Preclinical Safety Assessment of Immunostimulatory Antibodies for Cancer Therapy

Noël Dybdal, DVM, PhD, DACVP
Steven Laing BVMS(Hons) PhD DACVP MRCVS

gRED Development Sciences

Genentech
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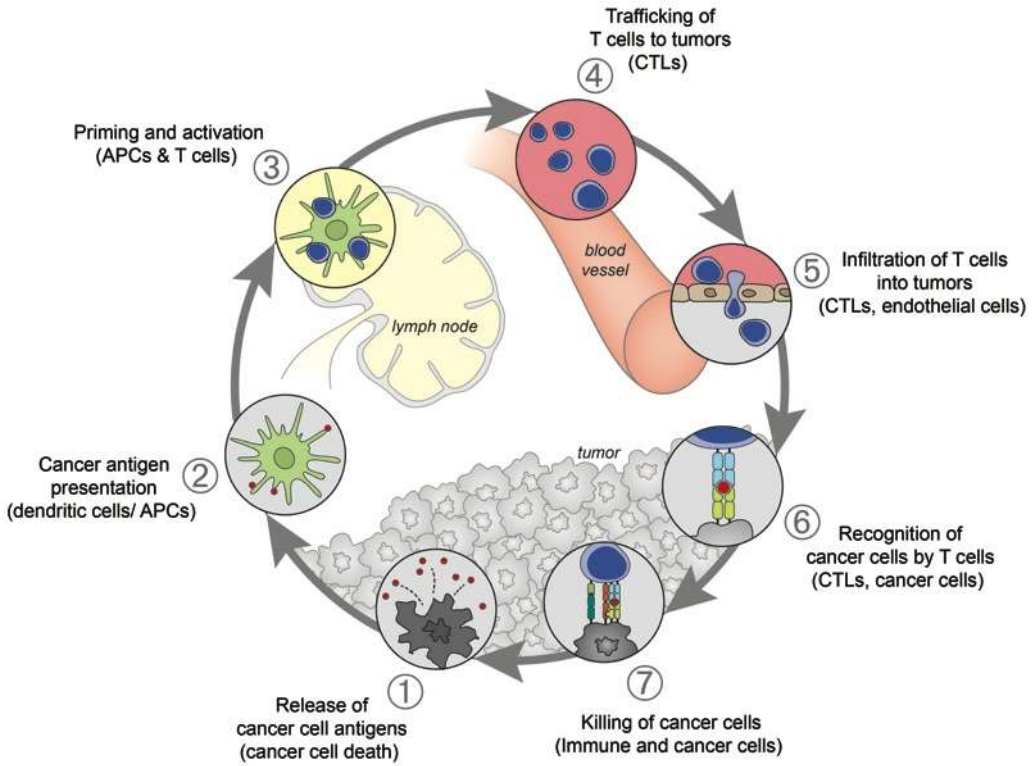
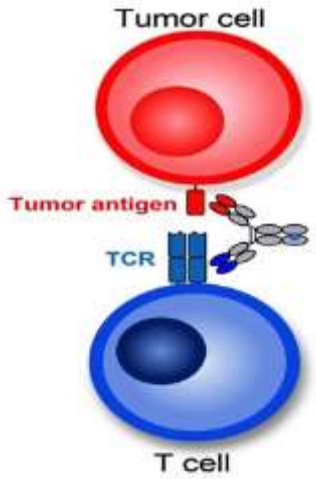
Harnessing the immune system to kill cancer cells

Enhance cancer immunity cycle



Requires pre-existing anti-cancer immune cells

Recruit cytotoxic immune cells



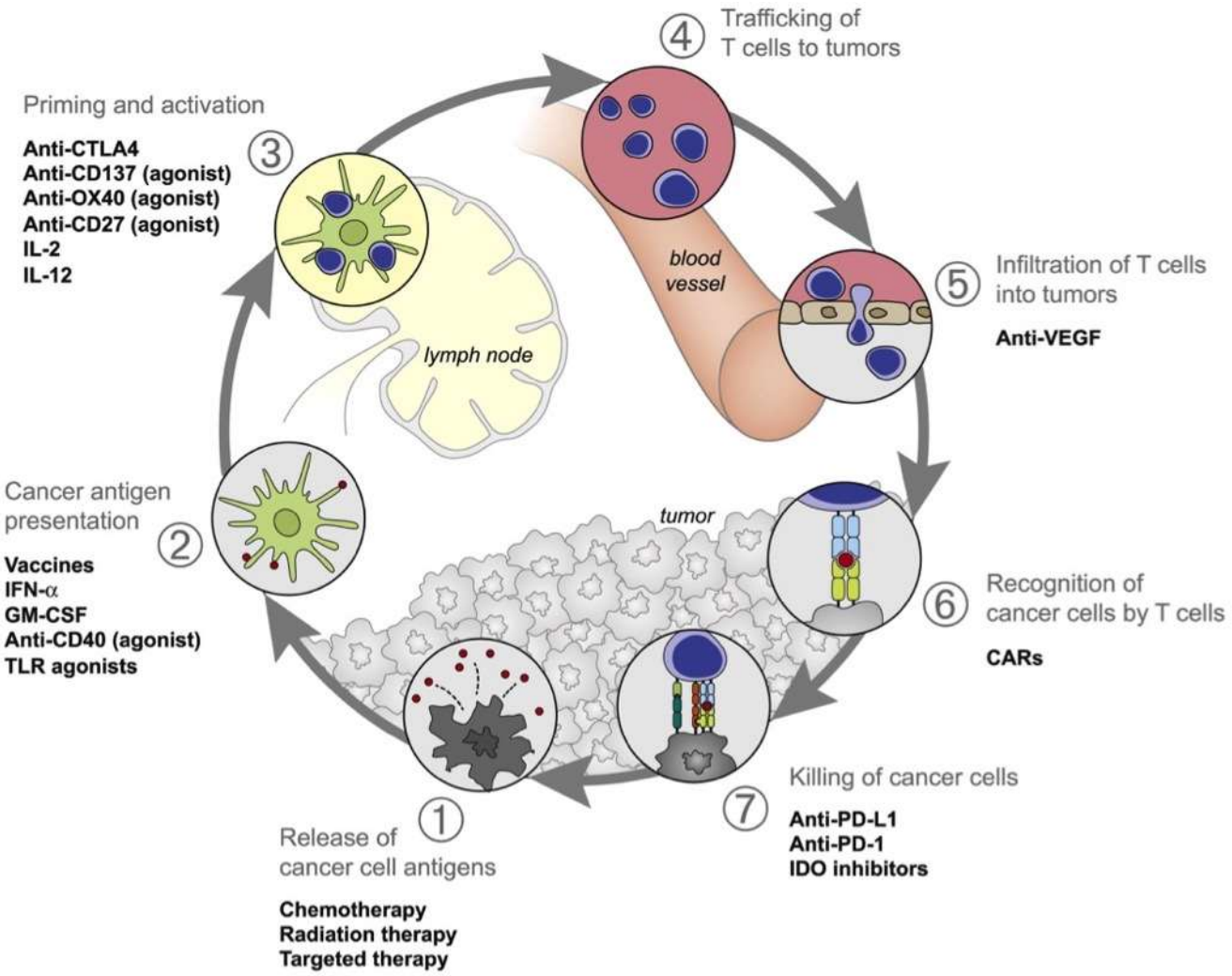
Bispecifics:

- **BiTE**
- **DART**
- **TDB**
- **TCB**

Kills cancer cells in a non-TCR dependent manner

Prell, R et al. FDA-AACR 2016

Enhancement of cancer immunity
(check point inhibitors and costimulatory molecules)



Enhances pre-existing immune processes

- Anti tumor and other

Most experience in the clinic

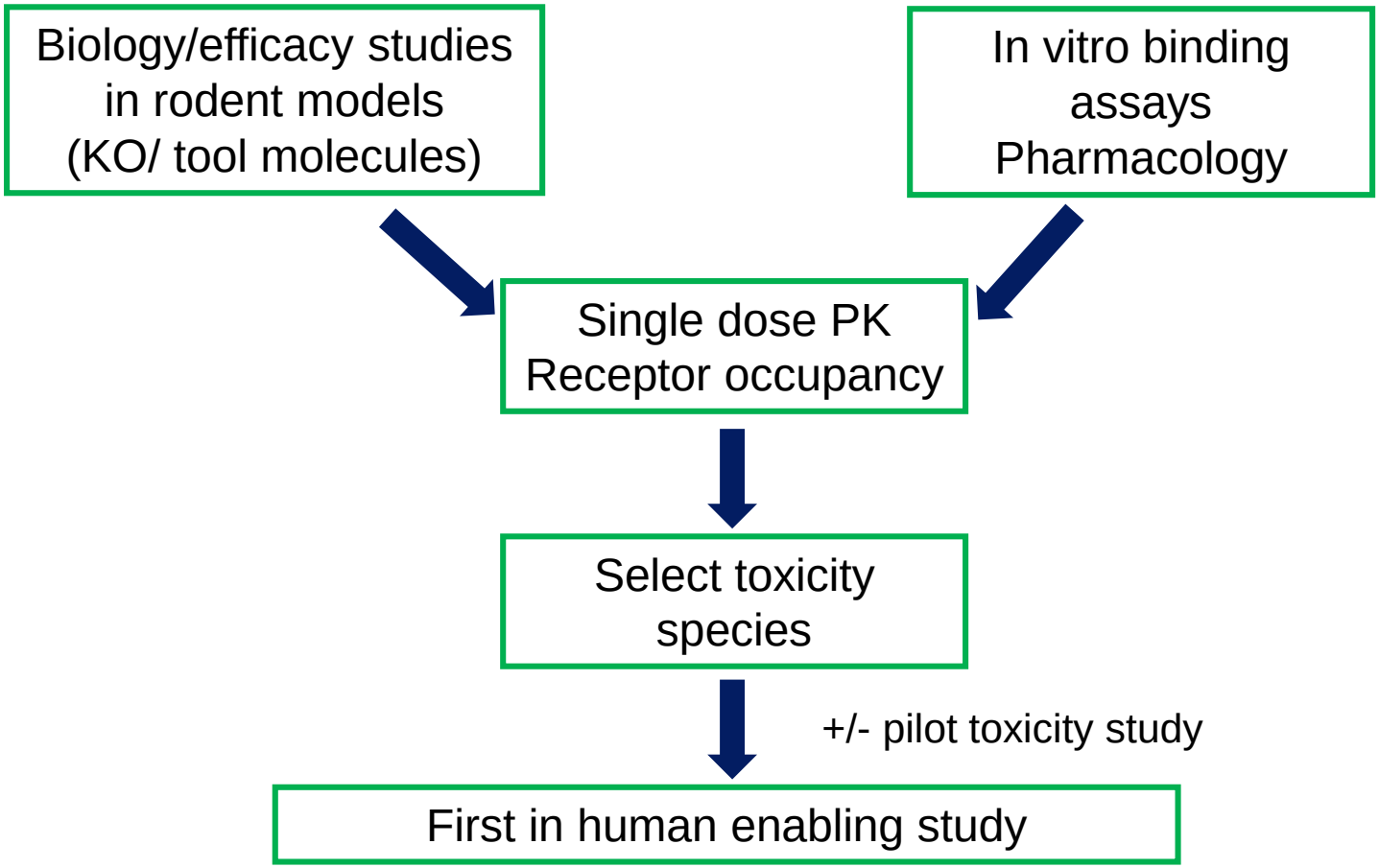
Adverse events tend to be related to PD activity

- Autoimmunity/
- enhanced immunity

Chen and Mellman, 2013

Development path for simple Antibodies

Typically antibodies result in minimal toxicity in healthy animals



Example IND enabling GLP cyno toxicity study

Group No.	No. of Males/Females	Dose Level	Route of Administration	No. Necropsied:	
				Terminal	Recovery
1*	5/5	0 (vehicle)	IV/SC	3/3	2/2
2	3/3	Low	IV	3/3	N/A
3	3/3	Mid	IV	3/3	N/A
4*	5/5	High	IV	3/3	2/2

* Recovery animals in Groups 1 and 4 used for CV safety pharmacology assessments

Weekly to q3w administration for up to 13 weeks (3 months)
Doses selected based on pharmacokinetics and receptor occupancy

- Toxicokinetics, receptor occupancy, anti-drug antibody (ADA) assessment
- Standard safety endpoints
  - body weights, clinical examinations, ophthalmic examinations, clinical pathology, anatomic pathology
- Safety Pharmacology
  - CV-telemetry/external leads, respiratory examination, neurological examination
- Exploratory assessment of pharmacodynamics (PD)
  - Immunophenotyping w/ T-cell activation markers
  - Serum cytokines
  - +/- others dependent on target pathway biology and anticipated pharmacology

Typical in-life toxicity findings are minimal

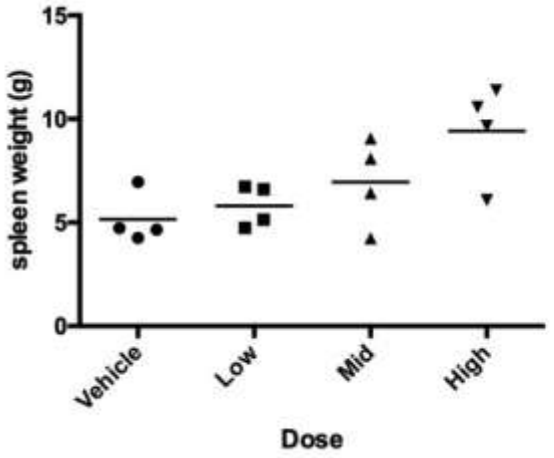
- Pharmacodynamics
  - Typically no change or only minimal elevation in cytokines in individual animals (associated with Anti-Drug Antibodies [ADA])
  - No or only transient effect on blood cell subtypes or T-cell activation
- No effect on safety pharmacology endpoints
- No effect on clinical pathology parameters
  - Evidence of inflammation associated with ADA response in individual animals

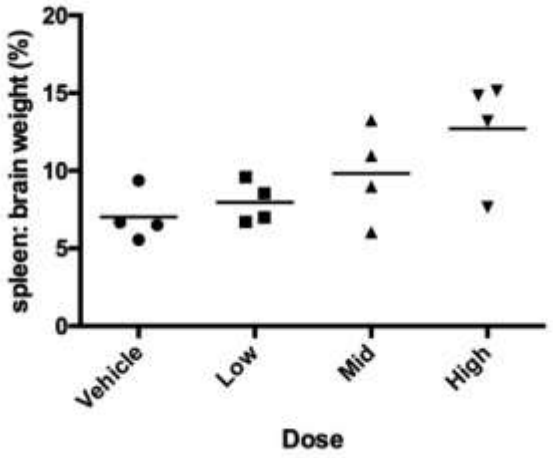
Receptor Occupancy data are integral to provide evidence of target engagement

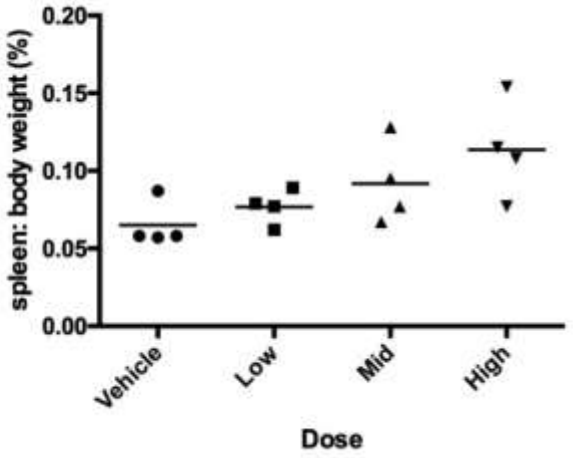
Better models of PD are a major area of interest

Test-article related AP findings with a costimulatory molecule

- Increased spleen weights correlated to increased size/number of germinal centers





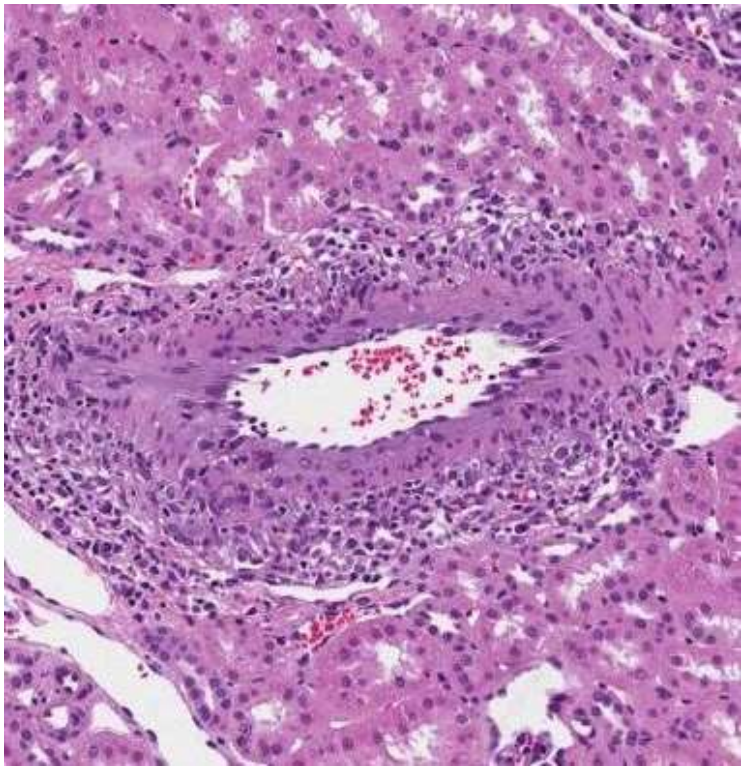


- 4 week study with weekly dosing of anti-OX40 (agonist)
- Clear dose response in spleen weight
- Correlated to increased lymphoblasts within germinal centers +/- increased cellularity of PAL

Arteritis/ periarteritis is a common finding

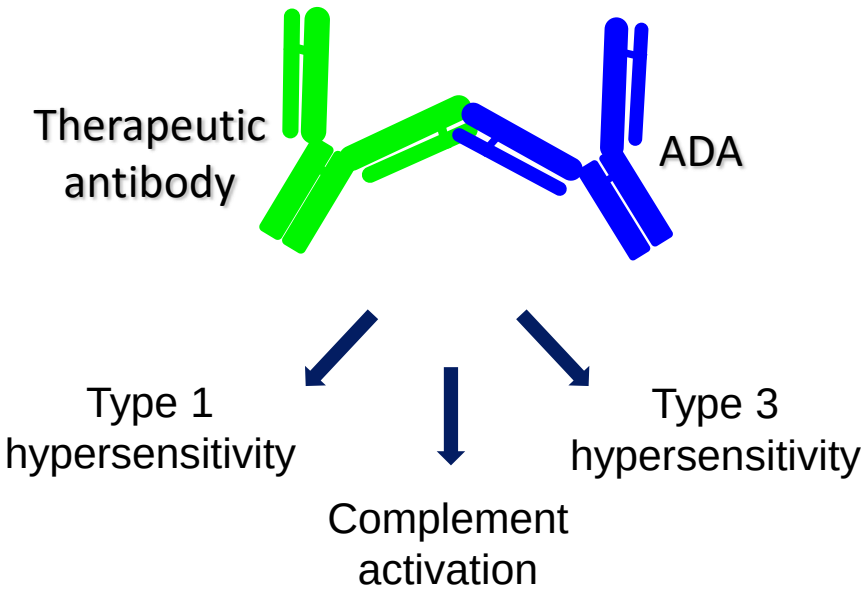
- Noted in multiple studies with both agonistic (e.g. OX40) and anti-inhibitory (e.g. PD-L1) antibodies
- Multiple tissues may be affected
  - Heart, liver, pancreas, GI tract, reproductive tract
- Higher incidence than background finding
- Exacerbation of an underlying immune-mediated arteritis?

Kidney, 8 weekly IV doses aPD-L1 50mg/kg (High dose)

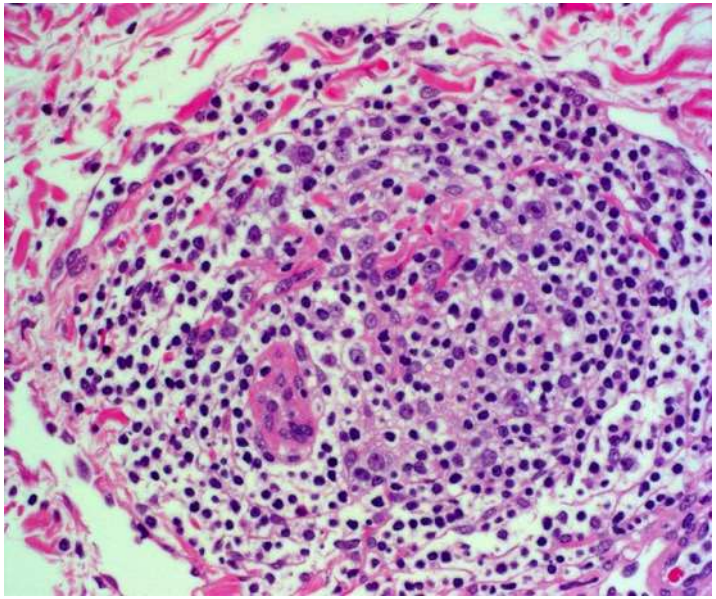


Interference of Anti-Drug Antibodies?

- Common in non clinical species
- May result in acute infusion reactions
  - Typically not until multiple doses
  - IL-6, IL-1ra, MCP-1 elevations with aOX40



- **May result in vasculitis/ glomerulonephritis**
  - Typically random and more frequent at mid dose
- May <u>reduce exposure</u>



Vasculitis in animal w/ high ADA titer following dosing with anti-inflammatory antibody

E.g. anti-PD-L1 (8-week study, weekly dosing)

Dose Level (mg/kg)	Route	Incidence of vasculitis	Tissues affected	ADA rate
0	IV/SC	0/6	N/A	0/6
5	IV	0/6	N/A	6/6
15	IV	0/6	N/A	6/6
50	IV	1/6	Heart, periaortic connective tissue, tongue, Stomach, Pancreas, Cecum, Rectum, Reproductive tract	4/6
15	SC	1/6	Heart, liver	6/6
50	SC	2/6	Kidney, stomach, epididymis	5/6

Vasculitis considered test-article related and adverse
No observed adverse effect level (NOAEL) = 5 mg/kg

Similar findings identified in the chronic (26 week) study

Checkpoint inhibitor combinations

- Published data suggests additive effect similar to that seen in the clinic

Cynomolgus Toxicology Signals with Ipilimumab and Nivolumab Combination.

Group	M/F	Treatment	Dose	Diarrhea[a]	Mean Spleen Weight[b] (g)		Spleen Pathology[c]	Gastrointestinal Pathology[d]
			mg/kg	n/N	Day 30 M/F	Day 59 M/F	n/N	n/N
1	5/5	saline control	—	0/10	3.9/2.8	3.5/3.7	0/6	0/6
2	5/5	nivolumab + ipilimumab	10 3	2/10	4.0/3.6	4.3/2.4	2/6	2/6
3	5/5	nivolumab + ipilimumab	50 10	4/10	6.1/4.47	7.5/3.2	4/5	3/5

[a] Incidence of repeated diarrhea (number of animals with finding/number of animal examined).

[b] Mean spleen weight on days 30 and 59; at day 30, 3 monkeys per sex per group with the exception of 2 males in Group 3; at day 59, 2 monkeys per sex per group.

[c] Incidence of lymphoid follicle hypertrophy or marginal zone expansion: number of animals with finding (n) / number of animals examined (N).

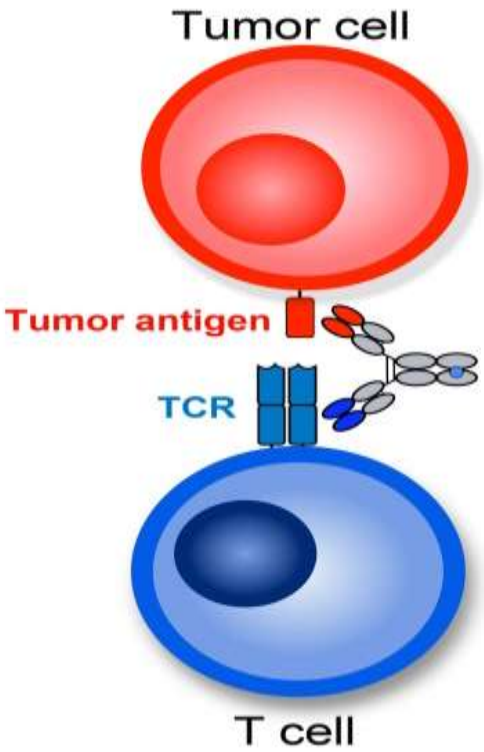
[d] Minimal, diffuse lymphoplasmacytic inflammation in the lamina propria with concurrent enlargement of the colonic or pelvic lymph nodes: number of animals with finding (n) / number of animal examined (N).

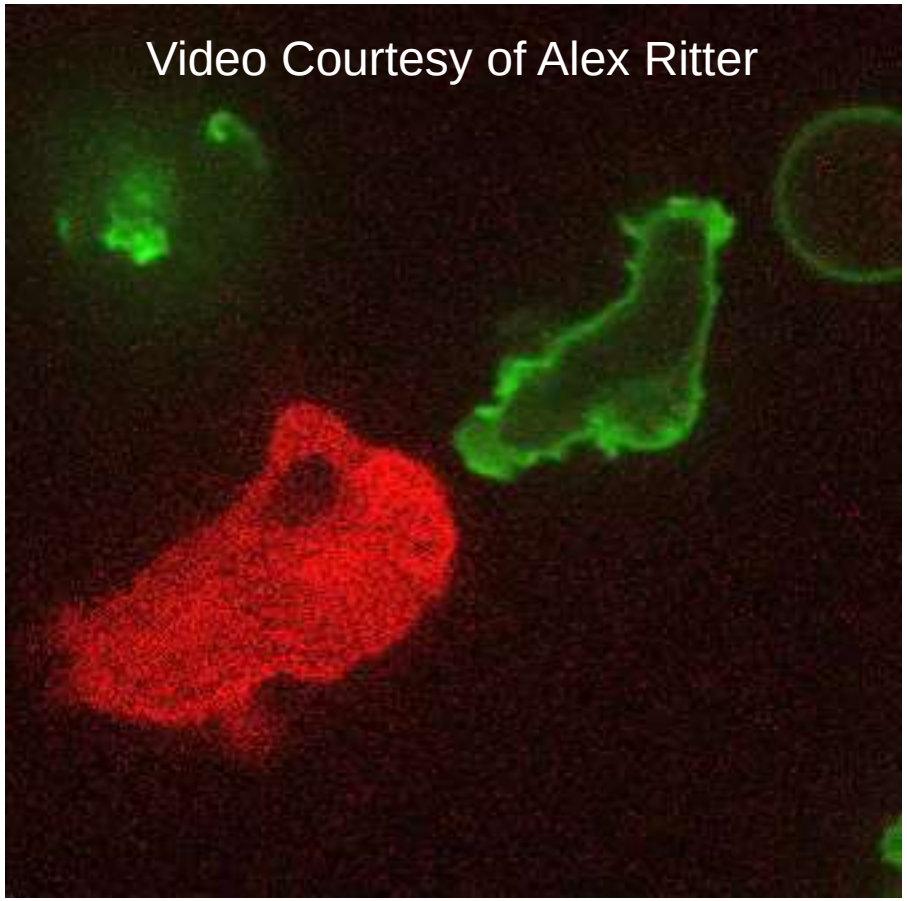
Selby et al. PLoS one. 2016

Summary: Check Point Inhibitors/ Costimulatory Molecules

- Molecules targeting check point inhibitors tend to result in minimal toxicity in macaques
- Demonstrating PD activity is challenging and receptor occupancy data is critical in setting the FIH dose
- Vasculitis/ perivascular infiltrates are common, but ADA related effects must be considered
- Cynomolgus macaques are generally considered to be a good model for type of adverse events in the clinic
  - Do not model tissues affected as well

T-cell recruiting therapies





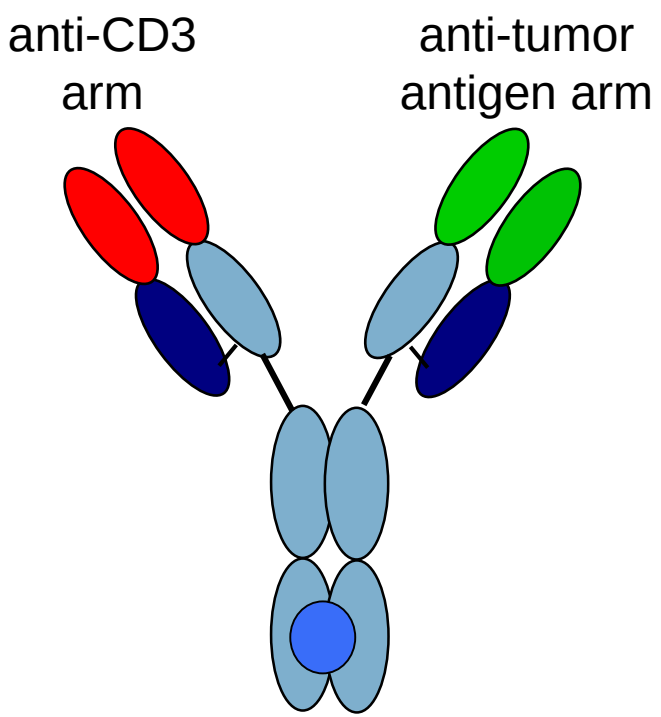
Bypass usual MHC guided activation of T-cells

Rapid effect and increased acute toxicity (preclinically and in clinic)

Acute toxicity guides dose selection for toxicity studies

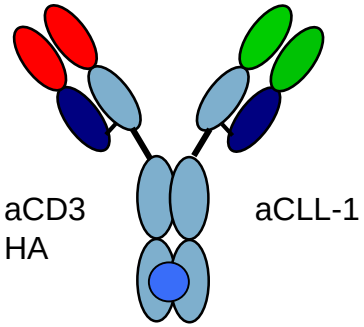
- In vivo cytokine release difficult to predict
- Severity depends on:
  - Target density
  - Affinity of target arm
  - Affinity of CD3 arm

- ***Pilot studies with sentinel animal dosing advised for novel targets/ molecules***



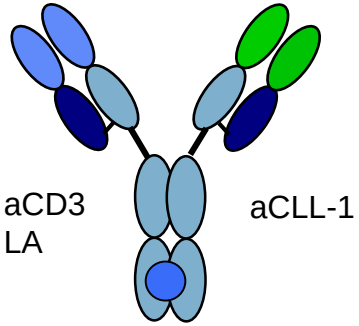
T-cell dependent bispecific antibody (TDB)

e.g. CLL-1 TDBs with varying CD3 affinity



aCLL1-H
High affinity
CD3 arm

Vs.



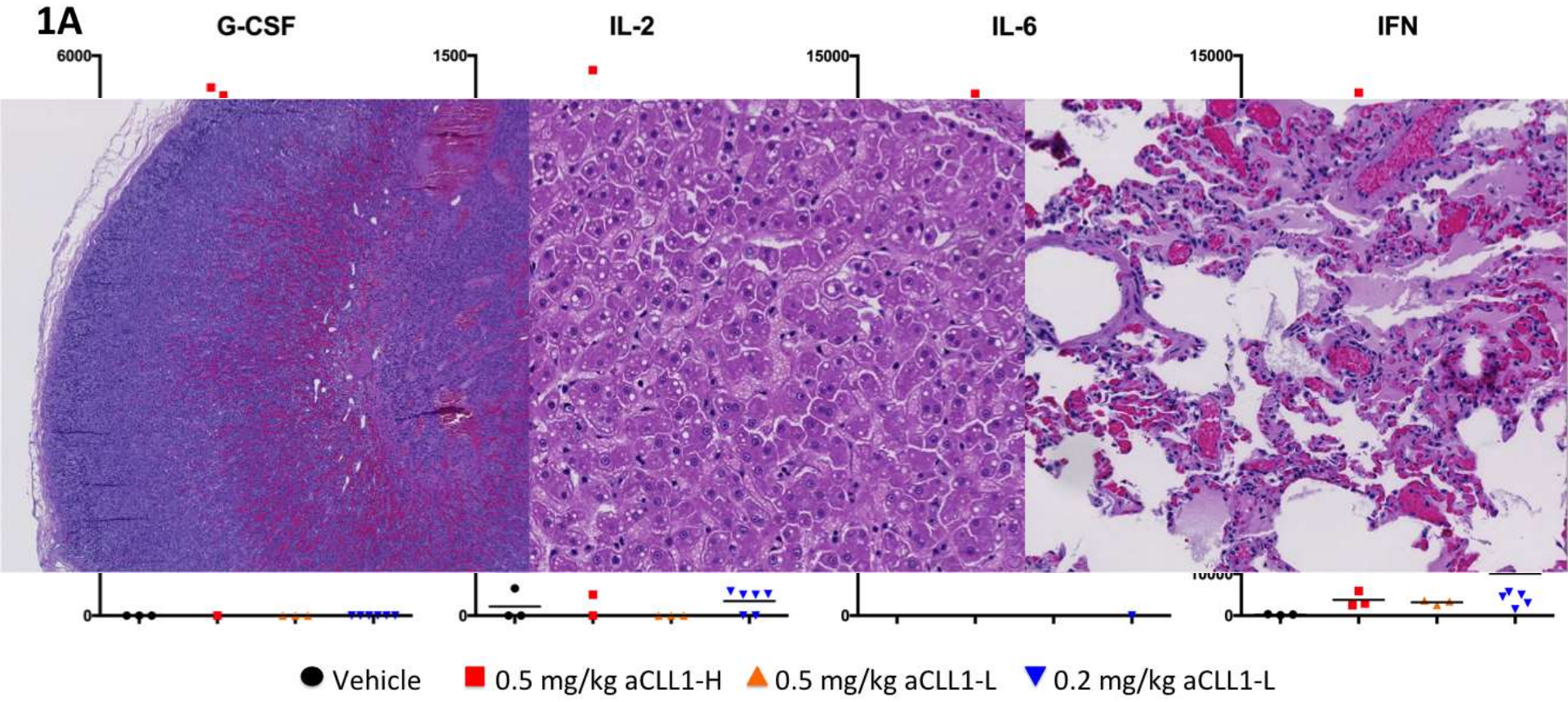
aCLL1-L
Low affinity
CD3 arm

- CLL-1 is expressed on acute myeloid leukemia (AML) cells and normal myeloid cells
- Both TDBs have similar affinity to human and cynomolgus macaque CLL-1 and CD3
- Humanized mouse model revealed no difference in toxicity or PD
- Macaques administered a single, 1 hour IV infusion of 0.2-0.5 mg/kg CLL1-H or CLL1-L
- Sentinel animals dosed 24 hours prior to remainder of cohort (n=3 per group)

CLL-1= C-type lectin-like molecule 1

CLL1-H results in severe acute toxicity

- 0.5 mg/kg CLL1-H resulted in severe clinical signs within 6-36 hours of dosing
  - Fever, reduced activity, shock associated with cytokine release



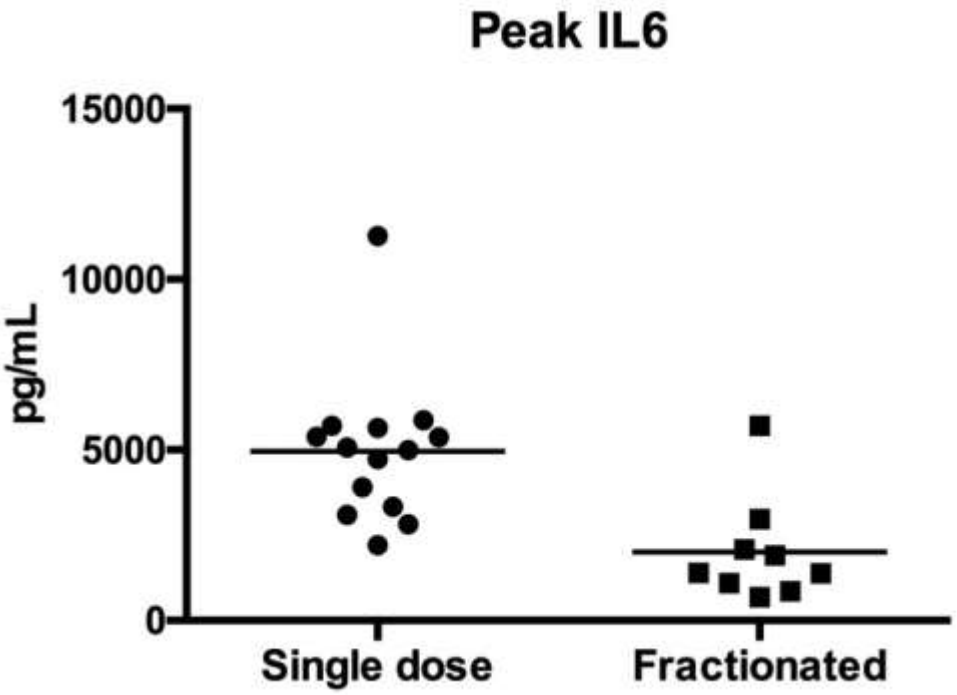
Leong et al. Blood. 2017

Steven Laing/ Julie Schwartz

Getting past cytokine release

Minimizing impact of cytokine release

- Longer infusions
- Dose fractionation
- Anti-inflammatories

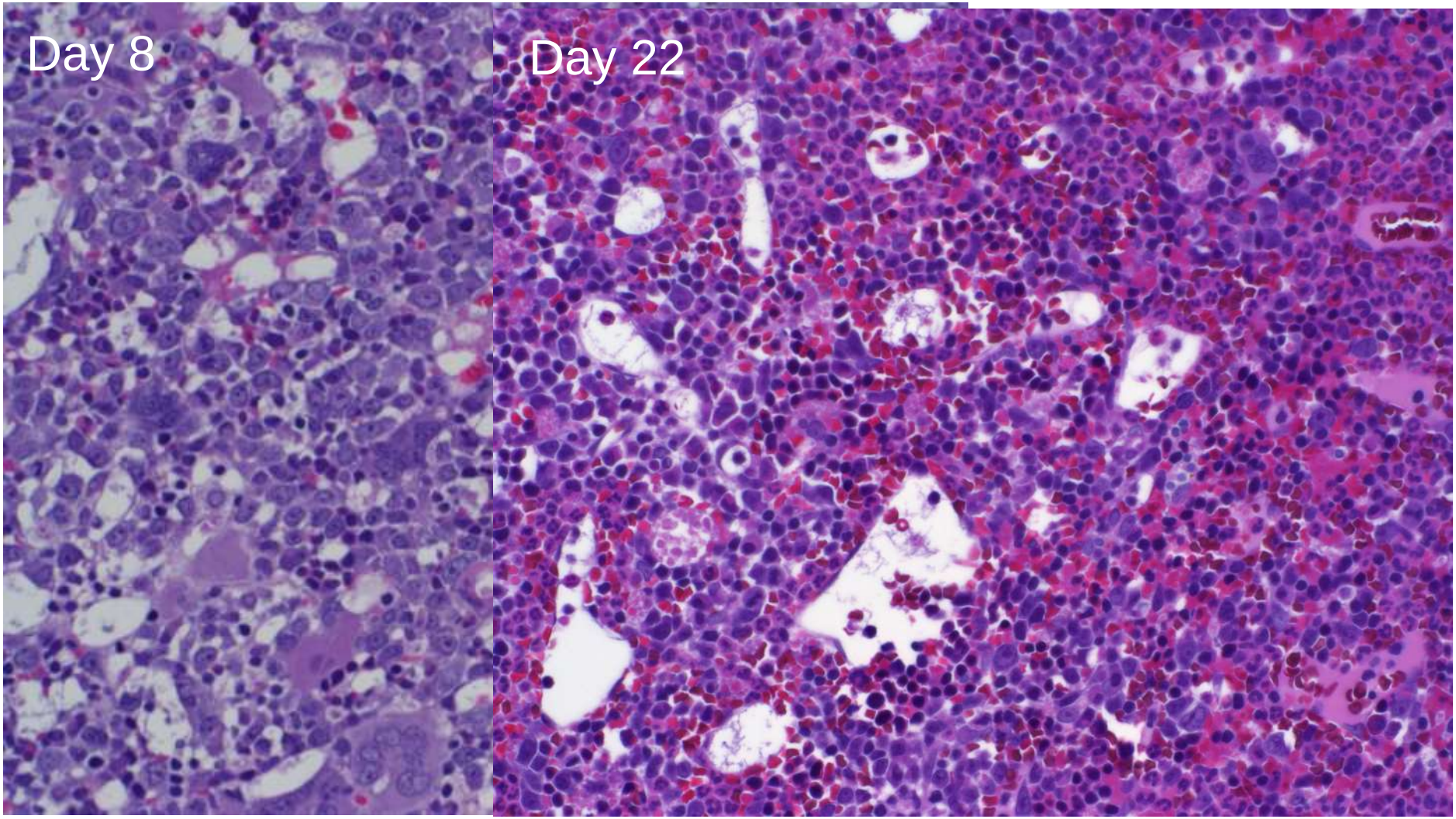


Single dose:
1mg/kg day 1
1 hour infusion

Fractionated:
0.2 mg/kg Day 1
0.8 mg/kg Day 2
1 hour infusion

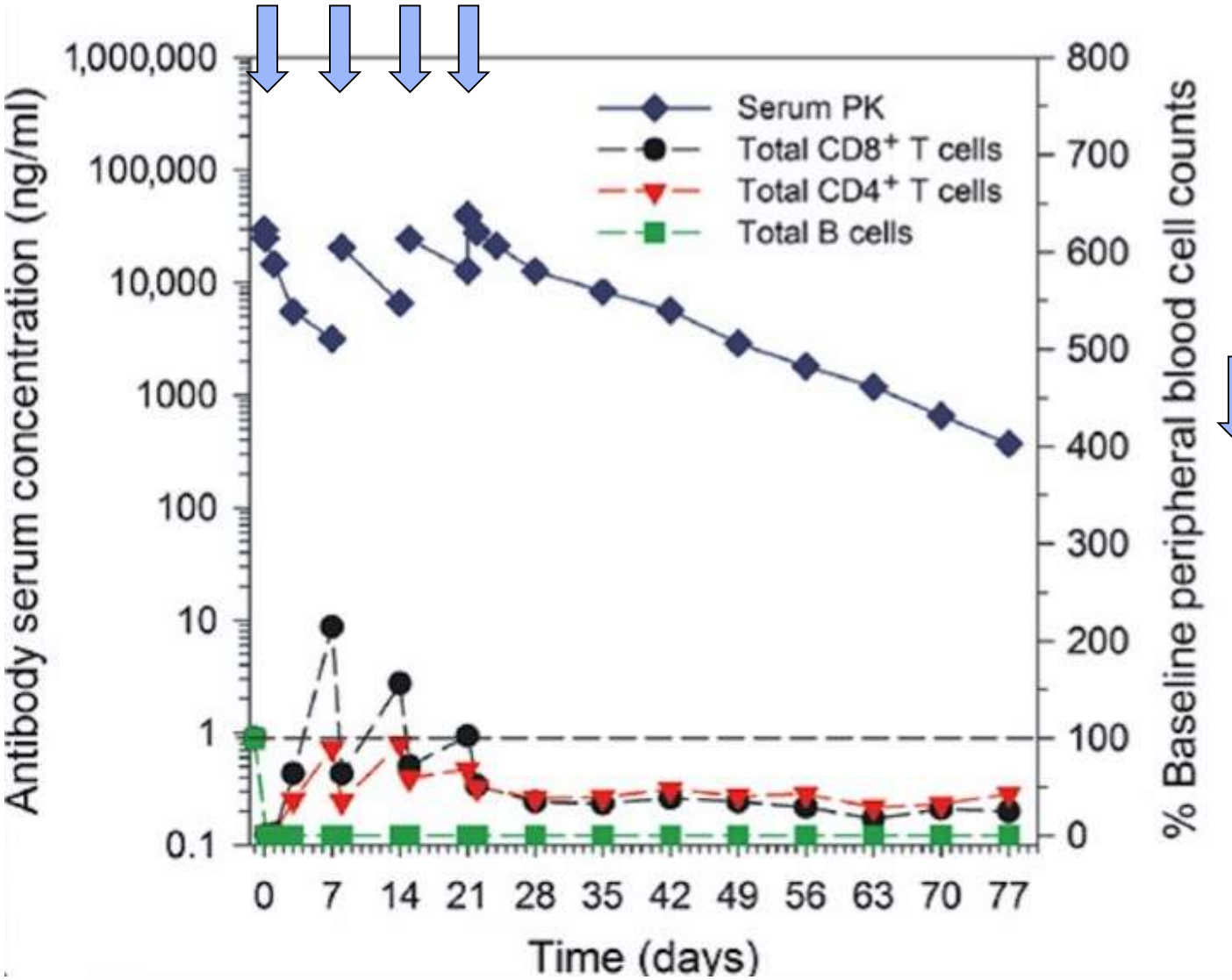
ent.

PD related effects- CLL1 TDB



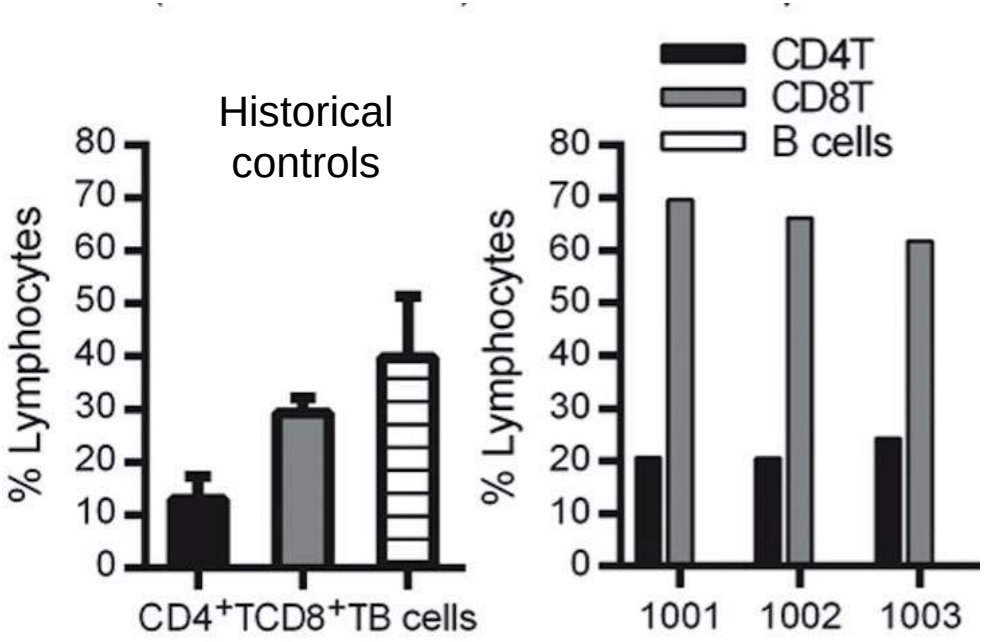
Steven Laing/ Julie Schwartz

PD-related effects- B-cell target TDB

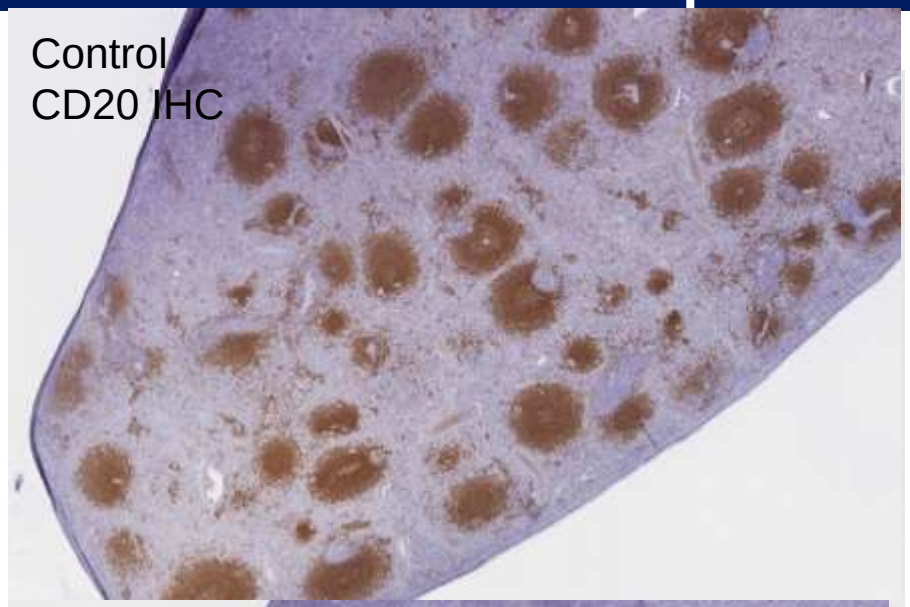


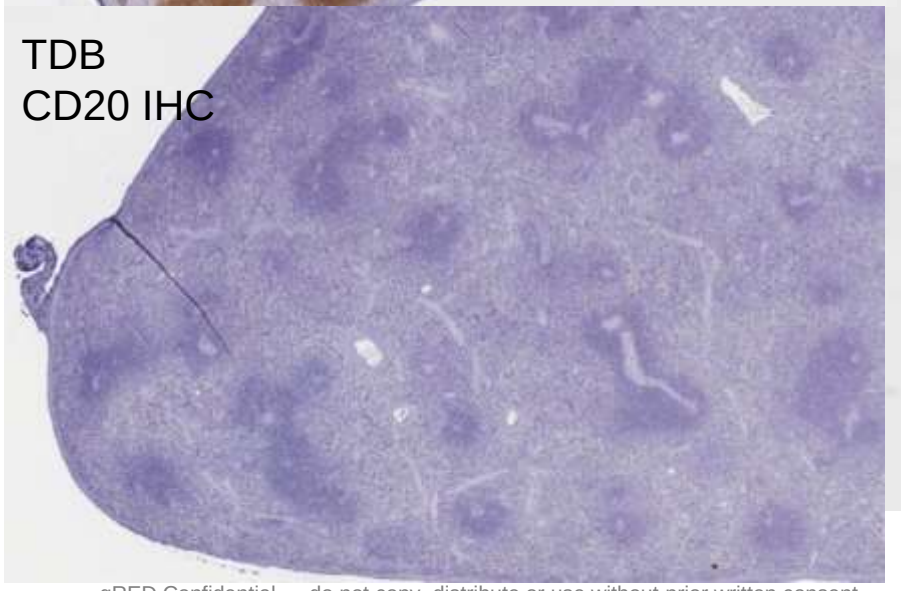
Sun et al. Science translational medicine 2015

PD-related effects- B-cell target TDB



Spleen Immunophenotyping following 4 weekly doses

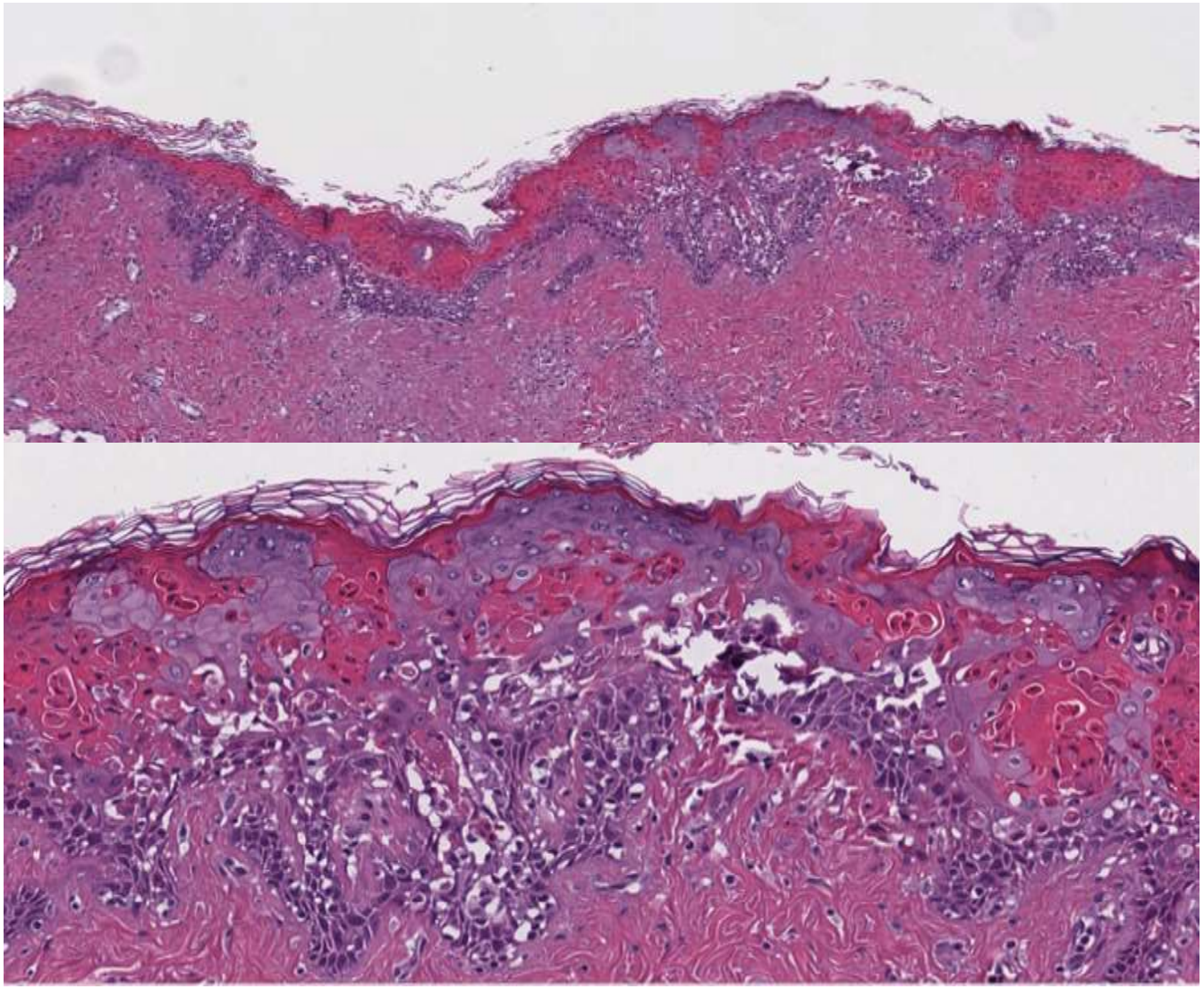




Sun et al. Science translational medicine 2015

Tanja Zabka/ Jennifer Chilton

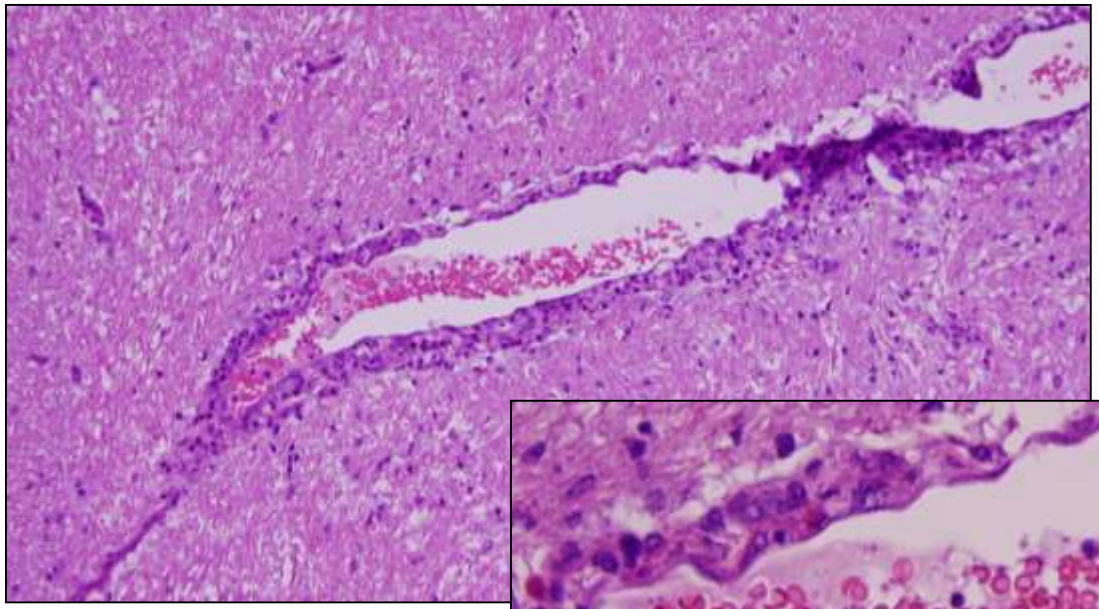
PD-related effects- Epithelial target TDB



Erythema multiforme- like
Single cell and widespread keratinocyte necrosis with epidermal spongiosis

Noel Dybdal/ Matt Smith

Other findings: More inflammatory cell infiltrates



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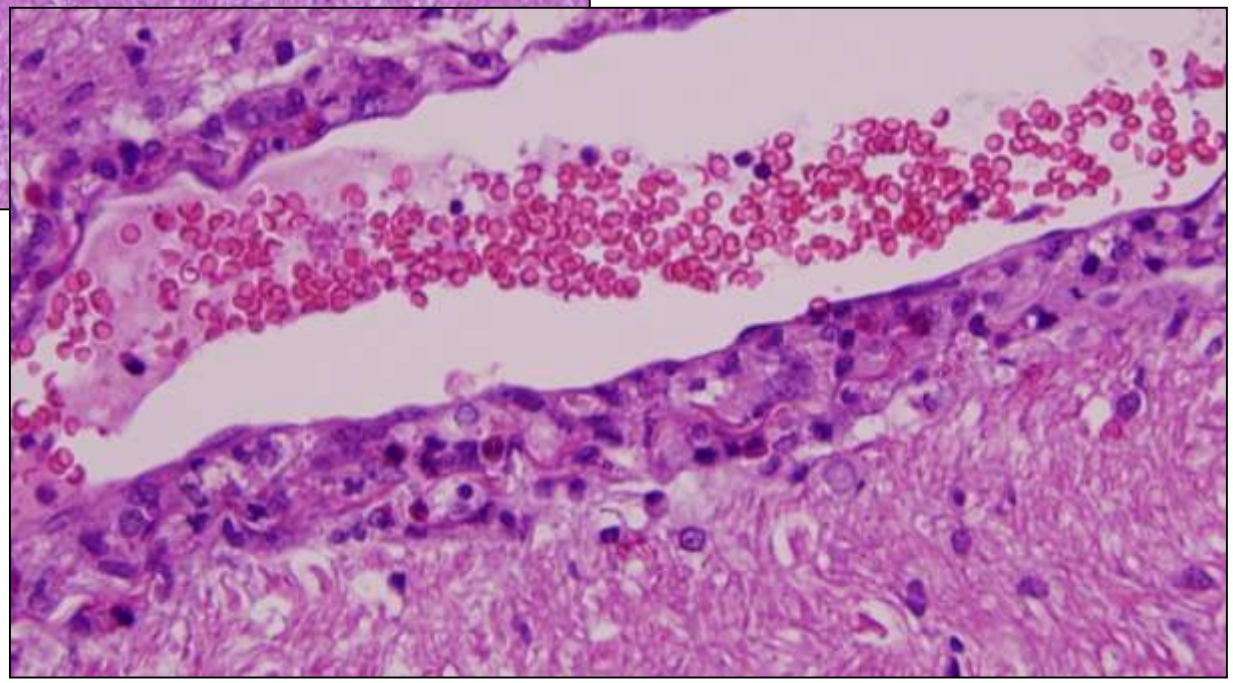

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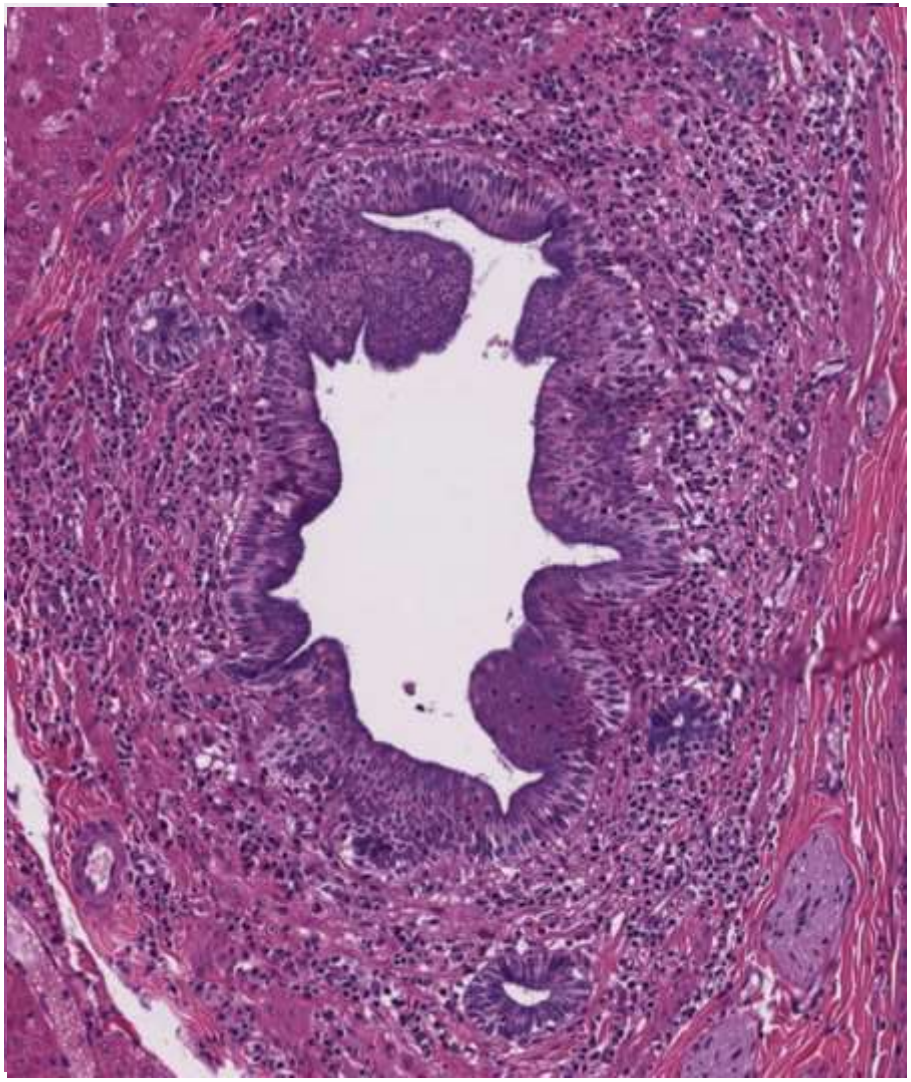
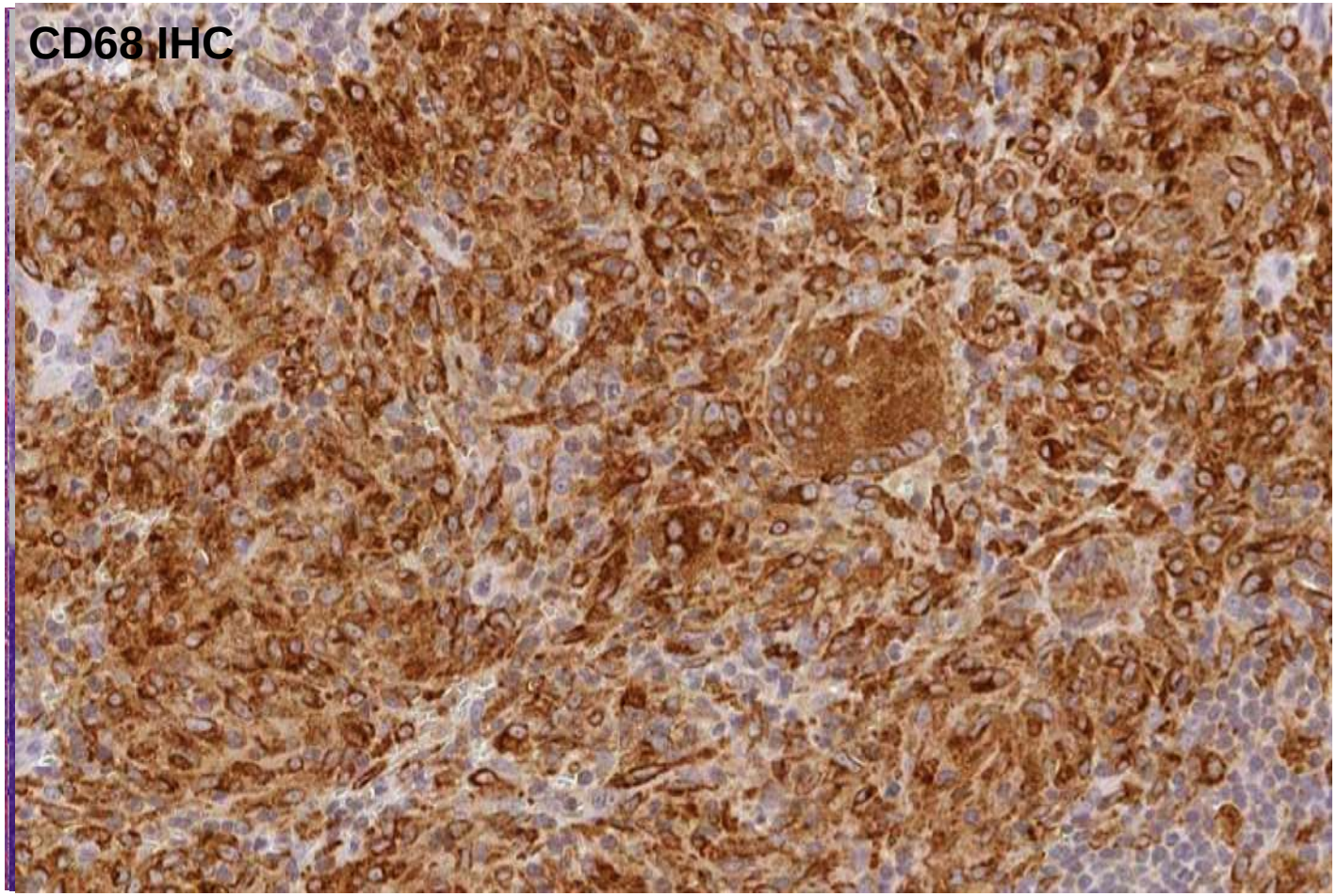
Other findings: More inflammatory cell infiltrates



- Seen with all TDBs tested thus far
- Multiple tissues affected
- Thought to represent increased inflammatory cell trafficking secondary to cytokine release/ activation

Unusual findings: granulomatous inflammation



Single animal

4 weekly doses of B-cell targeting TDB

Infiltrates CD68+

Negative for infectious agent stains
(Giemsa/ PAS/ AF/ Gram)

Tanja Zabka/ Jennifer Chilton

Summary: TDBs

- Acute cytokine release limits first dose
  - Extending infusion time/ dose fractionating can allow higher doses to be explored
- Much of toxicity is consistent with PD- know your target biology
- Perivascular infiltrates may be consistent with increased lymphocyte trafficking
- Cynomolgus macaques are generally considered to be a good model for adverse events in the clinic
- Unusual findings are thus far sporadic and effect single animals
  - Pathogenesis and relevance to test-article still to be elucidated

Thank you! Questions?

nod@gene.com; 1-650-225-1425