# Immunotherapeutic barriers at the level of the tumor microenvironment

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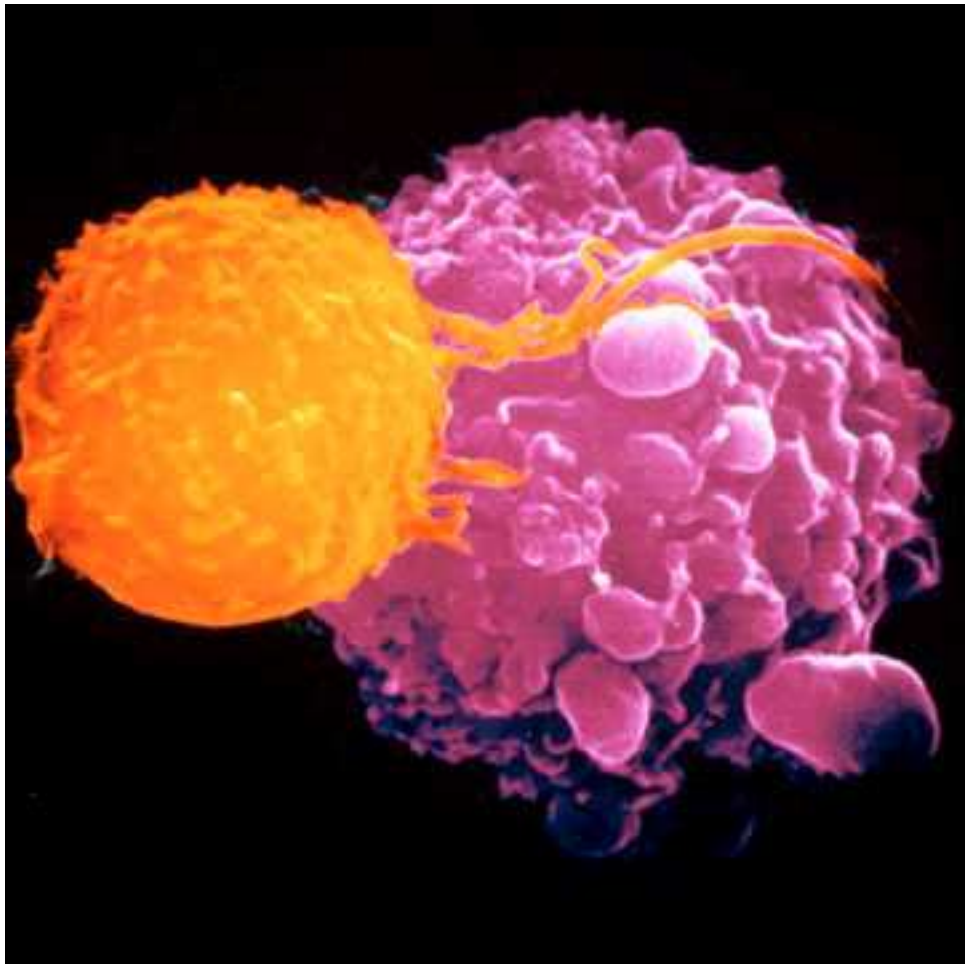
# Disclosures

- Advisory boards:
  - GSK-Bio, Roche-Genentech, Merck, BMS, Abbvie
  - Co-founder: Jounce
- Research support:
  - GSK-Bio, Eisai, Roche-Genentech, BMS, Curetech, Morphotek, Incyte

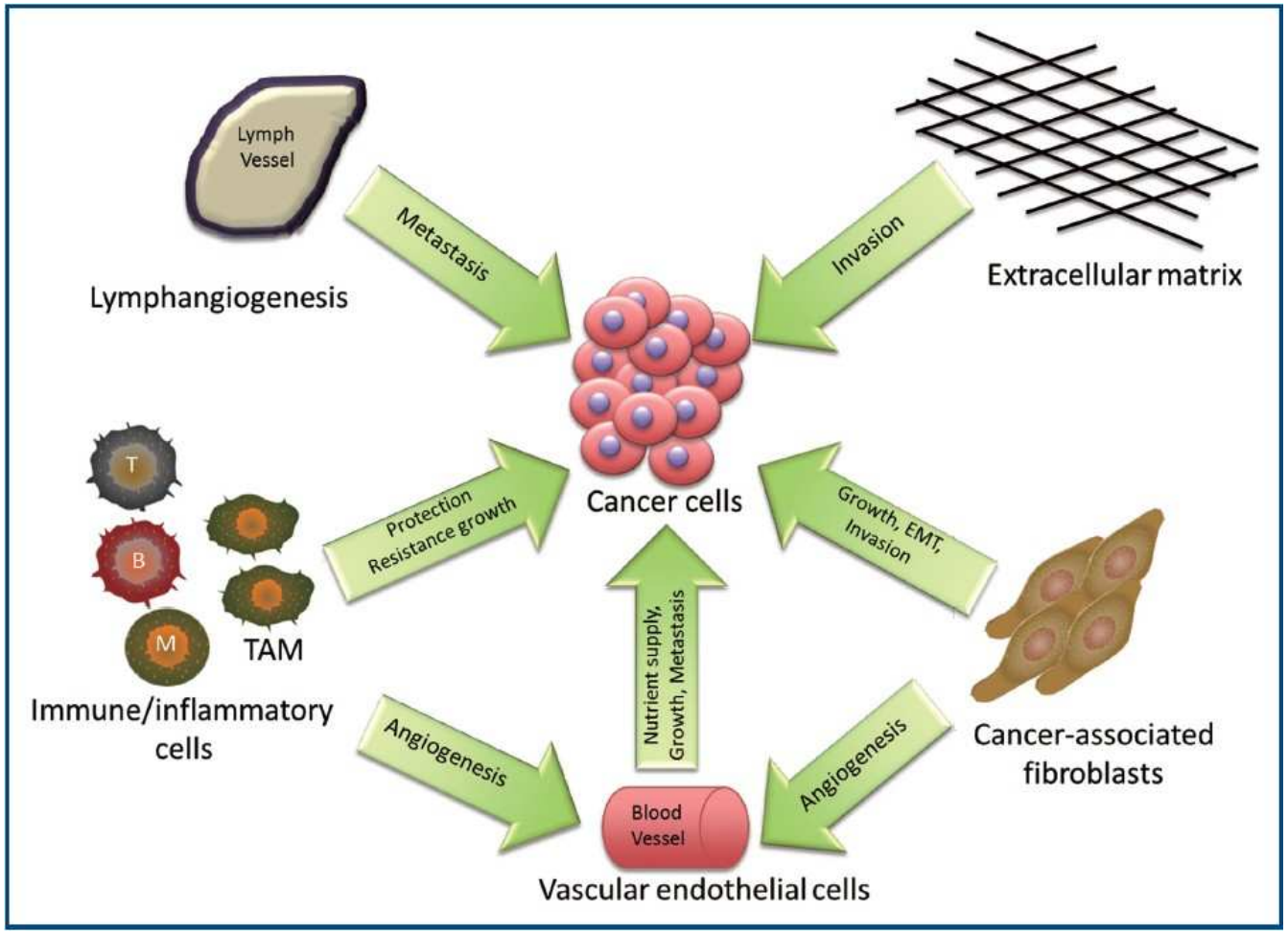
# $CD8^+$ cytotoxic T lymphocyte killing an antigen-expressing tumor cell



# In vivo, a tumor is more than tumor cells

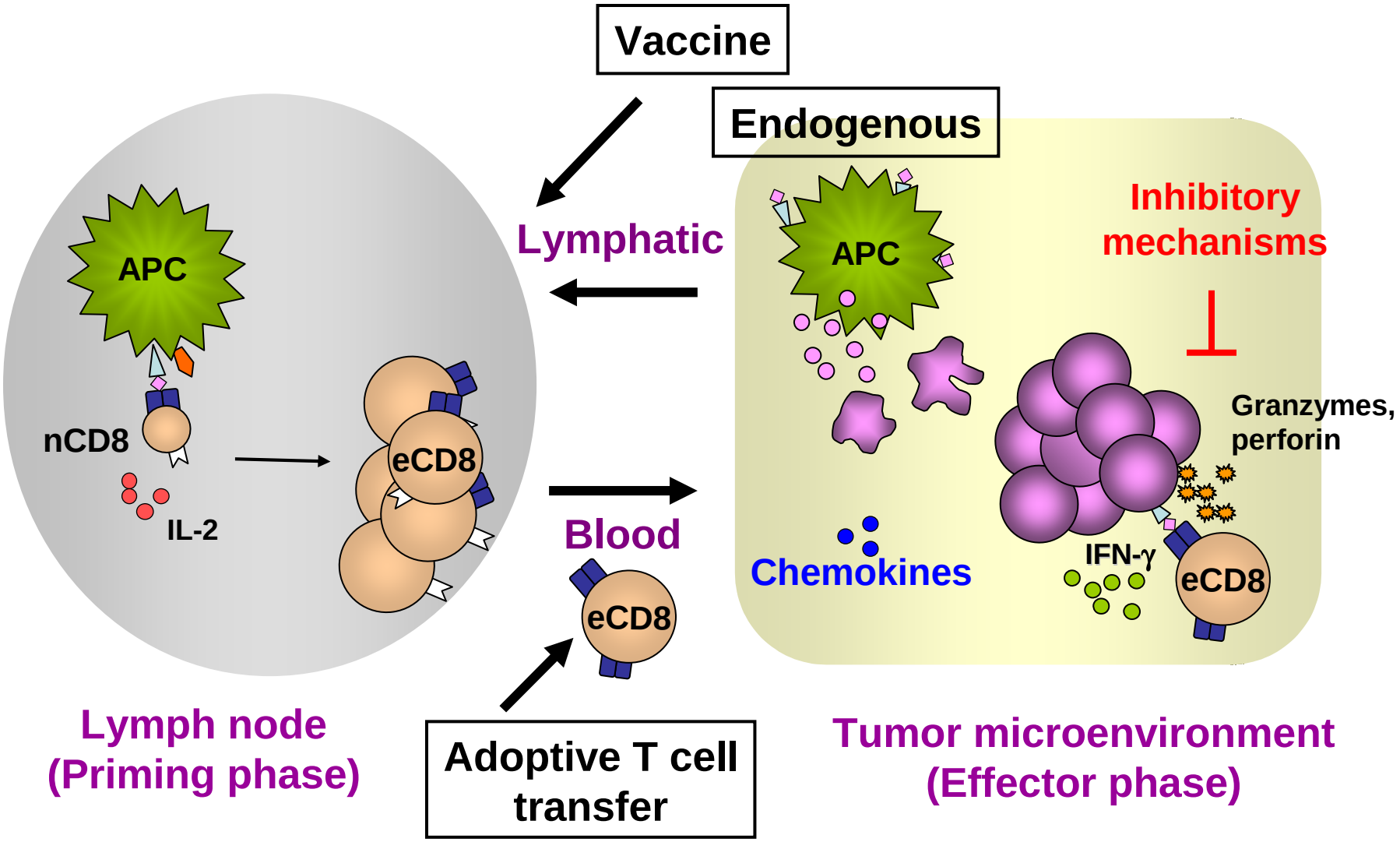
- Three dimensional mass
- Extracellular matrix
- Supported by the neovasculature, fibroblasts, macrophages
- Variable presence of inflammatory cells
  - T cells (and subsets thereof)
  - B cells/plasma cells
  - NK/NKT cells
  - Dendritic cell subsets
- The functional phenotypes of these cells may or may not be permissive for an effective anti-tumor immune response (either priming phase or effector phase)
- Also, likely need for dynamic interaction with draining lymph node compartment for optimal anti-tumor immunity➔added complexity

# Complexity of stromal elements in solid tumors




*DeMorrow et al. 2011*

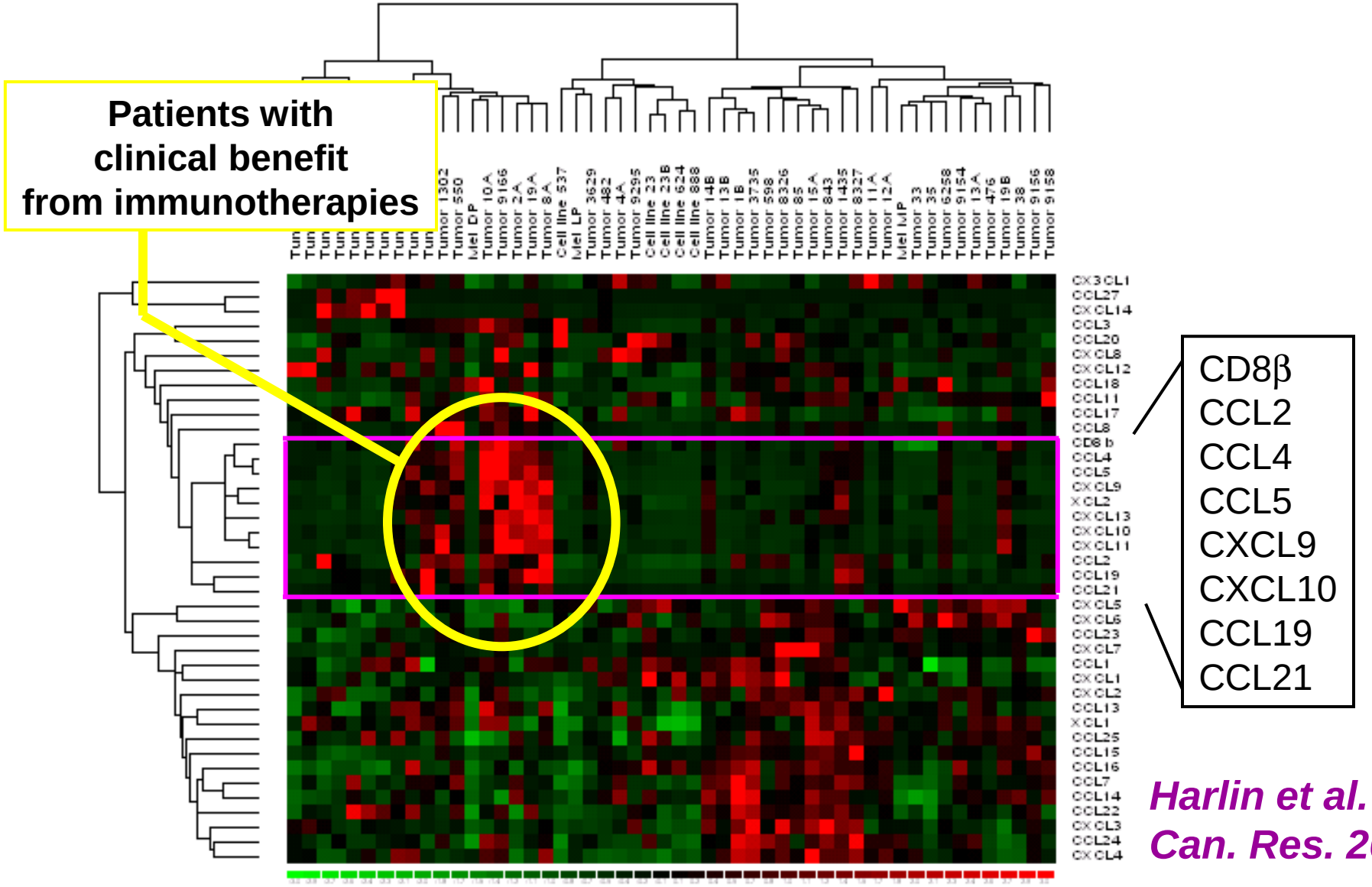
# Anti-tumor immune responses in vivo: Taking into account the tumor microenvironment



# Features of subsets of solid tumors that might mediate poor immune recognition or lack of immune destruction

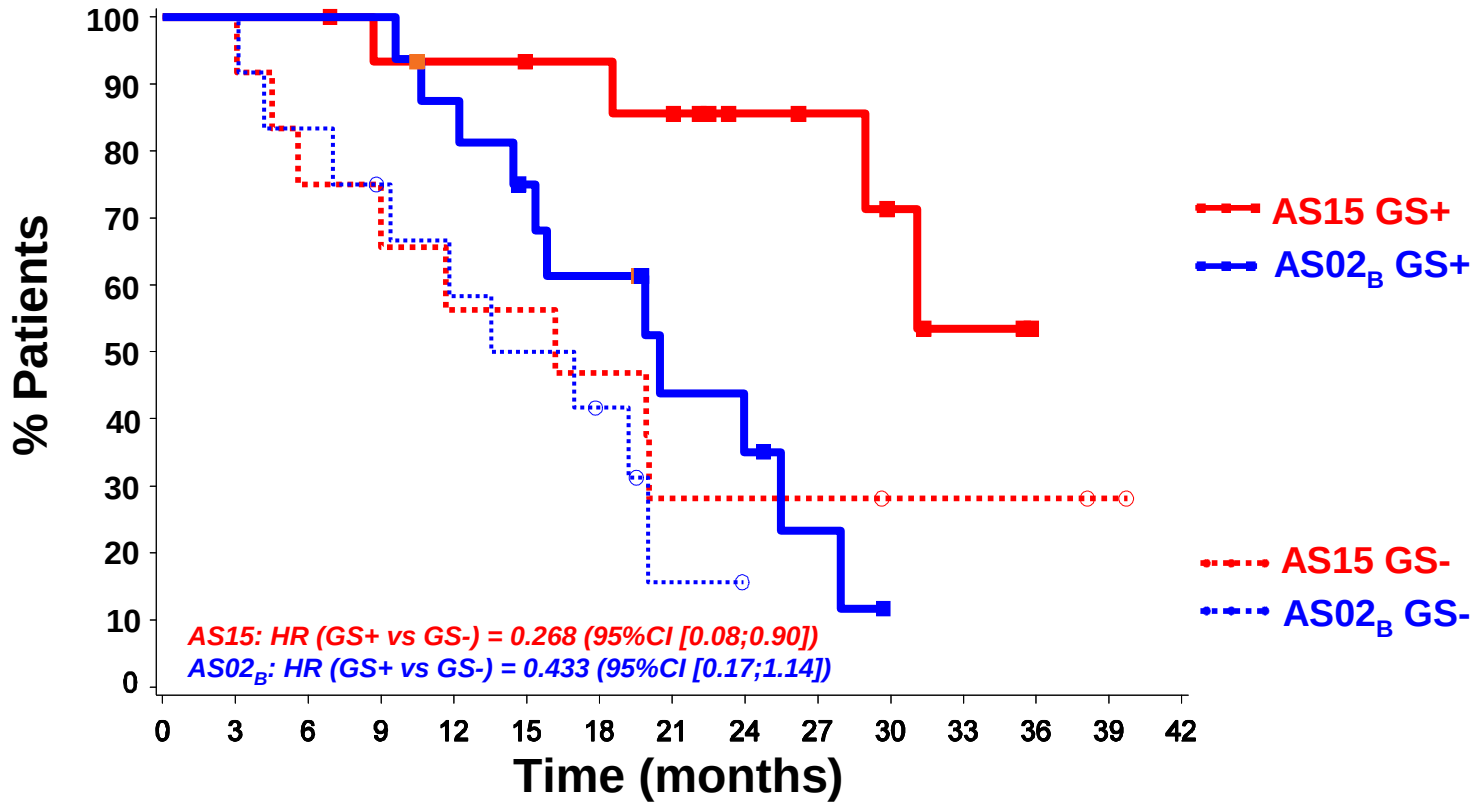
- Priming phase
  - Lack of innate immune-activating “danger” signals
  - Poor recruitment of the critical APC subsets for cross-presentation of antigens to T cells
  - Inadequate expression of costimulatory ligands on tumor cells or on infiltrating APCs
- Effector phase
  - Inadequate recruitment of activated effector T cells
    - Vascular endothelial cells/homing receptors
    - Chemokines
  - Presence of dominant immune inhibitory mechanisms that suppress T cell effector functions
    - Inhibitory receptors (e.g. PD-L1/PD-1)
    - Extrinsic suppressive cells (e.g. Tregs, MDSCs)
    - Metabolic inhibitors (e.g. IDO, arginase)
    - Inhibitory cytokines (e.g. IL-10, TGF-β)

# Expression of a subset of chemokine genes is associated with presence of CD8$^+$ T cells in melanoma metastases



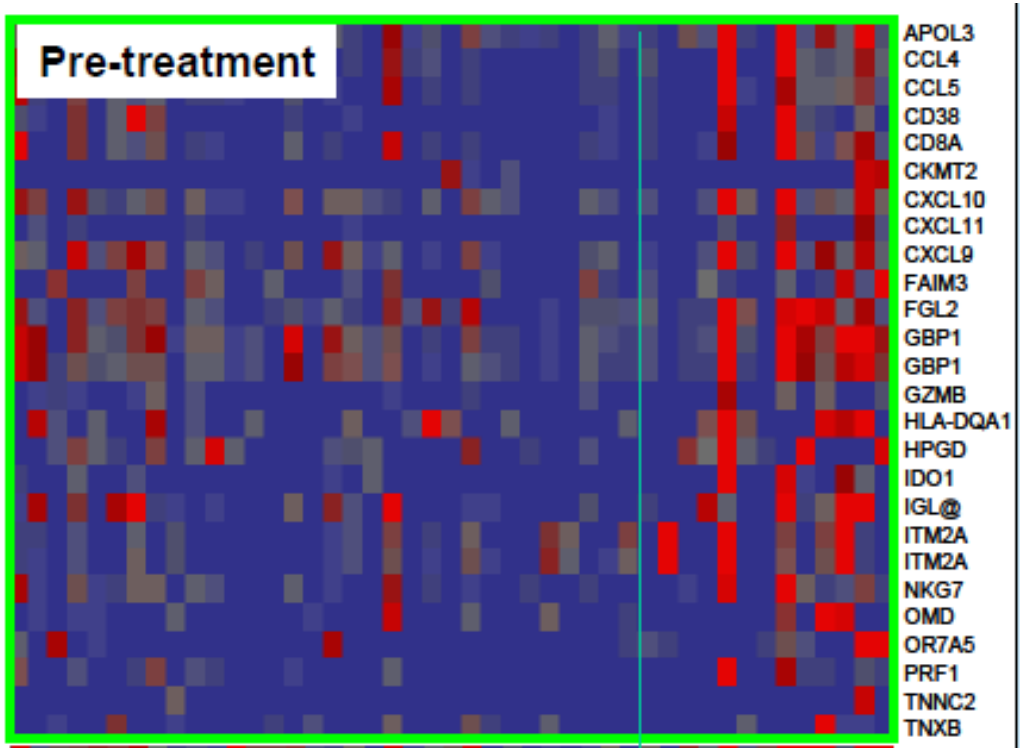

*Harlin et al. Can. Res. 2009*

# Chemokine/T cell gene expression signature is associated with survival following GSK MAGE3 protein vaccine




*Louahed et al., EORTC-NCI-AACR 2009*

# Ipilimumab clinical responders also show a chemokine/T cell gene expression profile in tumor microenvironment




- CXCL9, 10, 11
- CCL4, CCL5
- Granzyme B
- Perforin
- CD8α

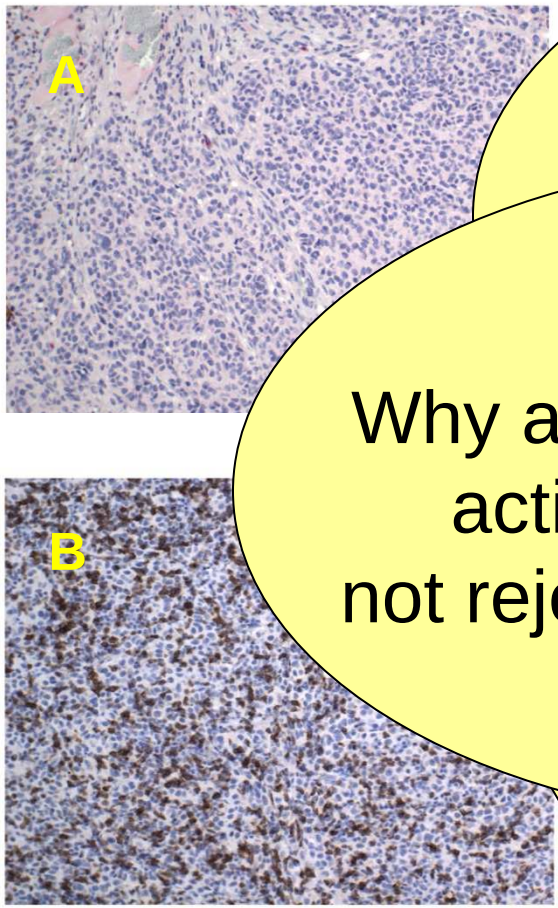
***Ji et al, Cancer Immunol. Immunoth. 2012***

# Implication of melanoma gene array results for patient-specific therapy

- Gene expression profiling of the melanoma tumor microenvironment has revealed reproducible patterns associated with clinical benefit➔should be explored as predictive biomarker in prospective trials
  - Already being pursued by GSK-Bio in context of multicenter MAGE3 vaccine studies
- Ideally, this strategy should allow enrichment for the potentially responsive patient population in the future
  - Think Her2 equivalent for T cell immunotherapies
- These observations also highlight critical aspects of tumor/immune system biology, and suggest specific strategies for overcoming immunologic barriers at the level of the tumor microenvironment

# Two broad categories of tumor microenvironments defined by gene expression profiling and confirmatory assays

CD8 IHC




What dictates
ivated CD8+
es?

Why are tumors that contain activated CD8+ T cells not rejected spontaneously?

mune
omote
ell priming
in a subset of patients?

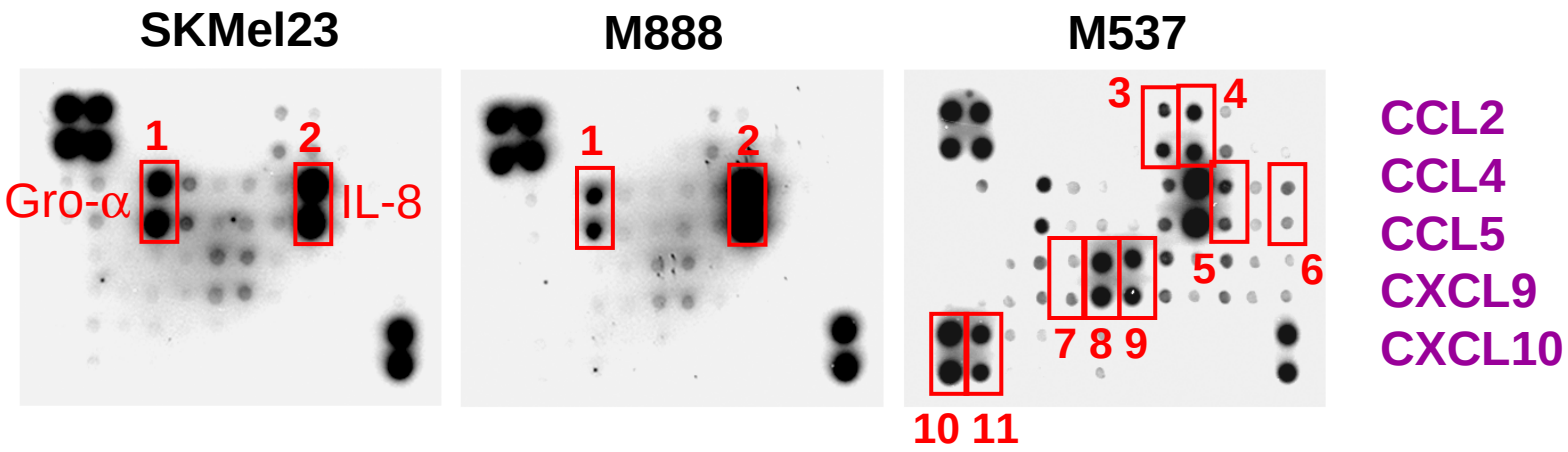
nes

*Gajewski, Brichard; Cancer J. 2010*

# 1. Chemokines, vascular endothelium, and T cell migration into tumor sites

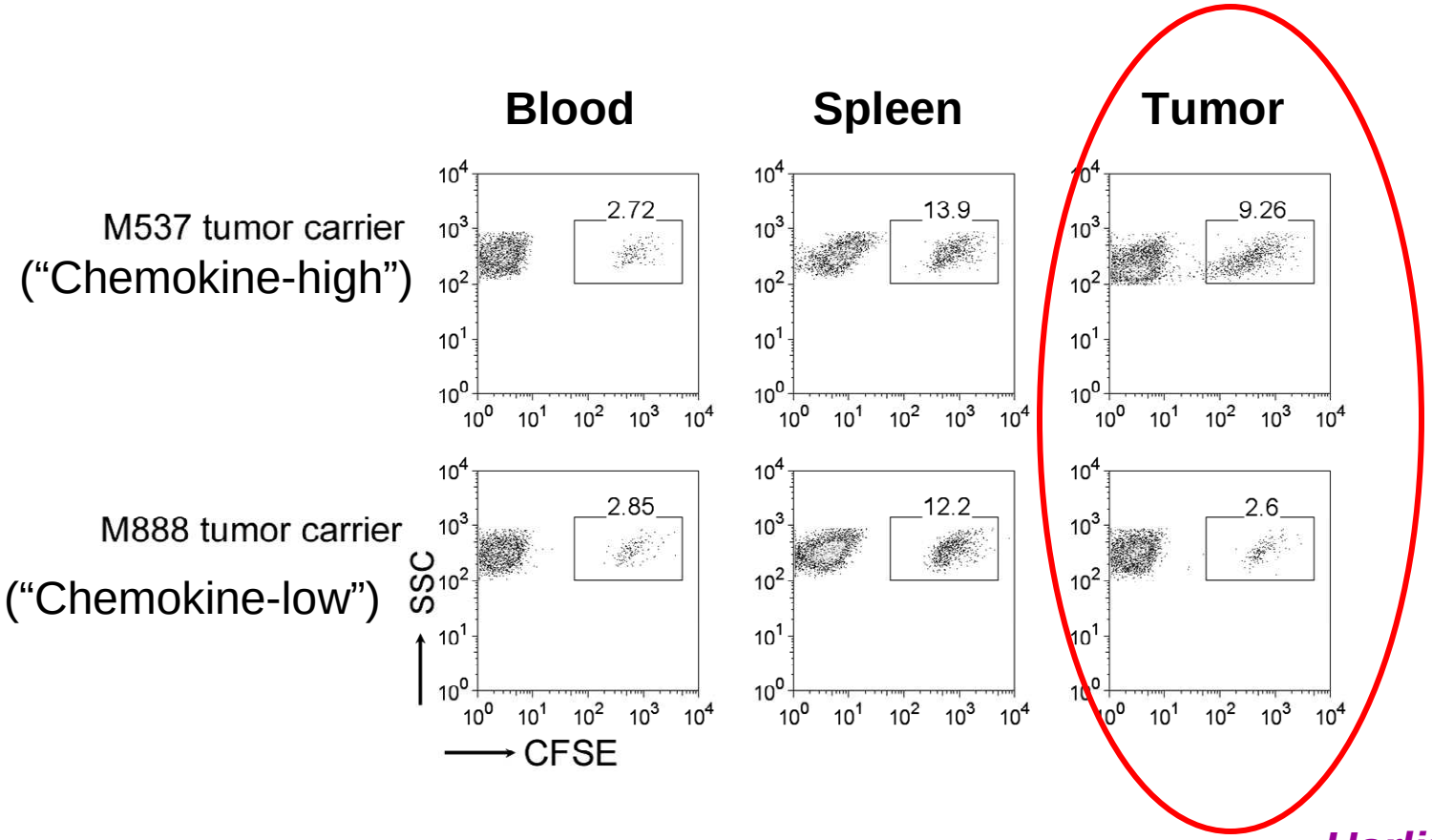
What is attracting T cells into some tumors? Can we mimic this in the tumors that fail to achieve it spontaneously?

# A subset of melanoma cell lines expresses a broad array of chemokines



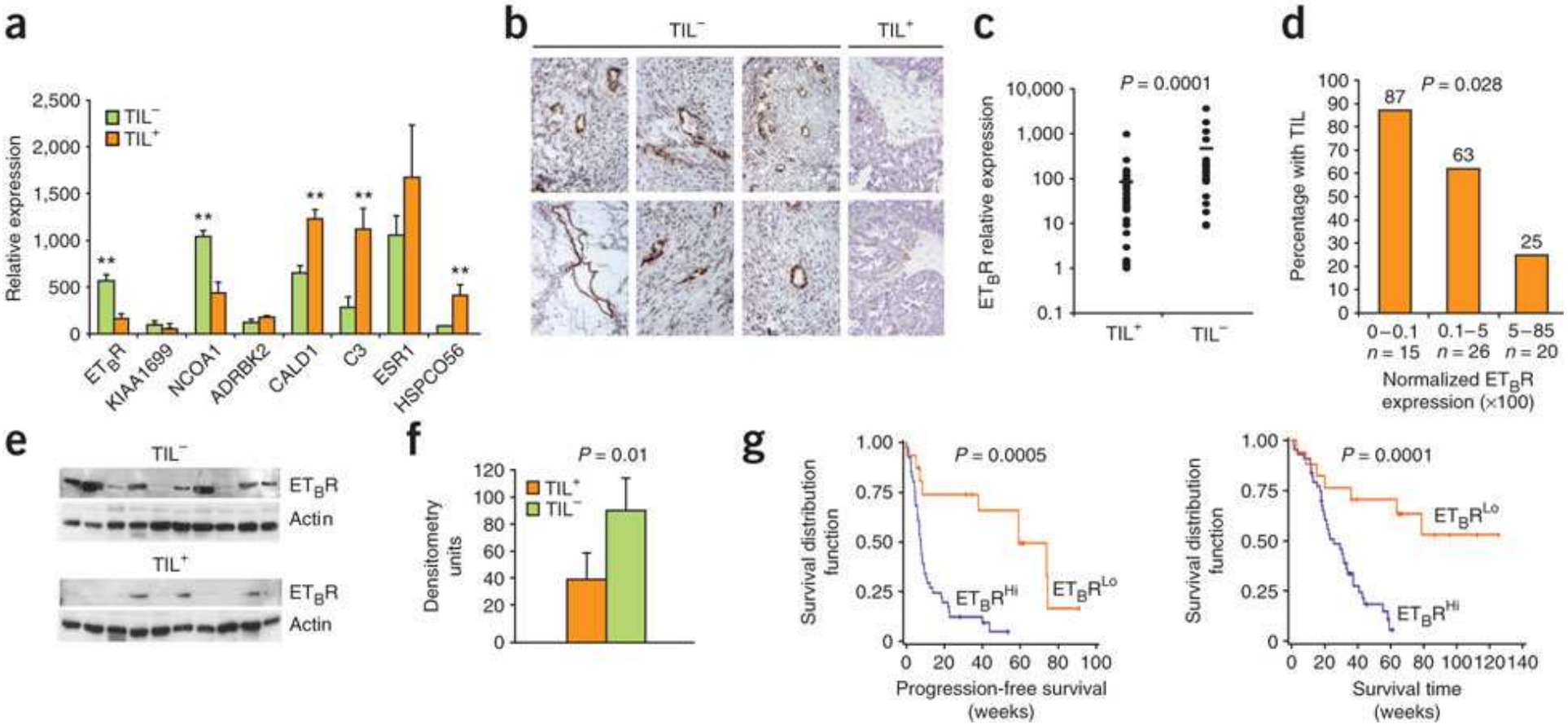

• Implies that in some cases, the melanoma tumor cells themselves can produce a broad panel of key chemokines for T cell migration

# Superior recruitment of human CD8$^+$ effector T cells in NOD/scid mice bearing "chemokine-high" M537 melanomas




*Harlin et al.*
*Can. Res. 2009*

# Features of vascular endothelial cells also regulate T cell homing: $ET_BR$




***Buckanovic, Coukos et al. Nat. Med. 2008***

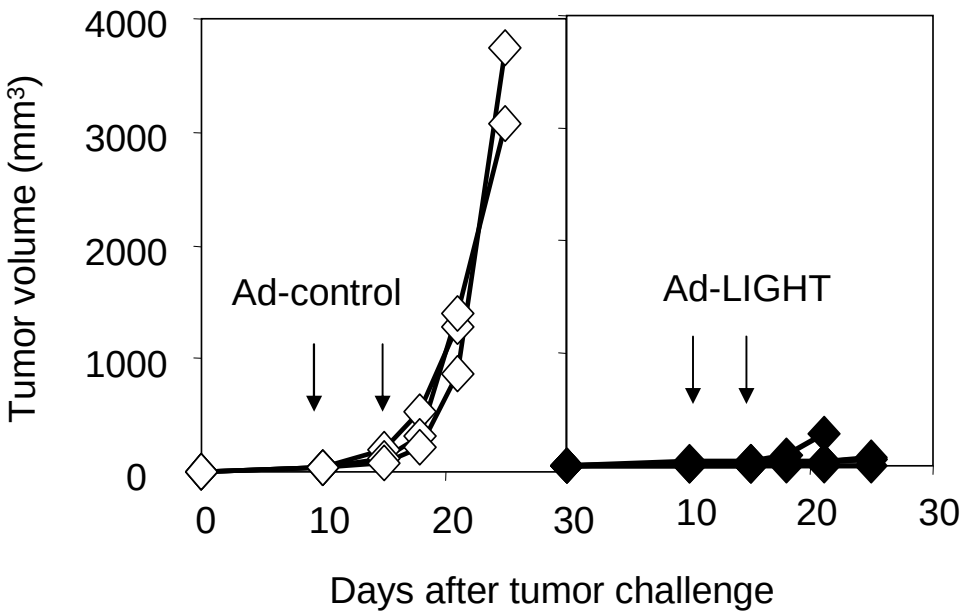
# Candidate strategies to promote effector T cell migration into tumor sites

- Introduce chemokines directly
  - CXCR3-binding chemokines (CXCL9, CXCL10)
  - Others (CCL2, CCL3, CCL4, CCL5)
- Induce chemokine production from stromal cells
  - LIGHT, lymphotoxin: bind LTβR
- Elicit appropriate local inflammation that includes chemokine production
  - Type I IFNs
  - TLR agonists
  - Radiation
- Alter signaling pathways in melanoma cells themselves to enable chemokine gene expression by tumor cells

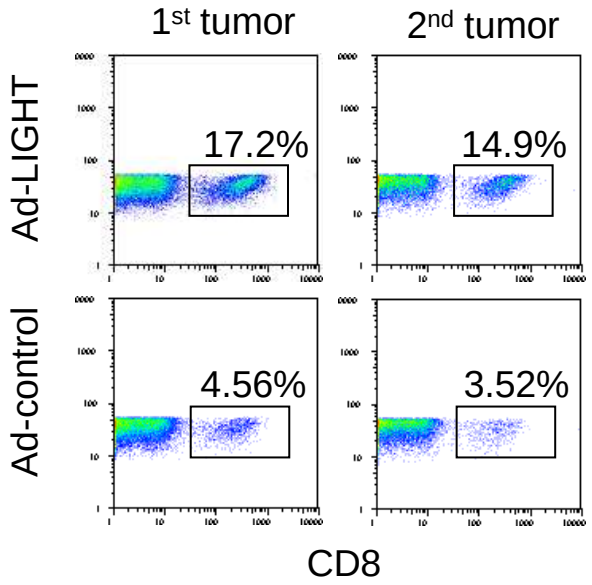
# Intratumoral LIGHT adenovirus in B16 melanoma:

## Promotes chemokine production, CD8$^+$ T cell recruitment, primary tumor control, and rejection of non-injected distant metastases

### Tumor rejection




### CD8$^+$ T cell infiltrate

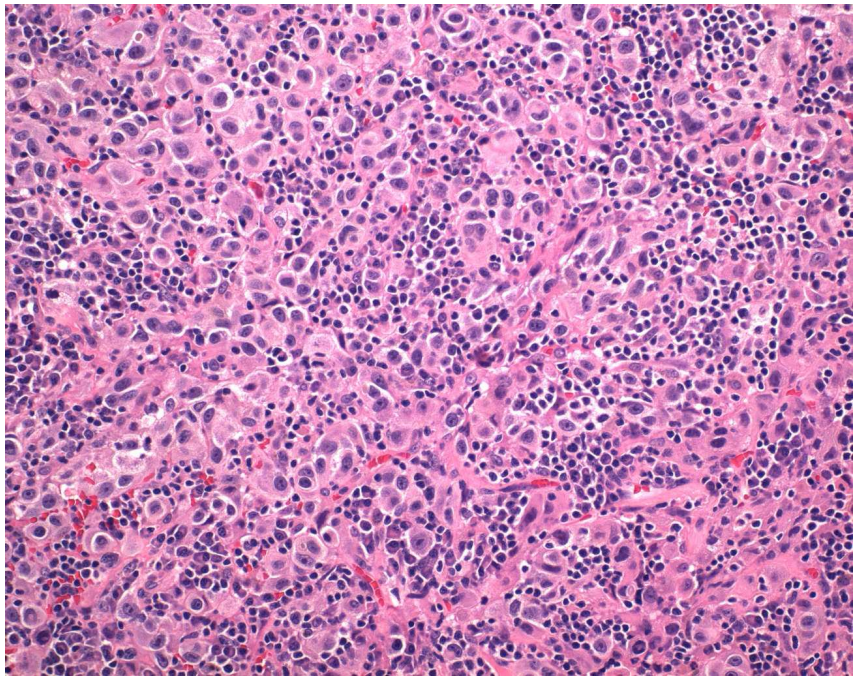



*Yu et al, J. Immunol. 2007*

# 2. T cell suppressive mechanisms

Why are TIL not eliminating the tumor cells they are infiltrating?  Can we overcome this defect and restore tumor rejection?

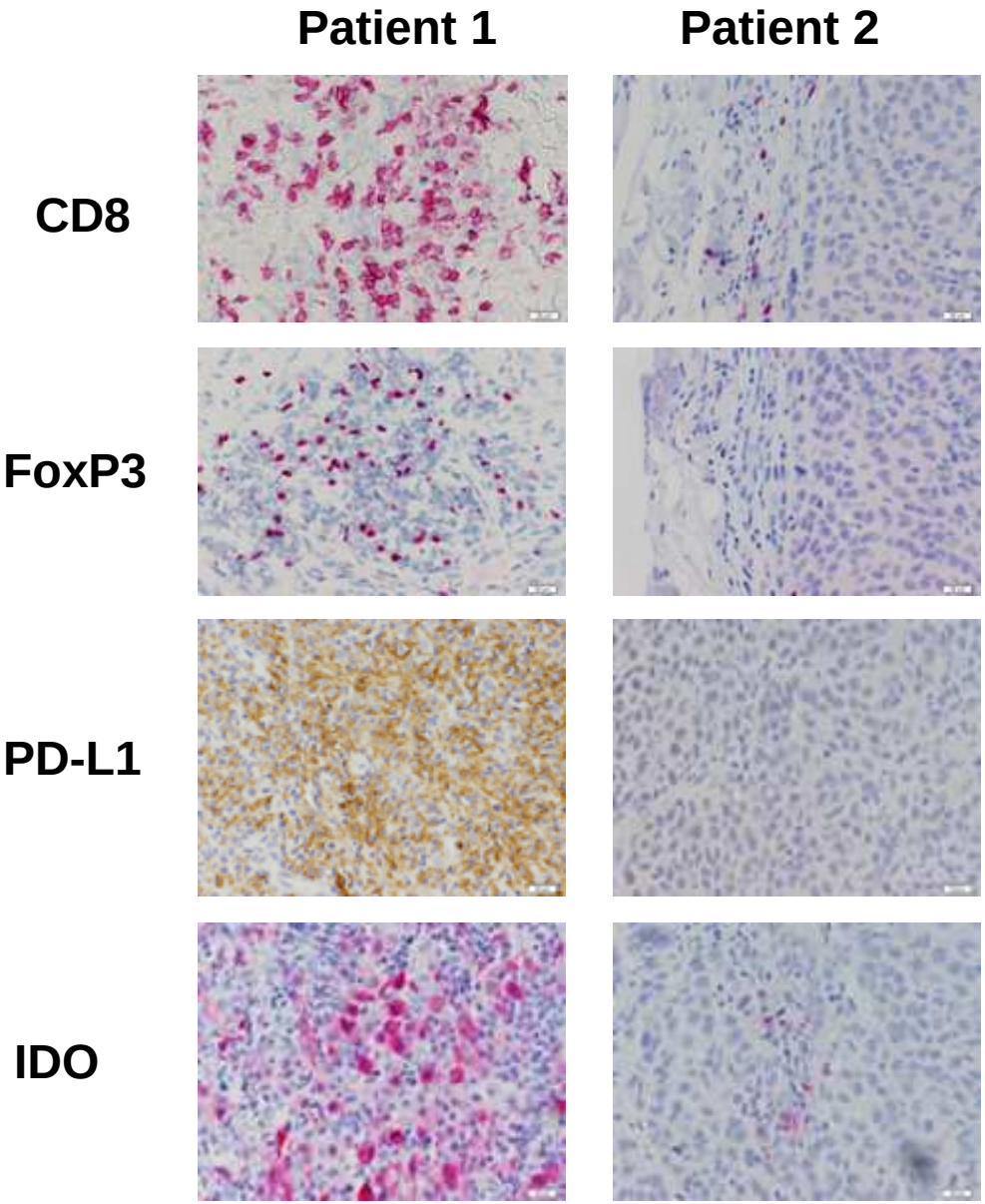
# Inflamed melanomas containing $CD8^+$ T cells have highest expression of immune inhibitory pathways

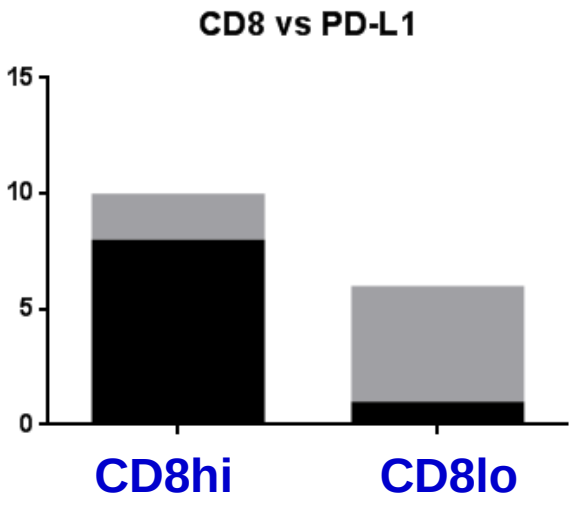


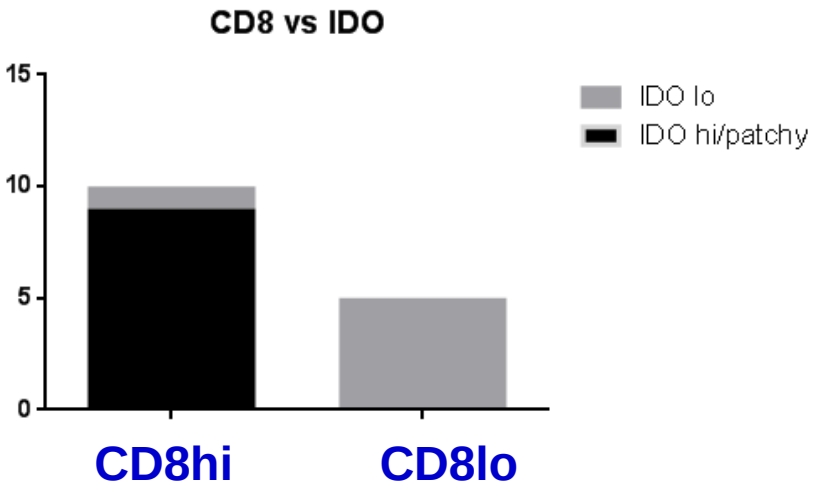
- **IDO** (indoleamine-2,3-dioxygenase)
  - Tryptophan depletion
- **PD-L1**
  - Engages PD-1 on T cells
- $CD4^+CD25^+FoxP3^+$**Tregs**
  - Extrinsic suppression
- T cell **anergy** (B7-poor)
  - T cell intrinsic TCR signaling defect

***Immunol. Rev. 2006,***
***Clin. Can. Res. 2007***

# Presence of Tregs and expression of PD-L1 and IDO are associated with a CD8+ T cell infiltrate



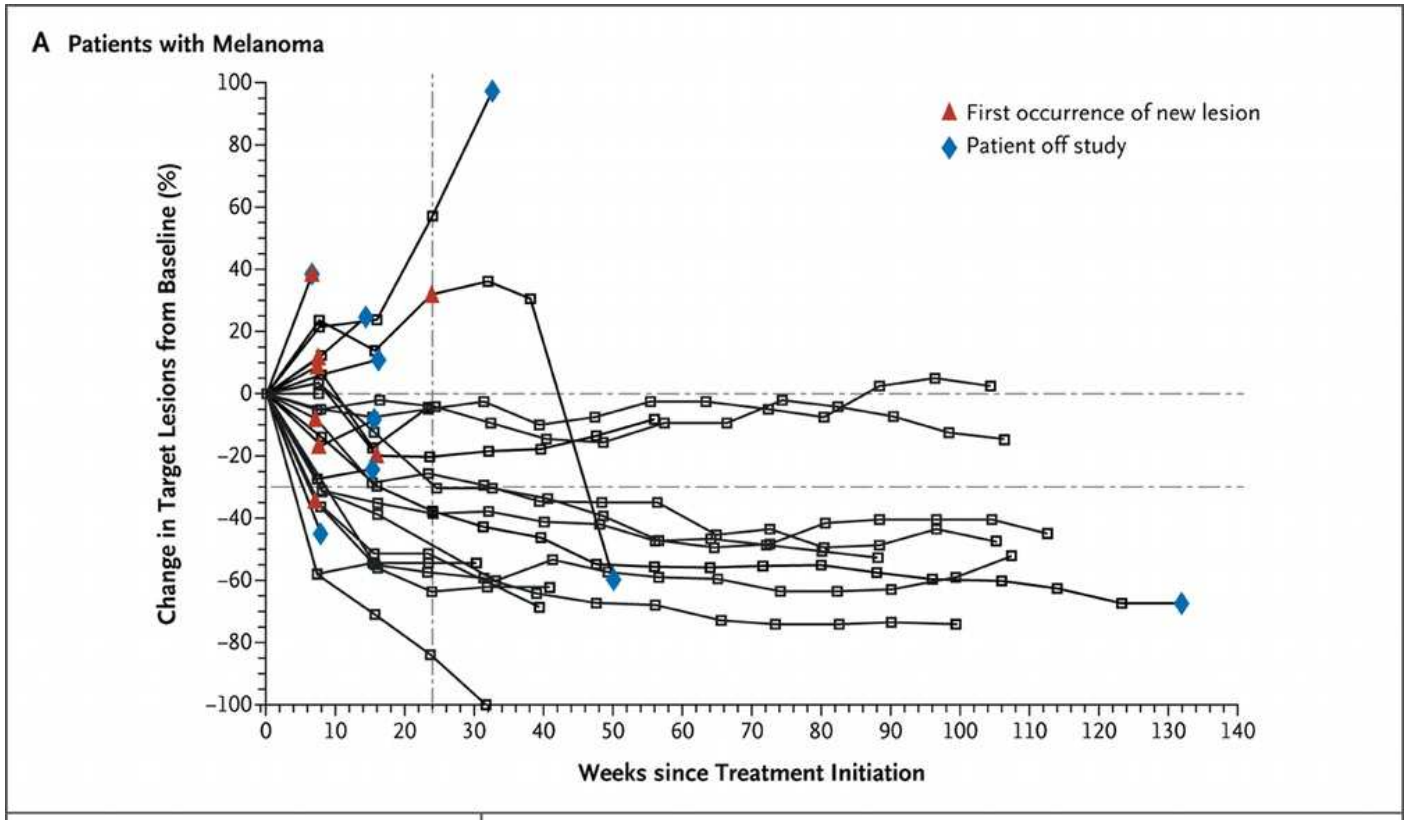



# Strategies to block immune inhibitory mechanisms validated in mouse models and being translated to the clinic

- **Blockade of PD-L1/PD-1 interactions**
  - Anti-PD-1 and anti-PD-L1 mAbs (BMS, Merck, Genentech, Curetech)
- **IDO inhibition**
  - Potent IDO small molecule inhibitors (Incyte)
- **Depletion of $CD4^+CD25^+FoxP3^+$ Tregs**
  - Denileukin diftitox (IL-2/DT fusion)
  - Daclizumab, Basiliximab (anti-IL-2R mAbs)
  - Ex vivo bead depletion of $CD25^+$ cells from T cell product for adoptive transfer
- **Anergy reversal**
  - Introduction of B7-1 into tumor sites
  - Homeostatic cytokine-driven proliferation
    - T cell adoptive transfer into lymphopenic recipient
    - Exogenous IL-7 (Future: IL-15, IL-21)
- **Combinations of negative regulatory pathway blockade**
  - Synergy between blockade of 2 or more pathways

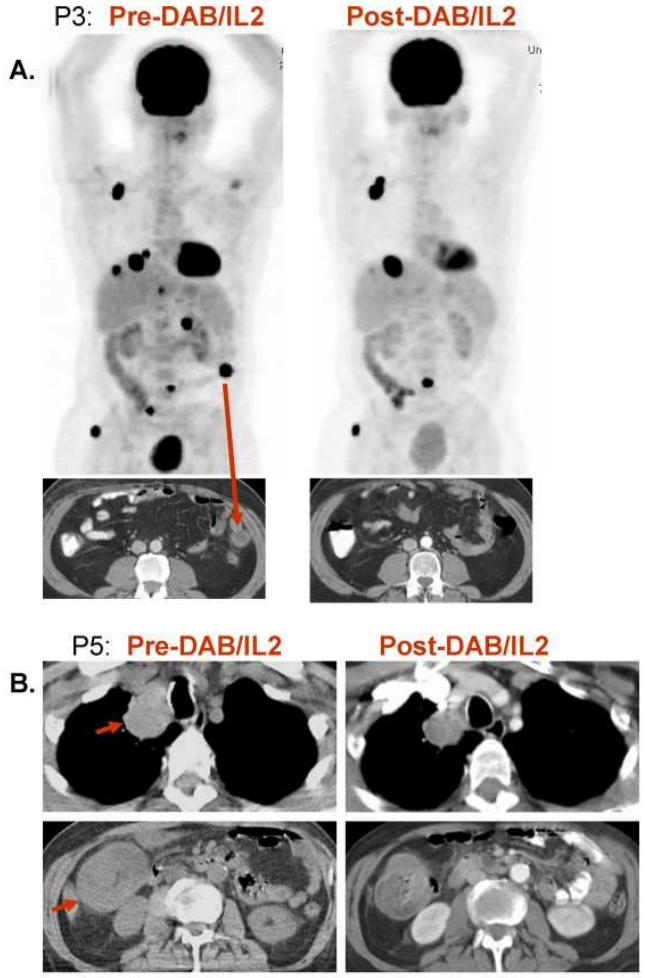
# Clinical activity of anti-PD-1 mAb in metastatic melanoma




**27% RR among 95 melanoma patients**

***Topalian et al. NEJM. 2012***

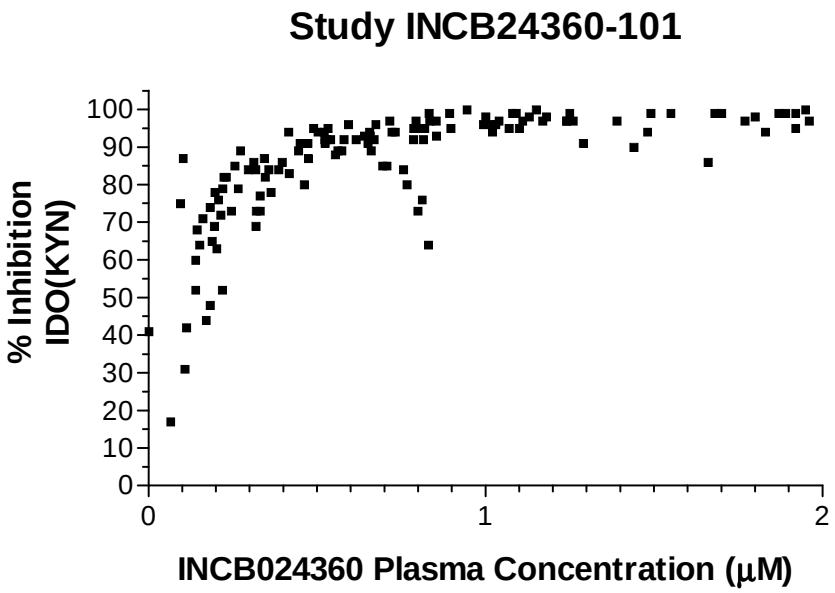
# Reduction of Treg number using Denileukin diftitox can have clinical activity in melanoma

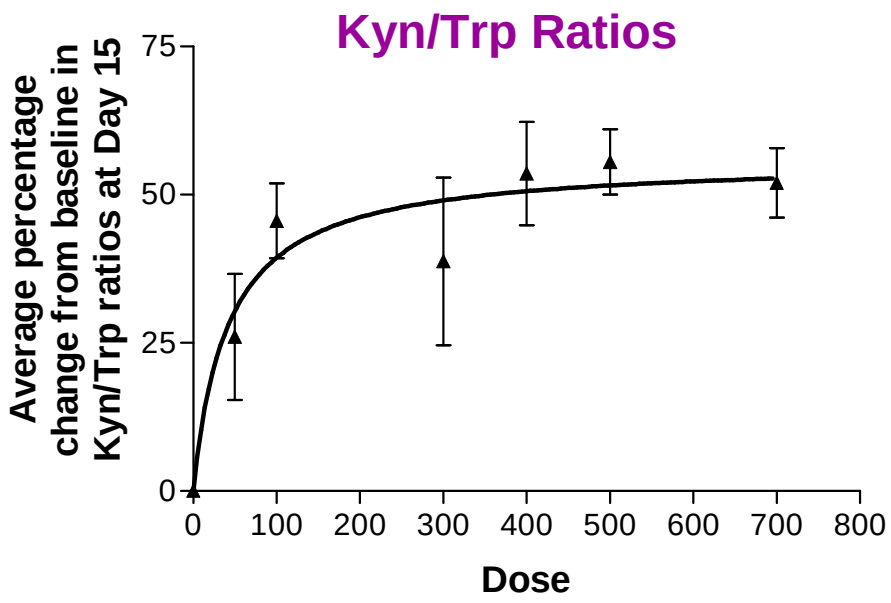



***Rasku et al***
***J. Trans. Med. 2008***

Multicenter phase II study currently ongoing

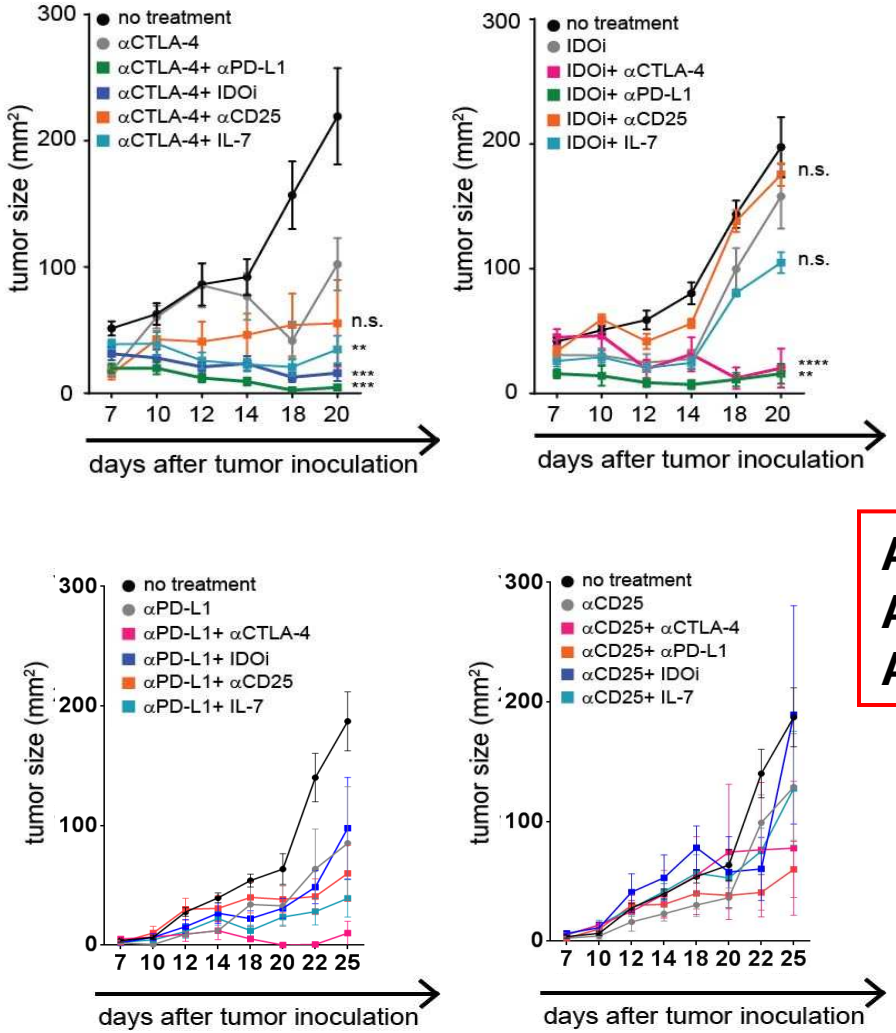
# Dose-dependent inhibition of IDO activity as assessed by kynurenine/tryptophan ratios in treated patients




***Newton et al. ASCO 2012***

# Combinatorial blockade of selected inhibitory pathways is therapeutically synergistic in vivo

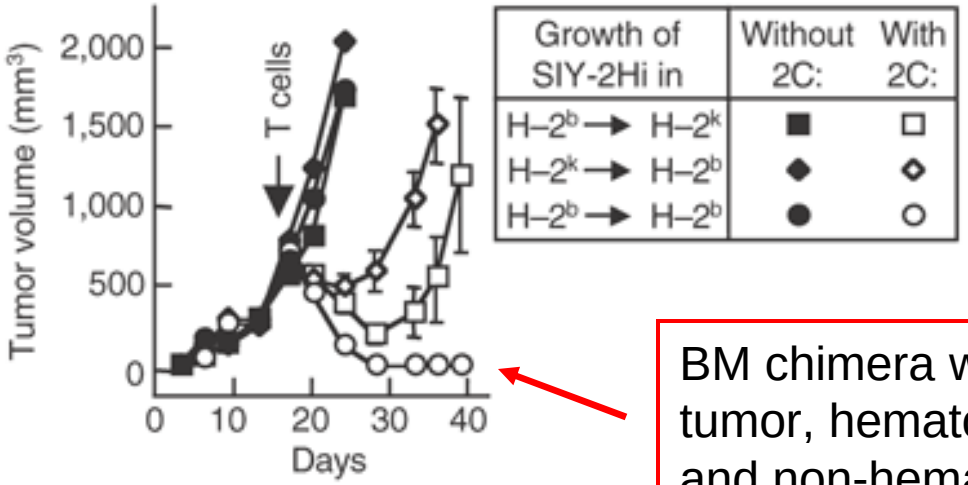



**Anti-CTLA-4 + anti-PD-L1**
**Anti-CTLA-4 + IDOi**
**Anti-PD-L1 + IDOi**

# 2b. Solid tumor stroma as a barrier

How do stromal components that support tumor growth interface with host immune response?

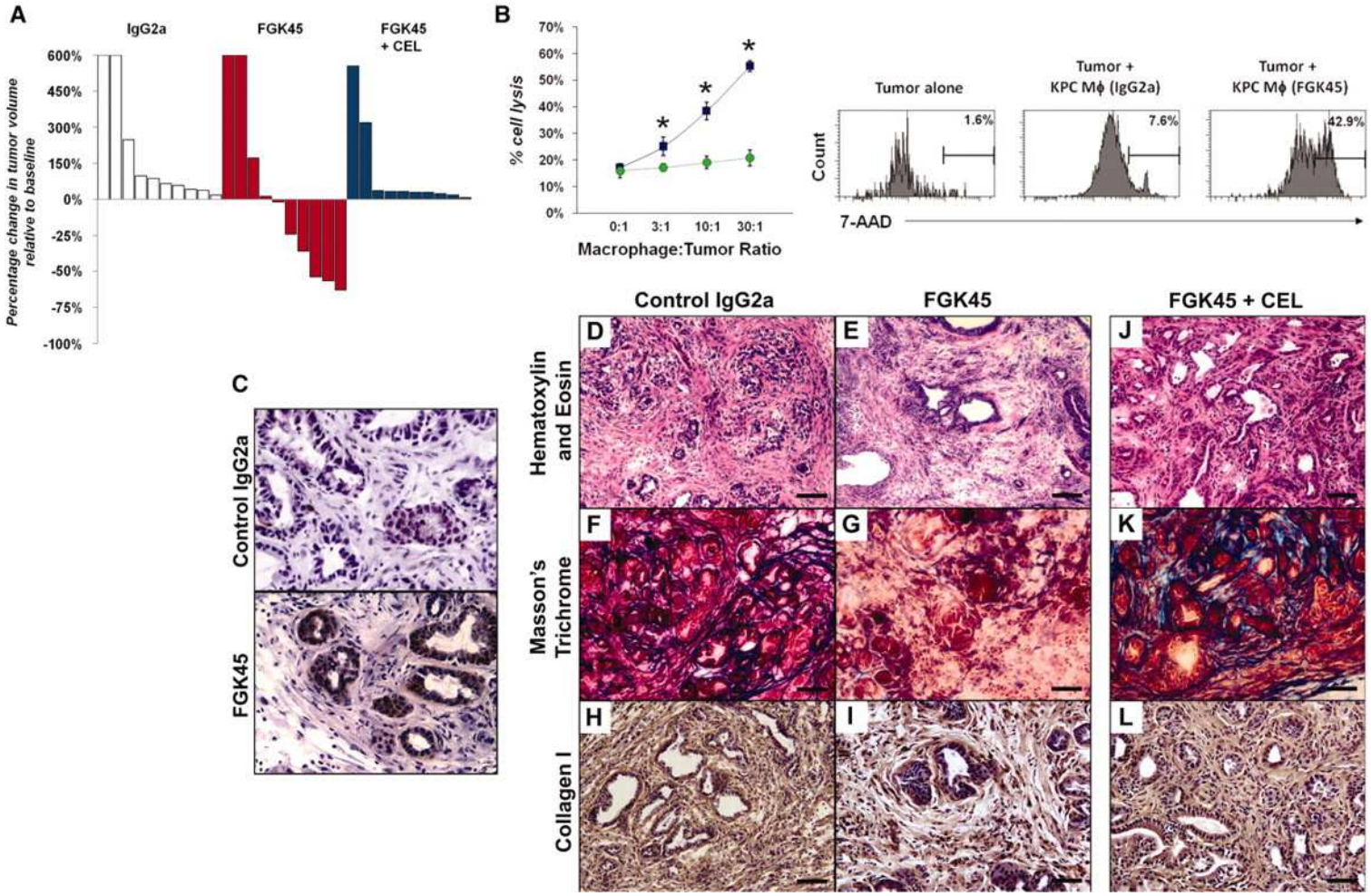
# Targeting tumor stroma immunologically may be the key to durable complete responses




BM chimera with MHC matched tumor, hematopoietic stroma, and non-hematopoietic stroma

***Spiotto, Schreiber et al. Nature Medicine 10:294, 2004***

# Anti-CD40 mAb promotes tumor shrinkage by altering intratumoral macrophages in pancreatic cancer

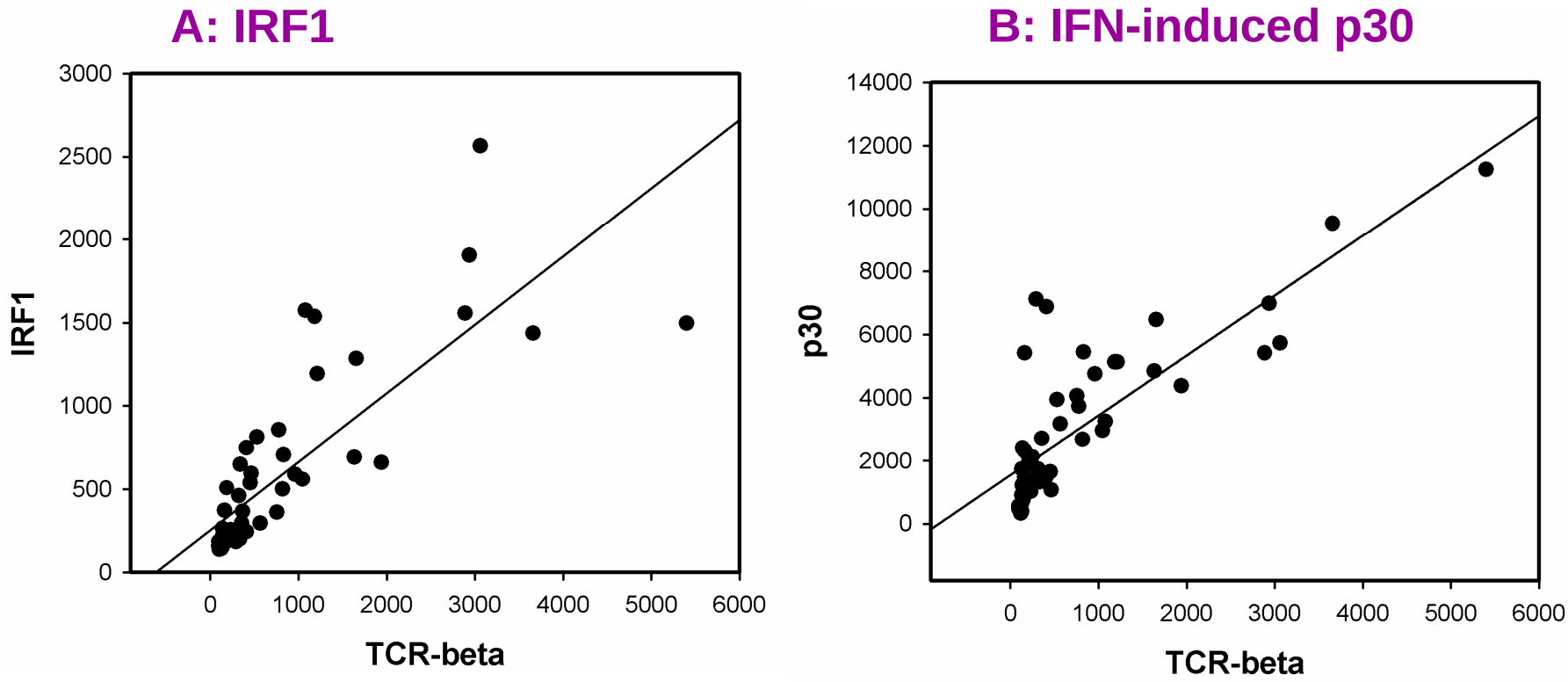



*Beatty, Vonderheide et al. Science 2011*

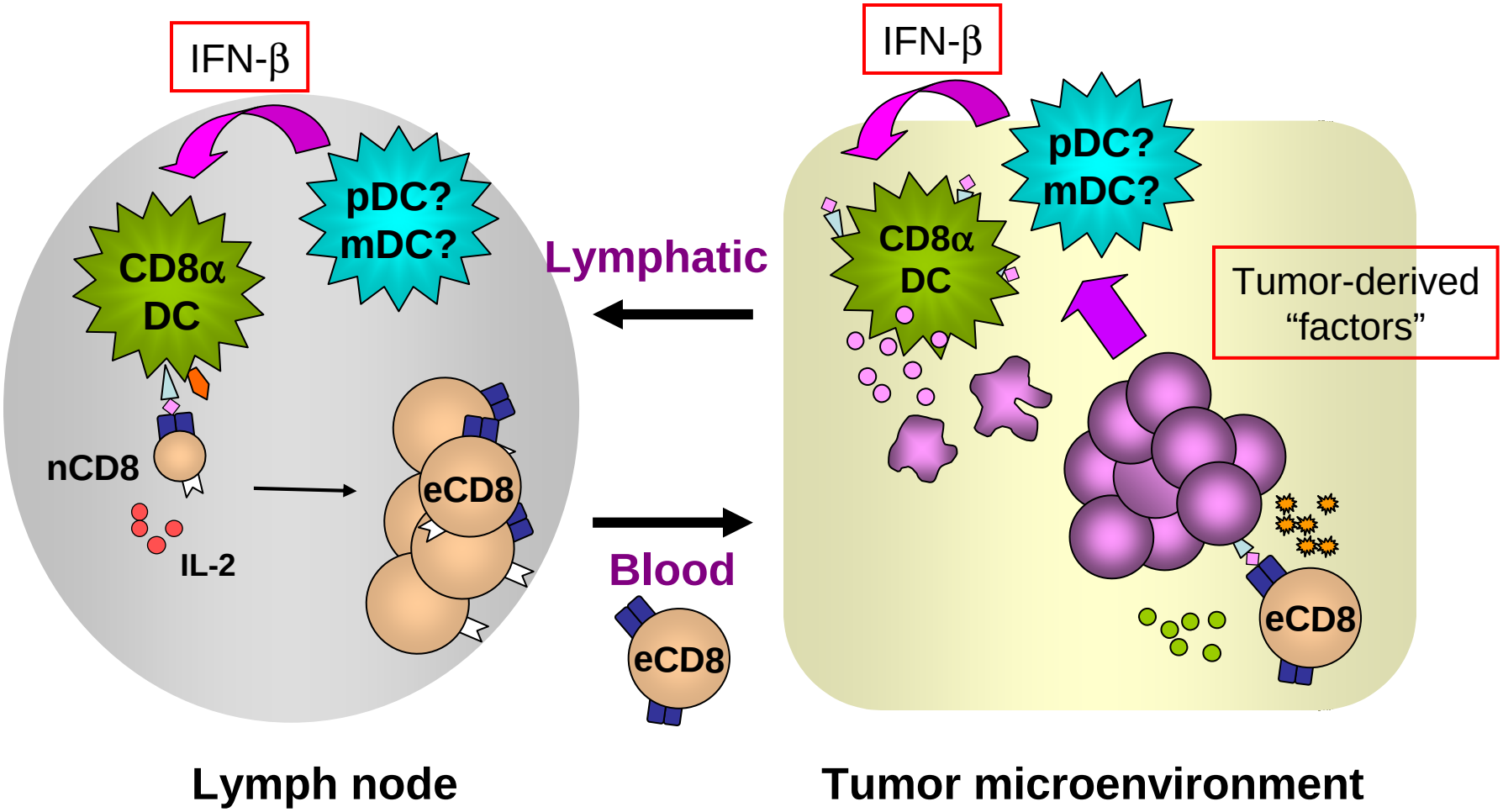
# 3. Innate immune sensing of tumor—type I IFNs

How are anti-tumor T cells sometimes becoming spontaneously primed? Can we improve endogenous T cell priming in the tumors that fail to do so alone?

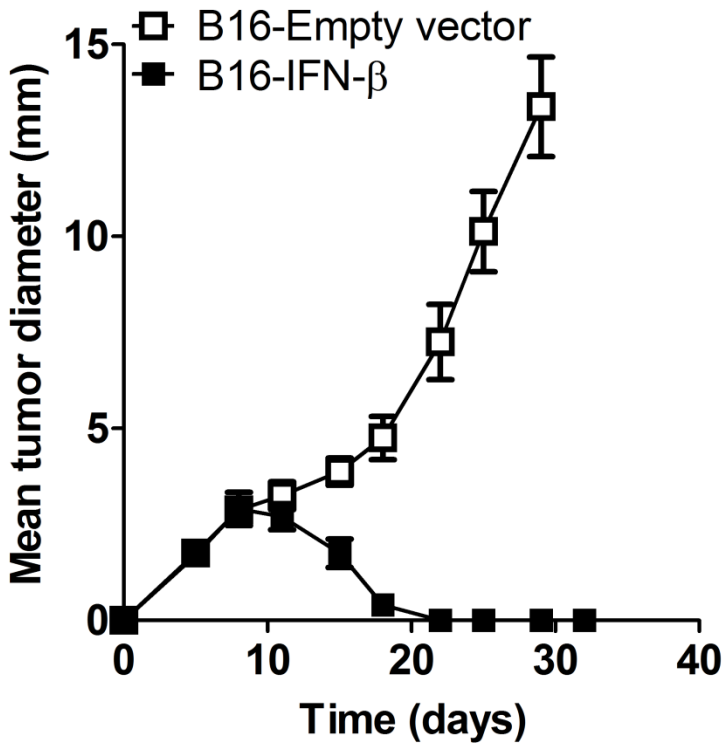
# Melanoma metastases that contain T cell transcripts also contain transcripts known to be induced by type I IFNs



# Innate immune sensing of tumors drives host type I IFN production and cross-priming of CD8$^+$ T cells via CD8α DCs



# Provision of exogenous IFN-β intratumorally can potently induce tumor rejection




**Should we develop strategies for intratumoral administration of IFN-α/β, to modify the tumor microenvironment?**

# Conclusions

- There is heterogeneity in patient outcome to immune-based therapies for cancer such as melanoma vaccines, IL-2, and anti-CTLA-4 mAb
- One component of that heterogeneity is derived from differences at the level of the tumor microenvironment
- Key determining factors in melanoma microenvironment include chemokine-mediated recruitment of effector $CD8^+$ T cells, local immune suppressive mechanisms, and innate immune activation including type I IFNs
- Understanding these aspects is enabling improved patient selection for Rx with immunotherapies (predictive biomarker), and also development of new interventions to modify the microenvironment to better support T cell-mediated rejection
- Targeting the tumor stroma immunologically may be just as critical as targeting the tumor cells

# Acknowledgments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