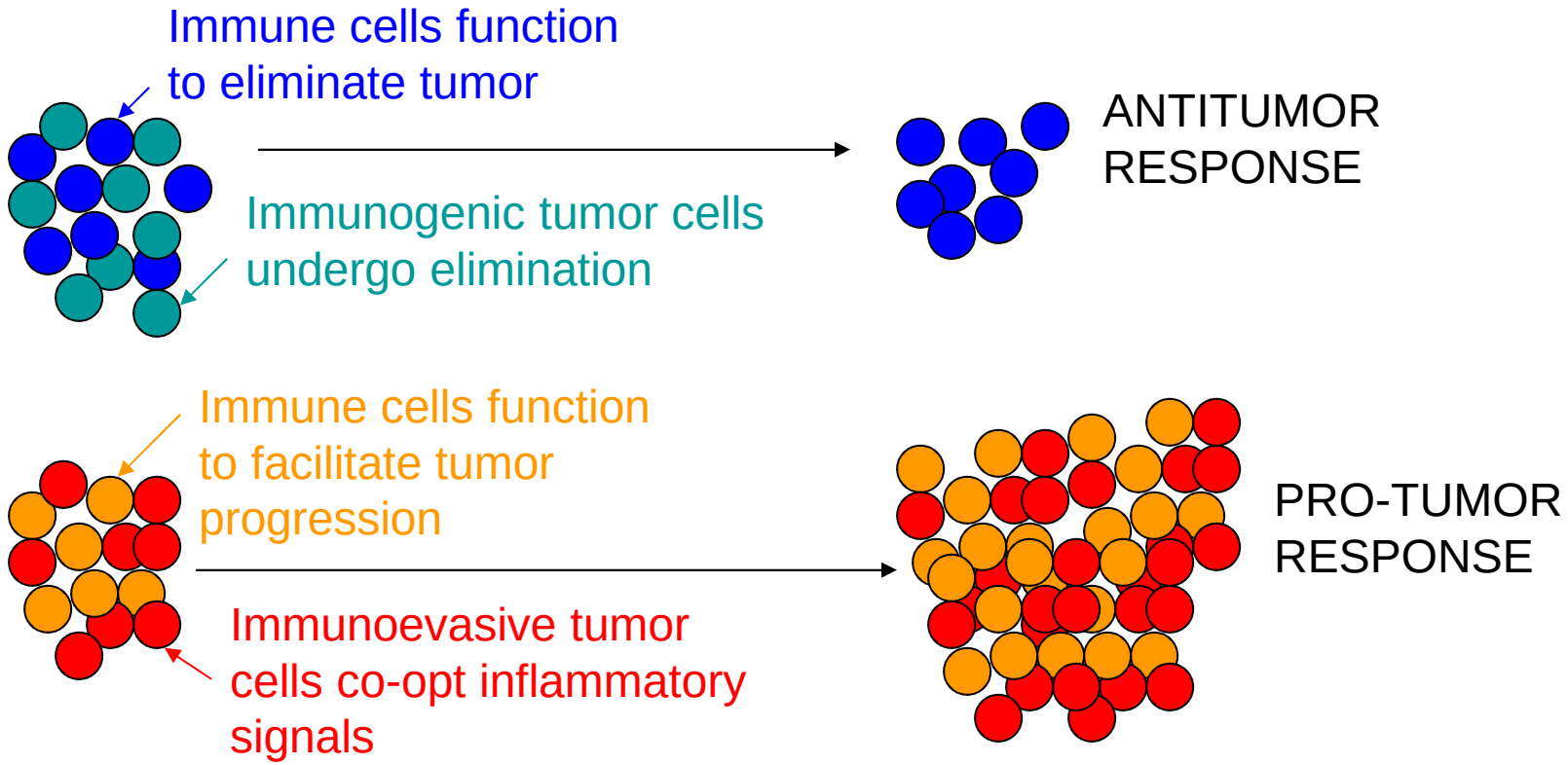
Outline of talk:

1. **Discuss antitumor and pro-tumor immune responses.**
2. **Illustrate a mouse model of tumor regression versus progression.**
3. **Cite limited human studies of tumor regression versus progression at the site of the tumor. (Biomarkers in the blood are NOT discussed in this session.)**

Learning objectives:

1. **Recognize antitumor and protumor immune effector molecules/cells.**
2. **Understand the T-reg to T-effector ratio in predicting immune responses in tumors.**

Immune cell interactions with tumor cells can lead to disparate outcomes

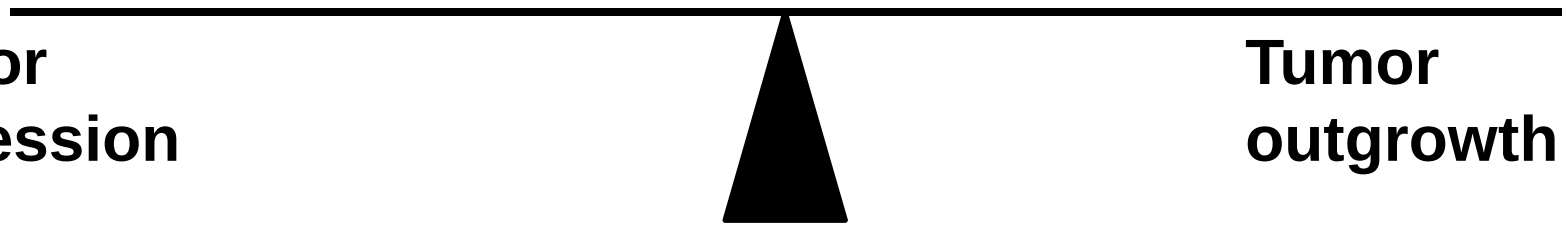


Bui and Schreiber, Curr Op Imm, 2007

What cells/molecules promote vs inhibit tumor formation? A curtailed list is shown.

DCs, M1 macs, N1 neuts, NK cells, Th1 cells, CD8 killer cells, IFNγ, IFNα, IL-2, IL-12, STAT1, perforin, NKG2D

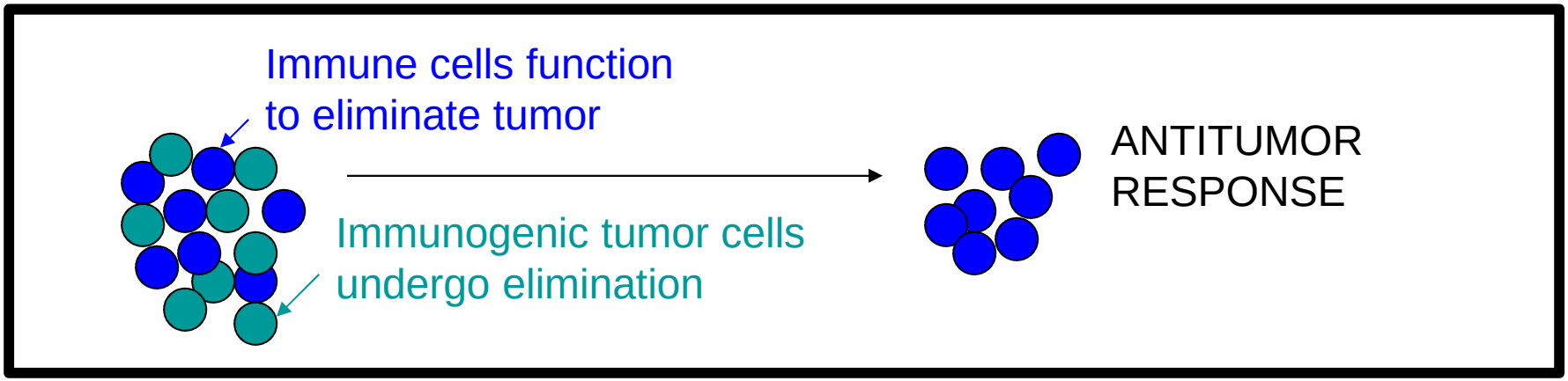
M2 macs, N2 neuts, B cells, T-regulatory cells, Th2 cells, NF-κB, FOXP3, TGF-β, IL-4, IL-10, STAT3, MMPs, CTLA4, PD-1/PD-L1

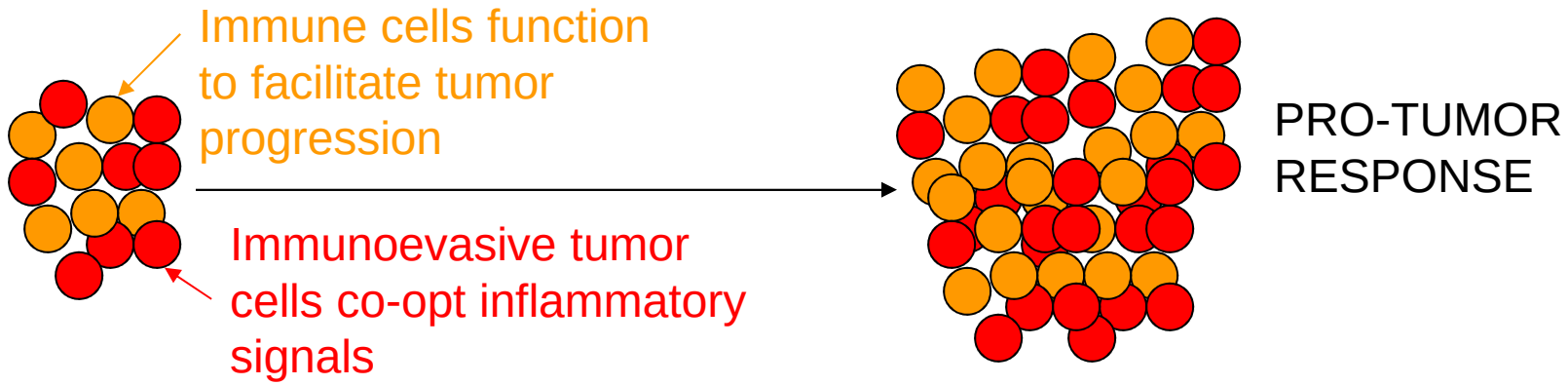


Tumor regression

Tumor outgrowth

Bui Lab Goal – enhance antitumor immune responses





Bui and Schreiber, Curr Op Imm, 2007

Types of naturally occuring antitumor immune responses

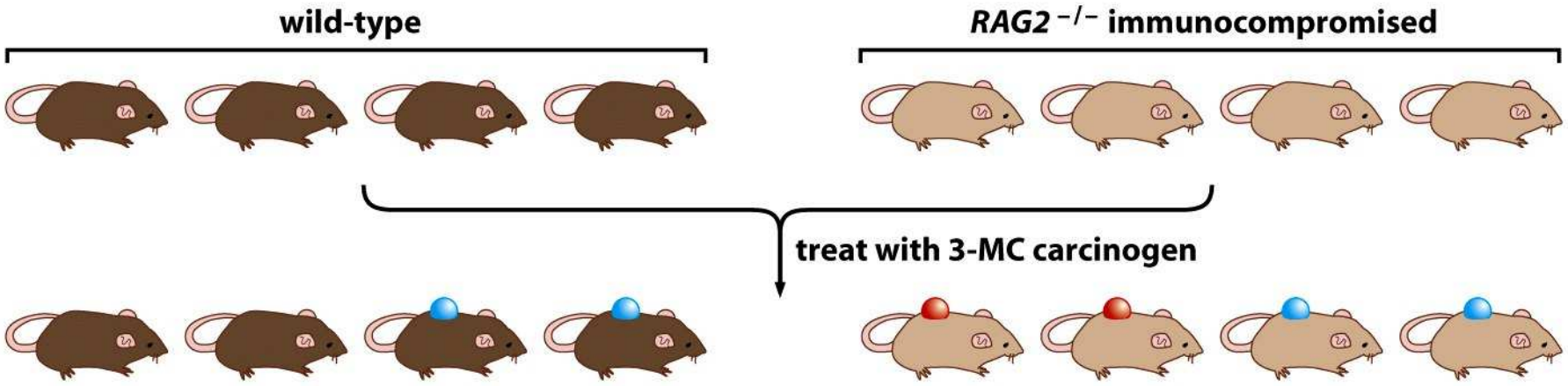
- **Spontaneous regression**
  - Tumor regresses without treatment
  - Documented cancer (clinically evident)
  - Rare: 1 in 100,000
  - Mechanism involves immune cells, but other mechanisms proposed
- **Tumor surveillance**
  - Tumor regresses without treatment
  - Clinically silent
  - Incidence not known, but probably common for certain cancer
  - Mechanism involves adaptive and innate immunity

Understanding tumor surveillance mechanisms can guide tumor immune therapy

Obstacles to studying tumor surveillance / regression

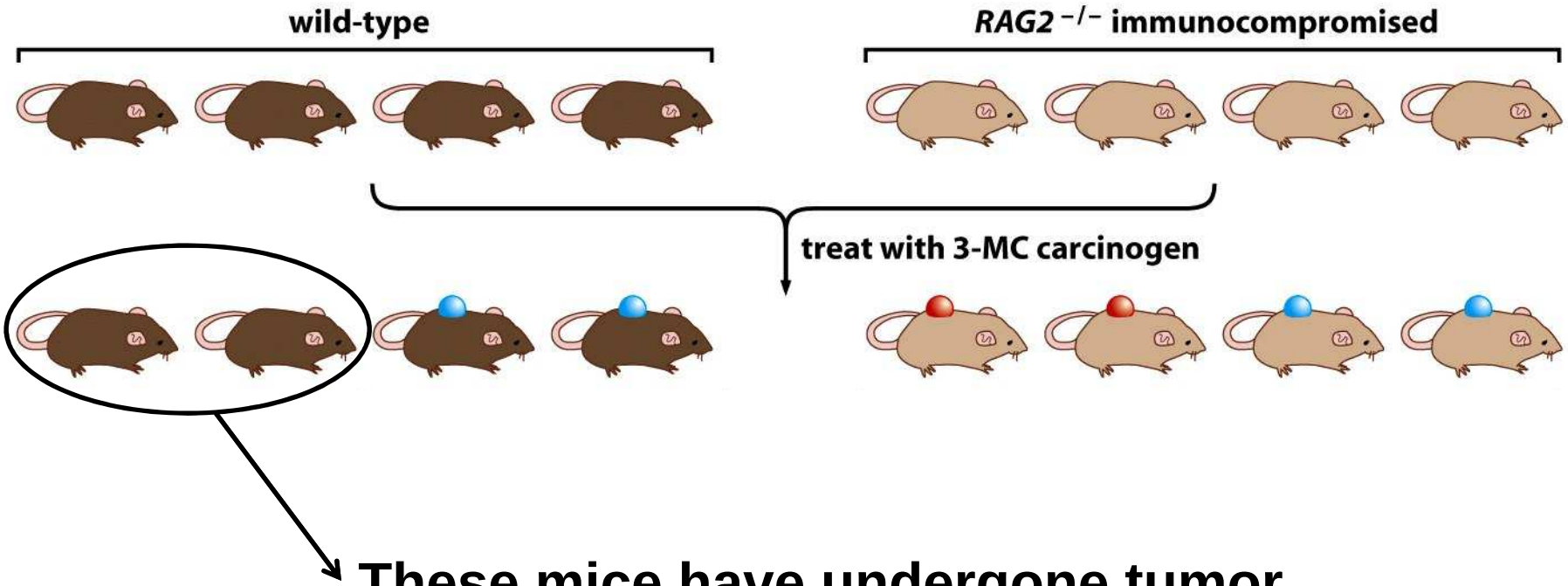
- How do we study a clinically silent process?
- How do we study tumors that are highly immunogenic when they are destroyed by the immune system?
- Open questions regarding tumor surveillance:
  - Where does it occur?
  - How often does it occur?
  - What are the cells and molecules involved?

Model system for studying tumor surveillance



Schreiber, Smyth, Bui Labs
Biology of Cancer. R. Weinberg

Model system for studying tumor surveillance

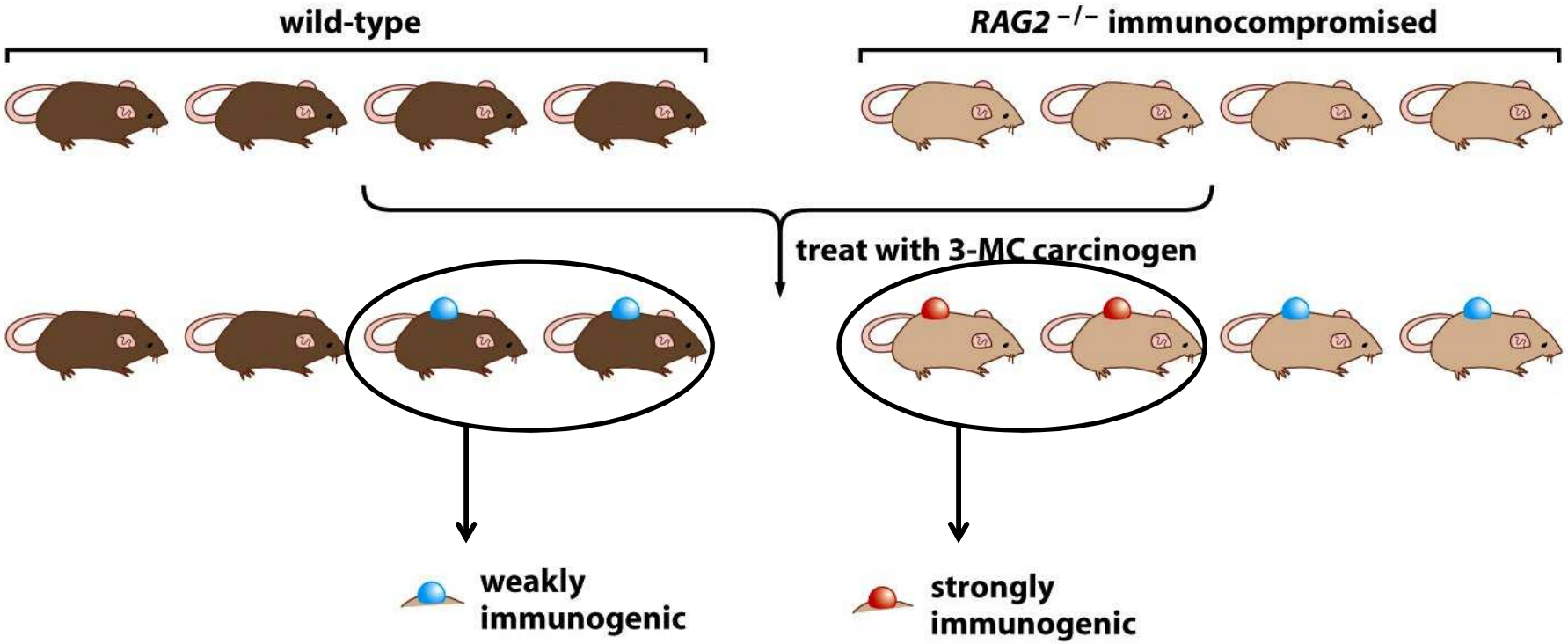


These mice have undergone tumor surveillance and their immune system has eradicated their tumor.

-Hypothesis: the tumor that forms in these mice are highly immunogenic

Schreiber, Smyth, Bui Labs
Biology of Cancer. R. Weinberg

Model system for studying tumor surveillance



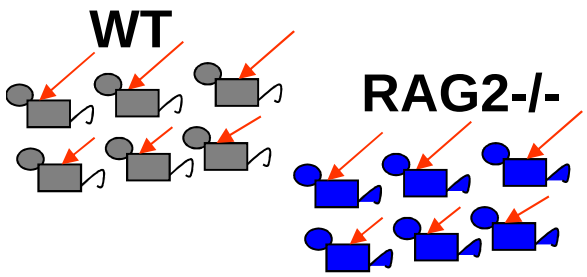
-weakly immunogenic tumor cell lines are generated in WT mice
-these are termed "progressors"

-strongly immunogenic tumor cell lines are generated in RAG$^{-/-}$ mice
-these are termed "regressors"

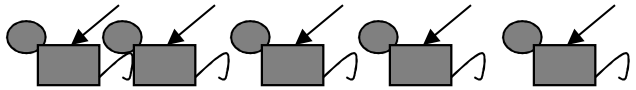
Schreiber, Smyth, Bui Labs
Biology of Cancer. R. Weinberg

Experimental design for generating regressor and progressor cell lines

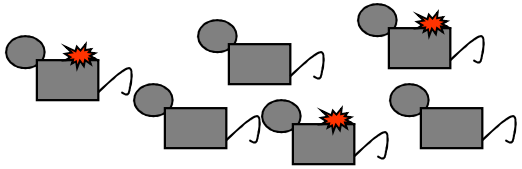
1a. Inject MCA s.c.

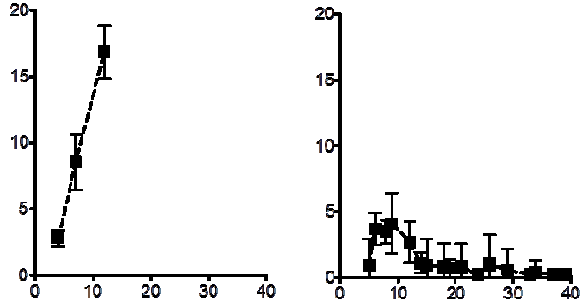


2. Transplant tumor cell line into naïve syngeneic WT or RAG2$^{-/-}$ mice

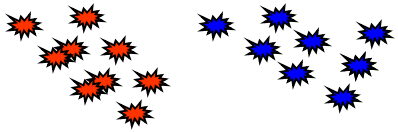


1b. Wait for tumors to develop at the site of injection (2-6 months)



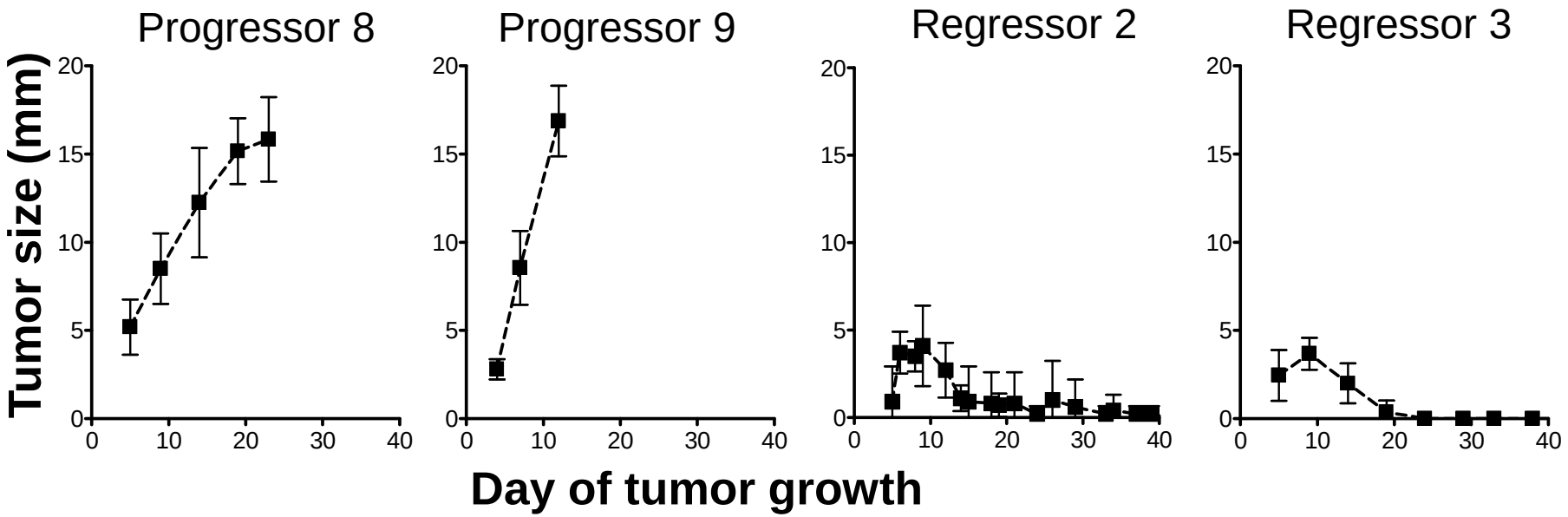


1c. Excise and disaggregate tumor chunk to generate tumor cell lines



Shankaran et al., Nature 2001; O'Sullivan et al., JEM 2012

Regressor cell lines undergo tumor surveillance



Data not shown: regressors will grow in immune deficient mice but not in WT syngeneic mice, confirming that the regression is due to immune recognition

Schreiber, Smyth Labs

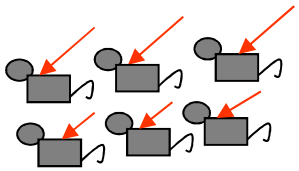
O'Sullivan et al., JEM 2012

What are the differences in immune responses to regressors and progressors?

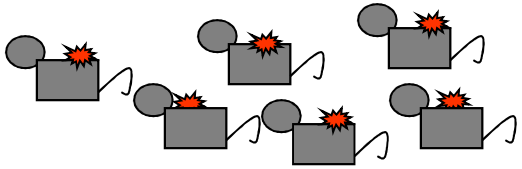
Model system – transplant regressor and progressor cell lines into syngeneic mice and examine immune responses at various timepoints in the tumor

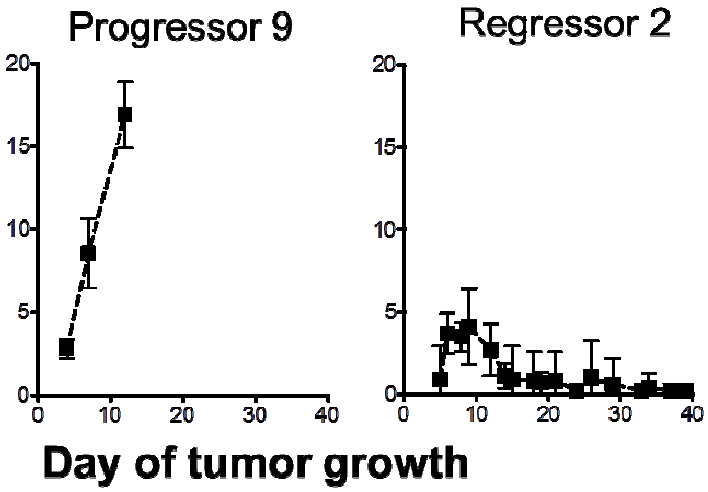
Experimental design to measure immune responses during tumor regression and progression

1. Inject regressor or progressor s.c. (into the flank of WT syngeneic mice).



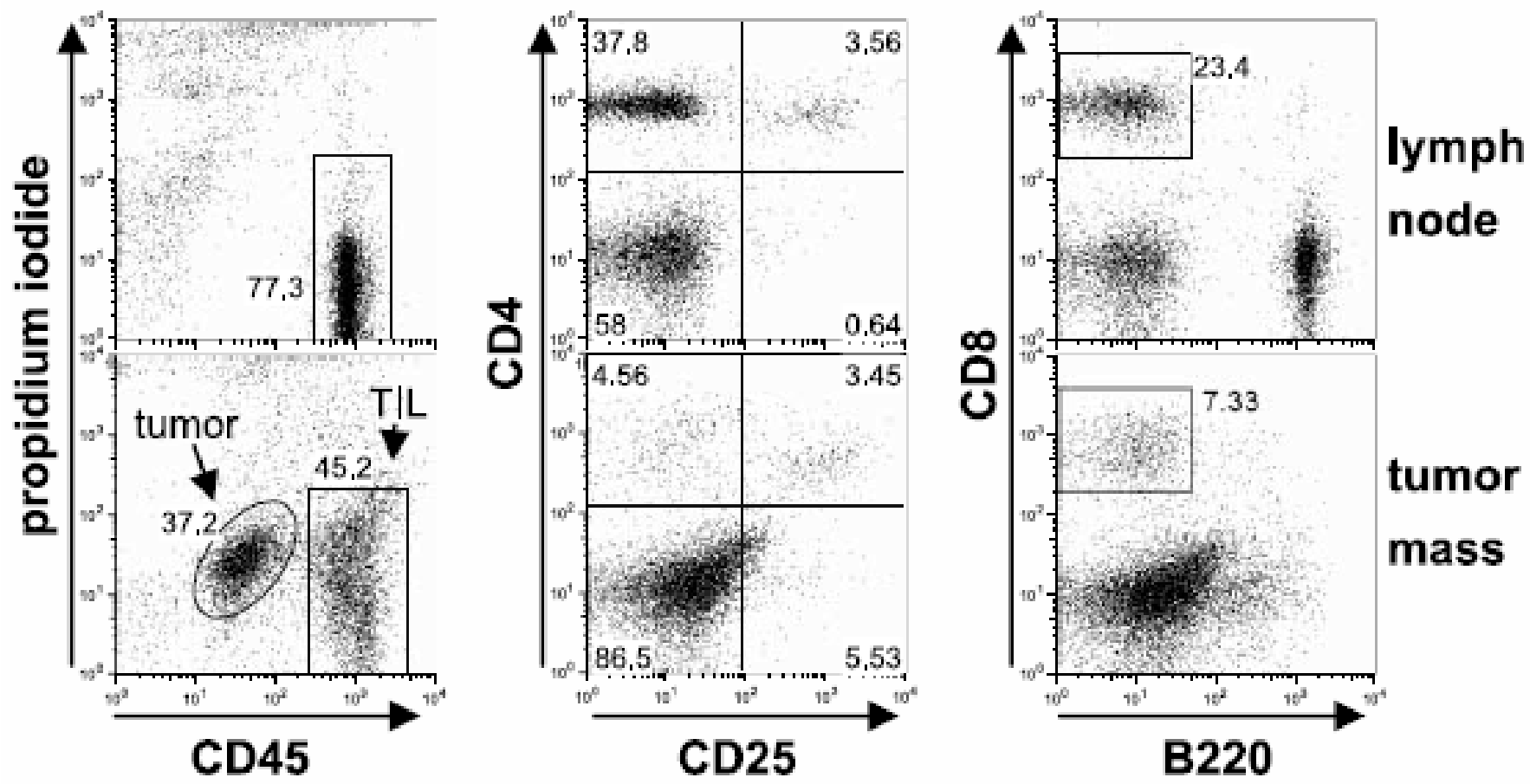
2. Wait for tumors to develop at the site of injection but prior to regression (3-12 days).





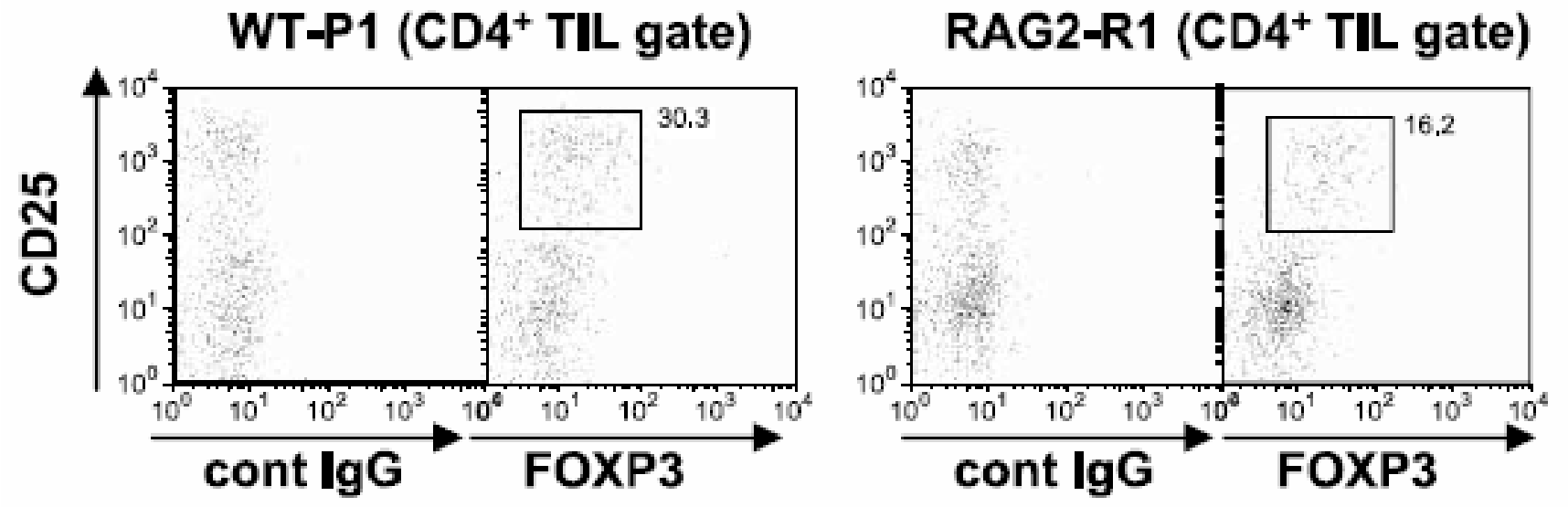
1c. Excise and disaggregate tumor chunk for flow cytometry studies. Also examine draining and non-draining lymph nodes.

Flow cytometric analysis of tumor cell suspensions allows for identification of TILS (tumor infiltrating leukocytes)



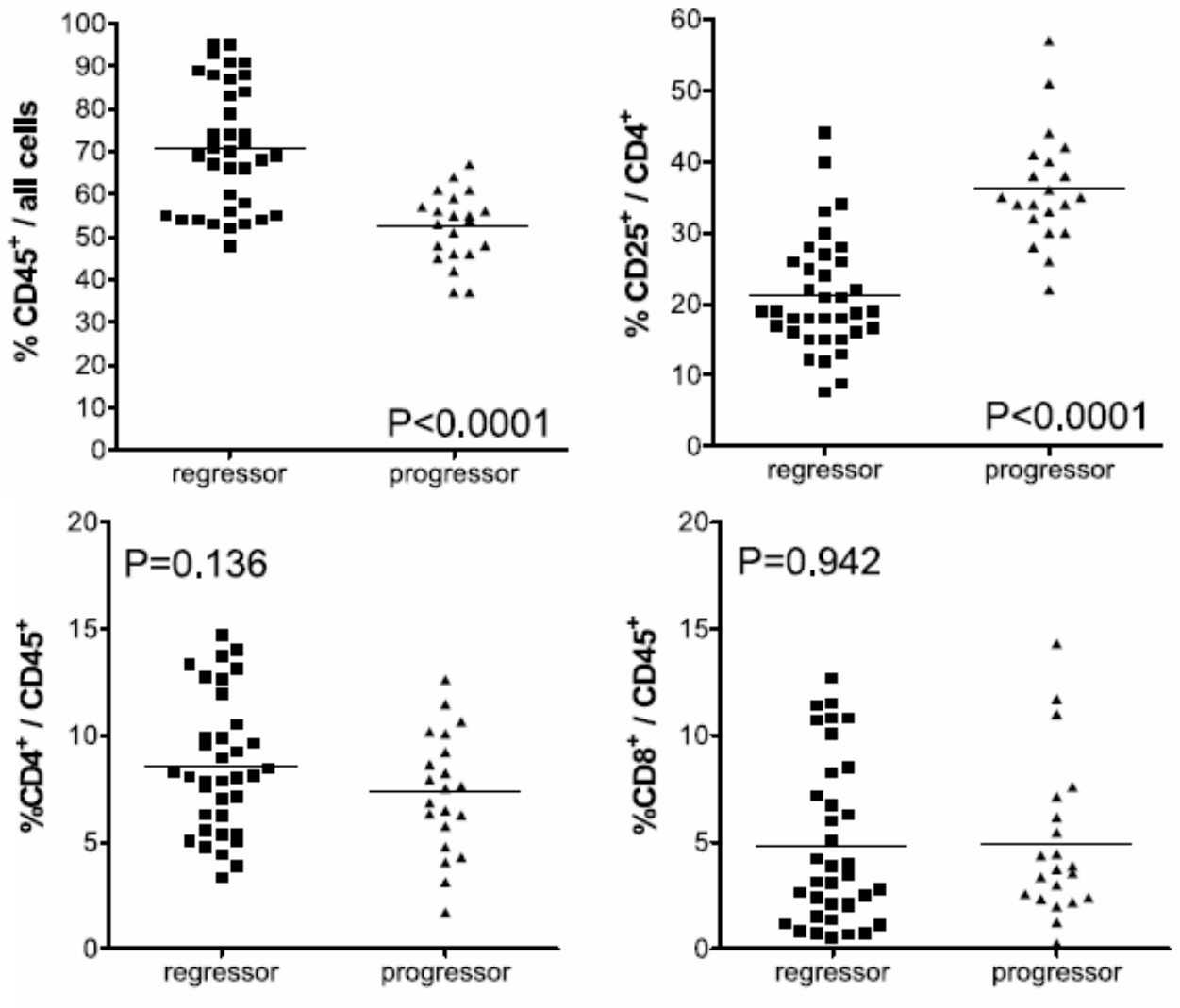
Bui et al., Cancer Research 2006

CD25 is a suitable marker of FOXP3+ T-regs in our mouse model system



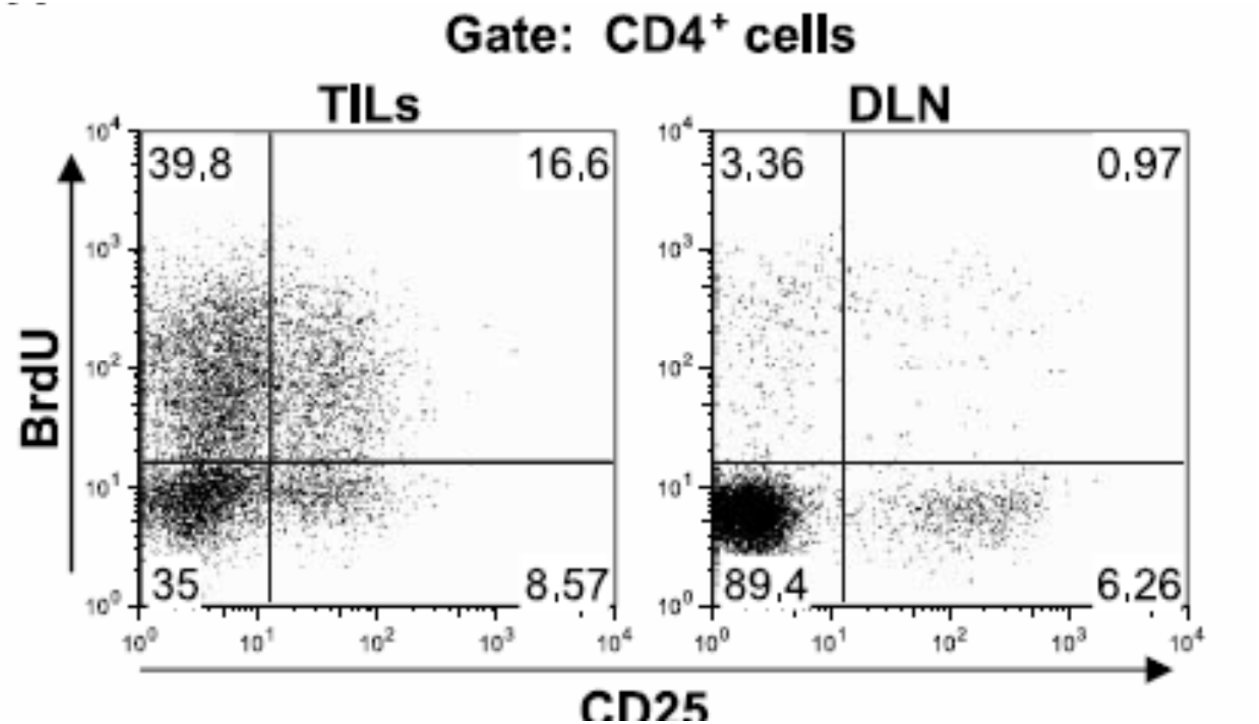
Bui et al., Cancer Research 2006

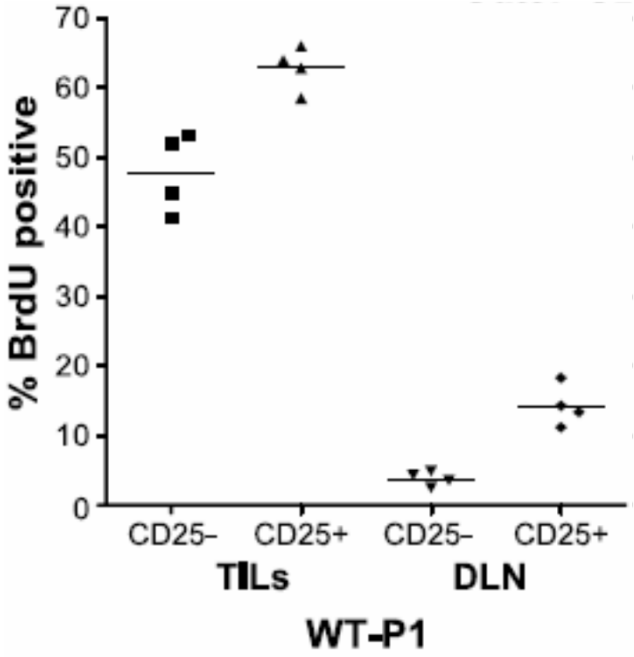
Regressing tumors have increase TILs and decreased T-regs



Bui et al., Cancer Research 2006

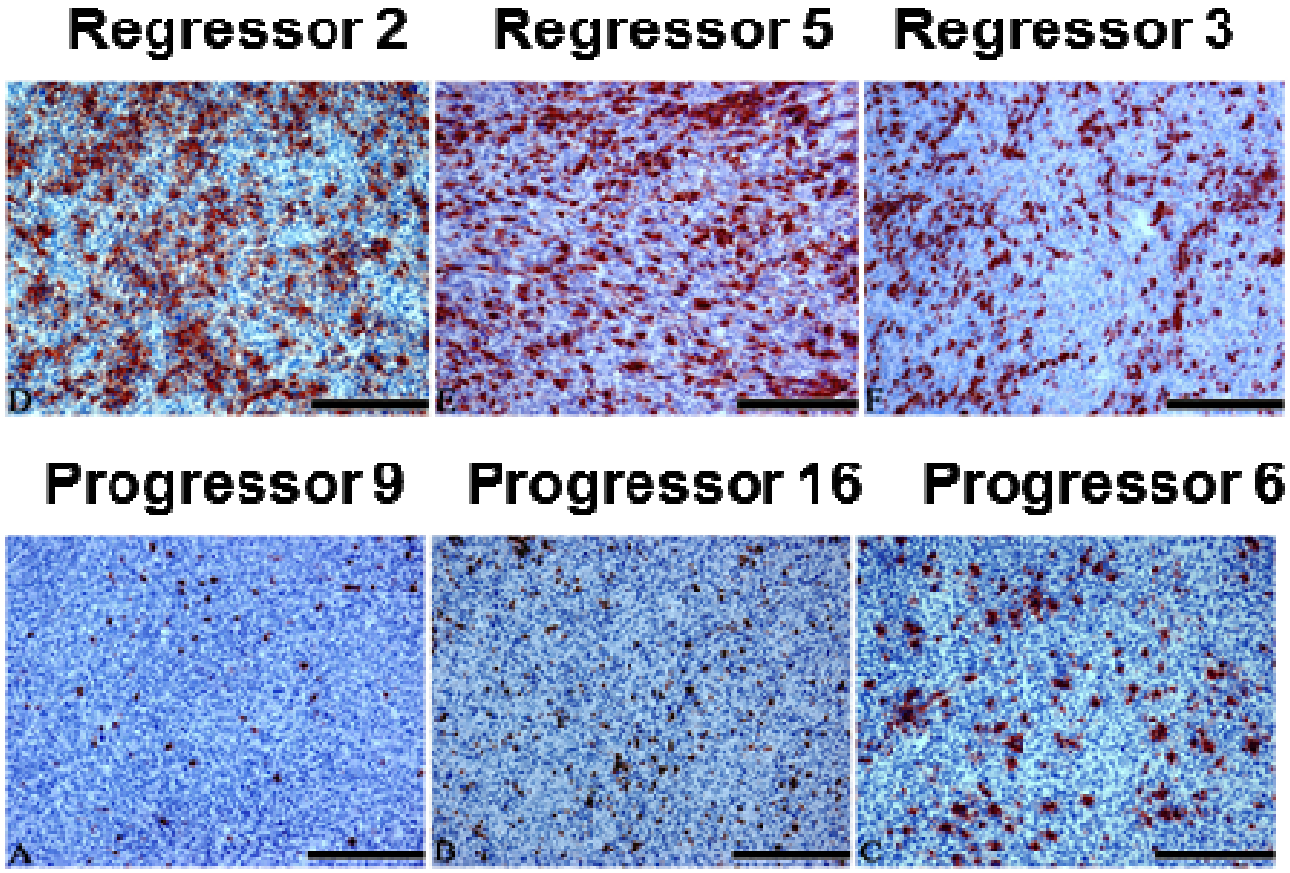
CD25+ T-regs are actively proliferating in the tumor and DLN

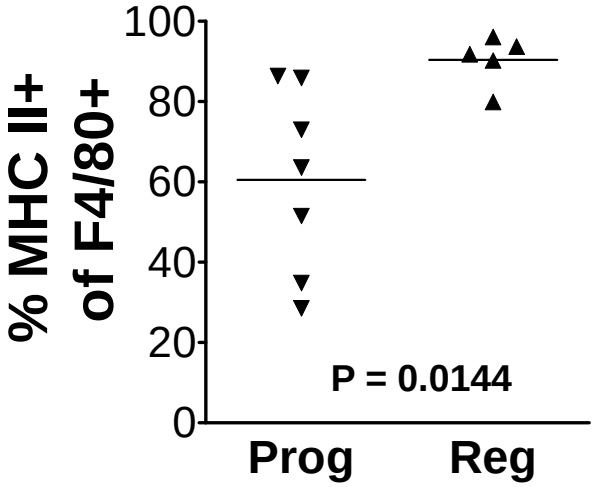




Bui et al., Cancer Research 2006

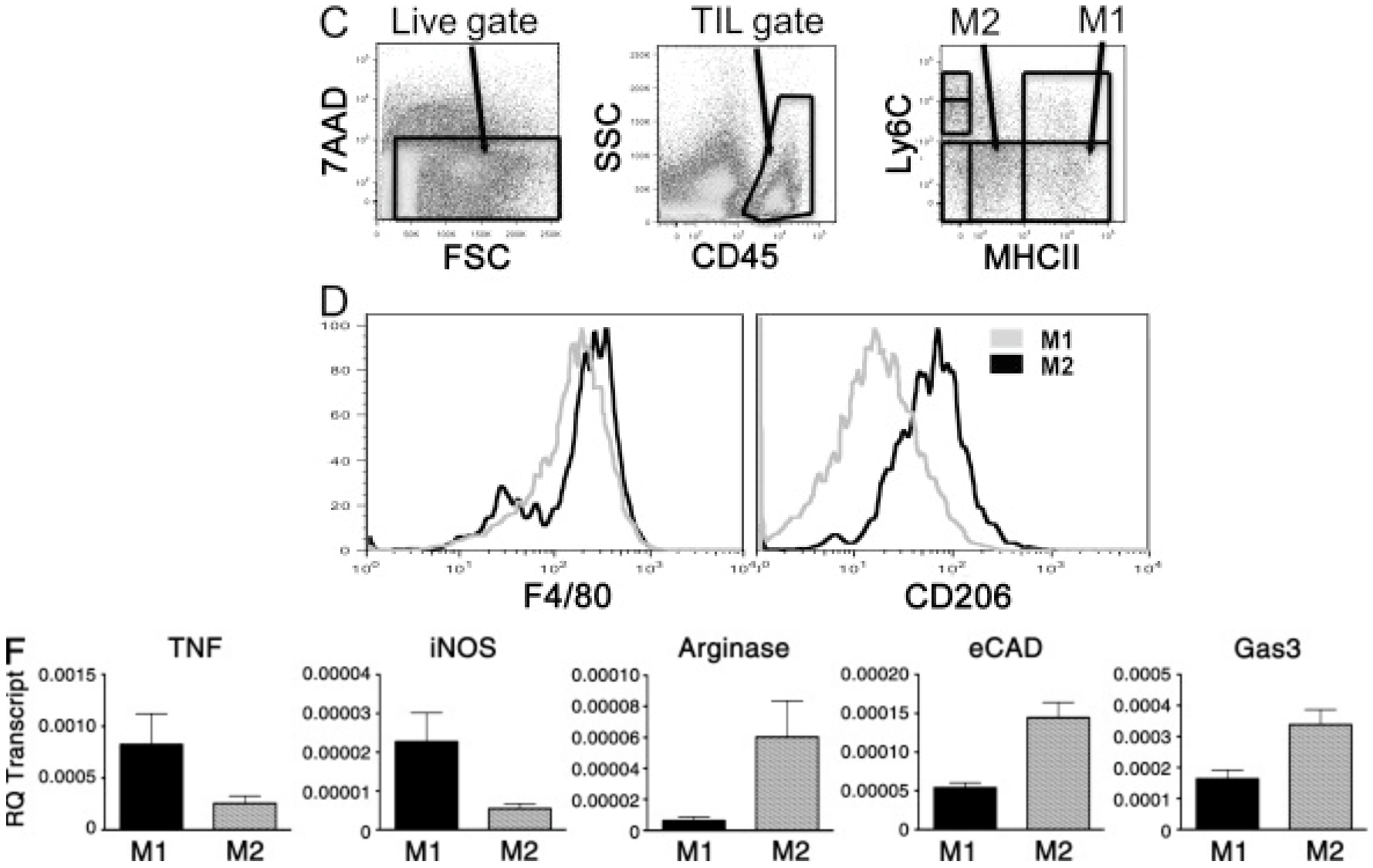
Heavy infiltration of MHC class II-positive macrophages in regressor tumors





Anti MHC class II staining

MHC class II as a marker of M1-type macrophages in tumors



O'Sullivan et al., JEM 2012 (first published by Movahedi et al., CR 2010

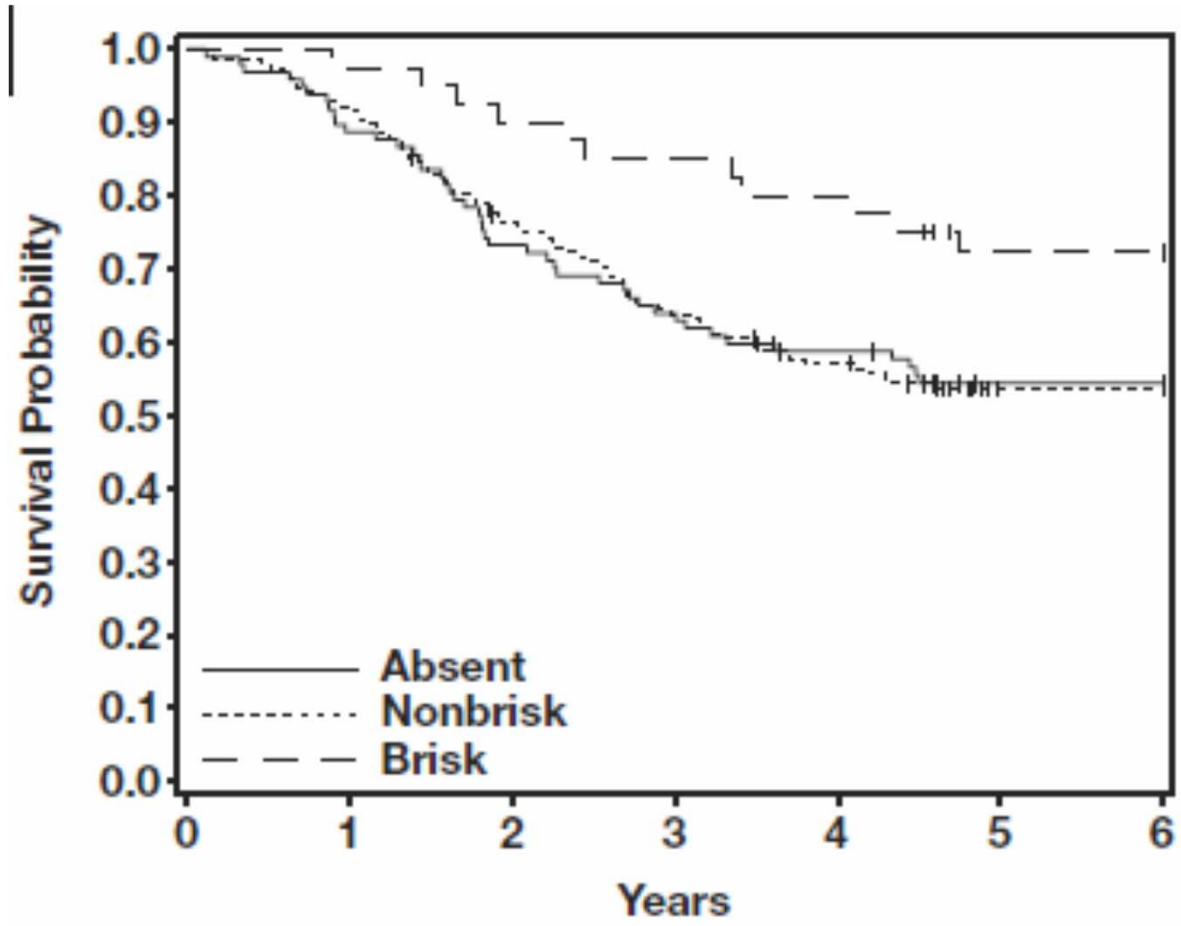
Summary of mouse model of tumor regression

Regression is associated with:

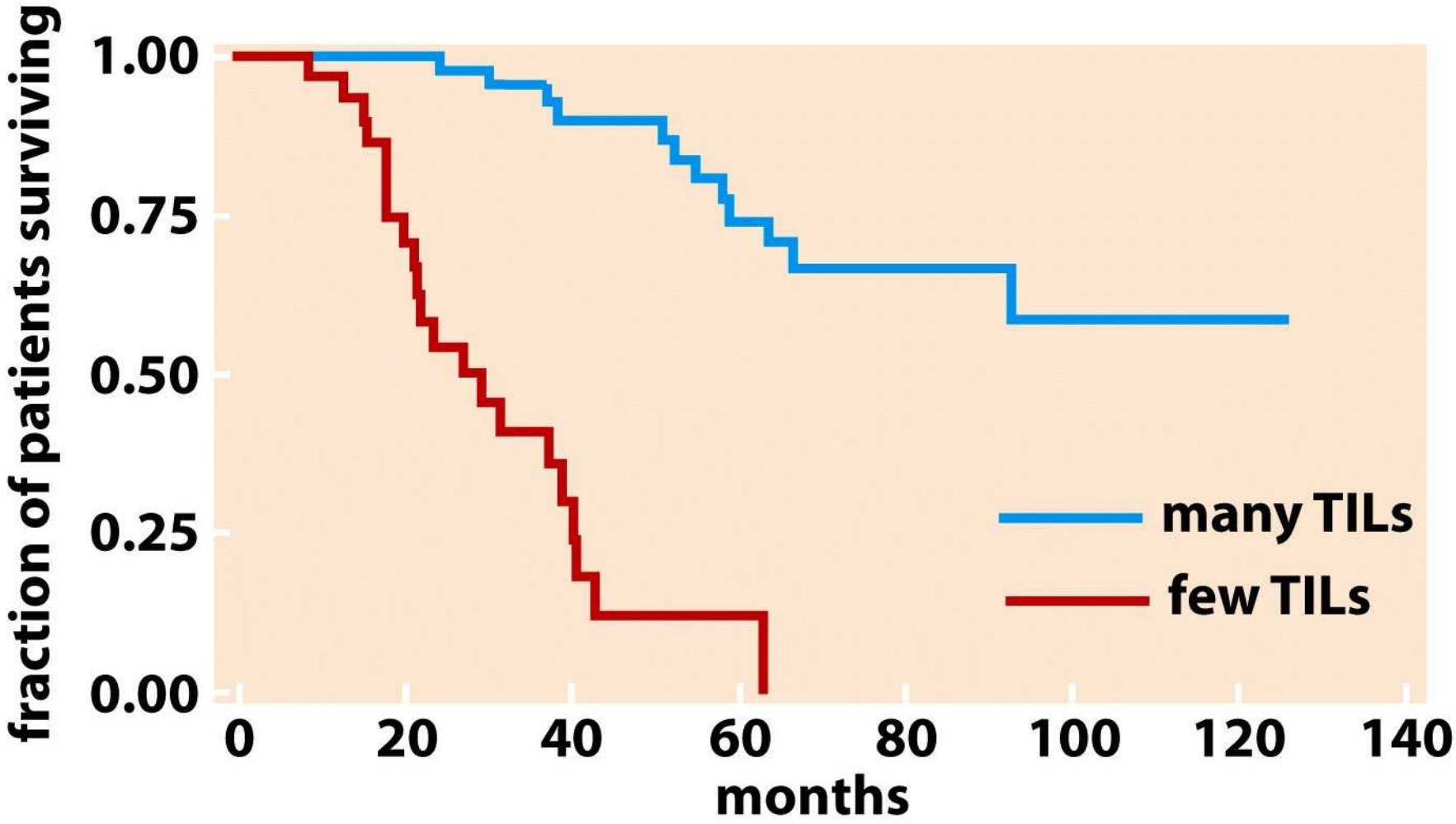
1. **Increased CD45+ cells**
2. **Decreased FOXP3+CD25+CD4+ T-regs**
3. **Increased class II+ macrophages (M1 versus M2)**

Is this seen in human cancer?

Increase immune infiltration correlates with better survival (thick T4 primary melanoma)



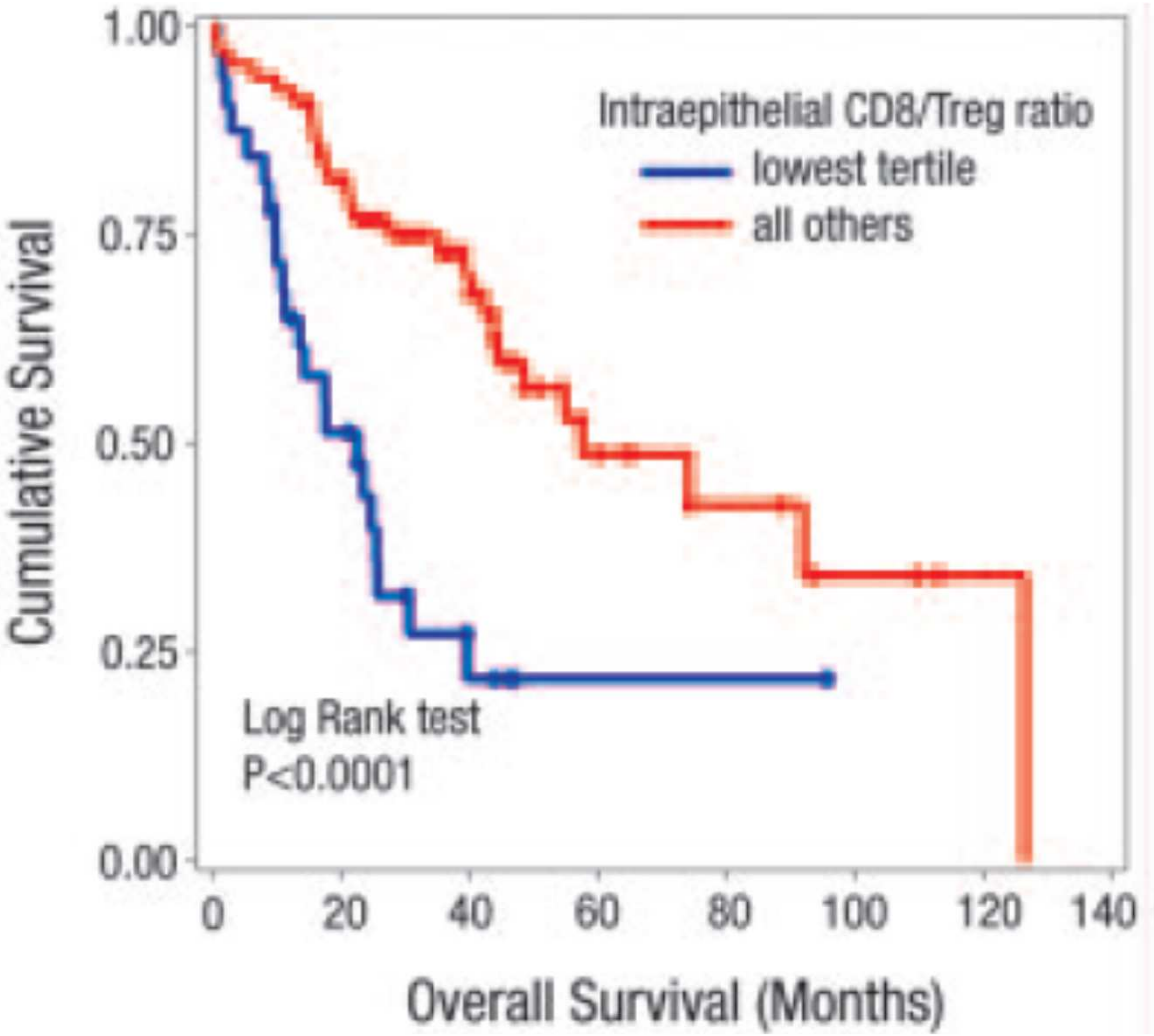
Rao et al., AJCP 2010



Shown is survival in ovarian cancer cohort. Similar findings have been shown for colon cancer and melanoma cohorts.

Figure 15.21d *The Biology of Cancer* (© Garland Science 2007) (Curiel et al., Nature Medicine 2004)

Tumors with increased CD8/Treg ratio are associated with better prognosis



IHC analysis of 117 cases of epithelial ovarian cancer. PNAS 2005 Sato and Odunsi; also see Curiel et al., Nat Med 2004

Simply counting TILs is probably not enough to prognosticate all human cancers

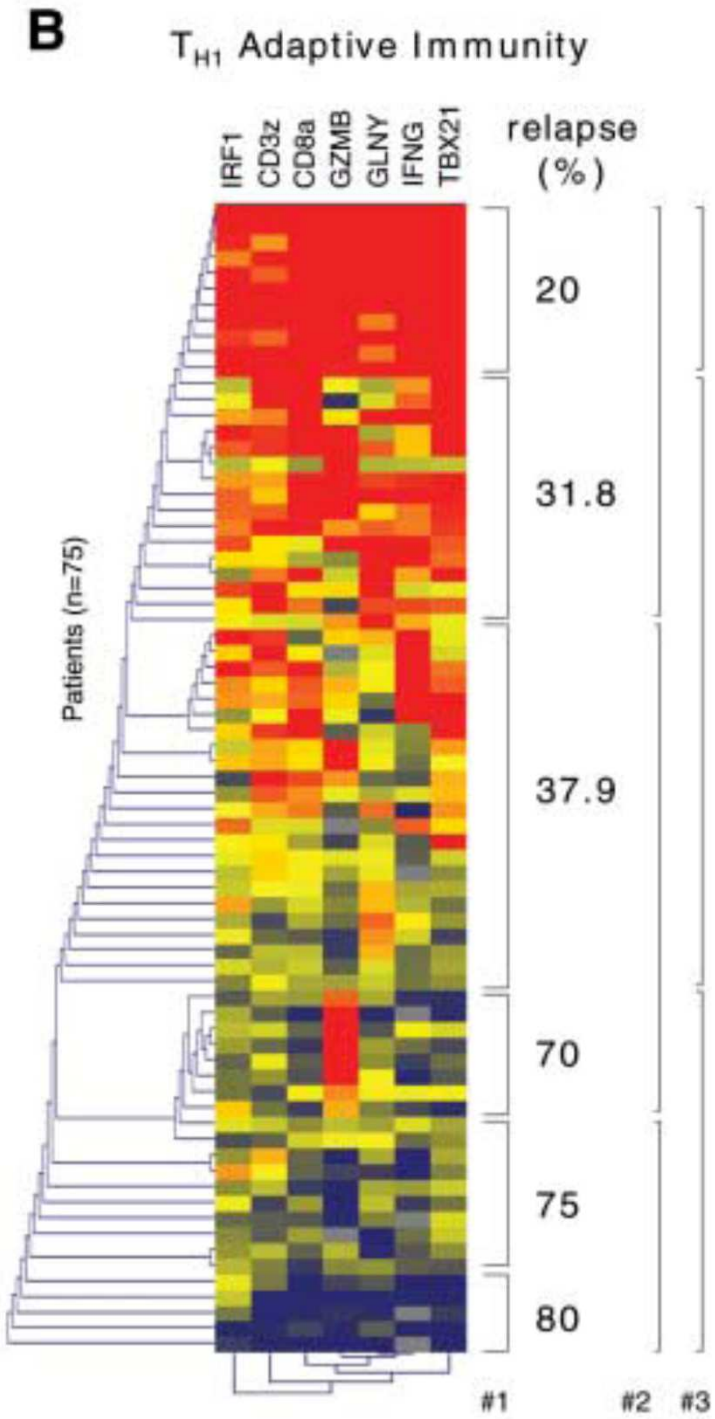
- **Uppaluri et al., 2008 examined 8 studies of TILs in over 600 HNSCC cases and concluded “no definitive studies exist supporting or dismissing the prognostic relevance of TIL analysis in HNSCC…”**

- **Dunn et al., 2007 examined 6 studies of TILS in over 1200 cases of glioma concluded “additional study is necessary to determine whether there is any clinical relevance to the presence of TILS in glioma…”**

- **Both studies recognized that TILs in melanoma and ovarian cancer has better correlation with survival than in HNSCC or glioma.**

Tumors with Th1 gene signature are associated with better prognosis

Galon et al., Science 2006
-gene expression microarray study of 75 patients with colorectal cancer
-Th1 associated genes could predict prognosis independent of conventional staging



Increased expression of Th1 genes (red) is associated with lower rate of relapse in colon cancer.

Galon et al., Science 2006

Summary of mouse model of tumor regression

Regression is associated with:

1. **Increased CD45+ cells**
2. **Decreased FOXP3+CD25+CD4+ T-regs**
3. **Increased class II+ macrophages (M1 versus M2)**

Is this seen in human cancer?

1. **Yes, brisk infiltration in melanoma and ovarian cancer is associated with better prognosis.**
2. **Increased CD8+ T cells is associated with better prognosis in ovarian cancer**
3. **Th1 gene signature is associated with better survival in colon cancer**

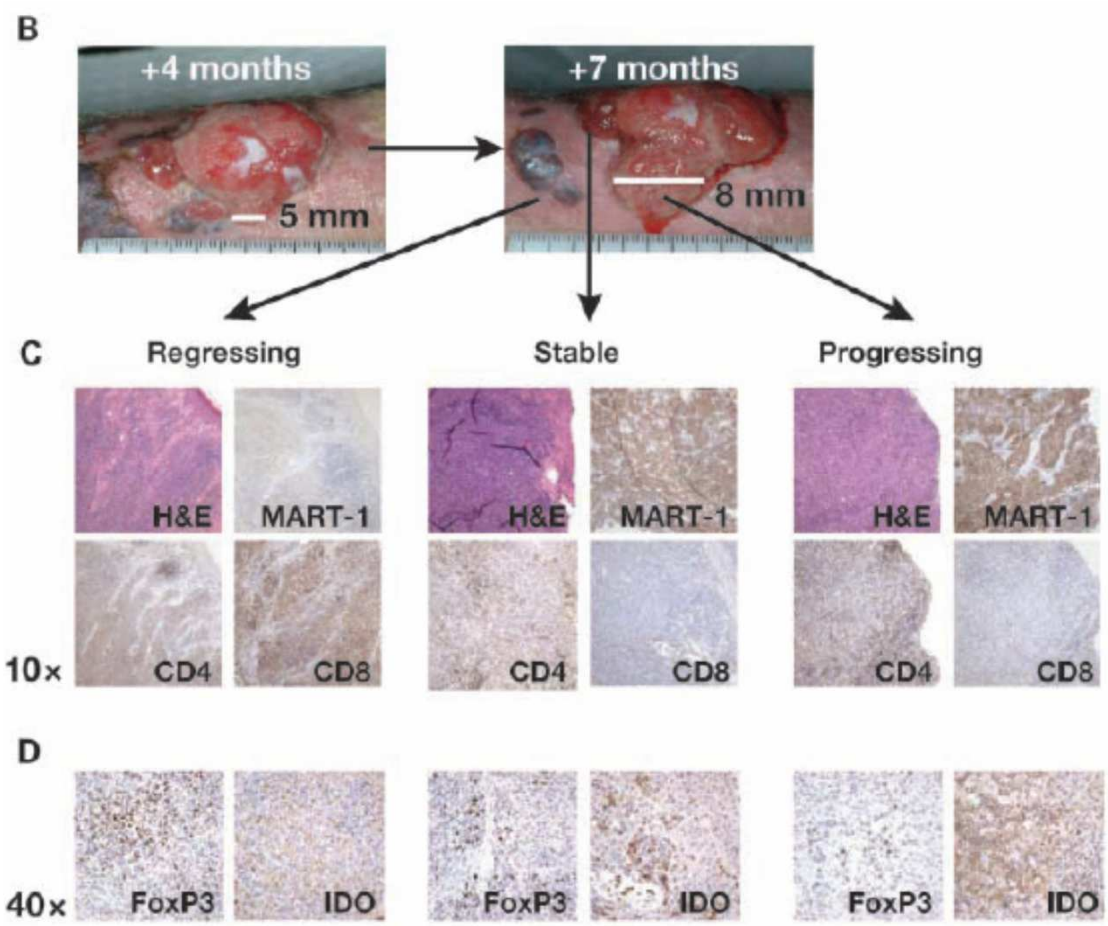
What are the immune changes associated with *therapy-induced regression* of an established tumor?

-Limited TIL studies of anti-CTLA4 induced tumor regression

-Recent mouse studies indicate that anti-CTLA4 works in part by depleting T-regs

-Mouse model of T-reg depletion shows increase proliferation of CD4 and CD8 cells as indicative of tumor regression

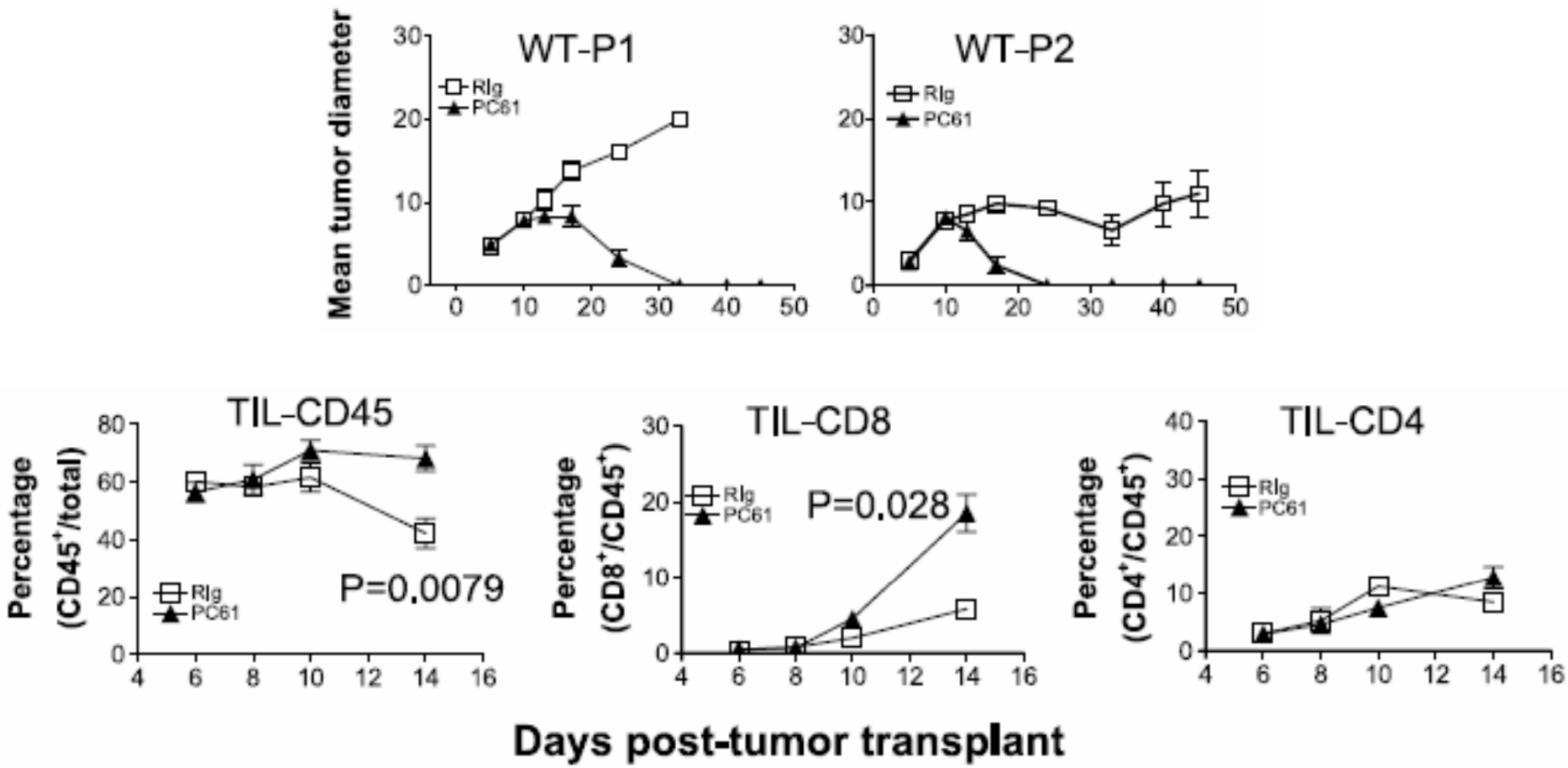
-ALC as best current marker of response to anti-CTLA4



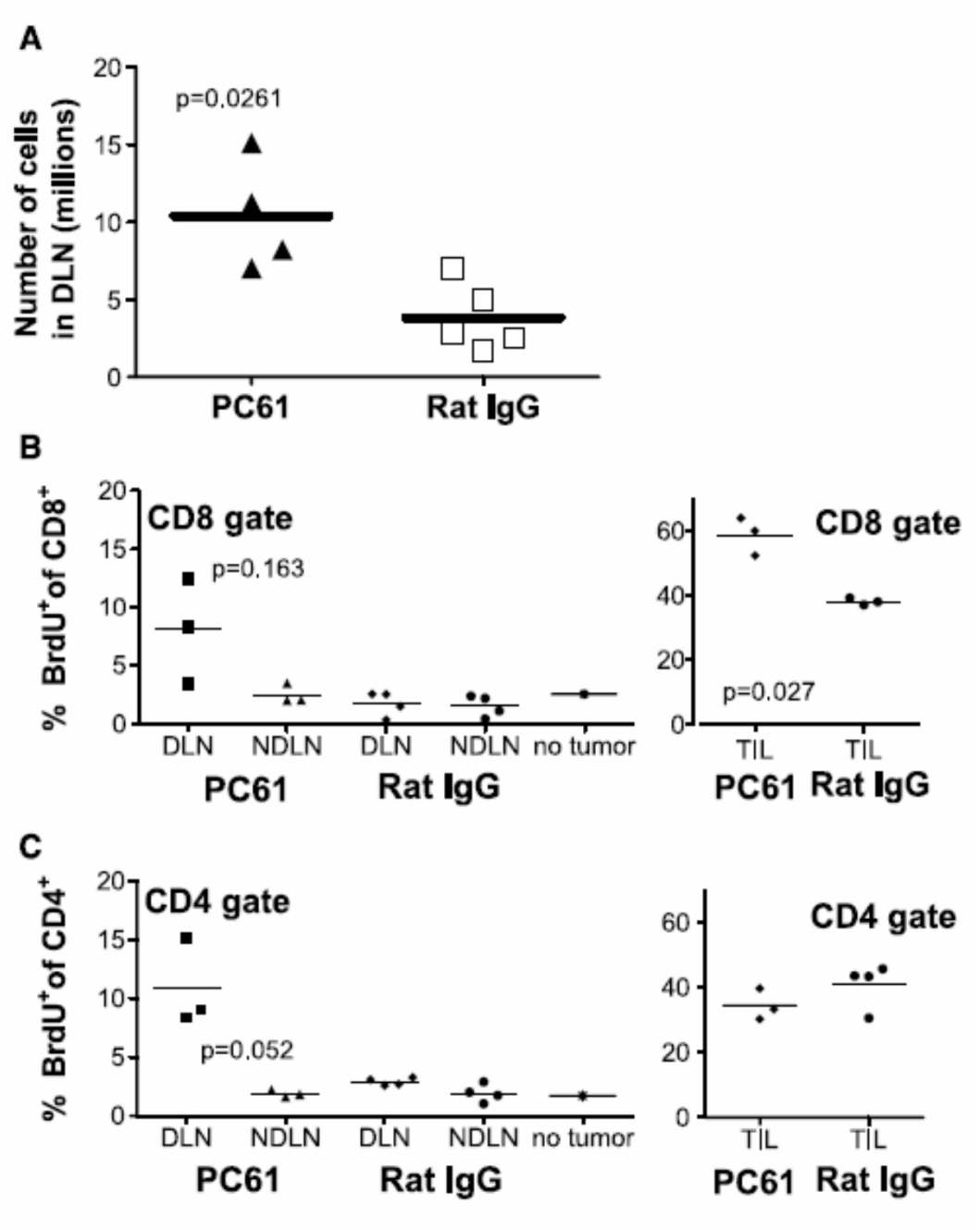
Conclusions: Administration of tremelimumab was associated with massive intratumoral infiltrates of CD8+ CTLs in patients with regressing tumors but had varying effects on intratumoral infiltrates of CD4+ and Foxp3+ cells or intratumoral expression of IDO.

Ribas Clin Canc Res 2009

Mouse model of therapy-induced tumor regression shows increased CD8+ cells in the tumor during regression



Bui et al., Cancer Research 2006

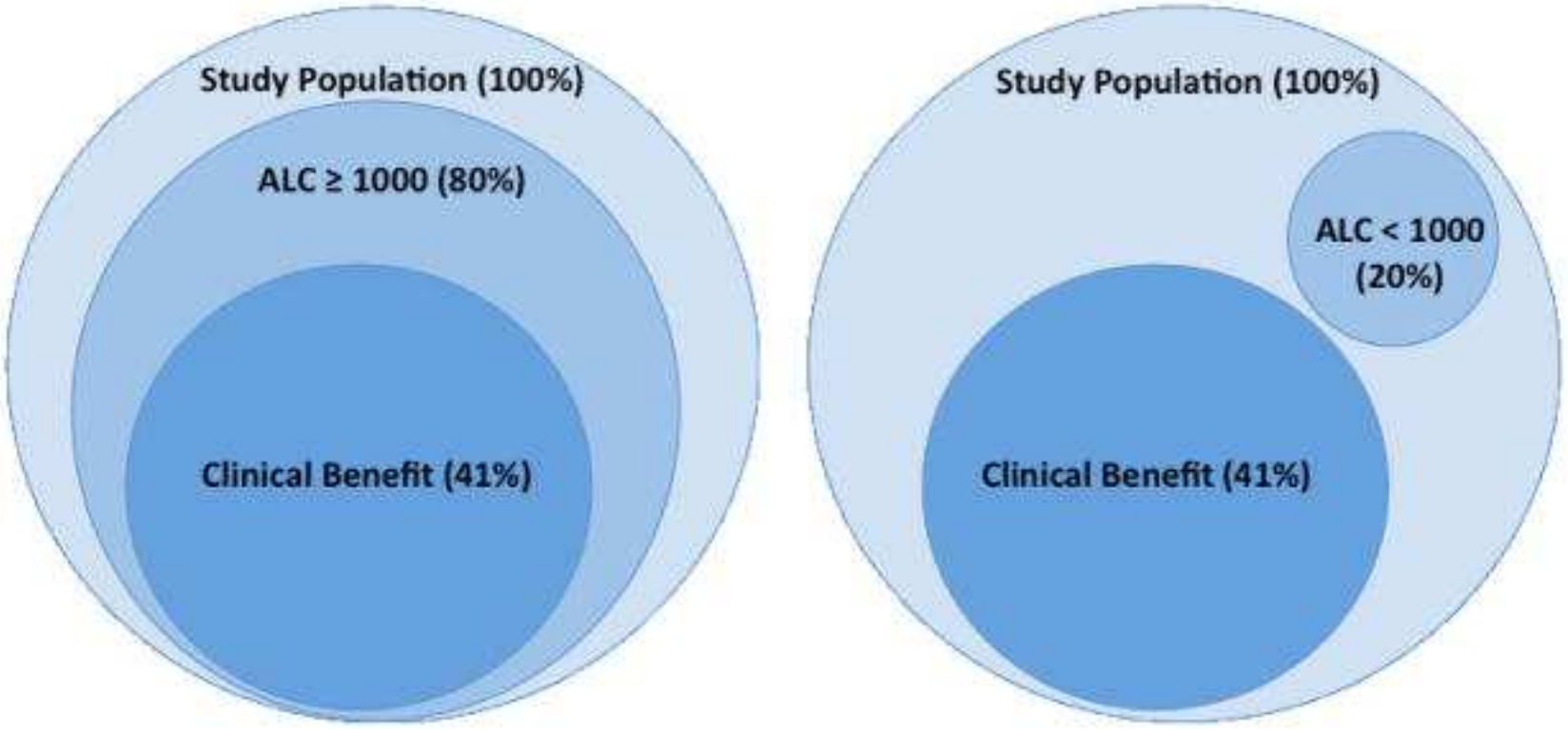


Increased CD4+ and CD8+ T cell proliferation in the draining lymph node during regression.

Increased CD8+ TIL proliferation in tumors that are regressing.

Bui et al., Cancer Research 2006

Absolute lymphocyte count (ALC) in the blood as a marker or clinical benefit from anti-CTLA4 treatment



Callahan, Wolchock, Allison 2010

What are the immune changes associated with *therapy-induced regression* of an established tumor?

-Limited TIL studies of anti-CTLA4 induced tumor regression show increased intratumoral CD8+ cells

-Recent mouse studies indicate that anti-CTLA4 works in part by depleting T-regs

-Mouse model of T-reg depletion shows increase proliferation of CD4 and CD8 cells as indicative of tumor regression

-ALC as best current marker of response to anti-CTLA4

Conclusions

- Antitumor effectors include CD8+ and CD4+Th1 T cells.
- FOXP3+ T-regs inhibit tumor immunity and are often associated with cancer progression.
- More sophisticated analysis of TILs is needed in order to determine prognostic relevance of TILs in all human cancer.

Acknowledgments

Bui Lab: Robert Saddawi-Konefka, Emilie Gross, Steve Searles, Allen Washington, Jr., Ruth Seelige, Carlos Peinado, Yu-Jin Jung, Isis Perez,

Collaborators: Robert Schreiber, Mark Smyth, Wayne Yokoyama

Funding: NCI, Hartwell Foundation, V Foundation, CRCC, CRI

What is the prognostic significance of increased intratumoral CD8+ T cells in human cancer?

- A. There is no prognostic significance of intratumoral CD8+ T cells in any human cancer.
- B. Increased CD8+ T cells is always associated with poor prognosis in human cancer.
- C. Increased CD8+ T cells is sometimes associated with better prognosis in human cancer.
- D. Decreased CD8+ T cells is associated with decreased T-regs in human cancer.

- Correct answer = C

What immune cells exert antitumor versus pro-tumor effects?

- A. Both CD8+ T cells and T-regs exert antitumor effects.
- B. Both Th1 T cells and T-regs exert pro-tumor effects.
- C. CD8+ T cells exert antitumor and T-regs exert pro-tumor effects.
- D. M1 and M2 macrophages inhibit cancer progression.

- Correct answer = C