Studies of Immune Checkpoint Toxicity:

The Achilles Heal of Immunotherapy

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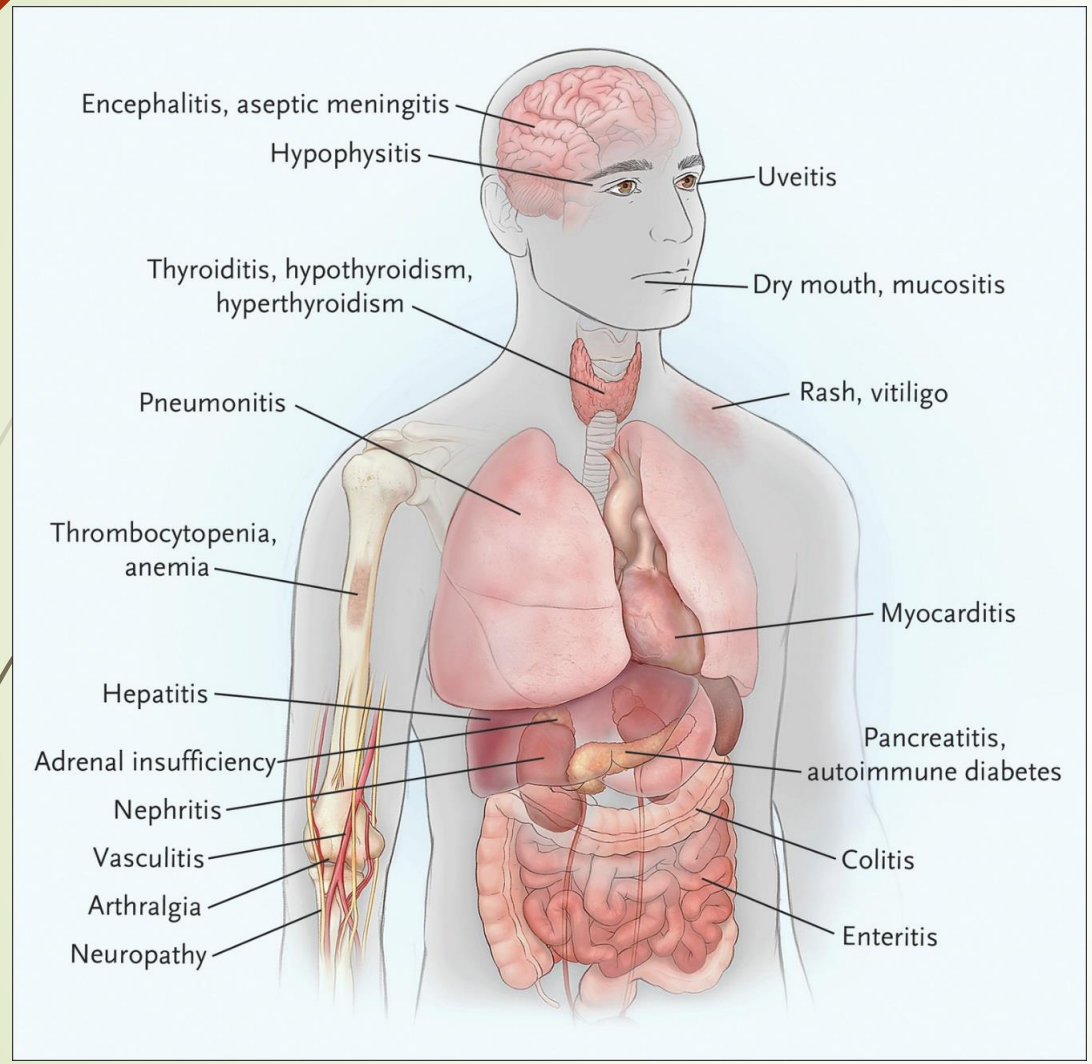
Disclosures

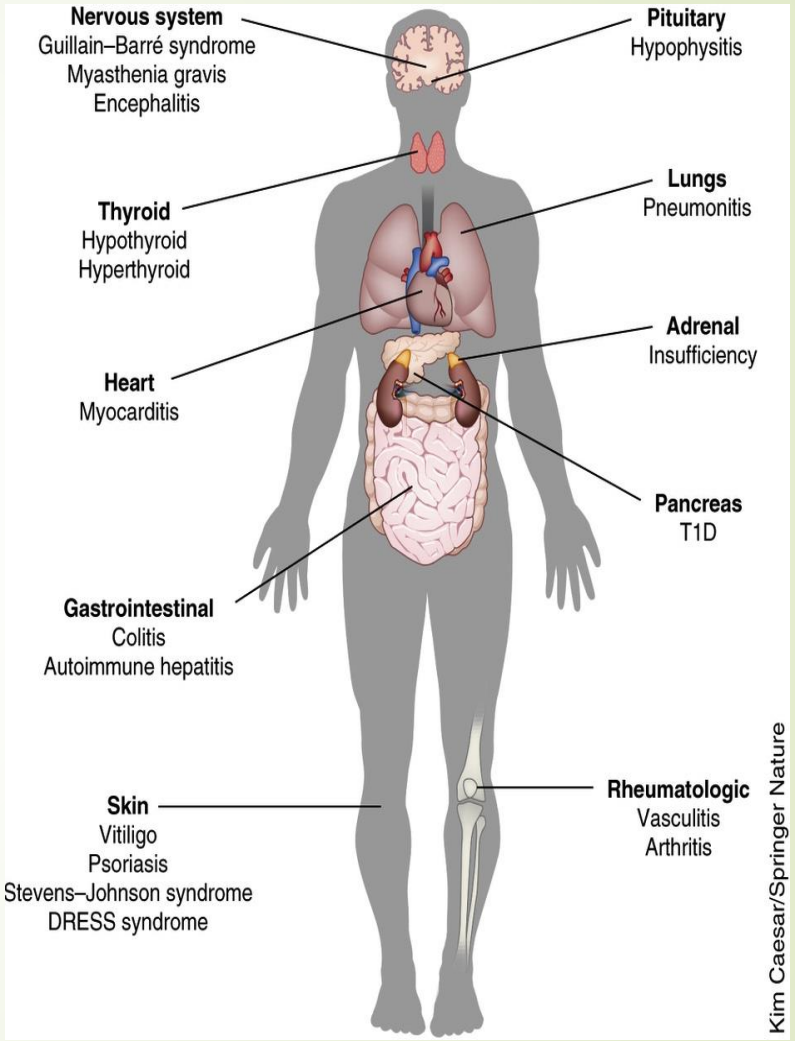
- Consulting Fees:
  - BMS, Genentech
- Contracted Research:
  - BMS, Corvus, Curis, Genentech

- I will be discussing non-FDA approved indications during my presentation.

Sites of Immune Checkpoint Inhibitor Toxicity (irAE- AE of special interest)

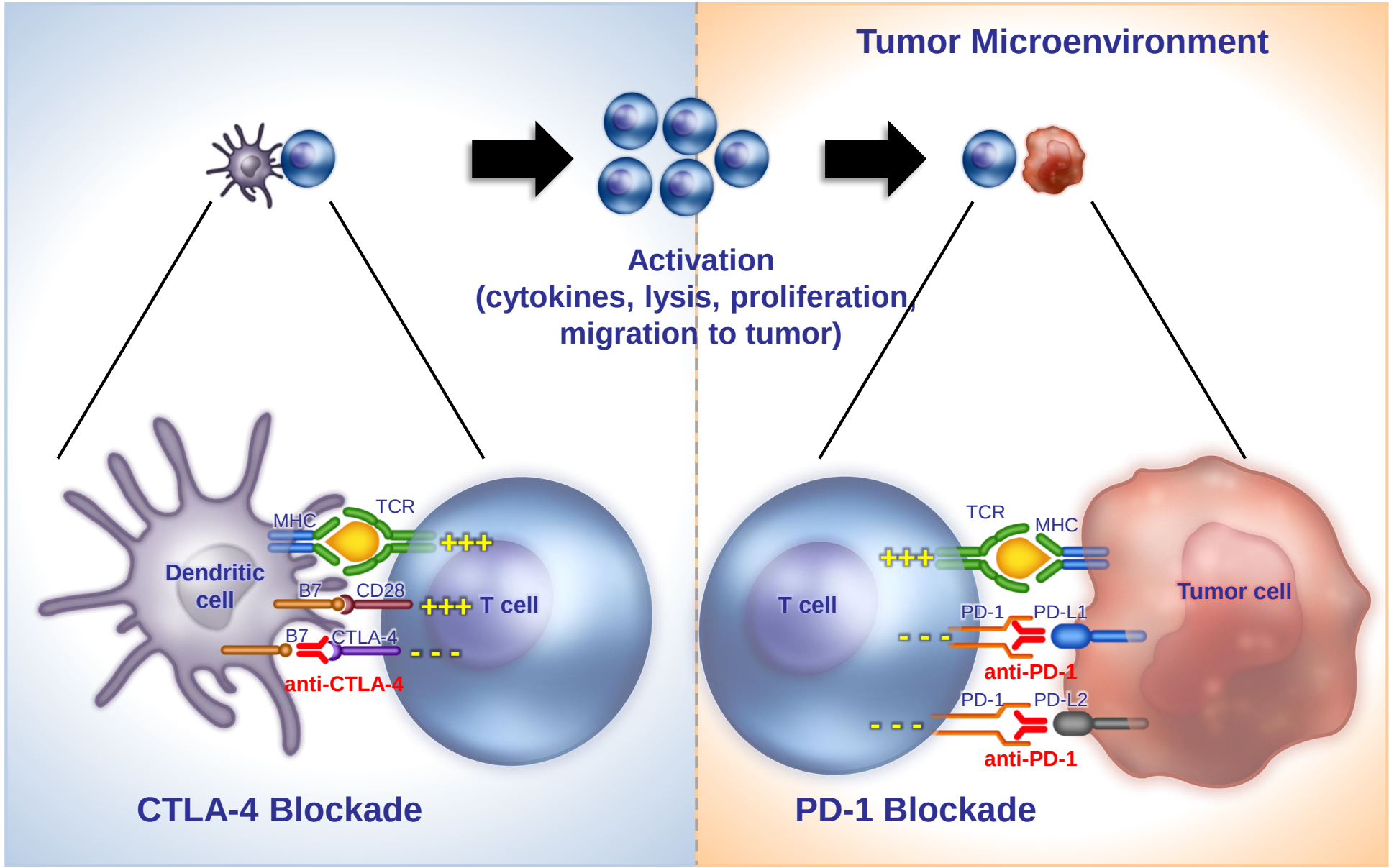


Postow et.al., N Engl J Med 2018.



June et.al , Nature Med, 2017

Blocking CTLA-4 and PD-1



Spectrum of PD-1/PD-L1 Antagonist Activity

Active

- **Melanoma**
- **Renal cancer (clear cell)**
- **NSCLC – adenocarcinoma and Squamous cell**
- **Head and neck cancer**
- **Small cell lung cancer**
- **Gastric and GE junction**
- **Mismatch repair deficient tumors (colon, cholangiocarcinoma)**
- **Urothelial cancer**
- **Triple negative breast cancer**
- Ovarian cancer
- **Hepatocellular carcinoma**
- Thymic carcinoma
- Mesothelioma
- Cervical cancer
- **Hodgkin Lymphoma**
- **Diffuse large cell lymphoma**
- Follicular lymphoma
- T-cell lymphoma (CTCL, PTCL)
- **Merkel Cell**

Minimal to no activity:

- Prostate cancer
- MMR+ Colon cancer
- Myeloma
- Pancreatic Cancer
- ER+ breast cancer
- Glioblastoma

Major PD-1/PD-L1 antagonists (approved)

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

Model to Predict patients likely to respond to Immunotherapies i.e., anti–PD-1

Host Factors

- Microbiome composition of GI tract
- Germline polymorphisms in immune regulatory genes

Tumor Factors

- Tumor Genomics
- Tumor Mutation Burden (Neoantigens)
- Specific Genetic Alterations
  - PTEN loss, Wnt/β- catenin pathway activation, Myc, RAS mutation
- PD-L1 expression (affected by factors above)
- MHC processing components-alterations

The Cancer Immunity Cycle

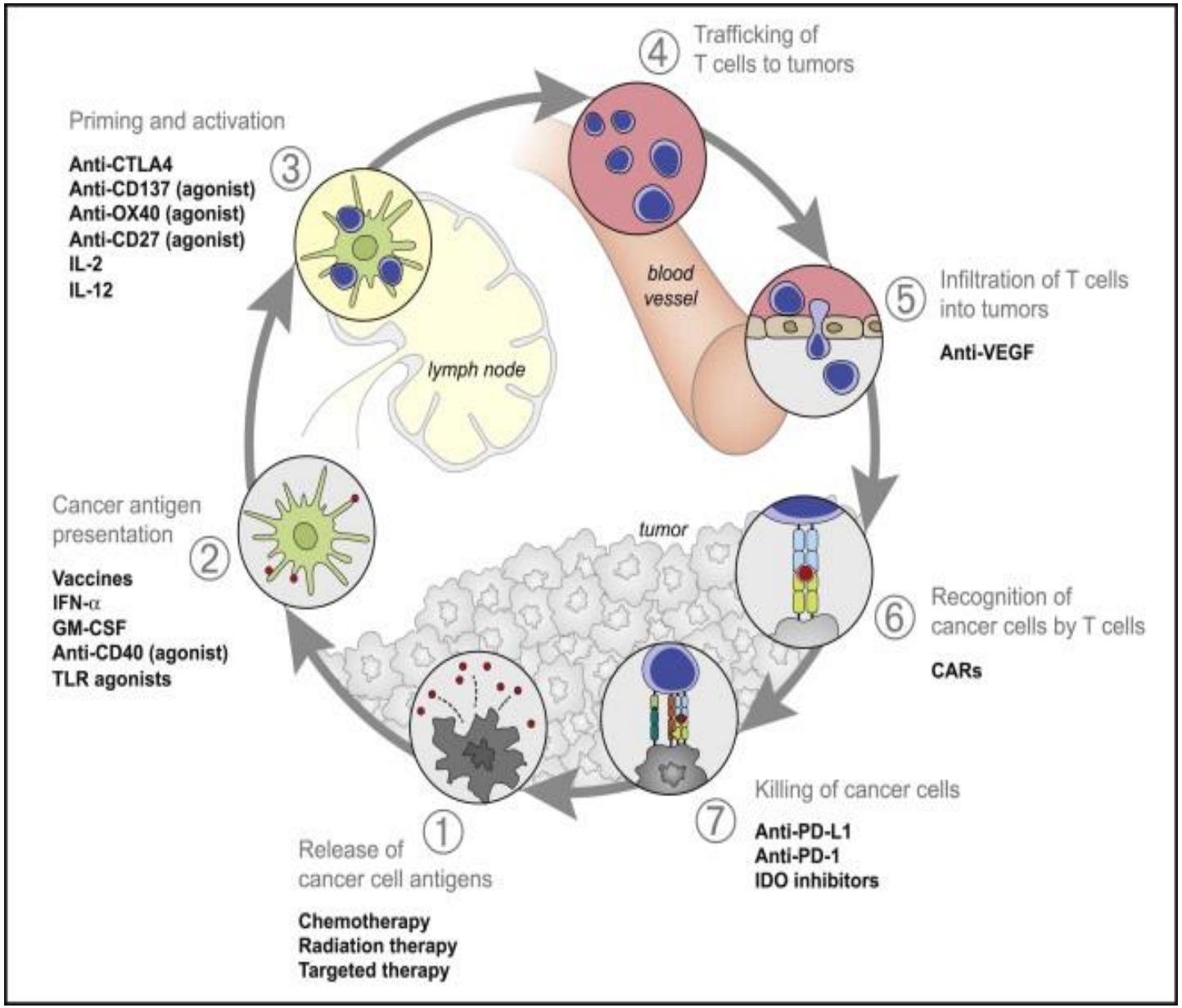
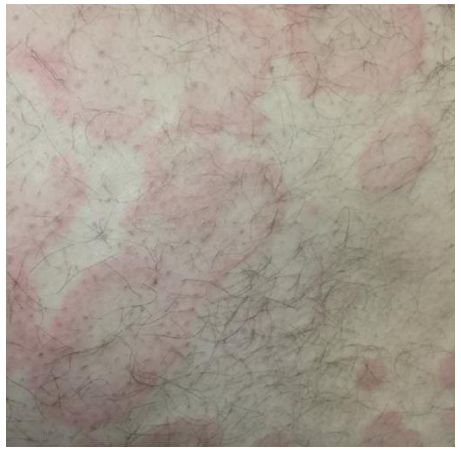


Figure 3 Therapies that Might Affect the Cancer-Immunity Cycle The numerous factors that come into play in the Cancer-Immunity Cycle provide a wide range of potential therapeutic targets. This figure highlights examples of some of the therapies currently ...

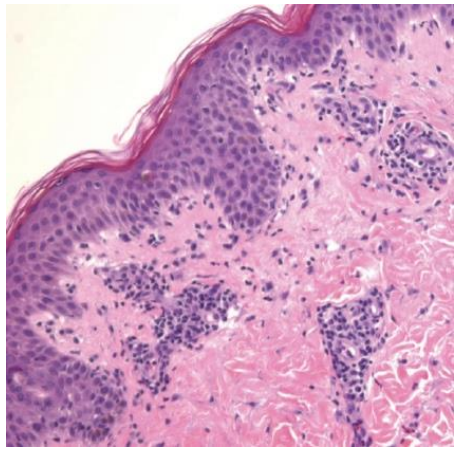
Immune-mediated adverse reactions

Skin: Dermatitis

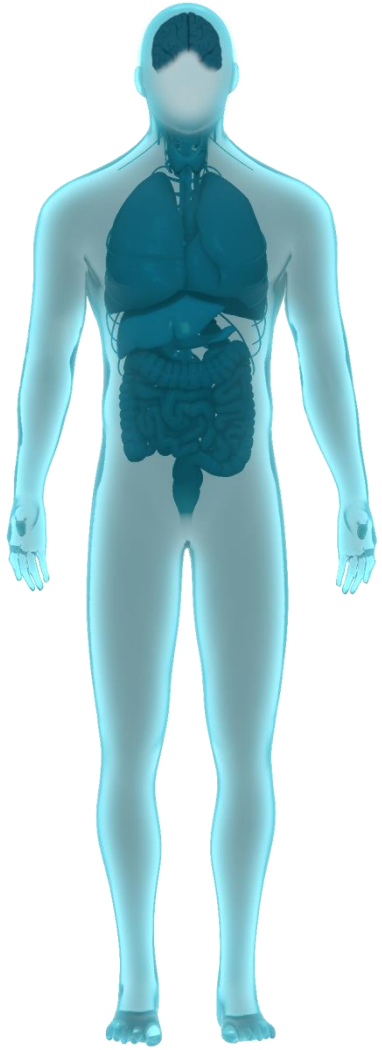
- Symptoms: pruritus, rash, dermatitis, erythema, photosensitivity, toxic epidermal necrolysis, urticaria, and vitiligo



Immune-mediated dermatitis



Histology of dermatitis

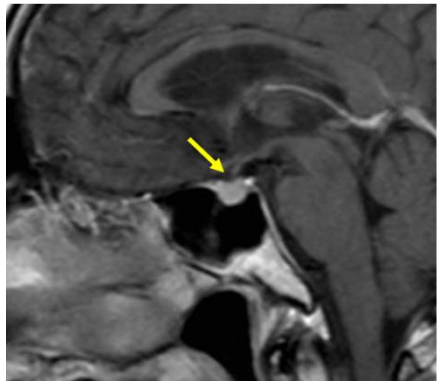


Left image courtesy of Dr Matthew Taylor. Histology image used by permission from John Wiley & Sons, Inc, James T et al, *J Cutan Pathol*, 2016.
Eigentler TK et al. *Cancer Treatment Rev*. 2016;45:7-18.

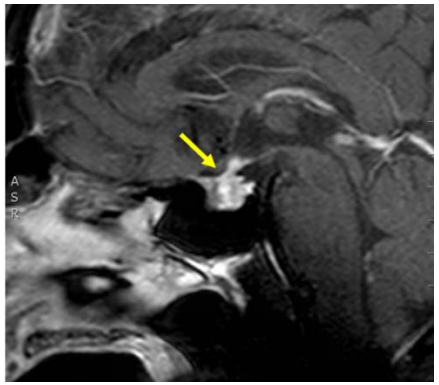
Immune-mediated adverse reactions

Endocrine: Adrenal insufficiency, Diabetes, thyroiditis/hypothyroidism, Hyperthyroidism, hypophysitis

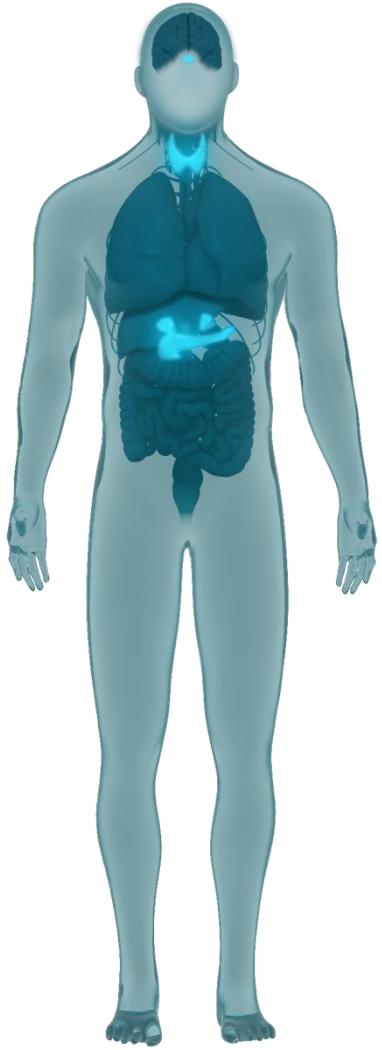
- Endocrine toxicities commonly manifest as hypothyroidism and hyperthyroidism
- Symptoms: unusual headaches, extreme tiredness, changes in mood/behavior, and weight changes



Prior to immune-mediated adverse reaction



With immune-mediated hypophysitis



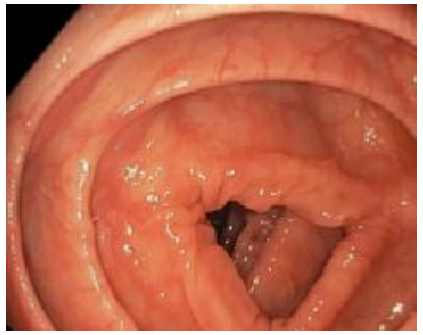
Images courtesy of Dr Matthew Taylor.

Eigentler TK et al. *Cancer Treatment Rev*. 2016;45:7-18.

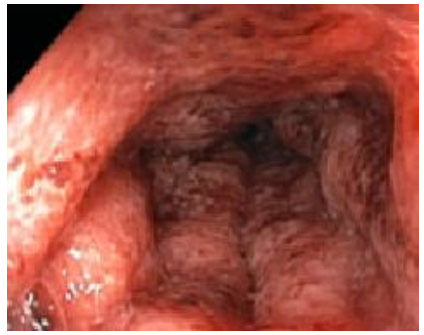
Immune-mediated adverse reactions

Digestive: Gastrointestinal events

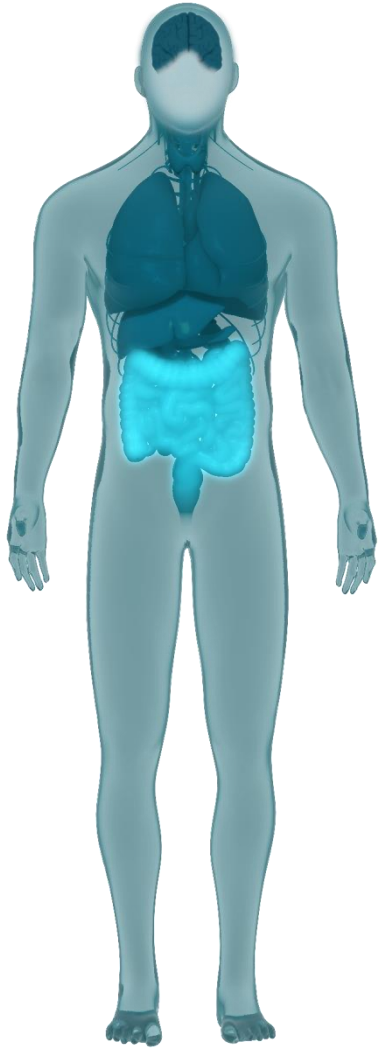
- Symptoms: diarrhea, abdominal pain, fever, anal pain, rectal bleeding, weight loss, and nausea/vomiting



Prior to immune-mediated adverse reaction



With immune-mediated colitis



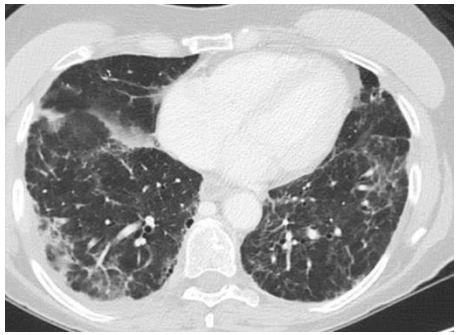
Images courtesy of Dr Matthew Taylor.

Eigentler TK et al. *Cancer Treatment Rev*. 2016;45:7-18.

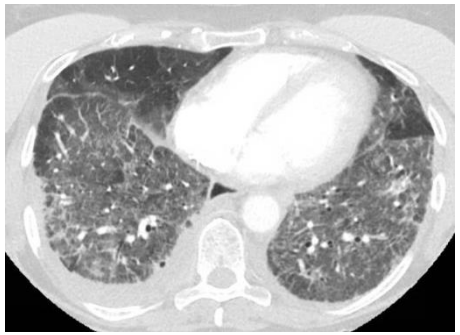
Immune-mediated adverse reactions

respiratory: pneumonitis

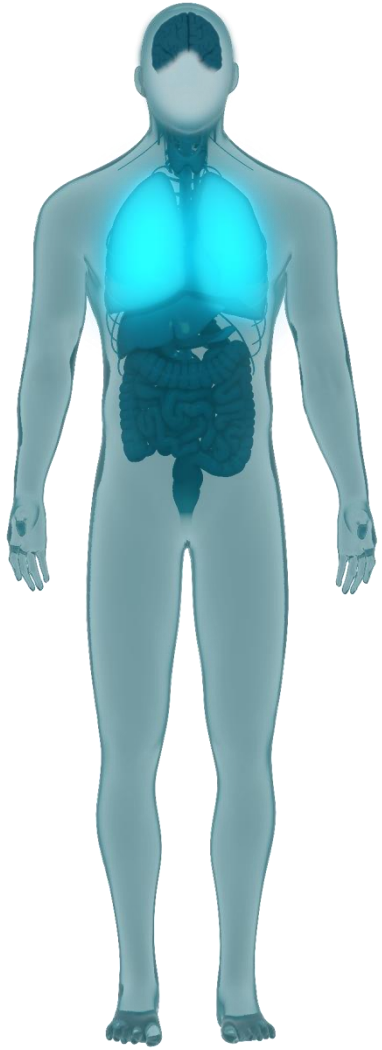
- Symptoms: dyspnea, cough, fever, and chest pain
- CT imaging can show a spectrum of findings



Prior to immune-mediated adverse reaction



With immune-mediated pneumonitis



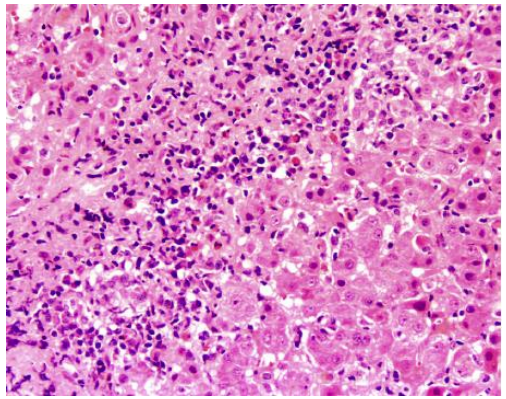
Images courtesy of Dr Matthew Taylor.

Naidoo J et al. *J Clin Oncol*. 2006 Sep 19. pii: JCO682005. [Epub ahead of print].

Immune-mediated adverse reactions

Liver: Hepatic Events

- Symptoms: jaundice, fatigue, arthralgia, fever, and increased serum aminotransferase levels



Histology of hepatitis

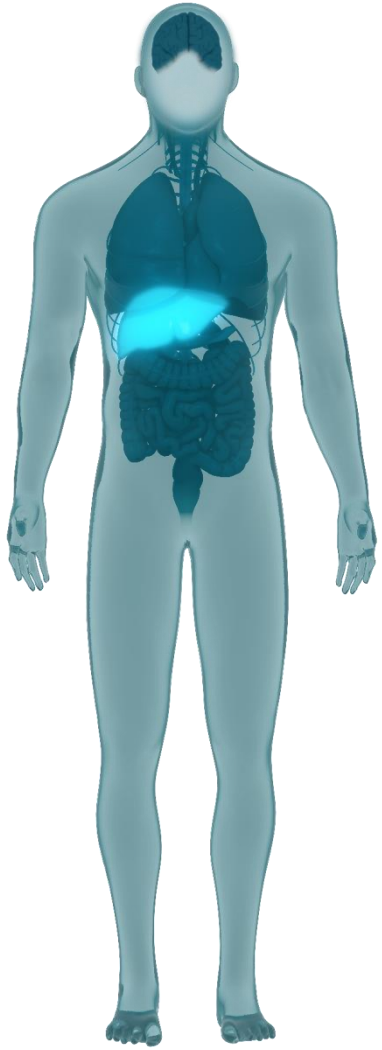
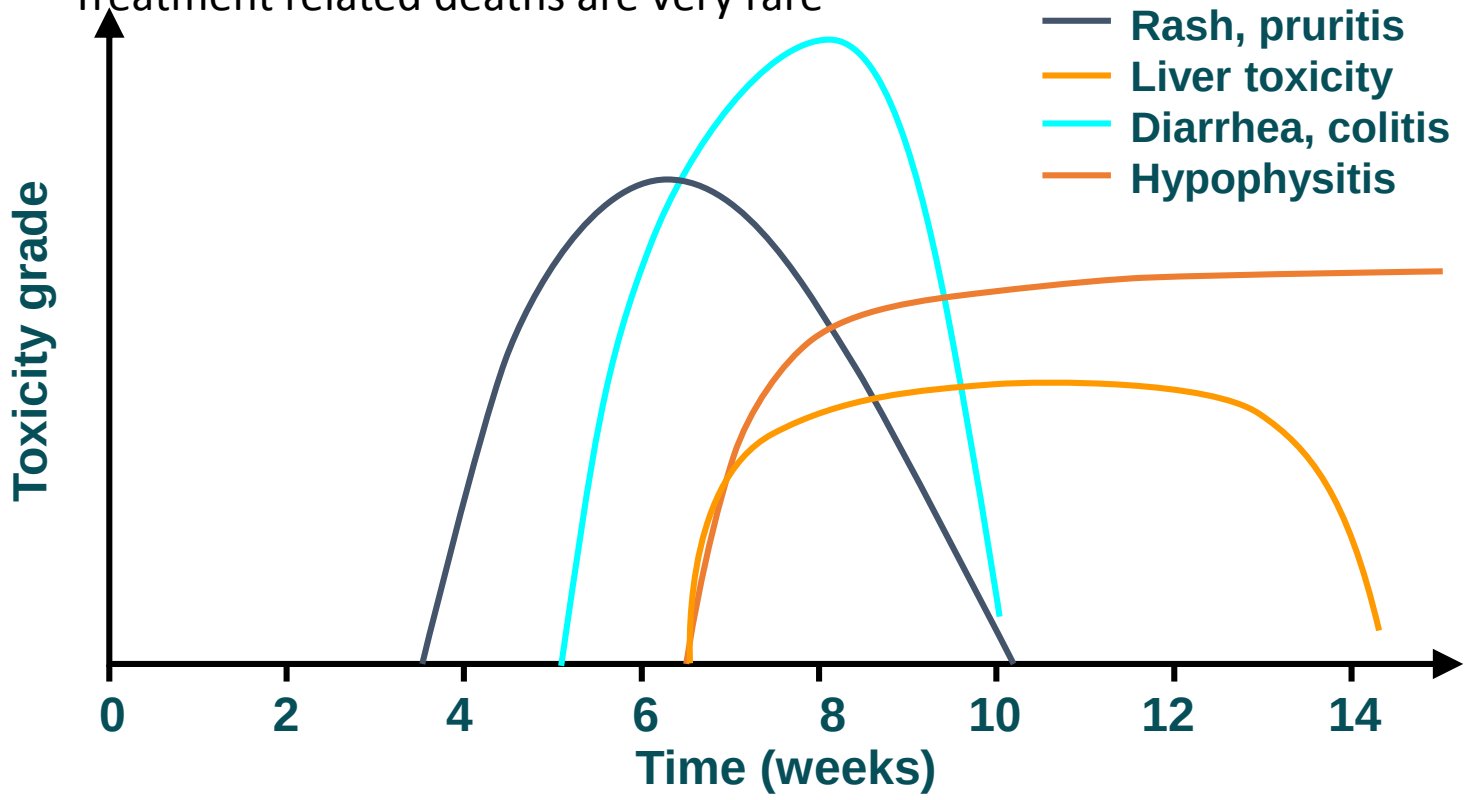


Image reprinted from *J Autoimmun*, Vol 46, Floreani A et al, Autoimmune hepatitis: contrasts and comparisons in children and adults – a comprehensive review, pages 7-16, Copyright 2013, with permission from Elsevier.

Czaja AJ. *Dig Dis Sci*. 2013;58(4):897-914.

Immune checkpoint inhibitors: immune-related adverse event (irAE) onset

- Each irAE has different kinetics of onset
- Rash first, followed by colitis, hypophysitis and finally hepatitis
- Related to aberrant T cell activation against self
- “long tail” of rare side effects (neuro, heme, cardiac, musculoskeletal, etc)
- Treatment related deaths are very rare



Weber JS, et al. *J Clin Oncol.* 2012;30:2691–2697.

Adverse Events from Immune Checkpoint Inhibitors

- Generally do not induce cytokine like effects
- Autoimmunity can affect any organ system
  - But skin, GI, liver, and endocrine organs most common
  - Multiple organ systems can be affected (concurrently or serially)
- Incidence/severity anti-CTLA-4+ anti-PD-1> anti-CTLA-4 > PD-1/PD-L1 antagonists
- Dose-relationship for anti-CTLA-4; not evident for active range of anti-PD-1/PD-L1
- Re-challenge with same agent often (but not always) leads to recurrent toxicity
- High grade AE to one class does not preclude safe administration of the other class
- Vast majority of events (except endocrine) completely reversible over time

Summary of CTLA-4 Blockade Immune-Mediated Toxicities

Common (> 20%)

- Rash, pruritus
- Fevers, chills, lethargy
- **Diarrhea/colitis**

Occasional (3% to 20%)

- Hepatitis/liver enzyme abnormalities
- Endocrinopathies: hypophysitis, thyroiditis (hypothyroid), adrenal insufficiency

Rare (< 2%)

- Episcleritis/uveitis
- Pneumonitis
- Pancreatitis
- Nephritis
- Neuropathies, Guillain-Barré, myasthenia gravis
- Limbic Encephalitis
- Lymphadenopathy (sarcoid)
- Myocarditis
- Thrombocytopenia
- Toxic epidermal necrolysis, Stevens-Johnson syndrome

Weber JS, et al. J Clin Oncol. 2012;30:2691-2697

Weber JS, et al. J Clin Oncol. 2015.

Summary of PD-1/PD-L1 Blockade Immune-Mediated Toxicities

- Toxicity less common than anti–CTLA-4 but can be severe and life threatening

Occasional (5% to 20%)

- Fatigue, headache, arthralgia, fevers, chills, lethargy
- Rash: maculopapular, pruritus, lichenoid rash, vitiligo
- Endocrinopathies:
  - **thyroid (hyperthyroid/hypothyroid),**
  - adrenal, hypophysitis
  - Diarrhea/colitis
- Hepatitis, liver/pancreatic enzyme abnormalities
- Infusion reactions

Rare (< 5%)

- Pneumonitis
  - Grade 3/4 toxicities uncommon
  - Low grade reversible with steroids and discontinuation
- Anemia
- Myocarditis
- Diabetic Ketoacidosis (Type I DM)
- Arthritis

Weber JS, et al. J Clin Oncol. 2012;30:2691-2697. Weber JS, et al. J Clin Oncol. 2015.

Combination Therapy With Ipilimumab and Nivolumab: Toxicity Summary

- The safety profile of ipilimumab and nivolumab is characterized by immune related adverse events
- Increase frequency of drug related adverse events with nivolumab combined with ipilimumab over either agent as monotherapy, in particular colitis and AST/ALT
- Skin toxicity, uveitis, neurological, renal
- No new toxicities have been identified with the combination treatment
- Toxicities with the combination have been manageable and reversible following intervention with systemic steroids in alignment with established AE management algorithms

Principles of AE Management

- Onset of adverse effects not predictable for individuals
- Close follow-up of patients, and timely management necessary to minimize morbidity
- Set of basic clinical decisions
  - Autoimmune or other cause?
  - Hold or continue treatment?
  - When to start steroids?
  - Dose? Duration?
  - PO or IV?
  - Inpatient versus outpatient?
  - When to start second-line immune suppressive?

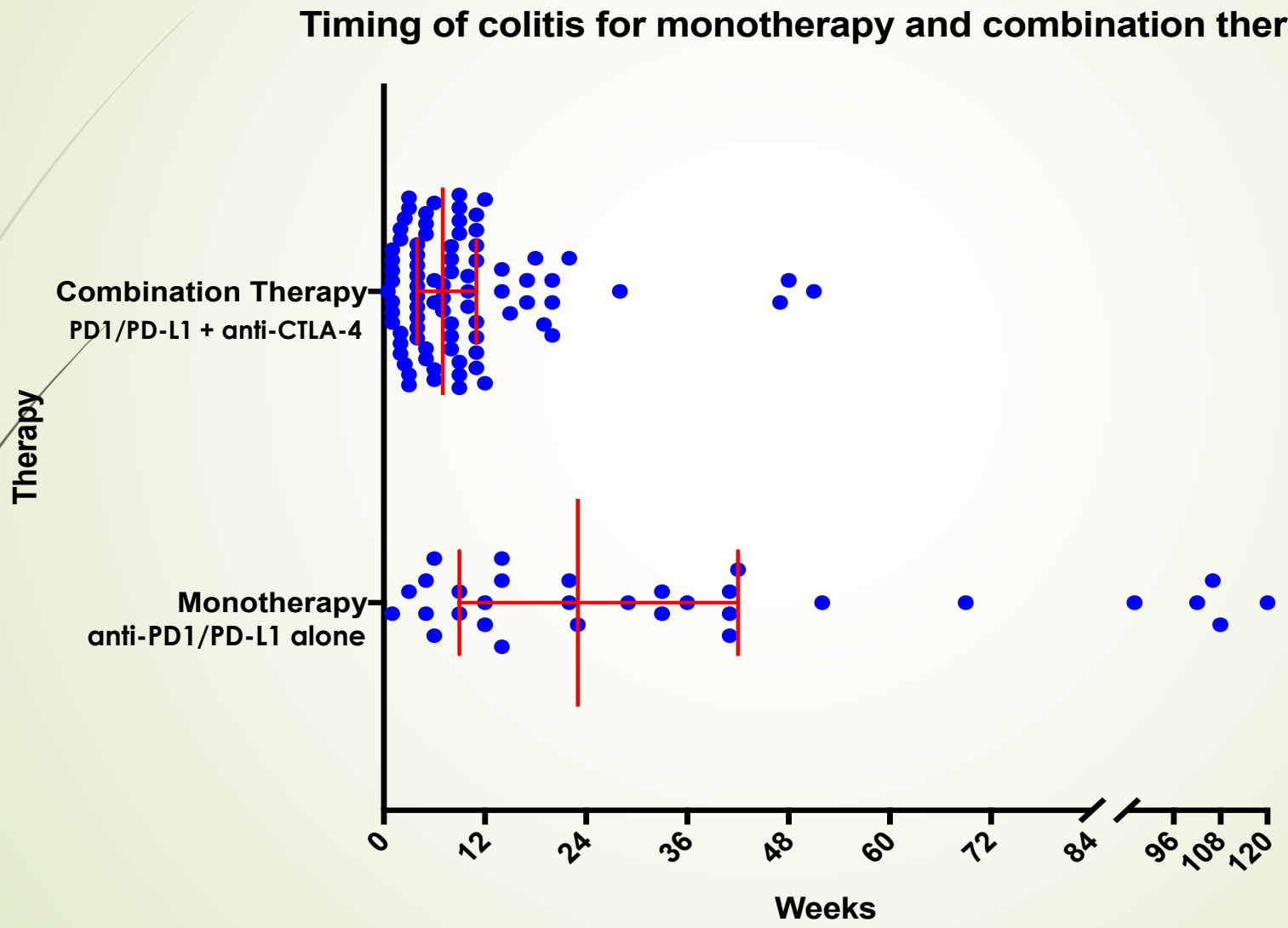
Efficacy in Patients With Advanced Melanoma Who Discontinued Treatment With Nivolumab and Ipilimumab Because of Adverse Events

- Median PFS
  - 10.8 mos for pts who discontinued treatment because of AEs
  - 9.4 mos for pts who did not discontinue because of AEs (P = .97).
- At 18 mos f/u median OS had not been reached in either group (P = .23).
- The ORR:
  - 58% for pts who discontinued due to AEs during the induction phase
  - 50% for pts who did not discontinue because of AEs .

Characteristics of Colitis with anti-PD-1+/- anti-CTLA-4

Treatment Regimens	Single Agent anti-PD-1	Combination anti-PD-1+ anti-CTLA-4	Total Patients
Number of Melanoma Pts	937	324	1261
Number of patients with clinically significant Colitis	31 (3.3%)	83 (25.6%)	114 (9.0%)
Median Time of Onset of Colitis	7 weeks	23 weeks	
Need for second line Immunosuppression (infliximab)	29%	42%	
Objective Response Rate	78%	57%	

Colitis in Melanoma Patient Receiving with anti-PD1/PD-L1 alone or in combination with anti-CTLA-4



Unusual Immune Checkpoint Adverse Events

- Systemic inflammatory syndrome
- Severe arthritis
- **Myositis**
- **Myocarditis**
- Pneumonitis
- Bowel perforation
- Meningitis
- **Insulin-dependent DM**
- Myasthenia Gravis
- Ascending polyneuropathy (Guillan-Barre)
- Limbic Encephalitis
- Thrombocytopenia (ITP)
- Dry eye syndrome
- Lichen planus (more common with anti-PD-1
- Alopecia areata

Mechanisms of immune-checkpoint inhibitor-induced toxicity

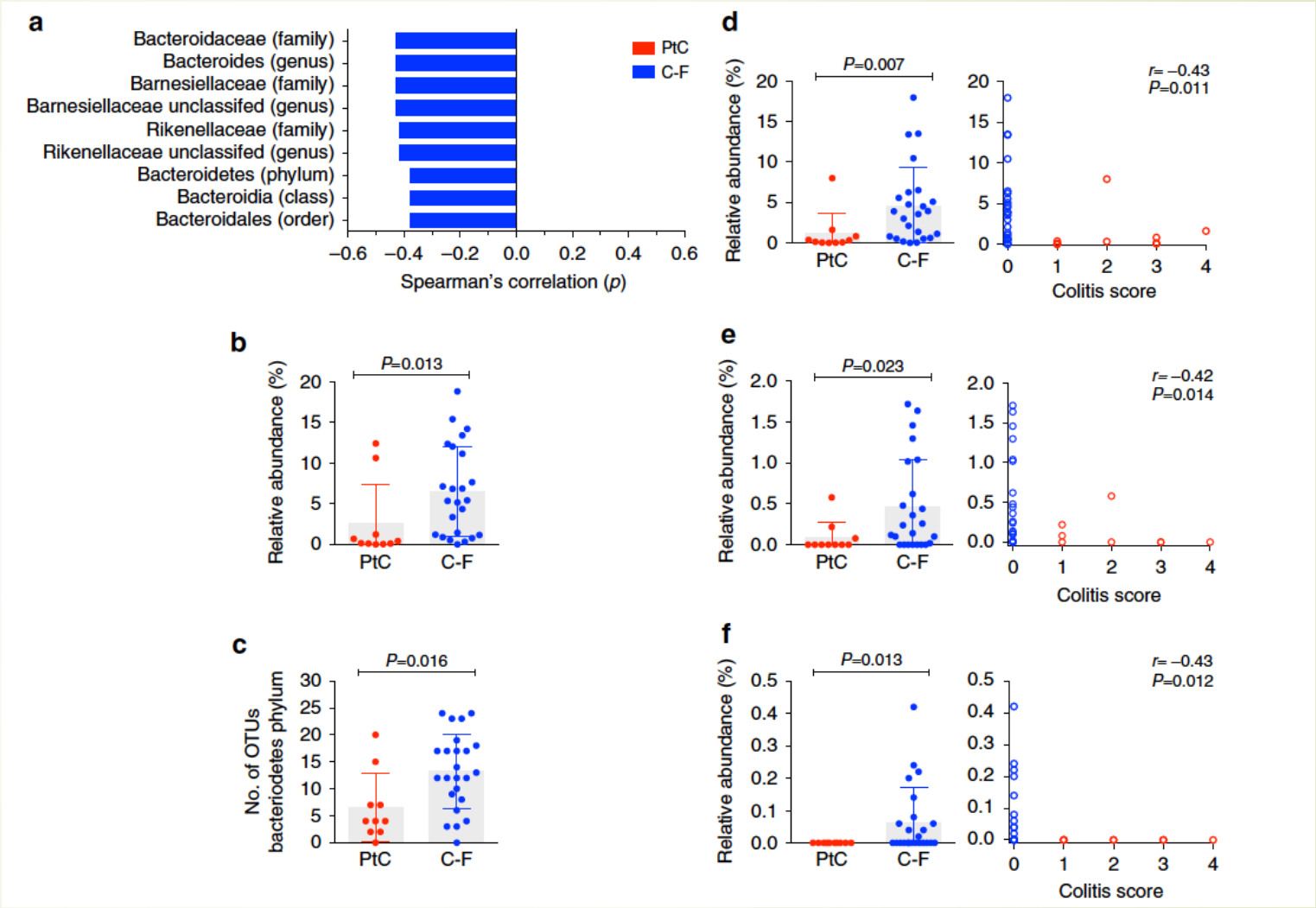
- A major issue limiting treatment with immunotherapy (Achilles heal of immunotherapy)
- Understanding how to intervene to treat and prevent immune related toxicity
- Understanding relationship of anti-tumor responses and autoimmune toxicity (scientifically)
- Insight into primary autoimmune disease (inflammatory bowel disease, endocrinopathy, autoimmune associated dermatitis

Mechanisms of Checkpoint Inhibitor Organ toxicity

Organ Effects	Mechanism
Hypophysitis	Expression of CTLA-4 on normal pituitary cells,
Thyroiditis	Anti-thyroid antibodies
Myocarditis	Clonal (TCR) T cells in Tumor & Myocardium
Colitis	Intestinal microbiome; Tregs, IL-17?
Dermatitis	T cells induce vitiligo (epitope spreading)
Insulin Dependent Diabetes	Autoimmune T cell repertoire; stress induces PD-L1 expression on inflamed organ

Checkpoint Induced Colitis and the Microbiome

Identification of biomarkers may enable interventions to reduce the risk of inflammatory complications with cancer immunotherapy



Prospective study of pts with metastatic melanoma undergoing ipilimumab treatment .

Correlate the pre-inflammation fecal microbiota and microbiome composition with subsequent colitis development.

Increased representation of bacteria belonging to the Bacteroidetes phylum is correlated with resistance to the development of checkpoint-blockade-induced colitis.

Role of the Microbiome in Checkpoint Toxicity and Efficacy

- Toxicity- Colitis due to anti-CTLA-4
  - Increased fecal abundance of the Bacteroidetes phylum families (Bacteroidaceae, Rikenellaceae and Barnesiellaceae)- **associated with decreased anti-CTLA-4 colitis**
  - Microbial genetic pathways involved in polyamine transport and B vitamin biosynthesis **associated with resistance to anti-CTLA-4 colitis**
- Clinical Efficacy – anti-PD1
  - high relative abundance of bacteria of the *Faecalibacterium* genus and diversity- associated with benefit
  - Increased relative abundance of *Akkermansia muciniphila* in patients associated with benefit. (antibiotics associated with worse outcome
  - Increased abundance of eight microbial species, including *Bifidobacterium longum* associated with benefit
  - Demonstrate of efficacy of fecal microbiome transplantation in animal models

Type I Diabetes Mellitus and anti-PD-1 Therapy

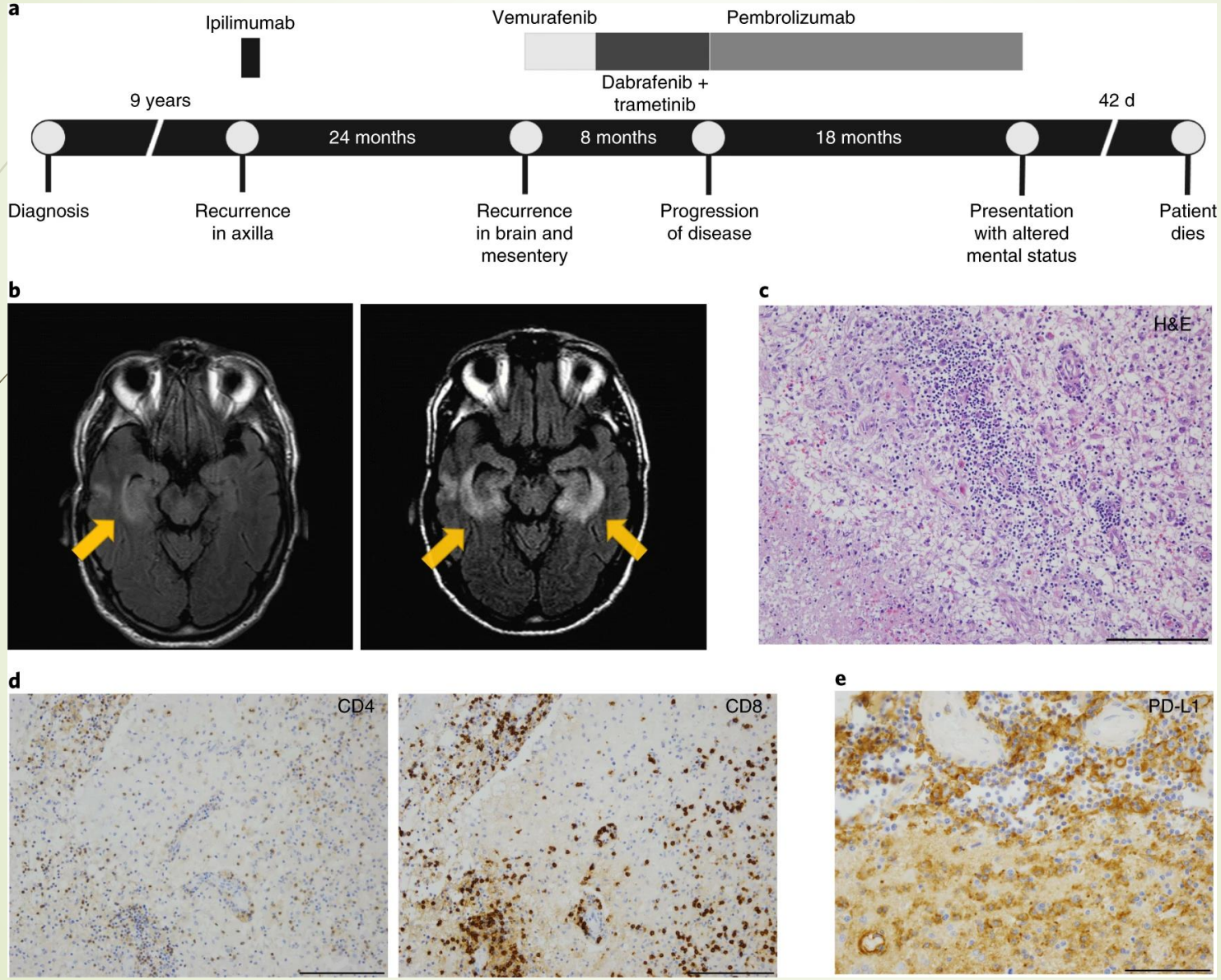
Table 1—Clinical history and key laboratory findings

Patient	Age/sex	Primary diagnosis	Pertinent history	Anti-PD-1 drug	Other chemotoxins	Diabetes presentation	Random C-peptide* and glucose	Time after anti-PD-1	Antibody positivity/titers ^	HLA	Diabetes antigen-specific T cells†
1	55/F	Melanoma	Autoimmune thyroid disease	Nivolumab	Ipilimumab, prednisone	DKA, glucose 532 mg/dL, HbA_{1c} 6.9% (52 mmol/mol)	<0.1 ng/mL and 52 mg/dL	5 months	None	$A2.1^+$, $DR4^+$	0.35%
2	83/F	Non–small-cell lung cancer	Remote smoker	Nivolumab	None	DKA, glucose 350 mg/dL, HbA_{1c} 7.7% (61 mmol/mol)	<0.1 ng/mL and 336 mg/dL	<1 month	GAD65/1.2	$A2.1^+$, $DR4^+$	0.28%
3	63/M	Renal cell carcinoma	Hypertension	Nivolumab	Proleukin, bevacizumab, interferon	Random glucose 247, 340 mg/dL; HbA_{1c} 8.2% (66 mmol/mol)	1.3 ng/mL and 79 mg/dL	4 months	GAD65/1.1, ICA512/1.2, Insulin (IAA)/47	$A2.1^+$, $DR4^+$	2.01%
4	58/M	Small-cell lung cancer	Type 2 diabetes	Nivolumab	Carboplatin/ etoposide, paclitaxel	DKA, glucose 749 mg/dL, HbA_{1c} 9.7% (83 mmol/mol) (from 8.5% [69 mmol/mol] prior)	<0.1 ng/mL and 284 mg/dL 0.6 ng/mL and 523 mg/dL	1 week	GAD65/13819	$A2.1^+$	0.89%
5	64/F	Melanoma	Autoimmune thyroid disease, psoriasis	Pembrolizumab	None	Ketonuria, glucose 703 mg/dL, HbA_{1c} 7.4% (57 mmol/mol)	0.5 ng/mL and 268 mg/dL	<1 month	None	$DR4^+$	N/A

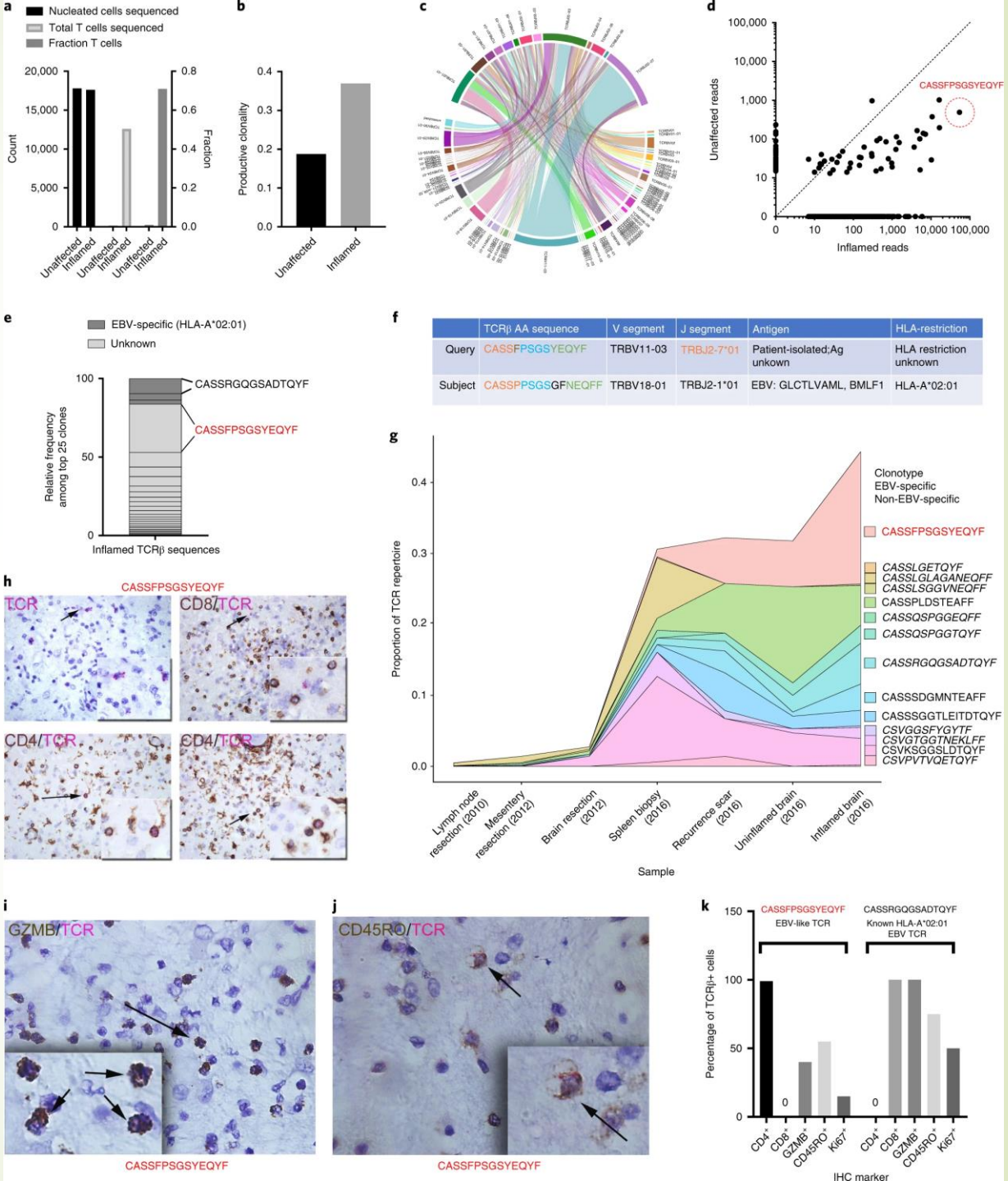
*C-peptide reference range: 1.1–4.4 ng/mL. †Patients 1, 2, 3, and 4 were positive for HLA-A2.1 from screening by flow cytometry using monoclonal antibody BB7.1 (Abcam, Cambridge, MA). HLA-A2.1 tetramers were obtained from the National Institutes of Health Tetramer Core Facility (Atlanta, GA) and loaded with peptides from five diabetes antigens: insulin A chain (GIVEQCCTSI), insulin B chain (HLVEALYLV), preproinsulin (ALWMRLLPL), GAD65 (VMNILLQYVV), and IGRP (LNIDLLWSV) (5). Peripheral blood mononuclear cells (PBMCs) were incubated with the five class I diabetes antigen-containing tetramers. The data shown represent positive staining after subtracting staining with a negative tetramer. PBMCs from HLA-$A2.1^+$ donors without diabetes served as negative control and showed staining (mean ± 2 SD) of 0.5%. PBMCs were also stained with monoclonal antibodies to CD45RO, CCR7, and CD45RA to identify cellular phenotypes. Flow data were analyzed using FlowJo software version 9.6.1 (Tree Star, Ashland, OR). ^ Diabetic autoantibodies to GAD65, ICA512, and insulin were performed at LabCorp, Burlington, NC. Normal GAD65 titers <0.5 U/mL, ICA512 <1.0 U/mL, and IAA <5.0 U/mL.

- Four of 5 Pts. developed acute DKA and low/undetectable C-peptide levels.
- Pts exhibited both cellular and humoral diabetes-associated autoimmunity, a generally rare finding in this age group (**>**55 yrs).

A case report of clonal EBV-like memory CD4+ T cell activation in fatal checkpoint inhibitor-induced encephalitis



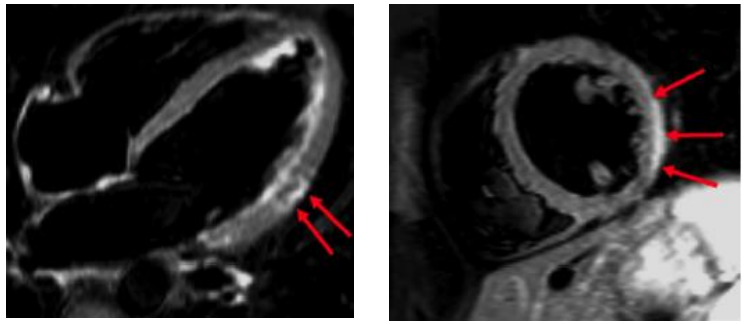
TCR sequencing identification of oligoclonal CD4+ cytotoxic T cells in inflamed encephalitic tissue.



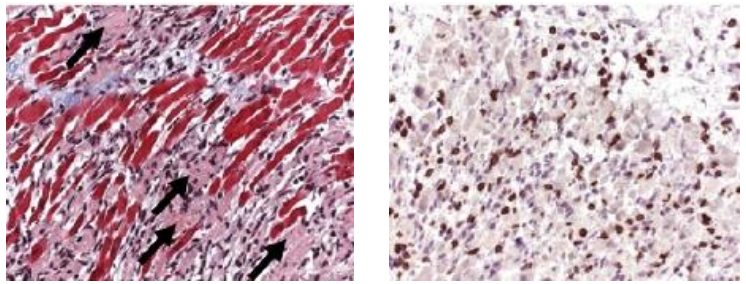
Immune-mediated adverse reactions

Heart: MYOCarditis

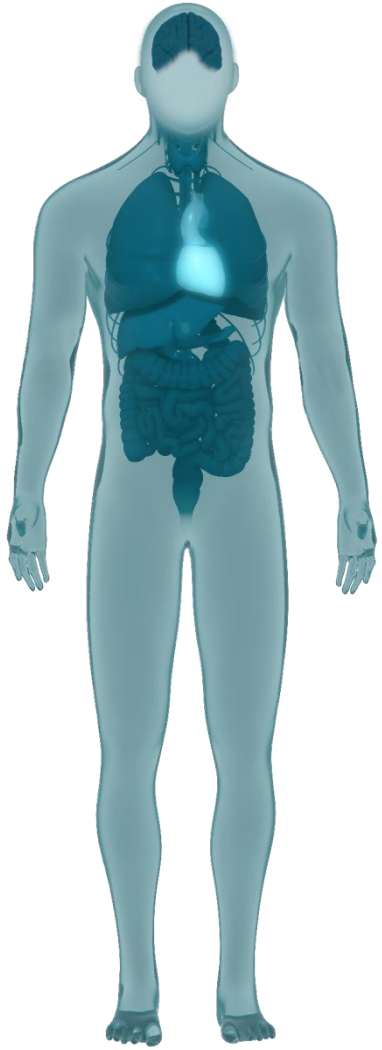
- Myocarditis can present as a broad spectrum of symptoms, from asymptomatic to signs of myocardial infarction to cardiogenic shock
- Symptoms: chest pain, cardiac arrhythmias, and acute or chronic heart failure



Magnetic resonance images of myocarditis



Histology of myocarditis



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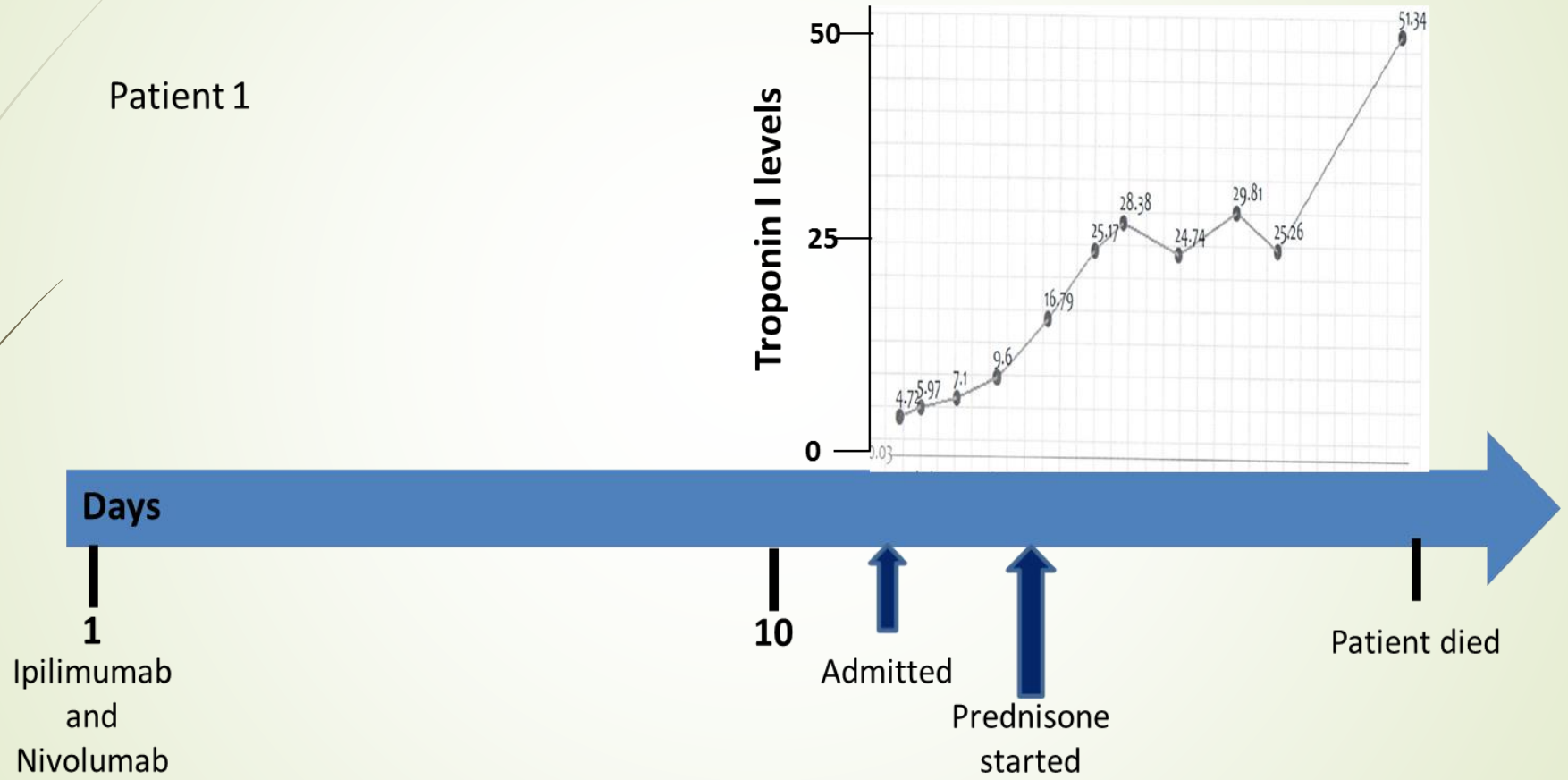
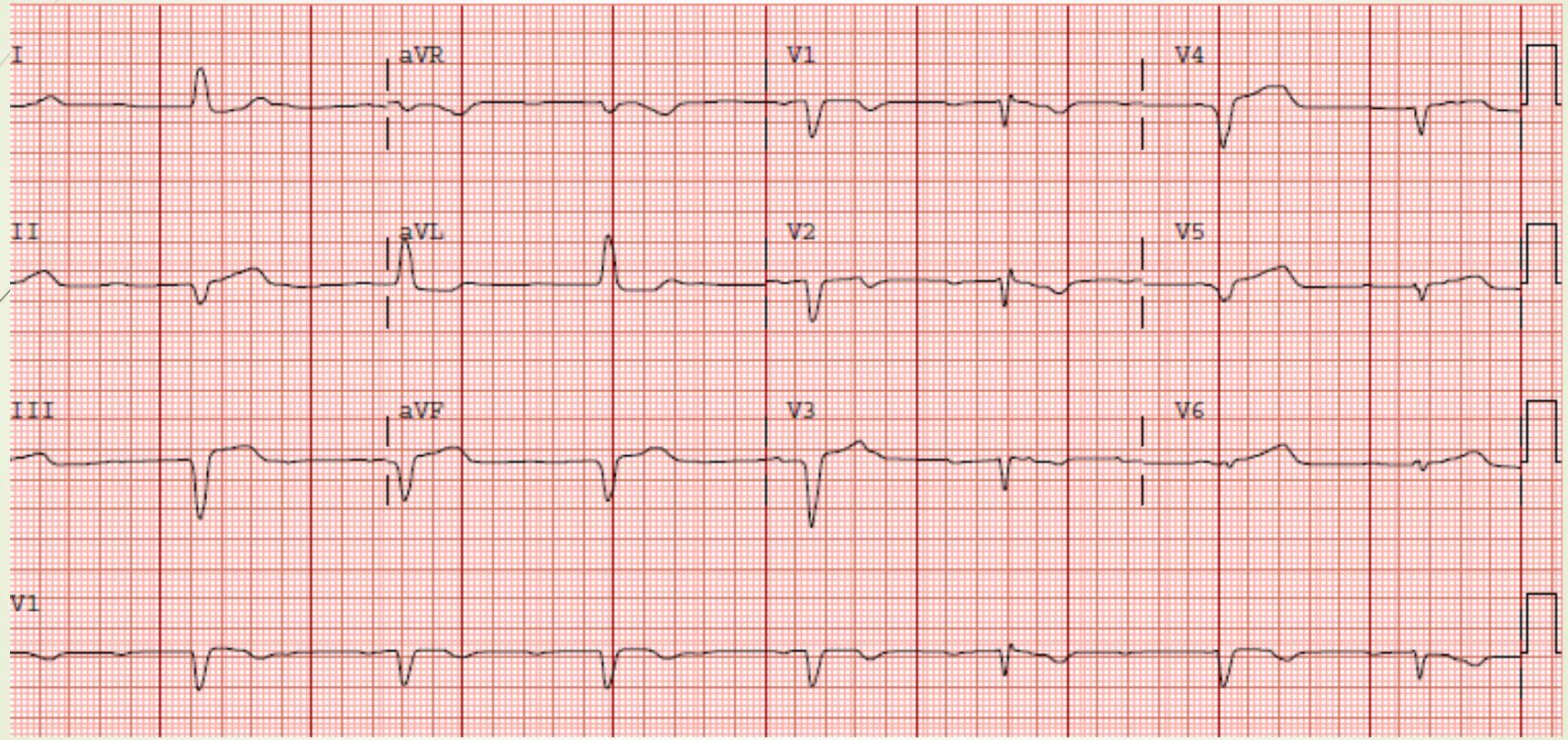
Kindermann I et al. *J Am Coll Cardiol*. 2012;59(9):770-792.

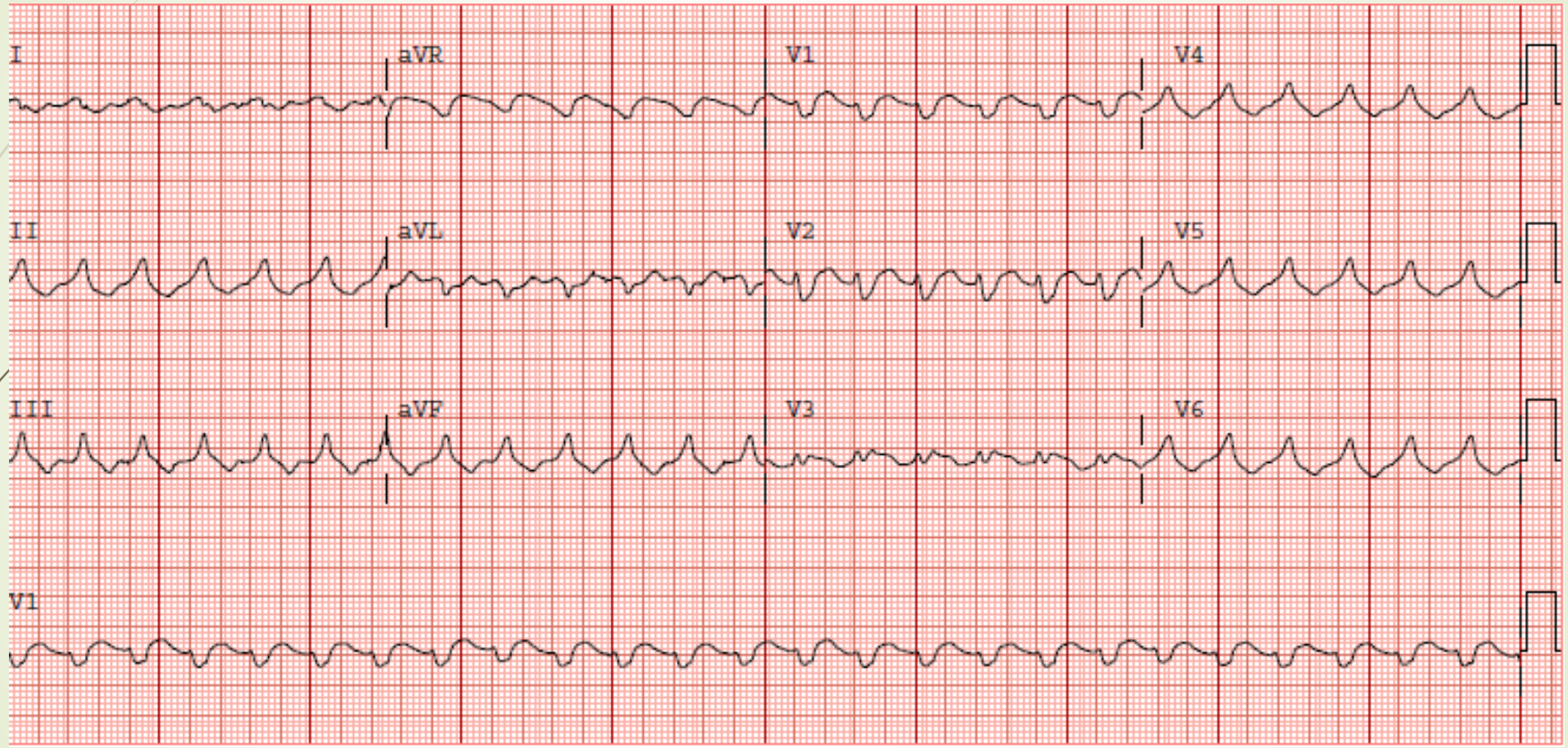
Case #1


Patient 1
Troponin I levels
50
25
0
4.72
5.97
7.1
9.6
16.79
25.17
28.38
24.74
29.81
25.26
51.34
Days
1
Ipilimumab and Nivolumab
10
Admitted
Prednisone started
Patient died

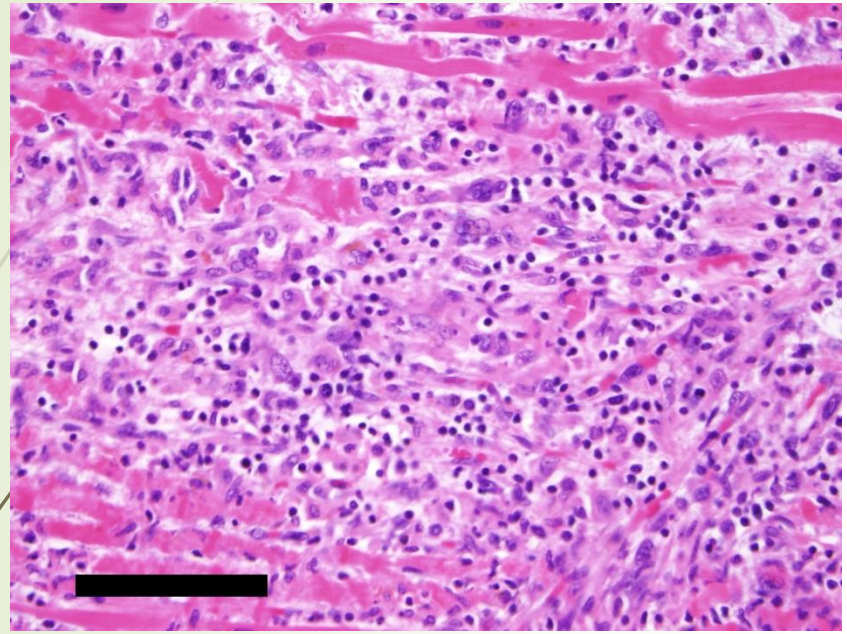
Initial EKG



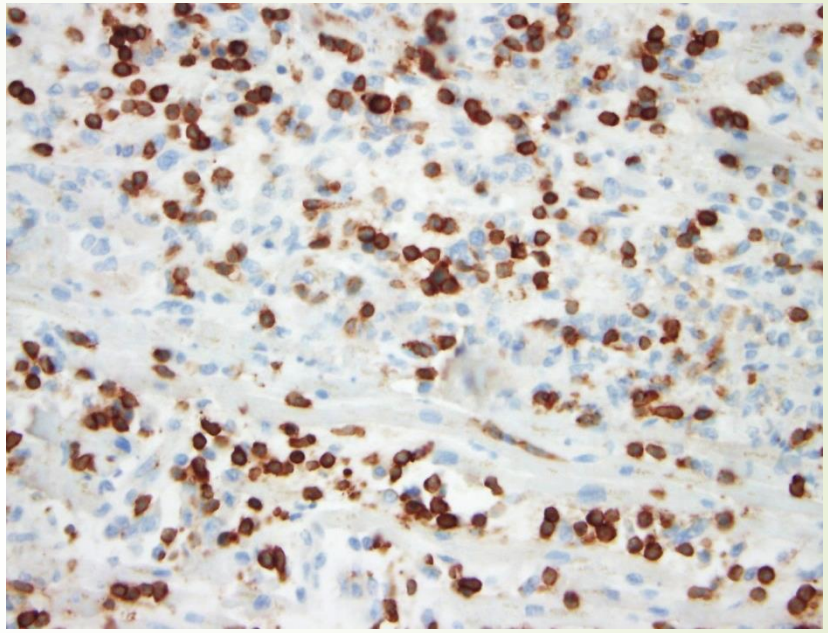
Subsequent EKG



Autopsy, Case 1



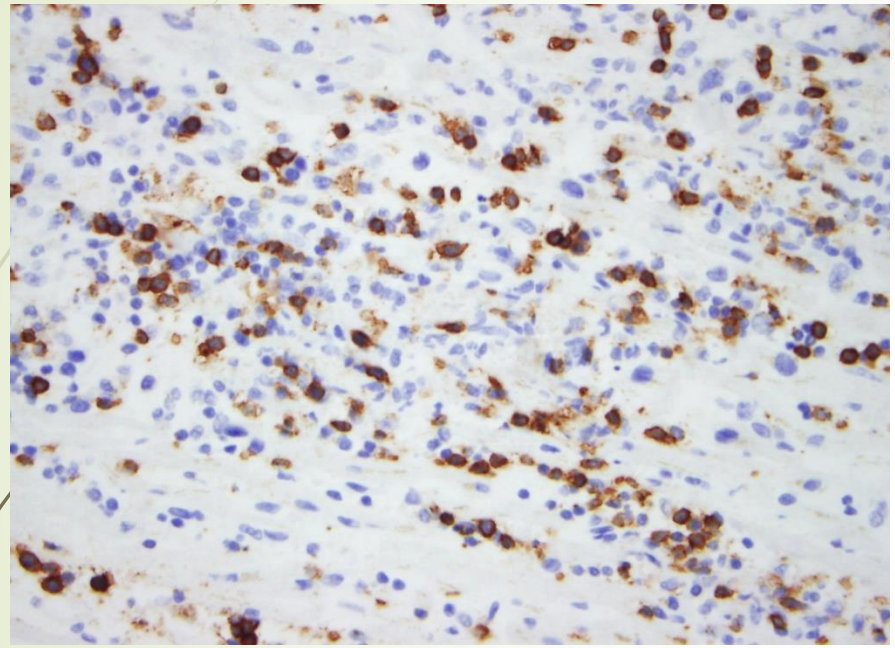
Myocardium, H&E



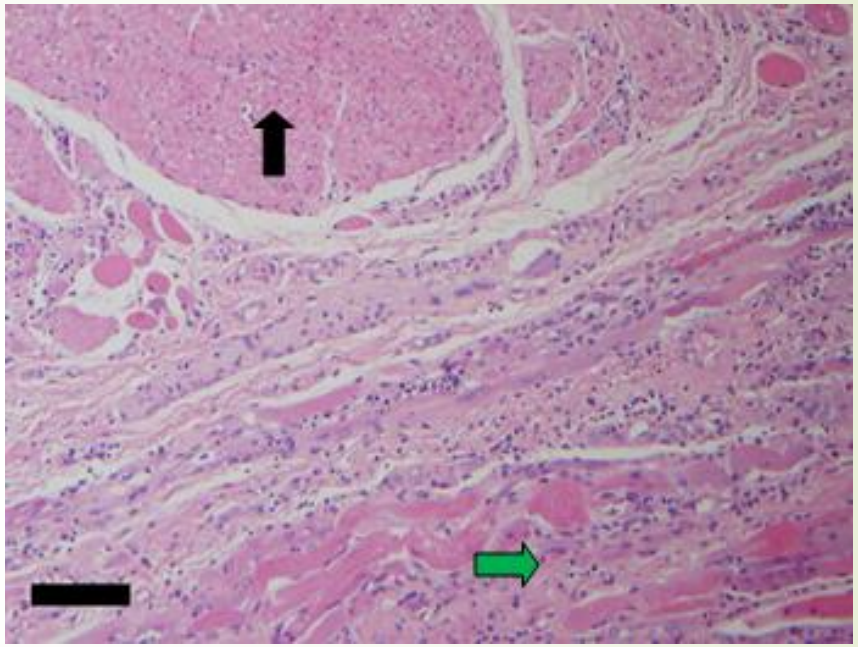
Myocardium, CD3

Johnson, et al, in press

Autopsy, Case 1



Myocardium, CD8

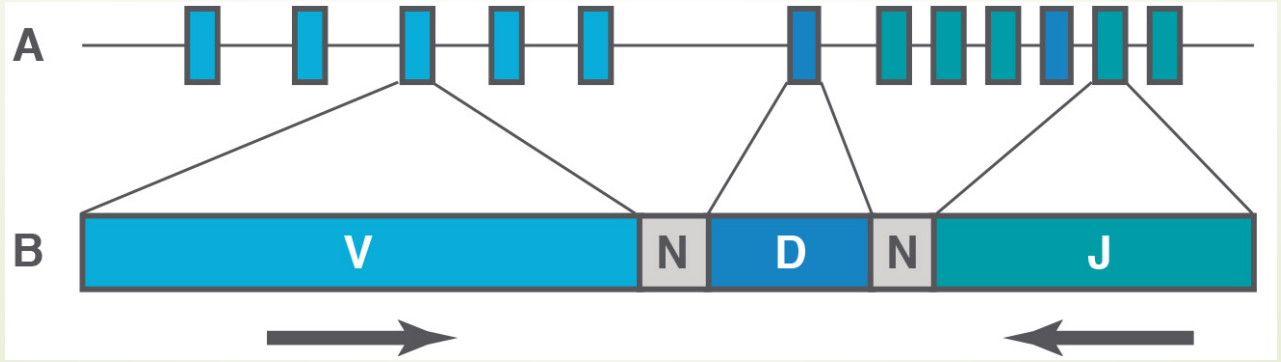


Esophagus, H&E

Johnson, et al, in press

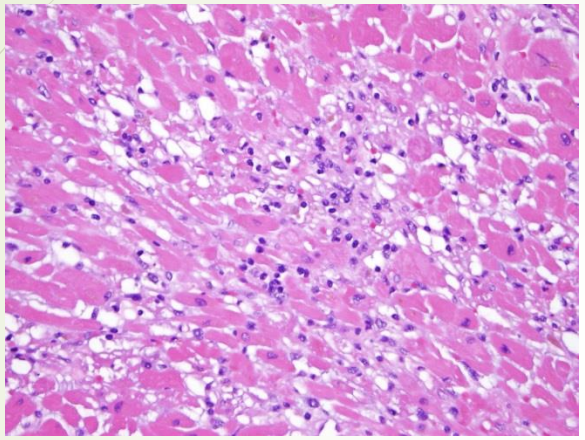
TCR sequencing

- NGS platform through Adaptive Biotechnologies
- Profile the diversity and clonality of infiltrating T cells – CDR3 region of beta chain
- Allow to assess whether shared T cell clones are present
- Begin to study mechanism

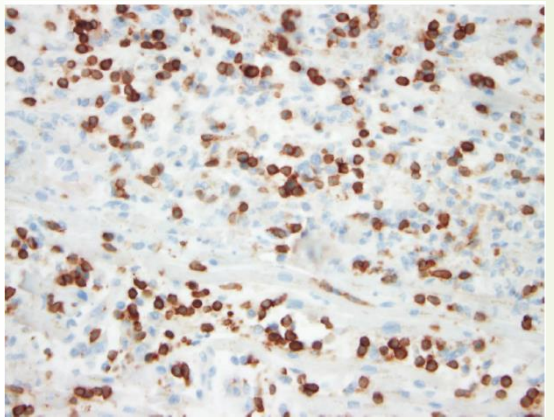


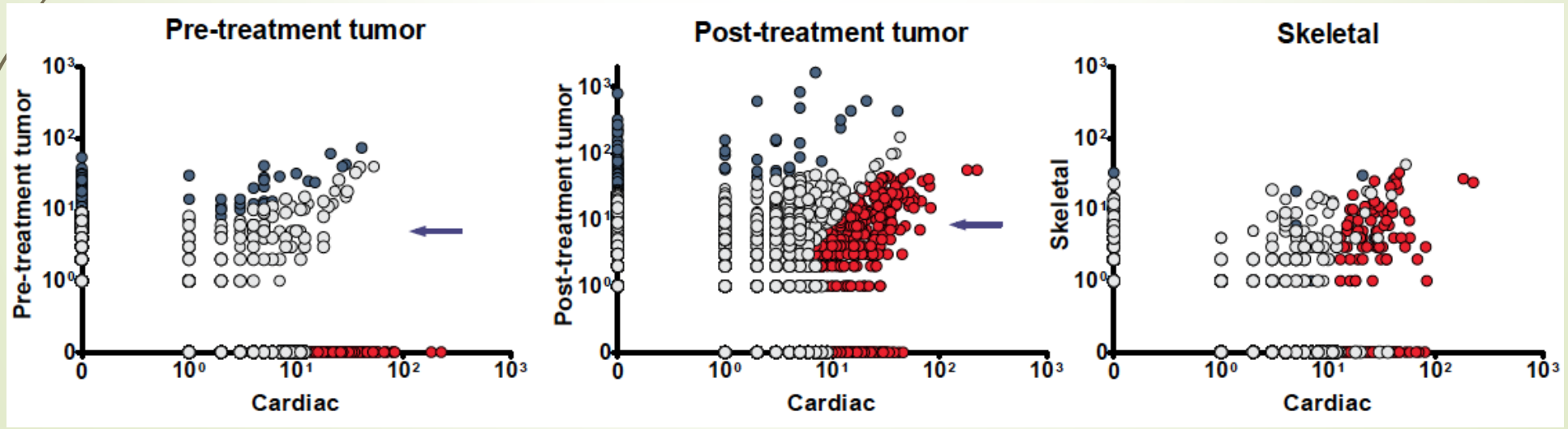
Immune Checkpoint Inhibitor Associated Myocarditis

H&E

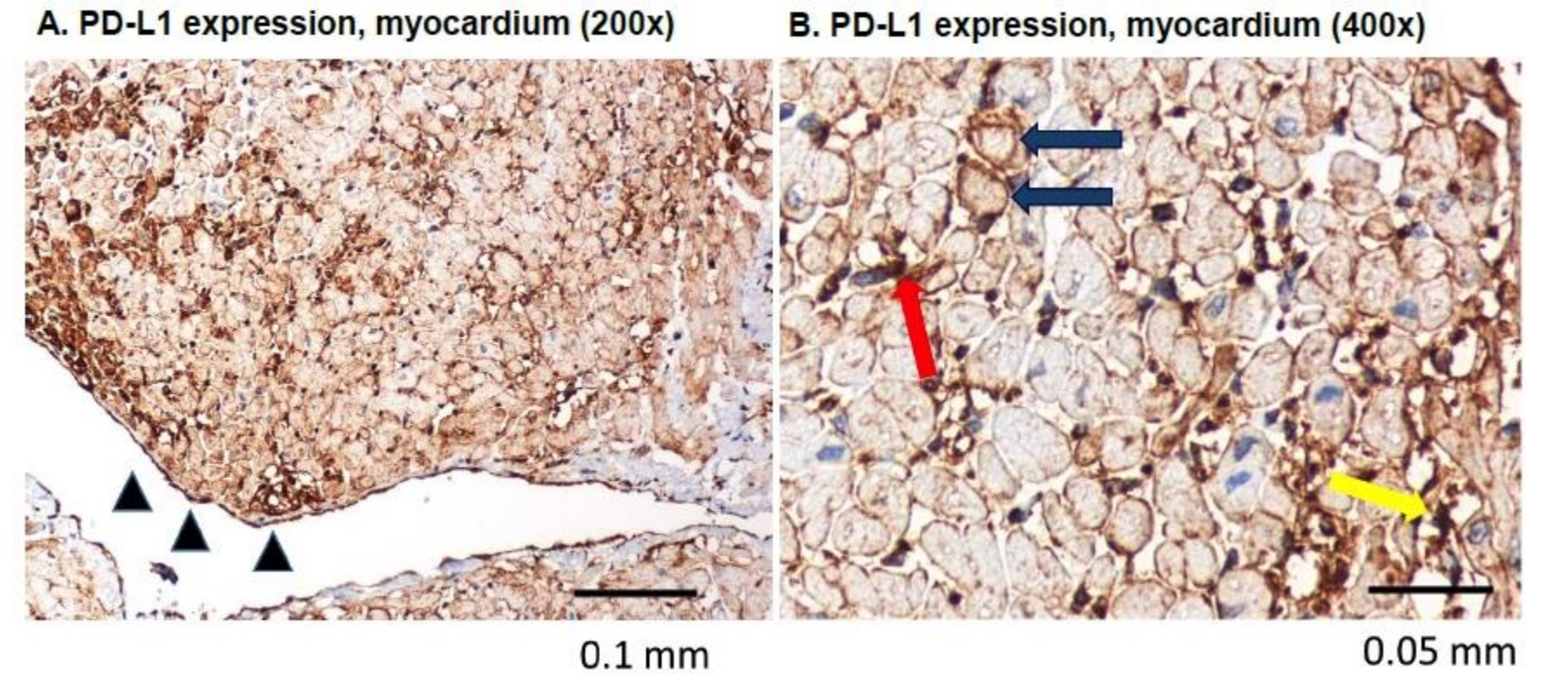


CD3





PD-L1 Staining



Incidence of myocarditis and myositis with ipilimumab and nivolumab treatment

C. Myocarditis and myositis incidence

Characteristic	Patients receiving nivolumab (N = 17,620)	Patients receiving nivolumab + ipilimumab (N = 2974)
Myocarditis* - no. (%)	10 (0.06%)	8 (0.27%)
Fatal events - no. (%)	1 (<0.01%)	5 (0.17%)
Myositis - no. (%)	27 (0.02%)	7 (0.24%)
Fatal events - no. (%)	2 (0.01%)	1 (0.03%)

*Includes 6 cases of concurrent myocarditis and myositis and/or rhabdomyolysis.

Myositis

- 34 cases listed as severe, 3 non-severe
- 5 patients on statins
- 3 cases fatal

Myocarditis

- 18 cases
- 6 fatal (5 of 8 with ipi + nivo)
- Time to onset 13-64 days
- 12 M, 5F
- 5 with prior cardiac disease

The World Health Organization (WHO) database of individual safety case reports, to identify 101 cases of severe myocarditis following treatment with ICIs

Table: Characteristics of patients with immune checkpoint inhibitor associated myocarditis (n=101)	
Characteristic	**Percent (%)**
Male gender	66
Cancer	
Melanoma	40
NSCLC	30
Renal	7
Other*	23
Region reporting	
Americas	54
Europe	33
Asia	11
Oceania	3
Concomitant medications	
Aspirin	11
Statin	11
Beta blocker	7
ACE/ARB	12
Diabetes medication	9
No CV/Diabetes medications	75
Regimen	
Anti-PD-1 monotherapy	
- Nivolumab	43
- Pembrolizumab	15
Anti-PD-L1 monotherapy#	3
Anti-CTLA-4 (Ipilimumab) monotherapy	5
* Combination anti-PD-1/PD-L1 + anti-CTLA-4	27
Combination anti-PD-1/PD-L1 + other agents**	8
Timing (median, range)	27 days (5-155)
Concurrent irAEs	
* Myositis/rhabdomyolysis	25
Myasthenia gravis	10
Colitis	4
Severe cutaneous events†	4
Other‡	5
Fatal outcome	46
Reporting year	
2010 – 2014	3
2015	6
2016	15
* 2017 (through Dec. 6)	76

NSCLC: non-small cell lung cancer; irAEs: immune-related adverse events; CV: cardiovascular

VigiBase (http://www.vigiaccess.org/)

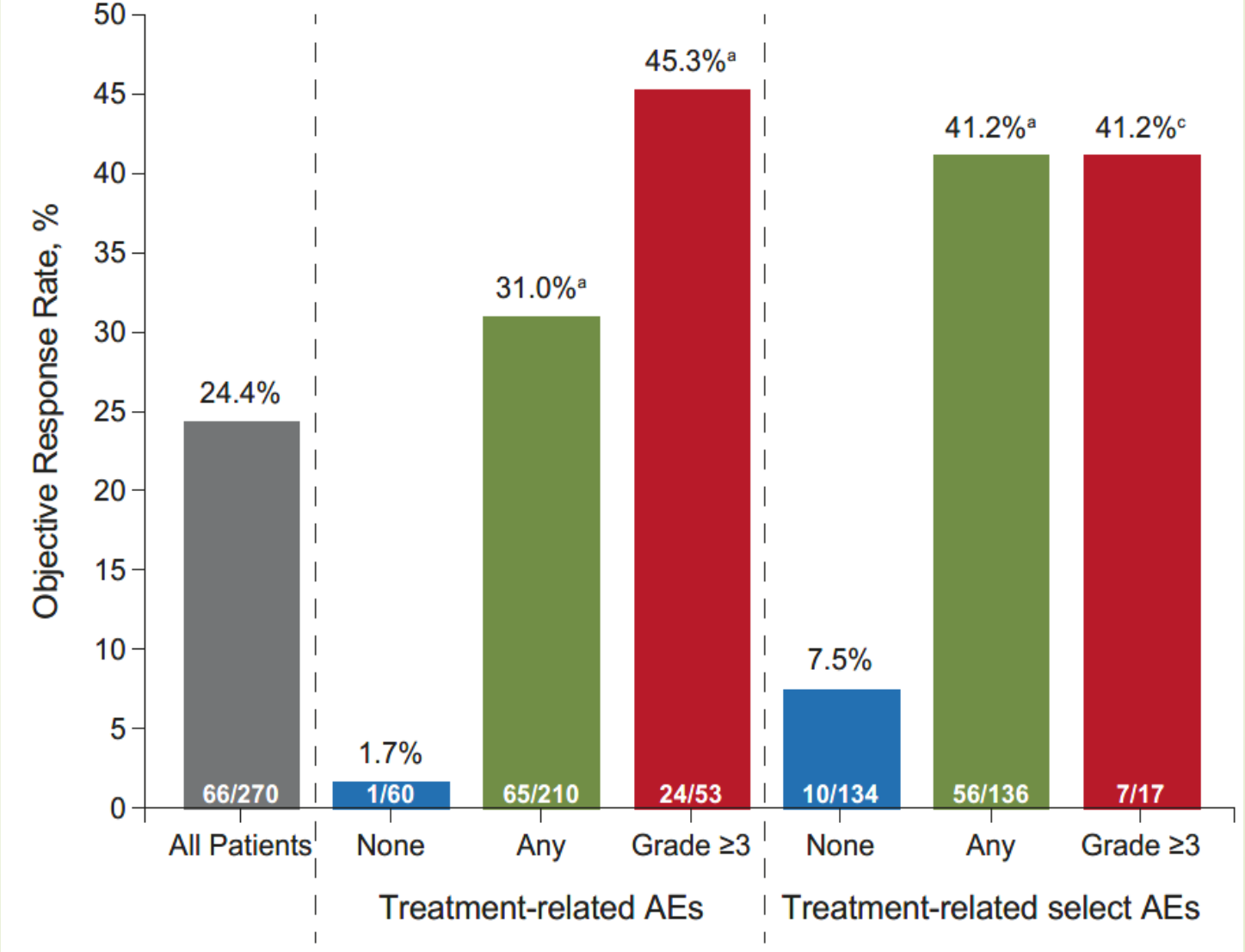
Approaches to Study Mechanisms of Checkpoint Inhibitor Toxicity

- Assess T cell clonal diversity and clonality in tumor or infectious agents and target organ (heart, colon, brain)- are there shared antigens
- Assess immune gene expression (cytokines, cell type, MHC associated molecules) in target organ
- Assess normal germline genome for overall (non-biased approach GWAS) polymorphisms, or emphasis on MHC polymorphism, or emphasis on immune/inflammatory genes
- Large or focused antibody screens and protein antigen arrays
- Peripheral blood- T cell or immune populations through flow cytometry, CyTOF

What are the predictive biomarkers for the onset of immune-related adverse events associated with checkpoint inhibition, and are they related to markers for efficacy?

Table 2: Data analysis and experimental questions (Aim 1)		
Primary Questions:	Correction factors:	Associated outcomes:
Are unique HLA haplotypes associated with outcome(s)?	• HLA-haplotype • ICI therapy type • Tumor type • Gender • Age • Prior autoimmune disease	• Grade 3/4 irAE (any) • Grade 3/4 irAE (specific site) • Clinical response
Are common autoimmune disease-associated SNPs associated with outcome(s)?		
Are autoimmune disease-associated autoantibodies identified at baseline associated with outcome(s)?		
Are TCRβ sequences in peripheral blood and/or tissue associated with outcomes?		

Association Between Incidence of Adverse Events and Objective Response Rate in 270 Patients With Melanoma, RCC, or NSCLC Receiving Nivolumab.



Association Between Incidence of Adverse Events and Overall Survival in 270 Patients With Melanoma, RCC, or NSCLC Receiving Nivolumab.