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Assays and endpoints for toxicology studies to assess immune-related adverse events

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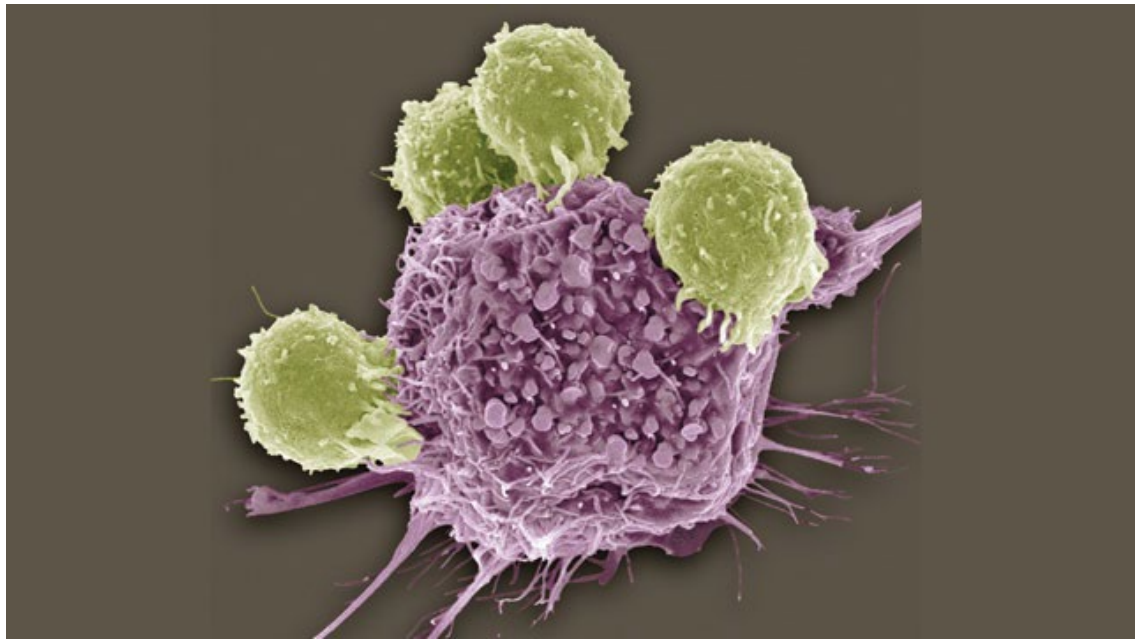

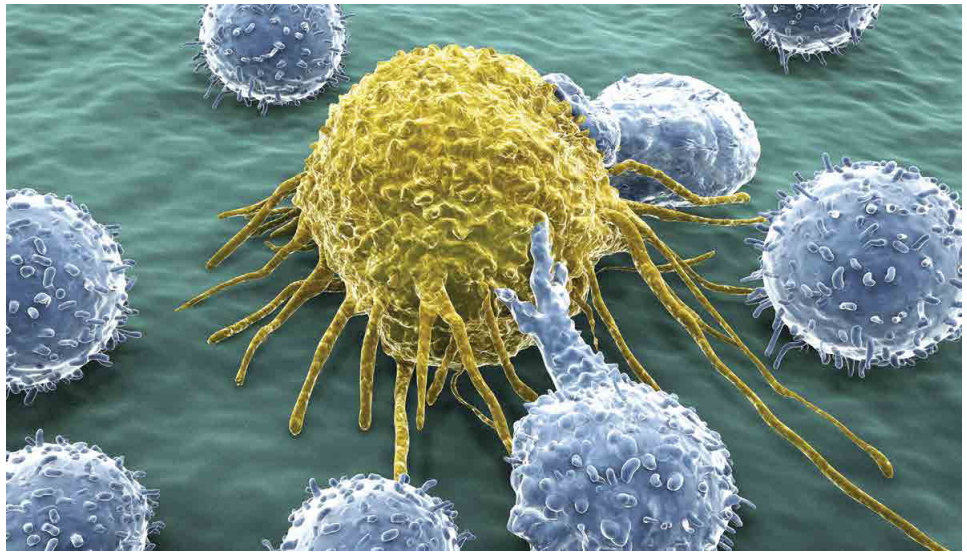

2021 NCI Drug Development Workshop
How to Advance A Therapeutic Candidate from Bench to Bedside

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Preclinical Assessment of IO Agents

- Why
- Challenges
- Learning from Disasters
- Approaches to Consider

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Why:
Pre-clinical Testing for New Agents

- Validation of target in *in vivo* animal model
- Confirmation of mechanism of action
- PK and PD analysis
- Antitumor effects
- Identification of Toxicity profile
- Identification of a starting dose, route, & schedule
- Small animals and non-human primates

Why: Determining unanticpated Toxicity of Novel Combinations

Hepatotoxicity with Combination of Vemurafenib and Ipilimumab

Table 1. Data for Patients with Grade 3 Elevations in Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) Levels While Receiving Combination Therapy with Vemurafenib and Ipilimumab.*

Study Cohort and Patient No.	No. of Doses of Ipilimumab before ALT–AST Elevation	Time to Onset of ALT–AST Elevation after First Dose of Ipilimumab	Treatment	Time to Resolution of ALT–AST Elevation	Toxicity Relapse with Repeated Ipilimumab
First cohort					
4	1	21 days	Glucocorticoids; vemurafenib discontinued for 5 days and then restarted with dose reduction; ipilimumab permanently discontinued	4 days	NA
5	2	36 days	Glucocorticoids; vemurafenib discontinued for 4 days and then restarted with dose reduction; ipilimumab continued (2 doses)	6 days	No
6†	1	21 days	Glucocorticoids; vemurafenib discontinued for 5 days and then restarted with dose reduction; ipilimumab continued (1 dose)	6 days	No
8	1	19 days	Glucocorticoids; vemurafenib discontinued for 4 days and then restarted with dose reduction; ipilimumab continued (1 dose)	12 days	Yes
Second cohort					
10	1	15 days	Glucocorticoids; vemurafenib discontinued for 7 days and then restarted with dose reduction; ipilimumab permanently discontinued	10 days	NA
16‡	1	13 days	Vemurafenib and ipilimumab permanently discontinued	20 days	NA

Ribas et al 2013 NEJM

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The Same Old and Same New Barriers in IO Drug Development

- 85-90% of new Cancer Agents that enter preclinical testing fail to achieve FDA approval
  - Economic cost
  - Social cost

- Models with Low predictive value
  - Cell lines
  - Immune competent Murine models spontaneous and induced
    - Leukemia L1210 P388
    - Solid tumor Col 38, B16 mel, Lewis Lung, M5076 sarcoma
  - Immunodeficient models
    - Xenografts
    - PDX

- **Remember that models are imperfect and can irreversibly alter:**
  - Cancer biological properties such as gain and loss of genetic information
  - Alterations in growth and invasive properties
  - Loss and gain of tumor heterogeneity
  - Interactions between human tumor and human stromal and immune cells

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Challenges for IO agents

- Species specificity of agents (e.g. cytokines, immune targets)
- Species-specific immune systems
- Identification of appropriate models
  - tumor bearing vs non-tumor bearing
  - Syngeneic vs. Xenografts in immune deficient animals
  - Humanized mice
  - Pet dogs

Central Role for the Effector Cell in Immunotherapy: Living Therapeutic

- Inflammatory pathways
- Immune Checkpoints
- Adoptive Cellular therapy

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DISASTER STRIKES!

- Northwick Hospital, London UK
- March 13, 2006
- TGN1412 First in Human Phase I Trial commences and closes. (TeGenero)

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Phase I Trial
Anti-CD28 Monoclonal Antibody TGN1412

- **TGN1412**
  - Super-agonistic anti-CD28 monoclonal antibody
  - IgG4κ
  - Stimulates and expands T cells independent of T cell receptor
  - Murine studies with murine counter part:
    - Preferentially expanded CD4 TH2, in particular, Tregs
    - Lymphocytosis and no detectable toxic or proinflammatory effects noted
  - Additional Preclinical studies in Cynomologus and Rhesus Monkeys revealed no toxicity signal

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TGN1412 Study Design

- Randomized Placebo controlled Phase I in healthy volunteers
  - 6 received TGN1412
  - 2 received placebo
- Within 90 minutes all 6 subjects had multi-organ failure and admitted to ICU

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TGN1412 Study Outcomes

- All Males
- Ages 19-34
- 0.1mg/kg of body weight I.V. over 3-6 minutes (@ 2mg/min rate)
- Headaches followed by lumbar myalgia @~60min
- Restless, Amnestic episode, Nausea, Vomiting, Diarrhea, Pyrexia

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TGN1412 Study Outcomes

- At 1-4 hours
- Vasodilation, Rigors
- Hypotension, Tachycardia, Respiratory Failure with Pulmonary Infiltrates on CXR
- Coagulopathy
- Lymphopenia and Monocytopenia, sparring neutrophils
- DIC, Renal Failure

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TGN1412 Study Outcomes

- Supportive therapy
- 200mg hydrocortisone (divided doses)
- Cholorpheniramine (10mg) & Odansetron
- metaraminol for BP support
- Empirically Rx (3days) with an anti–interleukin-2 receptor antagonist antibody, daclizumab (Roche)
- For possible histaminergic response ranitidine was used

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Lessons Learned from TGN1412

- Manufacturing Process was sound and the toxicity was related to the biological activity of the agent.
- Duff Report 2006:
  - Preclinical testing should be science based
  - Innovative technologies subject to regular review
  - Platform of information sharing of preclinical data & FIH studies relevant to toxicity
  - Early communication between developers and regulators
  - External expert review
  - Flexible time-scale of clinical trial appraisal for unusual toxicity
  - Special consideration to starting dose of agents for which the therapeutic effect cannot be demonstrated in animal models
  - Broader approach to determining "no observable adverse effects" in animal models based on mechanism Minimal Anticipated Biological Effect Level (MABEL)
  - When pre-clinical information is a poor guide, err on the side of caution
  - Rethinking FIH trial design, adequate period of monitoring, and selection of subjects
  - Qualifications of the treating team
  - Available antidotes for predictable risks
  - Specialized Centers

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Lessons Learned from TGN1412

- CD4+ T_{EM} mostly found in tissue
- CD4+ T_{EM} are the source of IFNγ, TNF, & IL-2
- T_{EM} accumulation over life
  - driven by exposure to infection,
  - NOT seen under clean conditions in lab animals
- Cynomologous Macaques and humans
  - have identical CD28 extracellular domains & bind TGN
  - BUT upon differentiation to T_{EM} cyno CD4+ lose CD28
- hPBMC do not proliferate or produce cytokine to soluble TGN unlike OKT3. BUT, this is culture density dependent.

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Lessons Learned from TGN1412

- Particularly for IO agents, understanding the difference between human and animal model immune systems in predicting outcome
  - Minor differences in the organization and regulation of T cell responses account for dramatic species-specific differences
- Validation of species specific pathways
- Need for Interdisciplinary preclinical team
- Recognize that even the most sophisticated and extensive *in vitro, in vivo, and in silico* analysis may fail to predict toxicity
- Defining the Minimal Anticipated Biological Effect Level (MABEL) for IO agents is better than the "no observed adverse effect level" (NOAEL) –based calculation.

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Approaches to Consider Preclinical Testing of IO agents

- Know your Target (s)
- Know your Model
- Central Role of Immune Cells

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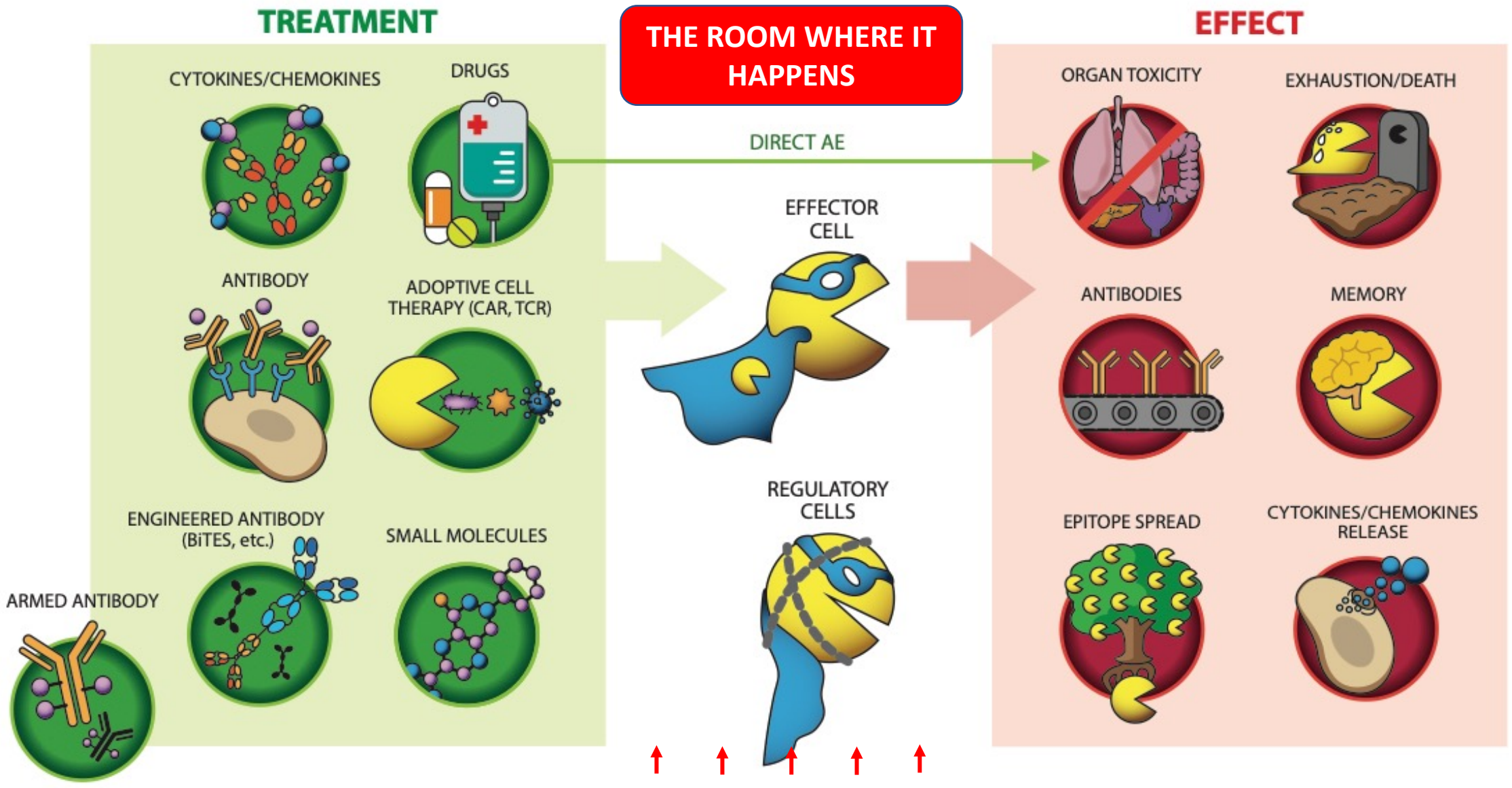
Immune Therapy as a live therapeutic & irAE



Blood Vessel TAF Soluble Factors Exosomes Nerves ECM

Immune Checkpoint Inhibitors on different Mouse Backgrounds

- Strain Specific Toxicities to ICI
- Response of tumors to ICI correlated with increase inflammatory response in organs

A novel mouse model for checkpoint inhibitor-induced adverse events

Inflammatory Infiltrates

Table 1

Characterization of immune related toxicities in various strains of mice treated with anti-PD-1 antibody and CFA boosters.

Mice strain	Immune infiltration			
	Liver	Colon	Lung	Pancreas
C57BL6	+	-	+	-
Balb/c	-	-	-	-
SWR	-	+	-	-
MRL/mpj	+	-	+	+

Table 2. Characterization of immune related toxicities in various strains of mice treated with anti-PD-1 and anti-CTLA-4 antibodies.

Mice strain	MC38	Immune infiltration			
	tumor	Liver	Colon	Lung	Pancreas
C57BL6	+	+	-	+	-
MRL/mpj	-	+	-	+	+
MRL/lpr	-	+	+	+	+
B6/lpr	+	+	+	+	+

Adam et al 2020

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Need for Species-Specific Reagents

ICI antibody targets are have similar between Human & Cynomolgus monkeys:

- Human anti PDL1 Atezolizumab and Durvalumab binds to PDL1 in both species
- Human anti PD1 Pembrolizumab and Nivolumab binds to PD1 in both species
- Human anti-CTLA4 to CTLA4 in both species

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IO Pathways

- Use of Knockout Models to determine type of toxicities

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CTLA-4 Knockout Mice

- Massive Polyclonal T cell proliferation
  - Multiorgan Tissue destruction and death in 2-3 weeks
  - Expansion & Activation of peripheral T cells (not thymic selection)
  - Activation of CD28-B7 pathway

Khattri, Auger, Griffin, Sharpe, Bluestone 1999

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PD1 Knockout Mice

- In normal mice
  - PD-1 expressed on small fraction of thymocytes
    - Transition from CD4-CD8- to CD4+CD8+ populations
  - In the periphery
    - Hardly on resting splenocytes
    - Strongly induced on activated T and B cells, and myeloid cells
- In knock out PD-1 -/-
  - Moderate splenomegaly
  - Augmented B cell proliferative response to anti-IgM and IgG3 ab response to T-independent antigen
  - PD-1 involved in the negative control of proliferation and class switching of B cells

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PD1 Knockout Mice

- Spontaneous autoimmune disease
- Different strains, different syndromes
  - BALB/c-*Pdcd1*$^{-/-}$
    - Cardiomyopathy anti troponin I abs
  - *C57BL/6 -Pdcd1*$^{-/-}$
    - Lupus like Glomerulonephritis and arthritis with IgG3 and C3 deposits
- Normal central tolerance in thymus in PD-1 deficient mice, but die @ 10 weeks from GVH-like disease
- PDL-1 expression β cell in NOD and in NOD-*Pdcd*$^{-/-}$ develop type 1 DM.
  - PDL-1 is expressed on insulin producing human β cell from T1DM but not in non-dabetics

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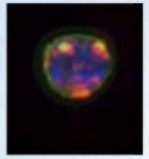
VISTA

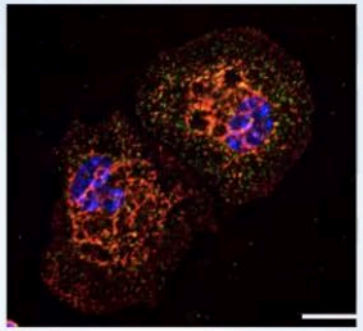
VISTA negatively regulates immunity

T cells



Red VISTA
Green CD4
Blue DAPI

Macrophages



Red VISTA
Green Clathrin
Blue DAPI

VISTA A New Immune Checkpoint in Cancer, Autoimmunity and Beyond

Phenotype of the VISTA KO

- Benign inflammatory phenotype/No overt autoimmunity on WT background
- Activated T and myeloid cells with age
- VISTA$^{-/-}$ exacerbates disease in autoimmune prone strains (EAE, lupus, GVHD, ConA hepatitis, IBD)
- Enhances anti-tumor responses and survival

T cells

- Enhanced T cell responses to antigen
- Enhanced cytokine responses
- Reduced tolerance

Myeloid

- Enhanced chemokine/cytokine production
- Enhanced activation phenotype
- Selective defects in chemotaxis
- Reduced uptake of apoptotic cells

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Target Function/Molecule characteristics: PD1 - Pembrolizumab

- **Pembrolizumab Does Not Spontaneously Activate T Lymphocytes**
- **Recall response assays, as well as in polyclonal human and primate SEB stimulation assays pembrolizumab does not stimulate detectable cellular proliferation or cytokine responses without specific concurrent stimulation of the T-cell receptor**

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Human T-cell Response to Staphylococcus Enterotoxin B as an Assay

- Enhances T-cell activity *in vitro* using healthy volunteer or cancer patient blood samples. Donor whole blood was stimulated

- Pembrolizumab enhanced IL-2 production over control human IgG4 on average 2-fold to 4-fold at the highest antibody concentration tested (25 µg/mL).

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Human Recall T-cell Response to Tetanus Toxoid Challenge

- Enhanced by Pembrolizumab
- Pembrolizumab potentiates an antigen-specific recall response to the TT antigen.
- Tetanus toxoid-induced IFNγ production was significantly enhanced by pembrolizumab

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Target Validation: Antigen

- For TCR, CAR, Vaccine, BiTEs, etc
- Evaluation of existing data bases
  - Human Protein Atlas
  - TCGA
- Confirm expression
  - Human Tissue based
  - Animal Model

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CAR-T Models

- Low protein homology between murine and tuman TAA
- Different impact in mice vs human
  - E.g. murine anti-CD-19 CAR-T lack persistence
- Other Models
  - Humanized mice
  - Pet Dogs
  - NHP

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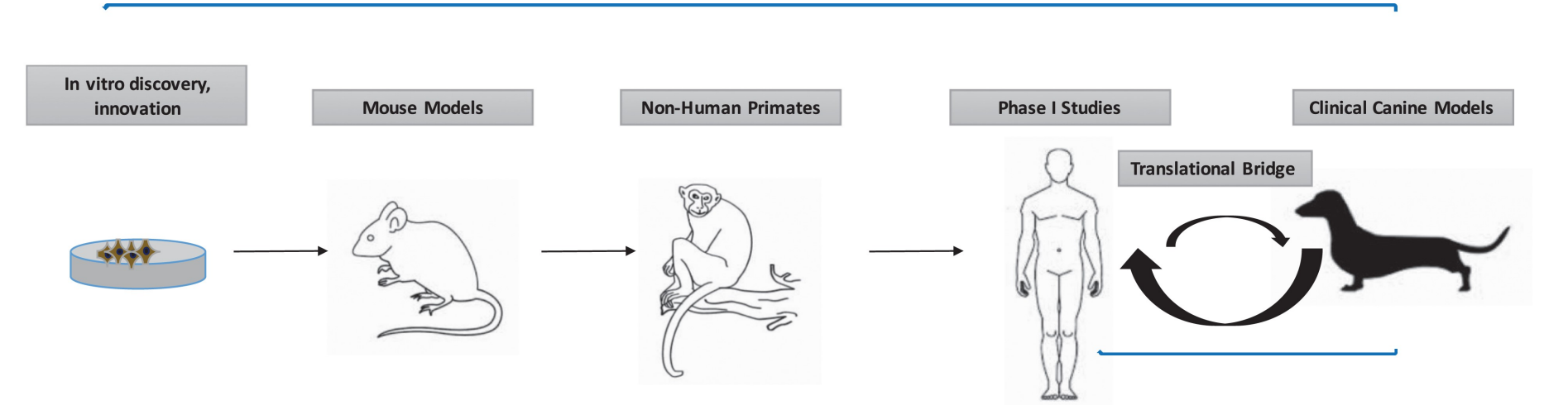
Figure 1. Representative steps of preclinical therapeutic validation and translation into human studies, including the advantages of each model system, as well as potential feedback through clinical canine models.



ILAR J, Volume 59, Issue 3, 2018, Pages 276–285, https://doi.org/10.1093/ilar/ilz009

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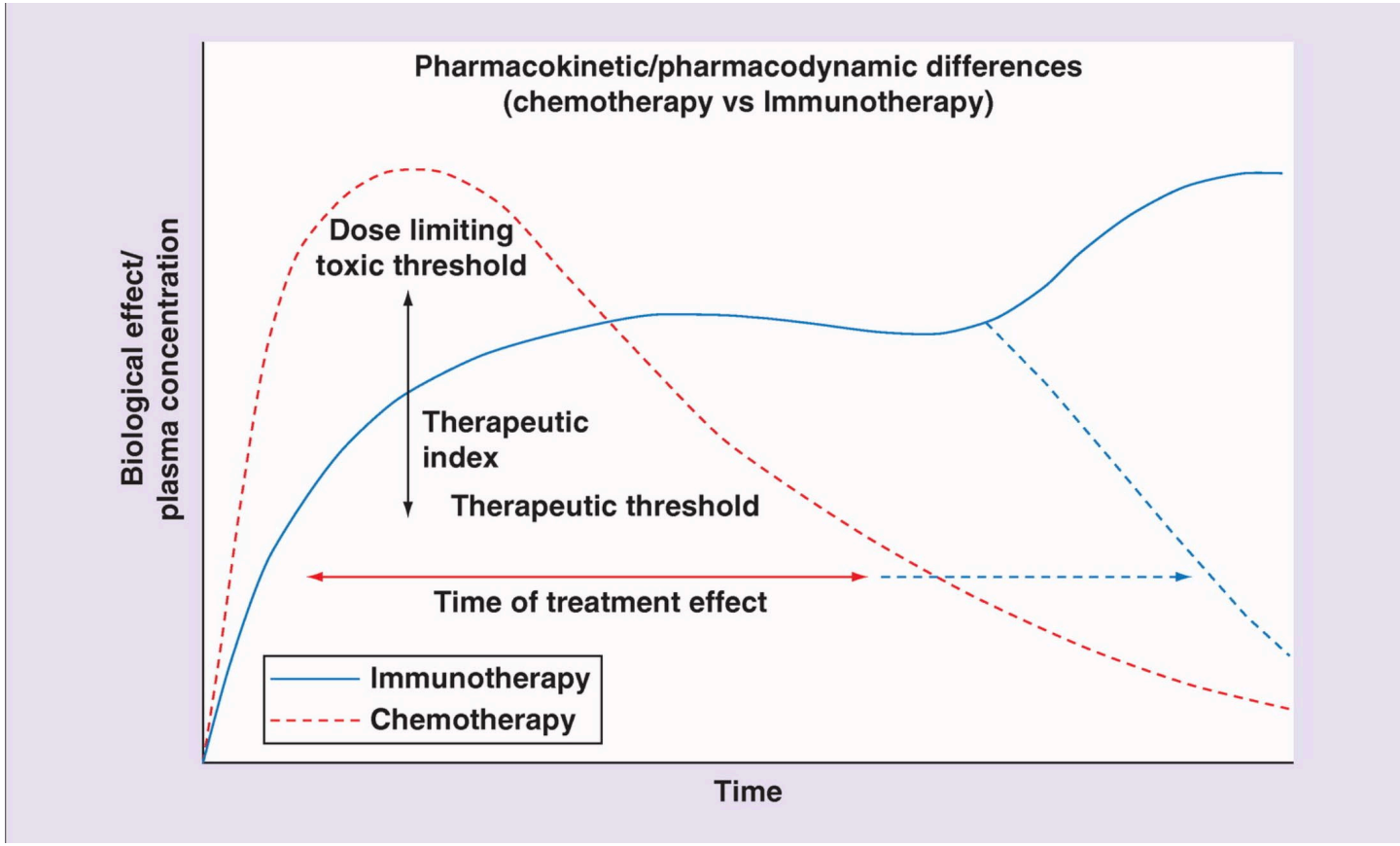
Monotonic vs non-monotonic PD
Dose quasi dependent



Ernstoff et al 2017

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IO Summary Comments

- A wide and increasing range of patients with cancers benefit from IO therapy
- Nevertheless, only ~20% of patients with advanced cancer have clinical benefit
- ~ 70% of patients receiving ICI Rx experience an irAE
- All organ systems have been associated with irAEs: Musculoskeletal, GI, C & PNS, Hepatic, Pancreatic, endocrine, Cardiovascular, ocular, skin

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irAE from ICI

- Pathways: on-target and onco-destructive, on-target and not onco-destructive, off-target
- Germline factors

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Conclusions

- Understand mechanism of action
- Consider on-target off tumor activity
- irAEs are species and environment specific
- Define MABEL vs NOAEL
- Use of histology assesments
- Cytokine production assays
- All models are imperfect