Updates in Canine and Feline Lymphoma
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Lymphoma
- Phenotyping
- Novel Therapies
  - Monoclonal antibodies
  - Tanovea
- Low grade lymphoma (Canine)
- Low Grade Lymphoma (Feline)

Why Phenotype?
- B cell LSA:
  - 2/3 of LSA case
  - Better response and **prognosis**
  - CHOP protocol – 12-18 month MST
  - Breeds:
    - Cocker Spaniel
    - King Charles Cav

Canine T cell LSA
- Represents: 10-38% of canine NHL
- Site predilection:
  - Skin (epitheliotrophic) CD8+
    - Fontaine et al. Vet Dermatol 2010
  - Gastrointestinal tract
    - Coyle KA et al. Vet Pathol 2004
  - Hepatic
    - Fry et al. Vet Pathol 2003
  - Mediastinal
    - Vail et al. Exp Hematol 1997
    - Ruslander et al. In Vivo 1997
    - Hypercalcemia (10-40%)

Canine T cell LSA: Etiology
- Breed predilection:
  - Boxer
  - Golden retriever
  - Asian lap dogs (Shih Tzu)
  - Siberian Husky
  - Australian Shepherd
    - Modiano et al. Cancer Res 2005
    - Dogue de Bordeaux?
- History of Atopic Dermatitis (AD)
  - Epitheliotropic LSA
    - Santoro et al. Vet Derm 2007
      - Retrospective case controlled study
      - Odds of having ELSA 12x higher in AD patients

T cell LSA Prognosis: Poor
- PFS 94–200 days
- OST 120–239 days
  - Vail et al. Experimental Hematology 1997
  - Ruslander et al. In vivo 1997
- Site specific:
  - Gastrointestinal:
    - Smit et al. JVM 2009
      - N=18; 63% T cell
      - OST 77d
    - Franz et al. JAAHA 2007
      - N=35; chemo n=23
      - OST: 13d
  - Hepatic:
    - Bank et al. JAVMA 2011
      - N=18
      - OST: 63d
Doxorubicin alone? : Beaver et al. JAVMA 2010
- B cell: n=26; CR: 25/29; Overall resp 80%
- T cell: n=12; CR: 2%; Overall 50%

MOPP+Elspar: Brodsky et al. JVIM 2009
- N=50 (3 not phenotyped but were hypercalcemic)
- Response rate: 98% (CR 78% PR 20%)
- Overall ST was 270 days; 25% @ 939 days.
  - 20% hospitalization rate

Best Protocol: Multicentric T cell LSA?

Phenotyping
- Immunohistochemistry (~$350)
  - Gold standard?
  - CD79a: B cell
  - CD3: T cell

Polymerase chain reaction (PCR)
- PCR-polymerase chain reaction:
  - Repetitive enzymatic reaction: generates ~10^9 copies of a particular DNA sequence from 1 original copy.
  - Utilizes heat-stable polymerases and sequence specific primers.
- PARR = PCR for Antigen Receptor Rearrangement (~$300)
  - PARR specifically amplifies the conserved regions of TCR or Ig genes

Lympocyte expansion in response to infectious organisms and self antigens is polyclonal

Neoplastic lymphocyte expansion is monoclonal

Neoplastic lymphocyte is no longer restricted by growth controls, and possesses immunoglobulin gene to daughter cells
PARR ($275)
- Determines if clonal vs. polyclonal
  - Is this cancer?
- Phenotypes lymphoma/leukemia
- Less expensive than biopsy + histo/IHC
- Blood, LN, CSF, mediastinal mass
- Molecular remission?

Flow Cytometry ($275)
- Common in human medicine for lymphoid malignancies
- Monoclonal antibodies + fluorescent markers
  - Large number of cells counted
  - Phenotype of circulating atypical cells
  - Aberrant surface marker expression
- Uses are still somewhat limited
  - Equipment (labs), antibodies, shipping
  - Allows
    - Categorization
    - Prognostication

Rao et al JVIM 2011 (MHC class II, cell size)→ prognostic

Basic markers to identify lymphocytes via FCM
- B cell
  - CD20, CD21, CD79a
- T cell
  - CD3 CD5
  - CD4 or CD8
- CD45: pan-leukocyte
- CD34: precursor cells
- MHC Class II

Flow cytometry -- principles of analysis
- Cells from patient
  - Fluorescently labeled antibodies against cell surface proteins
  - Flow cytometers detect light emitted by fluorochromes
- Antibodies will bind to only those lymphocyte subsets that express the proteins they recognize
- Laser light

Immunocytochemistry $255
- Thalheim et al. JVIM 2013
  - FCM and PARR to correctly immunophenotype as defined by IHC
  - Assess level of agreement among the 3 tests
  - The sensitivity of FCM vs. PARR:
    - B-cell (91% versus 67%; $P < 0.0072$)
    - T-cell (100% versus 75%; $P < 0.0312$)
  - The percent agreement:
    - FCM and IHC was 94%
    - PARR and IHC was 69%
    - FCM and PARR was 63%
**Why Phenotype?**

- **Protocol**
  - B cell CHOP (Madison Wisconsin)
  - 15, 19, 25 week discontinuous protocol
  - T cell Modified CHOP (CCNU for Dox) or MOPP

  **Monoclonal antibody therapy**
  (Finally!!!)

**CD20 in Cancer Cells**

- CD20 is an attractive therapeutic target for a number of reasons:
  - expressed by ~90 percent of all B cell non-Hodgkin lymphomas
  - CD20 is not present on the stem cells that give rise to B cells
  - B cells damaged by a CD20-targeted therapy can be replaced.
  - CD20 is not expressed on any other cells in the body

**Antibody Therapy:**

- **CD20**
  - Expressed on:
    - Normal B cells
    - Malignant B cells

- **CD52**
  - Expressed on:
    - T cells
    - B cells
    - Monocytes
    - Macrophages
    - Thymocytes

  Stable targets, not shed, found freely circulating in plasma, or internalized upon antibody binding

**Monoclonal: Mechanism of Action**

- Three proposed mechanisms
  - 1. **Antibody Dependent Cell Cytotoxicity:**
    - Natural killer cells, T cells, and macrophages are involved in recognizing and killing antibody-labeled target cells, leading to cell lysis

  **Mechanism of Action**

  - 2. **Complement Dependent Cytotoxicity:**
    - Binding of the antibody recruits complement proteins, which punch holes in the cell membrane leading to cell lysis

  **Monoclonal: Mechanism of Action**

  - Three proposed mechanisms
  - 3. **Apoptosis:**
    - Binding of the antibody signals the cell death
Monoclonal Antibodies In Veterinary Medicine

- In one year: 2 Conditionally Approved antibodies
  - CD20: B cell LSA (Novartis)
  - CD52: T cell LSA (Aratana)

- Safe with a reasonable expectation of efficacy
- IV administration over 15-30 minute
- Combined with chemotherapy

Clinical Trials
Conditionally Approved Antibodies

- B cell LSA:
  - 25 week CHOP + Monoclonal antibody
  - Partially funded (owner pays for chemo)

- Clinical Trial Sites (120 dogs):
  - Hope VS (PA)
  - Ketonah Bedford Veterinary Hospital (NY)
  - NC St (NC)
  - CSU (CO)

Anti-CD52 (T cell): Two Clinical Trials

Trial 1.
- 19 week CHOP + Monoclonal ab
- 19 week CHOP
- Fully Funded
- Hope VS (PA) only site in tri-state region

Trial 2.
- CCNU + Monoclonal ab
- CCNU alone
- Fully Funded
- Oradell only site in tri-state region

Tanovea in Canine Lymphoma

- 62 dogs with lymphoma treated with VDC-1101 monotherapy
  - 20 naïve; 42 refractory
  - 4 schedule (daily x 5, q7, q14, q21)

- 77% Overall Response Rate (ORR) (58% CR, 19% PR)
  - Naïve: 100% (70% CR, 30% PR)
  - Refractory: 74% (52% CR, 21% PR)

- Adverse events were generally mild and self-limiting – some pulmonary and cutaneous events

GS-9219 / VDC-1101/Tanovea

- Novel double prodrug of the anti-proliferative nucleotide analog 9-(2-phosphonylmethoxyethyl) guanine (PMEG)
- PMEG: known antiproliferative effects but severe DLTs when given systemically
- VDC-1101 effectively loads lymphoid cells while markedly reducing levels of PMEG in plasma and target organs of toxicity
- VDC-1101 inhibits proliferation of myeloid and lymphoid cell lines in vitro

Indolent Lymphoma

- High grade/Intermediate grade
  - Most common
  - Rapid onset
- Low grade
  - Indolent form

**Indolent LSA**

- True prevalence unknown
  - Estimated 5-30%
- Characteristics:
  - Indolent, slowly progressive
  - Incomplete responses to CHOP
  - Long survival times

**Indolent LSA: WHO Classification**

- **B cell types**:
  - Marginal Zone LSA
  - Follicular LSA
  - Mantle Zone LSA
- **T cell types**:
  - T Zone LSA

**Other Types Described**:

- Small Lymphocytic LSA
- Lymphoplasmocytic LSA
- T-cell rich B-cell LSA

**Indolent LSA: Limited Information**

  - 66 dogs
  - Survival data for 18
  - Indolent LSA = long survival
  - True incidence unknown, suspect these tumors are under-recognized
  - Evaluates:
    - Architectural features
    - Routine immunophenotyping
    - Molecular donality

  - n=5, retrospective study
  - 2/5 presented for hemoabdomen
  - 3/5 incidental finding (abdominal mass palpable)
  - 4/5 = splenectomy and doxorubicin
    - 3 died unrelated to LSA at 2, 2.5 and 5 yrs post dx
    - 1 still alive at 1 year (when manuscript submitted)
  - 1 of 5 = splenectomy alone
  - Relapse at 180 days post diagnosis
Indolent LSA: Limited Information

Veterinary Comparative Oncology. 2012
- N=75, retrospective study
- T zone lymphoma most common (62%)
- Marginal zone lymphoma 2nd most common (25%)
- Overall lymphoma-specific ST = 4 years
- No difference COP vs. Pred, Leukeran
  - Chemo helpful?

Obrien et al. Clinical Characteristics and Outcome in Dogs with Splenic Marginal Zone Lymphoma.
Journal of Vet Int Med 2013
- N=34, retrospective study
- All confirmed B cell marginal zone
- Splenectomy n=29
  - Asymptomatic (n=14): MST 1,153 days
  - Symptomatic (n=15): MST 309 days
- Chemotherapy had no influence on outcome

Indolent LSA: Clinical Presentation

- Middle-age to older dogs
- Substage “a”
- Incidental finding by owner/veterinarian
- No breed or sex predilection
- Local disease or multicentric
- No hypercalcemia reported, even with TZL

Diagnosis:
- “Indolent”: clinical history provides information
  - Present with chronically enlarged lymph nodes
  - Waxing and waning in size

Indolent LSA: Diagnosis

- Cytology is NOT enough
- Tissue biopsies and histopathology
  - Large tru-cut, or lymphadenectomy
  - Immunohistochemistry necessary
  - Splenectomy (MCL or MZL)

Recommendations
- Regular clinical staging

Indolent LSA: Therapy if Solitary

- Surgical resection
  - Lymph node, spleen
- Splenic marginal zone
  - Stefanello et al. JVIM 2011
    - All stage IV, Tumor rupture: n=2
    - Sx+chemo: n=4
    - MST>2 years
  - Flood-Knapik et al. VCO 2012
    - Is chemo necessary?
    - Obrien et al. JVIM 2013
    - Chemo did not help

Indolent LSA: Therapy for Multicentric

- Depends on variant
  - If treated, use a less aggressive protocol
    - Prednisone, Leukeran, CCNU
  - Mantle Zone LSA:
    - May progress to high-grade LSA late
  - T-Zone LSA
    - Often slow progression
    - May live years with lymphadenopathy
    - Is chemo necessary?
      - Flood-Knapik, et al.
Canine Indolent Lymphoma

- Lack of information in veterinary literature
  - Recent reports help define prognosis and optimal therapeutic approach
  - Still have a long way to go
- Underdiagnosed disease?
  - Lymphoid “hyperplasia”
- Diagnosis requires histopathology & IHC

Feline Low Grade Lymphoma

- AKA: Lymphocytic, well diff, small cell LSA
  - Likely represents 20-25% of feline LSA
- Pohlman et al. 2009 Vet Path (n=50)
  - Gastric (n=12): B cell, high grade
  - Intestinal (n=37): T cell (n=19), B cell (n=14)
    - Small cell/lymphocytic n=15
    - Often classified epitheliotropic

Clinical Signs
Weight loss (83%), Vomiting (73%), Anorexia (66%), Diarrhea (58%)
- Workup:
  - Anemia (25-50%), Leukocytosis (20%)
  - Low cobalamin (78%)
  - U/S: enlarged mesenteric LN, thickened bowel

Diagnosis
- Cytology: often “reactive or inflammatory”
  - Lymph node or intestine
- Biopsy:
  - Needed for a definitive diagnosis
    - Full thickness > endoscopic
      - Evans et al. JAVMA 2006
- LSA vs IBD: Kiupel et al. Vet Path 2011
  - Full thickness biopsy
  - Immunohistochemistry
  - Parr
Therapy

- Leukeran and Prednisone
  - 15 mg/m² PO daily for 4 days vs. q 21-day cycle
  - 2mg eod
  - Vit B12 injections

- Response rate: 90-96%
  - Majority a complete response
  - Response duration ~750 days
  - Survival ~ 2 years
  - Rescue:
    - Cytoxan q 2 wks
    - COP

Follow up??

- Resolution of clinical signs
  - Monthly recheck exams
  - CBC/chemistry panel
- Weight gain
- Cobalamin levels?
- Abdominal ultrasound:
  - If mesenteric lymphadenopathy or mass

IBD → low grade LSA

- Continuum of disease?
  - Often concurrent IBD
  - Treat as such….
    - Dietary Modifications
    - Appetite stimulants
    - Vit B supplementation
    - Antinausea
    - Probiotic

Questions?

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