Applications of IHC

- Determination of the primary site in **metastatic tumors of unknown origin**
- **Classification of tumors** that appear 'undifferentiated' by standard light microscopy
- Precise classification of **leukemias and lymphomas** in routine specimens
- Determination of estrogen **receptors**, progesterone receptors & HER-2 overexpression status in breast cancer patients
- Identification of **micrometastatic** disease in sentinel lymph node biopsies
- Identify markers of targeted therapy , **THERONOSTICS**.

THERONOSTICS

New title: ***Diagnostic Immunohistochemistry with Theranostic and Genomic Applications*.**

David J. Dabbs

"It is not easy to have a publisher change a title, but IHC is not just useful in diagnosing carcinoma, melanoma, germ cell tumors, lymphoma, and sarcoma. "

"There is a whole battery of new applications that is available now in solid tumors. We are using molecular morphology of IHC and tissue to set the stage for treatment and further genetic testing for which the patient may be eligible."

IHC is far more powerful than it was two or three decades ago.

"We pathologists need to think about how to incorporate new markers and knowledge into our daily clinical pathology practice."

Immunohistochemistry : Total test approach

Elements of testing process	Responsibility of
1.Test selection; the clinical question	Surgical pathologist; sometimes clinician
2. Specimen acquisition and management	Pathologist / technologist
3.Technology/methodology	Pathologist / technologist
4.Analytical issues	Pathologist / technologist
5. Results: validation/reporting	Pathologist / technologist
6.Interpretation, significance	Surgical pathologist and / or clinician
The total test approach (Taylor 1992a,1992b,1993,1994b)	

BREAST

"Highlights of the St Gallen International Expert Consensus on the primary therapy of Breast cancer 2009" in Annals of Oncology July 2009

	Indicators for chemotherapy	**Factors not useful for decision**	**Relative indicators for endocrine therapy alone**
Clinicopathological features			
ER & PR	Low ER & PR		High ER & PR
Histological grade	Grade 3	Grade 2	Grade 1
Proliferation (Mitoses & Ki-67)	High	Intermediate	Low
Nodes	Positive (4 or more involved)	Positive (1 to 3 involved)	Node negative
Peri- tumoral vascular invasion	Extensive		Absence
pT size	> 5 cm	1 – 5 cm	Less than or equal to 2cm
Patient preference	Use all available treatments		Avoid chemotherapy related side effects
Multigene assays Gene Signature (e.g. **Mammaprint**)	High score		

ER & PR

- **Quick score** : Scores are summed to give a maximum of 8 (Commonly used)

Score for proportion

- 0 = No staining
- 1 = <1% nuclei staining
- 2 = 1-10% nuclei staining
- 3 = 11-33% nuclei staining
- 4 = 34-66% nuclei staining
- 5 = 67-100% nuclei staining

Score for intensity

0 = No staining

1 = Weak staining

2 = Moderate staining

3 = strong staining

- Score– 2 or < 2, negligible chance of response (May benefit from adjuvant Rx)

- **H score** – Different degrees of reactivity (Maximum score of 300 if 100 cells show strong positivity)

BASIS FOR TREATMENT

Quick Score

- 0 – Hormonal therapy will not work
- 2-3 – A small 20% chance of treatment response
- 4-6 - An even 50% chance of treatment response
- 7-8 – A good 75% chance of treatment response

ASCO/CAP GUIDELINES FOR C-erbB2 / HER-2neu reporting

Score to report	Overexpression assessment	Staining pattern
0	Negative	No staining or membrane immunoreactivity in < 10% of tumour cells
1+	Negative	A weak incomplete membrane immunoreactivity in > 10% of tumour cells.
2+	Borderline/Equivocal	A weak to moderate complete membrane immunoreactivity in >10% of tumour cells.
3+	Positive/Overexpressed	A strong complete membrane staining is observed in > 30% of the tumor cells

Note: FISH should be used as a secondary test in the equivocal 2+ category.

CISH : > 6 Her 2 Signals /Nucleus
FISH : > Ratio of Her 2 gene copies to chromosome 17 centromers > 2.2

LYMPH NODE

COMMON QUESTIONS

? Reactive
? Hodgkin
? Non hodgkin, if yes whether CD 20 positive
? Burkitt's
? Lymphoma leukemia esp lymphobastic
? Metastasis

MALIGNANT ROUND CELL TUMOURS

MALIGNANT SMALL ROUND CELL TUMOUR

	LYMPHOMA	NEUROBLASTOMA	RHABDOMYOSARCOMA	EWING'S / PNET
LCA	+	-	-	-
Neural Markers Syn , NSE, Chromo	-	+	-	-/+
Muscle markers Desmin, Myo D	-	-	+	-
CD 99	+/-	-	-/+	+

IHC IN LUNG CARCINOMA

- Small cell carcinoma – Chromogranin, synaptophysin, CD56(NCAM), TTF-1, CK7, Ki-67 > 90%
- Squamous cell carcinoma – TTF-1-negative and p63/CK5/6/34βE12-diffuse
- Spindle cell carcinoma – CK, Vimentin, TTF-1
- Non – mucinous Adenocarcinoma – CK7, CEA, Vimentin, TTF-1, Napsin A
- Mucinous adenocarcinoma – Difficult to differentiate from pancreatic & upper GI mucinous as CK 7 , 20 may be Pos & TTF 1 Neg (Pos only in 10 – 20 %)

LUNG CANCER

- Treatment options are evolving with new therapeutic agents that target specific pathways in tumours that may be up-regulated by amplification or mutation [e.g. inhibitors of epidermal growth factor receptor (EGFR) and VEGF]

- Memorial Sloan Kettering – Reflex testing – EGFR exon 19 deletions & exon 21 L858R mutations ; If lacking EGFR then k ras ; if that too negative then very rarely ALK asscociated

- Patients with lung cancer may have biopsies for histological confirmation of a diagnosis of carcinoma and then a panel of genetic markers to determine expression of prognostic markers and markers of drug sensitivity

- Tissue biopsy specimens may be required rather than cytology specimens in order to yield sufficient material for examination, and parallel samples may have to be snap frozen or placed in special carrier buffers

Molecular Subtypes

Sub-type	Description	Pathway	Potentially relevant therapies	Relevant histological subtypes	Strength of evidence for clinical use*
1.1	EGFR sensitizing mutations	EGFR	TKIs & chemotherapy	Adenocarcinoma	High
1.2	EGFR resistance mutations including T790M	EGFR	Dual EGFR/HER2 TKI, c-MET inhibitors +/− 1st or 2nd generation EGFR TKIs, Hsp90 inhibitors, dual MET/VEGFR2 inhibitors, Chk1 inhibitors	Adenocarcinoma	High
1.3	VeriStrat proteomic signature	EGFR	TKIs & bevacizumab	Adenocarcinoma	High
2.1	K-ras mutations	K-ras	Dual MAPK & AKT/PI3K inhibitors, Hsp90 inhibitors	Adenocarcinoma	High
3.1	EML4-ALK	EML4-ALK	ALK inhibitors, Hsp90 inhibitors	Adenocarcinoma	High

doi:10.1371/journal.pone.0031906.t001

Carcinoma of Unknown Primary

CK 7 + CK 20 +	CK 7 – CK 20 -	CK 7 + CK 20 -	CK 7 – CK 20 +
Urothelial Ca	HCC	Breast Ca	Colorectal Ca
Pancreas Ca	RCC	Lung NSCA	
Ovarian Mucinous Ca	Prostatic Ca	Ovarian Serous Ca	
	Sq Ca	Mesothe-lioma	
	Neuroendo Ca	Endomet Ca	

KEY DIAGNOSTIC POINTS
Sex Cord–Stromal Tumors

- Can be either **positive or negative for CK**.
- Are almost always **EMA negative**. Positive staining for EMA suggests an epithelial tumor, either primary or metastatic, that is mimicking a sex cord–stromal tumor.
- **Inhibin** is a relatively specific marker.
- **Calretinin** is a more **sensitive**, but less specific marker than inhibin.
- Other markers include **CD56, WT1,** and **SF-1, Melan A** & **CD 99**.

PROSTATE : BENIGN vs MALIGNANT

- Confirming prostate carcinoma and/or distinguishing it from its many benign mimics
- Basal cell–associated markers p63, high-molecular-weight CK, 34βE12, CK 5/6 and prostate carcinoma–specific marker α-methylacyl coenzyme A (coA) racemase (AMACR) are useful in confirming prostate carcinoma
- PIN 4 COCKTAIL
- Distinguishing prostate carcinoma from non prostatic malignancies that secondarily involve prostate
- Depends on the question e.g. CK 7 & CK 20 if to be differentiated from bladder primary

CISH

Chromogenic in situ hybridization (CISH), advantages :

1. Interpreted by the use of a bright-field microscope

2. Visualization of the nucleus and is also able to distinguish invasive from in situ carcinomas.

3. Signals do not generally fade over time allowing the tissue samples to be archived and reviewed later as compared to FISH.

4. CISH resembles IHC to a large extent (as opposed to FISH) due to the use of conventional counter stains, e.g.hematoxylin, for visualization of tissue morphology.

Because CISH combines the genetic information from FISH with the visualization and interpretation resembling IHC, the CISH technique is a practical and user friendly alternative to FISH.

The first description of the CISH procedure as a practical alternative to FISH for the detection of genetic alternations was published in 2000.

EBV

- CISH and PCR are equally sensitive tests while IHC is an insensitive technique.

- The IHC antibody detects EBV encoded recombinant LMP.

- EBV DNA by PCR is detected using specific primers in the conserved BAM-H1-W region of EBV genome and CISH detects EBV RNA in Formalin fixed paraffin embedded tissue (FFPE).

- In contrast to PCR , CISH allows identification and distinction of infected cell types e.g The correct diagnosis of EBV hepatitis requires detection of EBER in parenchymal cells and not periportal lymphocytes.