Information on the Applied Markers

Immune cell identification markers		
CD45/LCA	positive in	leukocytes including granulocytes, lymphocytes, monocytes, macrophages/histiocytes, mast cells and plasma cells
	relevance	identification of leukocytes

Immune checkpoint markers		
PD-L1	definition / function	inhibitory immune checkpoint protein; binds to its receptor PD-1 on T cells; inhibits autoimmunity and T cell activation; expression on tumor cells is an immune escape mechanism
	positive in	variably expressed on immune and tumor cells
	relevance	estimation of the extent of inhibition of T cell mediated anticancer activity
LAG3	definition / function	inhibitory co-receptor expressed on lymphoid cells; suppresses T cell mediated immune response
	positive in	exhausted T cells (antigen-activated T cells)
	relevance	identification of exhausted T cells; estimation of the extent of inhibition of T cell mediated anticancer immunity

Markers to characterize signal transduction pathways		
p-mTOR	definition / function	protein which promotes cell growth, proliferation, differentiation, protein synthesis, angiogenesis, autophagy, and apoptosis; phosphorylated (activated) state of mTOR; dysregulation of the mTOR pathway has been linked with malignant transformation/ tumorigenesis
	positive in	cells expressing activated mTOR protein
	relevance	evaluation of Pi3K/AKT/mTOR pathway activation in tumor cells (H Score for cytoplasmic expression)
p-ERK	definition / function	extracellular signal-regulated kinases (ERKs) are members of the mitogen-activated protein kinase superfamily (MAPK); ERKs require phosphorylation for their full activity; when activated, ERKs regulate cell proliferation, differentiation, and survival; ERK signalling is frequently dysregulated in cancer cells
	positive in	cells expressing activated ERK protein
	relevance	evaluation of MAPK pathway activation in tumor cells (H Score for cytoplasmic expression)
p16	definition / function	tumor suppressor protein encoded by the *CDKN2A* gene; inhibitor of the cell cycle activating proteins CDK4 and CDK6; suppresses cell growth and cell division; loss of p16 function contributes to the bypass cell senescence and is associated with progression of malignant disease
	positive in	cells with intact *CDKN2A*/p16; nuclear and cytoplasmic overexpression in HPV-associated tumors; loss of nuclear staining in tumors with biallelic *CDKN2A* inactivation
	relevance	evaluation of p16 expression in *CDKN2A* altered tumors; evaluation of activation of the CDK pathway; surrogate marker for HPV-association
p53	definition / function	tumor suppressor protein encoded by the *TP53* gene; activated in response to DNA damage; induces cell cycle arrest to allow for DNA repair mechanisms, directs cell senescence, initiates apoptosis
	positive in	normal tissues (scattered staining of few nuclei in case of wildtype p53 expression); tumor cells with *TP53* alteration (strong nuclear staining in a high fraction of tumor cells in case of most missense mutations, complete absence of nuclear staining in case of truncating, frameshift alterations and/or deletions)

	relevance	verification of a *TP53* alteration on the protein level; tumor cell identification in *TP53*-altered entities
HER2	definition / function	human epidermal growth factor receptor 2 encoded by the *ERBB2* gene; tyrosine kinase involved in cell growth, cell division and cell repair mechanisms; constitutively active, when overexpressed
	positive in	membranes of epithelial cells of several organs including breast, skin, gastrointestinal, respiratory, reproductive, and urinary tract; overexpression is found in up to 20% of breast cancers, but also in colon, stomach, and esophageal cancer
	relevance	assessment of membranous HER2 expression in tumor cells; verification of HER2 amplification or overexpression detected by DNA/RNA sequencing on the protein level
TROP2	definition / function	transmembrane glycoprotein encoded by the *TACSTD2* gene; modulates self-renewal, proliferation, invasion, and survival
	positive in	normal epithelial tissue, overexpressed in many tumor entities, predominantly in breast and bladder cancer
	relevance	verification of *TACSTD2* RNA overexpression on the protein level, categorization of TROP2 expression levels (e.g., low vs. intermediate vs. high), confirmation of target expression for clinical trials or translational research

Markers to characterize tissue and tumor types		
Ki67	definition / function	marker of cell proliferation; the extent of Ki67 staining is known as proliferation index and is oftentimes used as a measure of tumor malignancy
	positive in	cells that have entered the cell cycle
	relevance	assessment of the percentage of proliferating nuclei in a tissue type, cell type or tumor entity

This table provides an overview of the markers used in this analysis and an explanation of their application in the given context. The listed specifications may therefore not be comprehensive or complete.