IHC Antibody Test Selection Using a Panel Approach

Steven Westra B.S.
Reagent Product Specialist
Leica Biosystems

IHC Panels as an **Aid** in Diagnostic Decision Making

Diagnostic Use of Tumors Using Algorithms

- Utilizes a panel of antibodies intended to solve a diagnostic problem
- Many diagnostic algorithms exist
- The panel of antibodies selected should be based on the morphological appearance of the tissue

Diagnosis of Tumors using Algorithms

- A diagnostic algorithm is a method which utilizes a panel of antibodies intended to solve a diagnostic problem
- Many different diagnostic algorithms exist and are available in journals and text books
- A diagnostic algorithm is followed by a selective markers for tumor sub classification.
- The panel of antibodies selected should be based on the morphological appearance of the tissue and the patient's clinical history provided by the physician.

Living up to Life

Why are panels important?

- Increase the number of diagnostic tools for the pathologists
- See a macroview of the disease state
- Faster turn around time
- No single antibody is 100% sensitive and specific
- More specific diagnosis leads to more specific treatment

Living up to Life

CD Antibodies for Phenotyping
(Formalin-Fixed Paraffin-Embedded Tissues)

Antibody	Neoplasm and/or Cell Target
CD3	Pan T-cell
CD5	T-cell/mantle cell
CD4	T-cell (helper/inducer)
CD8	Cell-mediated cytotoxicity
CD15	Hodgkin's (Reed Sternberg)
CD20	B-cell lymphoma
CD21	B-cell, follicular lymphoma
CD22	B-cell lymphoma
CD23	Small lymphocytic and follicular lymphoma
CD30	Anaplastic large cell and Hodgkin's

Living up to Life

CD Antibodies for Phenotyping
(Formalin-Fixed Paraffin-Embedded Tissues)

Antibody	Neoplasm and/or Cell Target
CD43	Pan T-cell and low grade B-cell
CD45	Pan marker for lymphoma
CD45RB	Pan marker for lymphoma
CD45R0	Pan T-cell
CD68	Macrophage and histiocytic
CD74	B-cell lymphoma
CDw75	B-cell lymphoma
CD79a	Pan B-cell

Living up to Life

CD Markers

- Most commonly used markers (CD = cluster designation)
  - B-cell – CD10, CD19, CD20, CD22, CD23, CD24, CD79b, CD103, Pax-5, kappa, lambda, CD200, cytoplasmic kappa, cytoplasmic lambda
  - T-cell – CD1, CD2, CD3, CD4, CD5, CD7, CD8, TCR α-β, TCR γ-δ, cytoplasmic CD3
  - Myeloid/monocyte – CD11b, CD13, CD14, CD14, CD15, CD33, CD64, CD117, myeloperoxidase
  - Miscellaneous – CD11c, CD16, CD25, CD30, CD34, CD38, CD41, CD42b, CD45, CD56, CD57, CD61, HLA-DR, glycophorin, TdT, bcl-2

Living up to Life

CD Markers

CD1a, CD207: Langerhan cell histiocytosis cells
CD2, CD3, CD4, CD5, CD7, CD8: T cells
CD10: Early pre-B cells (immature B cells)
CD11c, CD25, CD103, CD123: Hairy cell leukemia cells
CD13, CD33, CD117: Myeloid cells
CD14, CD64: Monocytic cells (positive in AML-M4 and AML-M5)
CD15 :Reed-Sternberg cells, neutrophils
CD16, CD56: Natural killer cells
CD19, CD20, CD21, CD22 : B cells
CD23 and CD5 : Chronic lymphocytic leukemia/small lymphocytic lymphoma
CD23 negative and CD5 positive: Mantle cell lymphoma cells

Living up to Life

CD Markers

CD30 and CD15: Reed-Sternberg cells
CD30 positive and CD15 negative: Anaplastic large cell lymphoma
CD31: Endothelial cells (positive in angiosarcoma)
CD33: Myeloid cells and precursors
CD34: Stem cells (also positive in angiosarcoma)
CD41, CD61: Megakaryocytes and platelets (positive in AML-M7)
CD45 : All leukocytes (except Reed-Sternberg cells!)
CD45 RO: Memory T cells
CD45 RA: Naive T cells
CD68: Histiocytes (positive in malignant fibrous histiocytosis)
CD99: Ewings sarcoma cells
CD117: Gastrointestinal stromal tumor (GIST) cells, mast cells (positive in mastocytosis), myeloid cells

Living up to Life

CD Markers

- CD71 All proliferating cells; erythroid precursors through reticulocytes, capillary endothelium in brain
- CD123 Acute Myeloid Leukemia
- CD138 B cell precursors, plasma cells, stratified squamous epithelium

Living up to Life

B-cell lymphoproliferative disorders

Probable if immunoglobulin light chain restriction is demonstrated by surface typing of kappa or lambda

B-cell CLL or mantle cell lymphomas (MCL) are suspected if CD5 is positive and CD10 is negative

Circulating MCL can be mistaken morphologically for B-cell CLL or B-cell prolymphocytic leukemia (B-PLL)

MCL considered in the following
- CD20, CD19 – strong intensity
- Surface immunoglobulin – strongly expressed
- CD23 – absent
- Diagnosis
  - Molecular and FISH testing
  - Requires t(11;14) translocation demonstration

CLL is more likely when
- CD20 – weak intensity
- Surface immunoglobulins – weakly expressed
- CD23 – present
- CD200 – present

Living up to Life

B-cell lymphoproliferative disorders

Circulating germinal center cell-derived lymphoma is probable if CD10 is positive and CD5 is negative

- **Germinal center lymphomas** – follicular, Burkitt lymphoma, diffuse large B-cell lymphoma (DLBCL)

Marginal zone lymphoma should be considered if both CD5 and CD10 are negative

Hairy cell leukemia (HCL) has a characteristic phenotype that is CD5-, CD10-, CD11c+, CD22+, CD25+, and CD103+

- CD103 antigen (also known as B-ly7) is present in virtually all cases
- CD11c and CD25 are less specific but present in almost all cases of hairy cell leukemia
- HCL variant can be considered in otherwise typical cases of HCL when CD25-

Living up to Life

Examples of common panels of Antibodies Used

Generic T-cell Vs B-Cell : CD3, CD20, CD45

Folicular Lymphoma Vs Hyperplasia: Bcl2, **Bcl6, CD3, CD10,CD20**

Low Grade B Lymphoma: CD3, CD5, CD10, CD20, CD23, CD43, Bcl2, Bcl6,

MALT Lymphoma: CD3, Cd5, CD20, Bcl2, ISH Kappa and Lambda

Hodgkin's Lymphoma: CD3, CD15, CD20, CD30, CD45

Myeloma: CD138, ISH Kappa and Lambda

Carcinoma Vs Lymphoma: CD3, CD20, CD45, PanCK

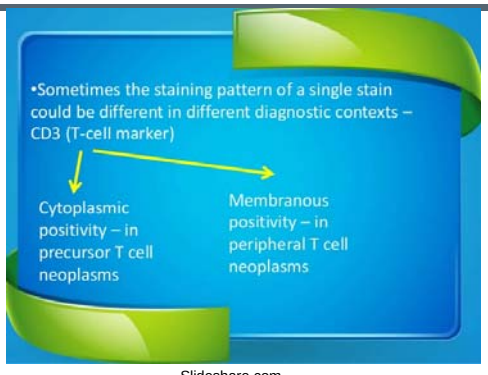
Metastatic Carcinoma: CK7, CK20, TTF-1

GIST: CD117, CD34, S100, Desmin, SMA

Mesothelioma: PanCK, CK5/6, Calret, TTF-1, CEA, CD15

If male add PSA/ if female add BRST2

Living up to Life



Slideshare.com

Living up to Life

Immunophenotypic Findings in Classical Hodgkin's Lymphoma

- ✓ positive for CD15 and CD30 and
- ✓ negative for LCA (CD45) and EMA .
- ✓ B-cell antigens—such as CD20, CD79A, PAX-5/BSAP, and MUM1/IRF4—are expressed in a subset of cases.
- ✓ CD20 expression is often weak.
- ➢ T-cell antigens are usually not expressed by the neoplastic cells.
- ➢ BCL-2 is positive in up to half the cases and has been correlated with poorer prognosis.
- ✓ EBV is common in the Reed-Sternberg and Hodgkin cells of classic HL

Living up to Life

Mantle Cell Lymphoma

- Mantle Cell Lymphoma has a generally poor prognosis
- Median survival is 3 to 4 years
- MCL is not curable by conventional chemotherapy and most patients succumb to organ dysfunction due to tumor infiltration

Living up to Life

Hairy Cell Leukemia

- CD20+
- CD5-
- CD10-
- CD23-
- CD11c++
- CD25+
- CD103+

Living up to Life

Chronic Lymphocytic Leukemia

- CD20+ (dim)
- CD5+
- CD23+
- Cyclin D1-
- CD3-

Living up to Life

Chronic Lymphocytic Leukemia

- The most common leukemia of adults in the Western world.
- Tumor cells resemble a small subset of circulating B-cells *that express CD5*.
- Most patients are males over 50 years of age
- Patients with CLL are often asymptomatic
- When symptoms are noticed, they are often non-specific (fatigue, weight loss)

Living up to Life

T-cell lymphoproliferative disorders

Most show abnormalities of pan T-cell antigens CD2, 3, 5, or CD7

T-cell disorders

Proliferating lymphocytes are usually positive for CD3
Most common form is large granular lymphocytosis

Large granular lymphocytosis is suspected if percentage of CD16+, CD56+, or CD57+ T cells is >50% or if absolute count of these cells >2,000/μL

Angioimmunoblastic lymphoma has characteristic CD10+ and CD4+, and CD52-, CD56-, and CD16-

Anaplastic large cell lymphoma – CD30+ and ALK(+)
Some pan T-cell antigens are frequently deleted

Living up to Life

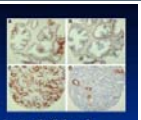
Immunohistochemistry

Cytokeratins (CKs)

- 20 sub-types of CK with different molecular weight and different expressions are seen in various cancers and cell types.
- Monoclonal antibodies to specific CK sub-types are used classify tumors according to site of origin.
- CK20/ CK7 are most useful and hence, most commonly used.
- *CK7* → seen in lung, breast, ovary and endometrium
  NOT seen in lower GI tract
- *CK20* → *seen in GI epithelium, urothelium and Merkel's cells.*
- *A pattern of CK20+/CK7- strongly suggests GI neoplasm*
- *A pattern of CK20-/CK7+ suggests cancer of lung, breast, ovary, endometrium and pancreatic biliary tract.*

Living up to Life

Cytokeratin phenotype



CK phenotype	Tumors
CK7 (-) / CK 20 (-)	HNC, Liver, Lung (SqCC and SmCC),Prostate, Renal
CK7 (+) / CK 20 (-)	Biliary and Pancreas, Breast, Cervical, EM, Lung(Ad ca), Ovarian (non-mucinous), thyroid
CK7 (-) / CK 20 (+)	Colon, Gastric, Markel C ca
CK7 (+) / CK 20 (+)	Biliary and Pancreas, Ovarian (Mucinous), Urothelial.

Source: Adopted from Dennis JL. Clin Cancer Res 2005[18]

Living up to Life

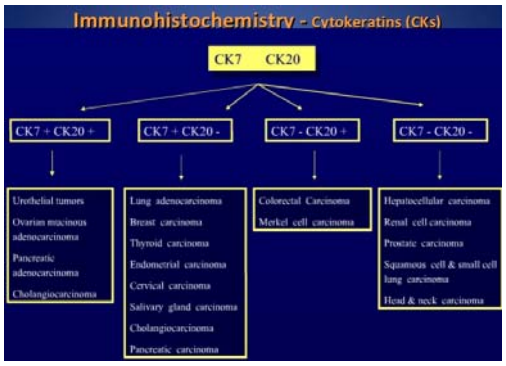
Differential Diagnosis

Bladder vs Prostate ca

Cytokeratin 7	+	(-)
Cytokeratin 20	+	(-)
CEA	+	(-)
PAP	(-)	+
PSA	(-)	+

Goldstein N.Immunohistochemical Antibody Panels to help Identify the Primary Sites of Various Carcinomas.ASCP Teleconference # 2109 16.

Living up to Life



Living up to Life

Infectious Organisms

- H. Pylori
- CMV
- HSV
- EBER

Living up to Life

Squamous and Basal Cell Carcinoma

- Most common cancer in the US
- 1% of all cancer deaths
- Excellent prognosis if early
- Fatal if neglected

Living up to Life

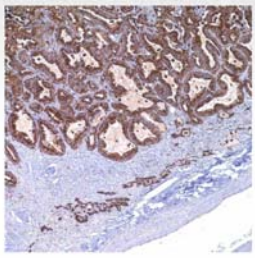
Lung Adenocarcinoma vs. Squamous Cell Carcinoma

Napsin A +	TTF-1 +	CK 5/14 -	Sox-2 -	Well Differentiated Lung Adenocarcinoma
Napsin A +	TTF-1 -	CK 5/14 -	Sox-2 -	Poorly Differentiated Lung Adenocarcinoma
Napsin A -	TTF-1 +	CK 5/14 -	Sox-2 -/+	Neuroendocrine Tumor (verify w/NE marker)
Napsin A -	TTF-1 -	CK 5/14 +	Sox-2 +	Squamous Cell Carcinoma

Living up to Life

CELL MARQUE

Napsin A

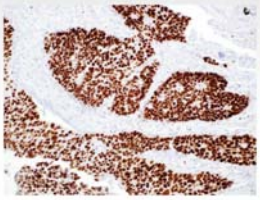


- Clone: Polyclonal
- Visualization: Cytoplasmic
- Lung adenocarcinoma
- Multiple panel applications
- Higher sensitivity and specificity compared to TTF-1
- USCAP 2010, IAP 2010

From Passion to Product to Patient®

CELL MARQUE

SOX-2



SOX-2 on Lung Squamous Cell Carcinoma

- Clone: SP76
- Visualization: Nuclear
- Rabbit Monoclonal
- Differentiates lung squamous cell carcinoma from lung adenocarcinoma
- Distinguishes embryonal carcinoma from other germ cell tumors
- Useful in the identification of astrocytomas
- Important for general pathologists and GU pathologists

From Passion to Product to Patient®

Master List for Immunohistochemistry

(Continued)

Macrophage	CD68, CD163, MAC387, D11, LN5, HAM56, AAT, AACT
Melanoma	S100, HMB45, Melan A (MART-1), Microphthalmia, P75, Tyrosinase, NCI/CB,
Ovary	CA125, PLAP, COTA, Pan Melanoma Cocktail, MAGE-1, Survivin
Pancreas	NSE, Chromogranin, Insulin, Synaptophysin, PDX-1, Mucin5AC
Pituitary	ACTH, FSH, TSH, LH, Prolactin, Human Growth Hormone
Prostate	PSA, PSAP, p63, PIN-4, P504S, Androgen Receptor, HMW CK, CD57, TURP 27, Vasculr Endothelial Growth Factor (VEGF)
Sarcoma	Pan Actin, Vimentin, Smooth Muscle Actin, Desmin, Myogenin, CD34, CD99. Fli.1
Skin	Pan CK, HMW CK, Neurofilament, Factor XIIIa, S100, HMB 45, Cytokeratins
Thyroid	Thyroglobulin, TTF-1, Calcitonin, LMW CK, CK19
Vascular	Factor VIII, CD34 (Qbend/10), CD31, VEGF, Ulex, CD105

Distribution of Cytokeratin

Cytokeratins are also expressed in pairs comprising of type 1 (9-20) and type II (1-8), and acidic and basic comprising of 1-6 (pH7.3 to 7.8) and 7-20 (pH 4.9 to 6.0).

Number	MW (kD)	Distribution in Tissues
1 & 10, 2	68, 56.5, 65.5	Cornifying stratified; skin, endocervix
3 & 12	63, 55	Human cornea
4 & 13	59, 58	Non-cornifying stratified squamous epithelia; tongue, esophagus
5 & 14, 15	58, 50, 50	Simple stratified epithelium; epidermis, squamous cell carcinoma
6 & 16	56, 48	Fast turnover cells; hair follicles, suprabasal cells, activated keratocytes
7 & 19	54, 40	Simple and glandular epithelium; breast, lung, bladder

(Continued)

Distribution of Cytokeratin

(Continued)

Number	MW (kD)	Distribution in Tissues
8, 18	52.5, 45	Simple and glandular epithelium
9, 10, 11	64, 56.5, 56	Cornified stratified epithelium, anal canal, epidermis, squamous cell carcinoma
17	46	Basal cells of complex epithelia; trachea, larynx, cervix, bronchi
19	40	Ductal and glandular epithelium; liver cancer, adenocarcinoma, hair follicles
20	46	Gastric and intestinal, urothelium, Merkel cell

Classification of Cytokeratin Antibodies

Type: Clone: Mol:	Pan Cytokeratin AE1/AE3 1, 2, 3, 4, 5, 6, 7, 8, 10, 14, 15, 16, 17, 19
Type: Clone: Mol:	Pan Cytokeratin Lu-5 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 13, 14, 15, 16, 17, 18, 19
Type: Clone: Mol:	Pan Cytokeratin MAK-6 8, 14, 15, 18, 19
Type: Clone: Mol:	Pan Cytokeratin (LMW) AE1 10, 14/16, 16/17, 19
Type: Clone: Mol:	Pan Cytokeratin (HMW) AE3 1, 2, 3, 4, 5, 6, 7, 8
Type: Clone: Mol:	LMW Cytokeratin 5D3 8/18
Type: Clone: Mol:	HMW Cytokeratin 34ßE12 (903) 1, 2, 5, 10, 14/15
Type: Clone: Mol:	HME Cytokeratin DE-SQ 13, 14, 15, 16

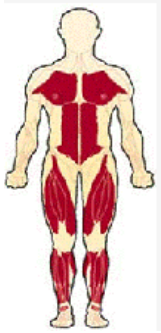
Living up to Life

Duchenne Muscular Dystrophy

Living up to Life

DMD and BMD

In the early stages, Duchene and Becker Muscular Dystrophy affect the pectoral muscles, the trunk, and the upper and lower legs. These weaknesses lead to difficulty in rising, climbing stairs and maintaining balance.



Living up to Life

Summary

- Consider “multi-purpose” antibodies
- Avoid employing antibodies if not ordered greater than three times per week
- Avoid employing novel antibodies until specificity is sufficiently established