

Immunotherapeutic barriers at the level of the tumor microenvironment

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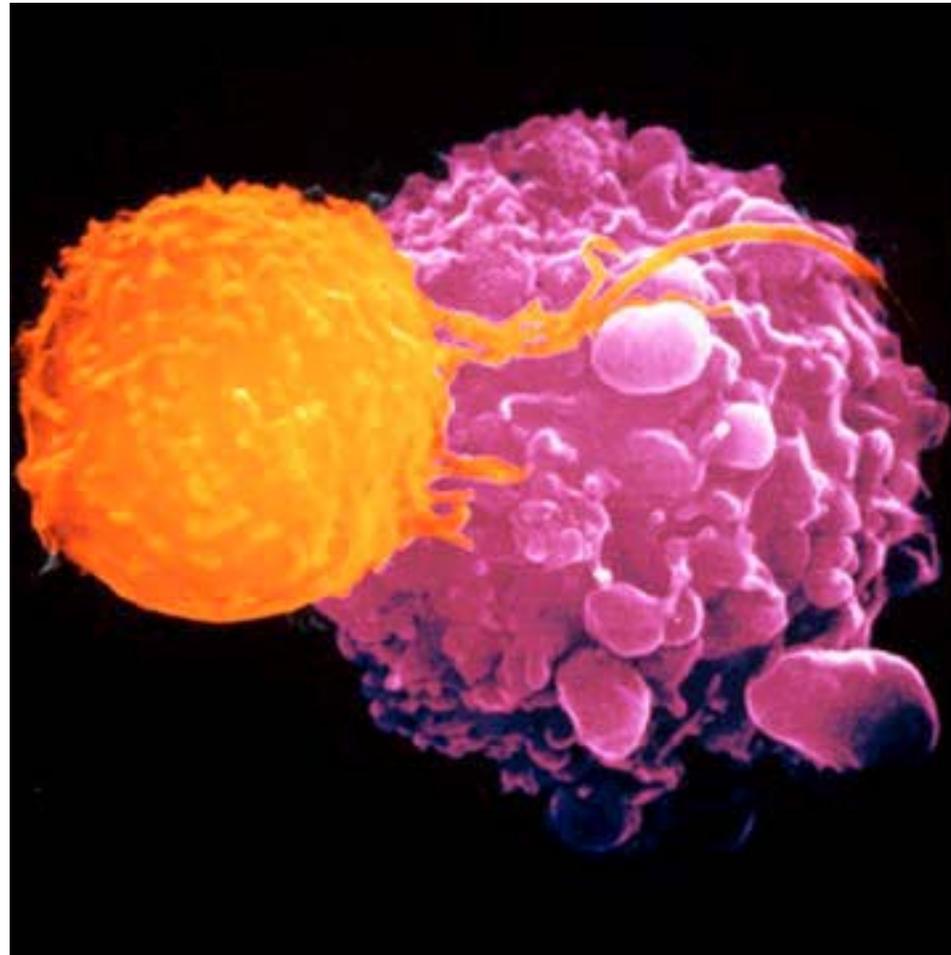


Disclosure Information

Thomas F. Gajewski, M.D., Ph.D.

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 - Eisai
 - Roche/Genentech
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 - Incyte
 - Roche/Genentech

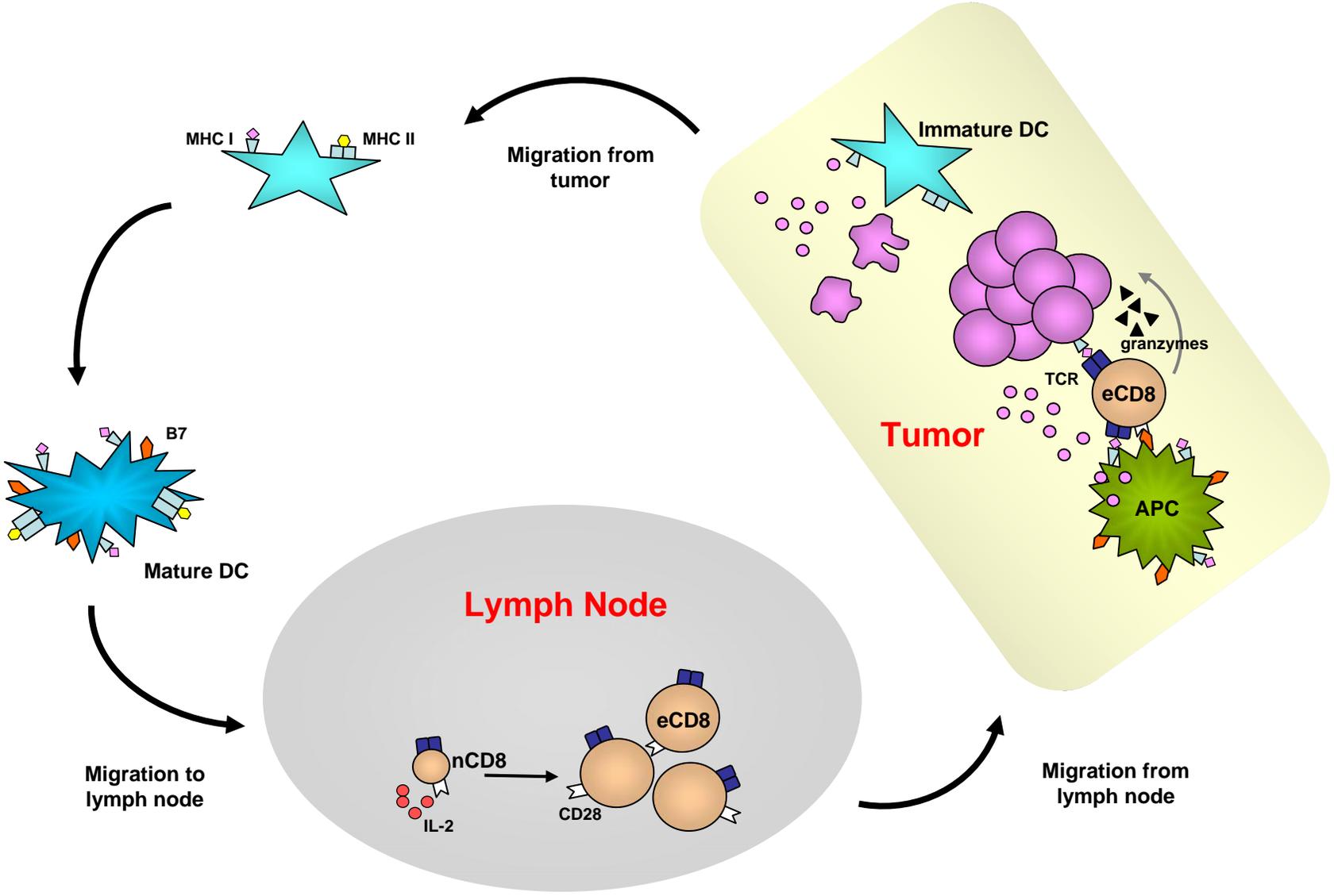
CD8⁺ cytotoxic T lymphocyte killing an antigen-expressing tumor cell



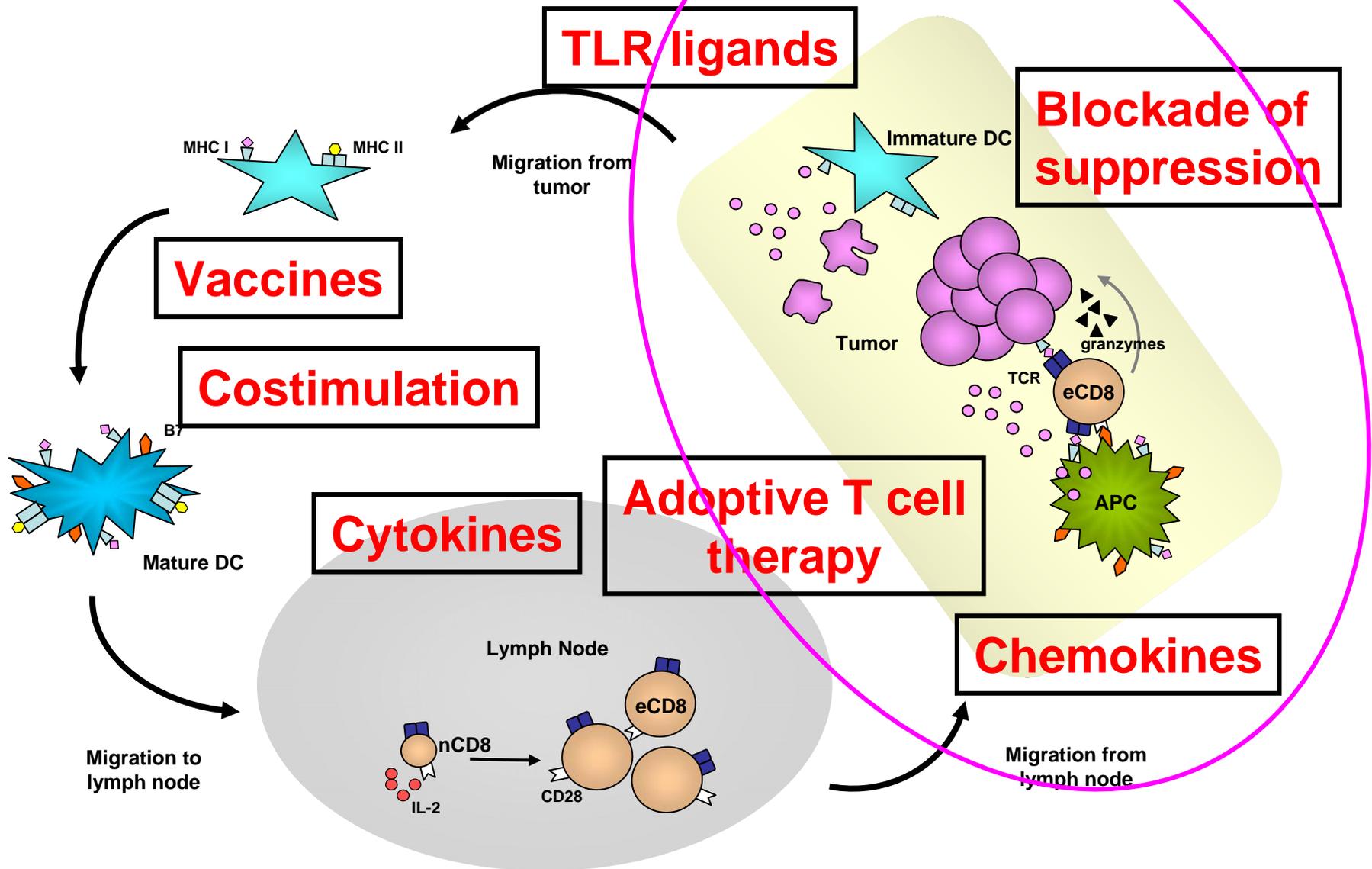
In vivo, a tumor is more than tumor cells

- 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

Model for spontaneous CD8⁺ T cell-mediated anti-tumor immune response *in vivo*



Model for CD8⁺ T cell-mediated anti-tumor immune response *in vivo*: Interventions



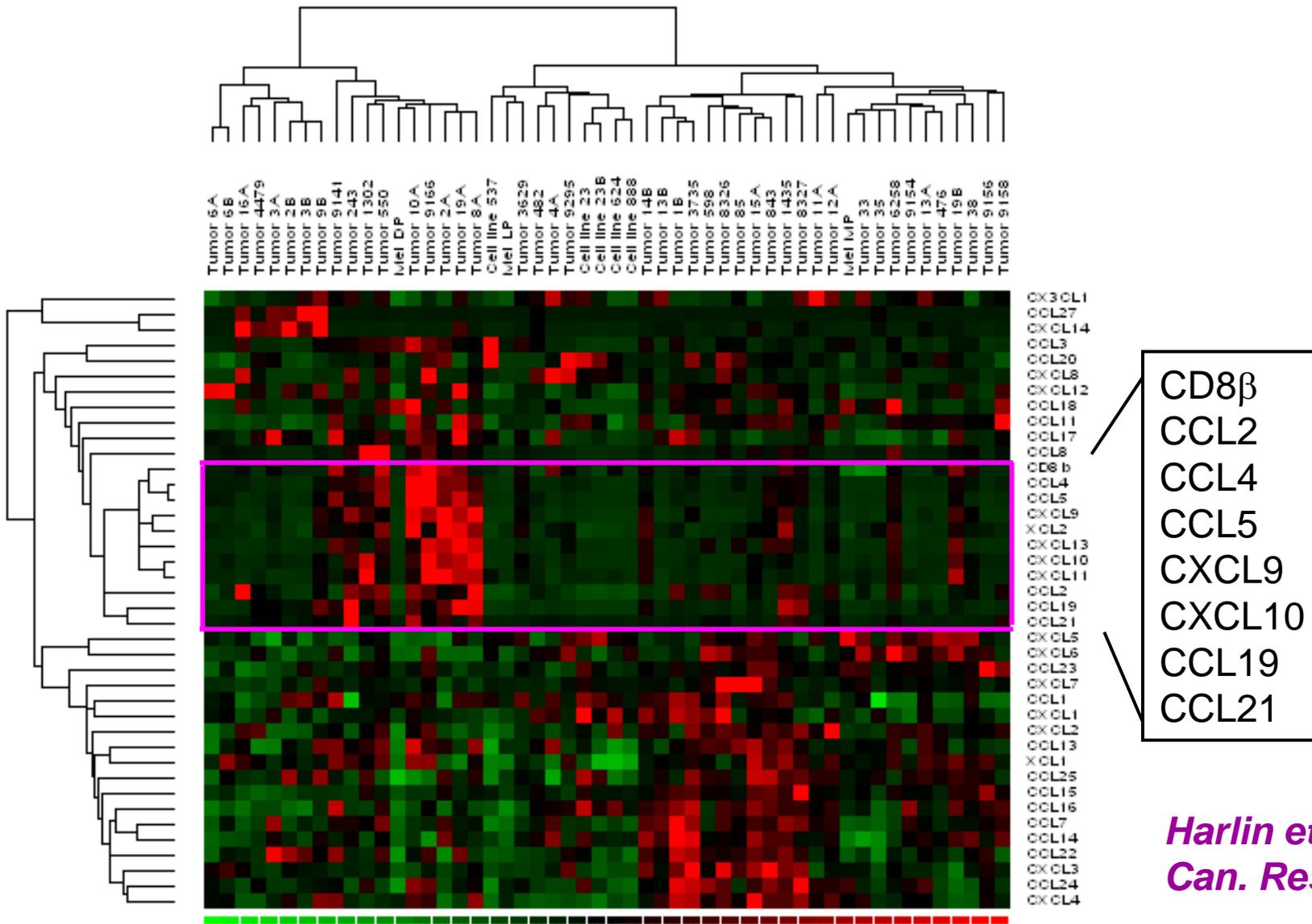
Features of subsets of solid tumors that might mediate poor immune recognition or lack of immune destruction

- 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
 - 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)

Hypothesis

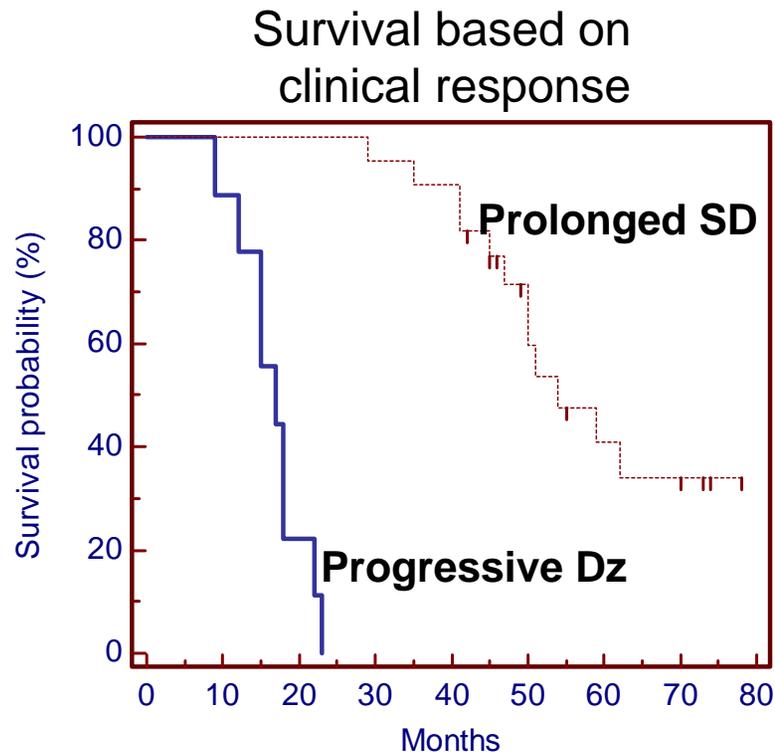
- Features of the tumor microenvironment could dominate at the effector phase of the anti-tumor T cell response and limit efficacy of current immunotherapies
 - T cell trafficking into tumor
 - Immune suppressive mechanisms at tumor site
 - Tumor cell biology and susceptibility to immune-mediated killing
 - Complexities of the tumor stroma (vasculature, fibrosis)
- Reasoned that these features could be interrogated through pre-treatment gene expression profiling of tumor site in each individual patient
- Such an analysis could identify a predictive biomarker profile associated with clinical response, and also highlight new biologic barriers that need to be overcome to optimize therapeutic efficacy of vaccines and other immunotherapies

Expression of a subset of chemokine genes is associated with presence of CD8 transcripts



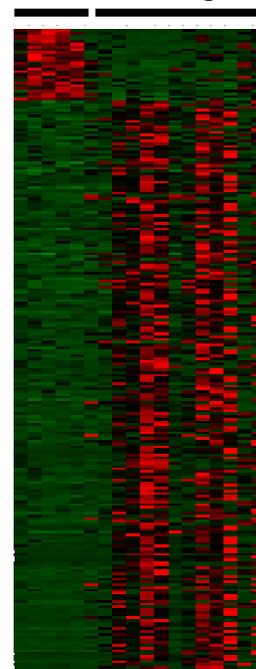
*Harlin et al.
Can. Res. 2009*

Gene expression pattern of tumor microenvironment associated with favorable clinical outcome to a dendritic cell vaccine



No correlation with immune response in blood

Survival groups

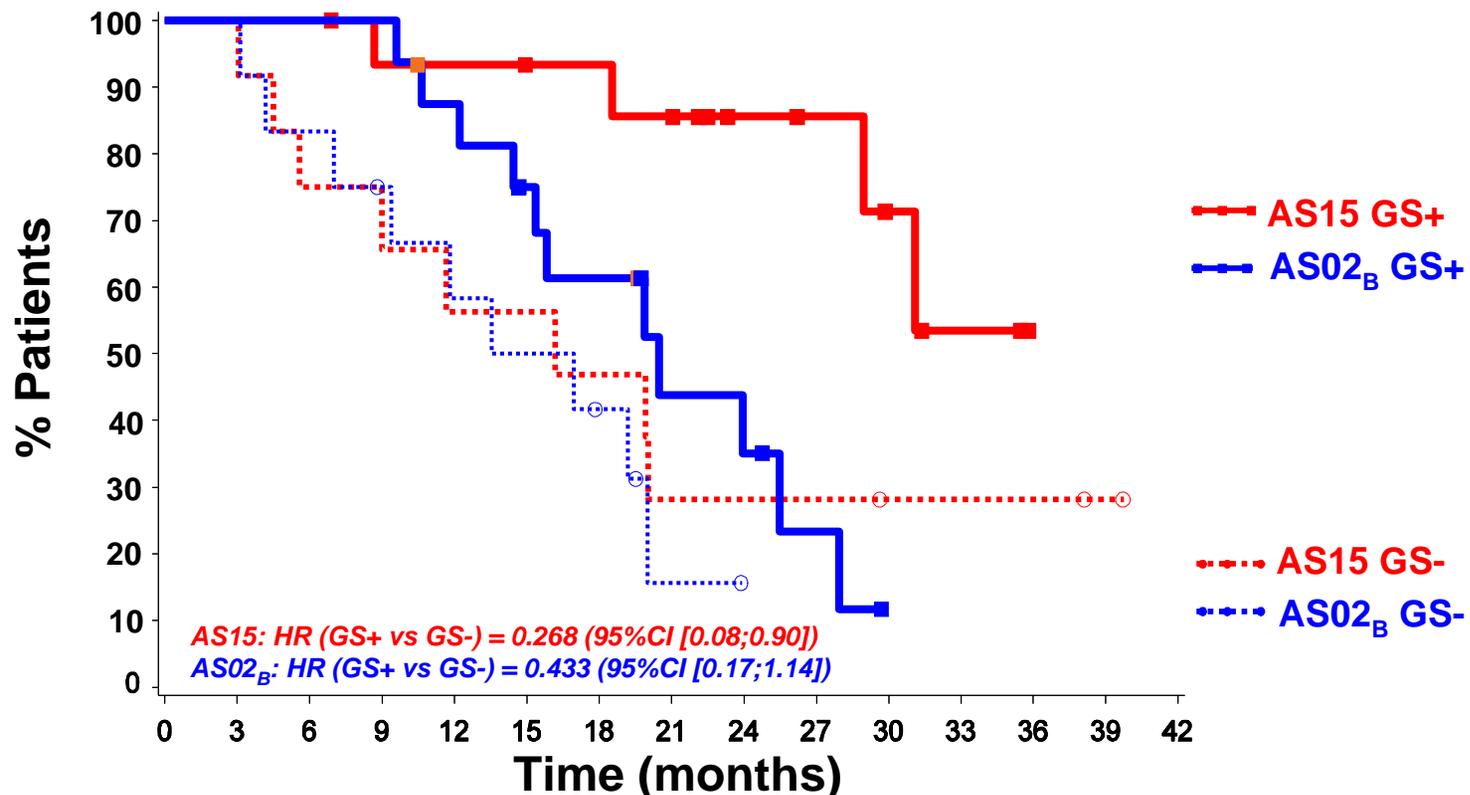


Thy1
CD28
CCL19
LTbeta
IL-27Ralpha
IL-1R

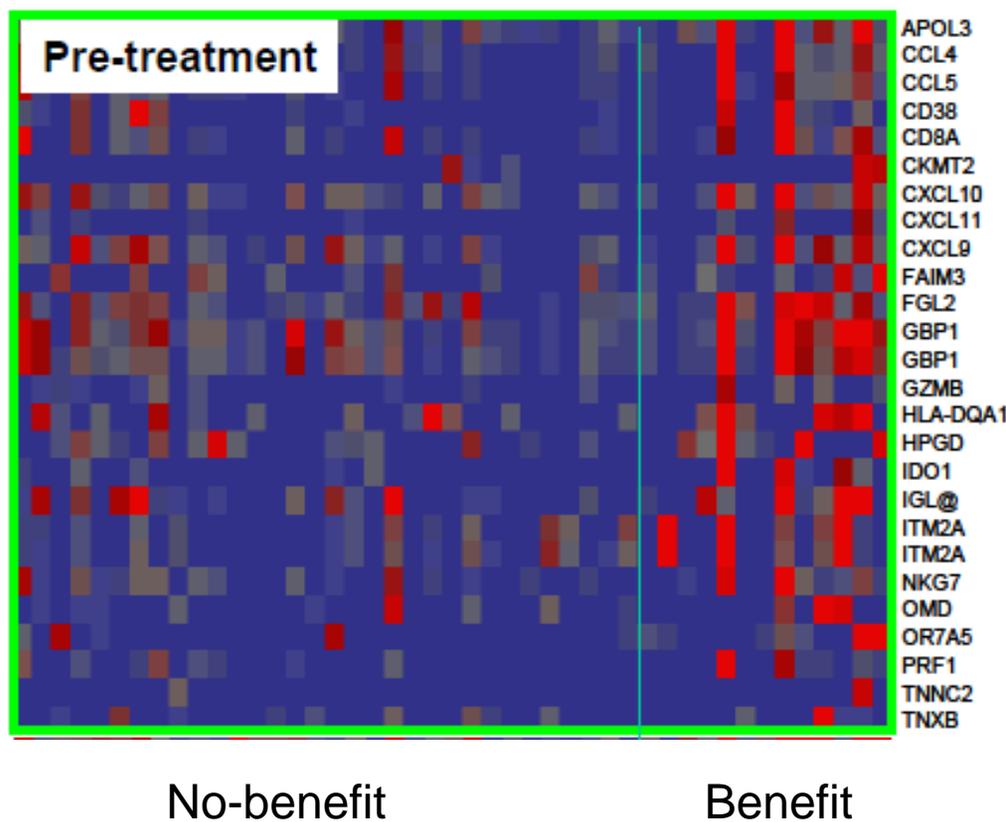
T cell markers
and chemokines

Schuler collaboration, ASCO 2009

“Inflamed” gene expression signature is associated with survival following GSK MAGE3 protein vaccine



Ipilimumab clinical responders also appear to show an “inflamed” tumor gene expression profile



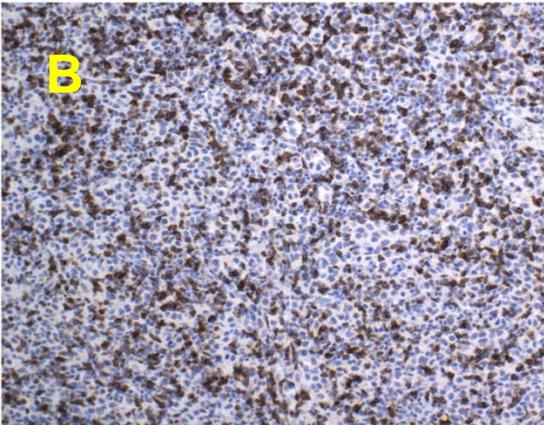
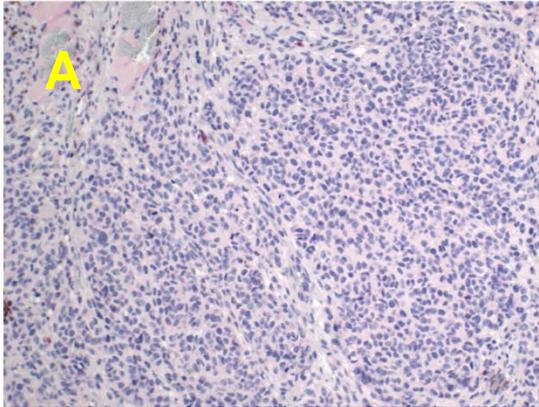
- CXCL9, 10, 11
- CCL4, CCL5
- Granzyme B
- Perforin
- CD8 α

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 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

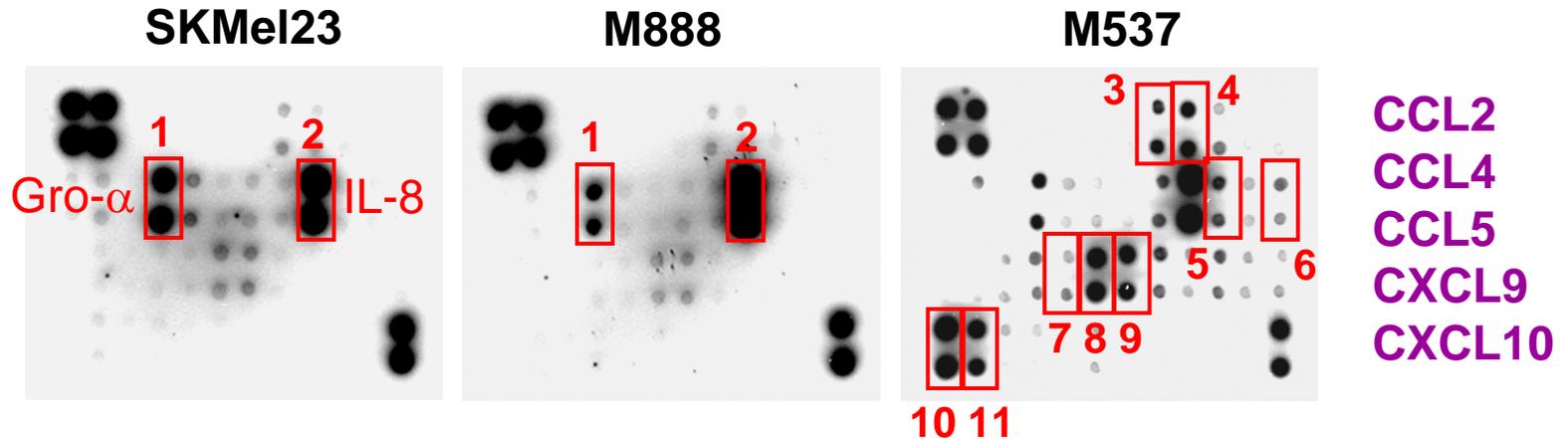


- **T cell “poor”**
 - Lack chemokines for recruitment
 - Low indicators of inflammation
- **T cell “rich”**
 - Chemokines for T cell recruitment
 - CD8⁺ T cells in tumor microenvironment
 - Broad inflammatory signature
 - Apparently predictive of clinical benefit to vaccines

1. Chemokines and T cell migration

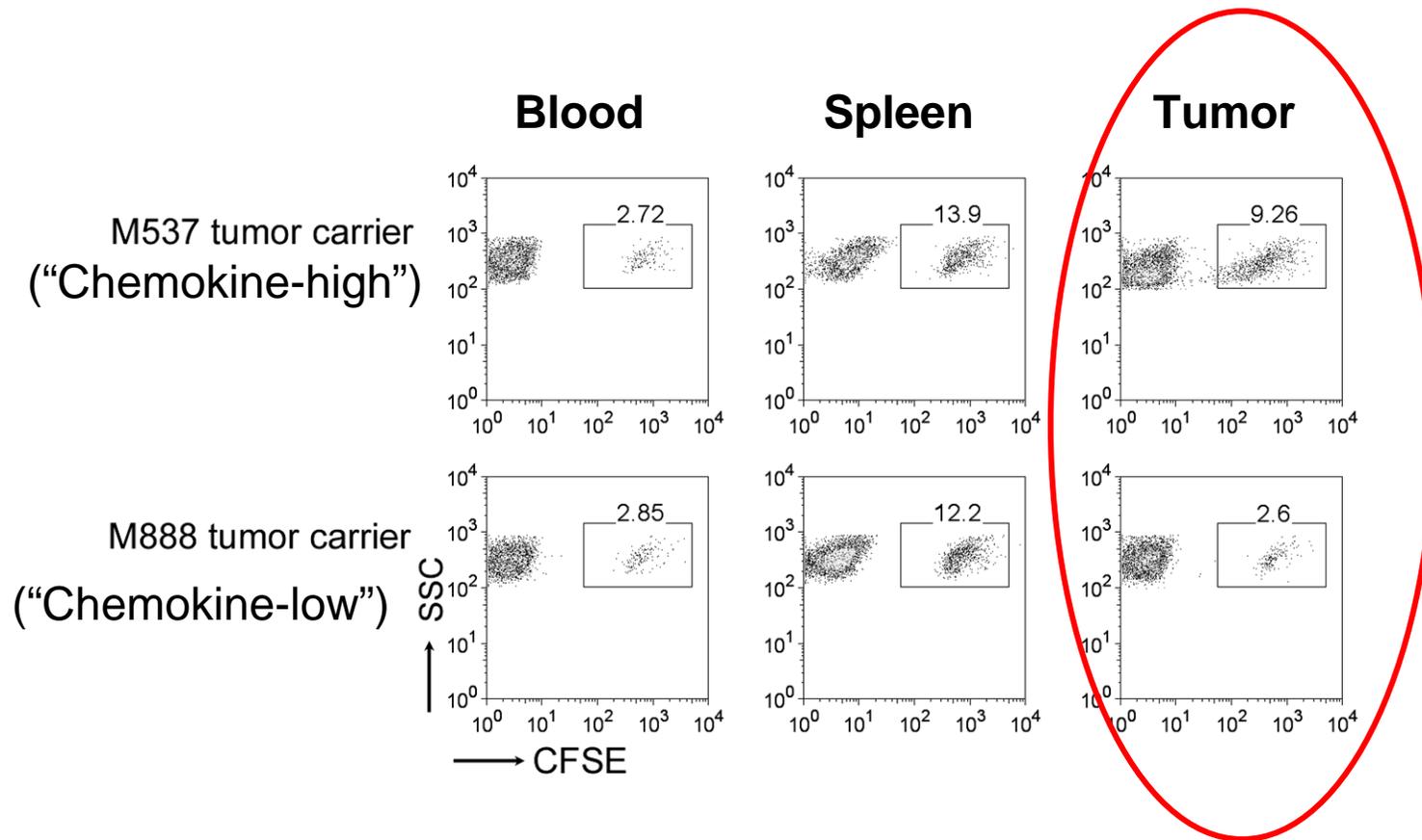
What is attracting T cells into some tumors? Can we mimic this in the tumors that fail to achieve it spontaneously?

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



- Implies that in some cases, the melanoma tumor cells themselves can produce the entire panel of key chemokines

Superior recruitment of human CD8⁺ effector T cells in NOD/scid mice bearing “chemokine-high” M537 melanomas

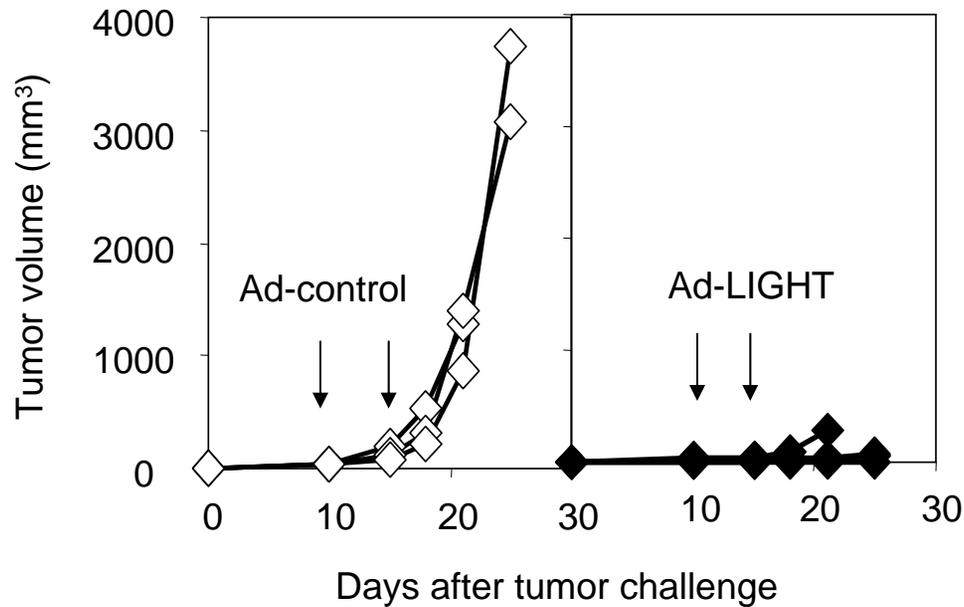


Candidate strategies to promote effector T cell migration into tumor sites

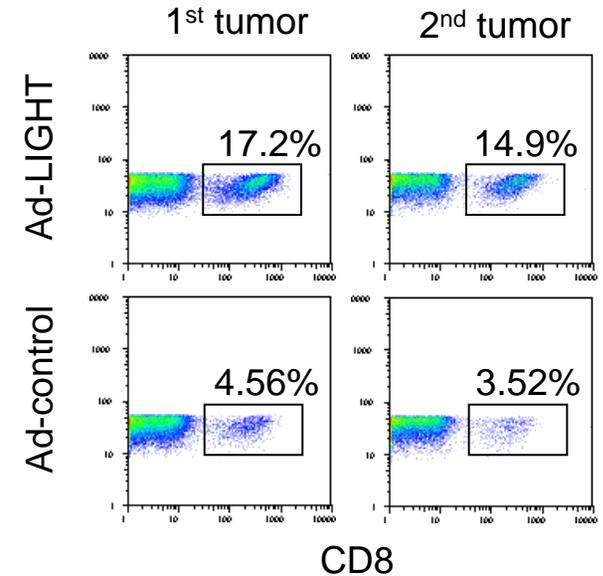
- Introduce chemokines directly
 - CXCR3-binding chemokines (CXCL9, CXCL10)
 - Others (CCL2, CCL3, CCL4, CCL5)
- Induce chemokine production from stromal cells
 - LIGHT, lymphotoxin: bind $LT\beta R$
- Elicit appropriate local inflammation that includes chemokine production
 - Type I IFNs
 - TLR agonists
 - 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

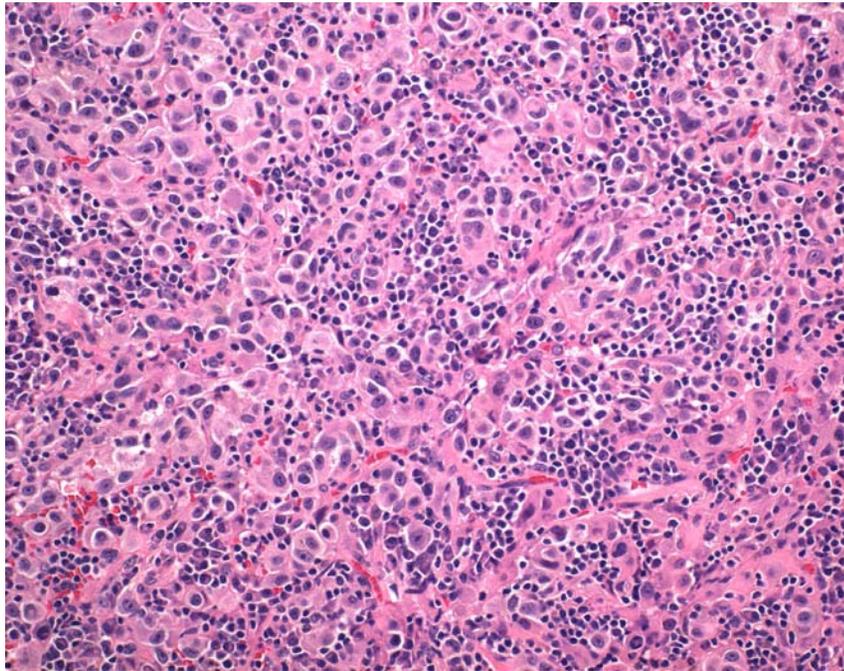


Yu et al, J. Immunol. 2007

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?

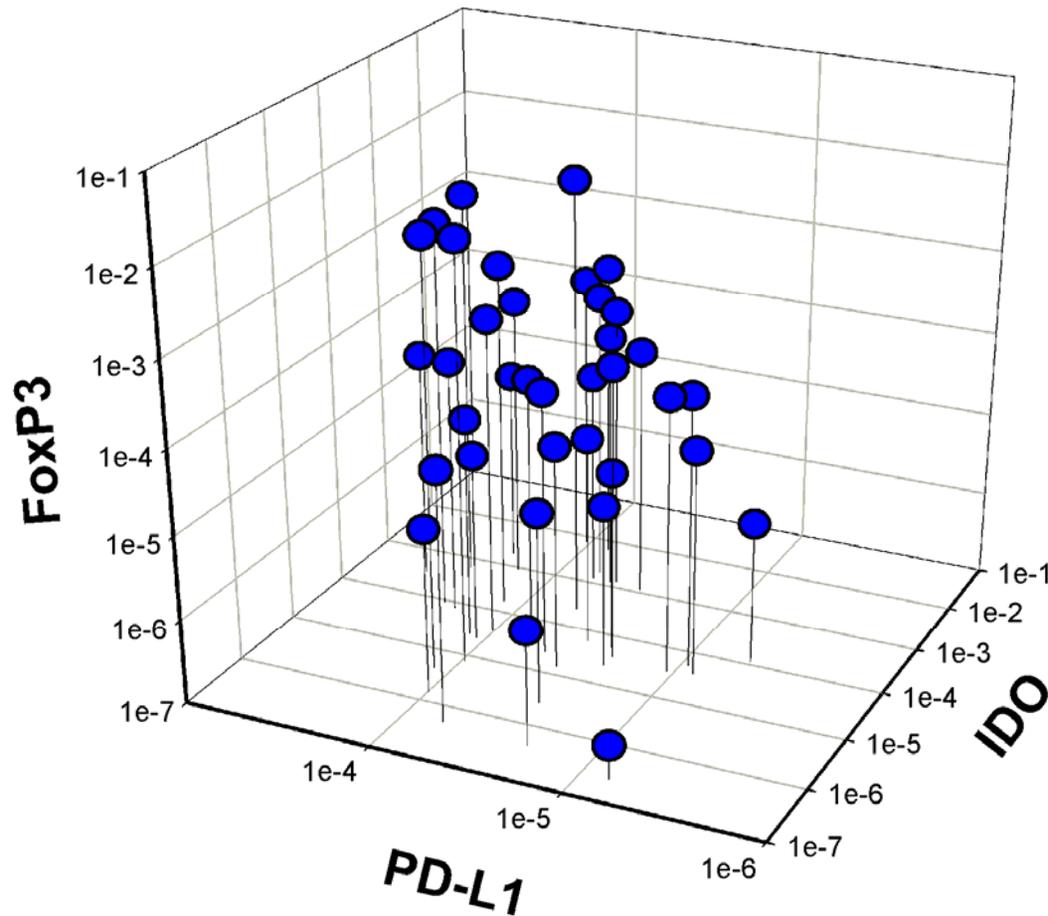
Inflamed melanomas containing CD8⁺ T cells have highest expression of immune inhibitory pathways



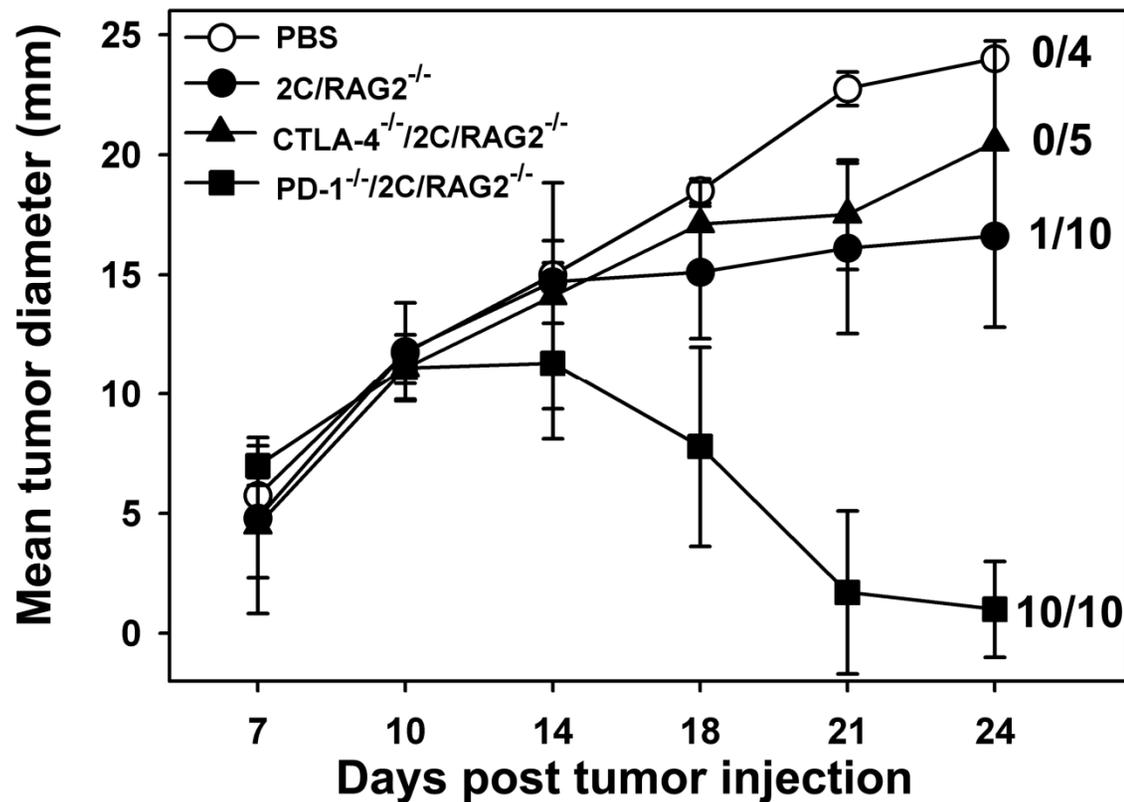
- **IDO** (indoleamine-2,3-dioxygenase)
 - Tryptophan depletion
- **PD-L1**
 - Engages PD-1 on T cells
- **CD4⁺CD25⁺FoxP3⁺Tregs**
 - Extrinsic suppression
- T cell **anergy** (B7-poor)
 - T cell intrinsic TCR signaling defect

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

Correlated expression of IDO, FoxP3, and PD-L1 transcripts in individual tumors

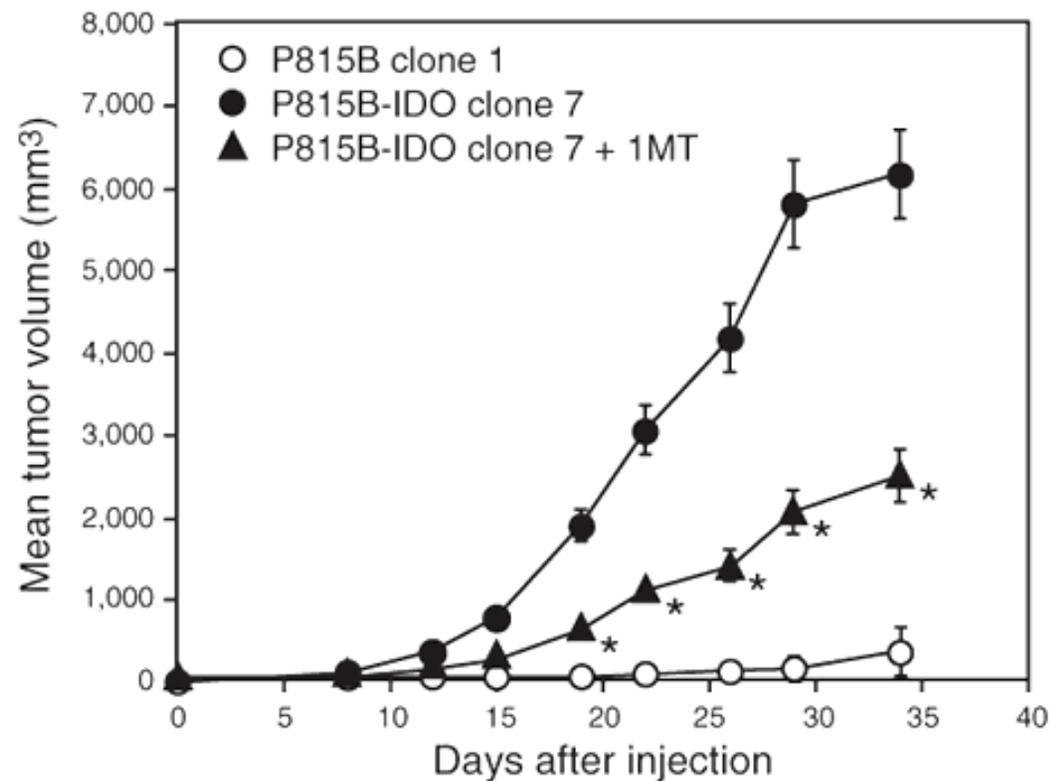


Interfering with PD-L1/PD-1 interactions can lead to tumor rejection in vivo



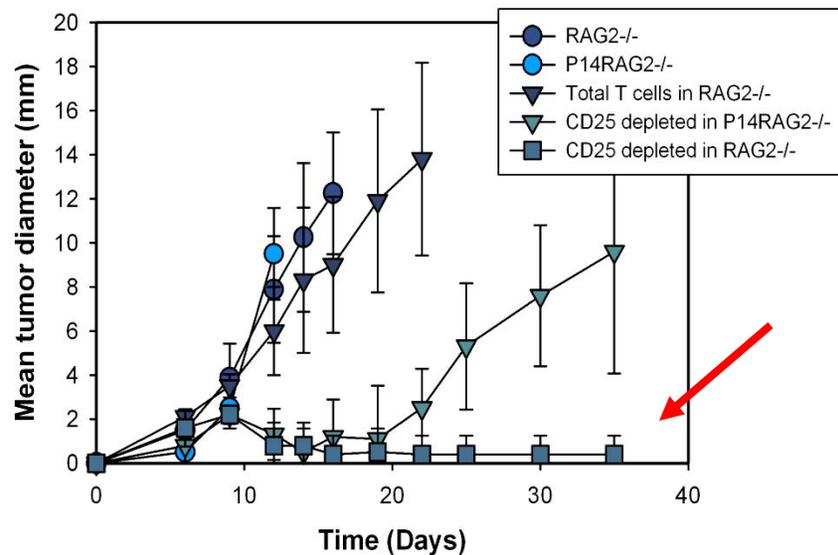
Blank et al, Cancer Research, 2004

1-methyltryptophan reverses immunosuppression by IDO and enables tumor control in vivo



Uyttenhove et al Nature Med. 9:1269, 2003

Uncoupling multiple immune suppressive mechanisms in combination: Treg depletion and anergy reversal synergize to promote rejection of B16 melanoma and vitiligo

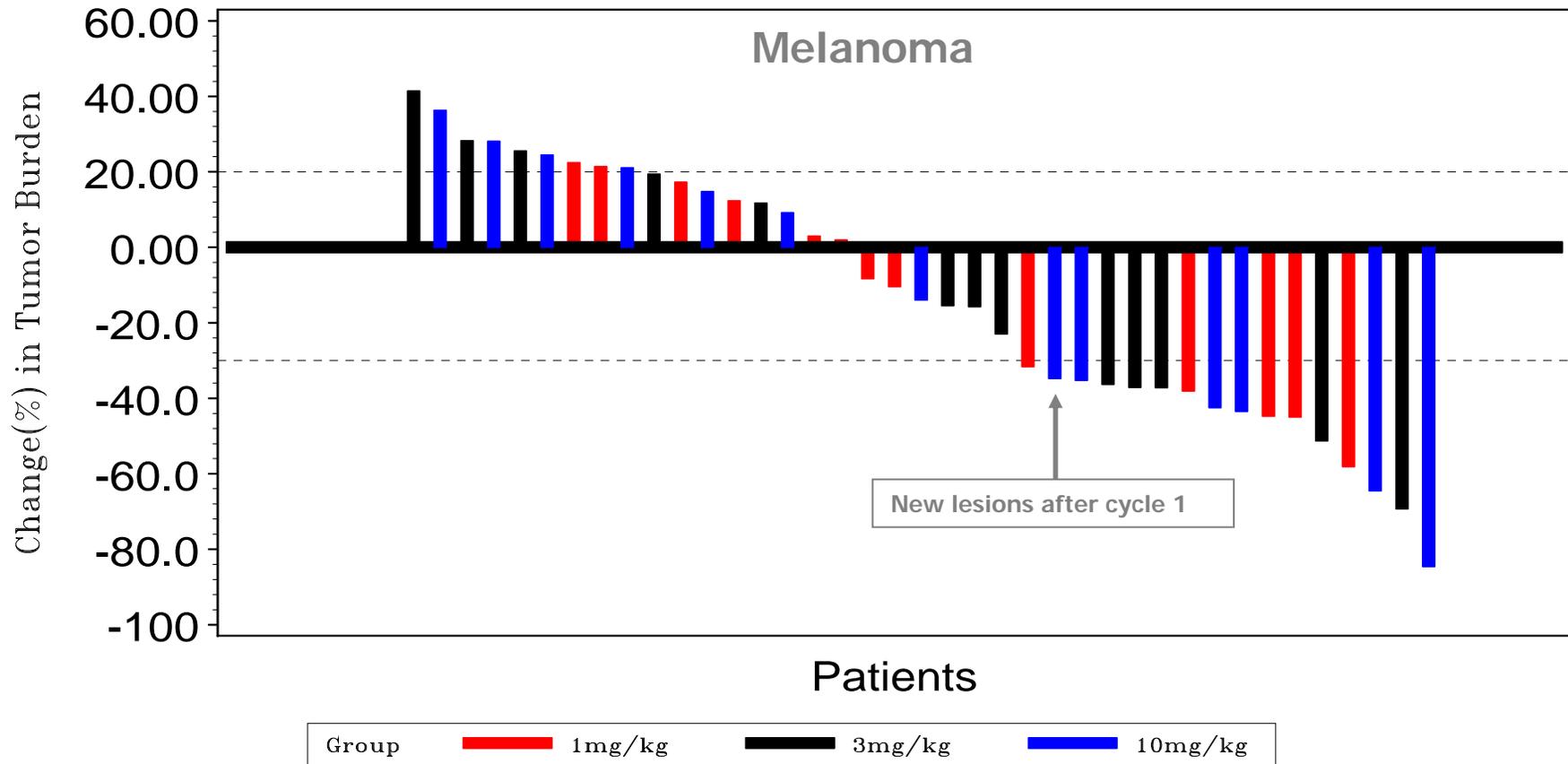


Kline et al., Clin. Can. Res. 2008

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

- **Blockade of PD-L1/PD-1 interactions**
 - Anti-PD-1 and anti-PD-L1 mAbs (Medarex/BMS; Curetech)
- **Depletion of CD4⁺CD25⁺FoxP3⁺ Tregs**
 - Denileukin diftitox (IL-2/DT fusion)
 - Daclizumab or Basiliximab (anti-IL-2R mAb)
 - Ex vivo bead depletion of CD25⁺ cells from T cell product for adoptive transfer
- **IDO inhibition**
 - 1-methyltryptophan (RAID program)
 - New more potent IDO inhibitors (Incyte)
- **Anergy reversal**
 - Introduction of B7-1 into tumor sites
 - Homeostatic cytokine-driven proliferation
 - T cell adoptive transfer into lymphopenic recipient
 - Exogenous IL-7 / IL-15
 - Decipher molecular mechanism and develop small molecule inhibitors to restore T cell function
- **Combinations of negative regulatory pathway blockade**
 - Synergy between blockade of 2 or more pathways

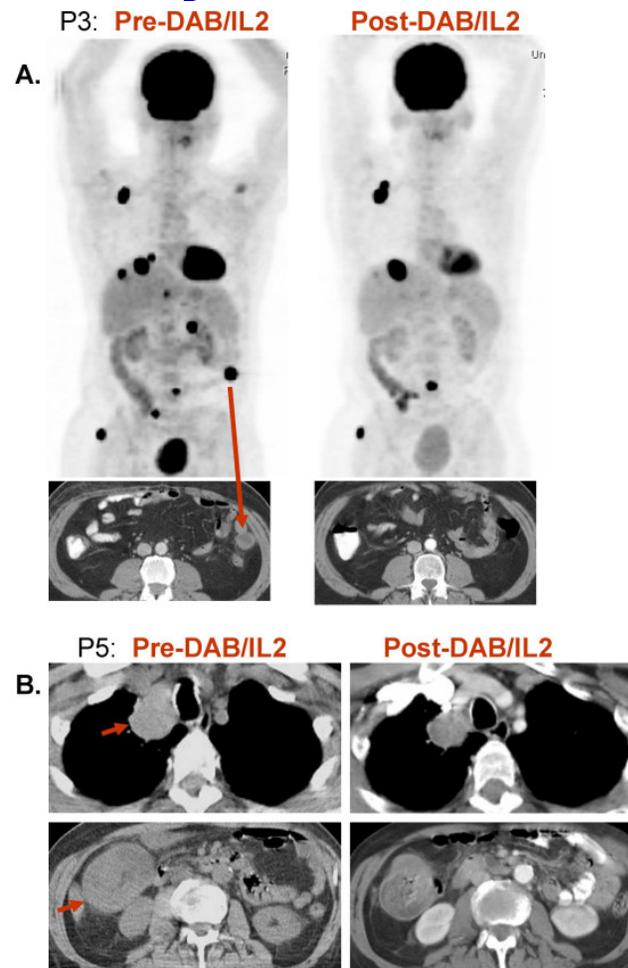
Anti-PD-1 mAb phase I (MDX-1106; BMS 936558): Tumor response



Sznol et al, ASCO 2010

Responses also seen in NSCLC and renal cell carcinoma

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



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

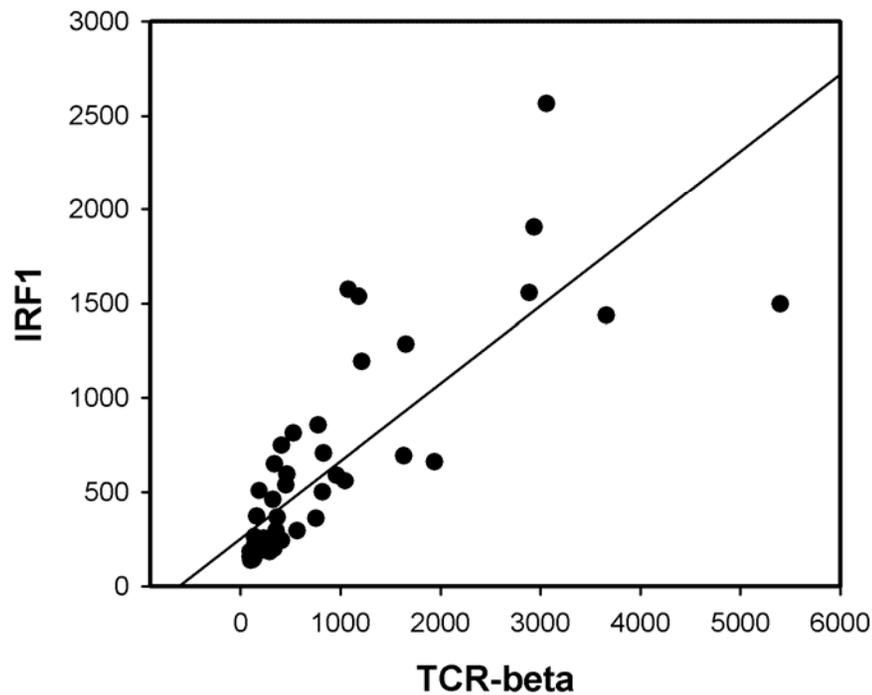
Multicenter phase II study currently ongoing

3. Innate immune signals— type I IFNs

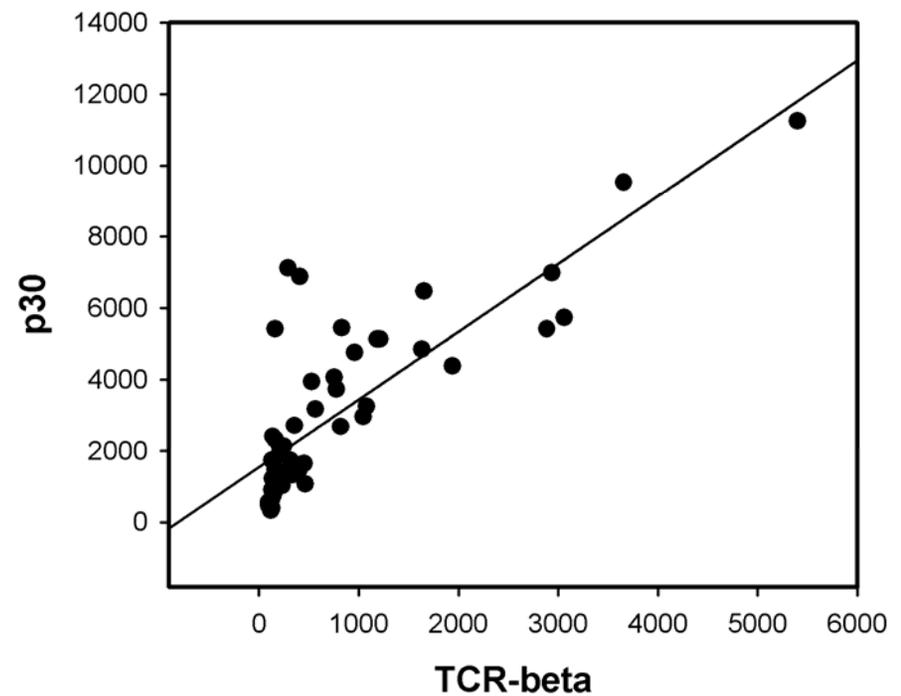
How are anti-tumor T cells sometimes becoming spontaneously primed? Can we improve endogenous T cell priming in the tumors that fail to do so alone?

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

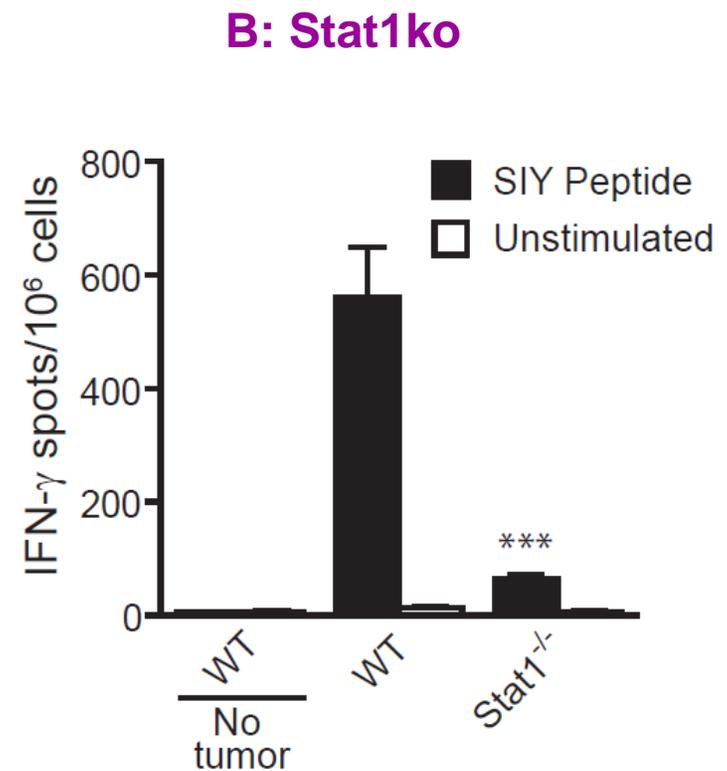
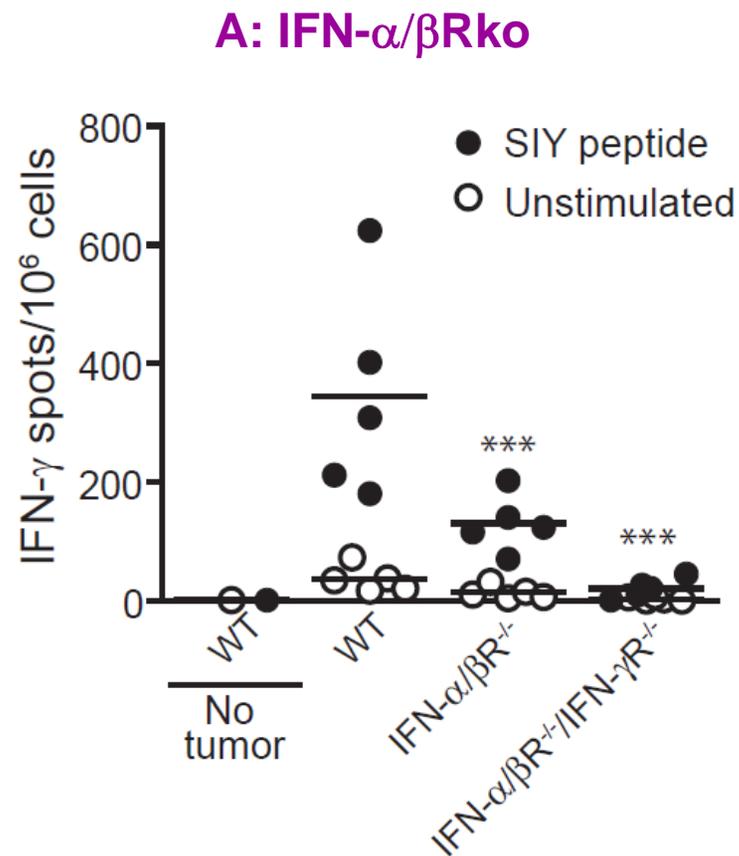
A: IRF1



B: IFN-induced p30

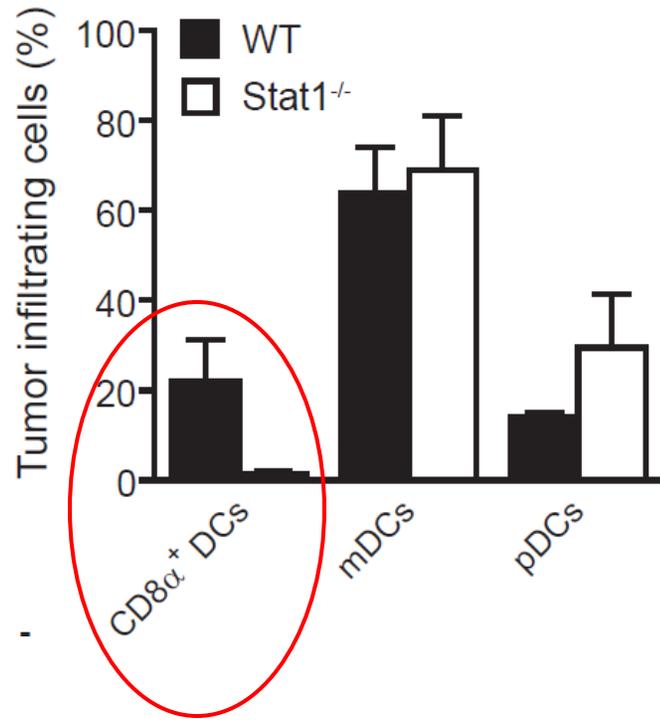


Host IFN- α/β R is critical for generating a spontaneous tumor-specific T cell response

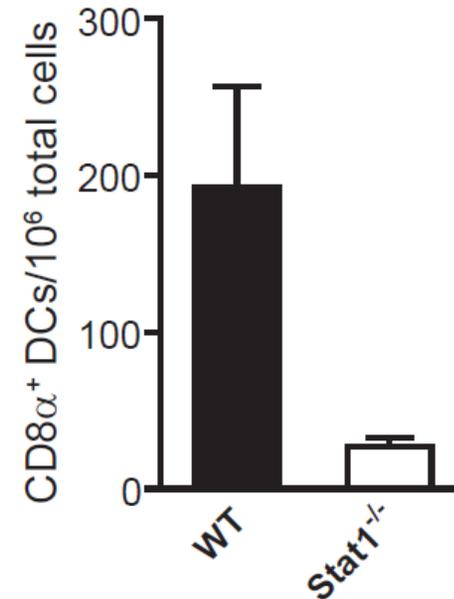


Mice deficient in IFN signaling fail to accumulate CD8 α^+ DC subset in tumor microenvironment

A: Percentage

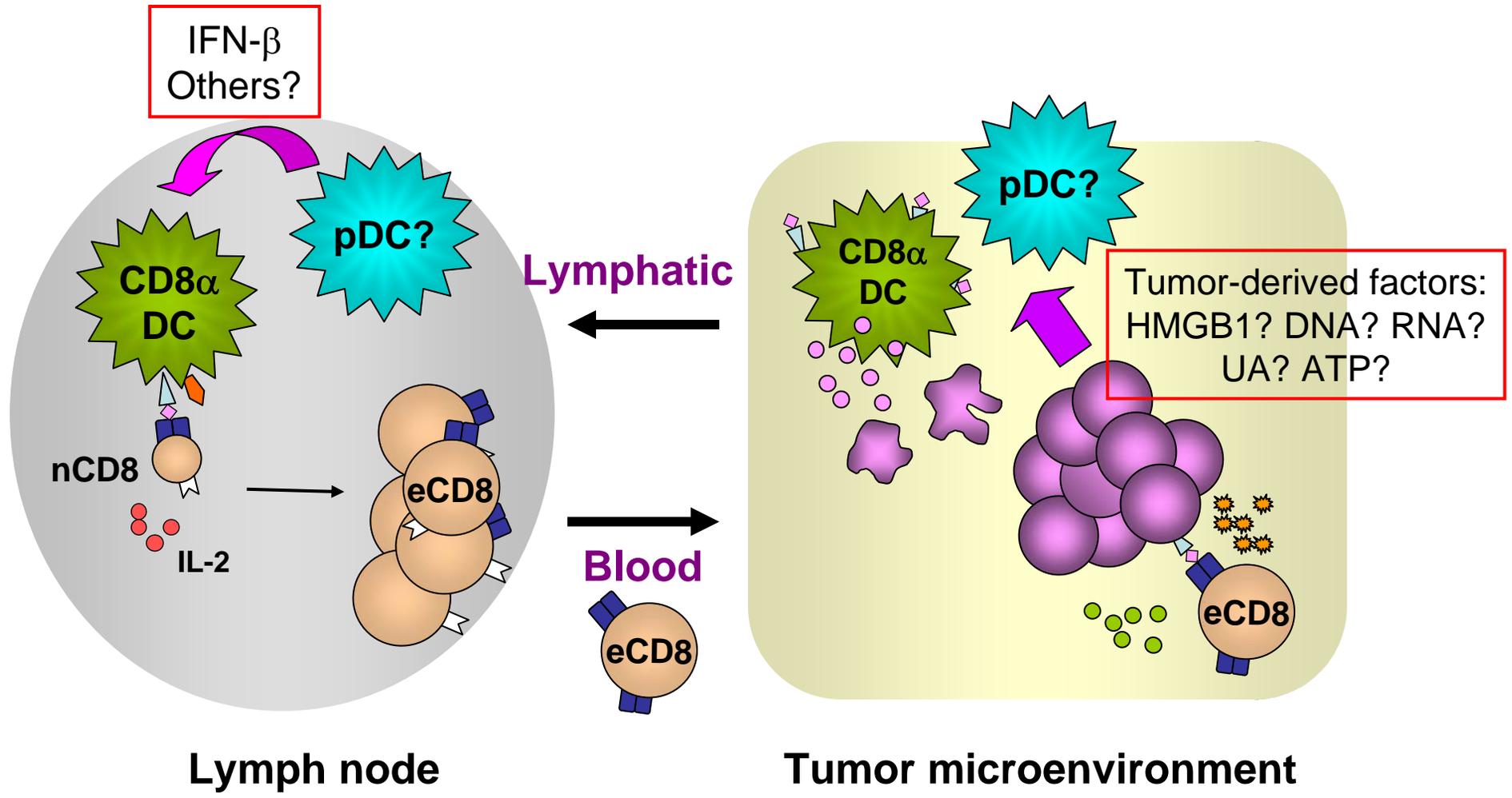


B: Absolute number

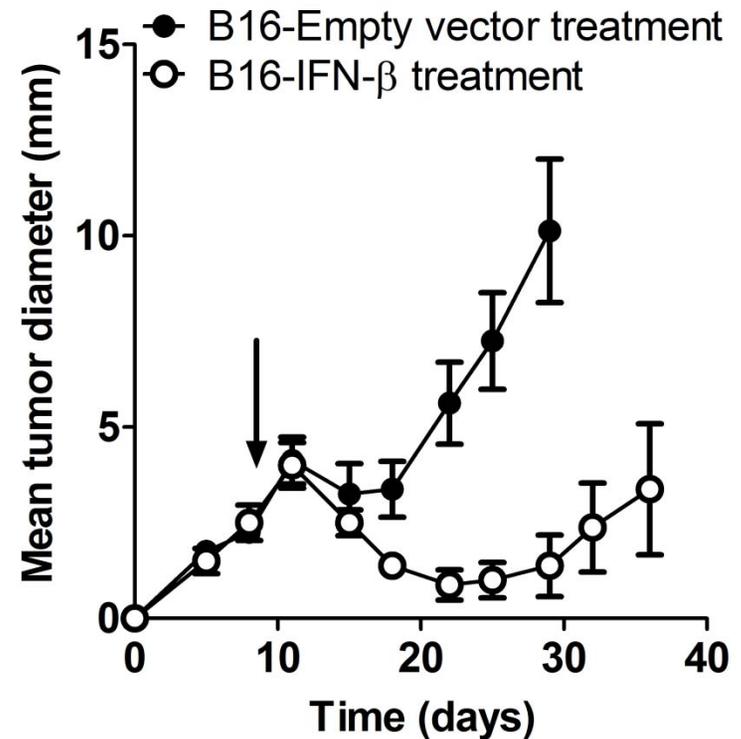
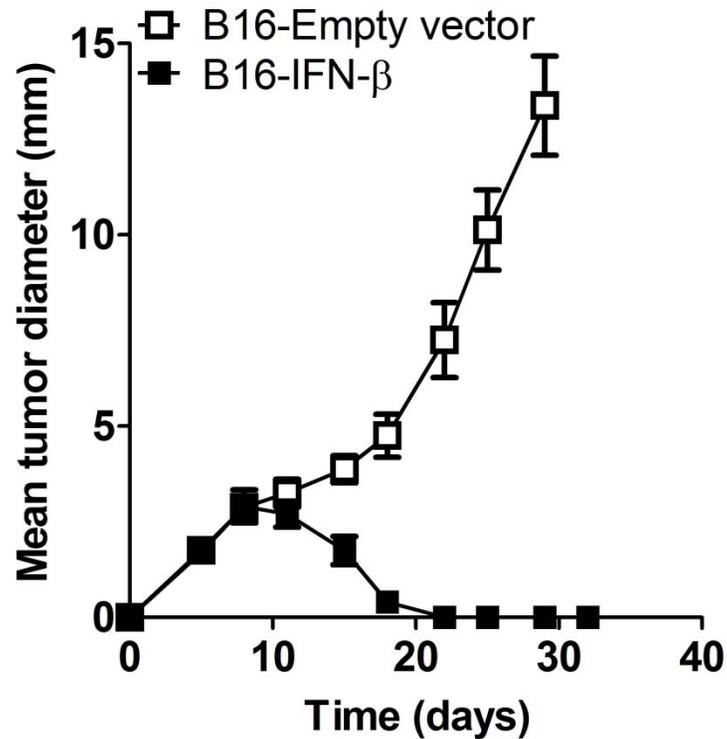


Fuertes et al; J. Exp. Med. 2011

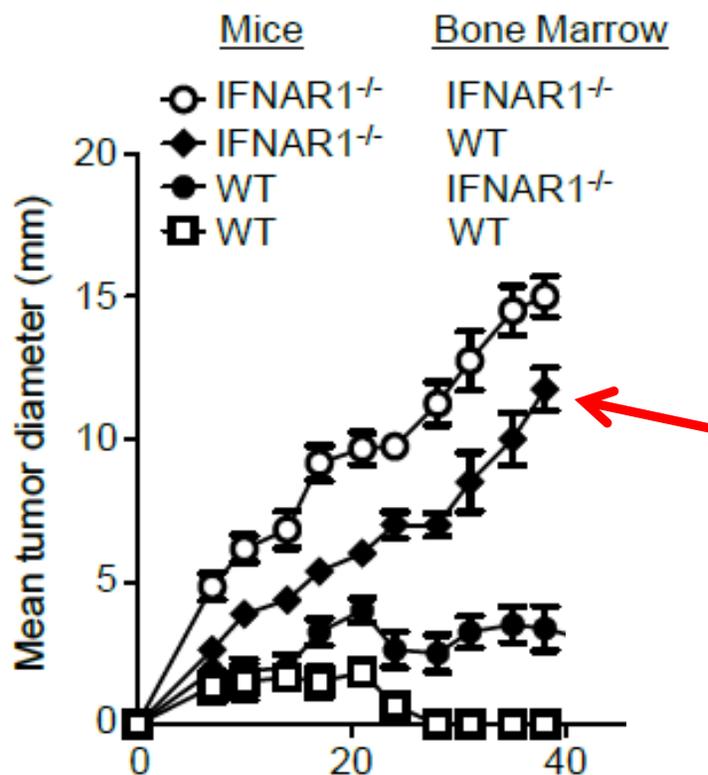
Anti-tumor immune responses: Working model for innate immune signals and spontaneous cross-presentation



Provision of exogenous IFN- β can potently induce tumor rejection

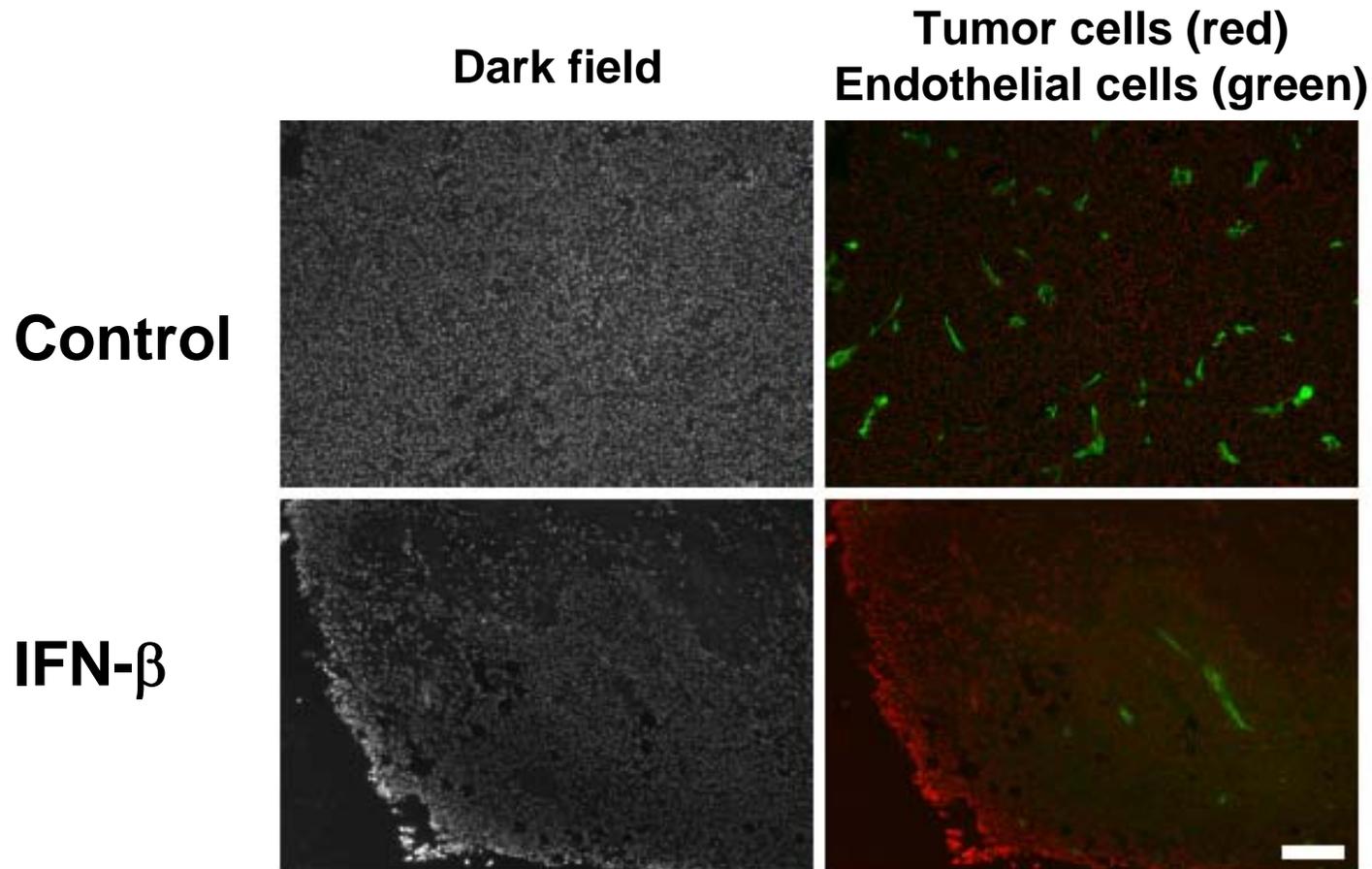


IFN- α/β signaling on host non-hematopoietic cells is necessary for control of B16 melanoma



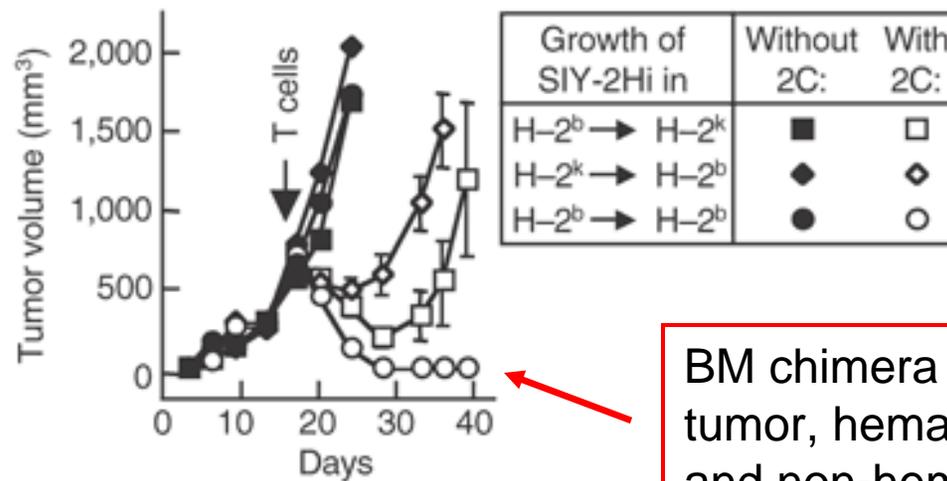
Implies effect at the level of non-hematopoietic stromal cells

Intratumoral IFN- β exerts a profound anti-angiogenic effect



*Spaapen et al.
Manuscript in preparation*

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

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



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Melanoma gene array/ chemokines

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Functional genomics core

LIGHT adenovirus

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Ping Yu

Hans Schreiber

Uncoupling negative regulation

Justin Kline

Robbert Spaapen

Yuan-yuan Zha

Christian Blank

Amy Peterson

Ian Brown

Type I IFNs

Mercedes Fuertes

Robbert Spaapen

Aalok Kacha

Justin Kline

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GSI project

Ruth Meng

Yuan-yuan Zha

Kim Margolin

SWOG, CTEP

Collaborative vaccine/gene array data

Gerold Schuler (Erlangen group)

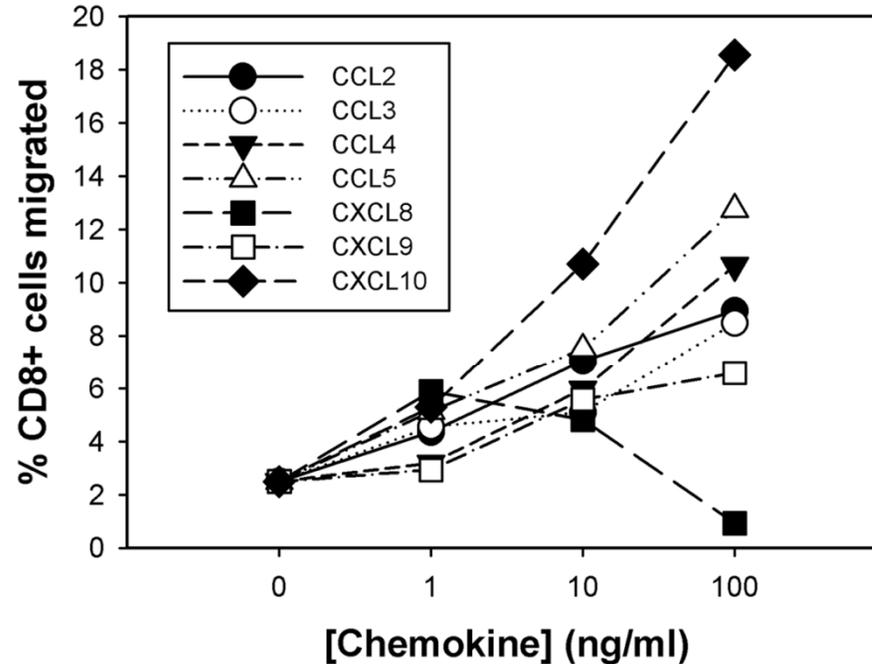
Vincent Brichard (GSK-Bio)

Translational research is like scuba diving...



Hawaii, 2011

Human CD8⁺ effector T cells can migrate to each of these 6 chemokines in vitro

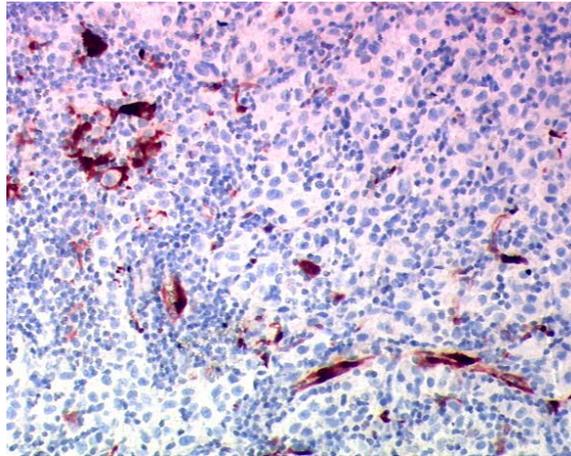


Mechanisms of negative regulation of T cell function within the melanoma tumor microenvironment

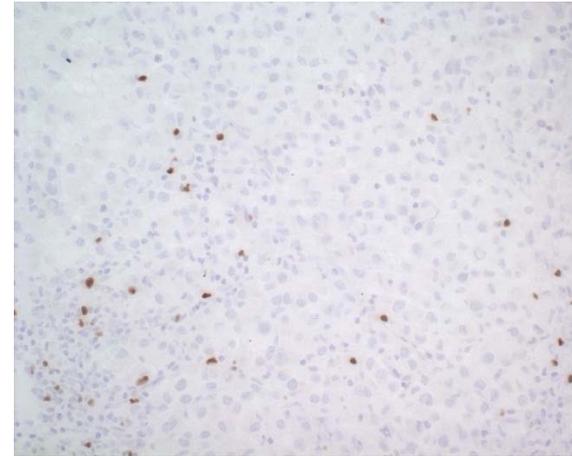
1. Indoleamine-2,3-dioxygenase (IDO → tryptophan catabolism)—inhibits T cell function
2. PD-L1 (inhibitory ligand expressed by tumor cells)—engages PD-1 on T cells
3. CD4⁺CD25⁺FoxP3⁺ Tregs (extrinsic suppression)—inhibit activation of effector T cells
4. T cell anergy (deficient B7 costimulation)—T cell intrinsic dysfunction

IHC for IDO, FoxP3, and PD-L1 shows expression in distinct cell subsets in melanoma metastases

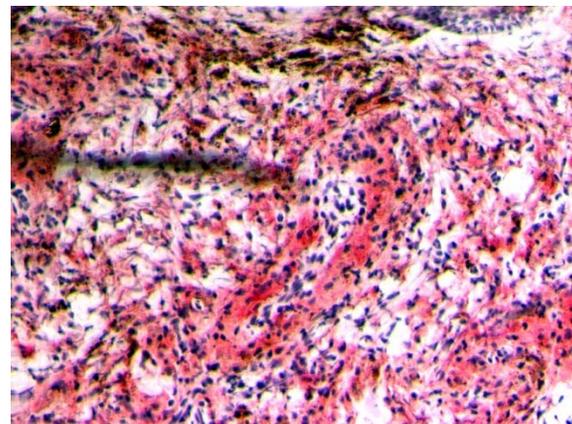
A: IDO



B: FoxP3

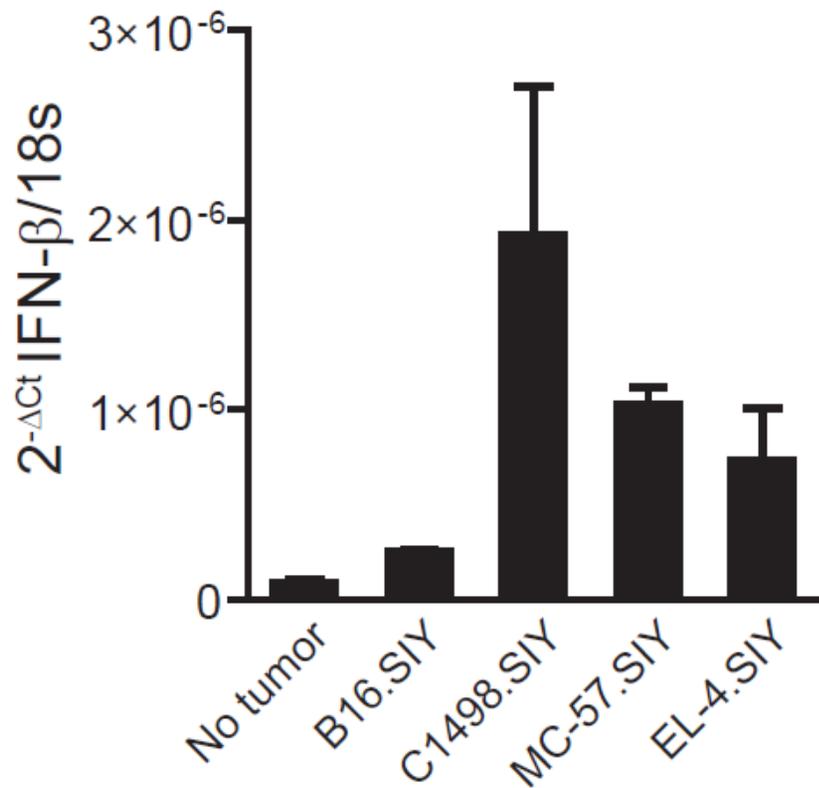


C: PD-L1



Implantation of tumors in vivo results in IFN- β production in the tumor-draining lymph node

A: IFN- β mRNA in DLN cells



B: IFN- β mRNA based on CD11c

