# APPLICATIONS OF IMMUNOHISTOCHEMISTRY IN CHARACTERIZATION OF PATIENT DERIVED XENOGRAFT MODELS

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## INTRODUCTION

**Well characterized patient derived xenograft models (PDX) are becoming the preferred pre-clinical tool in every aspect of translational cancer research, including biologic understanding of the disease, development of new treatments and identifying potential therapy predictive and resistance biomarkers. Establishing a repository of well characterized PDX models of different cancer types is a critical first step in using this platform for pre-clinical research. Characterization of PDX models using a multi-omic approach is most desirable, although such efforts can be very expensive and technically demanding. The continuous discoveries of tissue-specific biomarkers has made immunohistochemistry (IHC) an indispensable ancillary tool. Utilizing IHC can also be crucial during propagation of PDX models as certain aberrations in biological characteristics may affect the validity and reliability of the model for downstream research analysis. Here we present a panel of essential antibodies and the testing strategy employed in our lab for histopathologic assessment of PDX models and their subsequent passages. We also present certain commonly encountered challenges during PDX development to depict the utility of this IHC panel, including accurate histomorphologic classification of tumors, identifying subclonal outgrowth and tumor evolution, identifying murine tumor, identifying malignant transformation of lymphoid/stromal elements, and evaluation for the presence/absence of therapeutic or prognostic biomarkers.**

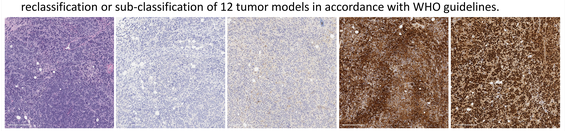
## IMMUNOHISTOCHEMISTRY PANEL FOR CHARACTERIZATION OF PDX

| ANTIBODY | CLONE | VENDOR | DILUTION | ANTIBODY | CLONE | VENDOR | DILUTION |
|---|---|---|---|---|---|---|---|
| **Androgen Receptor (AR)** | [EPR1535(2)] | abcam | 1:500 | **FOXP3** | (5H10L18) | Invitrogen | 1:20 |
| **B-Catenin (FFPE and FF)** | [E247] | abcam | 1:500 | **GATA3** | [EPR16651] | abcam | 1:100 |
| **CD19** | polyclonal | abcam | 1:200 | **GCDFP-15** | [EPR1582Y] | abcam | 1:250 |
| **CD3** | polyclonal | abcam | 1:200 | **GFAP** | polyclonal | DAKO/Agilent | 1:400 |
| **CD20** | [SP32] | abcam | 1:100 | **Ki-67** | [D2H10] | Cell Signaling | 1:100 |
| **CD34** | [EP373Y] | abcam | 1:2500 | **Ku80** | [EPR3468] | abcam | 1:250 |
| **CD45** | polyclonal | abcam | 1:1000 | **MelanA** | [EPR20380] | abcam | 1:500 |
| **CD56 (NCAM1)** | [EPR2566] | abcam | 1:100 | **MGMT** | MT3.1 | Millipore | 1:200 |
| **CD68** | [EPR20545] | abcam | 1:4000 | **Mitochondria Marker (Biotin)** | MTC02 | abcam | 1:250 |
| **CDX2** | [EPR2764Y] | abcam | 1:750 | **Myogenin** | [EPR4789] | abcam | 1:500 |
| **Chromogranin A** | [SP12] | abcam | 1:100 | **NAPSIN A** | [EPR6252] | abcam | 1:3000 |
| **CK7** | [EPR1619Y] | abcam | 1:750 | **p63** | polyclonal | GeneTex | 1:100 |
| **CK19** | [EPR1580Y] | abcam | 1:3000 | **PD-1** | [EPR4877(2)] | abcam | 1:500 |
| **CK20** | [EPR1622Y] | abcam | 1:200 | **PD-L1 (CD274)** | RBT-PDL1 | LifeSpan Biosciences | 1:25 |
| **Cytokeratin wide spectrum (CK)** | polyclonal | abcam | 1:200 | **Progesterone Receptor (PR)** | [SP2] | abcam | 1:100 |
| **Desmin** | [Y66] | abcam | 1:100 | **Prostate Specific Antigen (PSA)** | [EP1588Y] | abcam | 1:200 |
| **EBV LMP1** | [D24-G] | abcam | 1:500 | **S100** | [EPR19013] | abcam | 1:1000 |
| **ErbB2 (Her2)** | [SP3] | abcam | 1:100 | **Smooth Muscle Actin (SMA)** | polyclonal | abcam | 1:1000 |
| **ERG** | [EPR3864] | abcam | 1:750 | **Synaptophysin** | [SP11] | abcam | 1:50 |
| **Estrogen Receptor (ER)** | [SP1] | abcam | 1:100 | **TTF1** | [SP141] | abcam | 1:50 |
| **FOXP1** | monoclonal | LifeSpan Biosciences | 1:50 | **Vimentin** | [EPR3776] | abcam | 1:2000 |

- 43 IHC assays were validated on the Leica Bond RX automated staining platform to identify common inconsistencies in PDX development including markers for classifying carcinomas, lymphomas, sarcomas, murine tumors, and theragnostic biomarkers. Rabbit antibodies are used rather than mouse antibodies to prevent non-specific staining of murine tissue.
- The staining protocol used is the standard Leica Bond *IHC Protocol F without the Post Primary step and with a 30-minute primary antibody incubation.
- Other staining conditions, such as epitope/antigen retrieval, are assay specific.

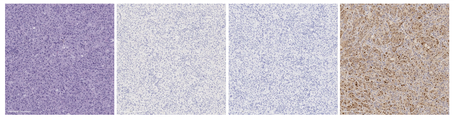
## ACCURATE HISTOMORPHIC CLASSIFICATION OF TUMORS

- IHC evaluation of models within NCI's Patient Derived Models Repository (pdmr.cancer.gov) led to reclassification or sub-classification of 12 tumor models in accordance with WHO guidelines.

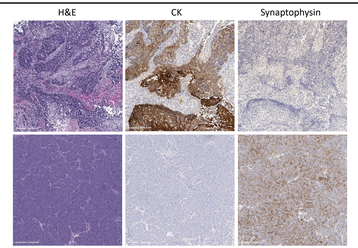



- Enrolled Patient diagnosis & subtype: Invasive breast carcinoma
- Morphological PDX assessment: Sarcoma or sarcomatoid carcinoma
  - Tumor consisted of sheets of elongated cells with scant cytoplasm and high-grade nuclear features (Passage 0-3).
- IHC Classification of PDX model: Angiosarcoma
  - Tumor was negative for cytokeratin and GATA-3, and positive for CD34 and ERG (Passage 1).
- On further review, the enrolling site confirmed presence of chest wall nodule consistent with post-radiation angiosarcoma.

## IDENTIFICATION OF SUBCLONE OUTGROWTH, TUMOR HETEROGENEITY, AND TUMOR EVOLUTION

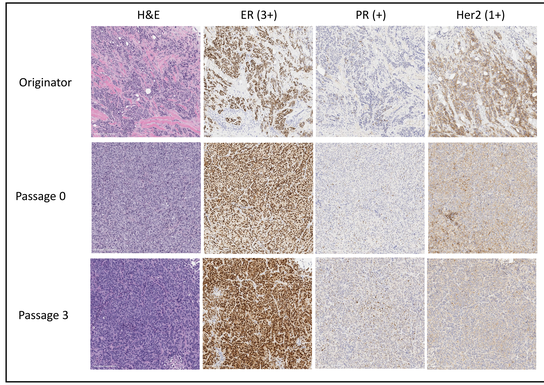


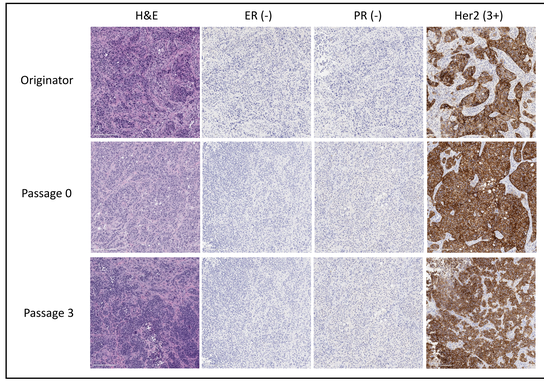

- Enrolled Patient diagnosis & subtype: Lung adenocarcinoma
- Morphological PDX assessment: High-grade carcinoma NOS
  - Tumor consisted of sheets of epithelial cells with abundant pink cytoplasm and round nuclei with prominent nucleoli (Passage 0-6).
- IHC Classification of PDX model: Lung Squamous Cell carcinoma
  - Tumor was negative for TTF-1 and NapsinA, and positive for p63 (nuclear), suggestive of clonal selection of squamous cell carcinoma during PDX development (Passage 3).




- Enrolled Patient diagnosis & subtype: Endometrioid endometrial adenocarcinoma
- Morphological PDX assessment: Poorly differentiated carcinoma
  - Tumor consists of sheets and nests of pleomorphic cells with scant cytoplasm and high grade nuclei showing salt & pepper chromatin. Abundant mitosis is present (Passage 0-2).
- IHC Classification of PDX model: High grade neuroendocrine carcinoma
  - PDX outgrowth is negative for cytokeratin and positive for synaptophysin consistent with high grade neuroendocrine carcinoma (Passage 2).

## ASSESSMENT OF STABILITY OF THERAGNOSTIC MARKERS THROUGH PASSAGING



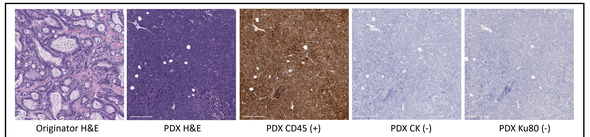




- Immunohistochemical analysis of ER,PR, and Her2 status in 22 invasive breast cancer models confirms stability of these markers with passaging in all models, the majority assessed through Passage 3.

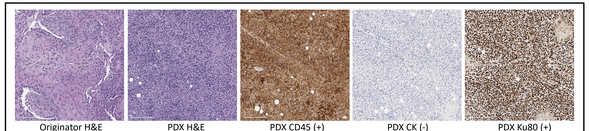
| IHC Analysis | #Models |
|---|---|
| ER+, PR-, Her2 (negative or equivocal) | 2 |
| ER+, PR+, Her2 (negative or equivocal) 1st example above | 2 |
| ER-, PR+, Her2 (negative or equivocal) | 1 |
| ER-, PR-, Her2 (negative or equivocal) | 16 |
| ER-, PR-, Her2 (**3+**) 2nd example above | 1 |

## IDENTIFICATION OF MALIGNANT TRANSFORMATION OF MURINE OR HUMAN LYMPHOID CELLS

- Malignant transformation of murine or human lymphoid cells is observed at a rate of 2.5%.




- Morphologic assessment of Originator specimen shows tumor consisting of poorly formed glands with elongated and hyperchromatic nuclei. Variable mucin production is identified. These features are consistent with pancreatic adenocarcinoma.
- However, on passaging, tumor is comprised of non-coherent cells with scant cytoplasm and pleomorphic nuclei with variable nucleoli. These cells are negative for cytokeratin and Ku80, and positive for CD45, consistent with lymphoma of murine origin.




- Morphologic assessment of Originator specimen shows tumor consisting of sheets of epithelial cells with abundant pink cytoplasm and round nuclei with prominent nucleoli within a background of stromal inflammatory infiltrate. These features are consistent with squamous cell carcinoma.
- However, on passaging, tumor is comprised of non-coherent cells with scant cytoplasm and pleomorphic nuclei with variable nucleoli. These cells are negative for cytokeratin and positive for CD45 and Ku80, consistent with lymphoma of human origin.

## SUMMARY

- Our validated panel of rabbit monoclonal antibodies demonstrate the exceptional specificity, sensitivity, and performance required for credible results in challenging applications such as IHC.
- IHC evaluation of models within NCI's Patient Derived Models Repository (pdmr.cancer.gov) led to reclassification or sub-classification of 12 tumor models in accordance with WHO guidelines.
- IHC evaluation of theragnostic markers in 22 breast cancer PDX models showed concordant results throughout passaging, suggesting stability of these biomarkers in our models.
- We observe malignant transformation of murine or human lymphoid cells at a rate of 2.5%. On IHC analysis, 52% were human lymphomas, 20% were murine lymphomas, and 28% were other murine tumors.
- IHC is a rapid, cost-effective tool that can be used for accurate tumor classification, identifying subclonal outgrowth and tumor evolution, assessing stability of biomarkers and identifying malignant transformation of benign tissue.

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