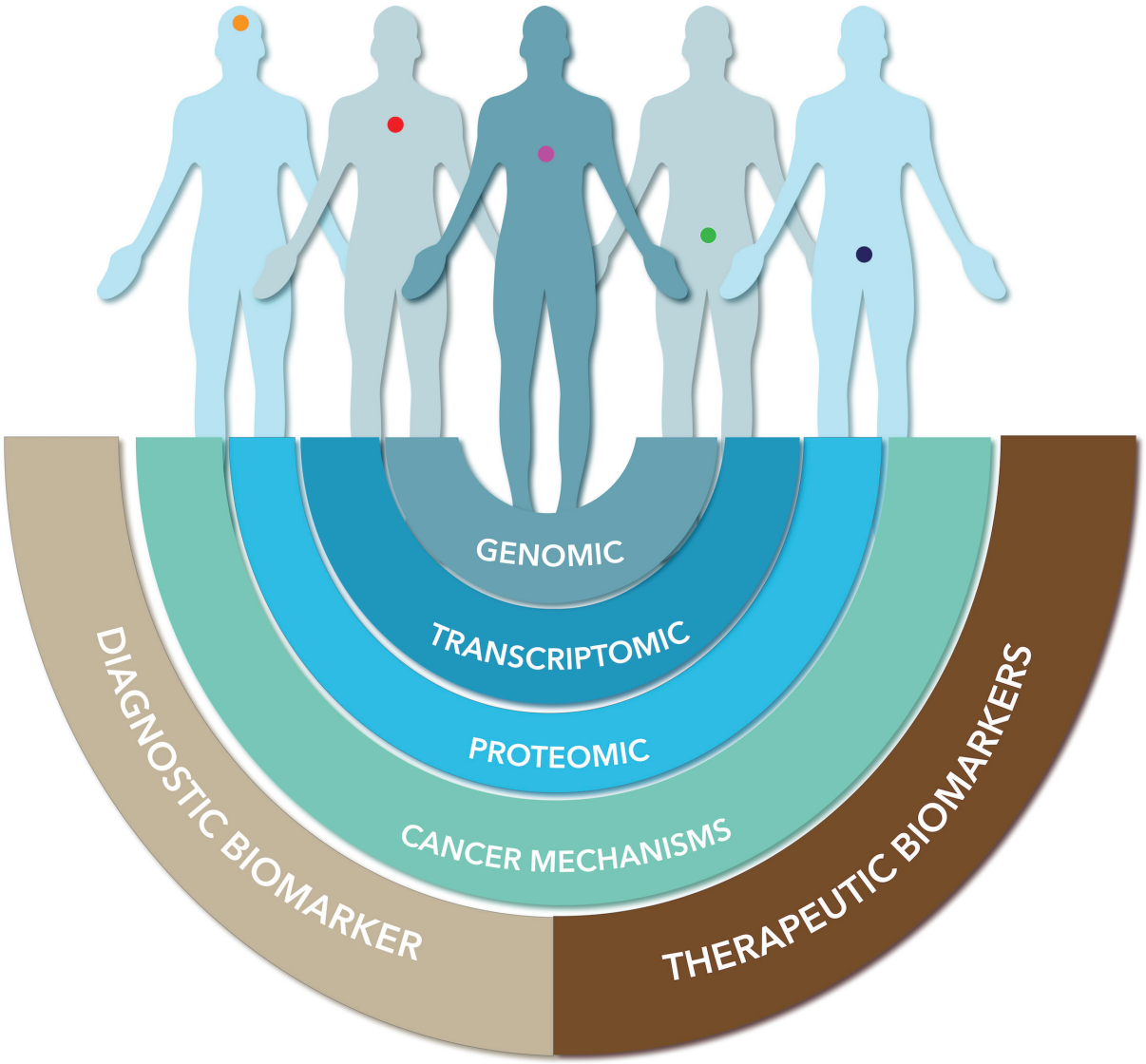
Pan-Cancer Markers for Proteomic Phenotyping



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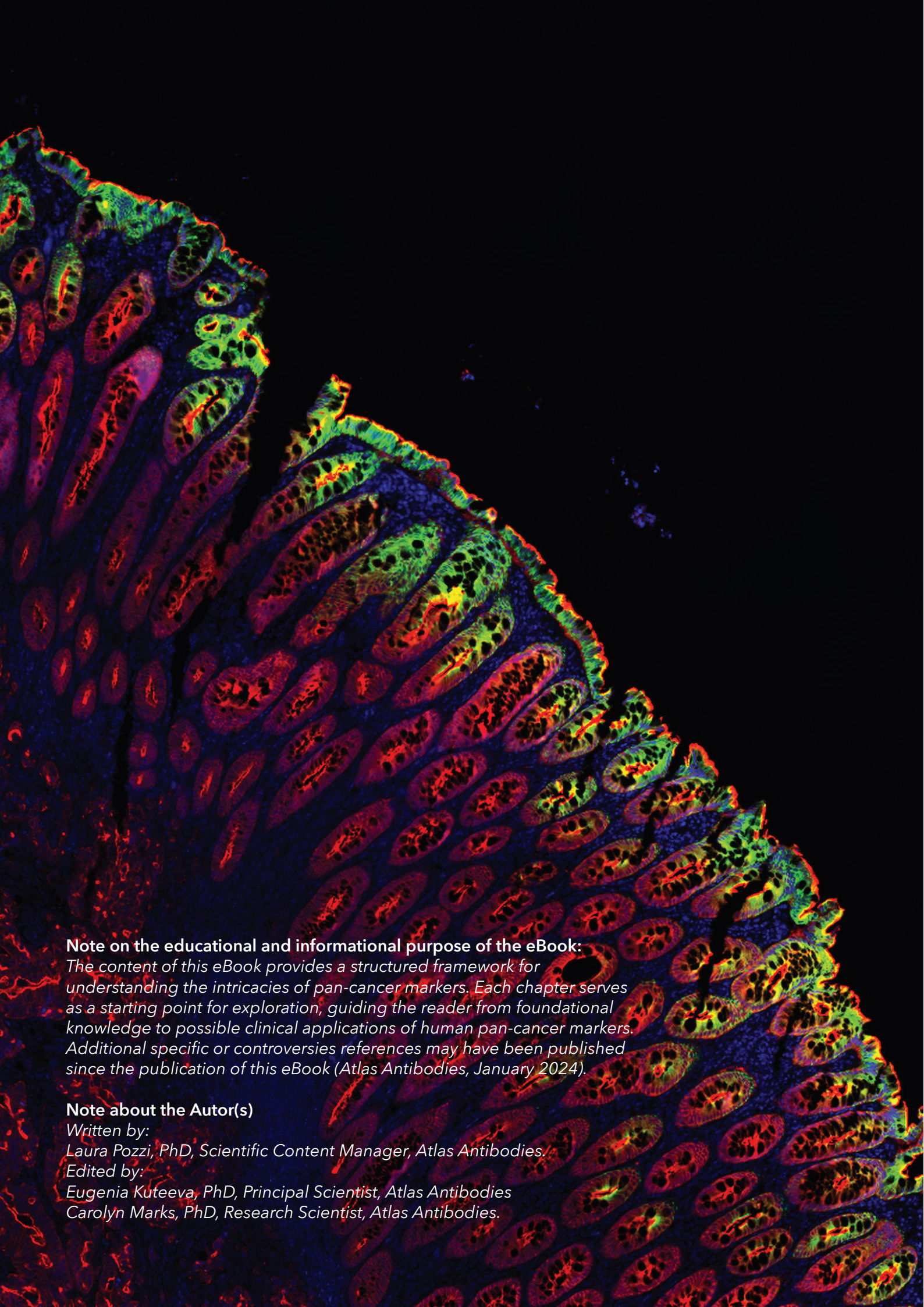

Note on the educational and informational purpose of the eBook:
The content of this eBook provides a structured framework for understanding the intricacies of pan-cancer markers. Each chapter serves as a starting point for exploration, guiding the reader from foundational knowledge to possible clinical applications of human pan-cancer markers. Additional specific or controversies references may have been published since the publication of this eBook (Atlas Antibodies, January 2024).

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Introduction

The field of cancer research is dynamic, and new biomarkers are continuously being discovered.

The number of known tumor markers and pan-cancer markers is substantial, and researchers continue to identify and validate new markers through advancements in technologies like genomics, proteomics, and metabolomics.

This dynamic process contributes to a deeper understanding of cancer biology and enhances the potential for more targeted and effective diagnostic and therapeutic approaches.

As we gain deeper knowledge into the intricacies of cancer, it becomes increasingly evident that the one-size-fits-all approach to diagnosis and treatment is no longer sufficient.

Cancer, as a complex and dynamic disease, exhibits remarkable heterogeneity, both within and between individuals. Understanding this heterogeneity is paramount for developing targeted and personalized therapeutic strategies.

In cancer diagnosis and monitoring, a range of biomarkers, including specific proteins, genetic mutations, and other molecular signatures, are used to provide a more accurate and comprehensive understanding of the disease. These biomarkers are often specific to certain types of cancer or even subtypes within a particular cancer type.

While each cancer possesses its unique molecular profile, several malignancies exhibit shared driver mutations. The concept of pan-cancer markers revolves around the examination of commonly mutated genes and genomic abnormalities present in diverse cancers, irrespective of their origin.

The identification and understanding of pan-cancer markers contribute to a broader understanding of shared pathways and mechanisms in cancer development, potentially leading to the development of universal diagnostic or therapeutic strategies applicable to multiple cancer types.

Utilizing advanced sequencing technologies like next-generation sequencing (NGS), initiatives such as The Cancer Genome Atlas TCGA have played a pivotal role in enhancing our comprehension of DNA and RNA variants across a multitude of cancer types.

However, to fully unravel the complexities of human cancer, we need a holistic approach that combines the wealth of genomic information with the nuanced insights provided by proteomic studies.

Navigating the intricate interplay between genes and proteins holds the key to unlocking a deeper understanding of cancer biology, paving the way for innovative diagnostics and targeted therapies.

In this dynamic landscape, the integration of genomics and proteomics presents both challenges and unparalleled opportunities.

The evolving landscape of pan-cancer markers holds promise for advancing precision medicine, allowing for more targeted and effective interventions across a diverse spectrum of malignancies.

This e-book takes you on an introductive journey through the concept of pan-cancer markers highlighting the need to a more comprehensive approach in cancer research by integrating the genomic and transcriptomic landscape with proteomic studies.

- Chapter 1 introduces the concept and the significance of biomarkers in deciphering cancer's complexity.

- In Chapter 2, we explore the genomic aspects, from the influential Cancer Genome Atlas to the Pan Cancer Atlas, identifying common molecular pathways in human cancer.

- Chapter 3 unravels the impact of proteome changes in tumorigenesis and the potential of proteomic approaches in cancer therapy.

1. Understanding Cancer Biomarkers

Cancer biomarkers serve as crucial indicators of the presence, progression, and characteristics of cancer.

While some biomarkers are specific to certain cancer types or tissues (cancer markers), providing valuable diagnostic and prognostic information, others exhibit pan-cancer characteristics (pan-cancer markers), offering insights into shared molecular features across diverse malignancies.

1:1
Tumor Markers: Specific for a Certain Cancer Type or Tissue

The role of biomarkers in understanding cancer heterogeneity cannot be overstated.

The field of cancer research is dynamic, and new biomarkers are continuously being discovered. The number of known tumor markers is substantial, and researchers continue to identify and validate new markers through advancements in technologies like genomics, transcriptomics, proteomics, and metabolomics.

Tumor markers specific to cancer types or tissues play a pivotal role in precision medicine. These molecular signatures not only aid in classifying tumors into distinct subtypes but also guide the selection of targeted therapies, thereby enhancing the precision and efficacy of cancer treatments **(Figure 1A, B).**

Tumor markers are often proteins, genetic mutations, or other molecular features unique to a specific cancer:

HER2 in Breast Cancer
The HER2 protein is a well-known biomarker for breast cancer, guiding the use of targeted therapies like trastuzumab.

IDH in Gliomas
In gliomas, particularly glioblastoma multiforme (GBM), the presence of mutations in the isocitrate dehydrogenase (IDH) gene serves as a crucial biomarker (Hartmann, 2009; Yan, 2009).

IDH mutations are associated with distinct molecular and clinical characteristics, influencing the prognosis and response to therapy in glioma patients.

KRAS in Colorectal Cancer
In colorectal cancer, the presence of mutations in the KRAS gene serves as a biomarker influencing treatment decisions. The assessment of KRAS mutation status has evolved into a routine and integral practice in the comprehensive management of colorectal cancer patients (Amando, 2008).

BRCA1 in Breast and Ovarian Cancers
The tumor markers BRCA1 and BRCA2 are genes associated with hereditary breast and ovarian cancers.

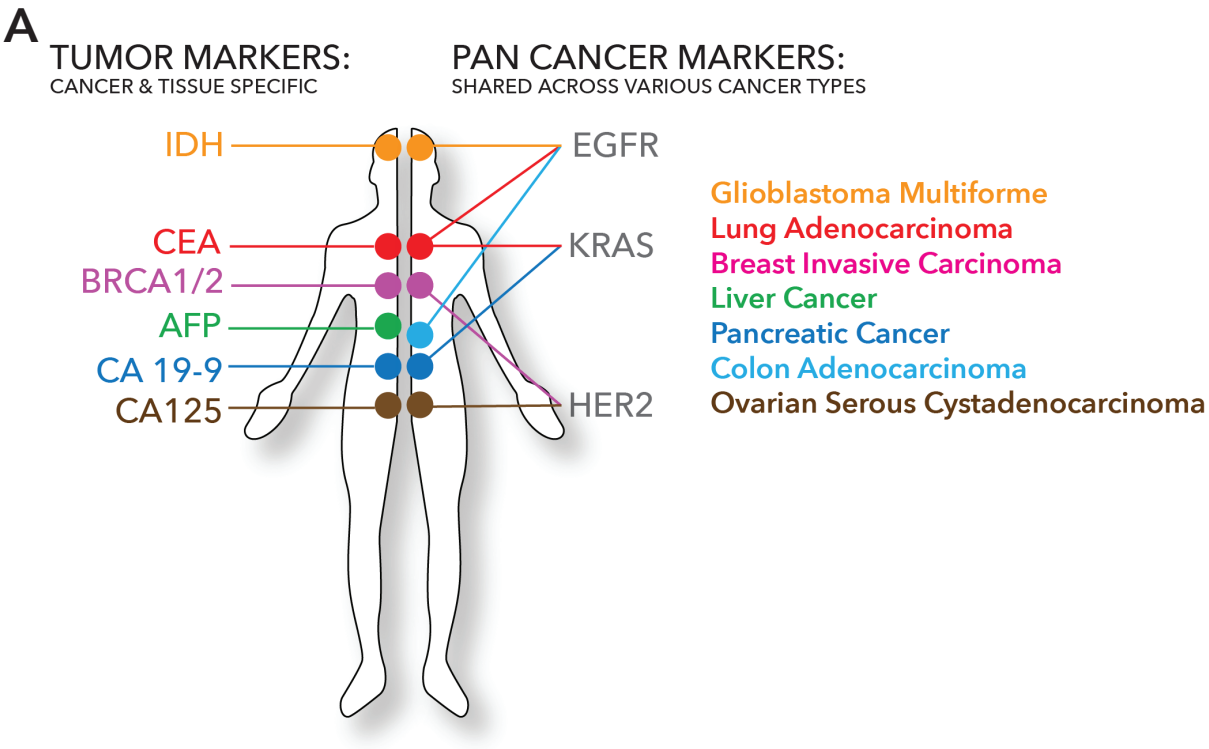
BRCA1/2 mutations contribute to the identification of subsets within triple-negative breast cancer, guiding personalized treatment strategies and highlighting diverse molecular profiles (Hall et al. 1990; Wooster et al. 1995).

In the context of ovarian cancer, mutations in BRCA1/2 have been identified as crucial indicators, serving both diagnostic and prognostic purposes. Detection of BRCA1/2 mutations in ovarian cancer patients has implications for treatment decisions, as well as for assessing familial cancer risks (Xu, 2017).

These specific biomarkers not only facilitate early detection but also enable tailored therapeutic interventions, improving patient outcomes.

Figure 2 shows some examples of immunohistochemical staining of various human cancers using tumor markers such as IDH1, BRCA1, KRAS, AFP, PSMB6 and BRAF.

A



B

Breast Cancer
BRCA1/2
Calponin
CD10
CEAD
CK5-8,14, 20
E-cadherin
ESR
GCDFP15
HER2
Mammaglobin A
p63
PGR
S100

Gastrointestinal Cancer
AMACR
CDX-2
CK7, 20
HepPar-1
Ki-67
MUC1,2
MUC5AC
P53
Villin

Lung Cancer
CEA
CK5-7
Napsin A
P63
TTF-1

Liver Cancer
AFP
ARG-1
BSEP
CD10,34
GPC-3
GS
HepPar-1
HSP70
LFABP
p-CEA
SAA

Prostate Cancer
ARG-1
ERG
Prostein
PSA
PSAP
PSMA

Kidney Cancer
ALK
AMACR
CA9
Cathepsin-K
CD10, 57, 82
CK7, 19
PAX2, 8
RCC Ma
S100A1
TFE3
TFEB
WT1

C

Pan Cancer Markers	Associated Cancers
p53	Breast, Lung, Colorectal and others
KRAS	Pancreatic, Colorectal, Lung
HER2	Breast, Ovarian, Gastric
EGFR	Lung, Colorectal, Glioblastoma
Cyclin D1	Breast, Colorectal, Lung
PTEN	Breast, Prostate, Endometrial
BRCA1	Breast, Ovarian, Pancreatic
PIK3CA	Breast, Colorectal, Ovarian

Figure 1.
Tumor markers vs Pan-Cancer markers

A. ***Left:*** Tumor markers can be broadly categorized into tissue-specific and cancer-specific markers, depending on their association with particular types of tissues or specific cancer types. Examples of tumor markers are IDH1 for glioma and BRCA1/2 for breast cancer.
Right: Pan-cancer markers, such as EGFR, KRAS, HER2, are not specific to a single cancer type but may be associated with general cancer characteristics.
B. Example of tumor markers specific for breast, gastrointestinal, lung, liver, prostate and kidney cancers.
C. Example of pan-cancer markers associated with (but not limited to) various human cancer types.

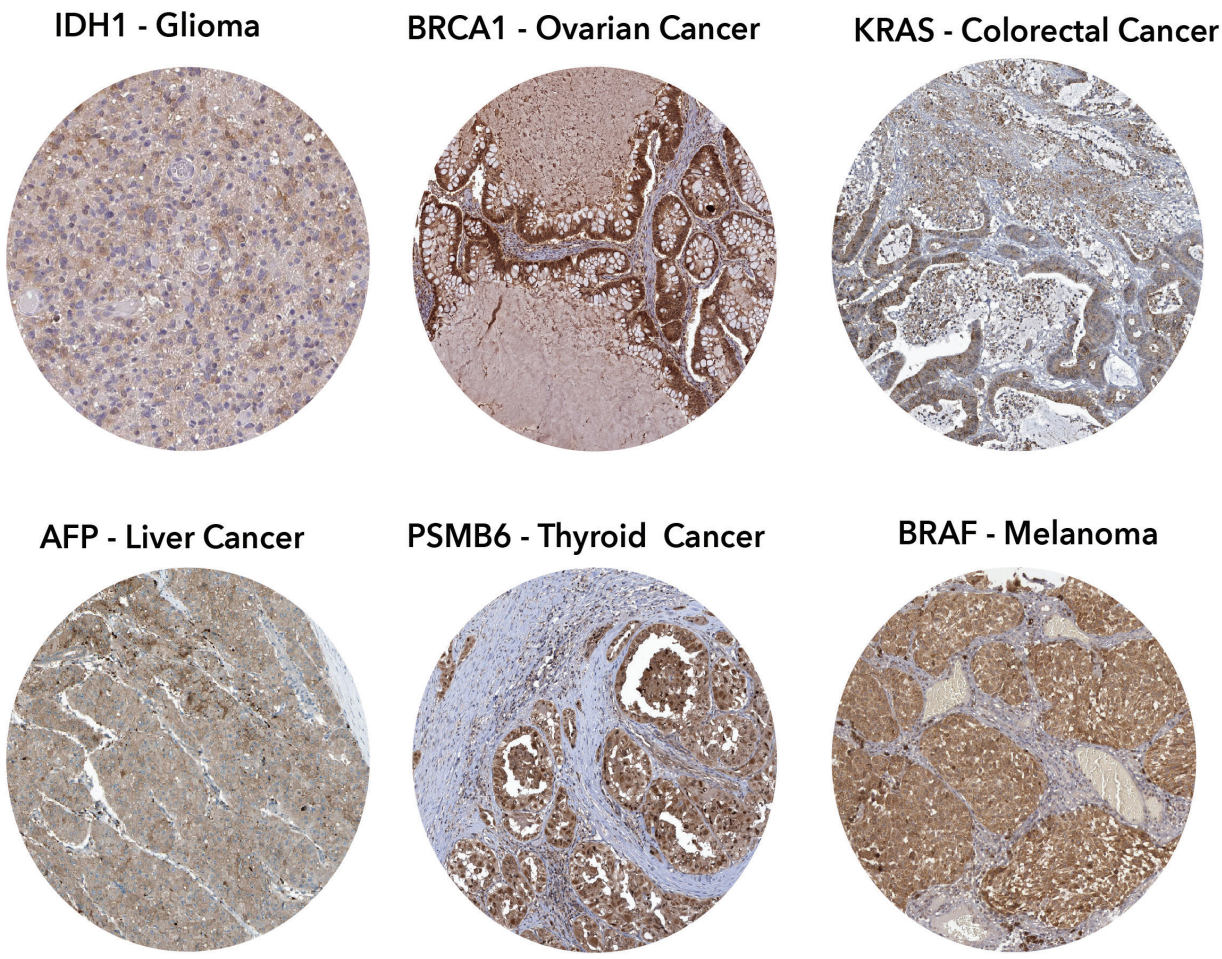


Figure 2.
Examples of immunohistochemical expression of tumor markers in various human cancerous tissue.

IHC staining of various human cancer using Triple A Polyclonals™ from Atlas Antibodies.
(A) Glioma: anti-IDH1 (HPA035248),
(B) Ovarian cancer: anti-BRCA1 (HPA034966)
(C) Colorectal cancer: anti-KRAS (HPA049830)
(D) Liver cancer: anti-AFP (HPA010607)
(E) Thyroid cancer: anti-PSMB6 (HPA023312)
(F) Melanoma: anti-BRAF (HPA001328)

Triple A Polyclonals (Atlas Antibodies Advanced Polyclonals) are research-grade rabbit polyclonals developed within the Human Protein Atlas project.

1:2
Pan-Cancer Markers: Shared Signatures Across Diverse Cancer Types

Pan-cancer markers refer to molecular characteristics, genetic mutations, altered gene expression patterns, or other molecular features consistently observed and shared across a spectrum of cancers **(Figure 1A, C).**

These markers, not specific to a single cancer type, transcend traditional cancer classification boundaries, holding the key to predicting treatment response across a spectrum of malignancies.

In the rapidly evolving landscape of precision medicine, the identification of pan-cancer markers represents a pivotal breakthrough in tailoring therapeutic interventions to individual patients and offer valuable insights into common pathways, potential therapeutic targets, and diagnostic strategies.

One prominent example is microsatellite instability (MSI), a pan-cancer biomarker indicative of defects in DNA mismatch repair. Tumors exhibiting MSI may respond favorably to immune checkpoint inhibitors, showcasing the potential for common therapeutic approaches in different cancers. Similarly, high tumor mutational burden (TMB) is a pan-cancer biomarker associated with increased neoantigen formation and improved response to immunotherapy.

The identification of pan-cancer biomarkers through initiatives like The Cancer Genome Atlas (TCGA) and other large-scale genomic studies has revolutionized our understanding of cancer biology (see Chapter 2). These markers offer a broader perspective, paving the way for the development of therapies that target shared vulnerabilities and pathways.

Integrating both specific and pan-cancer markers in clinical practice allows for a more comprehensive and personalized approach to cancer diagnosis, prognosis, and treatment, ultimately advancing the field of oncology and improving patient care.

Figure 3 shows some examples of pan-cancer markers expression in various human cancer tissue and cells.

As we continue to uncover the complexities of cancer biology, biomarkers will undoubtedly remain at the forefront of personalized medicine, shaping the future of cancer diagnosis and therapy.

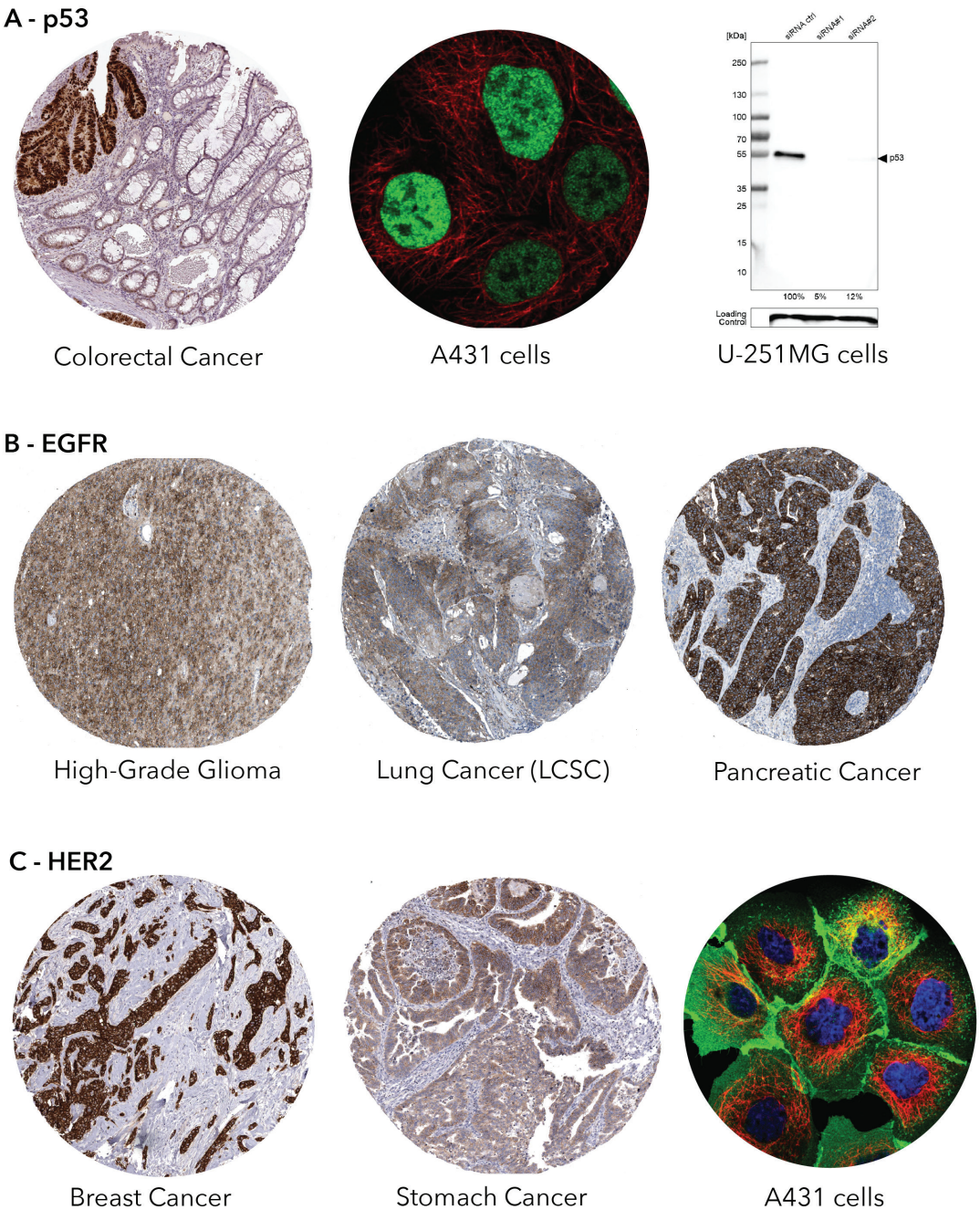


Figure 3.
Examples of Pan-Cancer markers in various human cancerous tissue.

A. p53 (anti-p53 monoclonal antibody AMAb90956, Atlas Antibodies)
Left: Immunohistochemical staining of colorectal cancer. Note strong overexpression of p53 protein in tumor tissue as compared to adjacent normal mucosa.
Middle: Immunofluorescence staining in A431 cell line showing cell cycle dependent nuclear staining in green. Microtubule probes are visualized in red.
Right: Western blot analysis in U-251MG cells transfected with control siRNA, target specific siRNA probe #1 and #2, Loading control: Anti-PPIB.

B. EGFR (anti-EGFR polyclonal antibody HPA018530, Atlas Antibodies)
Immunohistochemical staining of EGFR in high grade glioma (*left),* lung squamous cell carcinoma (*middle)* and pancreatic cancer (*right)*.

C. HER2 (anti-HER2 polyclonal antibody HPA001338, Atlas Antibodies)
Left, Middle: Immunohistochemical staining of breast and stomach cancer.
Right: Immunofluorescence staining in A431 cell line showing membranous staining in green. Microtubule and nuclear probes are visualized in red and blue respectively.

2. The Cancer Genome Atlas & The Pan-Cancer Atlas

The Cancer Genome Atlas (TCGA) and the Pan-Cancer Atlas (PCA) have identified the molecular subtypes and common genomic alterations across multiple cancer types revealing that tumors can be classified into molecular subtypes based on their genomic and molecular characteristics, transcending traditional tissue-based classifications.

2:1
Genomic Approaches of Cancer Markers

The Cancer Genome Atlas (TCGA) is a joint project of the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI).

TCGA began in 2006 as a pilot project focused on three cancer types: lung, ovarian, and glioblastoma. Due to the success of the initial efforts, TCGA was reauthorized for a full production phase in 2009.

In the following decade, TCGA collected more than 11,000 cases across 33 tumor types and generated a vast, comprehensive dataset describing the molecular changes that occur in cancer **(Table 1).**

It is difficult to overstate the value of the TCGA dataset. Its richness has enabled researchers to catalog specific genomic and molecular changes that occur in cancer, to define a more meaningful taxonomy of cancer types and subtypes, and to even investigate questions that were not imagined at the outset of the project.

2:2
The Pan-Cancer Atlas

The Pan-Cancer Atlas (PCA) initiative by the TCGA was undertaken to address several critical needs in cancer research such as the genomic alterations and molecular characteristics of various cancer types to improve our understanding of cancer biology.

The Pan-Cancer Atlas integrated molecular and genomic data from 12 different cancer types.

The integrated approach and collaborative nature of the project aimed to identify commonalities and differences across different malignancies, leading to a more comprehensive understanding of cancer biology and to accelerate advancements in cancer research and the development of personalized therapeutic strategies **(Figure 4).**

The Pan Cancer Atlas challenges traditional cancer classification based solely on the organ of origin and suggests that certain cancers share common underlying molecular features, opening new avenues for targeted therapies.

The proposed cancer classification system spans three overarching categories, each offering a unique lens into the intricate landscape of cancer biology:

(1) CELL OF ORIGIN
This classification organizes tumors based on their cellular origin. Tumor aggregation occurs through a comprehensive biological system approach, such as the Pan-Gynecological and Pan-Gastrointestinal systems, or by categorizing them according to histological subtypes, exemplified by Pan-Squamous Cell Carcinoma.

(2) ONCOGENIC PROCESSES
This category explores common oncogenic processes cutting across diverse tumor types. From immune system dynamics to mutation patterns and aneuploidy, a holistic understanding of these processes provides a unified perspective on the underlying mechanisms fueling cancer development.

(3) SIGNALING PATHWAYS
This classification highlights the unique roles played by various pathways in the development of different tumor types. By dissecting these signaling cascades, we gain valuable insights into the specific molecular events that contribute to the heterogeneity of cancer.

As with previous cross-cancer analysis, The Pan Cancer Atlas efforts uncover patterns across related tissue types and provide the scientific community with a wide-ranging overview of different aspects of cancer biology revealed through comprehensive analysis of a large collection of cases.

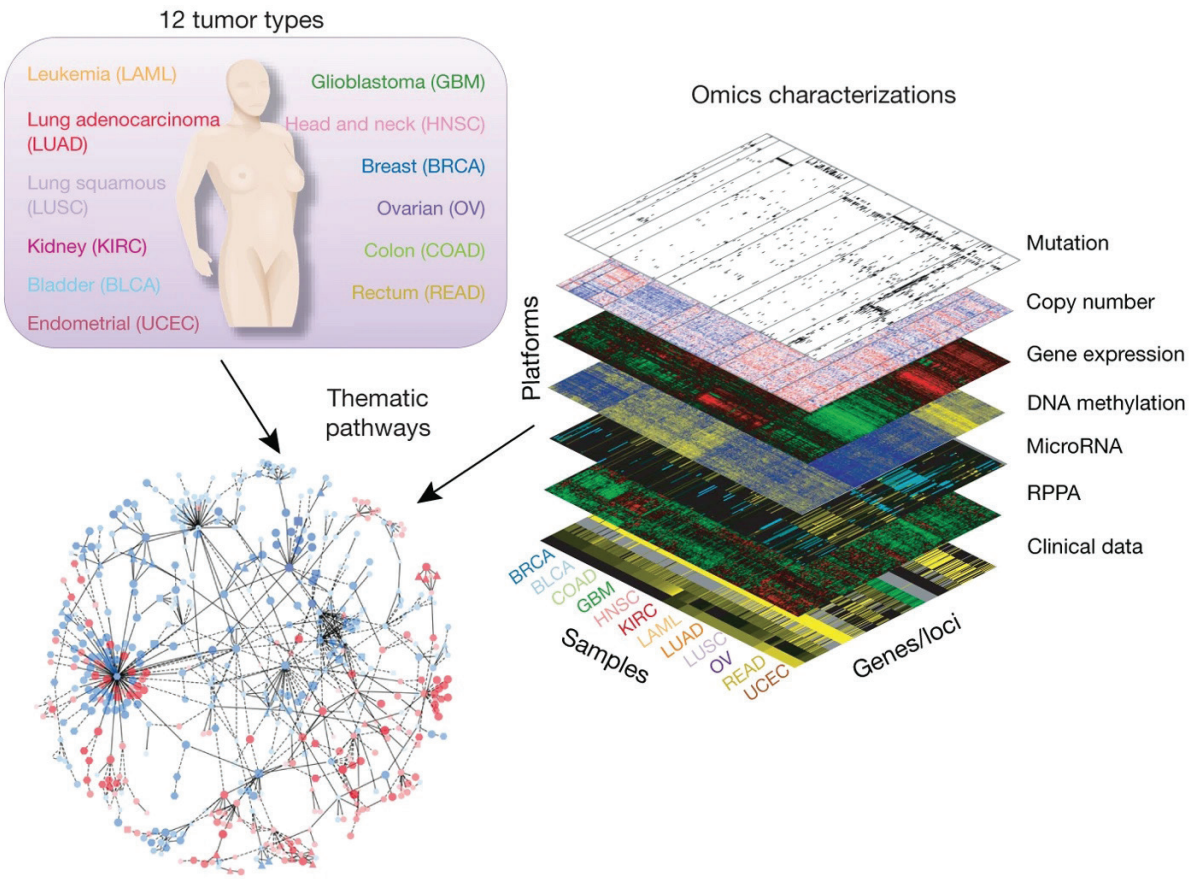


Figure 4.
Schematic of the TCGA Pan-Cancer framework project

12 tumor types (top left): The TCGA Pan-Cancer project assembled data from thousands of patients with primary tumors occurring in different sites of the body, covering 12 tumor types including glioblastoma multiformae (GBM), lymphoblastic acute myeloid leukemia (LAML), head and neck squamous carcinoma (HNSC), lung adenocarcinoma (LUAD), lung squamous carcinoma (LUSC), breast carcinoma (BRCA), kidney renal clear-cell carcinoma (KIRC), ovarian carcinoma (OV), bladder carcinoma (BLCA), colon adenocarcinoma (COAD), uterine cervical and endometrial carcinoma (UCEC) and rectal adenocarcinoma (READ).

Omics Characterization (right): Six types of omics characterization were performed creating a 'data stack' in which data elements across the platforms are linked by the fact that the same samples were used for each, thus maximizing the potential of integrative analysis.

Thematic Pathways (bottom left): Use of the data enables the identification of general trends, including common pathways, revealing master regulatory hubs activated (red) or deactivated (blue) across different tissue types.

Original image from:
The Cancer Genome Atlas Research Network., Weinstein, J., Collisson, E. et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat Genet 45, 1113–1120 (2013).

Table 1
The Cancer Genome Atlas: molecular subtypes and common genomic alterations across multiple cancer types.

CANCER TYPE	KEY FINDINGS
Breast lobular carcinoma	FOXA1 elevated in lobular carcinoma, GATA3 in ductal carcinoma; lobular enriched for PTEN loss and Akt activation
Breast ductal carcinoma	4 distinct subtypes: basal, Her2, luminal A, and luminal B; most common driver mutations: TP53, PIK3CA, GATA3; basal subtype similar to serous ovarian cancer
Prostate cancer	Highly heterogeneous with 26% driven by unknown alterations; ETS gene fusions or mutations in SPOP, FOXA1, or IDH1 define seven subtypes; actionable lesions in PI3K, MAPK, and DNA repair pathways
Colorectal adenocarcinoma	Colon and rectal cancers have similar genomic profiles; hypermutated subtype associated with favorable prognosis; new potential drivers: ARlD1A, SOX9, FAM123B/WTX
Cutaneous melanoma	Established 4 subtypes: BRAF mutant, RAS mutant, NF1 mutant, and triple wild-type based on driver mutations; higher levels of immune lymphocyte infiltration correlated with better survival
Thyroid carcinoma	Majority driven by RAS or BRAFV600E mutations
Endometrial carcinoma	Classified endometrial cancers into 4 categories: POLE ultramutated, MSI (microsatellite instability) hypermutated, copy-number low, copy-number high
Uterine carcinosarcoma	Strong and varied degree of epithelial-mesenchymal transition; TP53 mutations in 91% of samples; PI3K alterations in half
Invasive urothelial bladder carcinoma	Increased risk associated with smoking; frequently mutated; TP53 inactivated in 76% of tumors, ERBB2 (HER2), genes in the RTK/RAS pathways altered in 44%
Lung adenocarcinoma	High mutation burden; 76% have activation of receptor tyrosine kinase pathways
Lung squamous cell carcinoma	High average number of mutations and copy-number aberrations; almost all have mutation in TP53; many have inactivating mutations in HLA-A that may aid immune evasion.
Clear cell renal cell carcinoma	Commonly mutated genes: VHL, SED2, and the PI3K/AKT/mTOR pathway; metabolic shift similar to the "Warburg effect" correlates with a poor prognosis
Kidney papillary carcinoma	81% of type 1 tumors had MET alteration; type 2 tumors were heterogeneous, with alterations to CDKN2A, SETD2, TFE3, or increased expression of NRF2–ARE pathway; loss of CDKN2A expression and CpG island methylation phenotype associated with poor outcome
Chromophobe renal cell carcinoma	Extremely low mutation burden; metabolic shift distinct from the "Warburg effect" shift in clear cell carcinoma; TP53 and PTEN were frequently mutated; TERT gene promoter was frequently altered
Cervical cancer	Identification of HPV-negative, endometrial-like cancers with mutations in KRAS, ARID1A, and PTEN; amplification of CD274 and PDCD1LG2; frequent alterations in MED1, ERBB3, CASP8, HLA-A, and TGFBR2 and fusions involving lncRNA BCAR4; nearly threequarters had alterations in either or both of the PI3K/MAPK and TGF-beta pathways

Table 1 Cont.

Ref: SnapShot: TCGA-Analyzed Tumors
Amy Blum, Peggy Wang, and Jean C. Zenklusen
Cell. 2018 Apr 5;173(2):530.

CANCER TYPE	KEY FINDINGS
Testicular germ cell cancer	-
Ovarian serous adenocarcinoma	Mutations: TP53 (in 96%), BRCA1 and BRCA2 (in 21%) and are associated with more favorable outcomes
Glioblastoma multiforme	GBM subtypes Classical, Mesenchymal, and Proneural are defined by EGFR, NF1, and PDGFRA/IDH1 mutations, respectively; over 40% have mutations in chromatin-modifiers; frequently mutated: TP53, PIK3R1, PIK3CA, IDH1, PTEN, RB1, LZTR1
Lower-grade glioma	Defined three subtypes correlating with patient outcomes: IDH1 mutant with 1p/19q deletion, IDH mutant without 1p/19q deletion, and IDH wild-type
Stomach adenocarcinoma	Identified four subytpes characterized by EBV infection, microsatellite instability, genomic stability, and chromosomal instability
Liver hepatocellular carcinoma	TERT promoter mutations in 44%; TP53 commonly mutated or underexpressed; CTNNBB1 significantly mutated; many tumors had high levels of lymphocyte infiltration or overexpressed immune checkpoint genes
Cholangiocarcinoma	Low expression of CDKN2, BAP1, and ARID1 genes and overexpression of FGFR2 and IDH1/2 genes; 4 subtypes defined
Pancreatic ductal adenocarcinoma	KRAS mutations present in 93% of tumors; mutations in RREB1 or other members of RAS-MAPK signaling pathway
Esophageal carcinoma	Squamous cell carcinomas had frequent amplifications of CCND1, SOX2, and TP63; adenocarcinomas had frequent amplifications in ERBB2, VEGFA, GATA4, and GATA6
Acute myeloid leukemia	Low mutation burden—only 13 coding mutations on average per tumor; classified driver events into nine categories including transcription factor fusions, histone modifier mutations, and spliceosome mutations
Head and neck squamous cell carcinoma	HPV-positive associated with shortened or deleted TRAF3, HPV-negative characterized by co-amplification of 11q13 and 11q22, smoking-related characterized by TP53 mutations, CDKN2A inactivation, CNVs
Sarcoma	TP53, ATRX, and RB1 are recurrently mutated across all types; synovial sarcomas expressed fusions in SSX1 or SSX2 and TERT; JUN amplification associates with worse survival in dedifferentiated liposarcoma
Paraganglioma and pheochromocytoma	4 distinct subtypes: Wnt-altered, cortical admixture, pseudohypoxia, and kinase signaling; MAML3 fusion gene and CSDE1 somatic mutation define and drive the poor prognosis Wnt-altered subtype
Thymoma	-
Adrenocortical carcinoma	Overexpression of IGF2, mutations in TP53, PRKAR1A and other genes, and copy-number alterations were common; hypoploidy followed by whole-genome doubling may be a driving mechanism
Mesothelioma	-
Uveal melanoma	Complex mutation in BAP1 gene; identified distinct subdivisions of disomy 3 (D3) and monosomy 3 (M3) subtypes; in M3, mutually exclusive EIF1AX and SRSF2/SF3B1 mutations have distinct methylation profiles and prognoses.

While cancers share common characteristics and hallmarks, the absence of a universal treatment underscores the complexity of the disease.

Clinical oncologists traditionally focus on specific cancer types primarily based on their tissue origins.

However, advancements in genetics and cancer genomic research have unveiled profound distinctions in the origins and genetic changes of various cancers.

Simultaneously, shared features like key driver mutations, altered pathways, mutational patterns, immune responses, microbial signatures, and insights from pan-cancer studies suggest the intriguing potential clinical outcomes.

2:3 Overview of Pan-Cancer Markers

The Pan-Cancer Atlas initiative identified several molecular markers that play a significant role across multiple cancer types. These markers provide insights into common underlying biological processes and pathways shared by diverse cancers **Table 2** and **Figure 7.**

Here are some examples of pan-cancer markers:

p53
One notable example is the p53 protein (**Figure. 3A**), referred to as the "guardian of the genome." The TP53 gene, encoding the p53 protein, plays a pivotal role in regulating cell cycle progression and preventing the formation of tumors.

Mutations in TP53 are prevalent in a wide array of cancers, such as breast, lung, colorectal, and ovarian cancers. The aberrant function of p53, resulting from these mutations, contributes to uncontrolled cell growth and survival, underscoring its significance as a pan-cancer marker (Bellizzi, 2023).

PIK3CA
Similarly, the PIK3CA gene, encoding the p110α subunit of the PI3K enzyme, represents another pan-cancer marker with critical implications.

Activating mutations in PIK3CA are found in diverse cancers, including breast, colorectal, and endometrial cancers. Dysregulation of the PI3K/AKT/mTOR signaling pathway, driven by PIK3CA mutations, promotes cell proliferation and survival, making it a promising target for therapeutic intervention across multiple cancer types (Belli, 2023) **(Figure 5).**

SPAG5
An additional example is the sperm-associated antigen 5 (SPAG5). SPAG5, a mitotic spindle protein, is consistently overexpressed in diverse cancers (hepatocellular and several endometrial and renal cancers), indicating its potential oncogenic role **(Figure 6).**

A recent bioinformatic study (Gao, 2023) highlights its significance as a pan-cancer diagnostic marker, with elevated expression correlating strongly with poor prognosis. SPAG5 also shows promise in predicting immunosuppressive tumor microenvironments and immune therapy efficacy across various cancer types.

SPAG5 is hence positioned to be a valuable target for tumor immunotherapy serving as a versatile biomarker with diagnostic, prognostic, and immune-related implications, offering potential avenues for personalized cancer treatment.

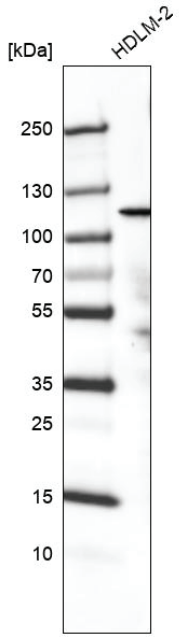


Figure 5.
Western blot analysis in human cell line HDLM-2 using the **anti-PIK3CA monoclonal antibody AMAb91513**.

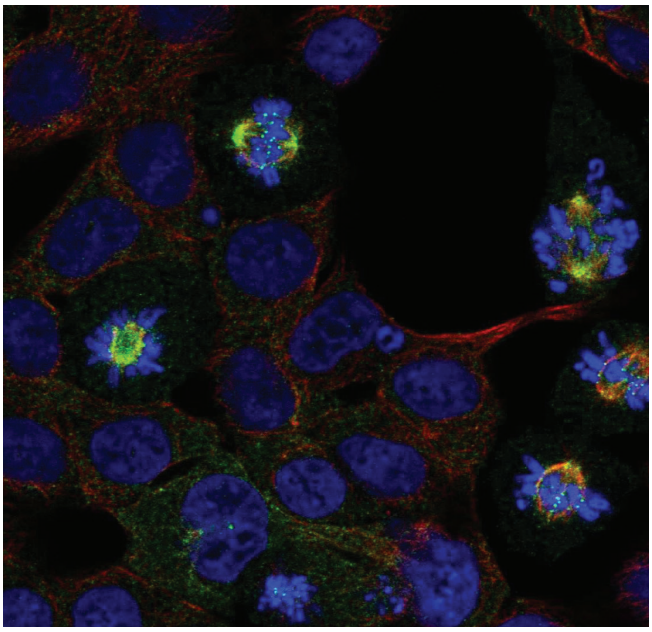


Figure 6.
Immunofluorescence staining of human cell line HAP1 using the **anti-SPAG5 polyclonal antibody** (HPA024418) shows protein localization to the nuclear bodies, cytosol & mitotic spindle. Microtubule and nuclear probes are visualized in red and blue respectively.

Microsatellite Instability
Moreover, the discovery of microsatellite instability (MSI) as a pan-cancer marker (Le, 2017) expanded our comprehension of molecular alterations that transcend specific cancer types and represents a pivotal breakthrough in our understanding of cancer biology.

The groundbreaking study by Le et al. demonstrates that MSI is not confined to a specific cancer type but is a shared molecular feature across various malignancies. Cancers with MSI are associated with a higher mutational load, leading to the production of neoantigens that can be recognized by the immune system.

This recognition has opened new avenues for the application of immunotherapeutic strategies, such as immune checkpoint inhibitors, which unleash the immune system to target and eliminate cancer cells.

By studying pan-cancer markers, researchers can identify patterns, potential therapeutic targets, and diagnostic strategies that transcend specific cancer types.

In essence, the role of pan-cancer markers is pivotal in paving the way for more precise and effective cancer diagnosis, prognosis, and treatment strategies.

Table 2.
Common pan-cancer markers: biological functions and relevance in human cancers.

MARKER	BIOLOGICAL FUNCTION	RELEVANCE (PREVALENT BUT NOT LIMITED TO)
p53 (TP53)	Tumor suppressor gene involved in cell cycle regulation)	Mutations in p53 are prevalent in breast, colorectal, lung and ovarian cancer.
EGFR (Epidermal Growth Factor Receptor)	Receptor involved in cell growth and division	Overexpression or mutations in EGFR are observed in lung (NSCLC), colorectal, glioblastoma, pancreatic, breast cancers
PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha)	Plays a key role in cell growth, survival, and metabolism by regulating the PI3K/AKT/mTOR signaling pathway.	Activating mutations in PIK3CA are found in breast, colorectal, endometrial and prostate cancers.
Ki-67 (MKI67)	Protein associated with cellular proliferation	Elevated Ki-67 levels are indicative of increased cell proliferation in many cancers.
BRAF (V-raf murine sarcoma viral oncogene homolog B)	Involved in the MAPK signaling pathway	BRAF mutations are found in various cancers, including melanoma and colorectal cancer.
HER2 (Human Epidermal Growth Factor Receptor 2)	Member of the EGFR family, involved in cell growth	Overexpression or amplification of HER2 is observed in breast, ovarian, and gastric cancers.
PD-L1 (Programmed Death-Ligand 1)	Immune checkpoint protein	Overexpression of PD-L1 can be observed in lung (NSCLC), melanoma, bladder and breast cancers, indicating immune evasion.

The longstanding debate between nature (original cell type) and nurture (mutations) is not exclusive to individuals but extends to cancer as well.

Advances in technology have enhanced our capacity to redefine cancers based on mutations rather than their organ of origin. Consequently, the focus shifts from treating, for instance, "breast cancer" to treateing "PI3K/AKT/PTEN cancer" addressing the specific PI3K/AKT/PTEN mutations.

As an additional example, the shift toward mutation-based characterization has been exemplified in the treatment of non-small cell lung cancer (NSCLC).

Rather than solely categorizing and treating NSCLC based on its lung origin, a molecular approach considers mutations like EGFR or ALK.

This approach underscores the importance of pan-cancer markers in steering targeted therapies and optimizing treatment outcomes in diverse cancer types.

Moreover, it tailors treatment strategies to the molecular underpinnings of the disease. An analogous shift from organ-based to mutation-based characterization fosters more precise and personalized cancer interventions.

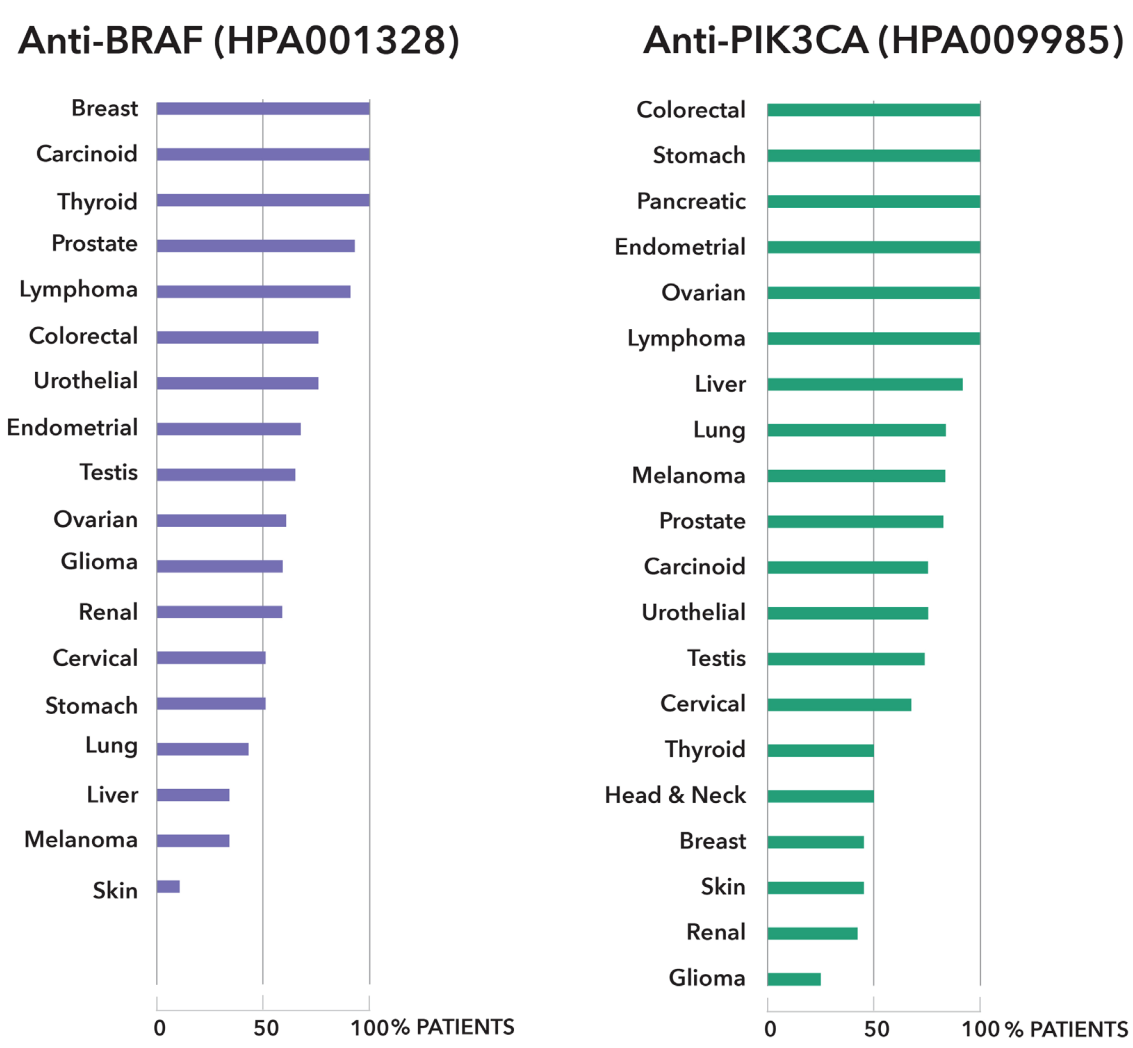


Figure 7.
Example of IHC protein expression of the pan-cancer markers BRAF and PIK3CA in 20 human cancers.

BRAF (left): IHC staining performed on 20 human cancer tissues using the anti-BRAF Triple A polyclonal antibody (HPA001328). The majority of cancer tissues exhibited moderate cytoplasmic staining, while strong immunoreactivity was observed in a few cases of prostate, breast, and thyroid cancer. *Not depicted in the schematic are pancreatic and head & neck cancers, which showed negative staining for BRAF.*

PIK3CA (right): IHC staining performed on 20 human cancer tissues using the anti-PIK3CA Triple A polyclonal antibody (HPA009985). Cancer cells showed moderate to strong cytoplasmic immunoreactivity with a granular pattern. Most gliomas and several cases of renal, testicular and breast cancers were weakly stained or negative.

2:4
Pan-Cancer Markers Tools for Precision Medicine

In the context of precision medicine and personalized cancer treatment, the identification of pan-cancer markers has significant implications for patient management. The following are few examples:

TP53 and BRCA1/2 Mutations and (PARP) Inhibitors
TP53, a tumor suppressor gene, plays a critical role in regulating the cell cycle and preventing the formation of tumors. Mutations in TP53 were found to be prevalent across various cancer types, such as breast, ovarian, and prostate cancer, indicating their importance in cancer development and progression (Lane, 1992).

BRCA1 and BRCA2 are genes involved in DNA repair. Mutations in these genes were found to be associated with an increased risk of breast and ovarian cancers. The Pan-Cancer Atlas highlighted the relevance of BRCA mutations in multiple cancer types, emphasizing their role in DNA repair mechanisms.

Many studies have shown that tumors with TP53 and BRCA1/2 mutations may be sensitive to poly (ADP-ribose) polymerase (PARP) inhibitors (Campeau, 2008; Zhang2014).

This information is crucial for tailoring the treatment strategy. In the past, a one-size-fits-all approach might have involved standard chemotherapy regimens. However, with the understanding of the TP53 mutation, a more precise and personalized treatment plan can be devised.

PIK3CA Mutations and PI3K Inhibitors
PIK3CA is a gene that codes for a subunit of the PI3K protein, which is involved in cell signaling and growth. Alterations in PIK3CA, including mutations and amplifications, were identified as common events in multiple cancers, suggesting a role in promoting cell survival and proliferation.

Based on the identified PIK3CA mutation, patients may be considered for treatment with a PI3K inhibitor, such as Alpelisib (André 2019).

KRAS Mutations and the Inefficacy of anti-EGFR Therapies
Another notable example involves the use of targeted therapies in metastatic colorectal cancer patients with specific KRAS mutations.
KRAS mutations are associated with uncontrolled cell growth, metastasis, and resistance to treatment.

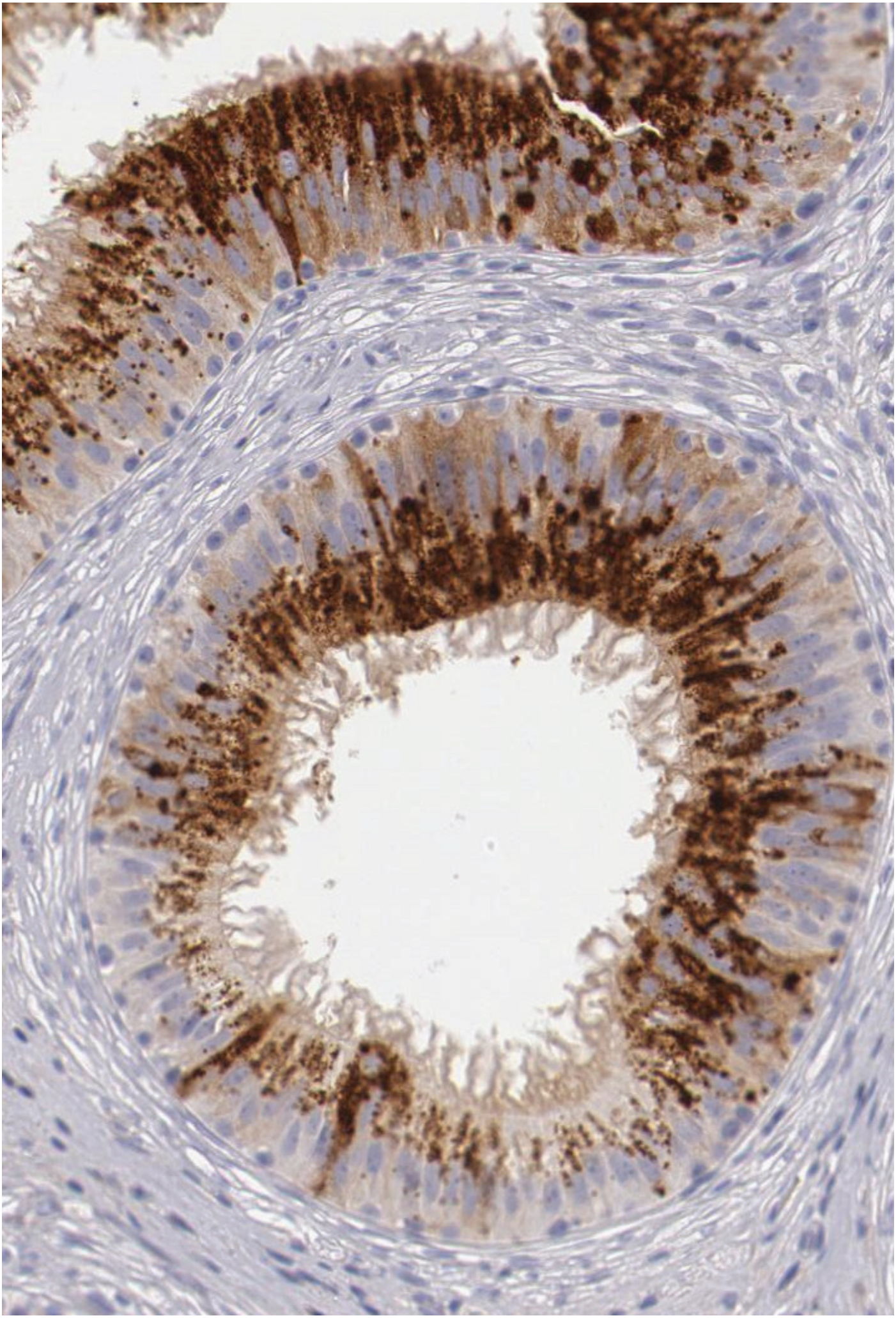
Historically, metastatic colorectal cancer was treated with anti-EGFR therapies (such as cetuximab and panitumumab). However, these therapies are generally effective only in patients with wild-type (non-mutated) KRAS. Patients with KRAS mutations, particularly in codons 12 and 13, do not benefit from anti-EGFR therapies and may experience resistance.

Following this discovery, researchers are studying alternative treatment strategies, such as combination therapies or other targeted agents, for patients with KRAS mutations.

Mismatch Repair Deficiency (MMR) and PD-1 Immune Checkpoint Inhibitors.
Deficiencies in DNA mismatch repair mechanisms were identified as a common feature in various cancers, leading to microsatellite instability (MSI). MMR deficiency is associated with increased mutational burden and has implications for immunotherapy response.

Therapies for MMR-deficient cancers often involve immune checkpoint inhibitors that target programmed cell death protein 1 (PD-1) or programmed death-ligand 1 (PD-L1). Pembrolizumab, for instance, has been approved for the treatment of MMR-deficient or MSI-High solid tumors across different cancer types (Le, 2017).

Although the prevalence of these mutations can vary within specific cancers subtypes and may differ in terms of prognosis and response to targeted therapies, these examples highlight the importance of specific genetic and molecular alterations that transcend individual cancer types, providing valuable information for the development of targeted therapies and precision medicine approaches in oncology.



3. Proteomic Approaches: Implementing the Pan-Cancer Atlas

As we move into an era of precision medicine, the significance of proteomics cannot be overstated.

While genetic information, such as RNA profiles, provides valuable insights into the blueprint of cellular processes, it is the dynamic world of proteins that truly governs the complexity of cancer.

Understanding not only the identity but also the abundance, localization, and interactions of proteins is essential for unraveling the mysteries of cancer biology and advancing targeted therapeutic interventions.

The molecular subtypes identified in various cancers often correlate with distinct protein expression patterns.

Proteomic analyses conducted as part of projects like the Pan-Cancer Atlas aim to uncover associations between specific molecular subtypes and alterations in protein expression.

Proteomic analyses, including mass spectrometry and other antibodies-based techniques, play a crucial role in identifying and quantifying protein expression patterns associated with specific molecular subtypes.

Understanding these associations is essential for gaining insights into the molecular mechanisms underlying each subtype and for developing targeted therapeutic strategies.

3:1 The Impact of Proteome Changes in Tumorigenesis

While genetic information serves as a foundational layer in understanding human cancer, it is the dynamic and multifaceted activity of proteins that holds the key to unraveling the complexity of it.

Proteomics provides a comprehensive view of the functional aspects of cellular processes, offering insights into disease mechanisms and paving the way for the development of targeted and personalized therapeutic strategies.

Cancer is fundamentally a disease of the proteome: cancer arises by and through changes in the protein signaling architecture, changes in the protein enzymes and structural protein machinery that cause and control cellular biology. The proteome is what produces the selective pressure that encourages specific changes in the genome, leading to the emergence, selection, and maintenance of permanent genetic alterations.

Ultimately it is the proteome that represents the drug targets for nearly all cancer therapeutics **(Table 3).**

3:2 CPTAC: 10 Proteome-Based Pan-Cancer Molecular Subtypes

Proteomics provides a comprehensive view of the functional aspects of cellular processes, offering insights into disease mechanisms and paving the way for the development of targeted and personalized therapeutic strategies.

The Clinical Proteomic Tumor Analysis Consortium (CPTAC) was established in 2011 with the aim of advancing the understanding of cancer at the molecular level through extensive proteome and genome analysis, known as proteogenomic.

Initially focusing on colorectal, breast, and ovarian cancer, CPTAC has made significant strides, uncovering proteomic-centric subtypes, prioritizing driver mutations, and exploring cancer-related pathways through posttranslational modifications.

Based on the CPTAC database, a study from Chen et al. (2019), classified 532 tissue-based types of cancers (breast, colon, ovarian, renal, uterine), into 10 proteome-based, pan-cancer subtypes that cut across tumor lineages.

The focus of the project was to determine what molecular subtypes would be discoverable from the proteome, as opposed to pan-cancer subtypes previously identified using the transcriptome.

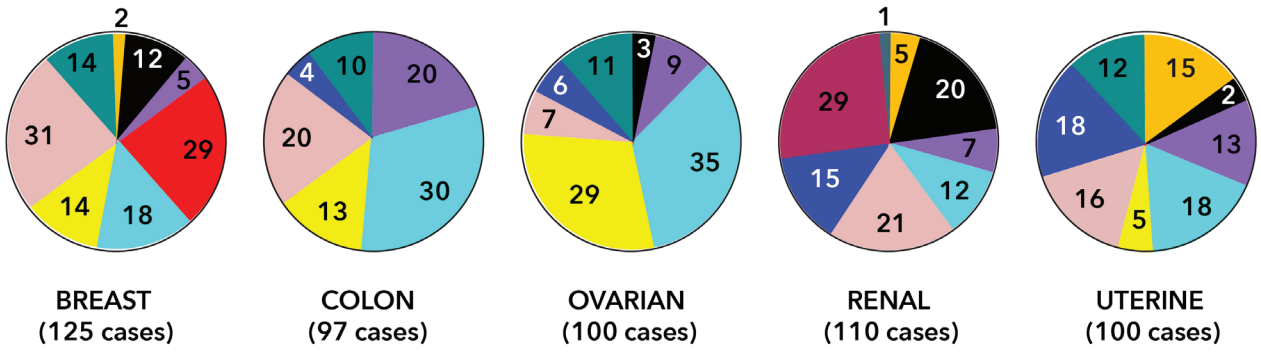
See **figure 8** for more details.

Table 3.
Pan-Cancer markers play a crucial role in generating actionable insights for cancer treatment.

Examples of monoclonal antibodies used in clinical therapy that have demonstrated efficacy in targeting specific proteins associated with cancer, leading to improved outcomes for patients in various clinical settings.

APPROVED TREATMENT	TARGET PROTEIN	CLINICAL APPLICATION
Trastuzumab (Herceptin)	Human Epidermal Growth Factor Receptor 2 (HER2)	Used in the treatment of HER2-positive breast cancer. (Slamon, 2001)
Bevacizumab (Avastin)	Vascular Endothelial Growth Factor (VEGF)	Employed in the treatment of various cancers, including colorectal, lung, and glioblastoma. (Hurwitz, 2004)
Rituximab (Rituxan)	CD20 antigen on B cells	Widely used in the treatment of non-Hodgkin lymphoma and certain autoimmune disorders. (Coiffier, 1998)
Cetuximab (Erbitux)	Epidermal Growth Factor Receptor (EGFR).	Utilized in the treatment of colorectal cancer and head and neck cancer. (Cunningham, 2004)
Pembrolizumab (Keytruda)	Programmed Death-1 (PD-1) receptor.	An immune checkpoint inhibitor used in the treatment of various cancers, including melanoma, non-small cell lung cancer, and head and neck cancer. (Robert, 2015)

The discovery of shared molecular features and the move toward a more molecularly driven classification of cancers has implications for developing targeted therapies that may be effective across multiple cancer types, potentially revolutionizing cancer treatment strategies.



- **Proteome 1**
  Over-expression of proteasome complex proteins, glycolysis proteins, and pentose phosphate pathway proteins (CALM1, PSMA2, PSMA5, PSMA6).
- **Proteome 2**
  Adaptive immune system-related; associated with T-cell activation; expression of major histocompatibility complex proteins (BTK, CD3E, CCD3G,CD4, CD38, CSK, FCER1G, HLA-B, IISG20, ITGAL, PIK3CD, PRKCB, PTPN1, PTPN6, WAS).
- **Proteome 3**
  Innate immune system-related; over-expression of complement system proteins; involvement of eosinophils, neutrophils, mast cells, and macrophages; hypoxia signature (C5, CDA, CDC42, CYBB, F2, IGHG1, ITGB2, MMP9, NCF2, NCF4, NCF1).
- **Proteome 4**
  Represents basal-like breast cancer; over-expression of YAP1 and MYC targets.
- **Proteome 5**
  Epithelial signature; normoxia signature; over-expression of YAP1 and MYC targets; over-expression of oxidative phosphorylation and TCA cycle proteins.
- **Proteome 6**
  Stromal-related; over-expression of matrix metallopeptidases; Wnt and Notch pathway signatures; hypoxia signature (MMP2, CCXCL12, TGFB3, FSTL1).
- **Proteome 7**
  Stromal-related; over-expression of collagen VI proteins; Wnt and Notch pathway signatures (DCN).
- **Proteome 8**
  Over-expression of Golgi apparatus-related proteins; Ras pathway signature (ERBB4, IINSR, KRT8, PPKAA2, PSMB8, MUC16).
- **Proteome 9**
  Found in clear cell renal cell carcinoma cases only; over-expression of hemoglobin complex proteins (PSMB4, SLC9A6).
- **Proteome 10**
  Over-expression of endoplasmic reticulum-related proteins and steroid biosynthesis pathway proteins (IGF2R, NSDHL).

Note:
The original paper by Chen et al. 2019 identifies the 10 proteome as k1, k2, k3...k10.

Figure 8.
10 proteome-based Pan-Cancer molecular subtypes

Chen et al. (2019), classified 532 tissue-based types of cancers (breast, colon, ovarian, renal, uterine), into 10 proteome-based, pan-cancer subtypes that cut across tumor lineages.

Top: Pie-chart distributions by proteome-based subtype (proteome 1-10) for each cancer type. The number of cases showing alterations in proteins associated to each pan-cancer molecular proteome subtype is indicated in each chart section.

For example, among the 125 cases of breast cancer tissue analyzed, 31 exhibit alterations in proteins associated with Proteome 7 (pink), while 14 cases display alterations in proteins associated with Proteome 6 (yellow), and so forth.

Bottom: Detailed description of proteins class associated with each pan-cancer molecular proteome subtype. The proteins that best distinguish between the 10 proteome-based subtypes are reported in parentheses.

3:3 Proteomic Approaches in Cancer Therapy

Genetic information offers a static snapshot of potential cellular functions, but the actual execution of these functions is orchestrated by proteins. Proteins, as the molecular machines of the cell, carry out vital tasks ranging from cellular signaling to DNA repair. Aberrations in protein expression, post-translational modifications, or interactions can profoundly impact cellular behavior, leading to the development and progression of cancer.

Understanding and manipulating the proteome can lead to the development of targeted cancer therapies. By elucidating the intricate signaling networks and protein interactions involved in cancer, researchers can identify key nodes within the proteome that serve as potential drug targets, allowing for more precise and effective treatment strategies.

The following cases exemplify the importance of targeting the proteome for therapeutic intervention.

Targeting HER2 protein in breast cancer
One illustrative example is the role of the HER2 protein in breast cancer. While the HER2 gene amplification can be identified through genetic analysis, it is the overexpression of the HER2 protein that marks a subset of breast cancers with aggressive behavior. Targeting HER2 protein overexpression with drugs like trastuzumab has revolutionized the treatment landscape, emphasizing the critical role of proteomic information in guiding therapeutic decisions (Swain 2023).

Inhibition of BRAF protein in melanoma
Another notable example that underscores the pivotal role of proteomics in cancer is the study of BRAF mutations in melanoma. While genetic analysis can identify the presence of BRAF mutations, it is the characterization of the activated BRAF protein that holds significant clinical implications. In melanoma, the BRAF gene mutation, particularly the V600E mutation, results in the constitutive activation of the BRAF protein, a key player in the MAPK signaling pathway. The dysregulated MAPK pathway drives uncontrolled cell proliferation, contributing to the aggressive nature of melanoma.

Understanding the specific protein alterations, such as the activation status of BRAF, is crucial for targeted therapeutic interventions. In this context, drugs like trametinib and dabrafenib have been developed to specifically inhibit the activated BRAF protein, thereby disrupting the aberrant signaling cascade and impeding melanoma growth (Robert, 2015).

EGFR and Protein Kinases in Cancer
The epidermal growth factor receptor (EGFR) is a well-studied receptor tyrosine kinase involved in regulating cell proliferation. Mutations in the EGFR gene lead to constitutive activation of the receptor, promoting tumor development. Targeted therapies, such as tyrosine kinase inhibitors (TKIs) like gefitinib and erlotinib, primarily target the protein rather than the gene itself. In the context of these specific TKIs, they are designed to inhibit the activity of the epidermal growth factor receptor (EGFR) protein (Lynch, 2004).

Proteasome Inhibition in Multiple Myeloma
Proteasomes are protein complexes responsible for degrading unwanted or damaged proteins by proteolysis. In some cancers, such as thyroid cancer and in multiple myeloma, inhibiting the proteasome can lead to the accumulation of misfolded proteins, inducing cell death. Bortezomib is a proteasome inhibitor that has shown significant clinical efficacy in treating multiple myeloma (Richardson, 2003). By blocking the proteasome, bortezomib disrupts the protein homeostasis in cancer cells, triggering apoptosis and inhibiting tumor growth.

In summary, while cancer therapy is influenced by the genetic alteration (mutations in the gene), the therapeutic intervention is directed at the product of the gene (e.g. HER2, BRAF, EGFR proteins) to impede their activity and mitigate the aberrant signaling associated with cancer growth.

These examples highlight how the assessment of proteins, in addition to genetic mutations, provides a more nuanced understanding of cancer biology, emphasizing the importance of proteomic information in tailoring precision therapies.

While the Pan-Cancer Atlas provides a comprehensive overview of genomic alterations across diverse cancers, targeted and detailed studies of specific proteins are essential.

Primary antibodies and associated techniques play a crucial role in unraveling the intricacies of protein dynamics, offering a focused perspective in understanding cancer biology.

3:4 Applications of Proteomic Studies for Pan-Cancer Targeting and Therapy: 5 Examples

Proteomics plays a pivotal role in elucidating the dynamic nature of cellular processes.

The localization of proteins within specific cellular compartments or their movement between different cellular regions provides insights into their functional roles. For instance, the dynamic interplay of proteins involved in cell cycle regulation can be better understood through proteomic approaches, shedding light on the mechanisms underlying uncontrolled cell proliferation in cancer.

While the Pan-Cancer Atlas offers a panoramic view of genomic alterations, primary antibodies and targeted approaches provide depth and specificity, enabling researchers to delve into the functional and clinical aspects of cancer biology. The two approaches are complementary and can be used synergistically to gain a more comprehensive understanding of cancer.

Here are 5 examples of how application of proteomic studies and primary antibodies could complement/implement the Pan-Cancer Atlas:

1. Protein-Protein Interactions

Protein-protein interactions constitute another layer of complexity that is crucial for comprehending cancer biology. The crosstalk between signaling pathways, mediated by intricate protein interactions, dictates cellular responses to external stimuli. By mapping these interactions through proteomic techniques, such as proximity ligation assays (PLA) and MolBoolean (Raykova 2022) researchers can identify key players in signaling cascades and potential vulnerabilities that can be targeted for therapeutic intervention **(Figure 9).**

2. Functional Validation

Primary antibodies allow for functional validation of genomic findings by confirming the expression and localization of specific proteins in cancer cells. For instance, if a genomic analysis indicates increased expression of a certain kinase in breast cancer, functional validation using primary antibodies would involve conducting immunohistochemistry or immunofluorescence experiments to verify the presence and subcellular localization of the kinase protein in breast cancer cells, providing tangible evidence to support the genomic findings.

3. Clinical Translation

Primary antibodies are integral to clinical diagnostics, aiding in the translation of research discoveries into practical applications for patient diagnosis and treatment.

Annese et al. (2022) exemplifies the significance of primary antibodies in clinical translation by employing the anti-KIT (c-KIT, CD117) primary antibody in immunohistochemistry (IHC).

The anti-KIT antibody played a crucial role in identifying and visualizing the expression of c-KIT in melanoma tissues, providing valuable information on the presence and distribution of c-KIT-positive mast cells. This data, in turn, contributes to our understanding of the autocrine/paracrine loop involving SCF+/c-KIT+ mast cells and its impact on cutaneous melanoma progression.

The clinical translation aspect is evident as the findings from such studies can potentially influence diagnostic and therapeutic strategies for melanoma. The identification of specific markers, like c-KIT, can have implications for patient diagnosis and the development of targeted treatments in the clinical setting. Therefore, the use of primary antibodies supports the translation of research insights into practical applications for the diagnosis and treatment of melanoma patients **(Figure 10).**

4. Detailed Pathway Analysis

Researchers can perform in-depth studies of specific pathways or proteins of interest using primary antibodies, providing detailed insights into molecular mechanisms.

In a study by Lam et al. (2022), detailed pathway analysis revealed a correlation between genetic KRAS mutation and immunohistochemical SOX9 protein expression in colorectal cancer (CRC).

The results demonstrate that, in instances of KRAS mutation, there is a notable upregulation of SOX9 protein expression in CRC tissues. Interestingly, the study also uncovered a KRAS mutation-independent downregulation of MAPK/PI3K signaling in CRC, suggesting a complex interplay between genetic alterations and signaling pathways that contribute to the molecular mechanisms underlying CRC progression **(Figure 11).**

This example showcases how proteomic studies can be instrumental in dissecting the intricate relationships between genetic mutations and protein expression, providing nuanced insights into the molecular intricacies of human cancer.

5. Drug Development

Primary antibody-based studies contribute valuable information for drug development by targeting specific proteins that may serve as therapeutic targets.

For instance, a study by Zhao et al. (2021) utilized proteomic studies to gain valuable insights into the role of MTHFD2 in oral squamous cell carcinoma (OSCC). Specifically, primary antibodies were employed to assess the expression levels and localization of MTHFD2 in OSCC cells.

The findings revealed that MTHFD2 inhibition had notable effects, including the inhibition of cell proliferation and promotion of apoptosis. This suggests that MTHFD2 may serve as a potential therapeutic target in OSCC **(Figure 12).**

In Table 4, we have compiled recommended tumor markers and pan-cancer markers that target proteins involved in cancer mechanisms.

The listing features Triple A Polyclonals™ and PrecisA Monoclonals™ from Atlas Antibodies.

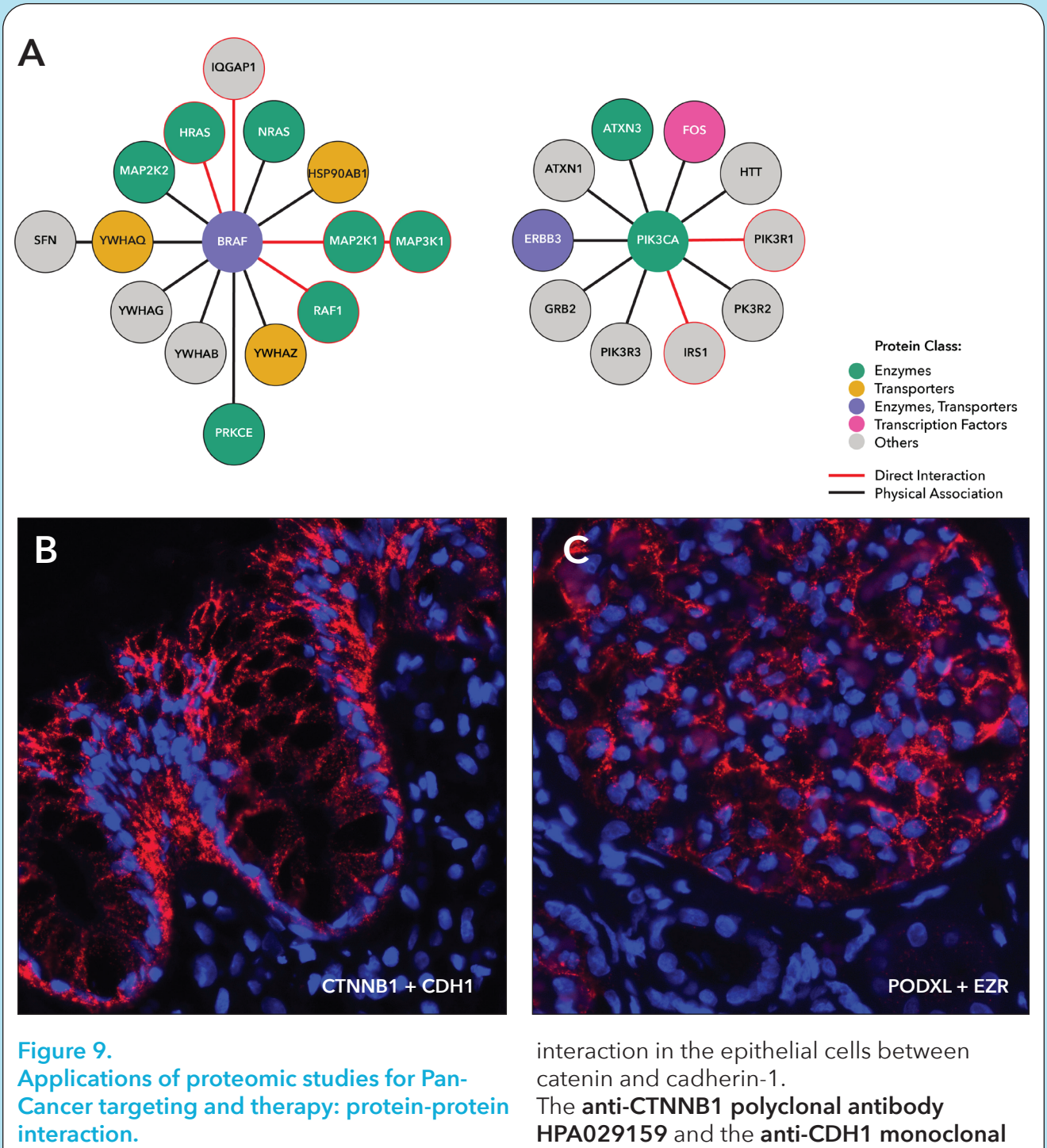


Figure 9.
Applications of proteomic studies for Pan-Cancer targeting and therapy: protein-protein interaction.

A. Node networks of protein interactions for the pan-cancer markers BRAF and PIK3CA. The type of interaction direct (**red**) or physical (**black**) is reflected by the color of the edges. Protein classes are colored according to the subcellular location based on data in the Subcellular section of the Human Protein Atlas (www.proteinatlas.org).

B. Example of direct protein-protein interaction (**red, 40x**) using in-situ proximity ligation assay (is-PLA) on human colon tissue, showing positive signal for protein-protein interaction in the epithelial cells between catenin and cadherin-1.
The **anti-CTNNB1 polyclonal antibody HPA029159** and the **anti-CDH1 monoclonal antibody AMAb90865** from Atlas Antibodies were used.

C. Example of direct protein-protein interaction (**red, 40x**) using in-situ proximity ligation assay (is-PLA) on human kidney tissue, showing positive signal for protein-protein interaction between podocalyxin-like and ezrin proteins in renal podicytes.
The **anti-PODXL monoclonal antibody AMAb90644** and the **anti-EZR polyclonal antibody HPA021616** from Atlas Antibodies were used.

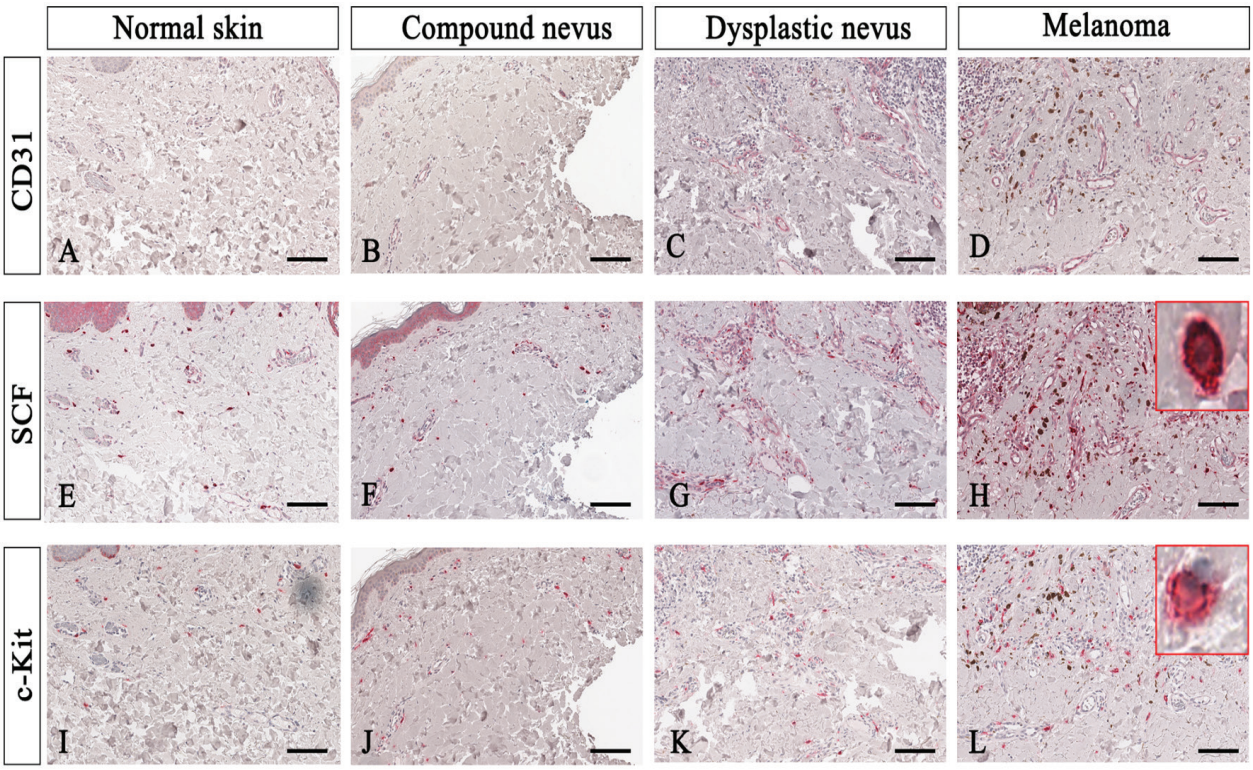


Figure 10.
Applications of proteomic studies for Pan-Cancer targeting and therapy: example of clinical translation.

Representative IHC of SCF or c-Kit on the same area of interest around CD31+ blood vessels in the normal skin (A, E, I), compound nevi (B, F, J), dysplastic nevi (C, G, K), and melanomas (D, H, L) tissue sections.

The micrographs show a significative gradual increased CD31, SCF, and c-Kit expression in malignant melanoma lesions compared to premalignant ones and normal skin (A–L).

The SCF- or c-Kit-positive mast cells appear mostly with a strong red-granulous membranous and moderate cytoplasmic staining in all the samples (see the inserts in the red rectangles; scale bar: 10 μm).

The **anti-KIT (c-KIT) AMAb90901 monoclonal** antibody from Atlas Antibody was used.

Image from Annese et al. 2022.

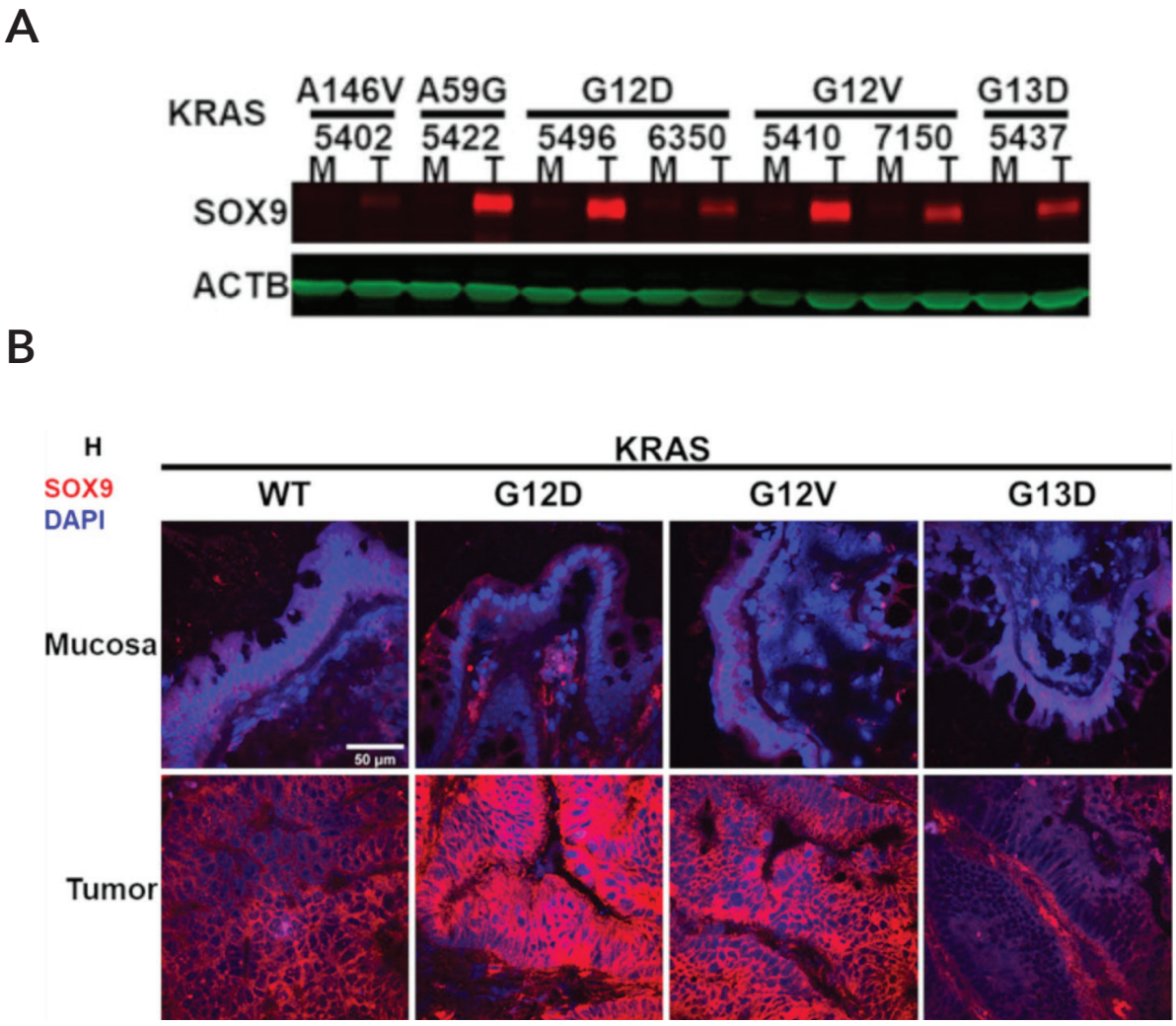


Figure 11.
Applications of proteomic studies for Pan-Cancer targeting and therapy: pathways analysis

Example of KRAS mutation-dependent upregulation of protein SOX9 in colorectal cancer (CRC) compared to matched mucosa.

A. Representative WB of SOX9 and ACTB for 7 pairs of CRC tumor (**T**) and matched mucosa (**M**).

B. Representative confocal images of IHC-IF of SOX9 (**red**) with nuclear DAPI (**blue**) counterstain of CRC-matched mucosa and tumor cryosections for each KRAS mutation status.

The **anti-SOX9 monoclonal antibody (AMAb90795)** from Atlas Antibodies was used to detect SOX9 in WB and IHC-IF.

Image from Lam et al. 2022.

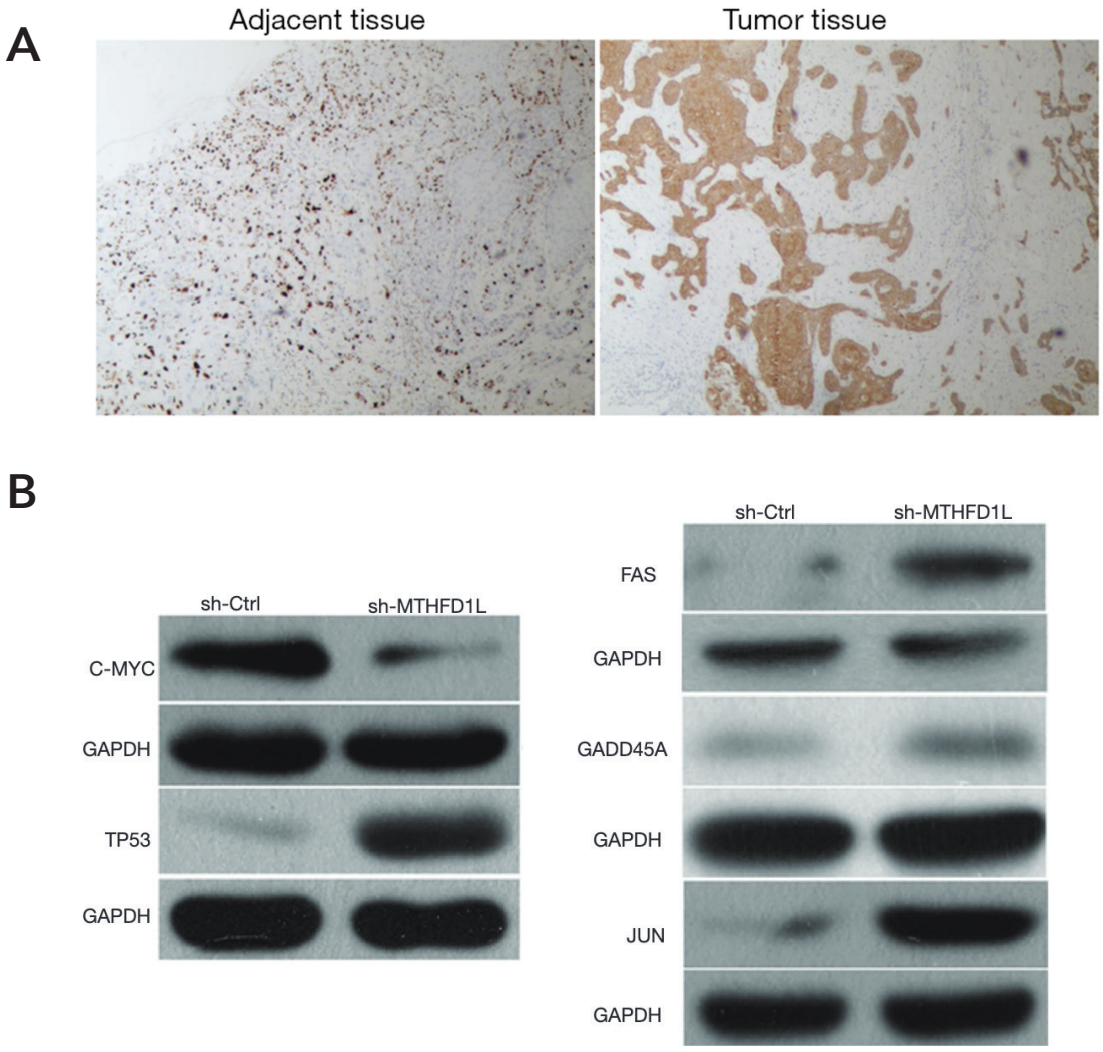


Figure 12.
Applications of proteomic studies for Pan-Cancer targeting and therapy: example of target for drug development.

A. Representative figures of IHC staining for MTHFD1L in oral squamous cell carcinoma (OSCC) tissues and paired adjacent normal tissue using the **anti-MTHFD1L (HPA0290409) polyclonal** antibody from Atlas Antibody.

The expression level of MTHFD1L in OSCC tissues from 96 patients was significantly higher than that in normal tissue. MTHFD1L expression in tumor tissue and normal tissues was reviewed using data from the Human Protein Atlas (HPA).

B. Confirmation of the microarray results using Western blot analysis of c-MYC, TP53, GADD45A, FAS and JUN in tumor tissue. GAPDH was used as the internal control.

The following primary antibodies from Atlas Antibodies were used for the IHC and WB assay:

- Anti-FAS (HPA027444)
- Anti-GAPDH (HPA040067)
- Anti-TP53 (HPA051244)
- Anti-JUN (HPA059474)
- Anti-GADD45A (HPA053420)

Images from Zhao et al. 2021.

Conclusion

Together, we're moving towards advancing precision medicine and reshaping the future of cancer research.

In concluding our journey through pan-cancer markers, this e-book underscores the imperative shift from a generic approach to a more nuanced and personalized paradigm in cancer research. The integration of genomics, transcriptomics, and proteomics provides a holistic understanding of cancer's complexity, unraveling shared pathways and mechanisms **(Figure 13).**

As we explored the significance of biomarkers in Chapter 1, investigated genomic landscapes in Chapter 2, and uncovered the potential of proteomic approaches for drug discovery and development in Chapter 3, we emphasized the need for comprehensive strategies that transcend traditional boundaries.

This e-book urges for holistic strategies beyond traditional limits, aiming for a more inclusive -omics approach that recognizes the unique nature of each cancer.

Atlas Antibodies, together with the Human Protein Atlas initiative, takes a leading role in cancer research by producing and supplying highly validated primary antibodies that specifically target essential protein markers associated with cancer.

Our antibodies play a pivotal role in advancing our understanding of cancer biology, aiding researchers and clinicians in accurately identifying and studying key proteins involved in various aspects of cancer development and progression.

This commitment to providing reliable tools positions Atlas Antibodies at the forefront of the ongoing efforts to unravel the complexities of cancer, ultimately contributing to the advancement of diagnostic and therapeutic strategies in the field.

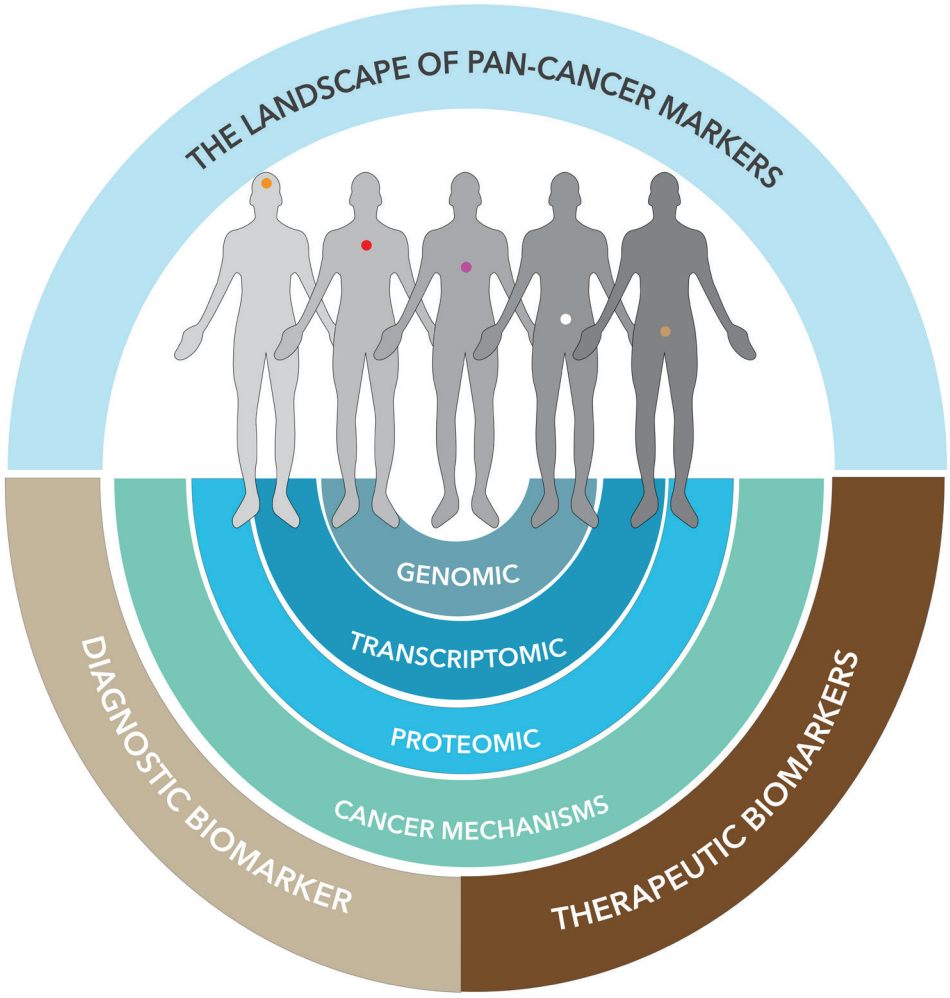


Figure 13.
The landscape of Pan-Cancer markers.

The exploration of pan-cancer markers represents a dynamic landscape that relies on the integration of diverse omics techniques, prominently featuring genomic, transcriptomic, and proteomic analyses.

Genomic studies play a crucial role in identifying DNA alterations, such as mutations, copy number variations, and structural rearrangements, that contribute to the initiation and progression of various cancers. For instance, the identification of common genomic alterations, like TP53 mutations, across different cancer types highlights shared pathways involved in tumorigenesis.

Transcriptomic analyses, which examine the expression patterns of genes, provide insights into the functional activity of genes and molecular pathways. An example of a pan-cancer marker derived from transcriptomic data is the elevated expression of certain oncogenes or the downregulation of tumor suppressor genes, reflecting dysregulation in cellular processes. The identification of overexpressed HER2/neu in breast, ovarian, and gastric cancers exemplify a pan-cancer marker with significant therapeutic implications.

Proteomic investigations focus on the study of proteins, reflecting the functional outcomes of genomic and transcriptomic changes. For instance, the overexpression of certain proteins involved in cell cycle regulation, like cyclin D1, across different cancer types underscores shared mechanisms driving uncontrolled cell proliferation.

Insights into **cancer mechanisms** by the integration of these omics approaches enhance our understanding of human cancers and provide a comprehensive view of the molecular landscape.

Such insights enable the development of more effective **diagnostic tools and targeted therapies** that can address the commonalities in cancer biology, fostering advancements in precision medicine across a spectrum of malignancies.

Table 4.
Suggested cancer protein markers from Atlas Antibodies (product name, catalog nr. and clonality)

ANGIOGENESIS		
Anti-AKT1	AMAb90834	Monoclonal
Anti-AKT1	AMAb90835	Monoclonal
Anti-CD34	HPA036722	Polyclonal
Anti-CD34	HPA036723	Polyclonal
Anti-EGFR	AMAb90816	Monoclonal
Anti-EGFR	AMAb90819	Monoclonal
Anti-EGFR	HPA001200	Polyclonal
Anti-ENG	AMAb90925	Monoclonal
Anti-ENG	HPA011862	Polyclonal
Anti-EPAS1	HPA031200	Polyclonal
Anti-GAPDH	HPA040067	Polyclonal
Anti-GAPDH	HPA061280	Polyclonal
Anti-HIF1A	HPA000907	Polyclonal
Anti-IDH1	AMAb90578	Monoclonal
Anti-IDH1	HPA057936	Polyclonal
Anti-MMP9	AMAb90804	Monoclonal
Anti-MMP9	AMAb90805	Monoclonal
Anti-MMP9	AMAb90806	Monoclonal
Anti-MMP9	HPA001238	Polyclonal
Anti-MMP9	HPA063909	Polyclonal
Anti-NOTCH1	HPA067168	Polyclonal
Anti-VEGFA	HPA069116	Polyclonal
Anti-VWF	AMAb90928	Monoclonal
Anti-VWF	AMAb90931	Monoclonal
Anti-VWF	HPA001815	Polyclonal
Anti-VWF	HPA002082	Polyclonal
METASTASIS		
Anti-KRT20	AMAb91564	Monoclonal
Anti-KRT7	AMAb91531	Monoclonal
Anti-MMP9	AMAb90804	Monoclonal
Anti-MMP9	AMAb90805	Monoclonal
Anti-MMP9	AMAb90806	Monoclonal
Anti-MMP9	HPA001238	Polyclonal
Anti-MMP9	HPA063909	Polyclonal
Anti-VIM	AMAb90516	Monoclonal

AUTOPHAGY/CELL DEATH/APOPTOSIS		
Anti-AKT1	AMAb90834	Monoclonal
Anti-AKT1	AMAb90835	Monoclonal
Anti-ANXA1	AMAb90558	Monoclonal
Anti-ANXA1	HPA011272	Polyclonal
Anti-BAD	HPA028185	Polyclonal
Anti-BAX	AMAb91490	Monoclonal
Anti-BAX	HPA027878	Polyclonal
Anti-BCL2	AMAb91492	Monoclonal
Anti-BCL2	HPA055295	Polyclonal
Anti-BID	HPA000722	Polyclonal
Anti-BIRC5	AMAb91761	Monoclonal
Anti-CASP8	HPA005688	Polyclonal
Anti-CASP9	HPA046488	Polyclonal
Anti-FAS	HPA027444	Polyclonal
Anti-MAP1LC3A	HPA052474	Polyclonal
Anti-MCL1	AMAb90859	Monoclonal
Anti-MTOR	AMAb91508	Monoclonal
Anti-PARP1	AMAb90959	Monoclonal
Anti-PARP1	AMAb90960	Monoclonal
Anti-PDCD1	AMAb91197	Monoclonal
Anti-PIK3CA	AMAb91513	Monoclonal
Anti-PIK3CA	AMAb91514	Monoclonal
METABOLIC REPROGRAMMING		
Anti-GAPDH	HPA040067	Polyclonal
Anti-GAPDH	HPA061280	Polyclonal
Anti-HIF1A	HPA000907	Polyclonal
Anti-IDH1	AMAb90578	Monoclonal
Anti-IDH1	HPA057936	Polyclonal
GENOMIC INSTABILITY & MUTATIONS		
Anti-CHEK2	AMAb91570	Monoclonal
Anti-CHEK2	HPA001878	Polyclonal
Anti-PLK1	AMAb91515	Monoclonal
Anti-PLK1	HPA051638	Polyclonal

Table 4.
Cont...

PROLIFERATION		
Anti-ABL1	HPA028409	Polyclonal
Anti-BRAF	AMAb91257	Monoclonal
Anti-BRAF	AMAb91258	Monoclonal
Anti-BRAF	HPA071048	Polyclonal
Anti-CSF1R	AMAb91718	Monoclonal
Anti-EGFR	AMAb90816	Monoclonal
Anti-EGFR	AMAb90819	Monoclonal
Anti-EGFR	HPA001200	Polyclonal
Anti-ERBB3	HPA045396	Polyclonal
Anti-ESR1	AMAb90867	Monoclonal
Anti-ESR1	HPA000449	Polyclonal
Anti-HER2	AMAb90627	Monoclonal
Anti-HER2	AMAb90628	Monoclonal
Anti-JUN	AMAb91587	Monoclonal
Anti-JUN	HPA059474	Polyclonal
Anti-KIT	AMAb90900	Monoclonal
Anti-KIT	AMAb90901	Monoclonal
Anti-KIT	AMAb90904	Monoclonal
Anti-KIT	HPA004471	Polyclonal
Anti-KIT	HPA073252	Polyclonal
Anti-MKI67	AMAb90870	Monoclonal
Anti-PCNA	HPA030522	Polyclonal
Anti-PGR	AMAb91529	Monoclonal
Anti-PGR	HPA004751	Polyclonal
Anti-PGR	HPA008428	Polyclonal
Anti-PTEN	AMAb91735	Monoclonal
Anti-PTEN	AMAb91736	Monoclonal
Anti-PTEN	HPA031335	Polyclonal
Anti-SMAD2	AMAb91520	Monoclonal
Anti-SMAD3	HPA046386	Polyclonal
Anti-SMAD3	HPA067203	Polyclonal
Anti-SMAD4	AMAb91594	Monoclonal
Anti-SMAD4	HPA019154	Polyclonal
Anti-VEGFA	HPA069116	Polyclonal

CELL CYCLE		
Anti-AURKA	HPA002636	Polyclonal
Anti-AURKB	HPA037708	Polyclonal
Anti-CCNA1	HPA060646	Polyclonal
Anti-CCNB1	HPA030741	Polyclonal
Anti-CCNB1	HPA061448	Polyclonal
Anti-CCNB2	HPA008873	Polyclonal
Anti-CCNB3	HPA000496	Polyclonal
Anti-CCND1	HPA027802	Polyclonal
Anti-CCND2	HPA049138	Polyclonal
Anti-CCND2	HPA054196	Polyclonal
Anti-CDK2	AMAb91497	Monoclonal
Anti-CDK4	AMAb91499	Monoclonal
Anti-CDK5	HPA064535	Polyclonal
Anti-CDK6	HPA002637	Polyclonal
Anti-CDKN2A	HPA047838	Polyclonal
Anti-CDKN2B	HPA063327	Polyclonal
Anti-CDKN2D	HPA043546	Polyclonal
Anti-CHEK1	HPA044364	Polyclonal
Anti-CHEK2	HPA001878	Polyclonal
Anti-CHEK2	AMAb91570	Monoclonal
Anti-E2F1	HPA008003	Polyclonal
Anti-E2F1	HPA029735	Polyclonal
Anti-p53/TP53	AMAb90956	Monoclonal
Anti-p53/TP53	HPA051244	Polyclonal
Anti-PARP1	AMAb90959	Monoclonal
Anti-PARP1	AMAb90960	Monoclonal
Anti-PARP1	HPA045168	Polyclonal
Anti-PLK1	HPA051638	Polyclonal
Anti-PLK1	HPA053229	Polyclonal
Anti-PLK1	AMAb91515	Monoclonal
Anti-RB1	HPA050082	Polyclonal
Anti-RIF1	HPA036887	Polyclonal
Anti-RIF1	HPA036888	Polyclonal

References

Alsop K, et al. BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. J Clin Oncol. 2012 Jul 20;30(21):2654-63.

Amado RG, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. 2008 Apr 1;26(10):1626-34

André F, et al. SOLAR-1 Study Group. Alpelisib for PIK3CA-Mutated, Hormone Receptor-Positive Advanced Breast Cancer. N Engl J Med. 2019 May 16;380(20):1929-1940.

Annese T, et al. Autocrine/Paracrine Loop Between SCF+/c-Kit+ Mast Cells Promotes Cutaneous Melanoma Progression. Front Immunol. 2022 Jan 24;13:794974.

Belli C, Repetto M, Anand S, Porta C, Subbiah V, Curigliano G. The emerging role of PI3K inhibitors for solid tumour treatment and beyond. Br J Cancer. 2023 Jun;128(12):2150-2162.

Bellizzi AM. p53 as Exemplar Next-Generation Immunohistochemical Marker: A Molecularly Informed, Pattern-Based Approach, Methodological Considerations, and Pan-Cancer Diagnostic Applications. Appl Immunohistochem Mol Morphol. 2023 Aug 1;31(7):507-530.

Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010 Jun;138(6):2073-2087.e3.

Campeau PM, et al. Hereditary breast cancer: new genetic developments, new therapeutic avenues. Hum Genet. 2008 Aug;124(1):31-42.

Chen, F., Chandrashekar, D.S., Varambally, S. et al. Pan-cancer molecular subtypes revealed by mass-spectrometry-based proteomic characterization of more than 500 human cancers. Nat Commun 10, 5679 (2019).

Coiffier B, et al. Rituximab (anti-CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma: a multicenter phase II study. Blood. 1998;92(6):1927-1932.

Cunningham D, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. New England Journal of Medicine. 2004;351(4):337-345.

Gao X, Bu H, Gao X, Wang Y, Wang L, Zhang Z. Pan-cancer analysis: SPAG5 is an immunological and prognostic biomarker for multiple cancers. FASEB J. 2023 Oct;37(10):e23159.

Hall JM, et al. Linkage of early-onset familial breast cancer to chromosome 17q21. Science. 1990 Dec 21;250(4988):1684-9.

Hartmann C, et al. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. Acta Neuropathol. 2009 Oct;118(4):469-74.

Hurwitz H, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. New England Journal of Medicine. 2004;350(23):2335-2342.

Lam KK, Tang CL, Tan E, Wong SH, Cheah PY. KRAS mutation-independent downregulation of MAPK/PI3K signaling in colorectal cancer. Mol Oncol, 2022 Jan 1; 16(5):1171-1183

Lane DP. Cancer. p53, guardian of the genome. Nature. 1992 Jul 2;358(6381):15-6.

Le, D. T., Durham, J. N., Smith, K. N., Wang, H., Bartlett, B. R., Aulakh, L. K., ... & Diaz Jr, L. A. (2017). Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science, 357(6349), 409-413.

Lynch TJ, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med. 2004 May 20;350(21):2129-39.

Ramus SJ, Gayther SA. The contribution of BRCA1 and BRCA2 to ovarian cancer. Mol Oncol. 2009 Apr;3(2):138-50.

Raykova D, et al. A method for Boolean analysis of protein interactions at a molecular level. Nat Commun. 2022 Aug 13;13(1):4755.

Richardson PG, et al. Bortezomib (PS-341): a novel, first-in-class proteasome inhibitor for the treatment of multiple myeloma and other cancers. Cancer Control. 2003 Sep-Oct;10(5):361-9.

Robert C, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med. 2015 Jan 1;372(1):30-9.

Robert C, et al. Pembrolizumab versus ipilimumab in advanced melanoma. New England Journal of Medicine. 2015;372(26):2521-2532.

Slamon D, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. New England Journal of Medicine. 2001;344(11):783-792

Swain SM, et al. Targeting HER2-positive breast cancer: advances and future directions. Nat Rev Drug Discov. 2023 Feb;22(2):101-126.

Wooster R, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature. 1995 Dec 21-28;378(6559):789-92.

Xu K, et al. Prognostic significance of BRCA mutations in ovarian cancer: an updated systematic review with meta-analysis. Oncotarget. 2017 Jan 3;8(1):285-302.

Yan H, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med. 2009 Feb 19;360(8):765-73.

Zhang J. Poly (ADP-ribose) polymerase inhibitor: an evolving paradigm in the treatment of prostate cancer. Asian J Androl. 2014 May-Jun;16(3):401-6.

Zhao L, et al. NAD-dependent methylenetetrahydrofolate dehydrogenase inhibits oral squamous cell carcinoma cell proliferation and promotes apoptosis. Transl Cancer Res, 2021 Mar; 10(3):1457-1469.

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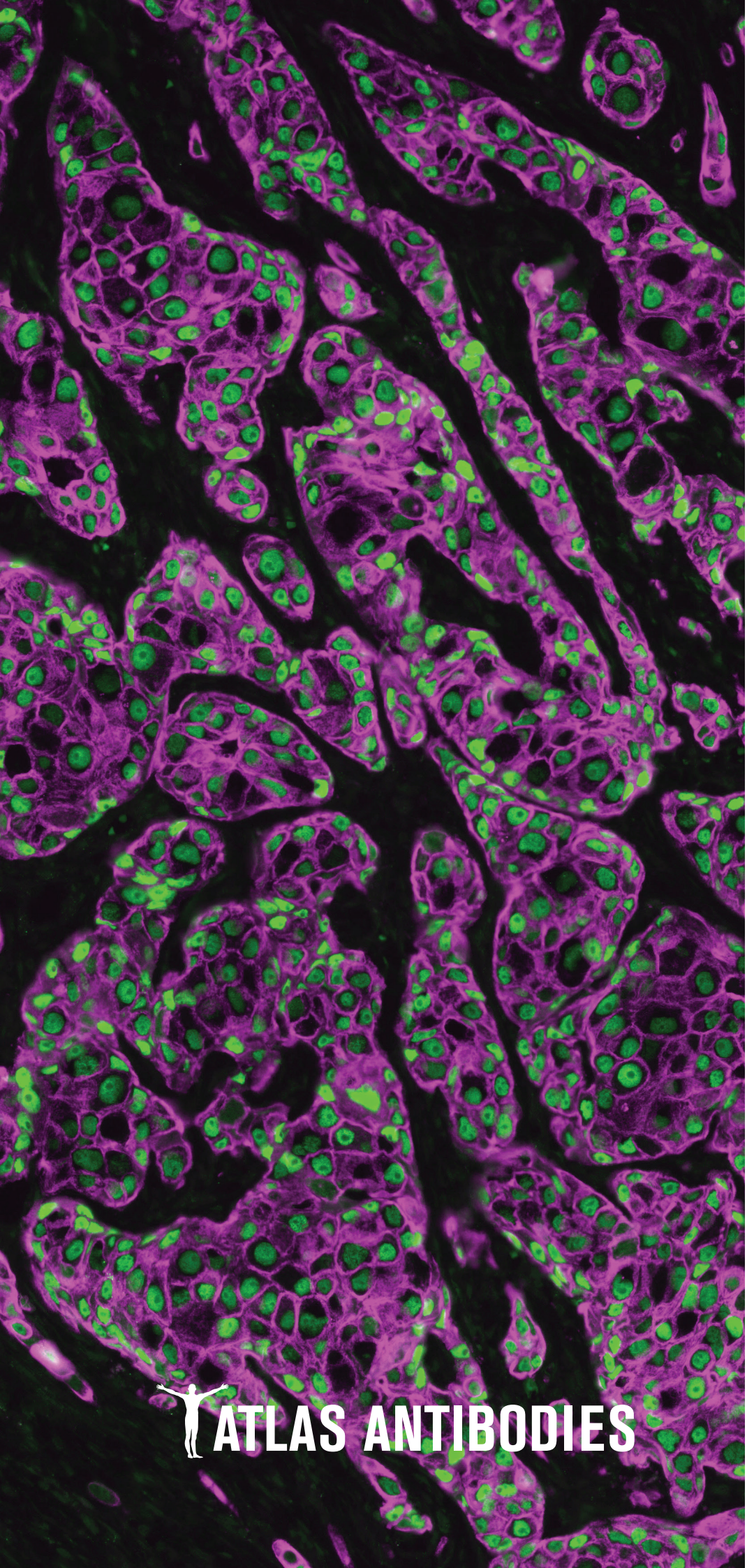

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