Immunopathology: Evaluation from Discovery to Safety Assessment

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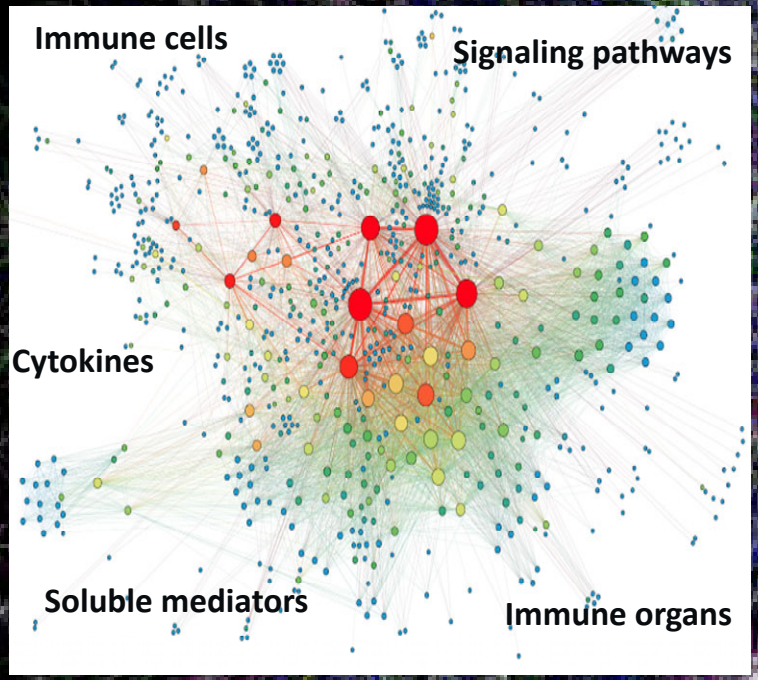
StageBio

Topics Presented

- Immune System: Complex and Powerful
- Immune-modulating Therapies and Drug Development
- Brief Overview of Immune System Basics
- Approaching Immune System Evaluation in Discovery and Safety
- Pathology Approaches and Considerations for Immune System Evaluation
- Integration and Integration of Pathology and Other Findings
- Adversity, Reporting and Regulatory Considerations for the Immune System
- Summary

The Immune System is Complex



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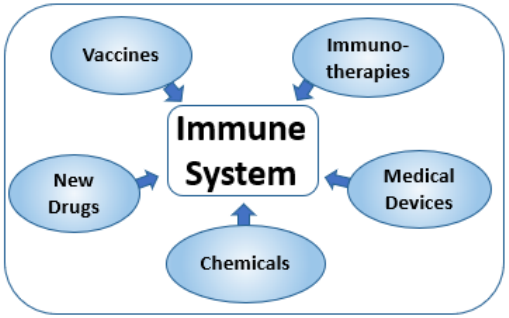
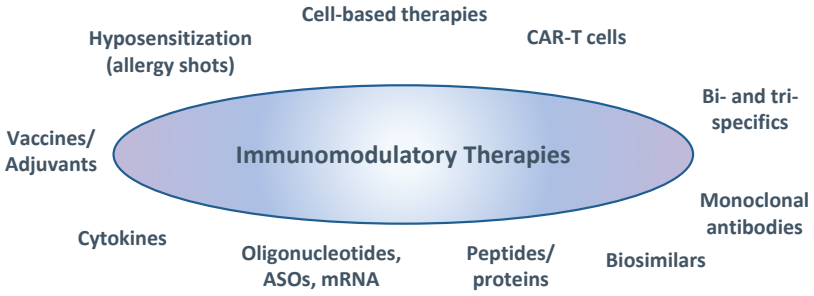
The Immune System is Powerful

- The immune system plays a role in many diseases
  - Even those not considered immune-mediated
- There are many immune pathways that can be targets for therapeutic intervention
- Therapies that affect the immune system have been used to treat diseases and medical conditions
  - Vaccines- against infectious agents, cancer
  - Immunosuppressants- to treat autoimmune and inflammatory diseases
  - Next generation- Immunomodulatory therapies
- Immunotherapies are being designed to specifically target immune function
  - Cancer immunotherapy (checkpoint inhibitors, cell therapies, etc.)
  - Immunomodulators (e.g. cytokine therapies, targeting pathways)
- Immunotherapies have changed the landscape of immune system evaluation

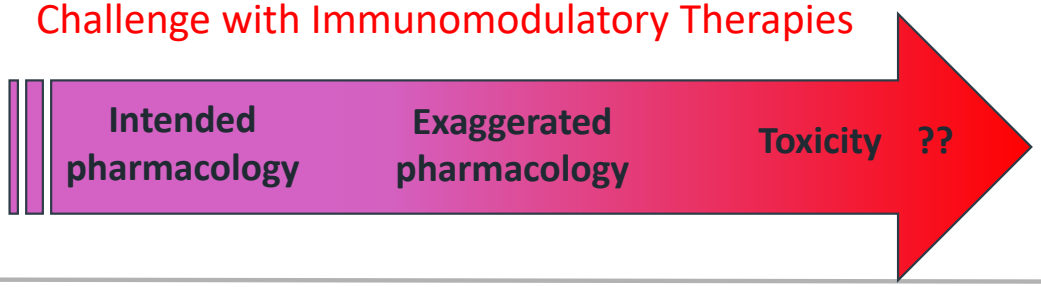
Range of therapies that affect the immune system

- Therapies, medical devices, chemicals can affect the immune system
  - From 2018-2022, 42-63% of all FDA approved therapies were immune-related
- Therapies can affect or be designed to modulate the immune system
  - Affect: Expected (due to MOA) or Unexpected
  - Designed: Biologics, small molecules, vaccines, cell therapies, etc.
- Immunomodulatory therapies have dramatically increased the last decade
  - Bring their unique challenges for immune system evaluation





Challenge with Immunomodulatory Therapies



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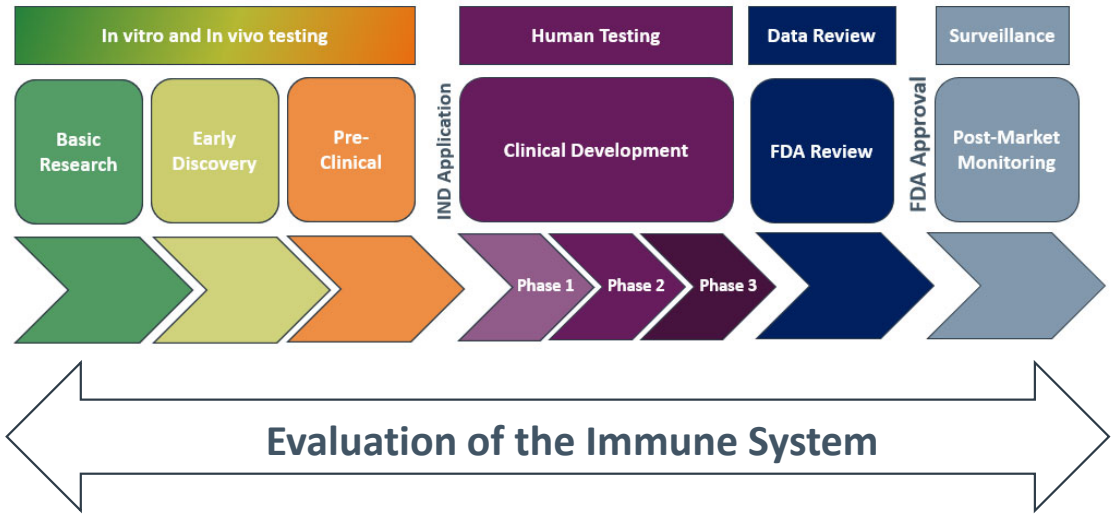
Immune modulation: Evaluating the Immune System

- Requires an understanding of:
  - Immunology
  - Pathology
  - Physiology
  - Pharmacology/Toxicology
  - Immune effects/changes due to disease, co-morbidities, other therapies, etc.

- Requires an integration across disciplines and the drug development pipeline
  - Immunology, toxicology, pathology, others
  - Immunotoxicology (*In vitro and in vivo),* immunophenotyping, immunopathology, etc.
  - Basic Research → Discovery → Preclinical Studies → Clinical Trials → Patients

Immune system evaluation occurs throughout drug development

- Drug Development
  - Occurs over years
  - Go/No-Go decisions made early
  - Data is integrated across development
  - Basic Research
  - Discovery
    - Model development
    - Efficacy
  - Pre-clinical Safety Assessment
    - Non-GLP
    - GLP
  - Clinical Development
  - FDA Approval
  - Patient Monitoring



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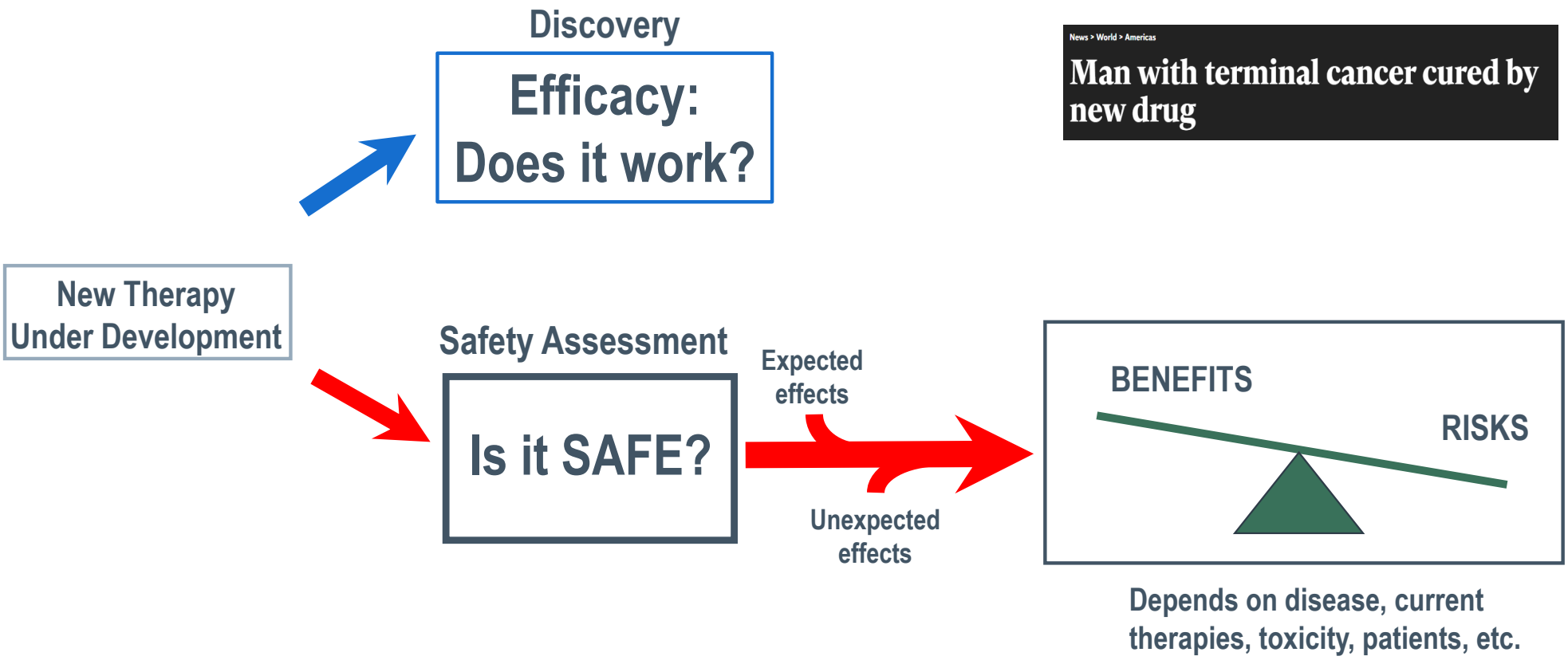
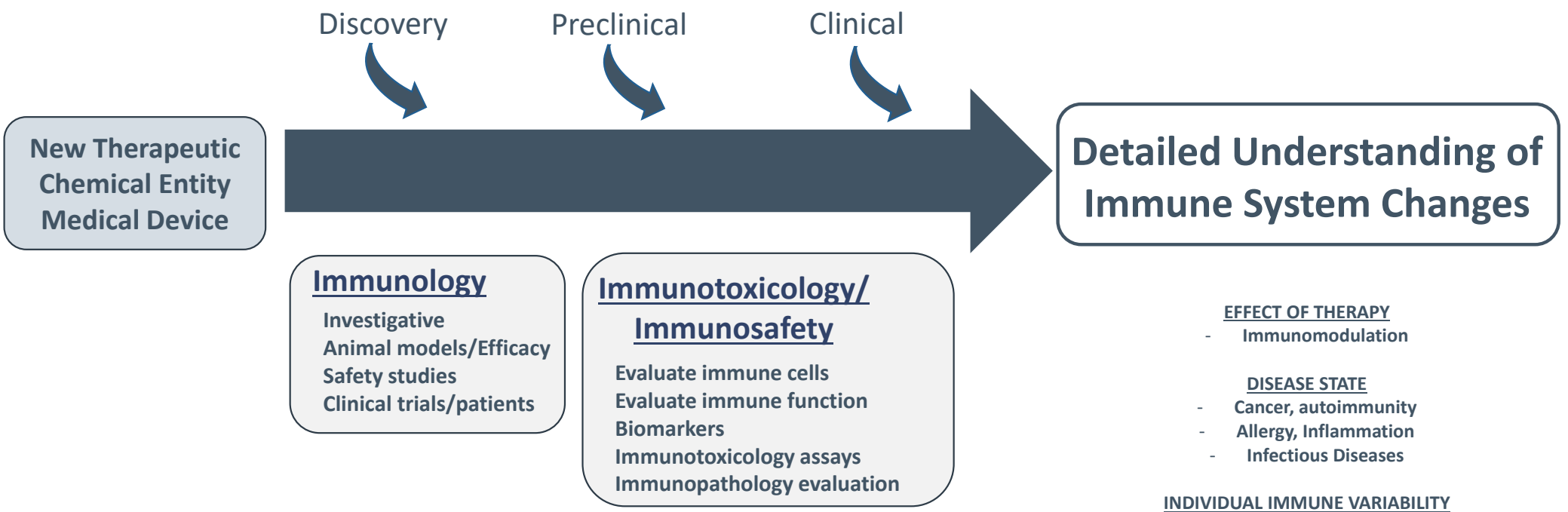
Drug Development Questions



Evaluating Immune Effects in Drug Development



Immune System Basics

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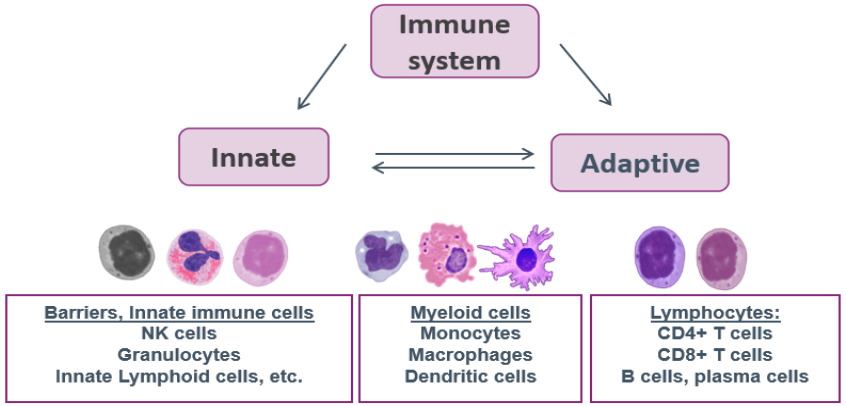
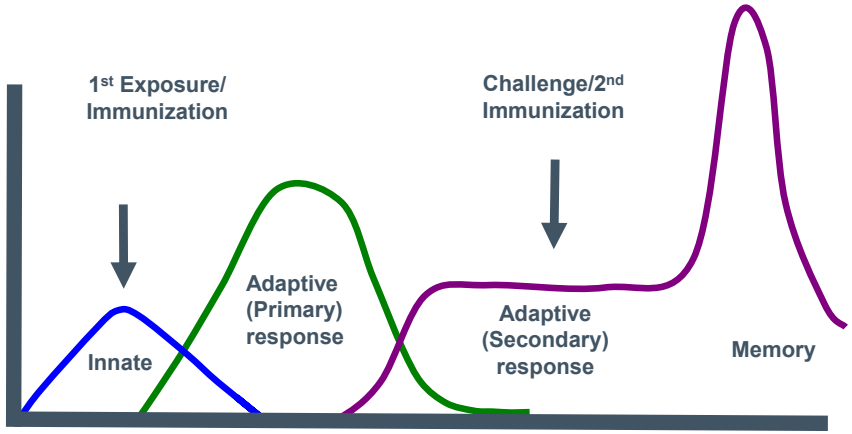
The Immune System

- A complex network of cells, tissues, organs, and the substances that they make to help the body fight infections, remove abnormal cells, and maintain overall homeostasis
- Present throughout the body
  - Immune organs
  - Non-immune organs
- Functionally dynamic and highly responsive
  - Protects against pathogens
  - Maintains homeostasis (prevents tumors, autoimmunity, etc.)
- Highly variable (differences between species, individuals, and across populations)
  - Age, sex, genetics
  - Environmental influences, previous exposures
  - Disease state/co-morbidities

Immune System Components

- Immune functions are accomplished via multiple components
  - Present in all organs and communication throughout the body
  - Cells and pathways for specific responses
- Two primary arms of the immune response
  - Innate- early, rapid, non-specific, no memory
  - Adaptive- slower to develop, specific, memory responses
  - They work cooperatively





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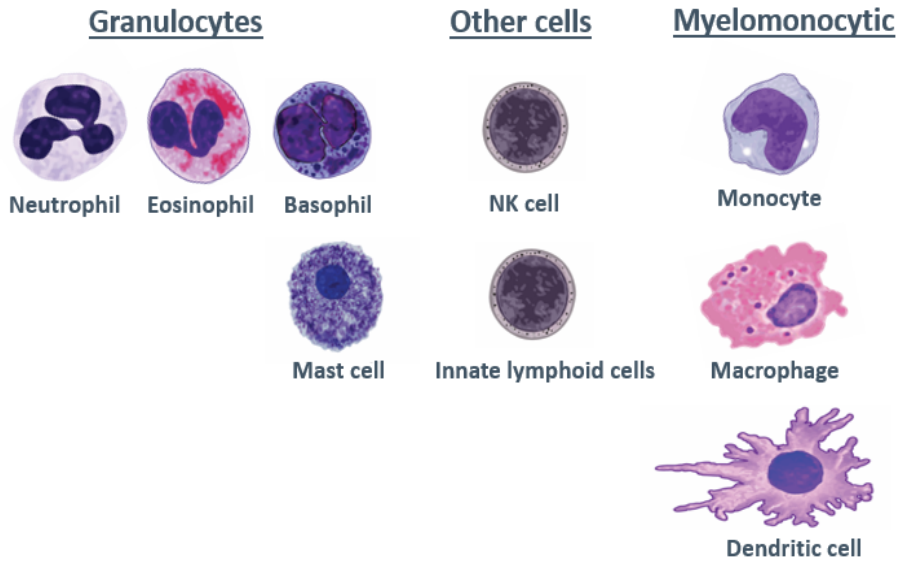
Innate Immunity

- Characteristics:
  - First line of defense
  - Non-specific response
  - No memory response generated

- Components
  - Non-cellular factors
    - Physical barriers (e.g. epithelium of skin, mucosa)
    - Mucus, anti-microbial peptides
    - Soluble factors (cytokines, chemokines, etc.)
  - Immune cells
    - Granulocytes
    - NK cells, γδ T cells, innate lymphoid cells
    - Monocytes, macrophages, dendritic cells (DCs)*
  - Other supporting cells, tissues, etc.



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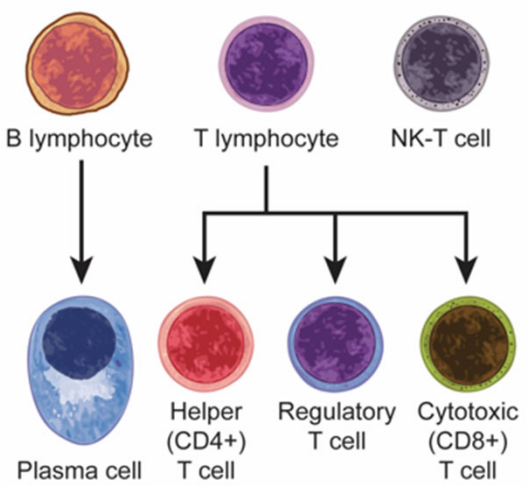
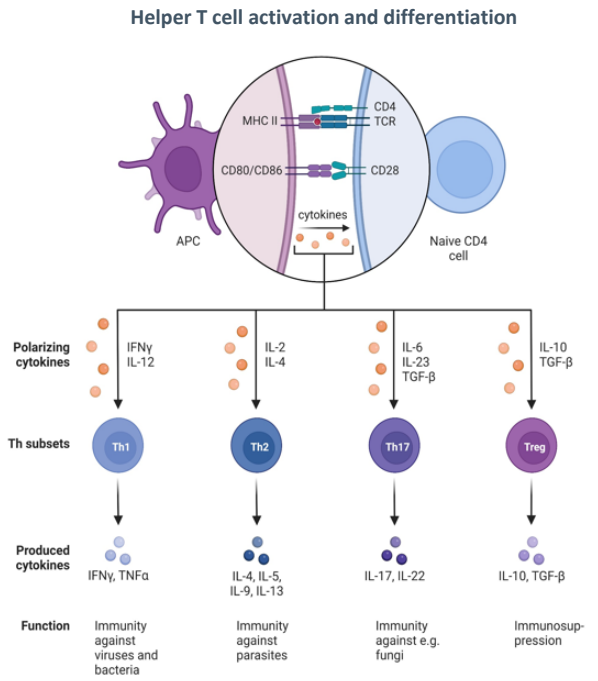
Adaptive Immunity

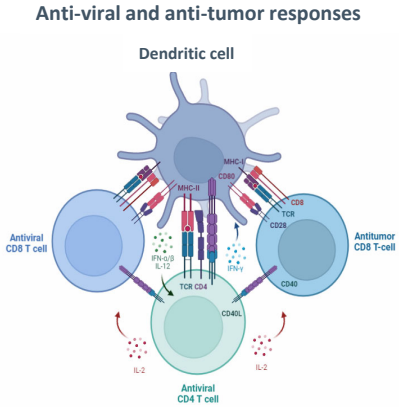
- Characteristics:
  - Catered response, takes time to develop
  - Antigen-specific, MHC class I or II restricted
  - Memory response generated
- Components:
  - B lymphocytes (plasma cells) = produce antibodies, act as APC
  - T lymphocytes (multiple subtypes → functions)
    - CD4+ (helper) T cells:
      - Coordinates humoral versus cell-mediated responses
      - Recognizes MHC class II antigens
      - Promote Th1, Th2, Th17, Tregs populations
      - Help support B cell development and activity (e.g. antibody production)
    - CD8+ (cytotoxic) T cells:
      - Kills target cells (e.g. tumor, infected or damaged cell)
      - Recognizes MHC class I antigens
      - Promote cytolysis/ apoptosis
  - Cytokines, antibodies, other soluble factors
  - Supporting cells, tissues, vessels, etc.



Innate-adaptive communication: Development of immune responses

- Antigen presenting cells interact with lymphocytes to promote humoral, cell-mediated, or regulatory responses
- Develops “appropriate” immune response for the threat
- Multiple signals are involved in generating
  - Signal 1) MHC – TCR interaction
  - Signal 2) Costimulatory molecule
  - Signal 3) Environmental signals (cytokines)
- Integration of these signals influences the nature of the immune response





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Immune System Responses

Considerations for Immune System Evaluation and Interpretation

- The immune system is highly responsive and can change rapidly
  - Significant trafficking of immune cells can occur within minutes to hours
  - Numerous cells, pathways, signaling cascades, and mediators are involved and change over time
- There is significant variability and a wide range of “normal” in immune responses
  - This makes determining functional considerations and determining what is adverse difficult
  - This is particularly true for therapies that are designed to modify the immune system and its responses
- Immune organs have a structure that is related to and dependent on function
  - But functional changes typically do not have anatomic changes
- The interpretation of immune endpoints depends on immune response kinetics
  - Key immune events may have already (or not yet) occurred at study timepoints
  - Cannot sample all data points so must determine ideal collection timepoints
  - An understanding of immune responses and therapy effects may be required for ideal interpretation

Approaches in Evaluating Immune Effects

Discovery → Preclinical → Clinical

New Therapeutic Chemical Entity Medical Device

IMMUNE EVALUATION

Detailed Understanding of Immune System Changes

Diagnostics/ Clinical signs

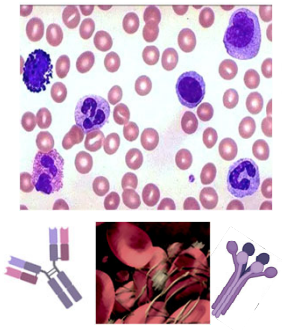
- Diagnostic tests
- Biomarker monitoring
- Prognostic indicators
- Disease State
- Clinical signs
  - Dyspnea, Rash, ….
  - Anaphylaxis
- Organ dysfunction
- Others

Immunology/ Immunotoxicology

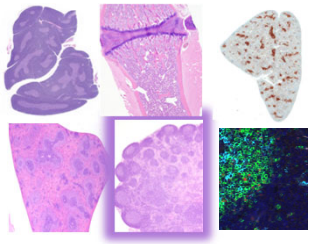
- Immunophenotyping
- TDAR
- Cytotoxicity (T, NK cell)
- Complement Assays
- Cytokines/Cytokine release
- Proliferation
- Biomarkers
- Predictive immunotoxicology
- Receptor occupancy
- Immunogenicity

Immunopathology

Clinical Pathology



Anatomic Pathology



Microscopic evaluation
Special stains (IHC, IF, etc.)
Multiplexing
Digital Pathology/AI

EFFECT OF THERAPY

- Immunomodulation

DISEASE STATE

- Cancer, autoimmunity
- Allergy, Inflammation
- Infectious Diseases

VARIABILITY IN IMMUNE RESPONSE

Diagnostic/Clinical Signs and Mortality

- **Diagnostics/Clinical Signs**
  - Numerous diagnostic tests, biomarker monitoring, and other prognostic indicators; varies based on therapy
  - Clinical signs consistent with immune activation/involvement (e.g. hypersensitivity, anaphylaxis, rash, dyspnea, infusion reactions, etc.)
  - Sudden death uncommon (unless hypersensitivity/anaphylaxis)
  - Influence on disease state ± prognostic indicators may be seen (e.g. efficacy studies)
  - May be absent; uncommonly included in pathology report for safety studies

- **Mortality (Survival)**
  - Unless hypersensitivity/anaphylaxis, mortality is uncommonly seen with immune toxicity
  - Clinical observations may be incorporated to provide supportive evidence of toxicity (hypersensitivity?) but often there are few to no correlative clinical observations

Immunotoxicology Assays I

- **Immunophenotyping**
  - Analysis of cell populations and subpopulations based on expression of specific markers
  - Cells routinely evaluated: T cells (including helper, cytotoxic and regulatory), B cells, NK cells
    - Identifies immune cell populations and subpopulations (e.g. Th1, Th2, Th17, Tregs, etc.)
  - Can evaluate activation status, functional changes (e.g. activation markers, cytokine production, transcription factors, etc.)
  - May be part of immunotoxicology or clinical pathology divisions

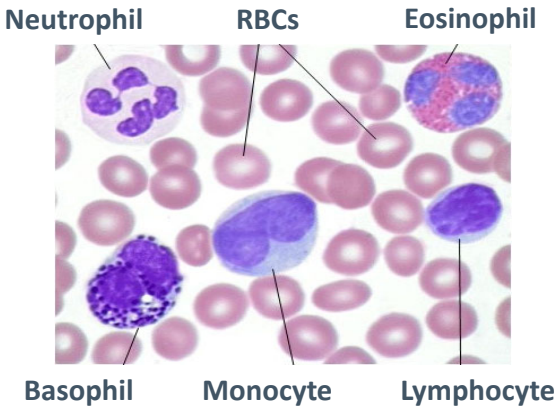
- **Cytokine/chemokine profiling**
  - Can evaluate overall levels or cell-specific production
  - Proinflammatory and anti-inflammatory cytokines evaluated (often panels)
  - Biological considerations: cytokine level changes may only be at cellular level, significance of levels/ratios often unknown, kinetic expression varies significantly (6-72+ hours)
  - **Cytokine release assay -** *In vitro* assay using human cells; predicts potential for cytokine release *in vivo*

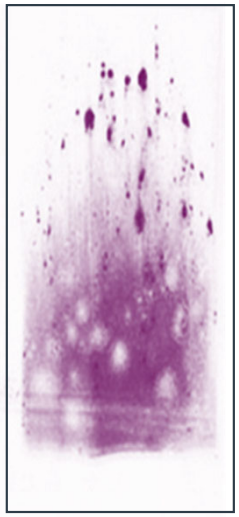
Immunotoxicology Assays II

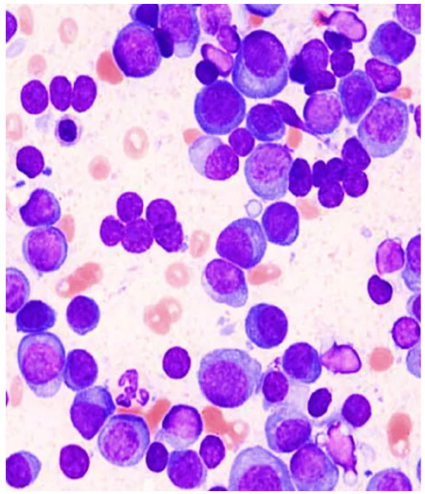
- **Functional Assays**
  - TDAR (T cell-dependent antibody response)- evaluates immune cells ability to develop antibody response
    - Evaluates innate-adaptive, helper T cells, and B cell interactions
    - Traditionally used to evaluate immunosuppression, can be used to evaluate immunostimulation
    - TIDAR (T cell-independent antibody responses)- evaluates antibody production to T-independent antigens, less common
  - Fewer assays available/utilized to evaluate cell-mediated immunity for immunotoxicology
  - Viral challenge models- evaluates CD8+ T cell function, many models
  - Cytotoxicity assays (NK cells, CD8+ T cells)
  - Phagocytosis/oxidative burst
  - Basophil or mast cell activation
  - Host resistance model- many challenge models
- **Others**
  - Various (e.g. immunogenicity, receptor occupancy, ADAs, etc.), driven by study-specific needs
- Correlate with other findings when possible

Clinical Pathology I

- **Complete blood cell counts (CBC)-** Determines the percentages of cell populations in the blood
  - Erythrocytes (RBCs), Leukocytes (WBCs): granulocytes, lymphocytes, monocytes, and platelets
- **Bone marrow smear/aspirate/biopsy**
  - Evaluates cellular elements of the bone marrow (precursors/mature RBCs, platelets, WBCs, iron stores, etc.
- **± Immunophenotyping**







Clinical Pathology II

- **Clinical (serum) chemistry** - Assesses physiological parameters/organ function
  - Immune-specific parameters (globulins, albumin/globulin ratio, acute phase response proteins)
- **Complement system analysis**- Evaluates complement cascade pathways and components
  - Classical, alternative, lectin pathways
  - Inactive precursors cleaved to form active proteins (opsonins, chemoattractants, MAC anaphylaxins, …)
- **Coagulation profile**- Assesses the ability of the blood to clot
- **Urinalysis** – Evaluates parameters in urine to assess renal function (and globulins)

- **Biomarkers**- Any measurable marker indicating effects on/changes in the immune system
  - Changes in cell populations (e.g. by CBC or immunophenotyping)
  - Cytokines, chemokines, complement, antibody levels, immune complexes, acute phase response proteins, etc.

Macroscopic (Gross) Pathology of Immune Organs

- Evaluation requires familiarity with location and normal appearance
  - May often be difficult to find during necropsy, especially in smaller animals or if depletion
  - Having prosector familiar with species-specific considerations and collection of immune organs is key
- May see immune-relevant gross changes in color, size, distribution
- Changes may be in specific sub-anatomical compartments in immune organ
  - Knowledge of anatomy and function of compartments is helpful
- Correlation with microscopic findings and OW changes may be helpful

Organ weights for the Immune System

- Organ weights (OWs) typically taken only for the thymus and spleen
- Can see significant variation in immune organs due to:
  - Stress, puberty and physiologic factors
  - Species, strain, sex, age effects
  - Collection/trimming considerations or procedure-related (e.g. euthanasia artifact) effects
- Comparison to historical controls may be difficult due to variability and limited data for some organs
- Immune-relevant changes best determined through comparison against age-matched controls (vs historical database)
- Immune-relevant OW changes (primarily spleen and thymus) can be influenced by:
  - Age, sex, puberty (e.g. thymic involution during puberty or stress), previous immune exposure
  - Physiologic or pathologic changes (e.g. changes in cell #s/composition, fluid composition, etc.)
- Organ weights may correlate with other findings

Microscopic (Histopathologic) Observations Assessment

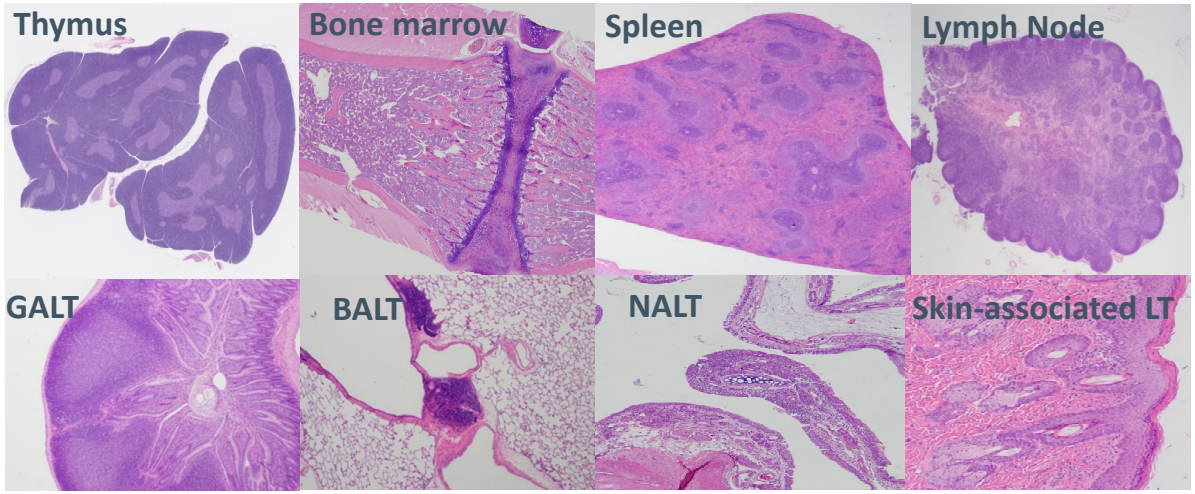
- **Histopathology (Hematoxalin/Eosin; H&E on PPFE)**
  - Determines microscopic alterations in tissue and cellular architecture and cellular composition
    - Evaluates immune and non-immune organs
    - Evaluates a single point in time
  - Requires a full understanding of normal tissue architecture and tissue-specific immune responses relevant to species within the context of the scientific question/test article MOA to:
    - Identify changes (and background findings)
    - Interpret the pathologic process and significance of changes
  - Does not evaluate immune function
  - Can correlate with other findings (to develop WOE) for significance of immune changes

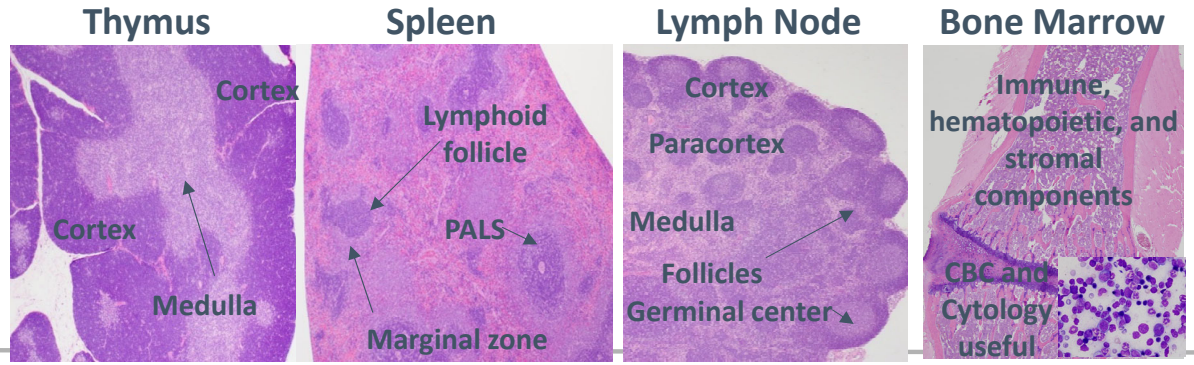
- Specialty histopathologic services
  - Specialty stains and ancillary diagnostics can provide additional information on
    - Identify or functionality of cells
    - Locations and compositions of cellularity constituents *in situ* (in the tissue)
  - Digital imaging and software can be used to identify, quantify, and interrogate immune system/cells
  - Enhanced Histopathology- semi-quantification compartment-aware evaluation
    - Not typically employed since routine pathology is compartment-aware with severity grades

Microscopic (Histopathologic) Observations Assessment

Histopathology of Immune Organs

- Discovery
- Safety Assessment



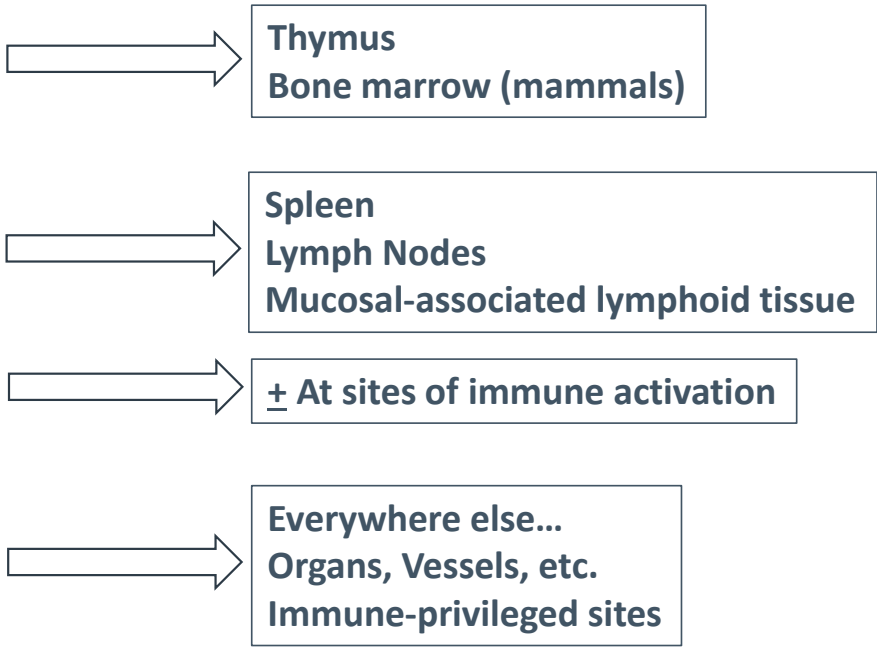


- Additional advanced evaluation (semi-quantitative and/or compartment-aware) can be provided by
  - Enhanced histopathology
  - Specialty staining, digital pathology, AI, etc.
- Uncommonly performed unless warranted

StageBio

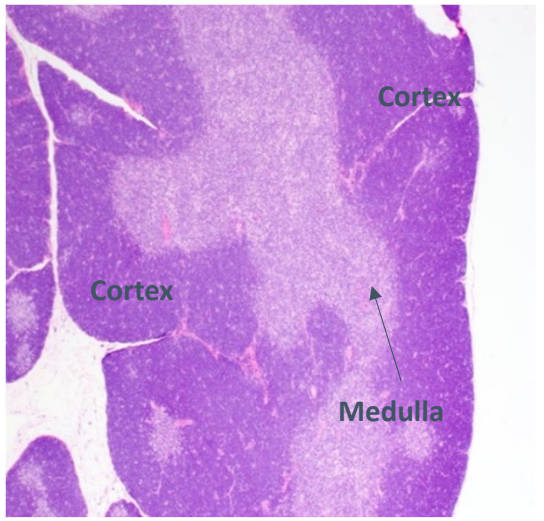
Immune and Non-Immune Organs

- Primary lymphoid/immune organs
  - Where lymphocytes are formed and mature
    - T cells develop in the thymus
    - B cells develop in the bone marrow* (Bursa of Fabrisicus in birds)
- Secondary lymphoid/immune organs
  - Where immune responses occur
  - Mature naïve and activated lymphocytes, memory cells
- Tertiary lymphoid/immune organs
  - Accumulations of immune cells
  - Often develop during chronic inflammation
  - May resemble lymph nodes
- Non-immune organs
  - Immune cells are present throughout the body
  - Surveillance and responses
  - Reflect changes in and/or responses of the immune system



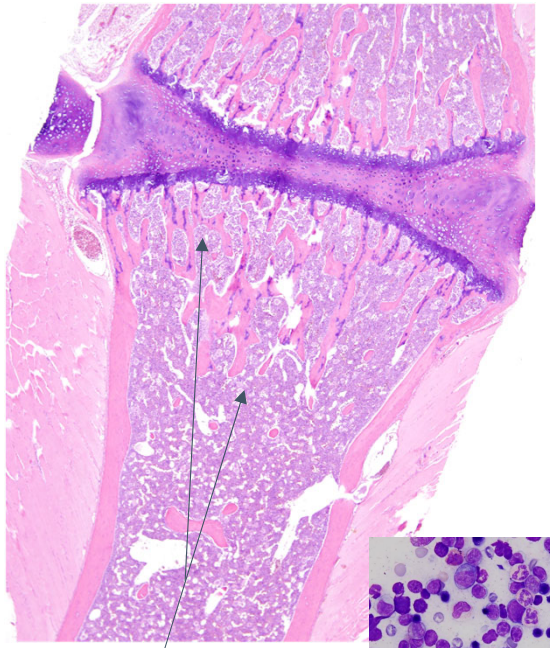
Thymus

- Immune Organ: Primary lymphoid organ
- Function:
  - Site of T cell development and maturation (positive and negative selection)
  - Normally decreases in size (involutes) during puberty
- Location: Thoracic inlet/cervical region
- Anatomic compartments:
  - Cortex: CD4+CD8+ (double positive)
  - Medulla- CD4+CD8- and CD4-CD8+ (single positive)
  - Vascular/stromal elements, epithelial/connective, adipose tissue, etc.

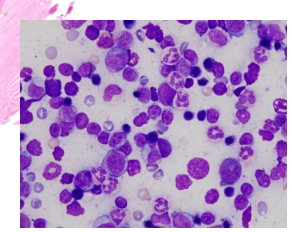


Bone Marrow

- Immune Organ: Primary lymphoid organ
- Function:
  - Site of B cell development and maturation (mammals)
  - Site of hematopoiesis
  - Amount of marrow (active “red”) decreases with age
- Location: Long bones and flat bones primarily
- Anatomic compartments:
  - Immune cell components
    - Granulocytes (neutrophils, eosinophils, basophils)
    - Myelomonocytic (monocytes, histiocytes, etc.)
    - B cells (precursors, plasma cells)
  - Blood cell components
    - Erythrocytes (RBCs) and iron stores
    - Megakaryocytes (and platelets)
  - Vascular/stromal elements, bone/connective/adipose tissue



Immune, hematopoietic, and stromal components



CBC and Cytology helpful

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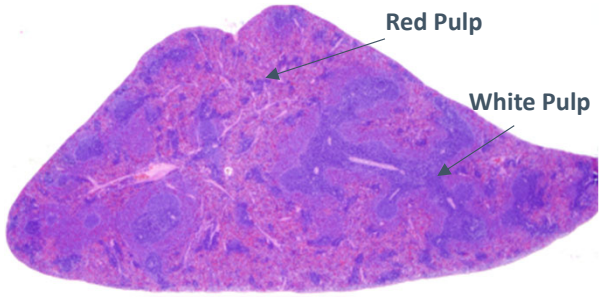
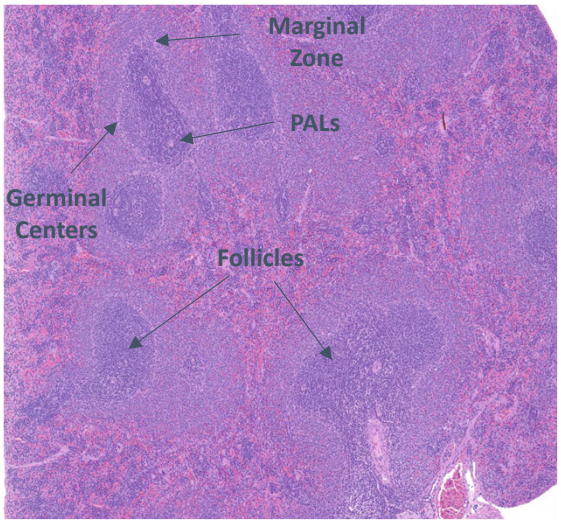
Spleen

- Immune Organ: Secondary lymphoid organ
- Function:
  - Surveillance of blood for pathogens
  - Filters and recycles blood components (erythrocytes)
  - Site of development of immune response
- Location: Abdominal cavity
- Anatomic compartments:
  - Red pulp: primarily erythroid/non-lymphoid constituents
  - White pulp: contains lymphoid constituents
    - Lymphoid follicles: Primarily B cells, continuous with PALs
      - May development into germinal centers after antigenic stimulation
    - Germinal centers: Where B cells mature, produce antibodies
    - Marginal zone: Primarily B cells and macrophages
      - Important surveillance role and in antigen processing/presentations
    - Periarteriolar lymphoid sheaths (PALs)- both T and B cells present
      - Where T cells are presented with antigen and initiative immune response
  - Vascular/stromal elements, connective tissue, etc.





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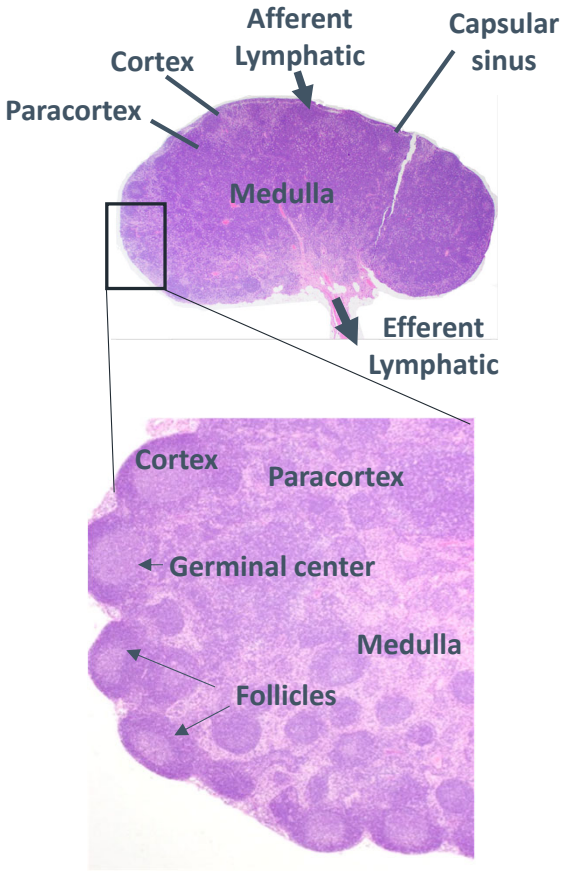
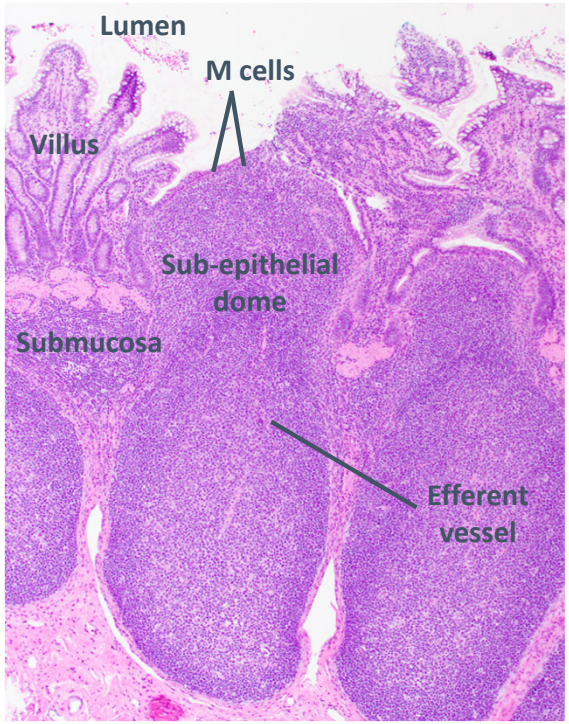
Lymph Nodes

- Immune Organ: Secondary lymphoid organ
- Function:
  - Filters lymphatics
  - Primary site of initial development of immune responses
- Location: Throughout the body
- Anatomic compartments
  - Lymphatics (afferent and efferent)
  - Cortex and paracortex: site of initiation of immune response
    - Outer cortex- Follicles (B cells) and geminal center development
    - Paracortex (deeper)- primarily T cells
    - Subcortical zone- where T cells interact with DCs
  - Medulla: contains sinusoids, medullary cords
    - Contains macrophages, B cells, and plasma cells primarily
  - Vascular/stromal elements, connective and other tissue, etc.



Mucosa-Associated Lymphoid Tissue

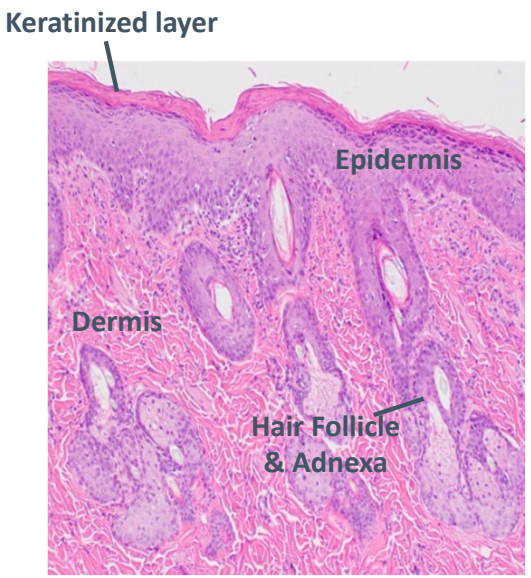
- Immune Organ: Secondary lymphoid organ
- Function:
  - Monitors mucosal sites, important in host defense
  - Develops/initiates immune response (mucosal), esp. IgA
- Location: Present in all mucosal sites
  - Gastrointestinal, respiratory, urogenital, mammary, ocular
  - Often named according to location
    - GALT = gut-associated (e.g. Peyer's patches, Appendix, etc.)
    - BALT = bronchus/bronchial-associated
    - NALT = nasal-associated
- Anatomic compartments
  - Variable degrees of organization
    - Organized (e.g. Peyer's patches, lymphoid follicles, etc.)
    - Diffuse (e.g. colonic cryptopatches)
    - Lymphatics (afferent and efferent)
  - Vascular/stromal elements, connective and other tissue, etc.



StageBio

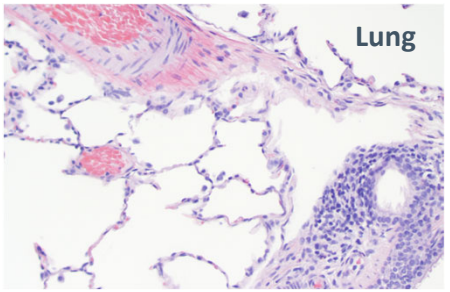
Skin and Skin-Associated Lymphoid Tissue

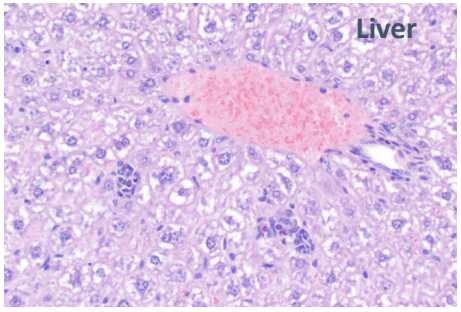
- Immune Organ: Secondary lymphoid organ
- Function:
  - Important in host defense, site of DC activation
  - DCs traffic to lymph nodes (spleen); initiate immune response
- Location: Present throughout all sites
- Anatomic compartments
  - Physical (and chemical) barriers
    - Keratinocytes, lipids, defensins, anti-microbials
  - Immune cells
    - Innate and adaptive cells
  - Stromal, vascular, lymphatic, and other elements to maintain immune cell location and facilitate trafficking

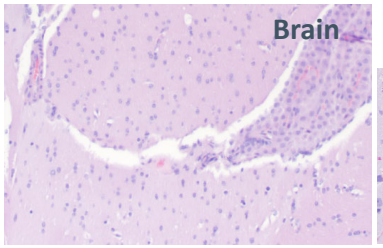


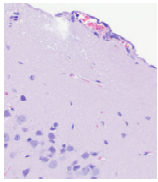
Non-Immune Organ Immune Components

- Non-Immune Organs
  - ALL organs (including immune-privileged) have immune components
- Function: Catered to organ-specific needs
  - Defense
  - Immune tolerance
  - Immune-privileged
- Location: Present throughout the body
- Anatomic compartments depends on organ
  - Lung: Capillaries with circulating immune cells can respond to pathogens (e.g. inflammation)
  - Liver: Kupffer cells lining sinusoids (tolerogenic)
  - CNS: Immune cells present to actively protect brain from self-reactive cells (i.e. immune-privilege)









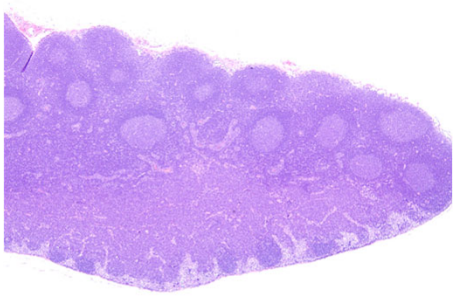
StageBio

Pathology Findings and Interpretation

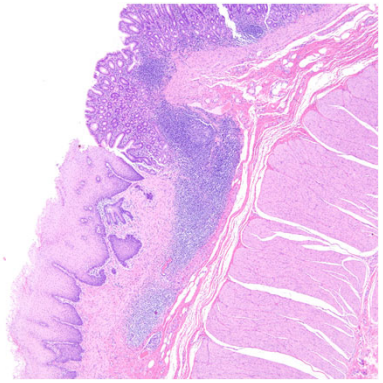
- Pathology findings (study-related) can be
  - Background (incidental) findings
  - Related to species, strain, age
  - Procedure-related (administration route, components of vehicle, etc.)
  - Test Article-related
    - Expected
    - Not Unexpected
  - Reversible
  - Adverse or nonadverse

- Successful interpretation of histopathology findings requires a knowledge of normal, abnormal, background, procedural, or artifactual findings to determine test article-related effects and significance

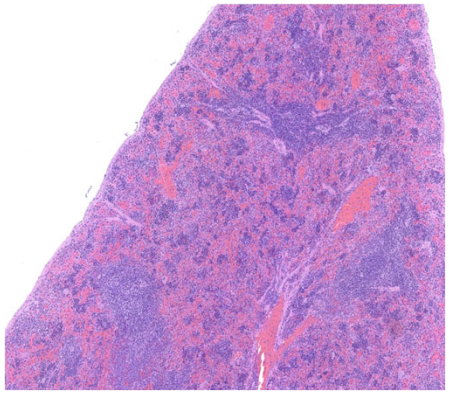
Immune System Pathology Changes: Potential Background Findings



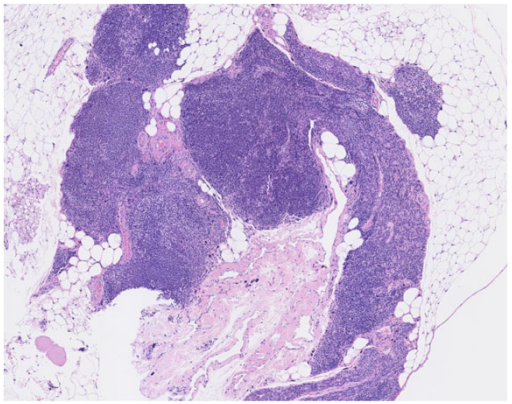
Prominent lymphoid follicles (Dog)



Prominent GALT in the stomach (Cynomolgus macaque)



Extra-medullary hematopoiesis in the spleen (Rat)

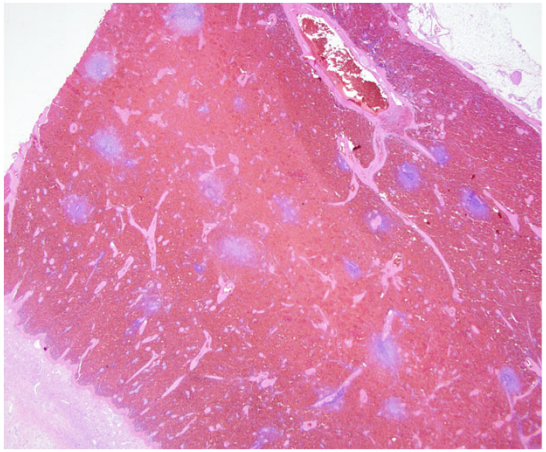


Thymic involution (Rat)

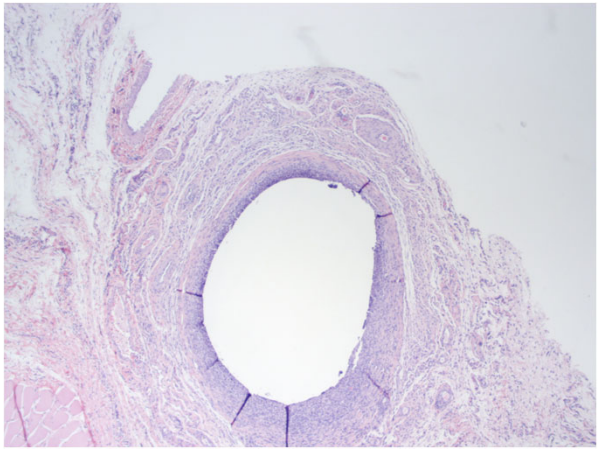
StageBio

Immune System Pathology Changes: Potential Procedure-Related

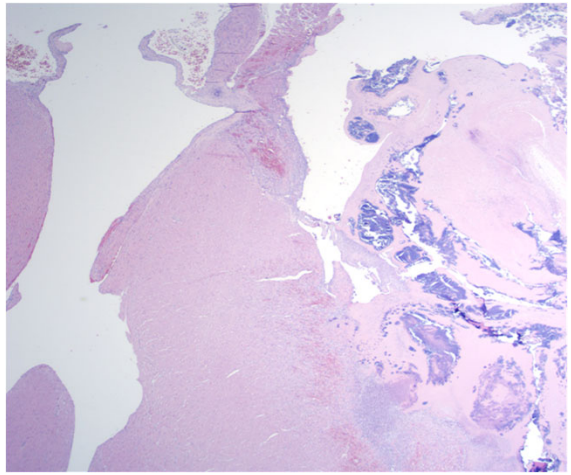
Expected Change: Congestion
Spleen: Euthanasia Artifact



Not Unexpected Changes: Fibrosis
Administration site: Catheter site



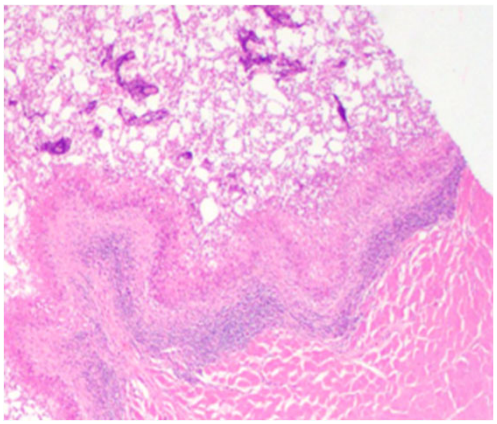
Unexpected Change: Bacterial infection
Multiple sites: Inflammation, abscess, bacteria



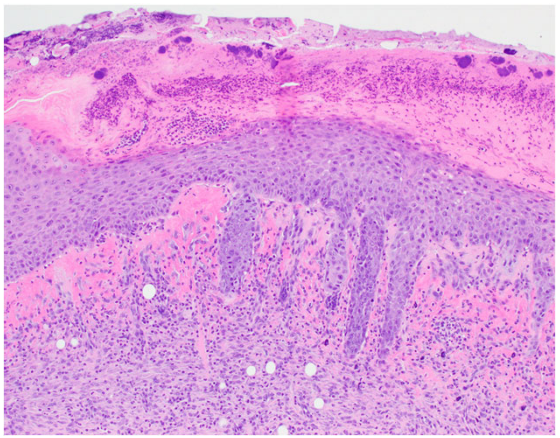
StageBio

Immune System Pathology Changes: Potential Test Article-Related

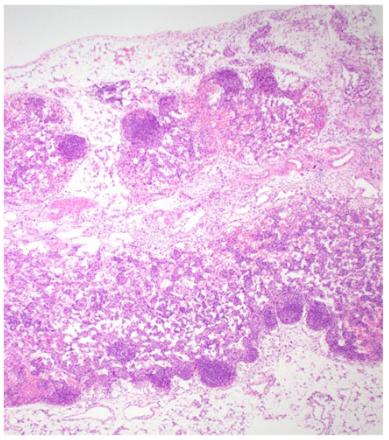
Expected Changes: Necrosis & Fibrosis (Skin)

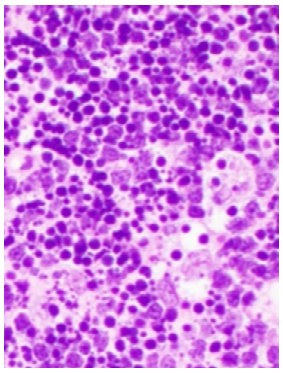


Not Unexpected Change: Inflammation (Skin)



(Not)Unexpected: Decreased lymphocytes
Lymph node: Decreased cellularity
Thymus: Apoptosis of lymphocytes





StageBio

Integrating and Contextualizing Pathology Findings

- The immune system is present throughout the body
  - In immune and non-immune organs
  - In circulation and disseminated for surveillance
- Findings in immune and non-immune sites can provide information on:
  - Immunopathology (pathogenic process)
  - How the immune system is being affected/altered
- Changes in immune cell #/function can impact changes in non-immune organs
  - Stress → Lymphocyte apoptosis/necrosis →Immune organ structure/function changes
    - May see secondary effects on the immune system (from stress):
      - Altered immune function (e.g. opportunistic infections → inflammatory infiltrations/organisms)
      - Immune-mediated effects (e.g. immune complex deposition)
    - Secondary effects often require sufficient time to develop so may not be apparent

Approaches in Evaluating Immune Effects

New Therapeutic Chemical Entity Medical Device

IMMUNE EVALUATION

Detailed Understanding of Immune System Changes

Diagnostics/ Clinical signs

Diagnostic tests
Biomarker monitoring
Prognostic indicators
Disease State
Clinical signs
- Dyspnea, Rash, ….
- Anaphylaxis

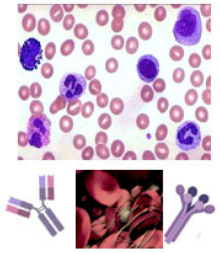
Organ dysfunction
Others

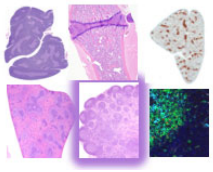
Immunology/ Immunotoxicology

Immunophenotyping
TDAR
Cytotoxicity (T, NK cell)
Complement Assays
Cytokines/Cytokine release
Proliferation
Biomarkers
Predictive immunotoxicology
Receptor occupancy
Immunogenicity

Immunopathology

Clinical Pathology **Anatomic Pathology**





Microscopic evaluation
Special stains (IHC, IF, etc.)
Multiplexing
Digital Pathology/AI

StageBio

Immunosuppression Example: Correlative Data

- **Chemotherapy (cytotoxic)** → targets rapidly dividing cells
  - Diagnostic/Clinical Data:
    - Clinical immunosuppression (e.g. increased (opportunistic) infections, tumors, etc.)
  - Immunotoxicology Data:
    - Decreased #s/ increased apoptotic lymphocytes (immunophenotyping)
    - Decreased function in various populations, TDAR, etc.
    - Decreased resistance to infections/challenge (host resistance) models, etc.
  - Immunopathology Data:
    - Decreased size of immune organs due to cell loss, ± Discoloration
    - Microscopic findings of lymphocyte apoptosis/necrosis/loss
    - Evidence of infections/tumors (supportive evidence of immunosuppression)

Immune stimulation (modulation) Example: Correlative Data

- **Cancer immunotherapy (checkpoint inhibitor)** → promotes anti-tumor response

  - Diagnostic/Clinical Data:
    - Varies: None → Immunogenicity, exaggerated pharmacology, toxic effects in test species, etc.
    - Clinically- therapeutic reduction of tumor burden (efficacy)

  - Immunotoxicology Data:
    - ± Changes in immune populations/subpopulations (immunophenotyping)
    - ± Increased functional responses
      - Immune stimulation due to "releasing the brakes" → exaggerated pharmacology/toxicity
    - May have evidence of systemic inflammation, cytokine release (cytokine "storm")

  - Immunopathology Data:
    - Often minimal/none in safety studies
    - Subtle increases in #s of immune cells at various sites
      - Commonly within the range of normal variability (background levels) of immune cells in various organs
      - Uptick in immune cell numbers may be suggestive of activation/enhancement of immune system
    - Evidence of immunogenicity (e.g. immune complex deposition)- may not be clinically relevant (humans)

Adversity Determination in Pathology

- Adversity is a term indicating harm
- In preclinical safety assessment, adversity determination is
  - Commonly assigned to findings in the pathology report
  - Used to evaluate the risk (or potential toxicity) for a given therapy
  - Guided by Best Practices publications (but may have limitations for immunotherapies)
- Assigning adversity (esp. for immune-related effects) can be challenging due to:
  - Lack of functional correlations
  - Variability in immune responses (responsive/reversible)
  - Study design-related limitations (limited timepoints evaluated, correlative data)
  - Species-specific differences and translational relevance to intended patient populations
  - Difficult to define when immune effects are expected (e.g immunotherapies)
    - Expected (or Intended) pharmacology → Exaggerated pharmacology → Toxicity?

Considerations for the Pathologist in Adversity Determination

- Pathology considerations
  - Organ weight, gross pathology, microscopic findings, clinical pathology, correlative data
  - Magnitude of effect, # of organs affected, reversibility, evidence of functional effects, are changes adaptive/species-specific?
  - All within context of background findings, expected immune responses, etc.
- Overall Study considerations for determining adversity/toxicity:
  - Pathology findings and adversity calls + Immunotoxicology endpoints
  - Translational and other considerations (e.g. MOA/target, intended patient population/acceptable level of toxicity/risk, etc.)
- While adversity determination is ideal, some additional considerations are:
  - Pharmacology should not necessarily preclude from an adversity determination
  - An adverse or nonadverse designation may not be possible (or necessary) for all findings.

Pathology Reporting

- Depends on the study, scientific question, and stage in development pipeline
- Discovery/Investigative Reports
  - Contents depend on the type of study
  - Study types (consultation, model development, early lead-op/dose-range finding, etc.)
- Standard Pathology Report (Toxicology (safety assessment) Studies)
  - Survival/Mortality, Macroscopic (Gross) Findings, Organ Weights
  - Microscopic (Histopathologic) Findings ± adversity determination
  - Correlates to other findings variably included (e.g. clinical pathology, immunotoxicology, etc.)
- Reports may have additional specialty components and evaluation as needed
  - Special stains (e.g. IHC, IF)
  - Ancillary and complement diagnostics (e.g. EM, laser-capture microdissections, etc.)
  - Digital imaging/AI (e.g. semi-quantitative/compartment-aware enhanced histopathology)

Pathology Reporting in Preclinical Drug Development

Discovery/Investigative Report

- Purpose: Varies depending on study:
- General SOW depends on study purpose
  - Model development
  - Efficacy
  - Lead optimization, dose range finding, etc.
- Components
  - Summary/conclusions of study findings
  - Interpretation and potential implications
  - Bespoke components as needed
- Scientific (expert) consultation often required, may be a significant component

Safety Assessment Report

- Purpose: Focused on identifying potential toxicities
- General SOW included
  - Non-GLP
  - GLP studies
  - Peer reviews
- Components
  - Summary and interpretation of study findings
  - Correlates with other data
  - Dose-related effects, assign adversity if relevant
- Consultation typically limited to pathology findings
  - May have additional follow-up (e.g. peer review, scientific consultation)

Integration for Understanding

↑ Data/information → ↑ Understanding → ↑ Ability to interpret significance

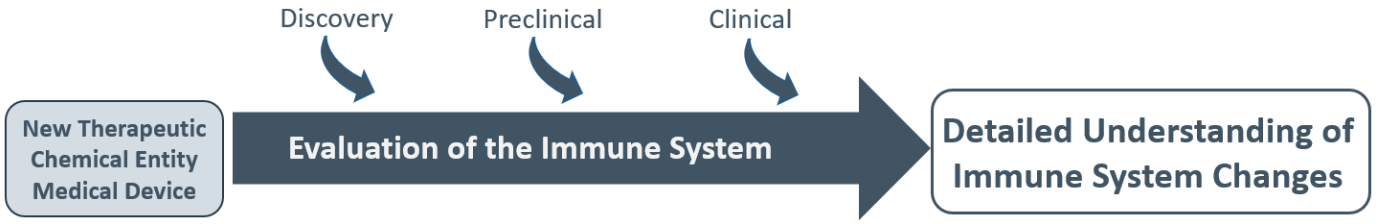
- Data and study findings can provide:
  - Information on immunopathogenic processes and effects on immune system
  - Indication of risk vs. benefits (toxicity, adversity, dosage levels, etc.) used in development and regulatory review
- Weight of evidence (WOE) approach
  - Includes many data sets (Diagnostics/clinical signs, immunotoxicology, immunopathology, translatability, etc.)
  - Requires an understanding of the immune system and significance of study-related effects
  - Requires communication between scientists (toxicologists, pathologists, immunologists, etc.)
  - Utilized by regulatory bodies (e.g. FDA) to make decisions regarding therapies
- WOE questions:
  - Are there immune-related effects?
  - Are the effects on-target/off-target?
  - Do the effects represent intended vs exaggerated pharmacology
  - Are the effects isolated or do they occur in multiple sites (are the additional lines of evidence)
  - Are additional immunotoxicology studies or immune data warranted?
  - Are the findings Adverse? (Rationale as to why/why not?) Adaptive? Reversible?
  - Regulatory bodies utilize WOE approach

Regulatory Considerations

- Potential safety concerns (categories (adverse) outlined by the FDA):
  - Hypersensitivity — Sensitization due to a drug and/or its metabolite
  - Immunogenicity — Humoral/cellular reactions elicited by a drug and/or its metabolites
  - Immunosuppression — Effects that result in decreased immune function
  - Immunostimulation — Adverse activation of immune system effector mechanisms
  - Autoimmunity — Immune reactions to self-antigens
- There are numerous guidance documents
  - Multiple regulatory agencies and guidances (FDA, EPA, EMEA, ISO, ICH, etc.) for different applications
    - Medical drugs and therapies, Medical devices, Environmental, Foods, Cosmetics, etc.
- Even within these areas, guidance documents may focus on some aspects over others
  - Nature of the therapy (e.g. biologics vs small molecules)
  - Nature of the intended use (e.g. cancer patient population, human vs animal)
- Often many gaps in coverage/focus (ongoing discussion)
- Important to know and understand guidance documents relevant to your program!

Summary

- The immune system is present in immune and non-immune organs/tissues
- The immune response is dynamic, highly responsive, and variable
- Immunotherapies have changed the landscape of immune system evaluation
- Evaluation of immune system should occur throughout drug development
- Immunotoxicology and pathology are key means to evaluating immune changes
- An understanding of immune changes requires an understanding of immunology and immunopathogenic processes and changes within the context of the study
  - Includes species considerations, the MOA/immune effects of the test article, study design, etc.
- Data integration is important to understanding, interpretation, and risk assessment
- Communication between toxicologists, immunologists, pathologists, and other scientists across drug development is critical for immune system evaluation



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Thank you!

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