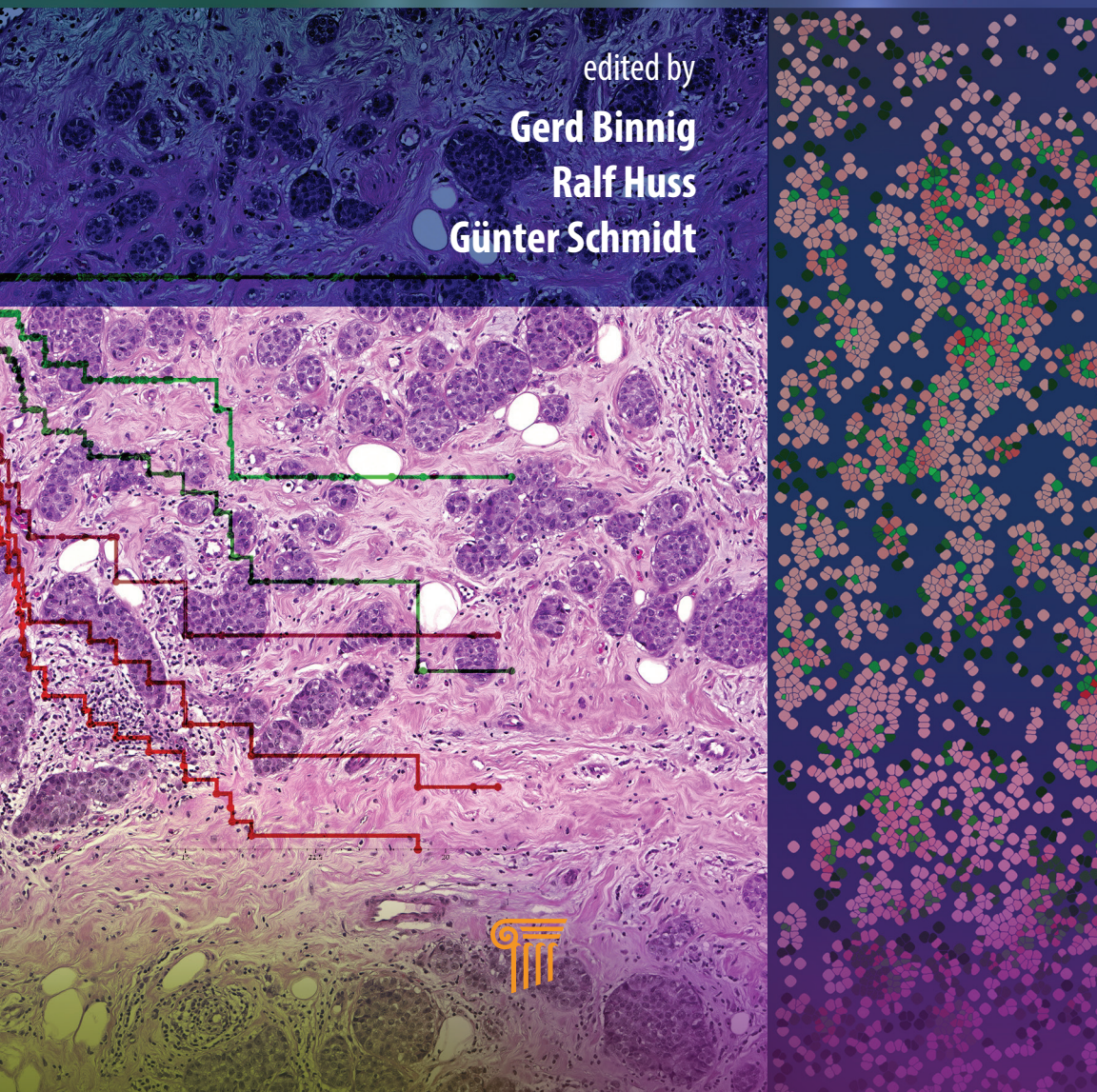
Jenny Stanford Series on Next-Generation Medicine Vol. 1

Tissue Phenomics

Profiling Cancer Patients for Treatment Decisions



edited by
Gerd Binnig
Ralf Huss
Günter Schmidt

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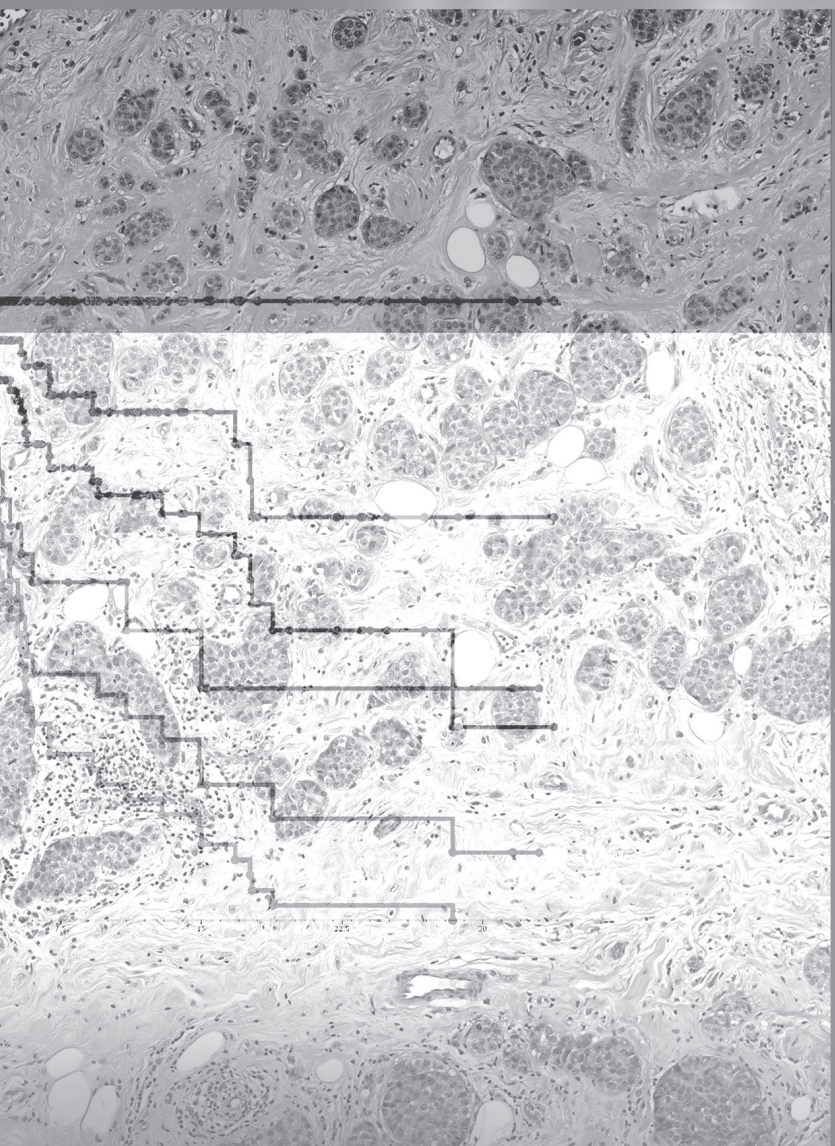

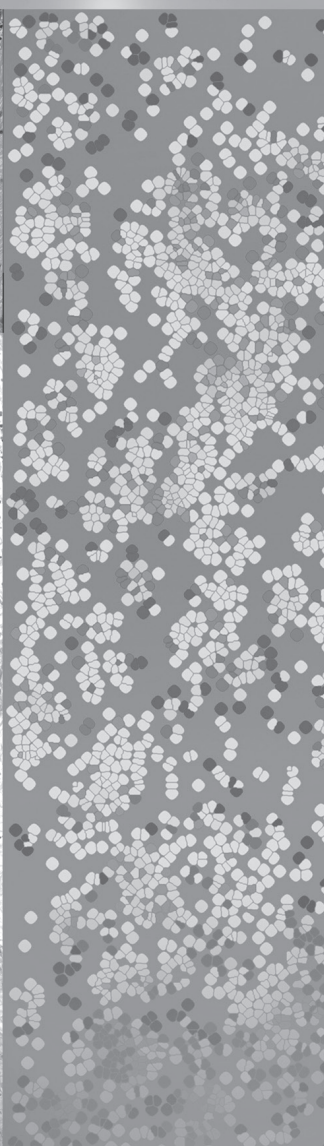

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Tissue Phenomics: Profiling Cancer Patients for Treatment Decisions

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Morphological and functional complexity of animal and human tissue is one of the most fascinating and simultaneously challenging topics in tissue-based science. The diverse organizational units of a normal liver, lung, or kidney, such as organ-specific cells, nerves, connective tissues, and different types of vessels, show so many variants that a systematic and comprehensive analysis by human eyes is not possible. In addition, time-related modifications and spatial distribution of the components as well as disease-related variants produce an even higher level of complexity, often termed hyper-complexity.

Today, the only way to find a solution for reliable and reproducible analyses of various tissues is based on multiplex digital systems that produce tissue-related big data. By applying suitable algorithms, these data can be sorted and used to answer different diagnostic, prognostic, predictive, and scientific questions. The concept of tissue phenomics is currently the most promising approach to answer many burning questions of cancer and other diseases.

—Prof. Dr. med. Dr. h. c. Manfred Dietel
Charité, Berlin, Germany

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Foreword

Evolution of Tissue Phenomics and Why It Is Critical to the War on Cancer

A view from a tumor immunologist and cancer immunotherapist

Those of us in biomedical research are witnessing an almost daily evolution of our science. Nowhere is this more obvious, or possessing greater impact, than in the field of cancer immunology and immunotherapy. Cancer, one of the great scourges on humanity, is having the veil of its secrets lifted. Digital imaging and objective assessment tools contribute substantial and solid evidence to document that immune cells are prognostic biomarkers of improved outcomes for patients with cancer. While anecdotal reports of associations between immune cell infiltrates and improved outcomes have been presented by pathologists for more than 100 years, the co-evolution of multiple science subspecialties has resulted in opportunities to better understand the disease and why it develops. Armed with this knowledge and evidence that checkpoint blockade therapies are capable of unleashing the immune system, increasing survival and possibly curing some patients with cancer, will lead to additional investment in this area of research, which will accelerate the pace at which we develop improved treatments for cancer. It is clear that digital imaging and assessment of complex relationships of cells within cancer, the very essence of tissue phenomics will play a central role in the development of the next generation of cancer immunotherapies. Ultimately, assessment of cancer tissue phenomics will be used to tailor immunotherapies to treat and eventually cure patients with cancer.

This is a very different time from when I began as an immunologist. Monoclonal antibodies, reagents capable of objectively assessing thousands of molecules, were not yet invented. To identify a subset of white blood cells, termed T cells, we used the binding of sheep erythrocytes to lymphocytes as the assay to characterize their numbers. The number of lymphocytes that formed *rosettes* was counted on a hemocytometer using a light microscope. This method was used to dose patients with immunosuppressive therapy to pre-

vent allograft rejection. In tissue sections and smears, we did not use immunohistochemistry (IHC); we used ocular annotation of a cell's morphological characteristics. My limited training in this area came at the hands of Dr. John W. Rebuck, a hematopathologist, who trained with Dr. Hal Downey, who trained with Professor Artur Pappenheim, at the University of Berlin. Professor Pappenheim, who developed the Pappenheim stains, educated his trainees into the subtleties of morphology, who then propagated the method to their trainees, and in this way the method spread.

Lymphocyte was my cell of interest; it was known to be a small round cell, with limited cytoplasm. Under Dr. Rebuck's tutelage, I denuded my skin with a scalpel, placed a drop of the diphtheria, pertussis, and tetanus vaccine on the area, covered it with a sterile glass coverslip, and attached it in place using a small piece of cardboard and adhesive tape. I would then change the coverslips every 3 h over a period of 48 h. Once the coverslips were removed and stained with Leishman's, I would sit at a multi-headed microscope and Dr. Rebuck would point out the monocytes or lymphocytes that were migrating into the site and onto the coverslip, identifying whether they were lymphocytes or monocytes that were morphing into large phagocytic cells. To support his description, Dr. Rebuck would describe the nuclear and cytoplasmic characteristics, as well as the other types of cells present in the area. That type of detailed evaluation has gone on for more than a century and remains the principal means to characterize disease.

As you read this book, it will become clear how the advances in image assessment technology allow subtle characteristics of cells to be evaluated in an objective and automated fashion. In addition to the morphological characteristics of cells, multiplex IHC provides simultaneous assessment of six or more markers on a single slide. Utilizing other technology, it is possible to stain a slide and then image and strip the slide of the reagents so that the cycle can be repeated as many as 60 times, allowing assessment of as many markers on a single slide. Coupled with the advent of tissue phenomics, all of this information can be evaluated in the context of whether it is inside the tumor, at the invasive margin, or in the stroma.

The molecular evaluation of disease is also advancing. Summaries of gene expression profiling data for tumor samples from hundreds of patients are available in The Cancer Genome

Atlas (TCGA) and other databases. These databases are being interrogated to evaluate how many cancers had high or low levels of genes associated with immune cells, as well as for expression of cancer-destroying molecules or of mechanisms cancer can use to evade immune-mediated destruction. While these interrogations of the data are providing important and powerful insights about the immune system's response to cancer, much of this information lacks the context of where these elements are expressed and the identity of which cells are expressing specific genes. Only by understanding the context of this information, specifically which cell is expressing what and which cells are nearby, can it be effectively used to guide a new generation of cancer immunotherapy trials.

Head and neck cancer will serve as an example to further clarify this point. For several years, it has been known that increased numbers of CD8(+) *cancer killer T cells* at the tumor were associated with improved survival. For example, in one study patients whose tumors had above the median number of CD8 T cells had around a 50% 5-year survival, while those whose tumors had CD8 T cell numbers below the median, had a 35% 5-year survival. This pattern was true for assessment of CD8(+) T cell numbers by IHC or gene expression profiling. As an immunologist who recognizes the important role that CD8 T cells can play in preclinical animal models, this made sense. However, it was also reported that an increased number of FOXP3(+) *suppressor* T cells were also associated with improved survival. Since these are the cells that can turn off the cancer killer cells, these results made no sense. As we began to apply the multiplex IHC method to visualize six markers on a single 4-micron section, it became clear that in some patients, immune cells associated with the tumor were organized in a specific pattern. In one case, the tumor, which uniformly expressed high levels of the immune checkpoint PD-L1, had excluded essentially all immune cells from inside the tumor. However, at the tumor–stroma barrier was a band of suppressor T cells, and outside of that band were the CD8(+) cancer killer T cells. Since several of the suppressor cells' immune inhibitory functions required cell contact, we reasoned that in order to be effective, the suppressor cells would need to be relatively close to the CD8(+) cancer killer cell that they were trying to inhibit. When we evaluated tumors with a high number of suppressor cells near the CD8(+) cancer killer cells, we found that these patients did significantly worse than patients with low numbers, not better. As

we evaluated another inhibitory molecule, PD-L1, which mediates inhibition by contact, we found the same pattern. While additional validation needs to be done, this illustrates the power of evaluating cell–cell relationships. It will be these types of assessments with panels of 20–25 markers that will provide critical insights into why tumors escape immune elimination and what hurdles will need to be addressed to improve patient outcomes. It is not going to be easy and after spending 5 years in the midst of digital imaging technologies, I realize there are substantial challenges ahead. There is no looking back! Since the immune system is the critical element that can cure patients of cancer, it is essential to assess the cancer that escapes and ultimately kills the patient. While multiple approaches must be applied, the *tissue is the issue* and tissue phenomics is the approach that will play the critical role in unraveling the amazing complexity of the cancer–immune system interphase and drive the development of treatments to cure patients with cancer.

Bernard A. Fox
Providence Cancer Center
Portland, OR, USA
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Ralf Huss
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