Immunotherapeutic barriers at the level of the tumor microenvironment

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Disclosures

- Advisory boards:
  - GSK-Bio, Roche-Genentech, Merck, BMS, Abbvie
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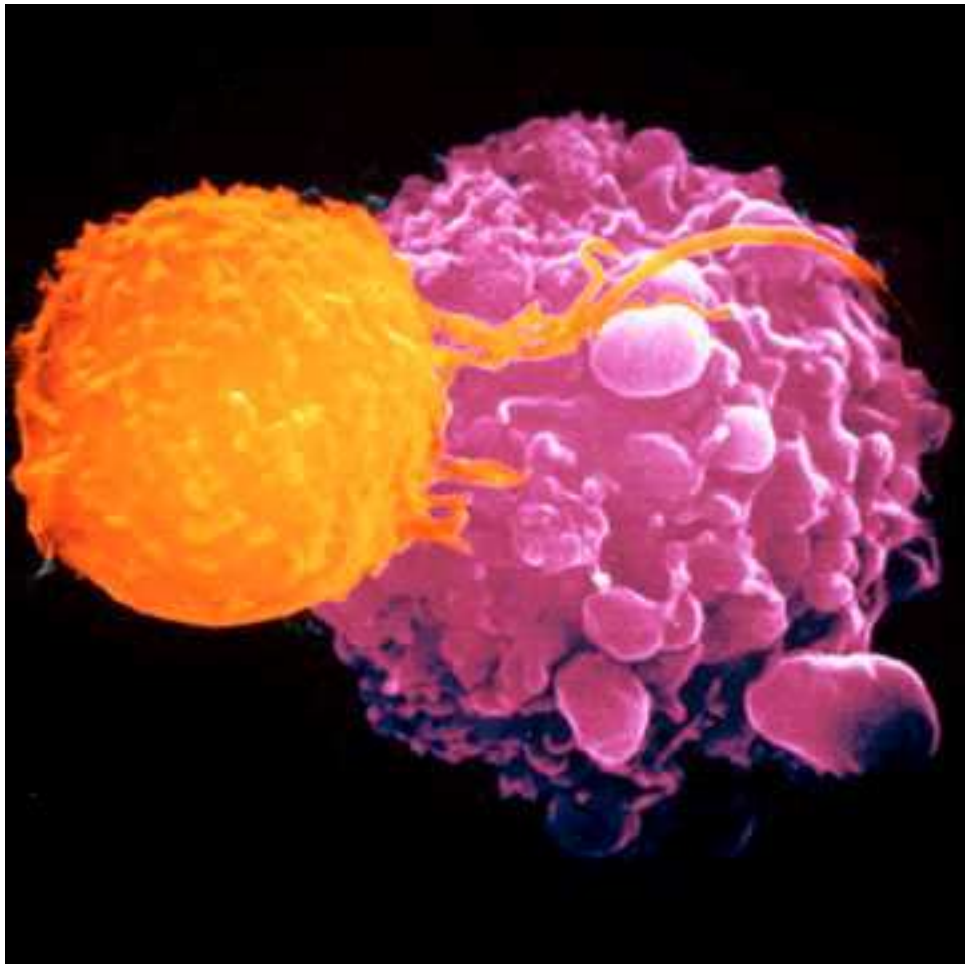
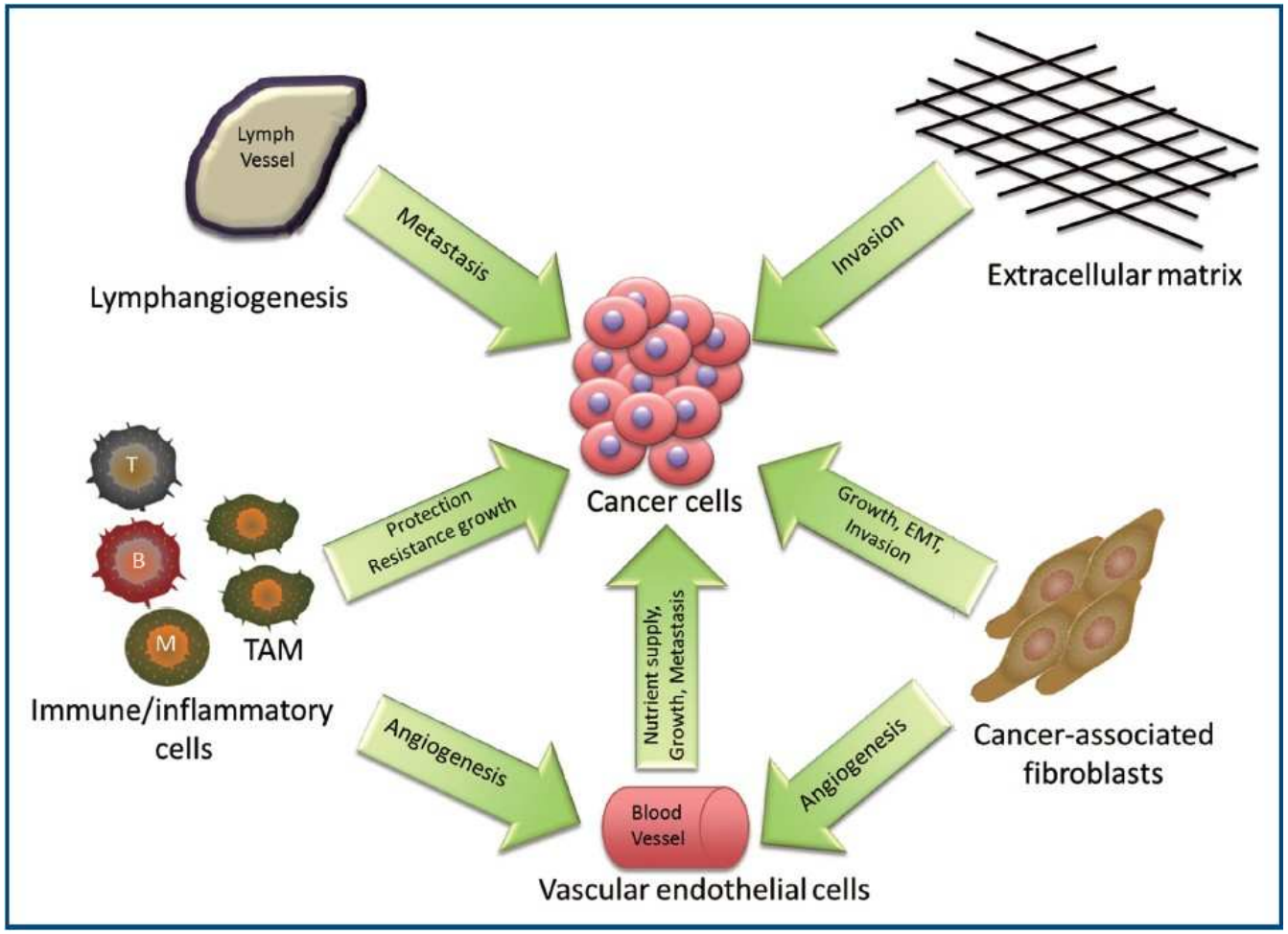
$CD8^+$ cytotoxic T lymphocyte killing an antigen-expressing tumor cell



In vivo, a tumor is more than tumor cells

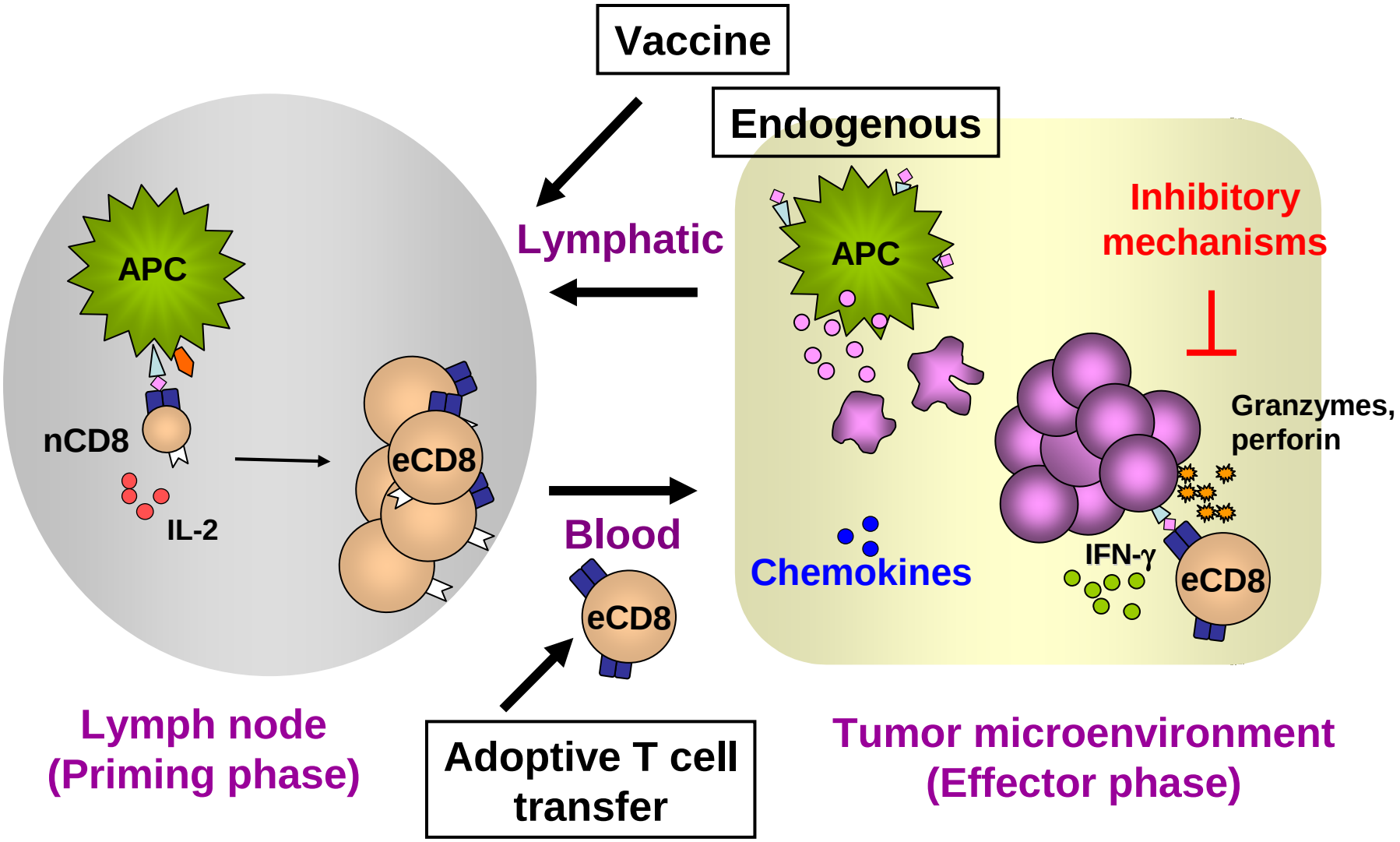
- Three dimensional mass
- Extracellular matrix
- Supported by the neovasculature, fibroblasts, macrophages
- Variable presence of inflammatory cells
  - T cells (and subsets thereof)
  - B cells/plasma cells
  - NK/NKT cells
  - Dendritic cell subsets
- The functional phenotypes of these cells may or 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

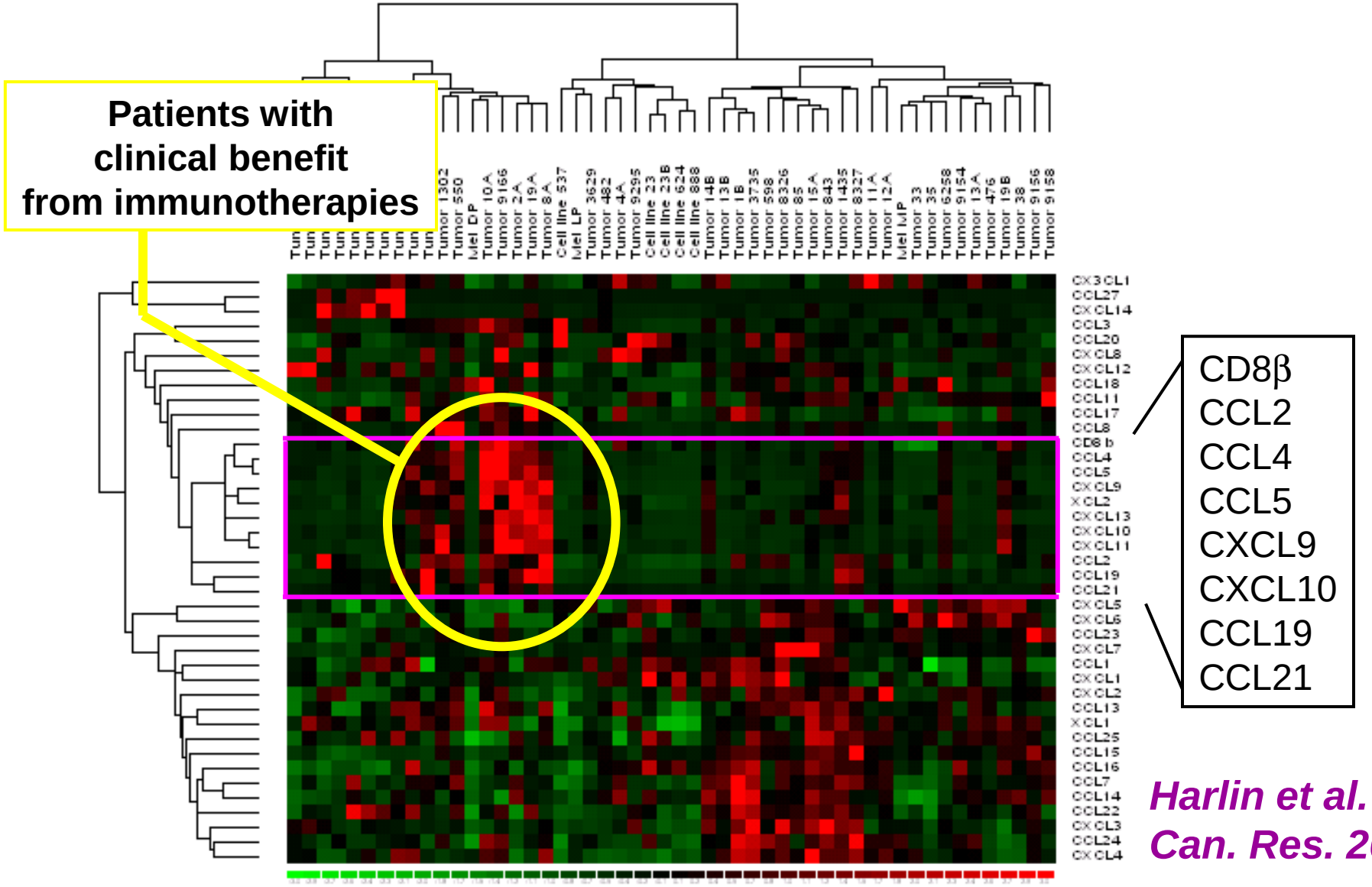
Anti-tumor immune responses in vivo: Taking into account the tumor microenvironment



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

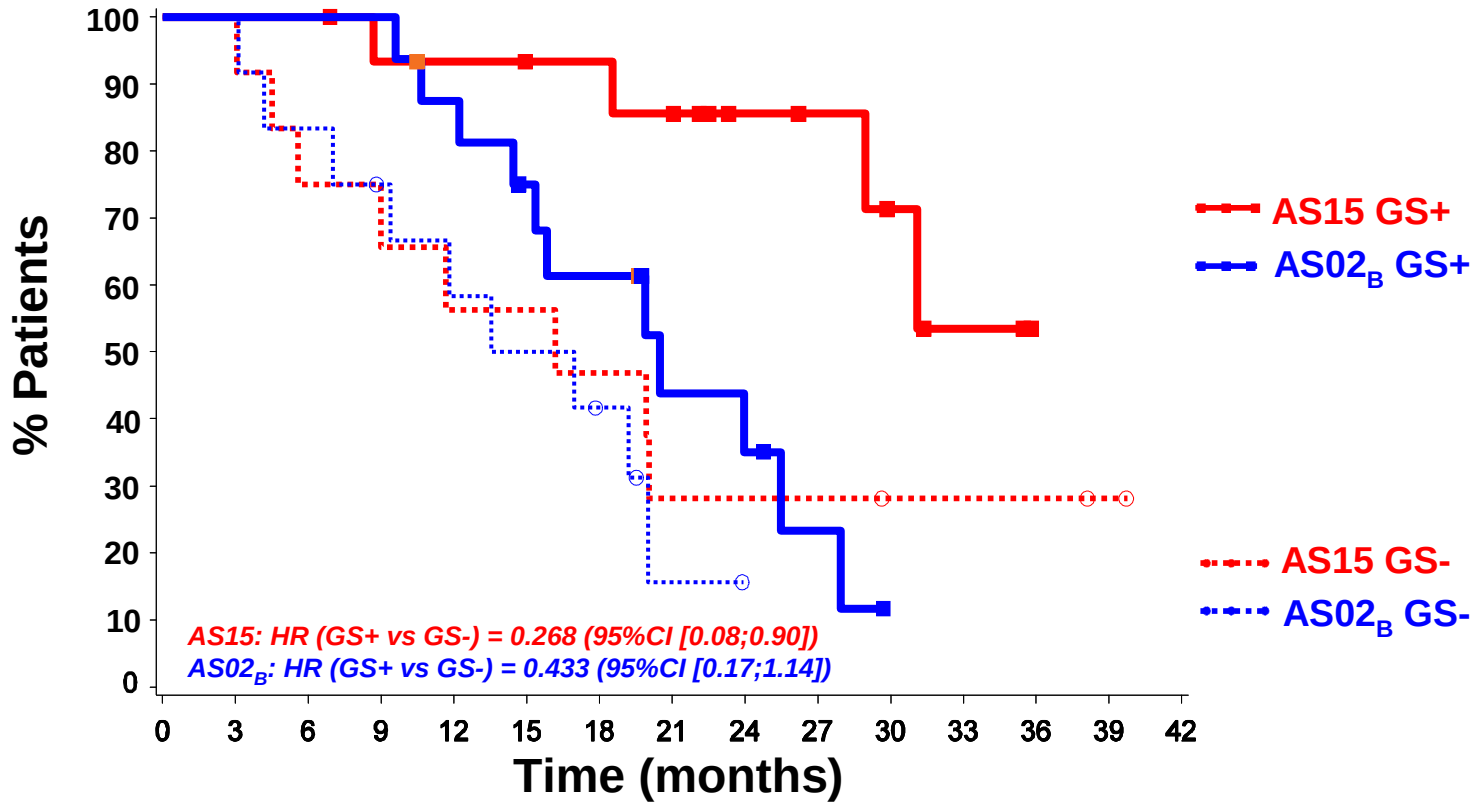
- Priming phase
  - Lack of innate immune-activating “danger” signals
  - Poor recruitment of the critical APC subsets for cross-presentation of antigens to T cells
  - Inadequate expression of costimulatory ligands on tumor cells or on infiltrating APCs
- Effector phase
  - Inadequate recruitment of 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



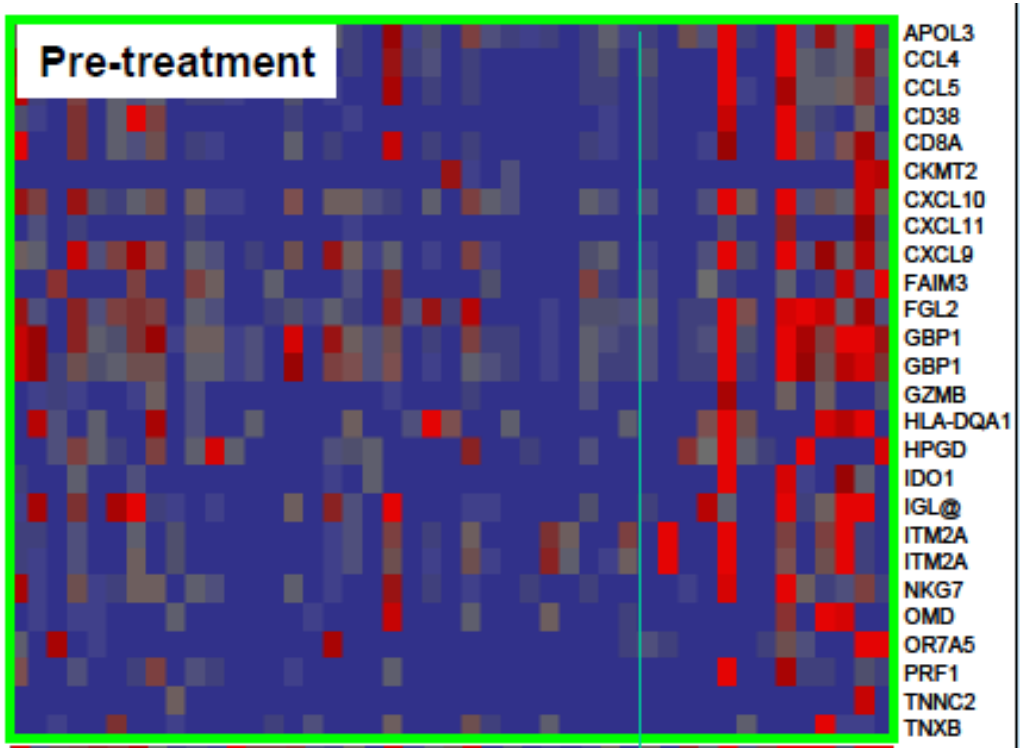
Harlin et al. Can. Res. 2009

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

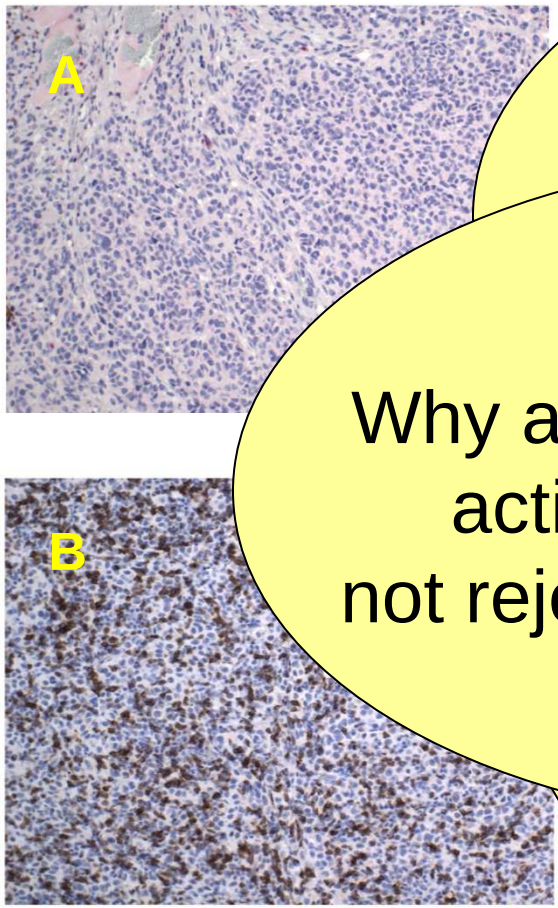
Ji et al, Cancer Immunol. Immunoth. 2012

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?

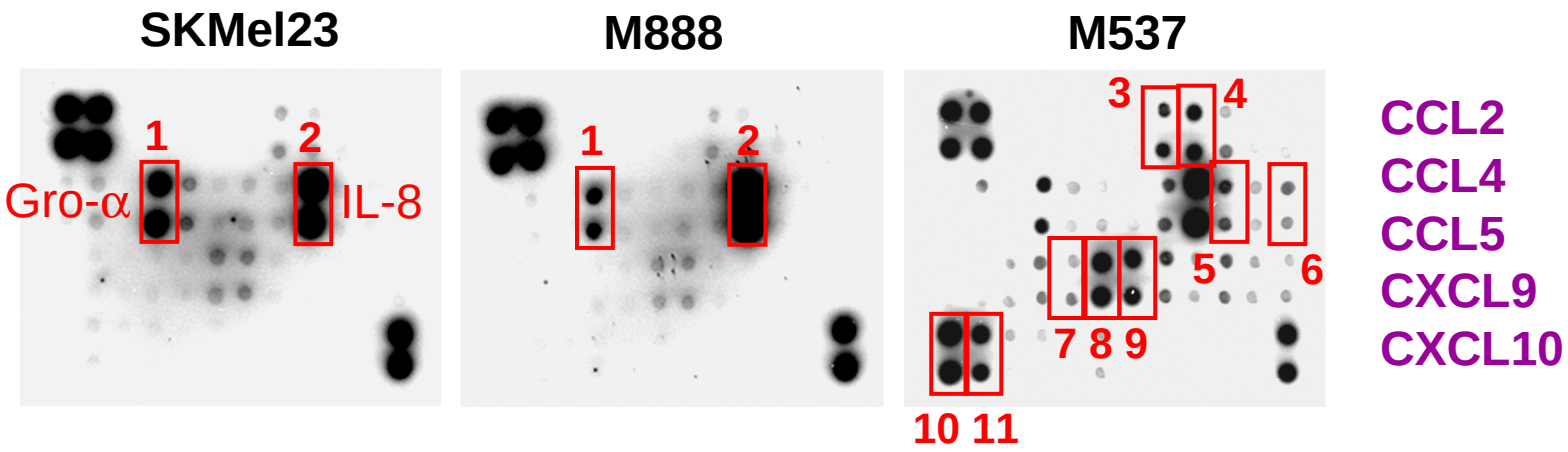
nes

Gajewski, Brichard; Cancer J. 2010

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

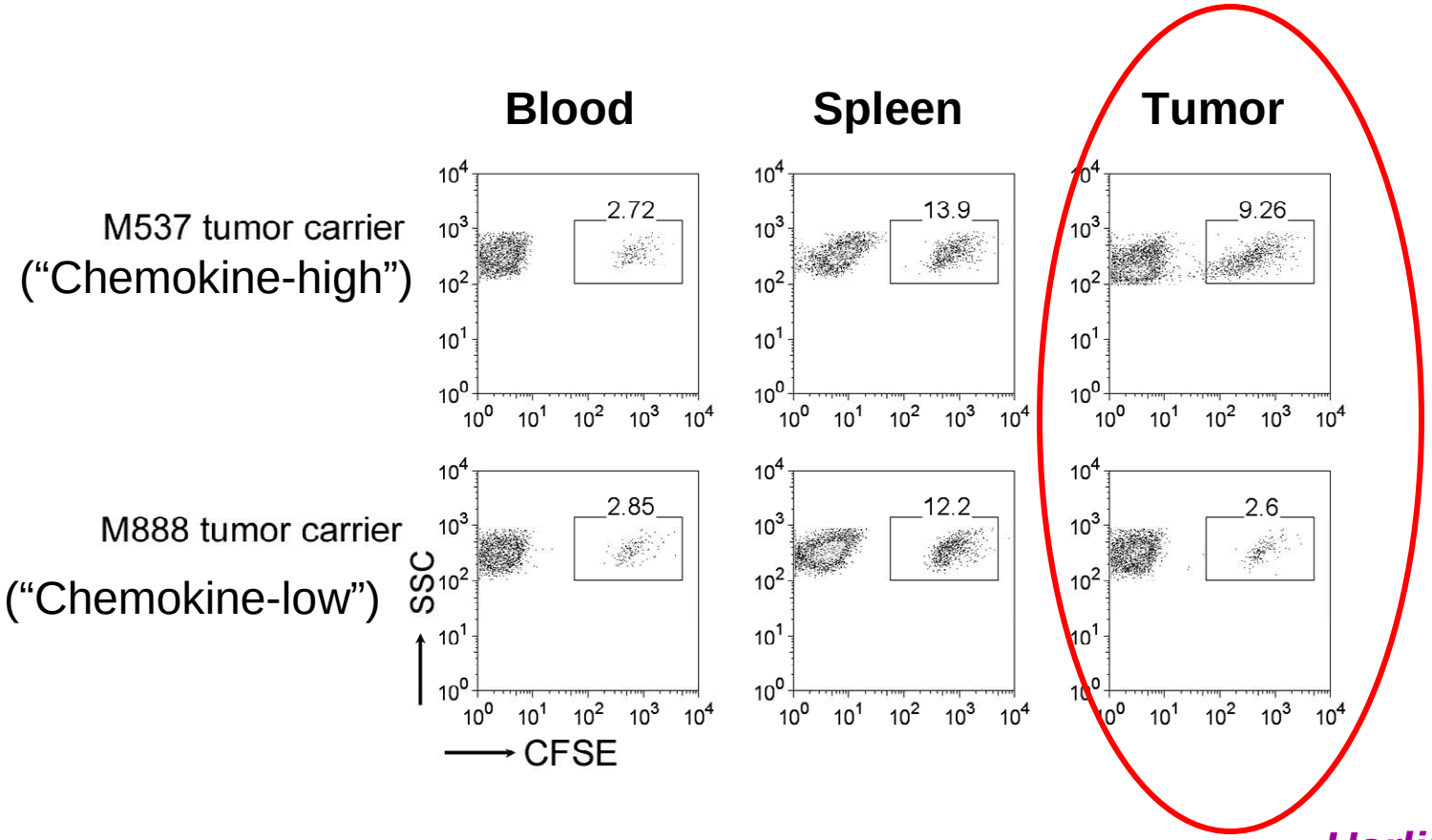
What is attracting T cells into some tumors? Can we mimic this in the tumors that fail to achieve it spontaneously?

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



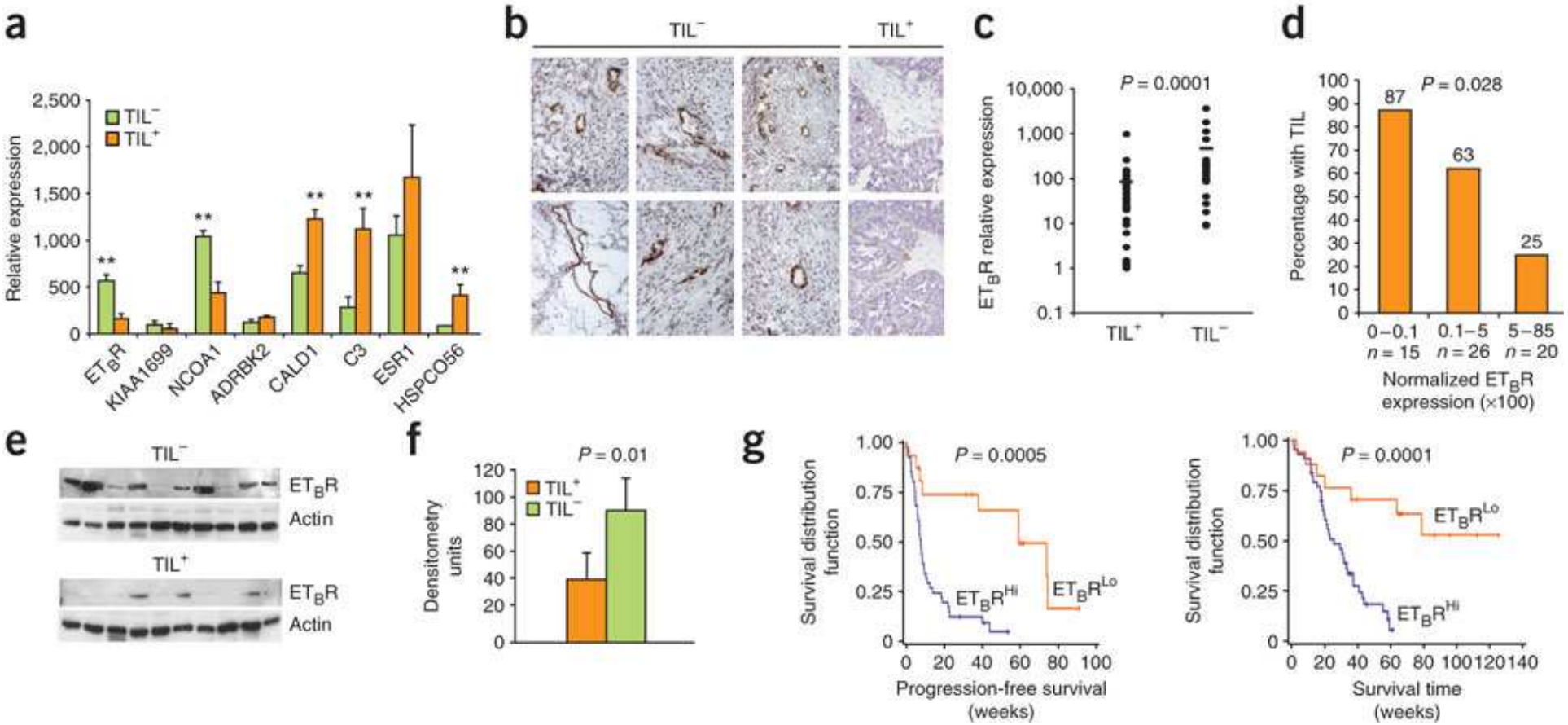
• Implies that in some cases, the melanoma tumor cells themselves can produce a broad panel of key chemokines for T cell migration

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

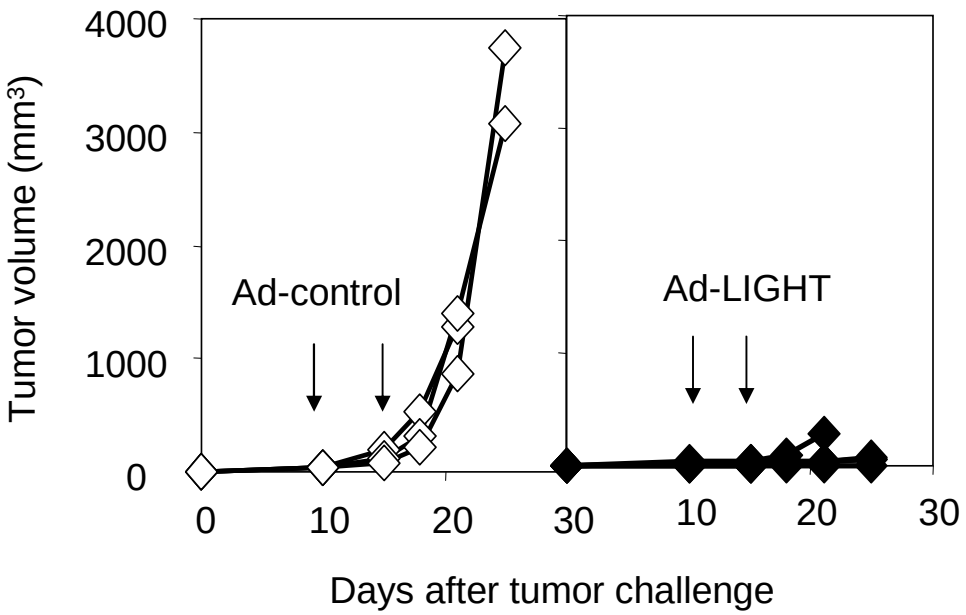
Candidate strategies to promote effector T cell migration into tumor sites

- 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

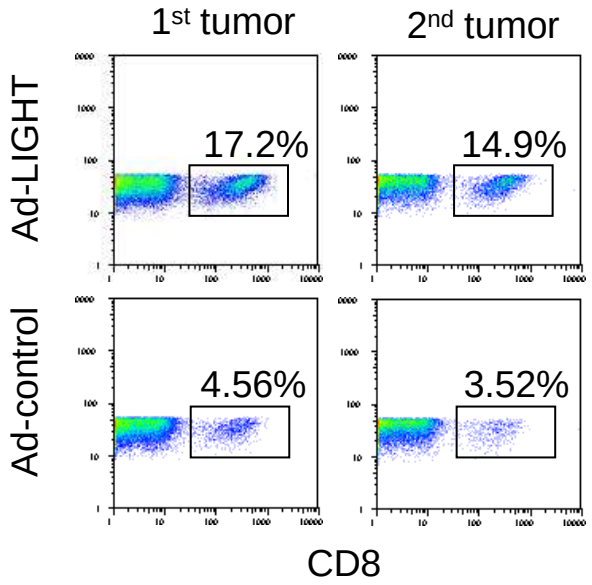
Intratumoral LIGHT adenovirus in B16 melanoma:

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

Tumor rejection



CD8$^+$ T cell infiltrate

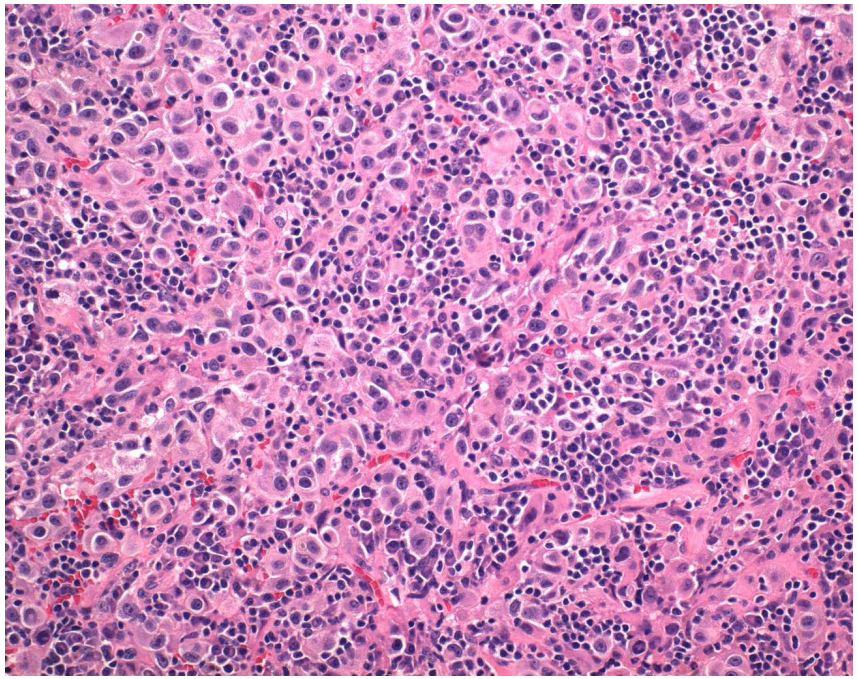


Yu et al, J. Immunol. 2007

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?

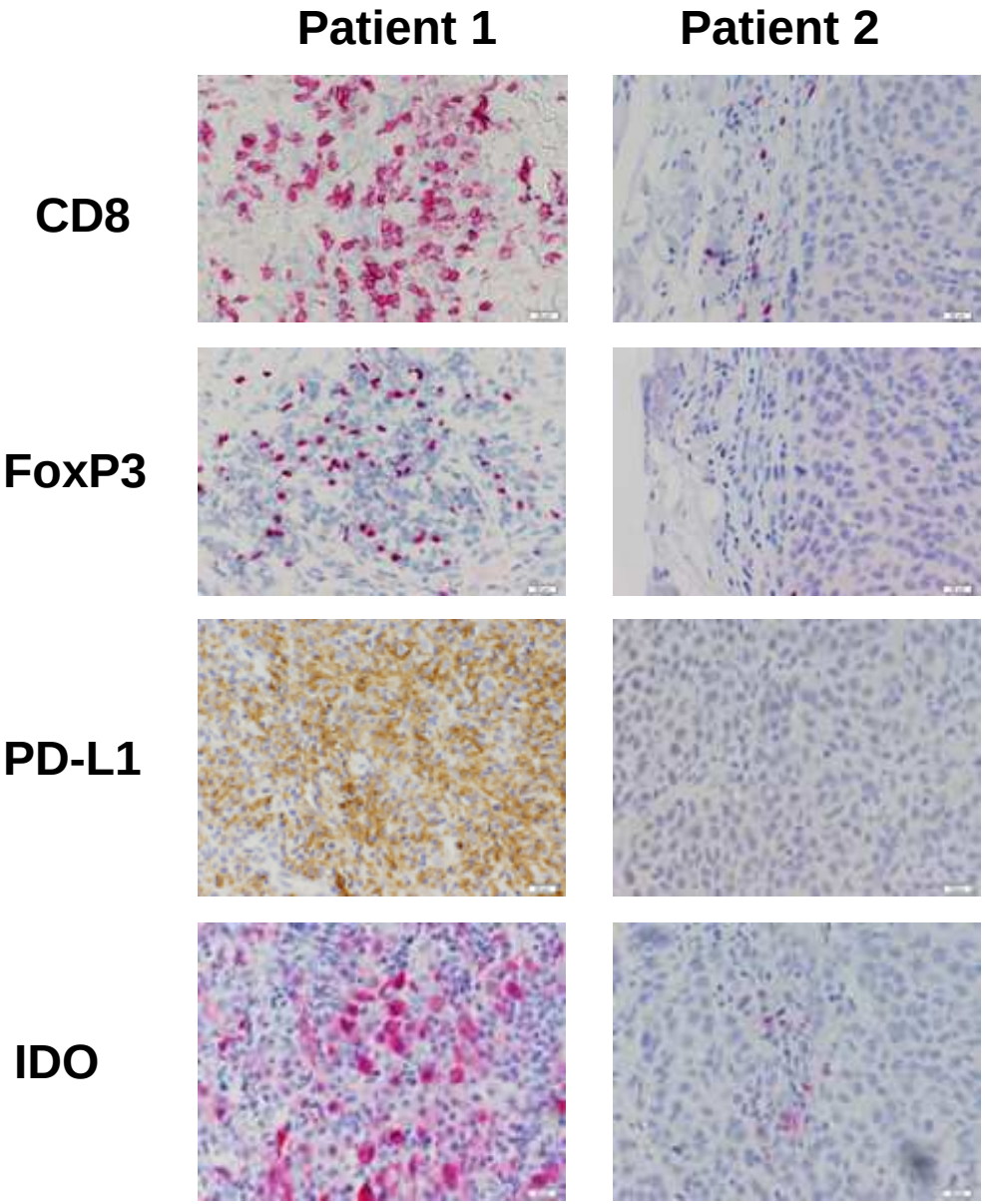
Inflamed melanomas containing $CD8^+$ T cells have highest expression of immune inhibitory pathways

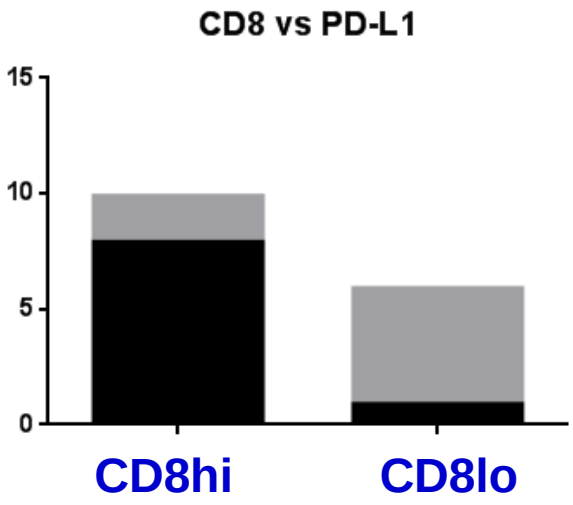


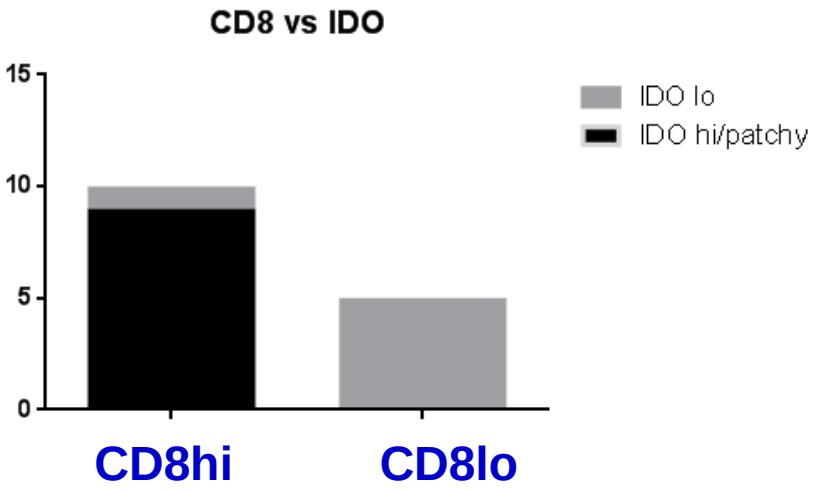
- **IDO** (indoleamine-2,3-dioxygenase)
  - Tryptophan depletion
- **PD-L1**
  - Engages PD-1 on T cells
- $CD4^+CD25^+FoxP3^+$**Tregs**
  - Extrinsic suppression
- T cell **anergy** (B7-poor)
  - T cell intrinsic TCR signaling defect

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



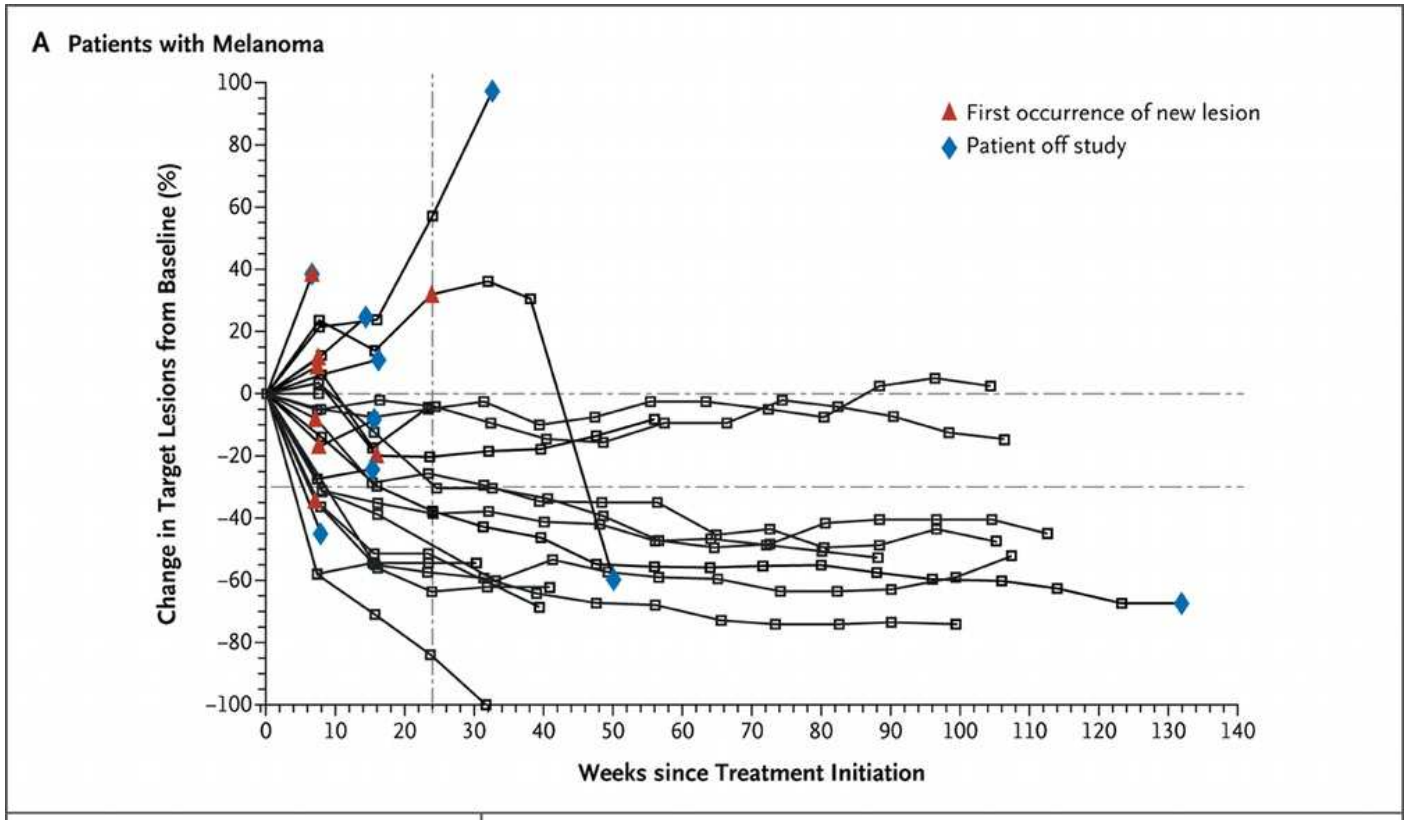




Strategies to block immune inhibitory mechanisms validated in mouse models and being translated to the clinic

- **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

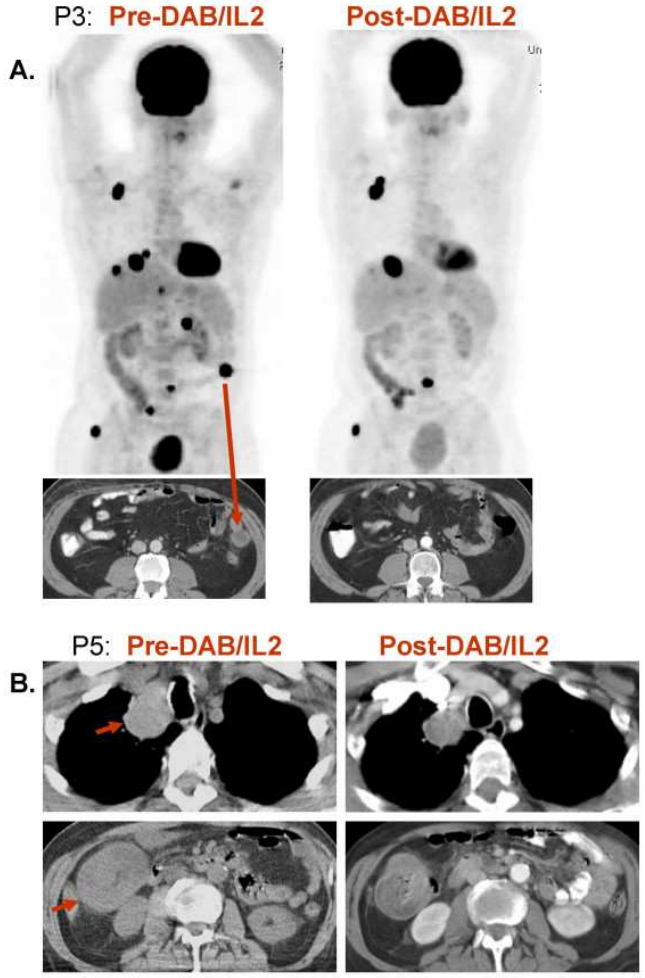
Clinical activity of anti-PD-1 mAb in metastatic melanoma



27% RR among 95 melanoma patients

Topalian et al. NEJM. 2012

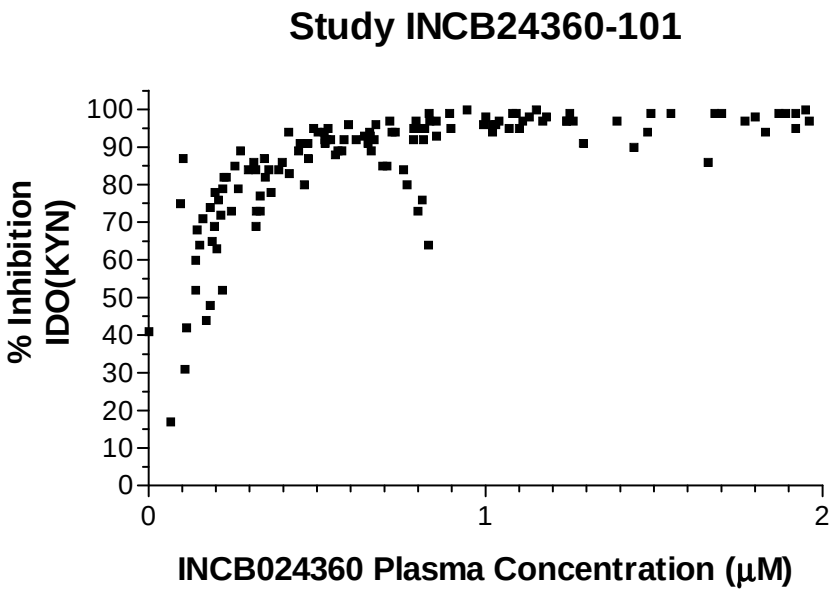
Reduction of Treg number using Denileukin diftitox can have clinical activity in melanoma

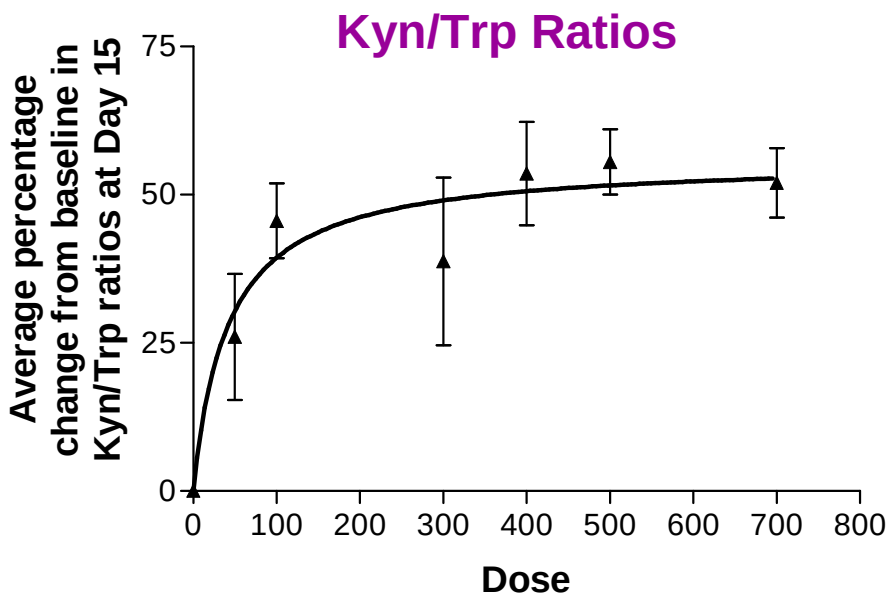


Rasku et al
J. Trans. Med. 2008

Multicenter phase II study currently ongoing

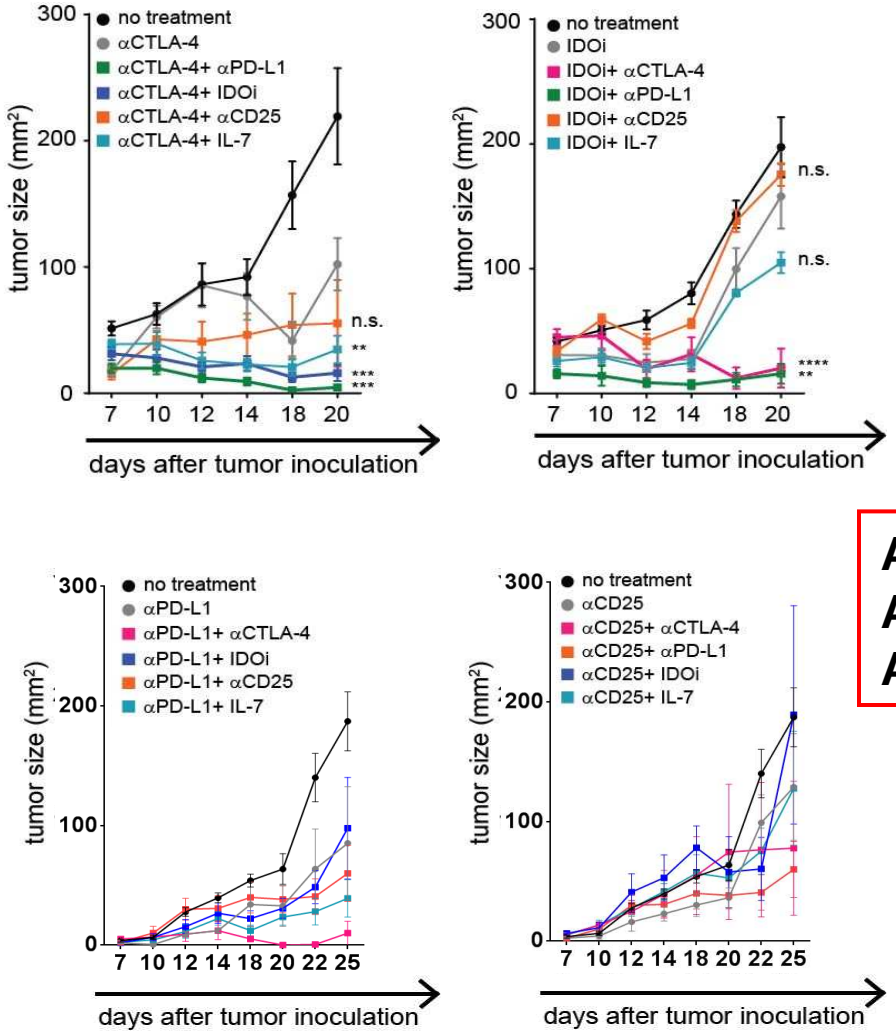
Dose-dependent inhibition of IDO activity as assessed by kynurenine/tryptophan ratios in treated patients





Newton et al. ASCO 2012

Combinatorial blockade of selected inhibitory pathways is therapeutically synergistic in vivo

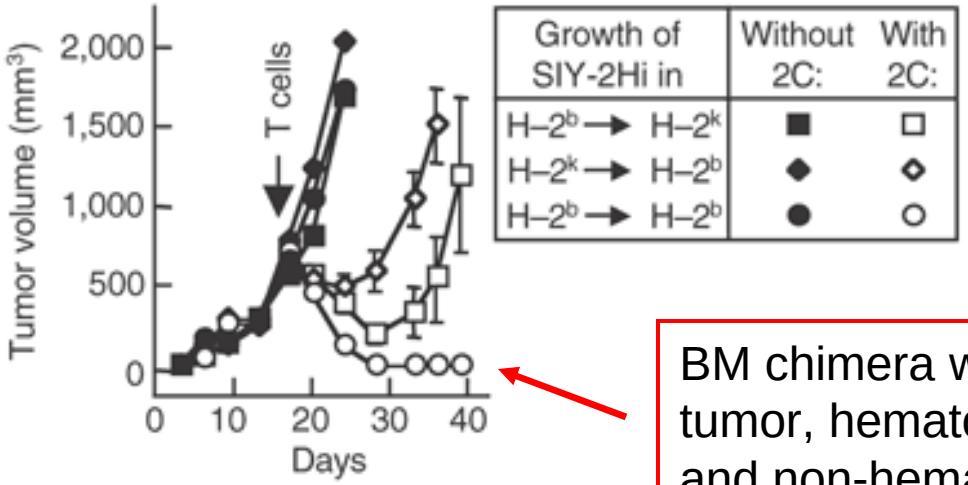


Anti-CTLA-4 + anti-PD-L1
Anti-CTLA-4 + IDOi
Anti-PD-L1 + IDOi

2b. Solid tumor stroma as a barrier

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

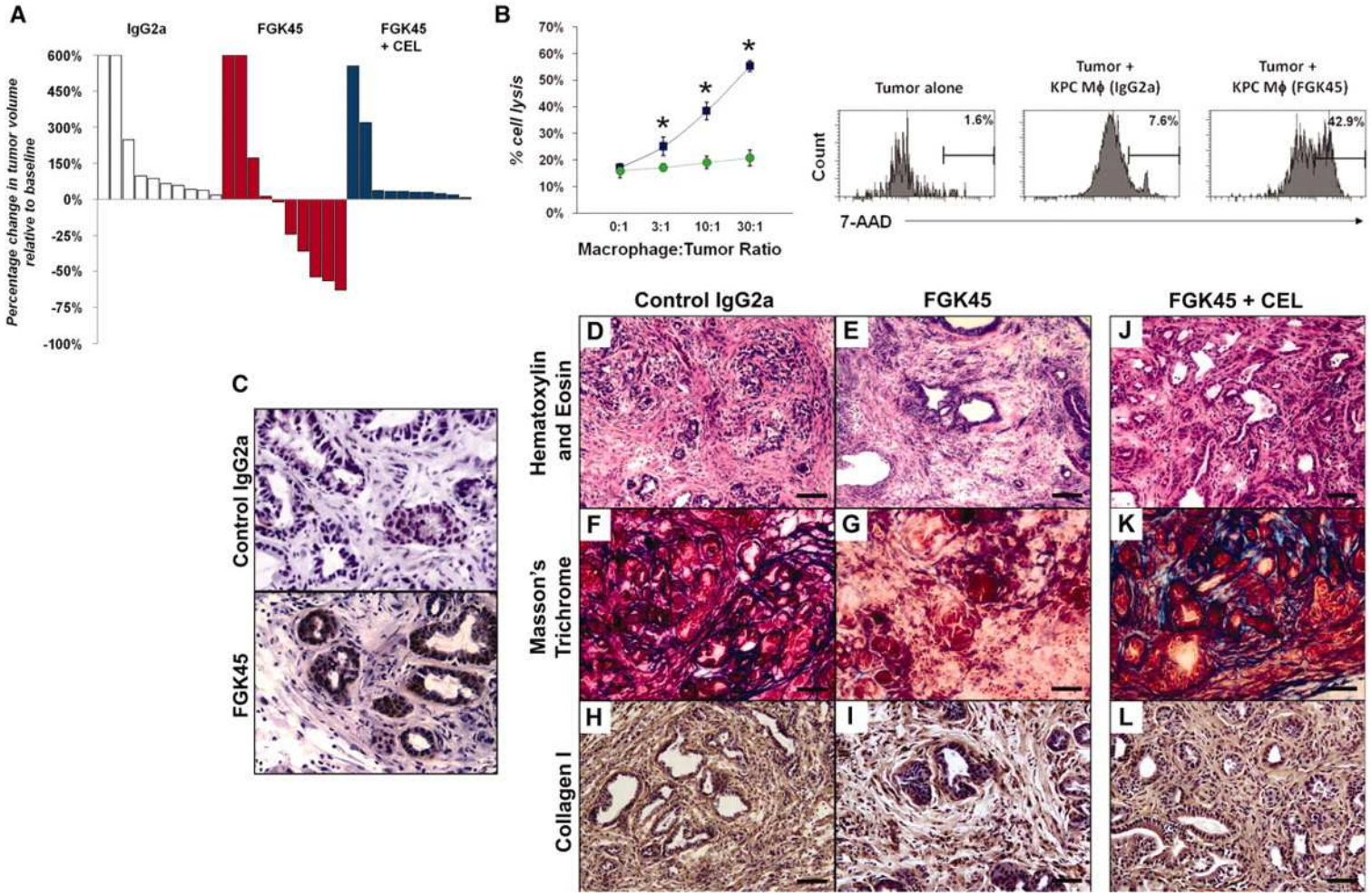
Targeting tumor stroma immunologically may be the key to durable complete responses



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

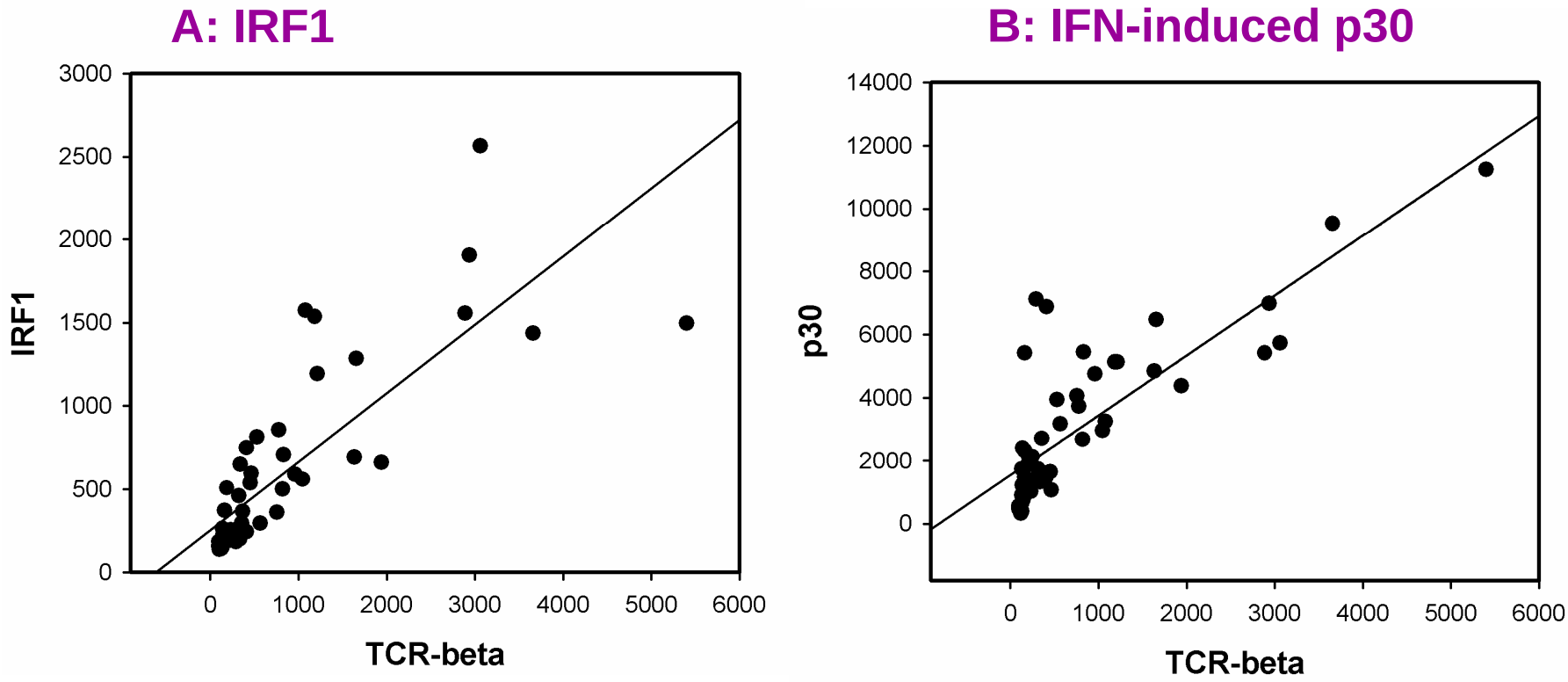


Beatty, Vonderheide et al. Science 2011

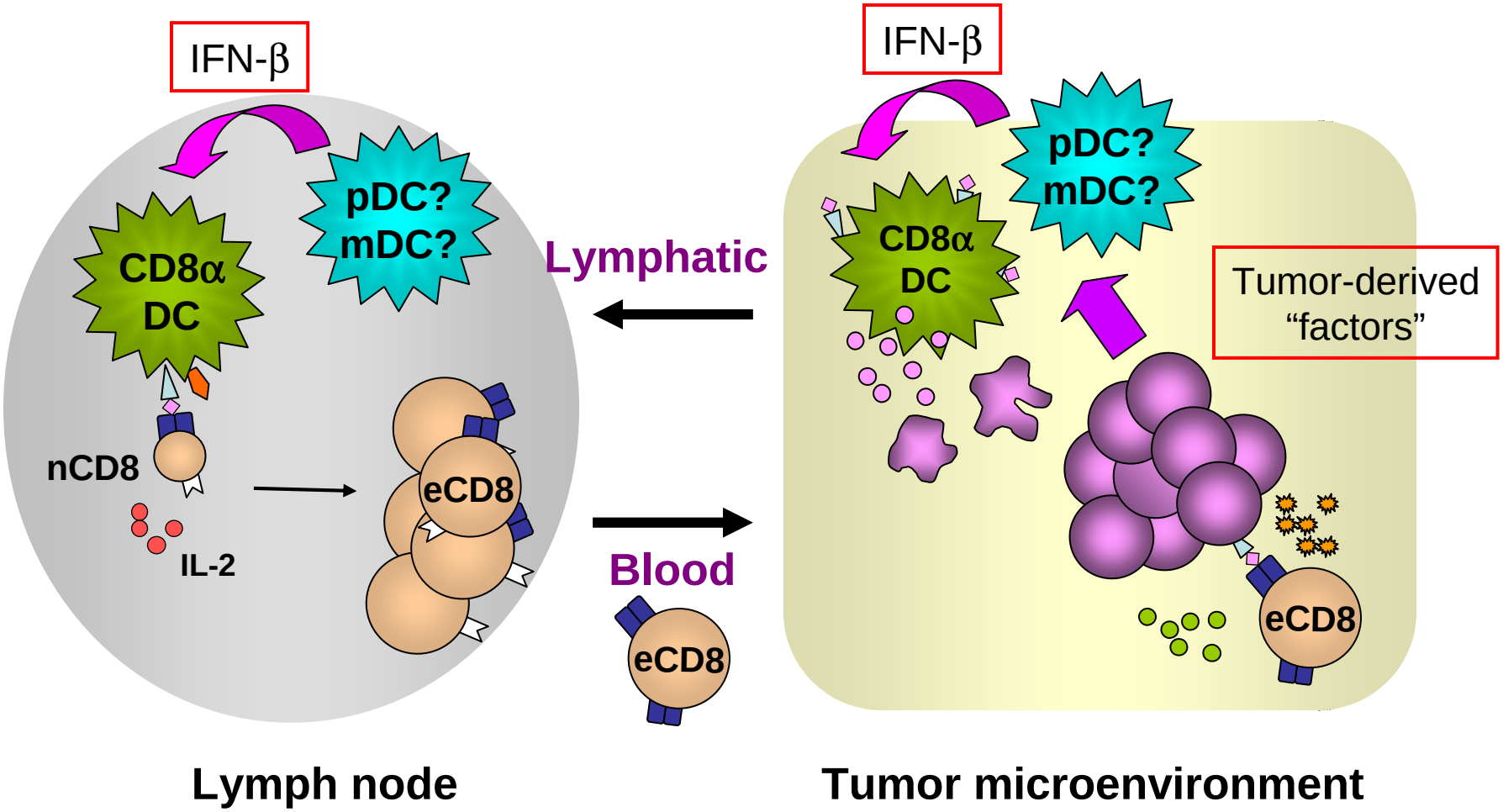
3. Innate immune sensing of tumor—type I IFNs

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?

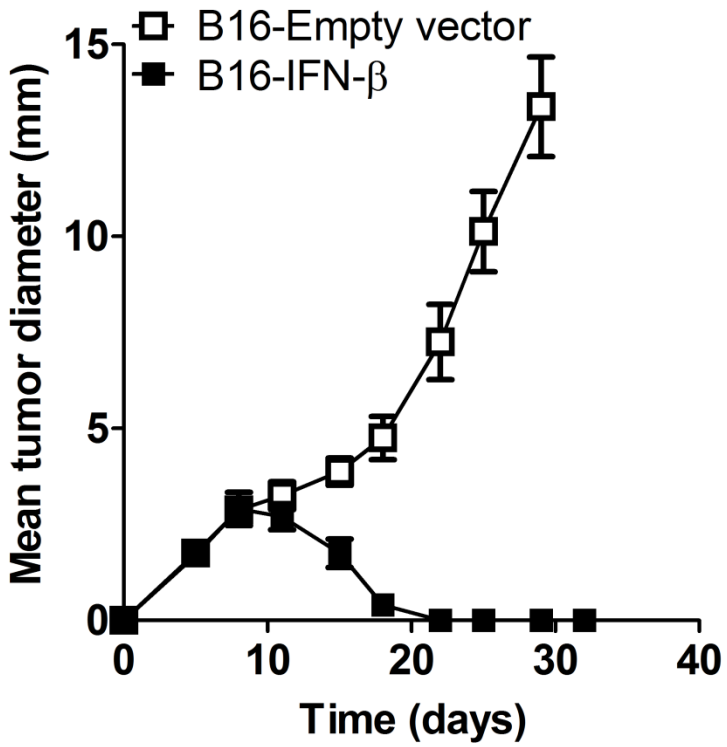
Melanoma metastases that contain T cell transcripts also contain transcripts known to be induced by type I IFNs



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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Type I IFNs
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Robbert Spaapen
Aalok Kacha
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