Cancer Immunometabolism: IDO Pathway and Its Therapeutic Correction

George C Prendergast, PhD
President & CEO, Lankenau Institute for Medical Research (LIMR)
Editor in Chief, *Cancer Research*

LIMR at a glance

- Faculty:
  16 Resident Faculty at Institute
  60 Affiliate Clinical & Nursing Faculty at Lankenau Medical Center

- Primary Research Programs:
  Cancer, Diabetes, Cardiovascular Disease

- Basic Science Theme:
  Disease Modifier Pathways

- Unique 'Acapreneurial' Research Model:
  14 Non-Profit Laboratories + 10 Biotech Start-Up Companies

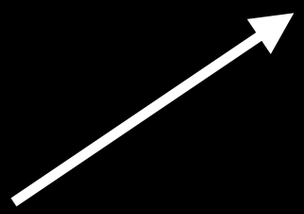
- Biotech Incubator (managed by LIMR Development Inc.):
  - LIMR Development Inc. (LDI) = Product Development & Business Affairs
  - 'Spin-out' and 'Spin-in' Biotech Companies = Drug & Device Development

COI STATEMENT

Learn more at www.limr.org

What is cancer?

- Cancer gene alterations — *Mainstream Theory*
- Permissive microenvironment — *Established Trend*
- Dysfunctional immunity — *Emerging Trend*



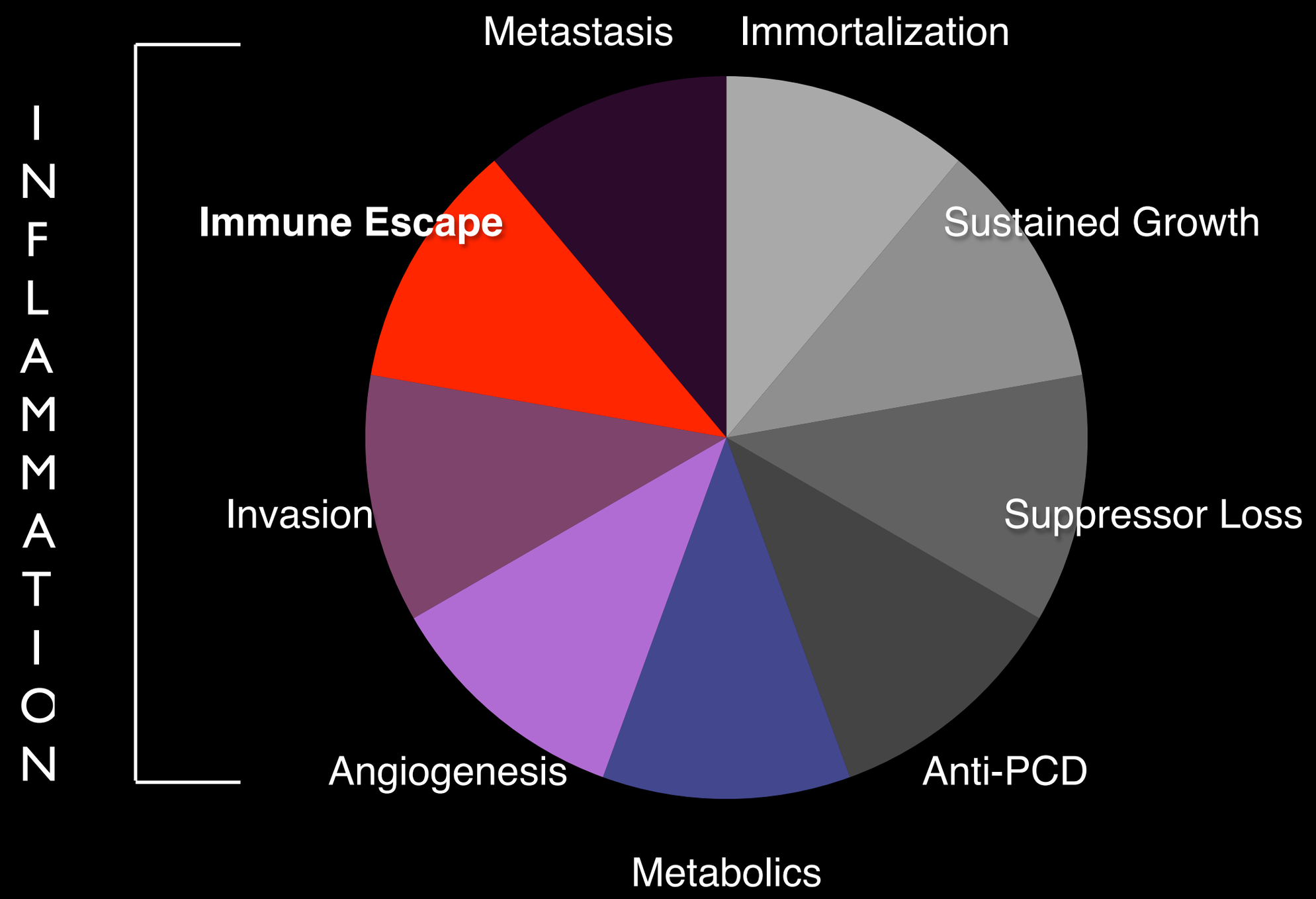
Back to the future !
Virchow, Erlich, Coley (1800s-1900s)

'Rogue' Cells

OR

Mismanagement of 'Rogue' Cells ?

Traits of Cancer vs. Hallmarks of ‘Rogue’ Cells



Failure of cancer screening to extend survival of screened populations argues the presence of ‘rogue‘ cells is not the same as the presence of cancer ...?

Immunology in the Historical Mainstream of Cancer Research: Divorce, Remarriage and Elective Affinities of the Future

1900s

Roots of cancer genetics
Animal tumor viruses (Rous)
Tissue culture (Eagle)
Cytogenetics (Nowell)

Cancer Genetics

1970

'Nude' Immune Deficient Mouse (the Divorce)

1980-2000

2000s

'Interferon Knockout' Mutant Mice (the Remarriage)

Cancer cell centric therapy

CANCER RESEARCH

Inflammation

Conceptual Synthesis

2010s

Host centric therapy

Immunochemotherapy

1800s

Roots of cancer immunology
Inflammation (Virchow)
Immune surveillance (Ehrlich)
Bacterial toxins (Coley)

Cancer Immunology

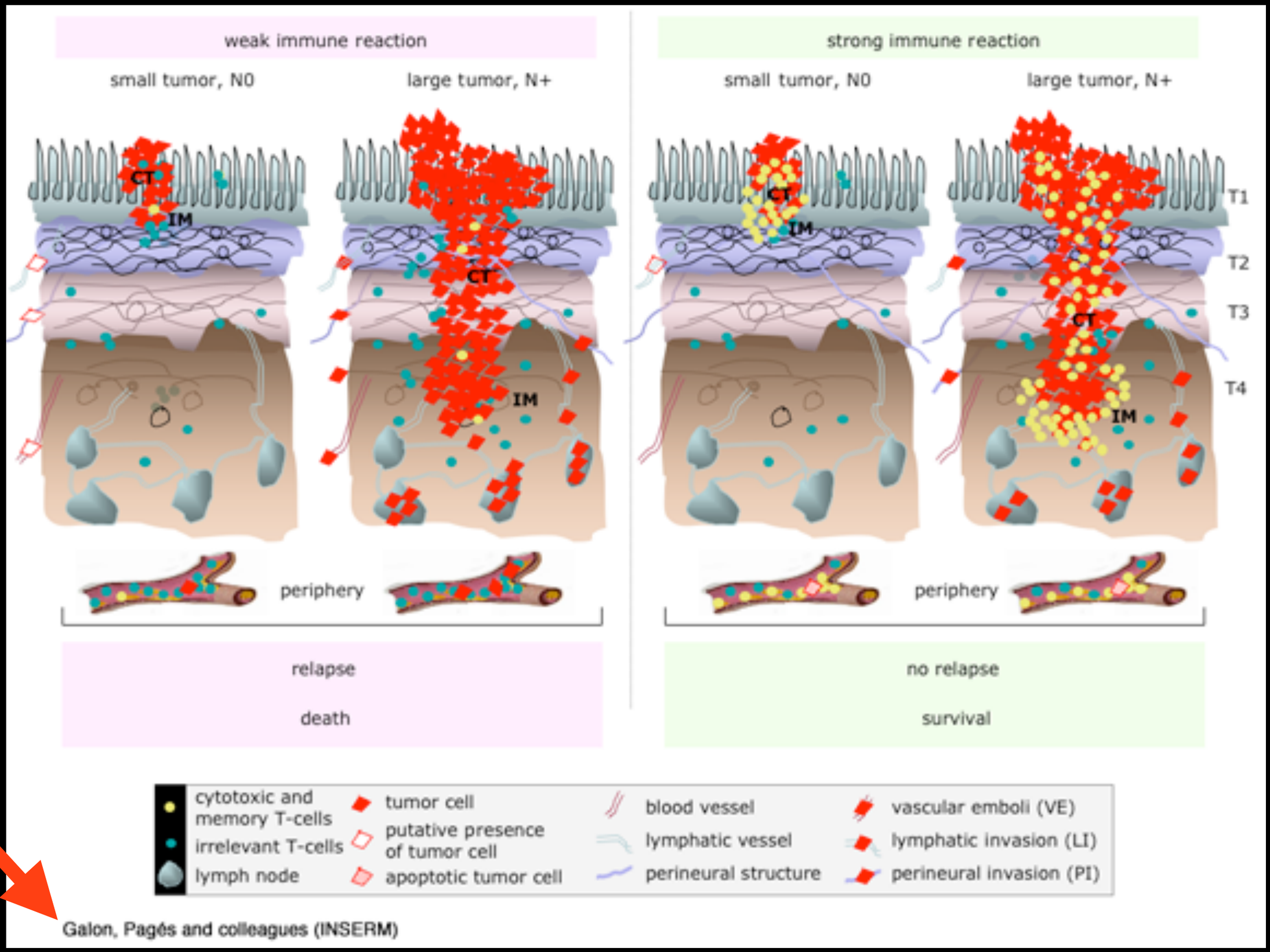
1980-2000

• Unchanged cancer risk despite diabled immunity (human tumor transplants OK)
• Immunity unimportant for control ... ?

• Elevated cancer risk caused by mutations in interferon pathway
• Immunity essential for control !

Prendergast and Jaffee, Cancer Res. (2007)
Prendergast, OncoImmunology (2012)

Superior Cancer Staging by Memory T Cells
CD45RO is a Key Marker



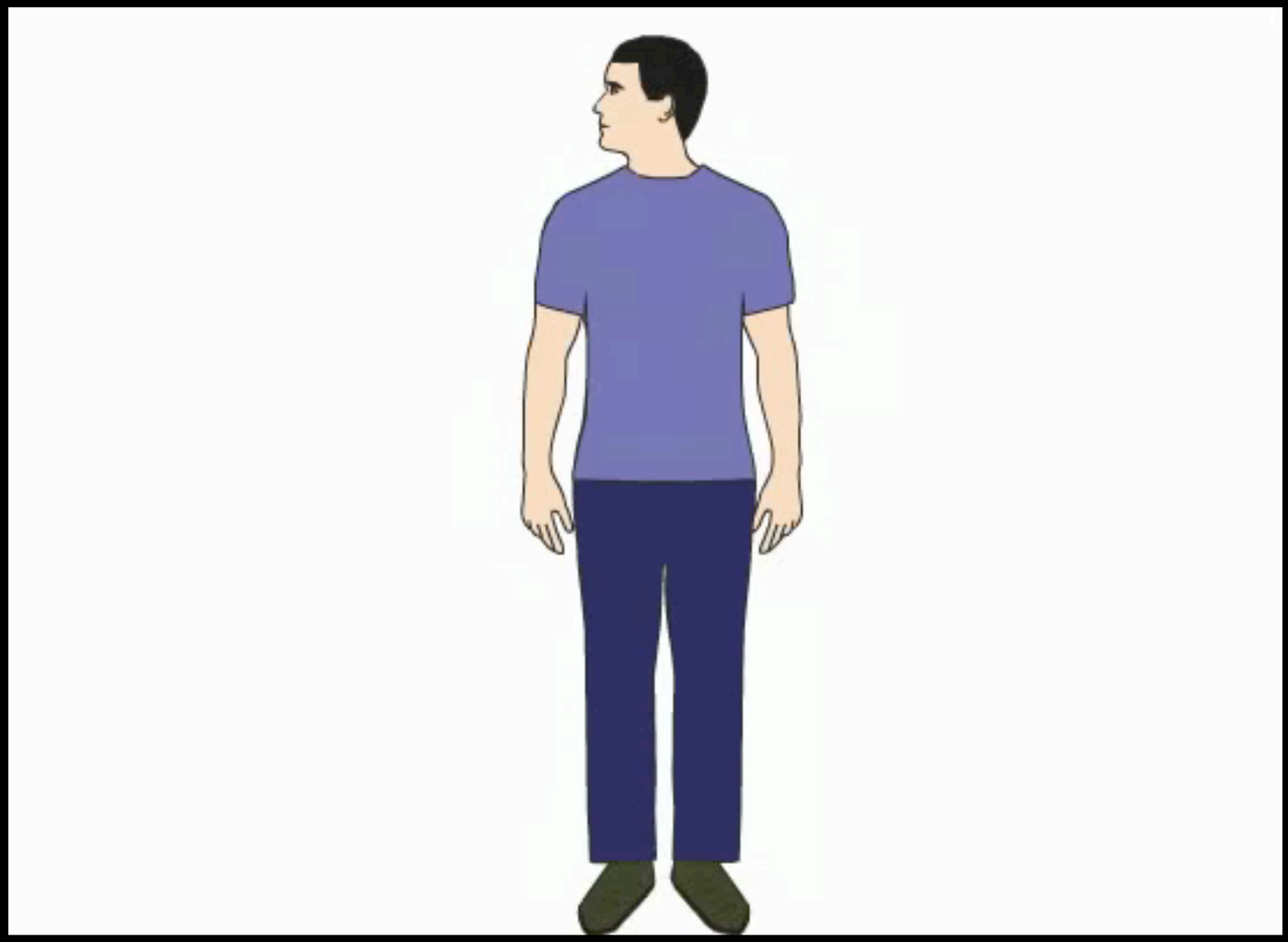
Cancer as a problem of ‘rogue cell’ mismanagement by the immune system

Can one restore immune management ?

What is Immunotherapy?

Drugs Which Recruit the Patient's Natural Immune System to Fight Disease

e.g.
Vaccination



Movie provided courtesy of Dr. Pooja Jain (Drexel University College of Medicine)

Active Immunotherapy
Adds
New Capabilities

Adoptive Cell Therapy
Vaccines (Cells, Biologics)
e.g. CART therapy, Provenge®

Gas

Passive Immunotherapy
Impedes or Promotes
Existing Capabilities

Antibodies
Other Biologics
e.g. Herceptin®, Yervoy®

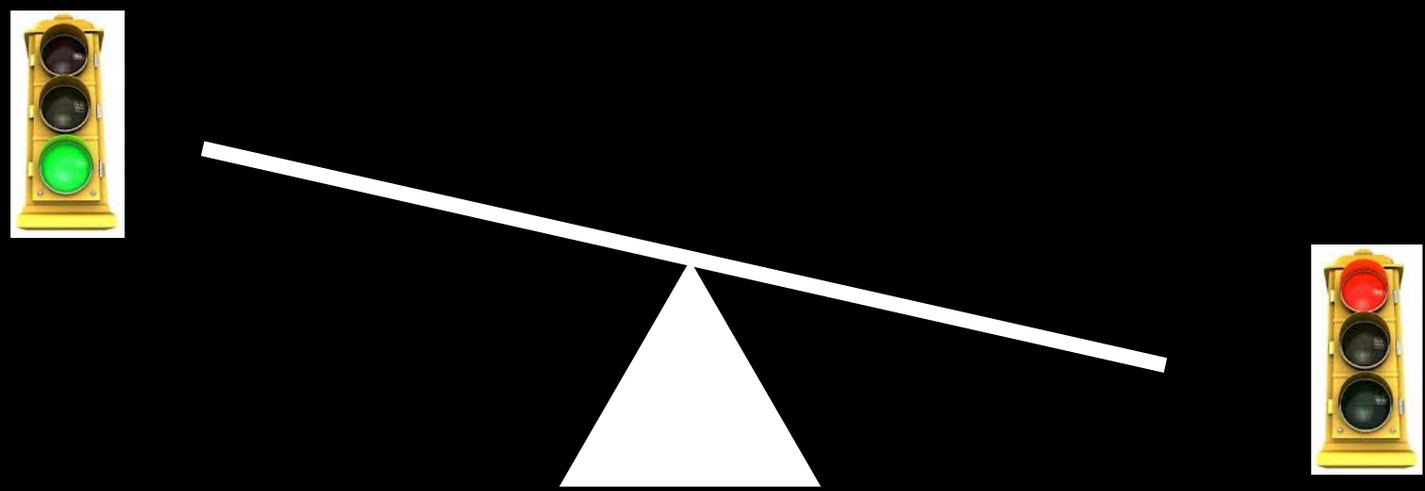
Brake & Clutch

Immunomodulation
Modifies
Existing Capabilities

Inflammatory Modifiers
Immune Adjuvants
e.g. COX2i, Alum (vaccine adjuvant)

Steering Wheel

Active Immunotherapy Has Mainly Failed Historically Because Immune Escape Was Not Understood



Immune Control

Cytotoxic Immune Cells
(T, NK, Innate)

Immune Escape

Supportive Inflammation
T Cell Suppression / Tolerance

Prendergast & Jaffee
Cancer Res. 67:3500 (2007)

“Manifesto”

Perspectives in Cancer Research

Cancer Immunologists and Cancer Biologists: Why We Didn't Talk Then but Need to Now

George C. Prendergast[1,2,3] and Elizabeth M. Jaffee[4,5]

[1]Lankenau Institute for Medical Research, Wynnewood, Pennsylvania; [2]Department of Pathology, Anatomy, and Cell Biology and [3]Kimmel Cancer Center, Jefferson Medical School, Thomas Jefferson University, Philadelphia, Pennsylvania; [4]The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins; and [5]Department of Oncology, Johns Hopkins University, Baltimore, Maryland

Abstract

What is cancer? Cancer is a disease initiated by a series of cumulative genetic and epigenetic changes that occur in a normal cell. However, in addition to the malignant cell itself, cancer is a disease of microenvironment and immunity. Although genetic and epigenetic alterations drive cellular transformation, genomic plasticity, and evolution, it has become increasingly apparent that multiple signals delivered within the tumor microenvironment by modifier genes, stromal and endothelial cells, and immune cells are critical factors in determining the progression versus dormancy or destruction of an initiated lesion and also whether metastasis may occur. With regard to the important roles of immune cells in cancer, a chasm exists between immunologists and biologists: although sharing a common disease interest, there is little history for workers to draw on based on shared perspectives or understanding. How did this disconnect arise? Here, we look at how these workers became separated in the past and address why it has now become critical to spur greater cross-fertilization. In particular, we highlight three ideas that we believe are important for discussion and debate. The first idea is that therapeutic strategies that fail to harness the immune system will always be defeated by tumor resistance, due to the large "genomic space" that genetically plastic tumor cells can readily access to evolve resistance mechanisms. Because all therapies drive tumor progression by imposing a selection for resistant cells, harnessing the adaptivity of the immune system will be indispensable to ultimately stanching the deadly adaptability of the tumor cell. The second idea is that using molecular targeted agents to reverse tumoral immune suppression may offer a powerful method to leverage the efficacy of most if not all therapeutic agents. We suggest that the mechanisms that support evolution of a "smoldering" inflammatory environment in cancer overlap with those that support evolution of tumoral immune escape. If true, relieving immune suppression will switch the inflammatory state from supportive to destructive for the tumor. The third idea is that by ablating immunosuppression mechanisms, cytotoxic chemotherapy might synergize with, rather than antagonize, active immunotherapy. Provocative preclinical studies in this area prompt clinical attention. We believe that increased efforts to intermingle the perspectives and work of cancer immunologists with cancer biologists and pharmacologists will be needed to realize the National Cancer Institute's goal of managing cancer in the clinic by 2015. [Cancer Res 2007;67(8):3500–4]

Historical Segregation of Cancer Immunology from Cancer Genetics and Cell Biology

"I can't understand why people are frightened of new ideas. I'm frightened of the old ones." (attributed to the composer John Cage, 1912-1992)

Starting about 1980, investigations in cancer genetics and cell biology began to assume the prominence in cancer research that they now hold today. Hatched initially from studies of avian and mammalian tumor viruses, the field of cancer genetics has been the dominant contributor to our understanding of the biological pathways involved in tumor development, identifying many specific targets for therapeutic intervention in cancer cells. With the discovery of oncogenes, the once radical idea that cancer was a disease of normal cellular genes gone wrong now became established as the dominant idea in the field. Importantly, this new concept began to strongly influence how to develop new cancer drugs, that being to attack the products of these altered genes. At the same time, these developments in the field outpaced concepts of cancer as a systemic disease involving perturbations in the immune system. Now, after decades of mutual skepticism, a historically important consensus among cancer researchers is emerging about the causality of chronic inflammation and altered immunity in driving malignant development and progression. Ironically, this synthesis is having the effect of making the "new" genetic ideas of the past two decades about cancer seem naive and outdated. In particular, it is becoming apparent that the tumor cell–centric focus championed by cancer genetics is unlikely to give a full understanding of clinical disease, in the absence of knowing about the systemic and localized tissue conditions that surround and control the growth and activity of the tumor cell. Perhaps contributing to some consternation about the conceptual weight of the "new" ideas since the 1980s, few of the molecular therapeutics developed from them have had much major clinical effect (the Bcr-Abl kinase inhibitor Gleevec being perhaps the most notable exception to the rule for a cancer driven essentially by a single oncogenic pathway).

Over the past 25 years, as a result of historical and scientific divisions, there has been limited communication, understanding, and collaboration among tumor immunologists, molecular geneticists, and cell biologists working in the field. For the latter groups, a major disconnect was the perception that the immune system did not seem to be very important to tumor development in laboratory animals, produced by experiments in nude mice in the 1960s that were argued to weaken the concept of tumoral

Note: E.M. Jaffee is the first recipient of the Dana and Albert "Cubby" Broccoli Professorship in Oncology at The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins.

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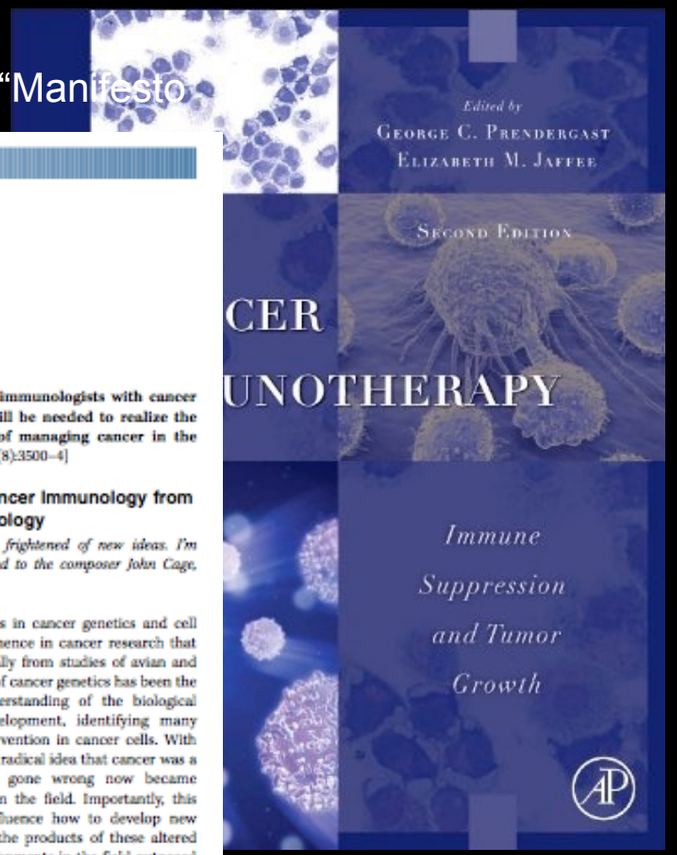
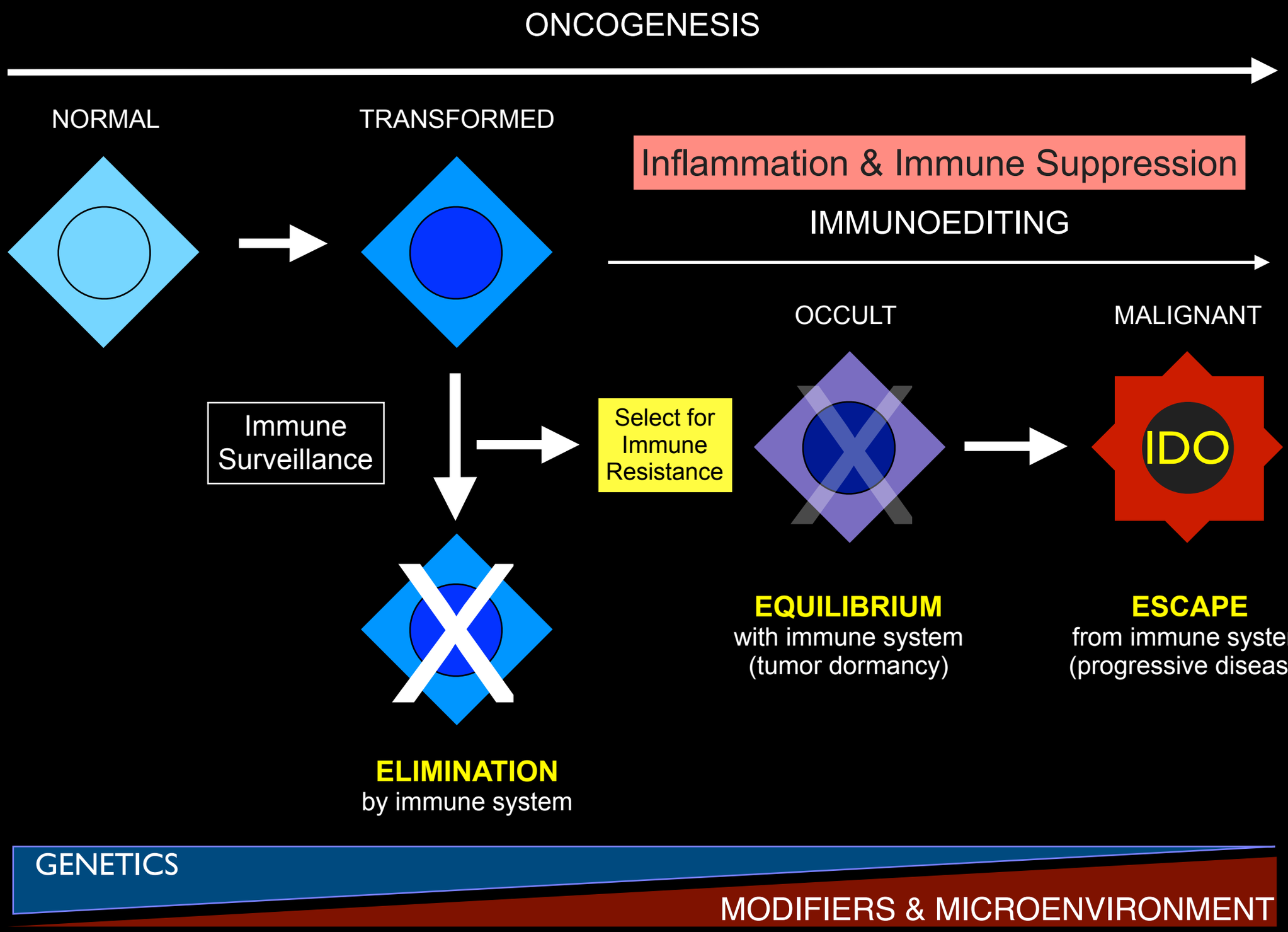
doi:10.1158/0008-5472.CAN-06-4626



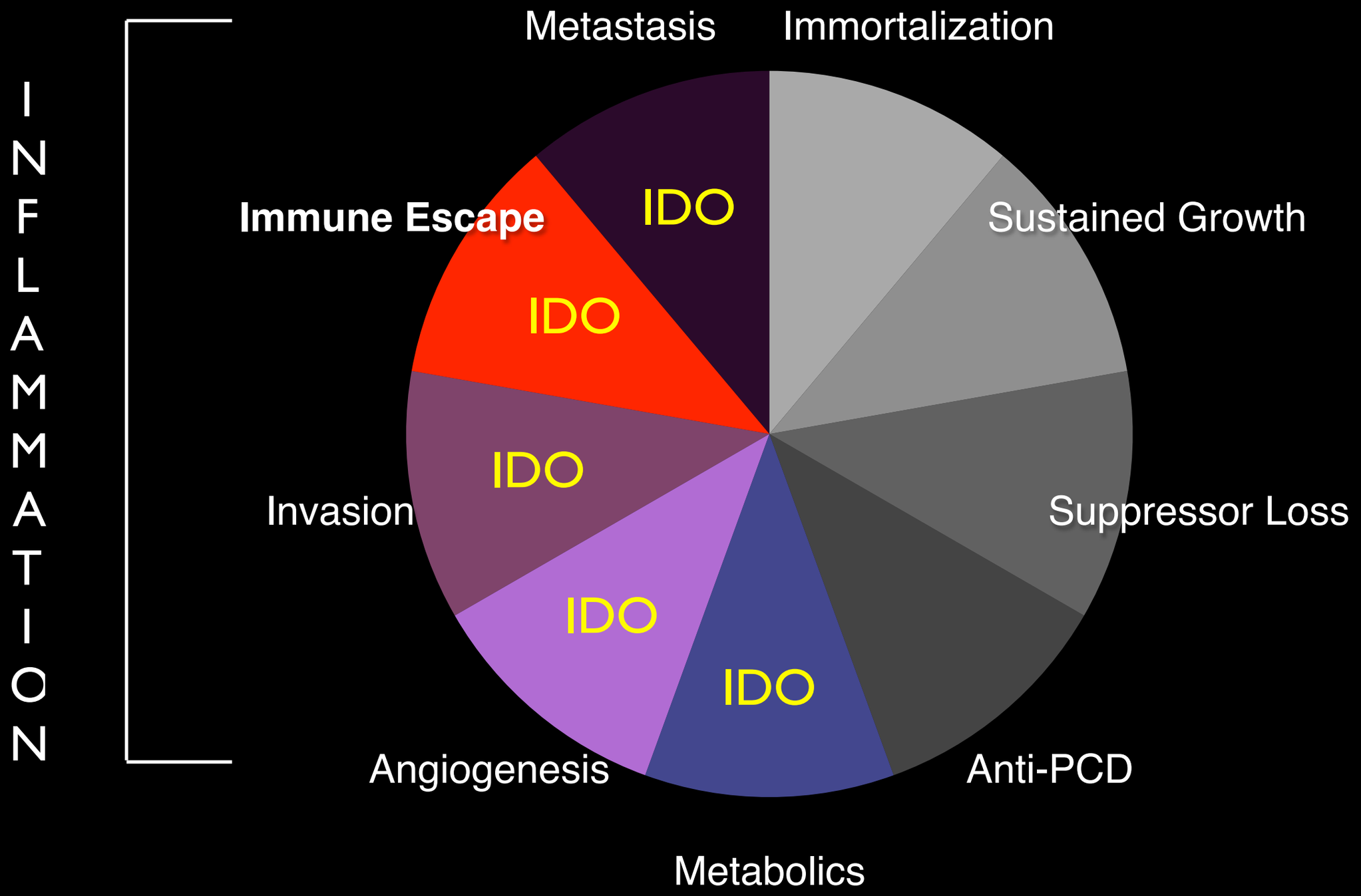
1. Cancer cell-centric therapy is inherently flawed due to the selection problem (therapy resistance)
2. Tumor microenvironment must be reprogrammed
3. Immune suppression must be relieved

How do cancer cells escape immune control ?

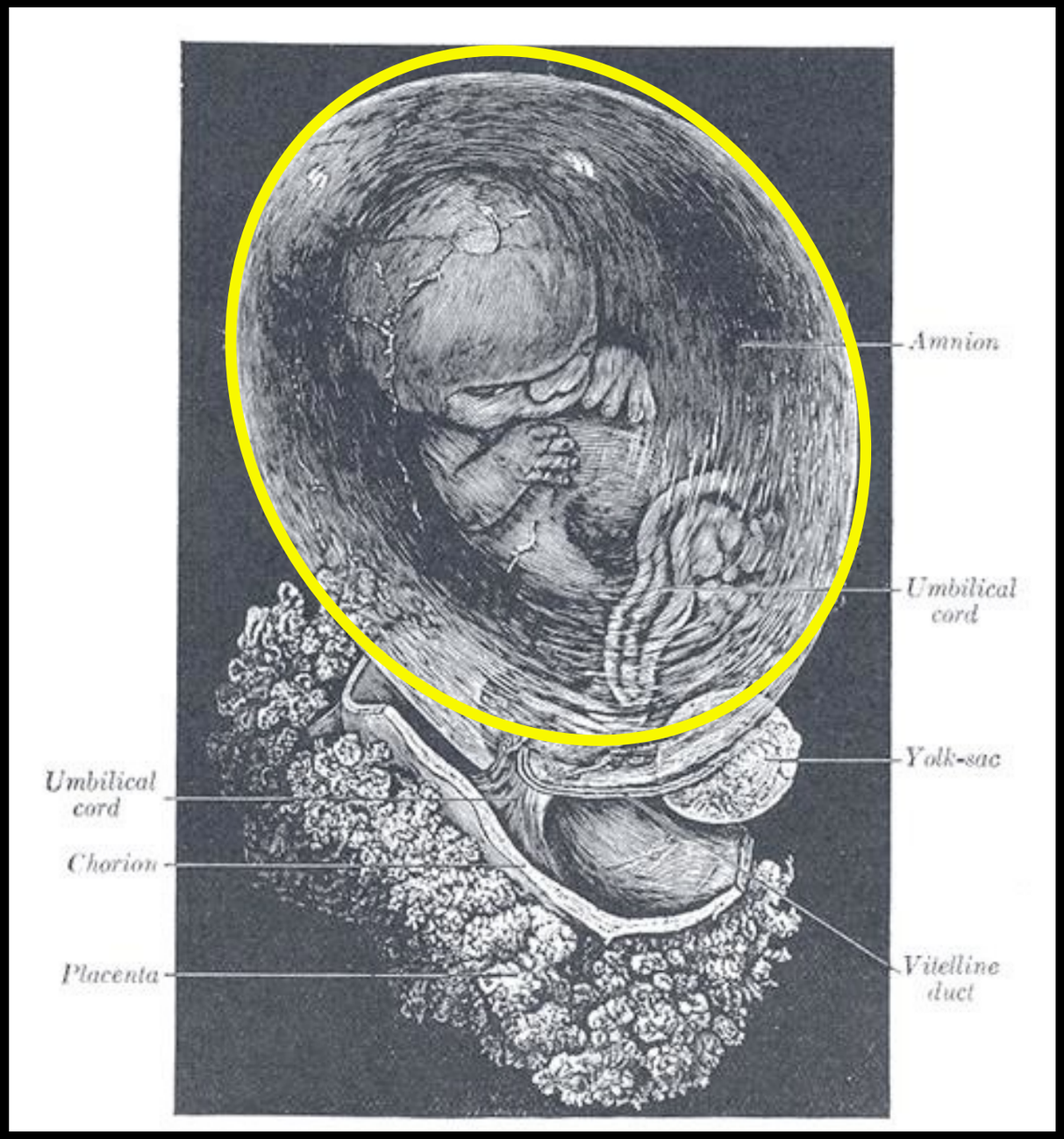


Prendergast, Oncogene (2008)

IDO may program an inflammatory state that supports several aspects of cancer progression



What is IDO ?



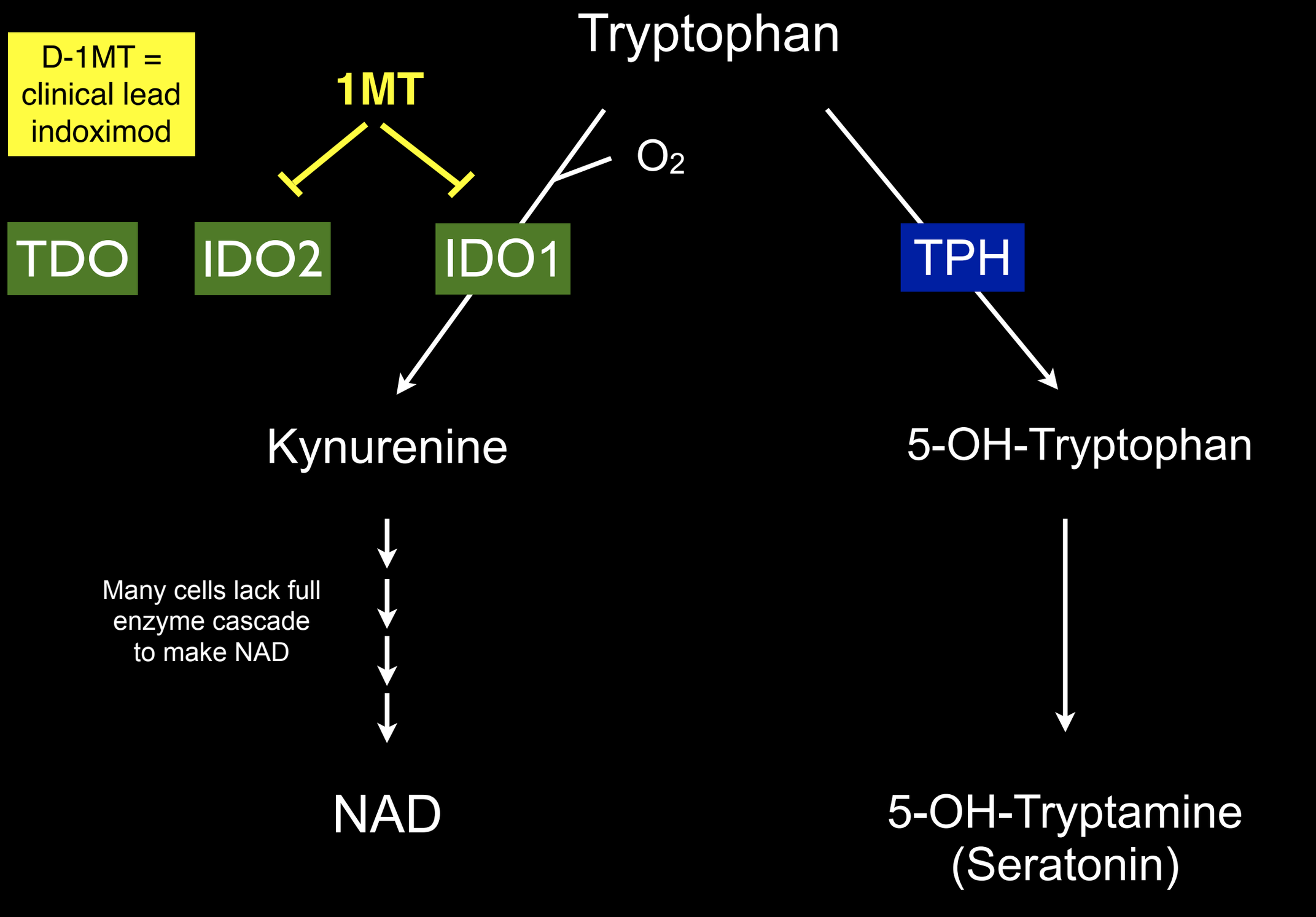
- Single-chain cytosolic enzyme that catabolizes tryptophan

- Implicated in T cell tolerance by evidence that IDO may protect allogeneic fetus against maternal immune attack

Munn, Mellor and colleagues, Science (1999)

IDO is one of four enzymes that catabolize Trp

All implicated in immune modulation



IDO is widely deregulated in human cancer

Table 1 Expression of IDO in human tumors

Tumor type	IDO-positive tumor samples[a] (no. positive per no. tested)	Proportion of IDO-positive tumor cells[b]		
		>50%	10–50%	<10%
Prostatic carcinomas	11/11	7	3	1
Colorectal carcinomas	10/10	5	3	2
Pancreatic carcinomas	10/10	8	2	0
Cervical carcinomas	10/10	0	4	6
Endometrial carcinomas	5/5	0	3	2
Gastric carcinomas	9/10	4	3	2
Glioblastomas	9/10	6	3	0
Non-small-cell lung carcinomas	9/11	1	1	7
Bladder carcinomas	8/10	3	1	4
Ovarian carcinomas	8/10	0	3	5
Head and neck carcinomas	7/11	0	3	4
Esophageal carcinomas	7/10	1	2	4
Mesotheliomas	6/10	2	1	3
Renal cell carcinomas	5/10	0	1	4
Melanomas	11/25	0	0	11
Breast carcinomas	3/10	2	0	1
Thyroid carcinomas	2/10	0	0	2
Lymphomas	4/18	0	0	4
Small-cell lung carcinomas	2/10	0	0	2
Sarcomas	2/10	0	1	1
Hepatocarcinomas	2/5	0	0	2
Adrenal carcinomas	2/5	1	0	1
Choriocarcinomas	1/5	0	0	1
Cutaneous basocellular carcinomas	1/5	0	0	1
Testicular seminomas	0/10	0	0	0

[a]Expression of IDO protein was detected by immunohistochemistry using purified IDO-specific rabbit antibodies. Specificity of staining was controlled by blocking with a synthetic peptide corresponding to the C terminus of IDO (**Fig. 2**). [b]Number of tumor samples with the indicated proportion of IDO-positive tumor cells is given in each column. The proportion of positive tumor cells was estimated visually.

Van den Eynde and colleagues, Ludwig Institute for Cancer Research, Brussels

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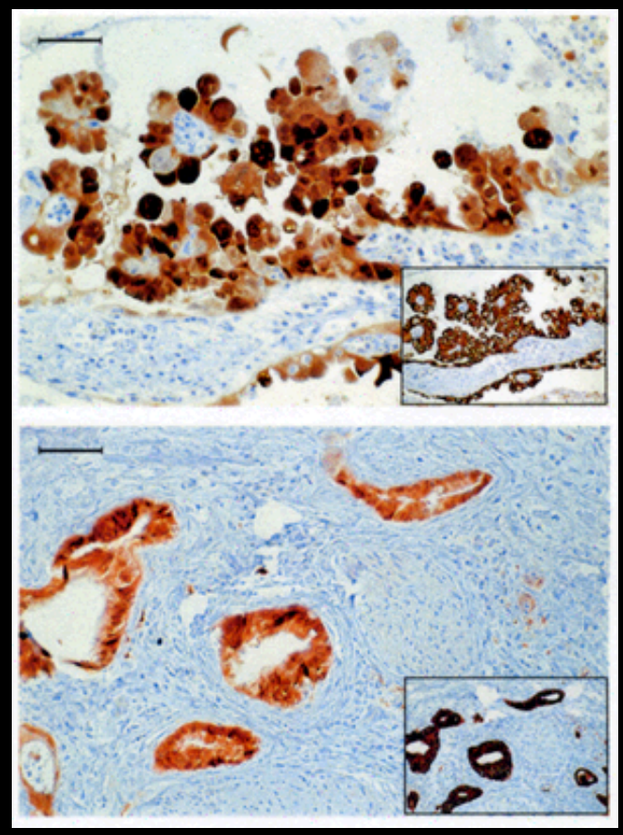
IDO overexpression in tumor cells is common



TDO and **IDO2** also found upregulated in certain cancers (not as common)

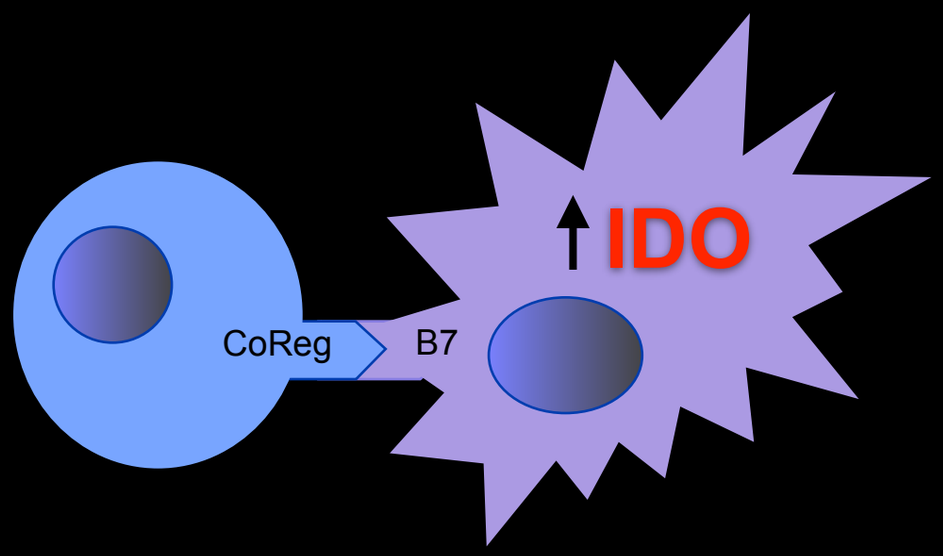
Suppressor gene Bin1 which is widely attenuated in human cancer normally acts to limit IDO1 overexpression

IDO may act at multiple sites to blunt antitumor immunity

TUMOR DRAINING LYMPH NODE

TUMOR

APC

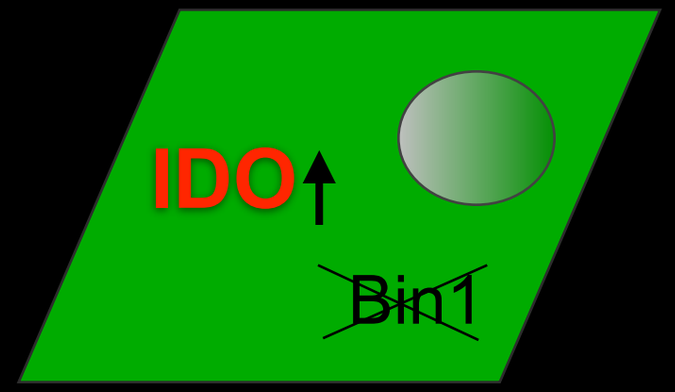


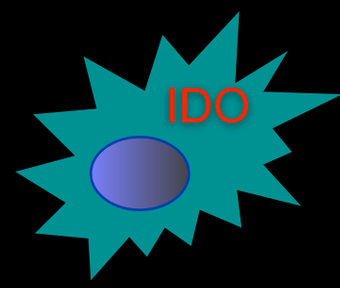
Trp

Kyn

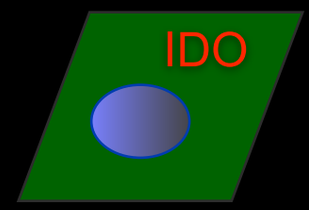
Cancer cell

Cancer stem-like cells



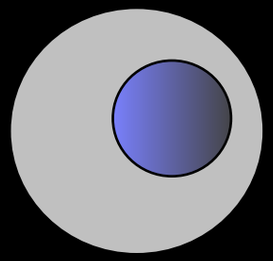


TAMs



Mesenchymal stem cells
Endothelial cells

↑ Treg

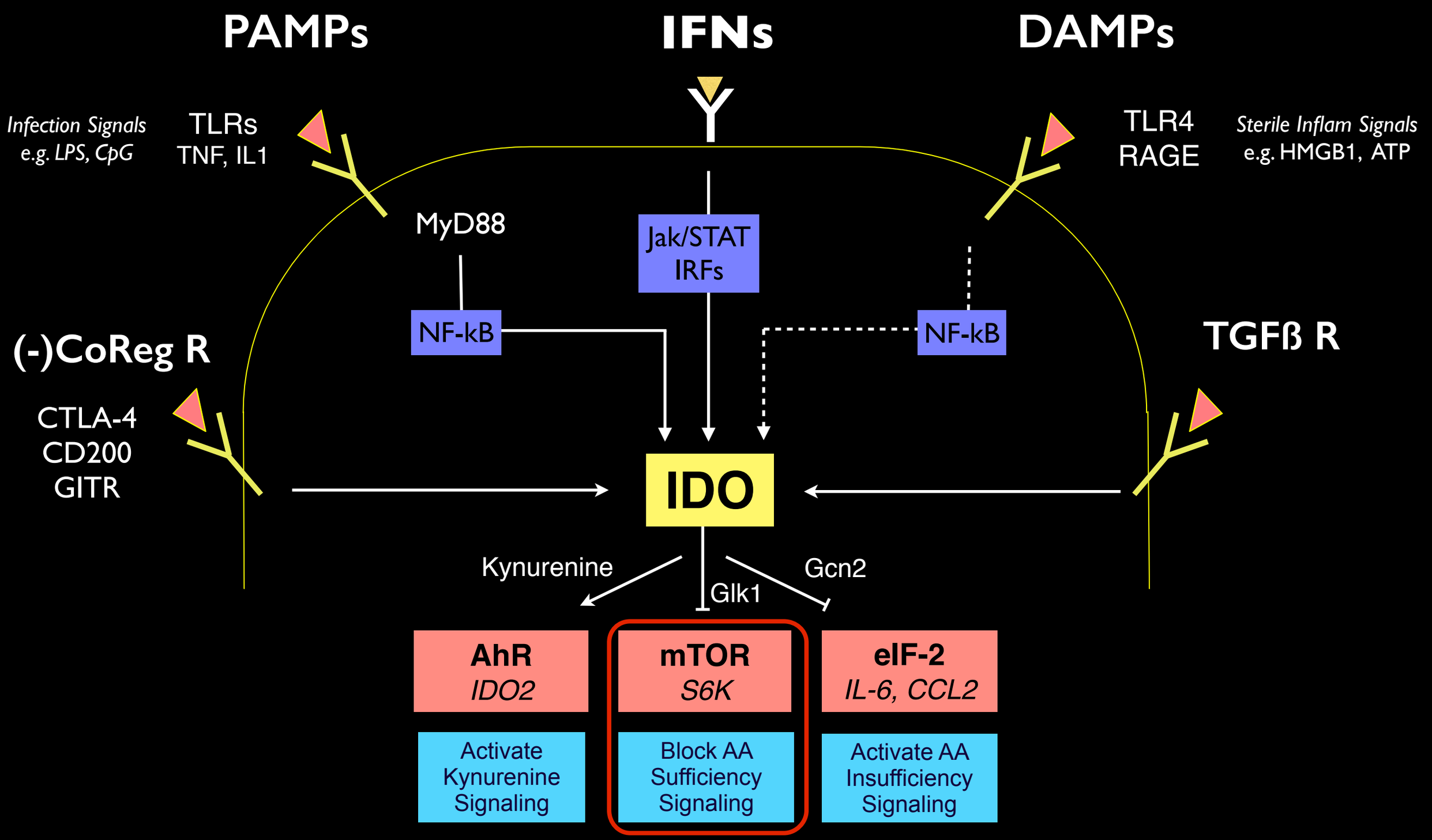


Effector T cells

↑ MDSC

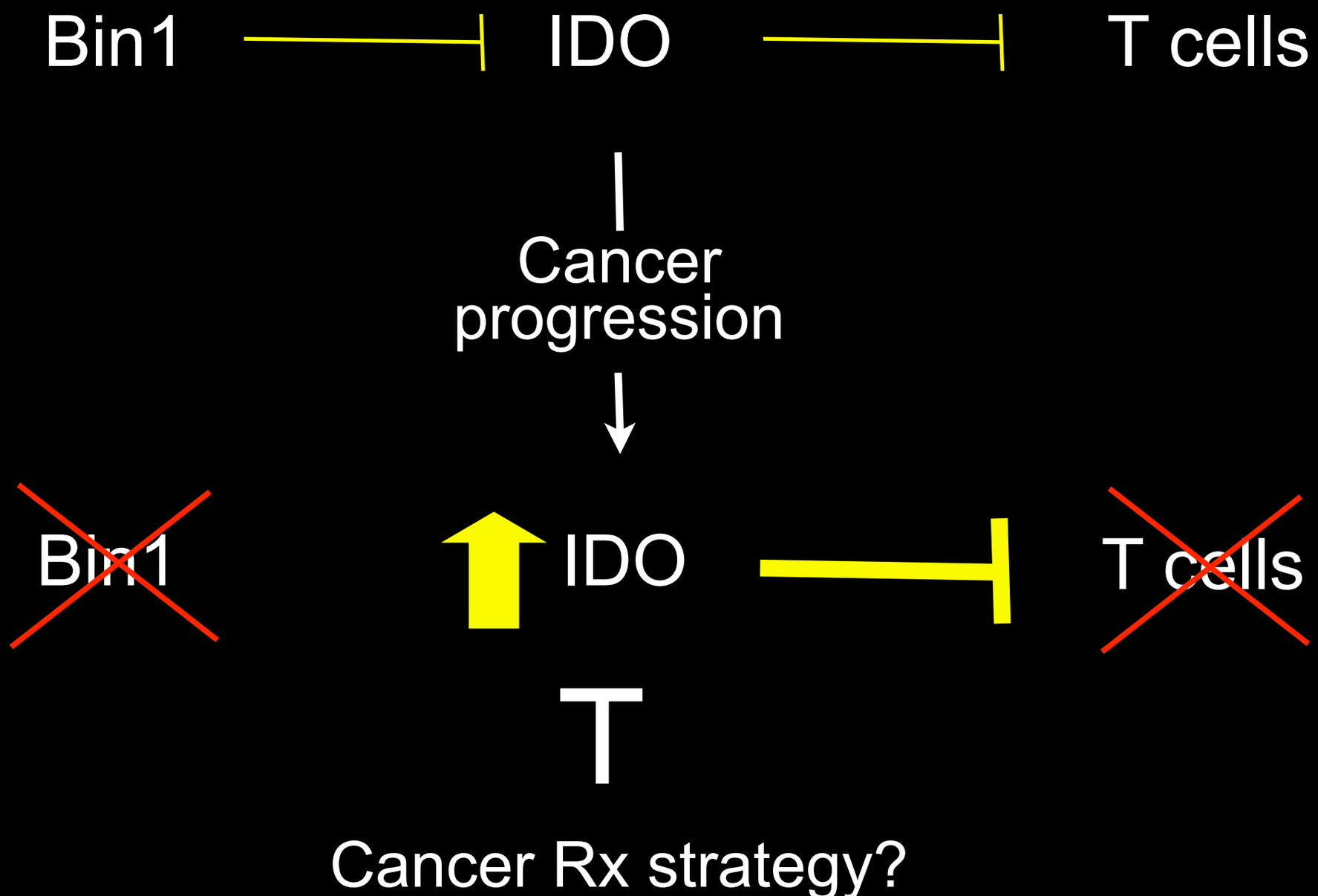
Munn, Mellor and colleagues
Fallarino, Grohmann, Orabona, Pucetti and colleagues

IDO is a modifier of inflammation and adaptive immunity



Metz et al. Oncoimmunology (2012)

Our studies of the tumor suppressor gene Bin1 led us to identify IDO as a critical target for Bin1 control



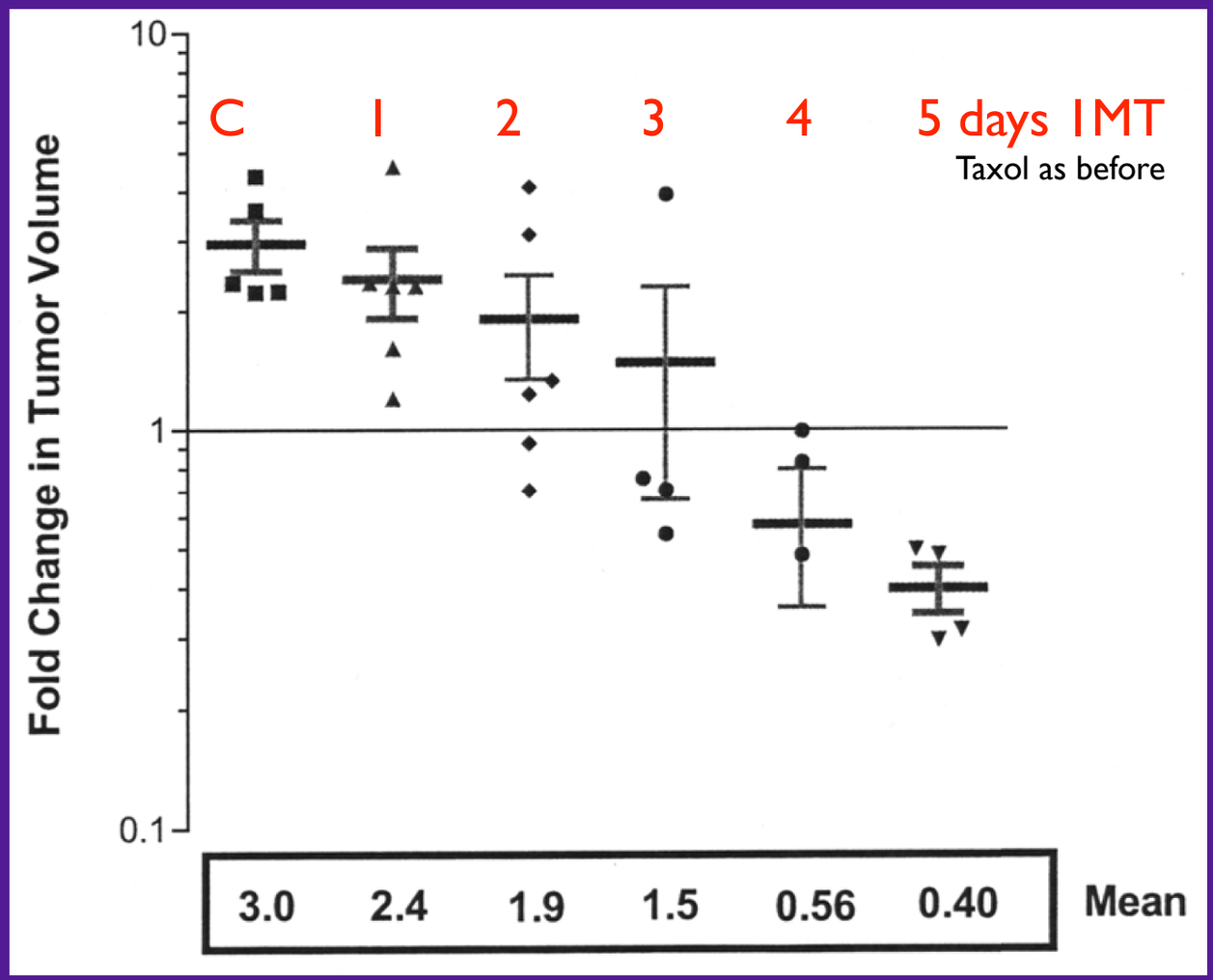
Cancer Rx strategy?

Muller et al., Nature Med. (2005)
Muller & Prendergast, Cancer Res. (2005)
Prendergast et al., BBA Cancer (2009)

IDO inhibitors powerfully enhance the efficacy of ‘immunogenic’ chemotherapy

MMTV-neu
Mouse model of HER2+ breast cancer

Short-term dosing causes regression at 2 wk



Response abolished by ablation of CD4+ or CD8+ T cells

Muller et al. Nature Med. (2005)

New classes of orally bioavailable IDO inhibitors we discovered displayed similar in vivo properties

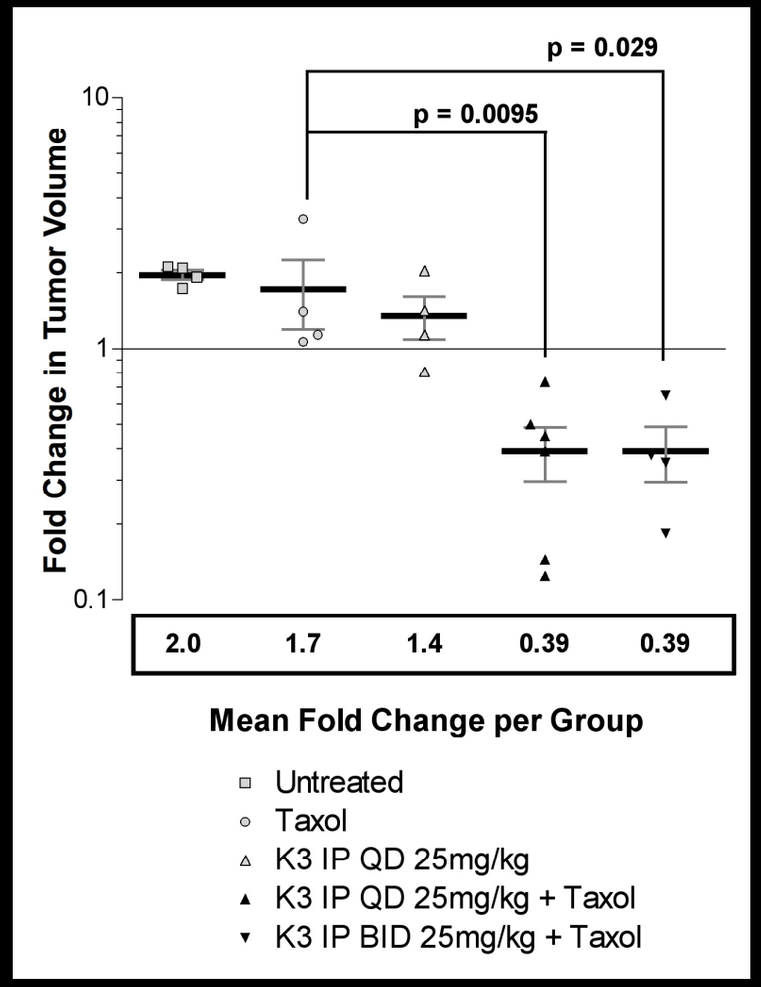
Thiohydantoins
Brassinins
- Plant phytoalexin, chemopreventive
- Some clinical experience

Naphthoquinones
- Known anticancer properties
- Clinical experience

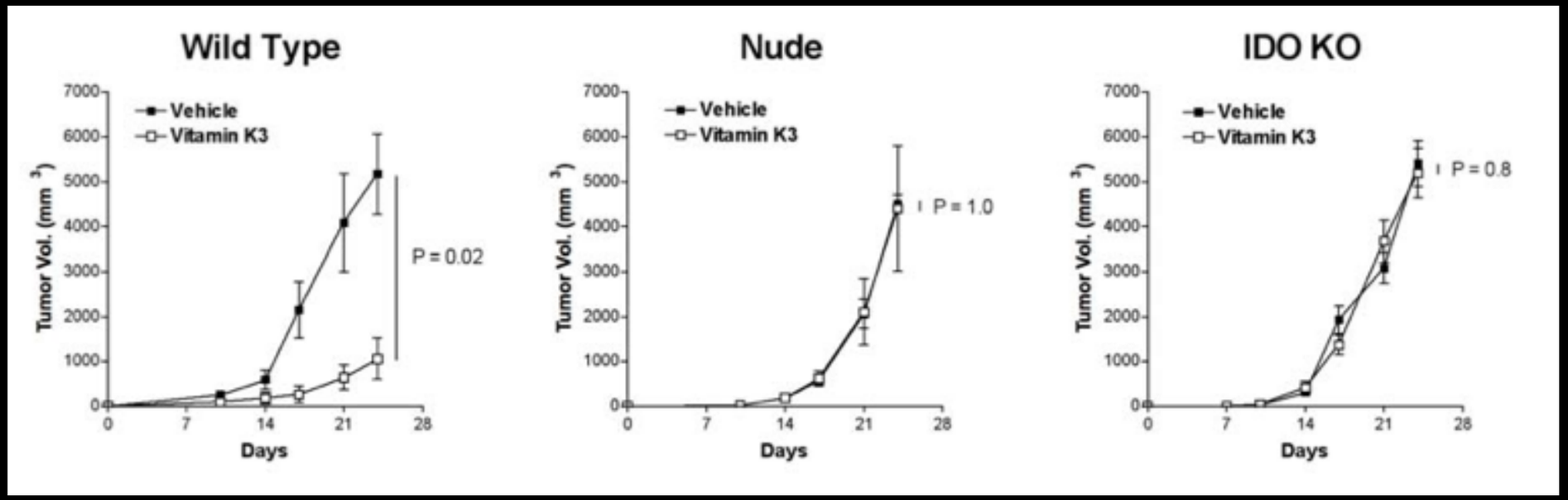
Phenylimidazoles
Hydroxylamines

Enter Phase II 2013 *(NewLink Genetics)*

MMTV-neu
BRCA model



B16 graft
(IDO negative tumor)



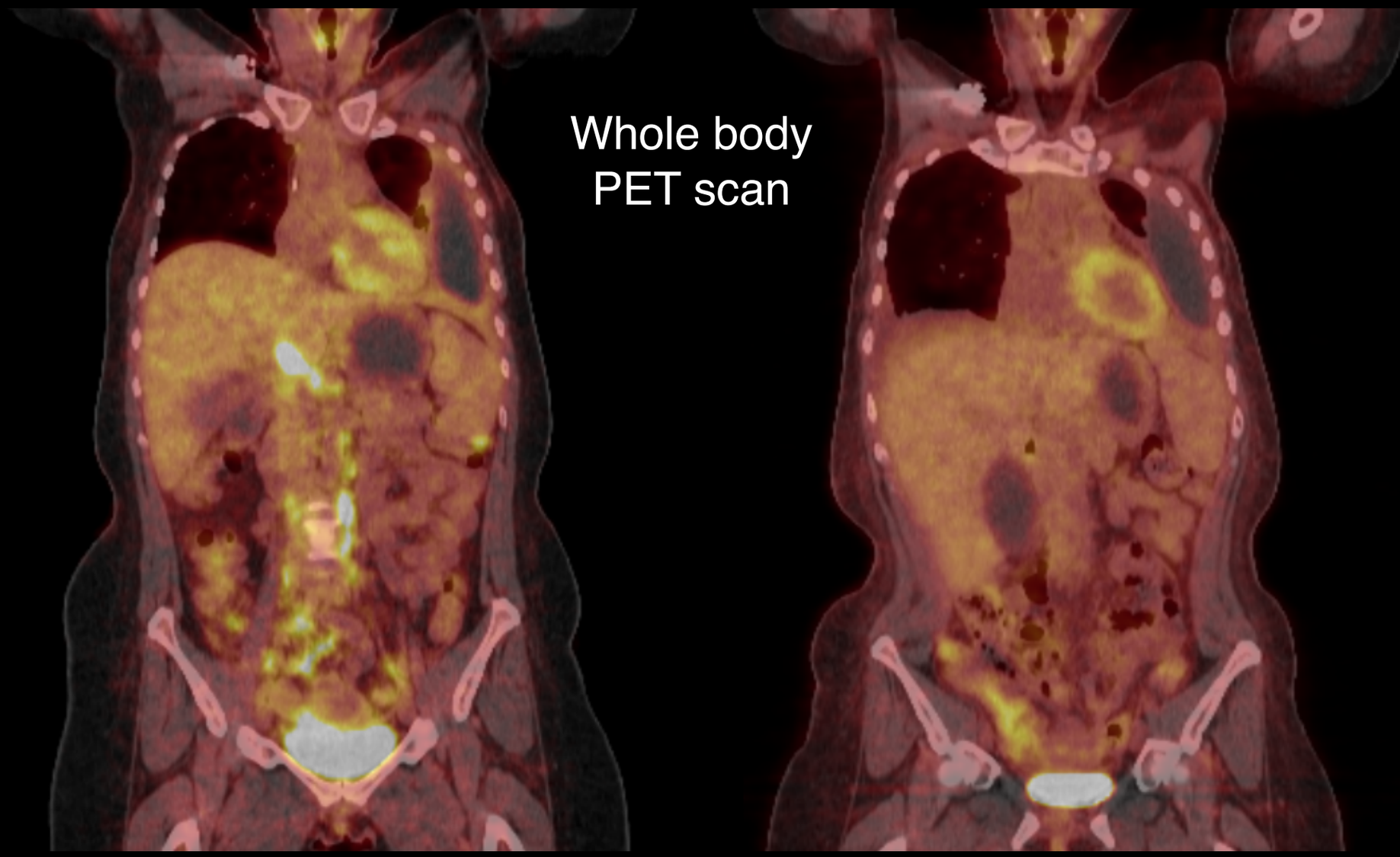
Nature Med. (2005)
J Med Chem (2006)
J Med Chem (2008a)
J Med Chem (2008b)
Oncogene (2008)

Phase 1B trial: Taxotere Combination with Indoximod

Intriguing responses in stage 4 BRCA patients

Pre-treatment

One month treatment



Whole body
PET scan

SOC taxotere +
800 mg p.o. indoximod q.d. (28 day cycles)

Courtesy of Hatem Soliman MD (Moffitt Cancer Center)

Is IDO critical for cancer development or progression?

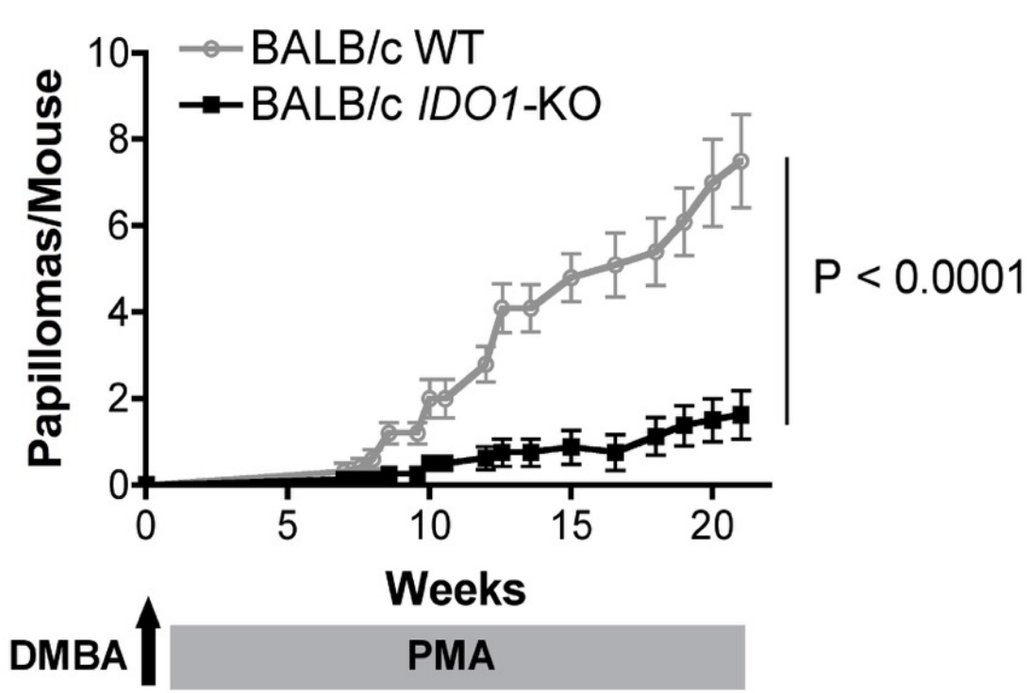
If so, how does it contribute to cancer?

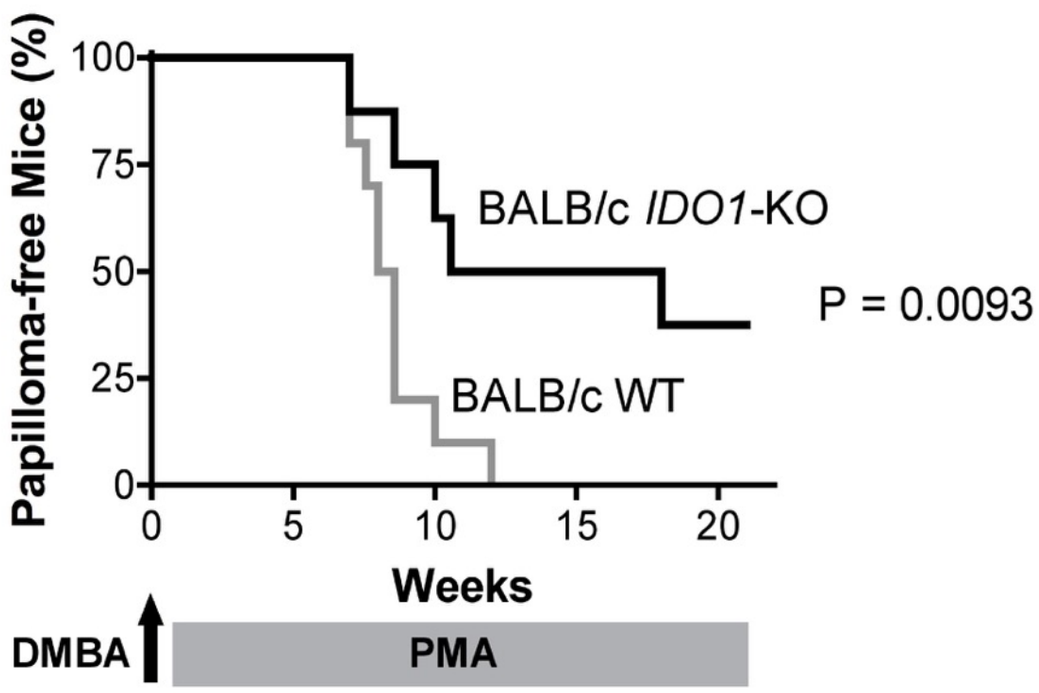
What is the basis for the anticancer effects of IDO inhibitors?

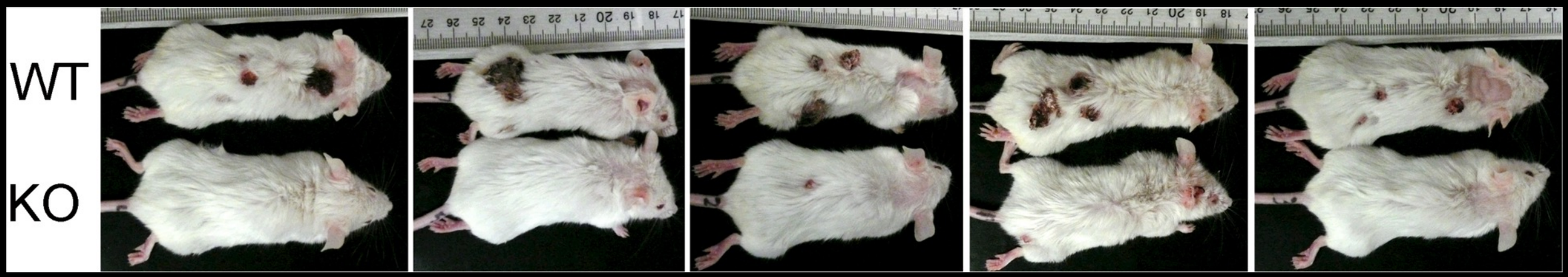
Genetic investigations in IDO deficient mice

IDO1 is essential for inflammatory carcinogenesis

- Classical model of inflammatory cancer: two-stage skin carcinogenesis
- No precocious autoimmunity or inflammation in IDO1–/– mouse





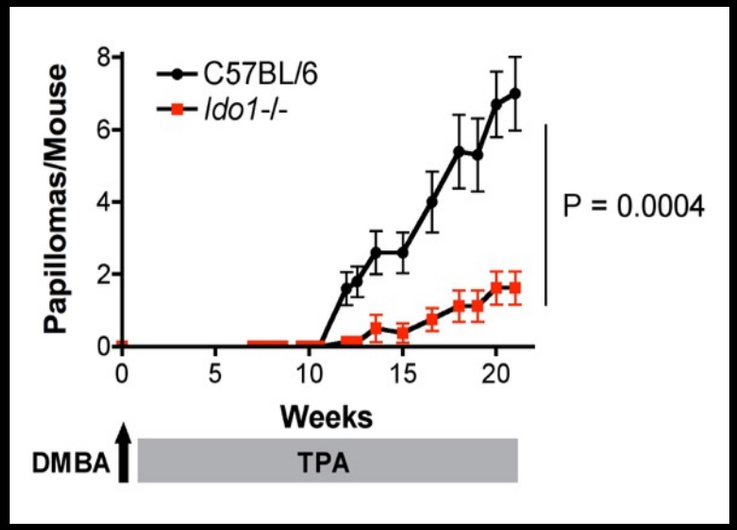


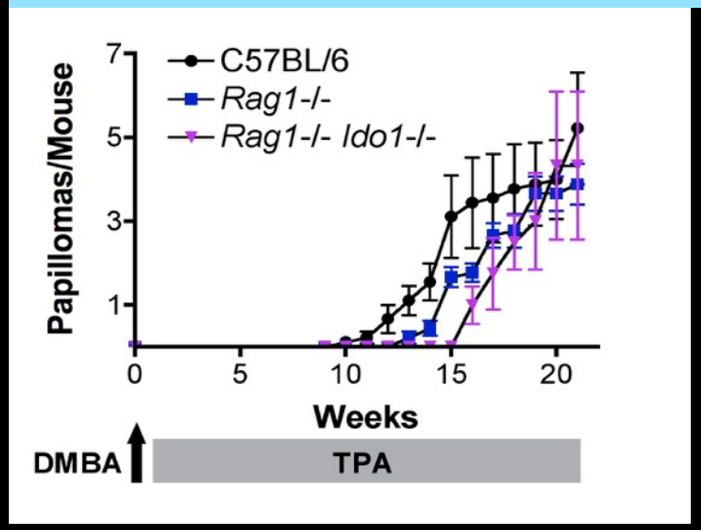
Muller et al. PNAS (2008)

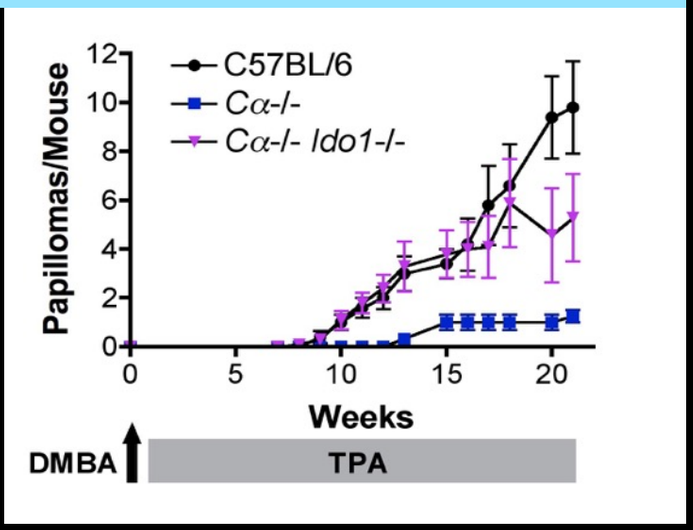
T cell immunity mediates the anticancer benefits of IDO loss

IDO1 double knockout mice

T







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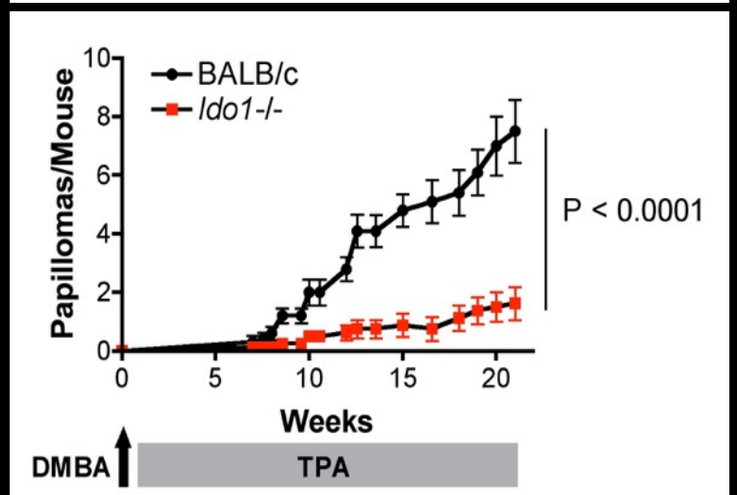
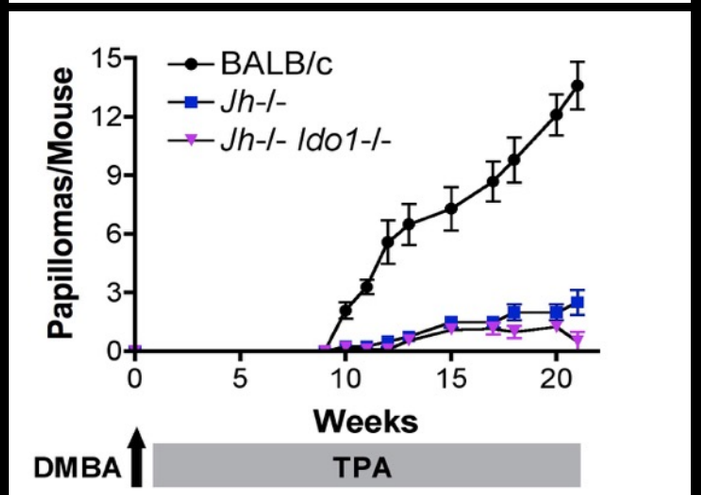
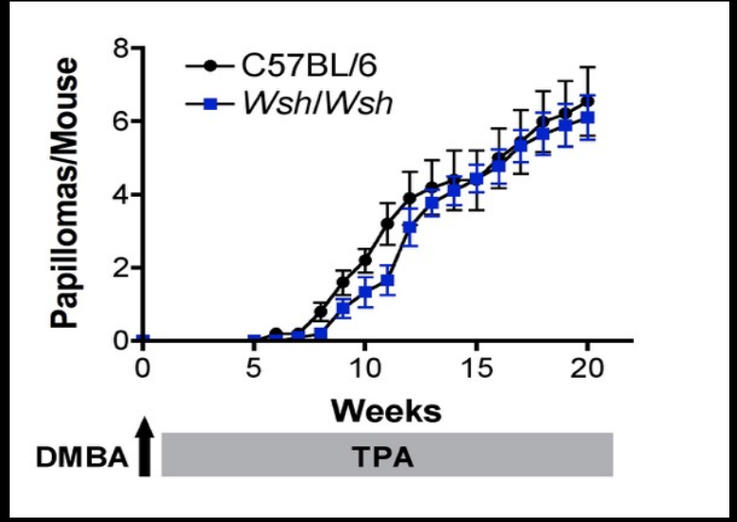
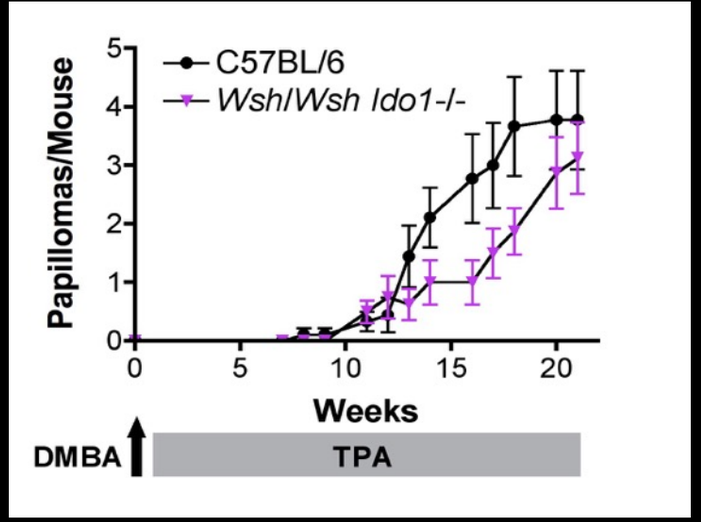




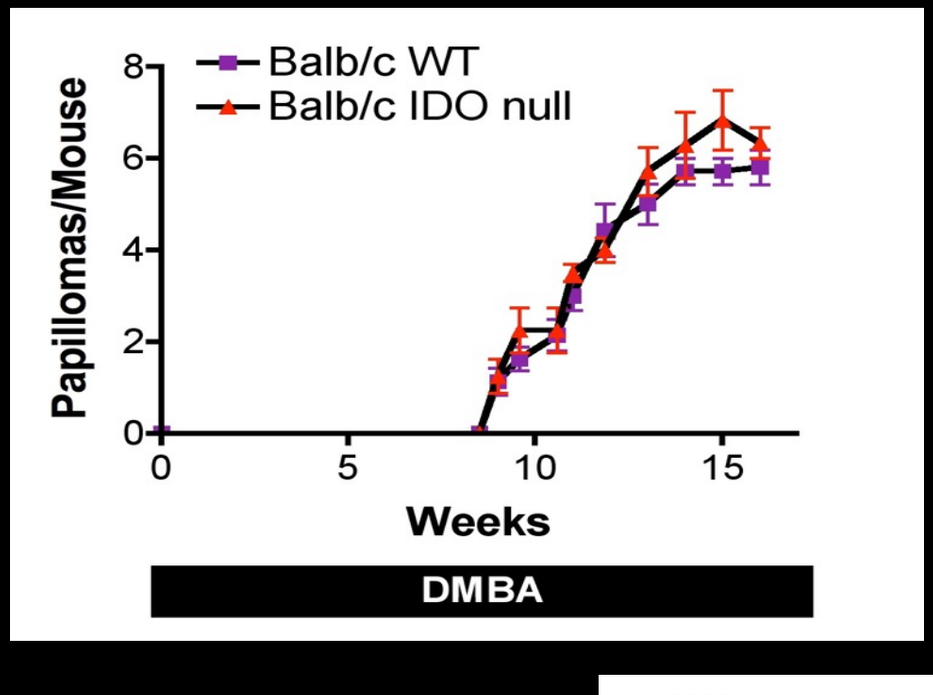
Mast



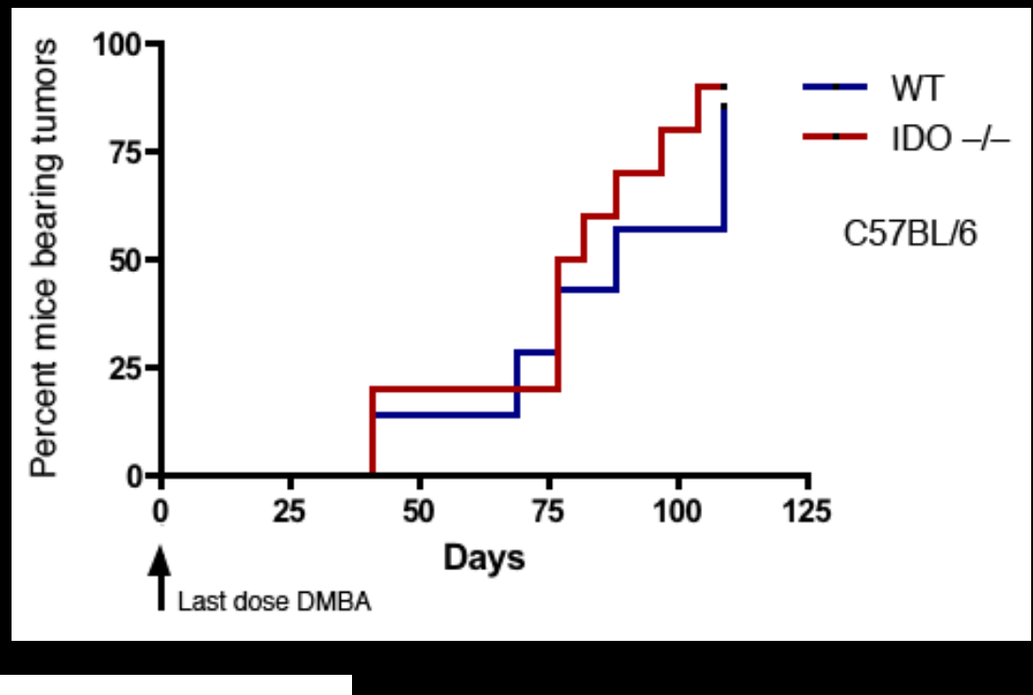


Is IDO critical for cancer *per se* ? No.

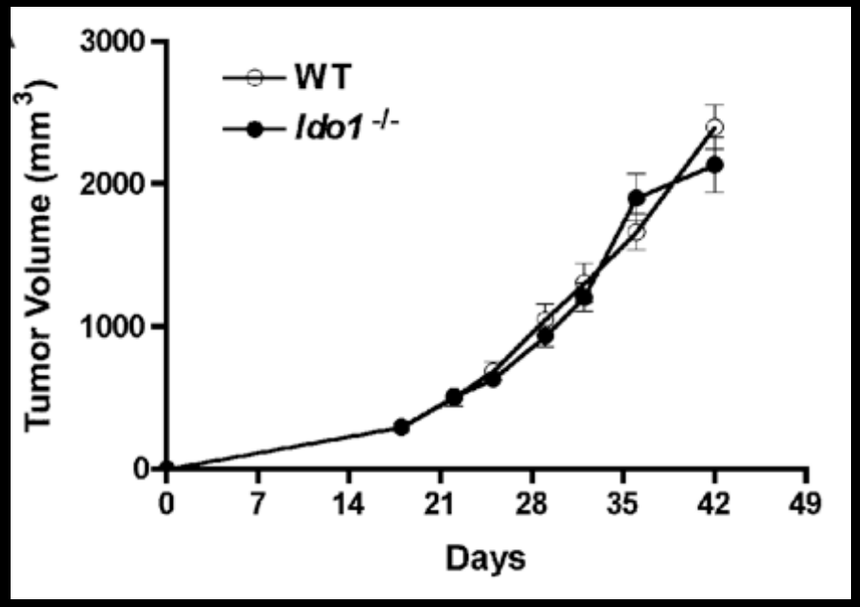
Complete skin carcinogenesis
(topical DMBA only)



Mammary carcinogenesis
(i.p. DMBA + progesterone)



No effect on growth of
primary tumor grafts
(e.g. 4T1 BRCA cells)



IDO programs a
‘cancerous
inflammation’

Muller et al. Cancer Immunol Immunother (2010)

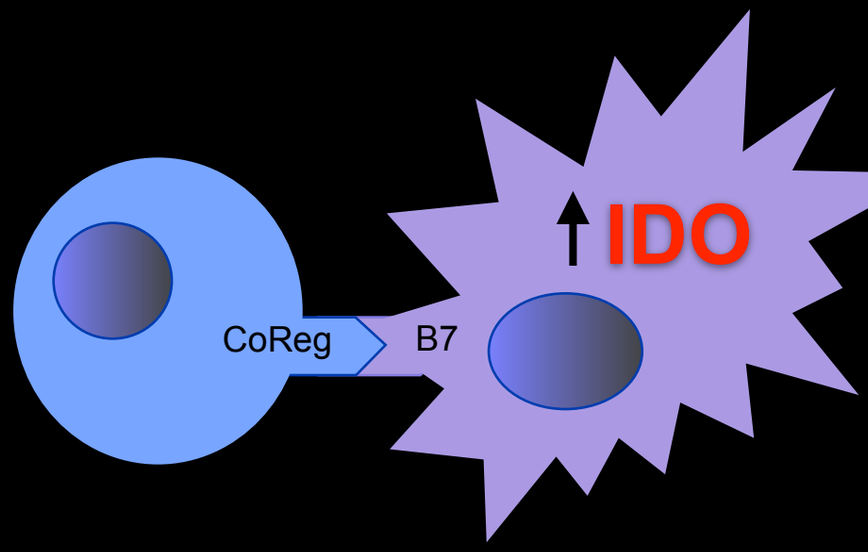
Where does IDO act to support inflammatory cancer ?

TUMOR DRAINING LYMPH NODE

TUMOR

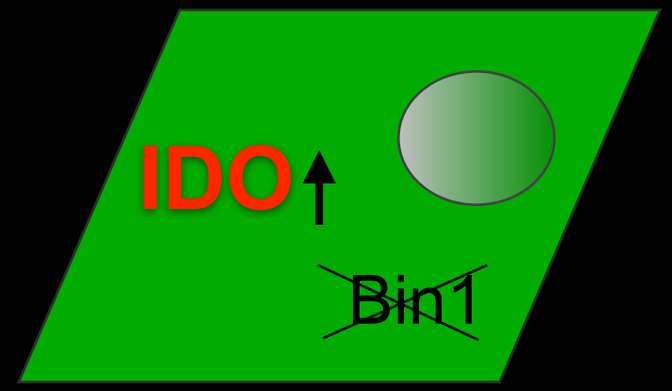
T cell

Antigen presenting cell



Trp

Kyn

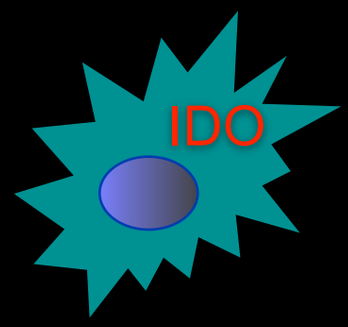


Cancer cells

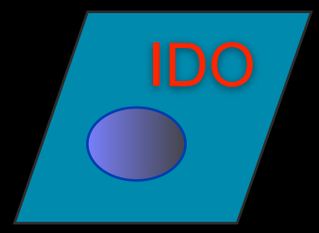
Cancer stem-like cells

Treg

Effector T cells



TAMs
TANs



Mesenchymal
stem cells
Endothelial cells

MDSC

IDO function crucial mainly outside hematopoietic cells ?

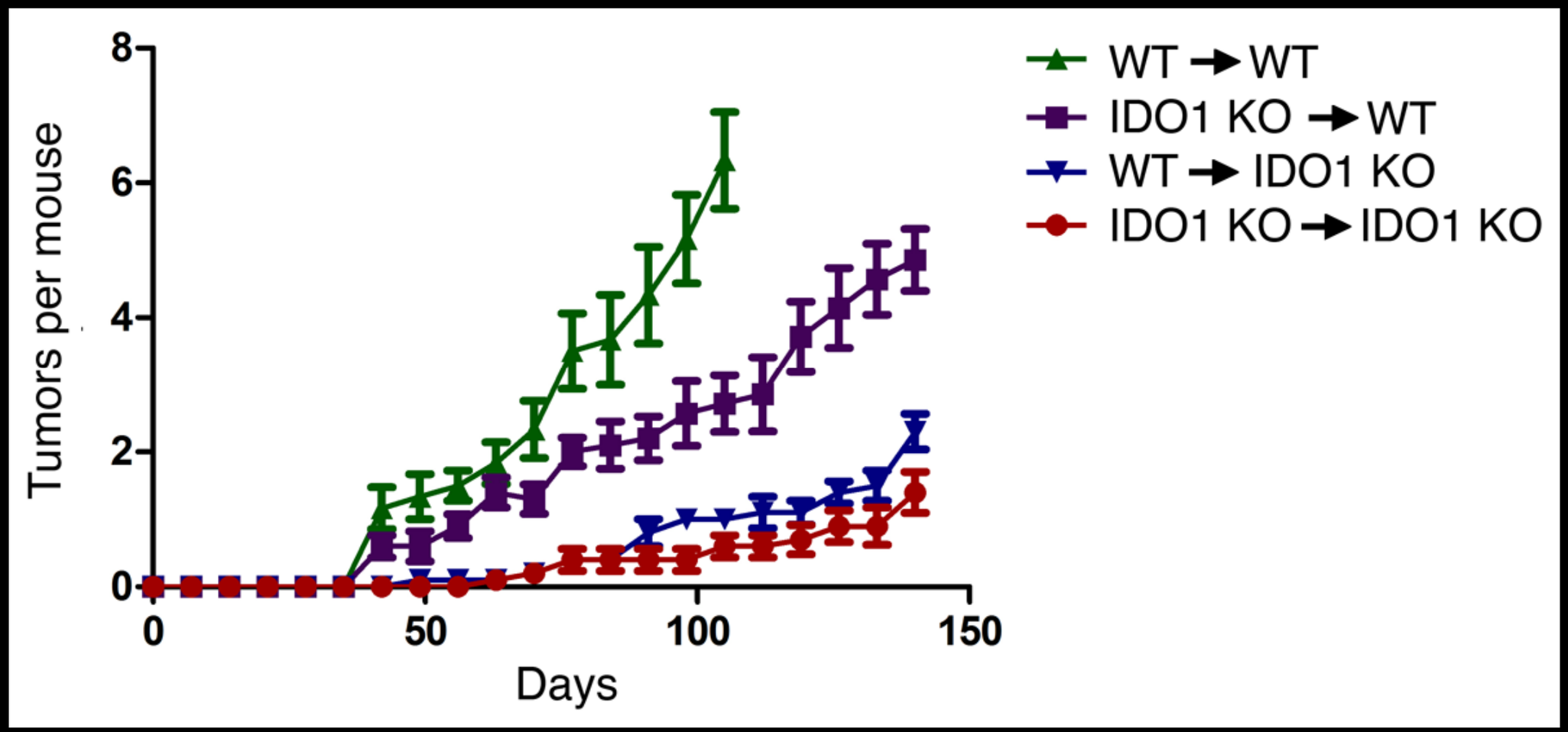
Irradiate (XRT)

Bone marrow transplant

Recover

Inflamm skin carcinogenesis

Score tumors



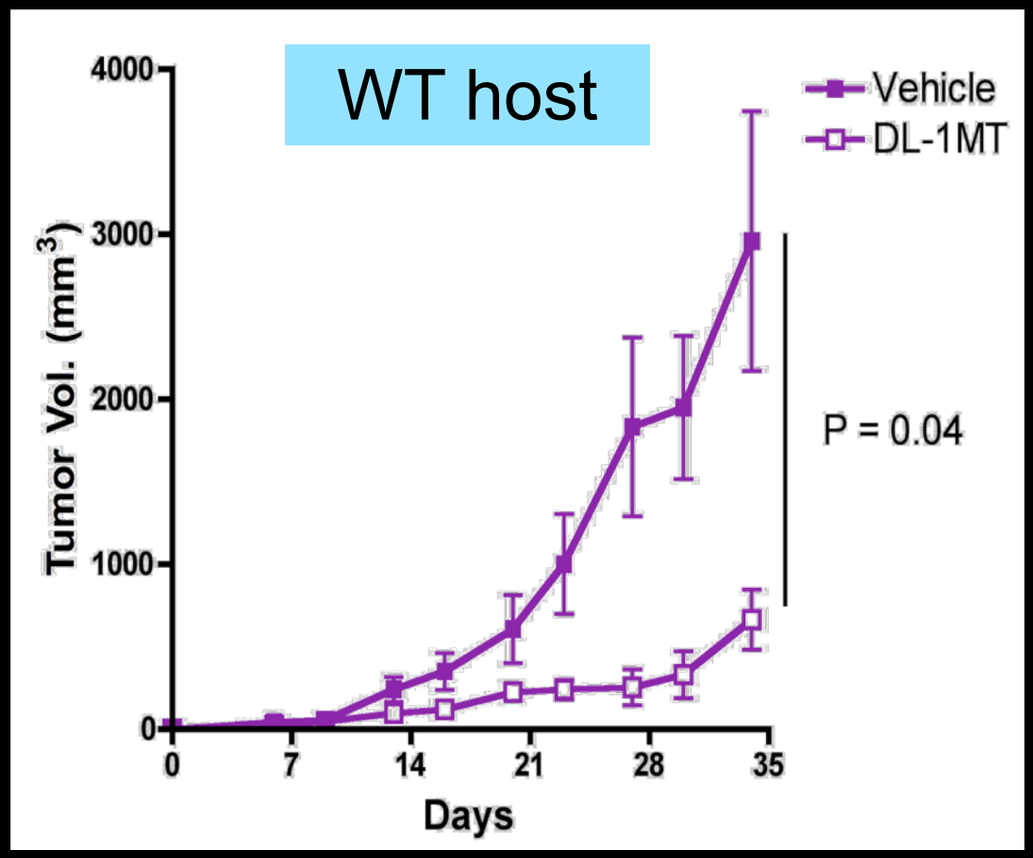
Muller et al. Cancer Immunol Immunother (2010)

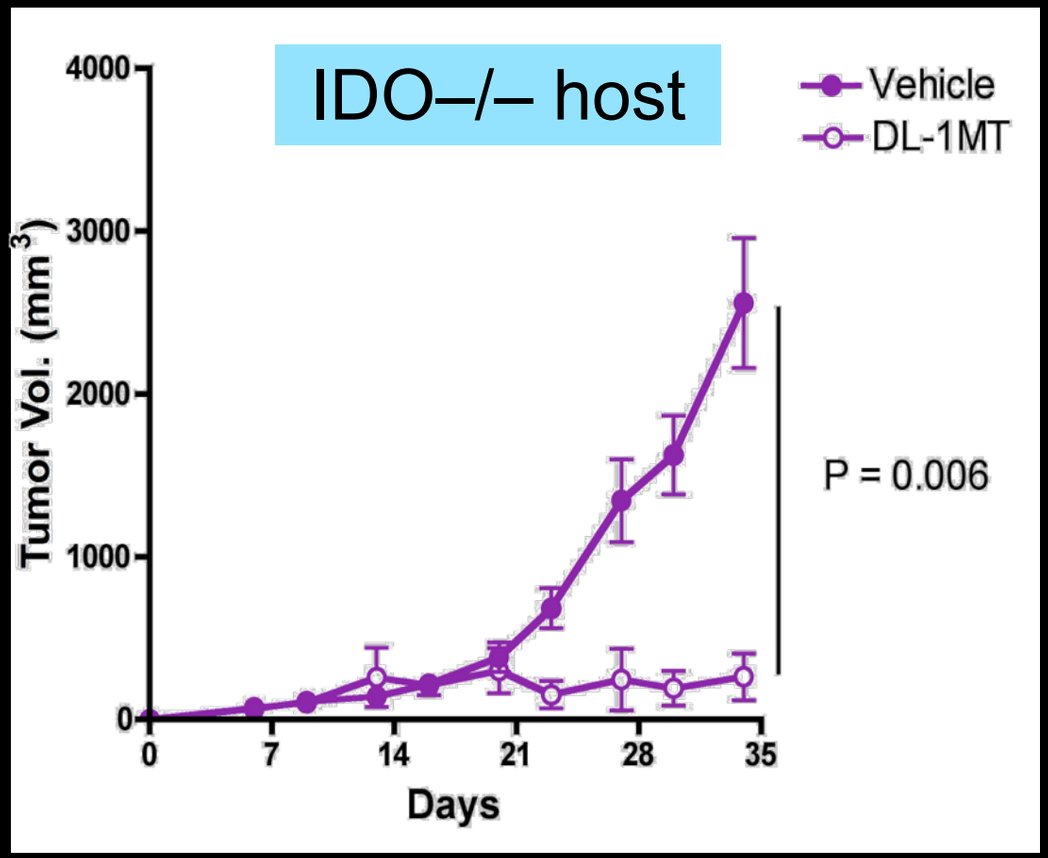
IDO function in cancer cells may be sufficient

Bin1–/–
neoplastically
transformed cells

Treat 1MT or placebo

Determine
tumor mass

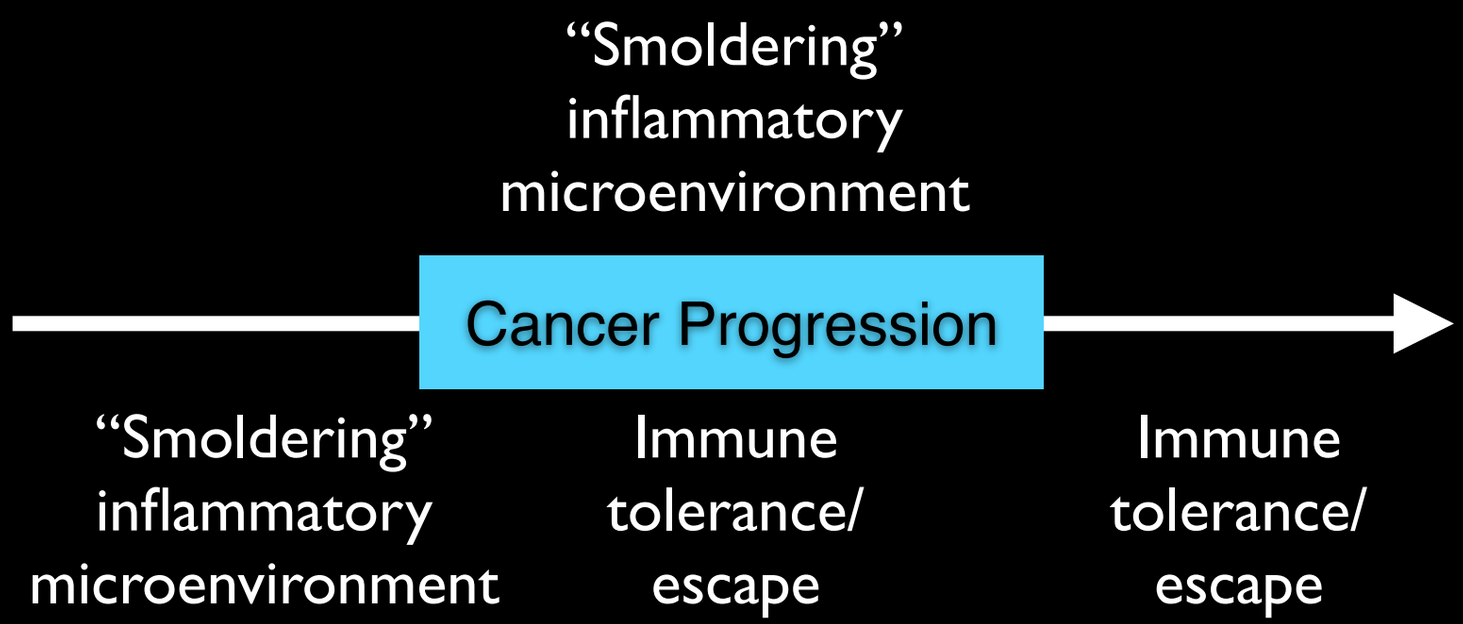




CD4+ T cell depletion abolishes response

Muller et al., Cancer Immunol. Immunother. (2010)

“Immune escape” and “cancer-associated inflammation” are genetically synonymous ?

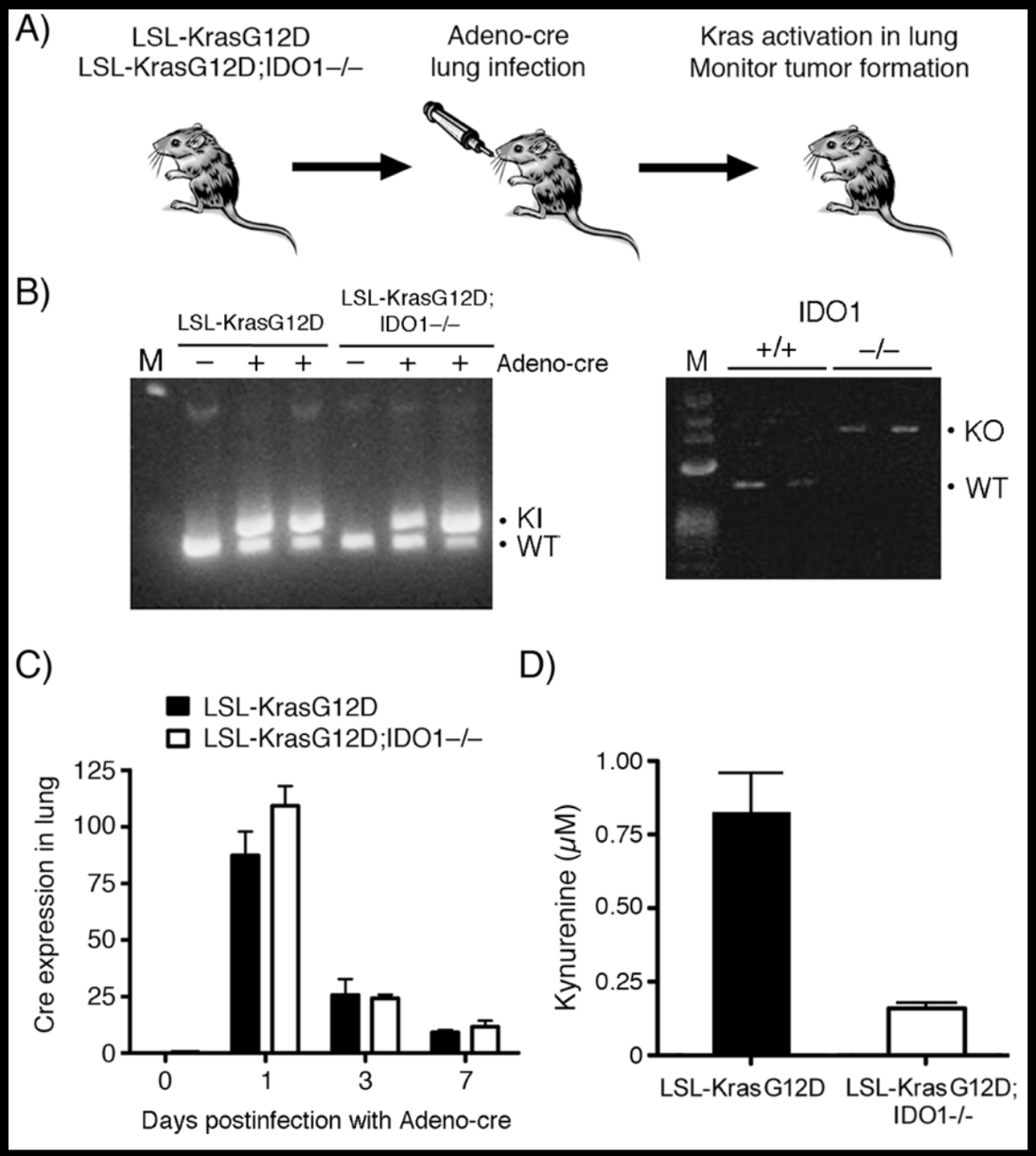


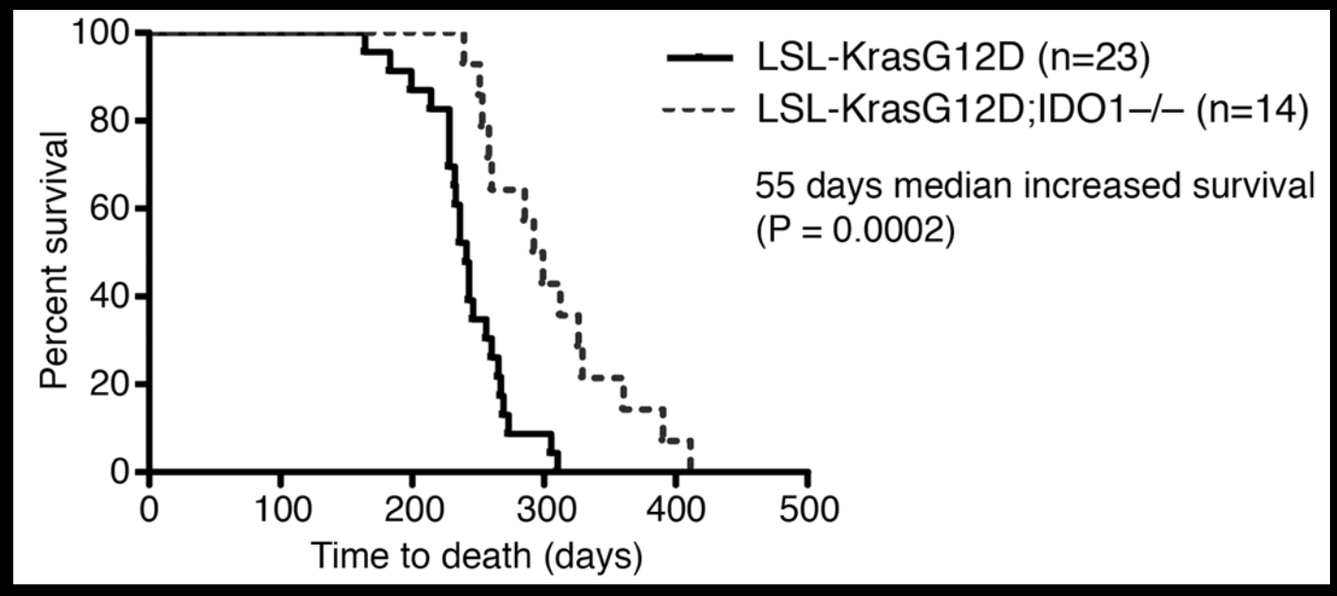
IDO programs inflammation to drive immune escape

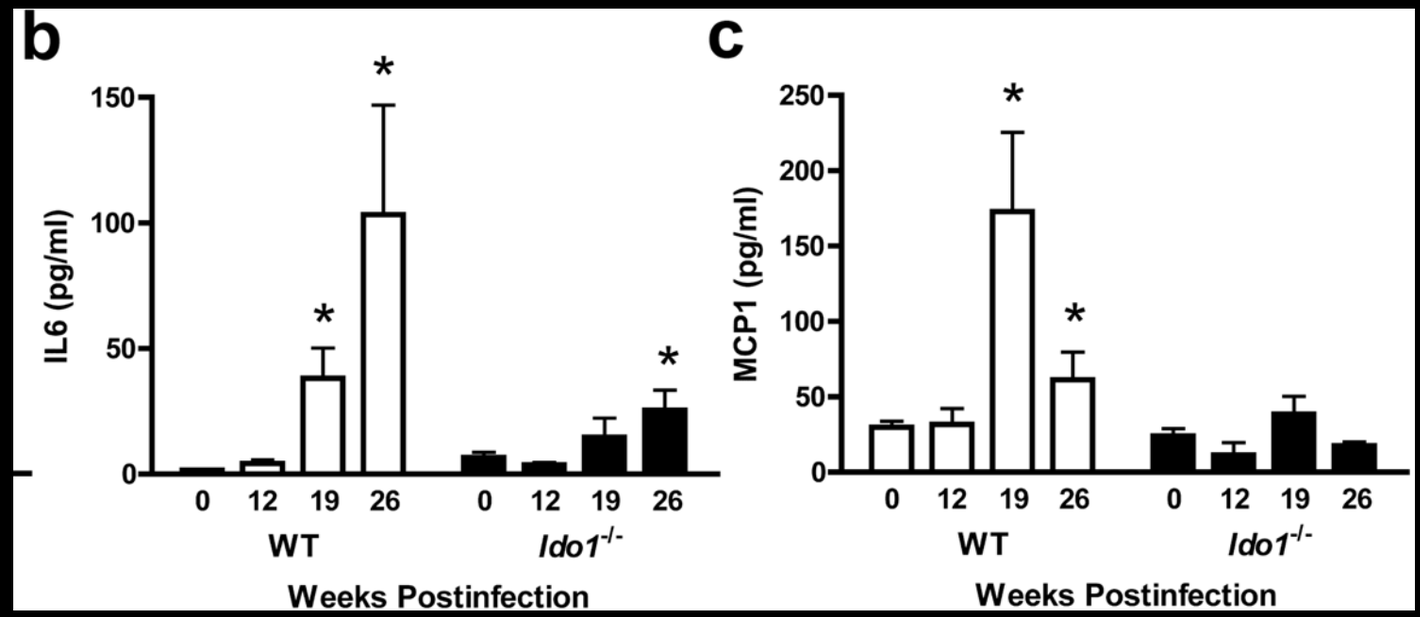
Prendergast et al., Amer. J. Path. (2010)

How broadly relevant is this inflammatory connection?

K-Ras model of lung adenocarcinoma: IDO blockade blunts progression and promotes survival





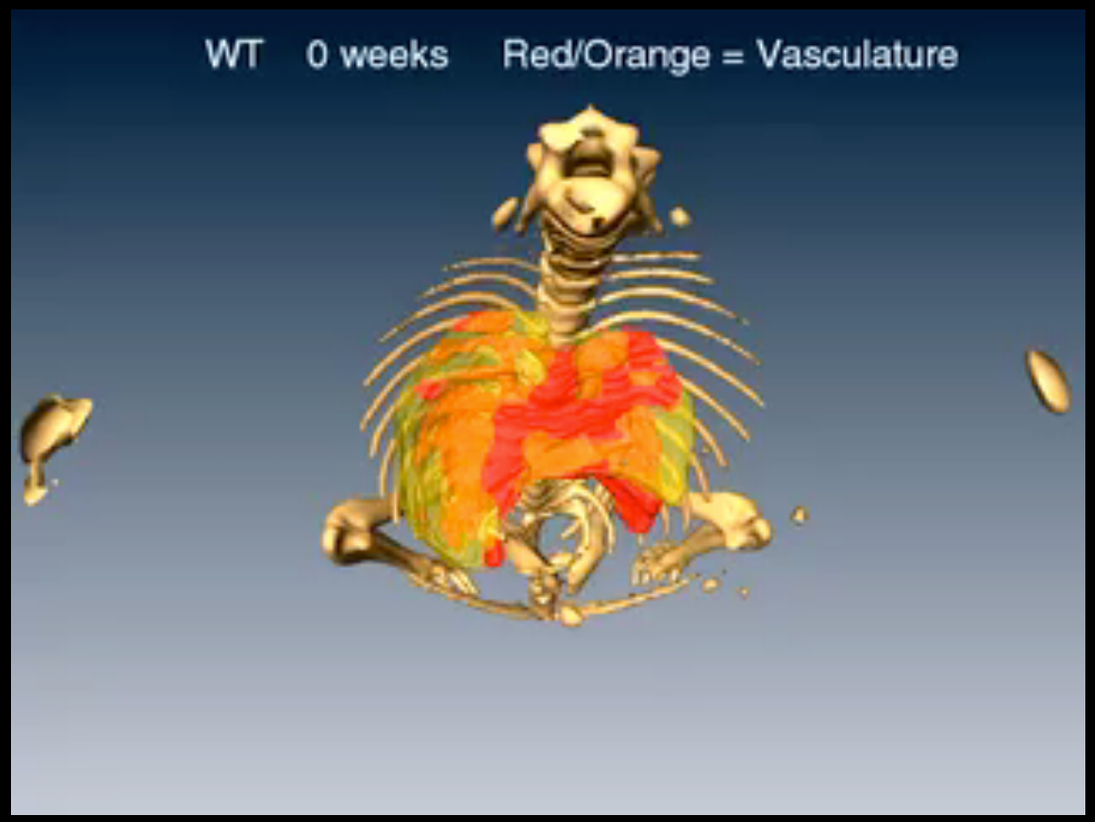


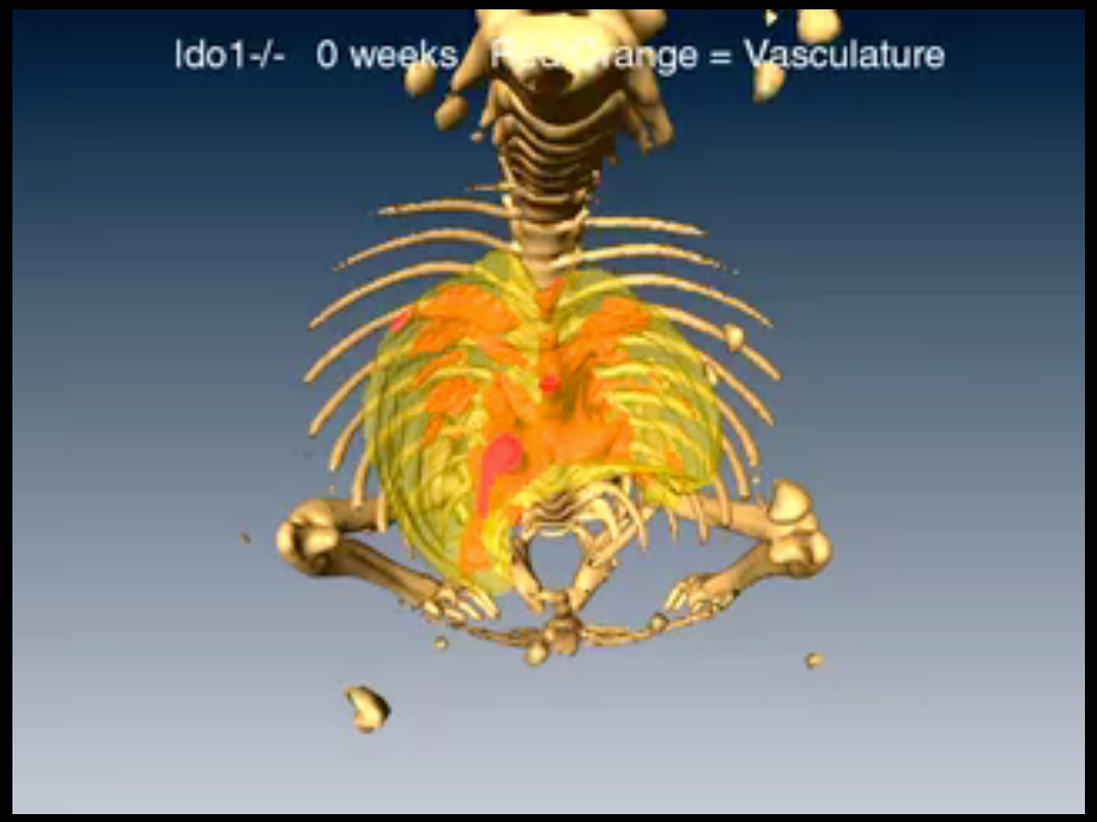
Defects in
Invasion & angiogenesis
IL-6 and CCL2 levels (myeloid attractants)
MDSC number & function

Smith, Chang et al. Cancer Discovey (2012)

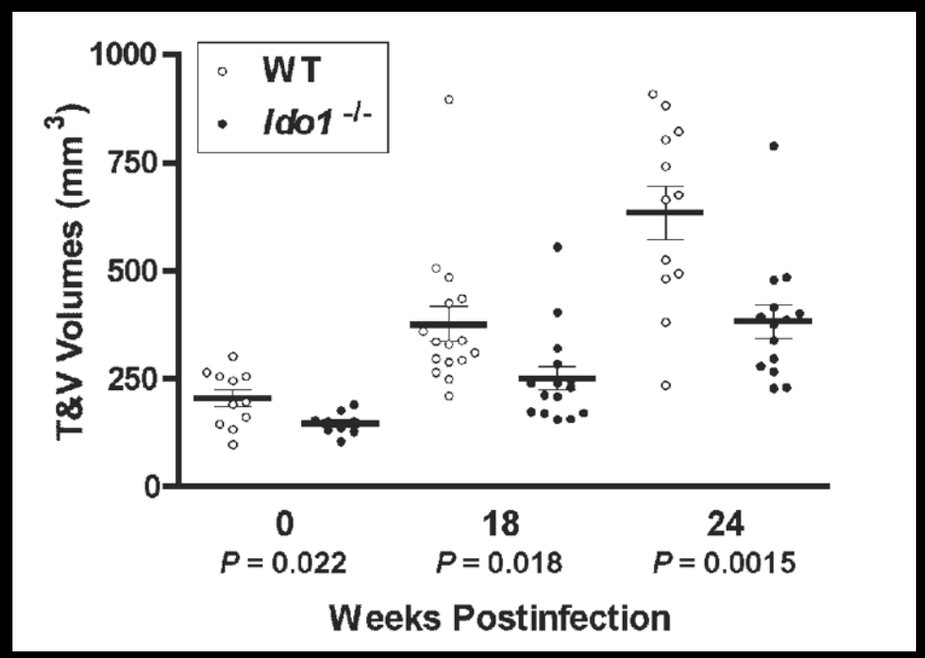
Lung angiogenic defect in IDO deficient mice

Tranverse microCAT





Effect accentuated in tumor-bearing animals

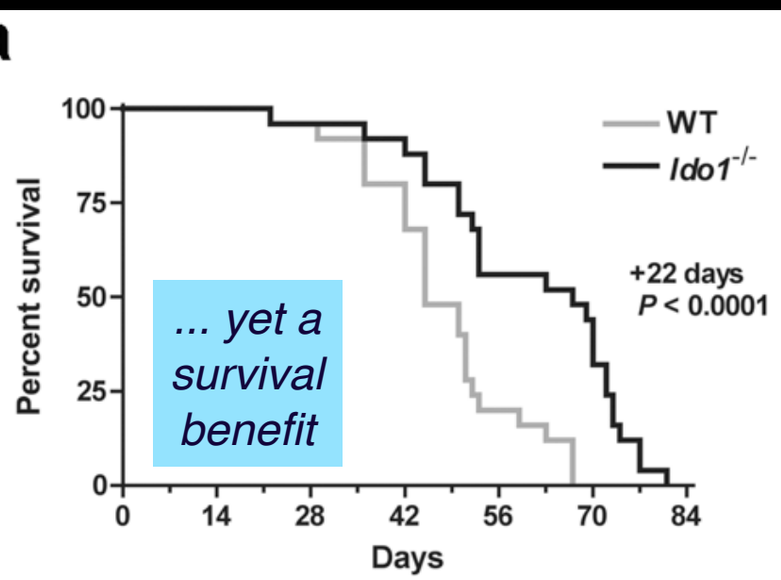


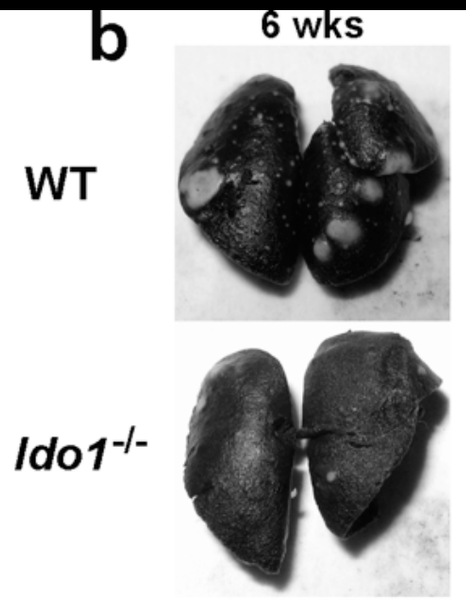
Smith, Chang et al. (2012) Cancer Discovery

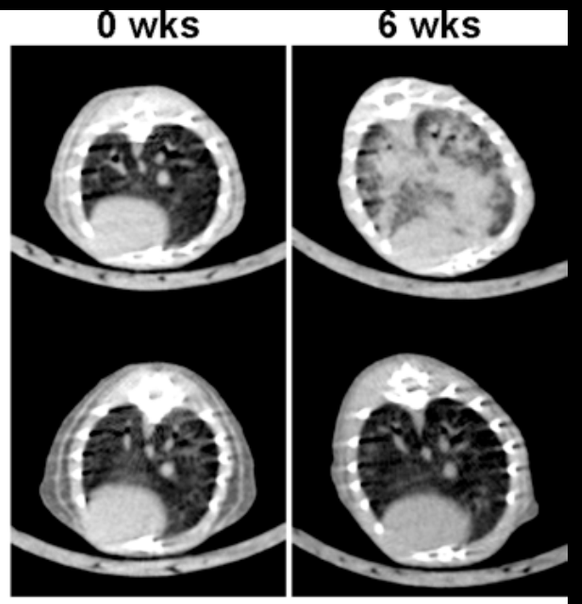
Lung metastatic defect in IDO deficient mice

4T1 breast cancer metastasizes to lung from orthotopic graft

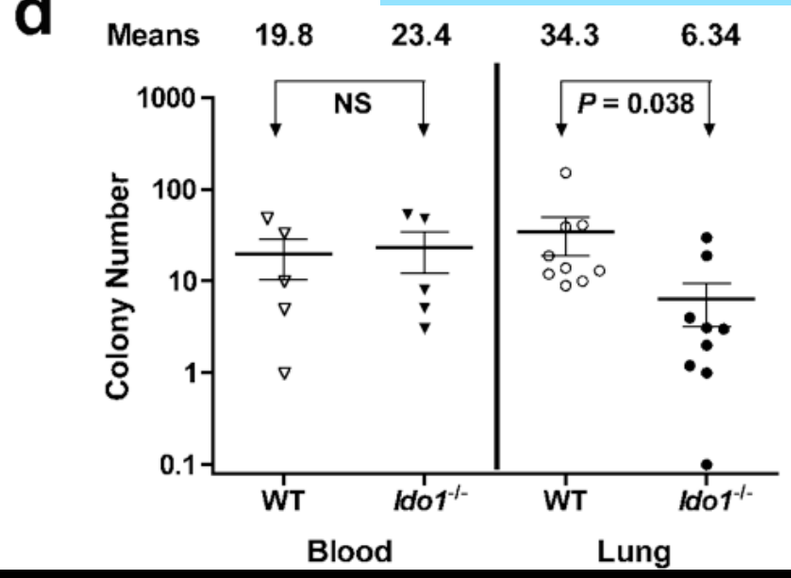
No difference in growth of primary tumor graft ...

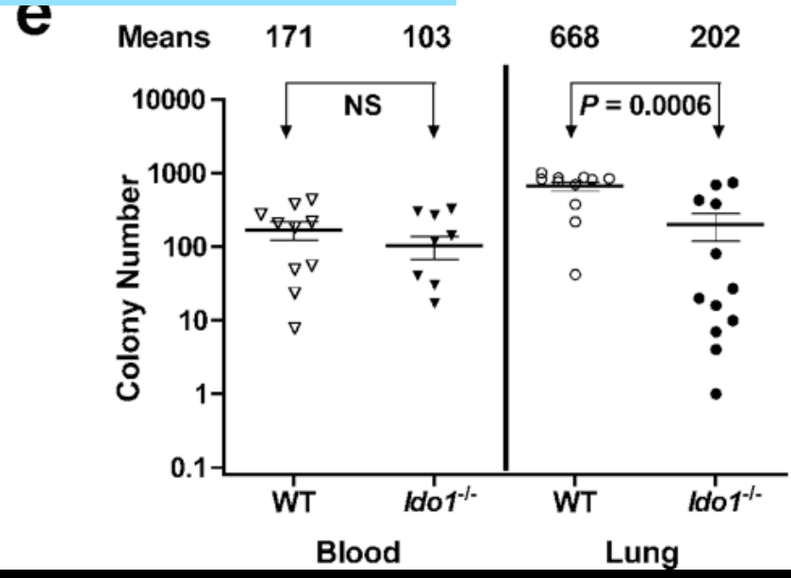






------ *Extravasion to blood unaffected* --------

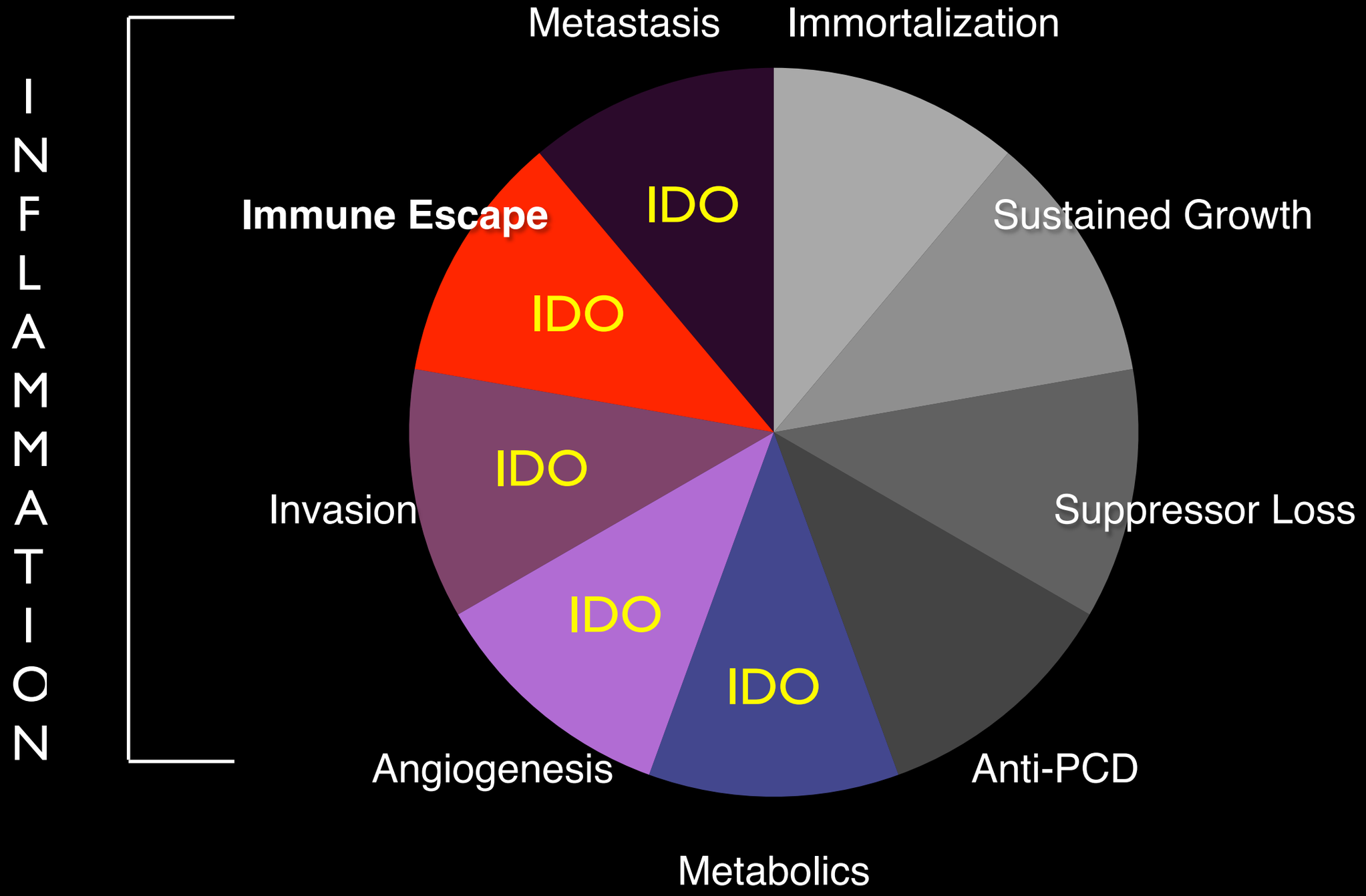




Defects in IL-6 and MDSC
Rescue MDSC and metastasis by restoring **IL-6**

Smith, Chang et al. (2012) Cancer Discovery

IDO programs an inflammatory state that supports several aspects of cancer progression



Re-programming inflammation :

IDO inhibition vs IDO pathway blockade ?

T

Block Expression -- Activity -- Effector signals

NFkB blockade
Ethyl Pyruvate
Cancer Res (2010)

Kinase inhibitors
Imatinib (Gleevec®)
Sorafinib
JAK inhibitors

Enzyme inhibitors
J Med Chem (2006-2008)

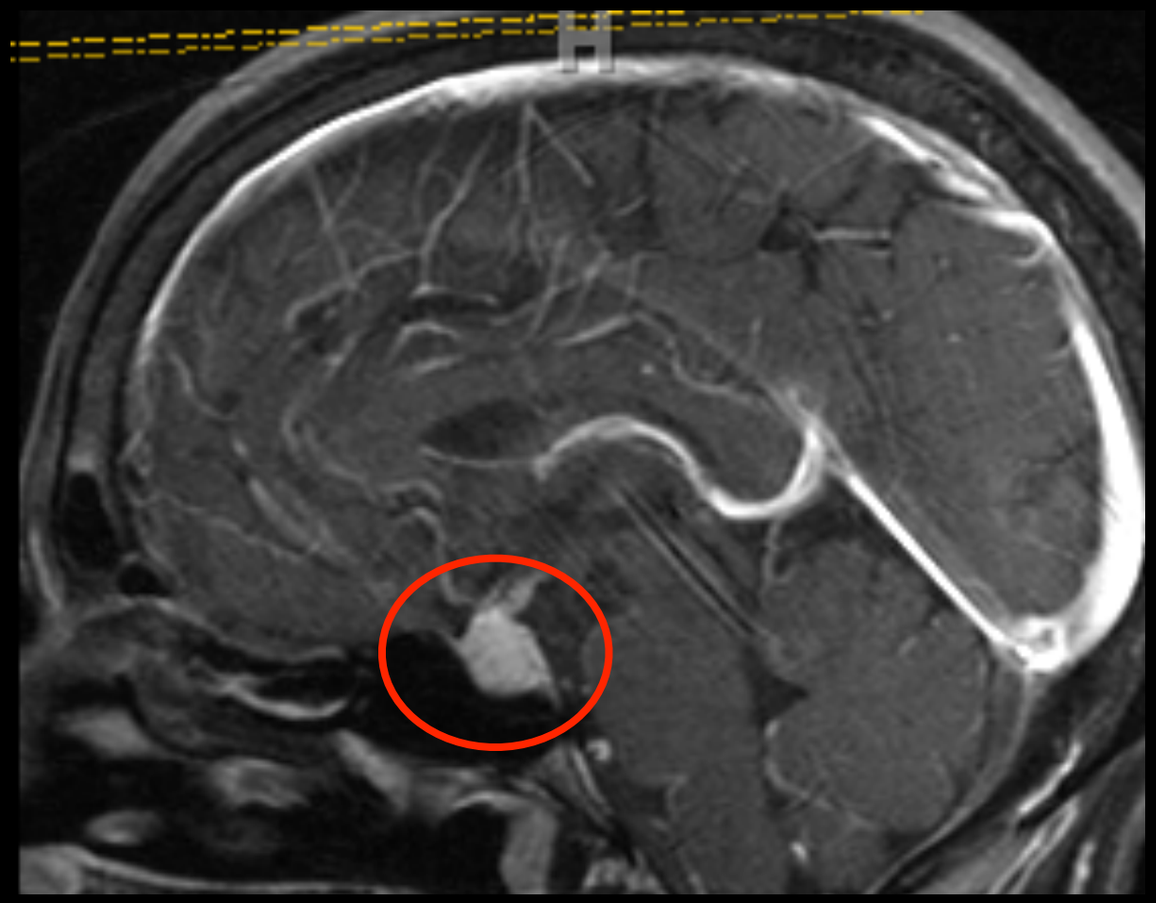
D-1MT (indoximod)
OncoImmunol (2012)

IND application (2009)
- Lankenau & Georgia (Preclinical)
- NCI (Pharm/Tox)
- New Link Genetics Corp. (GMP)
- Moffitt CC (Phase I site)

Indoximod safety findings

Hypophysitis

Pituitary gland inflammation



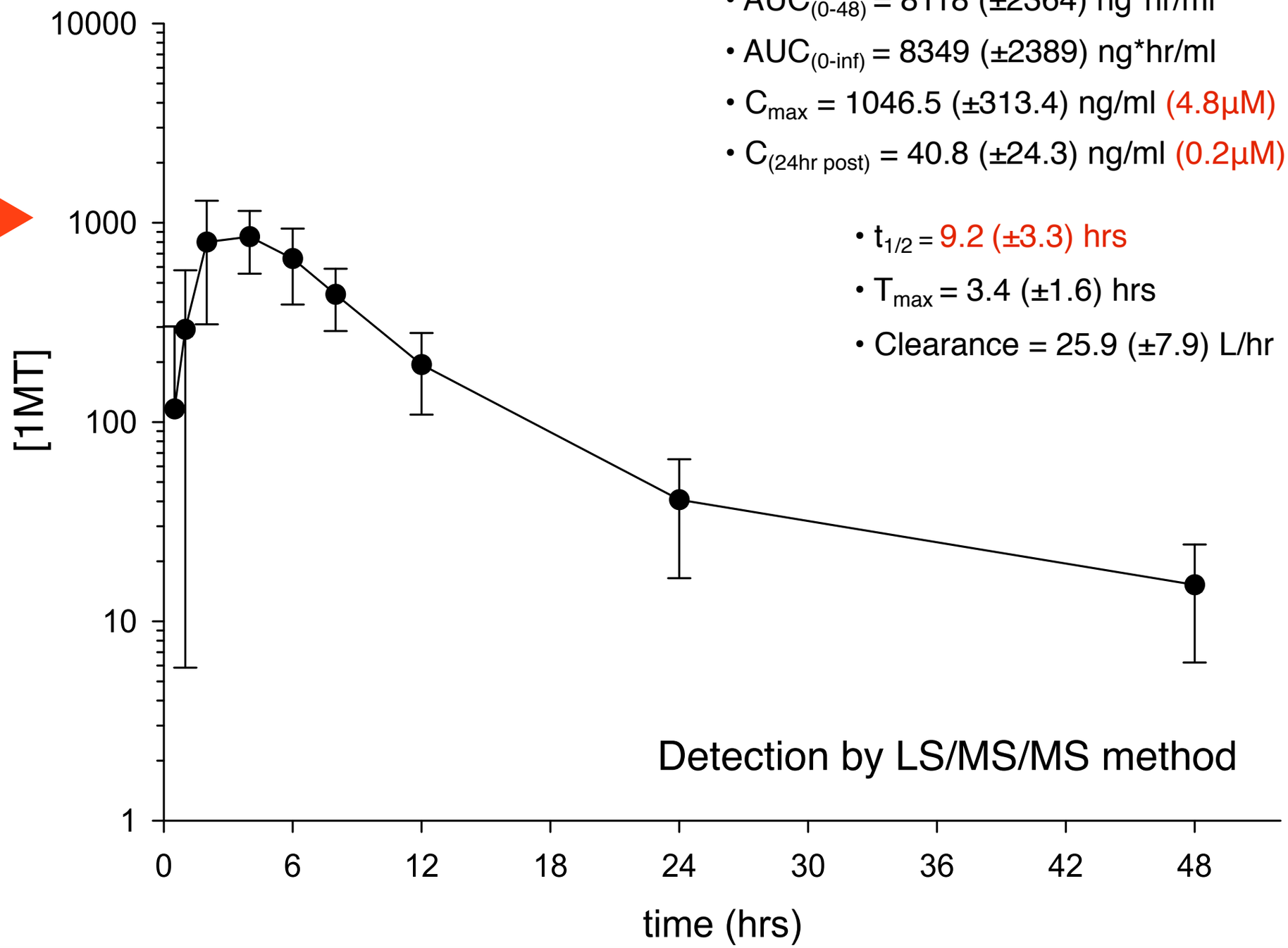
- Revealed by elevated TSH, ACTH
- Known side effect of anti-CTLA-4 *(CTLA-4 upregulates IDO in mice)*
- Emerges in all patients at highest doses - DLT in Phase I trial
- Encouraging as autoimmunity may correlate with beneficial responses

Soliman, Antonia, Link, Vahanian and colleagues, Moffitt Cancer Center & NewLink Genetics Corp.
ASCO abstracts 2011, 2012

PK: Clinical responses seen even at low exposure

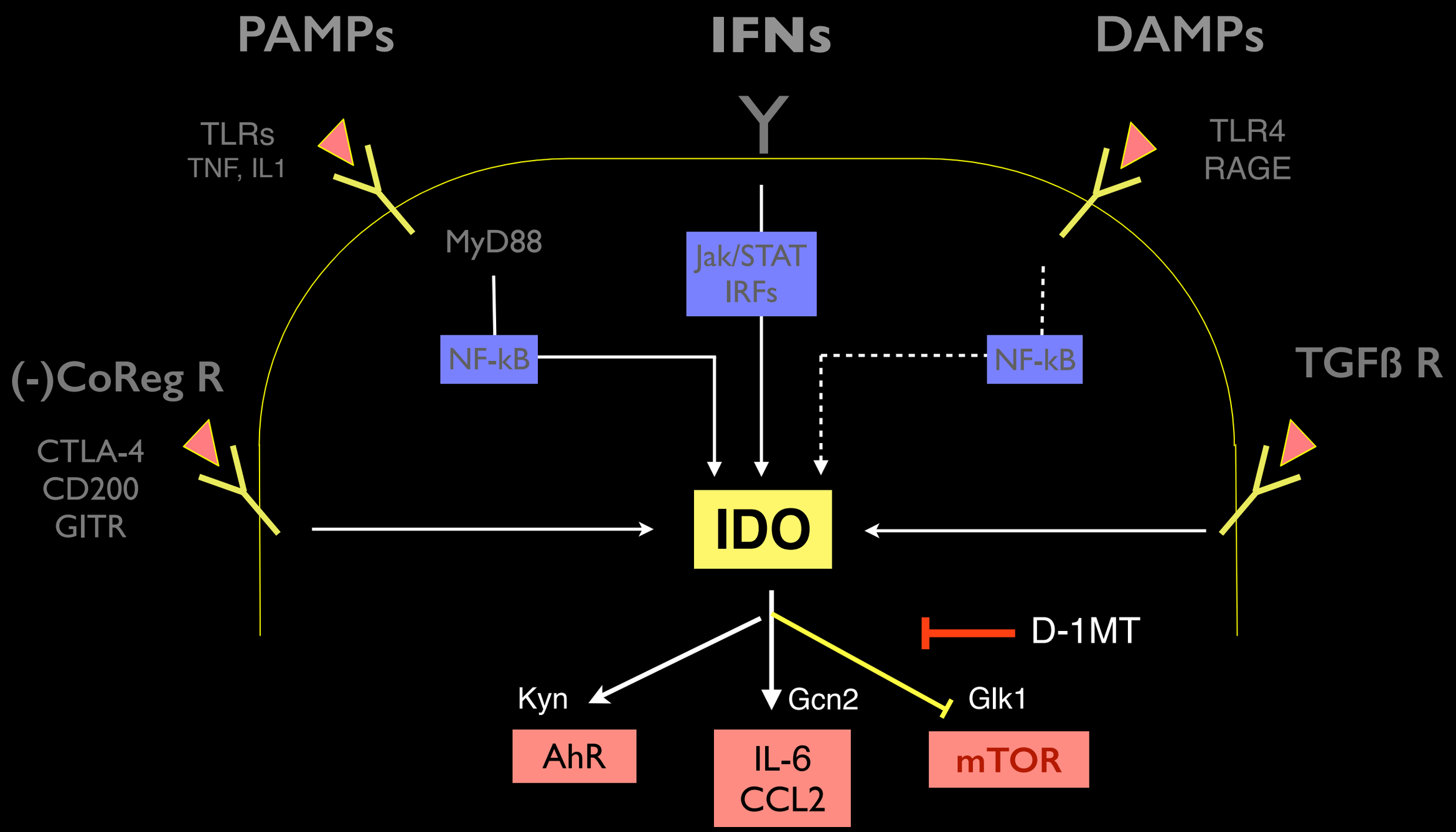
Time vs. Concentration (avg +/- stdev) for cohort 1 (n=10), 200 mg

< 1μM



- $AUC_{(0\text{-}48)}$ = 8118 (±2364) ng*hr/ml
- $AUC_{(0\text{-}inf)}$ = 8349 (±2389) ng*hr/ml
- C_{max} = 1046.5 (±313.4) ng/ml (4.8µM)
- $C_{(24hr\ post)}$ = 40.8 (±24.3) ng/ml (0.2µM)
- $t_{1/2}$ = 9.2 (±3.3) hrs
- T_{max} = 3.4 (±1.6) hrs
- Clearance = 25.9 (±7.9) L/hr

Recent findings suggest that D-1MT may act as a partial Trp mimetic to reverse mTOR blockade by IDO



Metz et al. Oncoimmunology (2012)

Trp depletion inhibits mTOR
D-1MT phenocopies Trp in relieving this effect

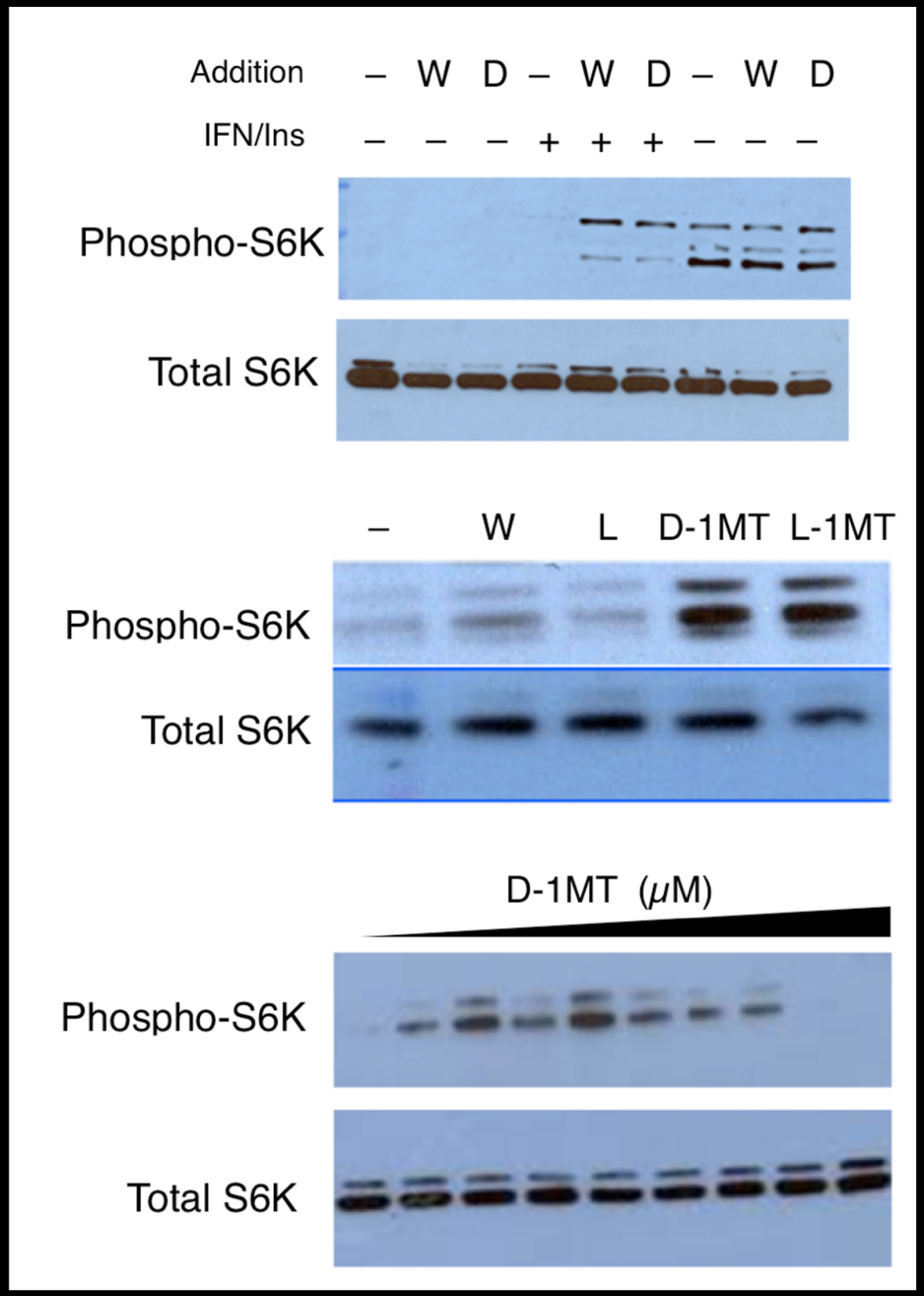
mTOR activity assay

Induce IDO

24 hr

± W or L or 1MT

Examine phospho-S6K 1-3 hr later

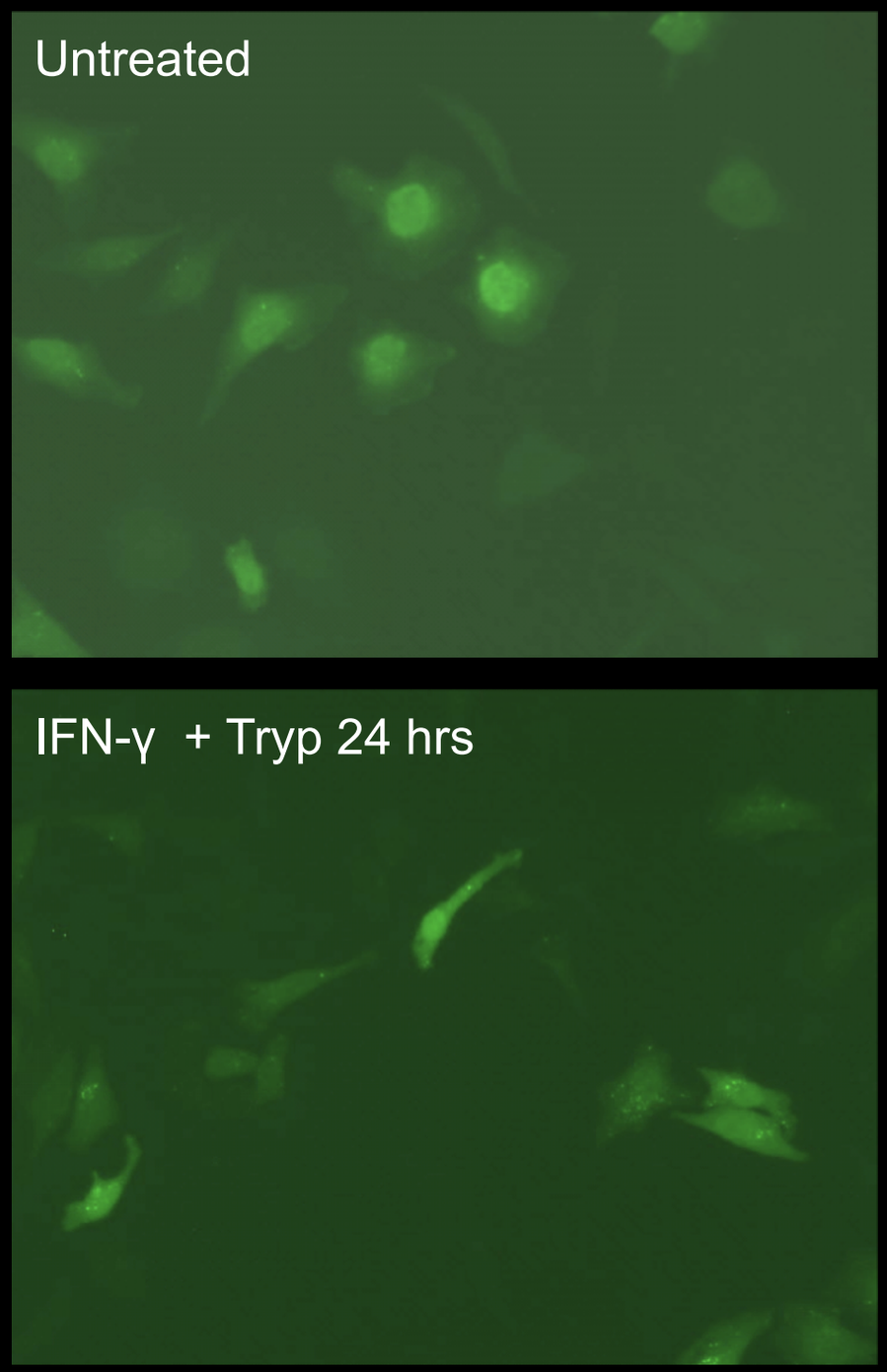


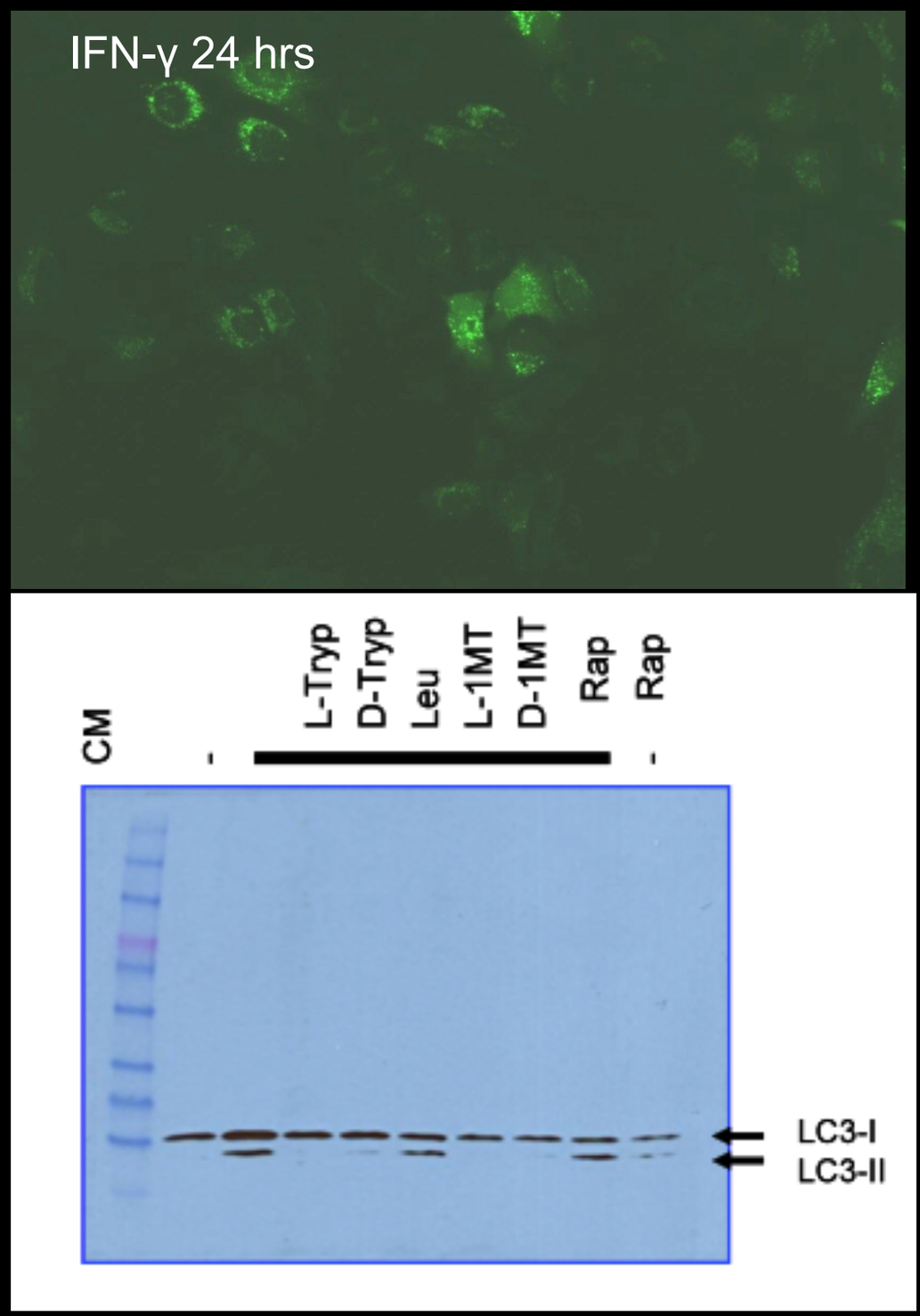
D-1MT a potent Trp mimetic (IC50 ~70 nM)

Metz et al. Oncoimmunology (2012)

IDO activates autophagy controlled by mTOR
D-1MT phenocopies Trp in relieving autophagy

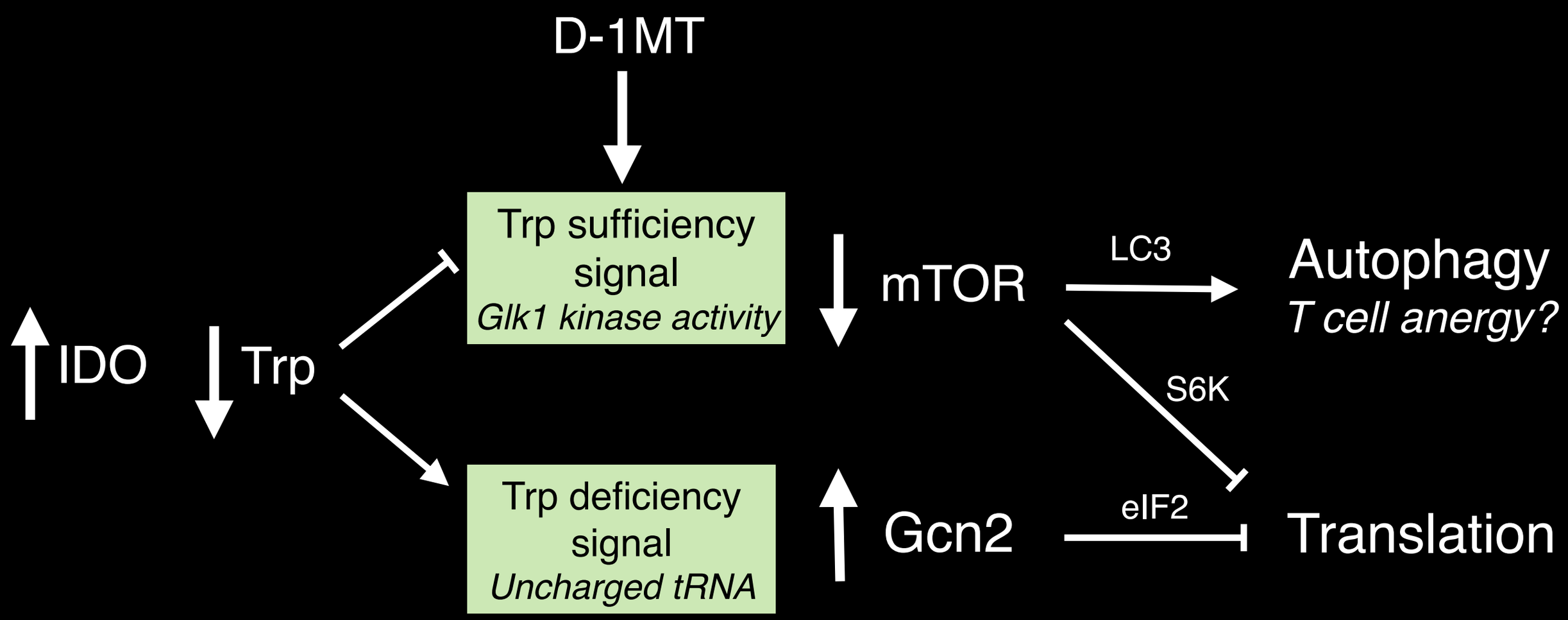
LC3-GFP localization





Metz et al. Oncoimmunology (2012)

D-1MT a Trp mimetic in Trp sufficiency signaling to mTORC1



Suggests S6K phosphorylation by mTOR as clinical PD marker to monitor indoximod response (blood draw)

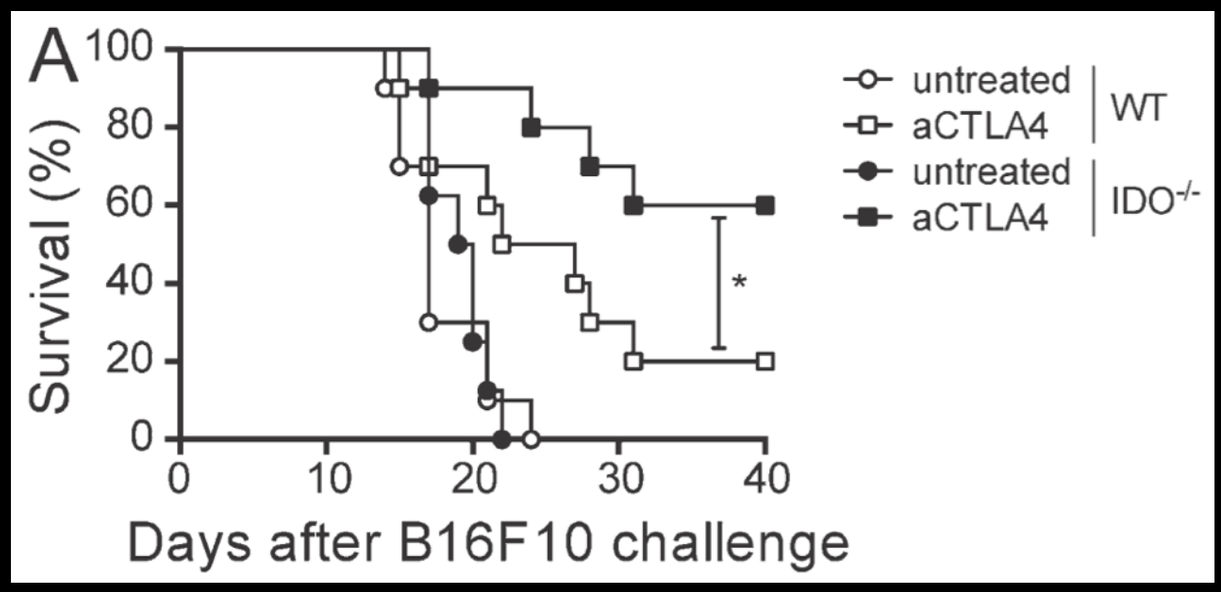
Implications

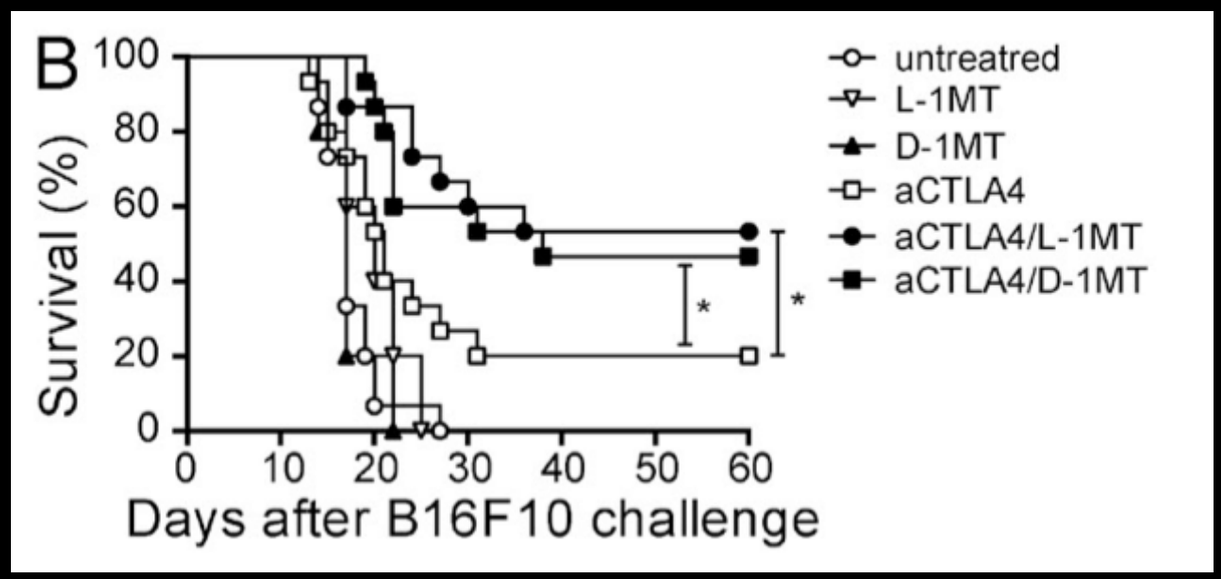
- D-1MT relieves mTOR inhibition by any Trp catabolic enzyme
  Rationale for different, perhaps broader use than IDOi
- D-1MT --> mTOR --> ICOS path
  Provides a mechanistic rationale for Ipilimumab combination

Indoleamine 2,3-dioxygenase is a critical resistance mechanism in antitumor T cell immunotherapy targeting CTLA-4

Rikke B. Holmgaard,[1,2] Dmitriy Zamarin,[1,2,3] David H. Munn,[4] Jedd D. Wolchok,[2,3,5,6] and James P. Allison[1,7]

[1]Howard Hughes Medical Institute, Department of Immunology, Memorial Sloan-Kettering Cancer Center, New York, NY 10065
[2]Swim Across America Laboratory/Ludwig Collaborative Research Laboratory, Immunology Program, Sloan-Kettering Institute for Cancer Research, New York, NY 10065
[3]Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY 10065
[4]Cancer Center and Department of Pediatrics, Georgia Regents University, Augusta, GA 30912
[5]Weill Cornell Medical College and Graduate School of Medical Sciences of Cornell University, New York, NY 10065
[6]Ludwig Institute for Cancer Research, New York, NY 10065
[7]The University of Texas, MD Anderson Cancer Center, Department of Immunology, Houston, TX 77030



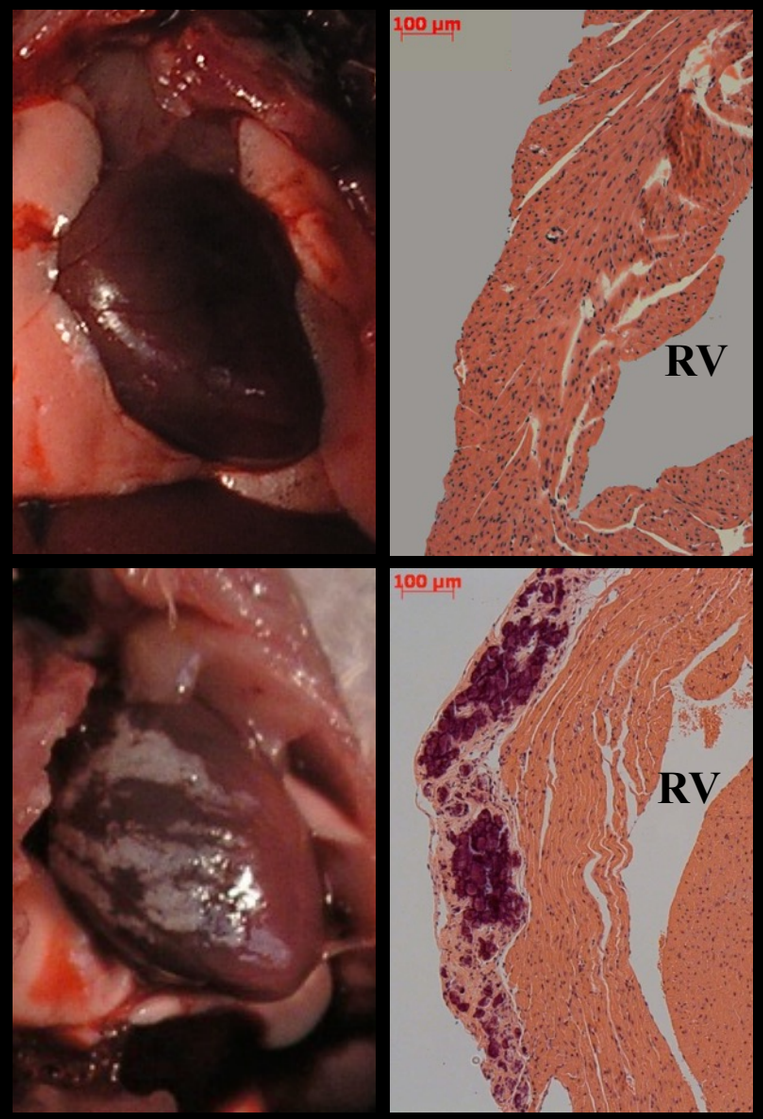


Safety concerns of enzymatic IDOi and indoximod may differ?
IDO–/– mouse phenotypes not seen with indoximod

Heart calcification (strain specific)

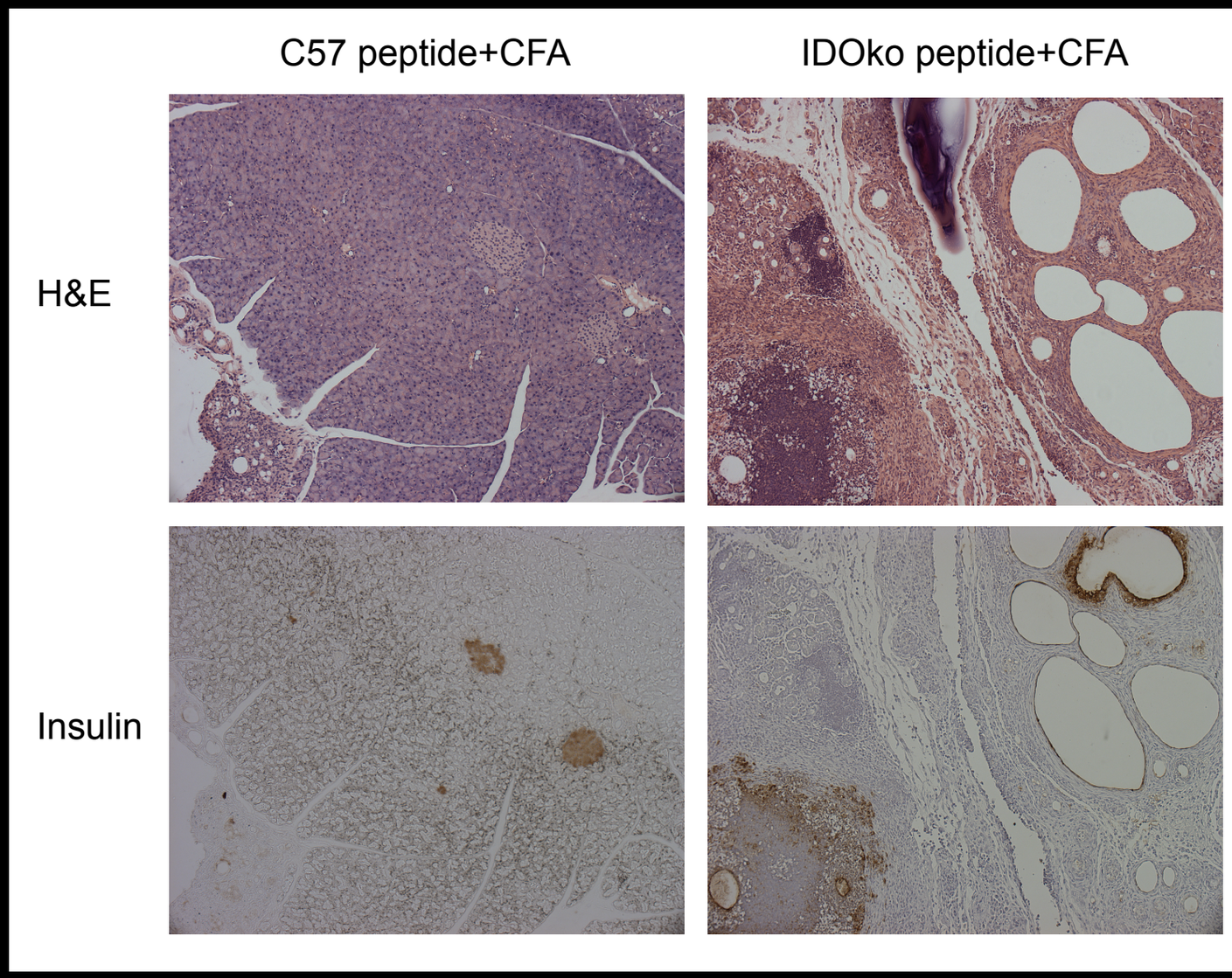
WT BALB/c

IDO–/– BALB/c



Partially penetrant by 3 months of age
Not associated with lethalty to 1 year

Acute pancreatitis after vaccination

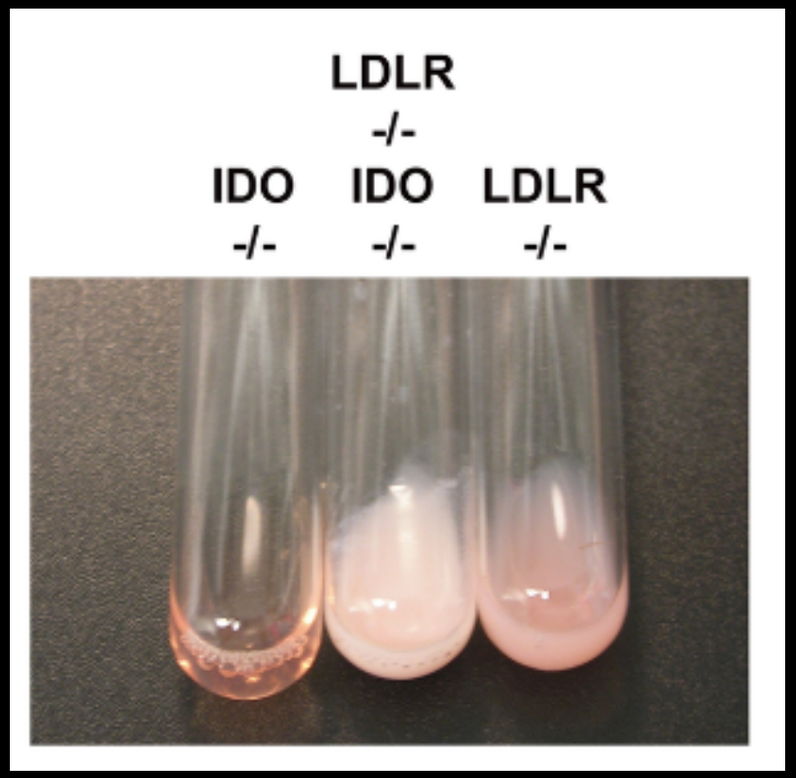


Observed in all vaccinated mice examined
which received complete Freund's adjuvant

Chang et al., Cancer Biol. Ther. (2011)

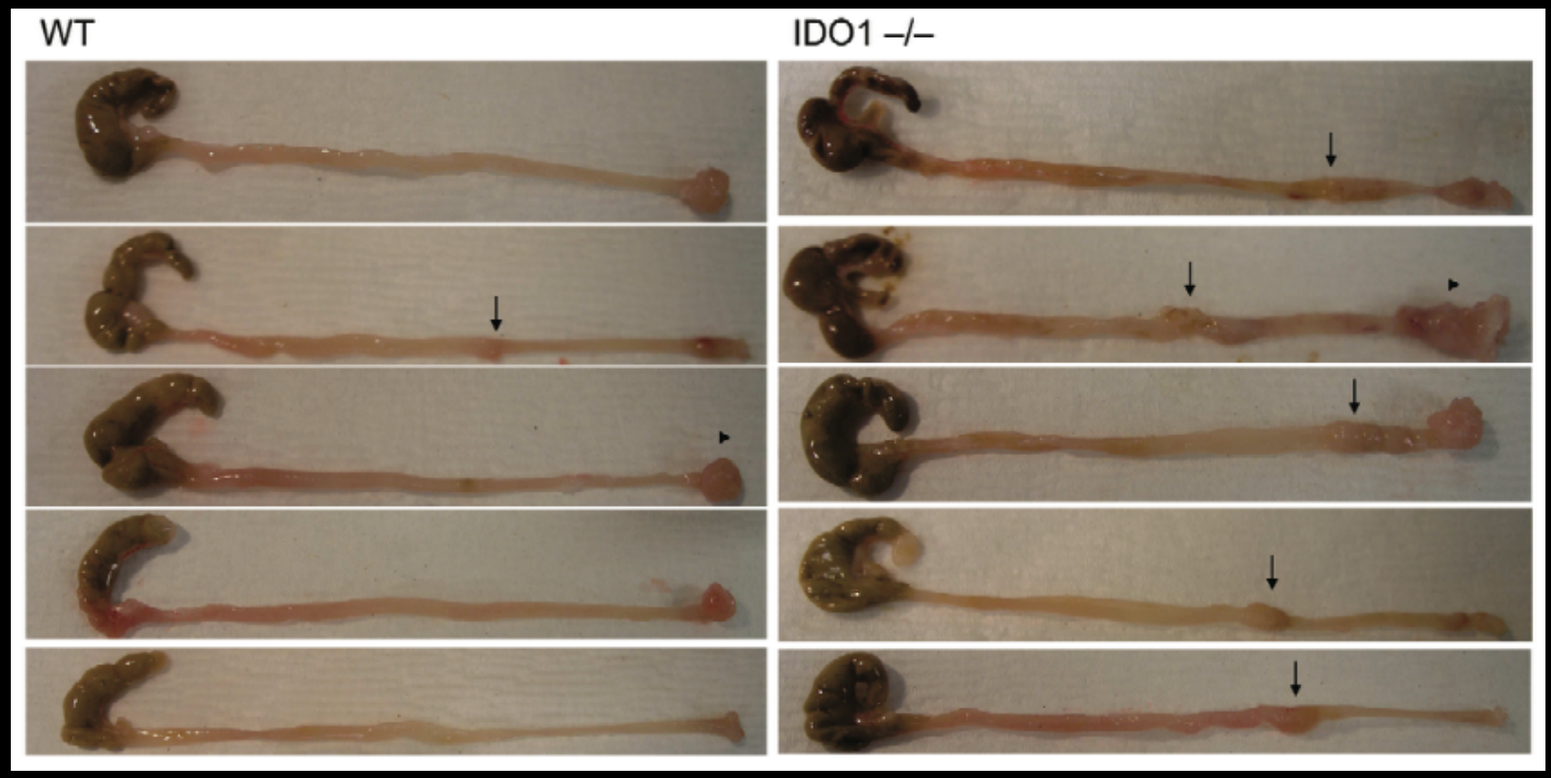
Safety concerns of IDO blockade based on IDO–/– mice not seen with indoximod

Exacerbates hyperlipidemia



Blood serum from naive animals

Heightens severity of colitis and
Elevates incidence of inflammatory
colon carcinogenesis



Gross pathology of colon carcinomas induced by a classical two-stage inflammatory protocol (DMH + DSS)

Chang et al., Cancer Biol. Ther. (2011)

Deeper insights from thinking about D-1MT ?

D-Amino Acids Trigger Biofilm Disassembly

Ilana Kolodkin-Gal,[1] Diego Romero,[2] Shugeng Cao,[3] Jon Clardy,[3] Roberto Kolter,[2] Richard Losick[1]*

Bacteria form communities known as biofilms, which disassemble over time. In our studies outlined here, we found that, before biofilm disassembly, *Bacillus subtilis* produced a factor that prevented biofilm formation and could break down existing biofilms. The factor was shown to be a mixture of D-leucine, D-methionine, D-tyrosine, and D-tryptophan that could act at nanomolar concentrations. D-Amino acid treatment caused the release of amyloid fibers that linked cells in the biofilm together. Mutants able to form biofilms in the presence of D-Amino acids contained alterations in a protein (YqxM) required for the formation and anchoring of the fibers to the cell. D-Amino acids also prevented biofilm formation by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. D-amino acids are produced by many bacteria and, thus, may be a widespread signal for biofilm disassembly.

Most bacteria form multicellular communities known as biofilms in which cells are protected from environmental insults (*1*, *2*). However, as biofilms age, nutrients become limiting, waste products accumulate, and it is advantageous for the biofilm-associated bacteria to return to a planktonic existence (*2*). Thus, biofilms have a finite lifetime, characterized by eventual disassembly. *Bacillus subtilis* forms communities on semi-solid surfaces and thick pellicles at the air/liquid interface of standing cultures (*1*, *3*–*5*). Cells in the biofilms are held together by an extracellular matrix consisting of exopolysaccharide and amyloid fibers composed of the protein TasA (*5*–*7*). The exopolysaccharide is produced by the *epsA-O* operon, and the TasA protein is encoded by the *yqxM-sipW-tasA* operon (*8*). After 3 days of incubation in a biofilm-inducing medium, *B. subtilis* formed thick pellicles at the air/liquid interface of standing cultures (Fig. 1A). Upon incubation for an additional 3 to 5 days, however, the pellicles lost their integrity (Fig. 1B). To investigate whether mature biofilms produce a factor that triggers biofilm disassembly, we asked whether a conditioned medium would prevent pellicle formation when added to a fresh medium (*9*). Medium from an 8-day-old culture was applied to a C18 column (Sep Pak, Waters, Milford, MA), and concentrated eluate from the column was added to a freshly inoculated culture. The eluate was sufficient to prevent pellicle formation (Fig. 1C). Concentrated eluate from a 3-day-old culture had little effect on pellicle formation (Fig. 1D). Further purification of the factor was achieved by eluting the cartridge stepwise with methanol. Elution with 40% methanol resulted in a fraction that was active in inhibiting pellicle formation (Fig. 1E), but had little effect on cell growth (fig. S1). The activity was resistant to heating at 100°C for 2 hours and proteinase K treatment (Fig. 1F).

Bacteria produce D-amino acids in stationary phase (*10*). We asked whether the biofilm-inhibiting factor was composed of one or more D-amino acids. Indeed, D-tyrosine, D-leucine, D-tryptophan, and D-methionine were active in inhibiting biofilm formation in a liquid medium, as well as on a solid medium (Fig. 1, G and H, and figs. S2 and S3). In contrast, the corresponding L-isomers and D-isomers of other amino acids (such as D-alanine and D-phenylalanine) were inert in our biofilm-inhibition assay. Next, we determined the minimum concentration needed to prevent biofilm formation. Individual D-amino acids varied in their activity, with D-tyrosine being more effective (3 μM) than D-methionine

[1]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA. [2]Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115, USA. [3]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA.

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OncoImmunology 1:6, 924-929; September 2012; © 2012 Landes Bioscience

A perspective on new immune adjuvant principles

Reprogramming inflammatory states to permit clearance of cancer cells and other age-associated cellular pathologies

George C. Prendergast[1]* and Richard Metz[2]
[1]Lankenau Institute for Medical Research; Wynnewood PA USA; [2]New Link Genetics Corporation; Ames, IA USA

Can indoximod be conceptualized as an immune adjuvant principle?
What is its target in the IDO-mTOR pathway ?

Summary

IDO

- Programs inflammation to support cancer
- Immune escape derivative of general inflammatory role
- Blocks Trp sufficiency signaling to mTOR, an IDO target

IDOi

- May reprogram inflammation
- Different MOA of enzymatic IDOi versus indoximod
- Indoximod acts like a Trp mimetic for mTOR pathway

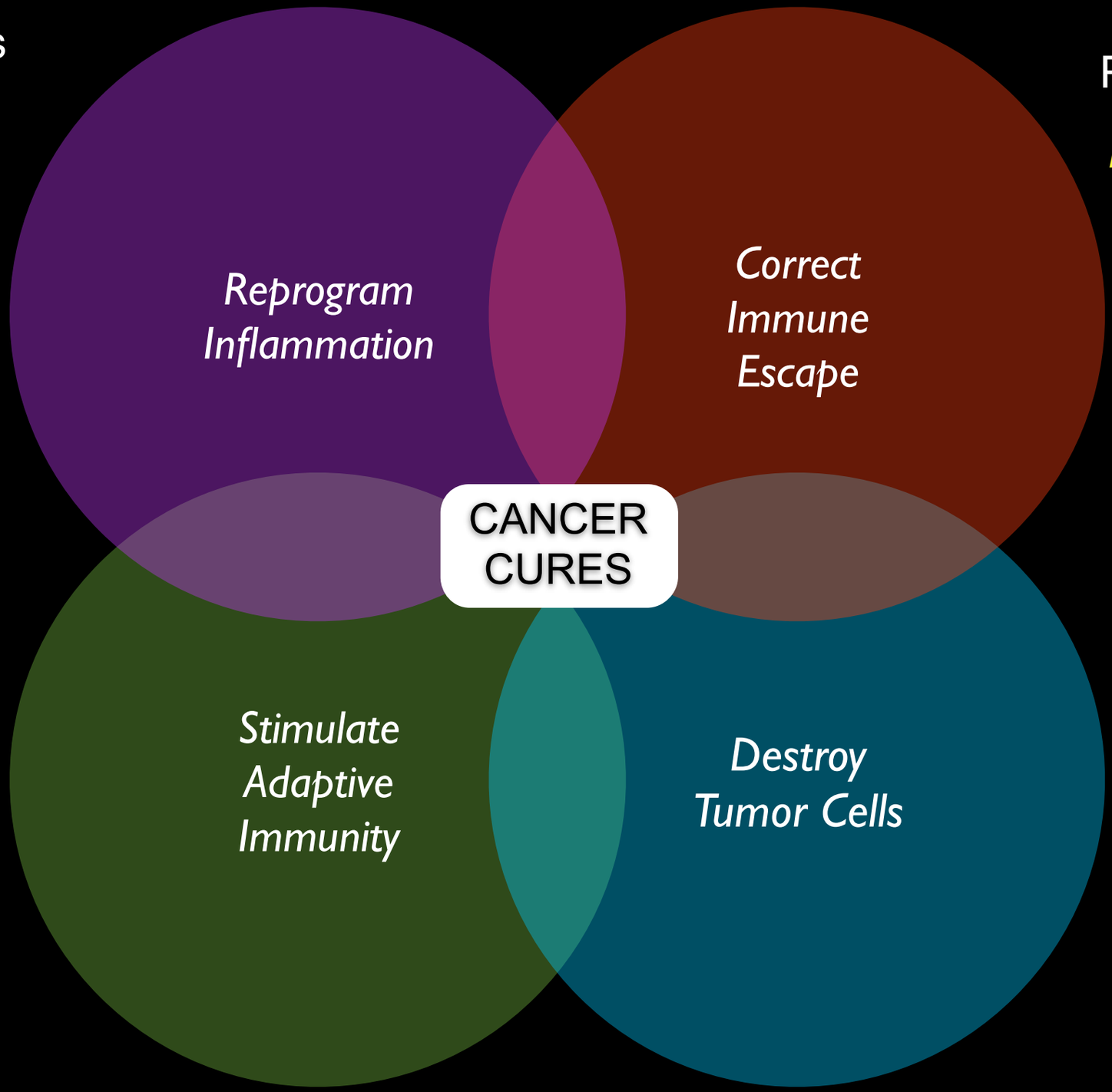
  Potently restores mTOR activity blocked by IDO

  Treat cancers driven by any Trp catabolic enzyme ?

Cancer Immunochemotherapy of the Future

Immunomodulators
Indoximod

Checkpoint
Pathway Inhibitors
Yervoy®, Anti-PD1
IDO inhibitors (e.g. 919)



Adoptive Cell
Therapies &
Vaccines
Provenge®, HyperAcute

Chemotherapy
Radiotherapy

Lankenau Inflammation ‘Orbit Group’

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and Charles Link
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