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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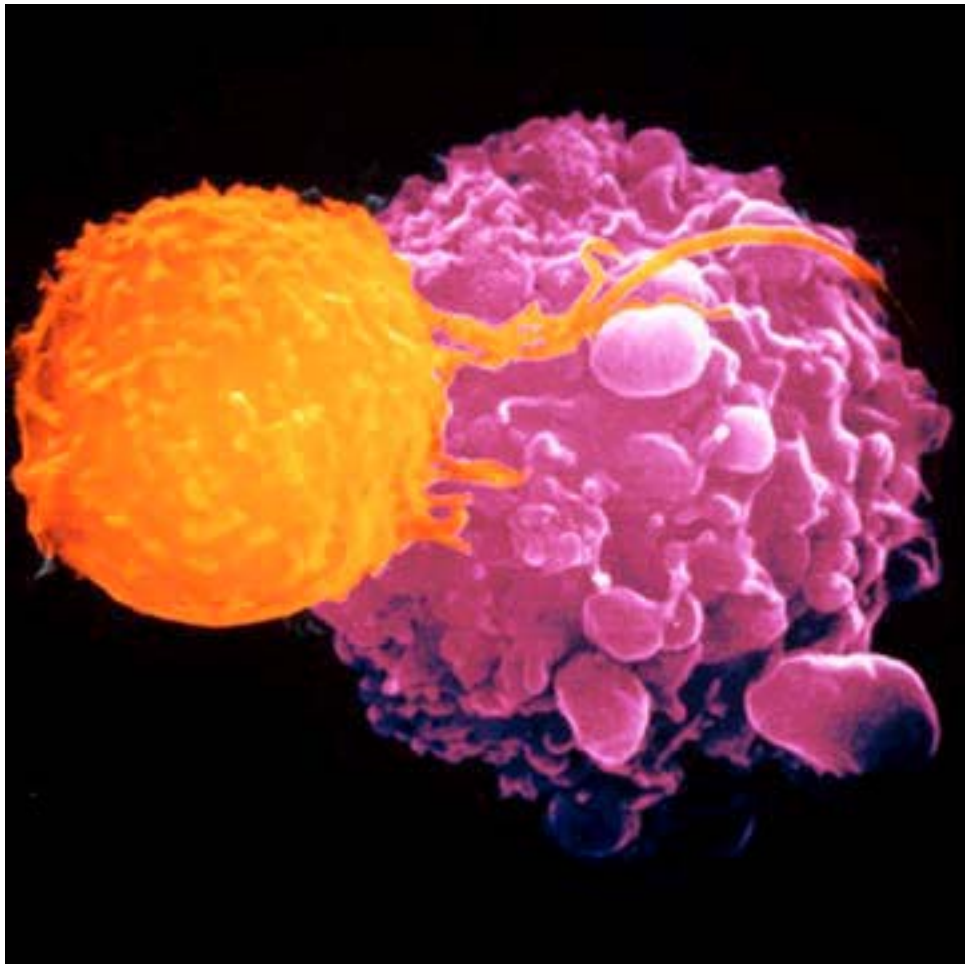
  - BMS
  - GSK-Bio
  - Eisai
  - Incyte
  - Roche
  - Novartis

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



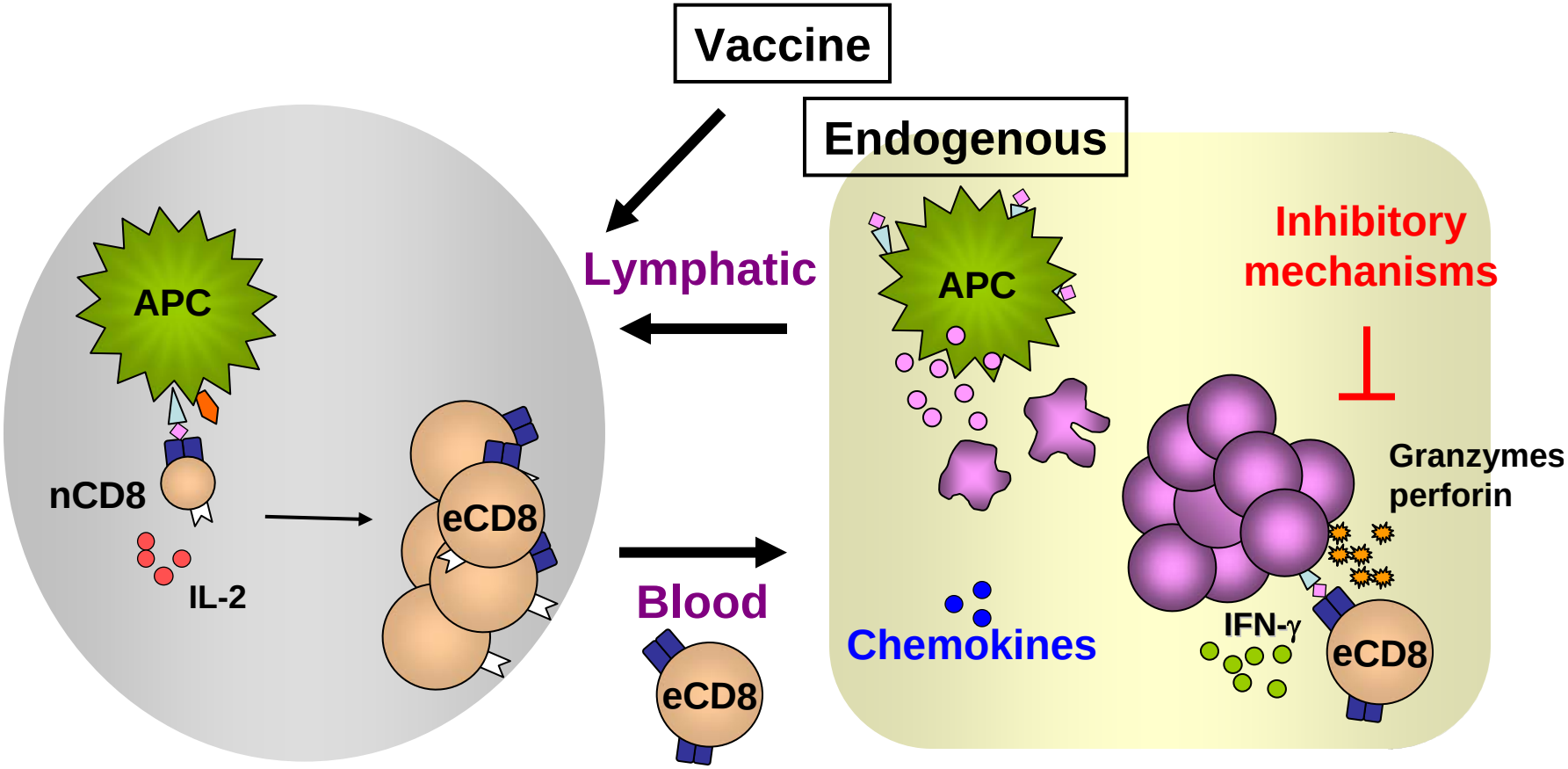
In vivo, a tumor is more than tumor cells

- 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 or effector phase)

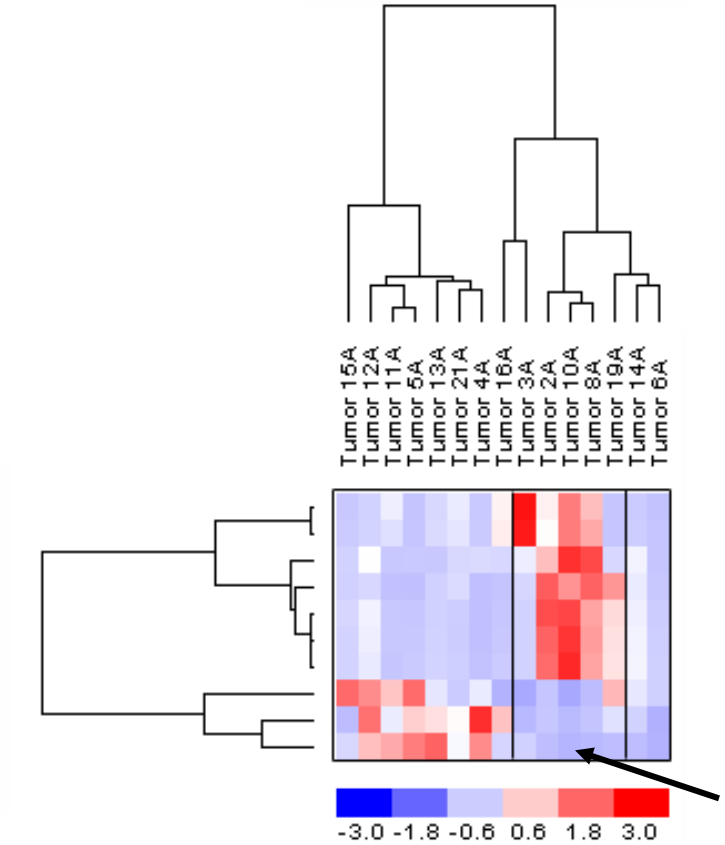
Hypothesis

- 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

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

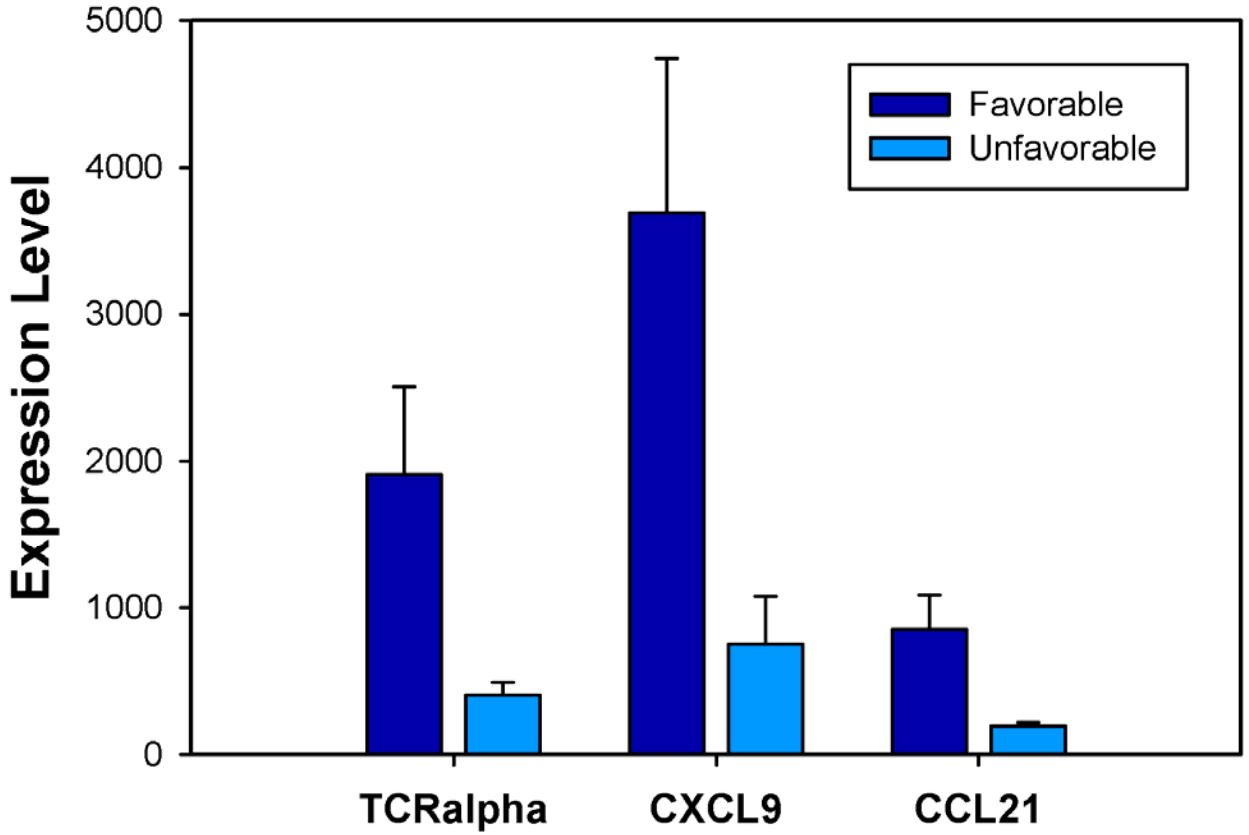


Affymetrix gene array analysis of pre-treatment biopsies from patients on melanoma vaccine sorted by clinical outcome

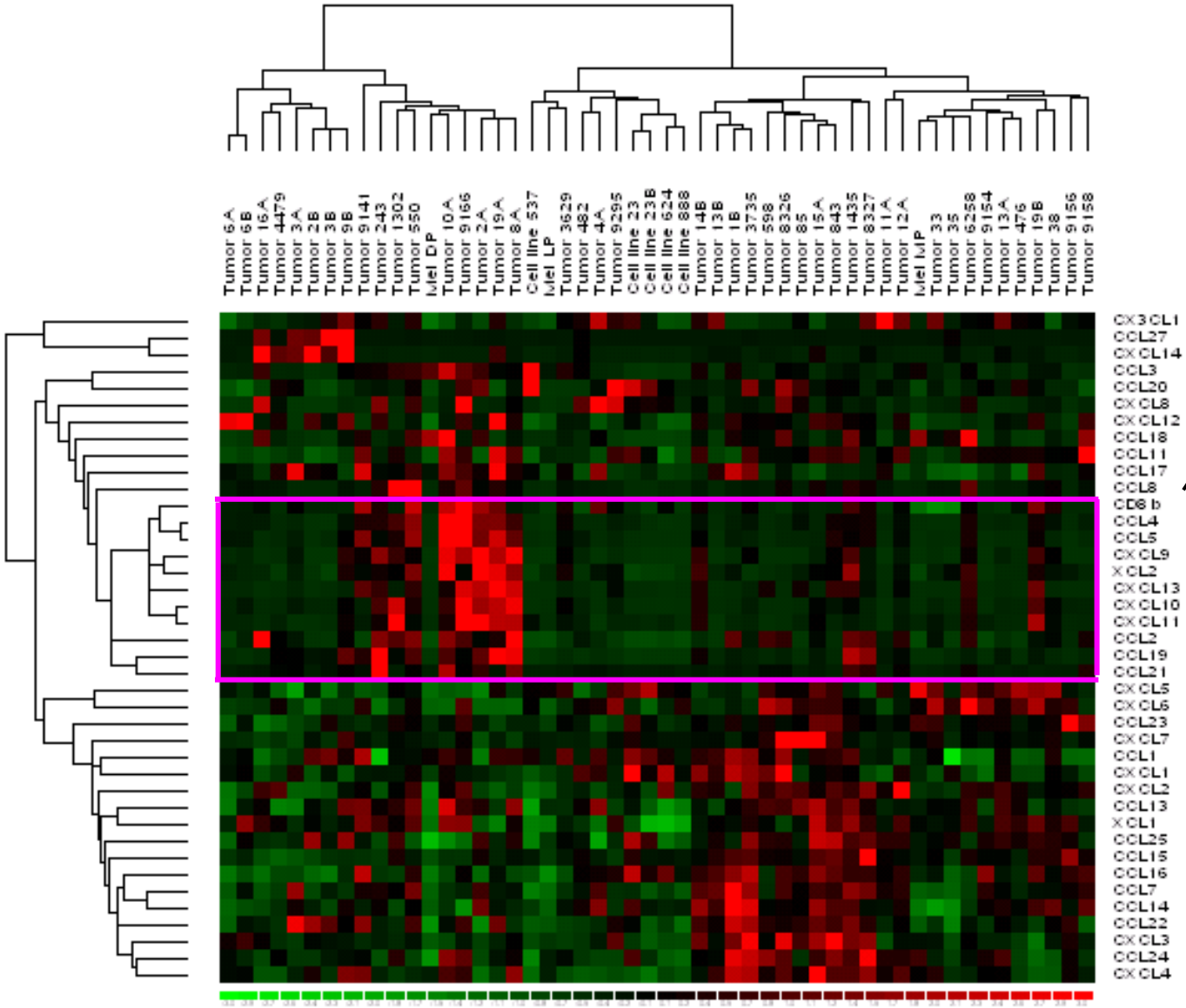


6 mos SD or better

Tumors from favorable clinical outcome patients express higher levels of TCRα, CXCL9, and CCL21



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

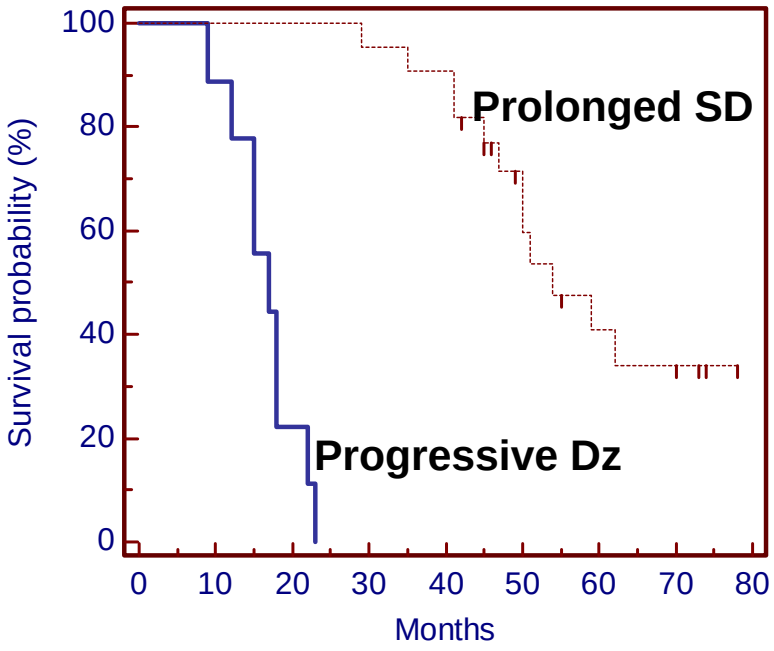


CD8β
CCL2
CCL4
CCL5
CXCL9
CXCL10
CCL19
CCL21

Harlin et al.
Can. Res. 2009

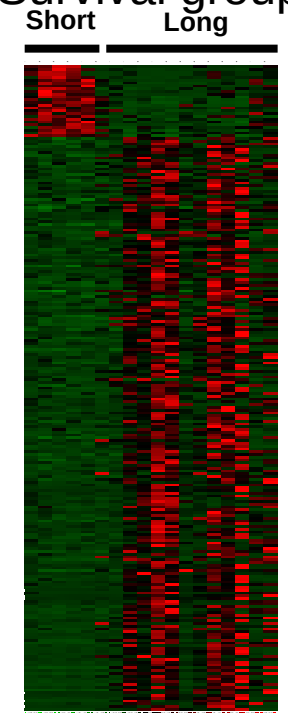
Gene expression pattern of tumor microenvironment associated with favorable clinical outcome to a dendritic cell vaccine

Survival based on clinical response



No correlation with immune response in blood

Survival groups

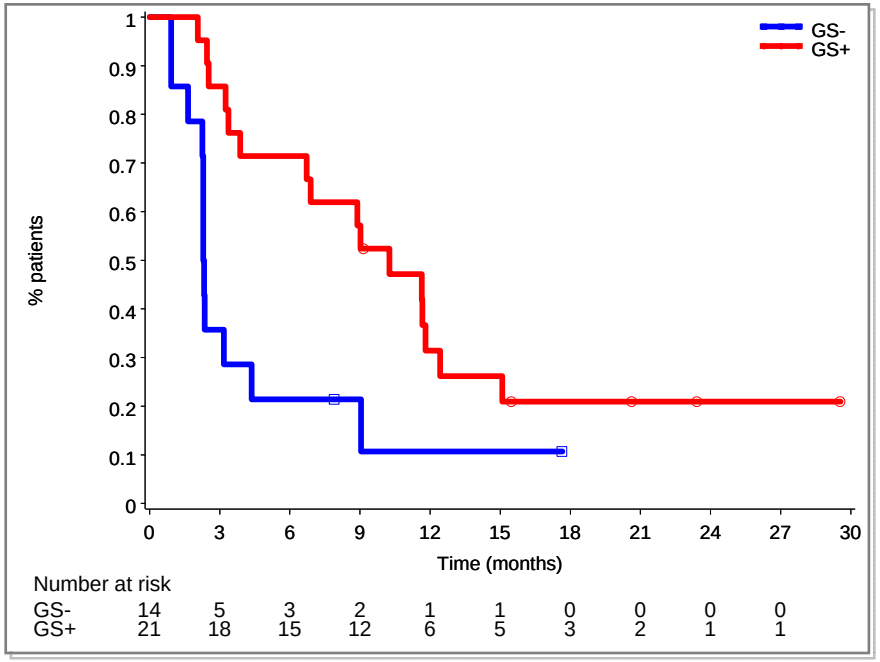


Thy1
CD28
CCL19
LTbeta
IL-27Ralpha
IL-1R

T cell markers and chemokines

Schuler collaboration, ASCO 2009

Impact of gene expression signature on clinical outcome in GSK MAGE3 protein vaccine trial in melanoma



Median

GS-: 2.3 months [95% CI: 2.3 - 4.4]

GS+: 10.3 months [95% CI: 6.7 - 12.4]

HR: 0.31 [95% CI: 0.13 - 0.76]

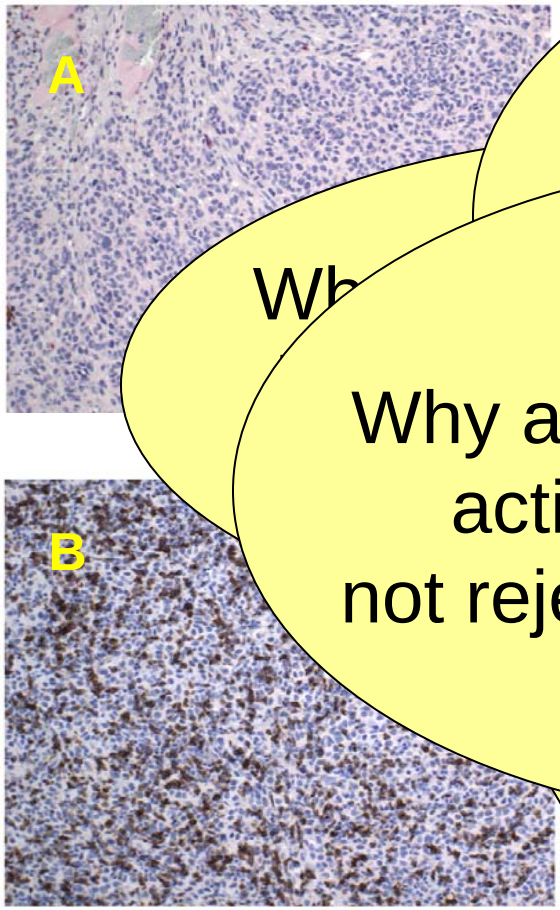
Louahed, ASCO 2008

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
- Analysis of the “inflamed” melanoma phenotype should be explored with respect to response to other immunotherapeutic approaches
  - Anti-CTLA4 mAb (Ipilimumab), anti-PD-1
  - Interleukin-2
- Ideally, this strategy should allow enrichment for the potentially responsive patient population in the future
- These observations also point toward 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?

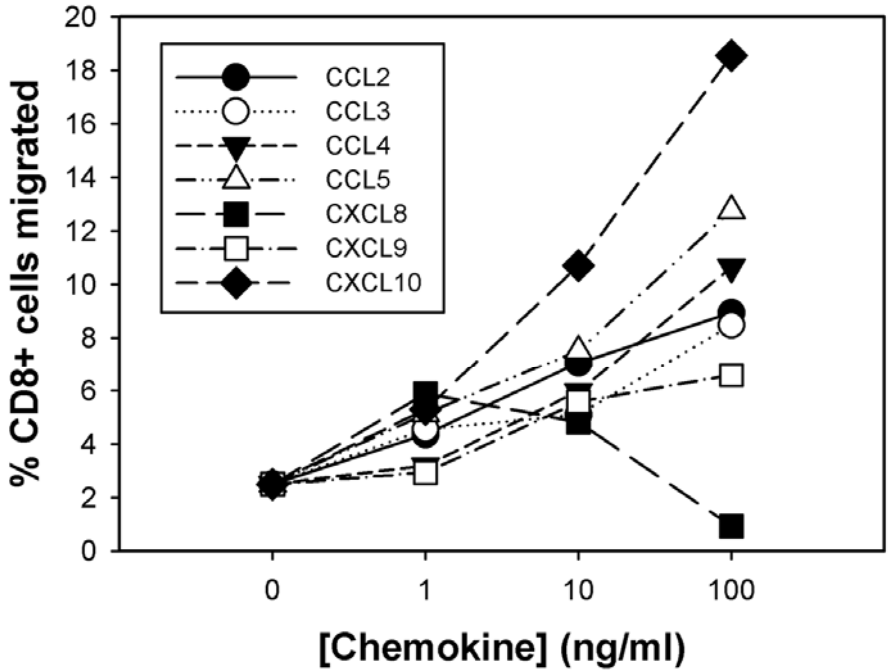
Why are tumors that contain
activated CD8+ T cells
not rejected spontaneously?

mune
omote
ell priming
in a subset of patients?

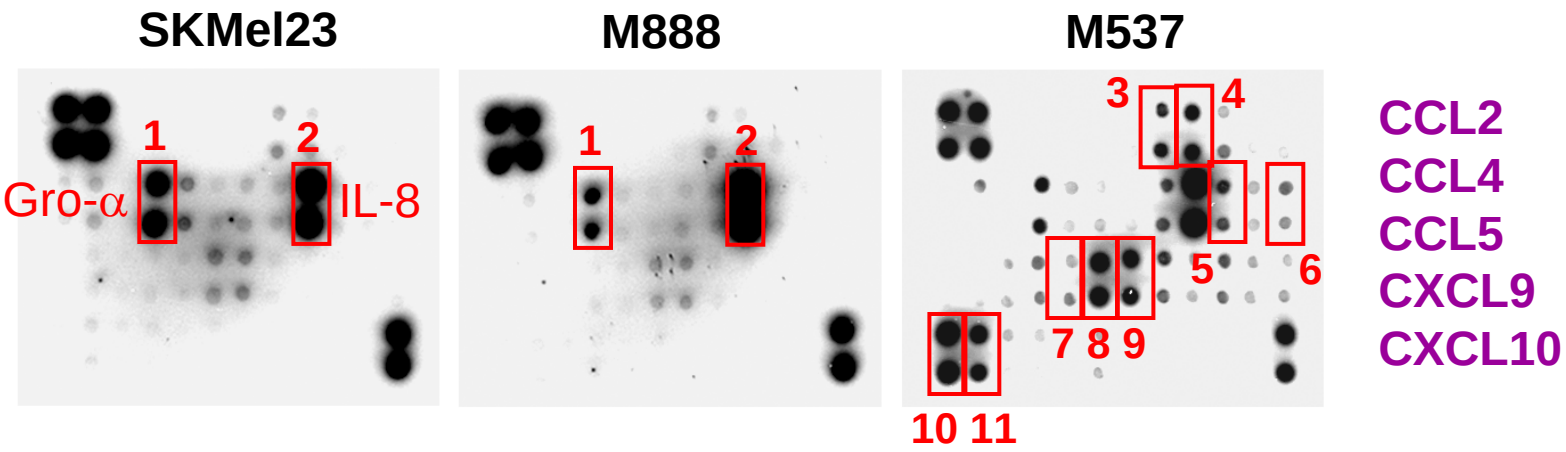
1. Chemokines and T cell migration

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

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

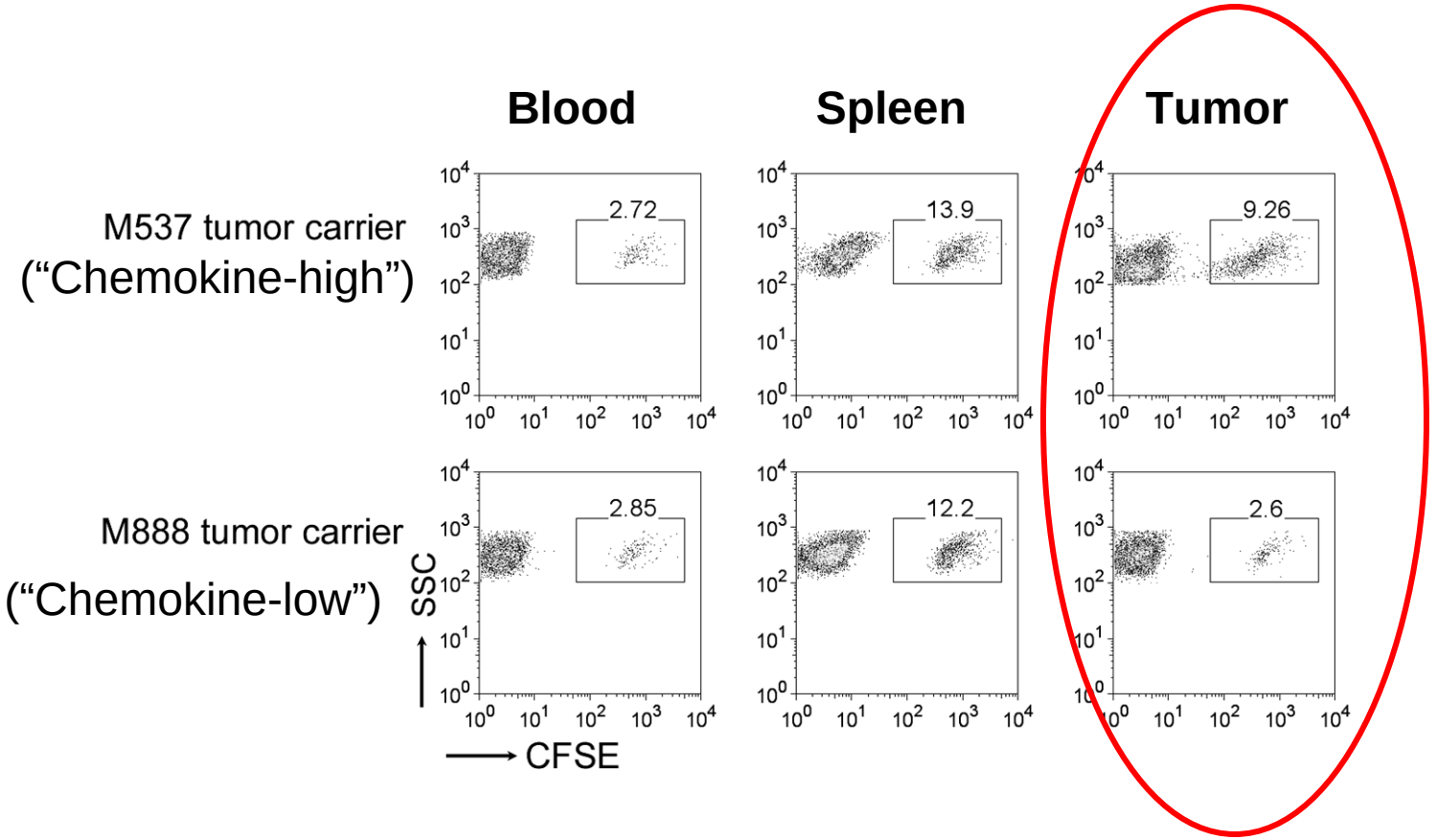


A subset of melanoma cell lines expresses a broad array of chemokines



- 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



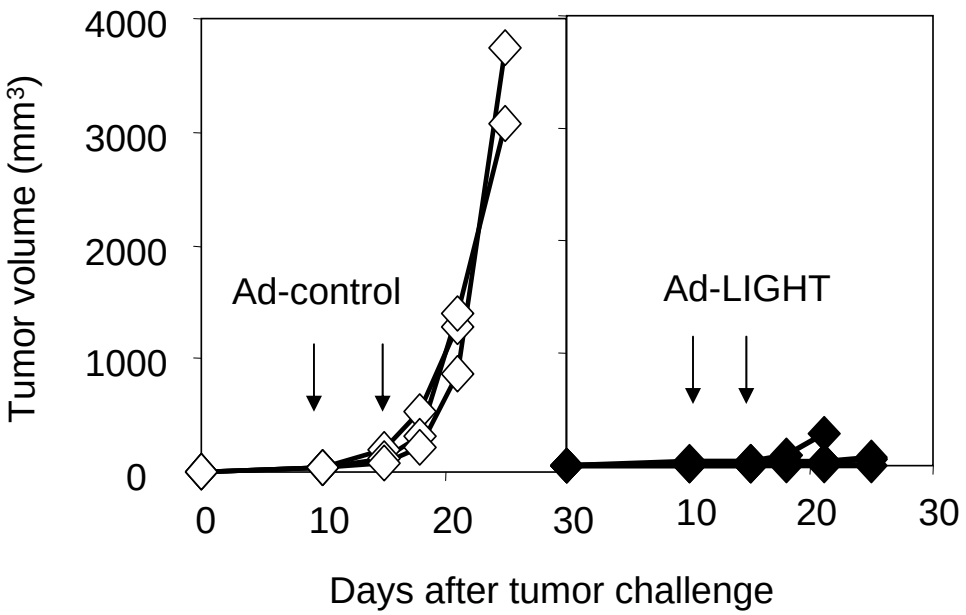
Candidate strategies to promote effector T cell migration into tumor sites

- Introduce chemokines directly
  - CXCR3-binding chemokines (CXCL9, CXCL10)
  - Others (CCL2, CCL3, CCL4, CCL5)
- Induce chemokine production from stromal cells
  - LIGHT, lymphotoxin: bind 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

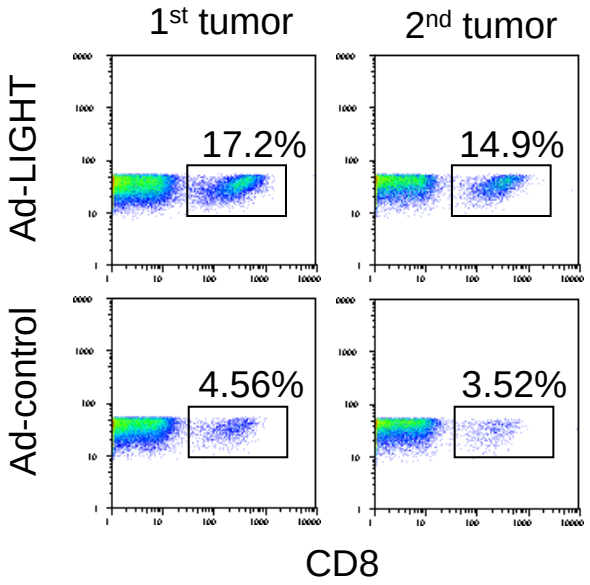
Intratumoral LIGHT adenovirus in B16 melanoma:

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

Tumor rejection



CD8$^+$ T cell infiltrate

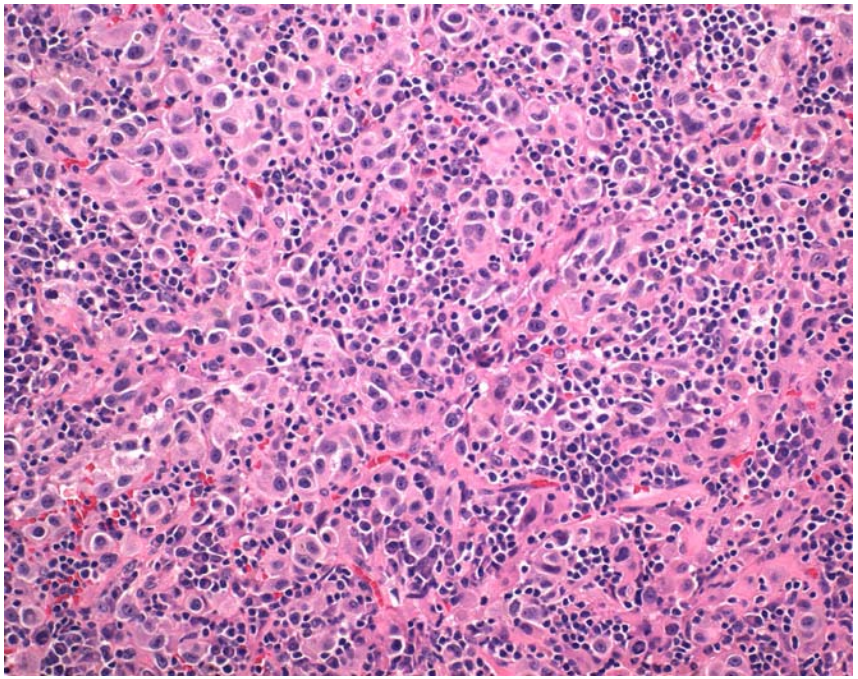


Yu et al, J. Immunol. 2007

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?

Why are melanomas that do attract $CD8^+$ T cell not rejected spontaneously?



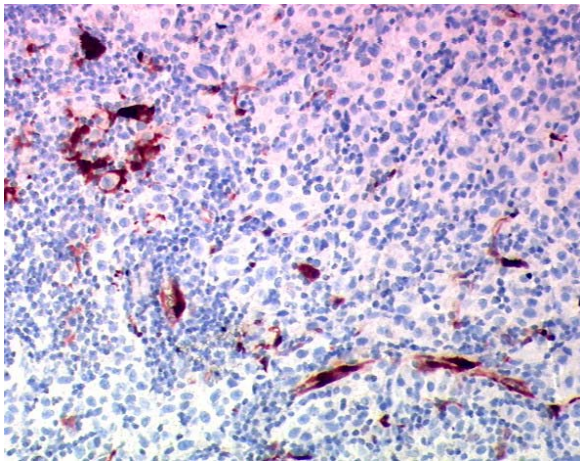
- **IDO** (indoleamine-2,3-dioxygenase)
- **PD-L1** (engages PD-1)
- $CD4^+CD25^+FoxP3^+$**Tregs**
- T cell **anergy** (B7-poor)

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

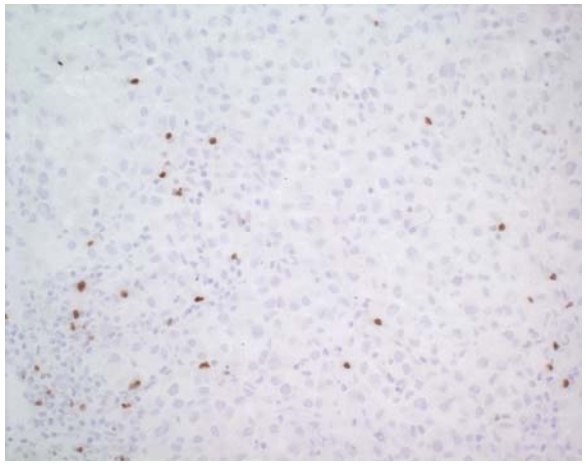
1. Indoleamine-2,3-dioxygenase (IDO➔ tryptophan catabolism)—inhibits T cell function
2. PD-L1 (inhibitory ligand expressed by tumor cells)—engages PD-1 on T cells
3. $CD4^{+}CD25^{+}FoxP3^{+}$ Tregs (extrinsic suppresion)—inhibit activation of effector T cells
4. T cell anergy (deficient B7 costimulation)—T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

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

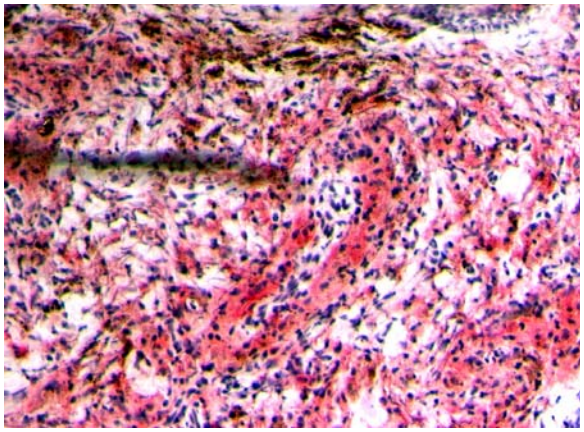
A: IDO



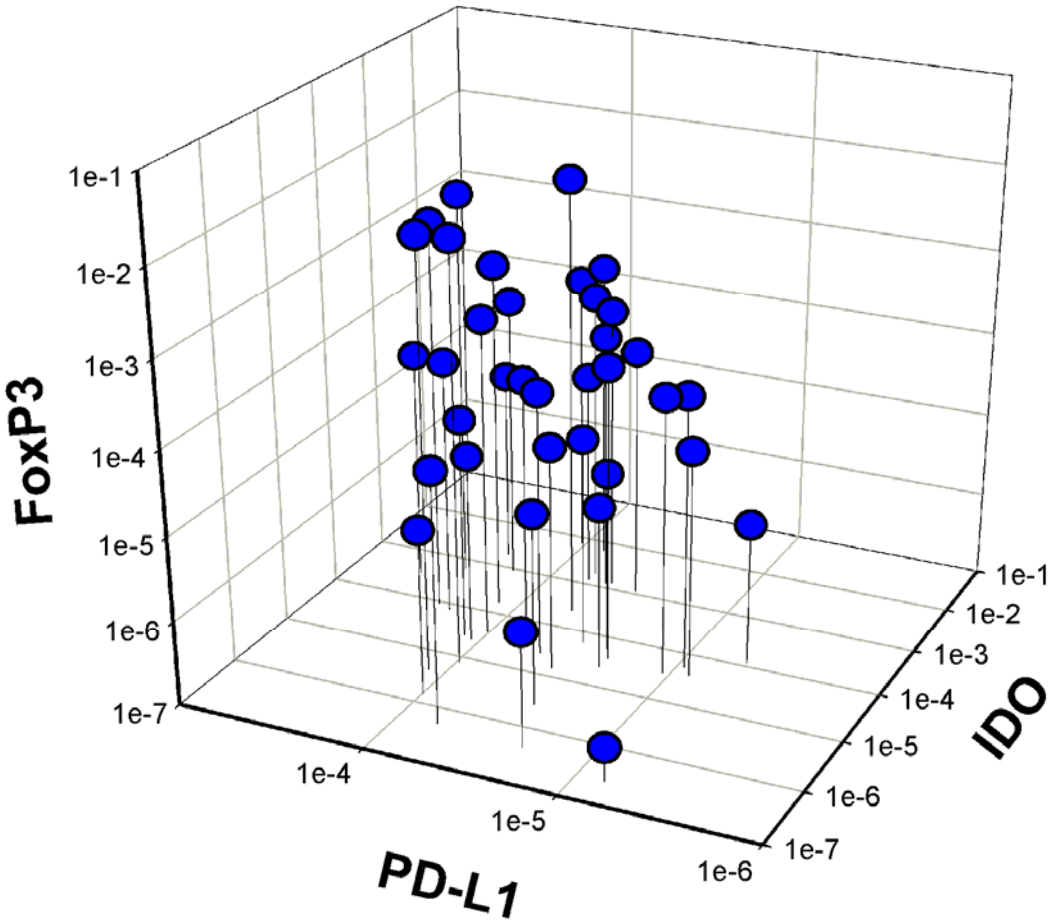
B: FoxP3



C: PD-L1



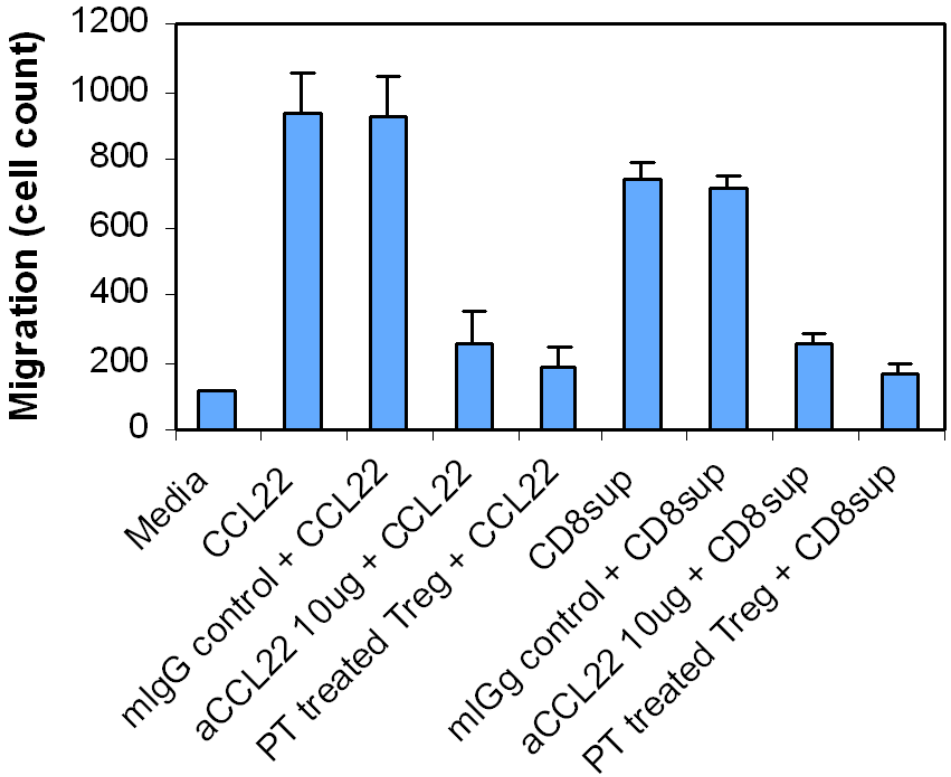
Correlated expression of IDO, FoxP3, and PD-L1 transcripts in individual tumors



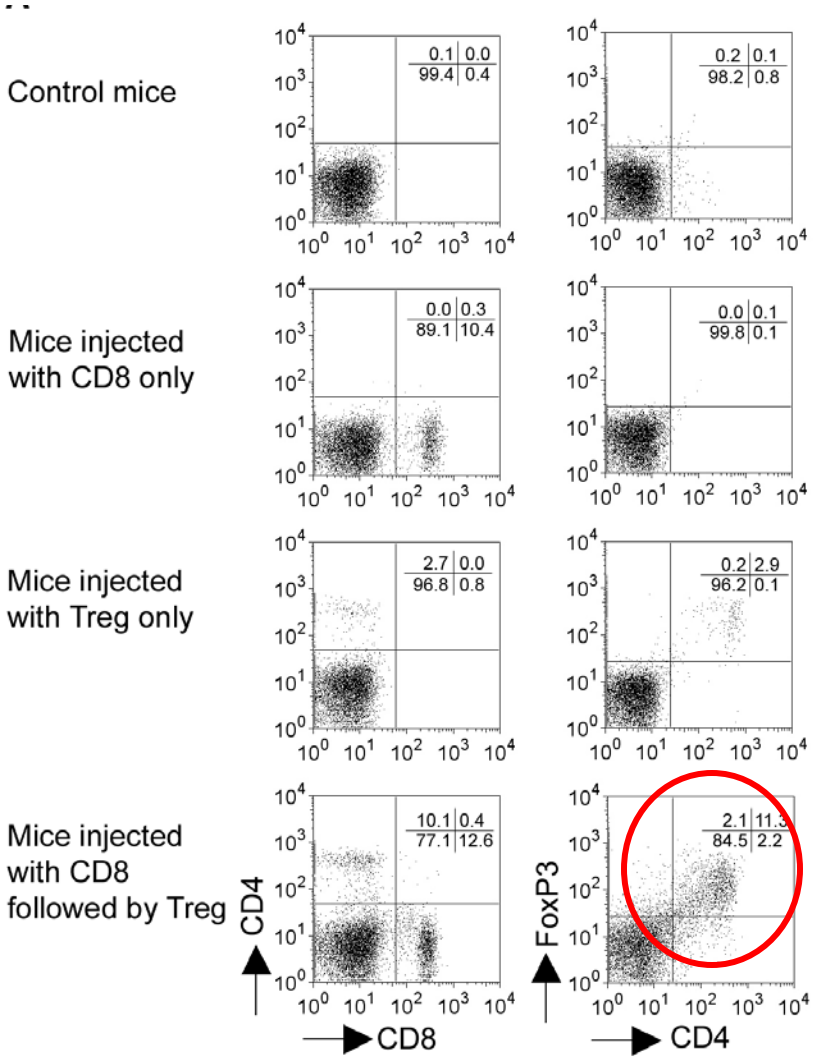
Is there a causal relationship between the accumulation of $CD8^+$ T cells and the presence of immune inhibitory pathways?

- It is assumed that the tumor establishes an immune suppressive microenvironment so that T cells that infiltrate become inhibited
- However, we observe higher expression of immune inhibitory pathways in tumors that contain T cells
- New hypothesis:
  - The expression of IDO and PD-L1, and the accumulation of Tregs, may depend upon the infiltration of $CD8^+$ T cells in the tumor site
  - These might be induced by specific factors produced by activated $CD8^+$ T cells
- To test these notions, in vivo mouse models were utilized

Supernatant from activated human $CD8^+$ T cells recruits sorted $CD4^+CD25^+$ T cells in a CCL22-dependent fashion



Superior migration of human Tregs into melanoma xenograft when CD8s are co-transferred



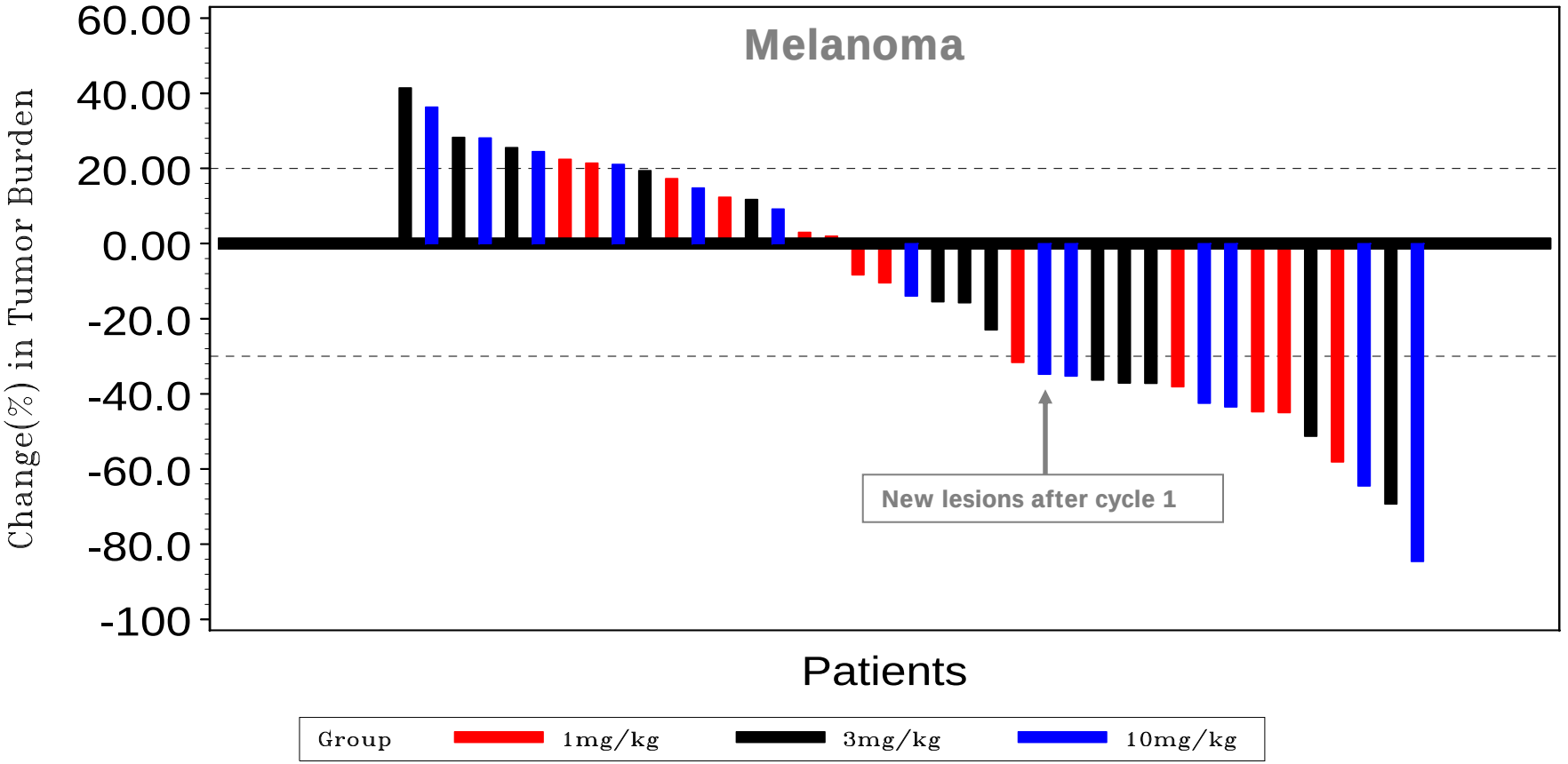
Summary of regulation of immune suppressive mechanisms in the tumor microenvironment

- The three major immune inhibitory mechanisms confirmed to be present in the melanoma tumor microenvironment appear to be immune-intrinsic, driven by $CD8^+$ T cells and not driven by the tumor
- For IDO and PD-L1, IFN-γ is the major mediator
- For Tregs, CCL22 production by $CD8^+$ effector cells is a major mediator
- Blockade of these mechanisms therefore represent attractive strategies to restore anti-tumor T cell function and promote tumor rejection in patients

Strategies to block immune inhibitory mechanisms being translated to the clinic

- IDO inhibition
  - 1-methyltryptophan (RAID program)
  - **New more potent IDO inhibitors** (Incyte)
- Blockade of PD-L1/PD-1 interactions
  - **Anti-PD-1 and anti-PD-L1 mAbs** (Medarex/BMS)
- Depletion of $CD4^+CD25^+FoxP3^+$ Tregs
  - **Ontak (IL-2/DT fusion)**
  - **Daclizumab (anti-IL-2R mAb)**
  - 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/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

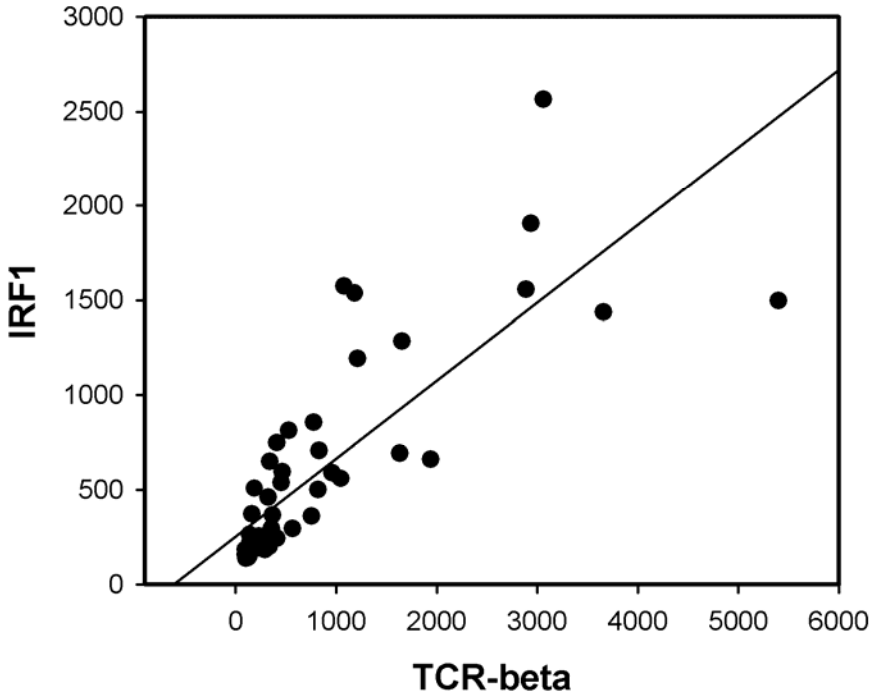
~30% response rate also seen in NSCLC and renal cell carcinoma

3. Innate immune signals—type I IFNs

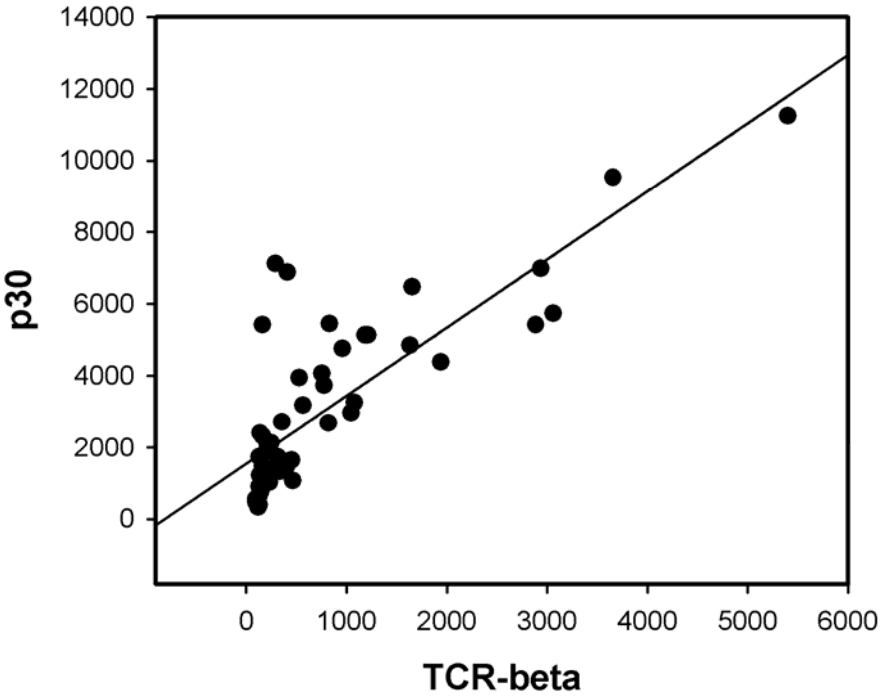
How are anti-tumor T cells sometimes becoming spontaneously primed? Can we improve endogenous T cell priming in the tumors that fail to do so alone?

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

A: IRF1

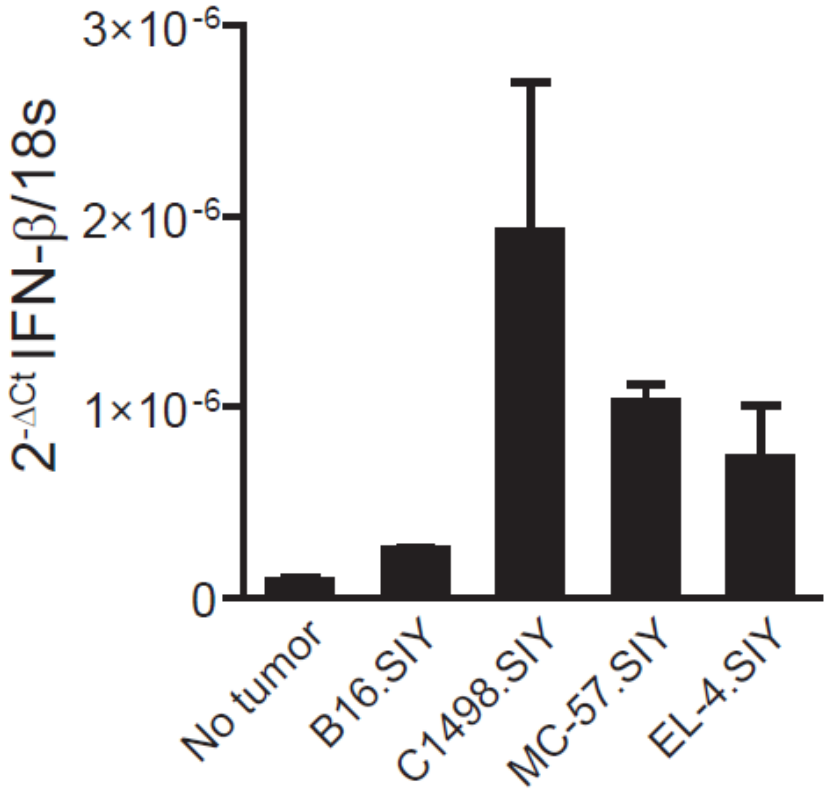


B: IFN-induced p30

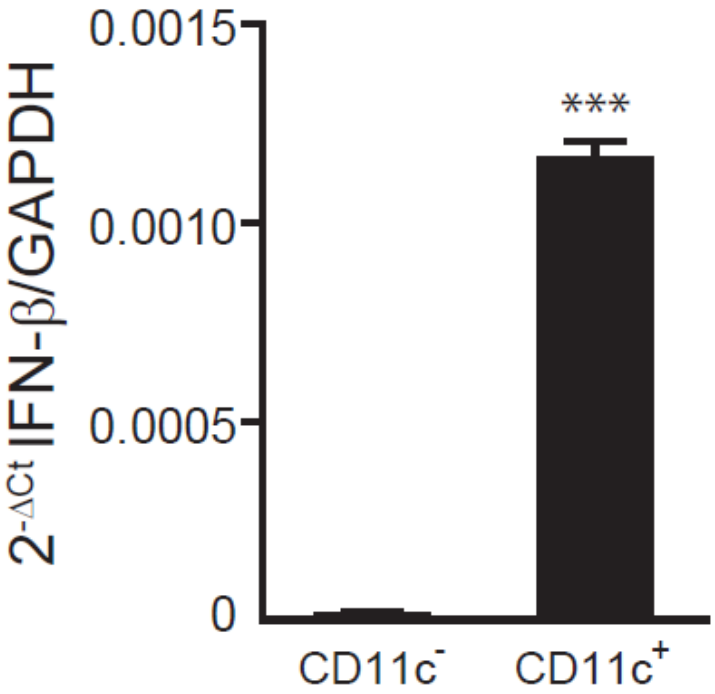


Implantation of tumors in vivo results in IFN-β production in the tumor-draining lymph node

A: IFN-β mRNA in DLN cells

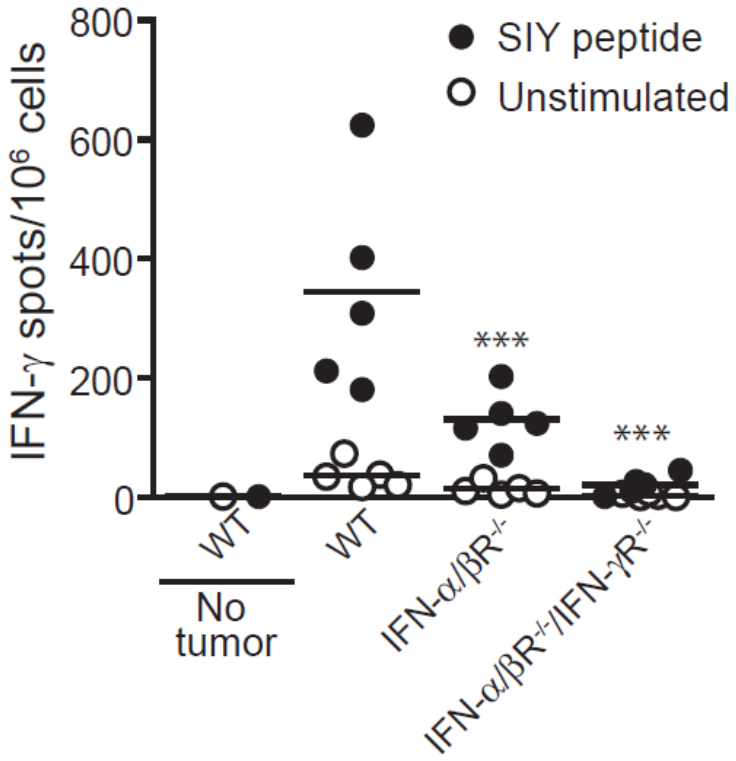


B: IFN-β mRNA based on CD11c

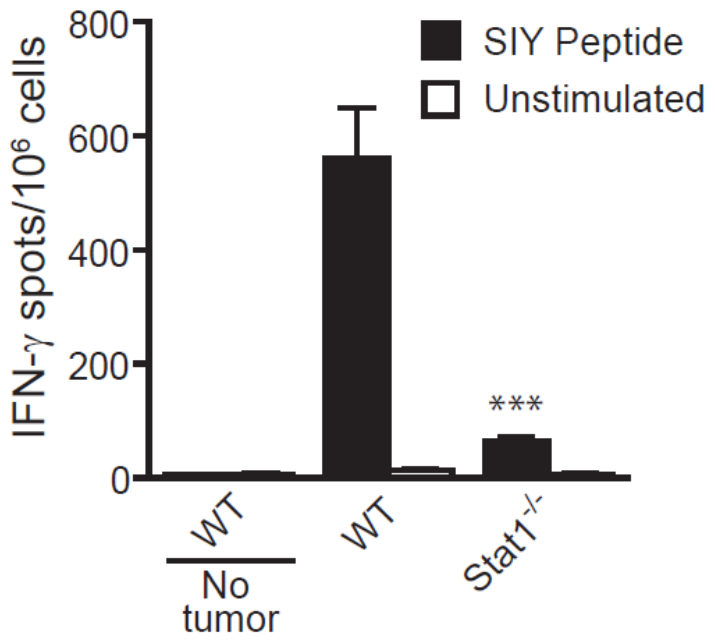


Host IFN-α/βR is critical for generating a spontaneous tumor-specific T cell response

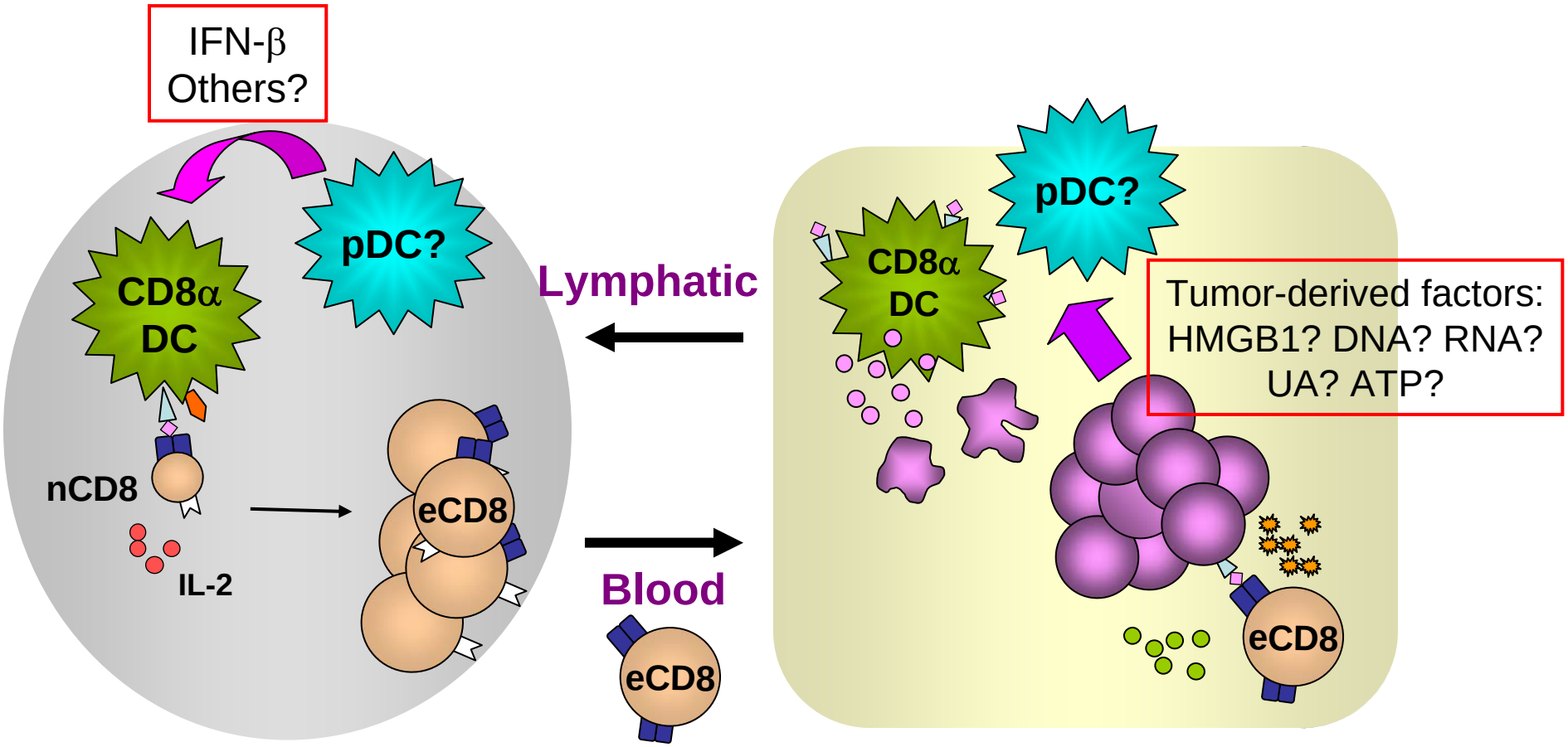
A: IFN-α/βRko



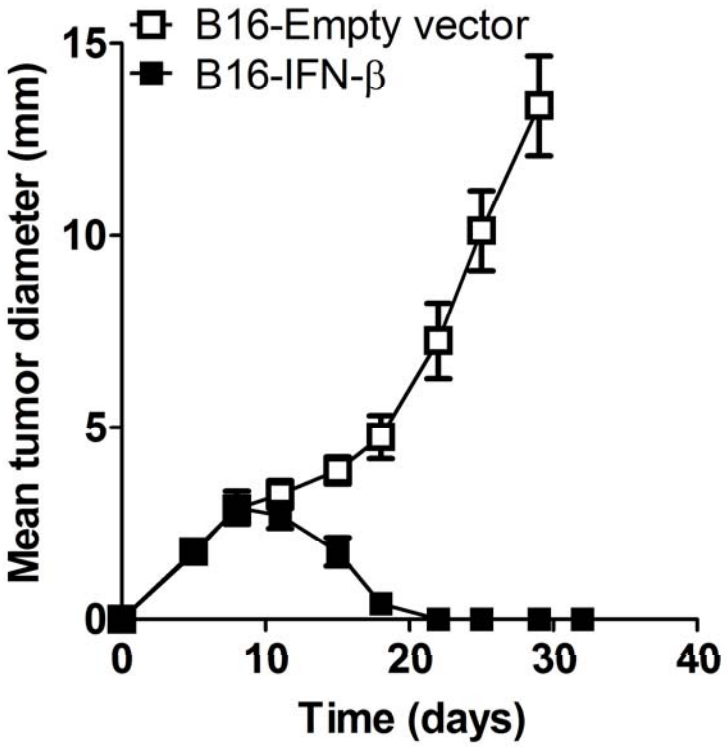
B: Stat1ko

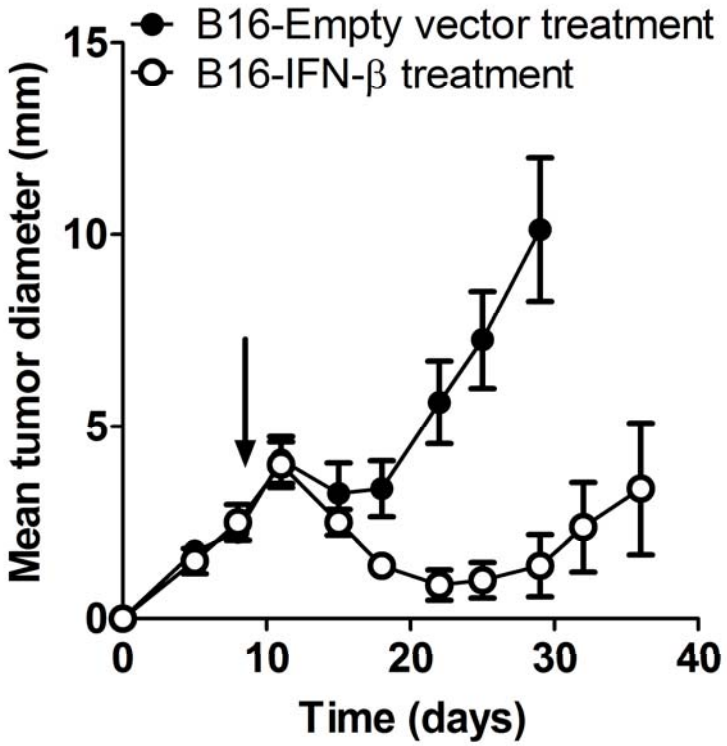


Anti-tumor immune responses: Working model for innate immune signals and spontaneous cross-presentation



Provision of exogenous IFN-β can potently induce tumor rejection





4. Candidate oncogenic pathways that may regulate the two major microenvironment subsets (inflamed vs. non-inflamed)

- Probably not Ras pathway (ubiquitously activated)
- Modifier pathways expressed in subsets of melanomas:
  - Stat3
  - beta-catenin
  - Notch
- Knockdown of Stat3 has been shown to promote chemokine production by tumor cells (Hua Yu)
- Notch pathway activation has been found to be highest in non-inflamed tumors (Gajewski)
- Future studies will determine whether the inflamed tumor microenvironment might be promoted by biochemical manipulation of one or more of these pathways

Conclusions

- There is heterogeneity in patient outcome to immune-based therapies for cancer such as melanoma vaccines
- 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 type I IFNs/innate immunity
- 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

Acknowledgments

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Craig Slingluff
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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
David Kranz
Hans Schreiber
Ken Murphy

GSI project
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Yuan-yuan Zha
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SWOG, CTEP

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Vincent Brichard (GSK-Bio)


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