^{89}Zr-labeled antibodies and fragments for imaging immune cells

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NCI/SNMMI/CTN Immune Modulation Therapy and Imaging workshop

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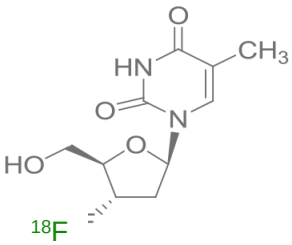
Disclosures

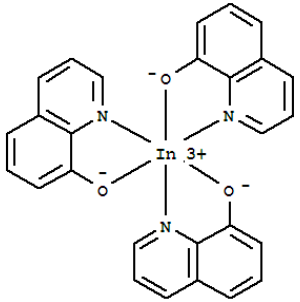
Anna M. Wu is a Founder, Board Member, and Consultant to ImaginAb, Inc.

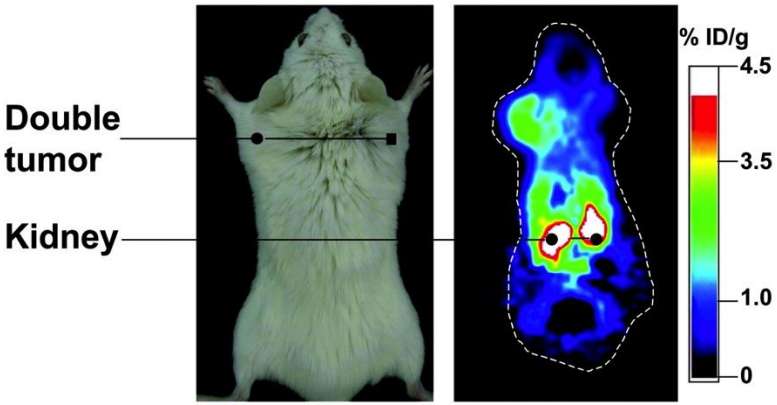
Dr. Wu is also a consultant to Avidity Nanomedicines.

Molecular imaging approaches
for imaging immune cells and immune responses

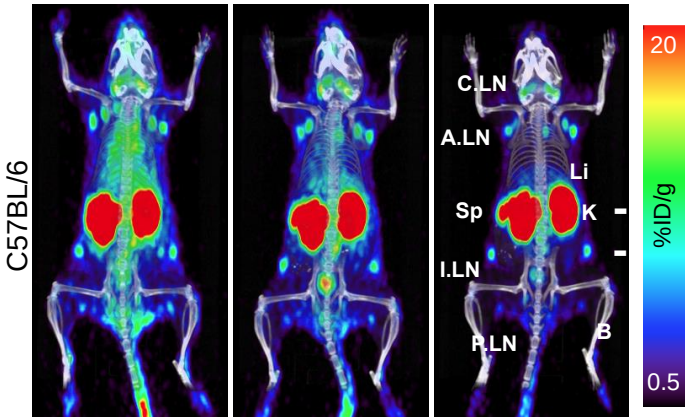
- Metabolic probes (*e.g,* [^{18}F]-fluorodeoxyglucose, FDG; [^{18}F] fluoro-thymidine; FLT, nucleoside analogs and others)
- Pre-labeling cells (^{111}In-oxine; ^{89}Zr-oxine; paramagnetic nanoparticles)
- Reporter genes (optical, PET)
- Direct imaging of cell surface targets using antibodies, nanobodies, etc.







HSV-tk PET reporter gene; Dubey et al. PNAS 2003



Anti-CD8 cys-diabody; Tavaré et al. J Nucl Med 2015

Molecular imaging approaches
for imaging immune cells and immune responses

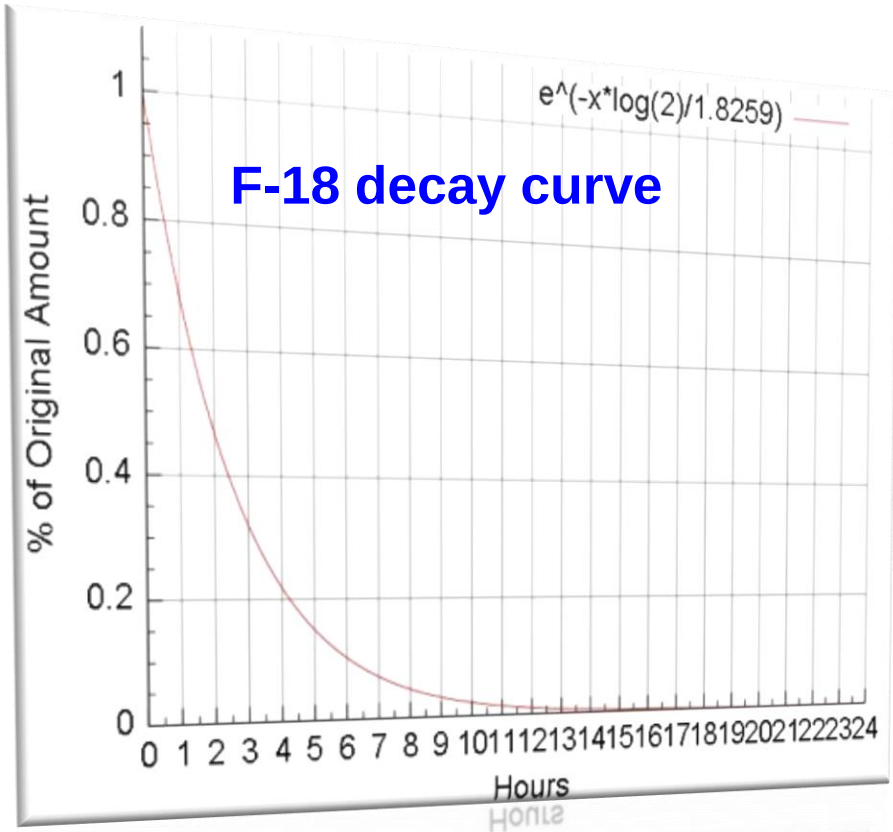
	Pros	Cons	Current and future clinical use
Metabolic probes	Detect metabolically active, proliferating cells	Relatively non-specific	FDG FLT and others
Pre-labeled cells	Low background	Need to remove, modify, reinfuse cells Dilution after cells replicate	In-111 oxine
Reporter genes	Potentially low background Can follow cells as they expand Cell surface tags	Need to modify cells (*in situ* or *ex vivo*) Potential immunogenicity of reporter genes	Non-immunogenic reporter genes (NaI symporter, huTK, etc.)
Probes for cell surface markers (antibodies, etc)	High specificity	Endogenous antigen sink Surface markers only	Human/humanized probes

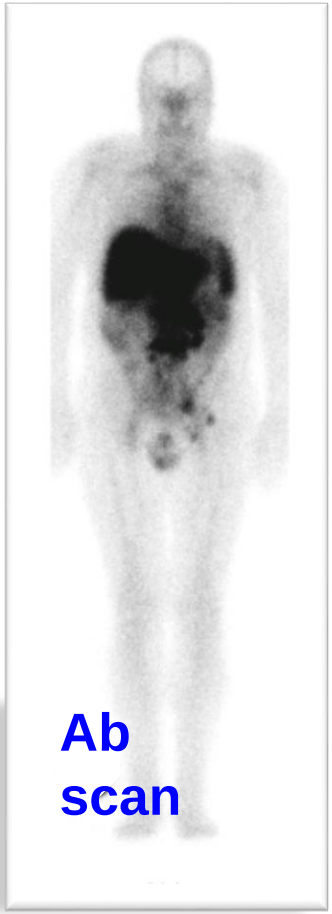
Overall challenges for molecular imaging:

- Complexity, cost, and time to develop; regulatory path; lack of financial incentives
- Inability to multiplex

Challenges in immunoPET

Irresistable force *vs.* Immovable object





$t_{1/2}$ = *109 min*

$t_{1/2}$ = 2 wks

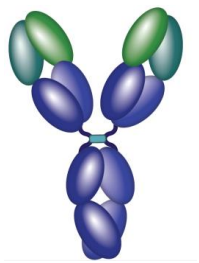
Positron-emitting radionuclides for ImmunoPET

Radio-nuclide	$T_{1/2}$ (h)	Positron yield (%)	□$^+$ max (MeV)	Additional considerations
^{68}Ga	1.1	89	1.89	Generator-produced
^{18}F	1.8	97	0.63	Common, cyclotron
^{64}Cu	12.7	19	0.66	Also beta, Auger e^-
^{86}Y	14.7	33	3.15	Also gamma
^{76}Br	16.2	23	3.98	Also gamma
^{89}Zr	78.5	23	0.90	Also gamma
^{124}I	100.3	23	2.14	Also gamma

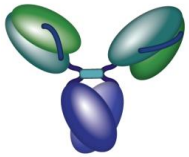
Wu, Methods 2014

Engineering antibodies for *in vivo* imaging

Intact Ab
150 kDa



Minibody
80 kDa

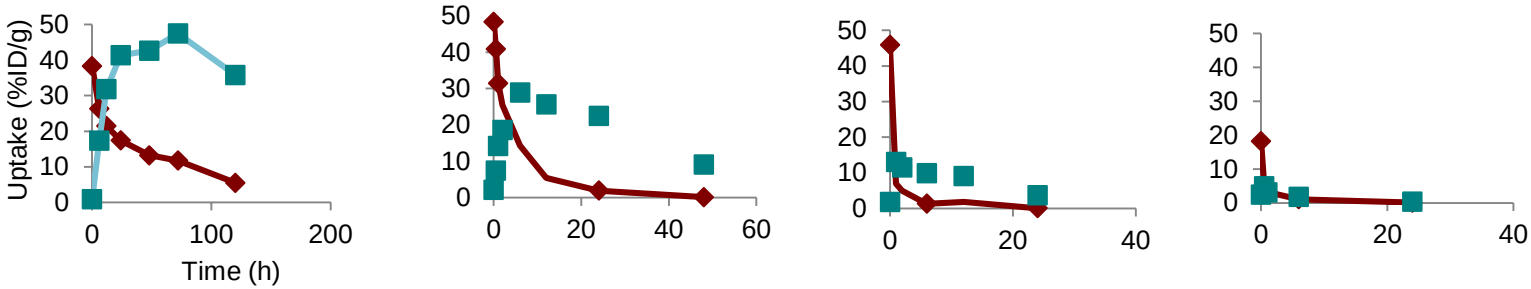


Diabody
55 kDa

scFv
25 kDa

Tumor

Blood



Minibody and diabody for *imaging*

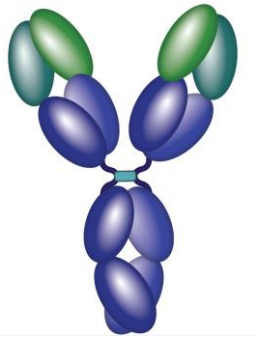
- Bivalent, retain specificity and affinity
- Half-life reduction / accelerated clearance (no FcRn interaction)
- Reduction of immunogenicity (humanized or human)
- Removal of effector functions (no C_H2/Fc; not glycosylated)
- Direct clearance to kidneys (< 60 kDa) or liver (>60 kDa)
- Improved diffusion / transport in target tissues
- Site-specific conjugation of imaging moieties

Biodistribution of anti-CEA fragments in LS174T xenografted mice

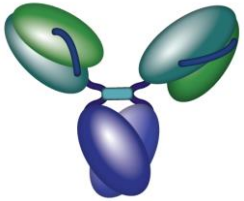
From Wu and Senter 2005

Rapid imaging using ^{124}I-anti-PSCA engineered antibody fragments

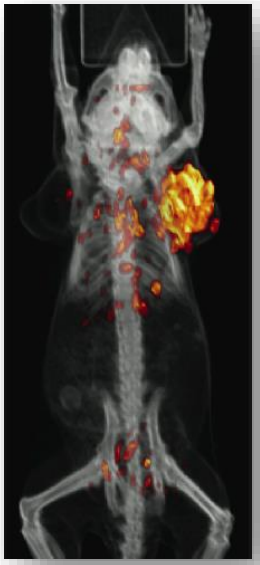
Intact Ab
150 kDa



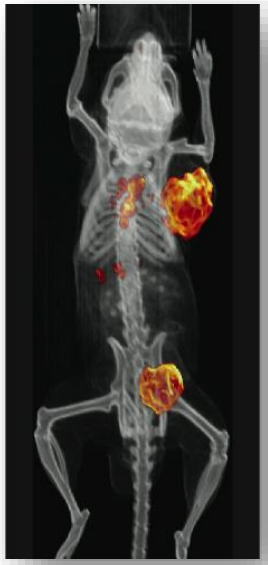
Minibody
80 kDa



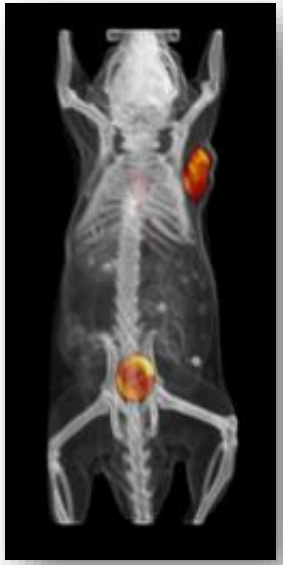
Diabody
55 kDa



168 h



21 h

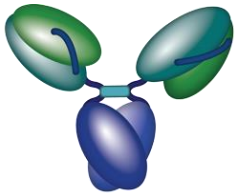


4-8 h

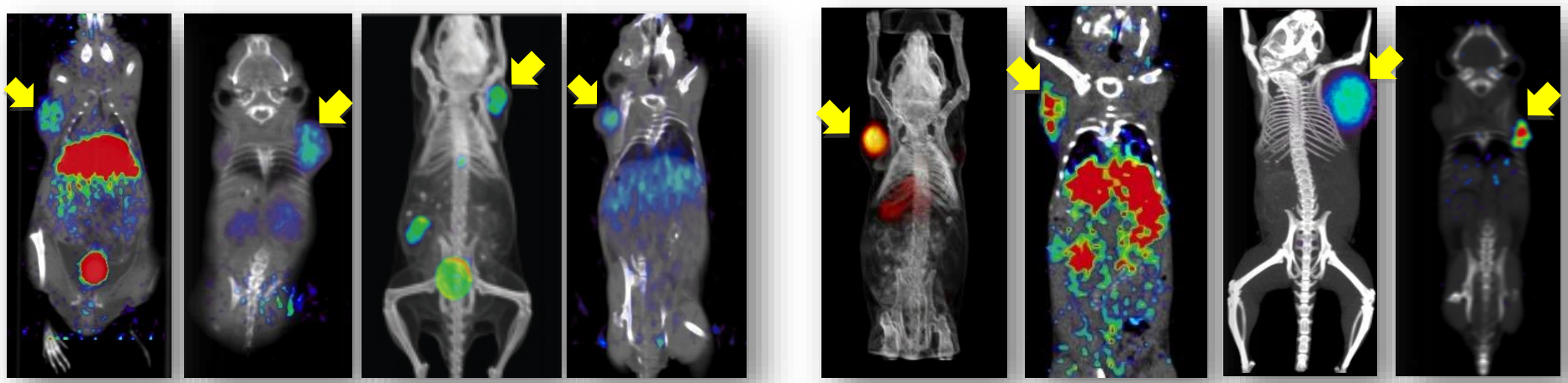
LAPC-9 prostate cancer xenografts in SCID mice; microPET/CT; images scaled individually

Knowles and Wu J. Clin. Oncol. 2012; K. Zettlitz.

Minibodies and diabodies as a platform for cell-surface imaging



ALCAM HER2 PSCA CA19-9 CEA EMP2 CD20 PSMA



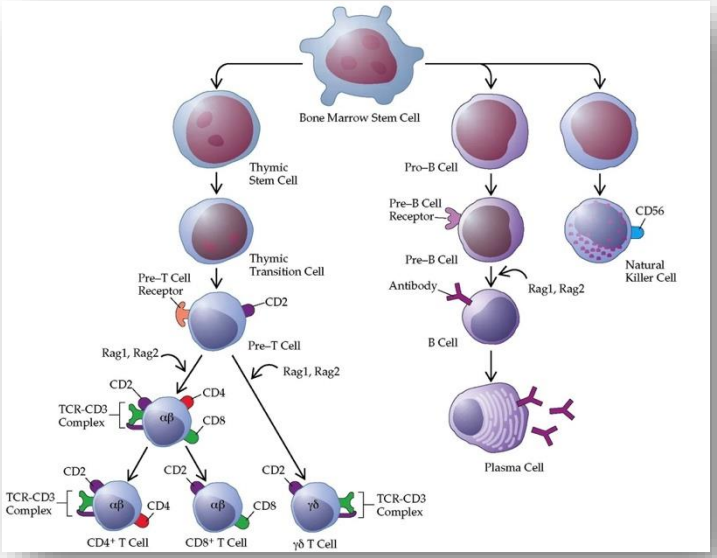
*ImmunoPET at **18-20 h** for minibodies; **1-4 h** for diabodies*

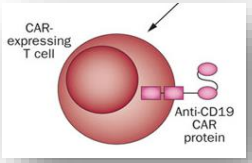
"In vivo immunohistochemistry"

Wu, Methods 2014

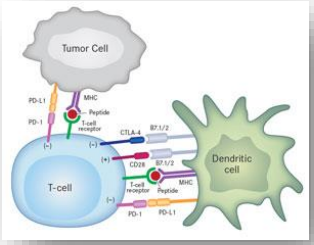
Beyond Oncology… Antibodies for Imaging Immunology

- FDG-PET non-specific
- CD antigens as markers of lineage, differentiation, activation
- Applications:
  - Immune responses and inflammation
  - Cancer immunotherapy



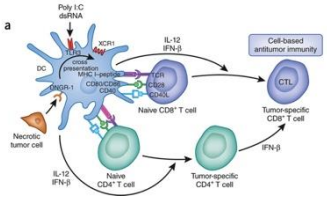


Cell-based therapies

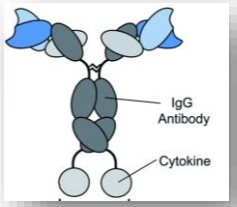


Checkpoint inhibitors

Bispecifics



Vaccines



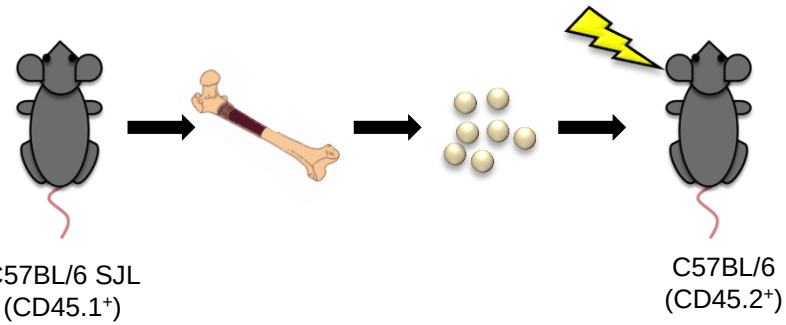
Immunocytokines

> *"The cytotoxic T cell is the drug."*
> *-Toni Ribas*

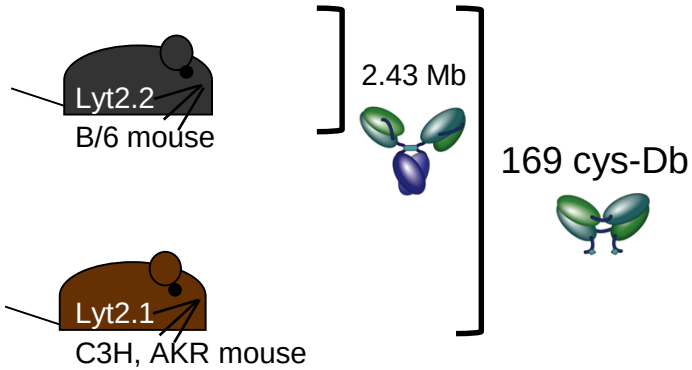
Collaborations with Owen Witte, Antoni Ribas, John Timmerman

Imaging CD8 T cell repopulation following HSC transplant

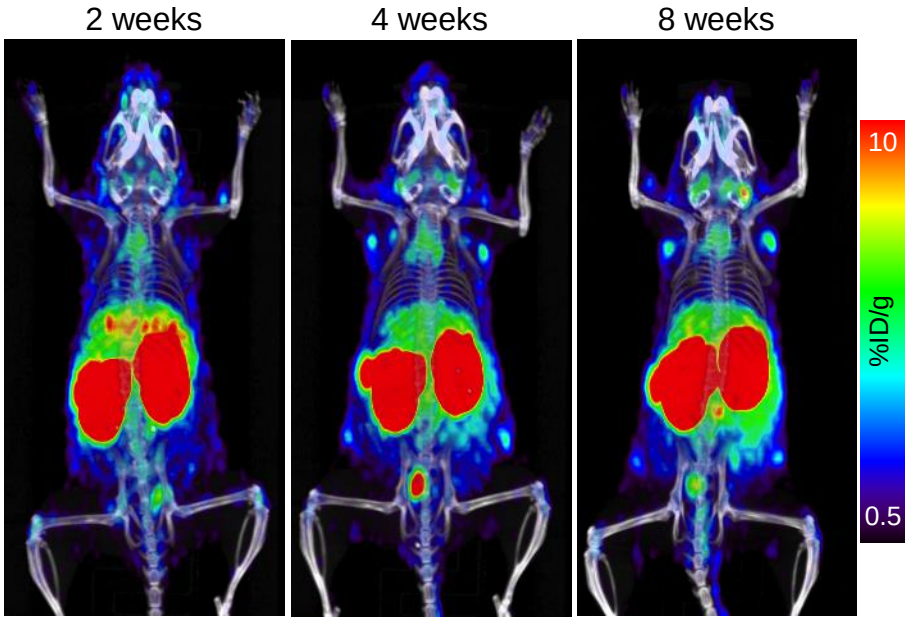
Preclinical hematopoietic stem cell (HSC) transplant model



Bone marrow derived HSCs are injected into lethally irradiated BL/6 mice



Time post-HSC transplantation

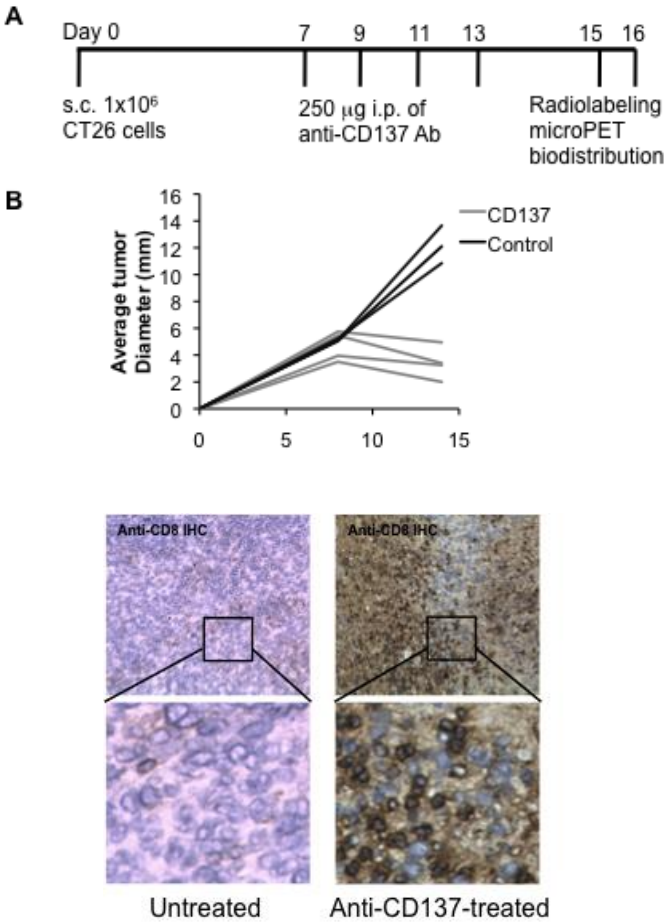


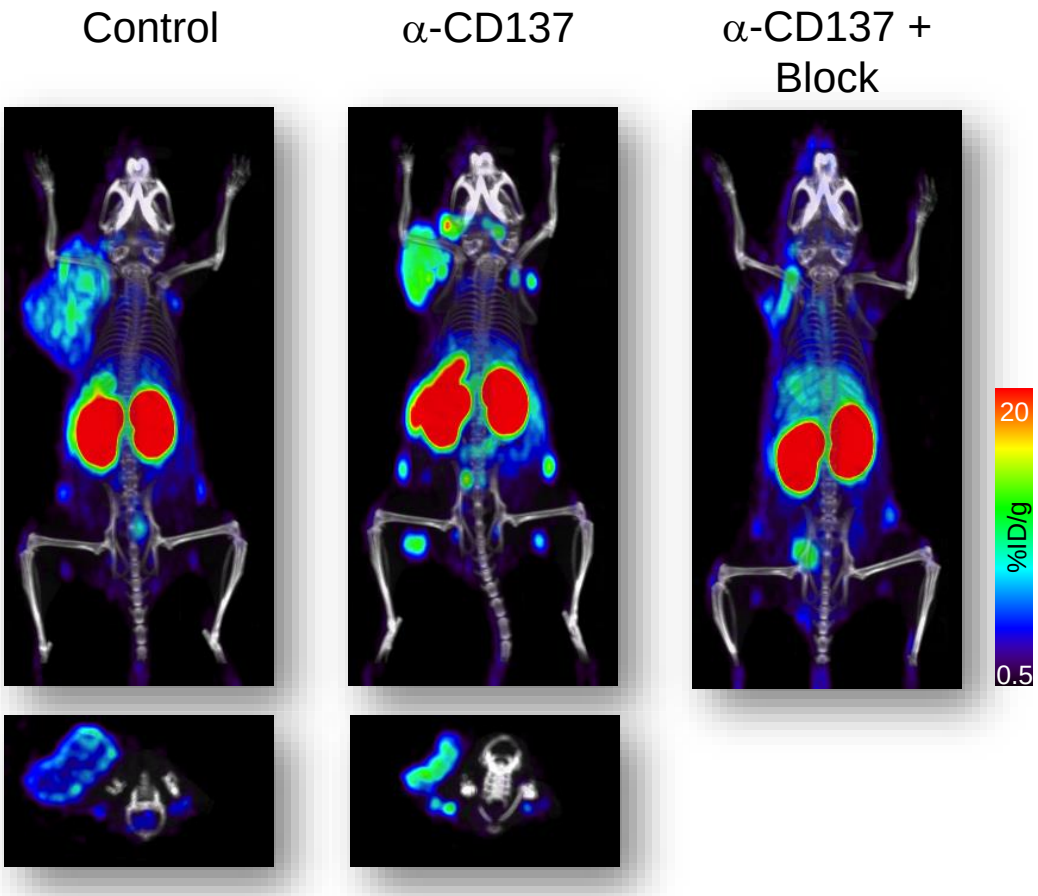
^{89}Zr-radiolabeled anti-CD8 169 cys-diabody successfully detects T cell repopulation over time

R. Tavaré, McCracken, M., Zettlitz, K.A., Salazar, F.B., Witte, O.N. and Wu, A.M., J.Nucl. Med. 2015

Imaging CD8 T cell infiltration in tumor immunotherapy

CT26 syngeneic tumor treated with anti-CD137 (4-1BB)



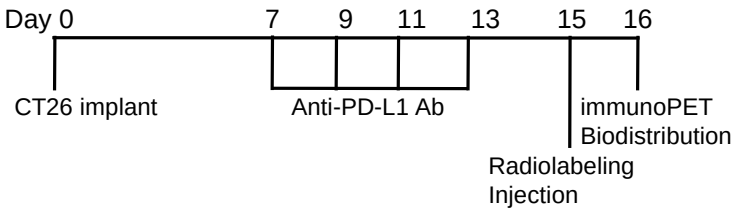


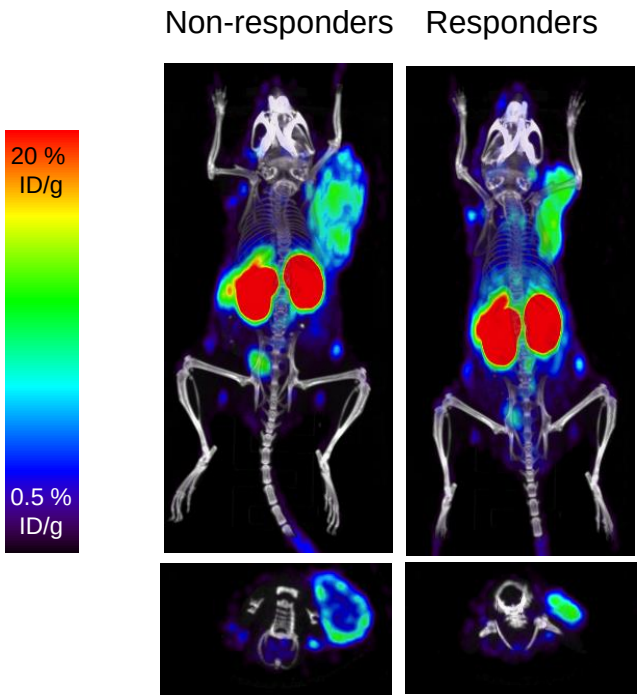
MicroPET imaging using ^{89}Zr anti-CD8 169 cys-diabody

Tavaré, R. et al. Cancer Research 2016

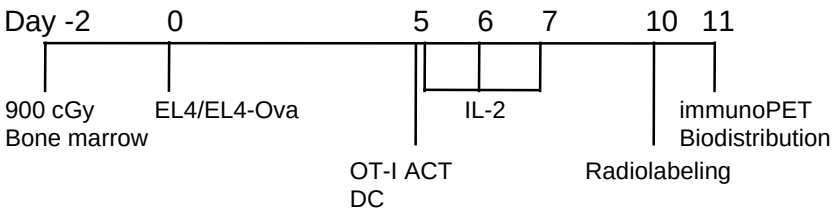
Imaging CD8 T cell infiltration in tumor immunotherapy

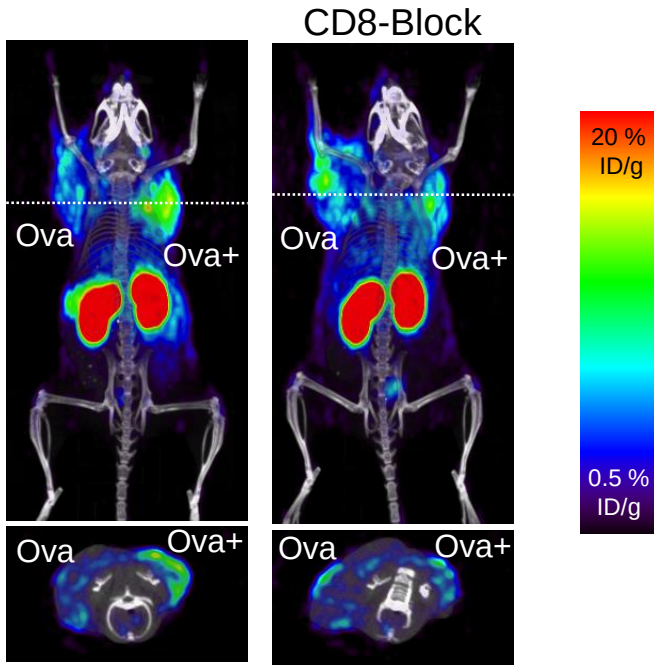
- CT26 tumor treated with anti-PD-L1
- 25-33% of treated mice respond





- EL4 murine lymphoma ± Ova
- Adoptive transfer of OT-I CD8^{+} T cells

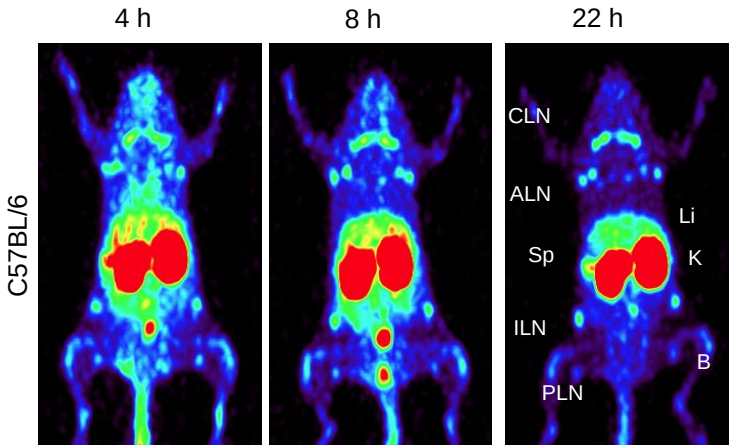


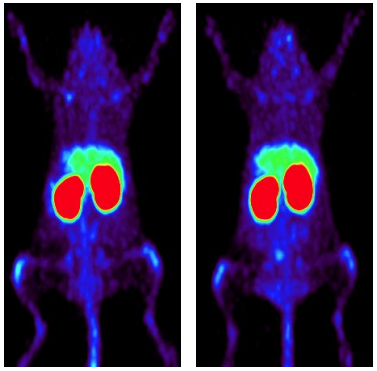


Tavaré, R. et al. Cancer Research 2016

Imaging **CD4** T cells in lymphoid tissues

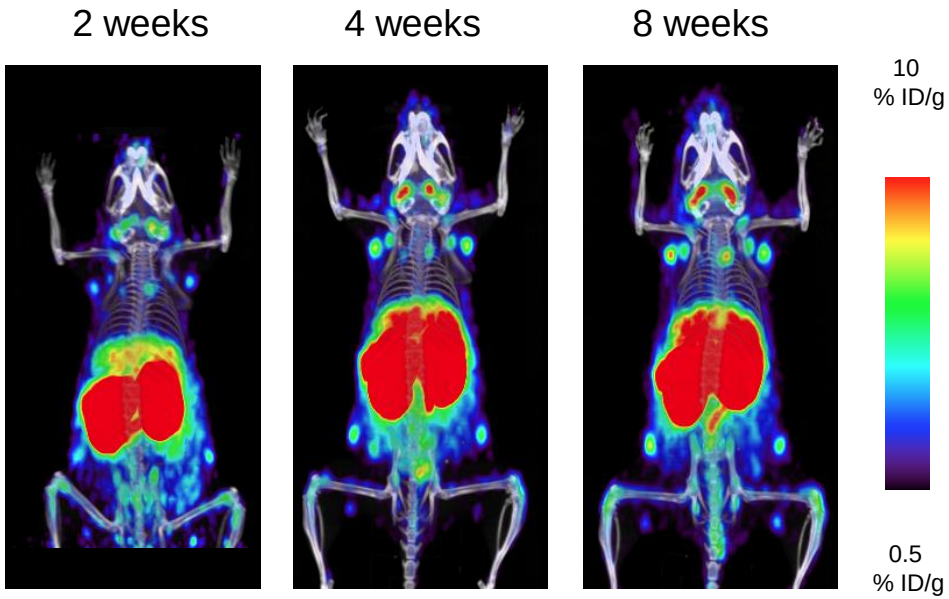
GK1.5 cys-diabody





Blocked Depleted

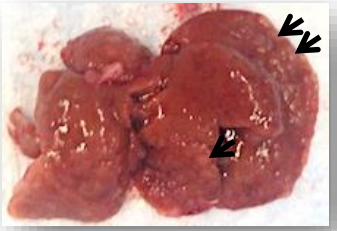
Time post-HSC transplantation



^{89}Zr-radiolabeled anti-CD4 GK1.5 cys-diabody detects T cell repopulation following HSC

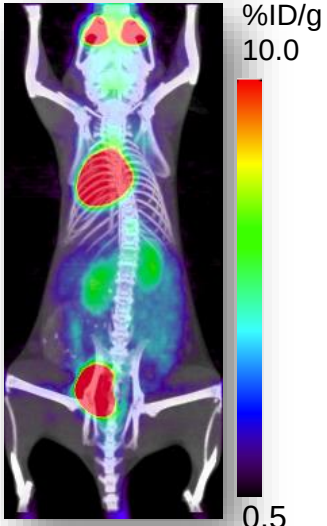
R. Tavaré, J.Nucl. Med. 2015

^{18}F-GA101 cDb imaging in A20-huCD20 metastatic lymphoma in huCD20TM mice

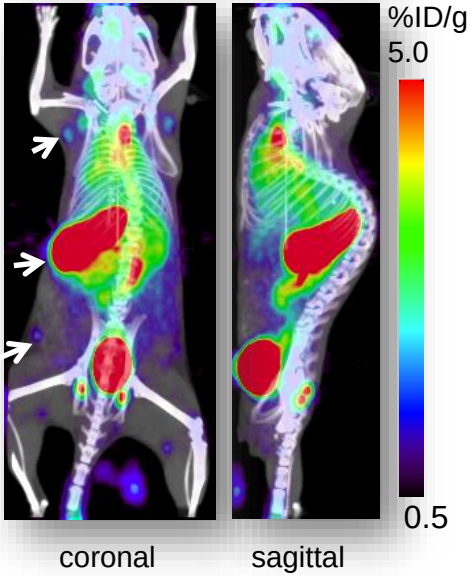


huCD20TM

^{18}F-FDG

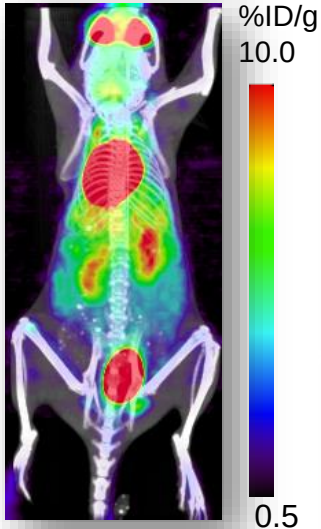


^{18}F-FB-GA101 cys-diabody

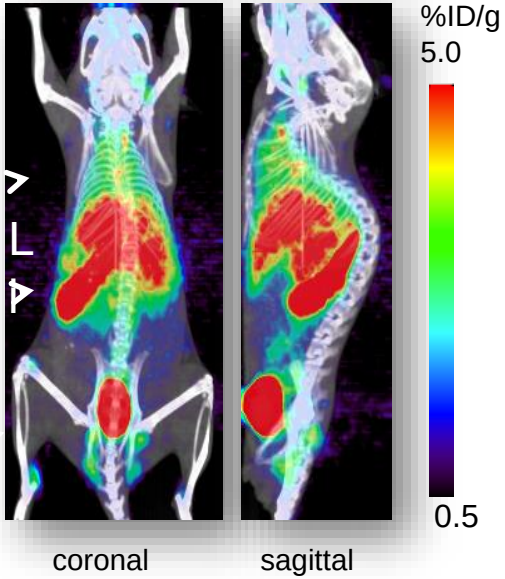


huCD20TM + A20-huCD20 i.v.

^{18}F-FDG



^{18}F-FB-GA101 cys-diabody



Zettlitz, K.A., Tavaré, R., Salazar, F.B., Steward, K.K., Yamada, R.E., Timmerman, J.M., and Wu, A.M. abstract WMIC 2014

Challenges to development of radiolabeled antibodies for immunoPET

Complex product:

- Biopharmaceutical – time consuming and costly to produce
- Radiopharmaceutical

Regulatory path – tox/safety

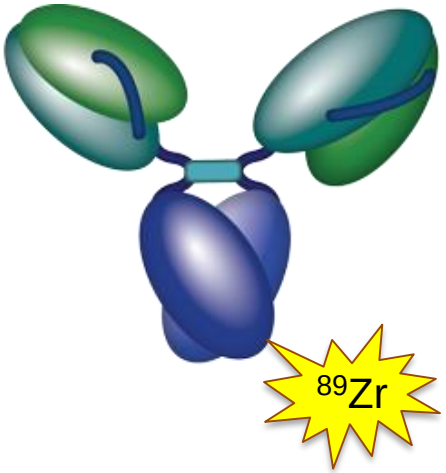
What is “efficacy” – requirements for approval; indication

Financial incentives/reimbursement

Selection of radionuclide: Go short or go long?

Radionuclide	Pros	Cons
^{68}Ga 68 min	Generator-produced 89% β+ Favorable dosimetry; radioactive waste not an issue Need rapidly targeting agent	Every site needs a generator High energy β+; poorer resolution
^{18}F 109 min	Cyclotron-produced 97% β+ Favorable dosimetry; radioactive waste not an issue Need rapidly targeting agent	Need cyclotron w/in 2h travel distance
^{89}Zr 3.2 d	Commercially available clinical grade (IBA, NCM, 3D Imaging, PE, etc.) 23% β+ Can be labeled centrally and shipped (e.g. across US)	Radiation dose (due to mixed emissions and half life)

Clinical development and translation

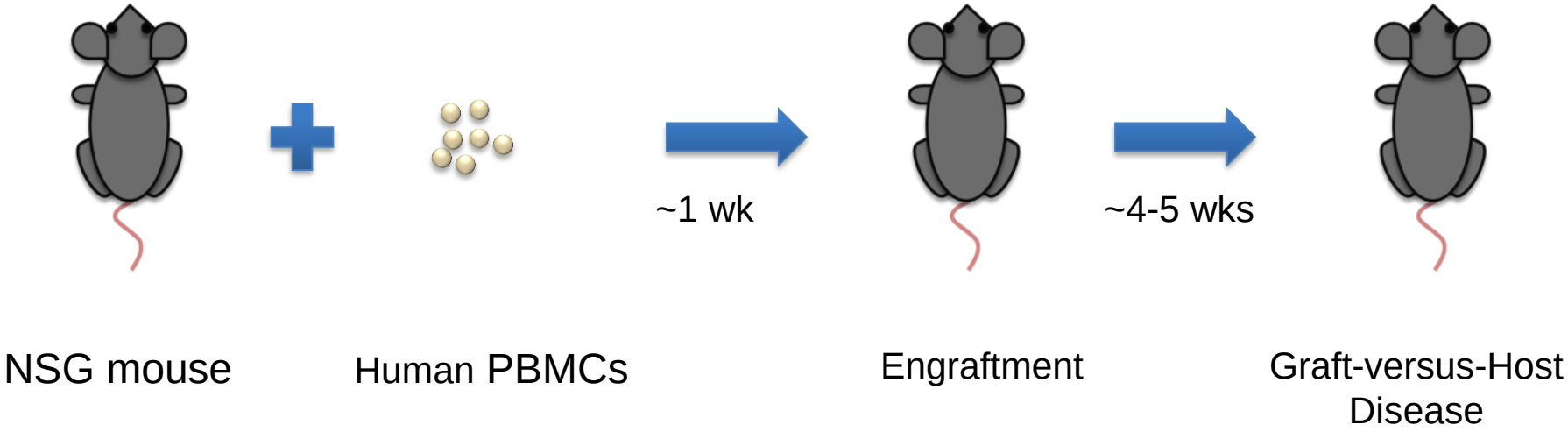


- Humanized minibodies
- Conjugated with desferrioximine
- ^{89}Zr for immunoPET
  - IAB22M CD8
  - IAB2M PSMA

ImaginAb, Inc.

IAB22M2C for detection and imaging of human CD8 T cells

- Cell surface marker on cytotoxic T cells
- Minibody: Does not contain full Fc; biologically inert (no T cell activation, cytokine release, etc.)
- Preclinical imaging in humanized mouse models



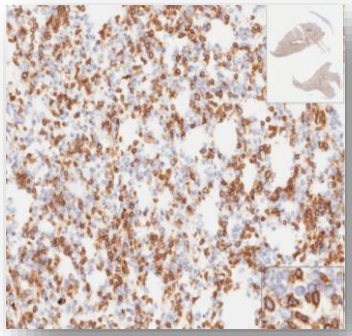
Infiltration of Human CD8 T cells into Lungs Can Be Followed in NSG Mice With GVHD

huCD8 immunoPET

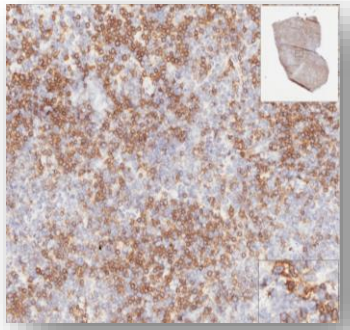
1 week - Engraftment

4 weeks - GVHD

huCD8 IHC



Lung



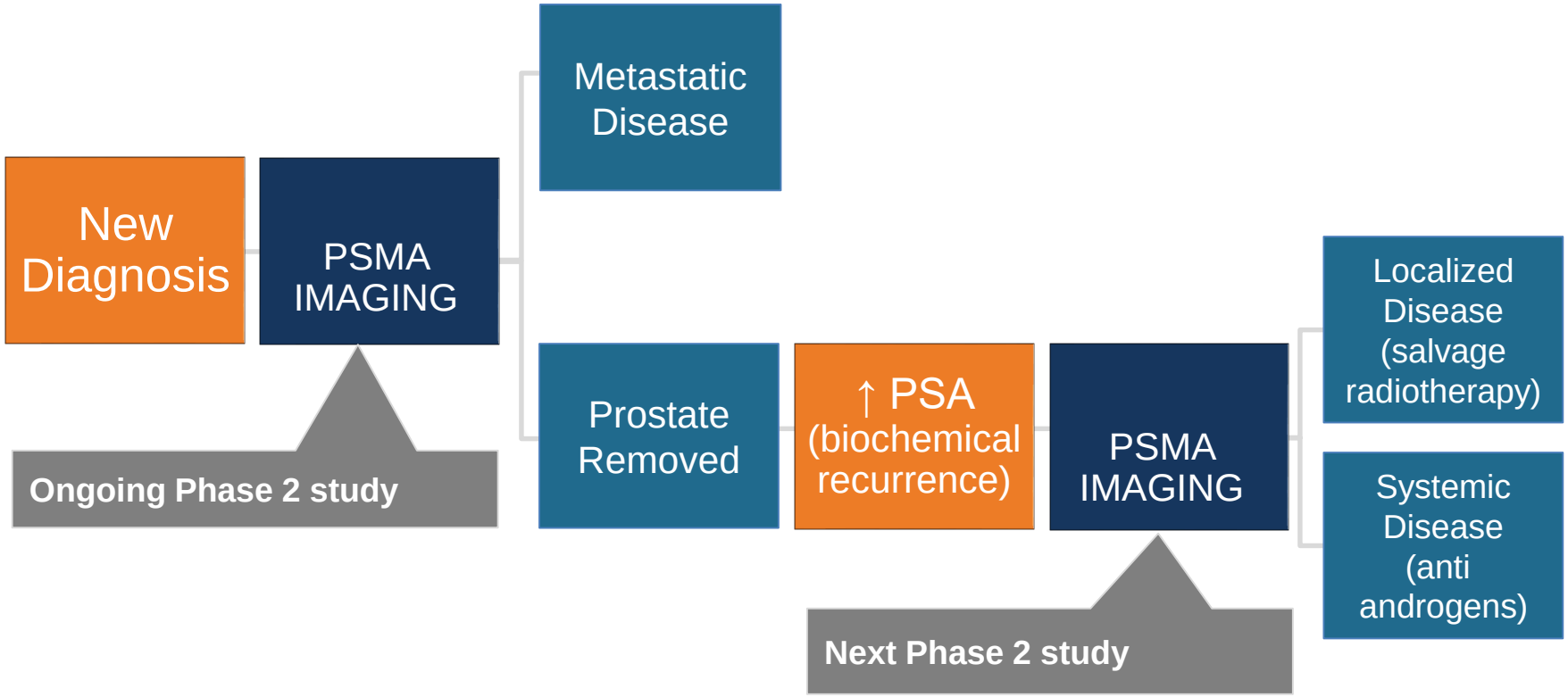
Spleen

Engraftment of NSG mice with 20 x 10^6 hu-PBMCs

IND Q3 2016

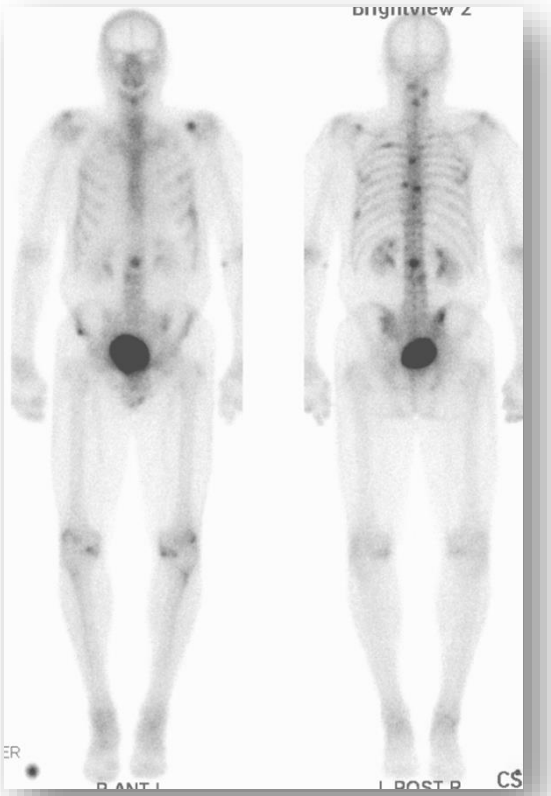
Olafsen et al. abstract Antibody Engineering 2015; AACR 2016 (ImaginAb, Inc.)

IAB2M anti-PSMA Targets Major Clinical Decision Points in Prostate Cancer

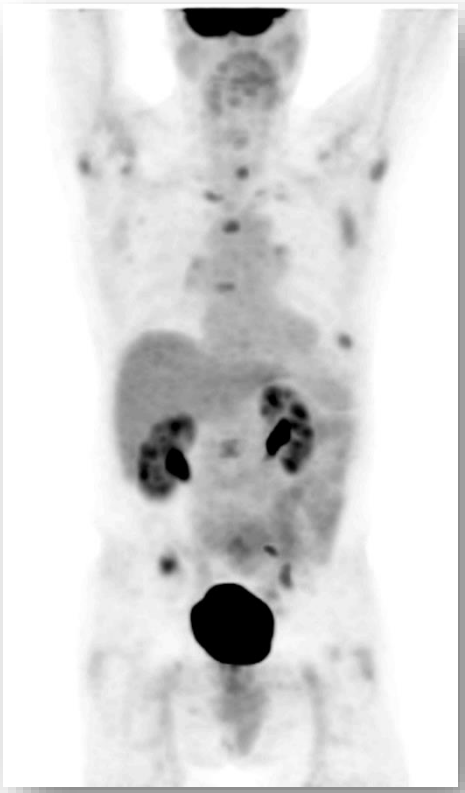


First-in-human imaging with ^{89}Zr-Df-IAb2M anti-PSMA minibody in patients with metastatic prostate cancer: Pharmacokinetics, dosimetry, and lesion uptake

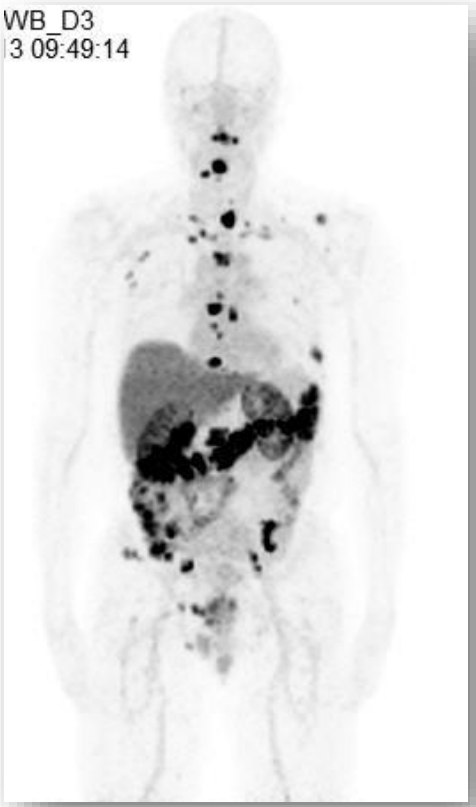
Pandit-Taskar, N., O'Donoghue, J., Lyashchenko, S., Shutian, R., Carrasquillo, J.A., Lewis, J.S., Lashley, A., Martinez, D., Keppler, J., Wu, A.M., Weber, W.A., Scher, H.I., Larson, S.M., Morris, M.J.



^{99m}Tc-MDP bone scan
Anterior and posterior



FDG PET scan
MIP

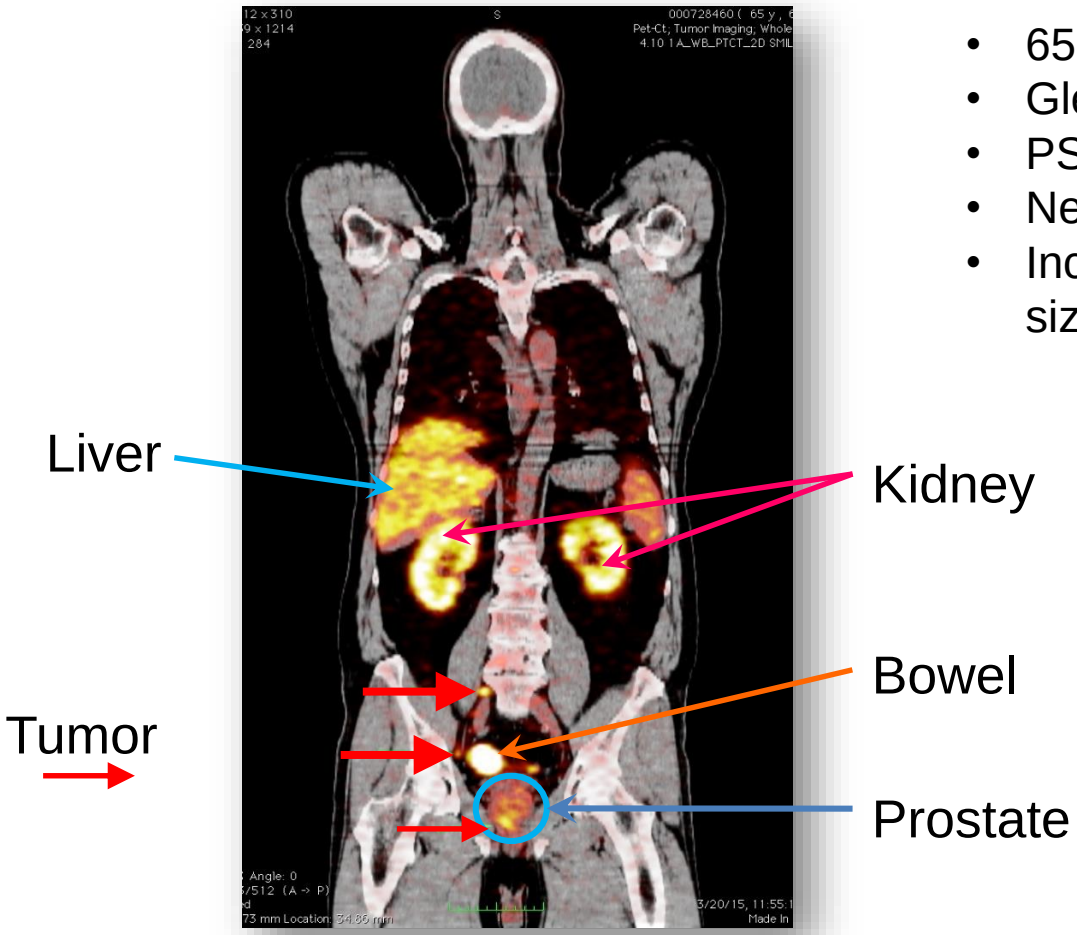


^{89}Zr-Df-IAB2M scan
MIP

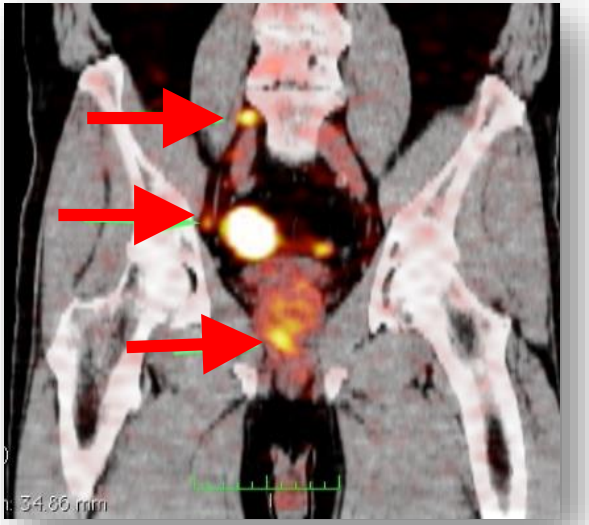
Pandit-Taskar et al., MSKCC, abstract, SNMMI 2015

Head-to-head comparison of ^{89}Zr-Df-IABM PET/CT to ^{111}In capromab pendetide SPECT/CT scans in the detection of occult prostate cancer in patients undergoing radical prostatectomy (RP) with negative conventional imaging (CI) studies

Bgurek, B.M., Woodruff, A.J., Wyman, B.T., Keppler, J., Wu, A.M., Masci, P., and Korn, R.L.



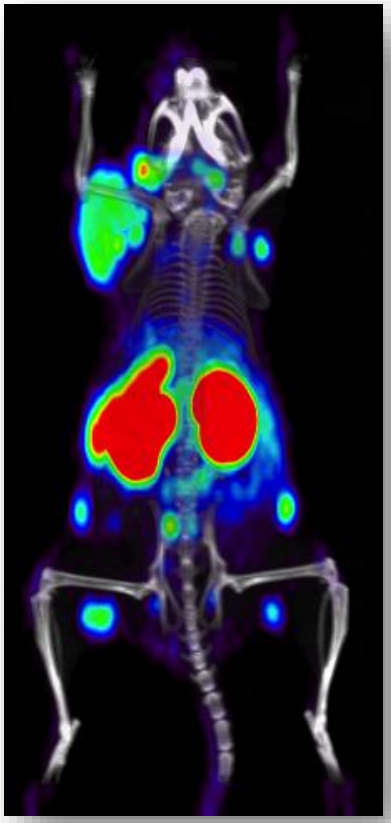
- 65 Year old male
- Gleason score 8
- PSA (at screening) 25.5 ng/mL
- Negative CI
- Increase ^{89}Zr-Df-IAB2M in normal size lymph nodes (red arrows)



Bgurek et al., Scottsdale Healthcare abstract, WMIC 2015

Summary and future: Non-invasive Imaging in Immuno-Oncology

- Powerful, specific, and whole-body approaches for monitoring immune cells and immune responses
  - Metabolic probes, pre-labeled cells, reporter genes
  - Engineered antibodies for immunoPET of cell surface markers
- Potential for profiling : immune cell subsets, expansion, trafficking, activation; biomarker microenvironment; potential role in patient selection and treatment monitoring
- Challenges:
  - Sensitivity: lower limit of detection (targets/cell and cells/cc) (AACR 2016)
  - Spatial resolution (macroscopic, not microscopic)
  - Multiplex imaging? Multiple cell types, subsets (e.g. T_{regs})
  - Endogenous vs adoptively transferred cells
  - Complex product, lengthy and expensive clinical development
  - Next targets – what do we need to assess *in vivo*?



CD8 T cell imaging

Imaging can enhance and complement in vitro biomarkers

“Antibody immunotherapy imaging”

Elisabeth de Vries
Department of Medical Oncology
University Medical Center Groningen
The Netherlands

umcg

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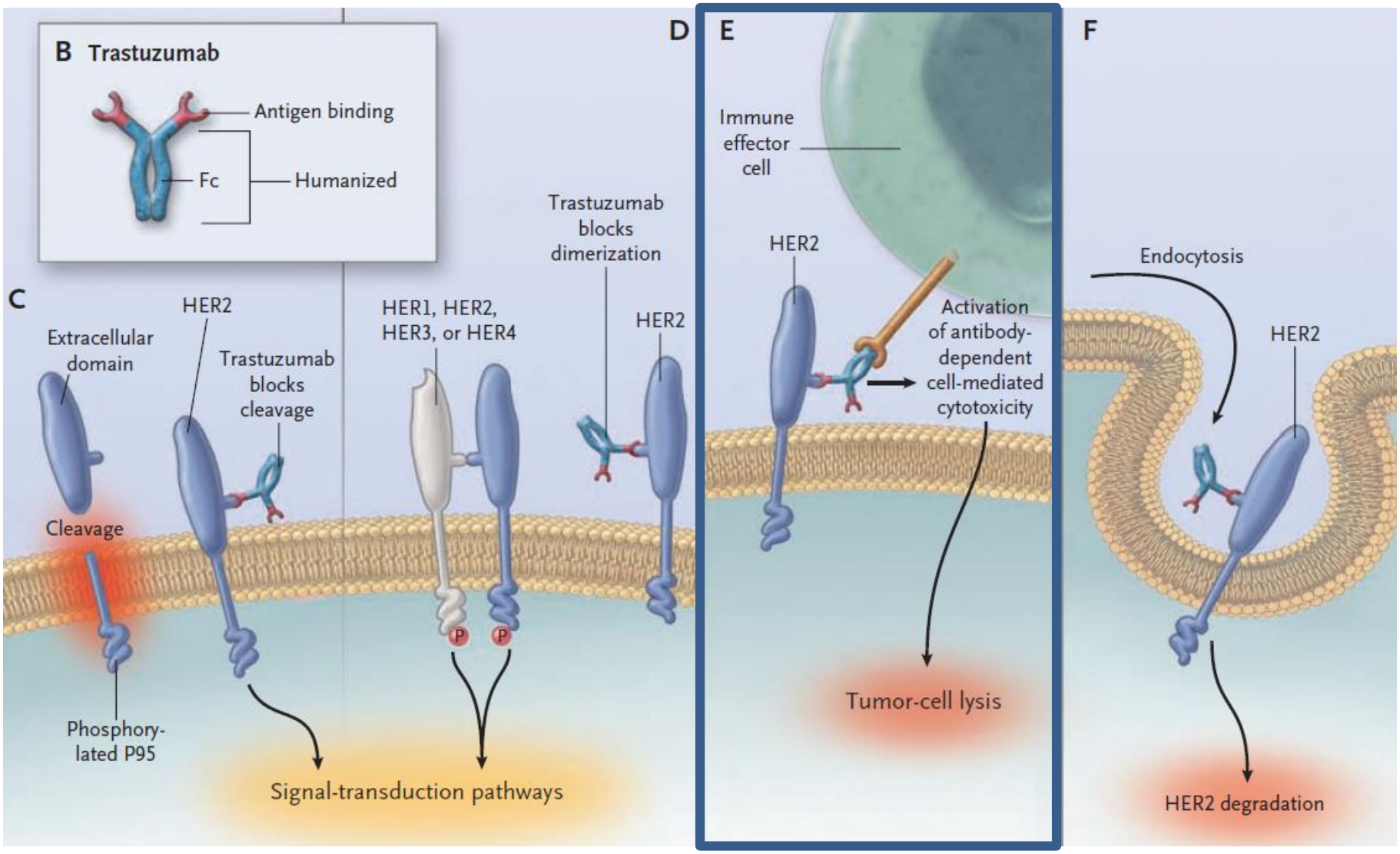
  - Roche/Genentech, Amgen, Novartis, Servier

Radionuclides for antibody imaging with SPECT & PET

SPECT		
radio-nuclide	half-life	residualization
^{111}In	67.3 h	+
^{131}I	192.5 h	-
^{123}I	13.2 h	-
^{99m}Tc	6.0 h	-

PET		
radio-nuclide	half-life	residualization
^{89}Zr	78.4 h	+
^{124}I	100.3 h	-
^{64}Cu	12.7 h	+
^{86}Y	14.7 h	+
^{18}F	109.7 min	-

Potential mechanisms of action of trastuzumab

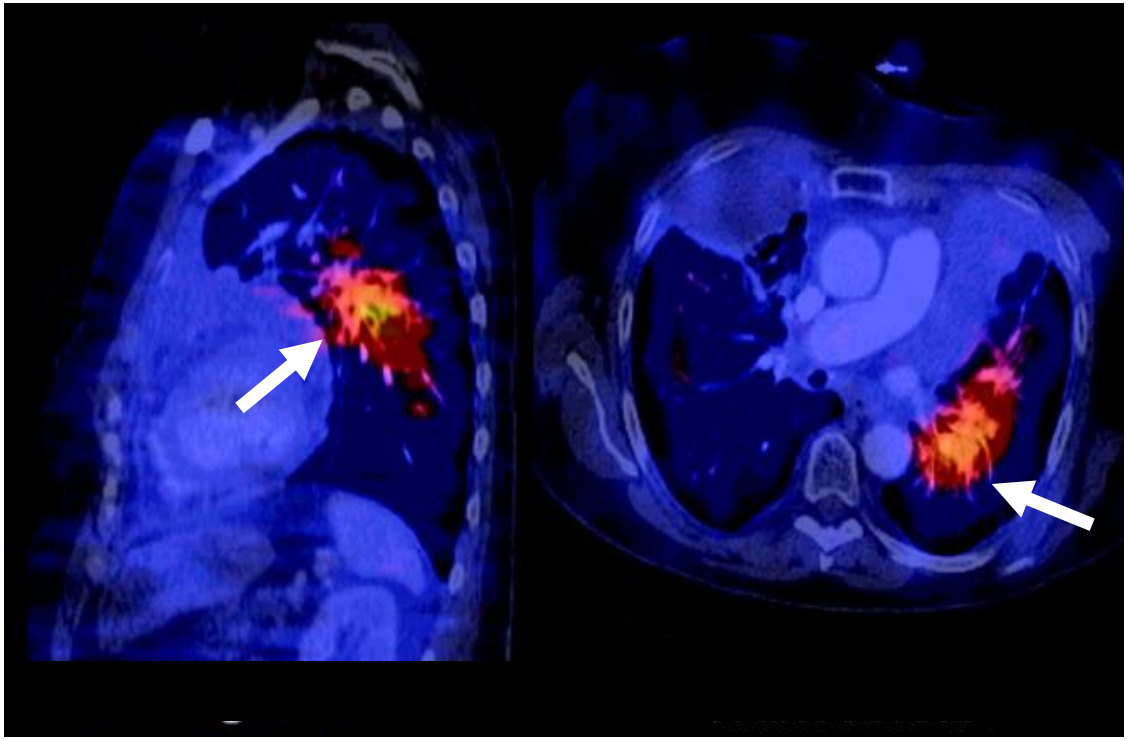


Hudis, New Eng j Med 2007

1

More lesions with ^{111}In-trastuzumab-SPECT in patients with HER2+++ metastatic breast cancer compared to conventional imaging

SPECT/CT



Overall results: Newly discovered tumor lesions in 13/15 patients

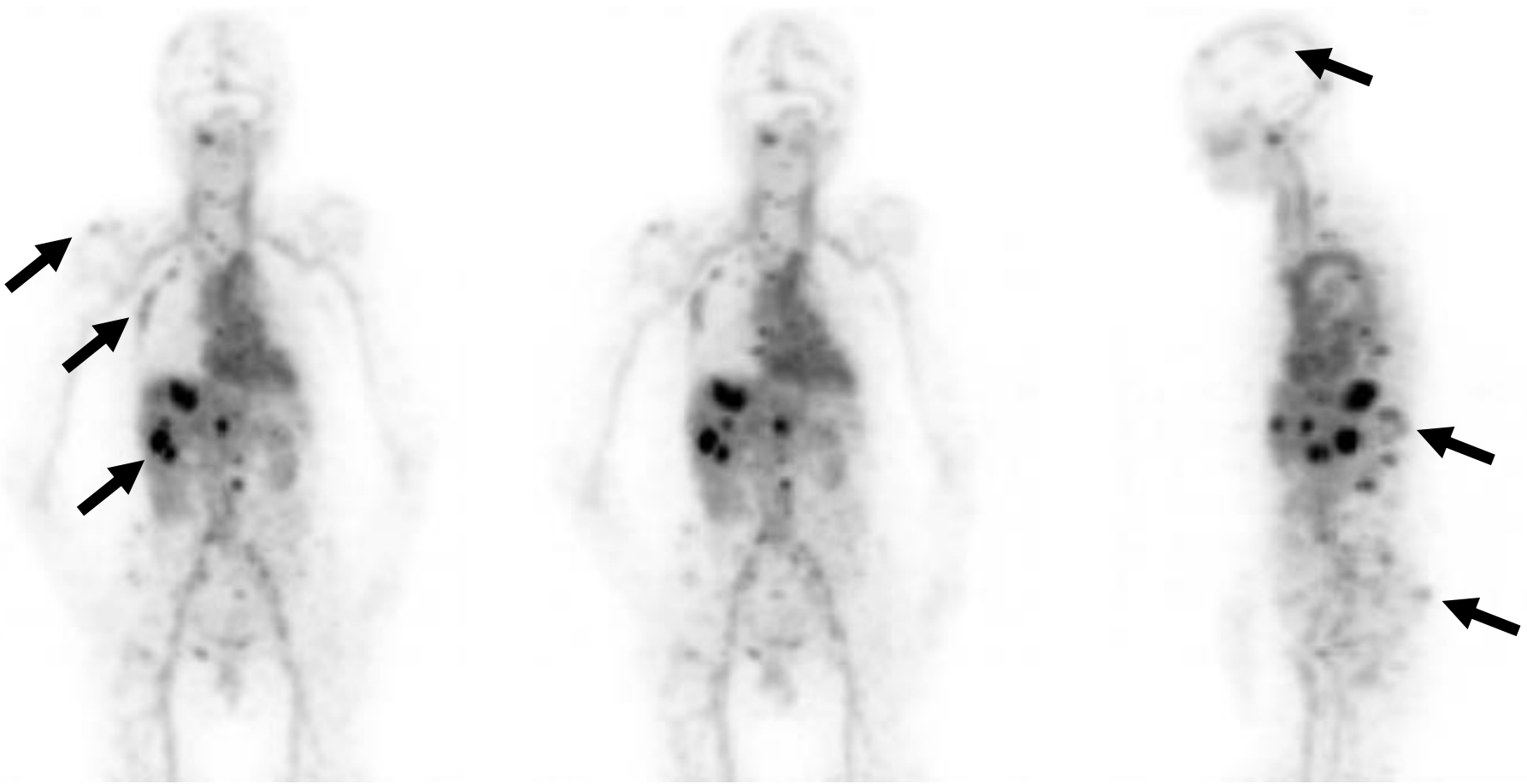
Perik et al, J Clin Oncol 2006

2 Limited trastuzumab tumor saturation: ^{111}In-trastuzumab

- Methods:
  - ^{111}In-trastuzumab administered day 1 of cycle 1 and day 15 of cycle 4 trastzumab plus paclitaxel.
- Results:
  - 25 tumor lesions in 12 patients visualized on both scintigraphy series
  - Tumor uptake decreased 19.6% (P = 0.03)
  - Residence times of normal organs remained similar

Perik et al, J Clin Oncol 2006
Gaykema et al, Mol Imaging 2014

^{89}Zr-trastuzumab tumor visualization

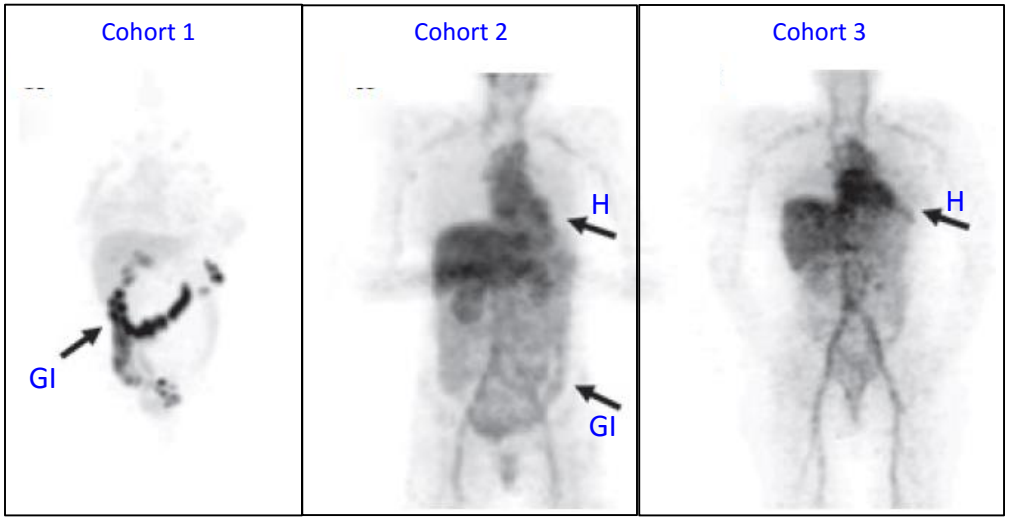


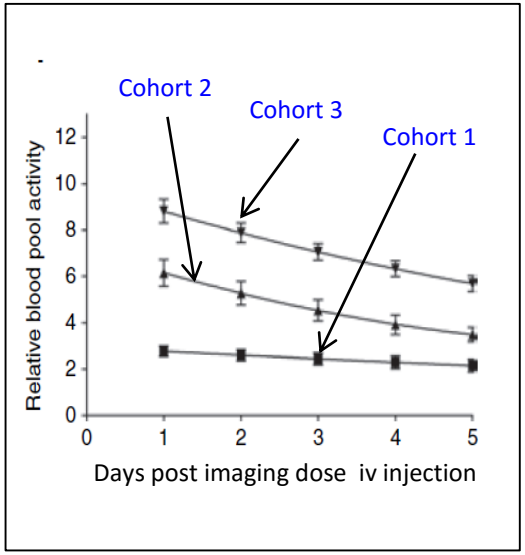
Day 4

Dijkers et al, Clin Pharmacol Ther 2011

3 ^{89}Zr-trastuzumab tumor accumulation dependent on total protein dose

H = heart
GI = intestines

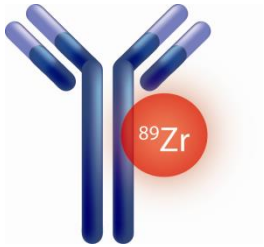




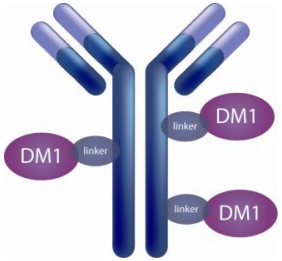
Imaging dose	n=2	n=5	n=7
^{89}Zr-trastuzumab	1.5 mg	1.5 mg	1.5 mg
trastuzumab	8.5 mg	48.5 mg	8.5 mg + up to 6 mg/kg therapy

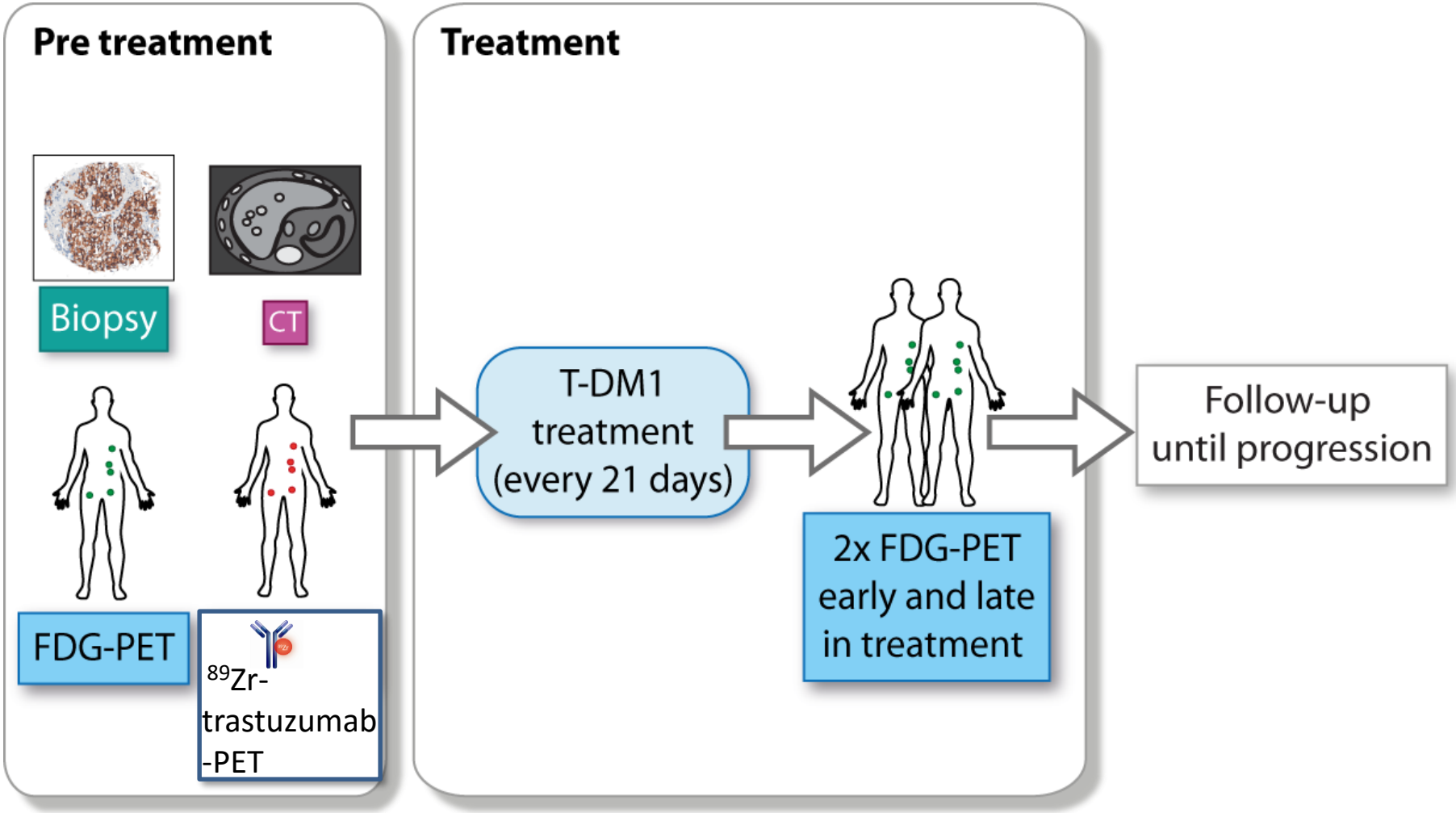
- Cohorts 2 & 3 have better ^{89}Zr tumor uptake than cohort 1
-

Dijkers et al, Clin Pharmacol Ther 2010



Zephir TDM-1 study

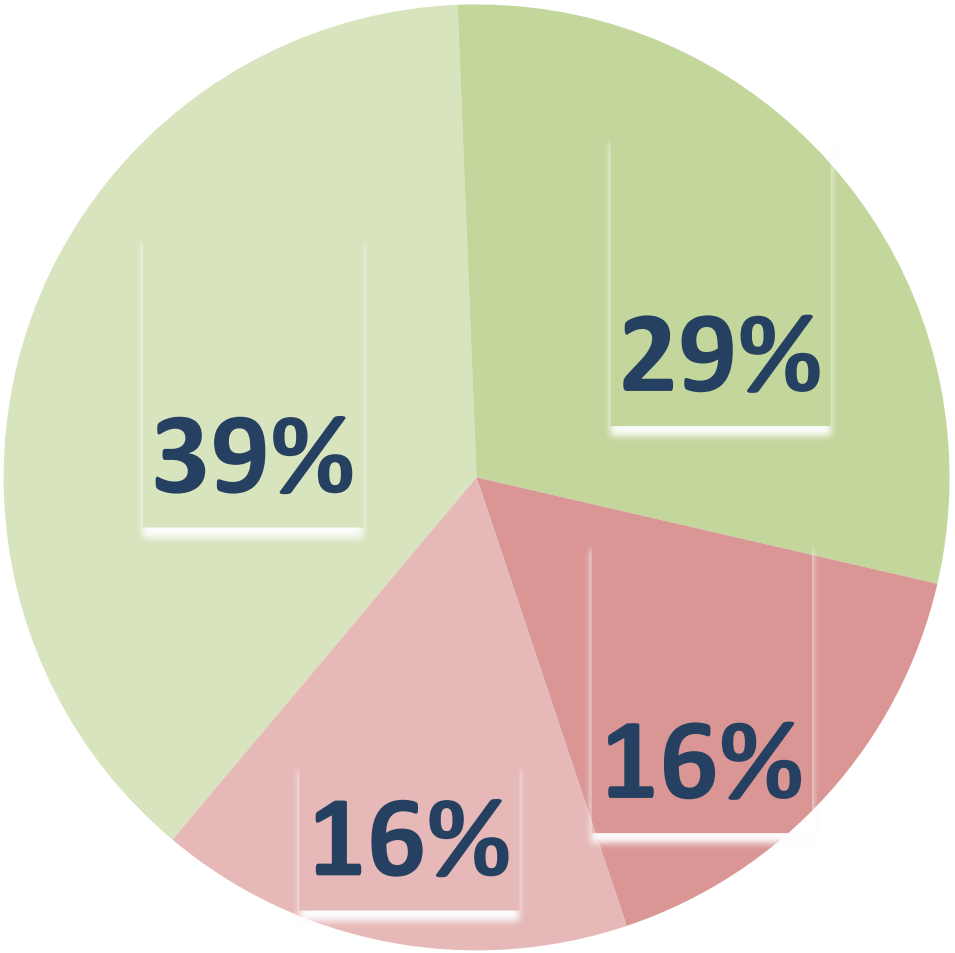




NCT01565200

4

Despite presence HER2, ^{89}Zr-trastuzumab does not always reach tumor (PET/CT n=52)



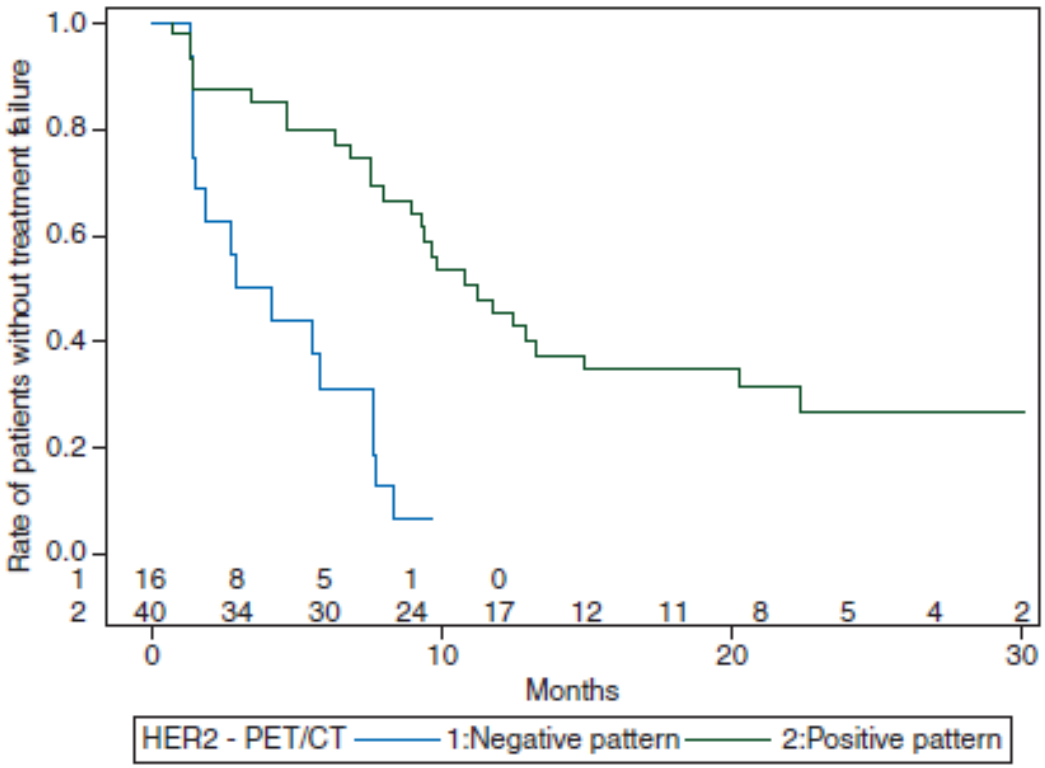
All or most of the tumor load is seen on ^{89}Zr-trastuzumab PET/CT

Minority of tumor load or no lesions are seen on ^{89}Zr-trastuzumab PET/CT

Gebhart et al, ASCO 2015 & Ann Oncol 2016

5 ^{89}Zr-trastuzumab tumor accumulation associates with T-DM1 time to treatment failure,

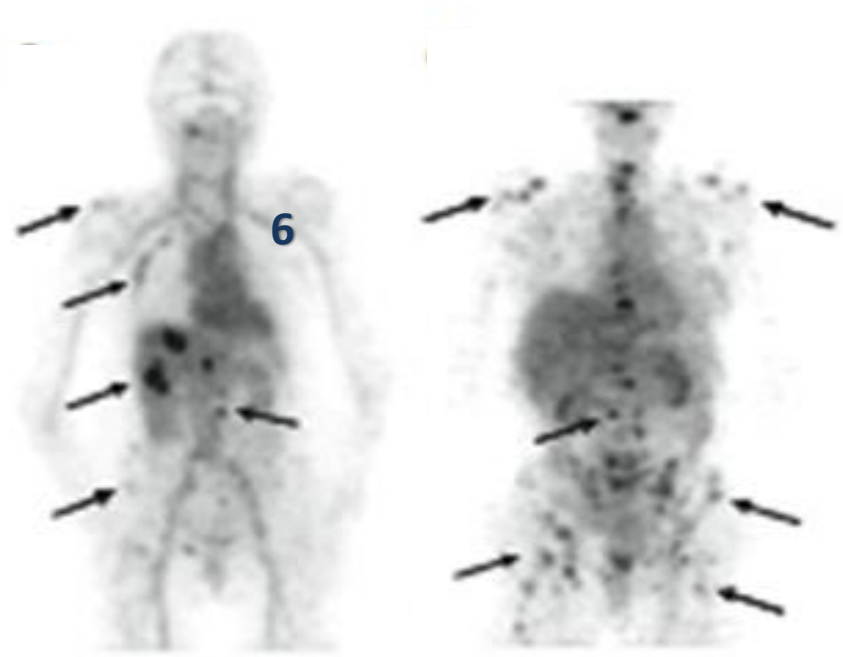
HER2+ (IHC/FISH) metastatic breast cancer patients



Gebhart G et al, Ann Oncol 2016

6

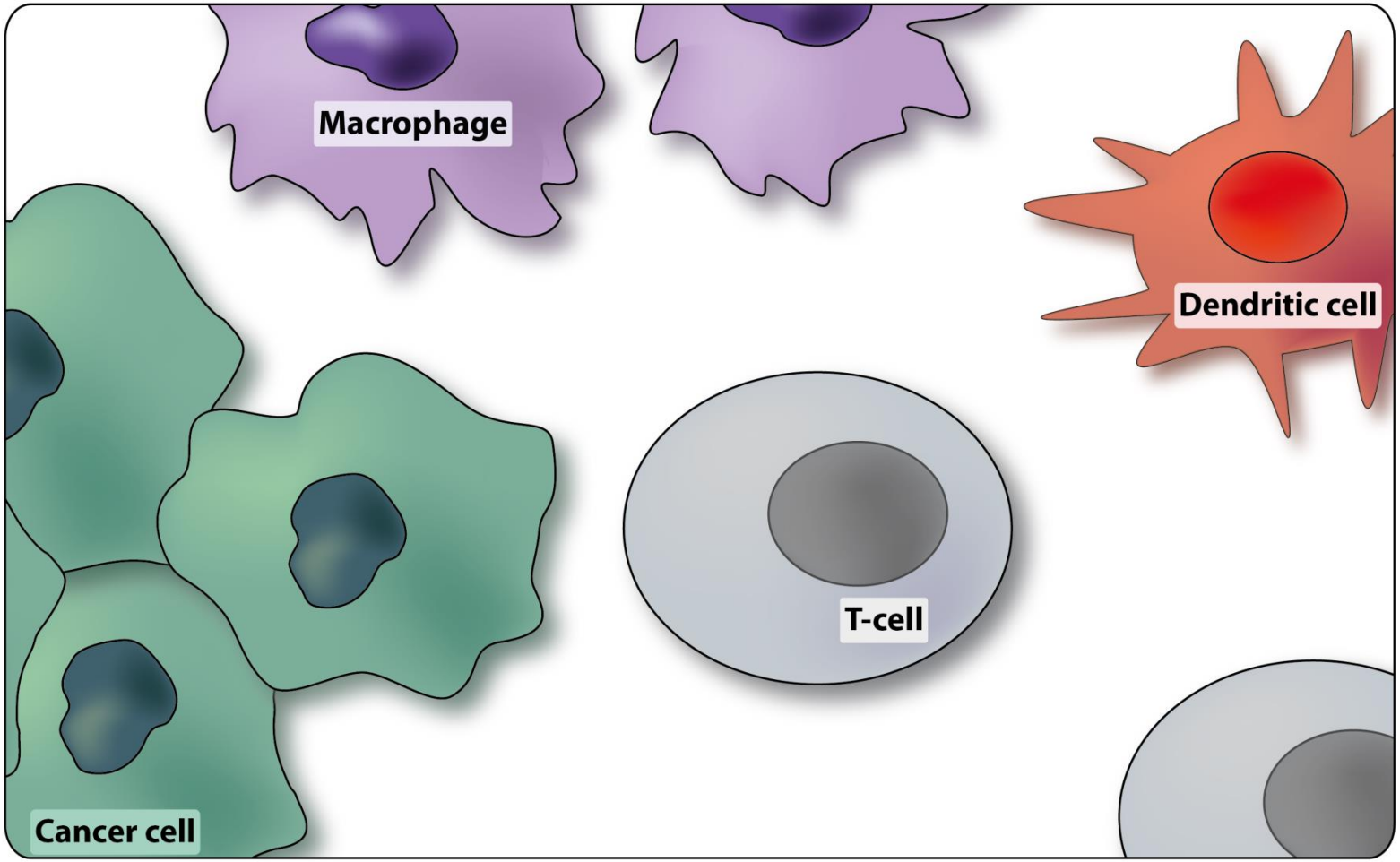
Heterogeneous ^{89}Zr-trastuzumab uptake in tumor lesions



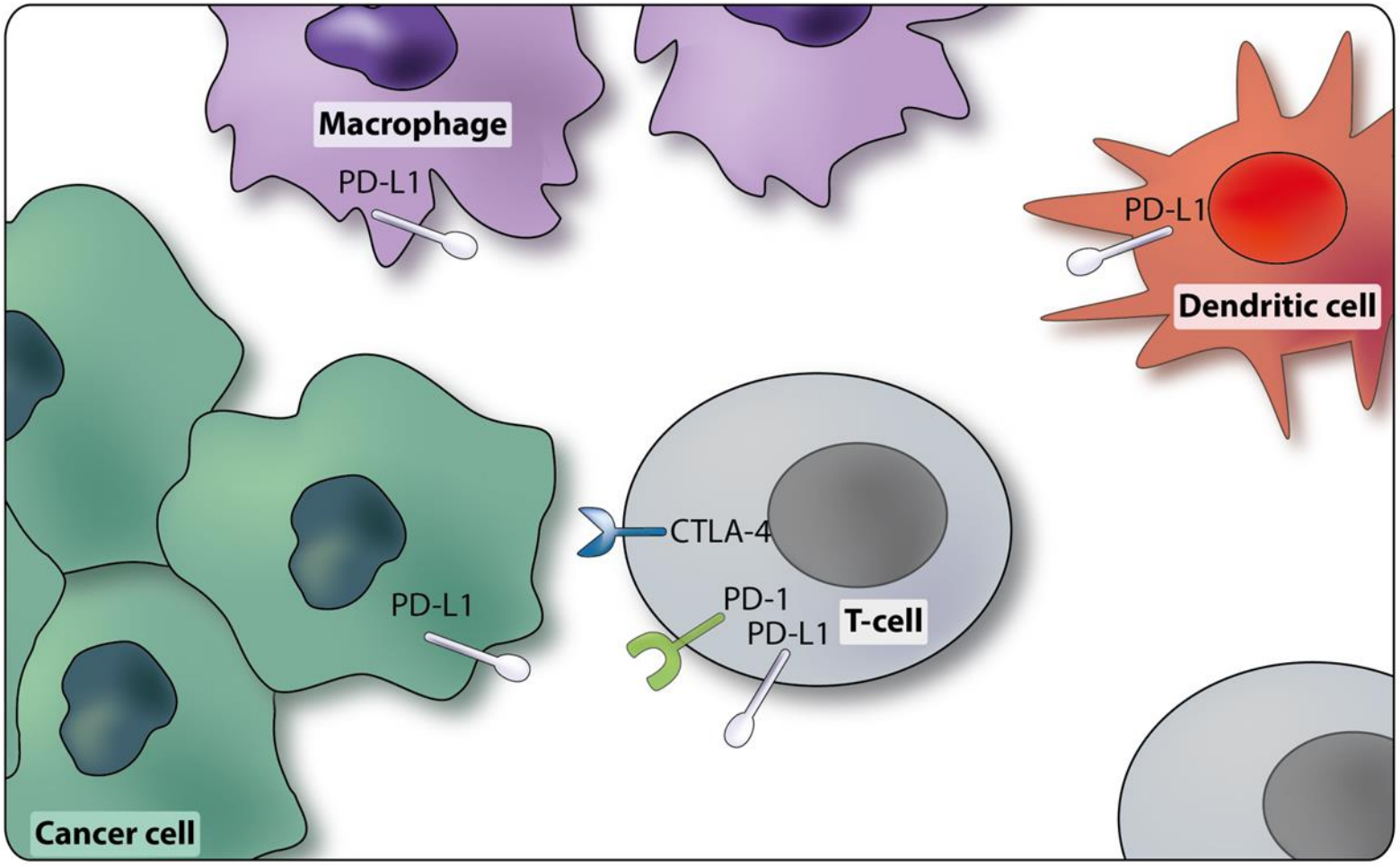
Different signal intensity in different lesions

Dijkers et al, Clin Pharmacol Ther. 2010

Tumor microenvironment



Targets immune checkpoint inhibitors



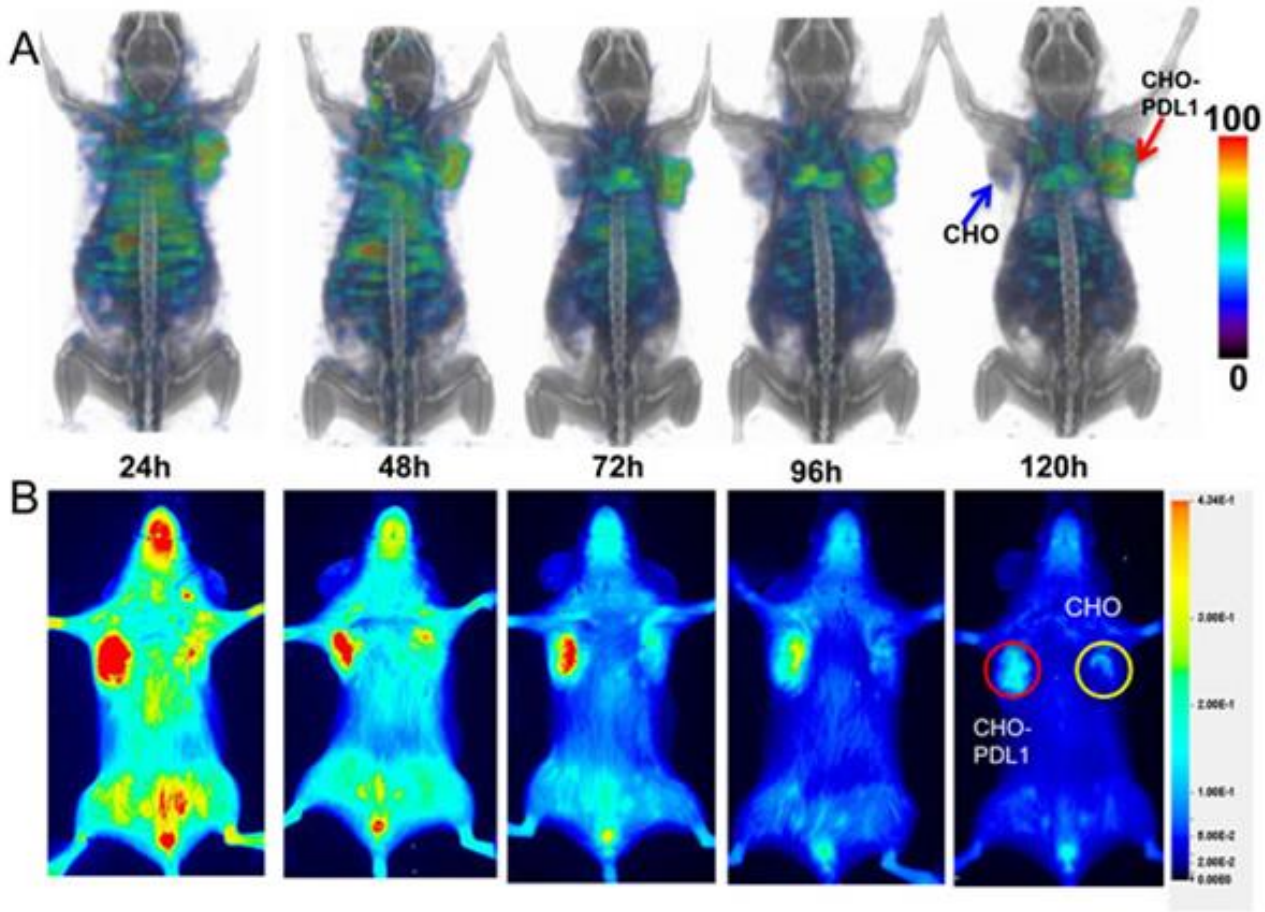
Preclinical imaging studies with radiolabeled immune checkpoint inhibiting antibodies

Tracer	Target	Origin	Model	Author	Journal
^{64}Cu-anti-CTLA-4	CTLA-4	Murine anti-mouse	CT26: mouse colon cancer	Higashikawa et al	PLoS One, 2014
^{111}In-anti-PD-L1	PD-L1	Humanized anti-human	Human cell lines	Chatterjee et al	Oncotarget, 2016
^{111}In-anti-PD-L1	PD-L1	Hamster anti-mouse	NT2.5: mouse mammary tumor	Josefsson et al	Cancer Res, 2016
^{125}I-anti PD-L1:PRO304397 biodistribution & autoradiography	PD-L1	Chimeric	Mouse	Deng et al	mAbs, 2016
^{111}In-anti-PD-L1	PD-L1	Murine anti-human	Human breast cancer cell lines	Heskamp et al	Cancer Res, 2015
^{64}Cu-anti-PD1	PD1	Hamster anti-mouse	B16F10: mouse melanoma	Natarajan et al	Bioconjug Chem, 2015
^{64}Cu-anti-PD1 ectodomain	PD1	Murine anti-mouse	CT26: mouse colon cancer	Maute et al	PNAS, 2015

Imaging with ^{111}In-PD-L1-mAb & NIR-PD-L1-mAb in sc CHO xenografts

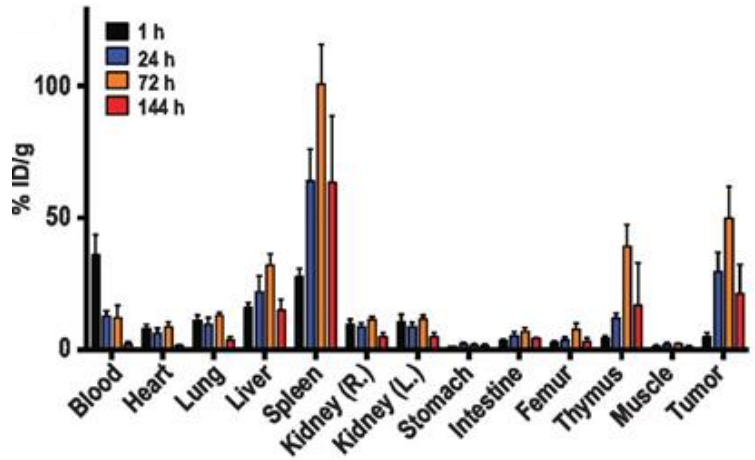
SPECT

Optical

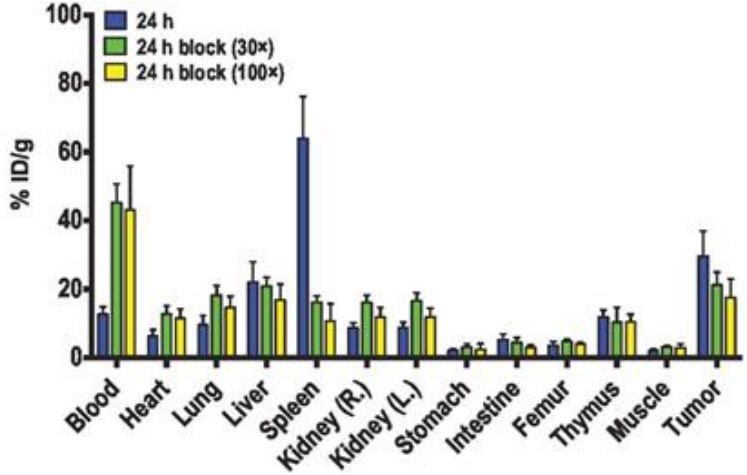


Chatterjee et al, Oncotarget 2016

^{111}In-PD-L1 antibody biodistribution in tumor-bearing transgenic neu-N mice for normal tissues

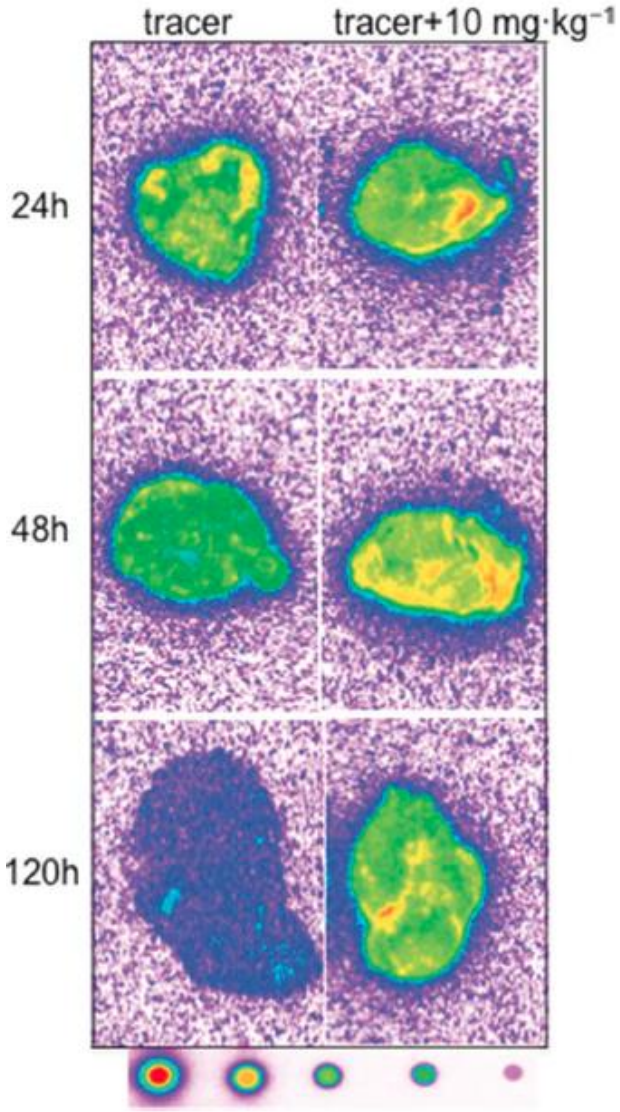


Coinjected for blocking with excess cold anti–PD-L1 Ab 30× (green) and 100× (yellow)



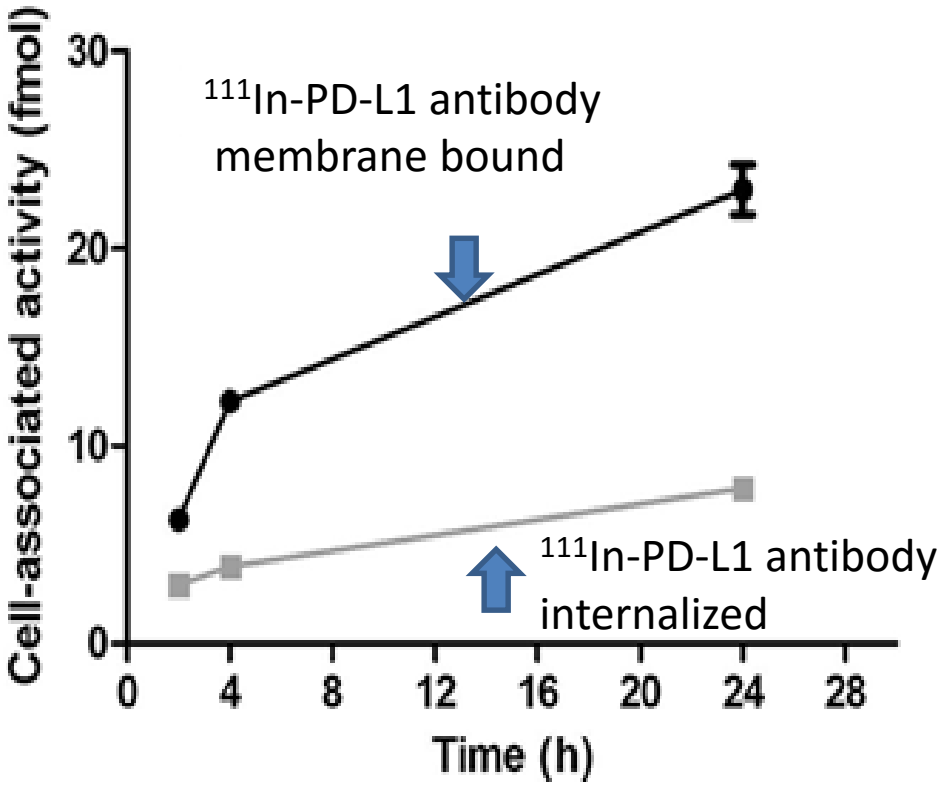
Josefsson et al. Cancer Res 2016

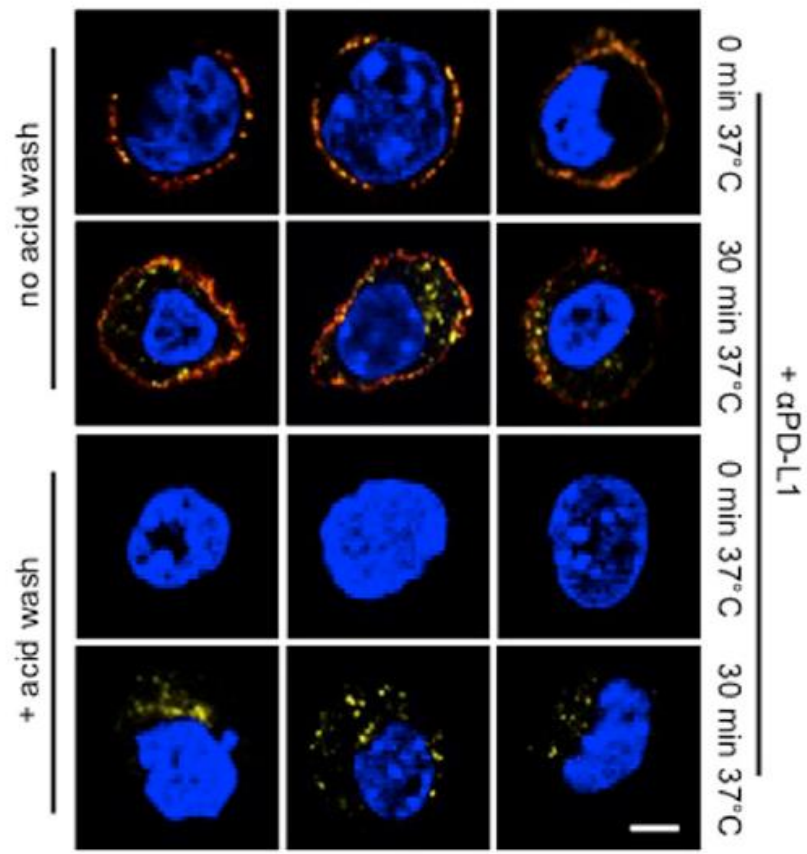
Autoradiography: distribution of ^{125}I-antiPD-L1 antibody PRO304397 in murine colorectal MC38 tumors



Deng et al, mAbs 2016

PD-L1 expressing tumor cells treated with anti-PD-L1 antibody, show PD-L1 antibody internalization

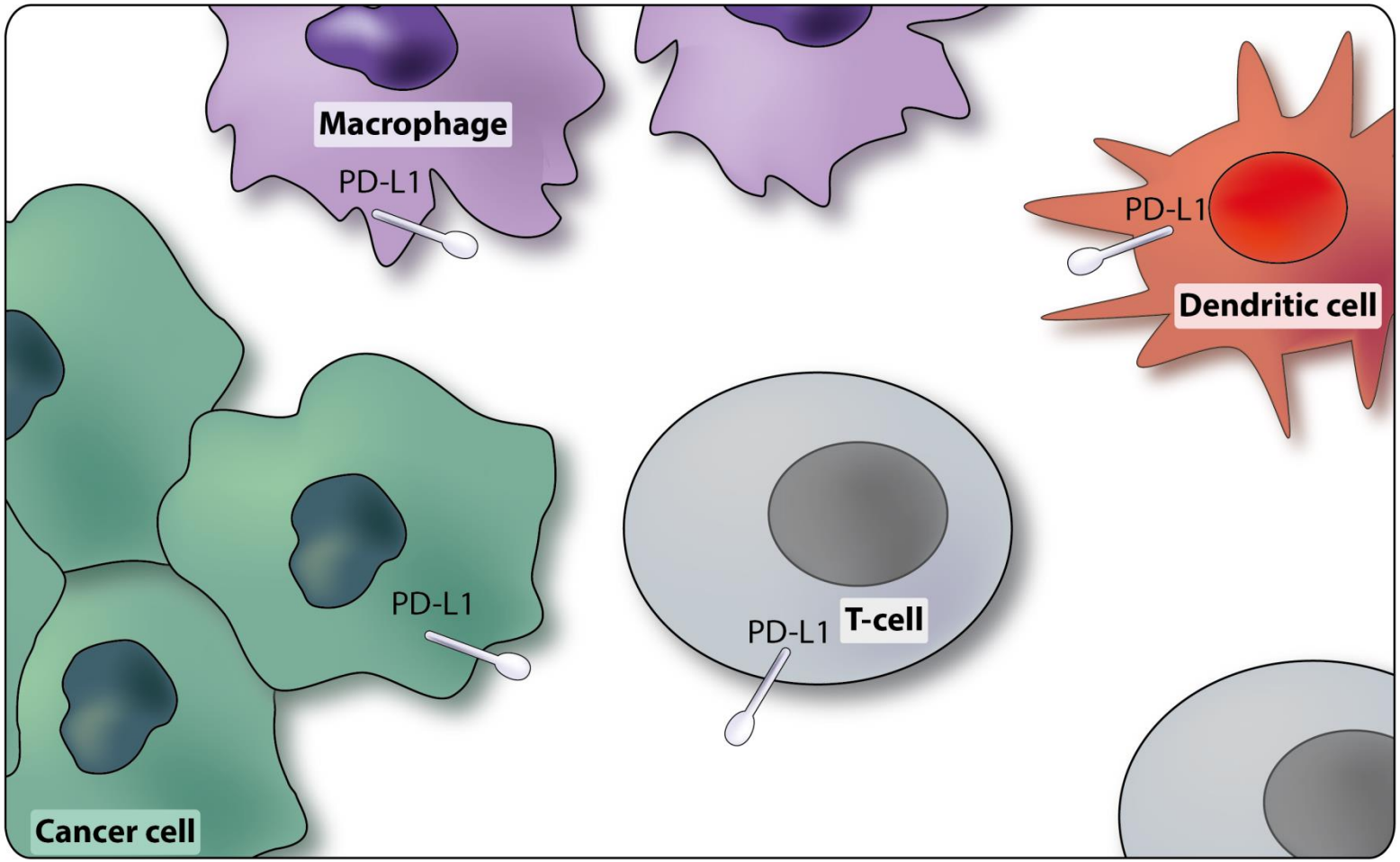




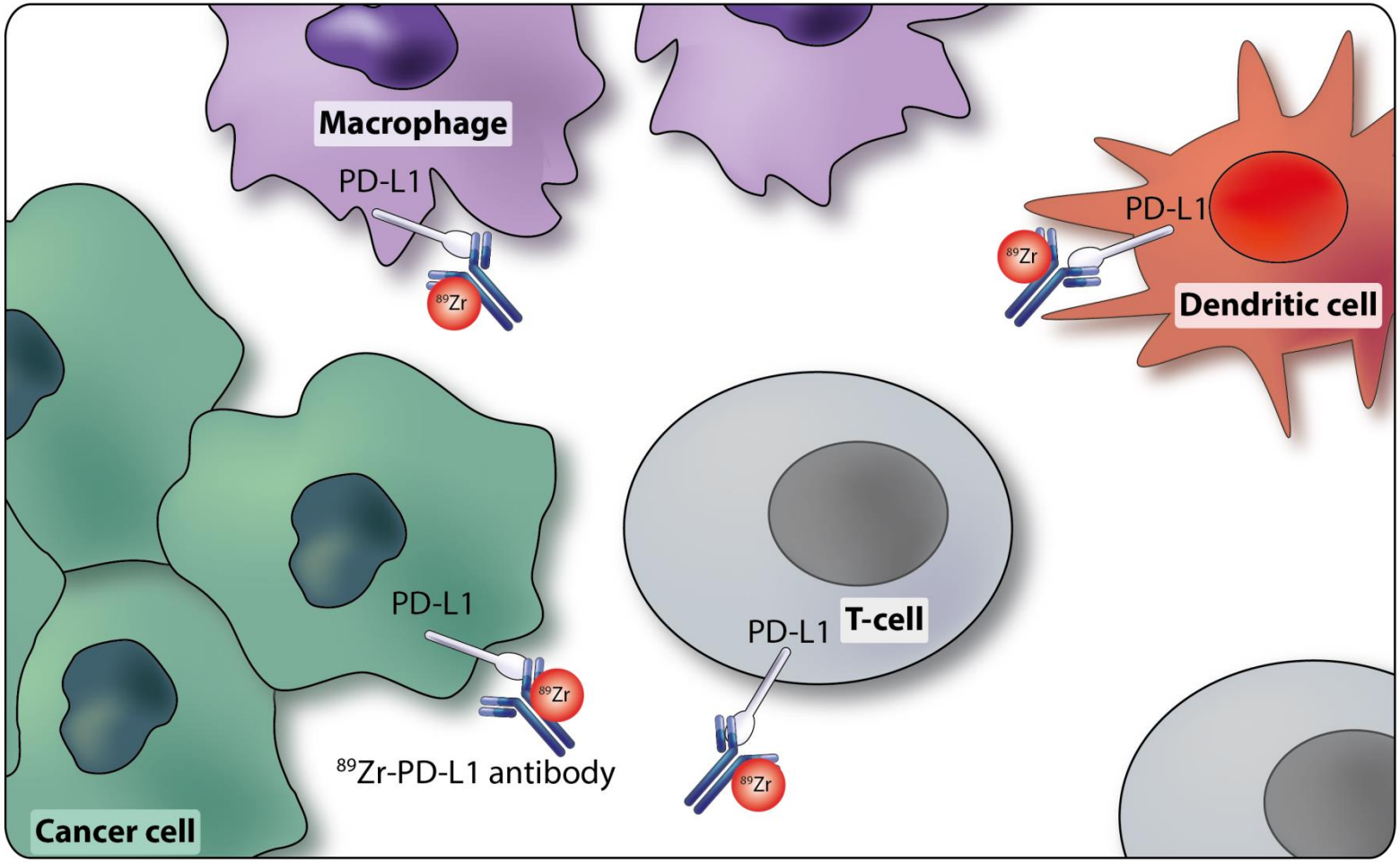
Yellow: Anti-PD-L1 antibody: αPD-L1

Heskamp et al, Cancer Res 2015: Chang et al, Cell, 2015

Immunotherapy:
^{89}Zr-labeled PD-L1 antibody

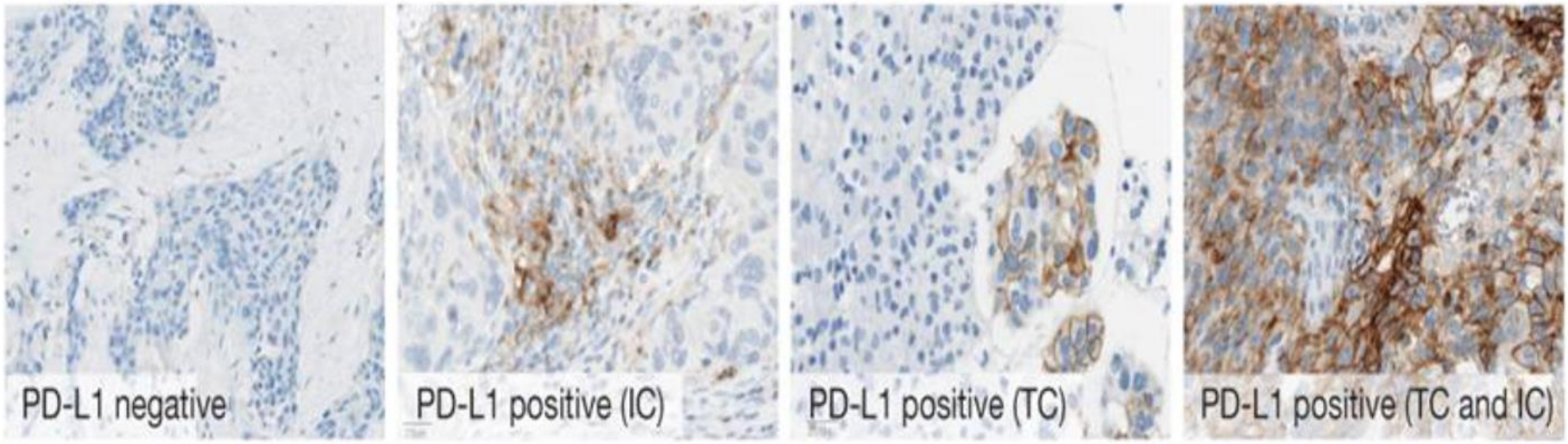


Immunotherapy: ^{89}Zr-labeled PD-L1 antibody



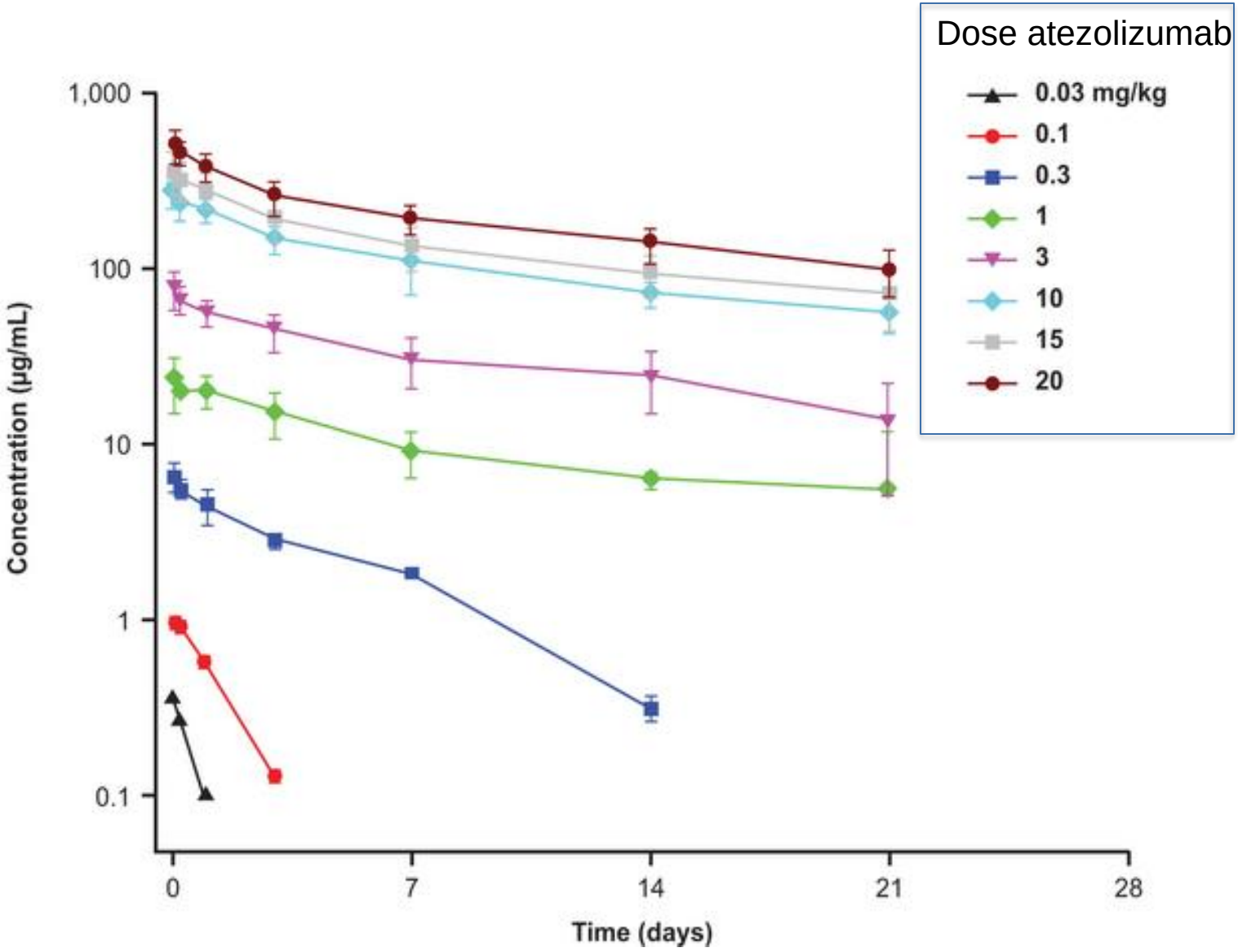
Biopsy provides a snapshot of 1 part of 1 lesions:

PD-L1 expression by tumor cells & tumor-infiltrating immune cells (macrophages, dendritic cells & lymphocytes)



Herbst et al, Nature 515, 563–567, 2014

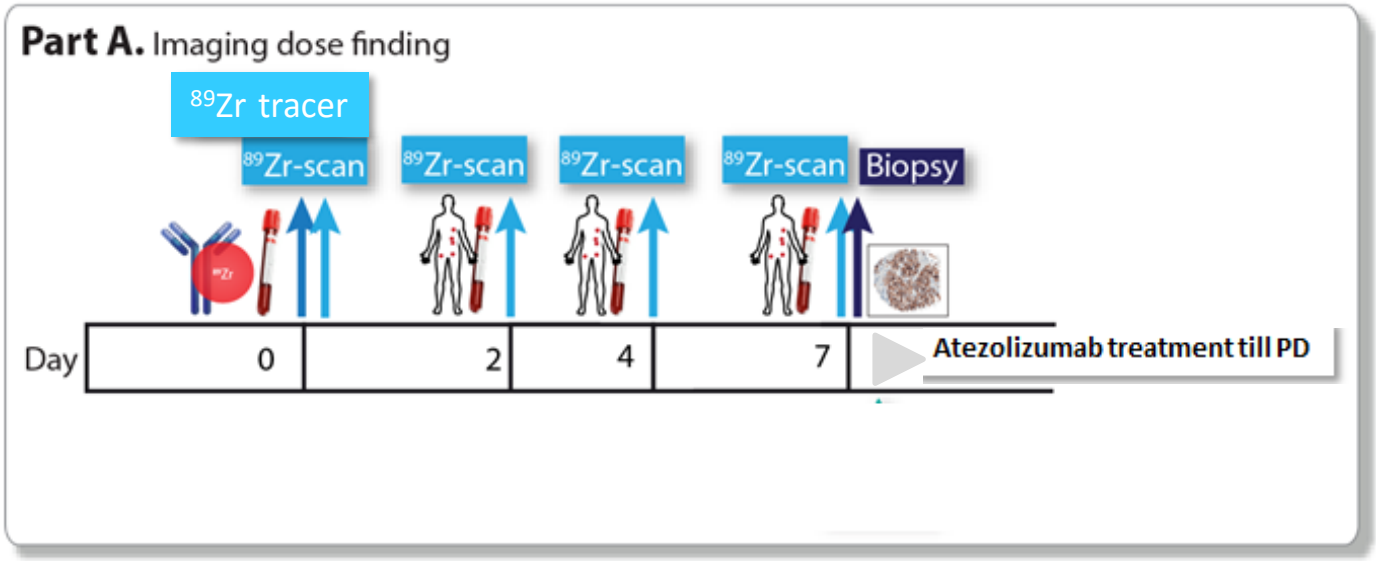
Serum pharmacokinetics cycle 1
PD-L1 antibody atezolizumab

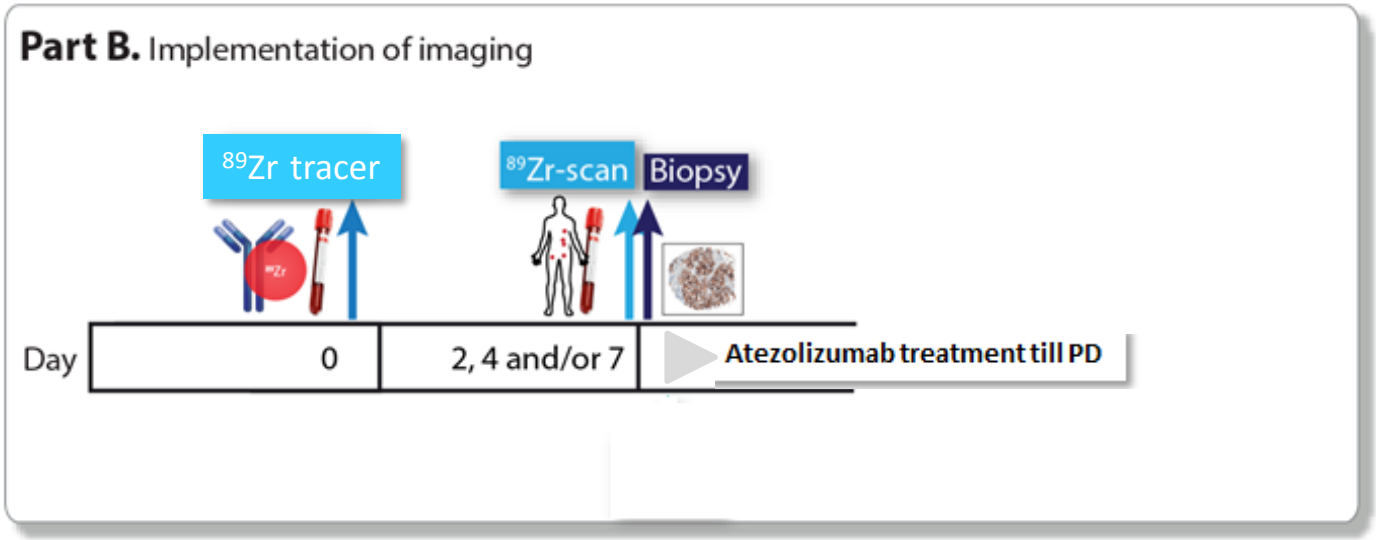


Herbst et al, Nature 515;563-567, 2014

Very preliminary PD-L1 antibody imaging results in patients: Primaries

Design trial with ^{89}Zr-atezolizumab in TNBC, bladder cancer and NSCLC patients

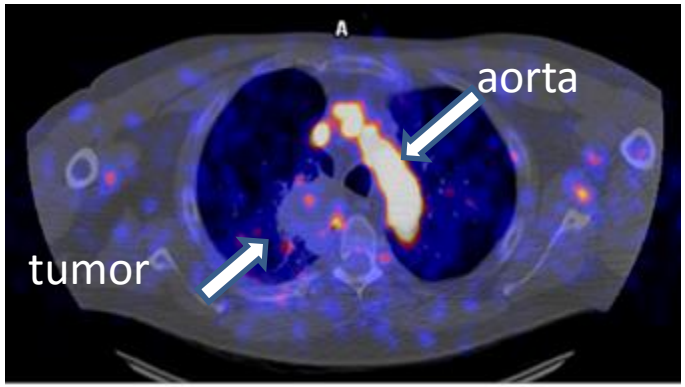




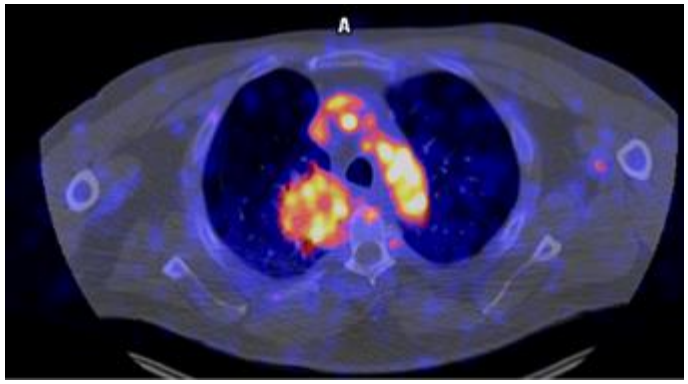
ClinicalTrials.gov Identifier: NCT02453984

^{89}Zr-atezolizumab uptake in NSCLC patient over time

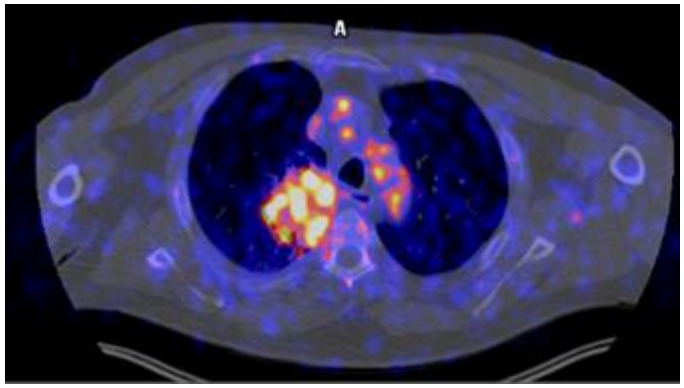
1 hour



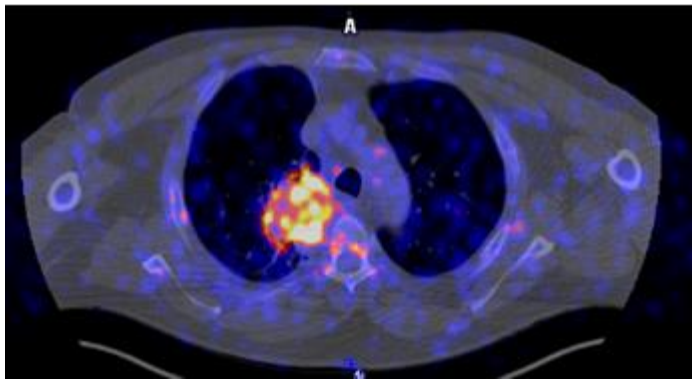
Day 2



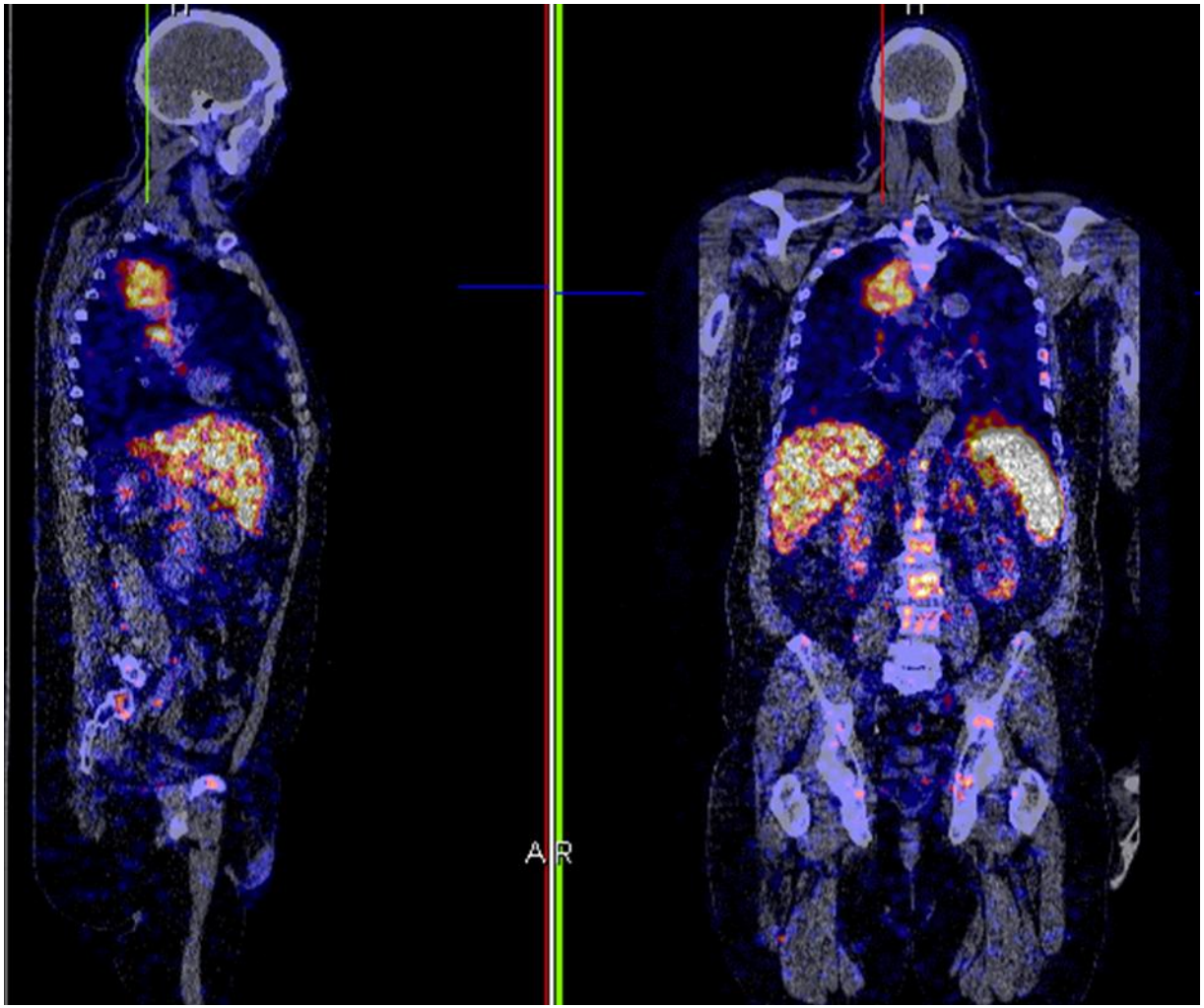
Day 4



Day 8



^{89}Zr-atezolizumab uptake in NSCLC patient day 8

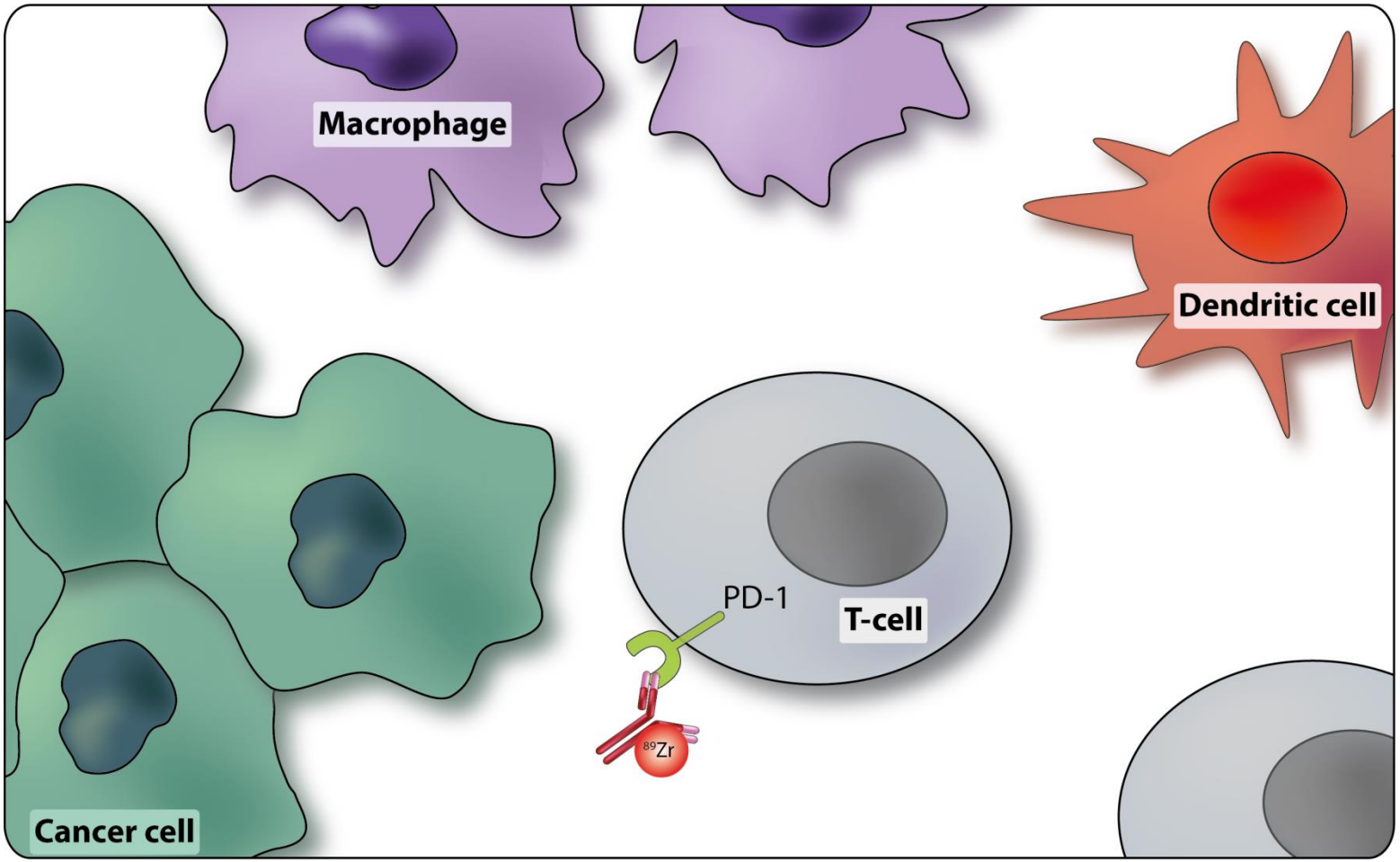


Conclusions PD-L1 antibody imaging

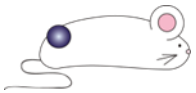
- **Preclinical:**
  - Specific tumor uptake of PD-L1 antibody (radioactive and fluorescent)
  - Biodistribution showed high specific PD-L1 antibody uptake in the spleen
  - PD-L1 antibody internalizes in tumor cells

- **Clinical:**
  - Immunohistochemical PD-L1 staining provides information of 1 part of the tumor at 1 moment
  - Ongoing ^{89}Zr-atezolizumab trial: currently collecting trial data

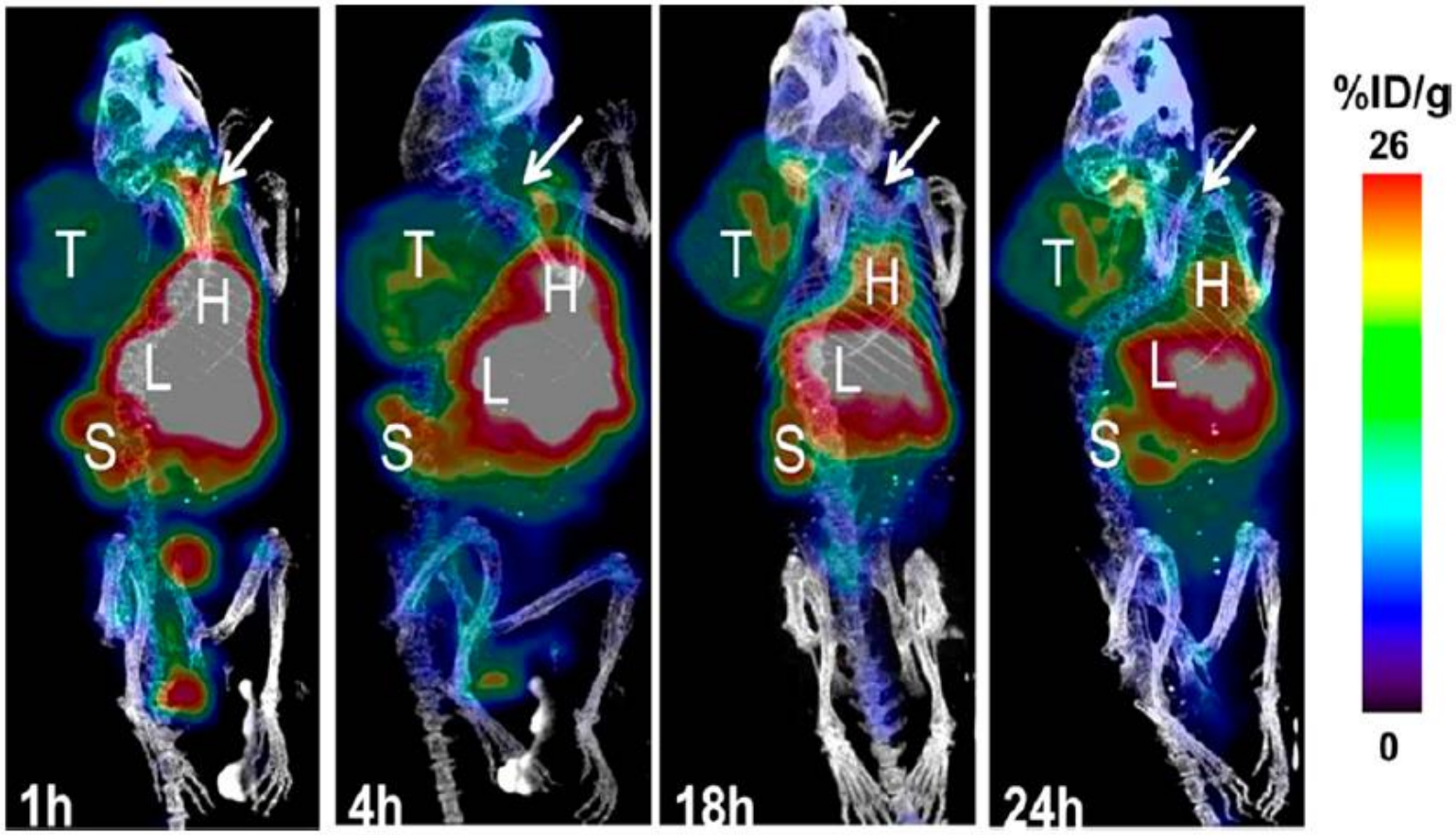
Immunotherapy: ^{89}Zr-labeled PD-1 antibody



^{64}Cu-anti-mouse antibody (IgG) PD-1 antibody tracer detecting in melanoma (B16F10) tumor bearing mice



PD-1 expressing murine TILs



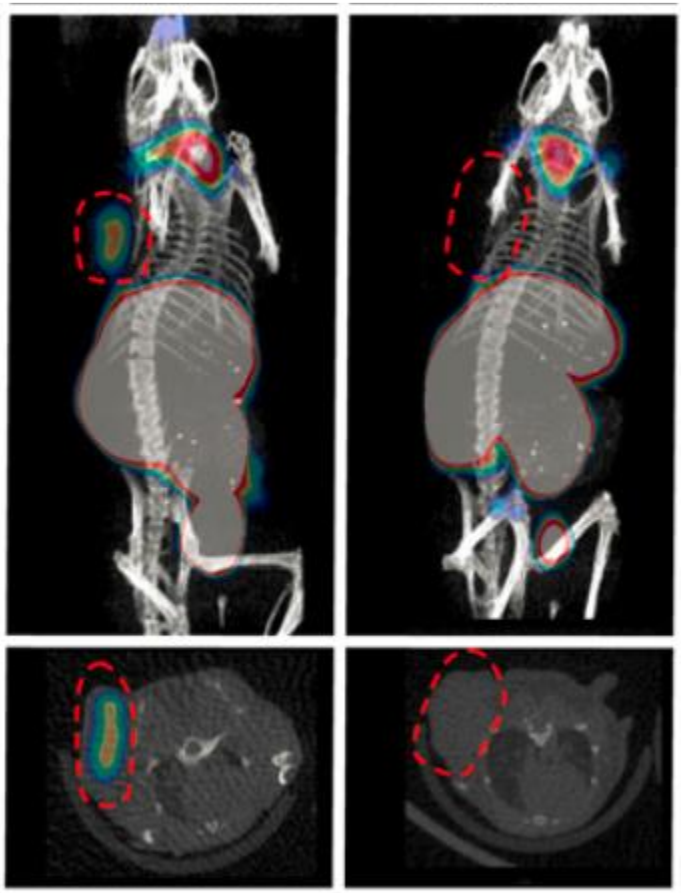
White Arrow = Thymus or lymph nodes, L = Liver, T = Tumor, H = Heart, S = Spleen.

Natarajan et al, Bioconjug Chem, 2015

Engineering high-affinity PD-1 variants for immuno-PET imaging with ^{64}Cu after 1 h

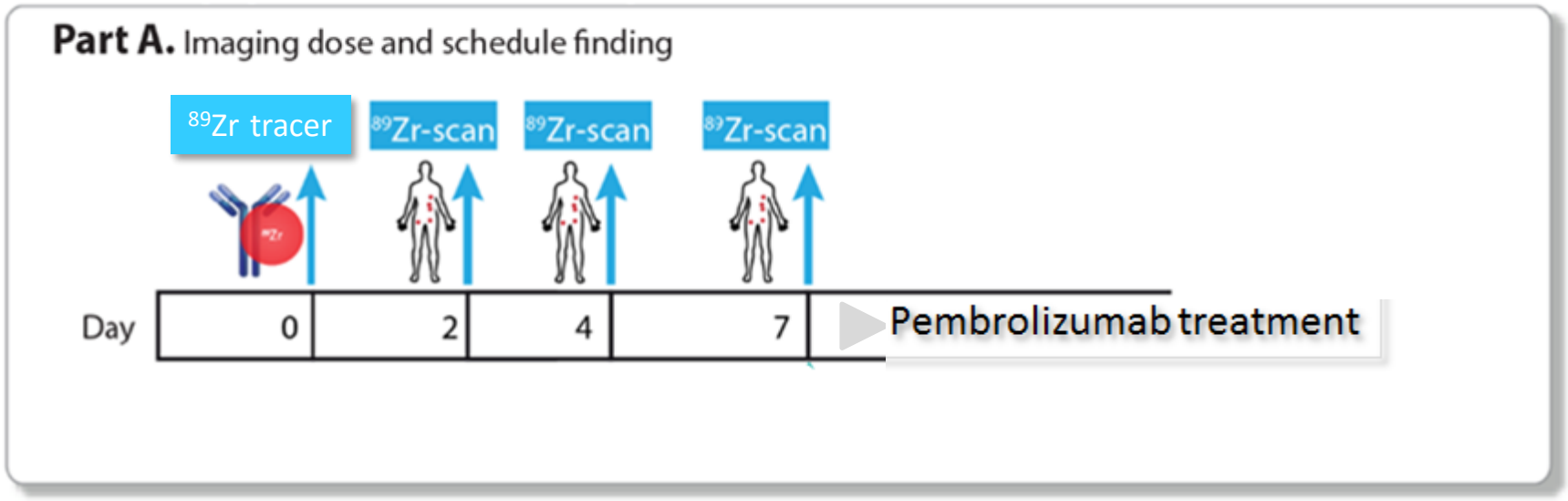
PD-L1 overexpressing tumor

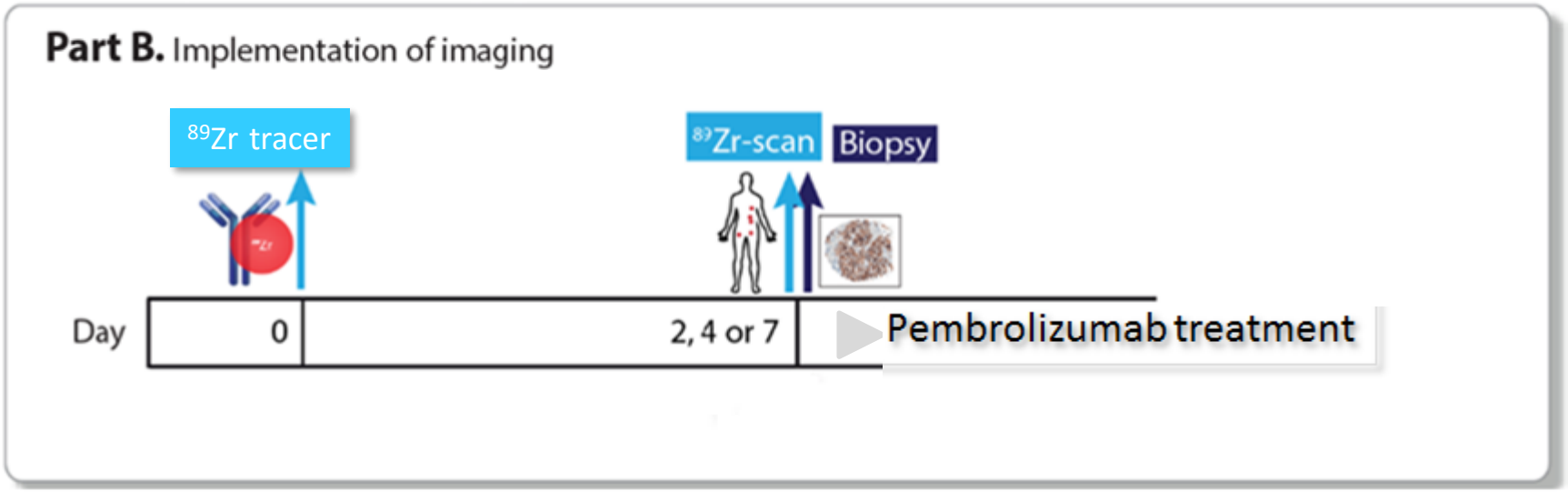
+PD1 antibody uptake



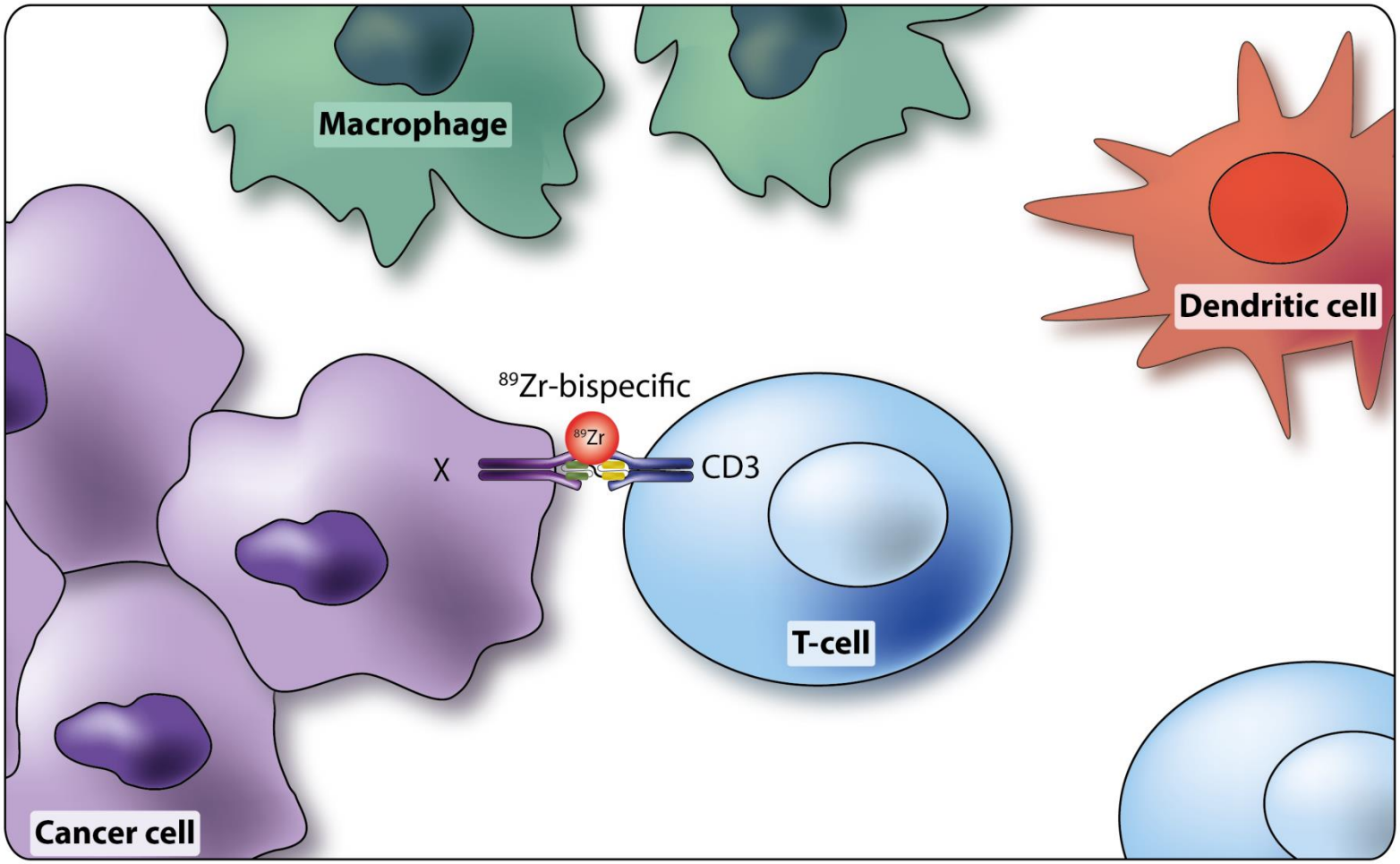
Maute et al, Proc Natl Acad Sciences 2016

^{89}Zr-pembrolizumab imaging in melanoma patients

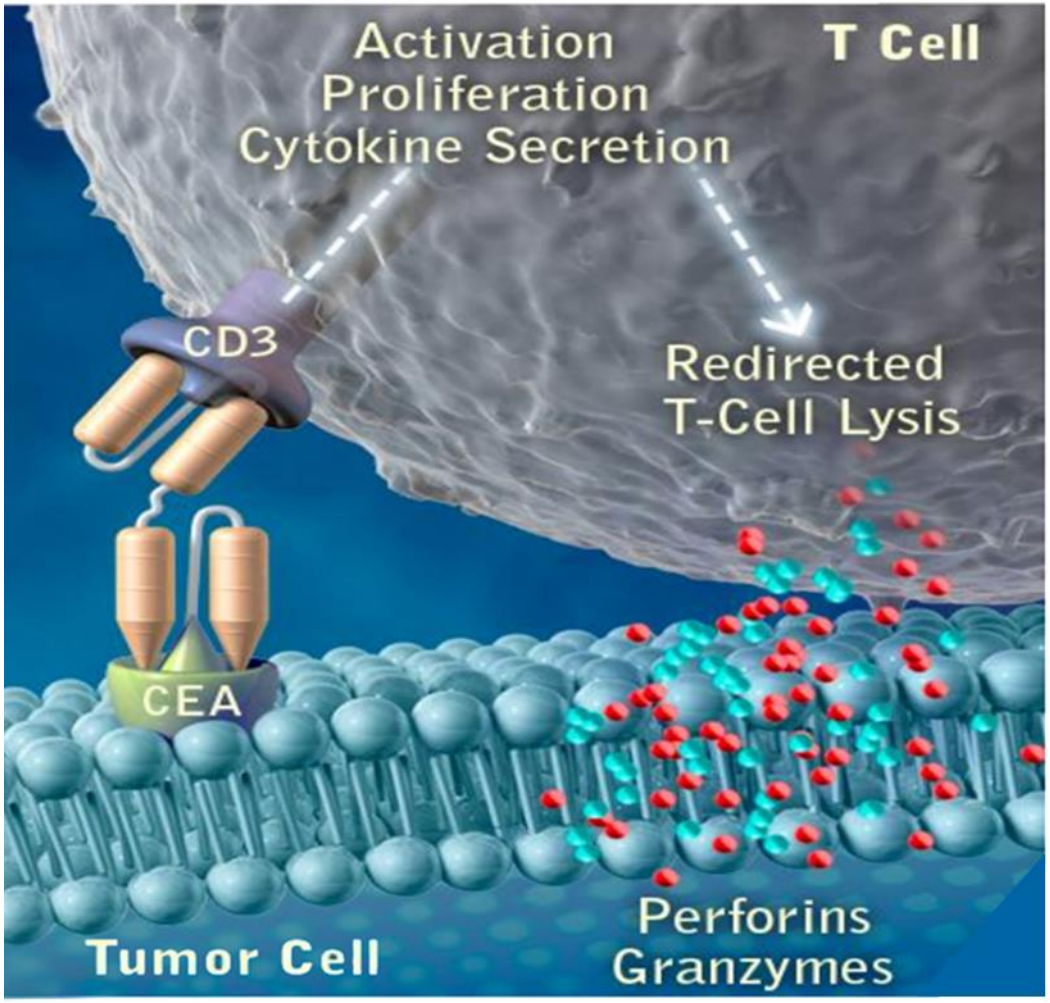




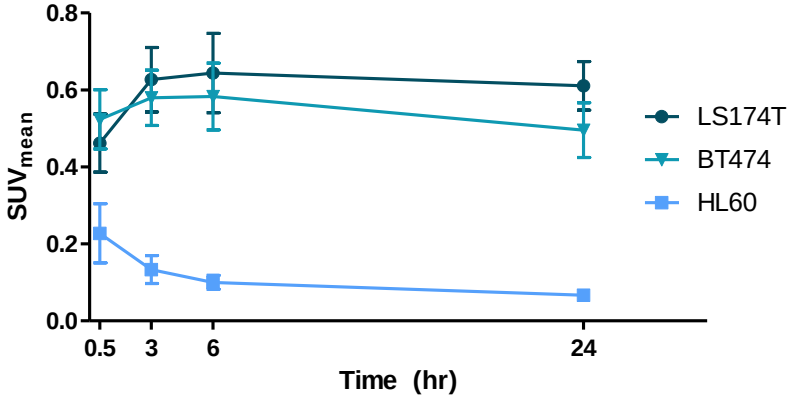
Labeled bispecific antibody (construct) tracers

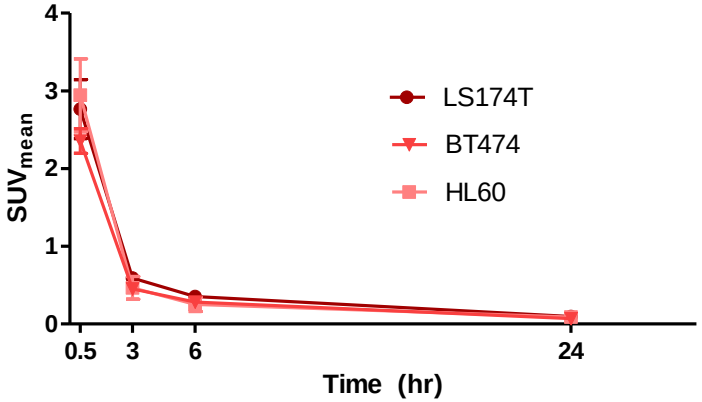


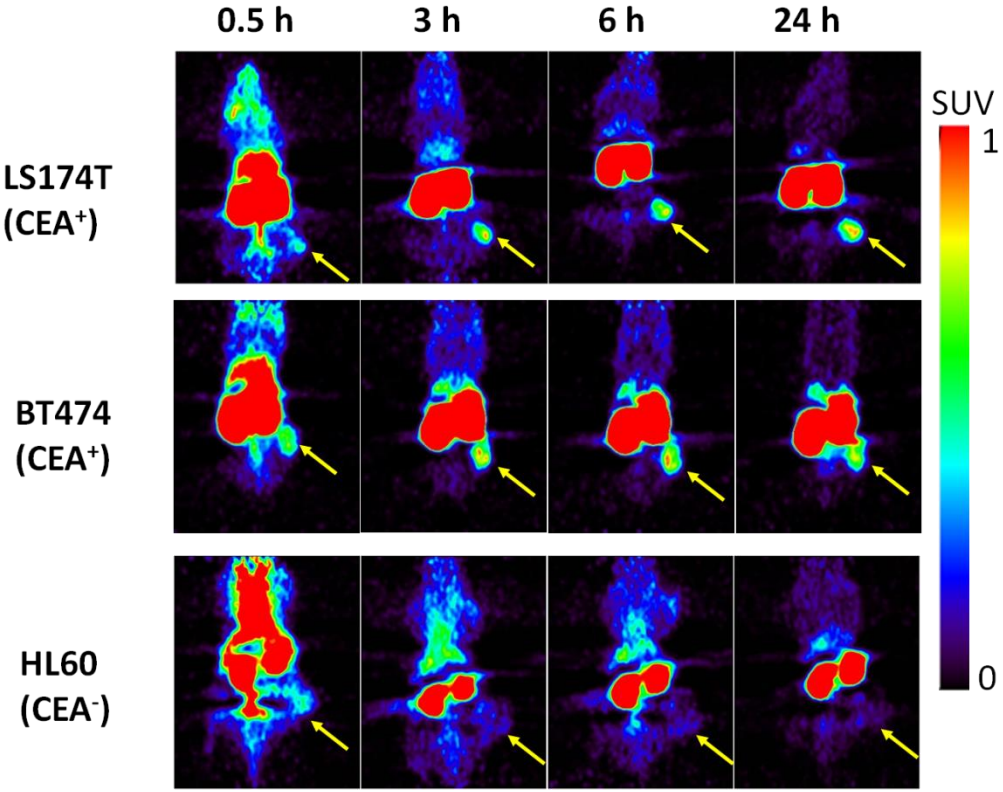
Labeled bispecific T cell engaging antibody targeting CEA



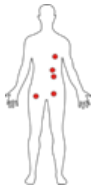
^{89}Zr-labeled bispecific T cell engaging antibody construct targeting CEA





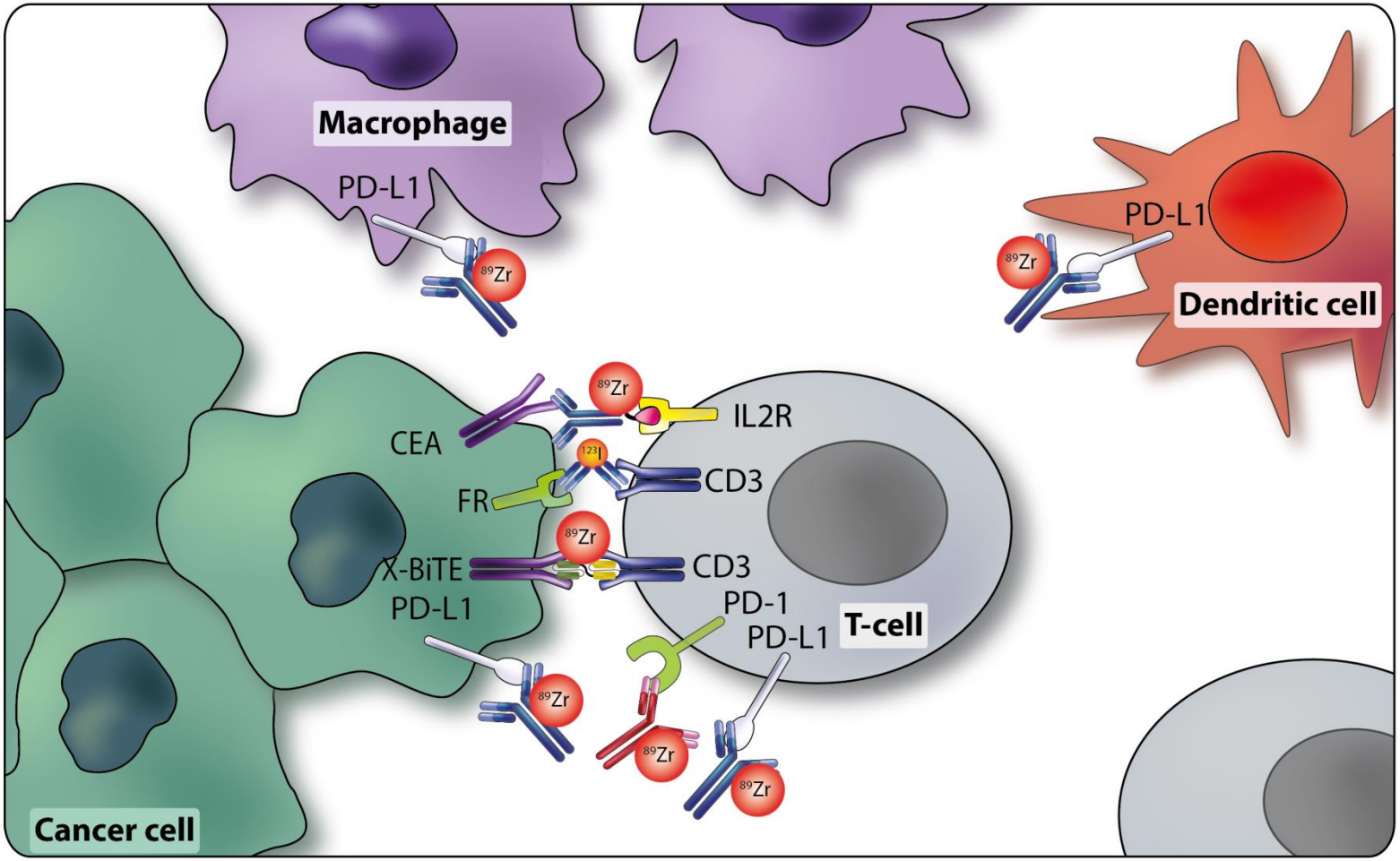


Waaijer et al, AACR-NCI-EORTC meeting Abstract # A85, 2015



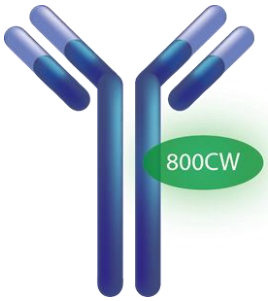
ClinicalTrials.gov Identifier: NCT02291614

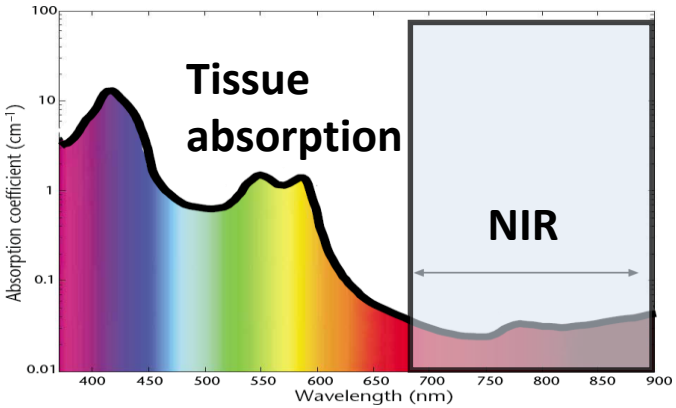
Antibody imaging with radionuclides in the clinic



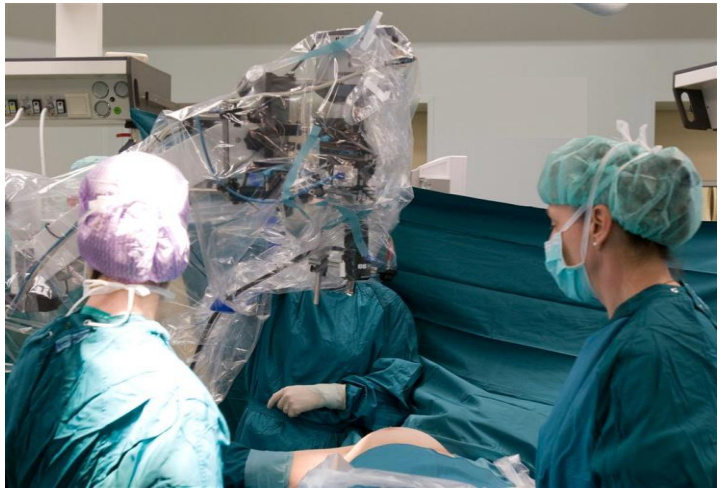
Optical imaging

- Optical imaging uses light
- High resolution (> PET)
- Non-radioactive
- Limited penetration
  - novel tracers
  - improved detection systems

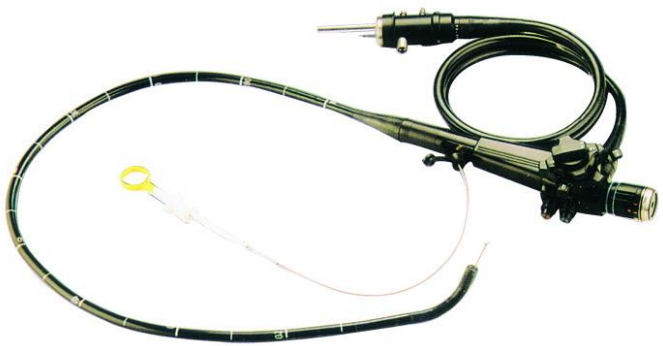




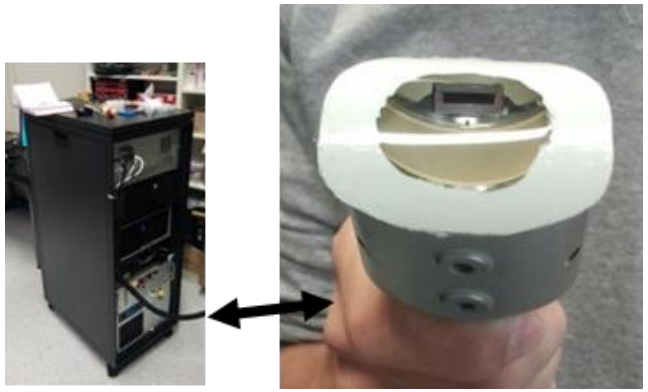
Intraoperative, endoscopic and hand held systems



Intraoperative camera

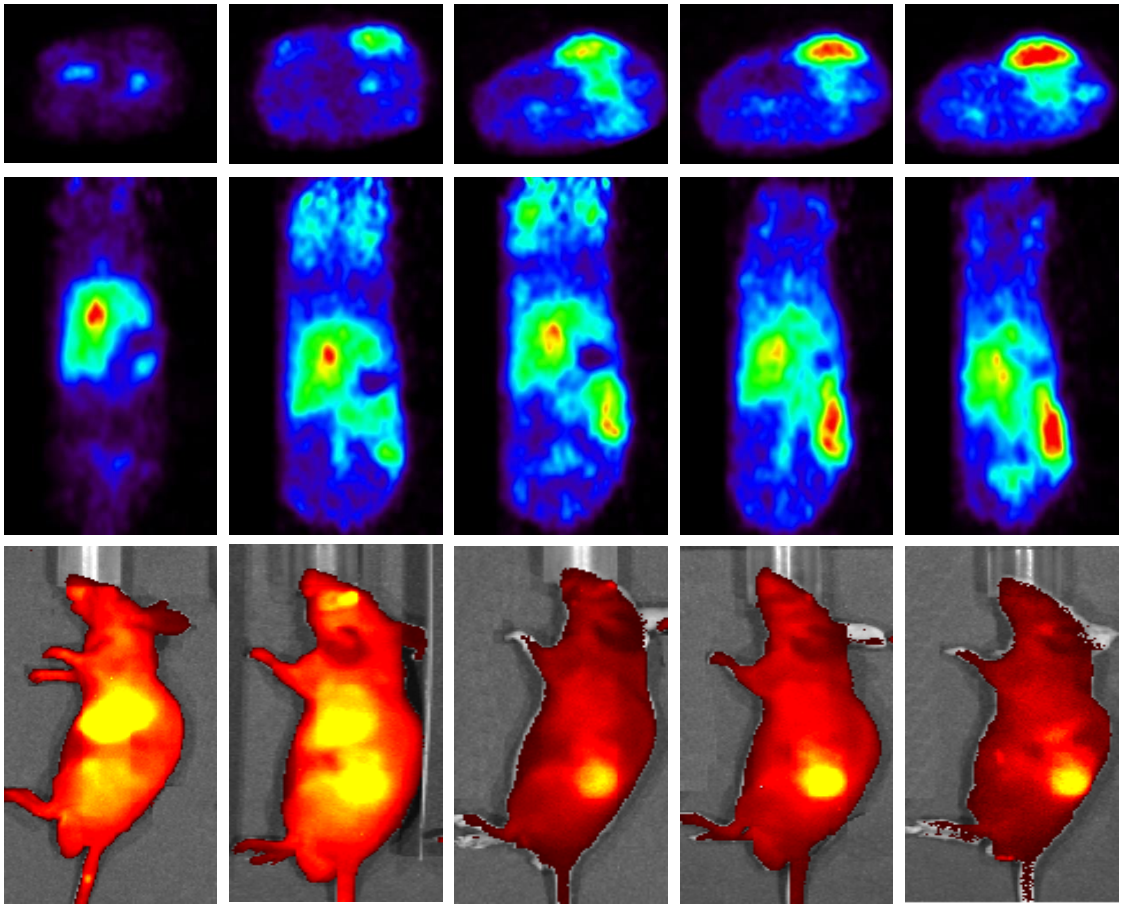


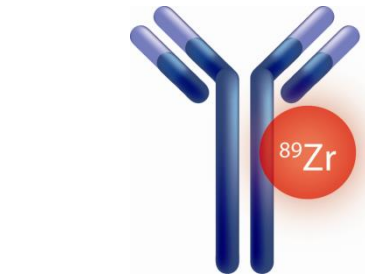
Optical fiber endoscope

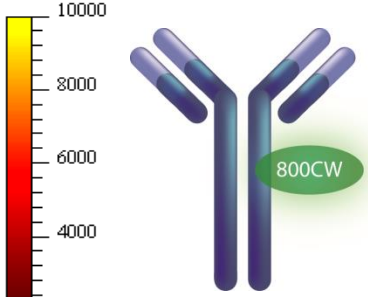


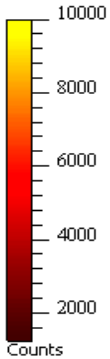
Optoacoustic handheld system

Dual imaging with ^{89}Zr-bevacizumab & IRDye800CW-bevacizumab



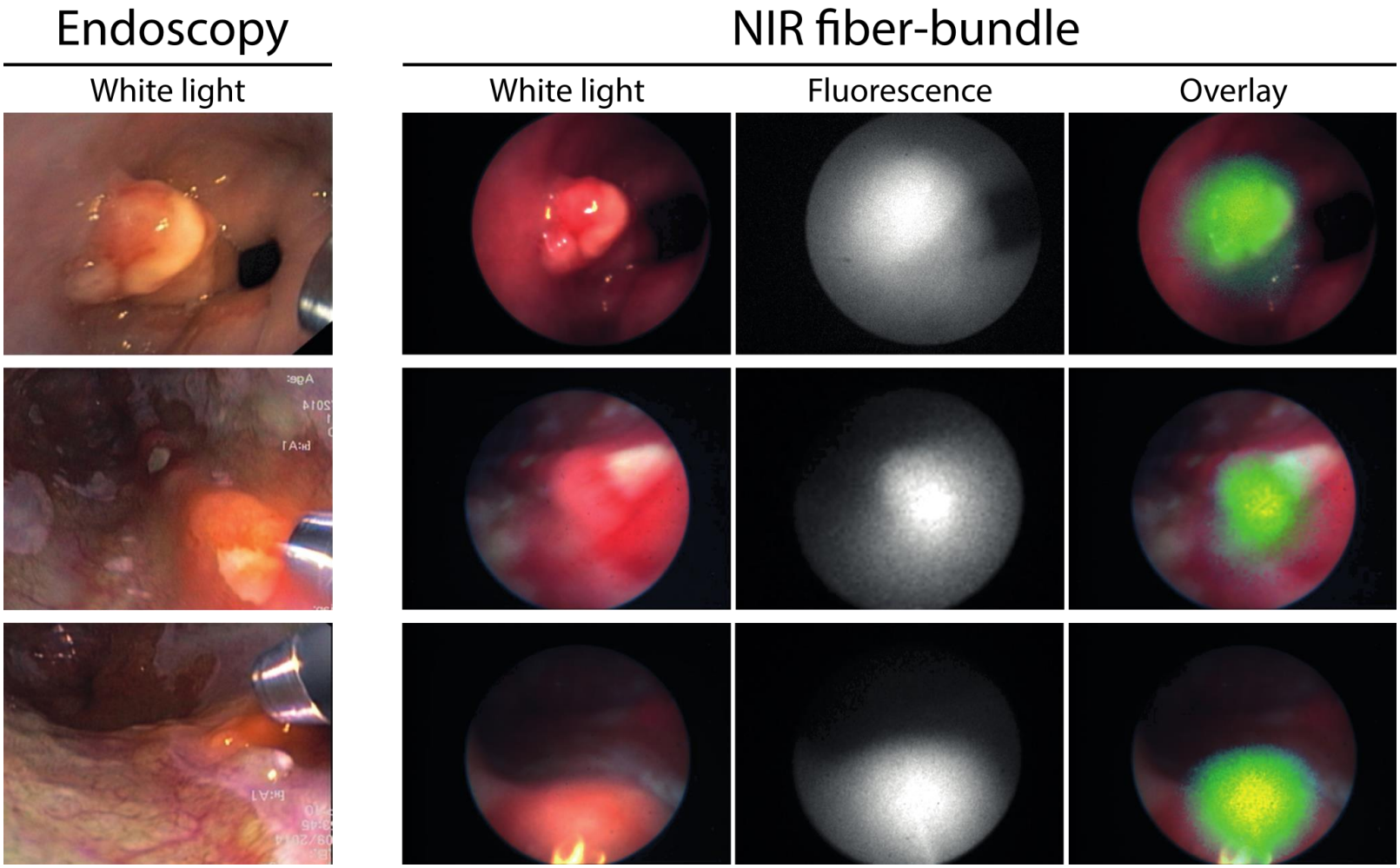




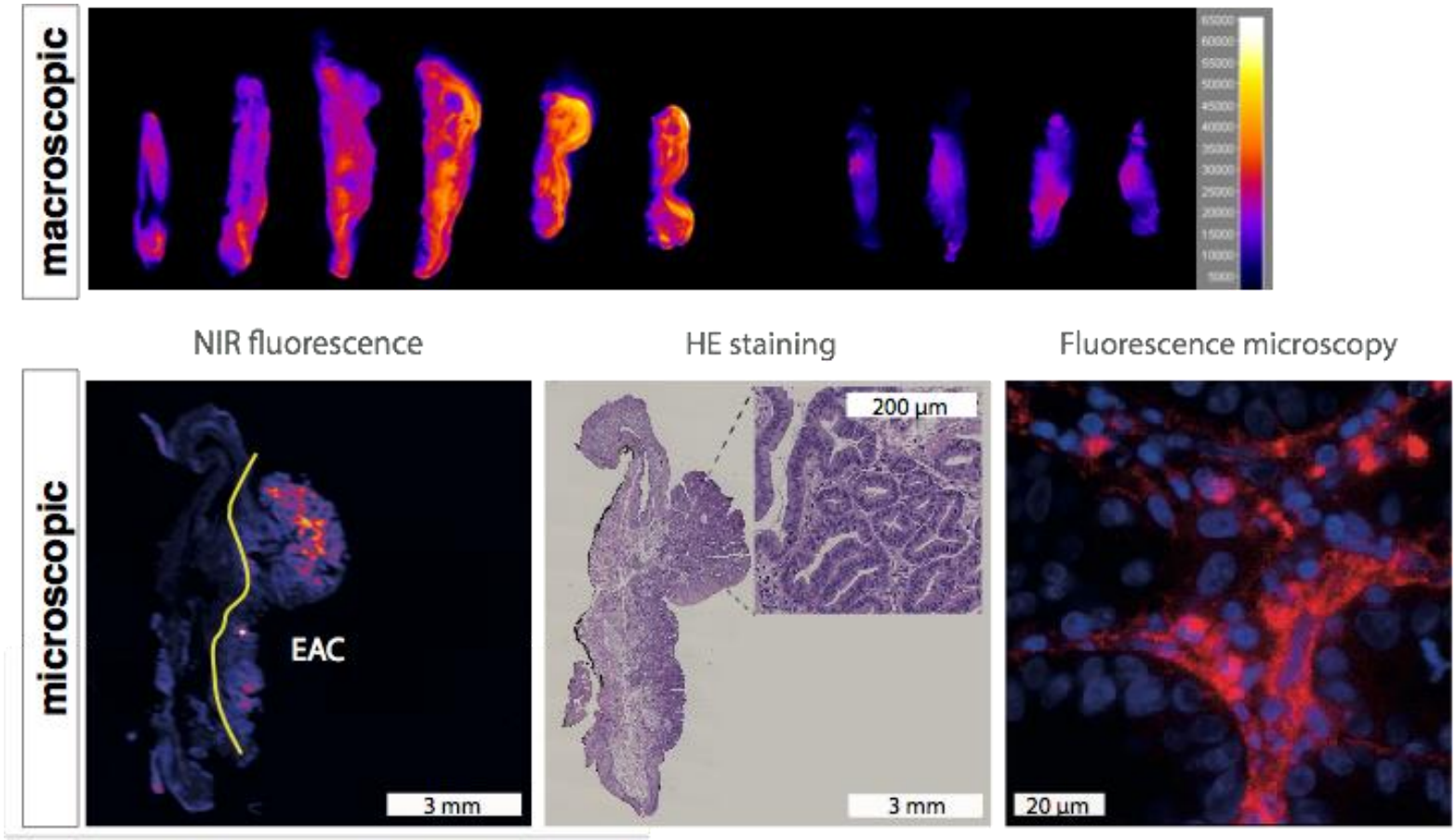


Terwisscha van Scheltinga et al, J Nucl Med 2011

First in human results IV CW800-bevacizumab in 3 small esophageal cancers

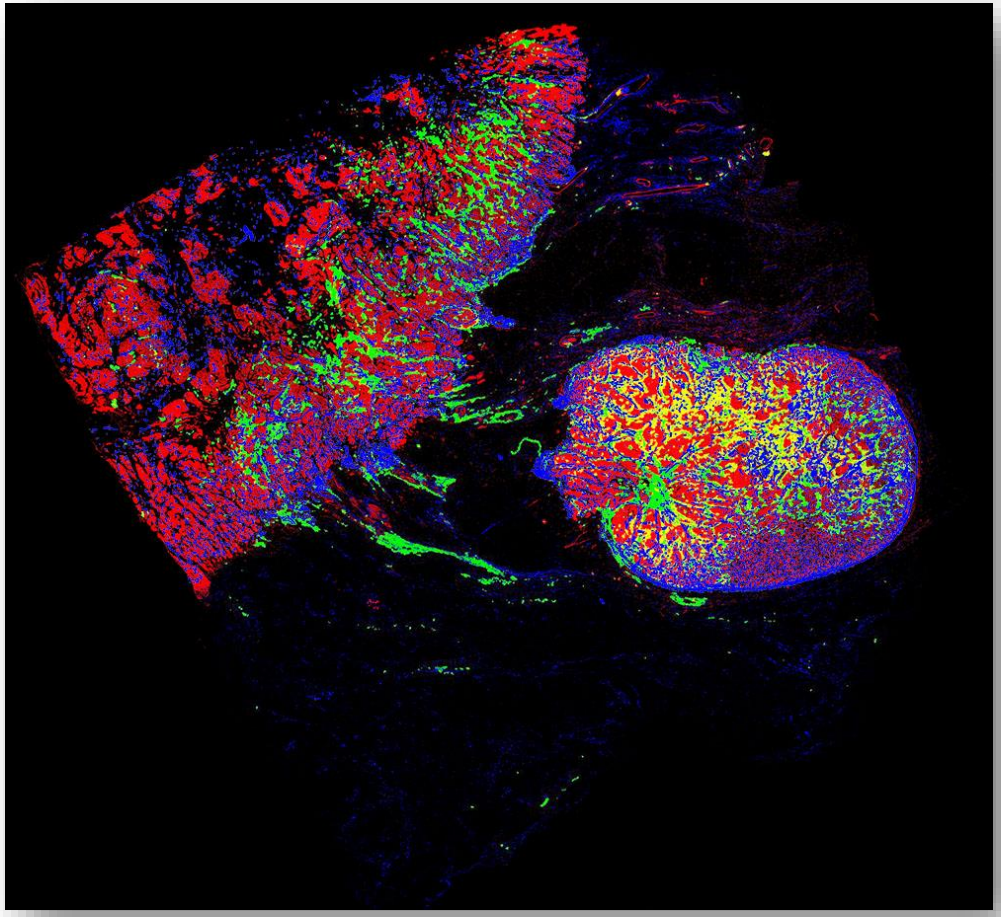


Endoscopic mucosal resection specimen including esophageal adenocarcinoma after IV CW800-bevacizumab

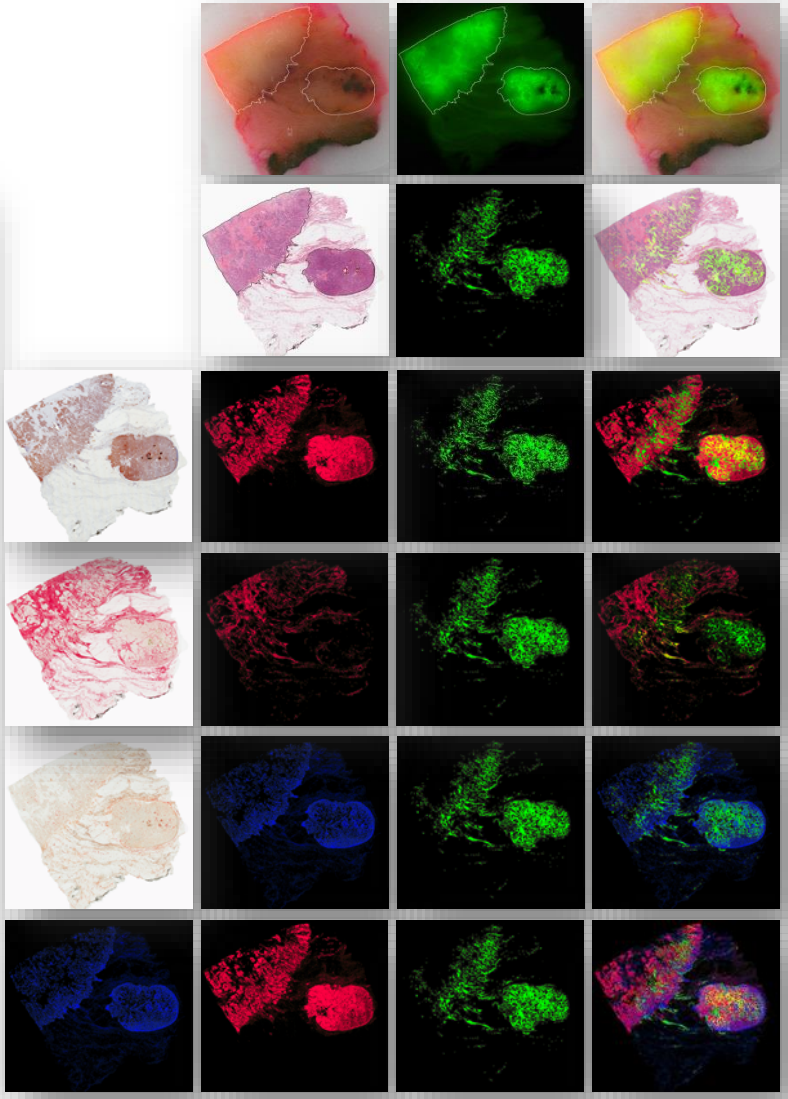


Multiplex Advanced Pathology Imaging (MAPI) for bevacizumab-IRDye800CW in breast cancer

CD3[illegible]tion



target NIR tracer overlay



Conclusions role molecular imaging

- Antibody imaging for immunotherapy can visualize drug distribution & tumor characteristics
- Provides insight in
  - Heterogeneity in tracer uptake by tumor lesions
  - Pharmacodynamic effects in the tumor
- Insight into localization of the drug in the tumor

Acknowledgments

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Gastroenterology

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Nuclear Medicine

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Andor Glaudemans

Hospital Pharmacy

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Martine Piccart
Patrick Flamen

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Simon Williams
Marcelle Bergeron
Christoph Mancao
Nathan McKnight
Sandra Sanabria
Simonetta Mocci
Luisa Veronese

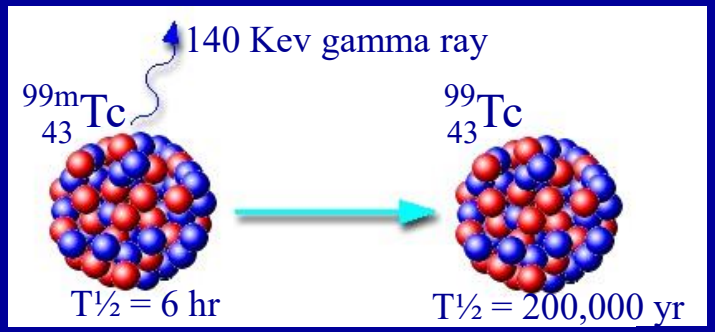
Overview of Nuclear Medicine Imaging Capabilities

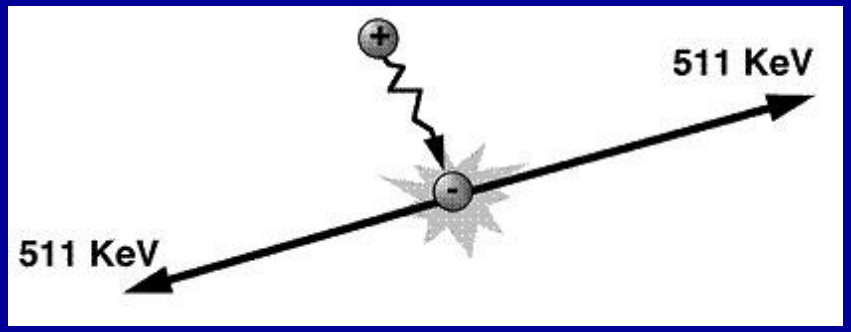
Michael M. Graham, PhD, MD
Director of Nuclear Medicine
University of Iowa

Major Instruments

Single Photon Emission Computed Tomography (SPECT)

Positron Emission Tomography (PET)





Major Instruments

Single Photon Emission Computed Tomography (SPECT)

Positron Emission Tomography (PET)

- Spatial resolution: 12 mm
- Temporal resolution:
  10 sec (planar)
  10 min (SPECT)
- ^{99m}Tc, ^{111}In, ^{123}I, ^{131}I

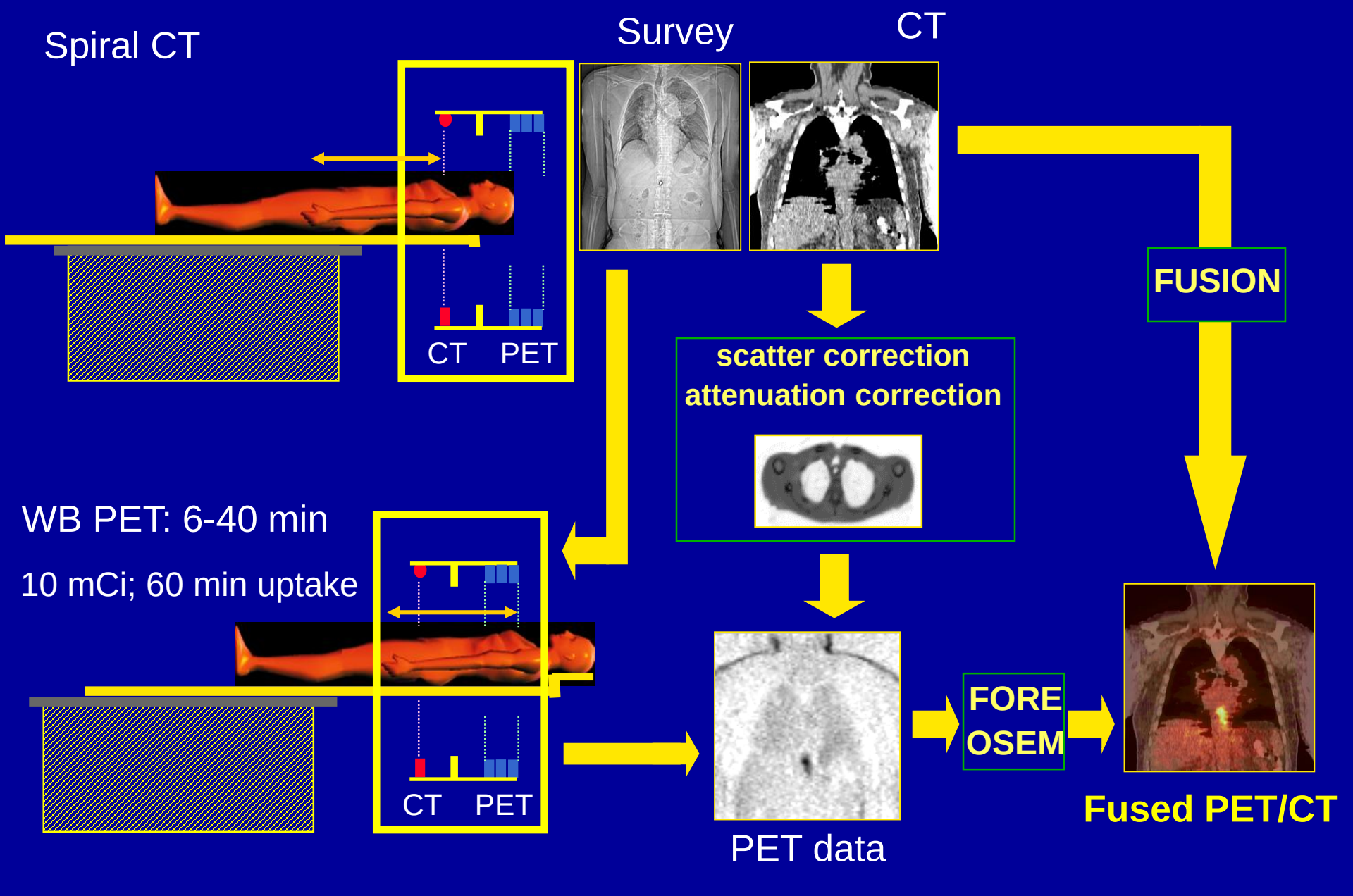
- Spatial resolution: 6 mm
- Temporal resolution: 2 min
- ^{11}C, ^{13}N, ^{15}O, ^{18}F
  ^{64}Cu, ^{68}Ga, ^{89}Zr, ^{124}I

SPECT/CT systems

GE Hawkeye

Siemens Symbia

PET/CT scan protocol



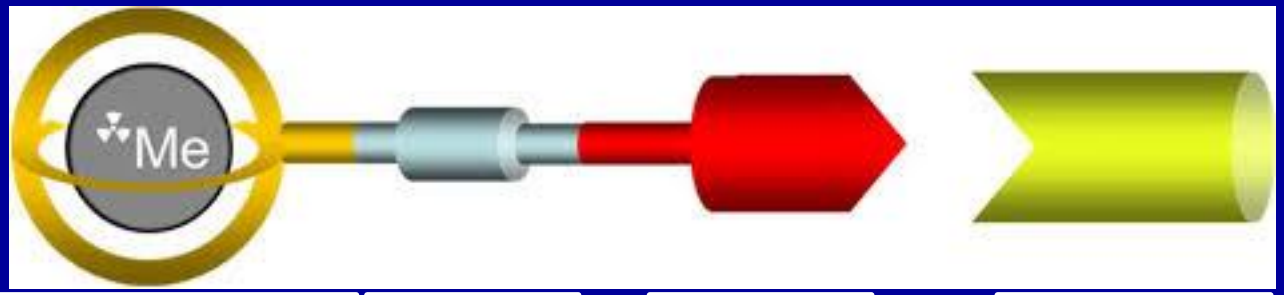
Types of studies
(Flow, Metabolism, Receptors, Cell Trafficking)

- Flow of material
  - Blood flow (brain, heart)
  - Gastric emptying
  - Lymphatic drainage
  - Bile
  - Urine
  - CSF
- Metabolism
  - Bone formation
  - Bile formation
  - Renal tubular function
  - Macrophage activity
    - Liver, spleen, bone marrow
  - Glucose metabolism
  - Fatty acid metabolism
  - Cell membrane synthesis
  - DNA synthesis
  - Protein synthesis
  - Iodine

Types of studies
(Flow, Metabolism, Receptors, Cell Trafficking)

- Receptor imaging
  - MIBG
  - Dopamine receptors
  - Somatostatin receptors
  - Prostate specific membrane antigen (PSMA)
  - CD20 (Zevalin)
  - Bombesin
  - Angiogenesis (RGD)
  - Folate receptor
  - CXCR4 (chemokine)
- Cell Trafficking
  - Red blood cells
  - White blood cells
  - Platelets
  - Lymphocytes
  - Eosinophils
  - Granulocytes
  - mesenchymal stem cells
- Hypoxia
  - FMISO, FAZA, EF-5, HX4
- Apoptosis
  - Annexin V, ML-10

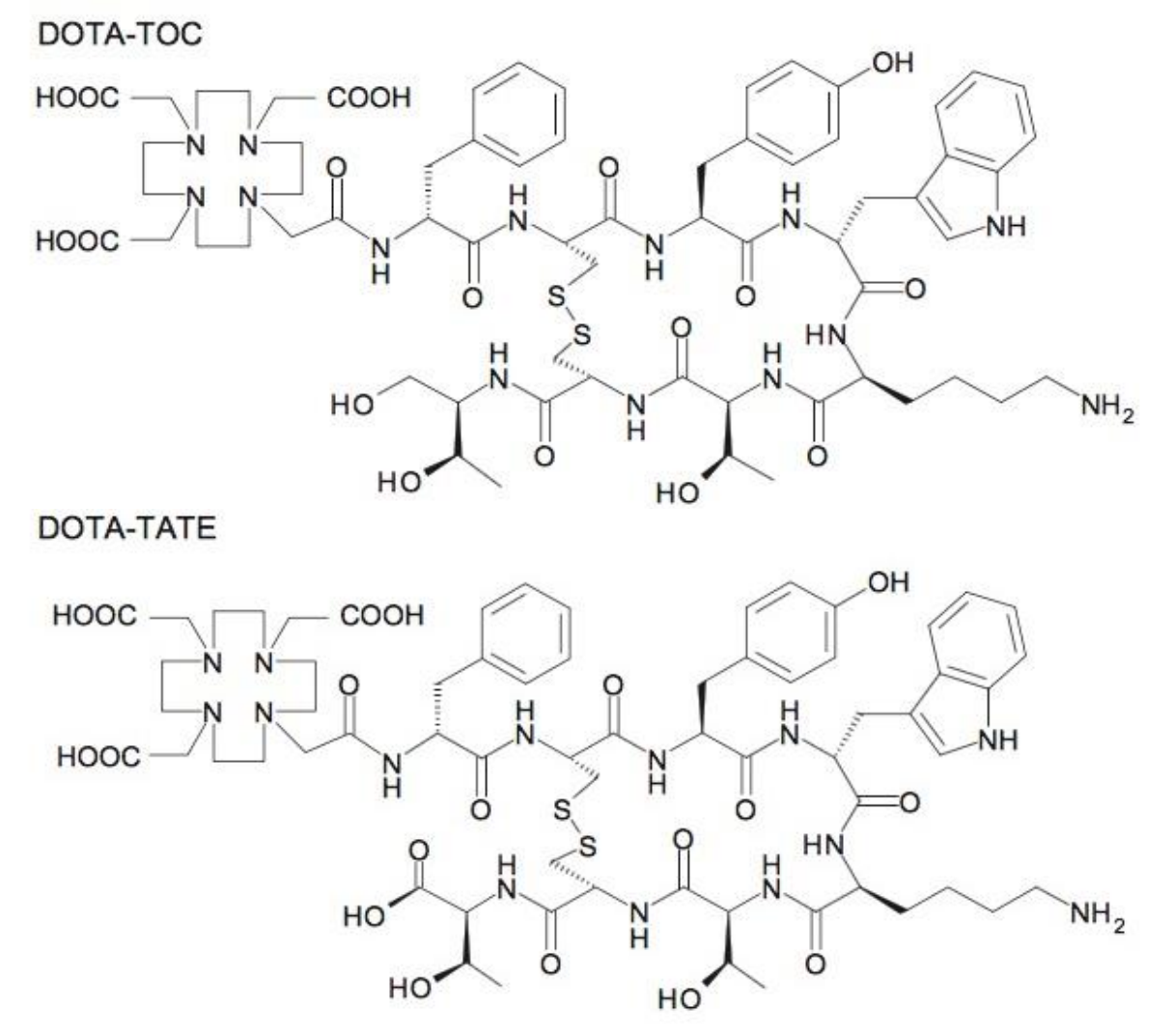
Radiolabeled Receptor Ligands



Chelation Cage | Linker | Ligand | Receptor

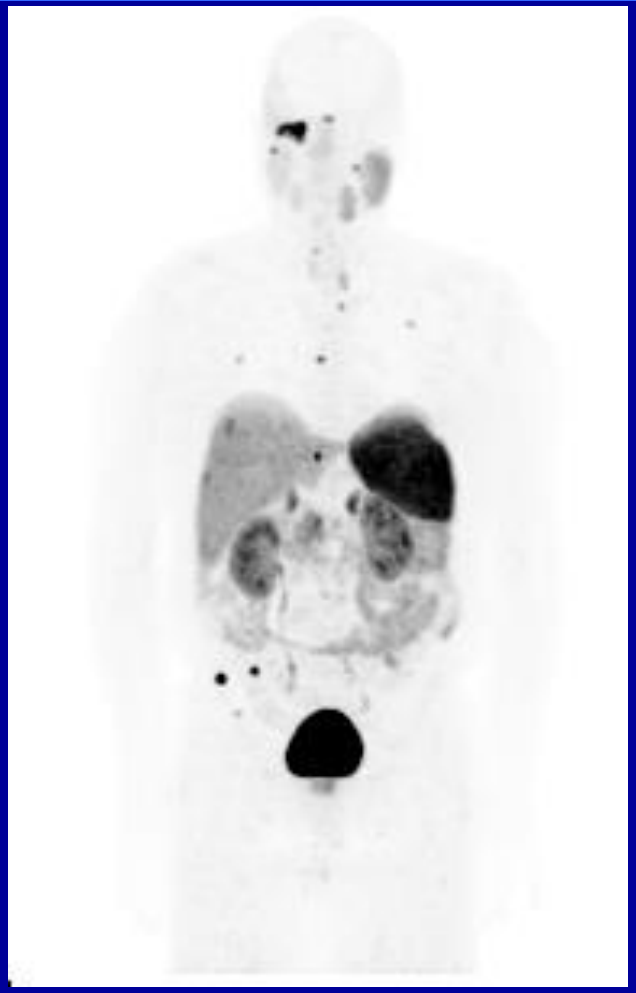
Radio-metal
^{68}Ga, ^{64}Cu, ^{89}Zr

DOTATOC and DOTATATE



The untapped potential of Gallium 68-PET: The next wave of ^{68}Ga-agents
D.L. Smith et al. / Applied Radiation and Isotopes 76 (2013) 14–23

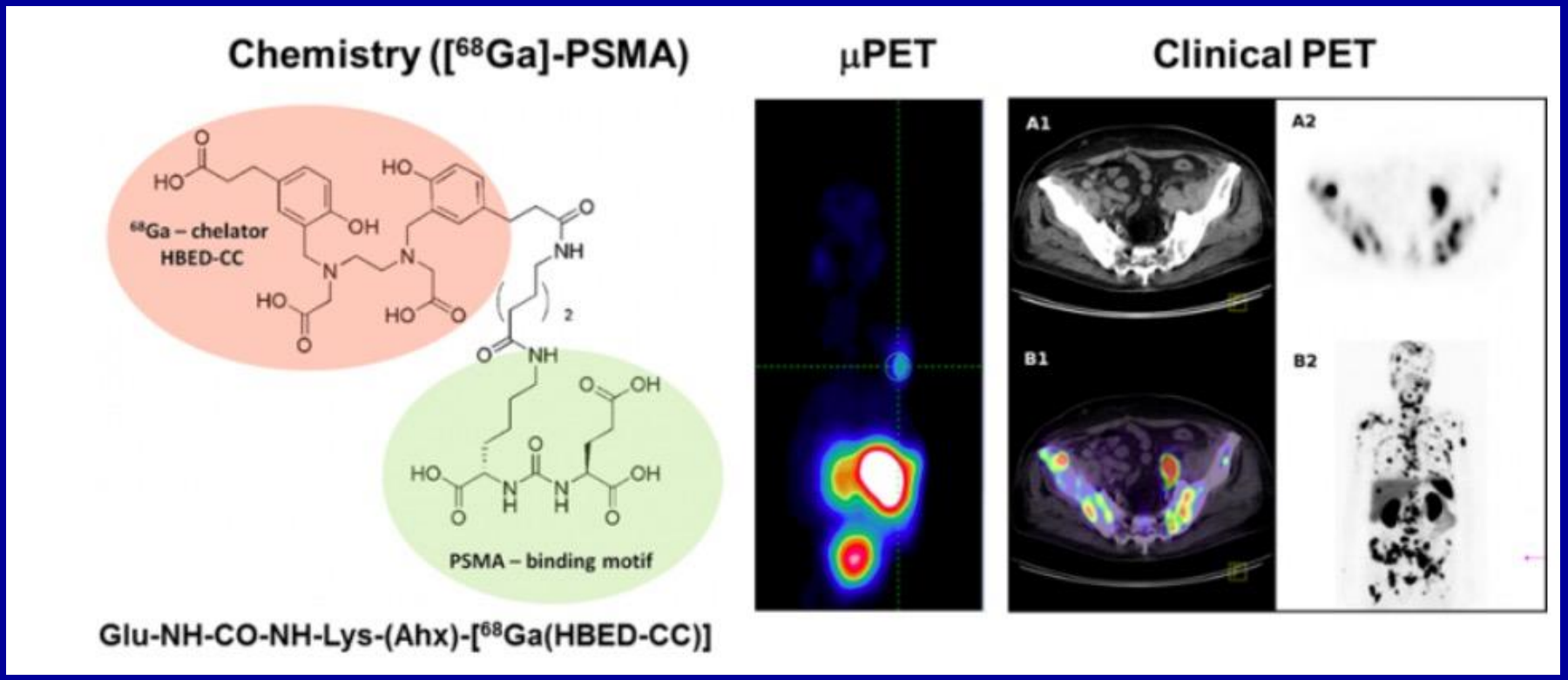
^{111}In Octreoscan®



^{68}Ga DOTA TATE

- Changes in management in 15 of the 20 patients who had ^{111}In-Octreoscan®
- Applying for funding to do ^{68}Ga DOTATATE PET/MR

^{68}Ga-PSMA



dkfz.
GERMAN CANCER RESEARCH CENTER
IN THE HELMHOLTZ ASSOCIATION
50 Years – Research for A Life Without Cancer

Methodology for Labeling Cells

- In vitro (requires separation of specified cell type)
  - ^{111}In oxine
  - ^{99m}Tc HMPAO
  - ^{99m}Tc pertechnetate after incubation with stannous chloride
  - ^{99m}Tc sulfur colloid
  - ^{64}Cu PTSM
  - ^{18}F FDG
  - ^{89}Zr-DBN
- In Vivo
  - ^{99m}Tc interleukin-8
  - ^{99m}Tc-Fanolesomab (targets CD15)

half-lives
^{64}Cu 12.7 h
^{111}In 2.8 d

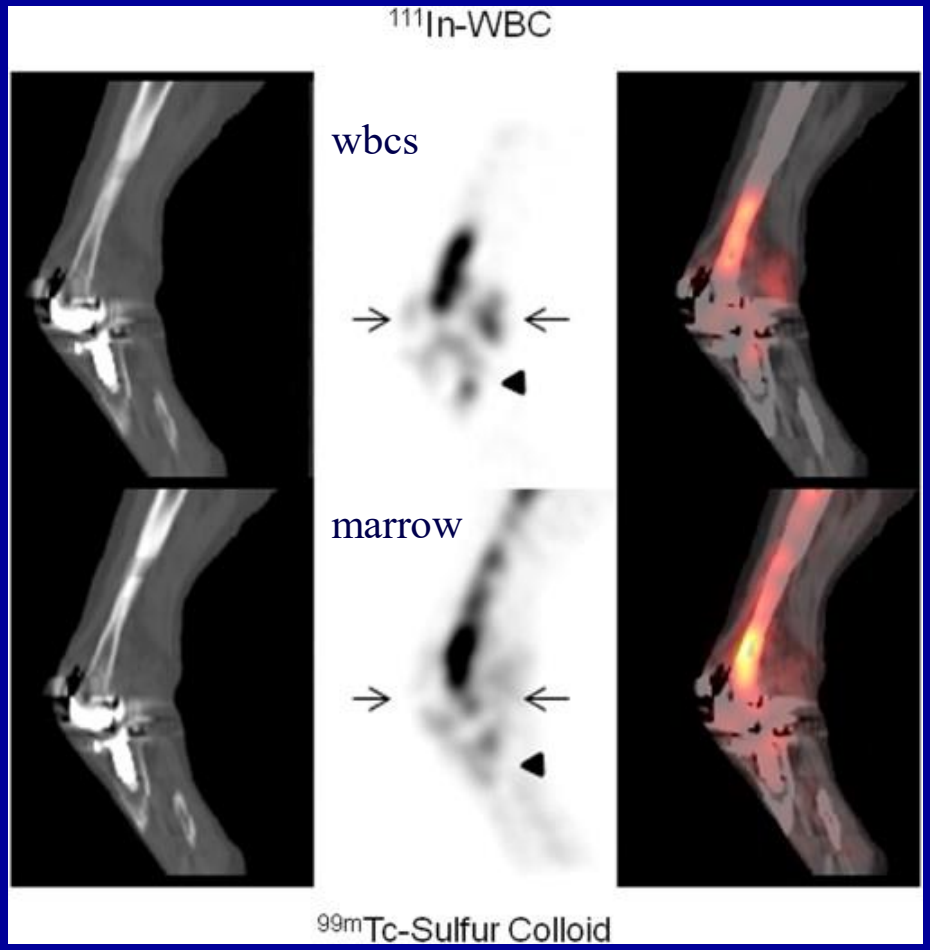


Figure 11. Infected right knee arthroplasty. On the sagittal images from the simultaneously acquired dual isotope SPECT CT, spatially incongruent distribution of activity on ^{111}In WBC (top) and marrow (bottom) images can be identified clearly anterior and posterior.

Christopher J. Palestro

Radionuclide Imaging of Osteomyelitis

Seminars in Nuclear Medicine, Volume 45, Issue 1, 2015, 32 46

Love C, Tronco GG, Palestro CJ.
Imaging of infection and inflammation with ^{99m}Tc-Fanolesomab.
Q J Nucl Med Mol Imaging. 2006; 50:113-20.

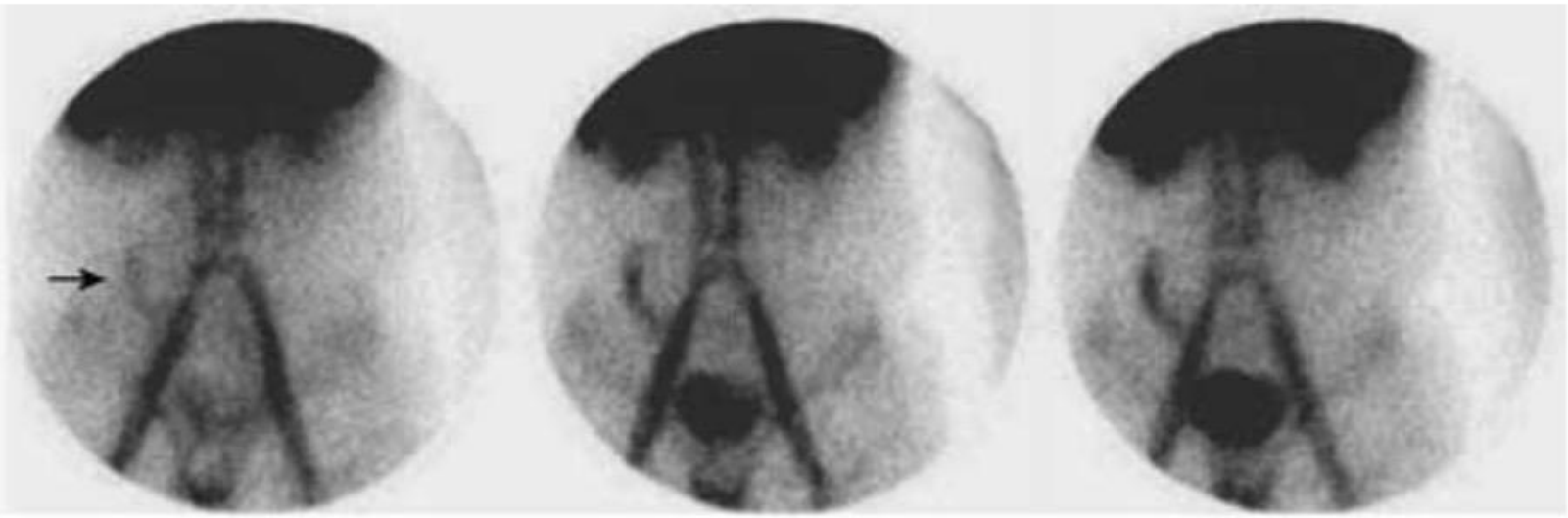


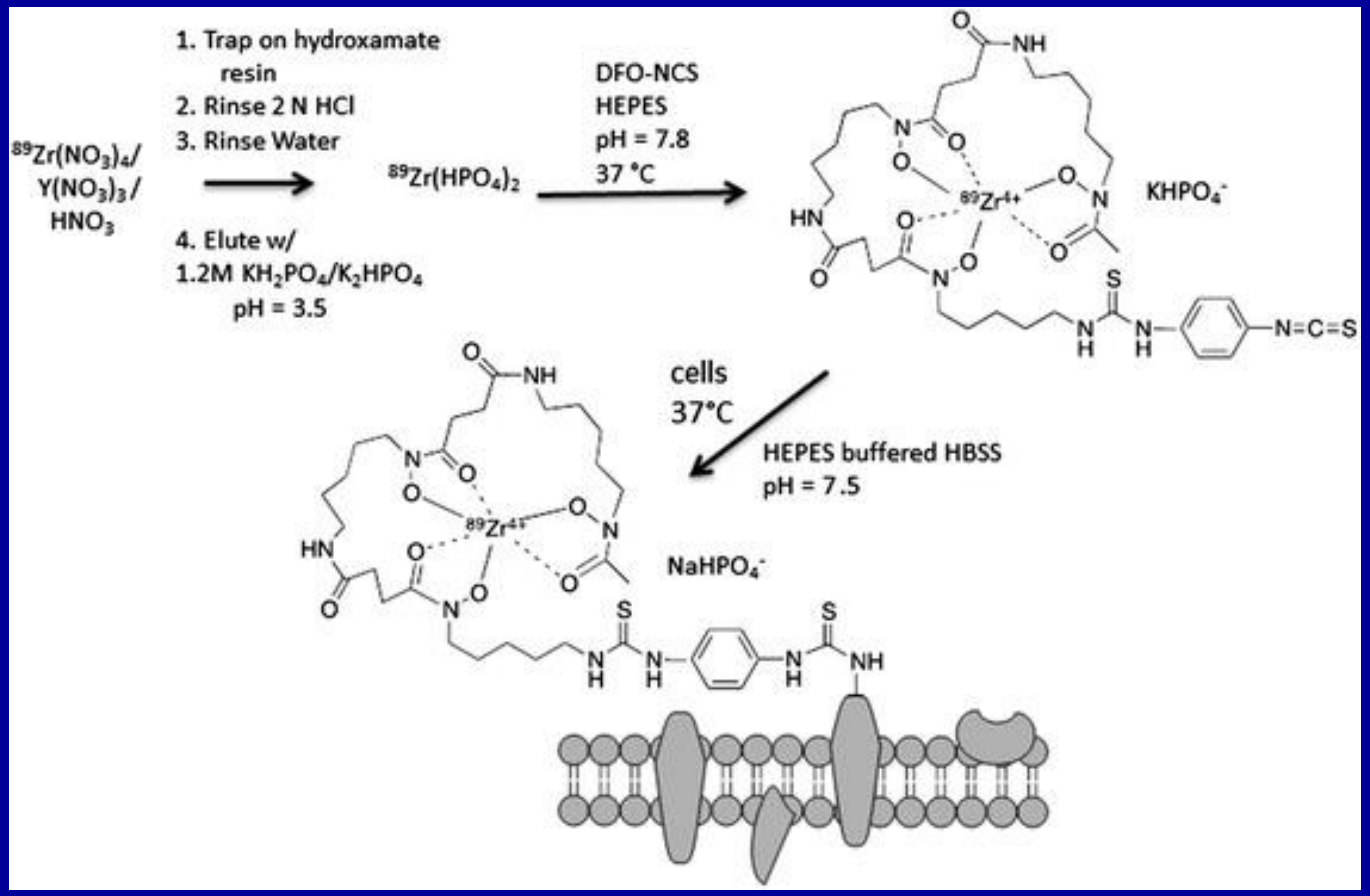
Figure 4.—Faint linearly increased activity lateral to the right iliac vessels (arrow), which increases in intensity over time, can be appreciated at 5 min after tracer injection. In about half of the patients with appendicitis, the appendix is visualized within 10 min after injection of (courtesy of Frederick L. Weiland, M.D.).

NeutroSpec™
Find it. Define it.™
[Technetium (99m Tc) fanolesomab]

Targets CD15

Bansal A, et al. Novel ^{89}Zr cell labeling approach for PET-based cell trafficking studies. EJNMMI Res. 2015 Mar 28;5:19.

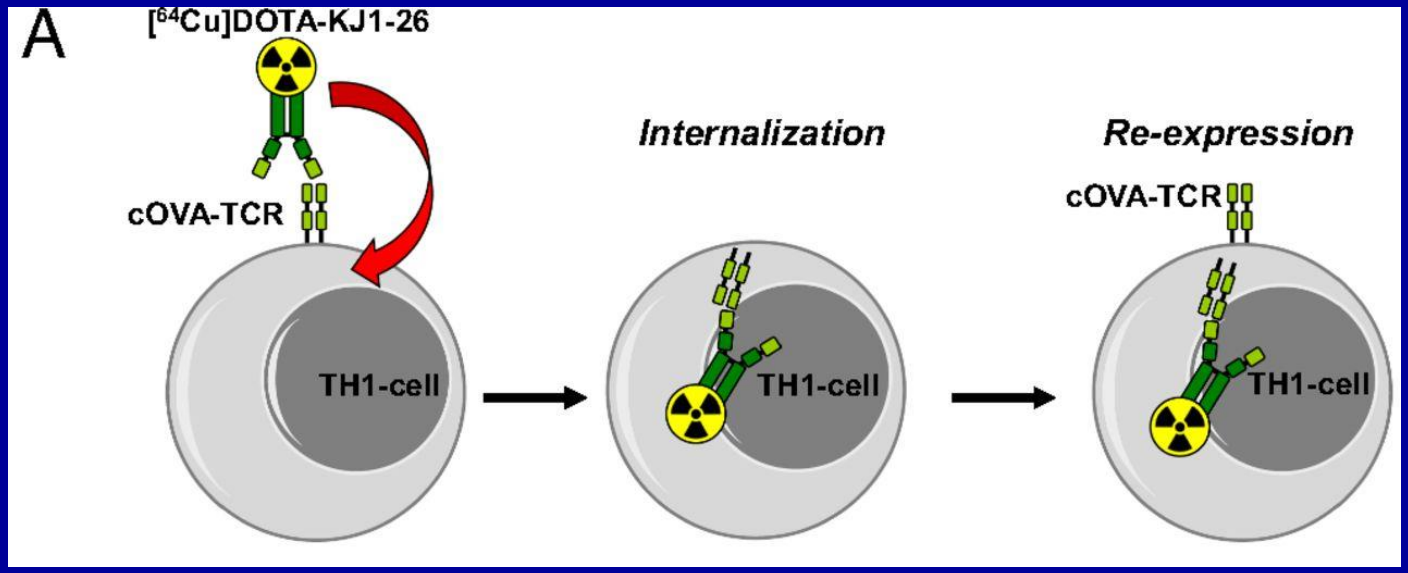
Mouse-derived melanoma cells, dendritic cells, and human mesenchymal stem cells were covalently labeled with ^{89}Zr-DBN via the reaction between the NCS group on ^{89}Zr-DBN and primary amine groups present on cell surface membrane protein.

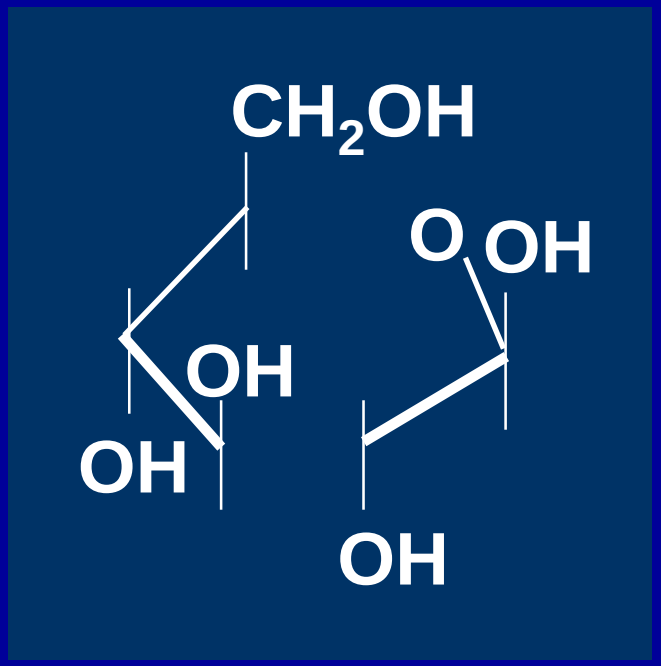


^{89}Zr half-life of 78.42 h

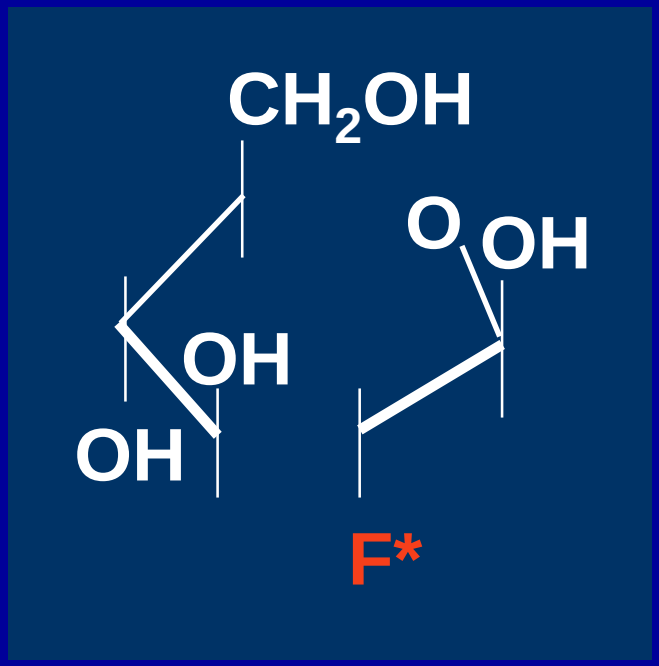
Griessinger CM et al. ^{64}Cu antibody-targeting of the T-cell receptor and subsequent internalization enables in vivo tracking of lymphocytes by PET. Proc Natl Acad Sci U S A. 2015; 112:1161-6.

We labeled chicken-ovalbumin-TCR-transgenic TH1 cells (cOVA-TCRtg-TH1) with ^{64}Cu-DOTA–modified cOVA-TCR–specific mAbs in vitro and investigated the endocytosis-dependent intracellular accumulation of the mAb–TCR complex.



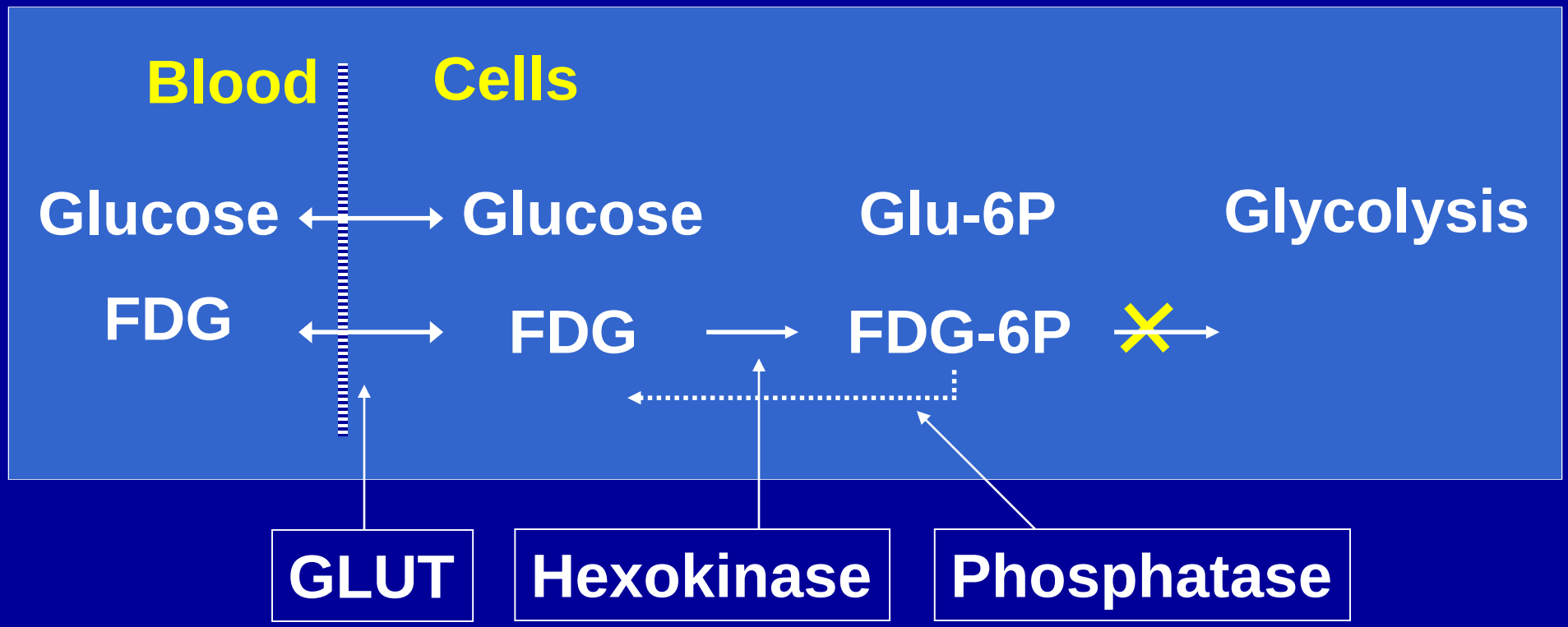


Glucose



Fluorodeoxyglucose (FDG)

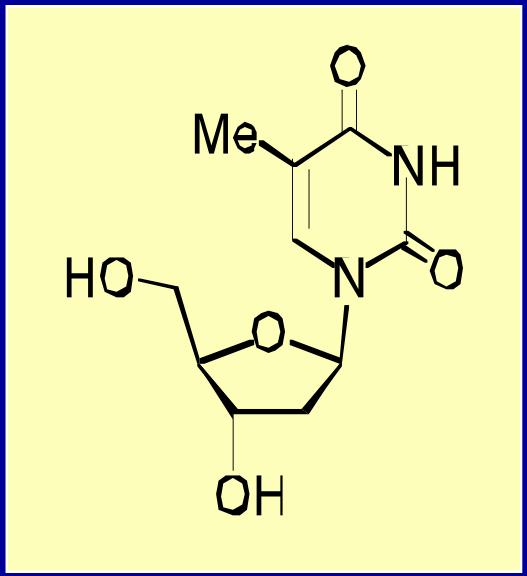
FDG Uptake and Retention



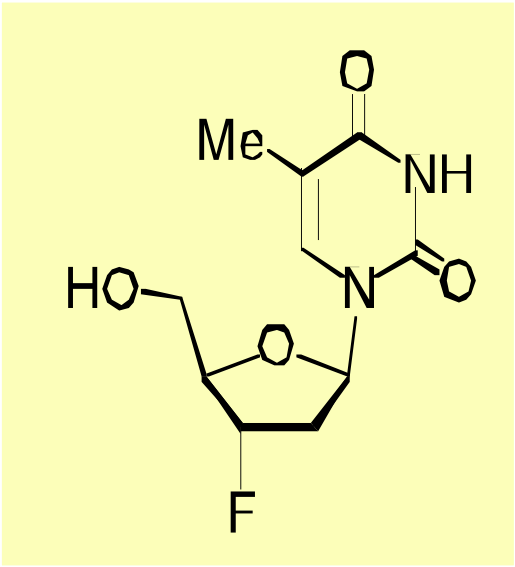
Tumors tend to have high levels of glucose transporters (GLUT) and hexokinase.

Fluorothymidine

DNA synthesis



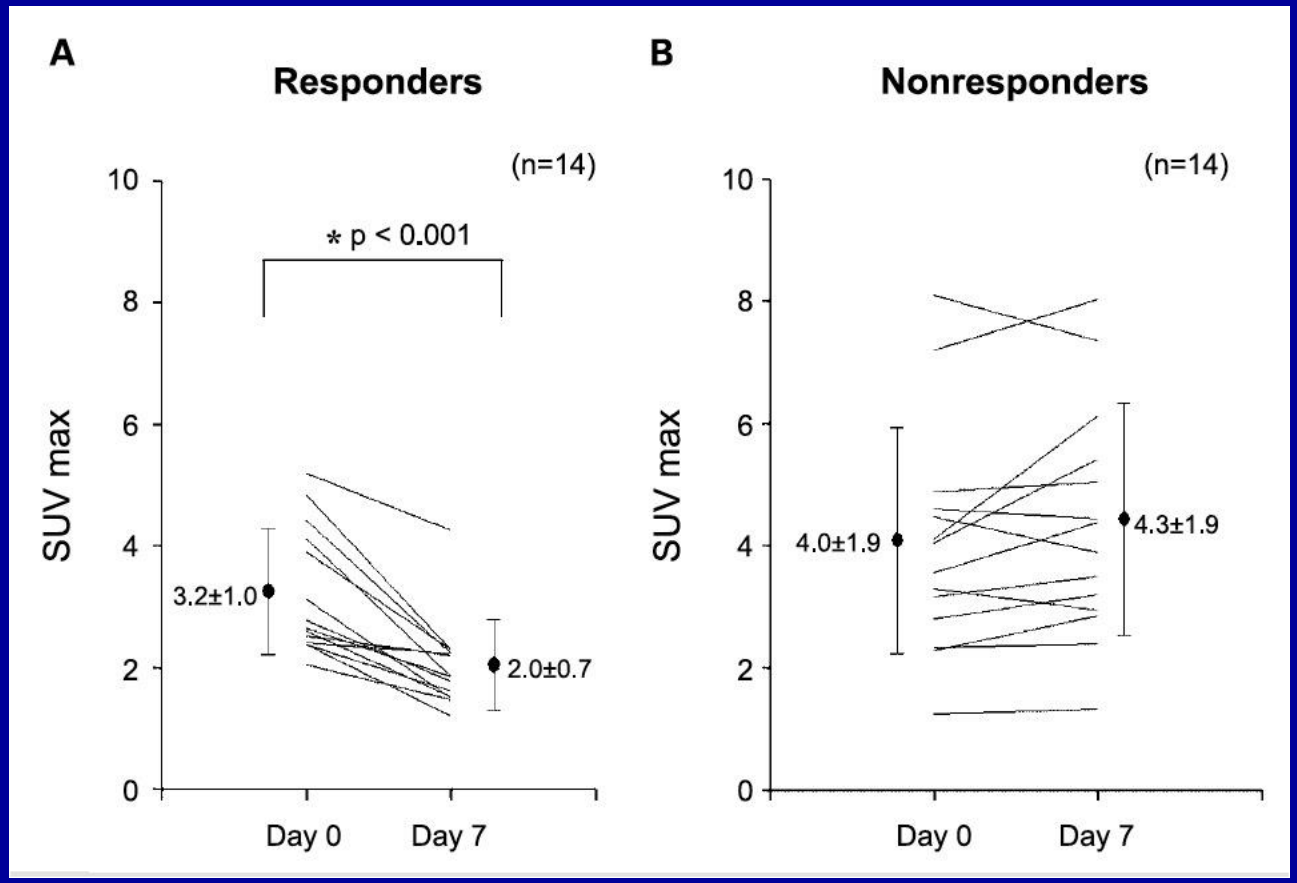
Thymidine



FLT
(3'-deoxy-3'-fluorothymidine)

John R. Grierson and Anthony F. Shields
University of Washington (Seattle) and Wayne State University (Detroit)

Sohn HJ, et al. FLT PET before and 7 days after gefitinib (EGFR inhibitor) treatment predicts response in patients with advanced lung adenocarcinoma. Clin Cancer Res. 2008; 14:7423-9.



Imaging at 1 hr p 15 mCi FLT

Threshold: decrease of >10.9% in SUVmax.

PPV & NPV were both 92.9%.

Korea

Apoptosis

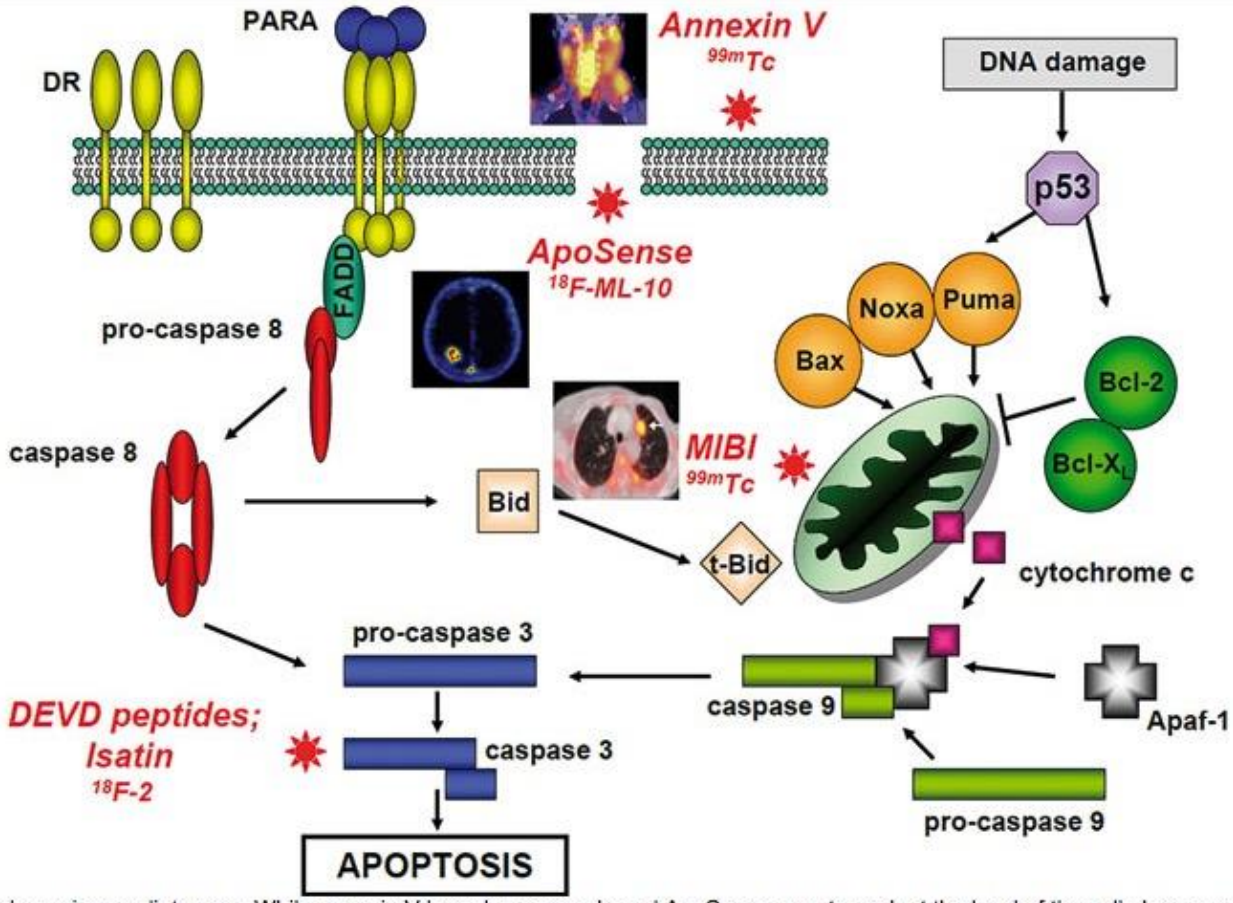


Fig. 1. Imaging of apoptosis by various radiotracers. While annexin V based compounds and ApoSense agents work at the level of the cell plasma membrane, MIBI acts at the mitochondria and Isatin targets executioner caspase 3 (DR=Death Receptor; PARA=Pro-Apoptotic Receptor Agonist). Inserts are examples of images acquired by the indicated modalities. Modified from: Haimovitz-Friedman et al., 2012 [94]

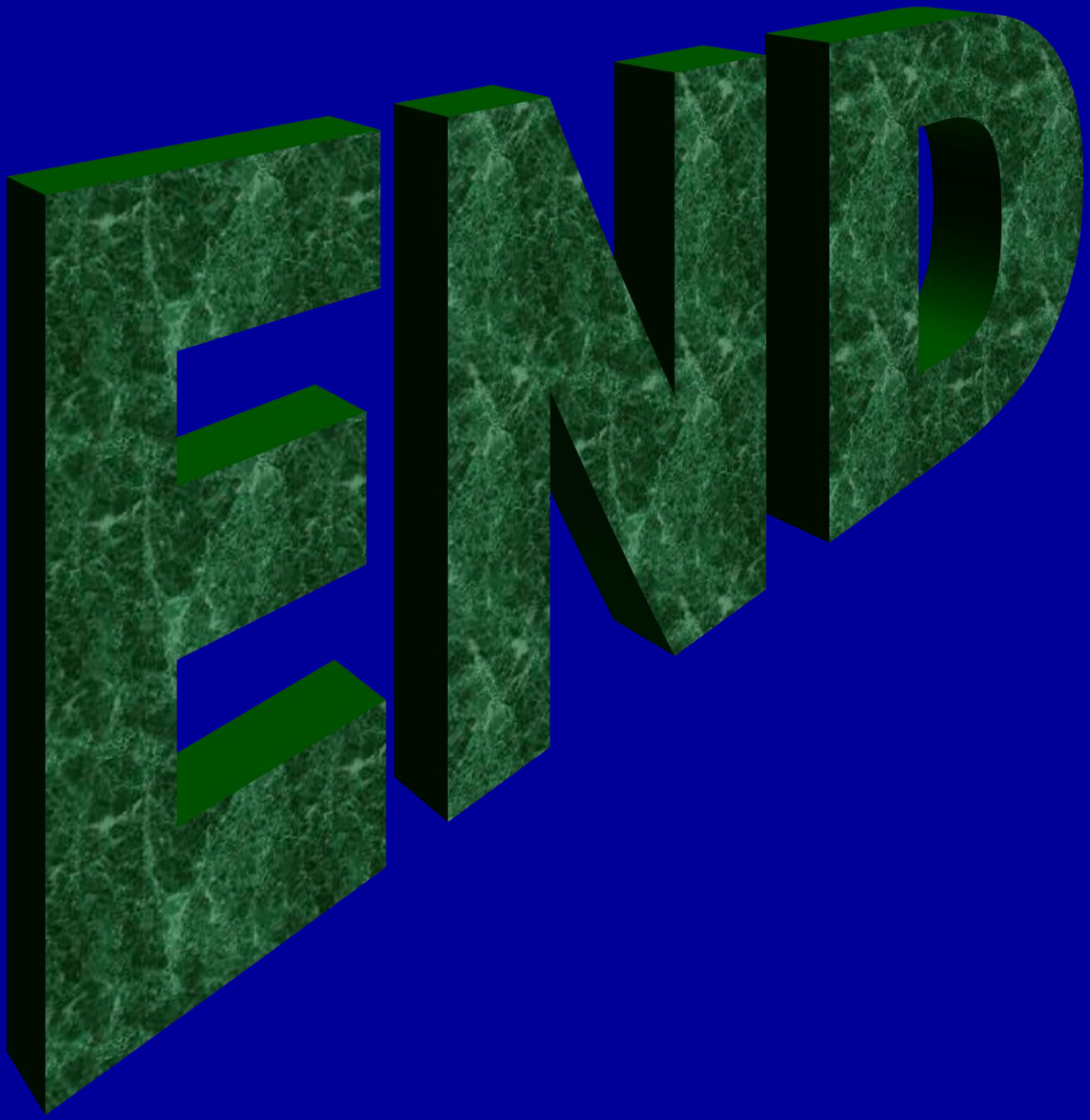
Yang, Haimovitz-Friedman, Verheij. Anticancer therapy and apoptosis imaging. Exp Oncol. 2012; 34:269-76.

Types of studies
(Metabolism, Receptors, Cell Trafficking)

- Metabolism
  - Bone formation
  - Bile formation
  - Renal tubular function
  - Macrophage activity
  - Glucose metabolism
  - Fatty acid metabolism
  - Cell membrane synthesis
  - DNA synthesis
  - Protein synthesis
  - Iodine
- Receptor imaging
  - MIBG
  - Dopamine receptors
  - Somatostatin receptors
  - PSMA
  - CD20 (Zevalin)
  - Bombesin
  - Angiogenesis (RGD)
  - Folate receptor
  - CXCR4
  - Apoptosis
- Cell Trafficking
  - Red blood cells
  - White blood cells
  - Platelets
  - Lymphocytes
  - Eosinophils
  - Granulocytes
  - mesenchymal stem cells

Goal

Identify specific metabolic pathways, up-regulated receptors, or cell trafficking that can either predict responders or assess response early in the course of therapy.


END

Bridging the Gap: Funding and Resources at NCI for Molecular Imaging Agents

Paula M. Jacobs, Ph.D.
Associate Director, Division of Cancer Treatment and Diagnosis, NCI
Cancer Imaging Program

NIH NATIONAL CANCER INSTITUTE

Outline

- Grants
  - General NIH funding
  - Specialized imaging funding
  - SBIR/STRR funding
- NCI Experimental Therapeutics Program (NExT)
- Cooperative Group Trials
- Regulatory advice

NIH Grant Funding

General Funding

- Funding Opportunities and Notices- NIH &NCI
  - http://grants.nih.gov/grants/guide/
  - http://www.cancer.gov/researchandfunding/funding/announcements
- Common types of grant
  - Unsolicited – R01, R03, R21
  - Request for applications (RFA)
  - Program announcement (PA/PAR)

Grant Funding For Imaging

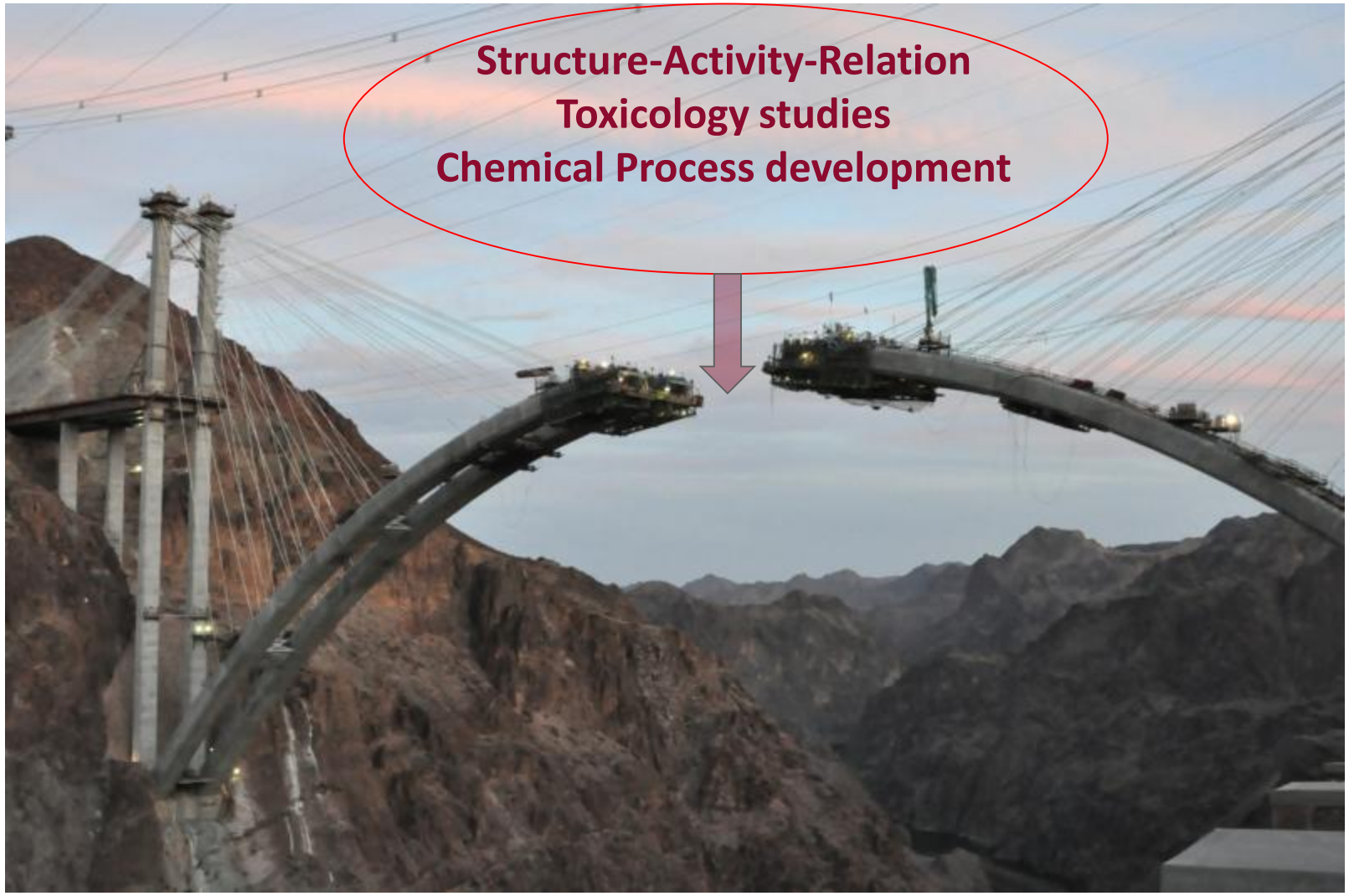
- Early Phase Clinical Trials in Imaging and IGI (R01): PAR-14-166
  - R01 - $500,000 total direct costs over 2 years
  - Supports early phase clinical trials
- Image-guided Drug Delivery in Cancer (R01): PAR-13-185
  - R01 – standard NIH policies
  - Encourages innovative translational research in image-guided drug delivery (IGDD) in cancer.
- Oncology co-clinical QI imaging research resources (U24) PAR 15-266
- Academic-Industrial Partnerships for Translation of in vivo Imaging Systems for Cancer Investigations (R01): PAR-13-169

Grant Funding For Imaging (2)

- SBIR & STTR
  - The Small Business Innovation Research (SBIR) PA-14-071; 2.9% set aside
  - Small Business Technology Transfer (STTR) PA-14-072; 0.4% set aside
  - ~$700M annually at NIH; $115 at NCI
- Not as relevant to imaging immunotherapy
  - Quantitative Imaging for Evaluation of Responses to Cancer Therapies: PAR 14-116
    - QIN – U01 – Cooperative Agreement
    - Develop and share quantitative imaging methods to measure tumor response to therapy
  - NCI Informatics (U01, R01, P01, U24): PAR 12-286-290

But grants don’t get you into the clinic…….

Bridging the "Valley of Death"



NCI Experimental Therapeutics Program (NExT)

NOT A GRANT PROGRAM

- Provides access to NCI resources and expertise – NCI performs the project
- Simple application process
- External expert review
- Internal expert review
- Full team support
- Applicant involved in project

NExT Development Resources

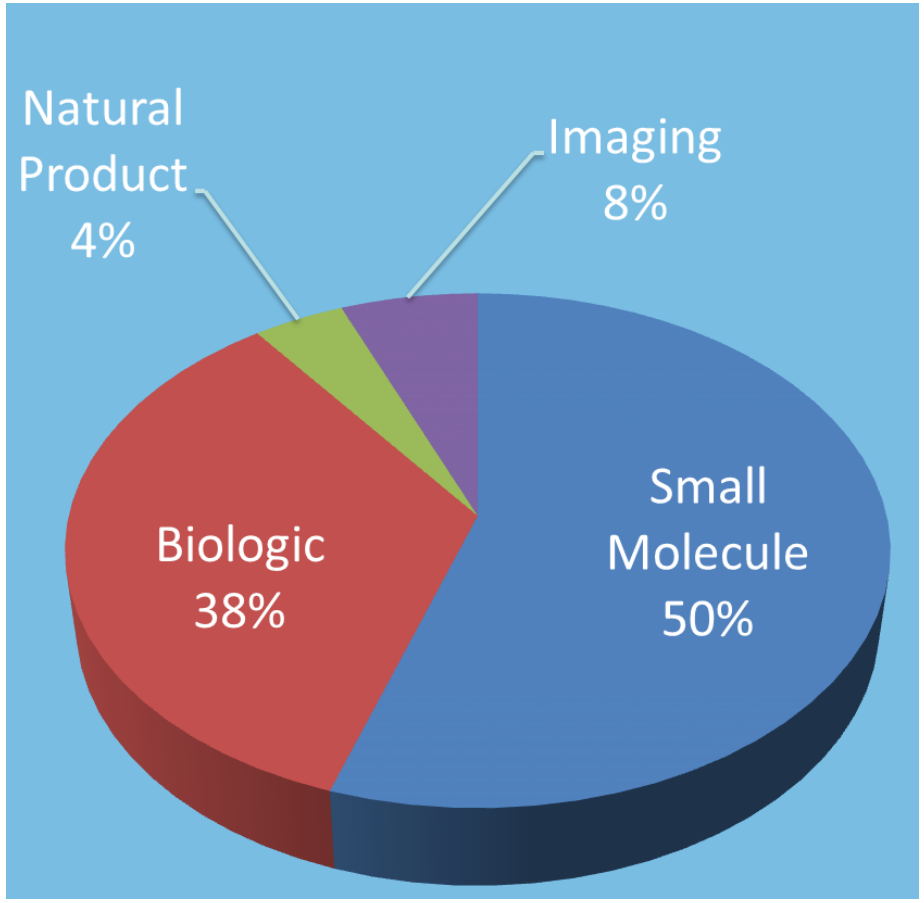
- Multi- and interdisciplinary clinical/translational teams
- Early access to leading-edge translational technologies
- PK/PD modeling and assay development
- Toxicology/Safety Pharmacology
- Formulation & GMP Scale-Up
- Imaging for biodistribution
- Development & validation of pharmacodynamics assays
- Development & validation of clinical assays
- Proof-of-concept or first in human studies

Next Resources Currently Support

- Investigational drugs and biologics
- Investigational imaging agents
- Academic, biotech and pharma projects
- Phase 0, 1 and 2 clinical trials
- HTS, Hit-to-Lead and Lead optimization

NOT basic research

Portfolio Stratified by Agent Class (Active Projects)



http://next.cancer.gov/

Choyke, Peter	CCR NCI	A Phase II Study of F-18 DCFBC, a Prostate Specific Membrane Antigen-Target
Frangioni, John	BIDMC	A NIR Fluorophore for Clinical Translation of Image-Guided Oncologic Surgery
Griffiths, Gary	CCR NCI	Large Scale Preparation of IR700-Panitumumab for Clinical Use
Kirsch, David	Duke	Using Molecular Imaging to Detect Microscopic Residual Cancer During Surgery
Rosenthal, Eben	UAB	Intraoperative Optical Imaging to Guide Surgical Resection of Cancer

Access to NExT

Who: Researchers in academia, government and industry, nationally and internationally.

http://next.cancer.gov/

Cooperative Group Trials and Regulatory Assistance

NCI Cooperative Groups

- A half-century old national clinical trial system for oncology
- Conduct large scale clinical trials
  - Disease oriented
  - Radiation therapy
  - Surgery
  - Imaging
- Restructured to 1 pediatric & 4 adult groups in 2014
  - COG – pediatrics
  - Alliance – oncology
  - ECOG-ACRIN – oncology, imaging
  - NRG – radiation therapy, surgery, gynecology
  - SWOG – oncology

CIP – Regulatory Advice/Resources

- File IND's for investigational trials, Group or CCR
  - FLT, FES, FMISO, Zr-panitumumab, NaF, DCFBC, ferumoxytol
  - Note that filings for Zr-antibody are posted on our web site
- Provide cross-file letters to independent PIs
  - Between 50 and 60 to date
- Provide full SOPs for tracer manufacture
  - FLT, FES, FMISO, Zr-panitumumab
- Advise on regulatory process
- File NDA's to permit ANDA's
  - ^{18}F-Sodium Fluoride 2012
  - Exploring ^{18}F Fluoromethylcholine

Thanks for your attention

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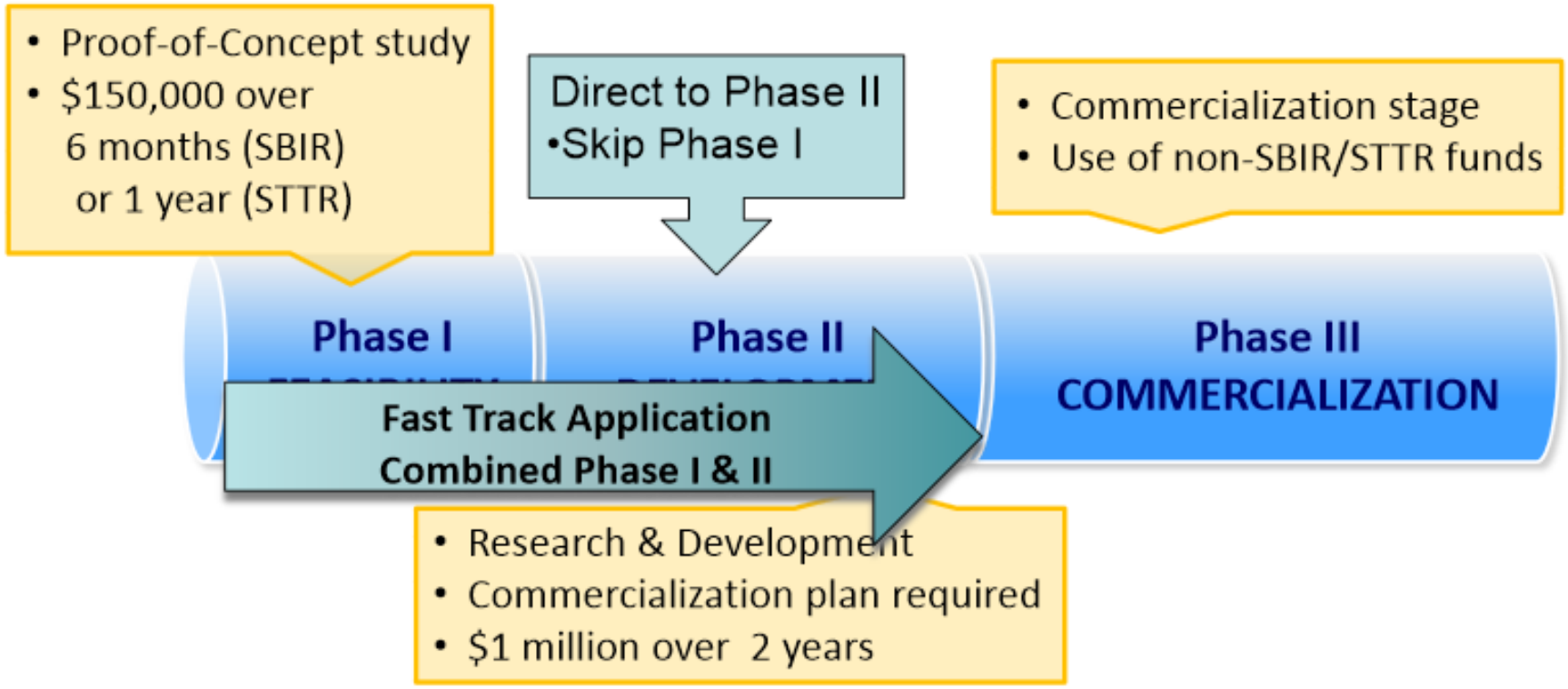
www.cancer.gov **www.cancer.gov/espanol**

SBIR & STTR: Three-Phase Program



- Hard caps on award sizes: $225,000 for Phase I; $1.5 million for Phase II
- Certain awards may exceed these caps if covered by topic-specific waivers
- Actual funding may vary by topic

SBIR Eligibility Requirements

- Applicant is a Small Business Concern (SBC) , organized for-profit U.S. business
- 500 or fewer employees, including affiliates
- PI's primary employment (>50%) must be with the SBC at time of award & for duration of project
- > 50% U.S.-owned by individuals and independently operated

  OR

- > 50% owned and controlled by other business concern/s that is/are > 50% owned and controlled by one or more individuals

  OR

- > 50% owned by multiple venture capital operating companies, hedge funds, private equity firms, or any combination of these

STTR Eligibility Requirements

- Applicant is a Small Business Concern (SBC), organized for-profit U.S. business
- Formal cooperative R&D effort
  - Minimum 40% by small business
  - Minimum 30% by US research institution
- US Research Institution: college or university; non-profit research organization; Federally-Funded R&D Center (FFRDC)
- Principal Investigator's primary employment may be with either the SBC or the research institution
- SBC must have right to IP to carry out follow-on R&D and commercialization

Reasons to seek SBIR/STTR Funding

- Provides seed funding for innovative technology development
- Not a Loan; repayment is not required
- Doesn't impact stock or shares in any way (i.e., non-dilutive)
- Intellectual property rights retained by the small business
- Provides recognition, verification, and visibility
- Helps provide leverage in attracting additional funding or support (e.g., venture capital, strategic partner)

Orphan Drugs – not NCI, but relevant

Orphan Drugs

- Drugs or biologics (not devices) intended to treat, diagnose, or prevent a rare disease or condition… or,
- A drug that will not be profitable within 7 years following FDA marketing approval (rare)
- Pathway for devices available, but not identical
- Can submit common application to EMA

Is the Disease or Condition Rare?

- The disease or condition prevalence <200,000 in the US
- Acute diseases or conditions: yearly incidence may be used in some cases to estimate the patient population (<200,000 in the US)
- Diagnostics and preventatives: may only be subjected to <200,000 patients in the US annually
- Medically plausible (orphan) subsets of common diseases (e.g. metastatic melanoma)
  - No salami slicing

Medically Plausible (Orphan) Subsets

- There is some property of the drug such that the use of the drug would be limited to the subset of the disease or condition
- E.g., toxicity profile, mechanism of action
- The drug would not be used in the full complement of the disease
- Regulatory term to delineate persons expected to use the drug
- Not a clinical definition

Benefits of Orphan Designation

- Purely financial in nature:
  - Seven years of market exclusivity
  - Up to 50% of tax credits for clinical research expenses
  - Waiver of marketing application fees
- However…
  - Often the first step in FDA communication
  - OOPD may provide informal guidance
  - May also attract venture capital
- Can apply for FDA grants to support clinical research

Request for Orphan Designation

- Possibly the simplest FDA submission
- The request must be made prior to the submission of a BLA or NDA
- An IND is not required for submission
- May be submitted from sponsors from any country
- May be private citizens, academic institutions, for-profit, non-profit, small biotech, industry, etc.

May 2, 2016

IMMUNOTHERAPY'S OTHER CHALLENGE: BIOMARKERS AND IMAGING TO DETERMINE WHO WILL BENEFIT?

Elizabeth M. Jaffee, M.D.
Dung Le, M.D.
Lei Zheng, M.D., Ph.D.
Eric Lutz, Ph.D.
Dan Laheru, M.D.

Disclosure Information

Elizabeth M. Jaffee, M.D.

I have the following financial relationships to disclose

I will be discussing the investigational use of:

- GVAX
- Listeria Monocytogenes – mesothelin

Both licensed to Aduro Biotech with potential to receive royalties

Consultation activity: BMS, Adaptive Biotech, MedImmune

Grants: Aduro, BMS, Roche

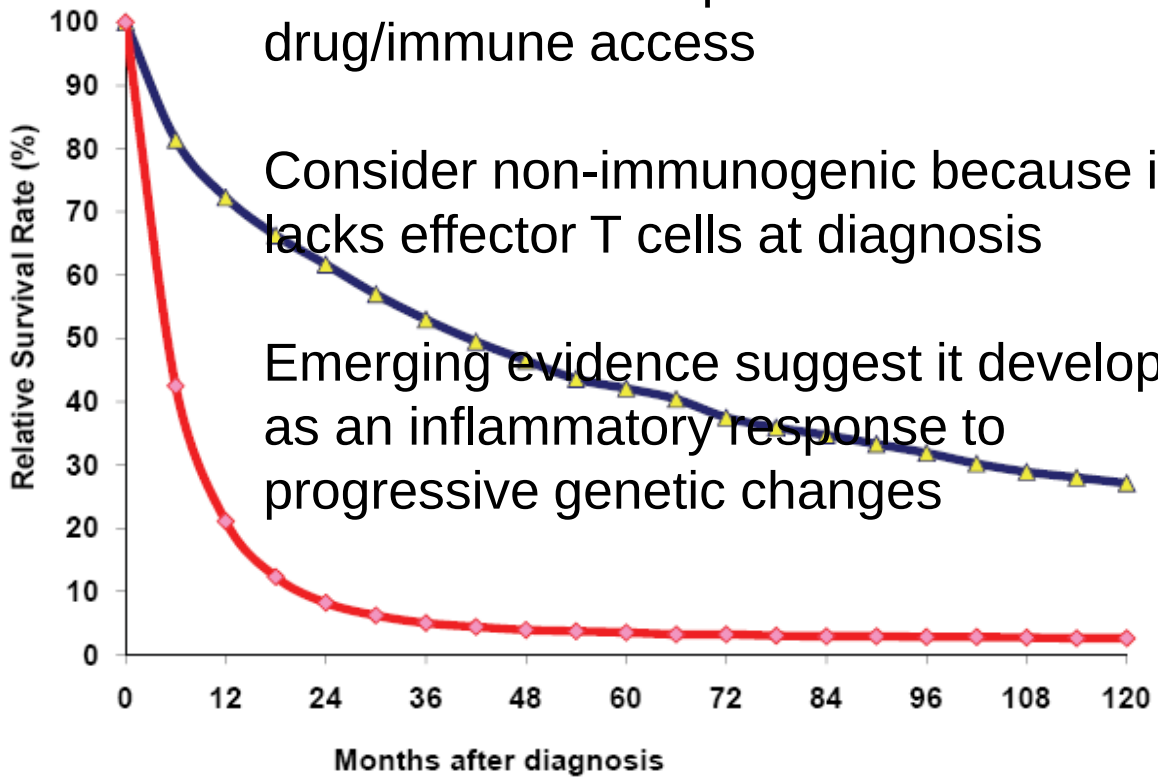
Immunotherapy has already changed the standard of care for patients with advanced prostate cancer and melanoma and NSCLC

Current immunotherapies work on up to 30% of all cancers

Why doesn't current immunotherapy work on all cancers?

Pancreatic Cancer: A model to study immunotherapy resistant cancers

Figure 7.1: Cancer of the Pancreas: Relative Survival Rates (%) by Histologic Subtype, Ages 20+, 12 SEER Areas, 1988-2001



Still among the deadliest cancers

Microenvironment provides barrier to drug/immune access

Consider non-immunogenic because it lacks effector T cells at diagnosis

Emerging evidence suggest it develops as an inflammatory response to progressive genetic changes

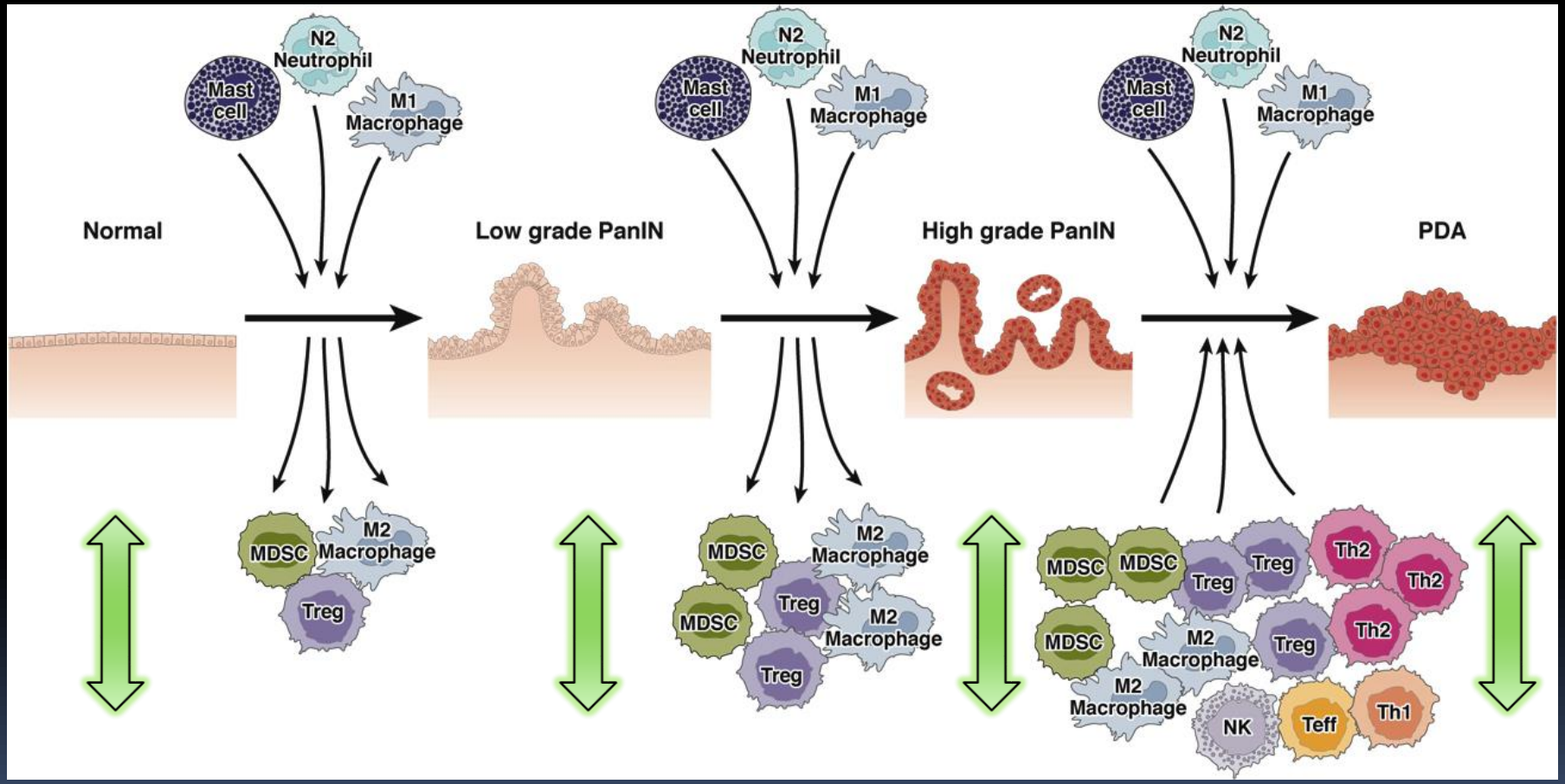
National Cancer Institute: SEER Survival Monograph

What have we learned from these successes?
Immune checkpoints are the game changer!

- Immune checkpoint agents act on T cells
- Only a minority of tumors have natural T cells
  - 50% of melanomas
  - 20-30% RCCS
  - 10-20% lung and colorectal tumors
- Pancreatic cancers and many other cancers are immunologically quiescent (lack effector T cells)
- For these cancers immune modulation alone is not enough – a T cell generating agent is also needed

Emerging concepts that explain why pancreatic cancers do not respond naturally to immunotherapy

There is an inflammatory response in pancreatic cancer that is a progressive, dynamic process, interrelated with cancer genetics

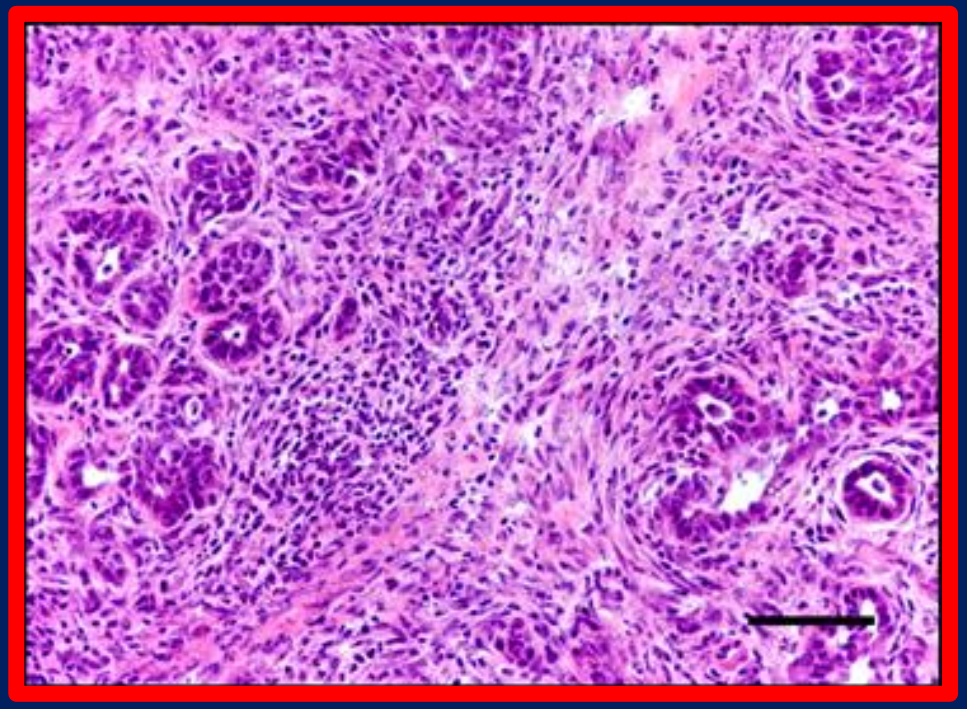


Telomere Shortening → Kras mutation → P16 Cyclin D1 → TP53 DPC4 BRCA2 → mesothelin

Immunobiology of pancreatic carcinoma

Fibroblasts

Extracellular matrix



Macrophages

Immature myeloid cells

B cells

Regulatory T cells

Desmoplastic stroma

But, minimal infiltration of effector T cells in the TME in most patients

Hypothesis:

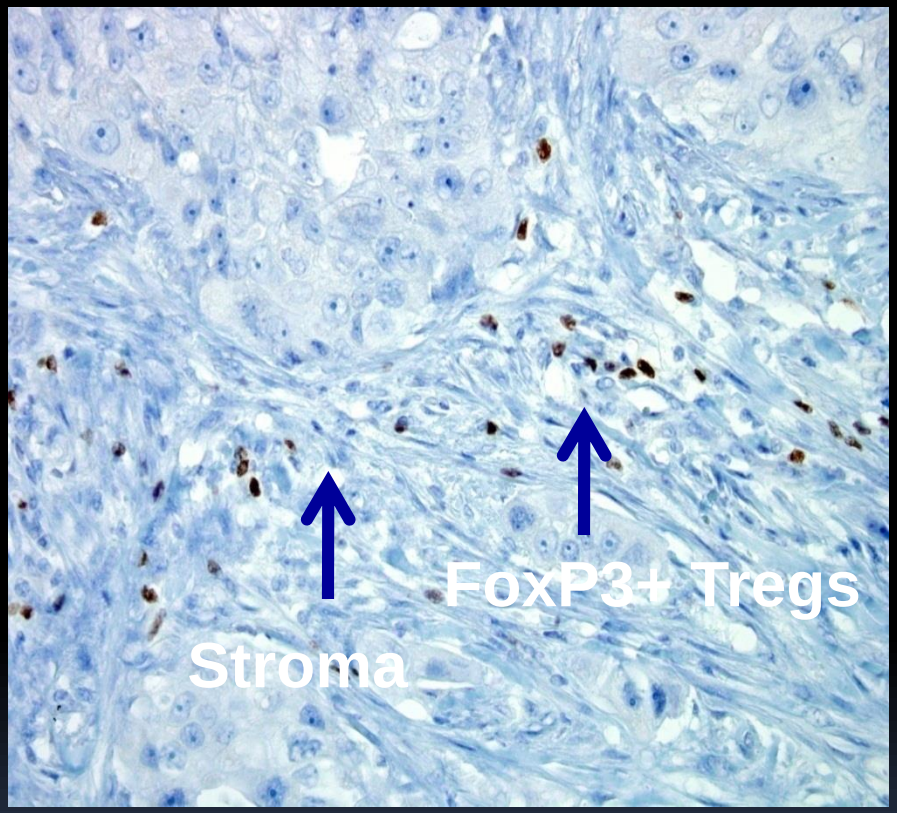
It's not the *physical* barrier of the stroma but rather an acquired network of oncogene-driven immunosuppression that excludes effector T cells in most of PDA

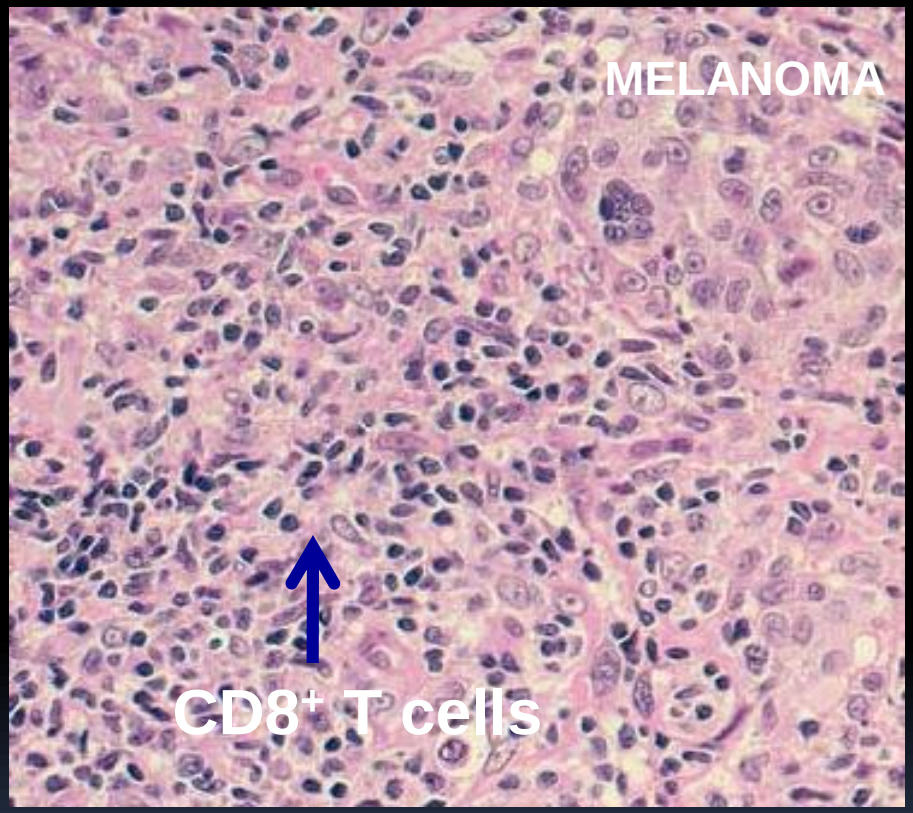
Implications:

- **Checkpoint blockade in PDA will be ineffective clinically**

- **Without Darwinian-like pressure from T cells, the underlying pancreatic tumor cells remain highly susceptible to T cells…. if these can be provoked**

Immunologically “resistant” tumors have inflammation but lack infiltration of effector T cells

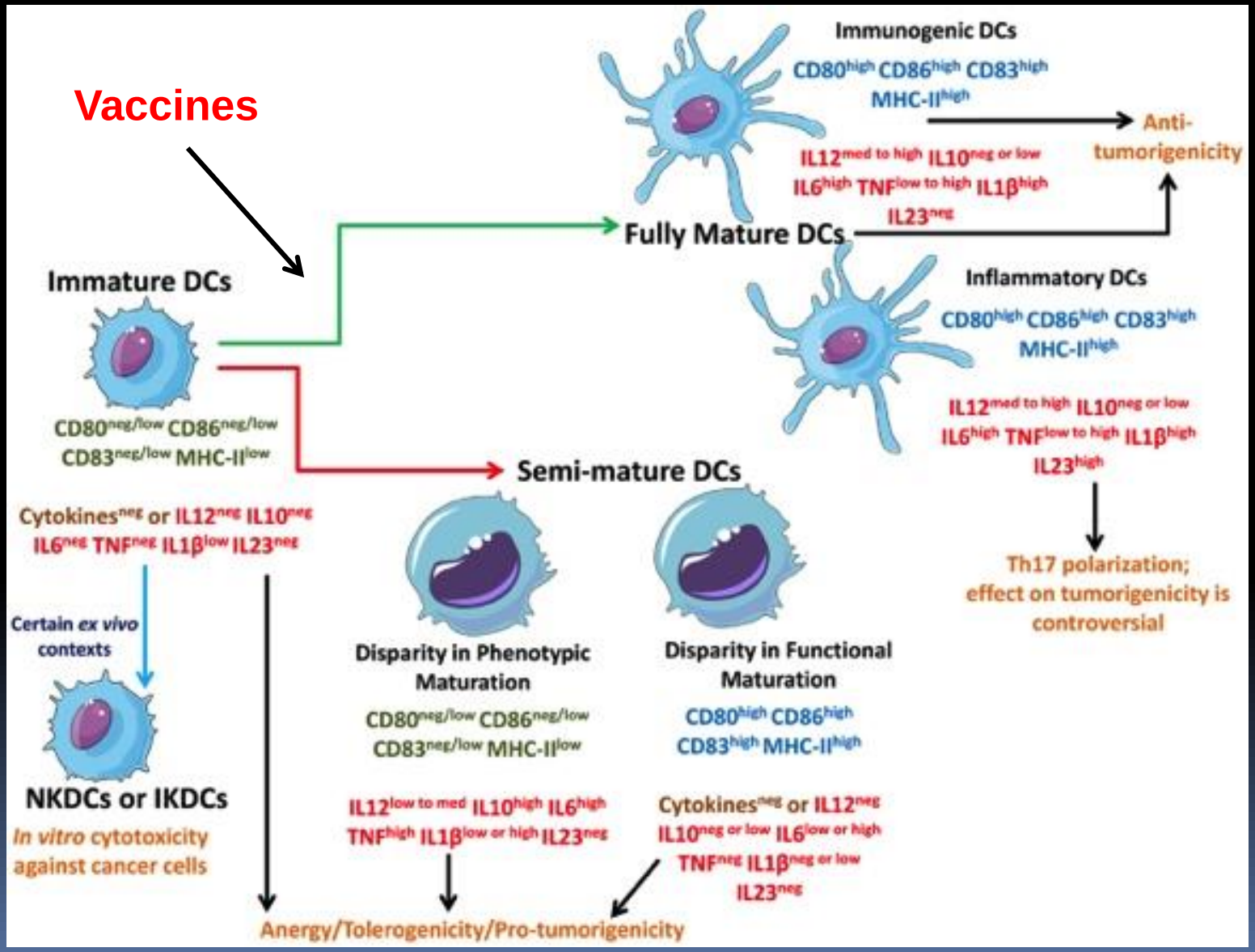




Pancreatic cancers are infiltrated with immune suppressive regulatory T cells (Tregs - shown), TAMS, Eos, B cells and MDSCS (not shown)

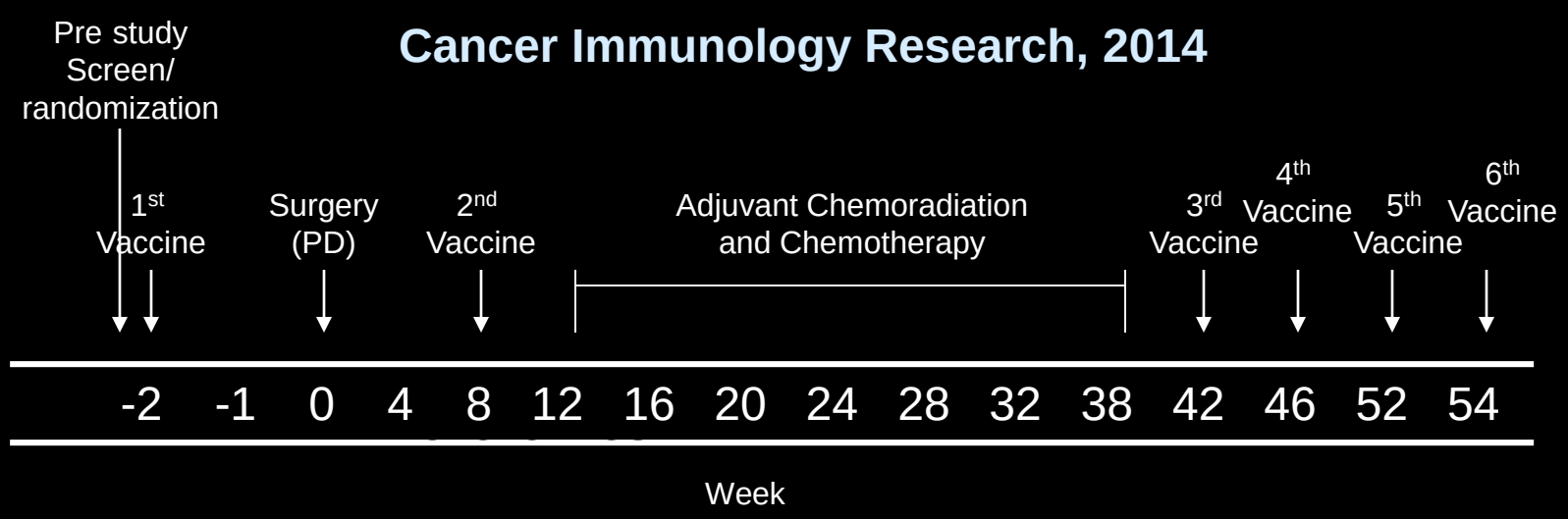
50% of Melanomas have spontaneous infiltration of effector T cells that can respond to checkpoint inhibitors

Dendritic cells exemplifies the divergent functional polarities of the different inflammatory cell populations



(Neo)adjuvant PDA vaccine study provides evidence vaccines can recruit T cells that traffic into immune resistant tumors

Cancer Immunology Research, 2014



Gal 3

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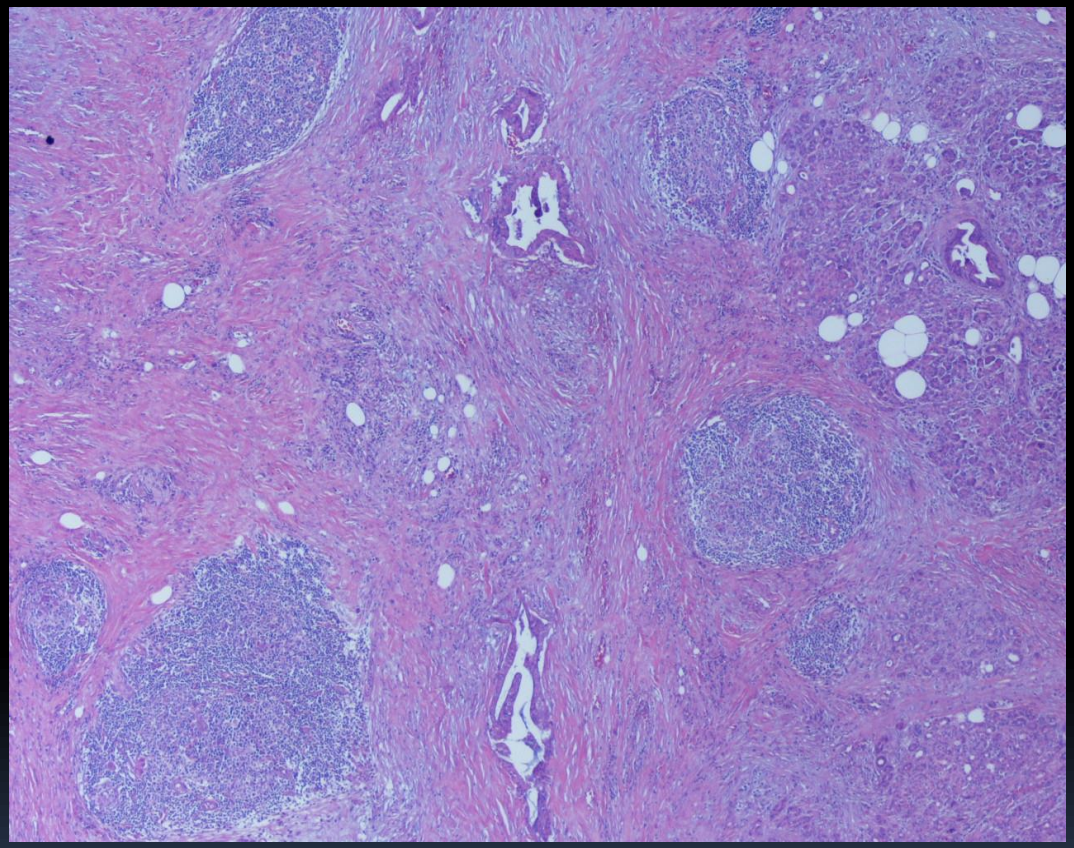
Chris Wolfgang M.D./Ph.D.

Dan Laheru, M.D.

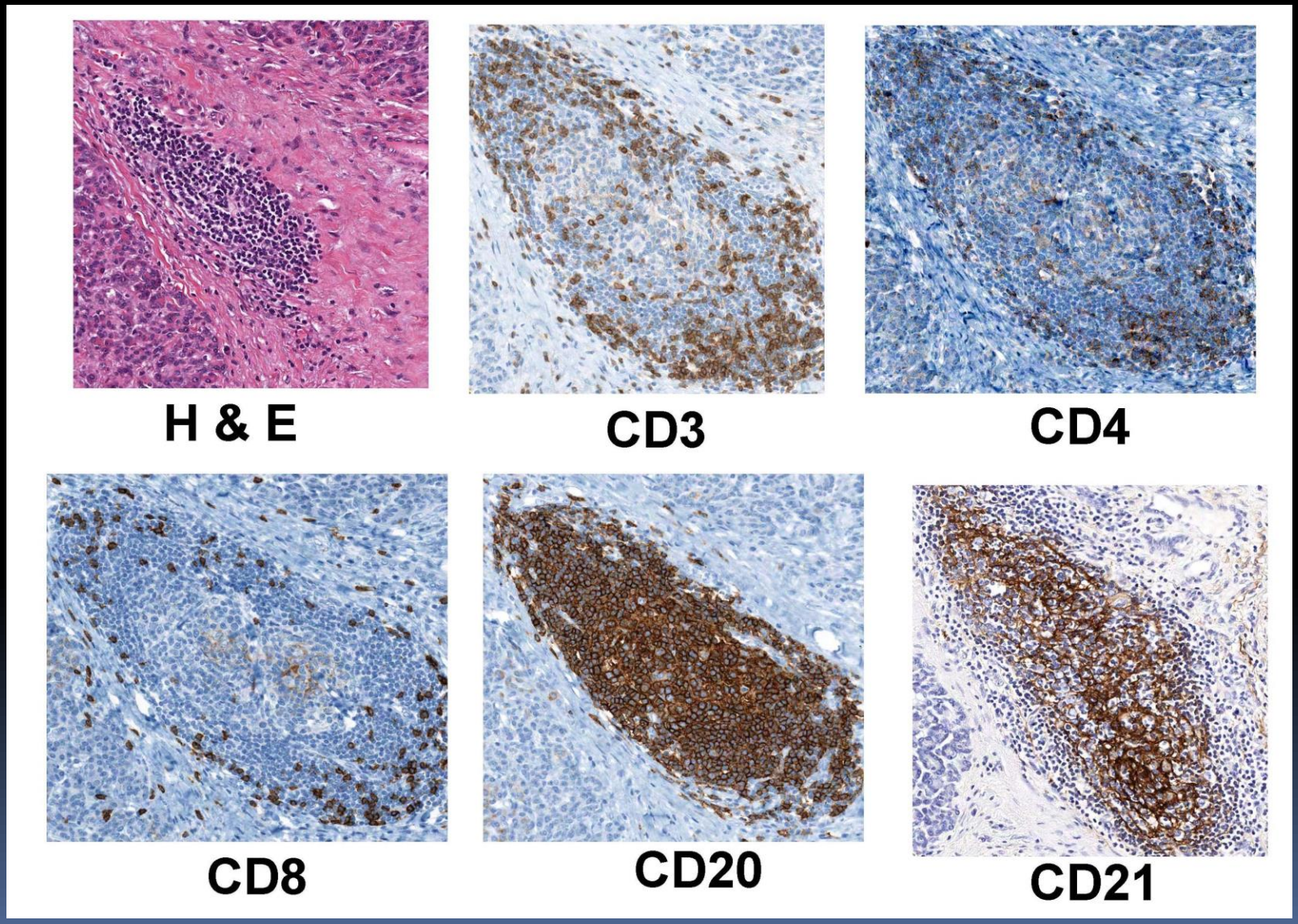
Eric Lutz, Ph.D.

Lymphoid Aggregates develop in tumors in vaccinated patients 2 weeks after a single vaccine

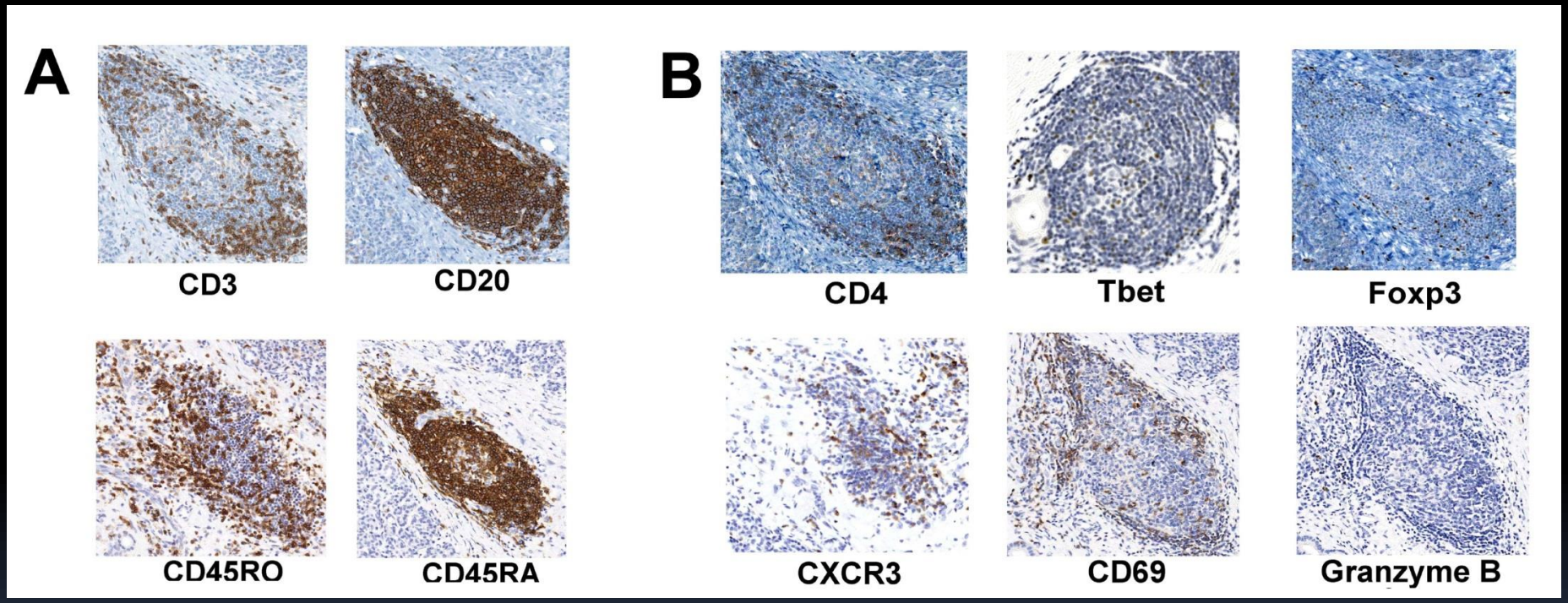


Intratumoral

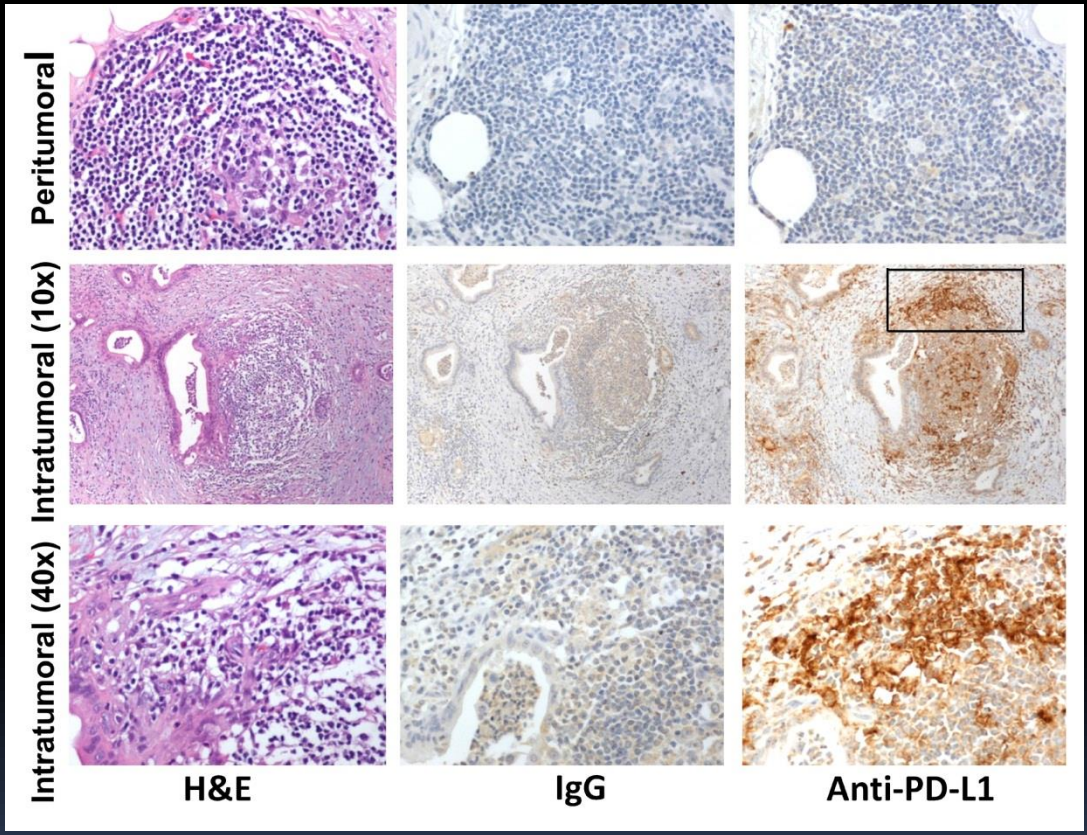
Lymphoid aggregates in PDAs are composed of organized T and B cell zones and a Germinal Centre-like structure



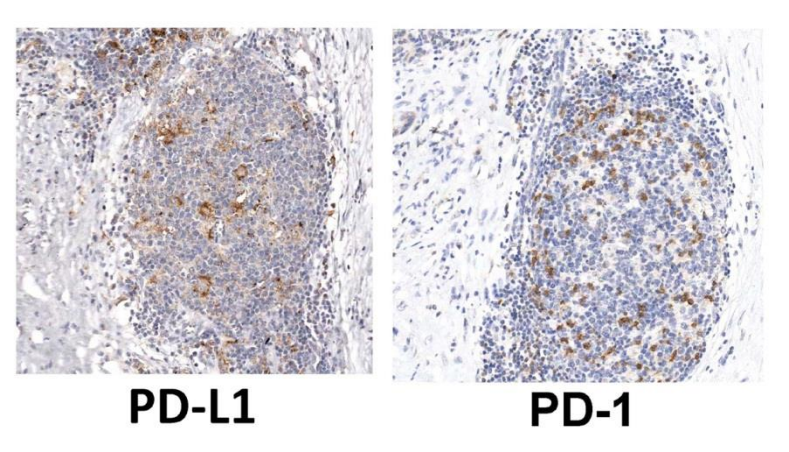
Lymphoid Aggregates Are Sites of Immune Activation and Regulation – Not Cytoloysis



PD-1/PD-L1 pathway is upregulated in vaccine induced lymphoid aggregates

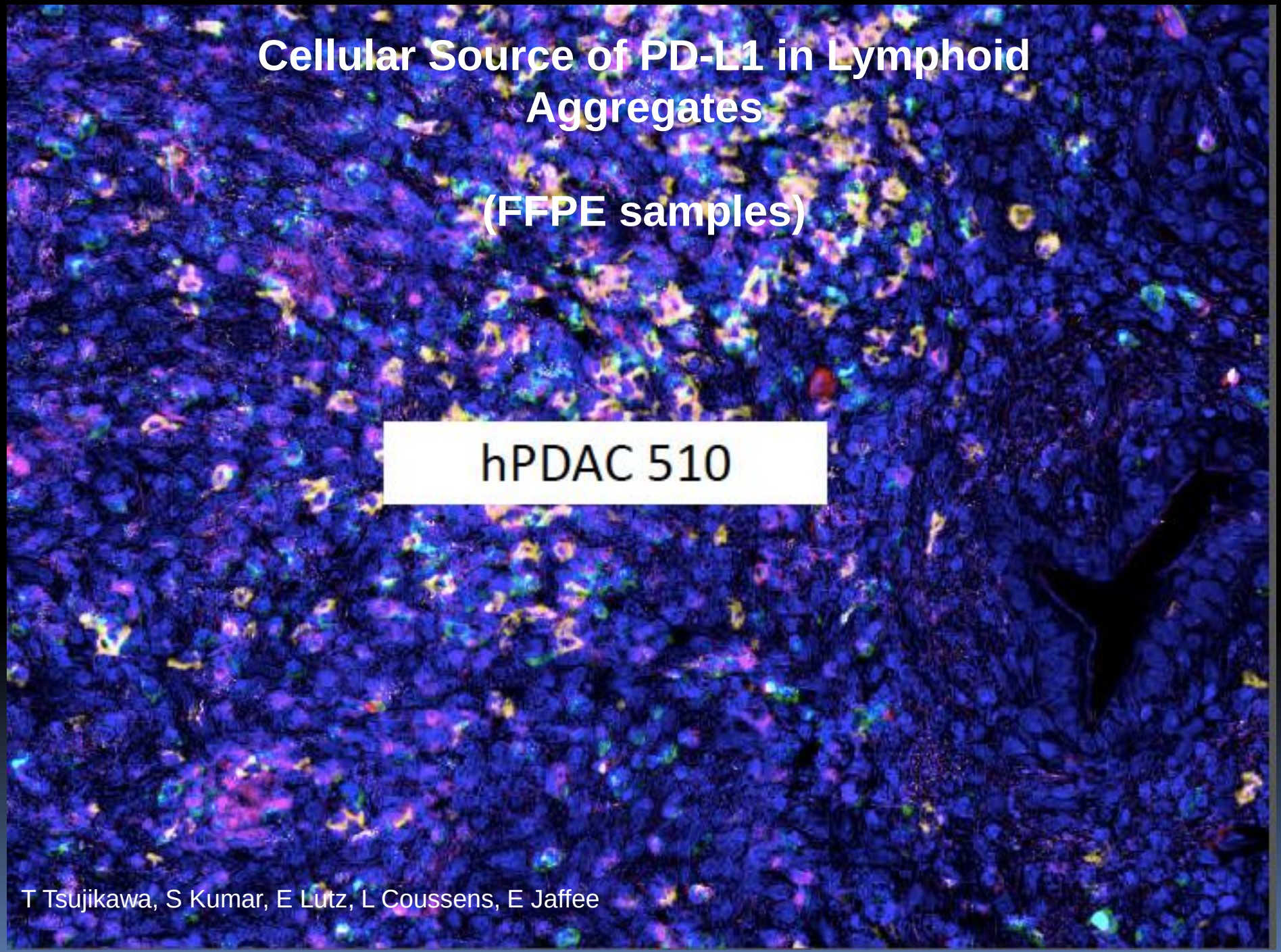


Co-localization



Cellular Source of PD-L1 in Lymphoid Aggregates

(FFPE samples)



T Tsujikawa, S Kumar, E Lutz, L Coussens, E Jaffee

Multiplex IHC enables detection of 12-different epitopes in a single FFPE section

Sequentia

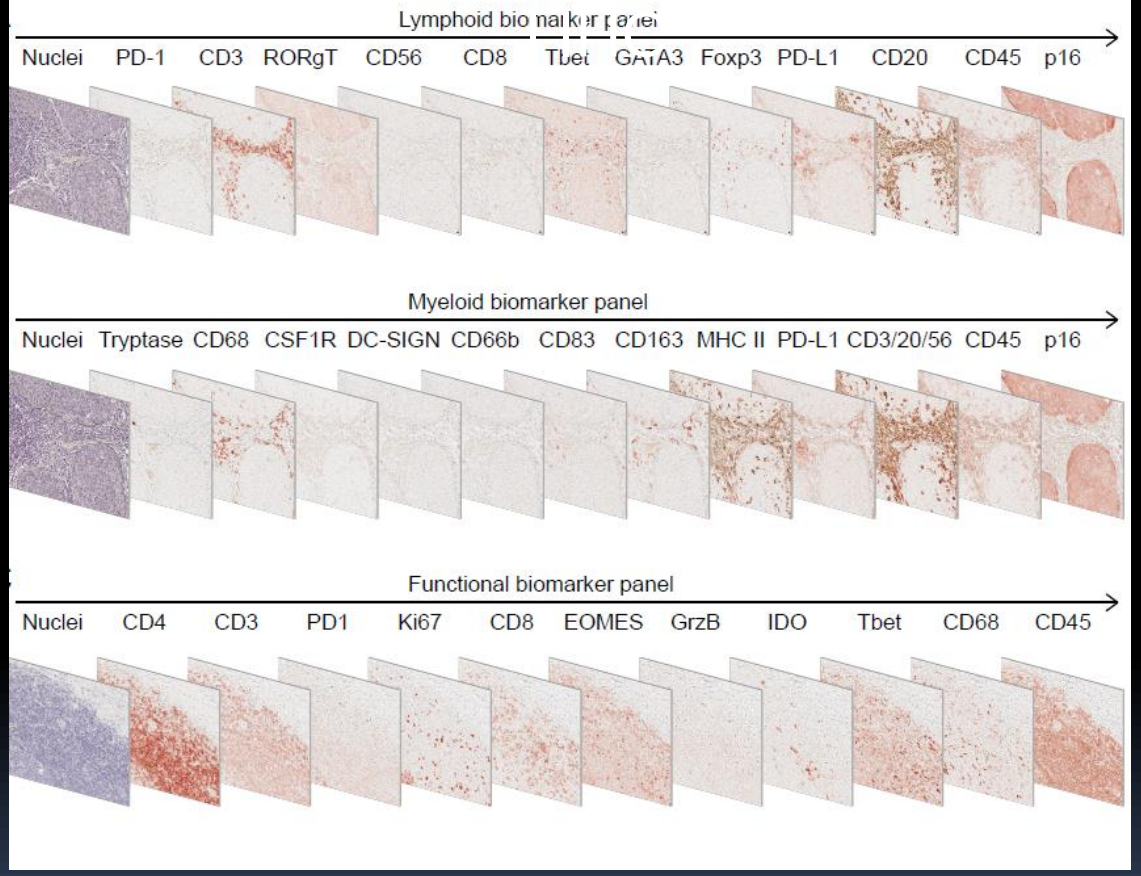
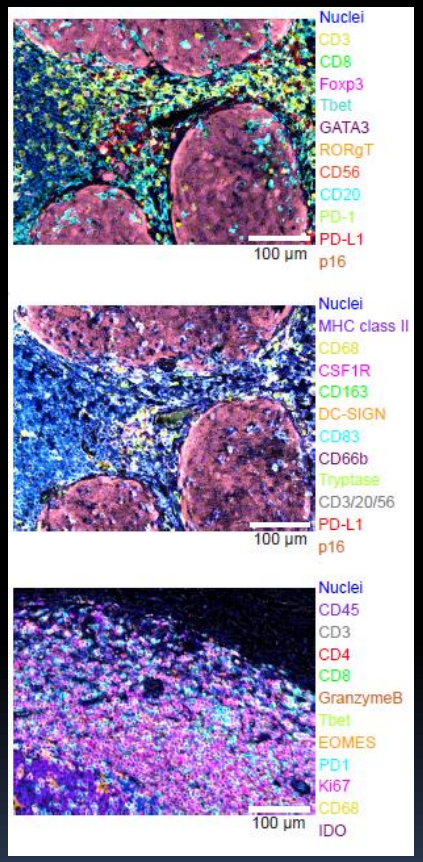


Image Co-registration

Color Deconvolution

Visualization



Tsujikawa T, et al. Manuscript in preparation

Tsujikawa T, et al. US Patent Pending 62/257,926,
filed November 20, 2015.

Two panels of 12-color multiplex IHC depicted tumor immune infiltrates in pancreatic ductal adenocarcinoma (PDAC) tissues

Human PDAC tissue, neoadjuvant GVAX

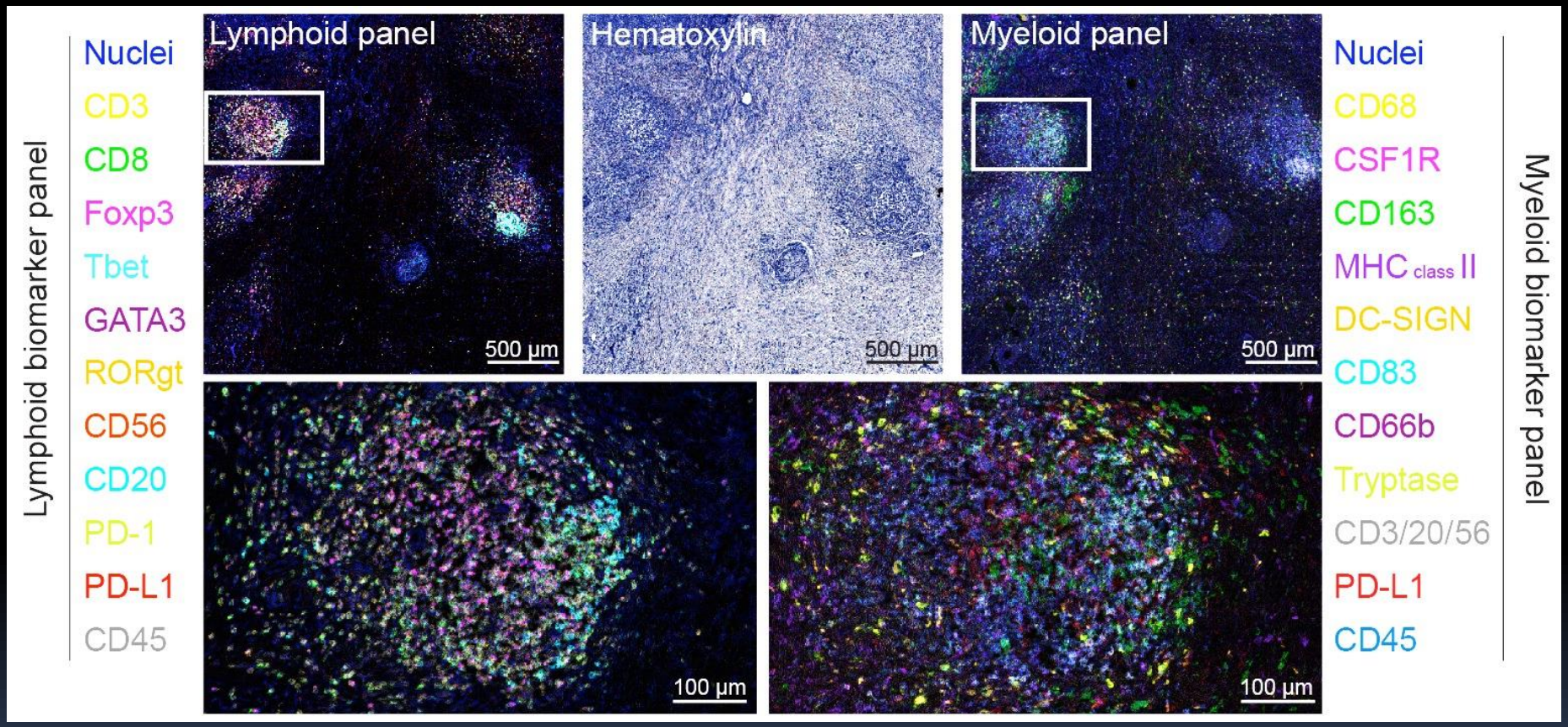
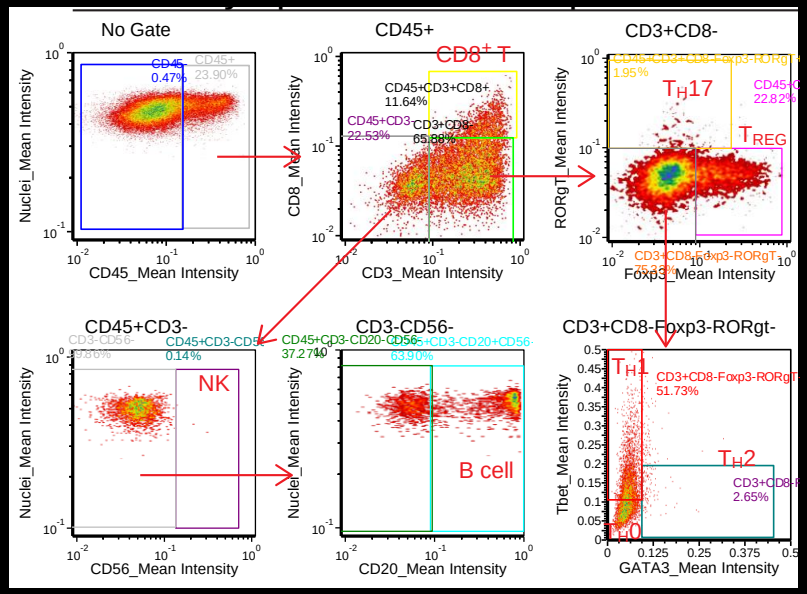
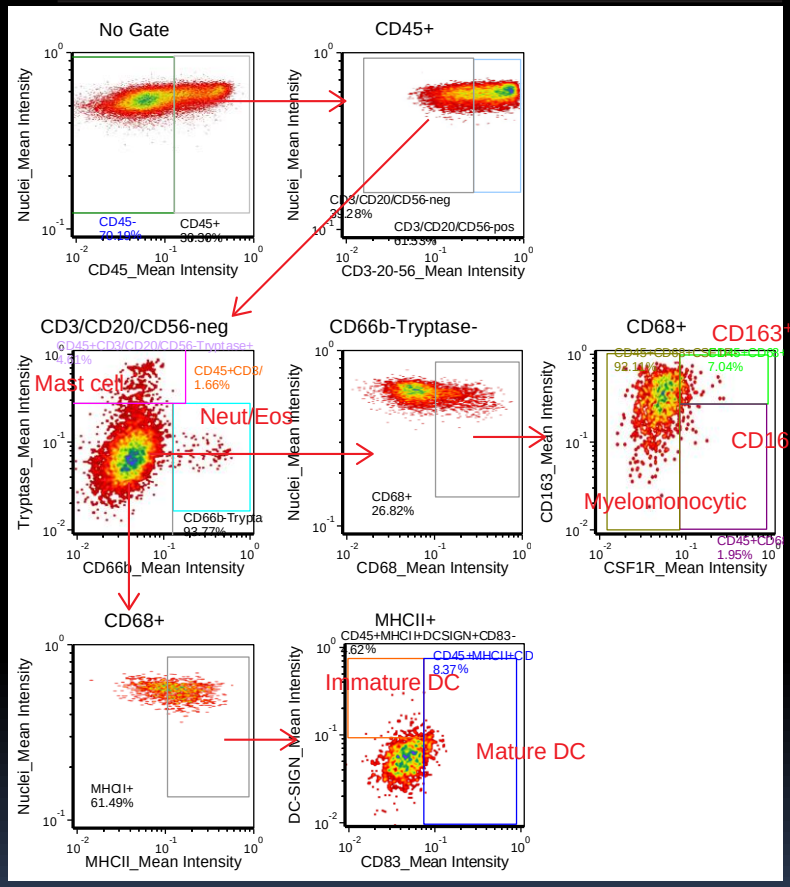


Image cytometry enables quantification of 16-different cell lineages

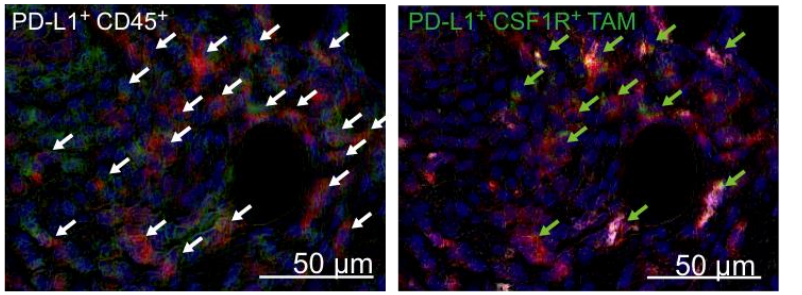




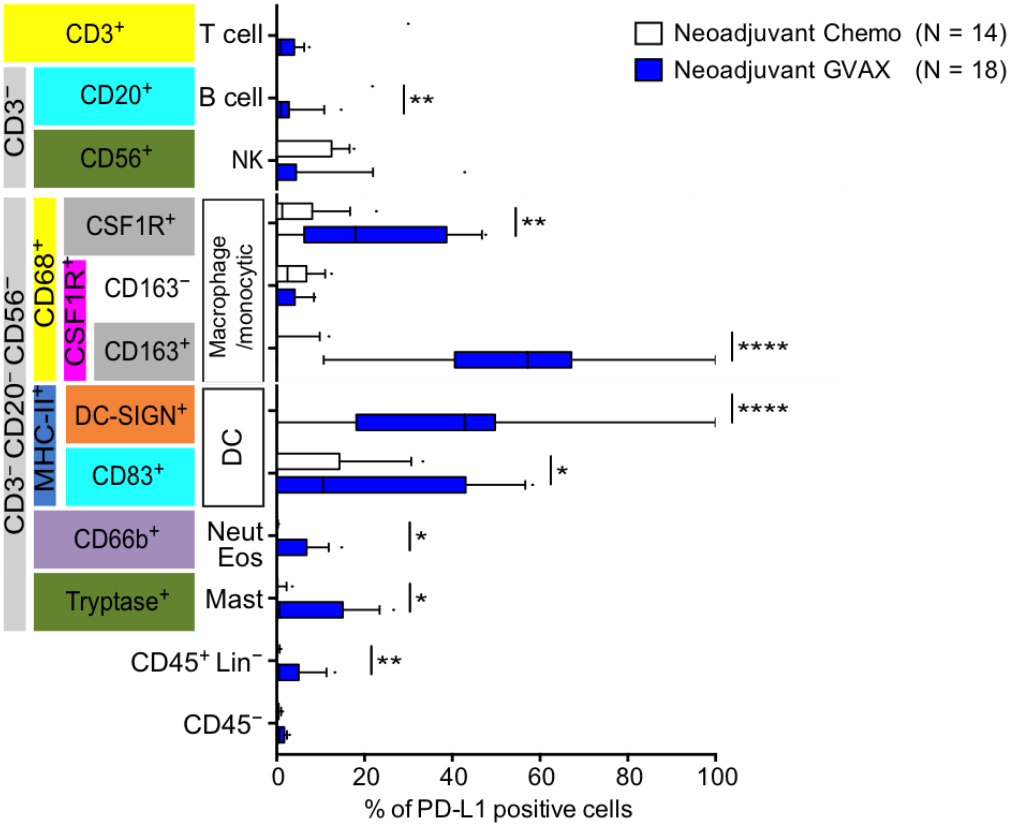
Tsujikawa T, et al. Manuscript in preparation

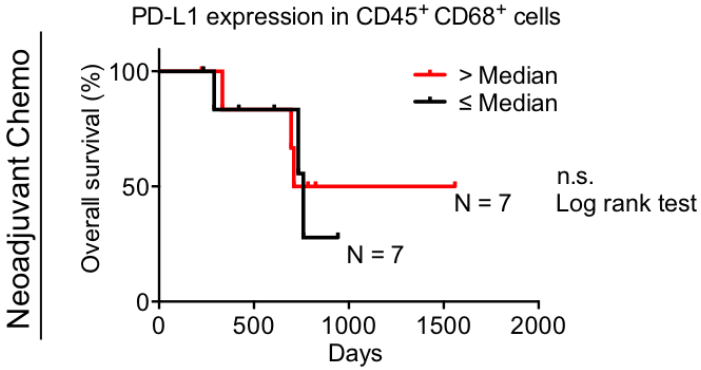
Neoadjuvant GVAX therapy is associated with PD-L1 upregulation in myeloid cell lineages, correlating with prognosis

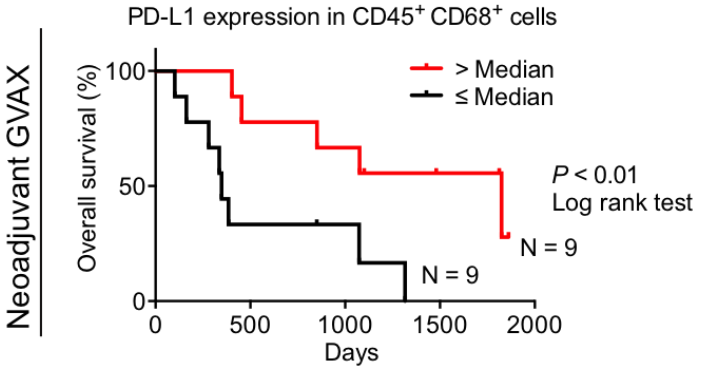
Myeloid biomarker panel



Nuclei PD-L1 CD45 Nuclei PD-L1 CSF1R CD68

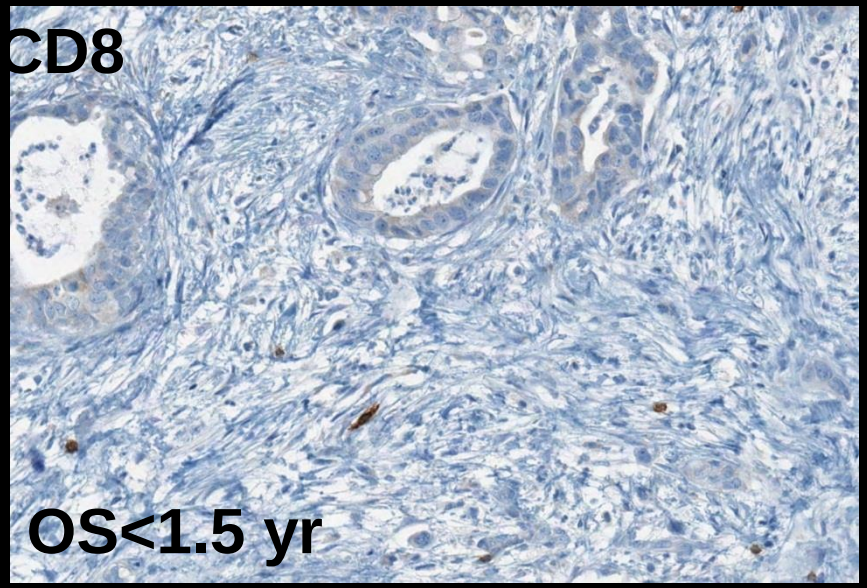


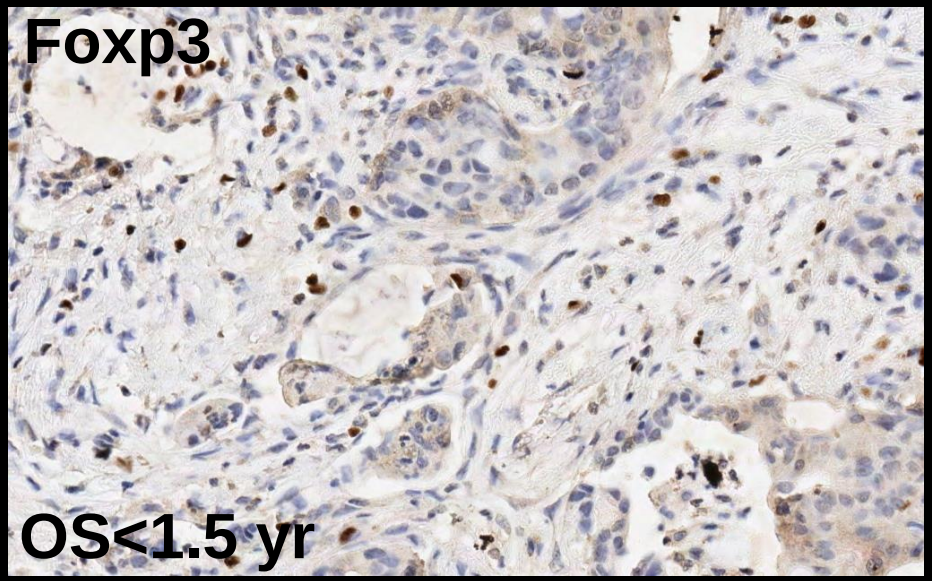


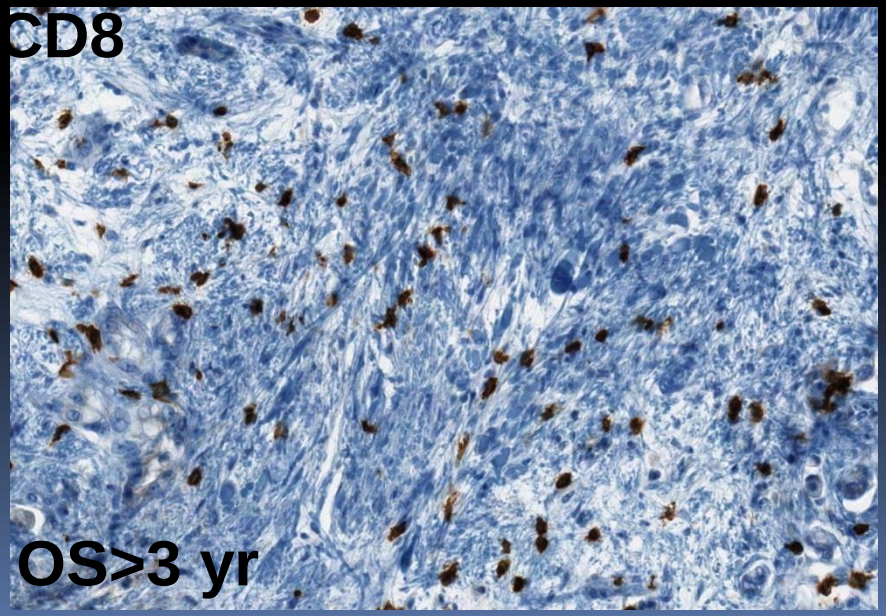


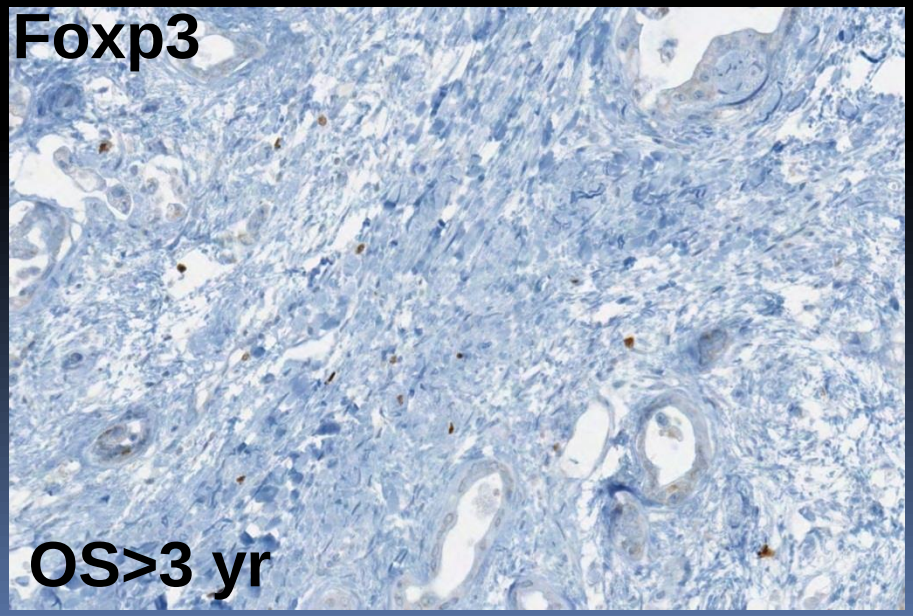
Tsujikawa T, et al. Unpublished data

T cells can be found infiltrating between lymphoid aggregates



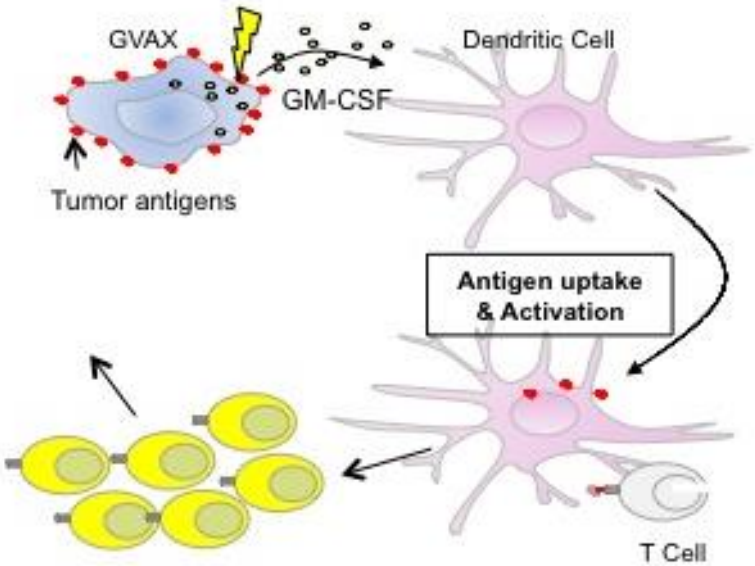






Neo-Adjuvant Study of Vaccine +/- PD-1 Blockade

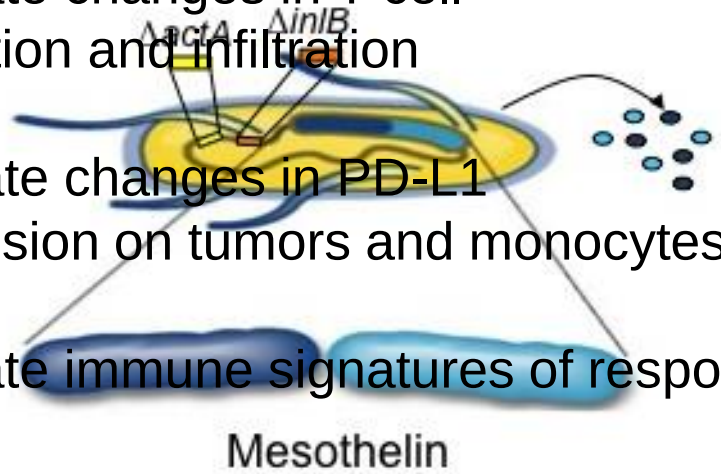
Immunotherapy Platforms
Vaccines +/- Checkpoint inhibitors



Evaluate changes in T cell Activation and infiltration

Evaluate changes in PD-L1 expression on tumors and monocytes

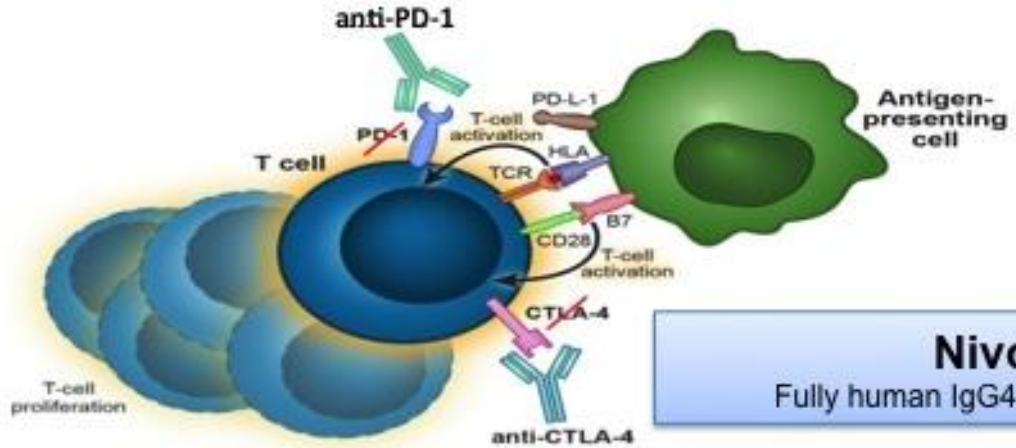
Evaluate immune signatures of response



GVAX Pancreas
Whole-cell tumor vaccine

LADD Listeria
Live-attenuated *Listeria monocytogenes*

+/-



Nivolumab
Fully human IgG4 antibody against PD-1

STAND UP TO CANCER

What are current challenges?

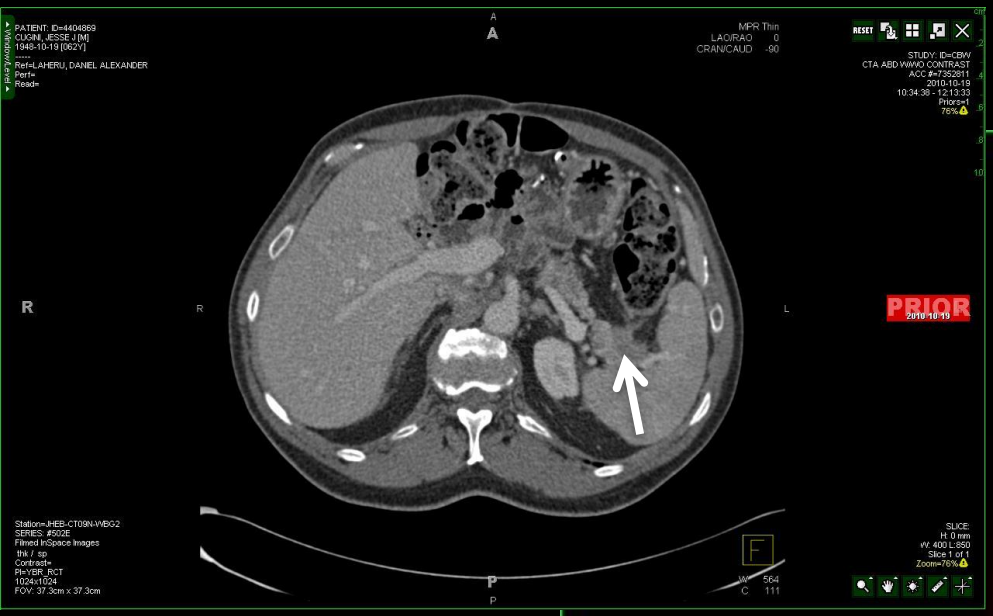
- Single agent immune modulatory agents work in 30-40% of immune responsive cancers (20% of all cancers)
- Combinations of a T cell inducing agent with immune modulators are likely needed to see responses in most other patients
- Measurable responses are often delayed by weeks to months
- Combinations of immune modulators increase efficacy but also increase toxicity
- Biomarkers and imaging techniques are needed to identify untreated patients who will respond to immunotherapy to avoid toxicity in non-responders
- Ideally biomarkers/imaging are needed to identify relevant checkpoint pathways in different patients to personalize treatment
- Biomarkers/imaging are needed to identify responders during treatment since some patients require months of treatment before exhibiting a radiographic and clinical response
- Biomarkers/imaging are needed to differentiate tumor progression from inflammation
- In vivo imaging of specific pathways are needed to avoid invasive biopsies

How do we distinguish inflammation from cancer recurrence in patients being treated with vaccine and/or immune modulating agents?

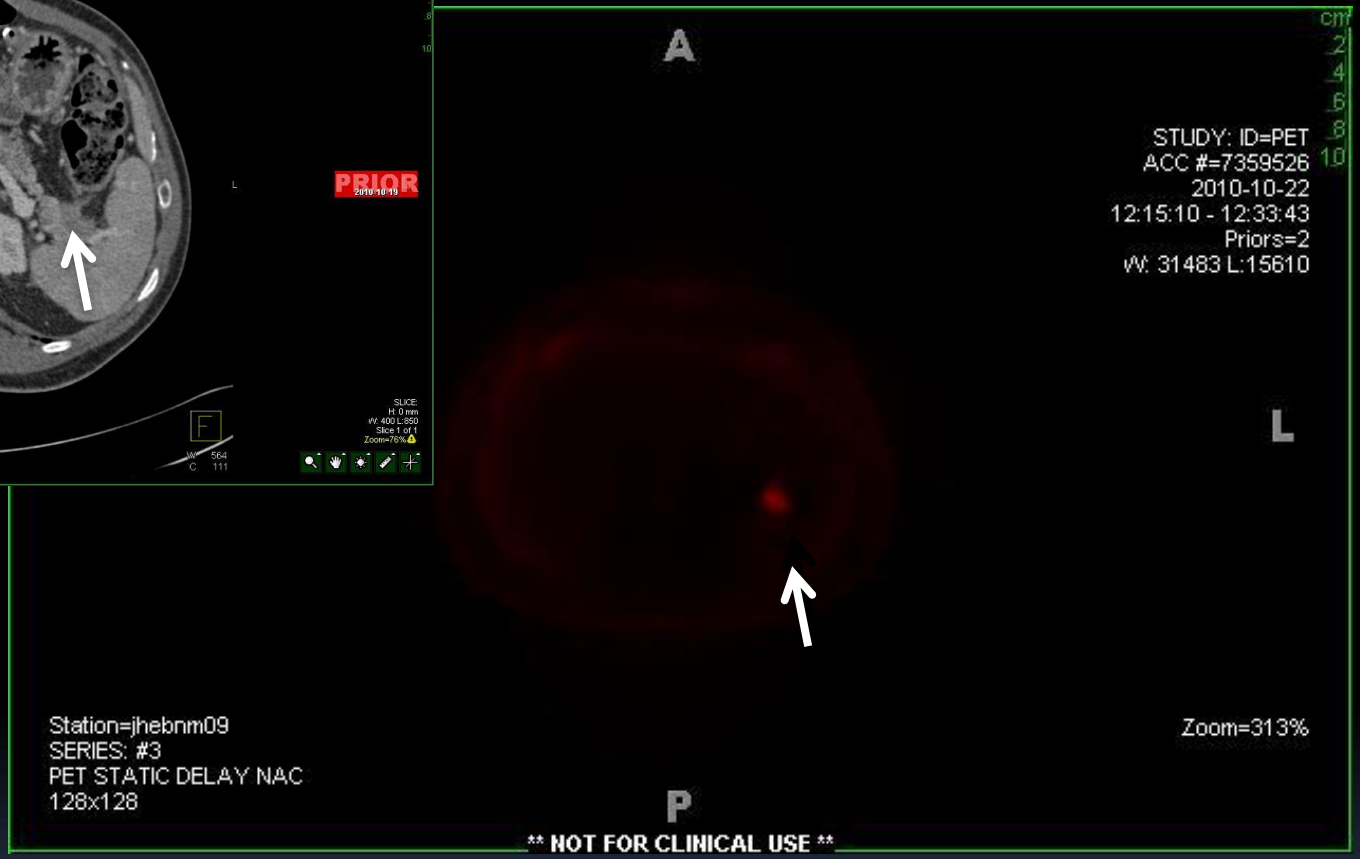
An Example

Some vaccinated patients demonstrate recurrent inflammatory reactions not associated with tumor recurrence

- First subject to complete neo-adjuvant and 4 adjuvant vaccines went on to long-term follow–up/boost study
- Boost given every 6 months
- Patient received 1st boost without problems
- Returns for 2nd boost (now at about 21/2 years since diagnosis)
- Patient feels great, no lab abnormalities
- Routine CAT scan evaluation for recurrence shows new mass in tail of pancreas

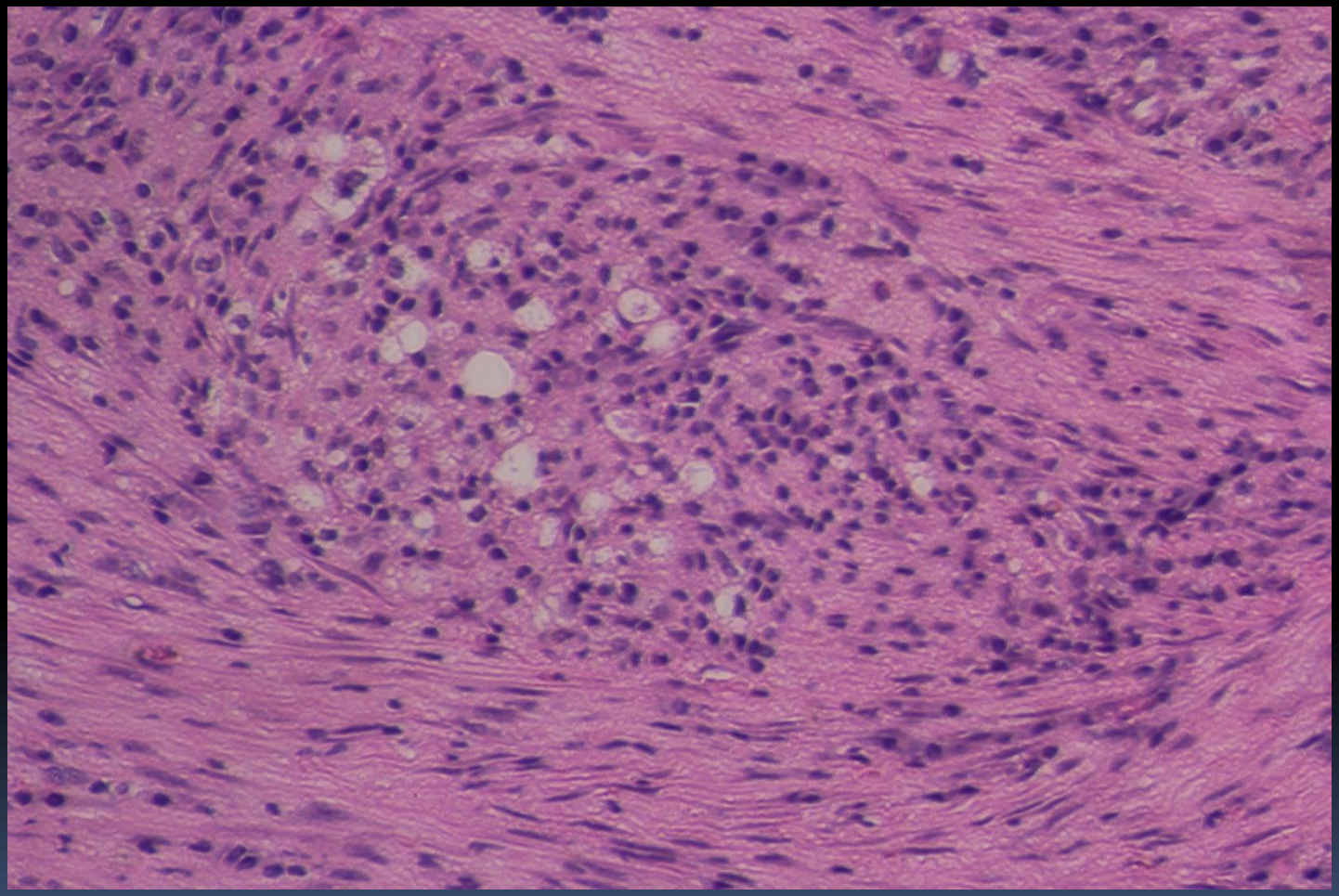


CT Scan



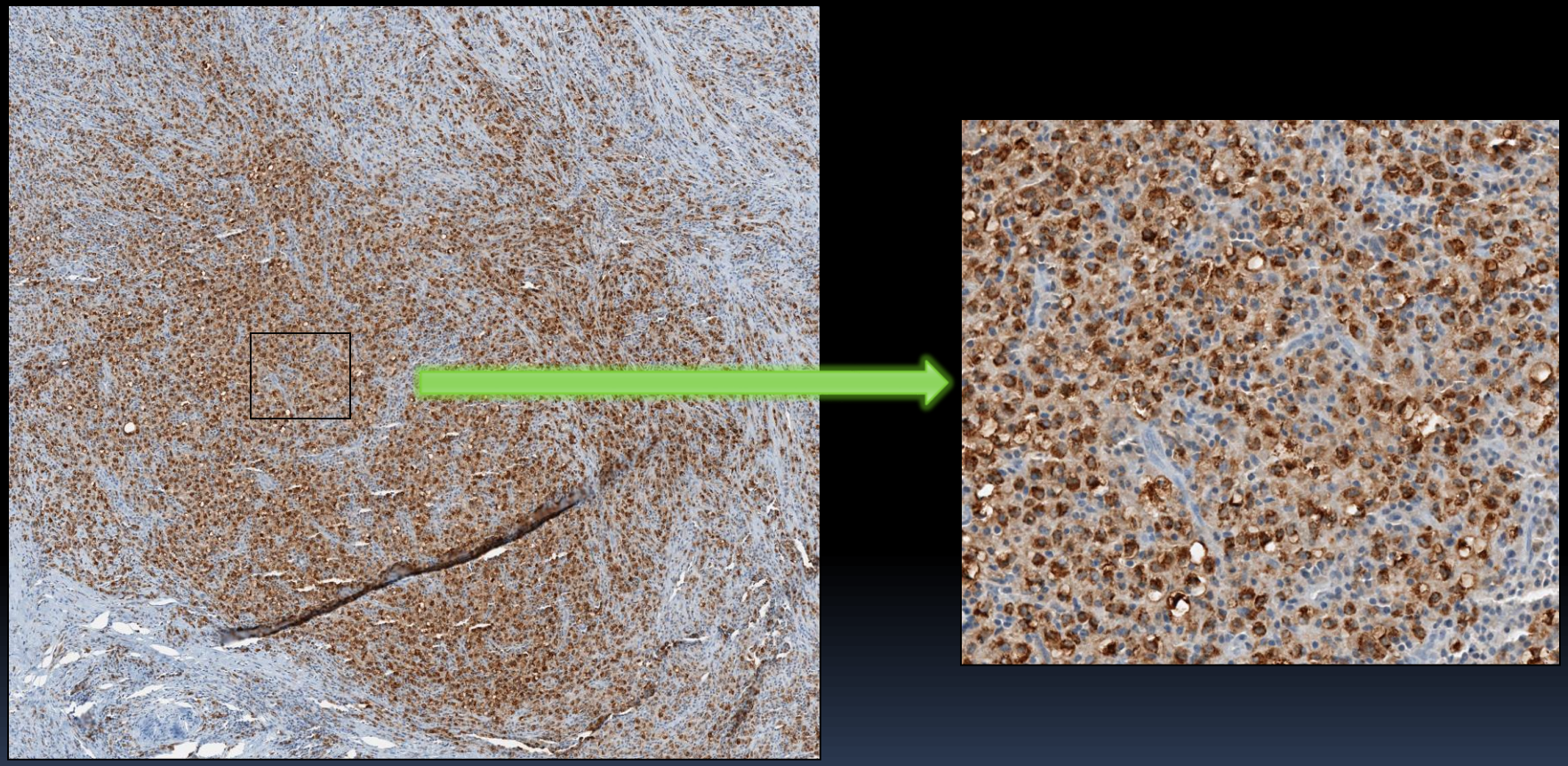
PET SCAN

Resected Lesion: H&E 20X



Chronic Inflammation – no tumor!

Predominantly macrophages



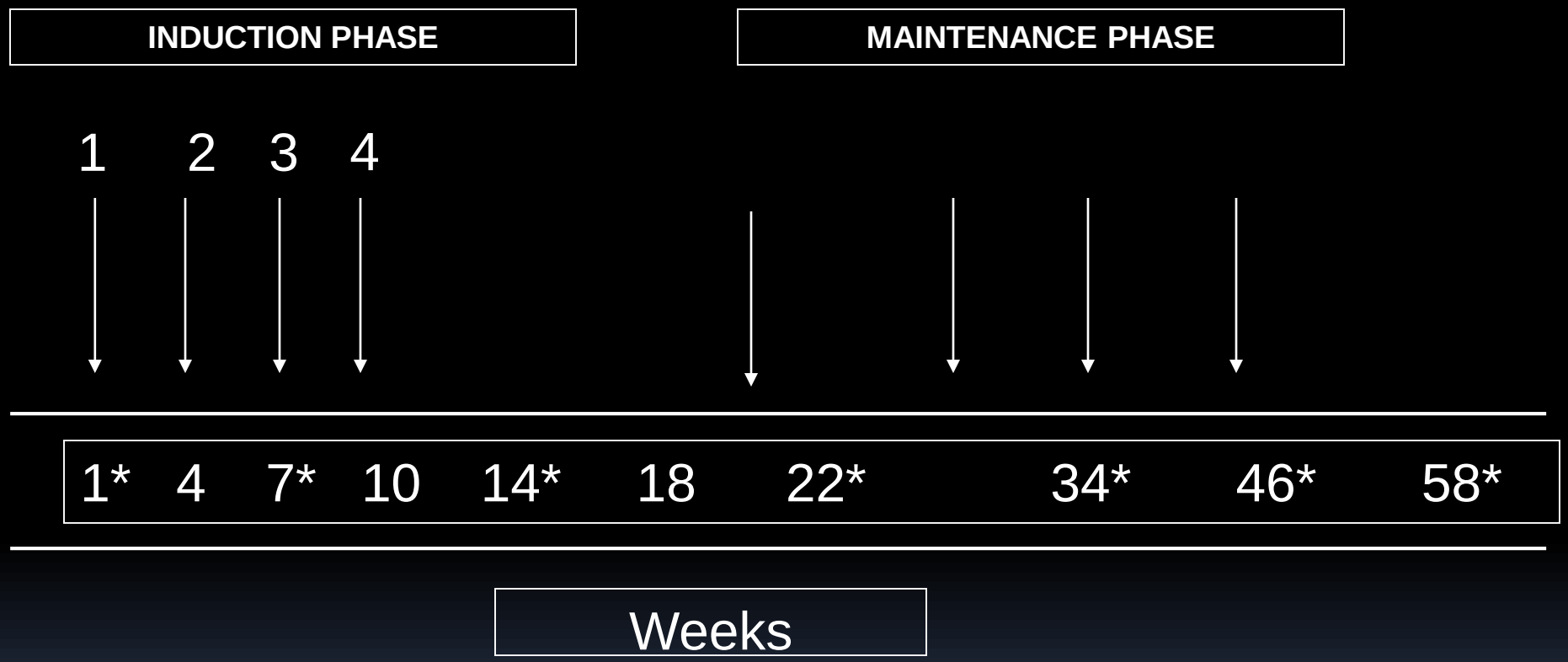
IHC using antiCD68

Pancreatic cancer patients can respond to vaccine + immune checkpoint inhibitors but take up to 6 months and often appear to progress before they regress

A Few Examples

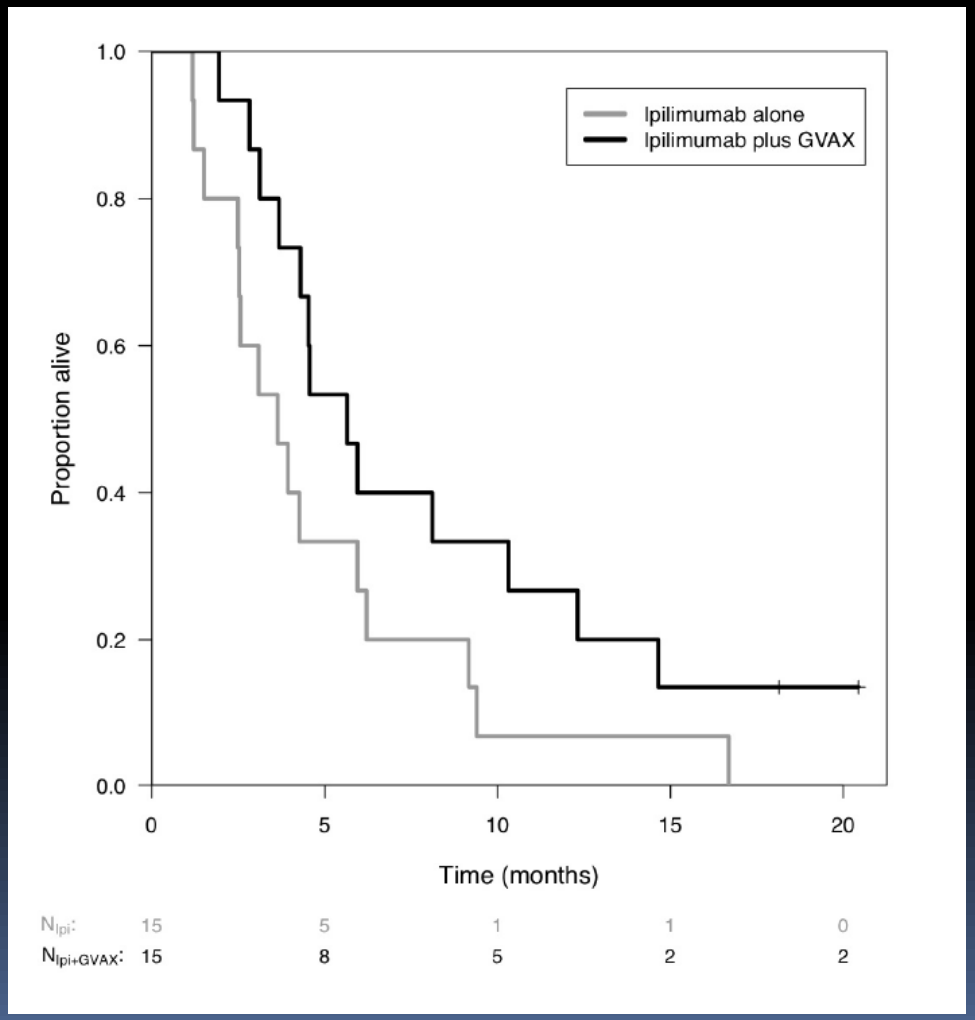
Phase Ib: Ipilimumab 10 mg/kg Alone or Ipi + Vaccine

Le, et al., J Immunother 2013



- **Vaccine** = 2.5 x 10^8 Panc 6.03 + 2.5 x 10^8 Panc 10.05 tumor cells
- *Tumor assessments (TA)
- Maintenance Phase Dosing And/Or TA q 12 weeks if SD or better at Week 22

Survival Favors GVAX + Ipilimumab Over Ipilimumab



Median OS:

3.6 vs. 5.7 Months

HR: 0.51 (0.23 to 1.08), p = 0.0723

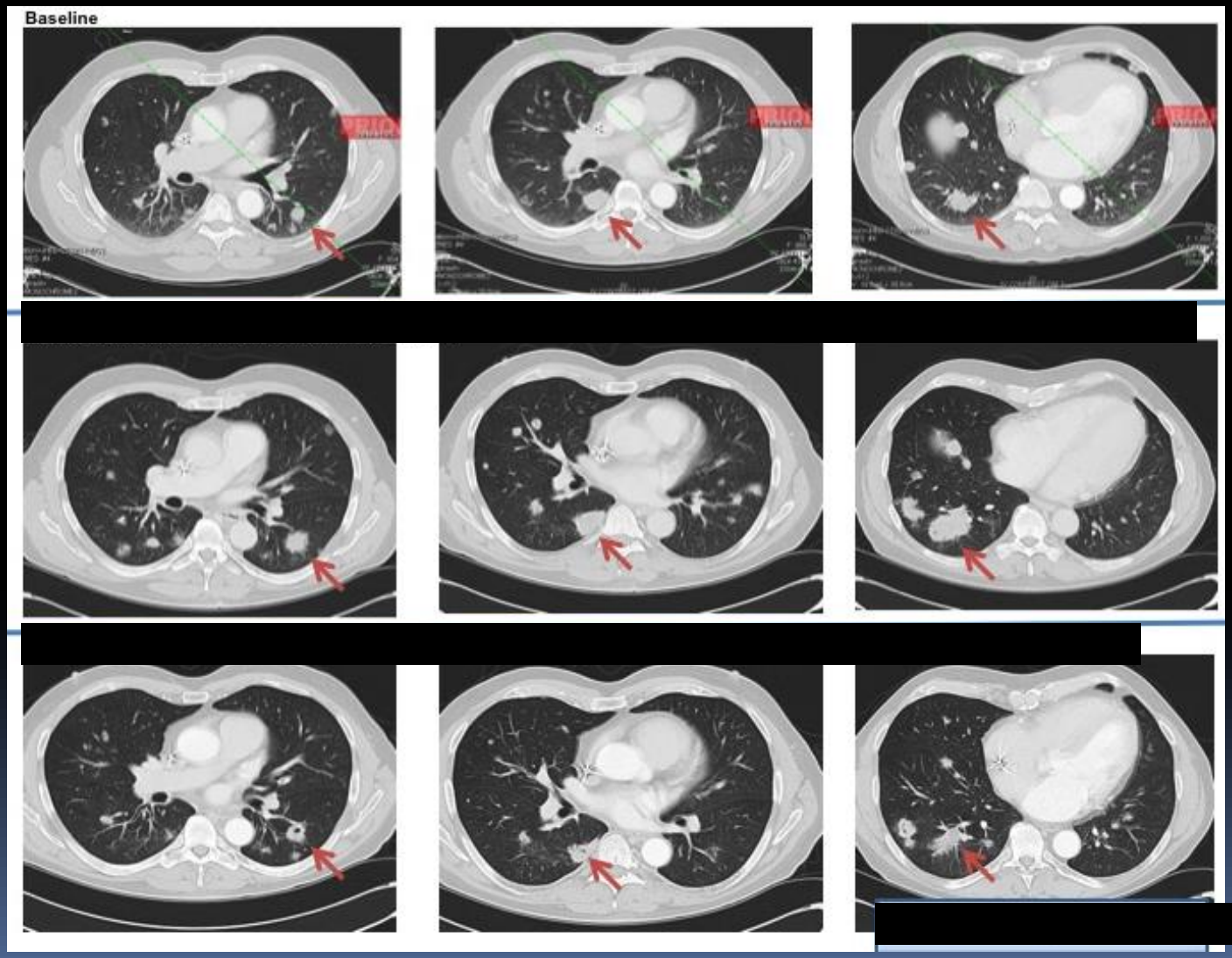
1 year OS: 7% vs. 27%

Radiographic Regressions After 14 Weeks Of Treatment with Ipilimumab (Ipi) + Vaccine

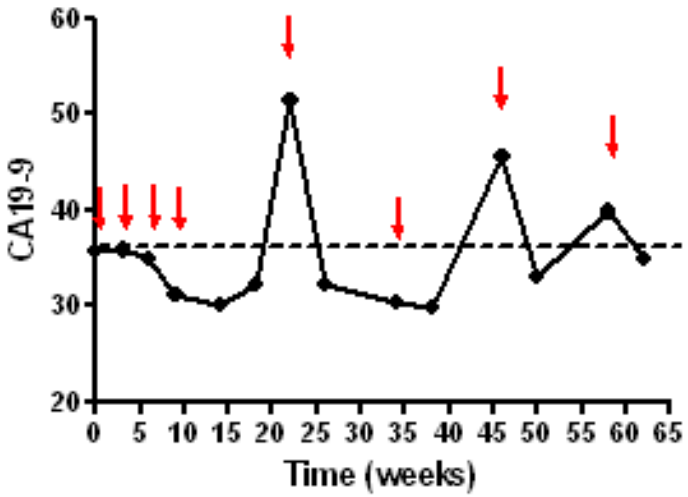
Baseline

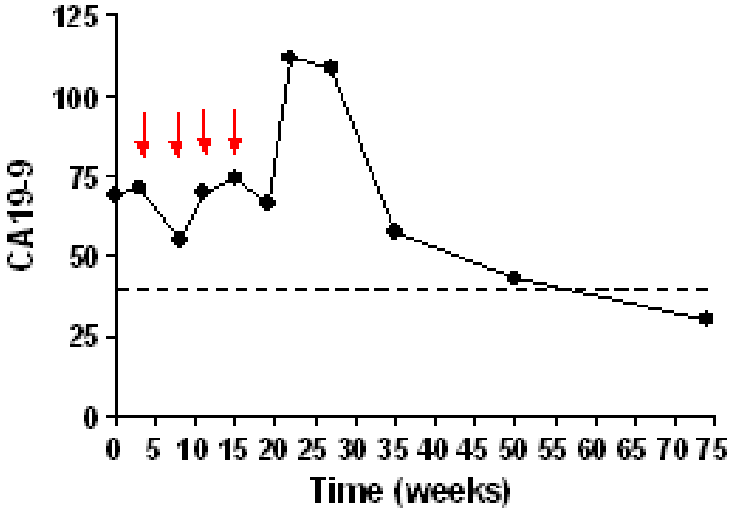
Week 7 Ipi/Vaccine

Week 14 Ipi/Vaccine



Tumor Marker Kinetics

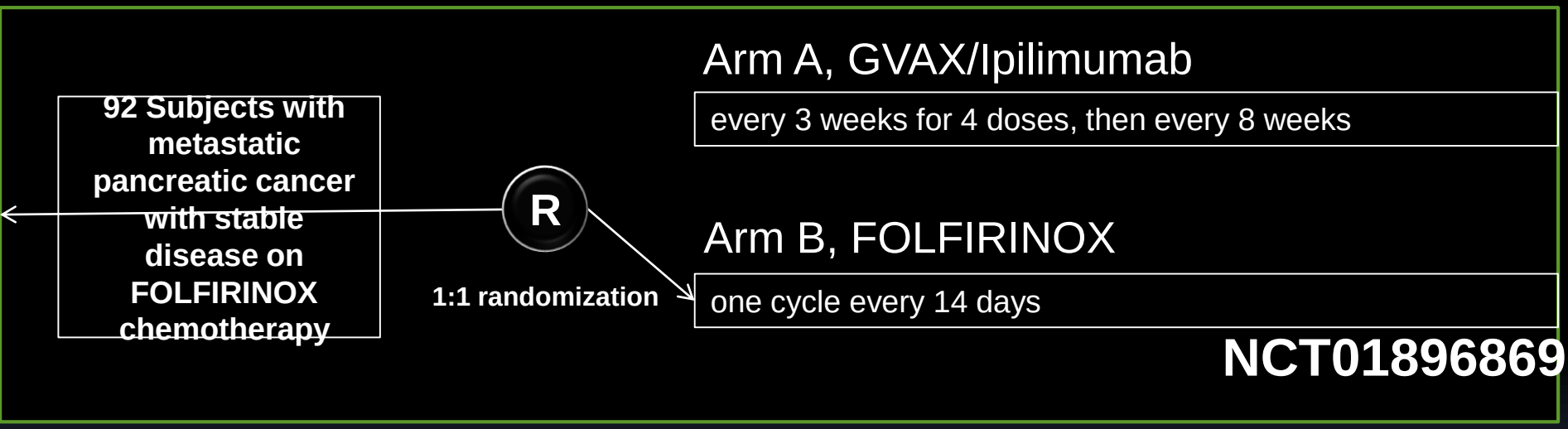




Arrows: Treatments with GVAX + Ipilimumab

GVAX/Ipi Frontline Maintenance Study

GVAX Pancreas + Ipilimumab vs. FOLFIRINOX



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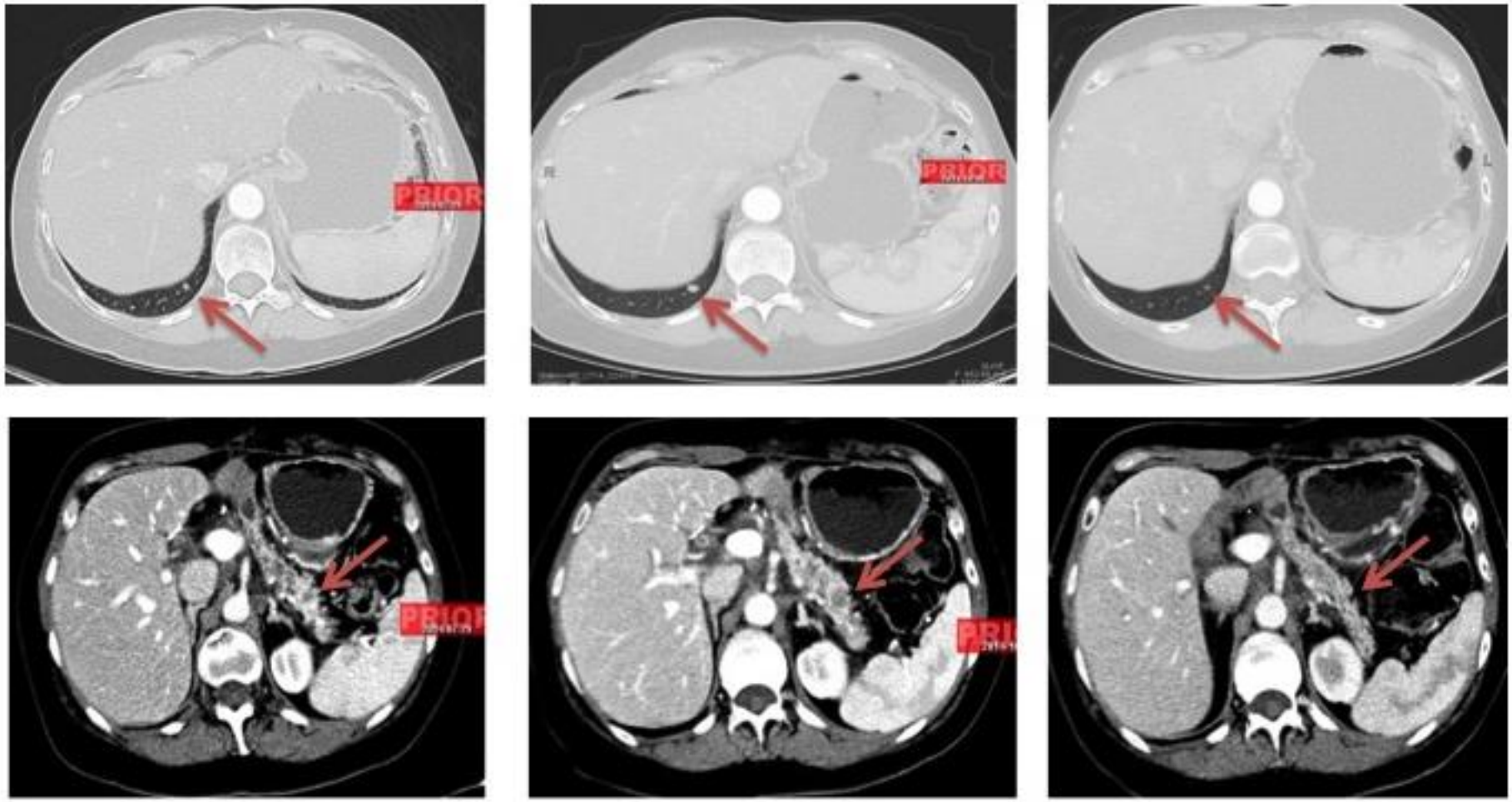
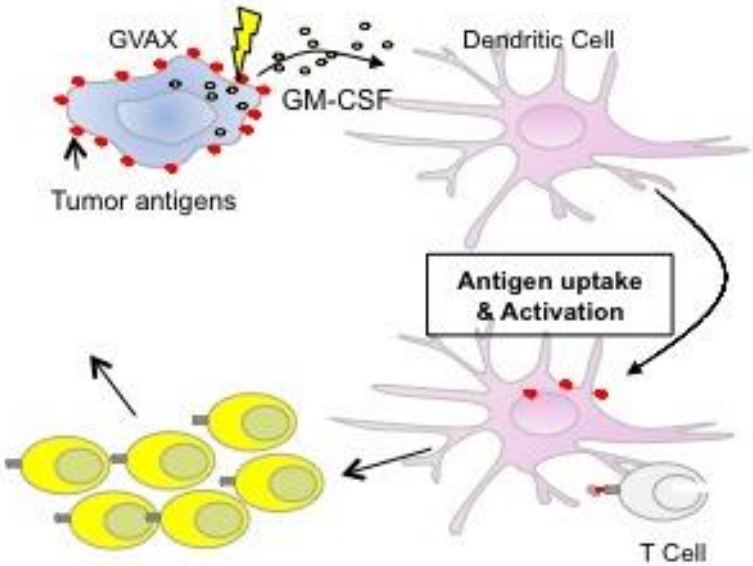
GVAX/Ipi Frontline Maintenance Study

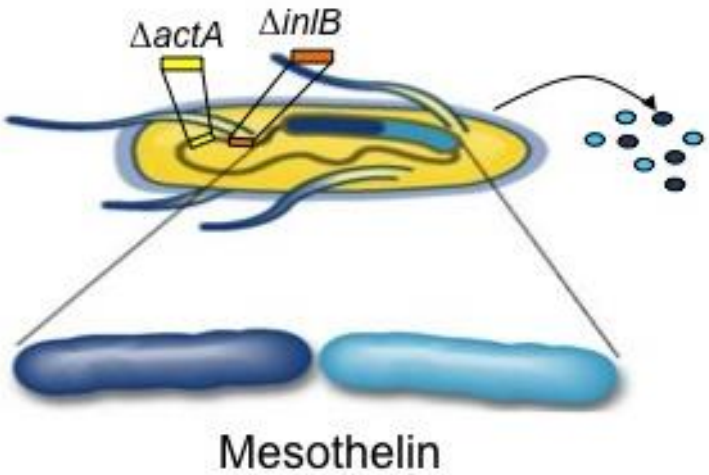
Delayed Response

BASELINE | **WEEK 10** | **WEEK 18**



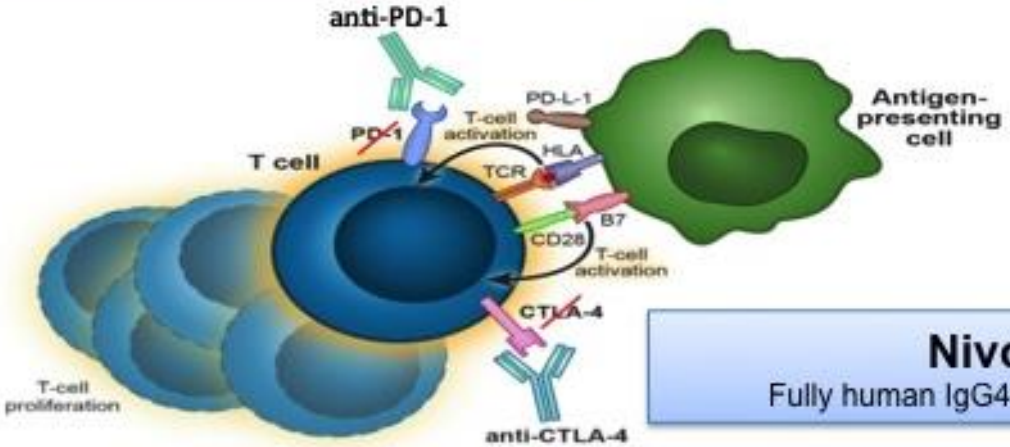
Immunotherapy Platforms
Vaccines +/- Checkpoint inhibitors





GVAX Pancreas
Whole-cell tumor vaccine

LADD Listeria
Live-attenuated *Listeria monocytogenes*

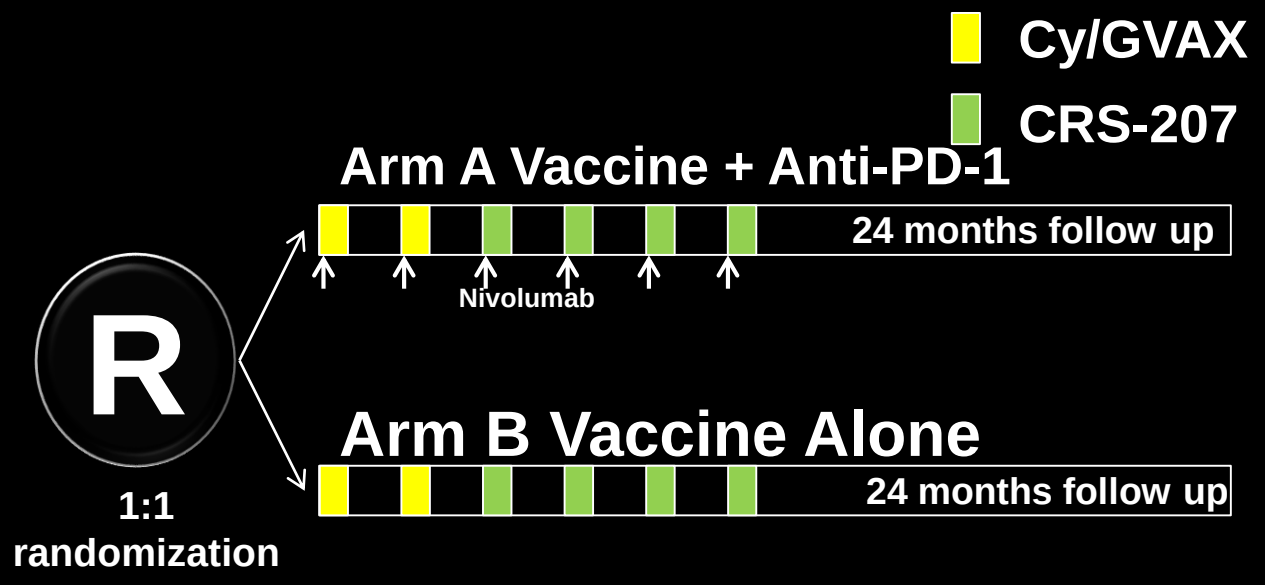


Nivolumab
Fully human IgG4 antibody against PD-1

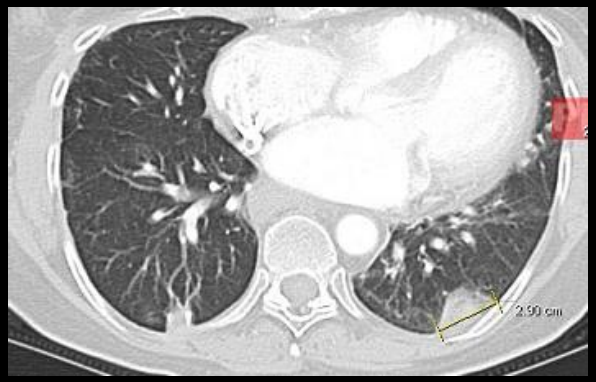
STAND UP TO CANCER

GVAX + CRS-207 Heterologous Prime Boost Vaccination with Programmed Death-1 (PD-1) Blockade

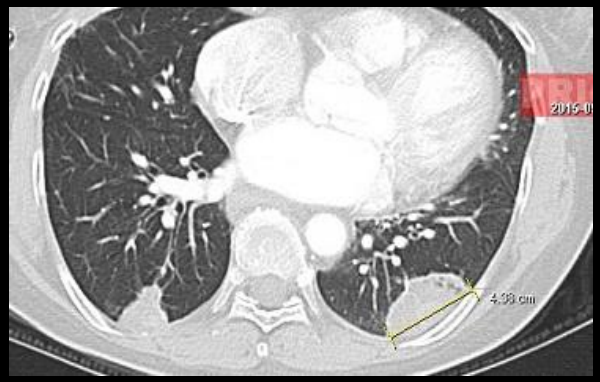
Patients with metastatic pancreatic cancer; progresssing after 1 prior chemotherapy for metastatic disease



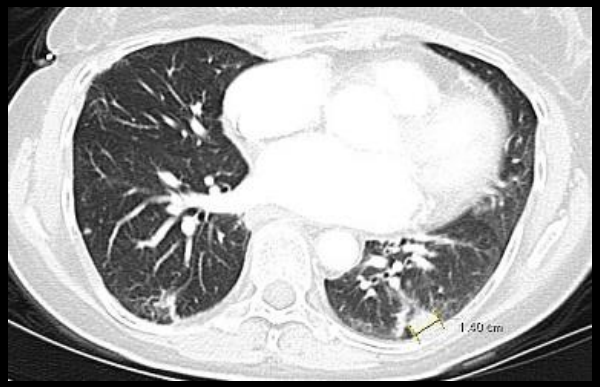
GVAX + CRS-207 Heterologous Prime Boost Vaccination with Programmed Death-1 (PD-1) Blockade



Baseline

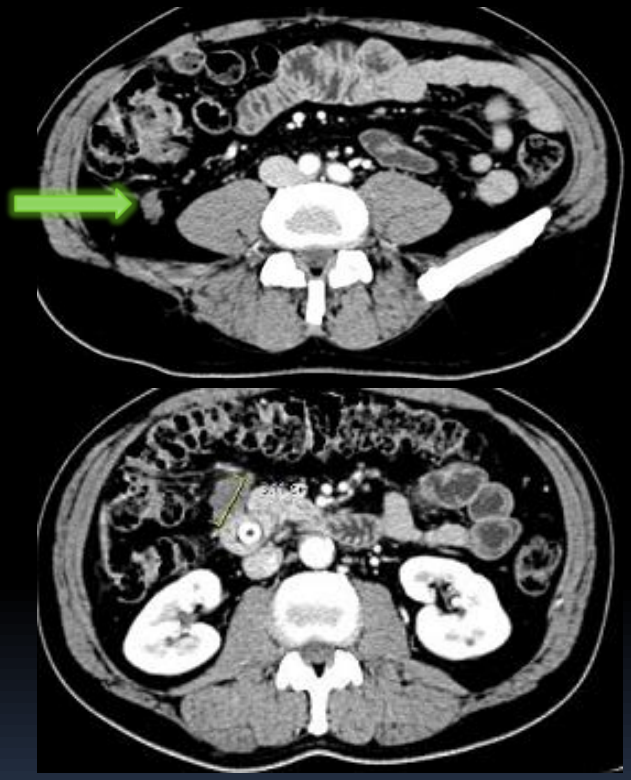


Week 10

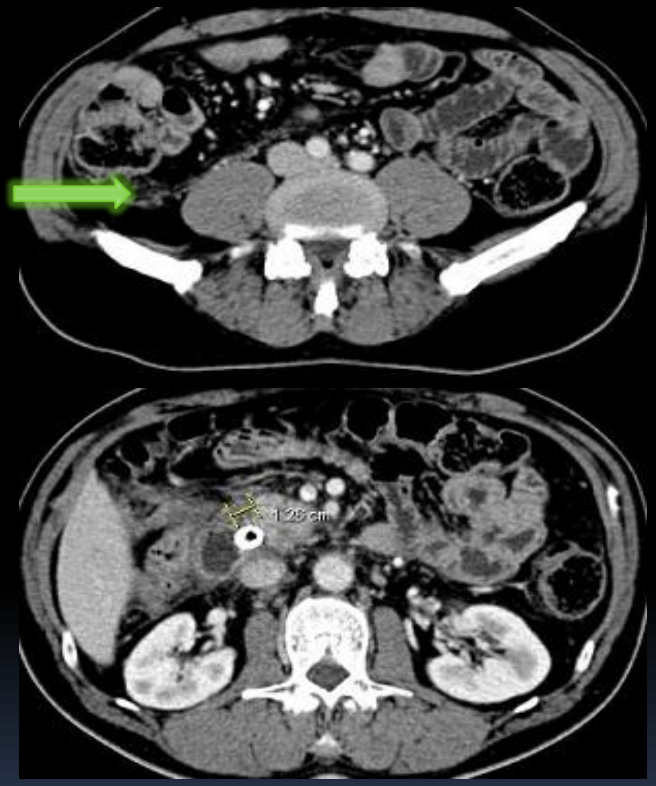


Week 30

GVAX + CRS-207 Heterologous Prime Boost Vaccination with Programmed Death-1 (PD-1) Blockade



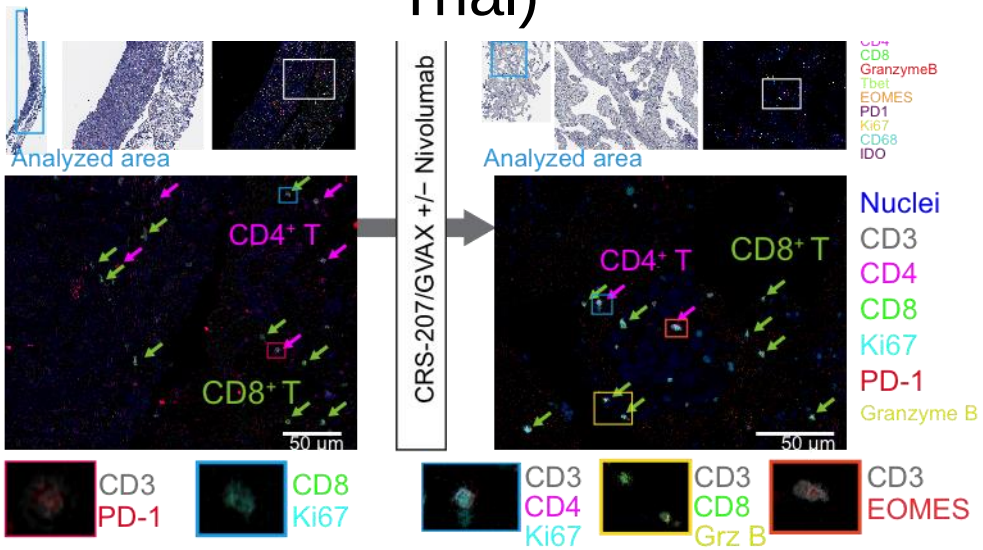
Baseline

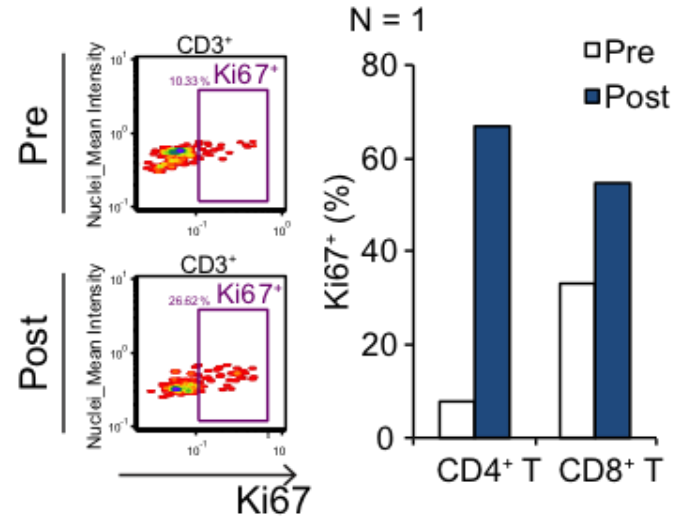


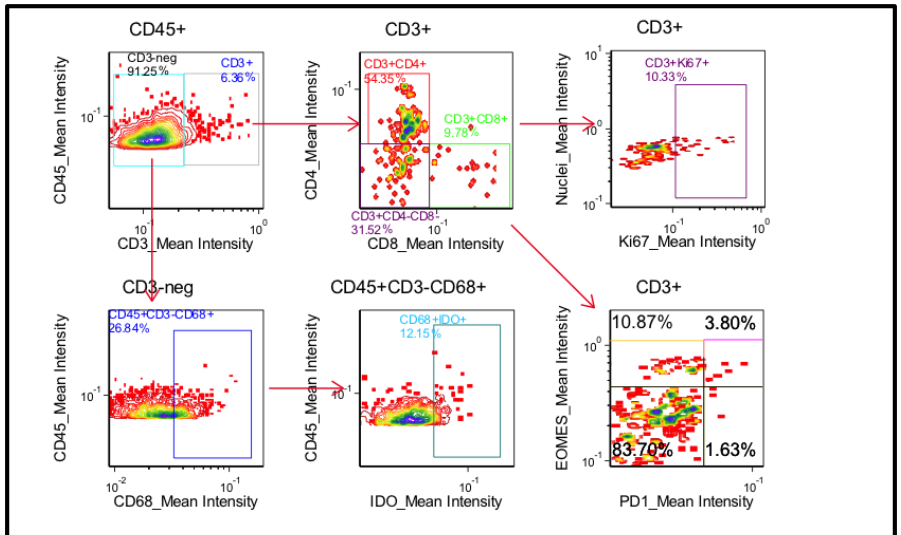
Week 30

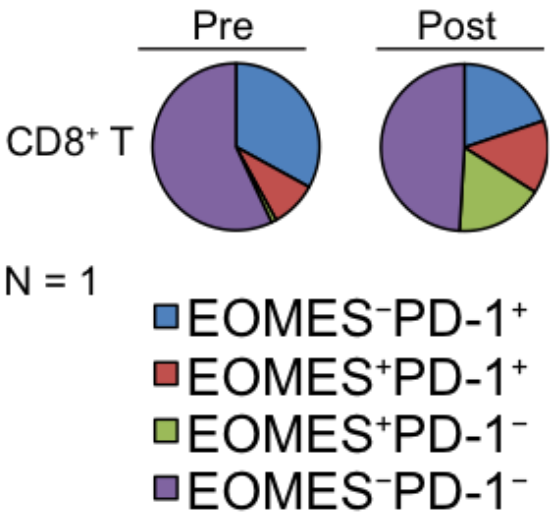
Multiplex IHC depicts evidence of T cell reinvigoration with GVAX/CRS207 + nivolumab

- Biopsy specimen (STELLAR Trial)









Tsujikawa T, et al. Unpublished data

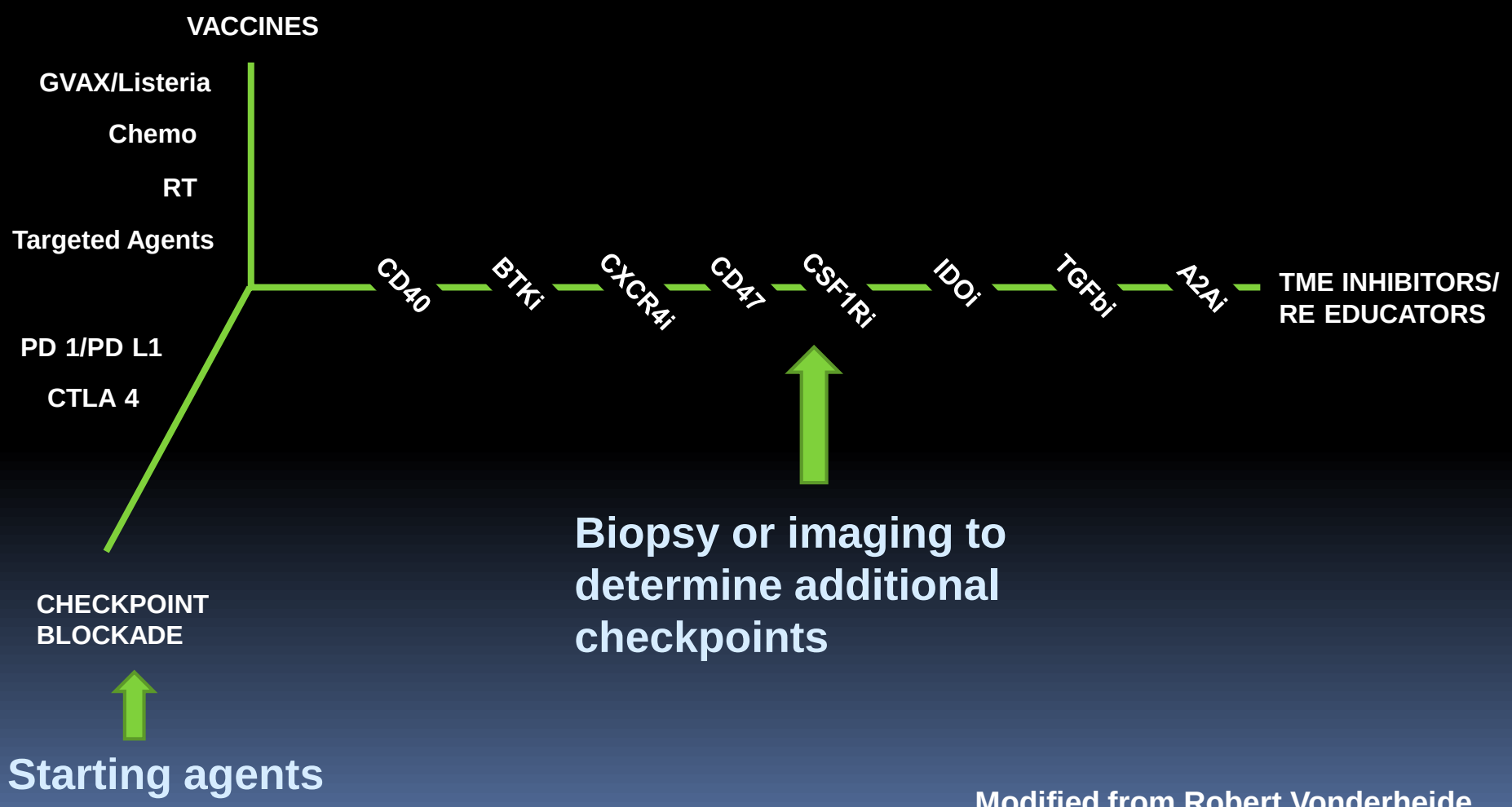
What more do we need to learn to effectively treat pancreatic cancers?

- What are the optimal combinations of immune modulators required to induce the most effective and durable immune response?
- Does every patient with a pancreatic cancer have the same immune checkpoint pathways inhibiting immune recognition of their tumors?
- Do patients who respond to inhibitors of PD-1/PD-L1 or CTLA-4 blockade eventually develop immune resistance?
- Are there other T cell regulatory pathways in pancreatic cancers that are inhibiting effective anticancer immunity?

In the future we will likely use repetitive biopsies to personalize each patients combinations

However in vivo imaging would provide a less invasive approach to identify combinations of immune modulators and also determine additional modulators needed over time

Personalizing Immunotherapy to each Patient's TME



Modified from Robert Vonderheide

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SU2C AACR Lustgarten DREAM TEAM
Aduro Biotech PANCAN AACR

Modernizing Tumor Response Assessment

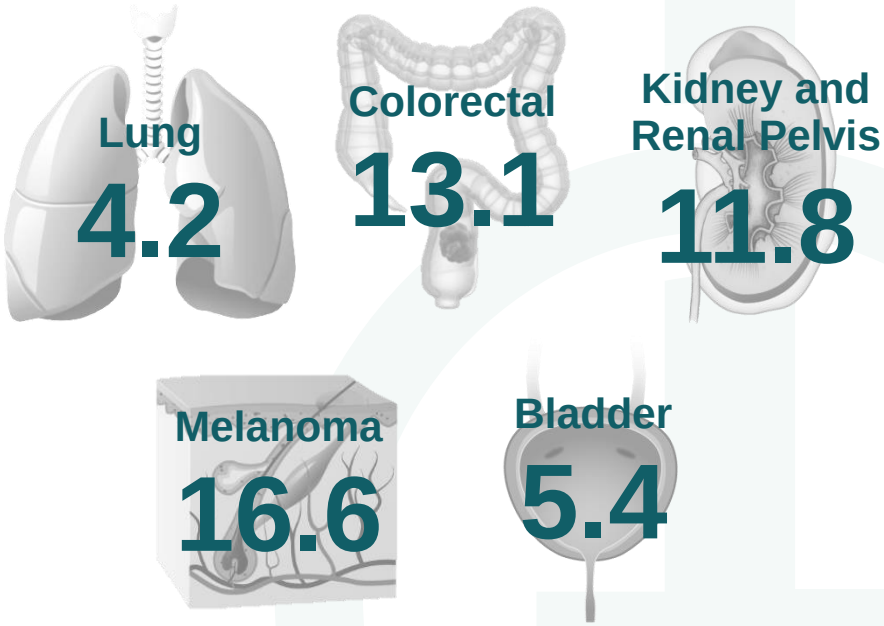
National Cancer Institute Workshop
Immune Modulation Therapy and Imaging:
What can we do now in clinical trials?
2 May 2016

David Leung, MD, PhD
Medical Director for Oncology Imaging
Exploratory Clinical and Translational Research
Bristol-Myers Squibb

Improved Survival Remains a Challenge

5-year Survival in Advanced Cancers (%)[1]

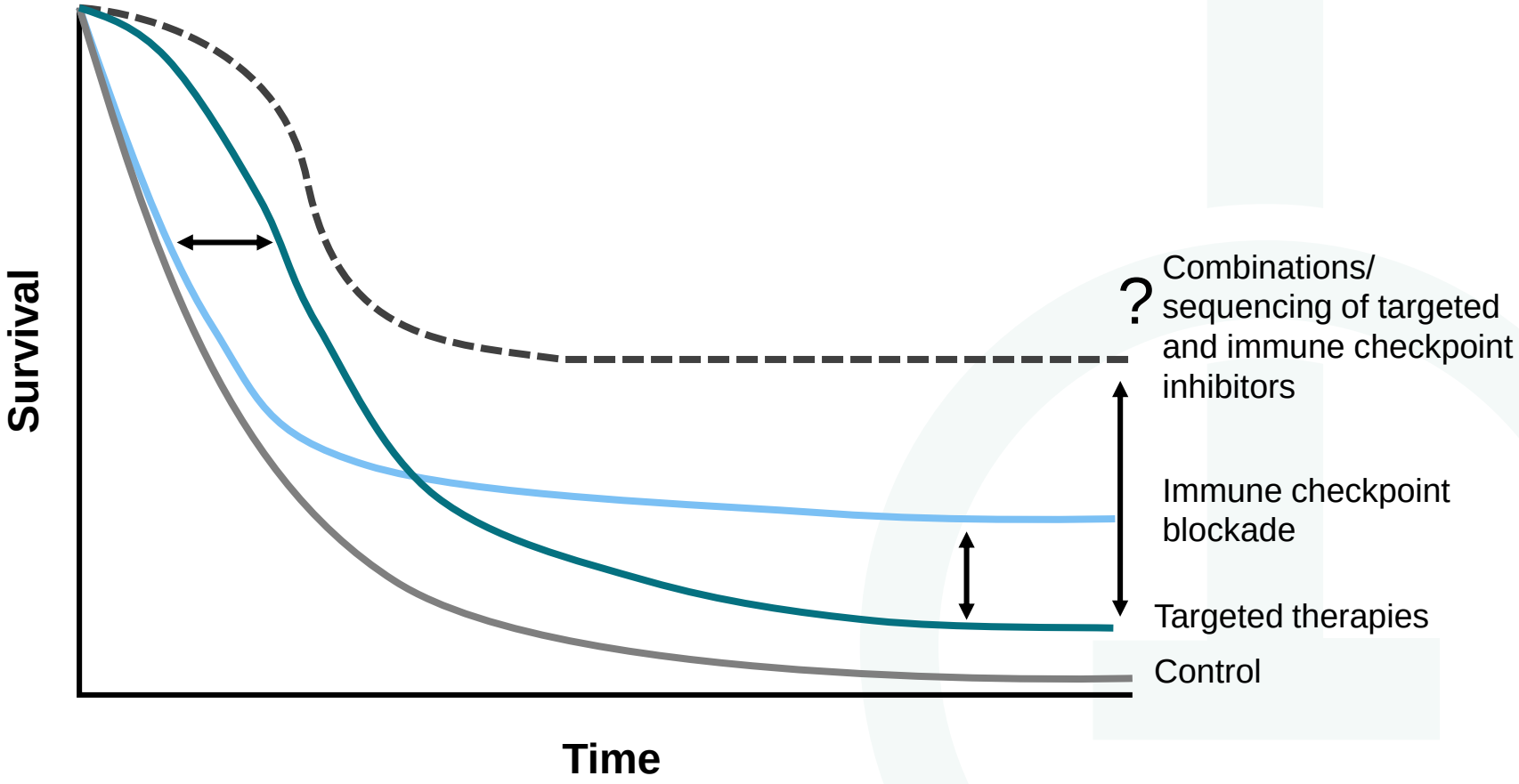
- 5-year survival remains poor for many patients with advanced metastatic solid tumors[1]
- In the US, it is estimated[2] that a total of 589,430 deaths due to cancer will occur in 2015



There is an ongoing need for new treatments and therapeutic modalities for patients with advanced cancers[3]

1. Surveillance, Epidemiology, and End Results (SEER) Stat Fact Sheets
2. Siegel RL et al. *CA Cancer J Clin.* 2015;65(1):5 29
3. Rosenberg SA. *Sci Transl Med*. 2012;4(127ps8):1 5

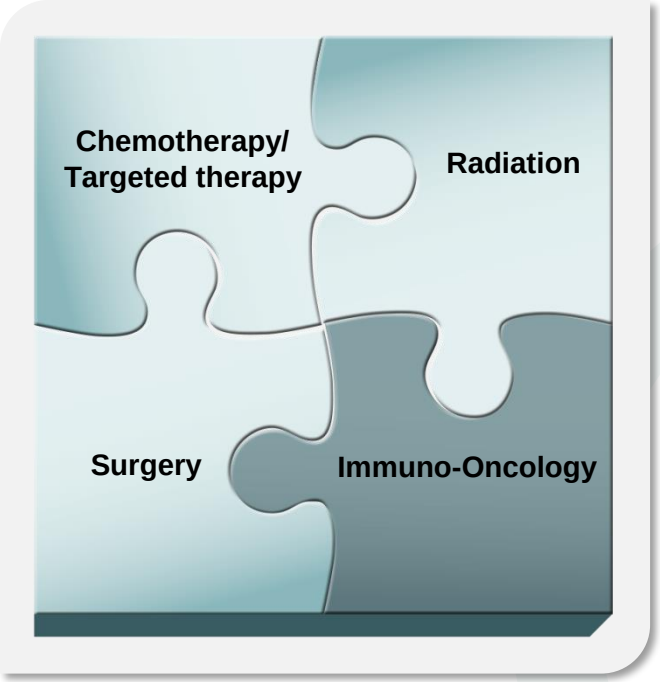
Aspirational Goals of I-O Therapies



Adapted from Sharma and Allison, Cell 2015;161(2):205 214

Immuno-Oncology is an Evolving Treatment Modality

- **Immuno-oncology is a fundamentally different approach to fighting cancer that harnesses the body's own immune system[1]**

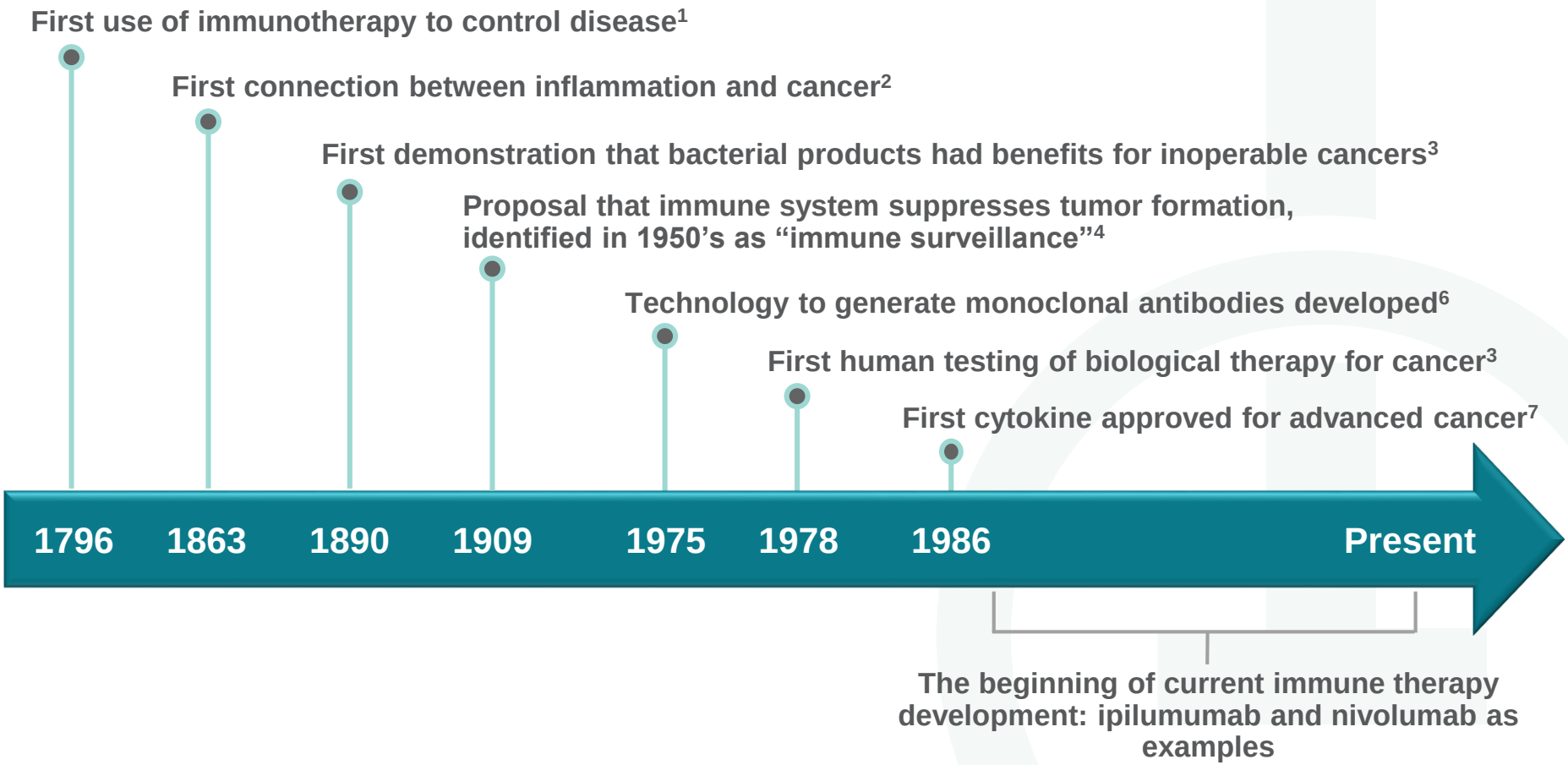


Through immuno-oncology research, therapies are being investigated in an attempt to utilize the body's own immune system to fight cancer[1 3]

1. Murphy JF. Oncology. 2010;4:67 80
2. Kirkwood JM et al. CA Cancer J Clin. 2012;62(5):309 335
3. Borghaei H et al. Eur J Pharmacol. 2009;625(1 3):41 54

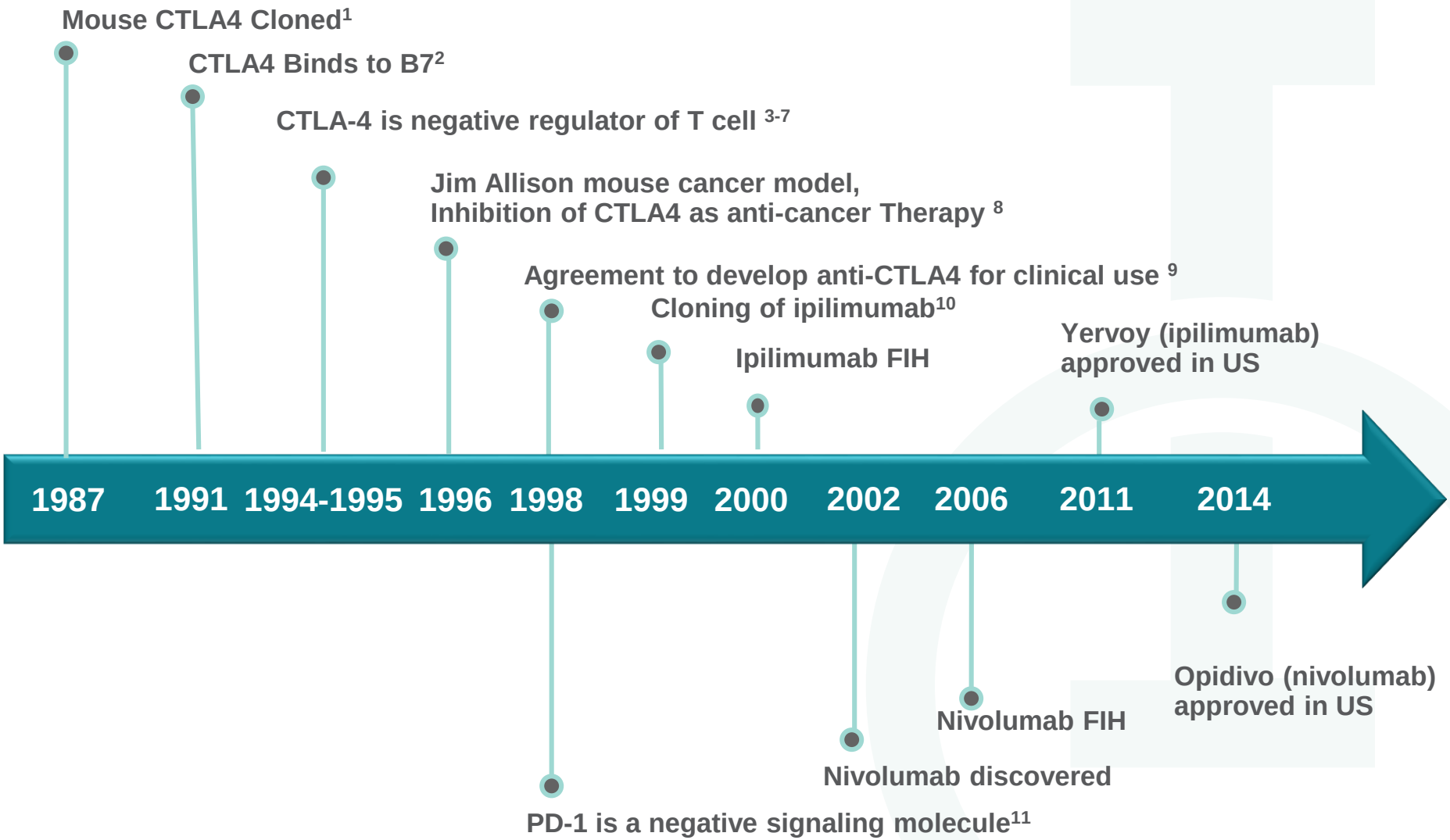
Bristol-Myers Squibb Immuno-Oncology

The Long Road in the Development of Immune Therapy for Cancer…



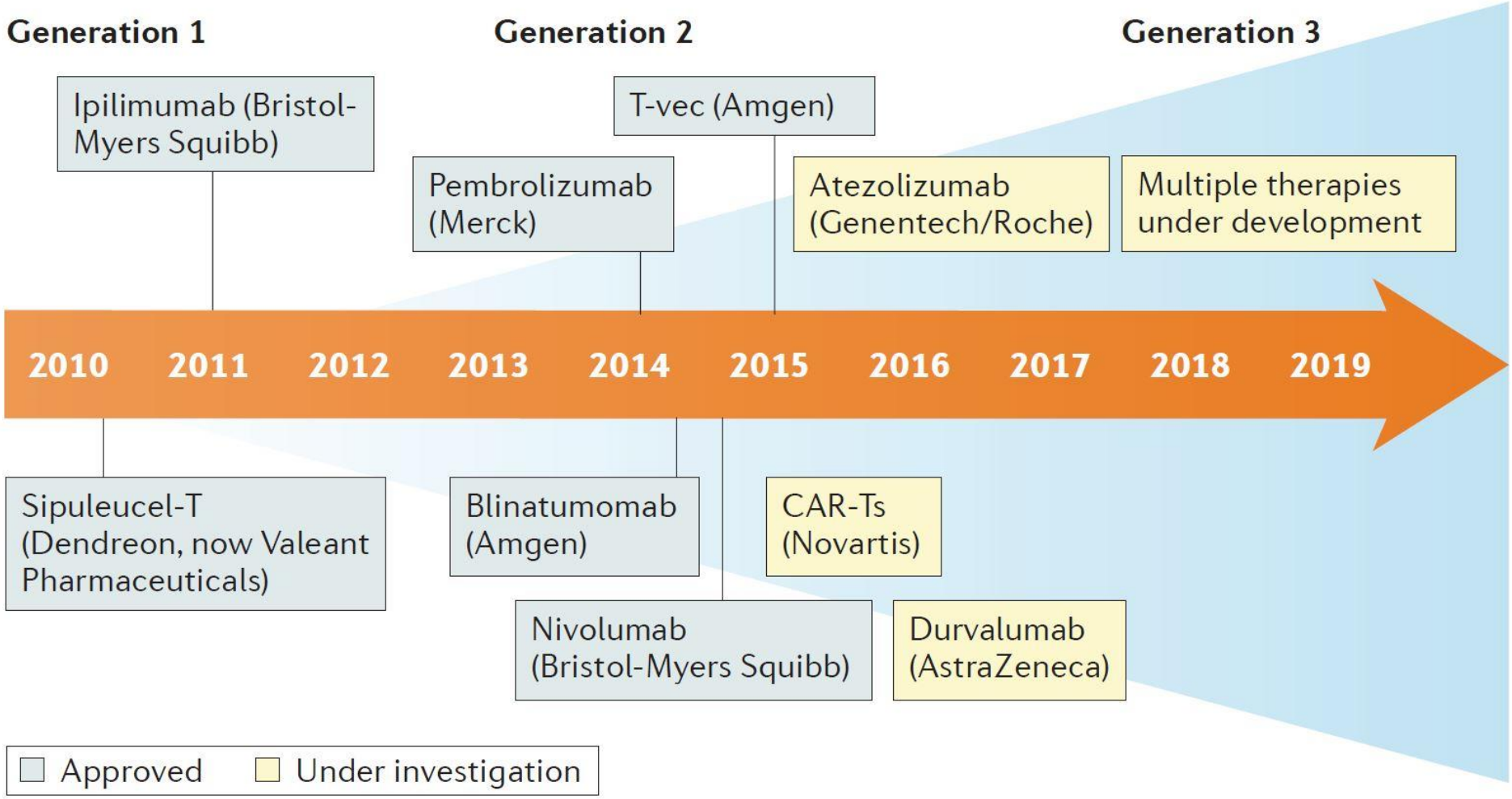
1. Murphy JF. *Oncology*. 2010;4:67-80; 2. National Cancer Institute. 150 Years of Advances Against Cancer 1860s to 1890s. www.cancer.gov/aboutnci/overview/150-years-advances. Accessed October 9. 2013; 3. Kirkwood JM, et al. *CA Cancer J Clin*. 2012;62:309-335; 4. National Cancer Institute. 150 Years of Advances Against Cancer 1900 to 1930s. www. cancer.gov/aboutnci/overview/150-years-advances. Accessed September 28, 2013; 5. Steinman RM, Cohn ZA. *J Exp Med*. 1973;137:1142-1162; 6. National Cancer Institute. 150 Years of Advances Against Cancer 1970s. www.cancer.gov/aboutnci/overview/150-years-advances. Accessed September 28, 2013; 7. Leach et al. *Science*. 1996. 8. CenterWatch. FDA Approved Drugs for Oncology. http://www.centerwatch.com/drug-information/fda-approvals/drug-areas.aspx?AreaID=12. Accessed January 20, 2015

The Discovery of Ipilimumab and Nivolumab



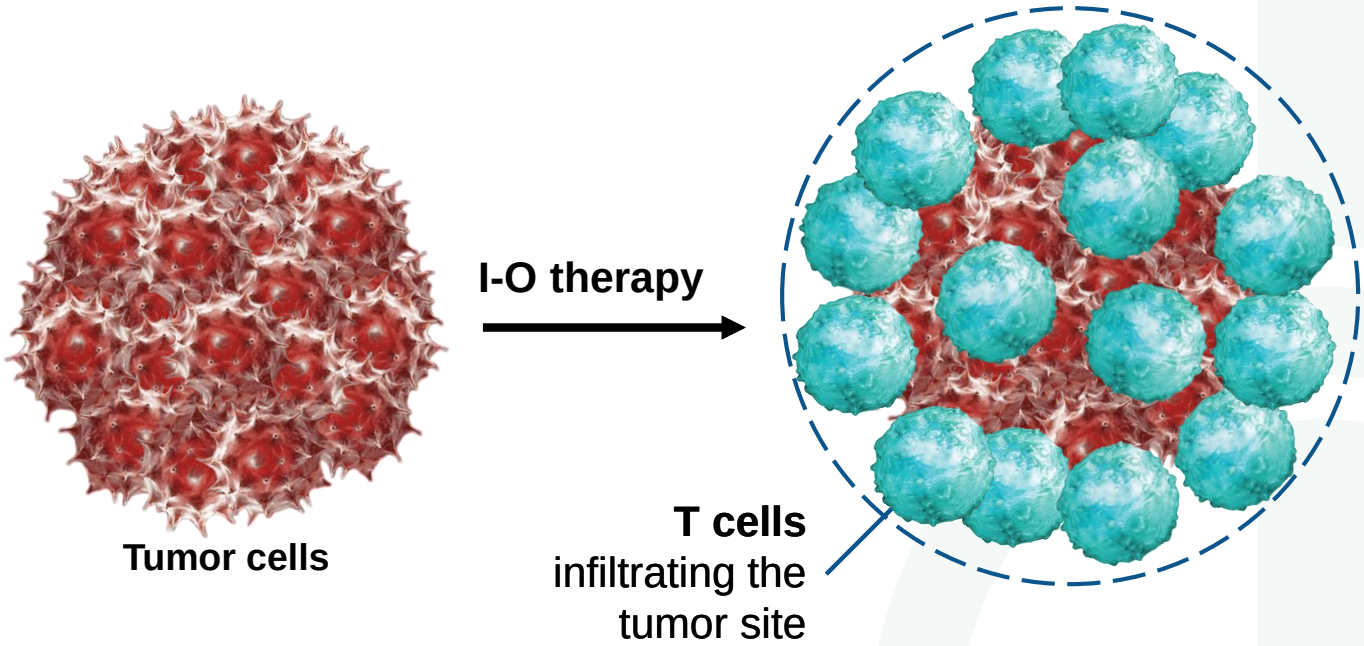
1. Brunet et al (INSERM, Marseille) Nature. 2. Linsely et al (BMS Seattle) JEM 1991 and 1992; 3.Walunas, Bluestone et al. Immunity, 4. Green et al. Immunity 1994, 5.Waterhouse, Mak et al Science, 6. Tivol, Bluestone, Sharp et al Immunity, 7.Krummel and Allison Jem 1995, 8. Leach, Krummel, Allison. Science 1996, 9. Korman, Lonberg, Allison, 10. Keler, Korman et al JIM 2003 (Medarex), 11. Honjo, KO

New generations of IO Agents



Hoos, A. Nature Review 2016

Unique Tumor Flare with Immunotherapy

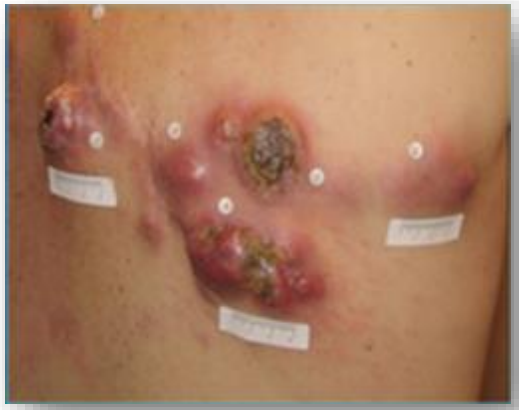


Tumor flare (growth of existing lesions or appearance of new lesions) may precede antitumor effects resulting in RECIST defined progression and premature discontinuation of therapy[1]

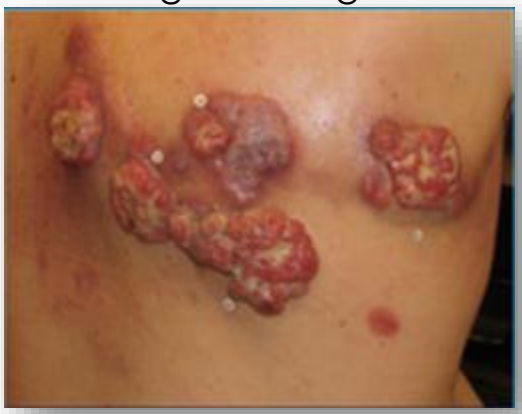
1. Wolchok, JD et al. Clin Cancer Res 2009

Tumor Flare Followed by Durable Response

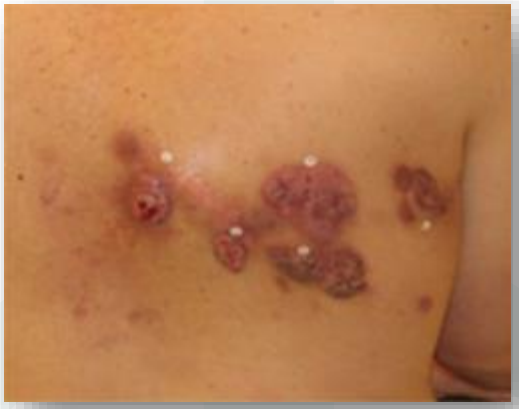
Screening



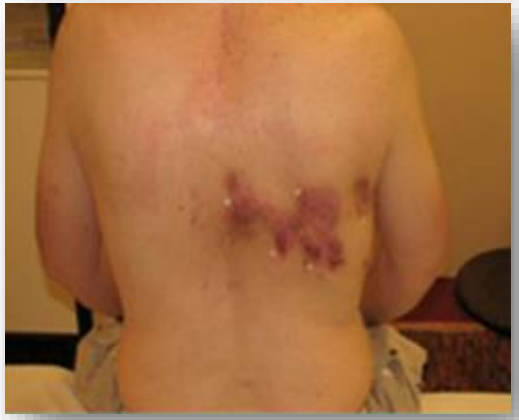
Week 12
Swelling and Progression



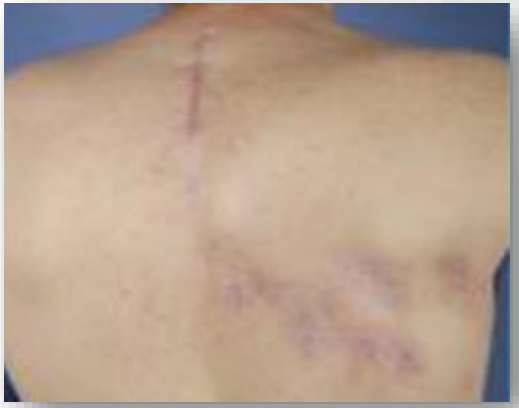
Week 14
Improved



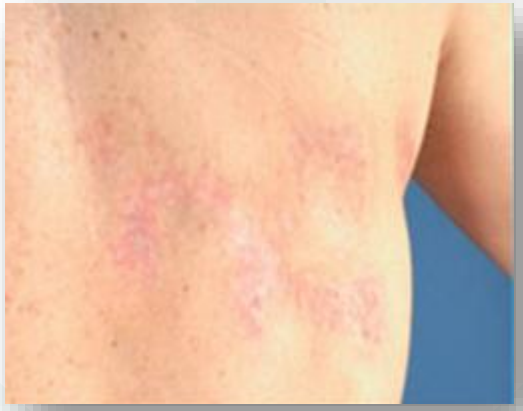
Week 16
Continued Improvement



Week 72
Complete Remission

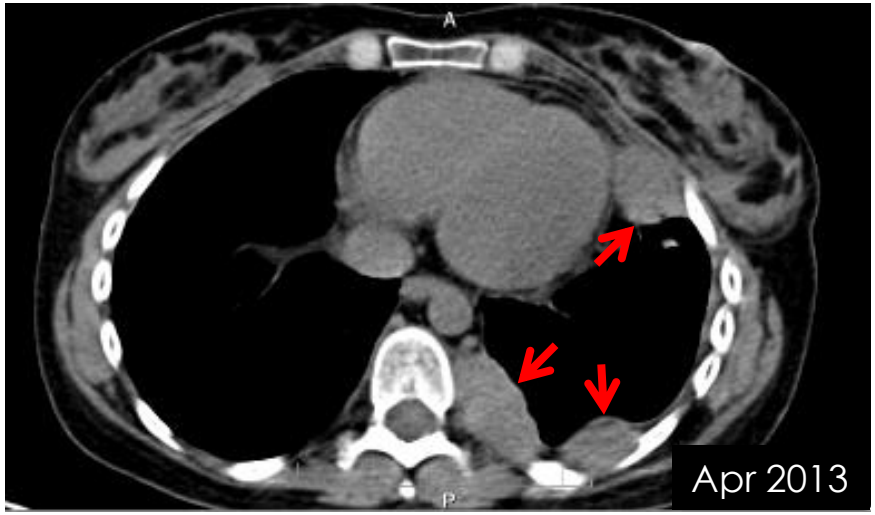


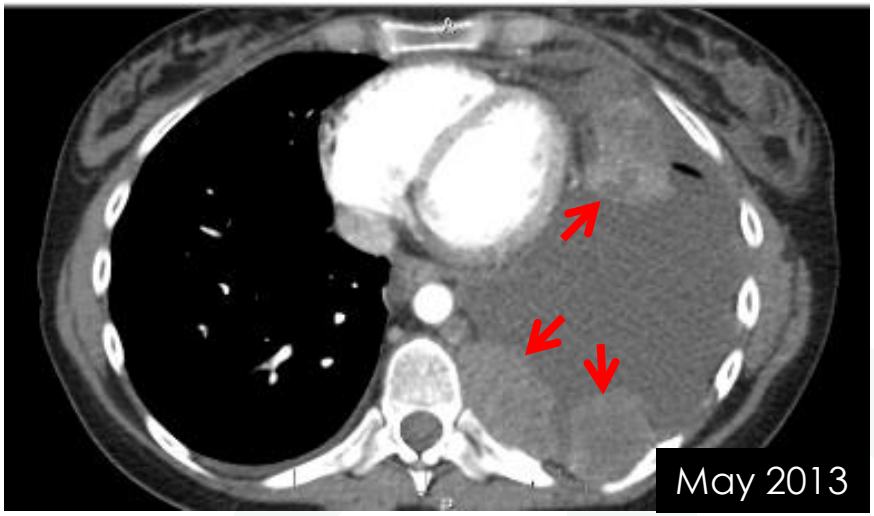
Week 108
Complete Remission

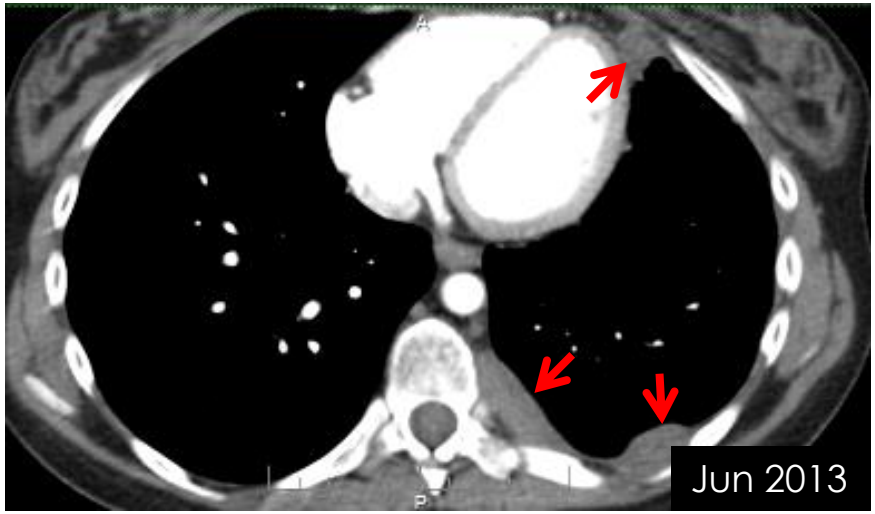


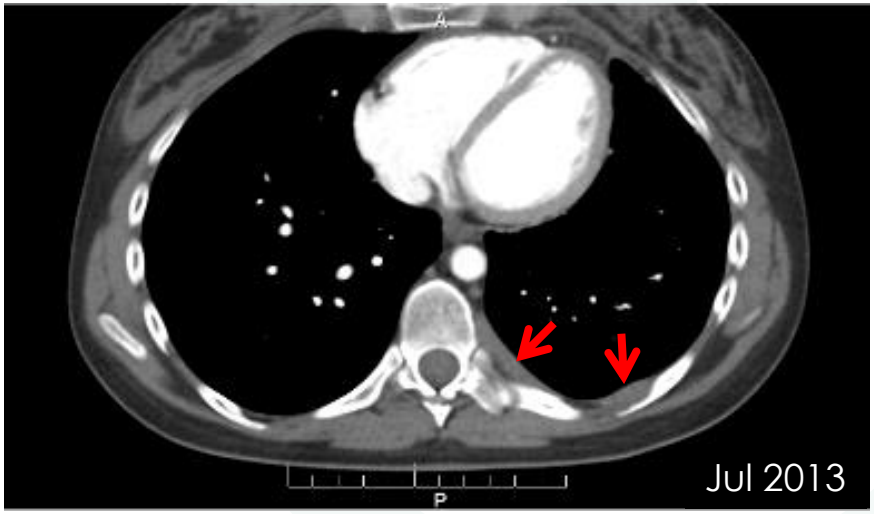
Courtesy of Jedd Wolchok. Yervoy patient

Patient with Hodgkin's Lymphoma on I-O Therapy









Efficacy and Safety of Nivolumab in Patients with Metastatic RCC Who were Treated Beyond Progression in a Randomized, Phase II Dose-ranging Trial

Objective: To evaluate the benefit of continuing nivolumab beyond first RECIST-defined progression in patients with mRCC

Key Criteria

- **mRCC with clear-cell component**
- **≥1 prior anti-angiogenic agent**
- **Karnofsky performance status (KPS) ≥70%**

→ **Randomize 1:1:1[a]** →

0.3, 2, or 10 mg/kg of nivolumab IV Q3W

Treat until progression or intolerable toxicity

Endpoints

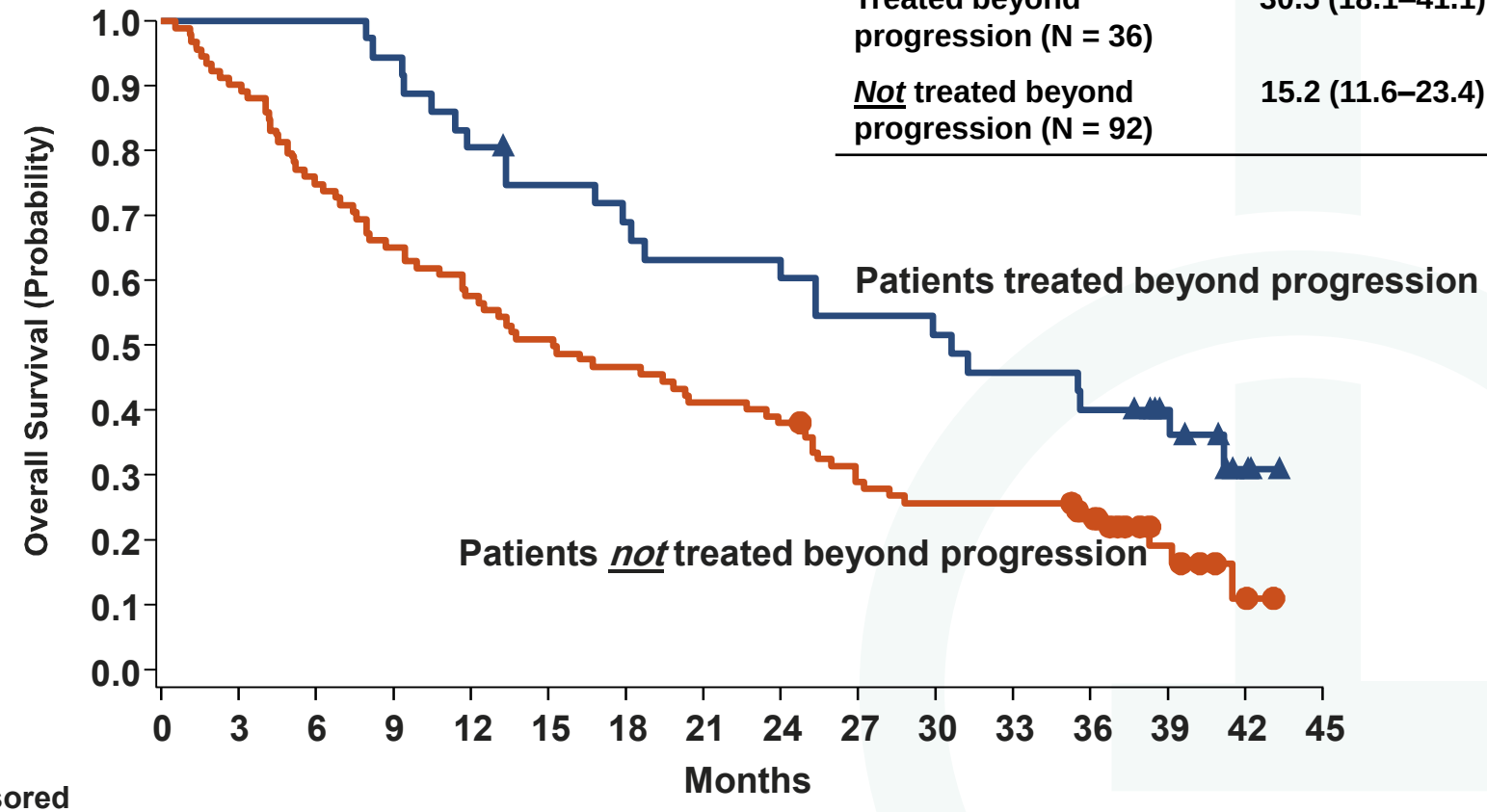
- **Primary: Dose response by PFS**
- **Key secondary: PFS, ORR, OS, safety**

Treatment beyond progression was permitted if nivolumab was tolerated and clinical benefit was noted

[a]Stratified by Memorial Sloan Kettering Cancer Center (MSKCC) risk group and number of prior therapies in metastatic setting.
Motzer, RJ. J Clin Oncol 2015

Overall survival

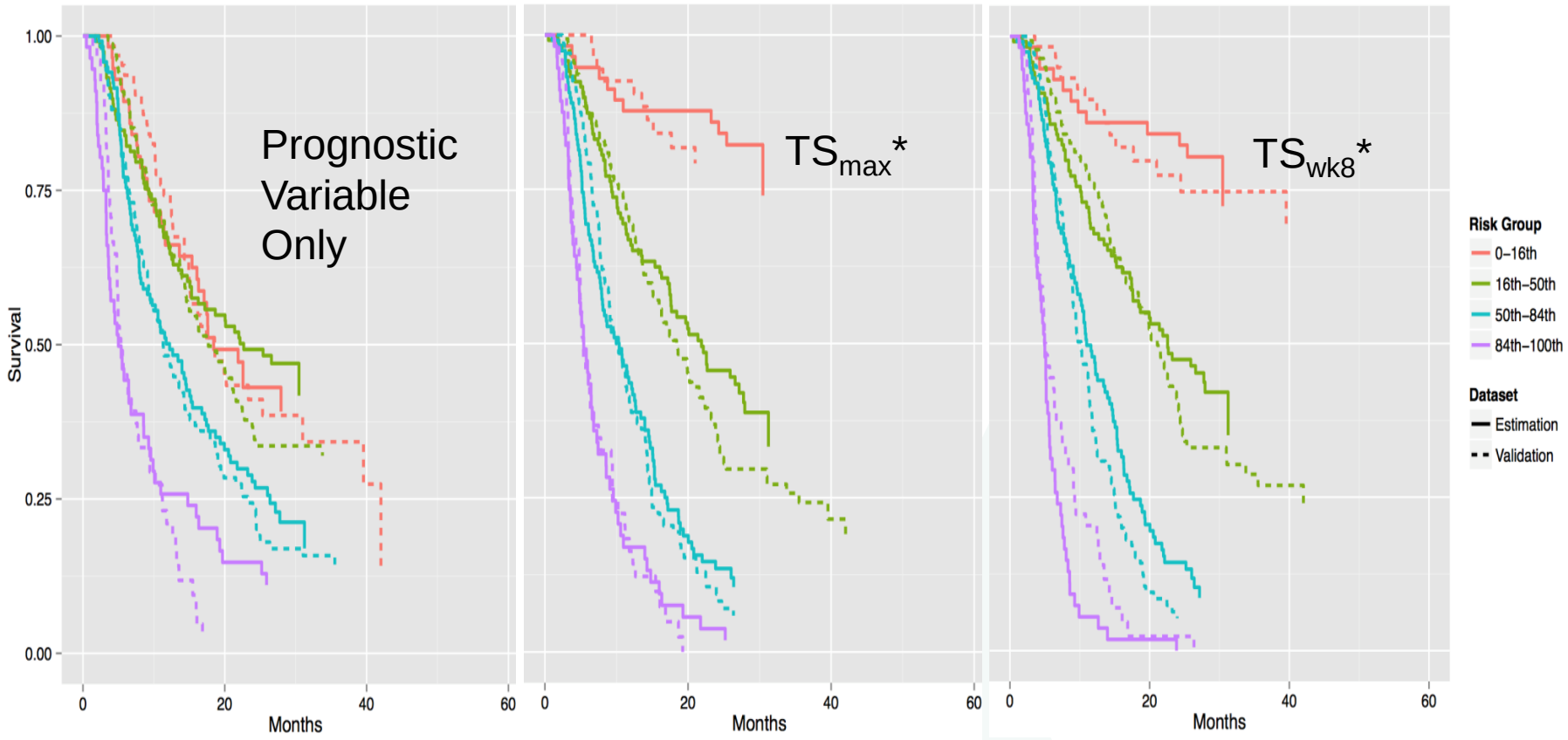
	Median OS, months (95% CI)
Treated beyond progression (N = 36)	30.5 (18.1–41.1)
Not treated beyond progression (N = 92)	15.2 (11.6–23.4)



▲ ● Censored

Patients treated beyond progression	36	36	36	34	29	26	24	22	21	19	18	16	14	10	3	0
Patients *not* treated beyond progression	92	83	69	60	53	47	43	38	35	26	23	23	19	7	2	0

Inclusion of Tumor Shrinkage Metrics Improves Discrimination of Survival Probability in Melanoma Patients



***Prognostic variables: M stage, Sex, ECOG, Albumin, LDH, Weight, Age, Baseline tumor burden.**

Suryawanshi, S. et al., presented at
ACOP Annual Meeting Oct 2015, Crystal City, VA

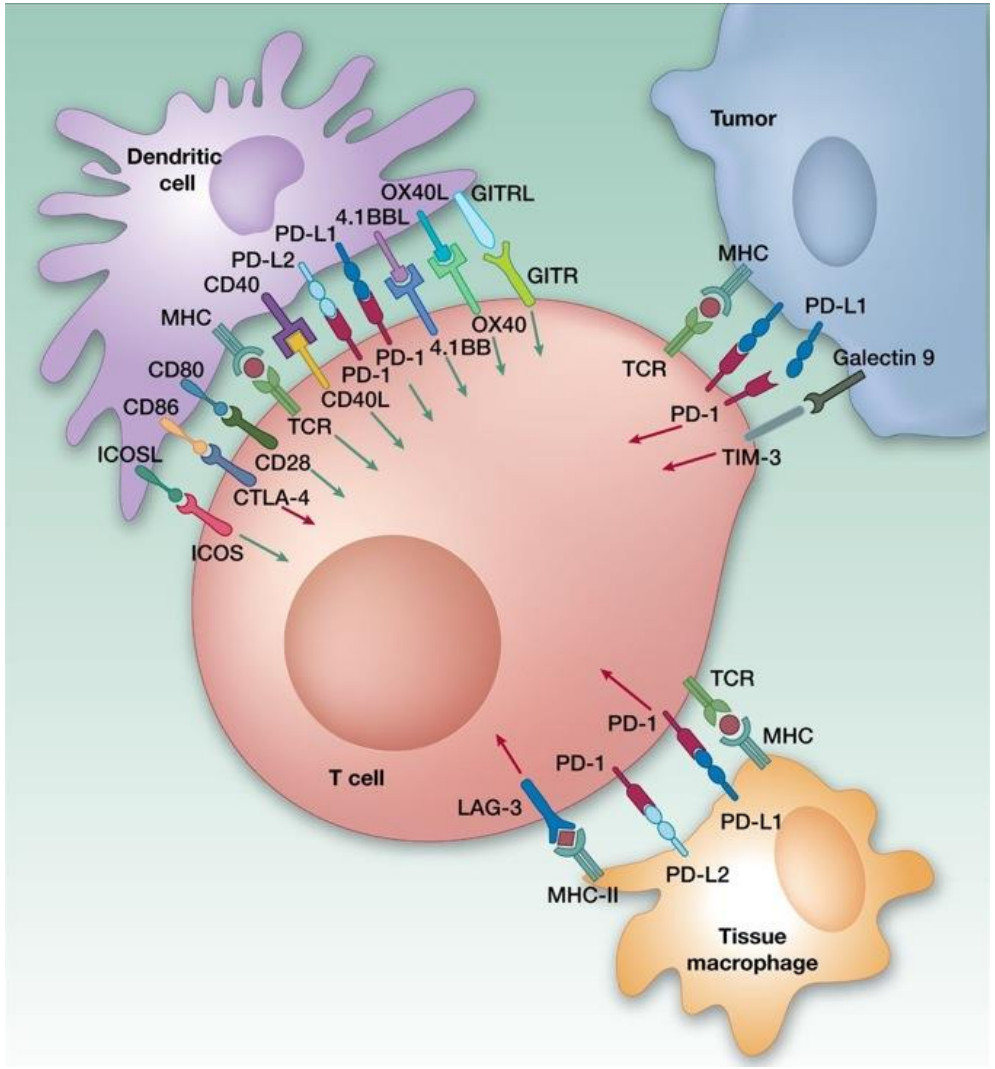
Bristol-Myers Squibb Immuno-Oncology

Why do we measure?

Need to determine relevant early measures of clinical activity predictive of clinical efficacy

- Are there early measurements of clinical activity to identify patients who may benefit from alternative or combination therapy?
- Can we predict long term survival based upon early clinical data allowing for limited sample size and follow-up?

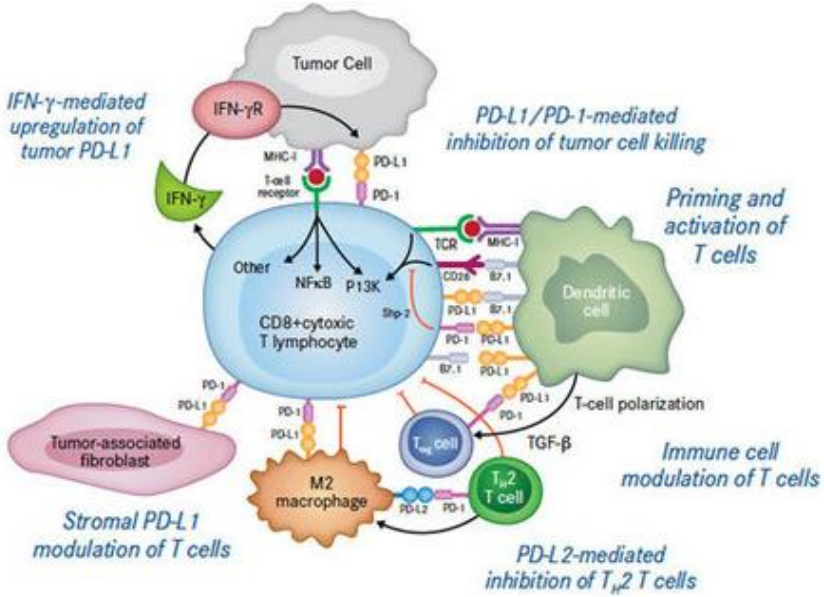
Increasing Complexity in the Future of Immune Modulation



- More immune targets
- More agents
- More combinations
- Immune modulation compared with other treatment modalities

Increasing Complexity in the Future of Immune Modulation

Complex Biology



Larger Tool Box

- Imaging
- Flow Cytometry
- Genomics
- Proteomics
- Pathology

Predictive Biomarkers

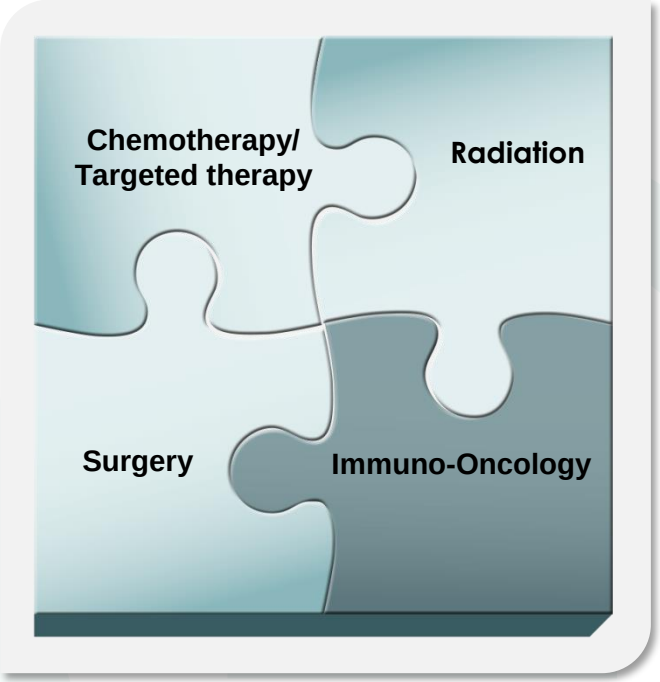
Resistance mechanisms

New targets and rational combinations

Optimal diagnostics

Moving Forward – An Evolving Landscape

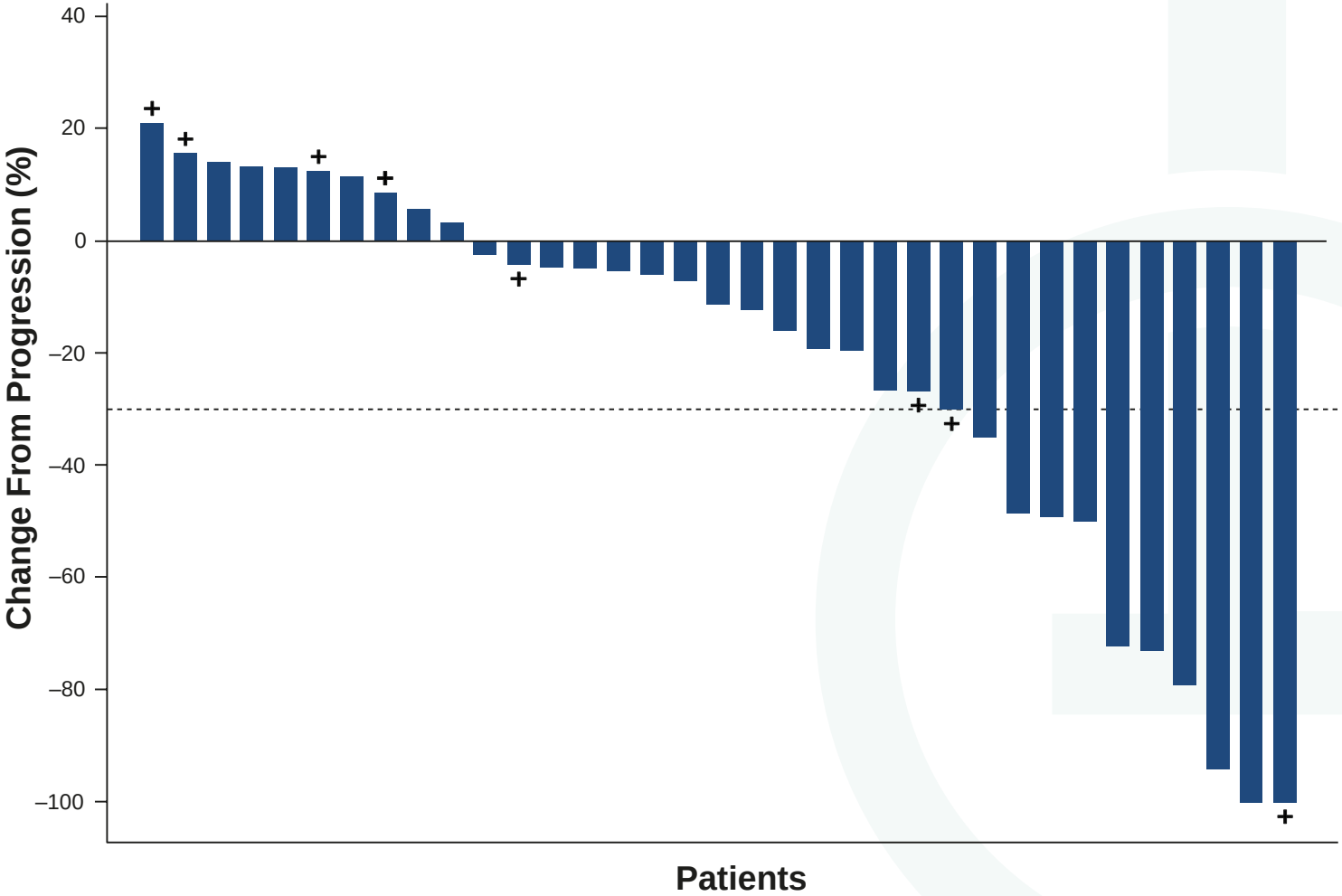
- Innovative novel therapies
- Comprehensive data analysis – FNIH VolPact, beyond anatomy
- Unified response criteria
- Reliable, robust assessment for optimal patient care



Backup Slides

Tumor Burden Change from First Progression in Patients Treated Beyond Progression

After first RECIST-defined progression, some patients continuing nivolumab treatment experienced subsequent tumor shrinkage and extended survival



Each + indicates patient who had at least a 20% increase in target lesions at time of first progression.
George S, Motzer RJ et al. ESMO 2015, Vienna

Bristol-Myers Squibb Immuno-Oncology

T Cell Therapies

Lawrence G. Lum, MD, DSc
Director of Cellular Therapy
Scientific Director of BMT
Emily Couric Cancer Center
Professor of Medicine
Department of Medicine
University of Virginia
Charlottesville, VA

NCI Workshop:
Immune Modulation Therapy and Imaging:
What can we do now In clinical trials?

Disclosure: Co-Founder of Transtarget, Inc
for Bispecific Antibody armed T cells (BATs)

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Bispecific antibody Armed T cells (BATs)

- Can low dose BiAb armed T cells (BATs) be used to target solid tumors?
- Can we avoid CRS related to engaging all T cells with BiAb infusions vivo while inducing long-term immune responses?
- Can we vaccinate with BATs and boost after HDC and SCT to enhance post SCT anti-breast cancer cellular and humoral immunity?
- Can BATs be tracked and imaged on tumors?
- Do BATs work on prostate, pancreatic or liquid tumors?

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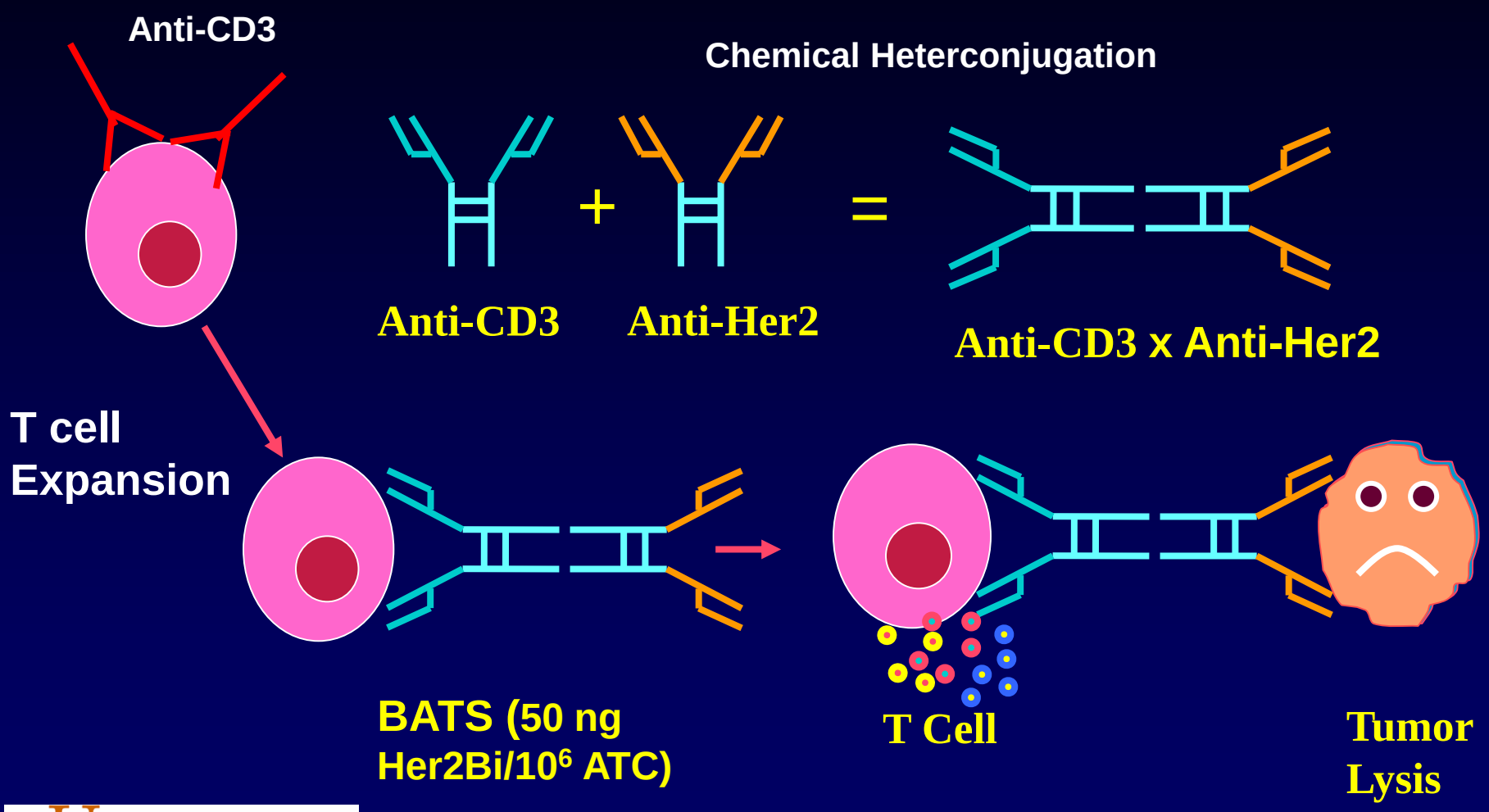
Targeted Killing by BiAb Armed T cells (BATs)



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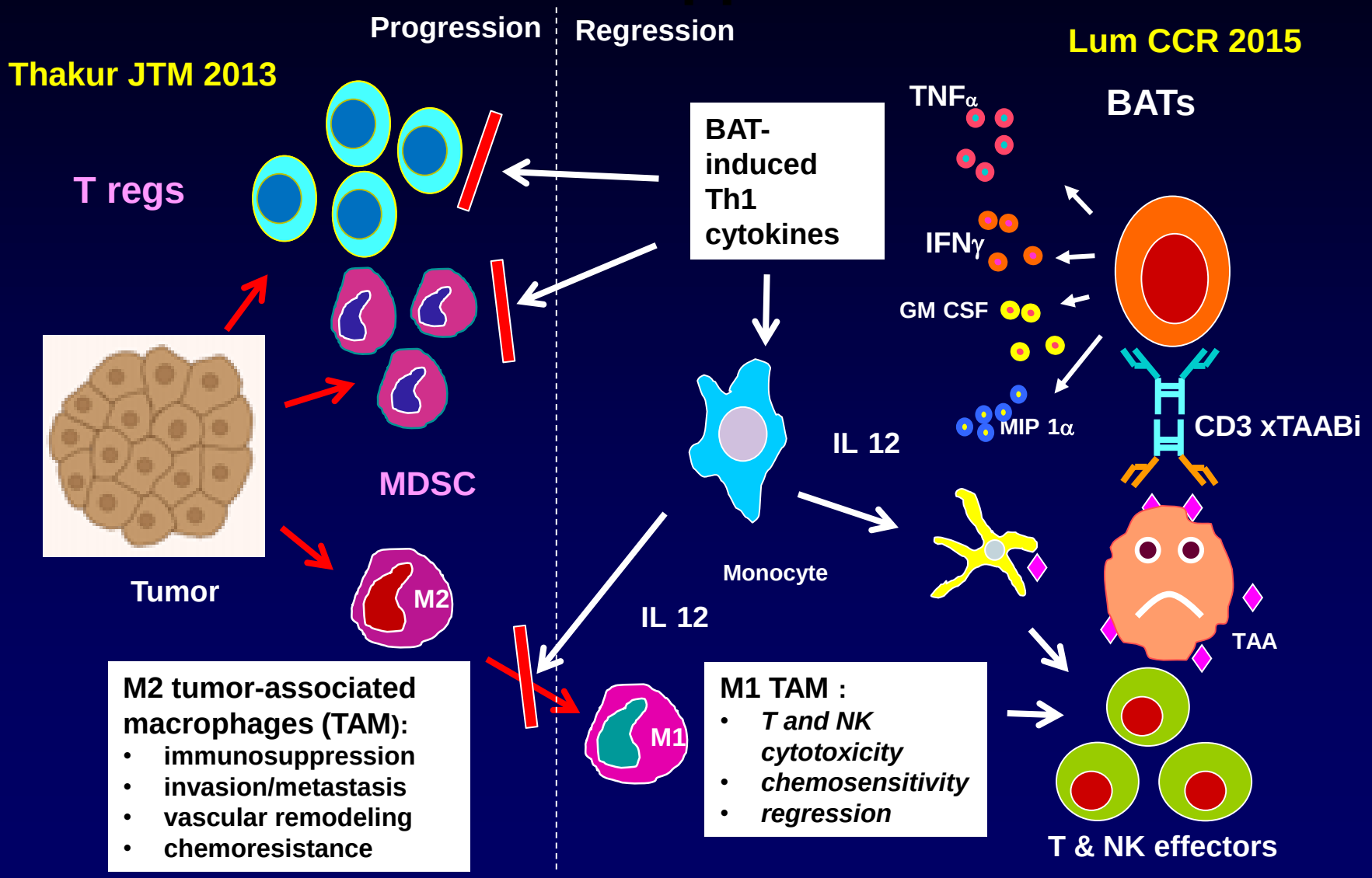
Mechanisms for BATs Overcoming Tumor Induced Suppression



BATS Target "Nil" Expression of Her2 on Sum 1315 Cells

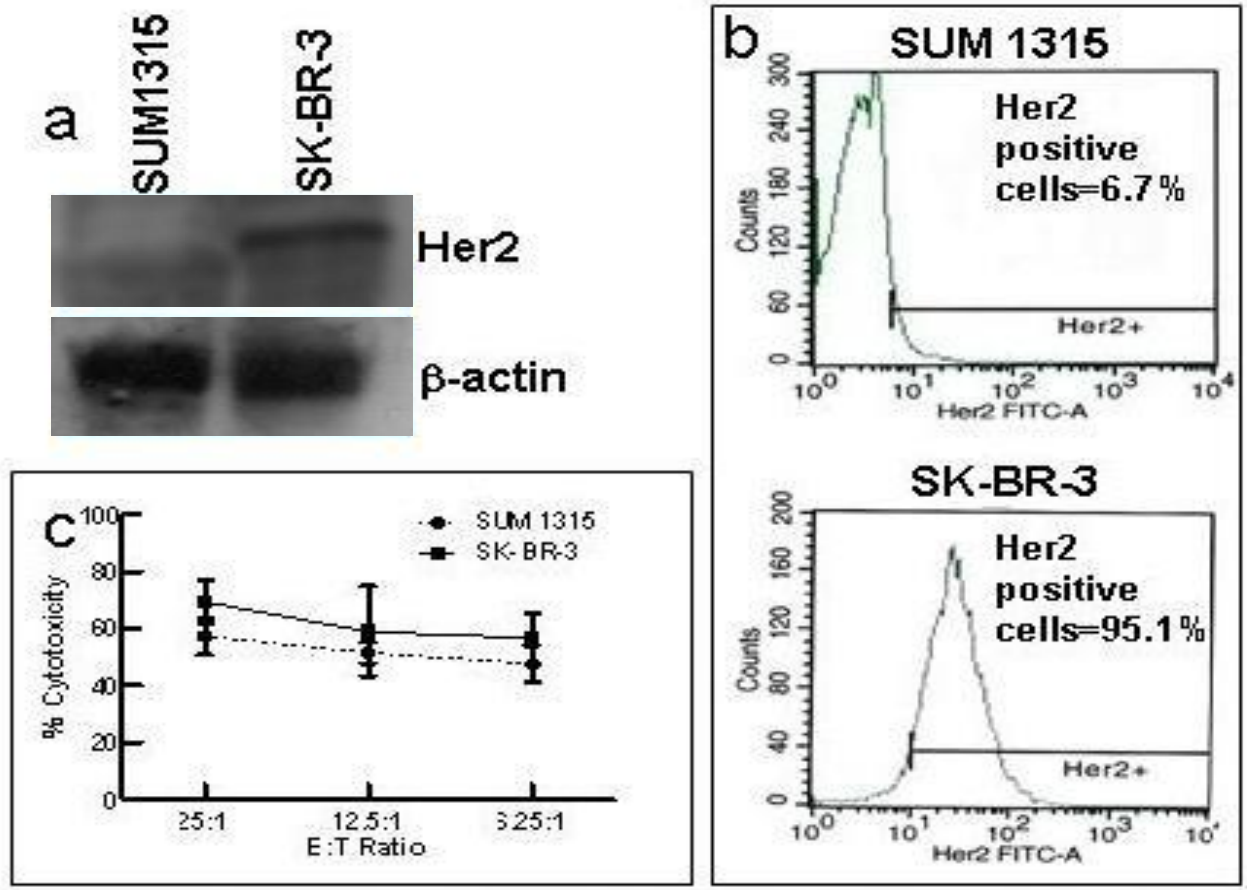


Fig 2: Cytotoxicity Remains High in Very Low Her2/neu Expressing Sum 1315. **a)** Western blot shows very low (SUM 1315) and high (SK-BR-3) HER2/*neu* expression in a Western blot; **b)** Flow cytometry shows surface expression of Her2/*neu* on 6.7% of Sum 1315 with very low mean fluorescent intensity (MFI) and 95.1 of SK-BR-3 with a high MFI; and **c)** Cytotoxicity remained high in the very low Her2/neu expressing Sum 1315 cell line.

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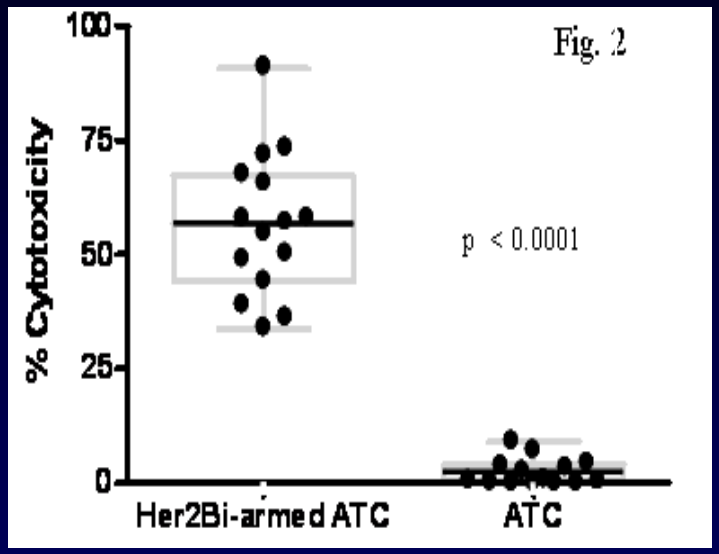
Treatment Schema for Stage IV Breast

Screening

Leukopheresis

ATC Expansion

ATC Infusions



Tumor Evaluation

Immune Evaluation

3 Wks

Wk8

Wk1 Wk2 Wk3 Wk4

GM-CSF 250 ug/m^2/dose

IL-2 300,000 IU/m^2/day

Dose escalation:
5, 10, 20, 40 in standard
3+3 design

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Stage IV Breast Cancer Patients

Table 1: Patient Characteristics		
	No.	**%**
Age		
< 50	**14**	**60.9**
≥ 50	**9**	**39.1**
Cancer Stage	**23**	**100**
Stage IV		
Performance Status		
(ECOG)	**18**	**78.3**
0	**5**	**21.7**
1	**0**	**0**
2		
ER/PR Status		
Positive	**14**	**60.9**
Negative	**8**	**34.8**
Unknown	**1**	**4.3**
HER2/neu Status		
0	**10**	**43.5**
1+	**2**	**8.7**
2+	**2**	**8.7**
3+	**8**	**34.8**
Unknown	**1**	**4.3**
Prior Treatment w/ Herceptin		
Yes	**8**	**26.0**
No	**15**	**74.0**

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Stage IV BrCa Phase I Toxicities

Toxicity Grade	Grade 1	Grade 2	Grade 3	Grade 4	Total Episodes	% of Total
Chills	0	4	36	0	40	51
Headache	0	3	14	0	17	22
N/V	8	1	2	0	11	14
Fever	3	1	0	0	4	5
Hypotension	1	3	0	0	4	5
Hypertension	0	0	0	1	1	1.3
SOB	0	1	0	0	1	1.3
Total	12	14	52	1	77	

1 patient died of CHF related to digoxin toxicity after IT was completed.1 patient developed a subdural hematoma that was evacuated without complications

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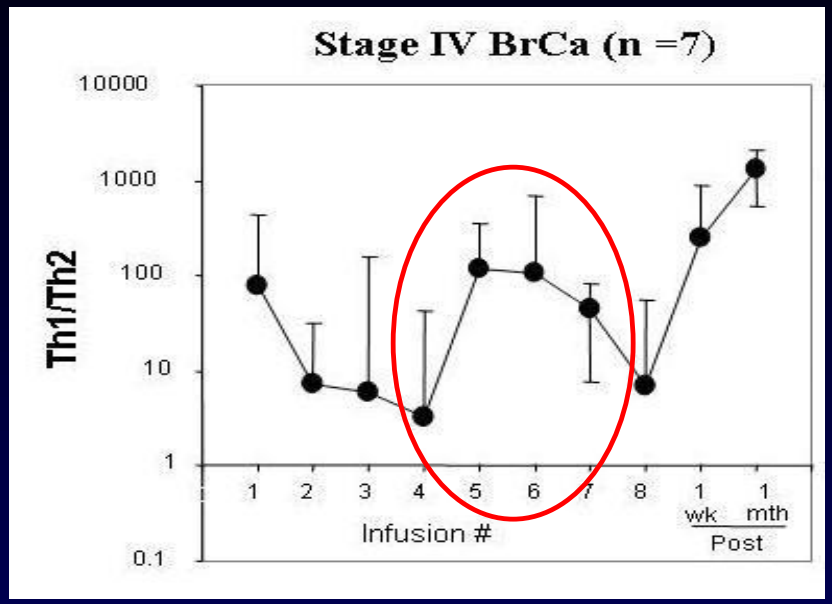
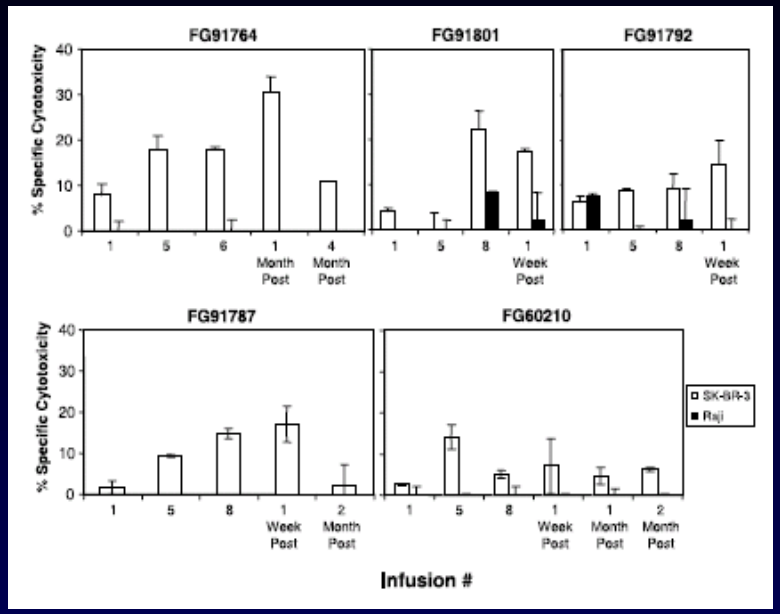
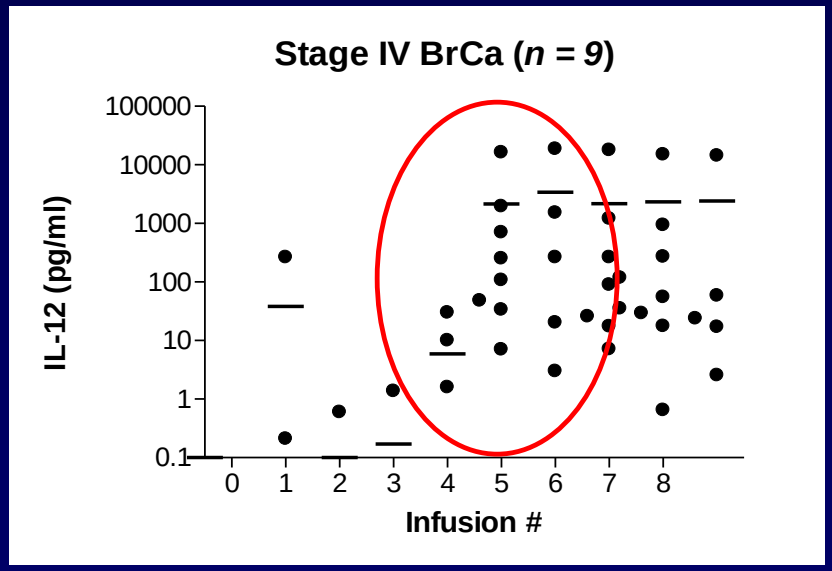
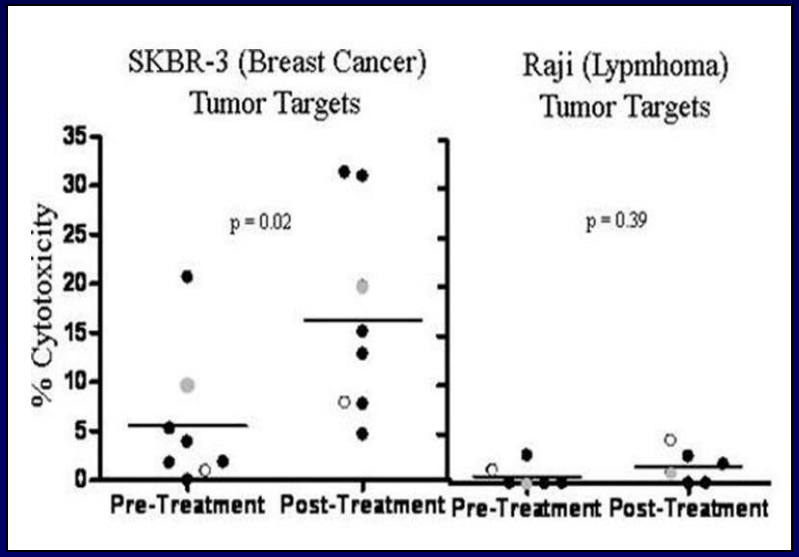
Immune Responses to Her2Bi-Armed ATC Infusions and Overall Survival









Clinical Responses to Her2Bi-Armed ATC Infusions

Clinical Responses to Her2Bi-armed ATC by Dose Level[a]						
Response (%)	All Pts #	All Pts %	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
PR	1	4.3	0	1(100)[c]	0	0
SD	12	52.2	4(33.3)	2(16.7)	6(50)	0
PD	8	34.8	4(50)	3(37.5)	1(12.5)	0
NE[b]	2	8.7	1(50)	0	1(50)	0

[a] At one month follow-up after the last infusion and 14.5 weeks after last Tx. [b]Did not complete infusion schedule or died before 1 month follow-up. [c]Pt received only 80 billion cells due to slow expansion. Evaluation 15 weeks after last chemotherapy/hormone therapy

These early results don't reflect effect on survival; patients Went on to receive dealer's choice – with prolonged survival; Delayed responses with a pt returning from hospice.

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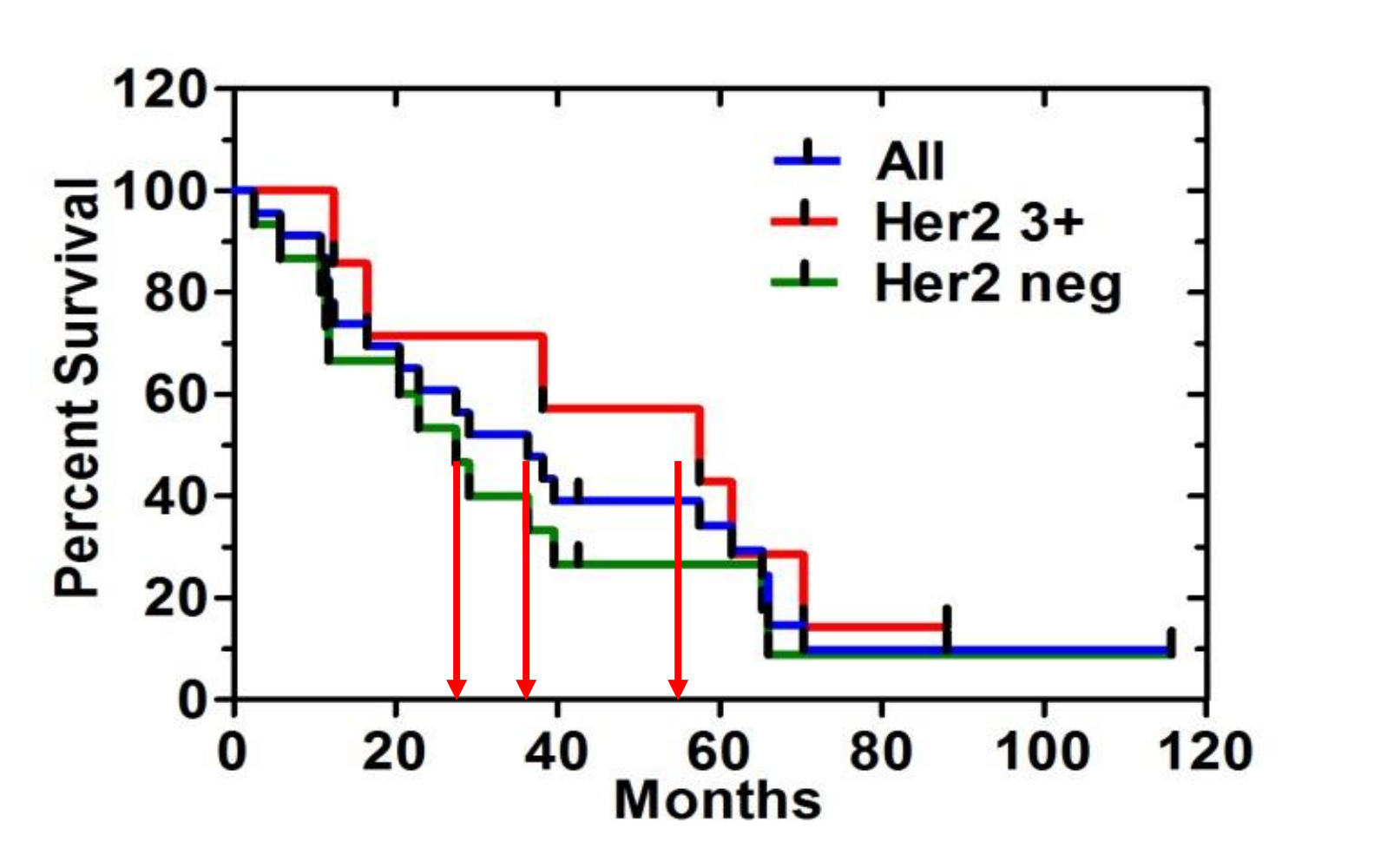
Phase I: Metastatic Breast



Lum 2015 Clin Cancer Research

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Her2/*neu* negative Pt: PR 7 months post IT

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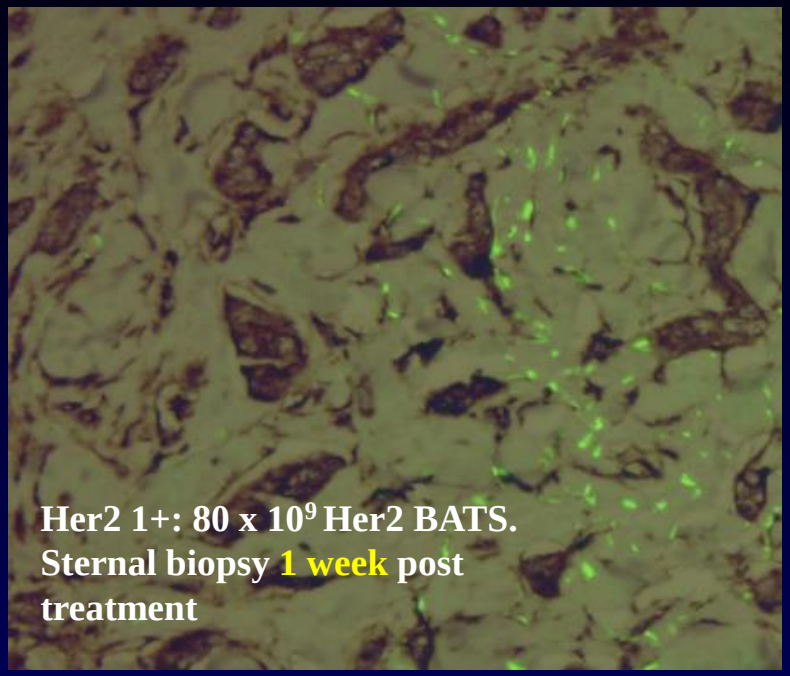
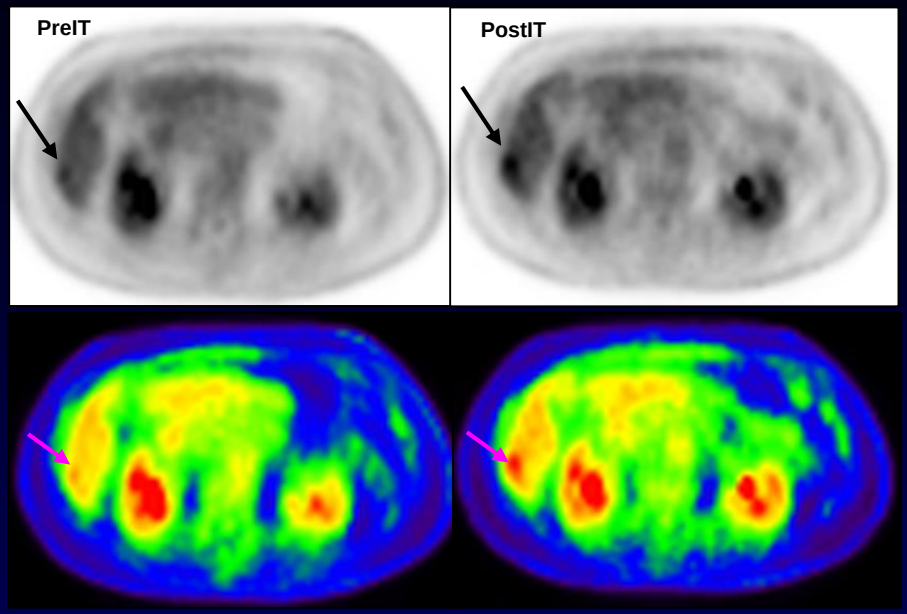
Trafficking of BATs in Breast Cancer Patients

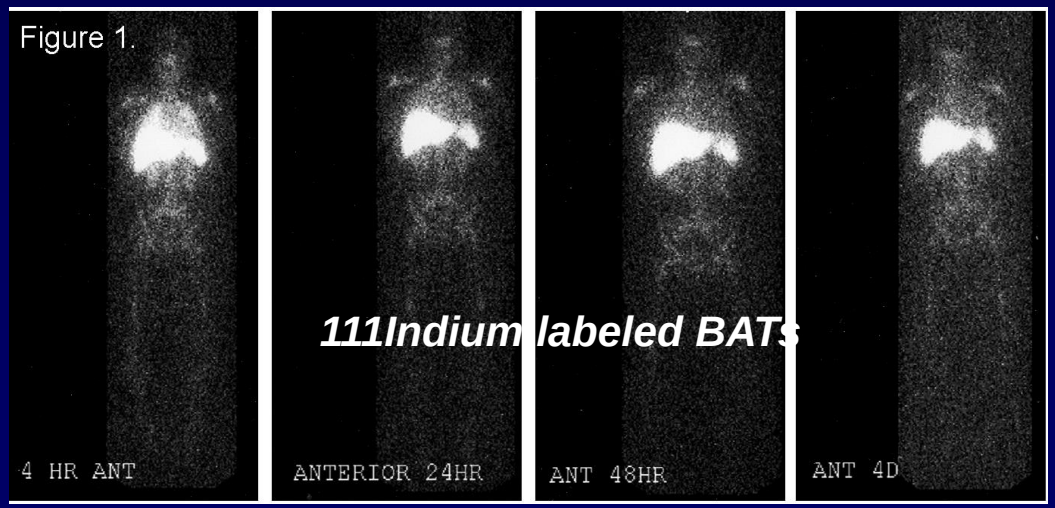


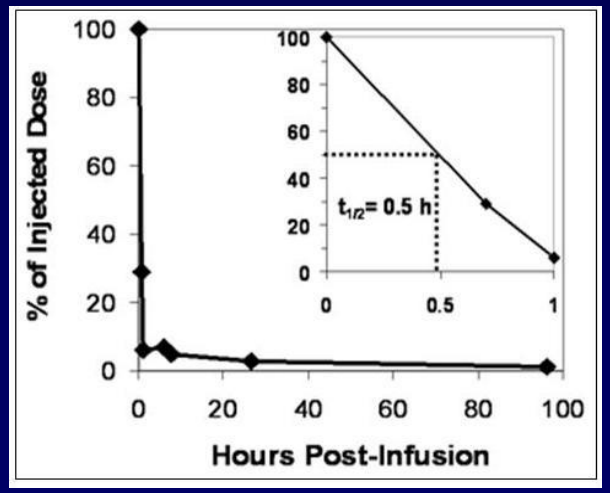


Scan 1, SUV max 3.65

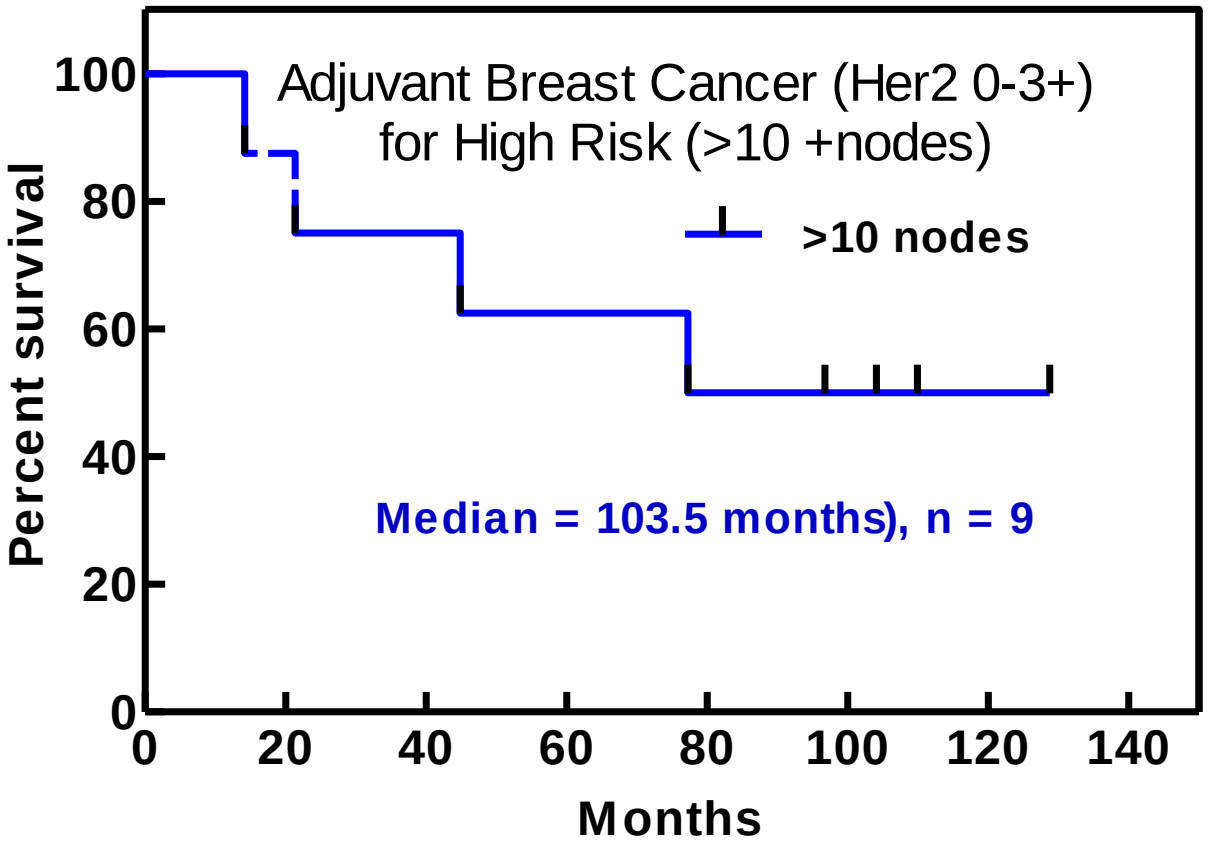
Scan 2, SUV max 5.75

Shields

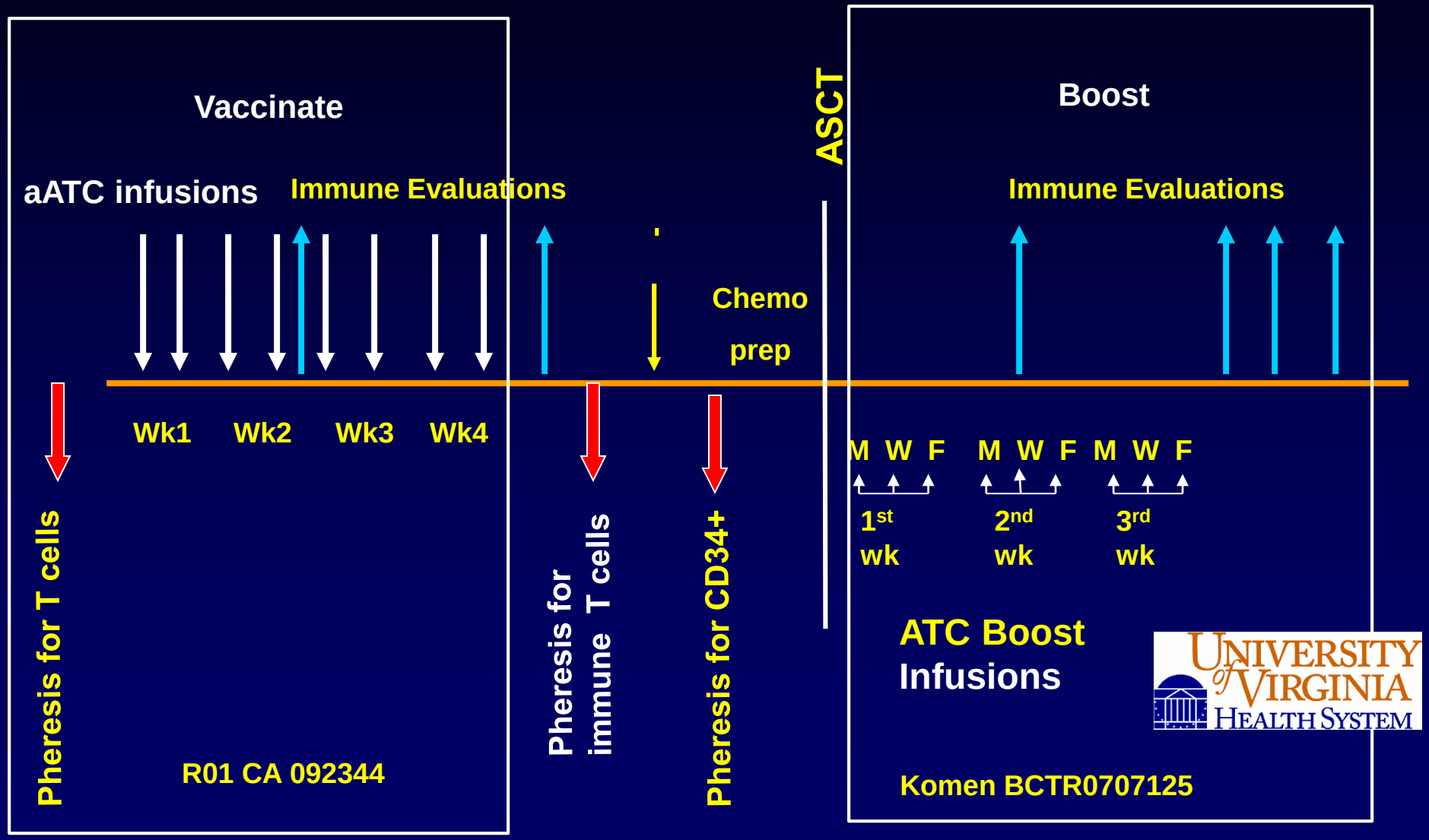




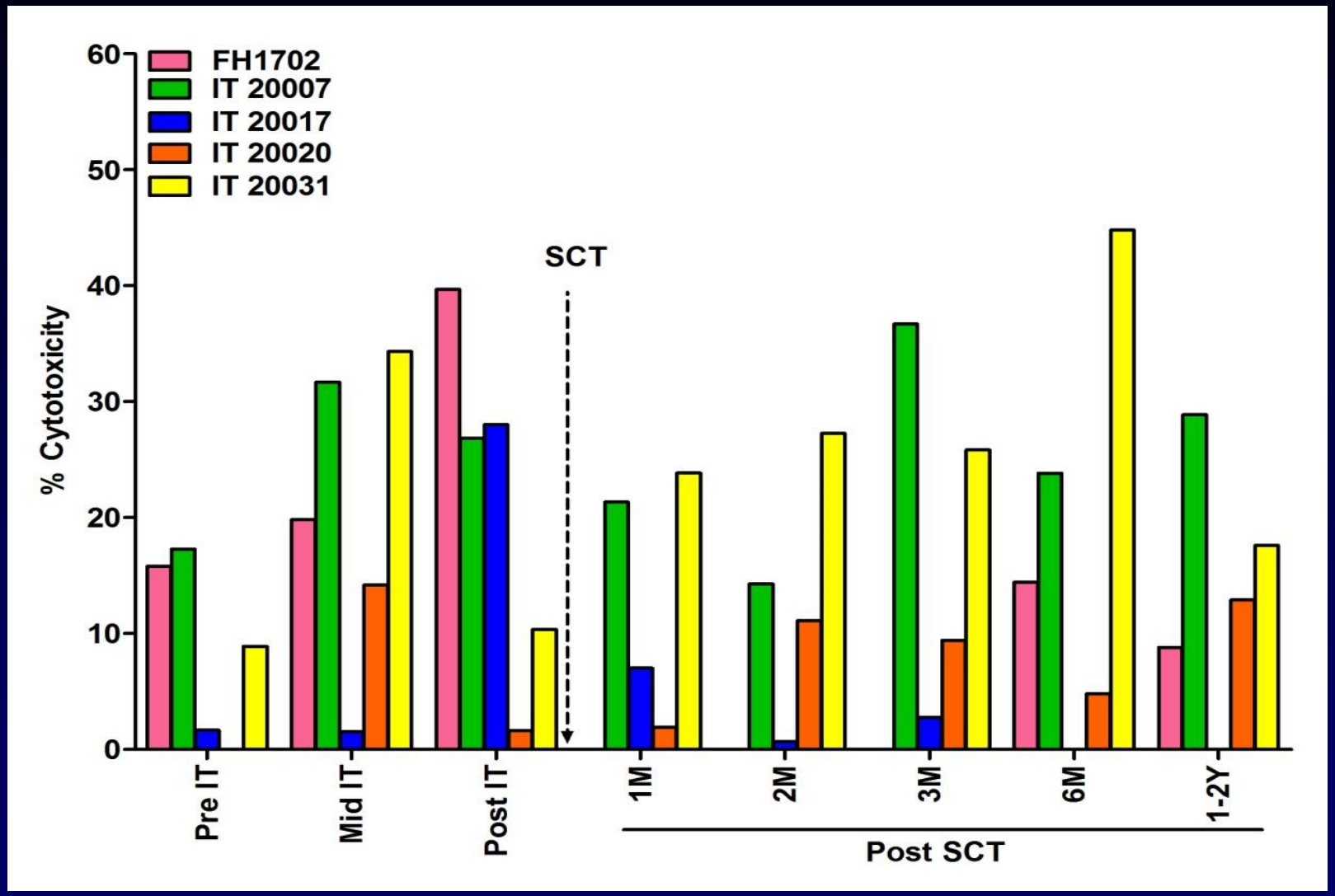
Survival Curves for High Risk Adjuvant BrCa (Her2 0-3+)



ATC Boost with "Immune Cells“ after PBSCT for Stage IV Breast Cancer



Cytotoxicity Directed at SK-BR-3 Pre and Post SCT



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Pancreatic Cancer (Phase I) EGFR BATS: 3/4 infusions and no IL-2 or GM-CSF

Pt	Age	Disease	Prior Tx	Dx Date	BATs x 10^9	TTP (days)	OS (days)	Comments
IT20087	58	Mets to liver	Folfirinox		47	186	Dead (409) 13.6 mos	Progressed after Immunotherapy
IT20091	63	T3 N1Mets to liver. Post Whipple	5FU,Leuk/5FU Folfirinox	1//2012	9.3 78.8	CR, 138	Dead (930) 31 mos	Chemo Induced CR after IT Treated Twice Progressing; Folfirinox restarted & responded again
IT20092	64	T2b Abd Nodes, post Whipple	Gemzar, 5FU, radiation,	2/2012	36	211	Dead (436) 14.5 mos	Had chronic diarrhea; Appendicitis From PC tumor with TILs
IT20102	56	T4, Mets to liver, lungs	Folfirinox	11/2013	74	Stable	Alive (626) 20.9 mos	No Treatment; Lesion decrease by 27% at 6 mos; no treatment, progressing chemorestarted, responded
IT20104	51	T4, Abd Nodes	FOLFOX stable 1 yr then Xeloda	9/2012	72	71, CR	Alive (577) 19.2 mos	Chemo Induced CR after IT; On Xeloda

Updated 3-14-16; median OS ~19 mos from ~6 mos

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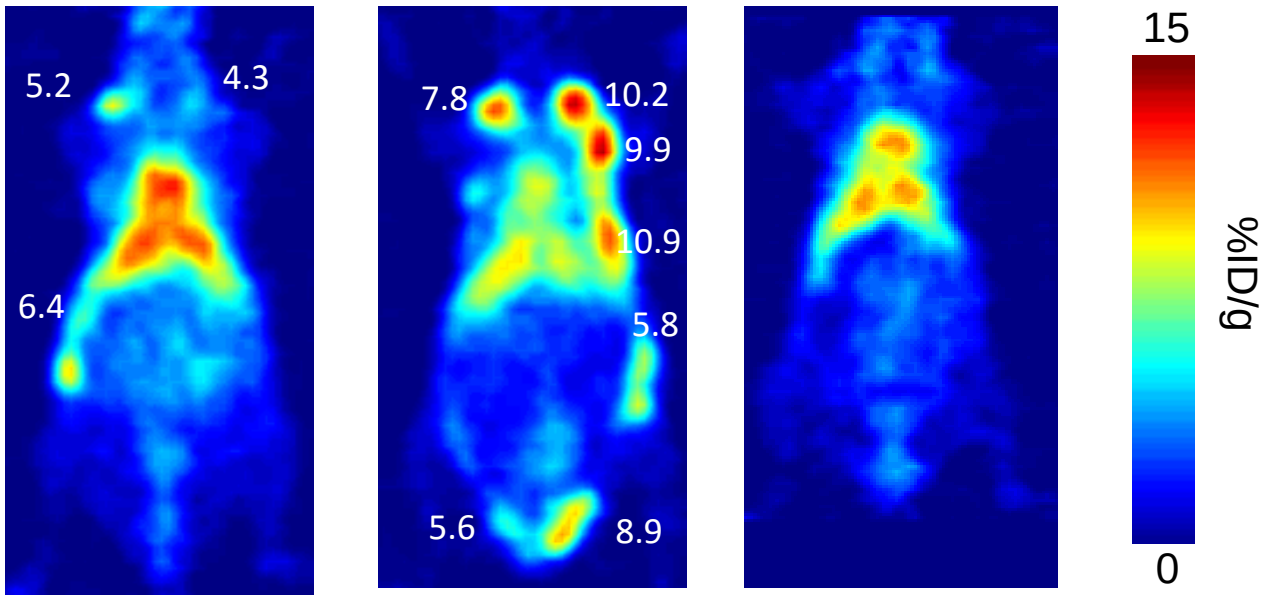
Summary of Clinical Trials using BATs

1. Hormone Refractory Prostate Cancer – BATs induce 1 of 7 PR and 2 minor responses in PSA and bone pain decreased by >80% in pts. Vaishampayan Pros Cancer 2015
2. High risk (>10 nodes) adjuvant breast cancer (Her2 0-3+) treated with HER2 BATs with 5 of 9 pts alive and NED 14 years later (Lum, unpublished).
3. Encouraging results in High risk NHL and Multiple Myeloma using CD20Bi BATs (Lum BMT 2013 and Lum 2013 BBMT).
4. Phase 2 in heavily pretreated (Her2 negative) MBC in 31 evaluable pts with median OS of 19 mos (Lum unpublished)

^{64}Cu-Ab4

Tumors are at different stages

Control, without tumor



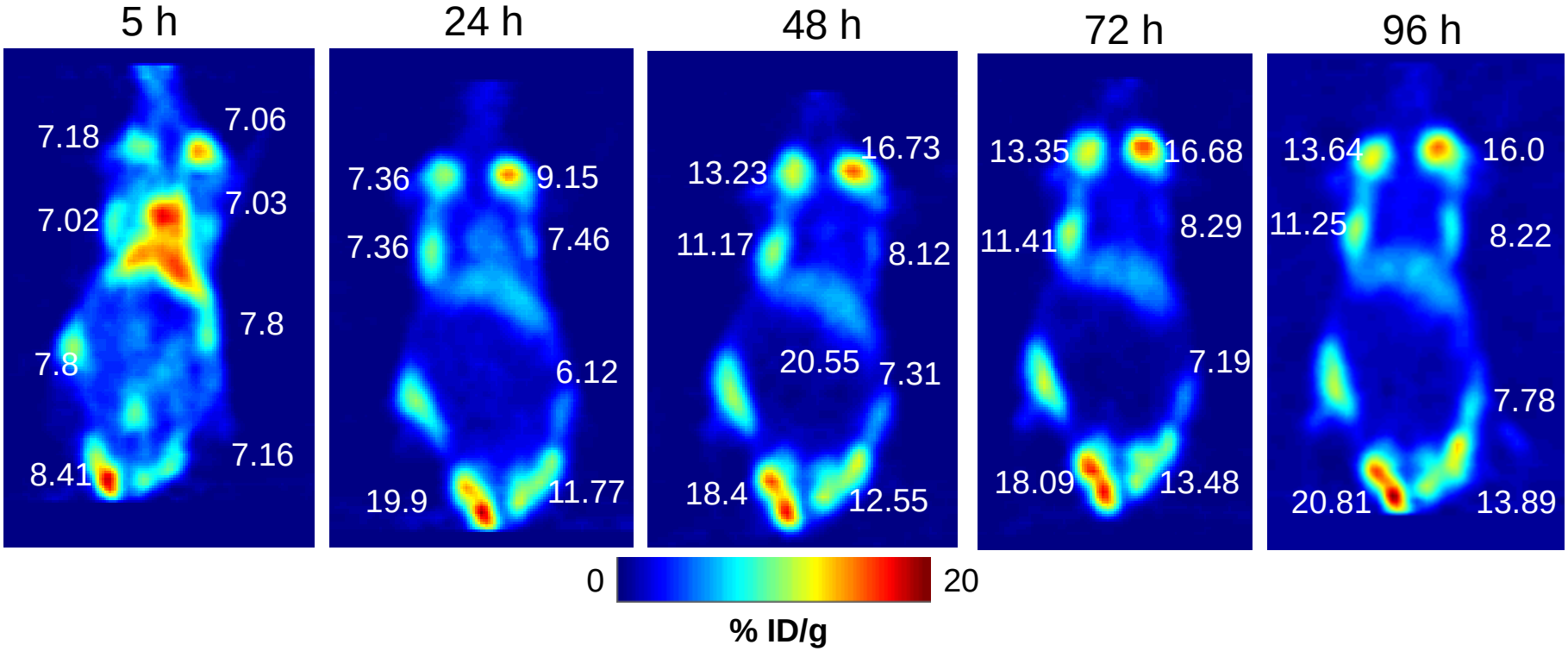
- Model: NeuT transgenic mice develop spontaneous tumors over time
- Ab4 is a murine antibody raised against Neu antigen
- ^{64}Cu ($t_{1/2}$ ~ 12.7 d) is labeled onto Ab4 using NOTA as chelator

Note: Numbers on the images reflect the tumor uptake of ^{64}Cu-Ab4.

** Unpublished data.*

Courtesy of Nerissa Viola-Villegas

^{89}Zr-Ab4



- ^{89}Zr ($t_{1/2}$ ~ 3.27 d) is labeled onto Ab4 using DFO as chelator
- Mouse injected with 4 micrograms=133 ng/ml

<u>* Unpublished data.</u>

Courtesy of Nerissa Viola-Villegas

Thanks to Those who made it Happen!

- ***BMT Team and Leukemia:*** R Rathore (RWMC), A Deol, L Ayash, M Abidi (UCD) Z Al-Kadhimi (Emory), V Ratanatharathorn (KCI), J Uberti (KCI), and J Zonder (KCI)
- ***Breast Cancer Team:*** **A Thakur (Uva), R Rathore (RWMC), F Cummings, Z Nahleh (TTU), E Gartner (SG), L Choi (KCI), A Weise, M Simon, L. Flaherty (KCI)**
- ***Neuroblastoma Team*****: M. Yankelevich (CHOM), S. Modak (MSKCC), NK Cheung (MSKCC)**
- ***GI and Imaging Team:*** **A Shields (KCI), M Choi (Stony Brook), N Viola-Villegas (KCI)**
- ***GU Team:*** **U Vaishampayan, E Heath (KCI)**
- ***Immune Evaluations:*** **A Thakur (Uva), V Kondadasula (KCI)**
- ***Lab Staff:*** **C Pray, Y Gall, P Davol, C Sorenson, E Tomaszewski (KCI), D Schalk (Uva), H Yano (U of Pittsburgh)**
- ***Nursing Staff:*** **W Young, L Hall, A Olson, P Steele, K Meyers , K Fields, M Dufresne, BMT and IV infusion nurses at RWMC, KCI**

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Imaging with chemokine receptors and small molecules

Sridhar Nimmagadda, Ph.D.

Opportunities

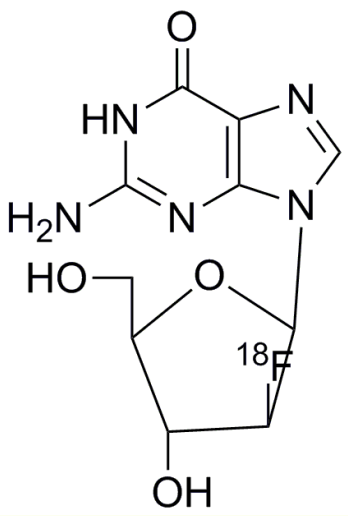
- Imaging immune cells (metabolic tracers, CD8, PD-1, PD-L1, chemokine receptors, chemokines?)

- Imaging the immunosuppressive tumor microenvironment (IDO, A2AR)

Deoxyguanosine kinase (dGK)

- dGK is a mitochondrial protein
- dGK activity is found in most tissues (liver, lymphoid tissues such as B and T cells, spleen, skin, and brain)
- dGK phosphorylates deoxyguanosine and exhibits broad substrate specificity (cladribine, fludarabine, cytarabine (Ara-C), gemcitabine, nelarabine (AraG) and clofarabine)
- Guanine-ß-d-arabinofuranoside (AraG) has a specific toxicity for T lymphocytes

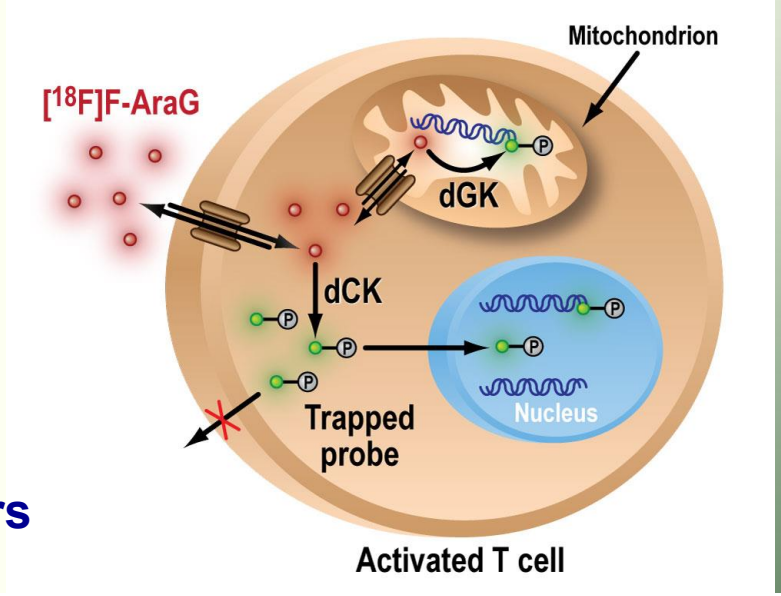
Imaging T-cell metabolism with [^{18}F]F-AraG



VisAcT: [^{18}F]F-AraG is Fluorine 18 labeled analog of an FDA approved drug AraG – ArabinoFuranosyl Guanine

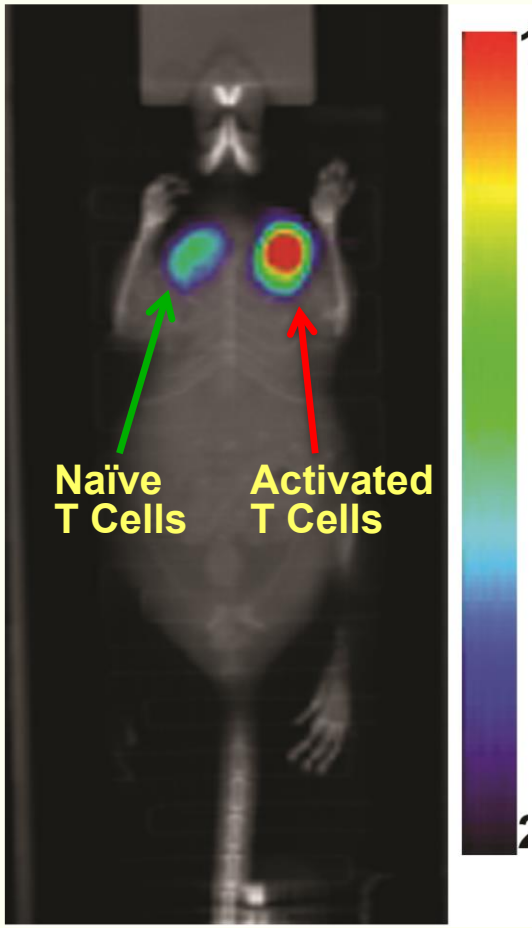
Mechanism of Action

- Activated T Cells overexpress dGK
- Tracer phosphorylated and trapped in cells with high levels of dGK
- Detected with existing PET scanners



Courtesy: CellSight Technologies

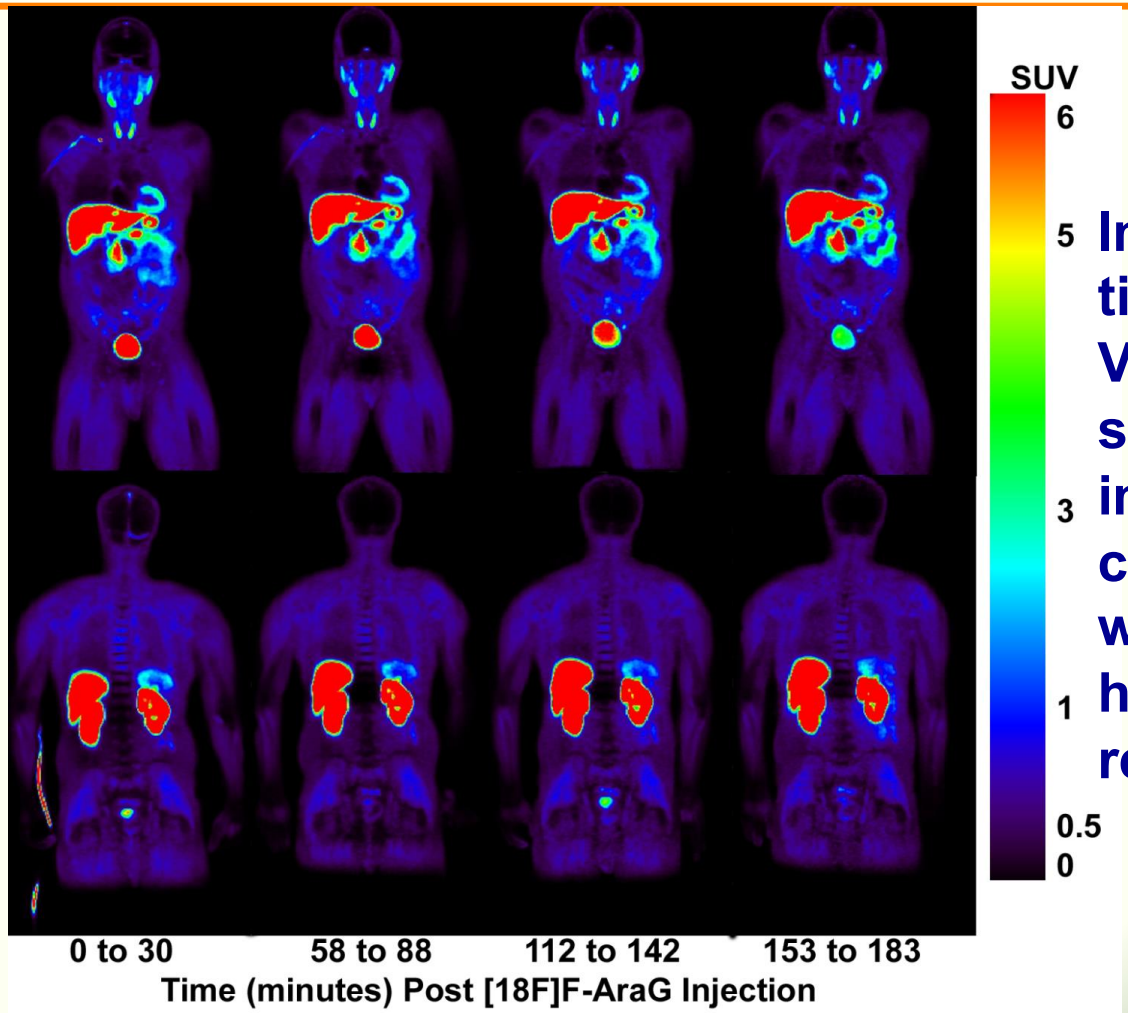
Visualizing activated T cells with [^{18}F]F-AraG



- Pan T cells isolated from spleen & lymph nodes of mice
- Cell group A treated with CD3/ CD28 to activate T cells
  Cell group B untreated
- 48 hrs post CD3/CD28 exposure both cell groups incubated with VisAcT
- Cells implanted subcutaneously
  left shoulder - naïve T cells
  right shoulder - activated T cells
- PET scanned

Courtesy: CellSight Technologies

[^{18}F]F-AraG in a Healthy Male



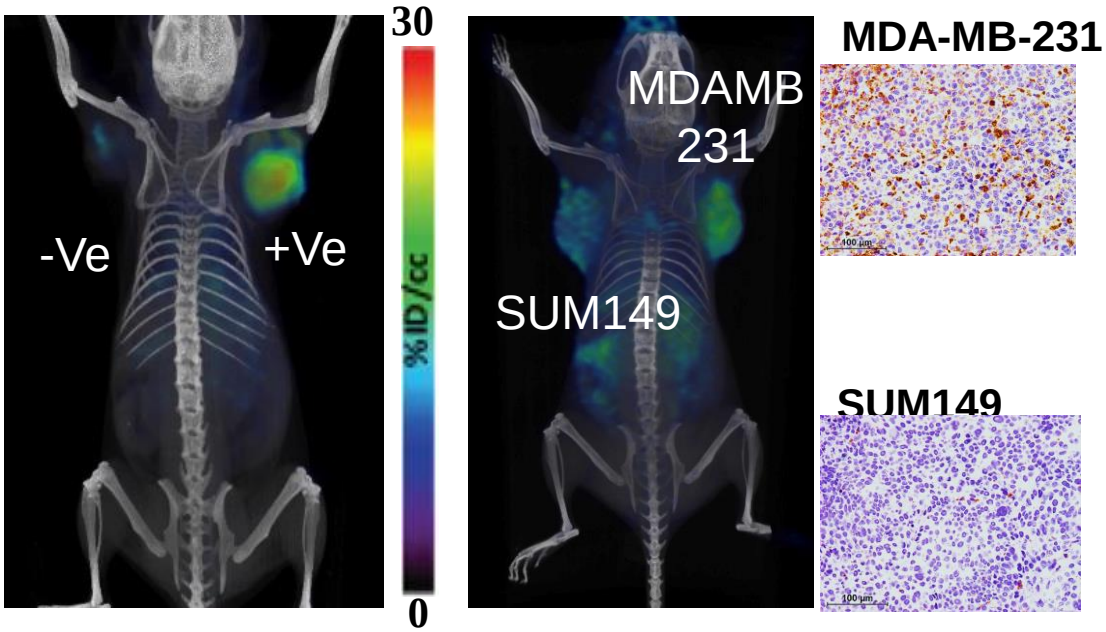
Images from 4 time points post VisAcT injection show ideal imaging characteristics with hepatobiliary and renal clearance

Courtesy: CellSight Technologies

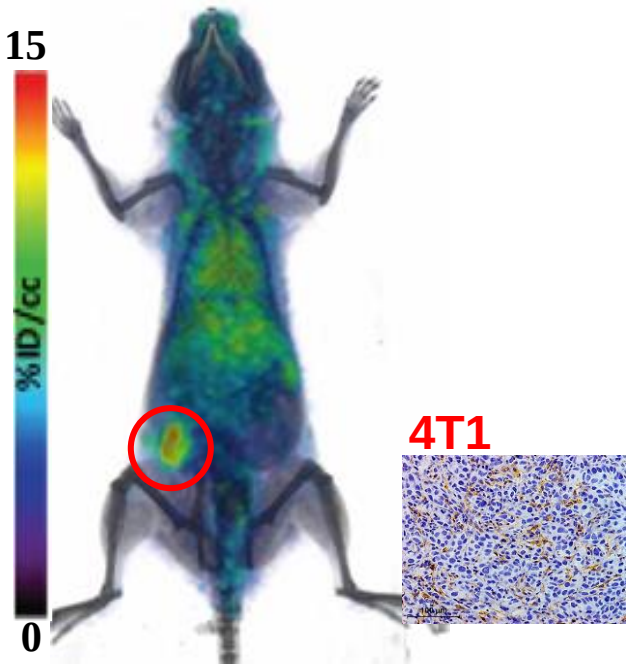
PD-L1 imaging with a humanized antibody
^{64}Cu-MPDL3280A-PET @ 24h

JOHNS HOPKINS MEDICINE

hPD-L1



mPD-L1



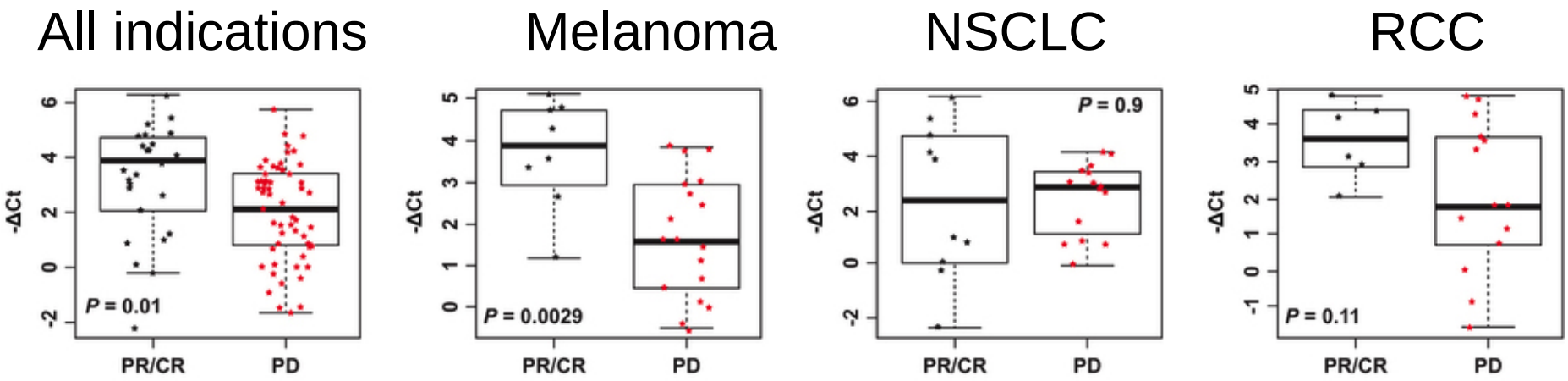
Human and mouse cross-reactive mAb

Chatterjee et al., Oncotarget, 2016
Chatterjee et al., unpublished

Chemokines/Chemokine receptors

- 48 chemokines and 20 chemokine receptors
- Involved in immune cell migration
- CXCL9, CXCL10, CCL5 and CXCL12 are emerging as important chemokines in the tumor microenvironment

Chemokines and immunotherapy response



CXCL9

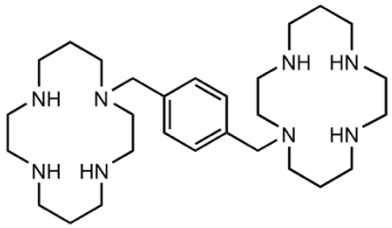
RS Herbst *et al. Nature* **515**, 563-567 (2014) doi:10.1038/nature14011

Chemokine Receptors

Expression and Function

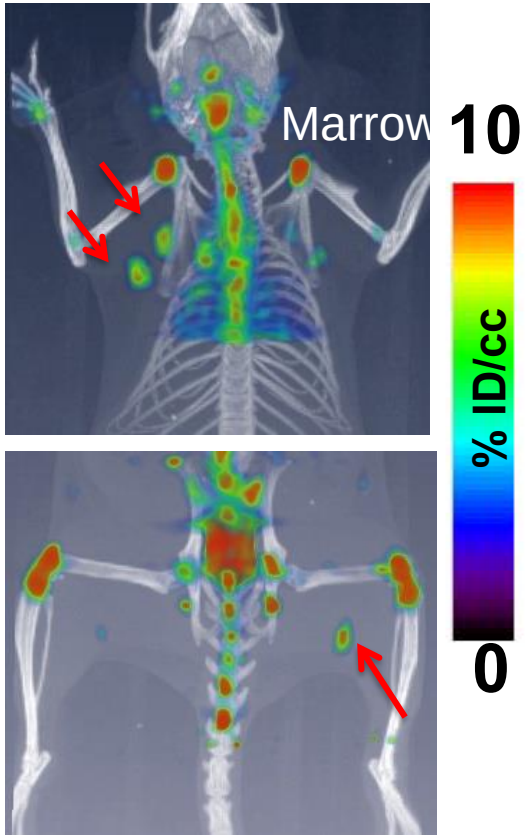
Receptor	Ligand	Expression	Function
CXCR3	CXCL9 CXCL10 CXCL11	Th1, CD8+ TCM and TEM, NK, NKT, pDC, B cell, Treg, Tfh	Th1-type adaptive immunity; Th1, CD8, NK trafficking
CXCR4	CXCL12	Most leukocytes	Hematopoiesis, organogenesis, bone marrow homing
CCR5	CCL5	Monocyte, macrophage, Th1, NK, Treg, CD8+ T, DC, neutrophil	Type 1 adaptive immunity Macrophage and NK cell migration; T cell–DC interactions

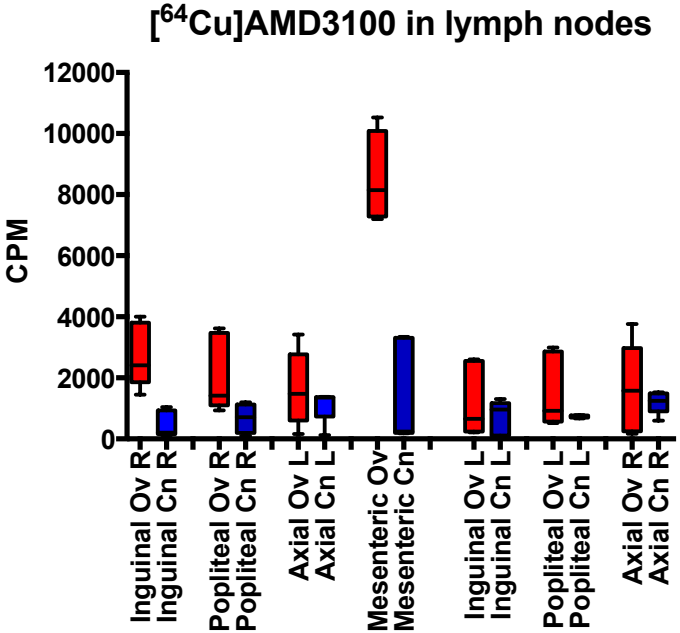
Imaging Ova induced immune response with a CXCR4 imaging agent [^{64}Cu]AMD3100



Plerixafor

IC_{50}: 651± 37nM



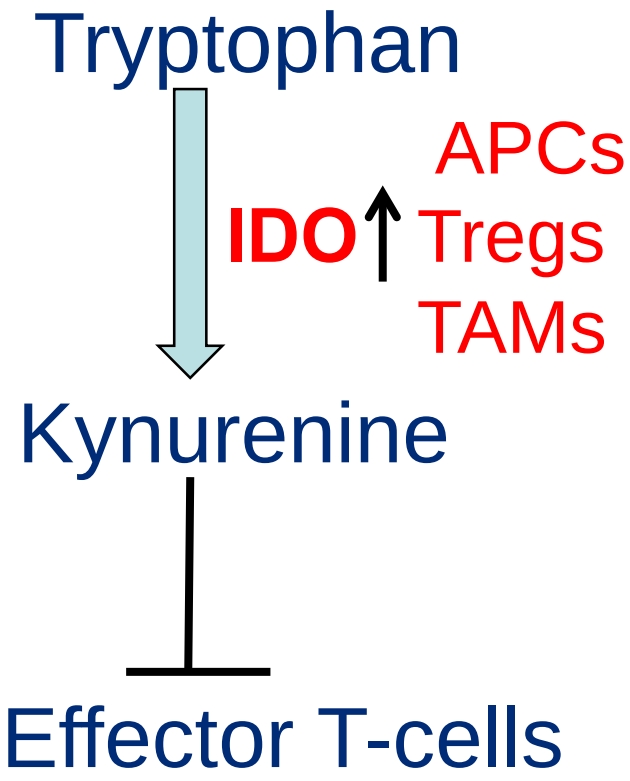


Imaging agents in the clinic (CPCR4-2)

What does change in tumor CXCR4 expression correlate with?

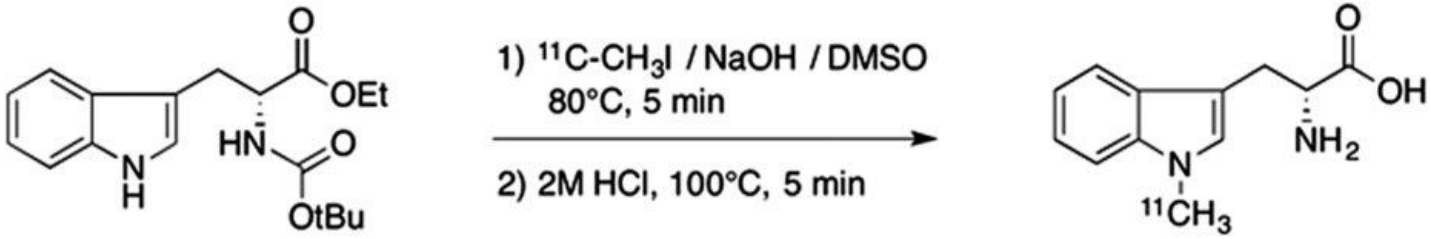
Indoleamine 2,3-dioxygenase (IDO1)

- Tumor induced tolerance is both acquired and active
- IDO catabolizes tryptophan
- Tryptophan metabolites blunt tumor immunity
- Deregulated in many cancers
- Small molecule inhibitors in clinical trials



Nat. Med. **9** (10): 1269–74. doi:10.1038/nm934

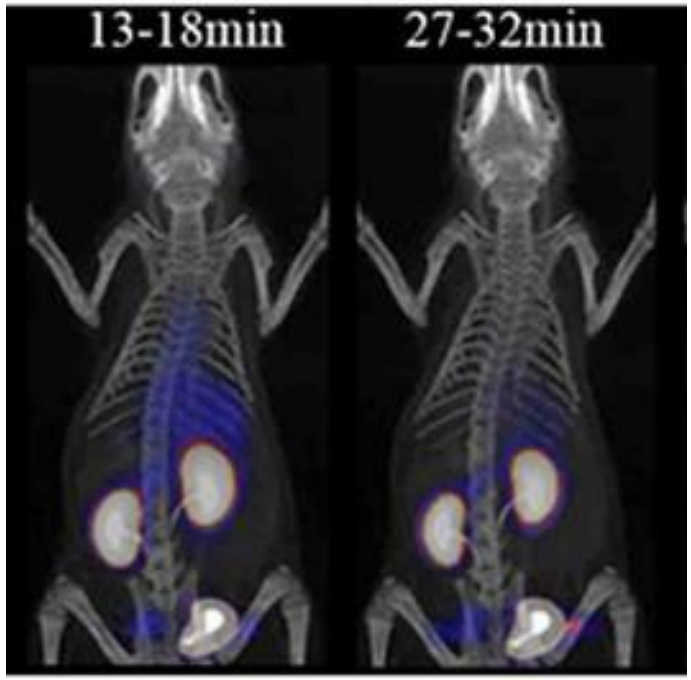
Imaging agents for IDO1



Boc-D-Trp-OEt

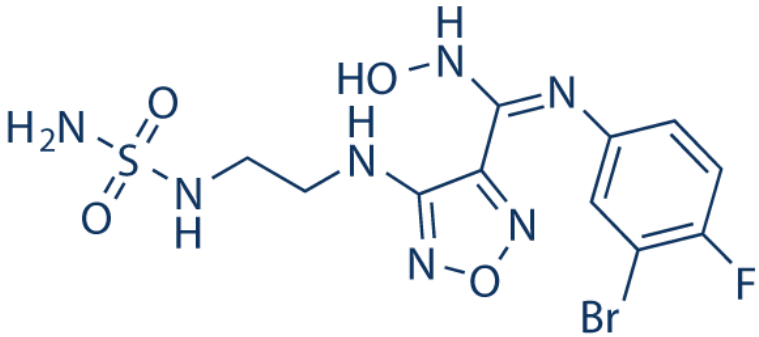
^{11}C-D-1MTrp

^{11}C-D-1MTrp

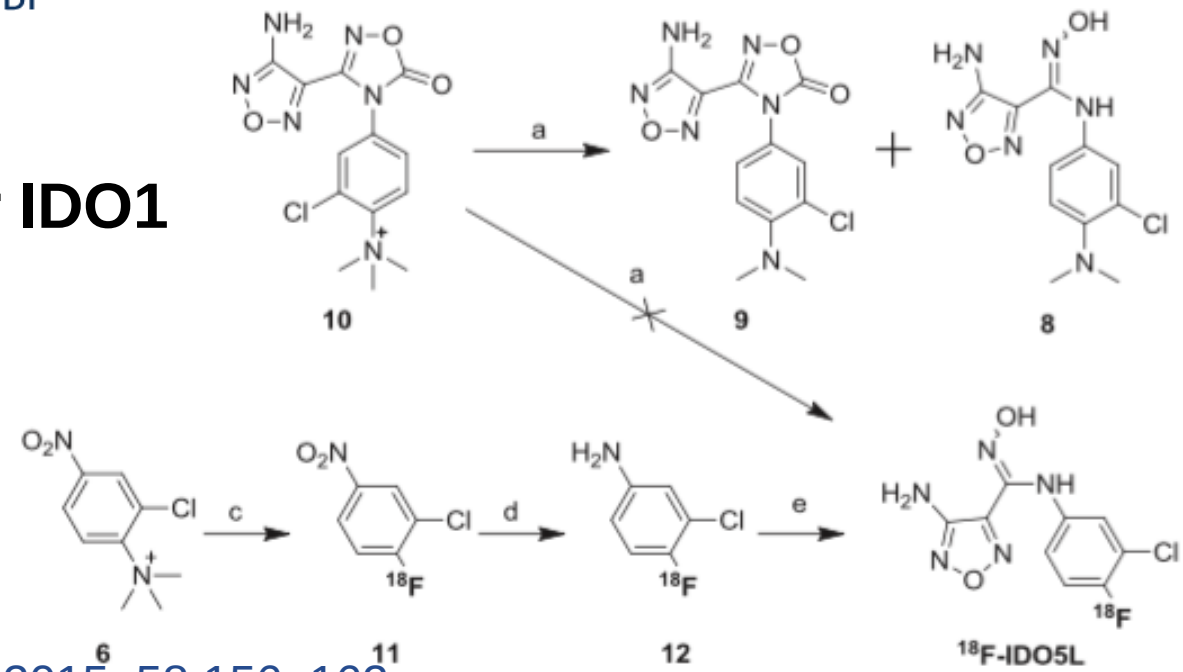


Scientific Reports **5**,
Article number: 16417 (2015)
doi:10.1038/srep16417

Imaging agents for IDO1



INCB024360
IC50=10 nM
High selectivity for IDO1



J. Label Compd. Radiopharm 2015, 58 156–162

Challenges

- A reliable biomarker for immunotherapy efficacy
- Dynamic nature of immune-tumor cell interactions→molecularly targeted imaging agent
- Most of the known targets are cell surface proteins
- Changes in target expression may not always correlate with changes in TME
- How to detect non-functional immune responses?
- Readily translatable
- Should we focus on two or more imaging markers?

Acknowledgements

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Leisha Emens, M.D., Ph.D.

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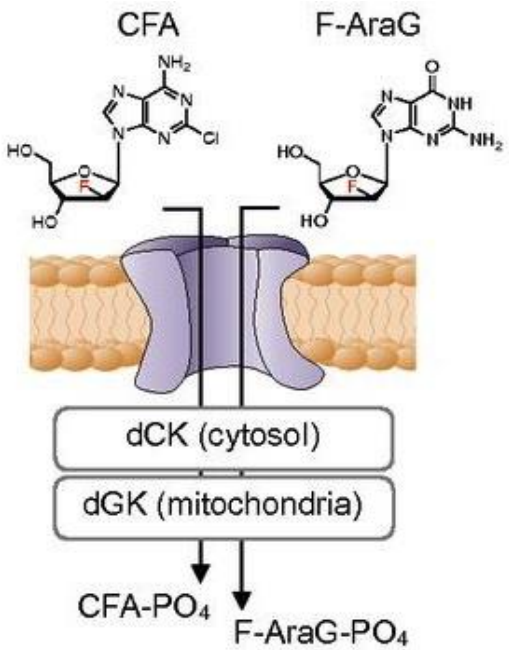
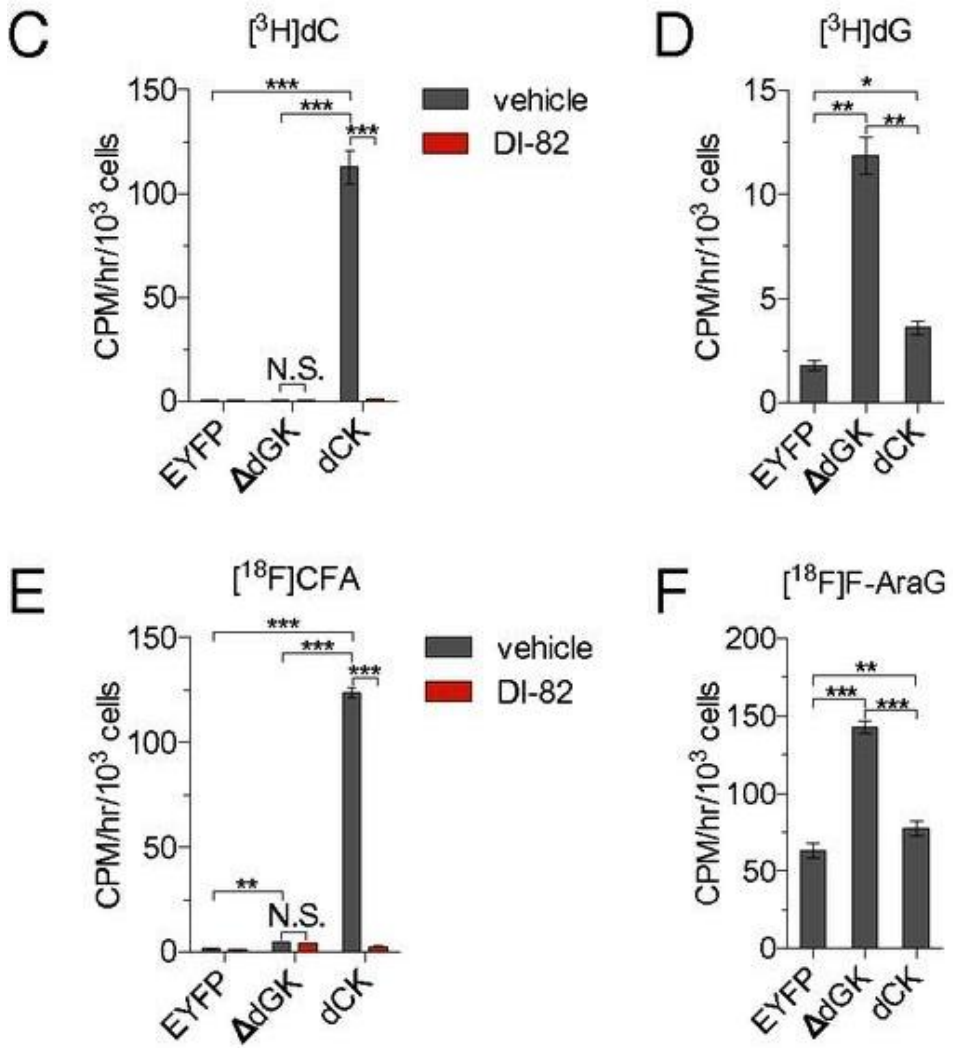
DOD BCRP Idea Award
JHU Career Catalyst Award
Hopkins-Allegheny Cancer Research Fund

Univ. of Wisconsin team for Cu-64 production

JOHNS HOPKINS
MEDICINE

[^{18}F]F-AraG accumulation in cells is dGK dependent





Woosuk Kim et al. PNAS 2016;113:4027-4032

National Cancer Institute

Activities and Opportunities in Cancer Immunotherapy at the NCI

Elad Sharon, M.D., M.P.H.

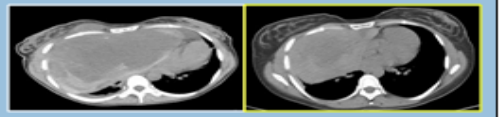
May 2, 2016

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

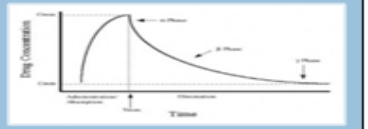
Clinical Translational Research and Cancer Biology: Bedside to Bench and Back

*Clinical observations:

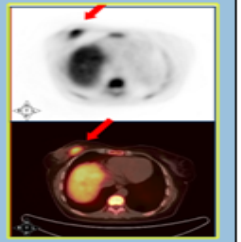
- Clinical response



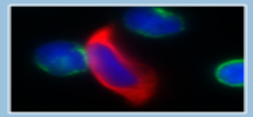
- PK



- Functional imaging



- Tumor and normal tissue PD markers
- CTCs, CECs



- Tumor-initiating cells

Patients eligible for early phase clinical trials

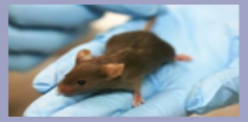
Analysis of tumor and other tissues for pathway activation or biomarker *

Patient assigned to trial based on molecular characterization of tumor

Patient monitoring *

Patient monitoring: Post-treatment molecular re-analysis for response/ resistance *

Non-clinical models for targets

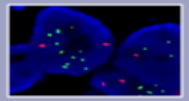


Translational research with clinical models

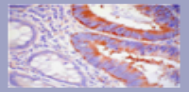
- Sequencing

- Methylation

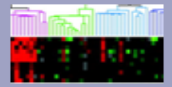
- FISH



- IHC



- Expression array



PMI – Oncology includes Immunotherapy

Rationale:

- **Precision Medicine Initiative (PMI) -Oncology - 4 parts**
  - **Clinical trials to advance precision oncology**
    - **Advanced sequencing for NCI-MATCH**
    - **Pediatric MATCH**
    - **Expand immunotherapy trials—combinations, molecular characterization, reagents**
  - **Develop better pre-clinical models for cancer treatment**
  - **Overcome therapeutic resistance in the clinic**
  - **Knowledge system for precision oncology**

National Cancer Institute

Inventory of NCI Funding for Cancer Immunology and Immunotherapy in Fiscal Year 2014

Definition of "Immunotherapy" used in this inventory –

- **Agents with the primary MOA mediated through modulation of cancer immunity and effected through the immune system/cells (e.g. cytokines, check point inhibitors, vaccines, adoptive cell therapy)**
- **Antibodies or agents directed at tumor cell targets/angiogenesis, with the primary MOA uncertain, or mediated through signal transduction or cytotoxic payload were NOT included in this analysis (e.g. bevacizumab, trastuzumab, immunotoxin, radioimmunotherapy)**

National Cancer Institute

NCI Extramural Funding for Immunotherapy – An inventory of projects funded in FY 2014

Single-project grants (# of grants)

	All grants[1, 2, 3]	Grants related to Immunotherapy	% for immunotherapy
DCB (Division of Cancer Biology) - ***Mostly basic science***	**1894**	**114**	**6%**
DCTD (Division of Cancer Treatment and Diagnosis) **- *Translational and clinical***	**1486**	**196**	**13%**
SBIR (Small Business Innovation Research Program)	**171**	**20**	**12%**
CCT (Center for Cancer Training) **- Training and Career Development Awards**	**977**	**79**	**8%**
DCP (Division of Cancer Prevention)	**391**	**4**	**1%**

1. Not included in this Table: Type 3's
2. Not included in this table – Multi-project grants - P01, P20, P30, P50, U19, U54, U10, UG1, UM1
3. Primary IC=CA

National Cancer Institute

NCI Extramural Funding for Immunotherapy – A list of projects funded in FY 2014

Multi-project grants or funding mechanisms

	All grants/subprojects	Immunotherapy	% for ImmunoRx
SPORE (P50)*	52 grants 209 subprojects	26 with ImmunoRx 49 for ImmunoRx	50% 23%
Program Project Grant (P01)	109 grants 708 subprojects	24 with ImmunoRx 66 with ImmunoRx	22% 9%
CTEP Clinical Trial Network New trials opened in 2014-2015	170 Trials (Phase 3 : 47 trials)	37 for ImmunoRx (Phase 3: 7 trials)	22% (15%)

*SPORE grants are based on FY 2015

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NCI Intramural (CCR) Projects on Immunotherapy – FY 2014

- **168 of 739 (23%) Intramural Research Projects (IRPs) were identified as being relevant to immunotherapy**

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Immunotherapy Trials in CTEP Clinical Trial Networks

CTEP Clinical trial network:

- NCTN (Cooperative Groups)
- ETCTN (Early clinical trials)
- CITN Cancer Immunotherapy Trials Network,
- Disease specific consortia (ABTC, PBTC)

		ImmunoRx	% of ImmunoRx
All CTEP trials	# of clinical trials	1274	12%
	(Phase 3)	*(128)*	*(6%)*
Before 2000	# of clinical trials	1002	12%
	(Phase 3)	(111)	(6%)
Activated between 2000-2009	# of clinical trials	184	8%
	(Phase 3)	*(10)*	*(3%)*
Activated between 2010-2013	# of clinical trials	51	9%
	(Phase 3)	*(2)*	*(3%)*
Activated between 2014- 2015	# of clinical trials	37	22%
	(Phase 3)	*(7)*	*(15%)*

*Trials without therapeutic interventions are excluded from the analysis

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Recent NCI-Supported Immunotherapy Trials

Between 2010 -2015

- **88 Phase I-III immunotherapy trials were activated in the DCTD Clinical Trial Network** (NCTN, ETCTN, CITN, and PBTC)
- **9 Phase III trials, 14 Randomized Phase 2 trials**
- **Clinical settings:** common, rare tumors; neoadjuvant, adjuvant and metastatic disease
- **Study regimens include single agent and novel combinations**

*Most randomized trials have mandatory collection of baseline tissues/blood
*Many early clinical trials include serial biopsies

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Immunotherapy agents under CRADA agreement with CTEP

(a partial list)

Check point inhibitors:

- **Anti- CTLA-4 (Ipilimumab)**
- **Anti-PD-1 Nivolumab, Anti-PD-1 Pembrolizumab**
- **Anti-PD-L1 (MEDI4736 and MPDL3280A)**

Cytokines:

- **IL-15**
- **IL-12**
- **Others:**

T-cell engaging bispecific antibody:

- **CD19 BiTE (Blinatumomab)**

Vaccines:

- **CDX1401** (against NY-ESO-1)
- **PSA PROSTVAC/TRICOM**
- **CEA TRICOM/PANVAC**
- **Other:** peptide (gp100, HPV, RAS, P53, MART and others)

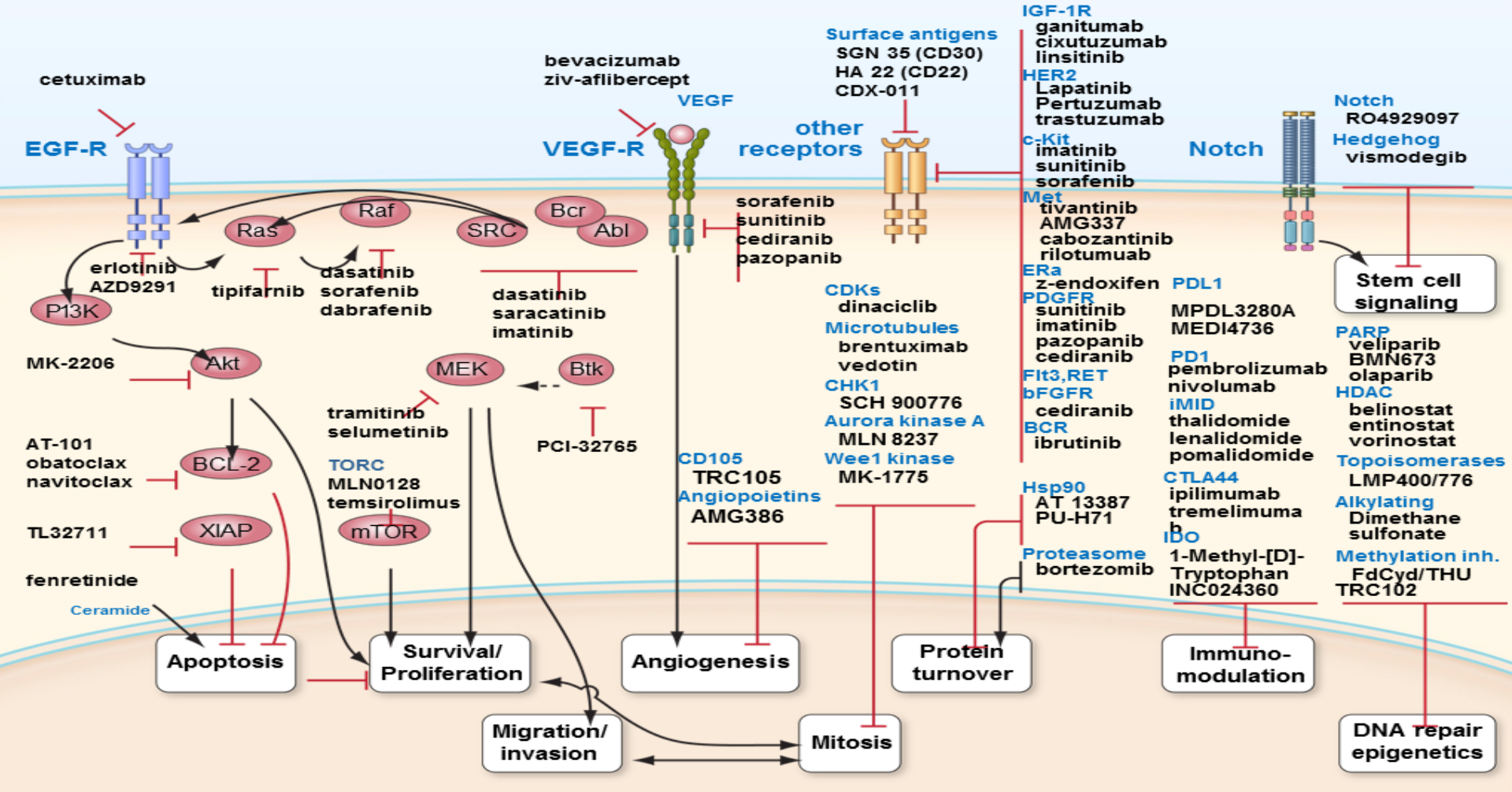
Other immune modulators:

- **IDO (INDB0243360) ~** 2 trials
- **Lenalidomide, Pomalidomide**: - not counted in the analysis
  FLT3 ligands
- **Anti-CD27 mAb (CellDex)**

Types of trials sponsored by CTEP:

- Rare indications
- Special populations (Pediatric, HIV)
- Novel combinations
- Phase III and registration trials
- Biomarkers as the primary endpoints

High Priority Targets and DCTD/CTEP Agents



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CTEP by the Numbers

- CTEP sponsors over 120 INDs
- Approximately 18,000 registered investigators at 3,300 institutions in the US and internationally
- Over 900 active protocol
  - 140 new protocols per year
  - Approximately 33,000 patients accrued per year
- Largest sponsor of cancer-related combination studies
  - Two-thirds of all combination studies in clinicaltrials.gov are CTEP-sponsored
- Over 100 collaborative agreements (CRADAs, CTAs, agent-CRADAs, and CSAs) with pharmaceutical companies

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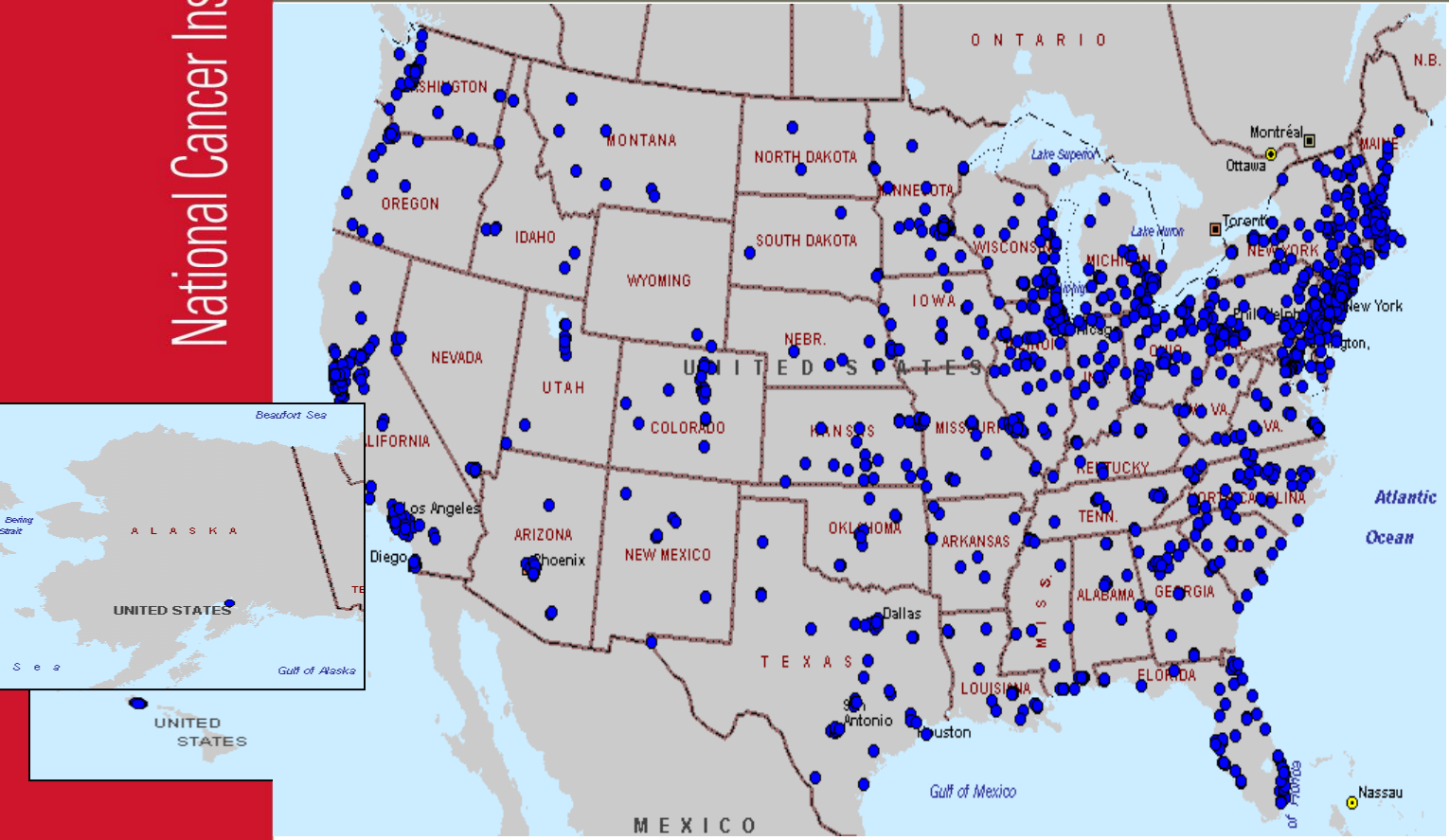
Cooperative Group Sites in US



3,300 Institutions

ECOG-ACRIN

SWOG

Alliance

NRG

COG

close collaboration with NCIC

Accrual Distribution:

Phase 3: 83.4%

Phase 2: 15.1%

Phase 1/Pilot: 1.5%

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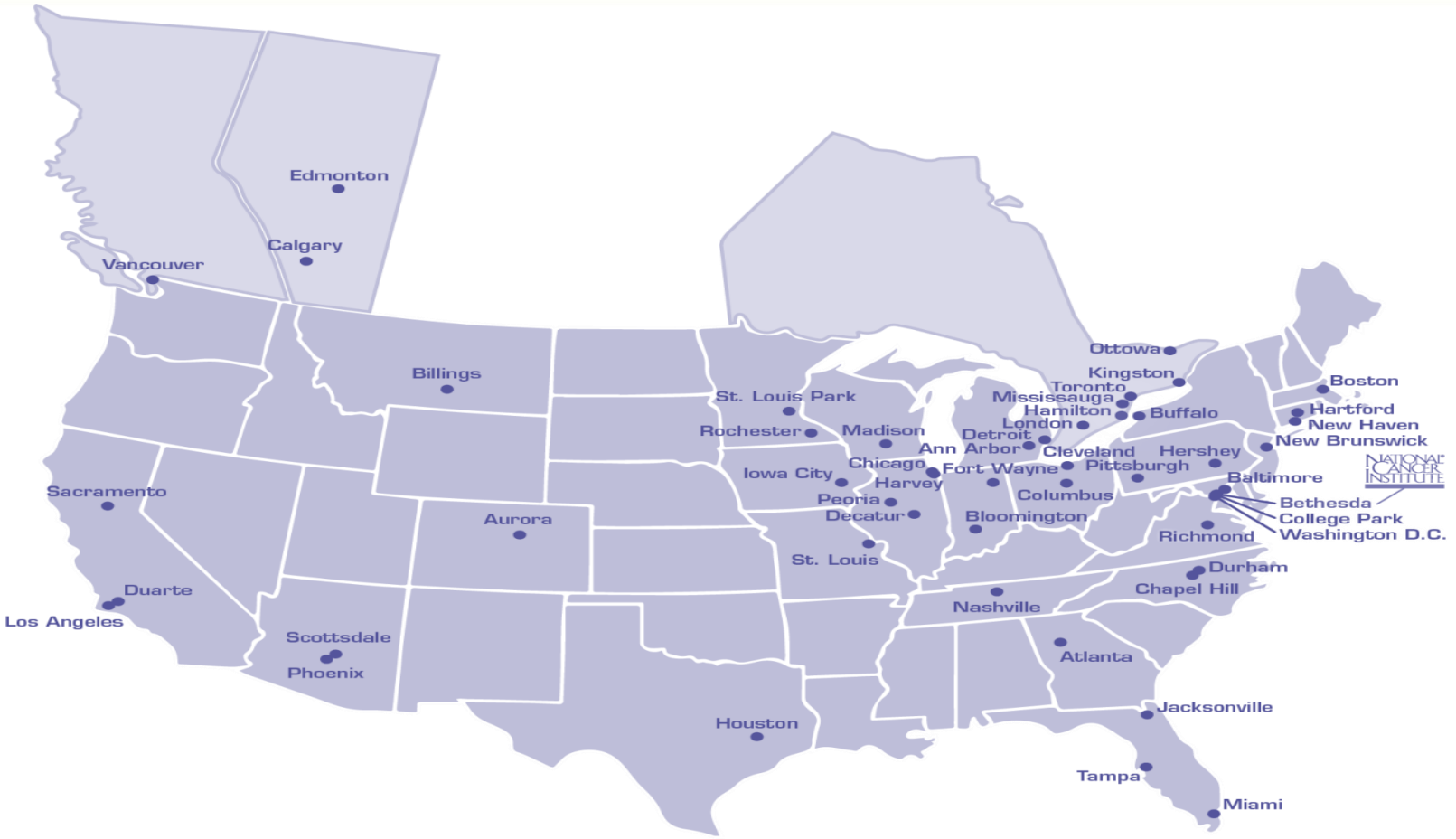
NCI Early Phase Drug Development Program: ETCTN



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Other Significant CTEP-Affiliated Groups

- NCI Intramural Research Program (IRP)
- Adult Brain Tumor Consortium (ABTC)
- Pediatric Brain Tumor Consortium (PBTC)
- Cancer Immunotherapy Trials Network (CITN)
- AIDS Malignancy Consortium (AMC)
- Agreements with France (INCa), South Korea, Japan, and Taiwan
  - NCI CGH has been instrumental in developing and maintaining these relationships

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Immunotherapy: A Rapidly Growing Part of NCI portfolio

- New agents being added to portfolio regularly
- Engaged with critical industry and academic stakeholders at the forefront of the field
- Efficient use of taxpayer dollars, with major results coming from small trials, shifting our understanding of immunotherapy
- Judicious use of Phase 3 trial resources to focus on critical unmet medical needs, which industry cannot or will not address on its own

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Biomarkers are critical to further development of cancer immunotherapy

- Immunotherapy has remarkable activity in many tumor types, but for most only a minority of patients benefit
  - Response rate to anti-PD1: Melanoma (30%); Renal cancer (20%), Lung ca (15-20%)
  - Even with ipilimumab-nivolumab in melanoma: ORR was 57%
  - Highest response rate thus far seen in Merkel Cell Cancer (NCI-sponsored trial, published in *NEJM*)
- Some tumors do not respond (pancreatic cancer, microsatellite stable colon cancer, myeloma) Mechanisms of intrinsic resistance poorly understood
- Combination is a potential strategy to improve outcome, however:
  - Which combination should be given to which patients to induce synergistic effect?
  - What is the optimal dose, sequence and schedule?
  - Understanding of pattern of immune receptors, tumor microenvironment and molecular characteristic of the tumor is critical to develop rational combinations.

Potential role of immune biomarker studies in the context of clinical trials:

- Explore and validate predictive markers of response and toxicity
- Reveal mechanisms of actions/resistance of individual agents, and guide selection of partners for combination regimens
- Enhance the understanding of cancer immunobiology

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Small trials with a big impact

- **ETCTN Trial of ipilimumab in patients who had already failed bone marrow transplant**
  - **First evidence of in patients with AML or in the post-transplant setting, including 5 complete responses**

DANA-FARBER CANCER INSTITUTE

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Home > Newsroom > News Releases > Immunotherapy shows clinical benefit in relapsed transplant recipients

Immunotherapy shows clinical benefit in relapsed transplant recipients

Like 0 Tweet Share 5

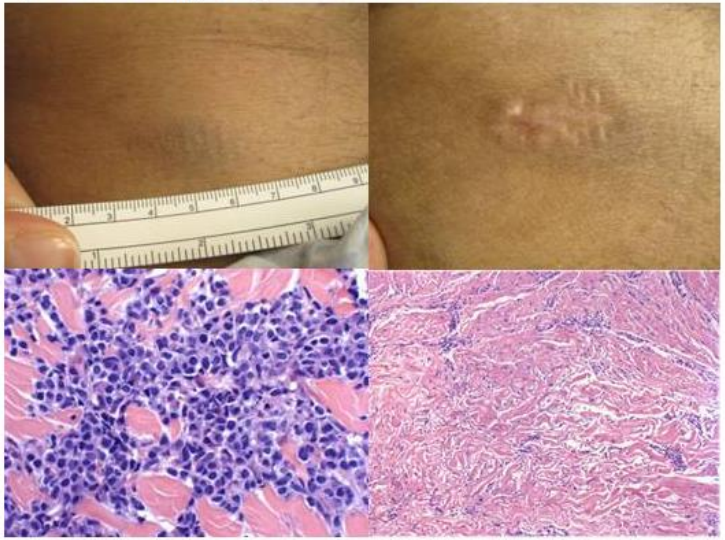
December 08, 2014 | **Tags:** MultipleMyeloma, Leukemia, Immunotherapy

A multicenter phase 1 trial of the immune checkpoint blocker ipilimumab found clinical benefit in nearly half of blood cancer patients who had relapsed following allogeneic stem cell transplantation, according to investigators from Dana-Farber Cancer Institute, who developed and lead the study.

The study reported at the American Society of Hematology annual meeting is the first in which ipilimumab was given in multiple doses over an extended time period, the researchers said.

At a median follow-up time of six months, "We have seen less toxicity than expected and a strong efficacy signal, and that's very encouraging in a population that does not have many therapeutic options," said Matthew S. Davids, MD, medical oncologist at Dana-Farber,

Figure 1. Clinical and histopathologic responses after 13 weeks of ipilimumab therapy in a patient with leukemia cutis.



Baseline **13 weeks**

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Small trials with a big impact

- **CITN Trial Merkel Cell Cancer (published online in NEJM on 4/19/16) had highest rate of response of any solid tumor**
  - **Responses in Merkel Cell Cancers that were virally-mediated as well as those that were non-viral**

The NEW ENGLAND JOURNAL of MEDICINE

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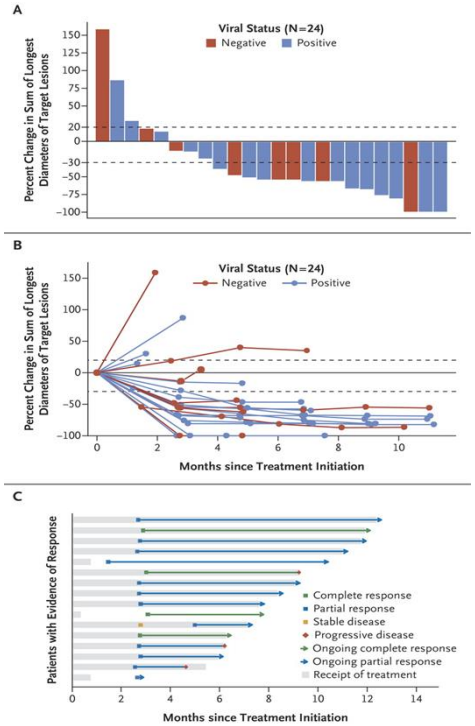
ORIGINAL ARTICLE

PD-1 Blockade with Pembrolizumab in Advanced Merkel-Cell Carcinoma

Paul T. Nghiem, M.D., Ph.D., Shailender Bhatia, M.D., Evan J. Lipson, M.D., Ragini R. Kudchadkar, M.D., Natalie J. Miller, B.A., Lakshmanan Annamalai, D.V.M., Ph.D, Sneha Berry, M.S., Elliot K. Chartash, M.D., Adil Daud, M.B., B.S., Steven P. Fling, Ph.D., Philip A. Friedlander, M.D., Harriet M. Kluger, M.D., Holbrook E. Kohrt, M.D., Ph.D., Lisa Lundgren, M.S., Kim Margolin, M.D., Alan Mitchell, M.Sc., Thomas Olencki, D.O., Drew M. Pardoll, M.D., Ph.D., Sunil A. Reddy, M.D., Erica M. Shantha, M.D., William H. Sharfman, M.D., Elad Sharon, M.D., M.P.H., Lynn R. Shemanski, Ph.D., Michi M. Shinohara, M.D., Joel C. Sunshine, M.D., Ph.D., Janis M. Taube, M.D., John A. Thompson, M.D., Steven M. Townson, Ph.D., Jennifer H. Yearley, D.V.M., Ph.D., Suzanne L. Topalian, M.D., and Martin A. Cheever, M.D.

April 19, 2016 | DOI: 10.1056/NEJMoa1603702



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Large Trial Efforts: NCTN Trials with Immunotherapy

- **Blinatumomab is a bispecific T-cell engaging (BiTE) antibody, now approved in relapsed Acute Lymphoblastic Leukemia (ALL)**
  - BiTE technology acts as an off-the-shelf version of adoptive cell transfer: BiTEs form a link between T cells and tumor cells and exerts an effect independent of the presence of MHC I or co-stimulatory molecules
  - Three trials, including two registration trials in NCTN
    - Up-front use in conjunction with chemotherapy for adults
    - Children with ALL and high-risk features
- Variety of Adjuvant Trials now underway or planned in bladder cancer, lung cancer, melanoma, head and neck cancer, renal cell cancer, and brain cancer in Phase 1, 2, and 3 settings in NCTN
  - Developing immunotherapy arms for MATCH and cognate trials for unmatched, but sequenced patients

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Examples of Phase 2/3 Trials in Melanoma

- Ipilimumab and bevacizumab randomized trial exploring angiogenesis/immunotherapy
- Nivolumab/Ipilimumab +/- GMCSF, following up on evidence of cytokine augmentation of checkpoint inhibitor effect
- Sequencing trial exploring BRAF/MEK inhibitor therapy versus combination nivolumab-ipilimumab
- Adjuvant trial of pembrolizumab versus ipilimumab or high-dose interferon for resected Stage 3 or 4 patients

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NCI-Supported Immunotherapy Trial in Pediatric Patients

- **A Phase I clinical trial of ipilimumab was conducted in pediatric patients with advanced solid tumors**
  - First time a checkpoint inhibitor was studied in children
  - Safety shown
  - Results: No objective responses were seen using this monotherapy but subjects with immune-related toxicities had an increased overall survival compared with those who showed no evidence of breaking tolerance
  - Future studies: combination immunotherapy strategies in pediatric patients

Published OnlineFirst November 3, 2015; DOI: 10.1158/1078-0432.CCR-15-0491

Cancer Therapy: Clinical

Clinical Cancer Research

Phase I Clinical Trial of Ipilimumab in Pediatric Patients with Advanced Solid Tumors

Melinda S. Merchant[1], Matthew Wright[1], Kristin Baird[1], Leonard H. Wexler[2], Carlos Rodriguez-Galindo[3], Donna Bernstein[1], Cindy Delbrook[1], Maya Lodish[4], Rachel Bishop[5], Jedd D. Wolchok[6,7], Howard Streicher[8], and Crystal L. Mackall[1]

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Correlating Imaging with Biopsies

- Trials available in all CTEP networks
- NCI Intramural Program and ETCTN are both funded for obtaining biopsies, and a variety of immunotherapy trials are currently planned and ongoing
- ABTC/PBTC have immunotherapy trials focused on gliomas, which can be biopsy-driven
- Variety of industry and academic partners, interested in utilizing our expertise and networks

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MPACT: Precision Medicine in ETCTN

- Randomized trial of treatment of patients according to molecular mutations vs. a more traditional approach
- Now undergoing expansion across all centers in ETCTN
- Immunotherapy arm being contemplated, which would treat screened patients without molecular targets
- Perfect timing to discuss a potential CTEP/CIP collaboration

Immunology Overview and Imaging's Current Role

Immune Modulation Therapy and Imaging:

What can we do in clinical trials now?

Monday May 2, 2016: 8:00 am – 5:30 pm

National Cancer Institute Shady Grove

Disclosures

Consulting

- Genentech-Roche, Bristol-Myers, Astra-Zeneca/Medimmune, Pfizer, Novartis , Kyowa-Kirin, Immune Design, Prometheus, Nektar, Pierre-Fabre, Lilly, Merck, Alexion, Theravance, Biodesix, Vaccinex, Janssen/Johnson and Johnson

Scientific Advisory Board (paid)

- Symphogen, Lion Biotechnologies, Amphivena (Stock options only), Adaptive Biotechnologies (stock options only), Intensity (stock options only), Lycera, Adaptimmune

Cancer Cell Antigens:
Mutations
Aberrant expression of developmental proteins
Tissue differentiation proteins
Stem cell ‘drivers’

Professional antigen presenting cells:
Dendritic cells (DC)

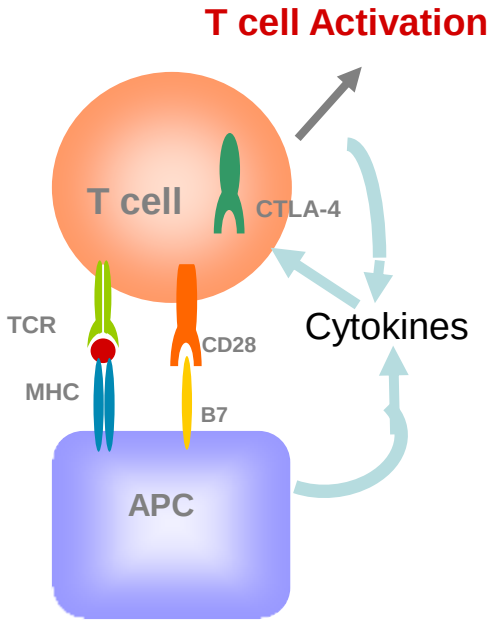
DC process proteins to peptides
Peptides bind to MHC molecules
Peptide-MHC complex presentation of antigen to T-cells

T-cell activation,
proliferation

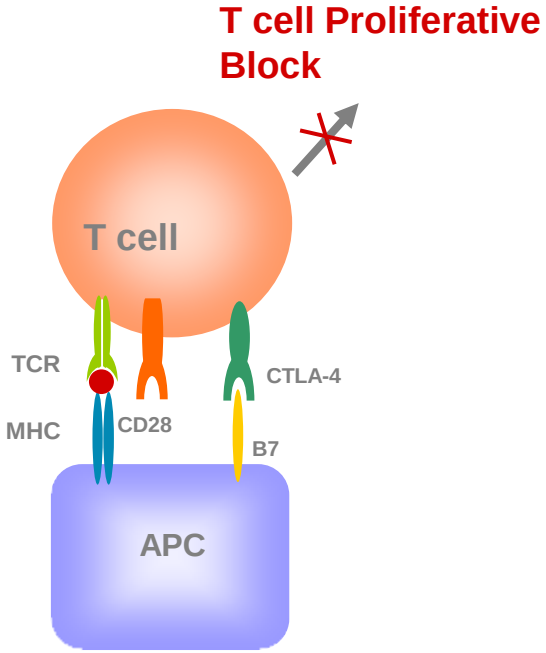
T-cells ‘find tumor’, kill cells
or secrete cytokines
to create anti-tumor
inflammatory response

T-cell Activation, Proliferation, and Function is Controlled by Multiple Agonist and Antagonist Signals

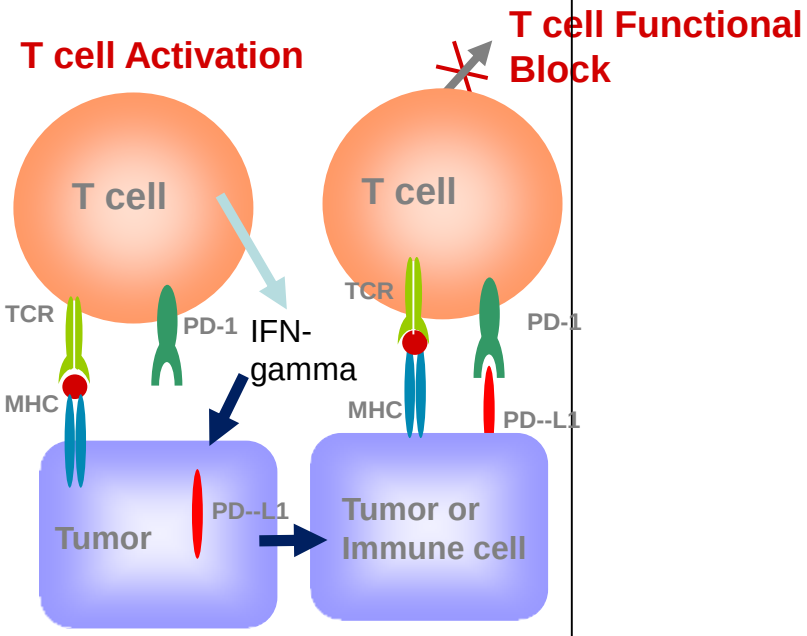
1. Co stimulation via CD28 ligation transduces T cell activating signals



2. CTLA 4 ligation on activated T cells down regulates T cell responses



3. T cell function in tissue is subject to feedback inhibition



Antigen Presenting Cell or Tumor	T lymphocyte	Function (excluding Treg)
Peptide-MHC	T cell receptor	Signal 1
CD80/CD86 (B7.1, B7.2)	CD28/CTLA-4	Stimulatory/inhibitory
CEACAM-1	CEACAM-1	inhibitory
CD70	CD27	stimulatory
LIGHT	HVEM	stimulatory
HVEM	BTLA, CD160	inhibitory
PD-L1 (B7-H1)	**PD-1** and CD80	Inhibitory (Th1)
PD-L2 (B7-DC)	PD1 and ?	Inhibitory (Th2) or stimulatory
OX40L	OX40	stimulatory
4-1BBL	CD137	stimulatory
CD40	CD40L	Stimulatory to DC/APC
B7-H3	?	Inhibitory or stimulatory
B7-H4	?	inhibitory
PD-1H (Vista)	?	inhibitory
GAL9	TIM-3	inhibitory
MHC class II	LAG-3	inhibitory
B7RP1	ICOS	stimulatory
MHC class I	KIR	Inhibitory or stimulatory
GITRL	GITR	stimulatory
CD48	2B4 (CD244)	inhibitory
HLA-G, HLA-E	ILT2, ILT4; NKG2a	inhibitory
MICA/B, ULBP-1, -2, -3, and -4+-	NKG2D	Inhibitory or stimulatory
CD200	CD200R	inhibitory
CD155	TIGIT/CD226	Inhibitory/stimulatory

Other Inhibitory Factors

IDO

Treg

MDSC

Macrophages

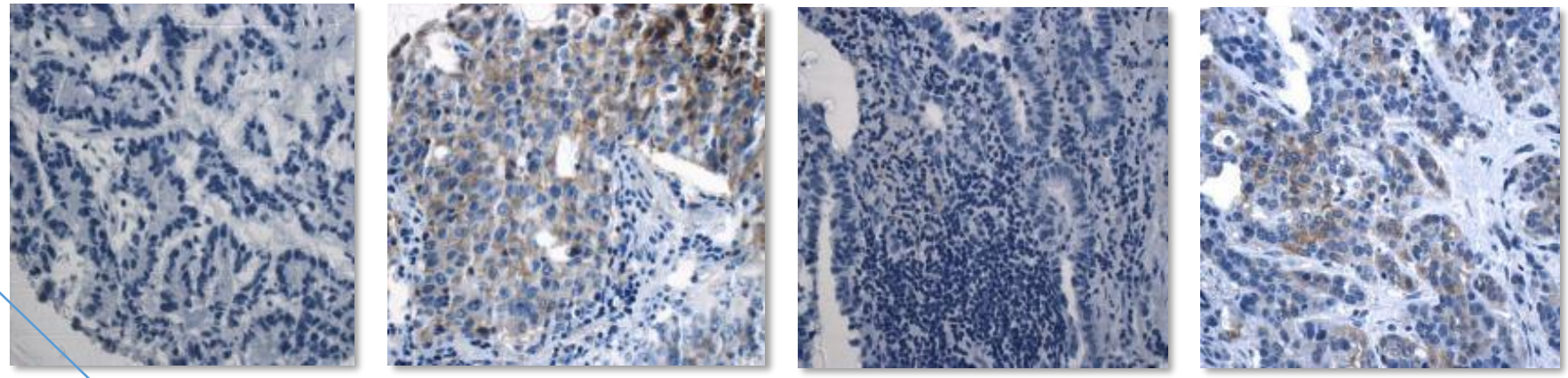
TGF-beta

IL-10?

VEGF

Presence of PD-L1 or TILs[1]

PD-L1⁻/TIL⁻	PD-L1⁺/TIL⁺	PD-L1⁻/TIL⁺	PD-L1⁺/TIL⁻



Schalper and Rimm, Yale University

NSCLC

45% Type 1	17% Type 2	26% Type 3	12% Type 4
45%	41%	13%	1%

Melanoma

Table 2. Correlation of B7-H1 expression by melanocytes with the presence of immune cell infiltration.

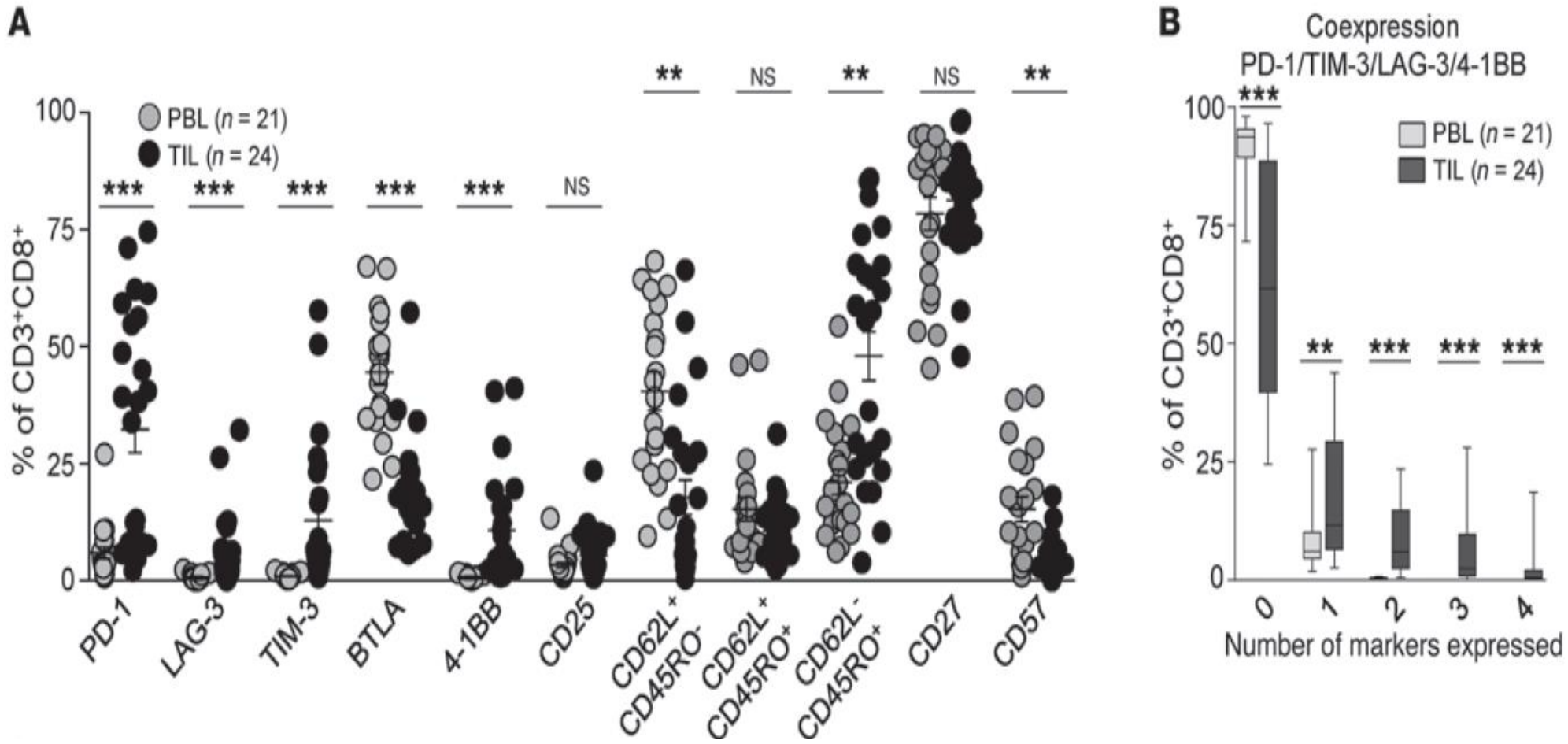
Histology	Total	Number of cases/total cases (%)				P*
		B7-H1⁺†		B7-H1⁻		
		TIL⁺‡	TIL⁻	TIL⁺	TIL⁻	
Benign nevi	40	14/14 (100)	0/14 (0)	4/26 (15)	22/26 (85)	<0.0001
Primary melanomas (in situ or invasive)	54	19/19 (100)	0/19 (0)	15/35 (43)	20/35 (57)	<0.0001
Metastases	56	23/24 (96)	1/24 (4)	7/32 (22)	25/32 (78)	<0.0001
All	150	56/57 (98)	1/57 (2)	26/93 (28)	67/93 (72)	<0.0001

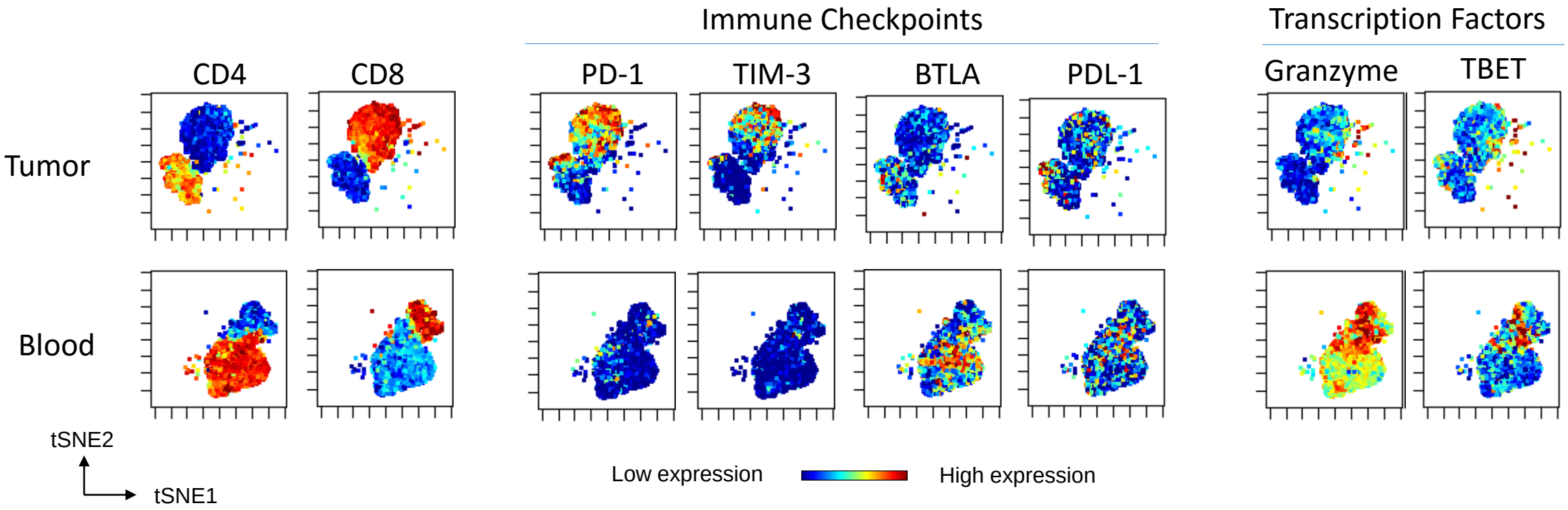
*Fisher's exact test, two-sided, was conducted on the 2 × 2 matrix defined by B7-H1 (±) expression and TIL (±) for each lesion type. †More than 5% melanocytes with membranous expression on IHC. ‡Including mild, moderate, and severe lymphocyte infiltrates and their associated histiocytes/macrophages.

Taube et al

Melanoma TIL – Expression of Co-inhibitory and Co-stimulatory Receptors (Gros et al)

The Journal of Clinical Investigation http://www.jci.org Volume 124 Number 5 May 2014

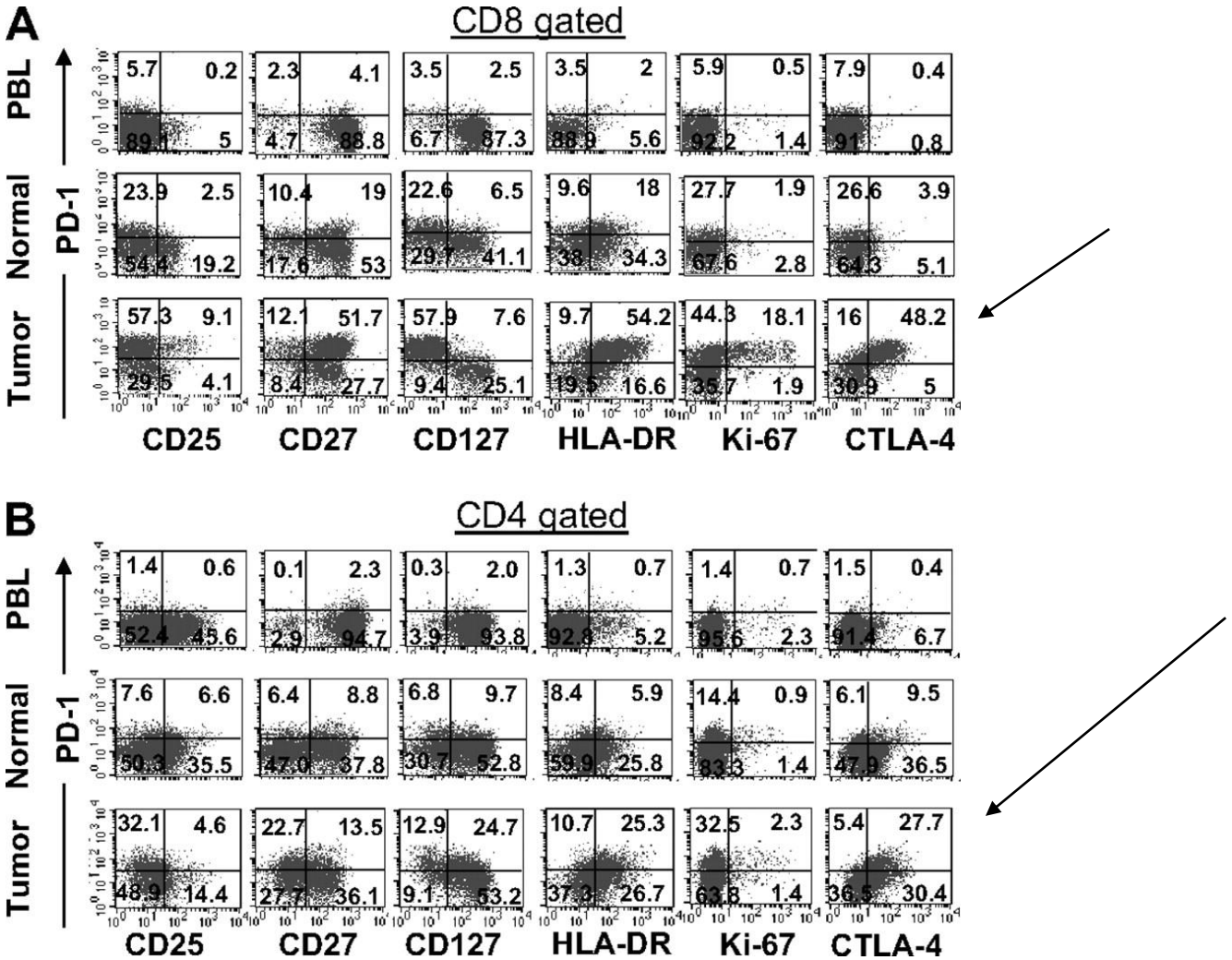




CD3 ViSNE

Provided by Kavita Dhodapkar, Yale University

Phenotypic comparison of CD8 and CD4 T cells infiltrating into tumor, normal tissue, and peripheral blood in the same patient.



Mojgan Ahmadzadeh et al. Blood 2009;114:1537-1544

Cytokine Production in TIL vs PBL in Metastatic Melanoma

2a

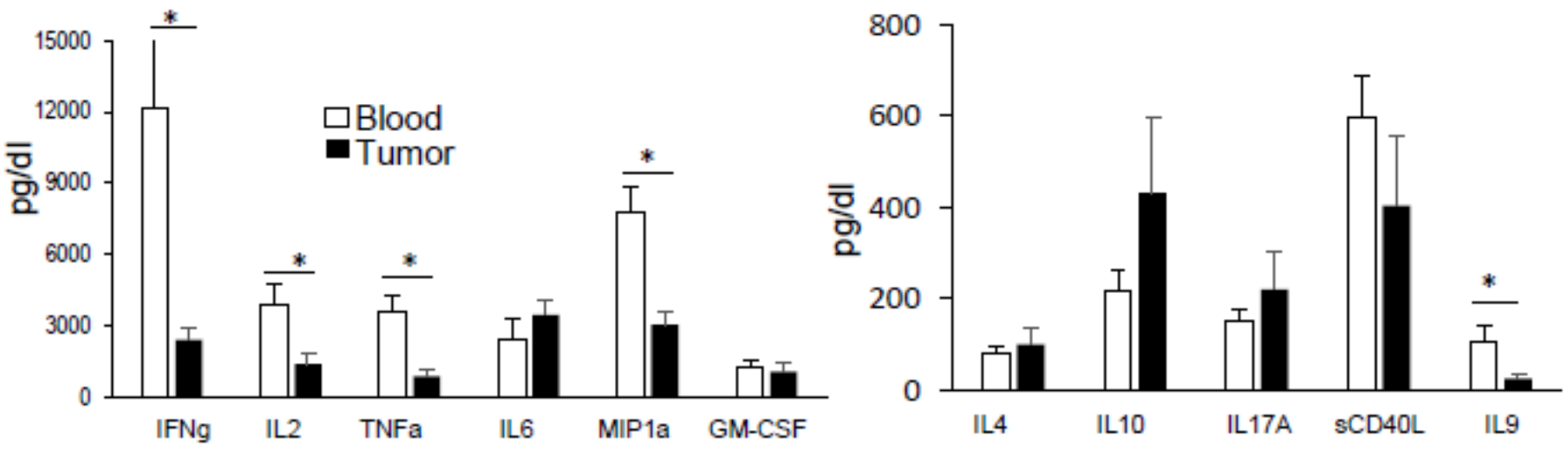


Figure shows Th1, Th2 and Th17 cytokines that were secreted by peripheral blood lymphocytes (n=15) and tumor tissue (n= 41), when incubated with anti-CD3/28 beads. Bar graph shows mean and standard error of mean (*; $p<0.05$)

Data provided by Kavita Dhodapkar, Yale University

Tumor antigen–specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired

(Blood. 2009;114:1537-1544)

Mojgan Ahmadzadeh,[1] Laura A. Johnson,[1] Bianca Heemskerk,[1] John R. Wunderlich,[1] Mark E. Dudley,[1] Donald E. White,[1] and Steven A. Rosenberg[1]

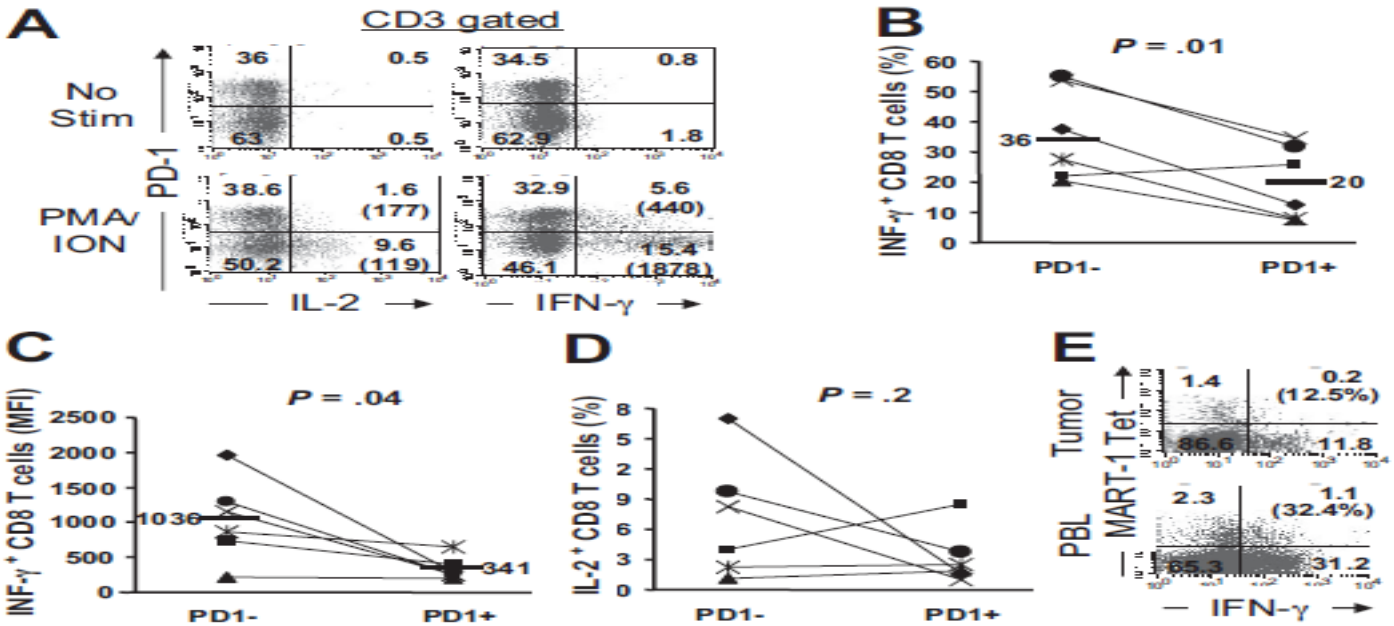


Figure 6. PD-1 expression on tumor-infiltrating T cells correlates with impaired effector function. Tumor digests and peripheral blood sample from patients with metastatic melanoma were thawed and immediately stimulated with PMA/I for 6 to 8 hours in the presence of monensin. Cells were subsequently stained with anti-CD3, anti-CD8, and anti–PD-1 mAb along with anti–IL-2 and anti–IFN-γ mAbs. (A) Dot plots were gated on $CD3^+$ T cells. The numbers represent the percentages of T cells in each quadrant and the value in parentheses represents the MFI for each quadrant. (B) The percentage of $CD3^+CD8^+$ T cells that were IFN-γ^+ is depicted for PD-1^+ and PD-1^- CD8 TILs. (C) The MFI for IFN-γ^+ $CD3^+CD8^+$ T cells are depicted for PD-1^+ and PD-1^- CD8 TILs. (D) The percentage of $CD3^+CD8^+$ T cells that were IL-2^+ is depicted for PD-1^+ and PD-1^- CD8 TILs for 6 patients. *P* values are calculated based on the paired *t* test. (E) IFN-γ production by MART-1 tetramer$^+$ CD8 T cells in tumor digests versus peripheral blood (PBL) from the same patient is shown. The percentage values represent the fraction of MART-1 tetramer$^+$ CD8 T cells that produced IFN-γ.

Tumor-specific T cells are contained in the PD-1+ TIL population and are functional after in vitro culture

The Journal of Clinical Investigation http://www.jci.org Volume 124 Number 5 May 2014

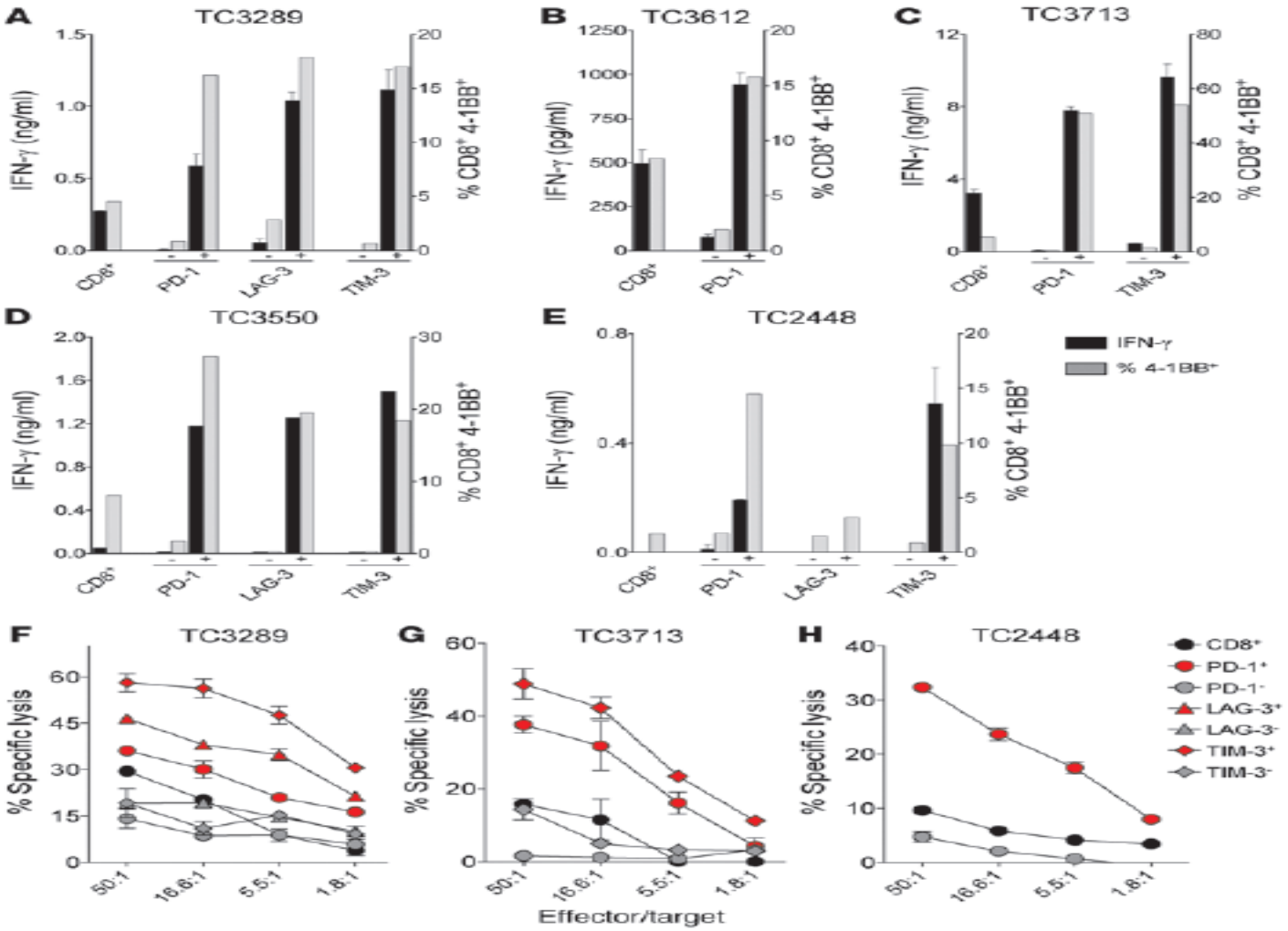
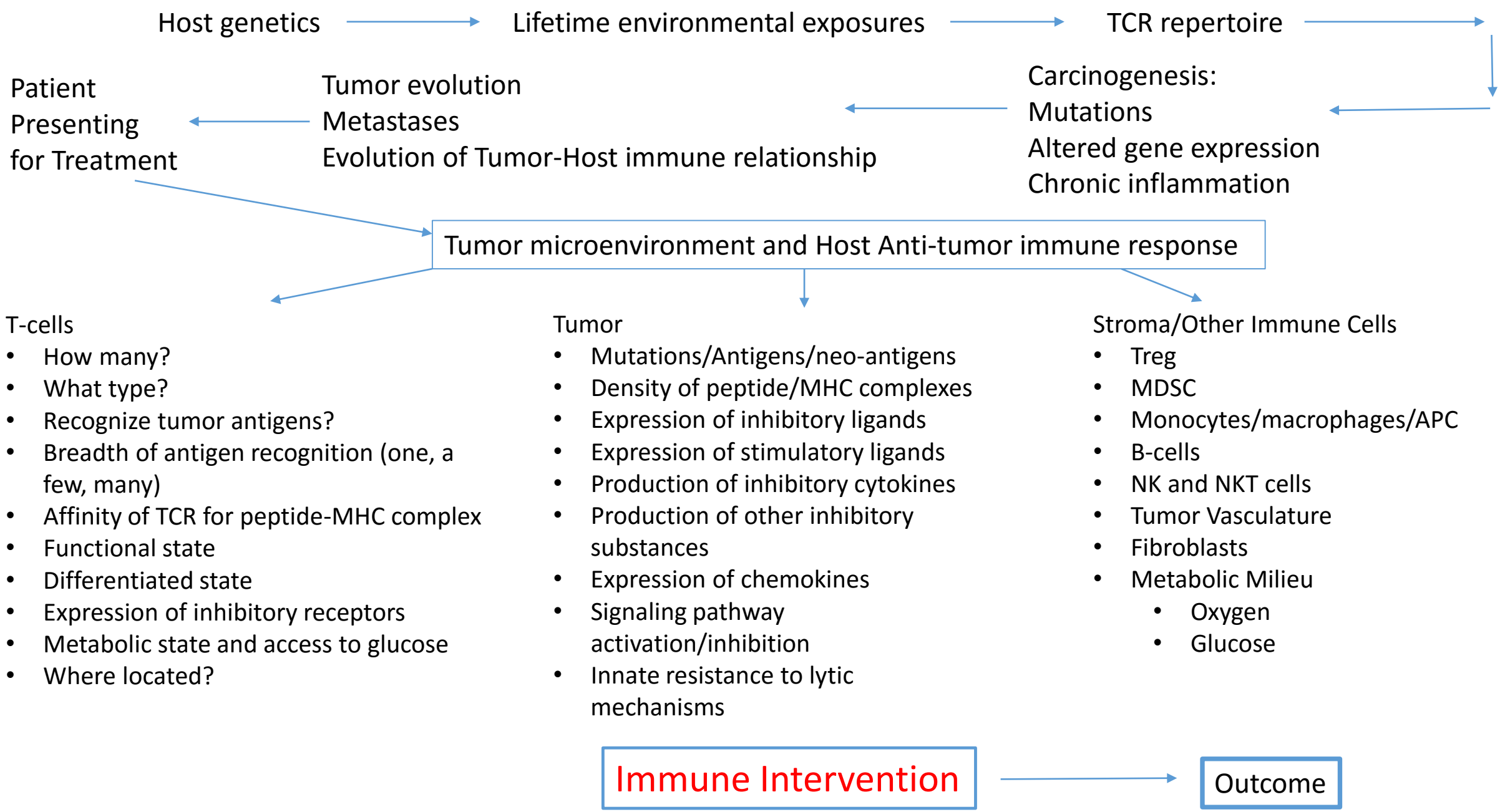


Figure 3
Recognition and lysis of autologous tumor by CD8+ TILs sorted based on PD-1, LAG-3, and TIM-3 expression. Bulk CD3+CD8+ TILs were sorted to high purity from FrTu3289, FrTu3612, FrTu3713, FrTu3550, and FrTu2448 based on positive or negative expression of PD-1, LAG-3 and/or TIM-3, and expanded in vitro for 15 days. (**A**–**E**) Response of fresh tumor–derived TILs to their respective autologous tumor cell lines, TC3289 (**A**), TC3612 (**B**), TC3713 (**C**), TC3550 (**D**) and TC2448 (**E**). Reactivity was assessed by measuring IFN-γ release (duplicates, mean ± SD) and frequency of 4-1BB upregulation. (**F**–**H**) Cytolytic activity of fresh tumor–derived TILs in response to their respective autologous tumor cell lines, TC3289 (**F**), TC3713 (**G**), and TC2448 (**H**). Percentage of specific lysis at different effector/target ratios is shown as mean ± SD.

Options for Immune Intervention in Cancer

- Vaccines (induce immune response against presumed cancer antigen)
  - Defined antigen and delivery method
  - Promote Ag presentation in vivo
- Cytokines to promote T-cell activation, proliferation and function
- Provide T cell co-stimulatory signals
- Block T cell inhibitory signals
- Modulate tumor signaling pathways that affect immune infiltration (STING, beta-catenin, VEGF, others)
- Adoptively transfer antigen-specific T cells
- Give antibodies that kill by CDC or ADCC
- Activate NK cell function to kill tumor cells


Host genetics
Lifetime environmental exposures
TCR repertoire
Carcinogenesis:
Mutations
Altered gene expression
Chronic inflammation
Tumor evolution
Metastases
Evolution of Tumor-Host immune relationship
Patient
Presenting
for Treatment
Tumor microenvironment and Host Anti-tumor immune response
T-cells
How many?
What type?
Recognize tumor antigens?
Breadth of antigen recognition (one, a few, many)
Affinity of TCR for peptide-MHC complex
Functional state
Differentiated state
Expression of inhibitory receptors
Metabolic state and access to glucose
Where located?
Tumor
Mutations/Antigens/neo-antigens
Density of peptide/MHC complexes
Expression of inhibitory ligands
Expression of stimulatory ligands
Production of inhibitory cytokines
Production of other inhibitory substances
Expression of chemokines
Signaling pathway activation/inhibition
Innate resistance to lytic mechanisms
Stroma/Other Immune Cells
Treg
MDSC
Monocytes/macrophages/APC
B-cells
NK and NKT cells
Tumor Vasculature
Fibroblasts
Metabolic Milieu
Oxygen
Glucose
Immune Intervention
Outcome

1970
- Autologous and allogeneic tumor cell cancer vaccines
- Intratumoral BCG

1980
- Interferon-alfa
- IL-2
- IL-2 and LAK cells
- Other cytokines (TNF, IFN-γ)
- IL-2 and TILs

1990
- Gene-transfected tumor cell vaccines
- Defined antigen vaccines, viral vectors, and DCs

2000
- Blockade of T-cell activation checkpoints (CTLA-4)
- Lymphocyte ablation + TIL
- T-cell and DC co-stimulatory antibodies
- Blockade of tumor immune suppressive mechanisms (PD-1)
- Gene (CAR, TCR, cytokine) modified lymphocytes for ACT

2010
- Combination of immune checkpoint inhibitors (CTLA-4, PD-1)

ACT = adoptive cell transfer; BCG = Bacillus Calmette-Guérin; CAR = chimeric antigen receptor; CTLA-4 = cytotoxic T-lymphocyte-associated protein 4; DC = dendritic cell; IL-2 interleukin-2; INF-g = interferon-gamma; LAK = lymphokine-activated killer cell; PD-1 = programmed cell death protein 1; TCR = T cell receptor; TILs = tumor infiltrating lymphocytes ; TNF = tumor necrosis factor.

CTLA-4

- Enhances T cell proliferation
- Increases T cell repertoire
- Causes ‘resistance’ of T-effectors to Treg suppression
- ‘killing’ of intratumoral Treg
- Causes tumor T cell infiltration
- Increases PD-1+ T cells

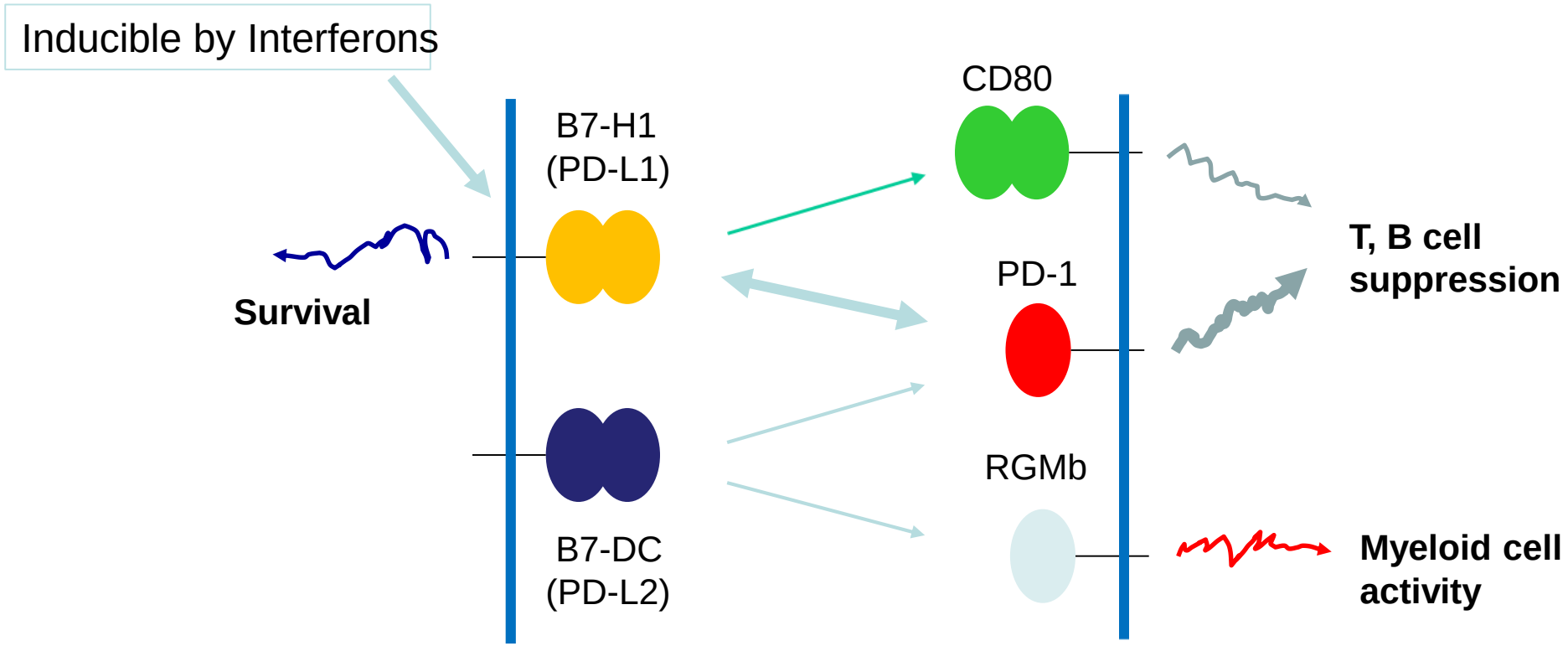
Anti-tumor activity

- Requires blockade on both CD4+ and CD8+
- Interaction with CTLA-4 on both effectors and Treg
- Isotype dependent in animal models (ADCC-dependent)

Key Aspects of Anti-CTLA4 Therapy

- Can be associated with autoimmune adverse events
  - Any organ, but rash, colitis, hepatitis and endocrinopathies are most common
  - May require steroids +/- additional immunosuppressive agents
- Unique kinetics of response in some patients
  - SD with slow, steady decline in total tumor volume
  - Response after initial increase in total tumor volume
  - Response in index plus new lesions at or after the appearance of new lesions
  - Continued benefit after Rx of discordant progressing lesions
- Possibility of second response with re-induction after PD

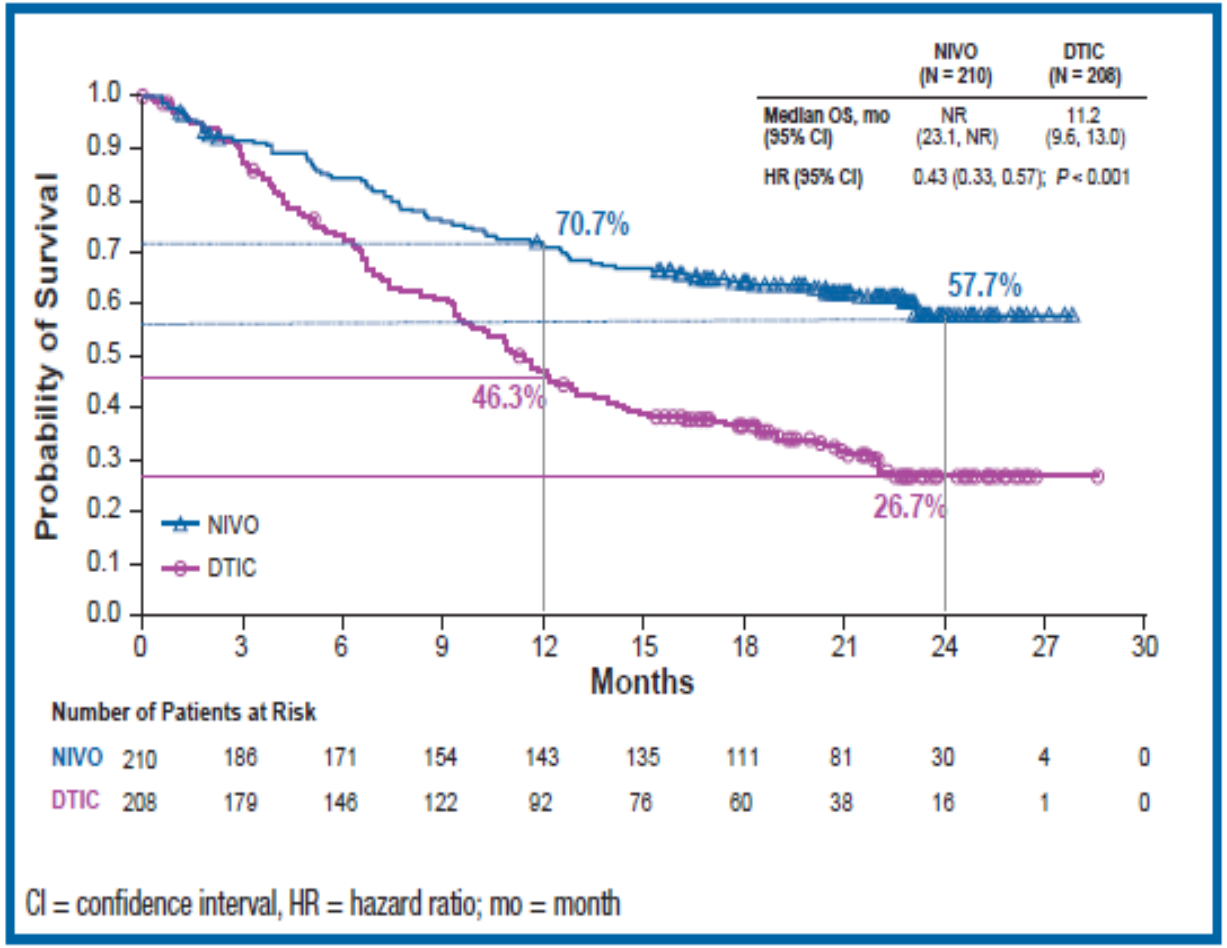
The PD-L1/PD-1 Pathway

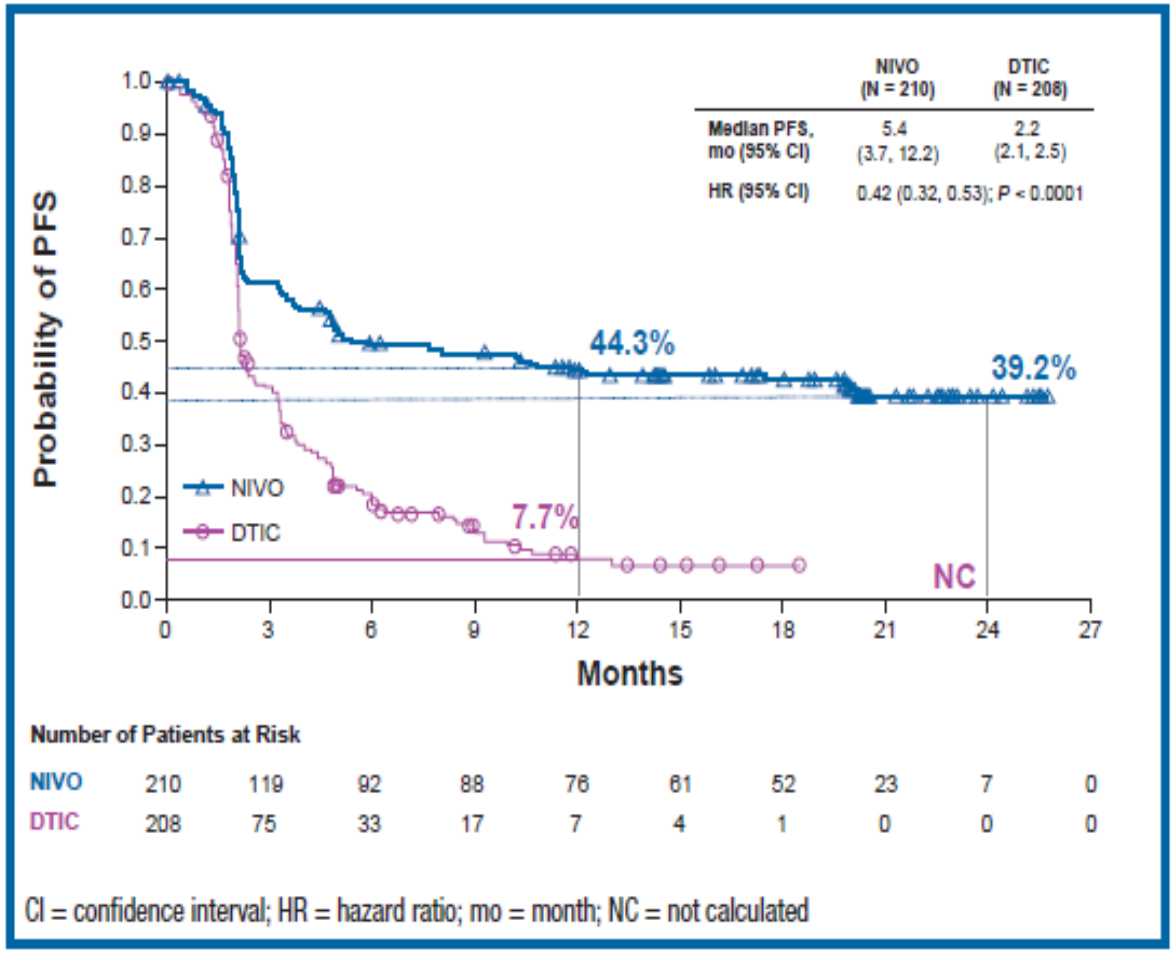


Slde courtesy of Lieping Chen

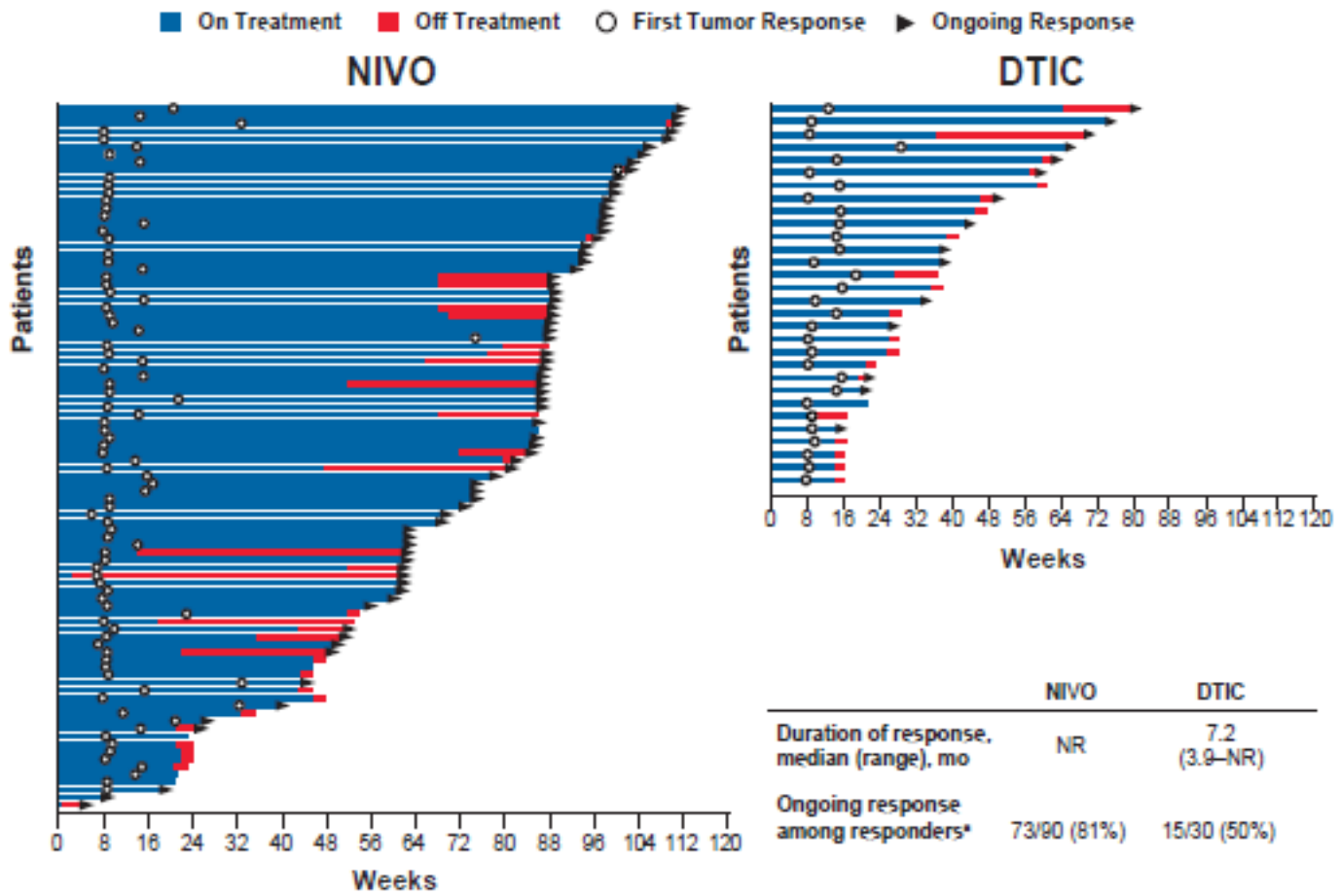
Nivolumab (anti-PD-1)versus DTIC – OSS and PFS

Atkinson et al, SMR 2015





Nivolumab versus DTIC- Duration of Response



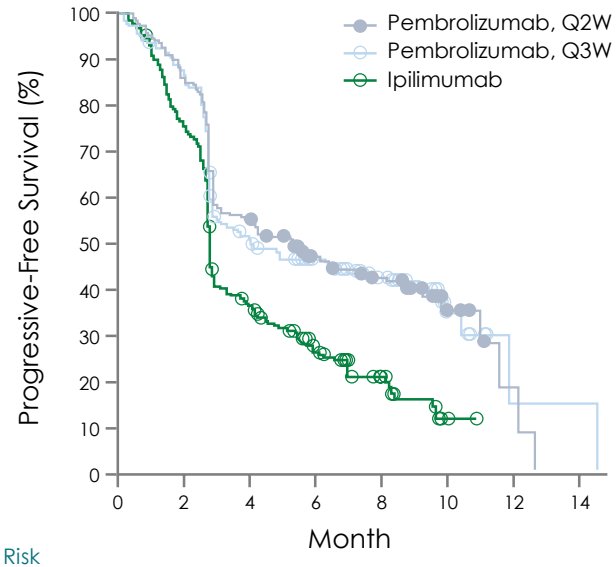
	NIVO	DTIC
Duration of response, median (range), mo	NR	7.2 (3.9–NR)
Ongoing response among responders[a]	73/90 (81%)	15/30 (50%)

[a]At the time of the last follow-up
mo = month; NR = not reached

Anti-PD-1 (Pembrolizumab) Versus Ipilimumab: Treatment of Advanced Disease

	n	ORR	Median PFS	OS at 12 months
Pembrolizumab 10 mg/kg Q2W	279	33.7	5.5	74.1%
Pembrolizumab 10 mg/kg Q3W	277	32.9	4.1	68.4%
Ipilimumab 3 mg/kg Q3W × 4	278	11.9	2.8	58.2%

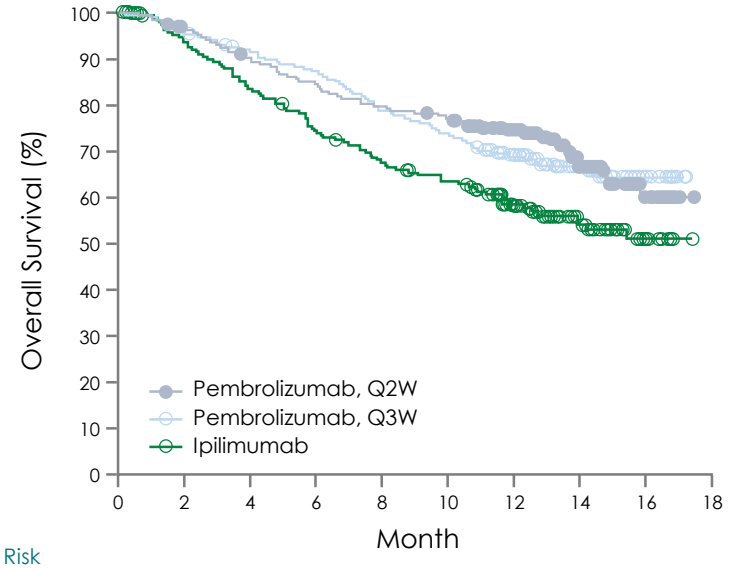
PFS



Patients at Risk

Pembrolizumab, Q2W	279	231	147	98	49	7	2	0
Pembrolizumab, Q3W	277	235	133	95	53	7	1	1
Ipilimumab	278	186	88	42	18	2	0	0

OS



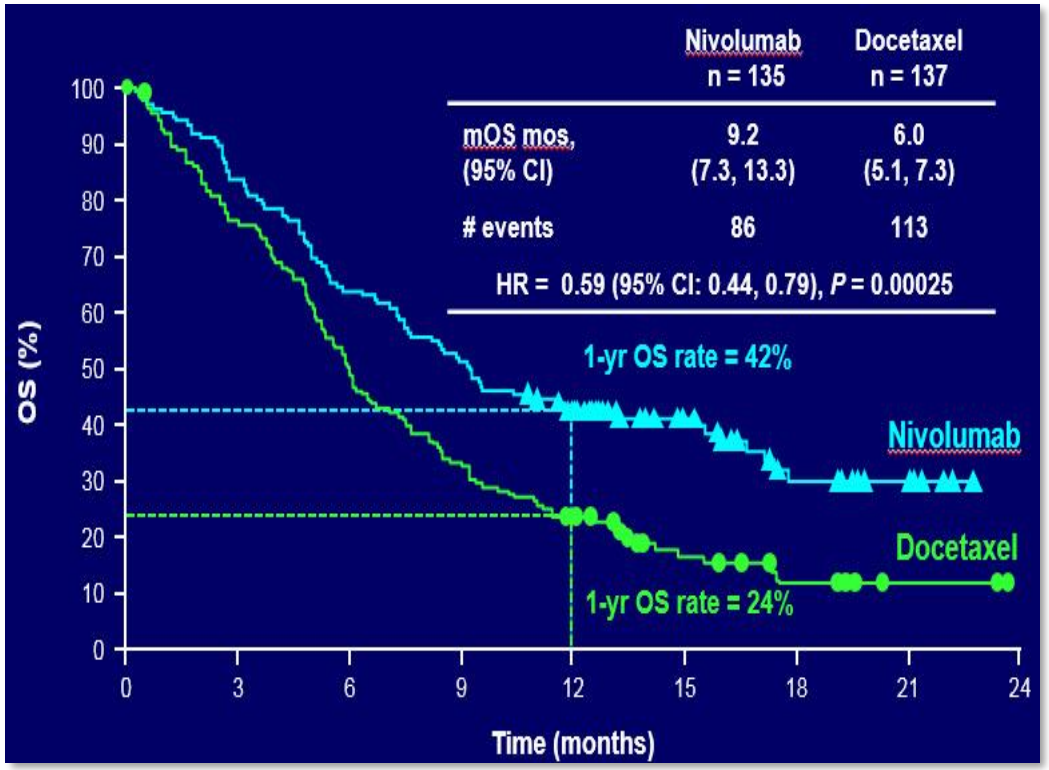
Patients at Risk

Pembrolizumab, Q2W	279	266	248	233	219	212	177	67	19	0
Pembrolizumab, Q3W	277	266	251	238	215	202	158	71	18	0
Ipilimumab	278	242	212	188	169	157	117	51	17	0

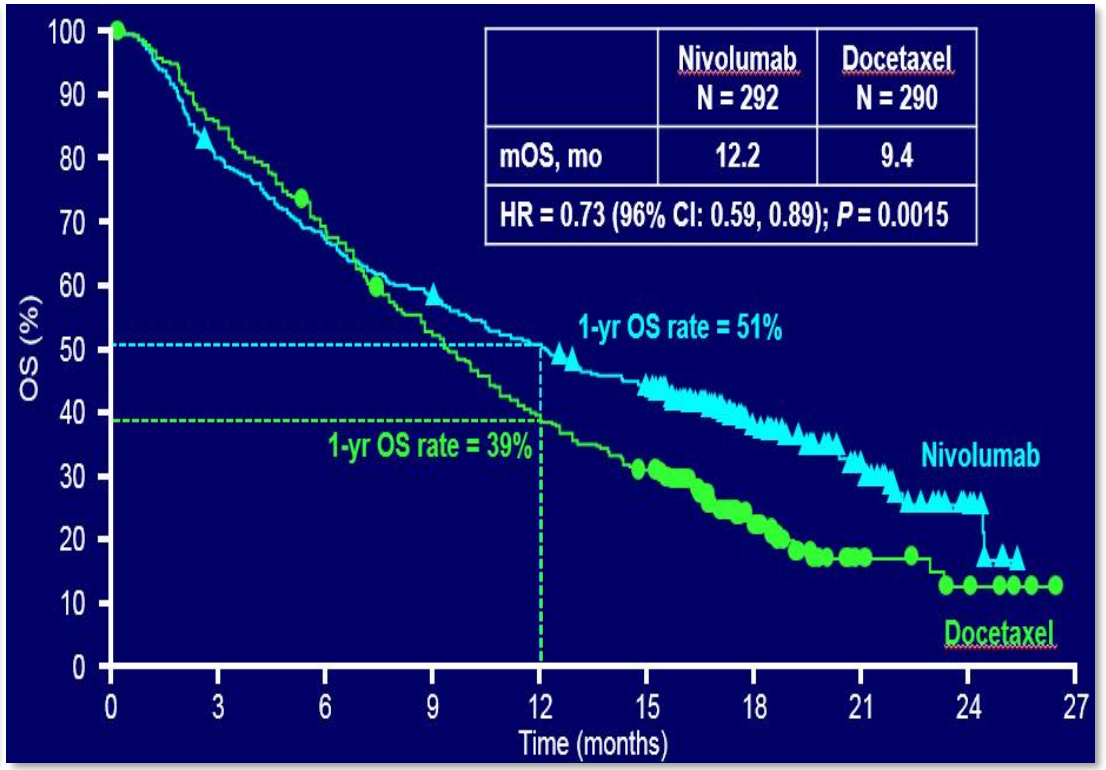
Robert C, et al. *N Engl J Med.* 2015;372:2521–2532.

Randomized phase III trials of nivolumab vs. docetaxel in NSCLC

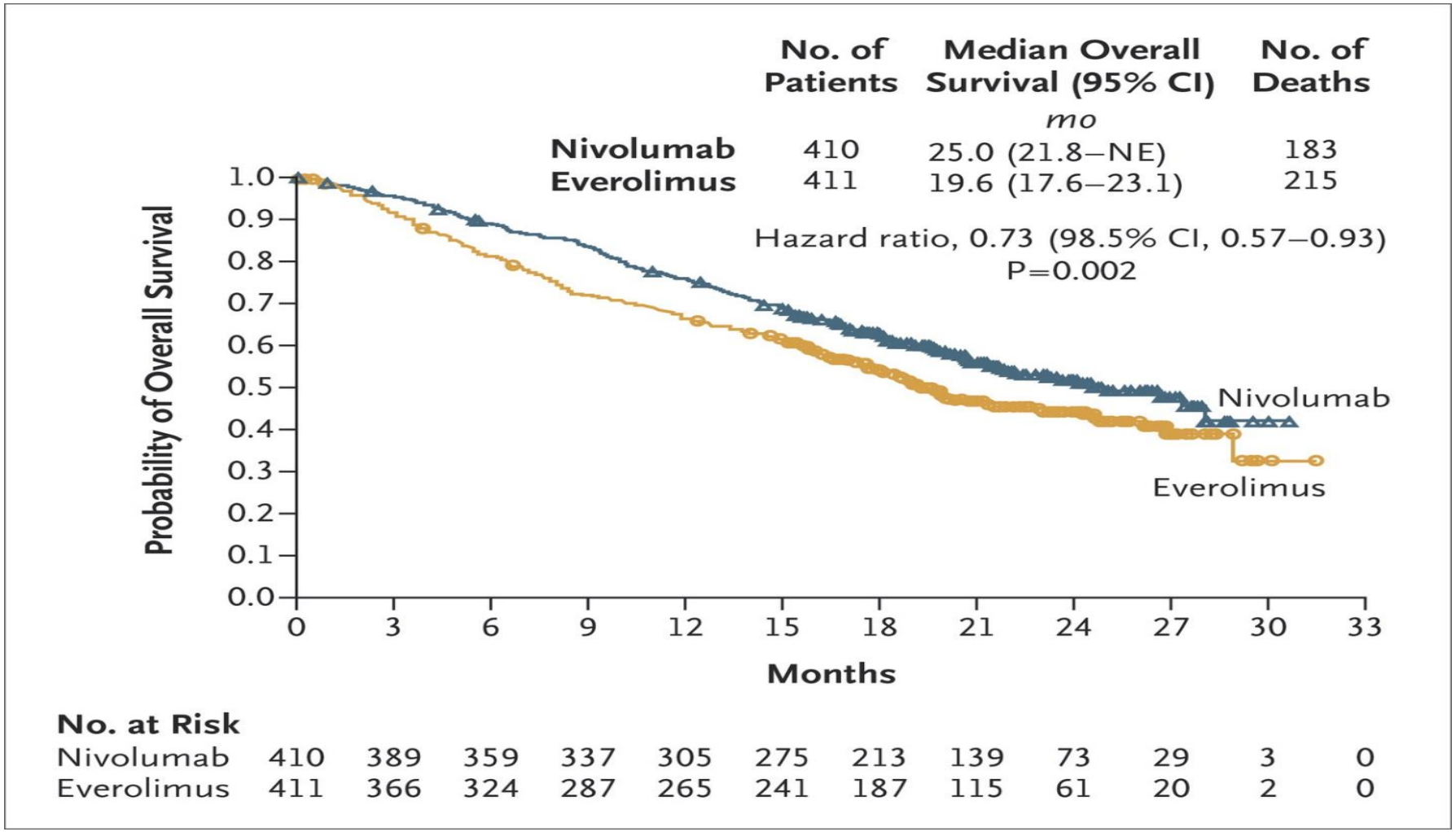
Trial 17: Squamous Cell Carcinoma



Trial 57: Non-Squamous Cell Carcinoma



Nivolumab Improves Overall Survival in mRCC



Motzer RJ et al. N Engl J Med 2015;373:1803-1813.

Spectrum of PD-1/PD-L1 Antagonist Activity

Active

- **Melanoma**
- **Renal cancer (clear cell and non-clear cell)**
- **NSCLC – adenocarcinoma and squamous cell**
- Small cell lung cancer
- **Head and neck cancer**
- Gastric and gastroesophageal junction
- **MMR-repair deficient tumors (colon, cholangiocarcinoma)**
- Bladder
- Triple negative breast cancer
- Ovarian
- Hepatocellular carcinoma
- Thymoma
- Mesothelioma
- Cervical
- **Hodgkin lymphoma**
- Diffuse large cell lymphoma
- Follicular lymphoma
- T-cell lymphoma (cutaneous T-cell lymphomas, peripheral T-cell lymphoma)
- **Merkel cell**

Minimal to no activity

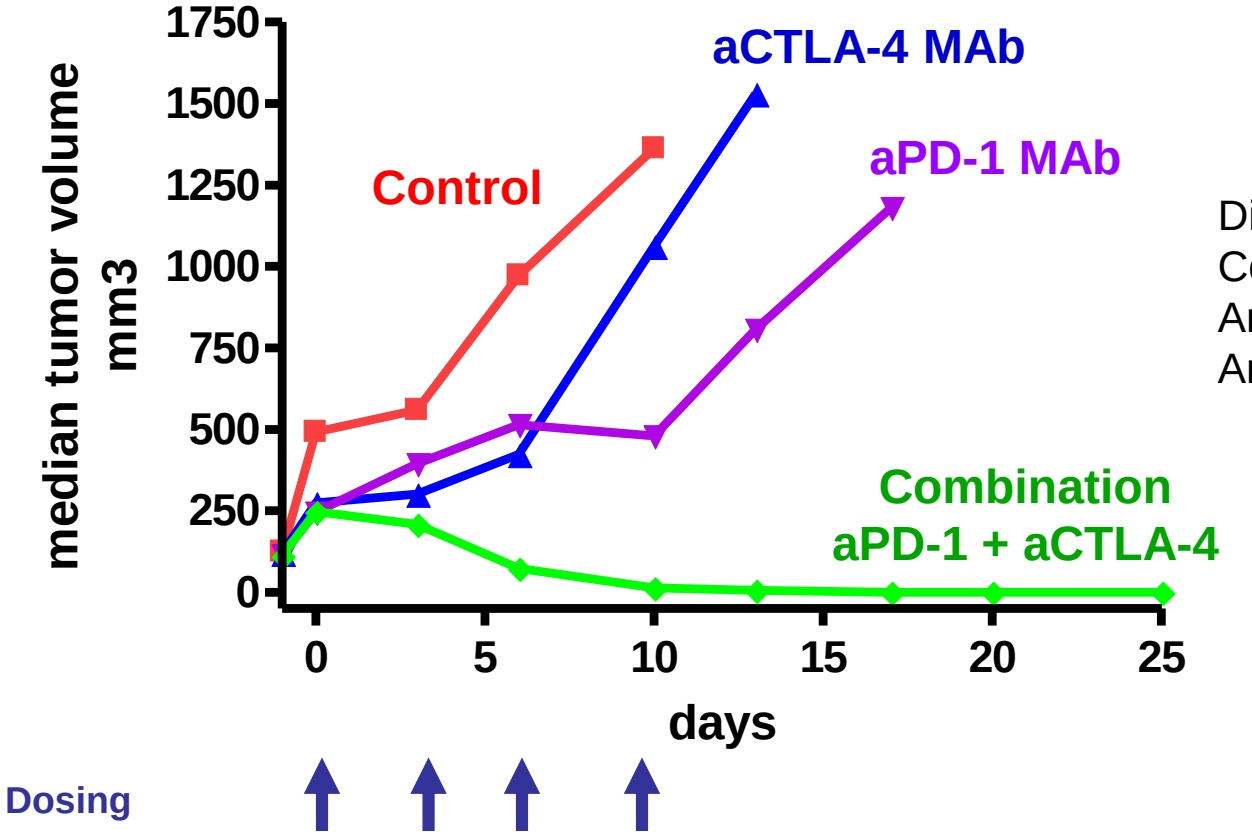
- Prostate cancer
- MMR+ (MSS) colon cancer
- Myeloma
- Pancreatic cancer

Major PD-1/PD-L1 antagonists

- Nivolumab (anti-PD-1)
- Pembrolizumab (anti-PD-1)
- Atezolizumab (MPDL3280, anti-PD-L1)
- Durvalumab (anti-PD-L1)
- Avelumab (anti-PD-L1)

Synergistic Activity with Anti-PD-1 and Anti-CTLA-4 Antibodies

Combination of Non-Efficacious Doses of anti-PD1 and anti-CTLA-4 Antibodies is Efficacious in Mouse Model



Different roles in T cell Differentiation-
Compensatory upregulation
Anti-CTLA4 elimination of tumor Treg
Anti-CTLA4 induced tumor T cell infiltration

Provided by Alan Korman, BMS

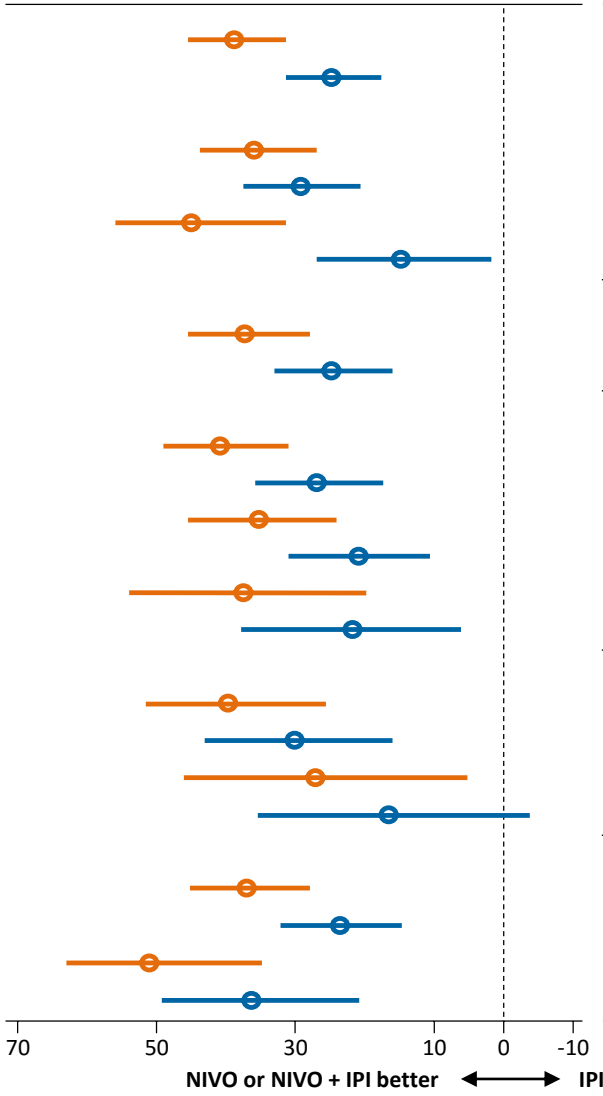
Study Design

Figure 1: Study CA209-004 concurrent cohorts

All units are mg/kg. Results from Cohorts 6 and 7 (sequenced treatment cohorts – IPI followed by NIVO) were reported previously[6]
[a]FDA approved regimen.
IPI = ipilimumab; NIVO = nivolumab; Q2W = every 2 weeks; Q3W = every 3 weeks; Q12W = every 12 weeks.

CA209-067: Ipi/Nivo vs. Nivolumab vs. Ipilimumab: Objective Response Rate

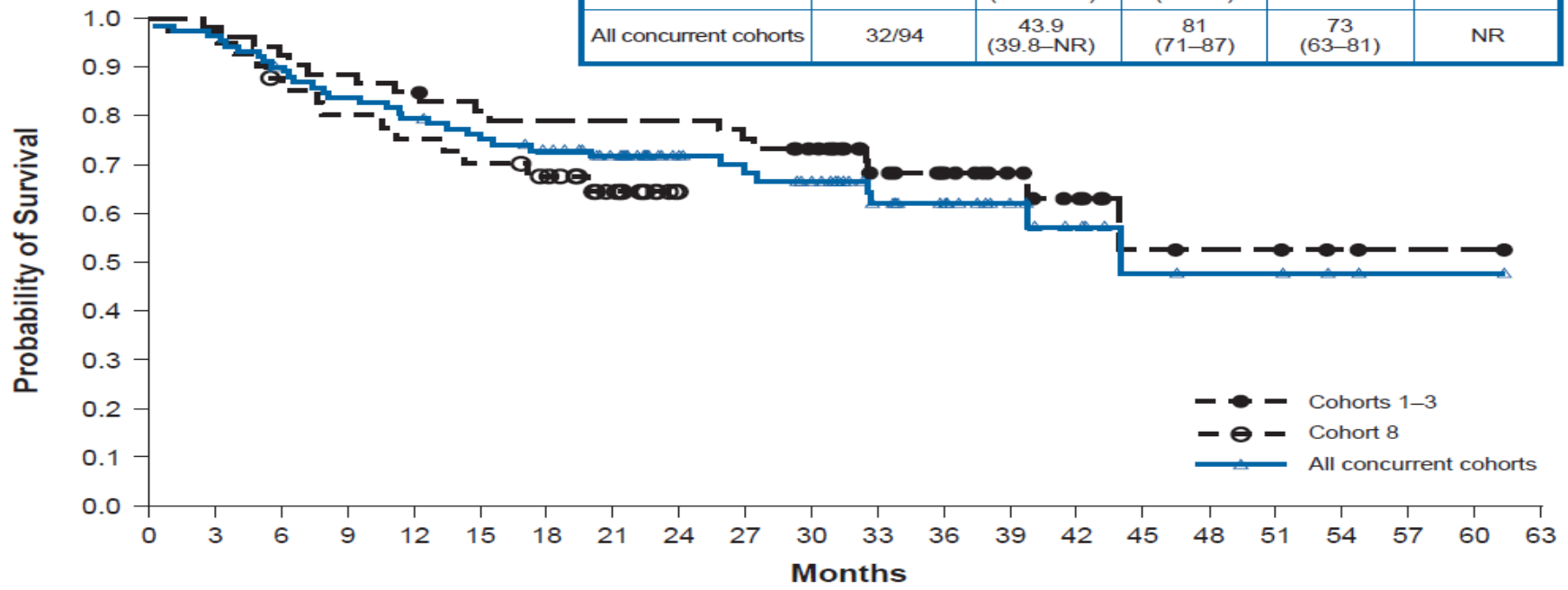
	ORR (Patients)		Unweighted ORR difference vs IPI (95% CI)
		NIVO + IPI / NIVO	
Total population	57.6% (314)		38.6% (31.3–45.2)
	43.7% (316)		24.6% (17.5–31.4)
BRAF			
Wild-type	53.3% (212)		35.6% (26.8–43.6)
	46.8% (218)		29.1% (20.5–37.1)
Mutant	66.7% (102)		44.7% (31.5–55.6)
	36.7% (98)		14.7% (2.0–26.8)
M Stage			
M1c	51.4% (185)		37.1% (27.9–45.4)
	38.9% (185)		24.6% (15.8–33.0)
Baseline LDH			
≤ULN	65.3% (199)		40.6% (31.1–48.9)
	51.5% (196)		26.8% (17.3–35.6)
>ULN	44.7% (114)		35.2% (24.1–45.2)
	30.4% (112)		20.8% (10.5–30.7)
>2x ULN	37.8% (37)		37.8% (20.0–53.9)
	21.6% (37)		21.6% (6.3–37.2)
Age (yr)			
≥65 and <75	57.4% (94)		39.5% (25.8–51.0)
	48.1% (79)		30.1% (16.0–42.8)
≥75	54.3% (35)		27.0% (5.3–45.8)
	43.6% (39)		16.3% (-4.1–35.2)
PD-L1 Expression Level			
<5%	54.8% (210)		36.9% (28.0–45.0)
	41.3% (208)		23.5% (14.8–31.8)
≥5%	72.1% (68)		50.7% (35.0–62.8)
	57.5% (80)		36.2% (21.0–49.0)



Larkin J, et al. Presented at ECC 2015, Abstract 3303.

Updated Survival CA209-004, Iplimumab + Nivolumab in Metastatic Melanoma

	Died/treated, n/N	Median OS, mo (95% CI)	OS rate, % (95% CI)		
			12 mo	24 mo	36 mo
Cohorts 1–3	18/53	NR (39.8–NR)	85 (72–92)	79 (65–88)	68 (53–79)
Cohort 8	14/41	NR (20.0–NR)	75 (59–86)	NR	NR
All concurrent cohorts	32/94	43.9 (39.8–NR)	81 (71–87)	73 (63–81)	NR



Number of Patients at Risk

Cohorts 1–3	53	52	49	47	45	42	41	41	41	39	35	26	21	14	10	5	4	4	2	1	1	0
Cohort 8	41	40	35	32	30	28	25	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0
All concurrent cohorts	94	92	84	79	75	70	66	58	41	39	35	26	21	14	10	5	4	4	2	1	1	0

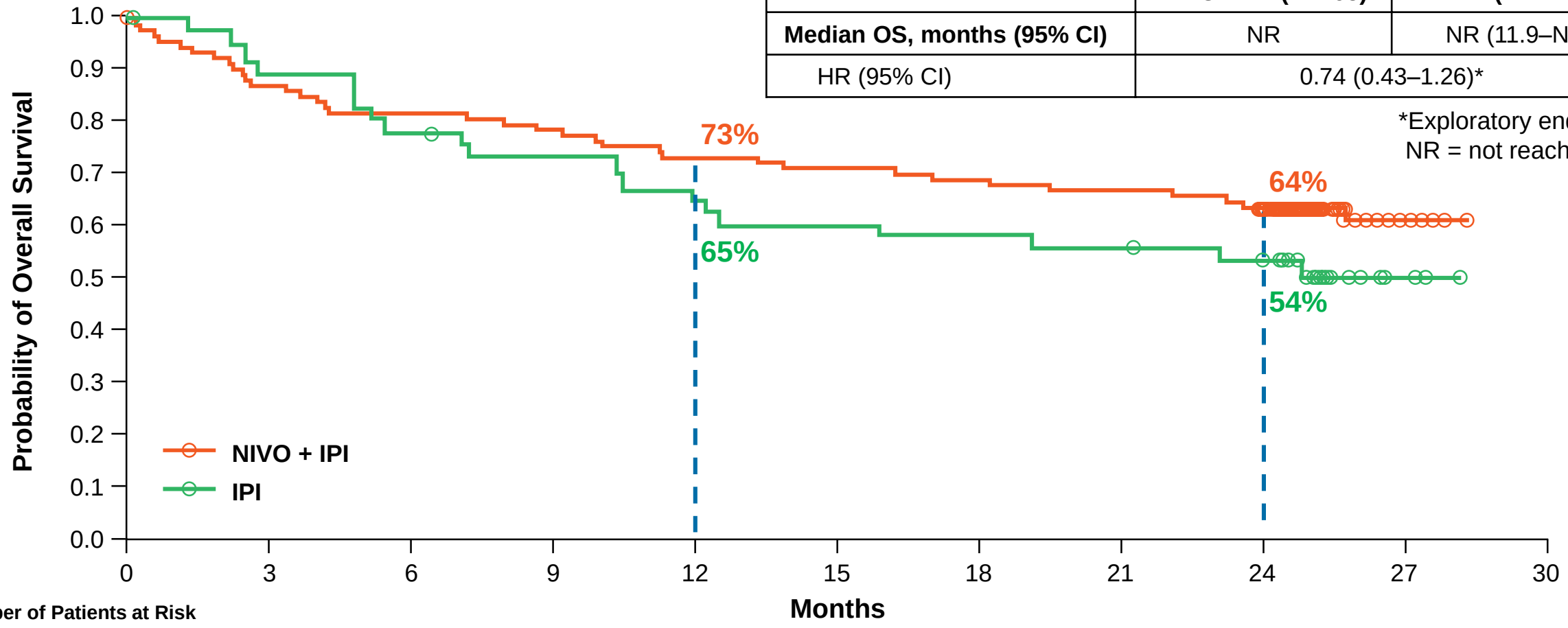
CI = confidence interval; mo = months; NR = not reached

Sznol et al, SMR 2015

CA209-069- OS at 2 Years of Follow-up (All Randomized Patients)

	NIVO + IPI (N = 95)	IPI (N = 47)
Median OS, months (95% CI)	NR	NR (11.9–NR)
HR (95% CI)	0.74 (0.43–1.26)*	

*Exploratory endpoint
NR = not reached



Number of Patients at Risk

NIVO+ IPI	95	82	77	74	69	67	65	63	57	6	0
IPI	47	41	36	33	29	27	26	25	22	3	0

- 30/47 (64%) of patients randomized to IPI crossed over to receive any systemic therapy at progression

Postow et al , AACR 2015

CA209-067: Adverse Events

Table 3. Adverse Events.*

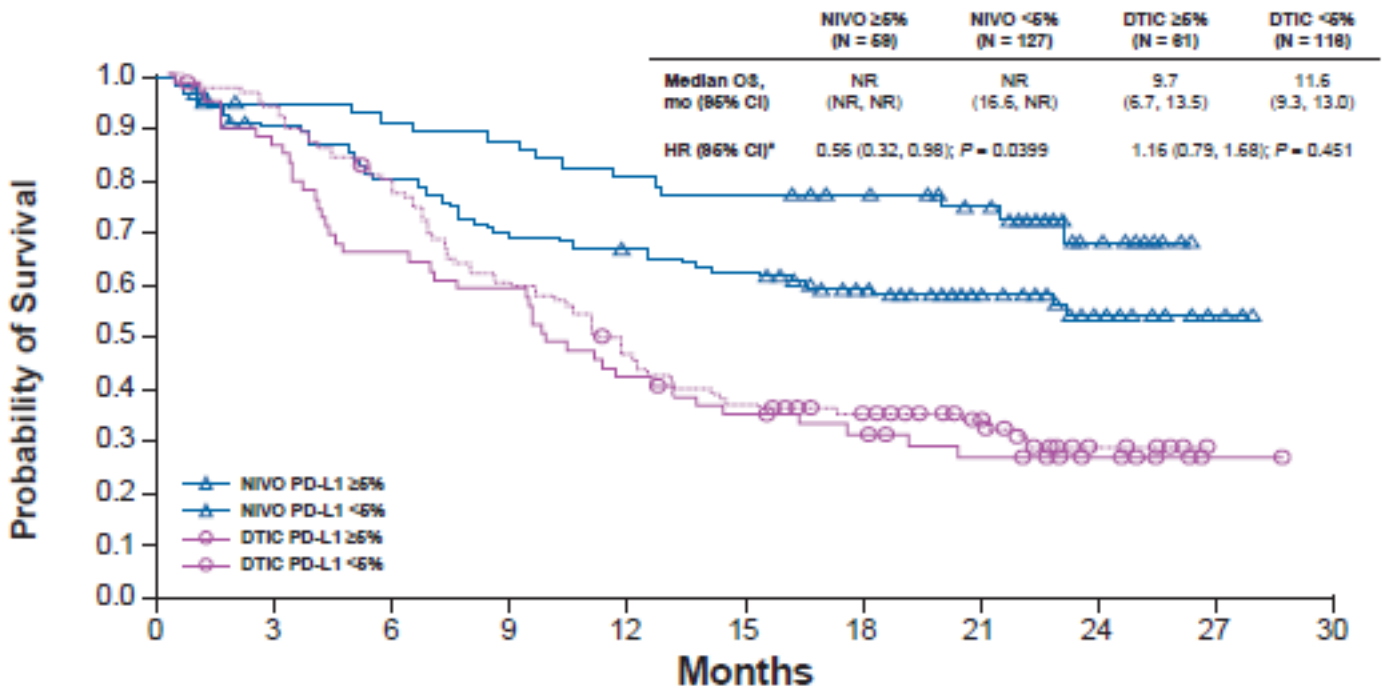
Event	Nivolumab (N=313)		Nivolumab plus Ipilimumab (N=313)		Ipilimumab (N=311)	
	Any	Grade 3 or 4	Any	Grade 3 or 4	Any	Grade 3 or 4
	number of patients with event (percent)					
Any adverse event	311 (99.4)	136 (43.5)	312 (99.7)	215 (68.7)	308 (99.0)	173 (55.6)
Treatment-related adverse event†	257 (82.1)	51 (16.3)	299 (95.5)	172 (55.0)	268 (86.2)	85 (27.3)
Diarrhea	60 (19.2)	7 (2.2)	138 (44.1)	29 (9.3)	103 (33.1)	19 (6.1)
Fatigue	107 (34.2)	4 (1.3)	110 (35.1)	13 (4.2)	87 (28.0)	3 (1.0)
Pruritus	59 (18.8)	0	104 (33.2)	6 (1.9)	110 (35.4)	1 (0.3)
Rash	81 (25.9)	2 (0.6)	126 (40.3)	15 (4.8)	102 (32.8)	6 (1.9)
Nausea	41 (13.1)	0	81 (25.9)	7 (2.2)	50 (16.1)	2 (0.6)
Pyrexia	18 (5.8)	0	58 (18.5)	2 (0.6)	21 (6.8)	1 (0.3)
Decreased appetite	34 (10.9)	0	56 (17.9)	4 (1.3)	39 (12.5)	1 (0.3)
Increase in alanine amino-transferase level	12 (3.8)	4 (1.3)	55 (17.6)	26 (8.3)	12 (3.9)	5 (1.6)
Vomiting	20 (6.4)	1 (0.3)	48 (15.3)	8 (2.6)	23 (7.4)	1 (0.3)
Increase in aspartate amino-transferase level	12 (3.8)	3 (1.0)	48 (15.3)	19 (6.1)	11 (3.5)	2 (0.6)
Hypothyroidism	27 (8.6)	0	47 (15.0)	1 (0.3)	13 (4.2)	0
Colitis	4 (1.3)	2 (0.6)	37 (11.8)	24 (7.7)	36 (11.6)	27 (8.7)
Arthralgia	24 (7.7)	0	33 (10.5)	1 (0.3)	19 (6.1)	0
Headache	23 (7.3)	0	32 (10.2)	1 (0.3)	24 (7.7)	1 (0.3)
Dyspnea	14 (4.5)	1 (0.3)	32 (10.2)	2 (0.6)	13 (4.2)	0
Treatment-related adverse event leading to discontinuation	24 (7.7)	16 (5.1)	114 (36.4)	92 (29.4)	46 (14.8)	41 (13.2)

* The safety population included all the patients who received at least one dose of study drug. The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

† The treatment-related adverse events listed here were those reported in at least 10% of the patients in any of the three study groups.

Nivolumab versus DTIC- OSS by PD-L1 Status

Atkinson et al, SMR 2015

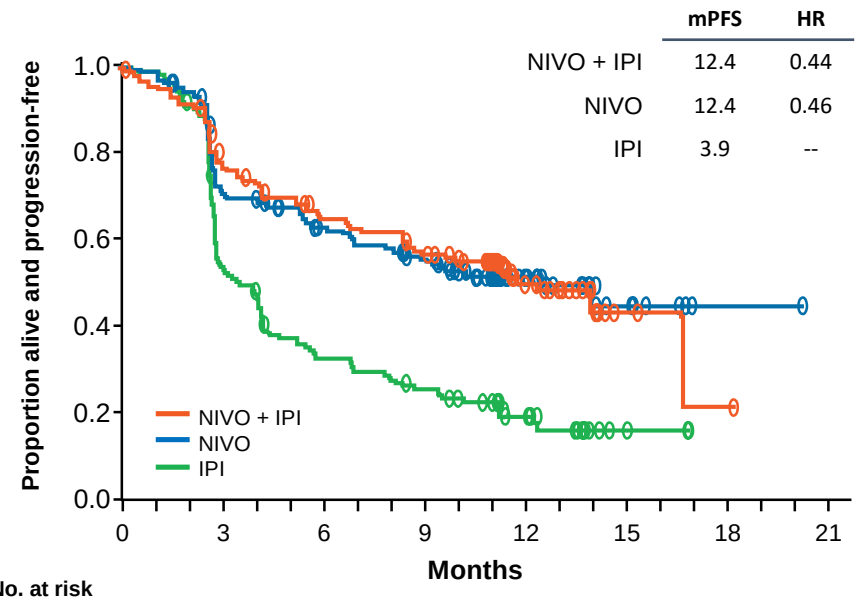


[a]Hazard ratios expressed as PD-L1 ≥5% over PD-L1 <5%

CI = confidence interval; HR = hazard ratio; mo = month; NR = not reached

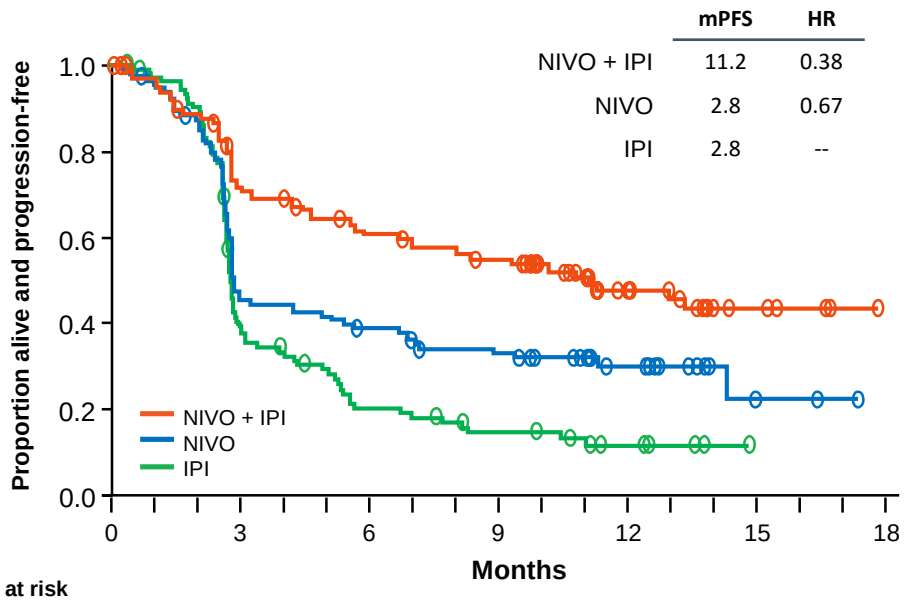
PFS by PD-L1 Expression Level (1%)

PD-L1 ≥1%*



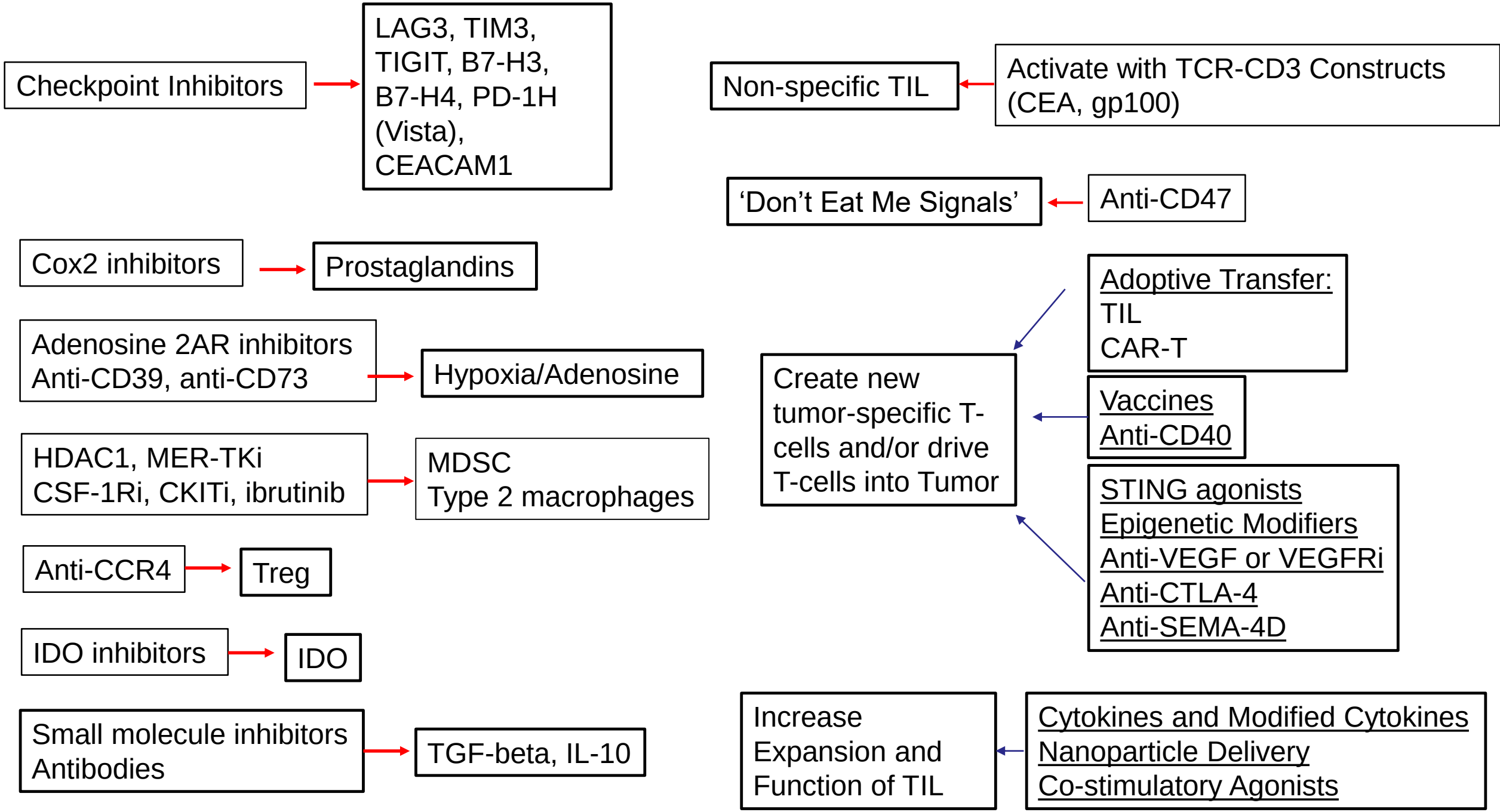
No. at risk								
NIVO + IPI	155	113	91	78	32	4	1	
NIVO	171	115	97	83	34	7	1	0
IPI	164	83	47	36	16	3		

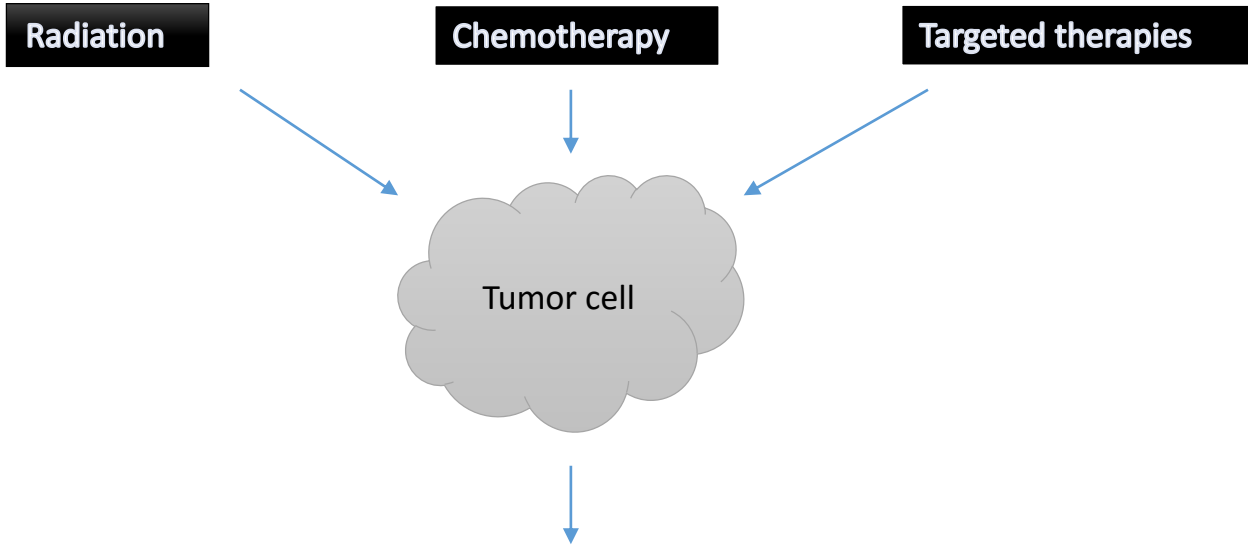
PD-L1 <1%*



No. at risk							
NIVO + IPI	123	82	65	57	26	6	0
NIVO	117	50	42	34	13	2	0
IPI	113	39	19	12	5	0	

*Per validated PD-L1 immunohistochemical assay with expression defined as ≥1% of tumor cells showing PD-L1 staining in a section of at least 100 evaluable tumor cells.


Checkpoint Inhibitors
LAG3, TIM3, TIGIT, B7-H3, B7-H4, PD-1H (Vista), CEACAM1
Cox2 inhibitors
Prostaglandins
Adenosine 2AR inhibitors
Anti-CD39, anti-CD73
Hypoxia/Adenosine
HDAC1, MER-TKi
CSF-1Ri, CKITi, ibrutinib
MDSC
Type 2 macrophages
Anti-CCR4
Treg
IDO inhibitors
IDO
Small molecule inhibitors
Antibodies
TGF-beta, IL-10
Non-specific TIL
Activate with TCR-CD3 Constructs (CEA, gp100)
'Don't Eat Me Signals'
Anti-CD47
Adoptive Transfer:
TIL
CAR-T
Create new tumor-specific T-cells and/or drive T-cells into Tumor
Vaccines
Anti-CD40
STING agonists
Epigenetic Modifiers
Anti-VEGF or VEGFRi
Anti-CTLA-4
Anti-SEMA-4D
Increase Expansion and Function of TIL
Cytokines and Modified Cytokines
Nanoparticle Delivery
Co-stimulatory Agonists

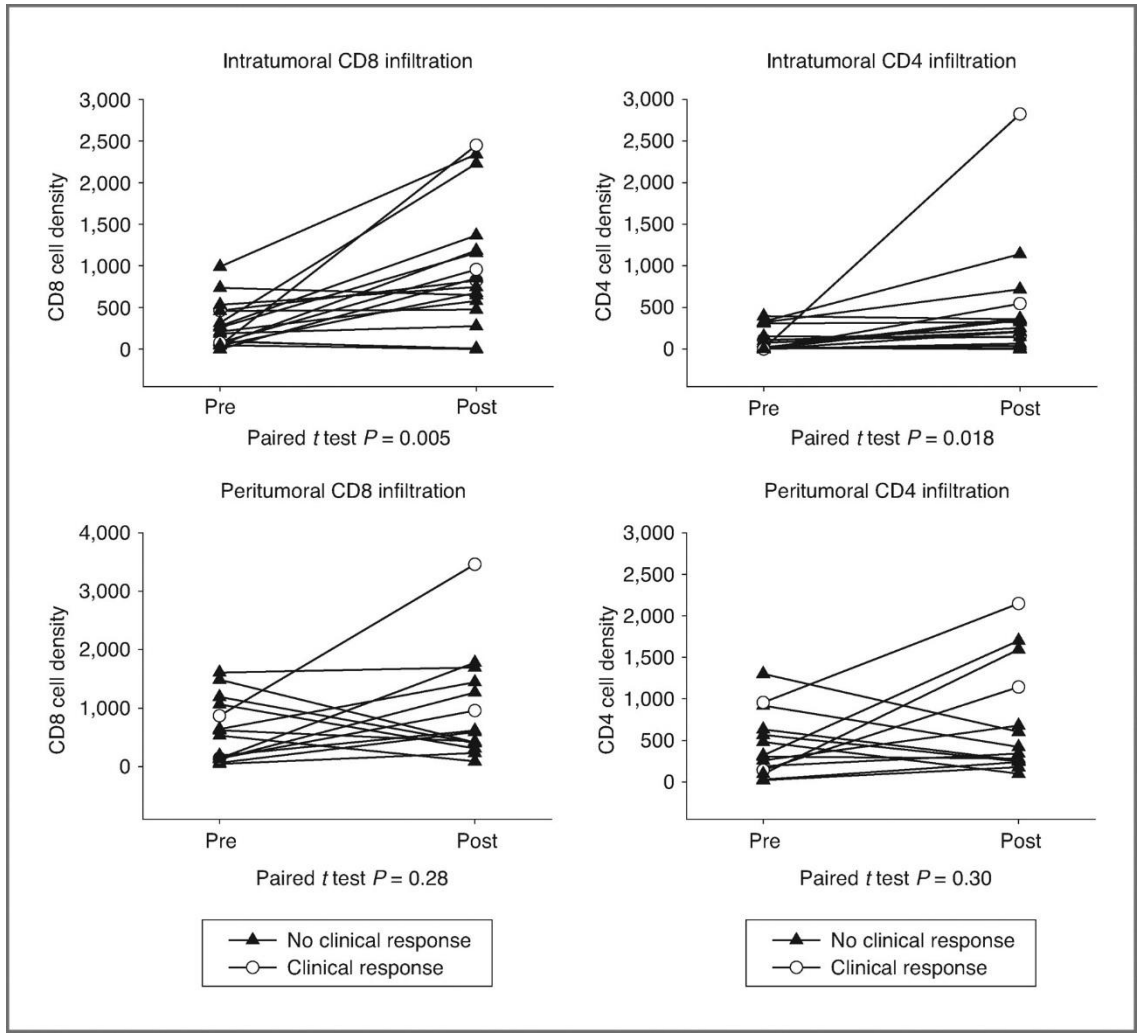


- Reduces Tumor bulk – Improves T-cell: tumor target ratio
- Separate mechanism of kill – 'synergize' with T-cell mechanism of killing
- Reduces T-cell inhibitory substances produced by tumor
- Alters tumor barriers (vasculature/pressure) to T-cell penetration
- Kills tumor cells in a manner that increases their recognition by T-cells and APC (vaccination)
- Alters T-cell signaling/gene expression to produce T-cell attractants

Imaging and immune therapy

- Predictive of Response
  - T-cell infiltration (extent, location, function, and type)
  - Other immune cells (MDSC, Treg?)
  - Expression of antibody targets (CD47, CD73, PD-L1, PD-1, TIM-3, PD-1H, etc)
  - Metabolisms/metabolic state (hypoxia, glucose consumption, other)
- Tumor response in the absence of regression
- Differentiate scar from residual tumor versus persistent inflammation without tumor
  - When to stop therapy?
- Differentiate pseudo-progression from true regression
- Biodistribution and pharmacodynamic endpoints
  - Receptor saturation
  - Tumor T-cell activation, T-cell infiltration, change in T cell ratios, cytokine production

Quantitative immunohistochemical analysis of ITI and PTI by CD4+ and CD8+ cells.



Huang R R et al. Clin Cancer Res 2011;17:4101-4109

Clinical Cancer Research

Abstract # 410

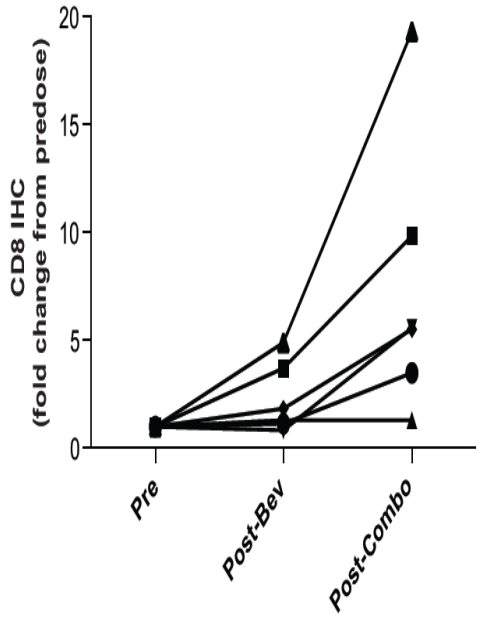
Phase 1b evaluation of MPDL3280A (anti-PDL1) in combination with bevacizumab (bev) in patients (pts) with metastatic renal cell carcinoma (mRCC)

Mario Sznol,[1] David F. McDermott,[2] Suzanne Jones,[3] James W. Mier,[2] Daniel Waterkamp,[4] Bo Liu,[4] Jeffrey Wallin,[4] Roel Funke,[4] Johanna Bendell[3]

[1]Yale Cancer Center, New Haven, CT, USA, [2]Beth Israel Deaconess Medical Center, Boston, MA, USA, [3]Sarah Cannon Research Institute, Nashville, TN, USA, [4]Genentech, Inc, South San Francisco, CA, USA

ASCO GU 2015

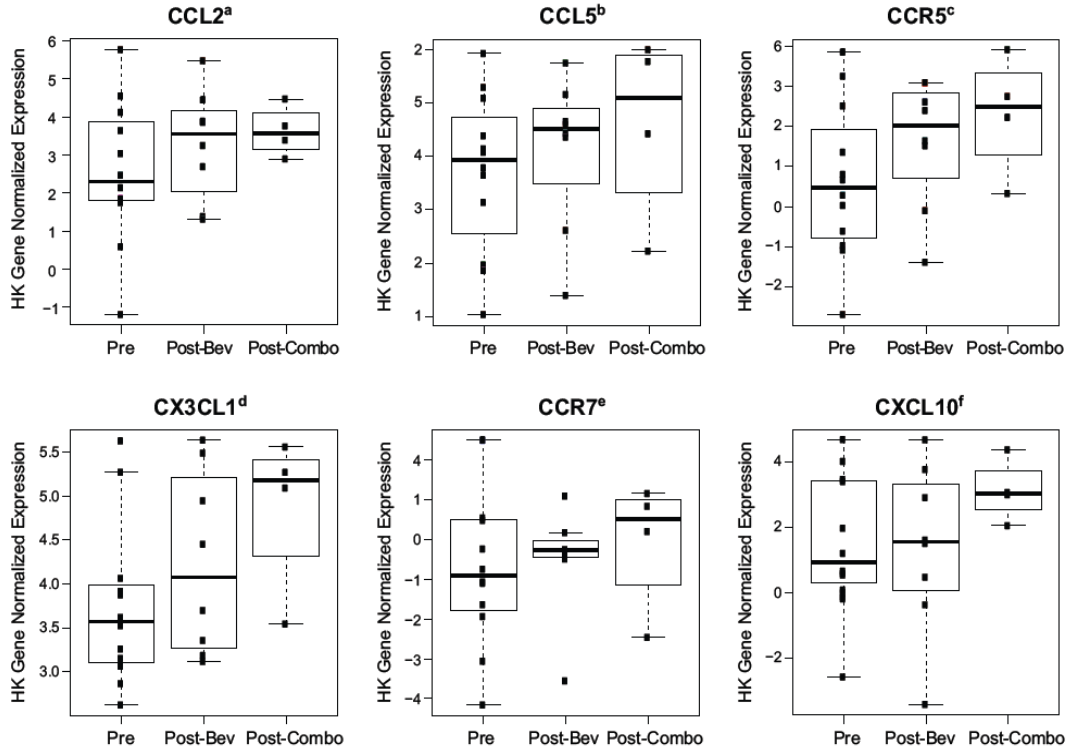
Figure 7. CD8 Staining in the Tumors of Patients With RCC After Treatment With MPDL3280A + Bevacizumab



IHC, immunohistochemistry.

- The increase in CD8+ cells was greatly enhanced in patients after treatment with MPDL3280A + bevacizumab

Figure 8. Chemokine Expression in the Tumors of Patients With RCC After Treatment With MPDL3280A + Bevacizumab



HK, housekeeping gene.

[a] CCL2 is generally produced by tissue injury or infection and serves as a chemoattractant for monocytes, T cells and dendritic cells.

[b] CCL5 is a chemoattractant for T cells, eosinophils and basophils.

[c] CCR5 is the receptor for CCL5.

[d] CX3CL1 is a potent chemoattractant for T cells and monocytes and is primarily expressed in endothelial cells.

[e] CCR7 is a chemoattractant for T cells and stimulates dendritic cell maturation.

[f] CXCL10 is secreted by monocytes, endothelial cells and fibroblasts in response to IFNγ and serves as a chemoattractant for immune cells.

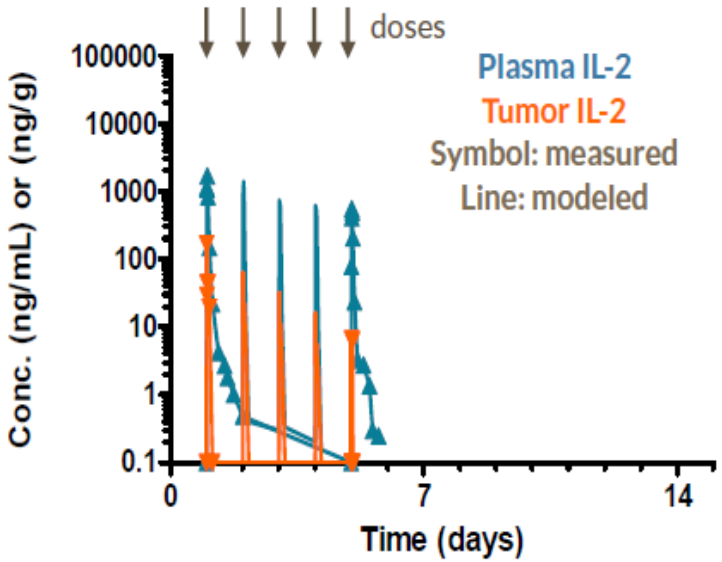
Nektar high MW releasable polymer located at strategic site

IL-2 cytokine Core

- Same protein sequence as clinically validated molecule (aldesleukin)

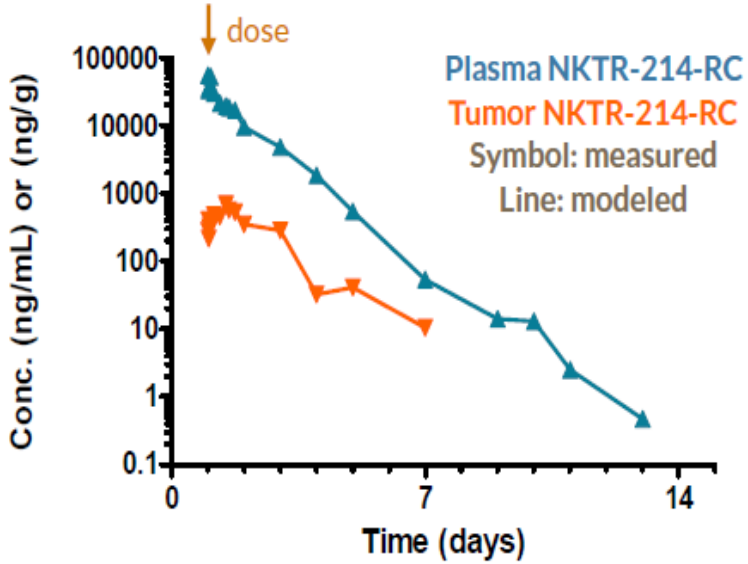
Aldesleukin

3 mg/kg, qdx5



NKTR-214

2 mg/kg, qdx1

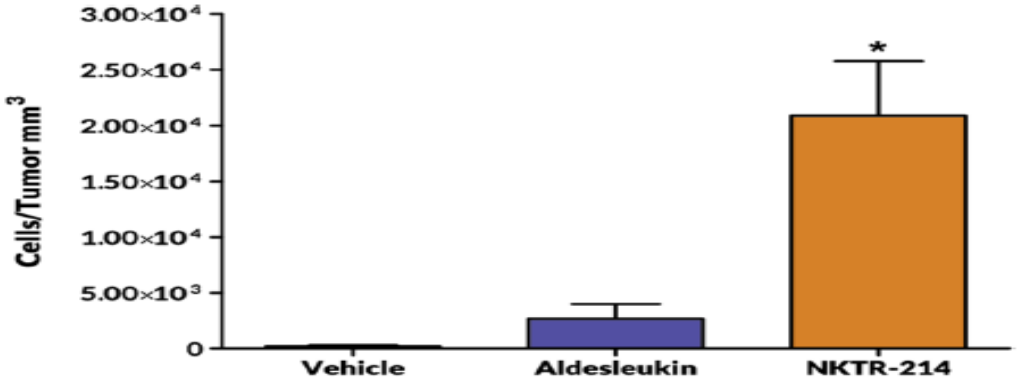


- A single dose of NKTR-214 leads to ~500-fold greater tumor exposure compared to an equivalent dose of aldesleukin
  - 62-fold higher tumor exposure compared to aldesleukin for 8-fold lower IL-2 equivalent dose

NKTR-214 Tips the Balance in Favor of Tumor Killing T-Cells Within the Tumor Microenvironment

Immune Cell Populations Isolated from Mouse Tumors

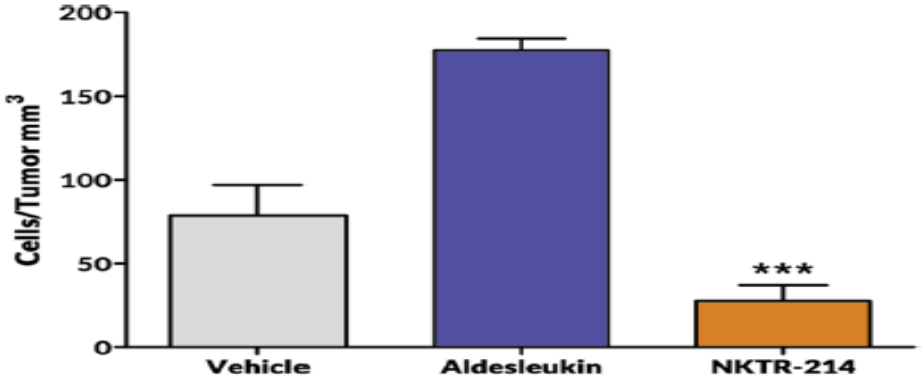
CD8+ Memory Effector T-Cells Increased After NKTR-214 Treatment



*P=0.0233 two tailed t test (NKTR-214 vs. aldesleukin)

Day 7 - Absolute Number Tumor Derived CD8+ Memory Effector T-Cells Per Tumor Volume Identified as CD8+CD122+CD44hi (N=3)

CD4+ Regulatory T-Cells Reduced After NKTR-214 Treatment



***P=0.0002 two tailed t test (NKTR-214 vs. aldesleukin)

Day 7 - Absolute Number Tumor Derived Regulatory T-Cells Per Tumor Volume Identified as CD4+CD25+FoxP3+ (N=3)

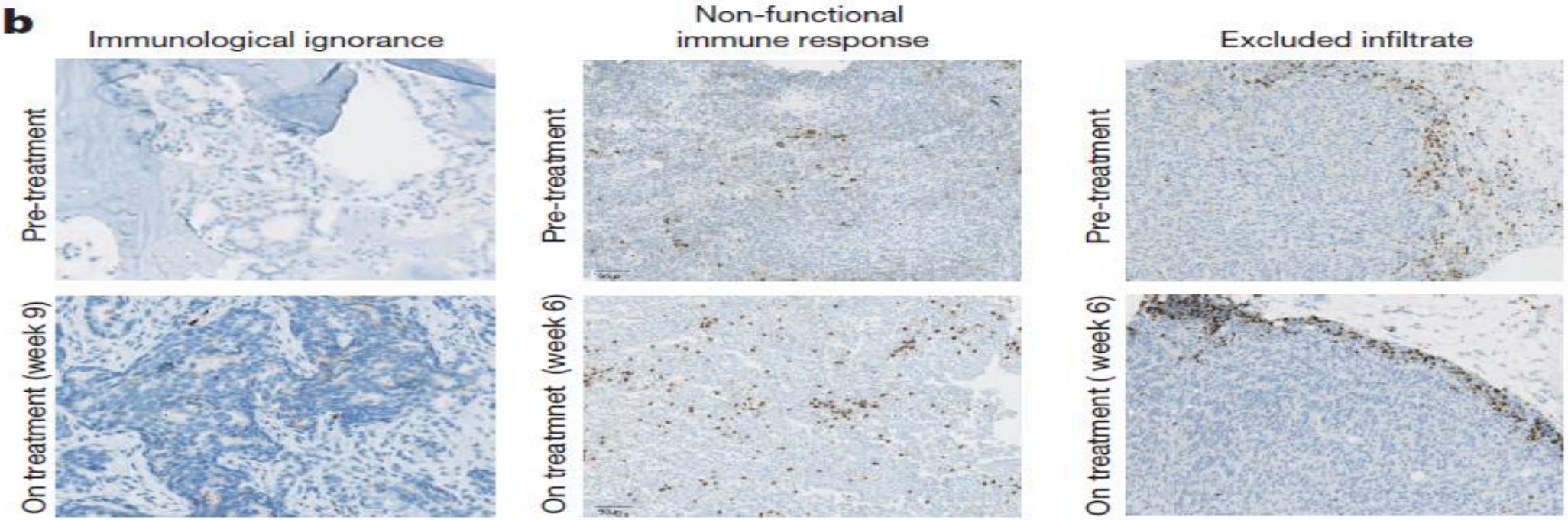
B16F10 mouse melanoma model

Herbst et al, Nature, Nov 2014

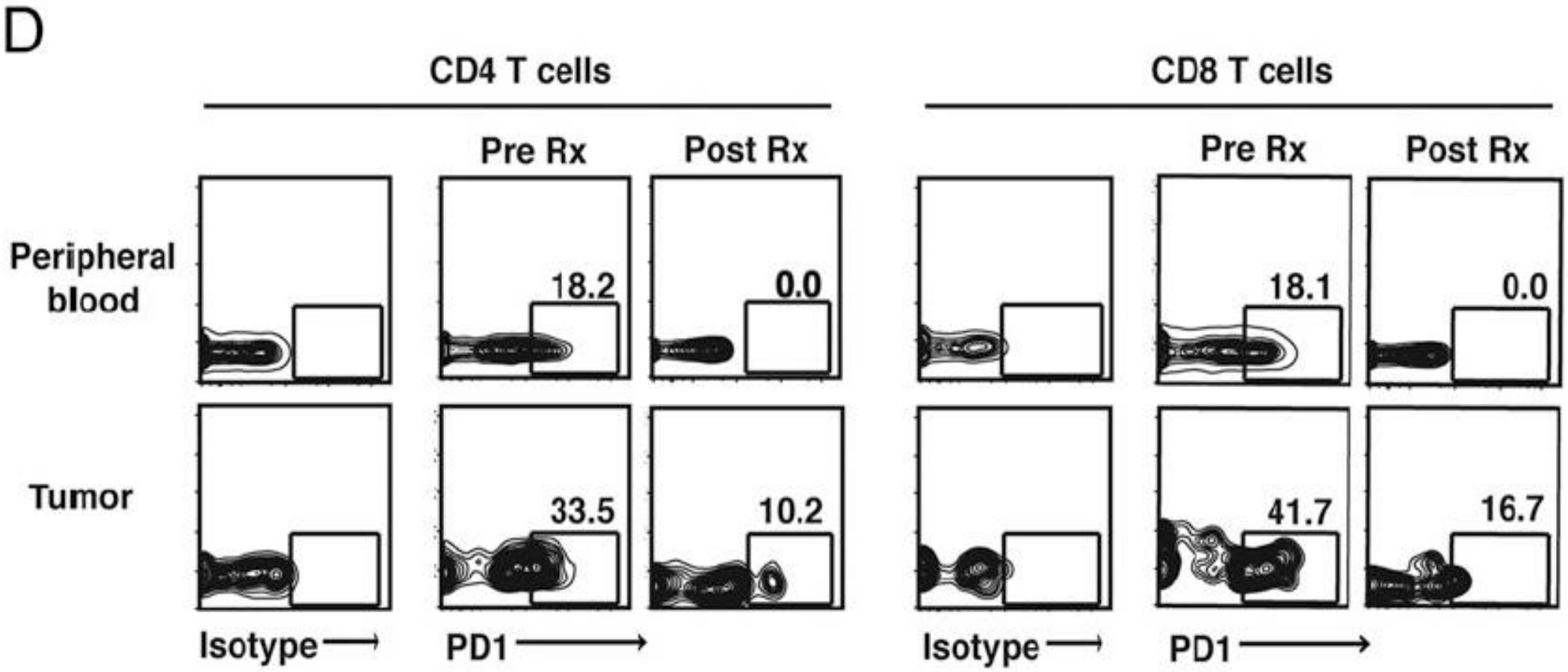
a Summary of responses to MPDL3280A in paired biopsies

	Increase in PD-L1 (TC) (no. (%))	Increase in PD-L1 (IC) (no. (%))
Maximum SLD decrease		
>30% reduction	3/6 (50)	5/6 (83)
0%–30% reduction	3/8 (37)	2/8 (25)
0%–20% increase	2/9 (22)	1/9 (11)
>20% increase	0/3 (0)	1/3 (33)
Unevaluable SLD	1/1 (100)	1/1 (100)
Objective response per RECIST v1.1		
Best response of PR	3/5 (60)	4/5 (80)
Best response of SD	5/12 (42)	2/12 (17)
Best response of PD	1/11 (9)	4/11 (36)

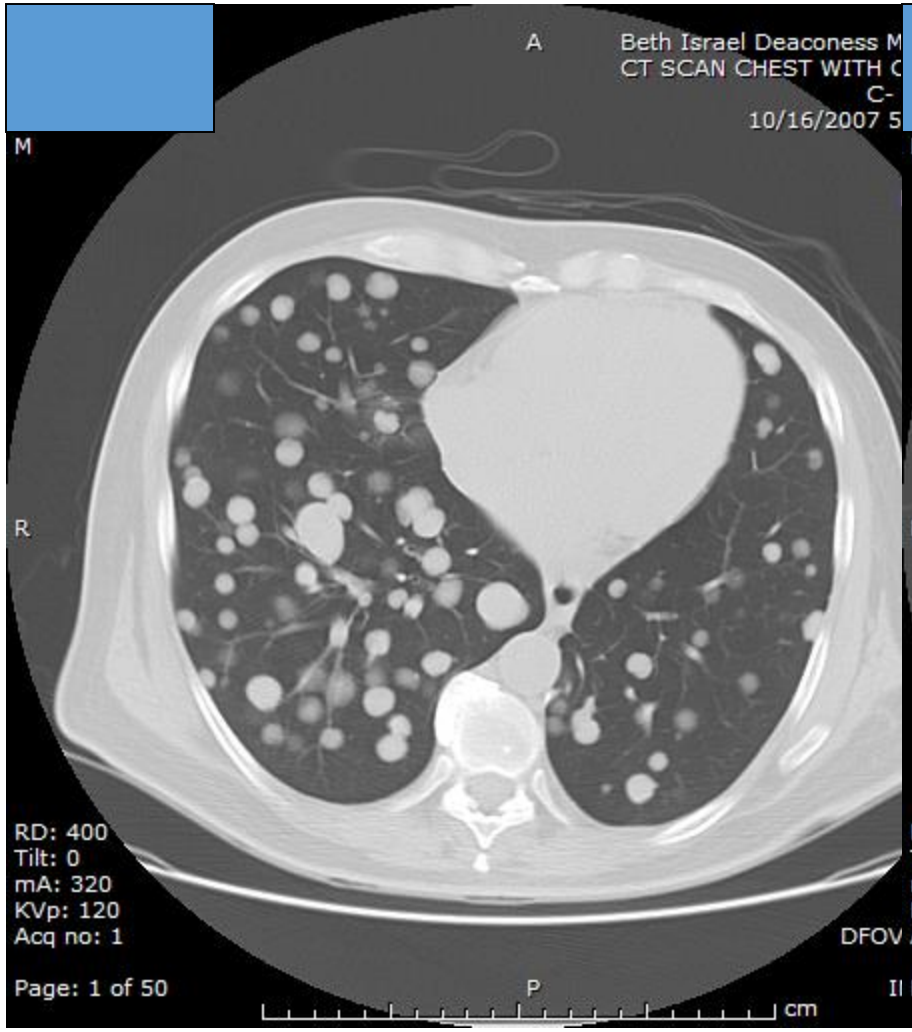
b

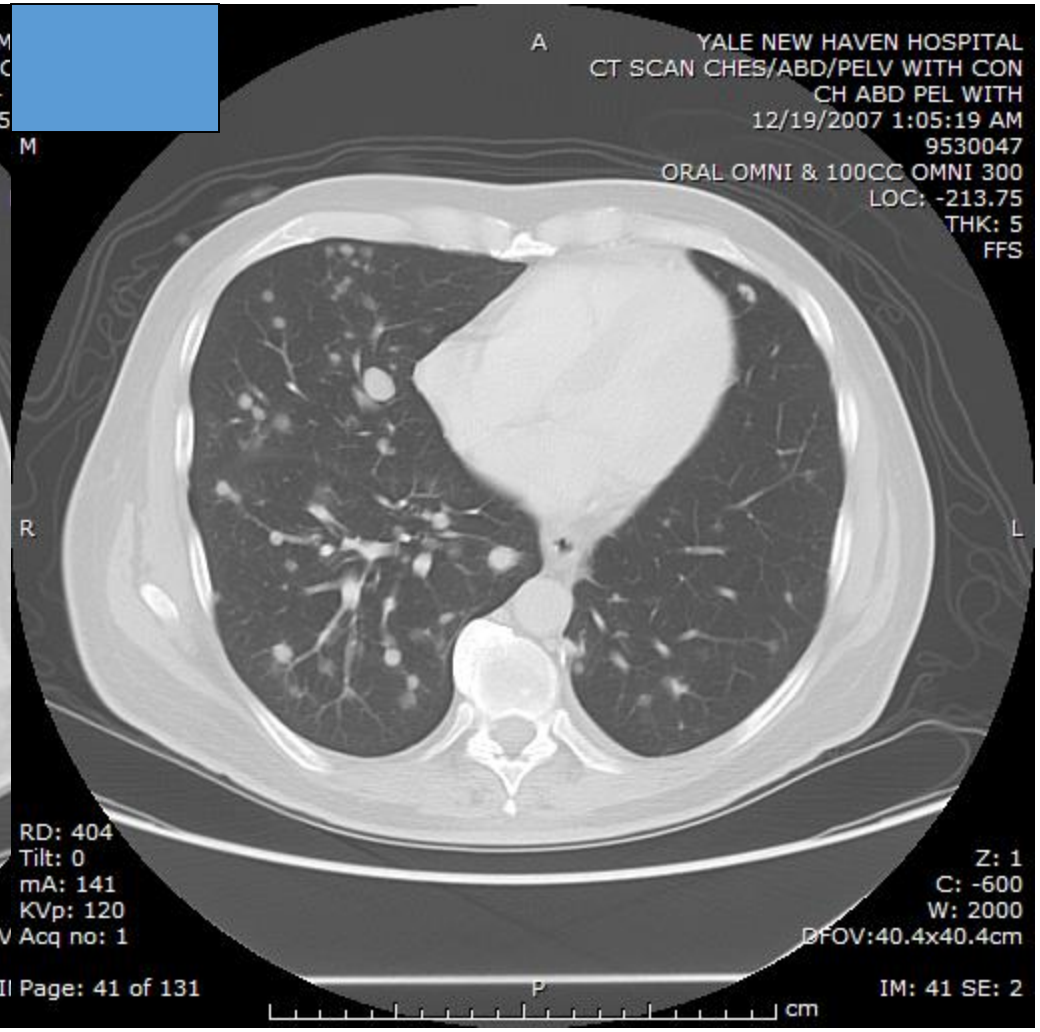


Is the dose of anti-PD-1 optimal in the combination?



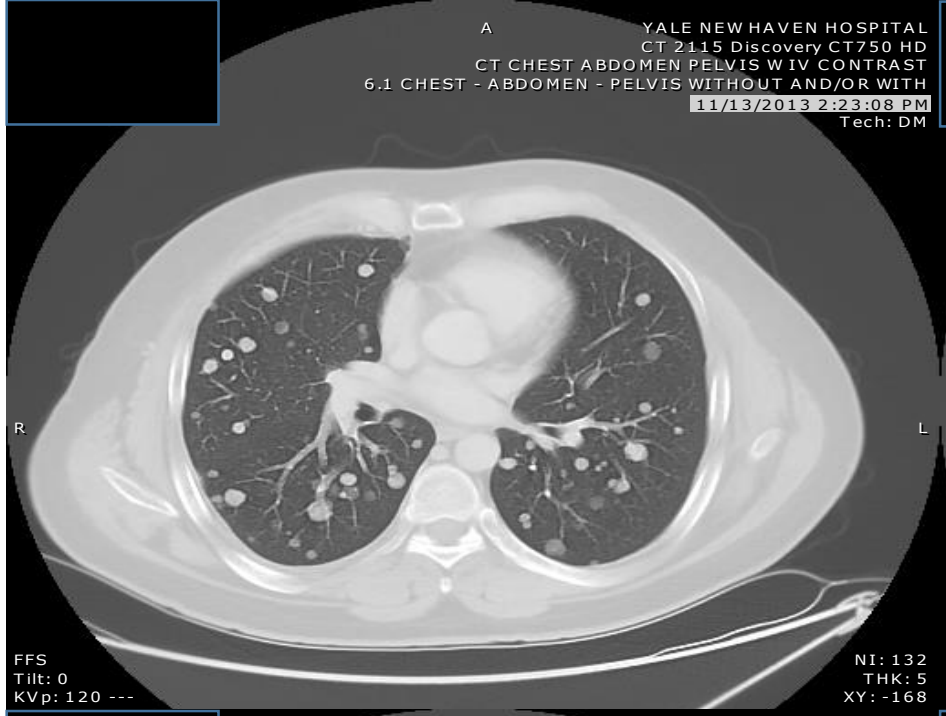
Response to Ipilimumab 10 mg/kg x 2 doses

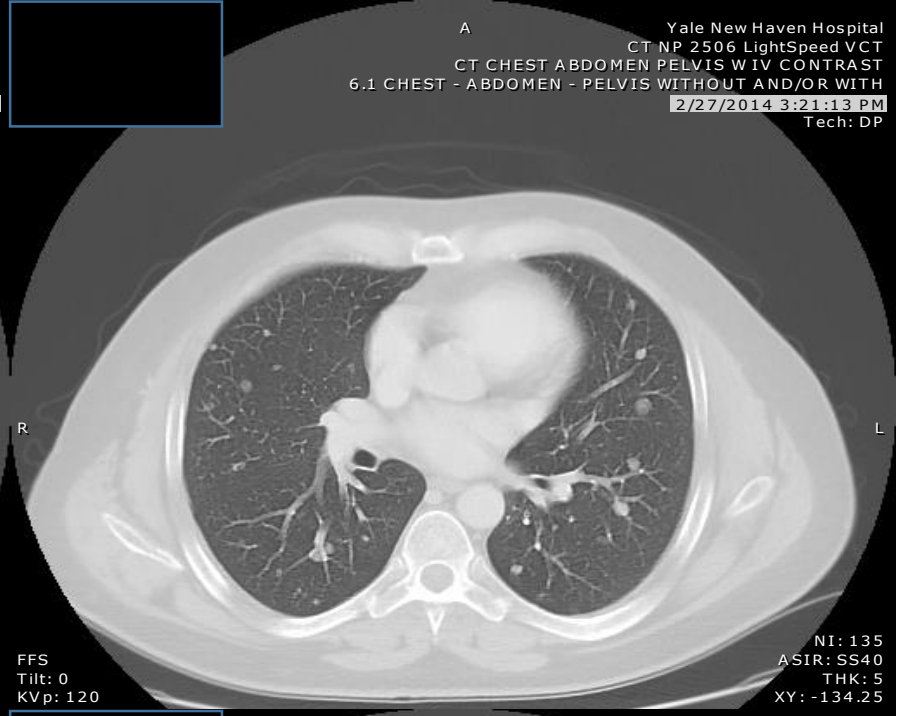


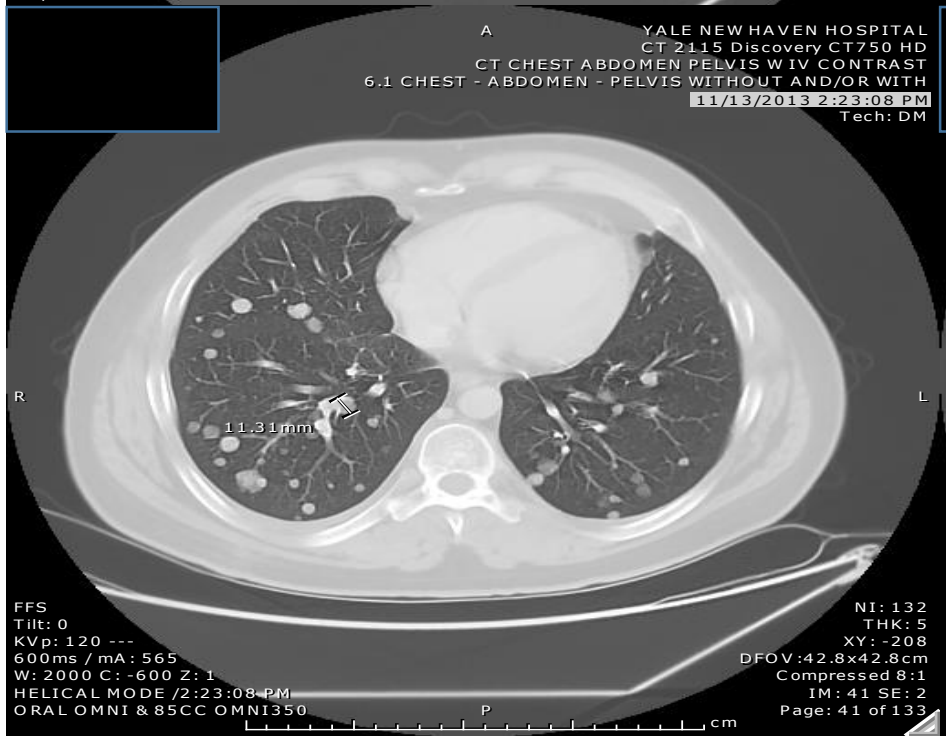


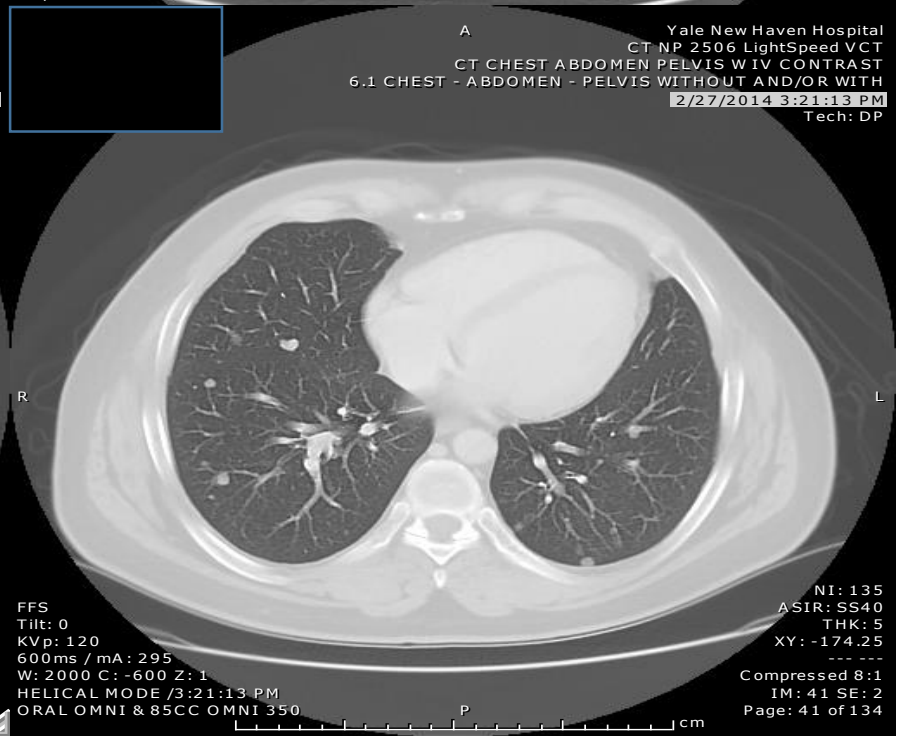
2 baseline brain mets regressed also:
No disease progression 8+ years

Baseline



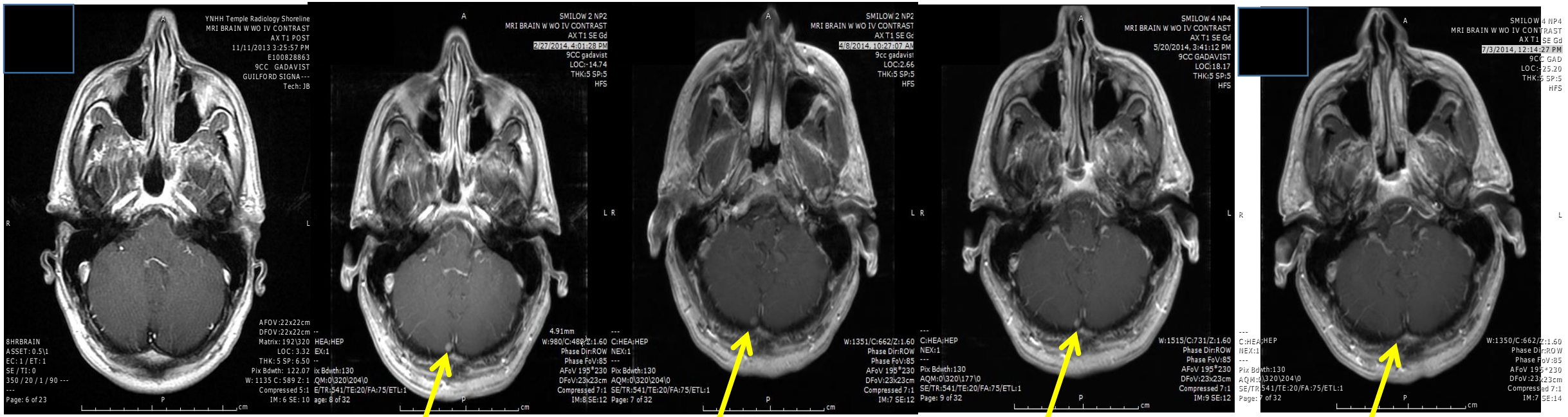


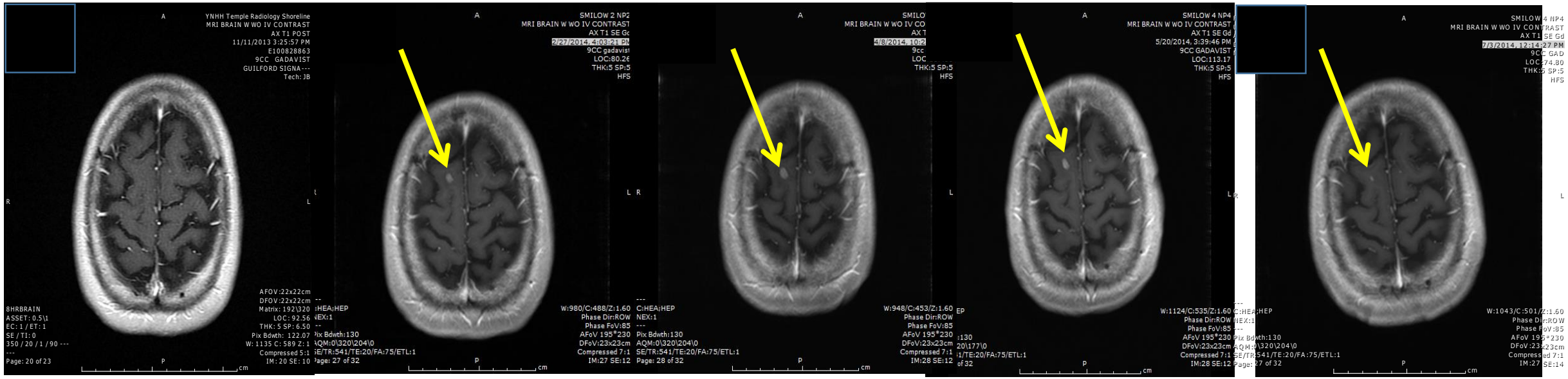




Week 13

Left untreated, brain lesions grew slightly at week 19 and began to regress at week 25





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LOC: -358.49
HFS

2/Volume 1
A 250
Yale New Haven Hospital
PET CT WHOLE BODY SUBSEQUENT
FUSED TRANS
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E103208639
Ex: Nov 13 2015

DFOV 50.0 cm
7.00
0.00
45 % PET
3.3/
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A
MGH Chelsea
OSF PET WHOLE BODY IMAGING
Art liver/ ch/ab/pel
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HFS

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W: 256
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P 250
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Tilt: 0
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C: 40
W: 400
DFOV: 49.2x49.2cm
Page: 103 of 268
P
IM: 103 SE: 10

R
L
FFS
Tilt: 0
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600ms / mA: 190
W: 400 C: 40 Z: 1
HELICAL MODE /1:47:11 PM
ORAL OMNI & 85CC OMNI 350
NI: 180
ASIR: SS50
THK: 5
XY: -225.75
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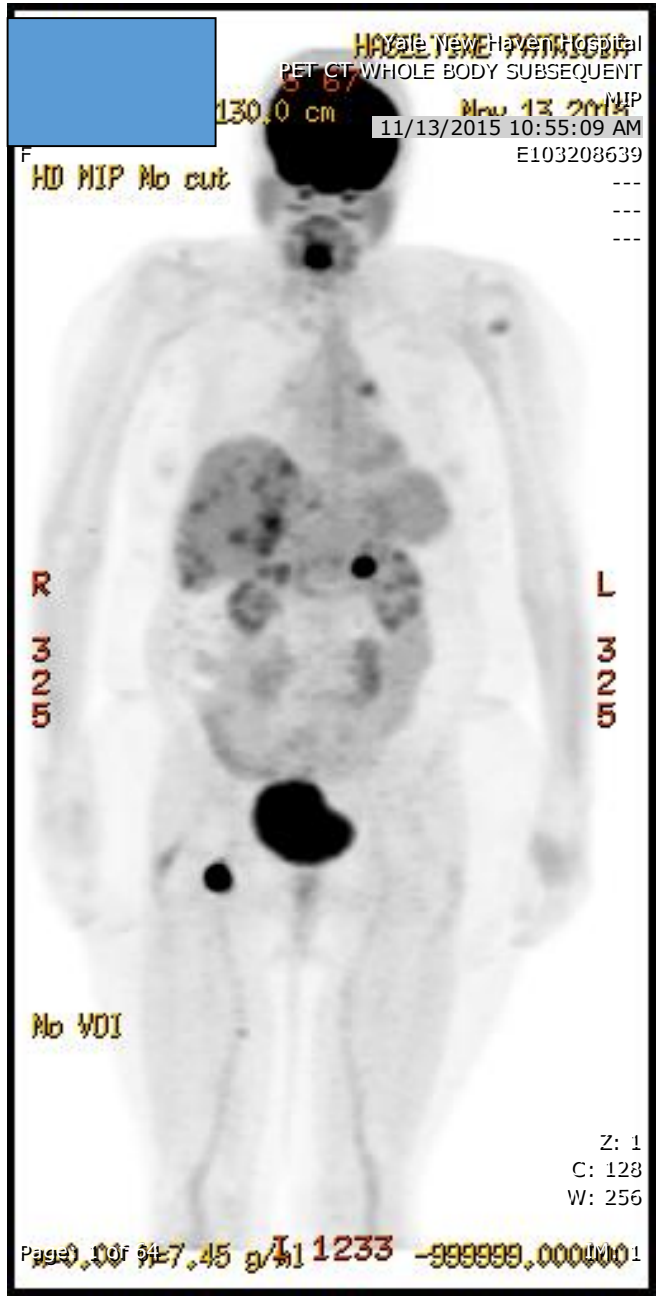
Compressed 8:1
IM: 51 SE: 2
Page: 51 of 132
P

After ipi/nivo x 4, nivo x one year:

- Marked clinical improvement, normalized LDH

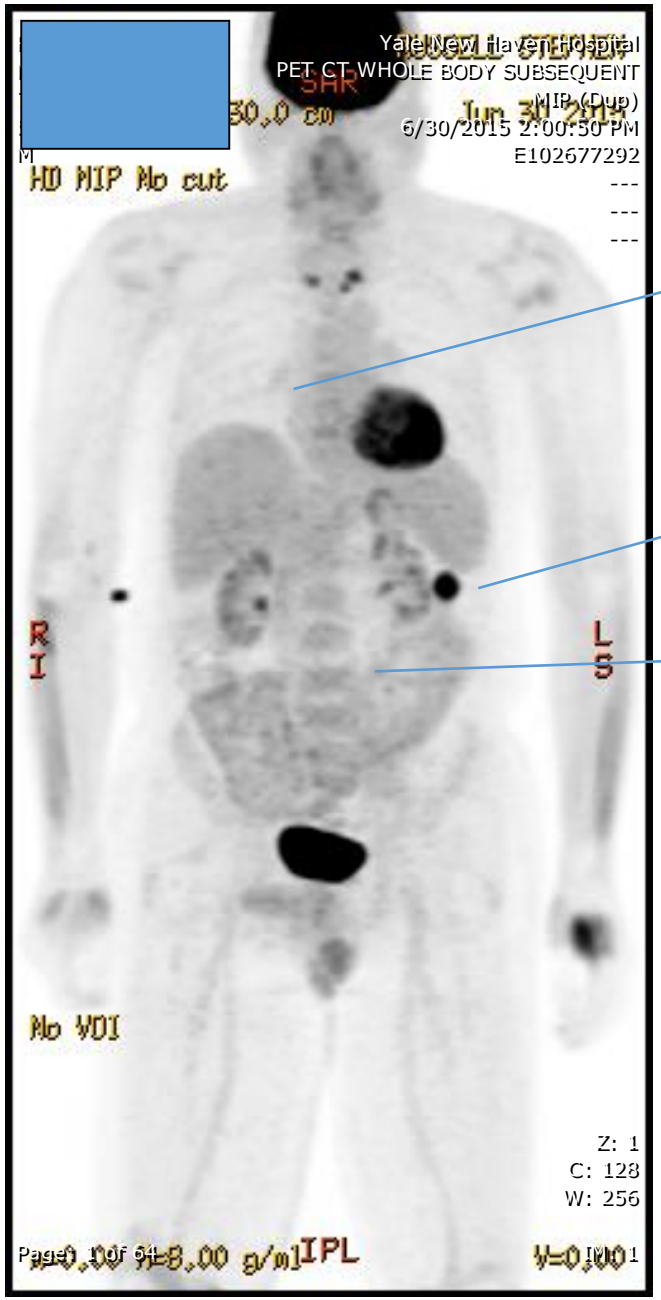
- MRI flair activity lateral left globe
- New FDG avid Left hilar node
- Increasing FDG avid Left adrenal
- Increasing avidity in right inguinal mass
- Improvement in hepatic lesions but still several with increased FDG uptake



- RAI plaque left lateral eye lesion
- Resected right inguinal mass
- Re-induction ipi/nivo

- Adrenal decreased in size and FDG uptake
- Left hilar node resolved
- Decrease FDG uptake in liver lesions

Ipi + Nivo x 4, Nivo q2w x 2 years, marked regression of most lesions (lung, LN, mesenteric and RP implants)

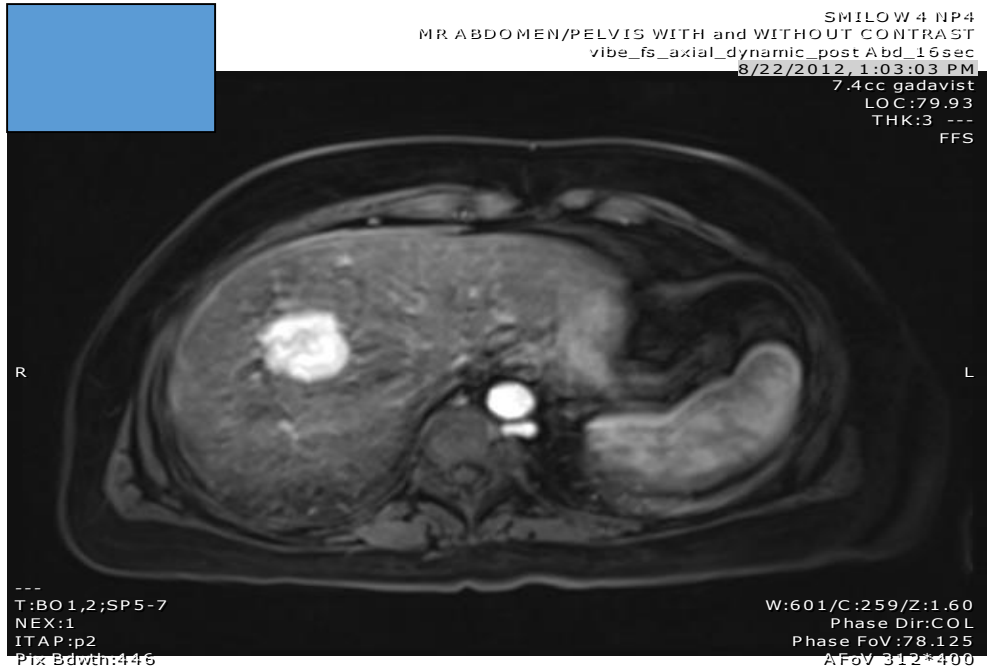


Persistent, slowly shrinking right middle lobe lesion, mildly FDG-avid

3 mesenteric nodules resected, 2/3 with active melanoma

Multiple other small residual lesions, not FDG-avid

Resect all FDG avid lesions?
Continue anti-Pd-1?
Re-induce with ipi/nivo?


SMILOW 4 NP4
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FFS
R
L

T:BO1,2;SP5-7
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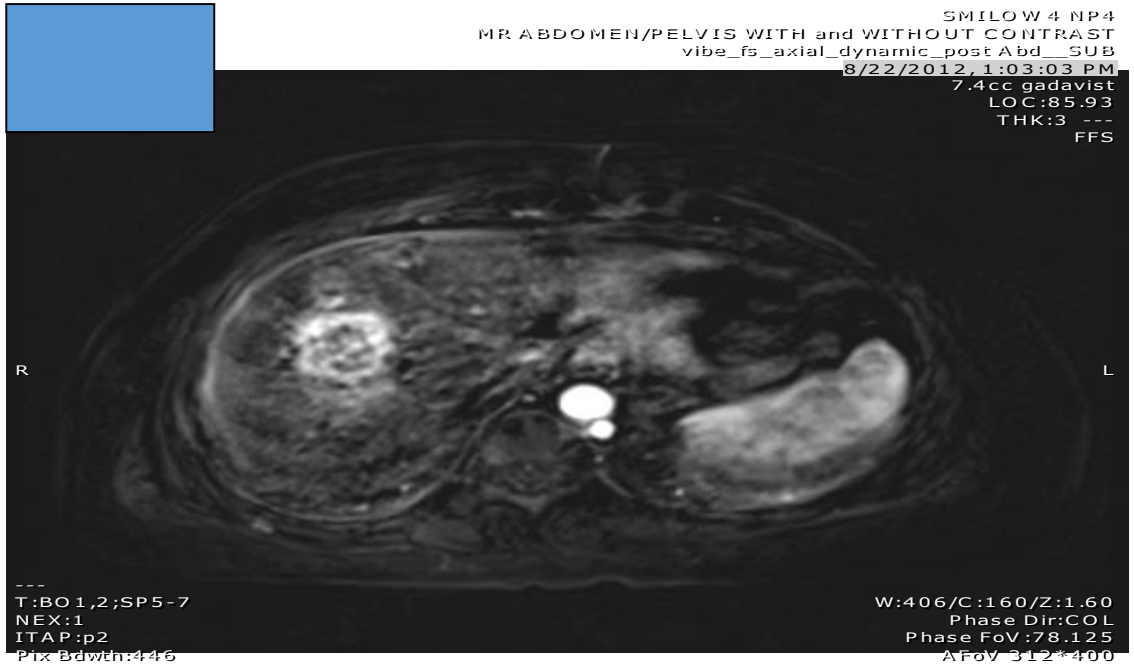
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MR ABDOMEN/PELVIS WITH and WITHOUT CONTRAST
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FFS
R
L

T:BO1,2;SP5-7
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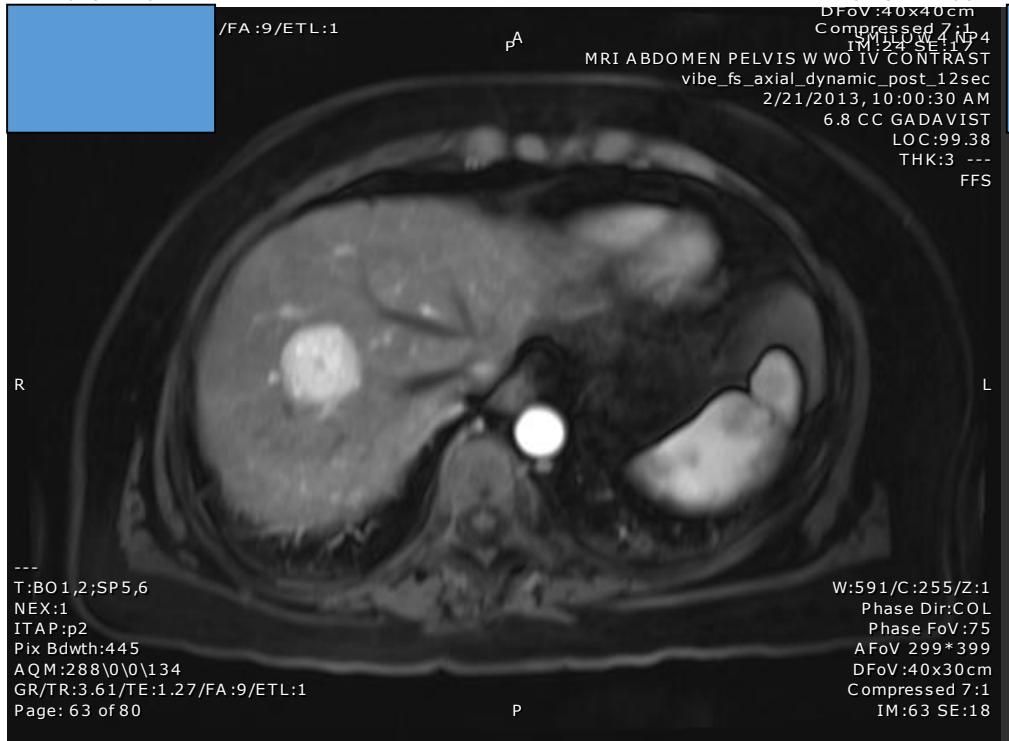
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MRI ABDOMEN PELVIS W WO IV CONTRAST
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6.8 CC GADAVIST
LOC:99.38
THK:3 ---
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T:BO1,2;SP5,6
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AQM:288\0\0\134
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Page: 63 of 80
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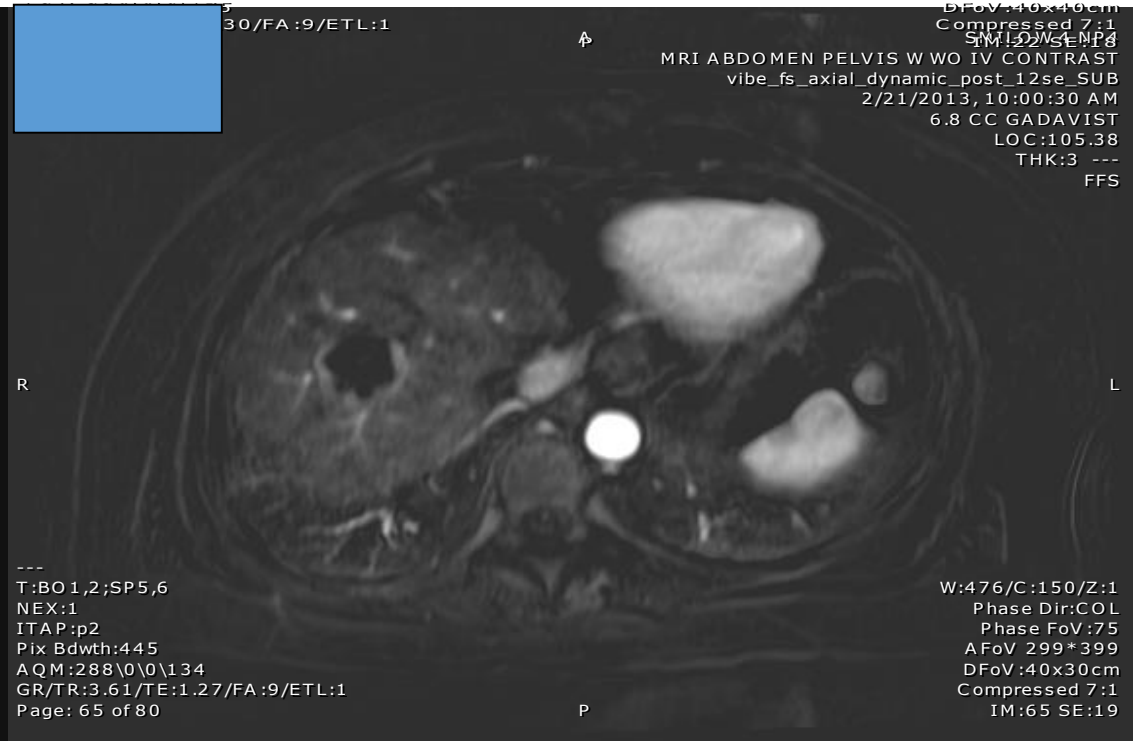
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SMILOW 4 NP4
MRI ABDOMEN PELVIS W WO IV CONTRAST
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6.8 CC GADAVIST
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FFS
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T:BO1,2;SP5,6
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Page: 65 of 80
P
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Phase Dir:COL
Phase FoV:75
AFoV 299*399
DFoV:40x30cm
Compressed 7:1
IM:65 SE:19

Imaging and immune therapy

High levels of clinical activity for immune therapy, complex biology, mechanisms of action and resistance poorly understood, complex patterns of clinical response, and innumerable agents and trials

HELP!!!!!

- Predictive of Response
  - T-cell infiltration (extent, location, function, type)
  - Other immune cells (MDSC, Treg?)
  - Expression of antibody targets (CD47, CD73, PD-L1, PD-1, TIM-3, PD-1H, etc)
  - Metabolisms/metabolic state (hypoxia, glucose consumption, other)
- Tumor response in the absence of regression
- Differentiate scar from residual tumor versus persistent inflammation without tumor
  - When to stop therapy?
- Differentiate pseudo-progression from true regression
- Biodistribution and pharmacodynamic endpoints
  - Receptor saturation
  - Tumor T-cell activation, T-cell infiltration, change in T cell ratios, cytokine production

DANA-FARBER CANCER INSTITUTE

BRIGHAM AND WOMEN'S HOSPITAL

HARVARD MEDICAL SCHOOL

Imaging Inflammation with FDG, FLT and Beyond

Annick D. Van den Abbeele, MD

Chief, Department of Imaging

Founding Director, Center for Biomedical Imaging in Oncology

Co-Director, Tumor Imaging Metrics Core

CBIO

Center for Biomedical Imaging in Oncology

NCI CCC DANA-FARBER/HARVARD CANCER CENTER
A Comprehensive Cancer Center
Designated by the National Cancer Institute

TUMOR IMAGING METRICS CORE

Disclosures for Annick D. Van den Abbeele, MD

- Research funding support to the Dana-Farber Cancer Institute from Novartis, Pfizer, Bayer, GSK, BMS

Immune Checkpoint Therapy: "A Game-Changer"

- Radical and disruptive change in cancer therapy:
  - Drugs are not designed to target the tumor cell, i.e., tissue of origin is becoming less relevant
  - Goal is to remove inhibitory pathways that block effective antitumor T cell responses
- Knowledge of the tumor microenvironment is becoming more important

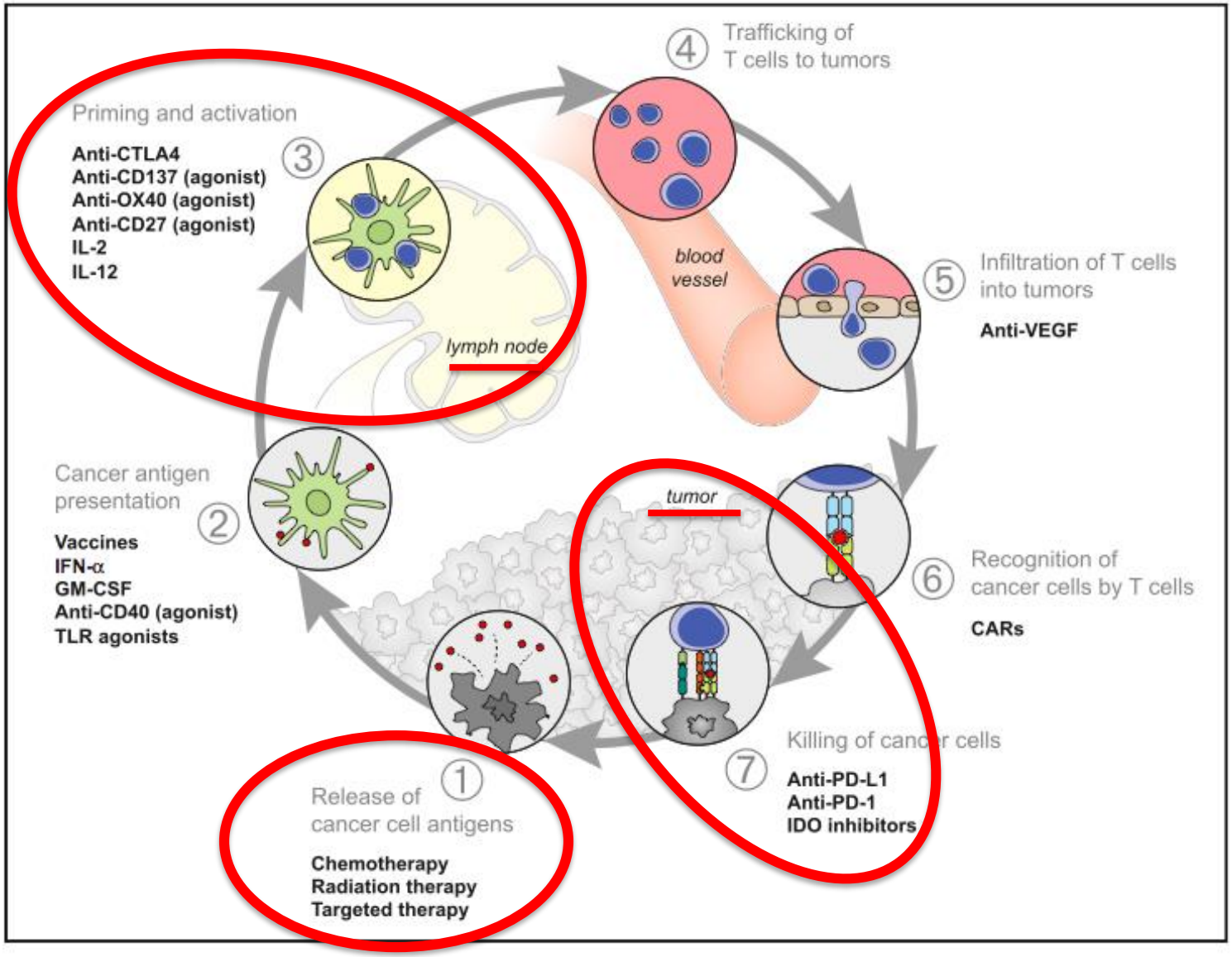
Sharma et al Science 348:6230, April 2015

Immune Checkpoint Therapy: "A Game-Changer" and a Challenge…

- Immune response is dynamic and changes rapidly
- A single biomarker may not be enough to predict response as with molecularly-targeted therapy
- Must be able to assess the effectiveness of an evolving immune response and define the response that contributes to clinical benefit

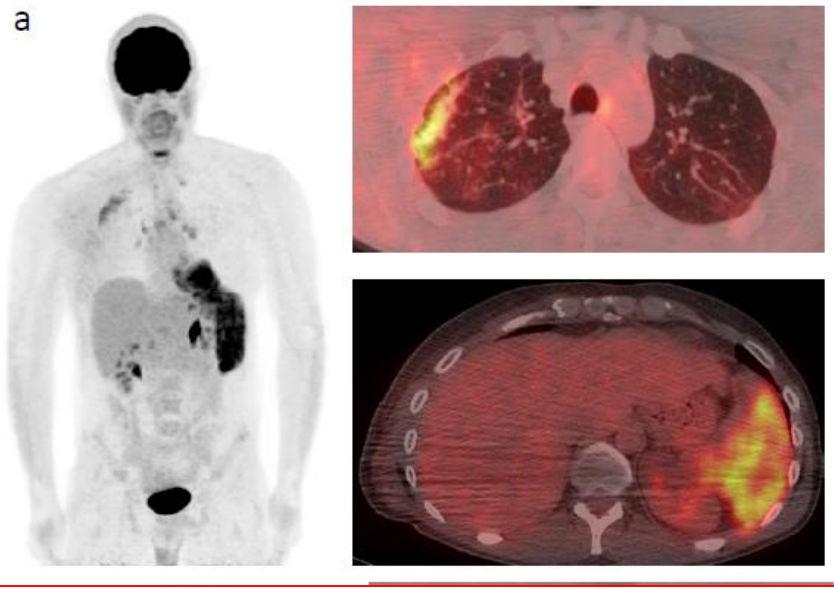
Sharma et al Science 348:6230, April 2015

Therapies that Might Affect the Cancer-Immunity Cycle



Immunity 39, July 25, 2013 a2013 Elsevier Inc.

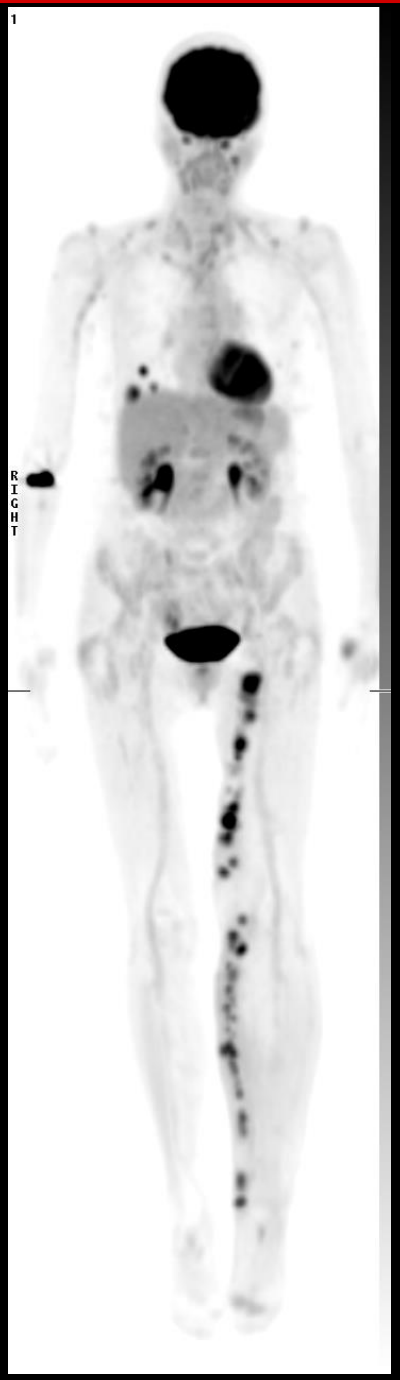
2 months after ipi



Is this an immune-related adverse event or a sign of qualitative and quantitative “immunocompetency” in spleen and draining lymph nodes?

Jen Kwak et al. Radiographics. 2015 Mar-Apr;35(2):424-37.

FDG



- Diagnosis
- Tumor characterization (prognostic value)
- Staging
- Restaging
- Assessment of response (predictive value)
- Tumor heterogeneity
- Guide biopsy to relevant tissue

IASLC
INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER

16TH WORLD CONFERENCE ON LUNG CANCER
SEPTEMBER 6–9, 2015 → DENVER, COLORADO, USA

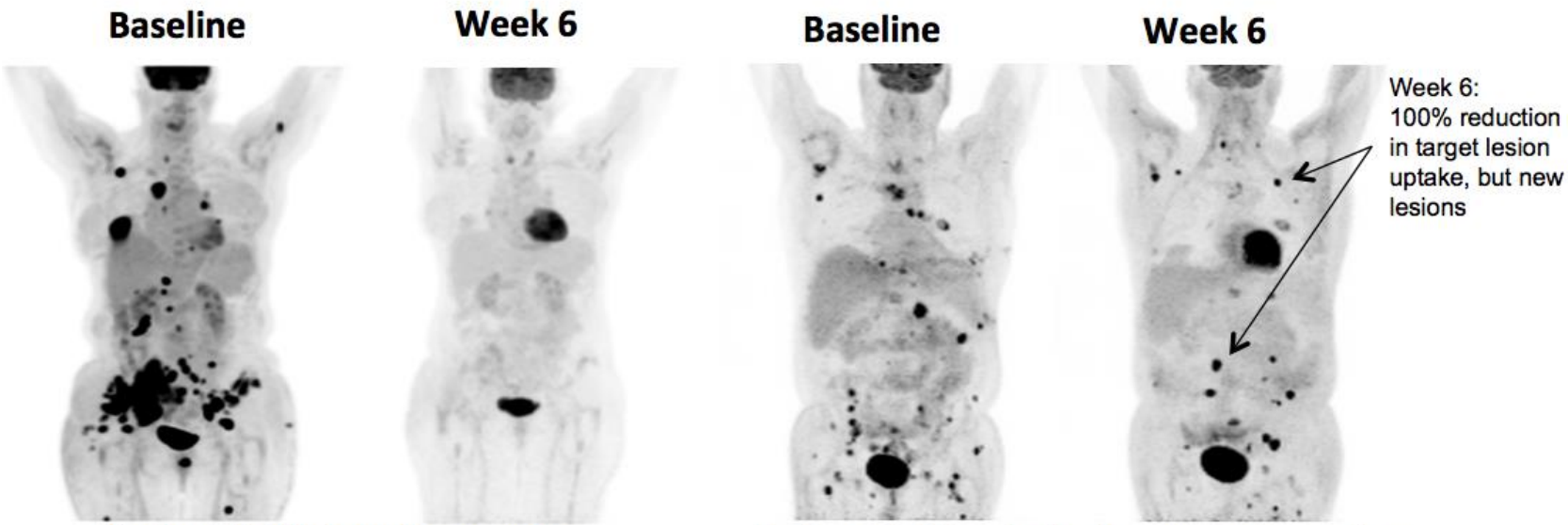
Evaluation of PD-L1 expression in metachronous tumor samples and FDG-PET as a predictive biomarker in Ph2 study (FIR) of atezolizumab (MPDL3280A)

Jamie Chaft,[1] Bo Chao,[2] Wallace Akerley,[3] Michael S. Gordon,[4] Scott J. Antonia,[5]
Jason Callahan,[6] Alan Sandler,[7] Roel Funke,[7] Larry Leon,[7] Jill Fredrickson,[7]
Marcin Kowanetz,[7] Scott Gettinger[8]

[1]Memorial Sloan-Kettering Cancer Center, New York, NY; [2]Ohio State University, Wexner Medical Center, Colombus, OH; [3]Huntsman Cancer Institute, Salt Lake City, UT; [4]Pinnacle Oncology Hematology, Scottsdale, AZ; [5]Moffit Cancer Center, Tampa, FL; [6]Peter MacCallum Cancer Center, East Melbourne, Australia; [7]Genentech Inc., South San Francisco, CA; [8]Yale School of Medicine, New Haven, CT

Courtesy of Bernard M. Fine, MD PhD

FDG-PET metrics and examples of response



Patient 1: Metabolic response

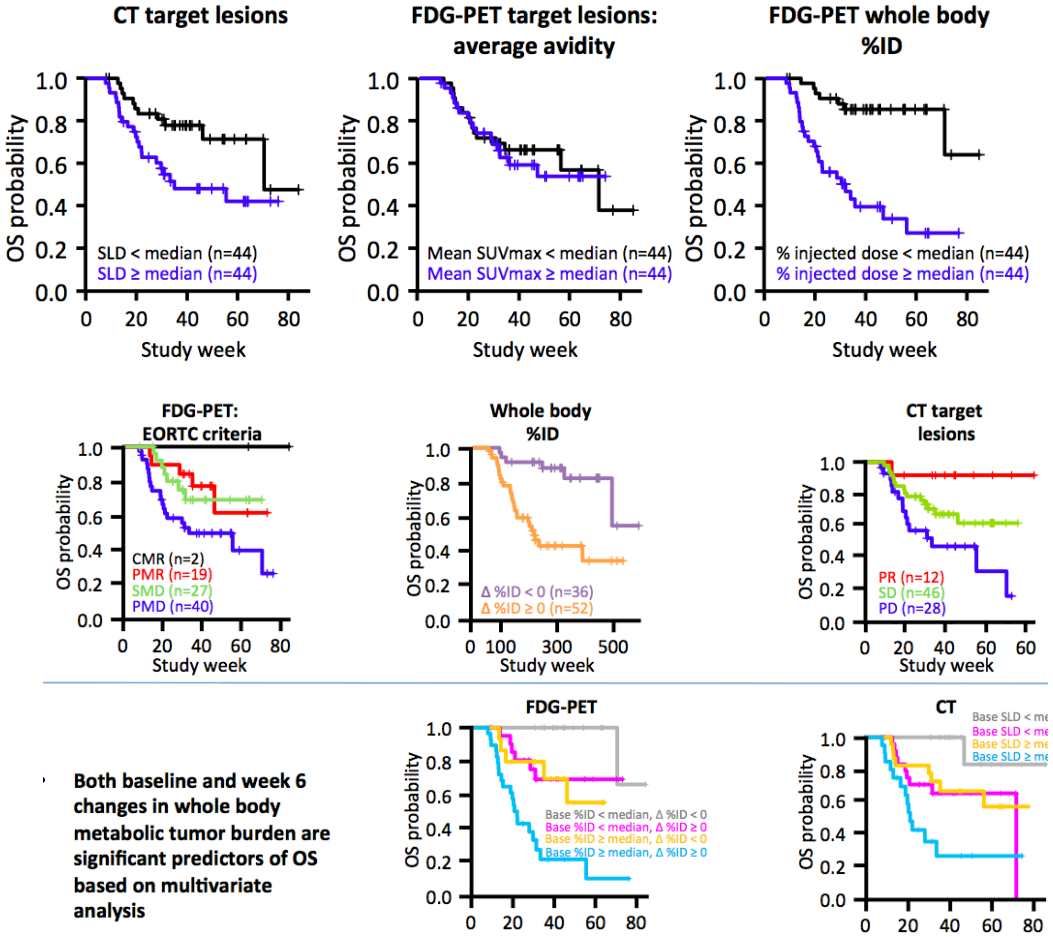
Patient 2: Metabolic disease progression

- **Metabolic response determined by EORTC criteria based on 5 target lesions**
- **Whole body analyses: metrics for metabolic tumor burden derived from automated volume of interest**
  - **Percentage Injected Dose (%ID): reflects both metabolic volume and intensity of FDG uptake**
- **Patients with metabolic response by EORTC criteria on week 6 scans had higher ORR by RECIST 1.1 than metabolic non-responders (71% [15/21] vs 4% [3/67])**

Courtesy of Bernard M. Fine, MD PhD

Conclusions

- Baseline metabolic tumor burden was a significant negative prognostic marker for OS
- Early metabolic response (week 6) was a significant predictor of OS



Both baseline and week 6 changes in whole body metabolic tumor burden are significant predictors of OS based on multivariate analysis

Courtesy of Bernard M. Fine, MD PhD

FDG and
Immune-Adverse Events (IAEs)

Ipilimumab Potential Side Effects

	Any Grade	>Grade3
• Dermatitis	40%	3%
• ***Diarrhea/Colitis***	30%	8%
• Hypophysitis/Thyroiditis	6%	1%
• Hepatitis and Pancreatitis	9%	6%
• Other	6%	2%
– Nephritis		
– Uveitis or Episcleritis		
– Neuritis		
• Overall	70%	20%

IRAEs can be waxing and waning

Courtesy of Steve Hodi, MD

Nivolumab Adverse Events

Drug-Related Adverse Event	All Grades		Grades 3-4	
	Tot Pop*	MEL	Tot Pop	MEL†
	No. (%) of Patients, All Doses			
Any adverse event	207 (70)	82 (79)	41 (14)	21 (20)
Fatigue	72 (24)	30 (29)	5 (2)	2 (2)
Rash	36 (12)	21 (20)	—	—
Diarrhea	33 (11)	18 (17)	3 (1)	2 (2)
Pruritus	28 (9)	15 (14)	1 (0.3)	—
Nausea	24 (8)	9 (9)	1 (0.3)	1 (1)
Appetite ↓	24 (8)	7 (7)	—	—
Hemoglobin ↓	19 (6)	7 (7)	1 (0.3)	1 (1)
Pyrexia	16 (5)	5 (5)	—	—

*AEs occurring in ≥5% of the total population.

†Common grade 3-4 AEs also included lymphopenia (3 pts) and abdominal pain and lipase increased (2 each). An additional 27 grade 3-4-related AEs were observed and one or more occurred in a single patient.

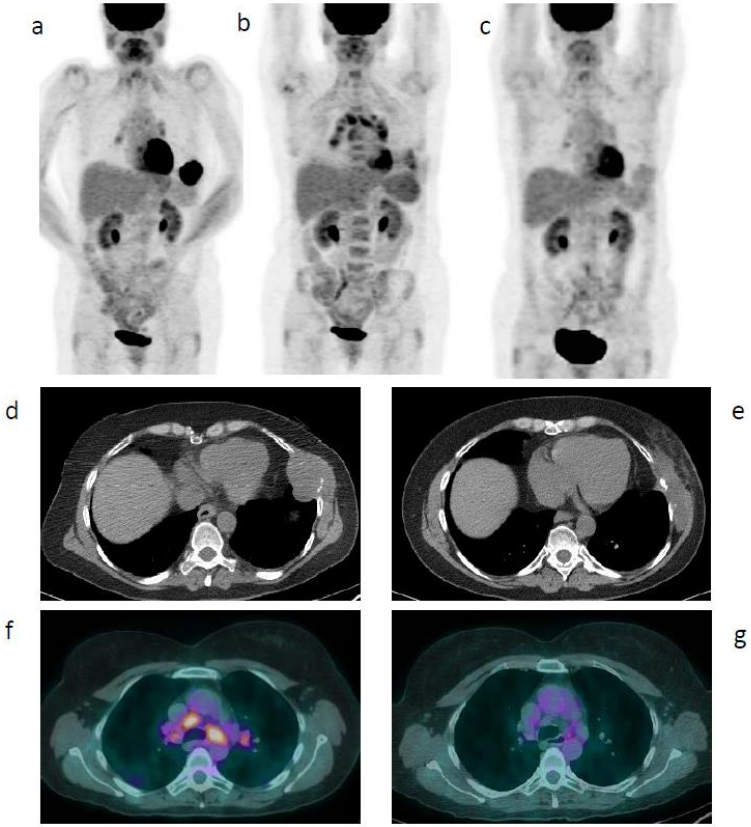
Courtesy of Steve Hodi, MD

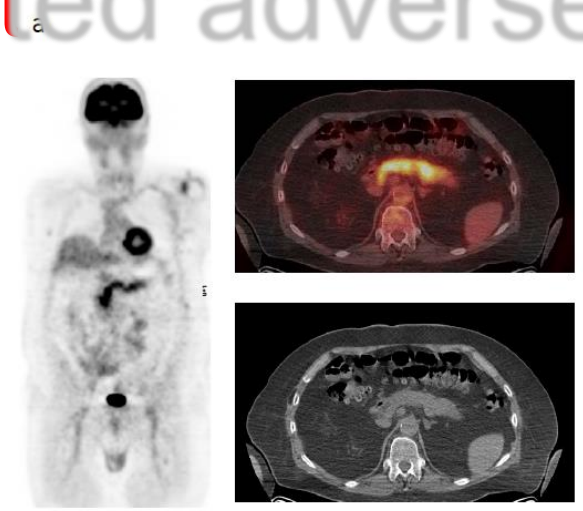
Treatment-Related Select Adverse Events Occurring in ≥1 Patient (ipilimumab and nivolumimab combination)

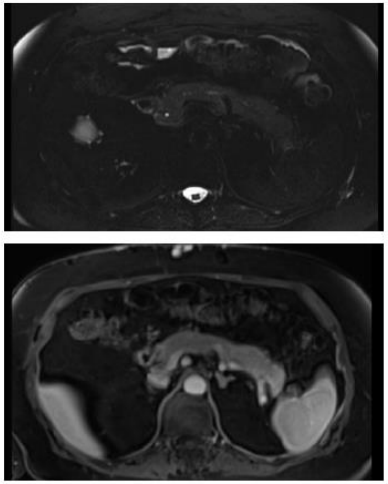
Select Adverse Event Number of Patients (%)	All Cohorts (n=53)	
	All Gr	Gr 3 4
Pulmonary	3 (6)	1 (2)
Renal	3 (6)	3 (6)
Endocrinopathies	7 (13)	1 (2)
Uveitis	3 (6)	2 (4)
Skin	37 (70)	2 (4)
Gastrointestinal	20 (38)	5 (9)
Hepatic	12 (23)	8 (15)
Infusion reaction	1 (2)	0
Lipase	10 (19)	7 (13)
Amylase	8 (15)	3 (6)

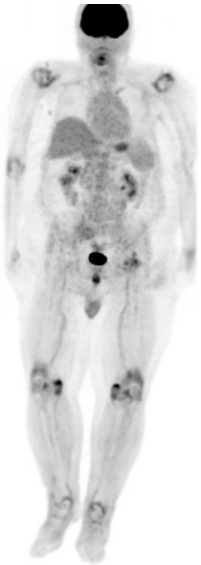
Courtesy of Steve Hodi, MD

Immune-related adverse events



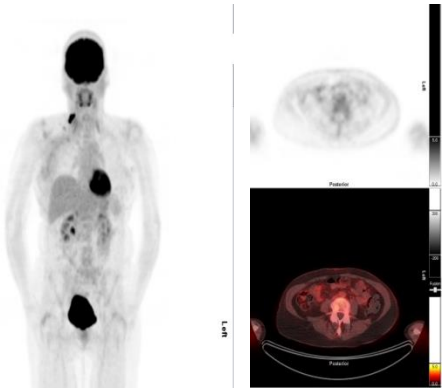




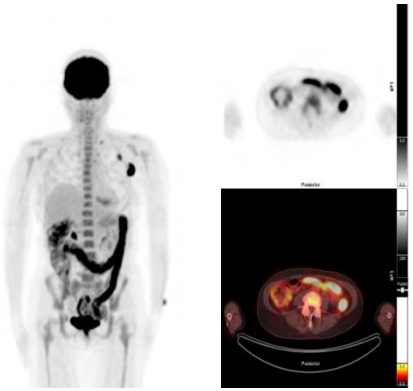


Jen Kwak et al. Radiographics. 2015 Mar-Apr;35(2):424-37

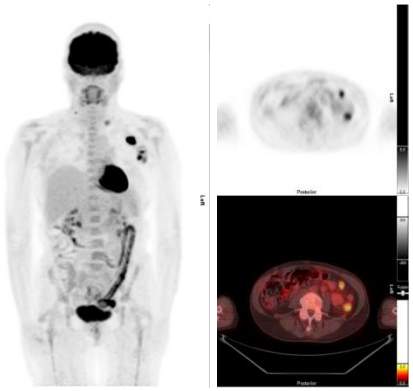
Immune-related adverse events



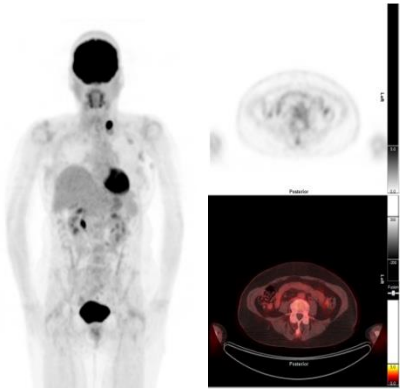
9/5/14



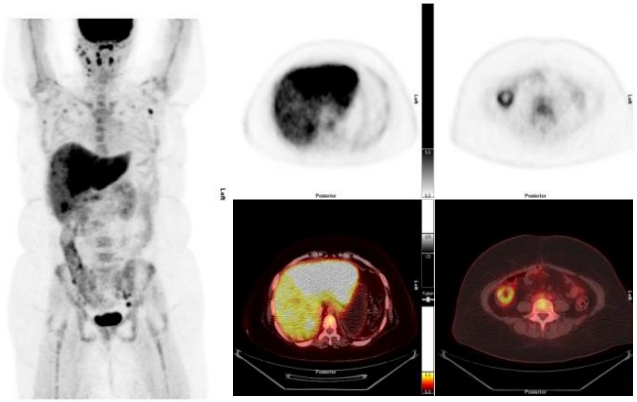
2/3/15



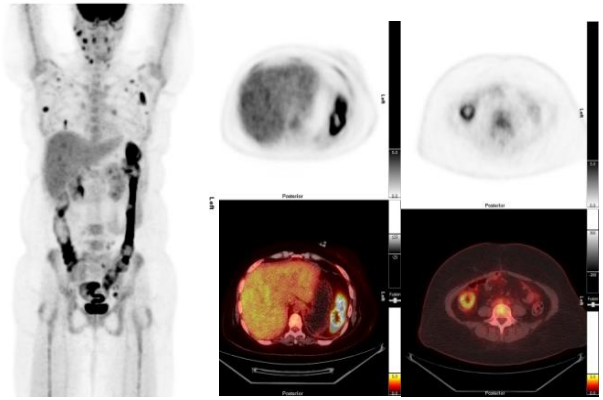
4/6/15



8/10/15



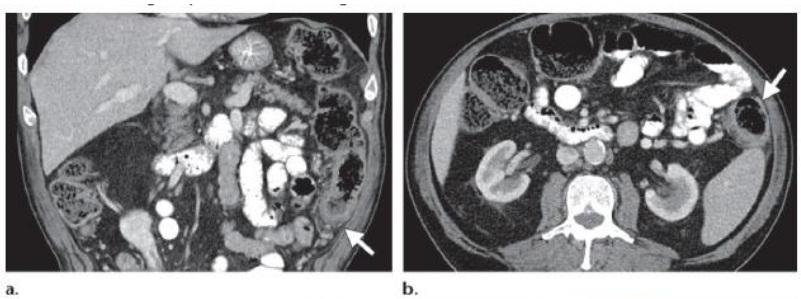
3/13/15



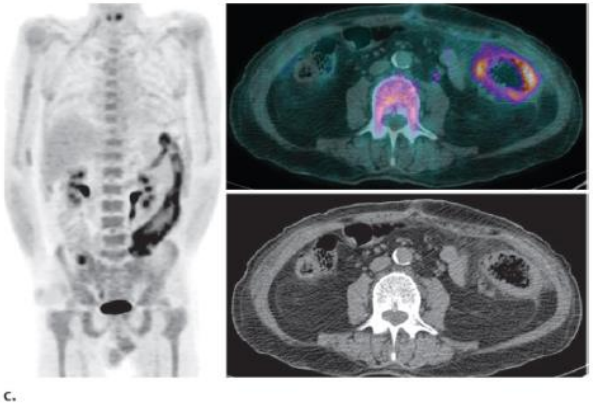
8/19/15

Immune-related adverse events

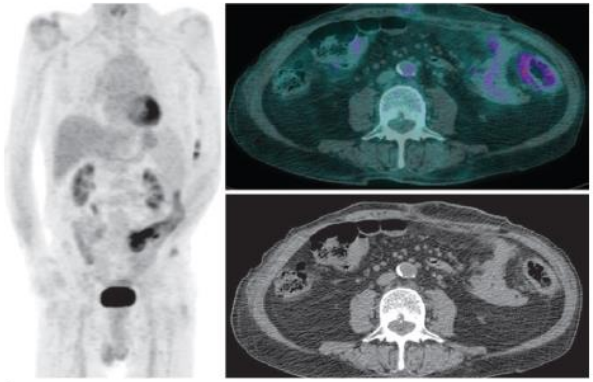
1 month
after ipilumimab
cycle 3



1 month
after corticosteroids,
started on infliximab

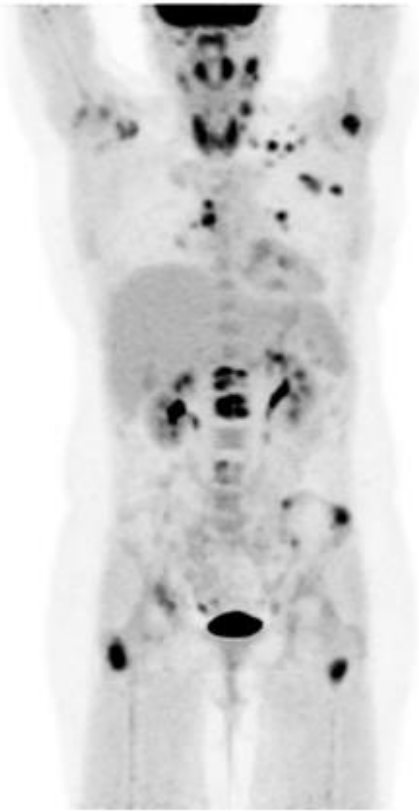


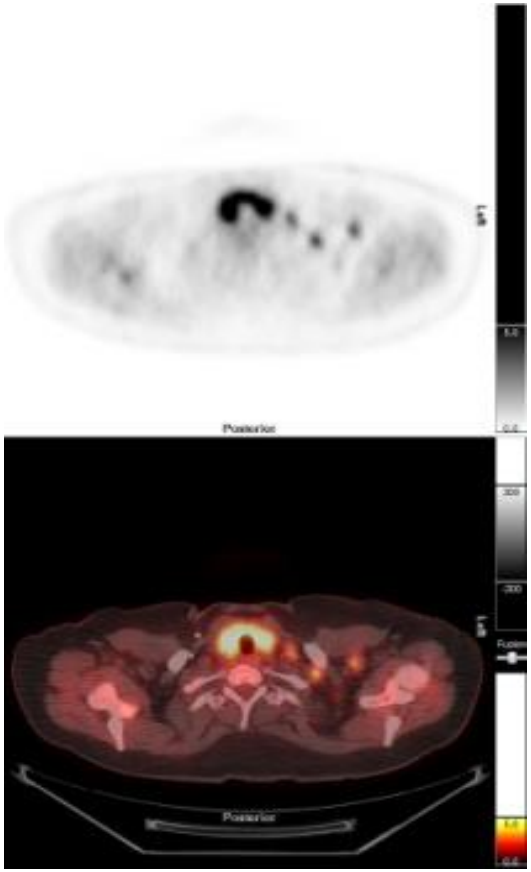
Follow-up

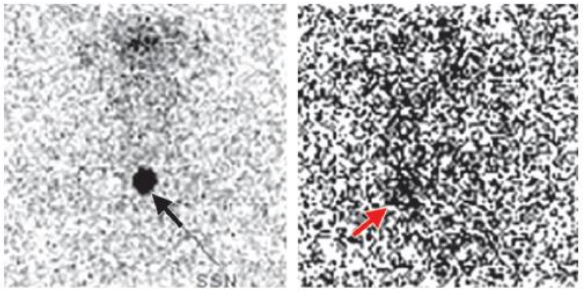


Jen Kwak et al. Radiographics. 2015 Mar-Apr;35(2):424-37.

Immune-related adverse events

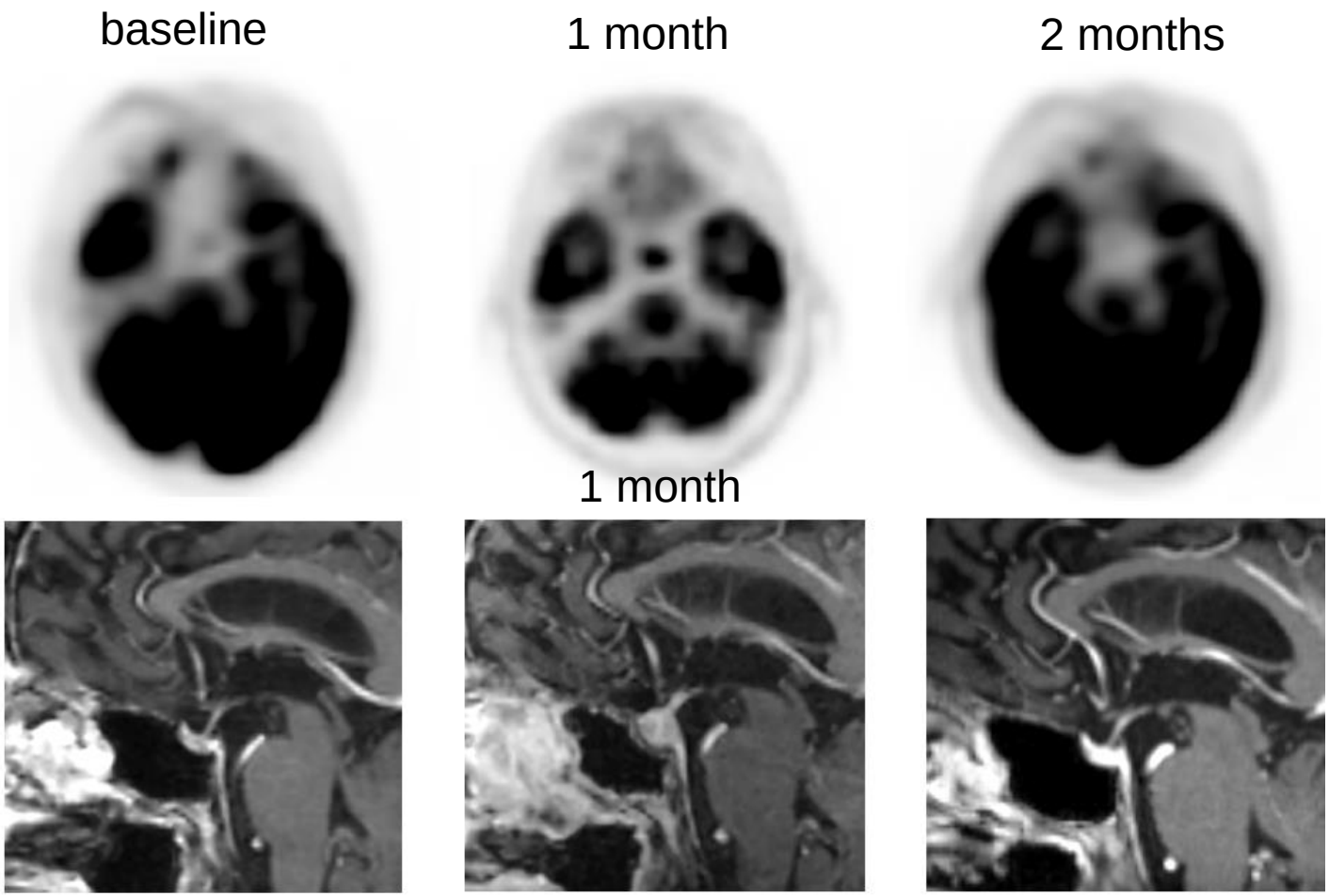






Jen Kwak et al. Radiographics. 2015 Mar-Apr;35(2):424-37.

Immune-related adverse events

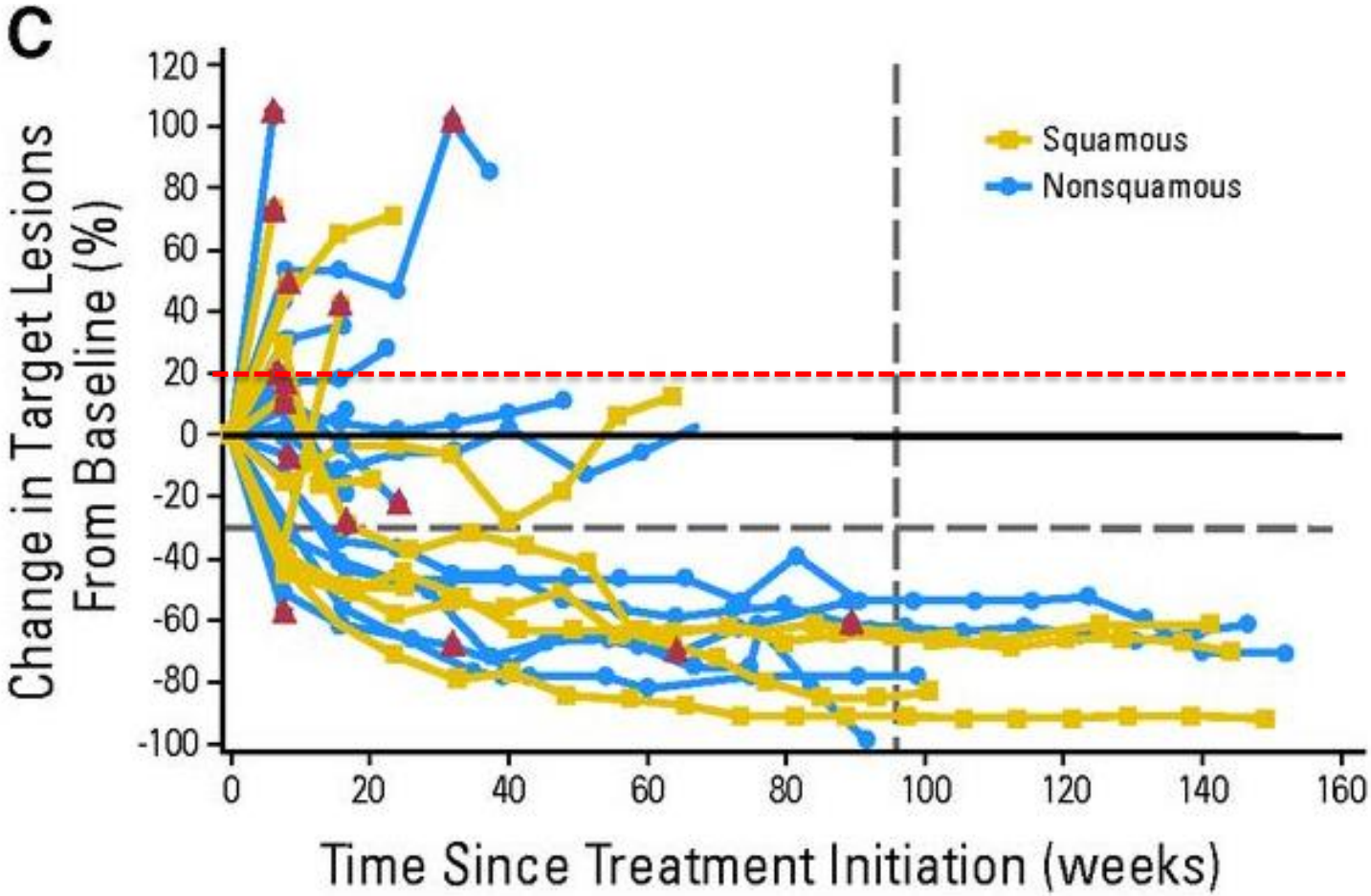


Jen Kwak et al. Radiographics. 2015 Mar-Apr;35(2):424-37.

Role of FDG in Immune-adverse events

- Clinically relevant
- May be seen months prior to symptom development
- Timely initiation of corticosteroid therapy may alleviate serious complications and life-long dependency on hormonal therapy

Clinical activity in patients with non–small-cell lung cancer (NSCLC) receiving nivolumab



Scott N. Gettinger et al. JCO doi:10.1200/JCO.2014.58.3708

JOURNAL OF CLINICAL ONCOLOGY ASCO

Evaluation of Treatment Response

- Cancer vaccines and immunomodulatory monoclonal antibodies have demonstrated
  - delayed response to treatment when compared to cytotoxic chemotherapy

Do we need to wait that long to assess response?

- In the longest follow-up study after ipilimumab treatment for metastatic melanoma, the average time to achieve response in complete responders was *30 months*

Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumors: Immune-Related Response Criteria. Clin Cancer Res. 2009;15(23):7412-20

Imaging Assessment Criteria are evolving

- WHO
- RECIST 1.0 and 1.1
- Volumetric Assessment
- Choi
- Cheson (Original and Revised)
- EORTC (European Organization for Research and Treatment of Cancer)
- PERCIST (PET Response Criteria in Solid Tumors)
- irRC (Immune-Related Response Criteria)
- Macdonald criteria (diagnostic criteria for multiple sclerosis)
- RANO (Revised Assessment in NeuroOncology) and iRANO
- PCWG for prostate cancer (Prostate Cancer Working Group)
- EBMT for myeloma (European Group for Blood and Marrow Transplantation)

Moertel et al Cancer 1976;38:388-394
Therasse et al. J Natl Cancer Inst 2000;92:205
Eisenhauer et al. Eur J Cancer 2009;45:228
Nishino et al. AJR 2010;195:281
Choi et al. J Clin Oncol 2007;25:1753-1759
Wolchok et al. Clin Cancer Res 2009

Polman et al Annals of Neurology 2011; 69:292
Wen et al JCO 2010;28:1963
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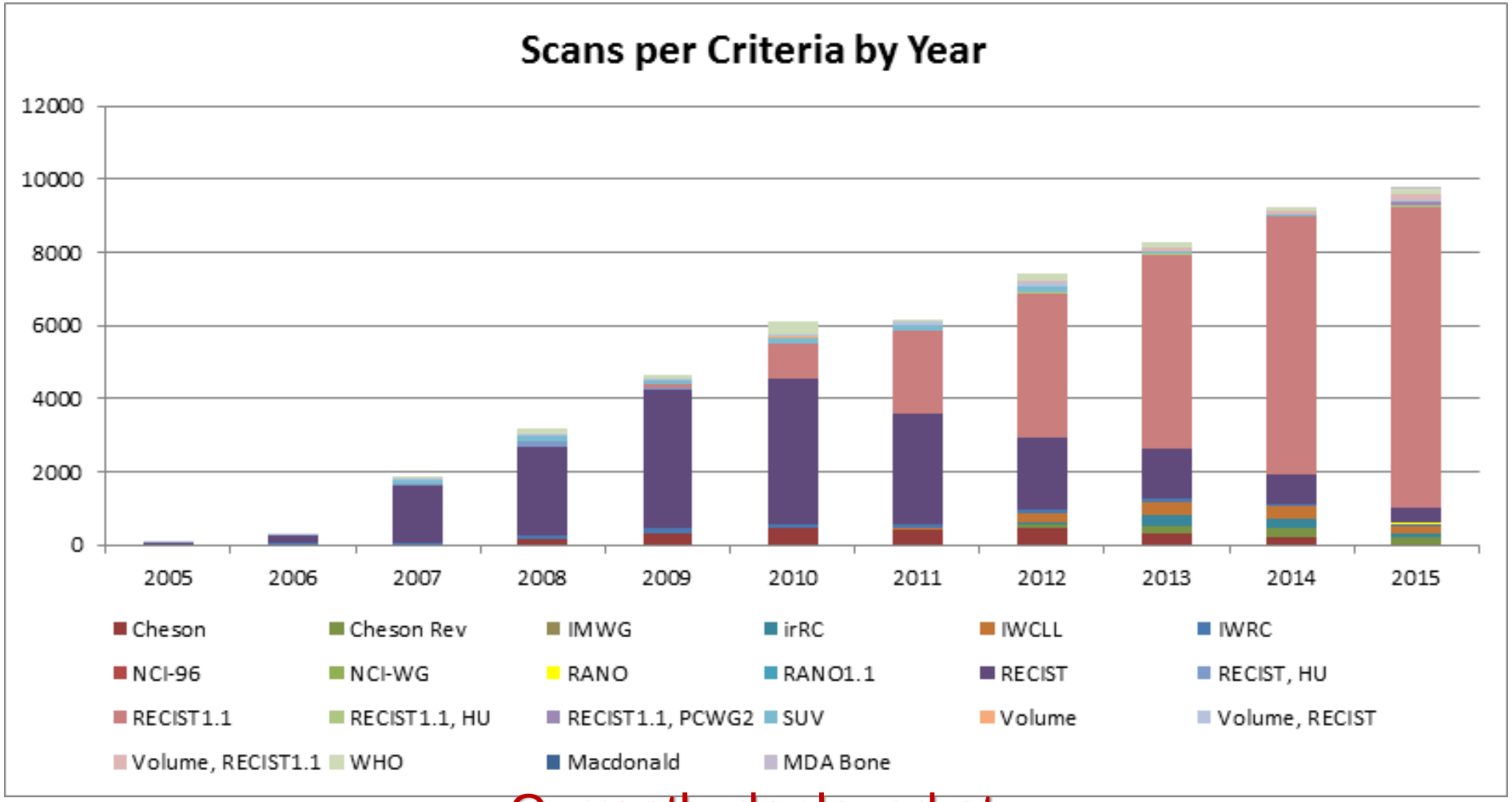
Young et al Eur J Cancer 1999; 35:1773
Wahl et al J Nucl Med 2009;50:122S
Scher et al JCO 2008; 26:1148
Durie et al Leukemia 2006; 20: 1467
Okada et al Lancet Oncology 2015

Tumor Imaging Metrics Core at the Dana-Farber/Harvard Cancer Center



Currently deployed at
5 NCI Comprehensive Cancer Centers

Imaging Assessment Criteria are evolving

- WHO
- RECIST 1.0 and 1.1, new criteria forthcoming for IT (ASCO 2016),
- Volumetric Assessment
- Choi
- Cheson (Original and Revised, new criteria forthcoming for IT)
- EORTC (European Organization for Research and Treatment of Cancer)
- PERCIST (PET Response Criteria in Solid Tumors)
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Durie et al Leukemia 2006; 20: 1467
Okada et al Lancet Oncology 2015

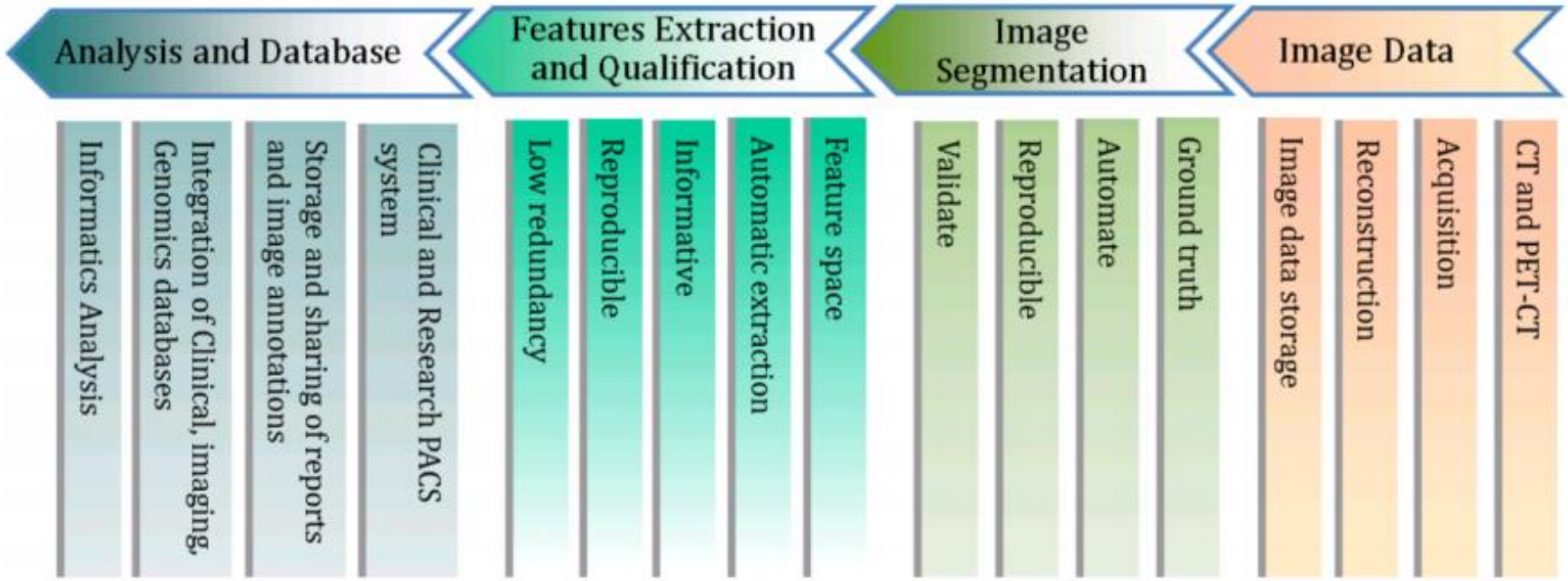
Decoding the Tumor Phenotype: Radiomics

Radiomics

- Data are designed to be extracted from standard-of-care images
- Extraction and analysis of large amounts of advanced quantitative imaging features with high throughput from medical images obtained with CT, PET or MRI

Magn Reson Imaging. 2012 Nov; 30(9): 1234–1248

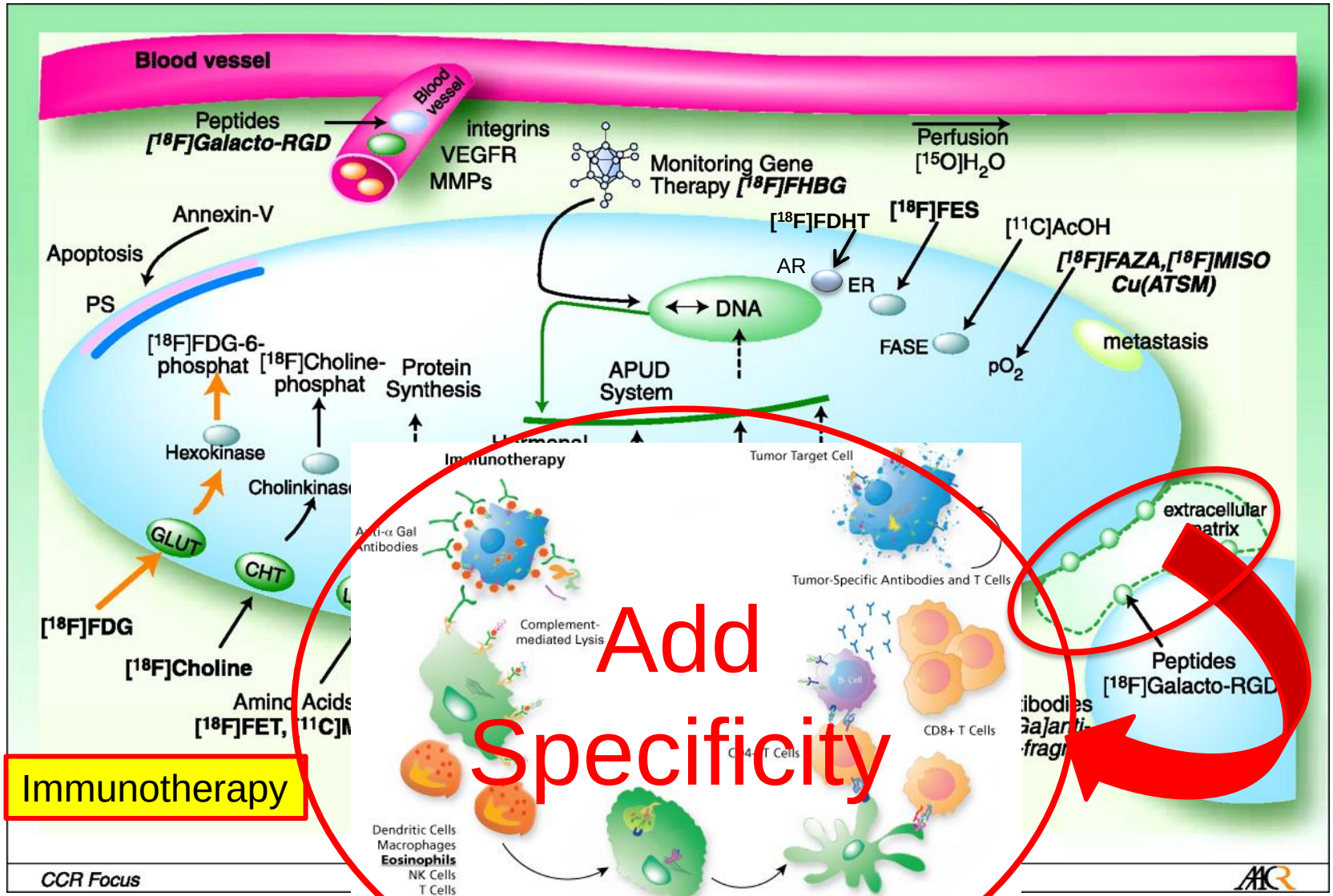
Radiomics Process and Challenges



Magn Reson Imaging. 2012 Nov; 30(9): 1234–1248

Beyond anatomy?

Molecular Imaging Explores the Hallmarks of Cancer Biology



Adapted from Wester, H.-J. Clin Cancer Res 2007;13:3470-3481

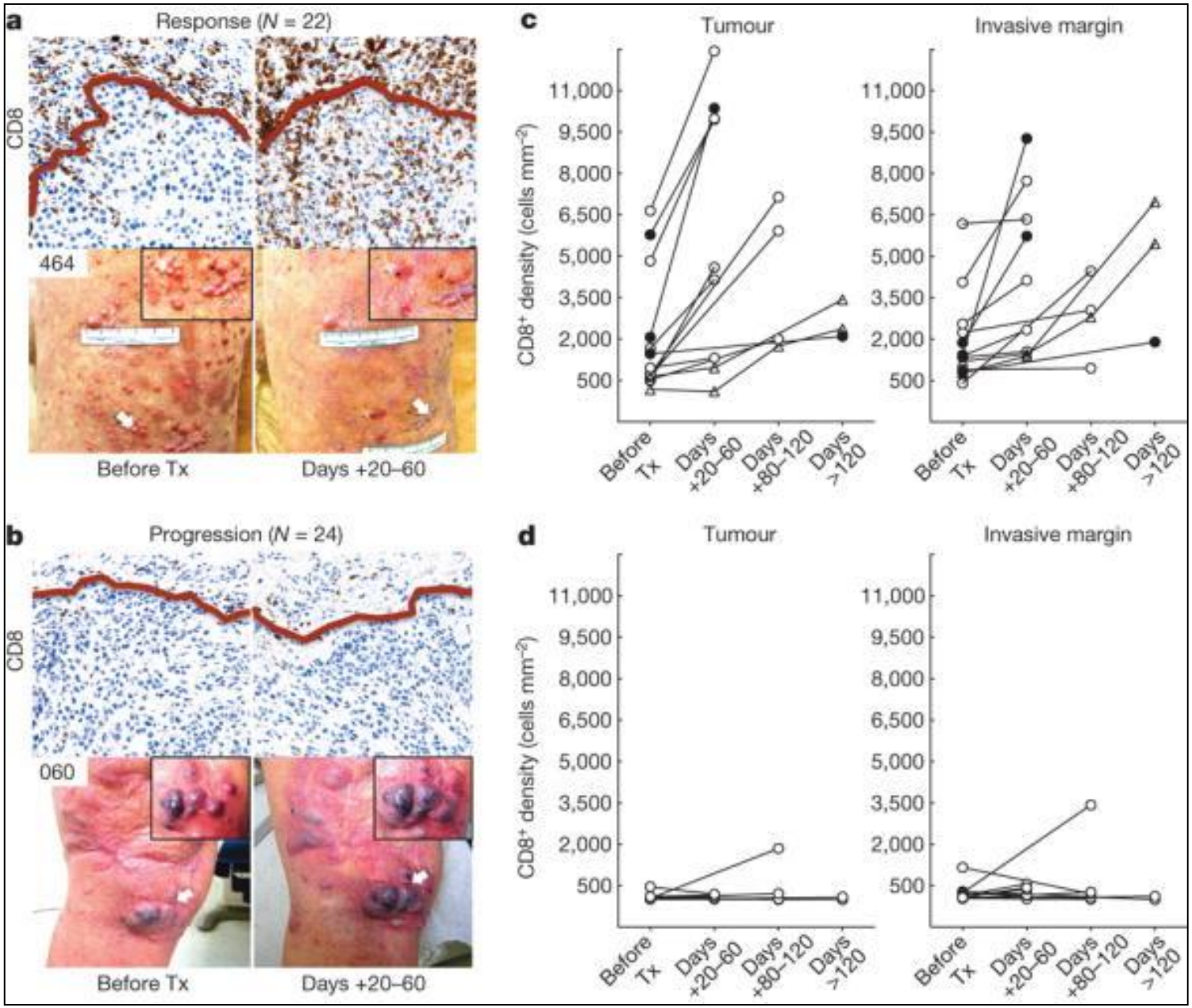
Clinical Cancer Research

“Burning” Questions from Investigators (Gordon Freeman, PhD, Steven Hodi, MD)

- Can we use imaging to:
  - characterize the tumor for the presence of inflammation (CD8 T cells) prior to treatment
  - determine if these CD8 T cells are activated

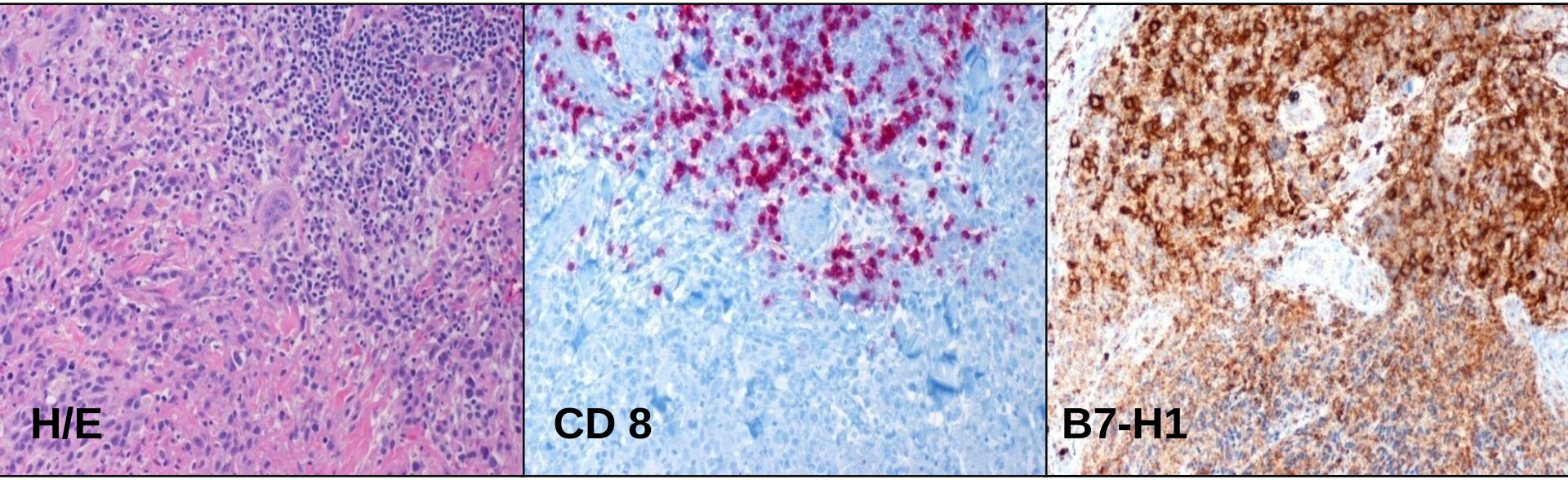
  (? is there a marker for CD107a?)
  - determine if CD69 T cells present?
  - evaluate PD1/PD-L1 axis expression (Zr-89-labeled PD-1, PD-L1, …) and compare it to IHC
  - Differentiate inflammatory response from tumor progression (new response criteria)

CD8+ T cells Before and during pembrolizumab treatment

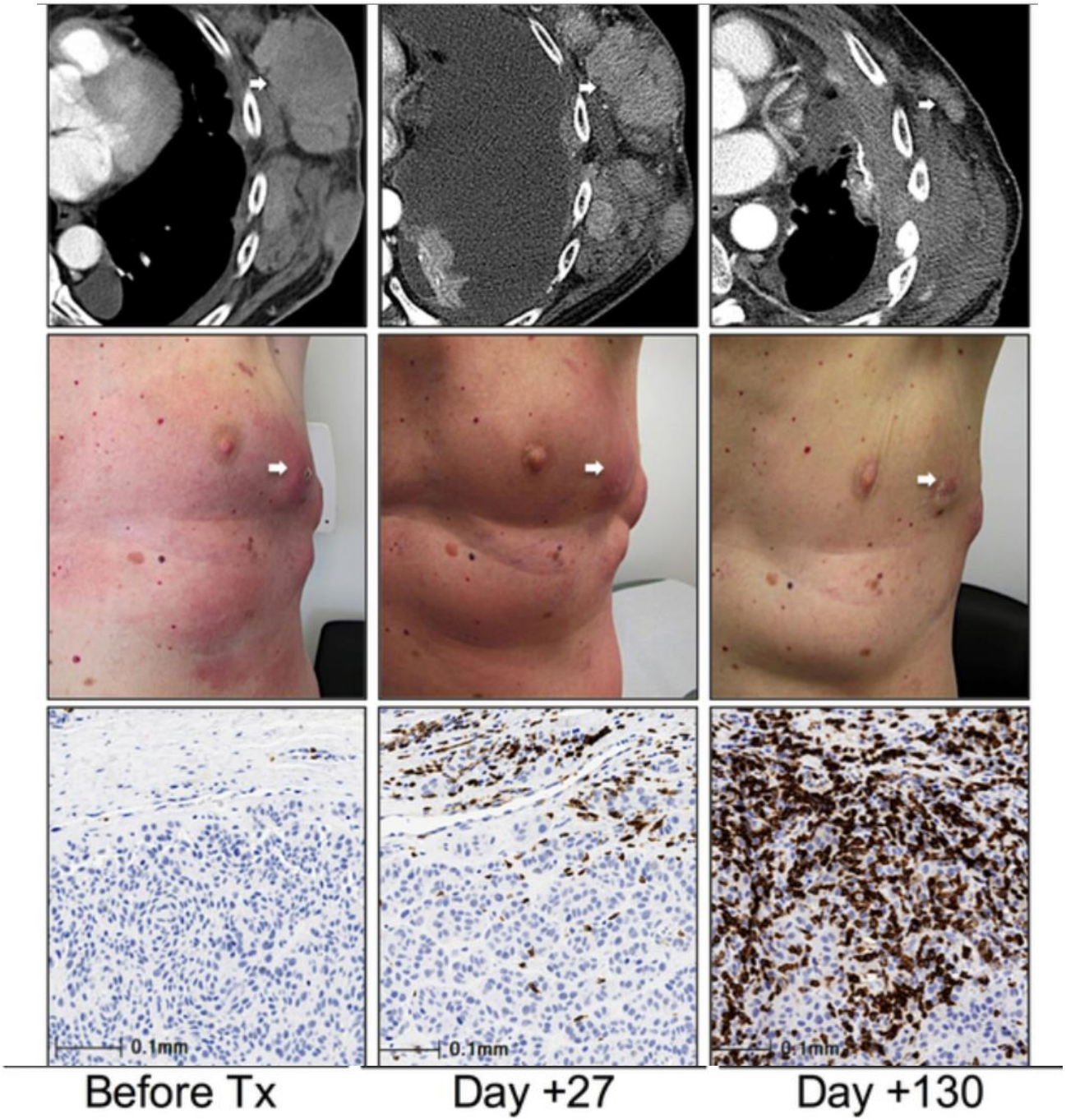


Nature Volume: 515:568–571 November 2014

Co-localization of PD-L1 and infiltrating T cells in melanoma

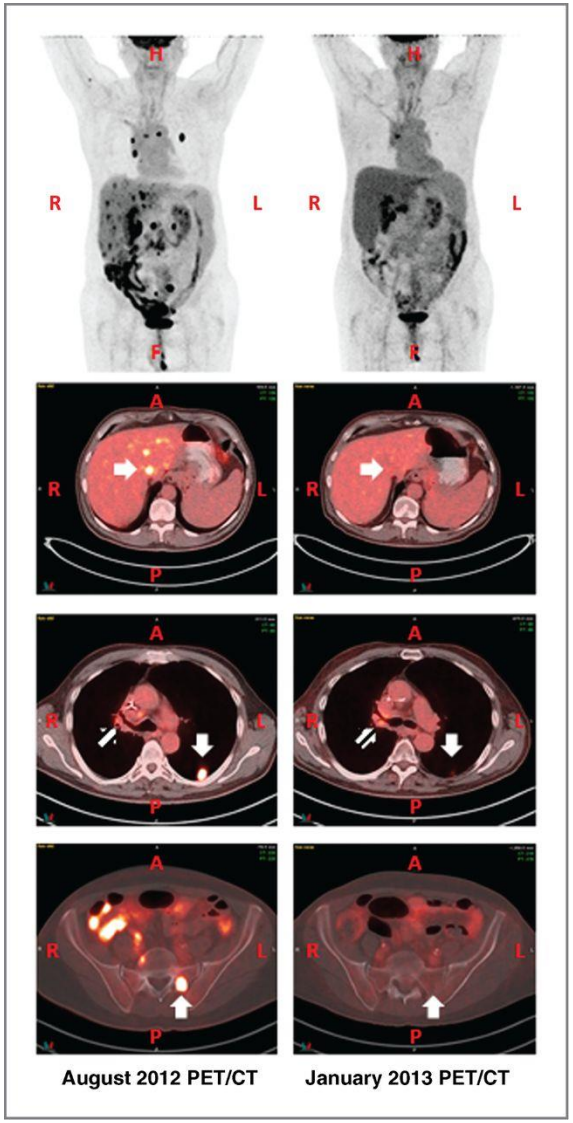


Taube et al, Sci Transl Med. 2012 Mar 28;4(127):127ra37. doi: 10.1126/scitranslmed.3003689.
Slide provided by Lieping Chen, courtesy of Steve Hodi, MD



Nature Volume: 515:568–571 November 2014

Ipilimumab and local radiotherapy result in an abscopal response

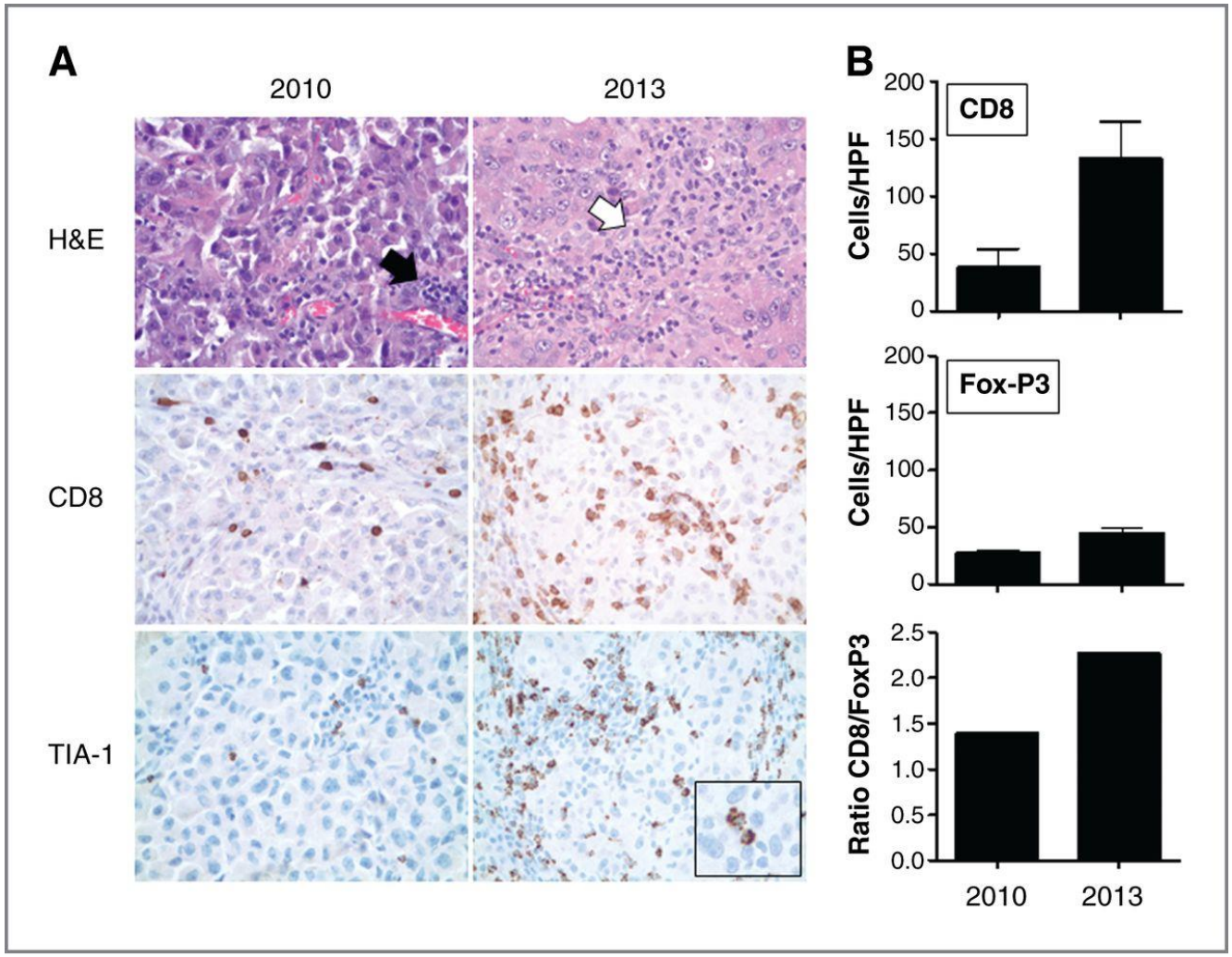


Abscopal:
ab-scopus,
"away from the target"

Encouse B. Golden et al. Cancer Immunol Res 2013;1:365-372

Cancer Immunology Research
AACR American Association for Cancer Research

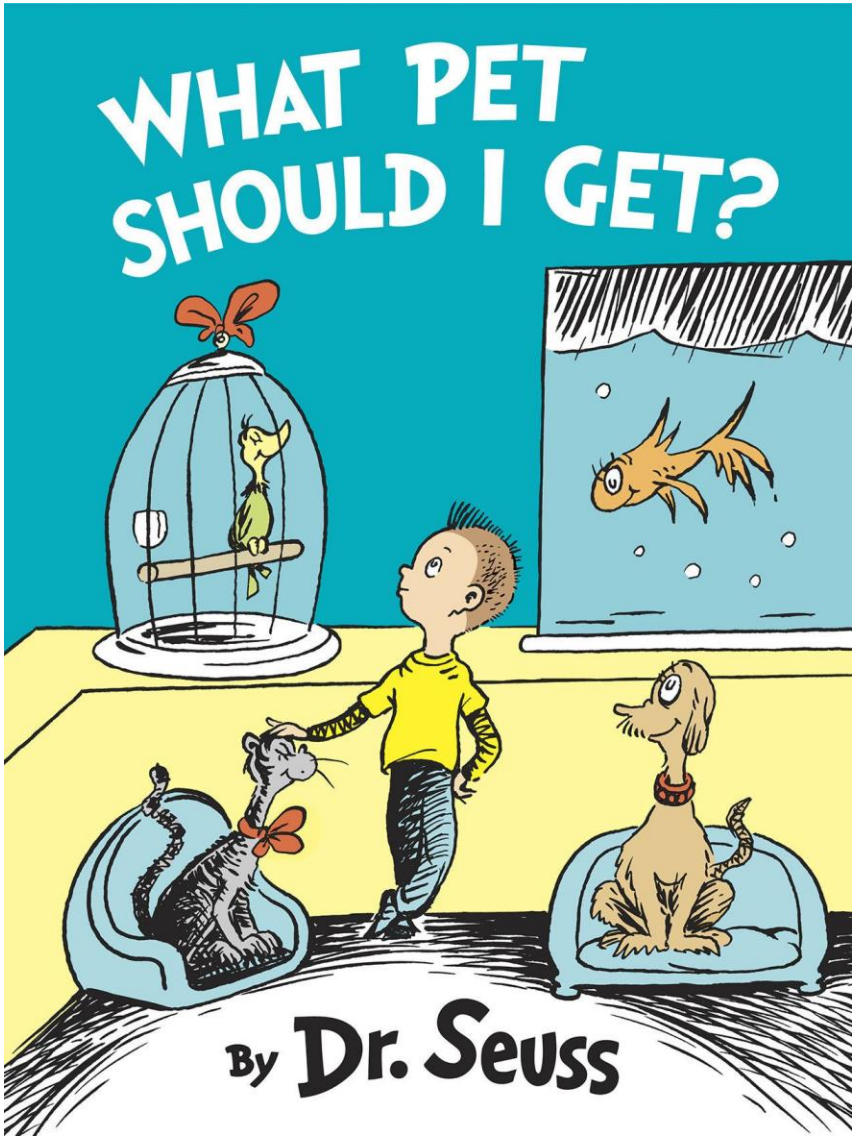
Enhanced tumor-infiltrating lymphocytes in an abscopal lesion (left supraclavicular node)



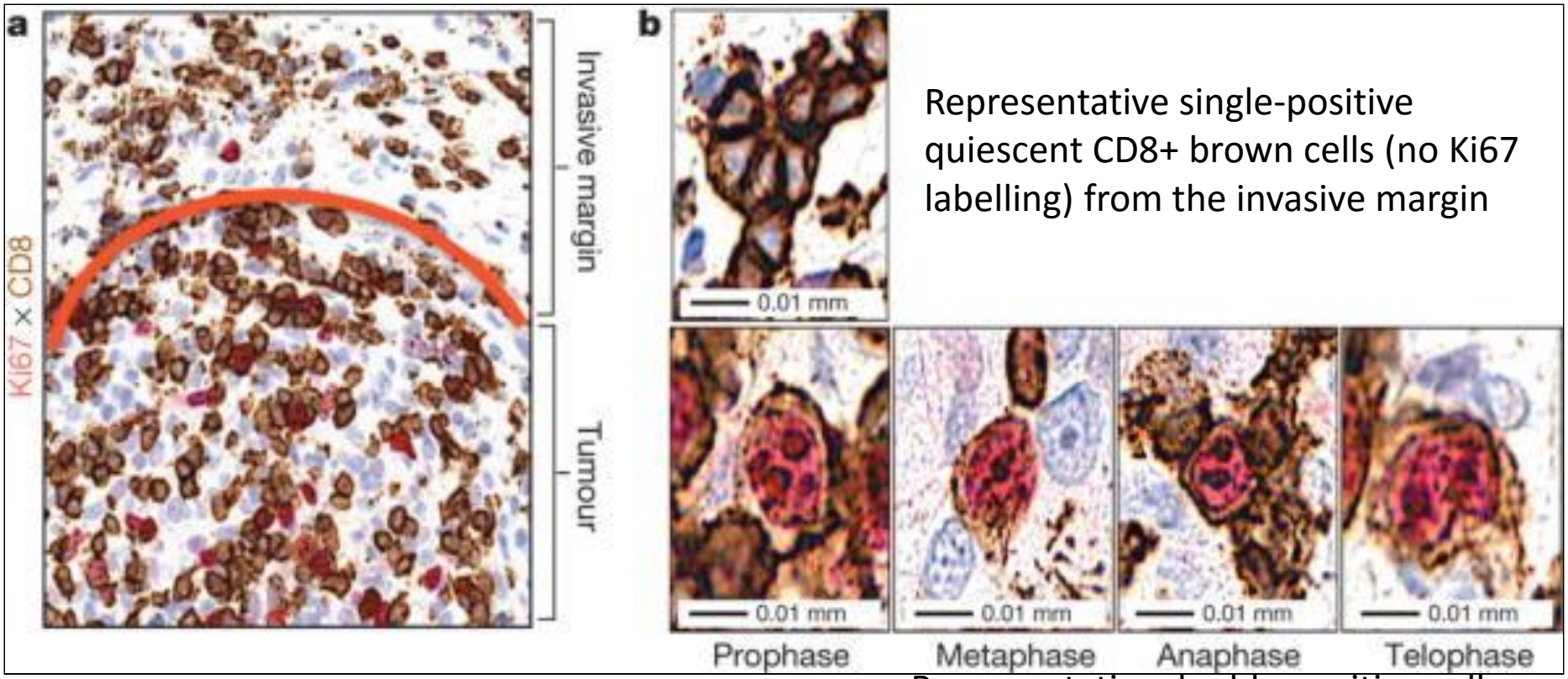
Encouse B. Golden et al. Cancer Immunol Res 2013;1:365-372

AACR American Association for Cancer Research
Cancer Immunology Research

SPECT vs. PET?



Regressing tumours during treatment are associated with proliferating CD8+ T cells that localize to the tumour



Representative single-positive quiescent CD8+ brown cells (no Ki67 labelling) from the invasive margin

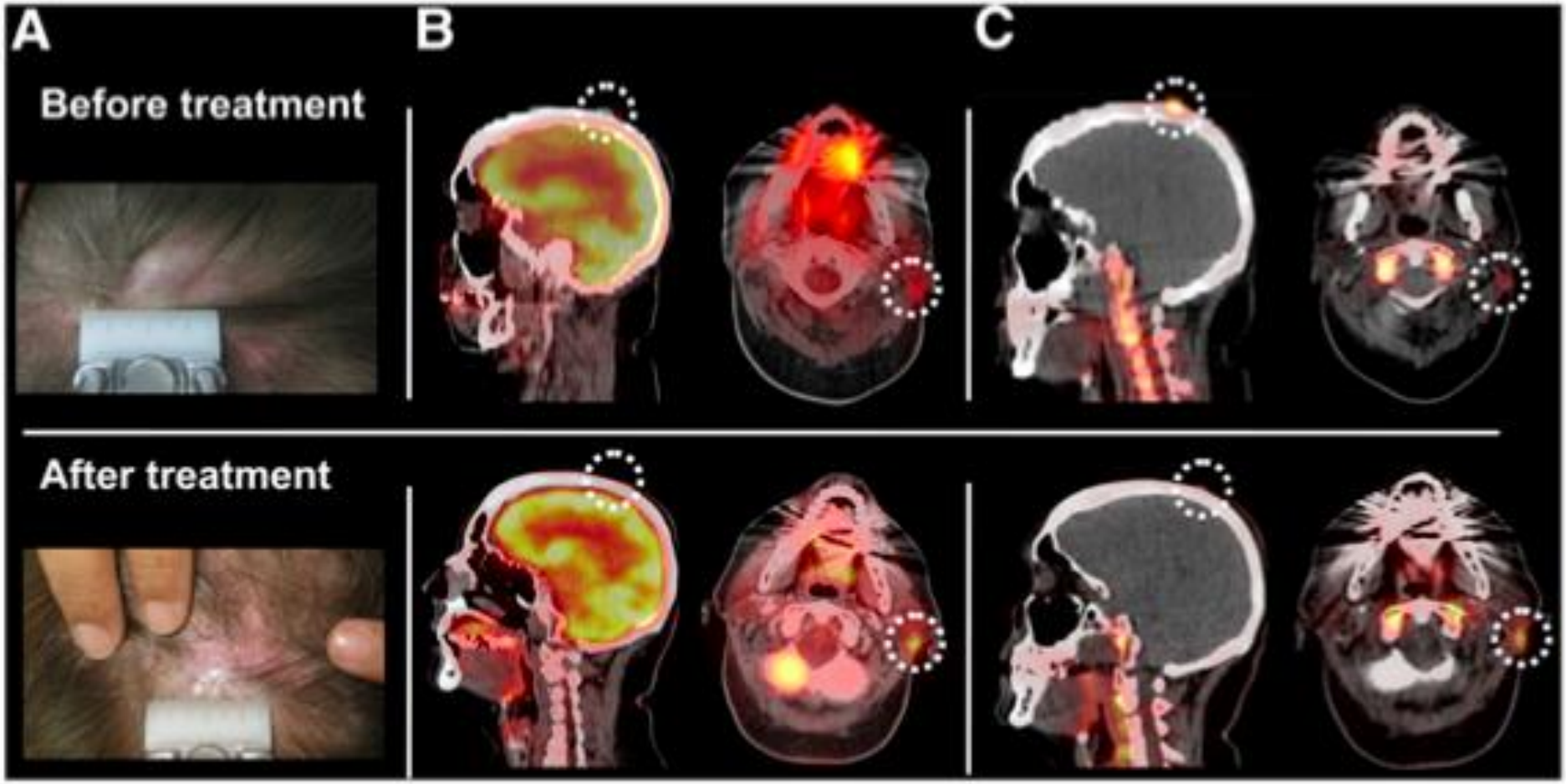
Sample obtained during tumour regression shows double-positive T cells localized to the tumour parenchyma.

Red line separates the invasive margin (above line) and tumour (below line)

Representative double-positive cells (red, labelled Ki67 nucleus; brown, labelled CD8 membrane) with characteristic chromatin patterns associated with sub-phases of mitosis

Nature Volume: 515:568–571 November 2014

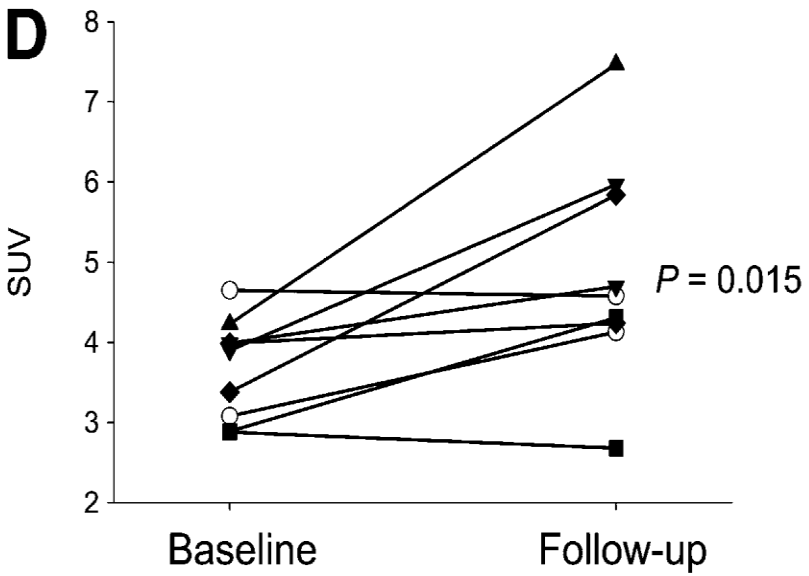
^{18}F-FDG and ^{18}F-FLT PET/CT scans in patient with metastatic melanoma with objective tumor response to tremelimumab (human IgG2 anti-CTLA4 monoclonal antibody)



Ribas et al. J Nuclear Med Vol. 51 • No. 3 • March 2010

FLT-PET: Antibody Therapy

- Advanced Melanoma patients: received Tremelimumab (CTLA4 blockade)
- PET Imaging of spleen at 1-2 months post-treatment
- SUV measurements:
  - Statistically significant difference in FLT uptake (SUV_{mean} and SUV_{max})
  - Variable response observed (2/9 had decreases)
  - No significant changes in FDG uptake
- FLT-PET was therefore able to detect cell activation in most patients (variable response)



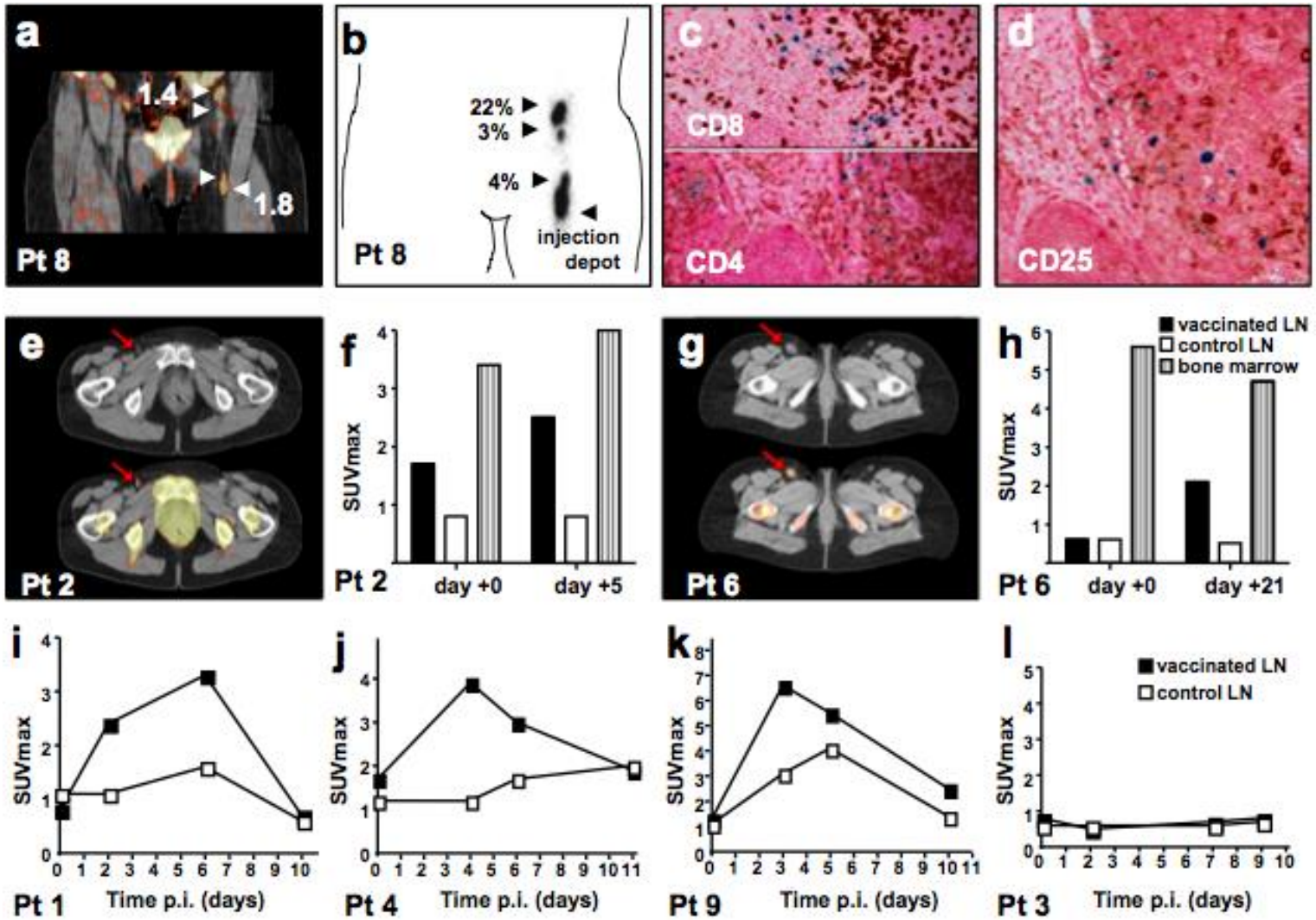
FLT spleen uptake: changes in SUV max

Ribas et al. J Nucl Med 2010; 51: 340

Courtesy of Anne Goodbody, PhD and John Valliant, PhD

Early identification of antigen-specific immune responses in vivo by [18F]-labeled 3′-fluoro- 3′-deoxy-thymidine ([18F]FLT) PET imaging

Erik H. J. G. Aarntzena,b, Mangala Srinivasa,1, Johannes H. W. De Wiltc,1, Joannes F. M. Jacobsa,b,d, W. Joost Lesterhuisa,b, Albert D. Windhorste, Esther G. Troostf, Johannes J. Bonenkampc, Michelle M. van Rossumg, Willeke A. M. Blokxh, Roel D. Musi, Otto C. Boermanj, Cornelis J. A. Puntb,2, Carl G. Figdora, Wim J. G. Oyenj, and I. Jolanda M. de Vriesa,b,3



8396–18399 | PNAS | November 8, 2011 | vol. 108 | no. 45

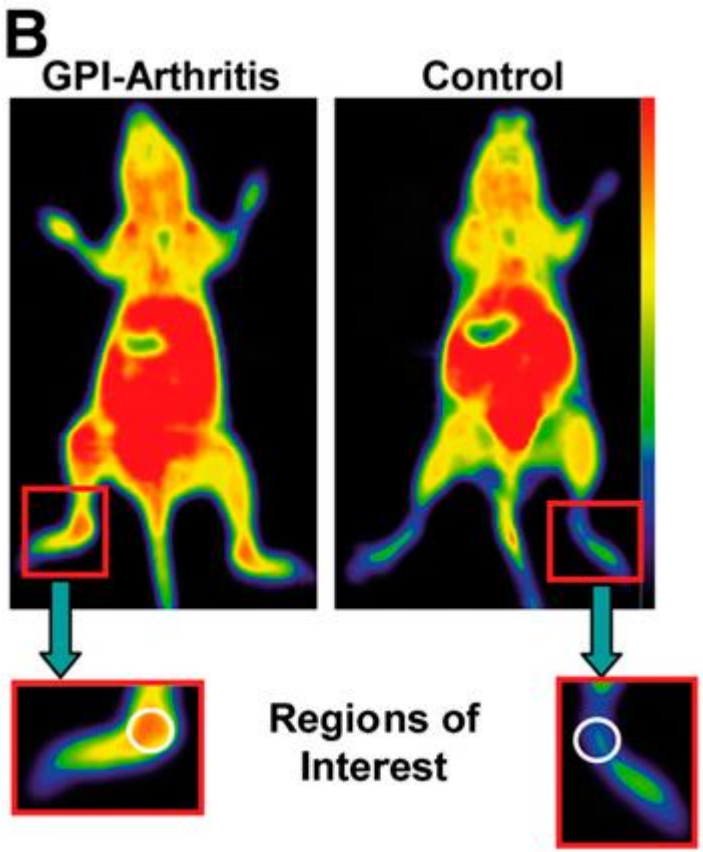
Potential applications of FLT-PET in Tumour Vaccination

- Sensitive tool to examine kinetics and localization of activation
  - Longitudinal monitoring by non-invasive imaging
  - Measurement of *in vivo* cell functionality to determine subsequent individualized treatment

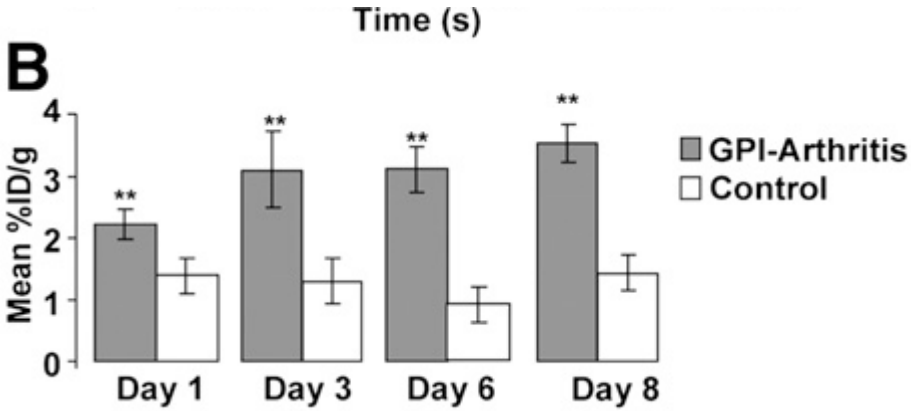
Aarntzen et al. PNAS 2011; 108: 18396

Courtesy of Anne Goodbody and John Valliant

FLT-PET: Autoimmune Rheumatoid Arthritis Preclinical Imaging



6 days post-induction of arthritis



^{18}F-FLT PET scans in arthritic (n = 5-7) and healthy ankles (n = 3-6). ** $p < 0.05$

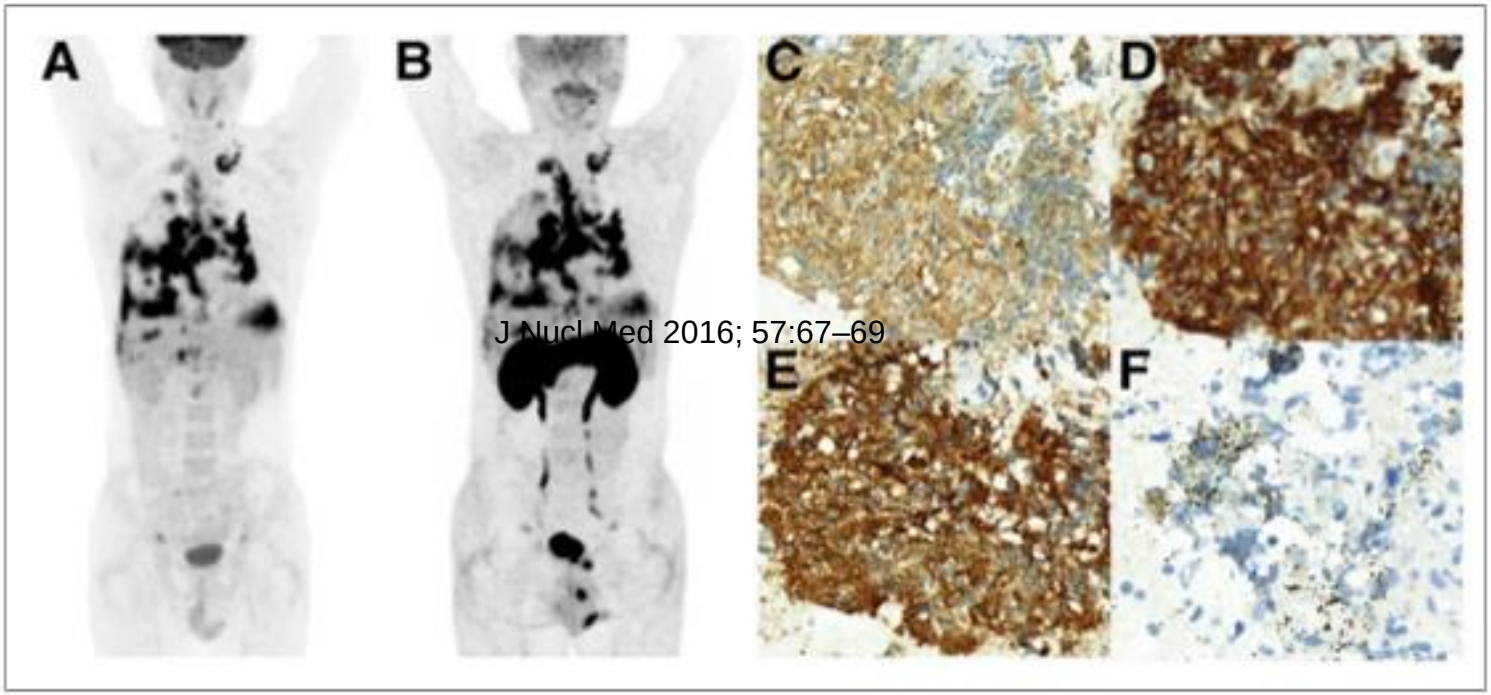
- Correspondence with Ki-67 expression
- Potential to detect sub-clinical arthritis, enabling early treatment, especially where classification criteria of RA are only partially met

Courtesy of Anne Goodbody, PhD and John Valliant, PhD

Fuchs et al. J Nucl Med 2013; 54: 151

Exploratory Clinical Investigation of (4S)-4-(3-18F- Fluoropropyl)-L-Glutamate PET of Inflammatory and Infectious Lesions

Sun Young Chae1, Chang-Min Choi2, Tae Sun Shim2, Yangsoon Park3, Chan-Sik Park3, Hyo Sang Lee1, Sang Ju Lee1, Seung Jun Oh1, Seog-Young Kim4, Sora Baek5, Norman Koglin6, Andrew W. Stephens6, Ludger M. Dinkelborg6, and Dae Hyuk Moon1



J Nucl Med 2016; 57:67–69

FIGURE 1. A 53-y-old man with sarcoidosis (patient 5). ^{18}F-FDG (A) and ^{18}F-FSPG (B) present similar major uptake involving pleura, supraclavicular lymph nodes, and thoracic lymph nodes. The only differences are normal physiologic uptake in brain, pancreas, and kidney. On immunohistochemistry evaluation (×400), proportion of inflammatory cells positive for xCT (C), CD44 (D), CD68 (E), and CD163 (F) was 80%, 80%, 80%, and 1%, respectively.

J Nucl Med 2016; 57:67–69

Table 3. Imaging agents for antitumor immune function in published preclinical studies.

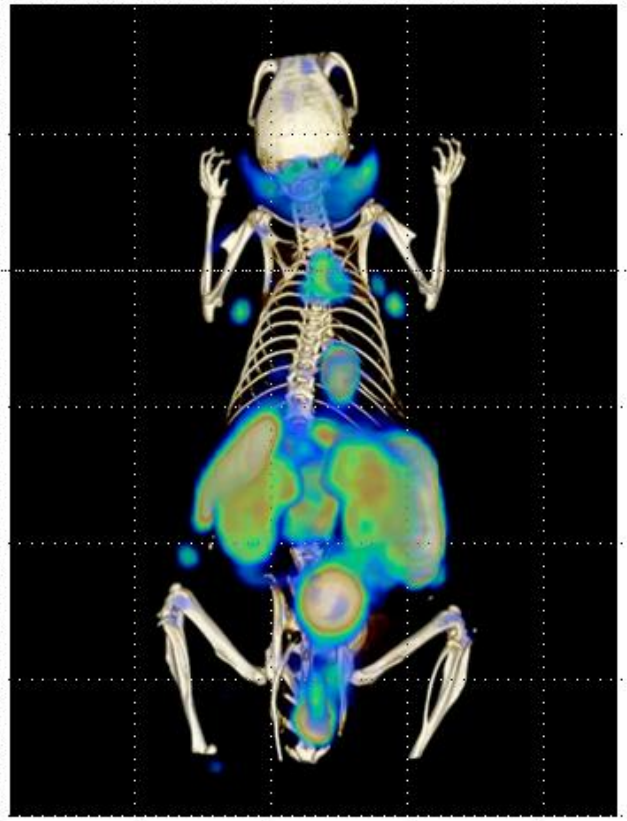
IMAGING AGENT	TARGETING CONCEPT	IMAGING TECHNOLOGY
^{18}F-/^{64}Cu anti-CD11b or MHC-II[81]	Labeled antibody fragments binding to CD11b or MHC II on tumor macrophage or myeloid cells	PET
^{64}Cu-anti-CD8[76]	Labeled antibody fragments binding to CD8 on tumor infiltrating cytotoxic T lymphocytes	PET
^{89}Zr-anti-CD8[78]	Labeled antibody fragments binding to CD8 on tumor infiltrating cytotoxic T lymphocytes	PET
^{18}F-FEAU[101]	Labeled ligand identifies viral transgene in activated CAR-T that are present in tumor	PET
^{111}I-anti-PD-L1[84]	Labeled monoclonal antibody binds to PD-L1 expressed on macrophage and tumor cells	SPECT
^{89}Zr-anti-CD47[83]	Labeled monoclonal antibody binds to CD47 expressed on cells within tumor	PET
^{64}Cu-Anti-CTLA-4[80]	Labeled monoclonal antibody binds to CTLA-4 expressed on cytotoxic T lymphocytes within tumor	PET
MB-anti-B7-H3[85]	Ultrasound microbubbles labeled with monoclonal antibody against B7-H3. Identifies cells expressing B7-H3 on macrophage and tumor cells	US
^{64}Cu-SPION[102]	CAR-T cells loaded with ^{64}Cu-SPION (iron nanoparticles). Image accumulation of therapeutic CAR-T	PET
DiR labeled T cells[103]	DiR fluorophore, activated by near-Infrared light, is used to label T cells. T cells that located in tumor are imaged	Fluorescence imaging

and many others…

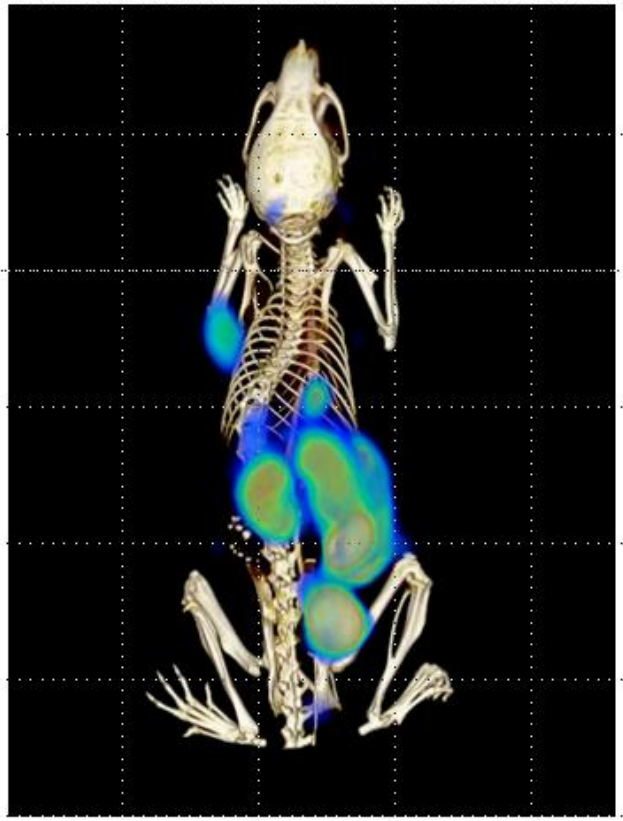
Juergens et a Biomarkers in CanCer 2016:8(s2)

Targeted imaging probe for immunotherapy

^{18}F-VHH7 (anti-mouse class II MHC)



^{18}F-VHHDC13 (anti-mouse CD11b)



Courtesy of Quang-Dé Nguyen, PhD

Rashidian M., Proc Natl Acad Sci U S A, 2015

Table 2. Imaging agents in current immunotherapy trials.

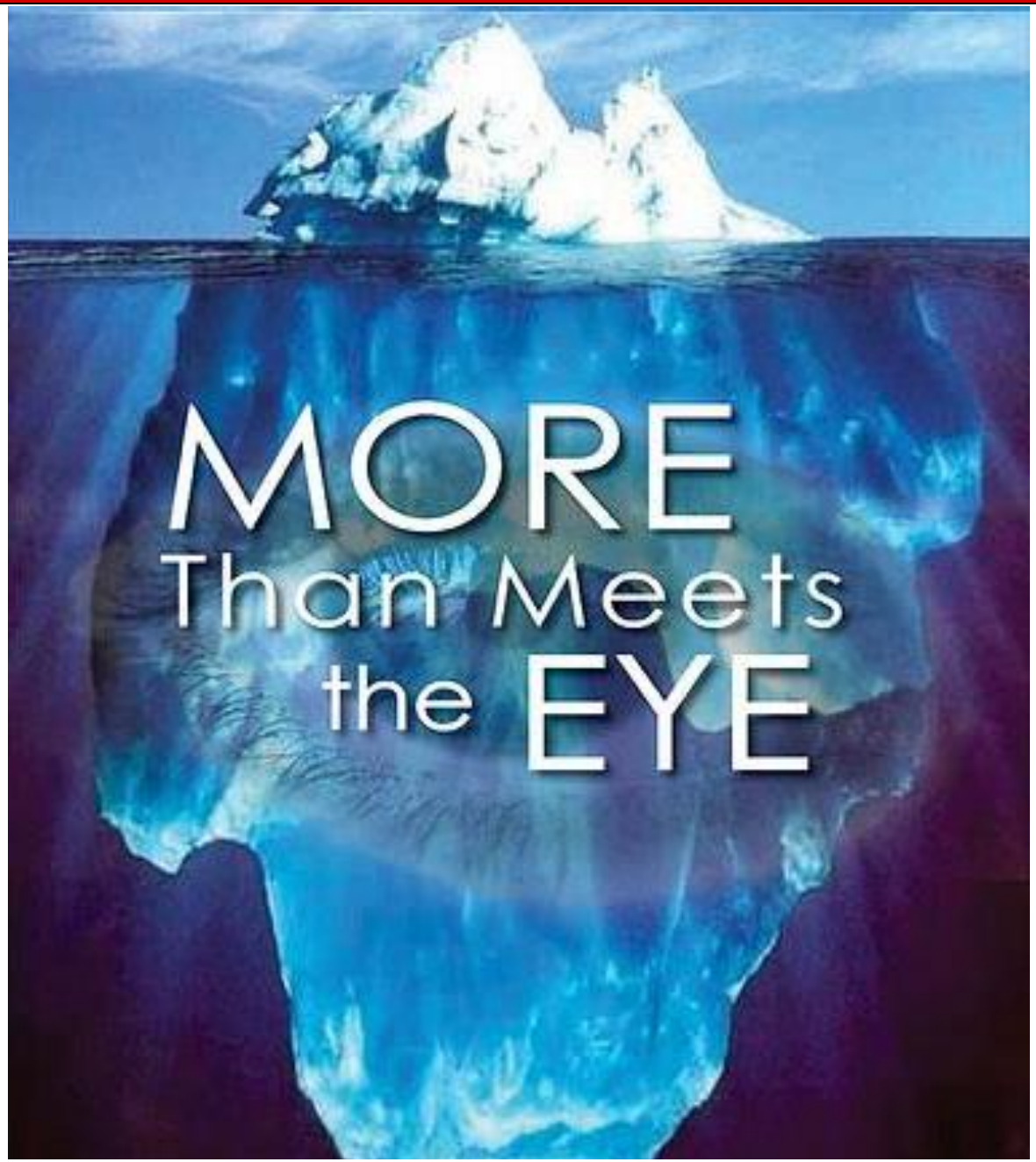
AGENT	CANCER	IMMUNOTHERAPY (IT) AND IMAGING TARGET	NATIONAL CLINICAL TRIALS (NCT) NUMBER	IMAGING TECHNOLOGY
^{18}F-FDG[99,100]	Melanoma, renal cell, lung	IT: anti-CTLA-4, anti-PD-1 Target: tumor metabolism	NCT01666353	PET/CT
^{18}F-FDG	Cervical, squamous cell	IT: anti-CTLA-4 Target: tumor metabolism	NCT01711515	PET/CT
^{18}F-FDG	Multiple Ca	IT: CAR-T, anti-CTLA-4, IL-2 Target: tumor metabolism	NCT02070406	PET
^{18}F-FDG	Renal cell	IT: IL-2 (plus chemo) Target: tumor metabolism	NCT01038778	PET/CT
^{18}F-FDG	Multiple Ca	IT: CAR-T, IL-2, DC vaccine Target: tumor metabolism	NCT01697527	PET
^{18}F-FDG or Na^{18}F	Prostate	IT: DC vaccine with GM-CSF Target: tumor metabolism	NCT02042053	PET/CT PET/MRI
^{18}F-FET	Brain melanoma metastases	IT: anti-PD-1, anti-CTLA-4 Target: tumor metabolism	NCT02374242	PET/MRI
^{11}C-PBR28a	Brain	IT: various IT treatments, Target: tumor benzodiazepine receptor	NCT02431572	PET
^{18}F-HBG[71]	Glioma	IT: CAR-T, IL-2 Target: CAR-T cells	NCT01082926	PET
^{89}Zr-MPDL3280A[74]	Multiple cancers	IT: anti-PD-L1 Target: PD-L1 on tumor or other cells	NCT02453984	PET
^{99}Tc-IL-2[68]	Melanoma	IT: anti-CTLA-4, anti-PD-1, IL-2 Target: TIL expressing IL-2 receptor	NCT01789827	SPECT
^{18}F-L-FAC[70]	Healthy volunteers and multiple cancers	IT: various immunotherapies Target: activated T-cells in tumor	NCT01180868 NCT01180907	PET
^{89}Zr-GC1008[72]	Brain glioma	IT: anti-TGF-β Target: TGF-β	NCT01472731	PET
Ferumoxytol[73] (iron nanoparticles)	Brain	IT: various immunotherapies Target: macrophage in tumors	NCT02452216	MRI
^{18}F F-AraG[69]	Healthy subjects	IT: prior to various cancer IT trials Target: activated T-cells	NCT02323893	PET

and many others…

Juergens et a Biomarkers in CanCer 2016:8(s2)

Adding More Specificity to Characterize the Immune Response

- Precision medicine era
- Will help:
  - characterize the tumor (prognostic value)
  - stratify patients
  - enrich patient population in clinical trials
  - define and validate biomarker(s) (predictive value)
  - to be relevant to the disease, immune process, immune therapy
- May become a clinically actionable test



http://www.allmystery.de/i/te8c190_5248593727_364ca32dff.jpg

Going Forward

- Response criteria will continue to evolve
- Pay particular attention to:
  - disease assessment time points to align with the natural course of the disease, the treatment and the response to the treatment
  - defining progression and duration of follow-up
  - immune-adverse events
  - discontinuation criteria
- Test Radiomics

Going Forward

- Imaging needs to be relevant to the immune system
- ?Co-develop novel immunotherapic drugs with companion diagnostics (PET probes specific to relevant mechanism of action and immune features) and co-validate them in prospective trials

All hands on deck!

- Learn from ongoing work in other specialties: neurodegeneration, atherosclerosis, CAD, vasculitis, myocardial inflammation, sarcoidosis, rheumatoid arthritis, infection or inflammation
- Create multidisciplinary teams of basic scientists, immunologists, infectious disease, oncologists, imaging, radiation therapy, surgeons…
- Think globally

Outlook for cancer patients has never been better

“Much to celebrate,
but even more to do”
(Nancy E Davison, MD)