Resistance to Targeted Anti-Cancer Therapeutics

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Resistance to Anti-Cancer Therapeutics Targeting Receptor Tyrosine Kinases and Downstream Pathways

Springer

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This volume is dedicated to the memory of the late Prof. Shraga Segal, our teacher, colleague, and dear friend.

Aims and Scope

For several decades, treatment of cancer consisted of chemotherapeutic drugs, radiation, and hormonal therapies. Those were not tumor specific and exhibited several toxicities. During the last several years, targeted cancer therapies (molecularly targeted drugs) have been developed and consist of immunotherapies (cell mediated and antibody), drugs, or biologicals that can block the growth and spread of cancer by interfering with surface receptors and with specific dysregulated gene products that control tumor cell growth and progression. These include several FDA-approved drugs/antibodies/inhibitors that interfere with cell growth signaling or tumor blood vessel development, promote the death of cancer cells, stimulate the immune system to destroy specific cancer cells, and deliver toxic drugs to cancer cells. Targeted cancer therapies are being used alone or in combination with conventional drugs and other targeted therapies.

One of the major problems that arise following treatment with both conventional therapies and targeted cancer therapies is the development of resistance, pre-existing in a subset of cancer cells or cancer stem cells and/or induced by the treatments. Tumor cell resistance to targeted therapies remains a major hurdle and, therefore, several strategies are being considered in delineating the underlining molecular mechanisms of resistance and the development of novel drugs to reverse both the innate and acquired resistance to various targeted therapeutic regimens.

The new series "*Resistance of Targeted Anti-Cancer Therapeutics*" was inaugurated and focuses on the clinical application of targeted cancer therapies (either approved by the FDA or in clinical trials) and the resistance observed by these therapies. Each book will consist of updated reviews on a specific target therapeutic and strategies to overcome resistance at the biochemical, molecular, and both genetic and epigenetic levels. This new series is timely and should be of significant interest to clinicians, scientists, trainees, students, and pharmaceutical companies.

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Preface

Unlike cytotoxic regimens, such as chemotherapy, which inhibit the ability of cells of all tumors to rapidly proliferate, molecular targeted therapies inhibit survival pathways or oncogenic mutations specific to each cancer's molecular subtype. The emerging ability to tailor a therapeutic drug to a patient is the outcome of deep knowledge of oncogenic circuitries, as well as detailed characterization of the specific repertoires of genetic aberrations driving individual tumors [1]. This new chapter of cancer pharmacology is attributable to two visionary concepts. The 1906 "Magic Bullet" concept of Paul Ehrlich, which was based on the use of histological dyes, predicted that it would be possible to target a pathological tissue while avoiding nearby healthy tissues [2]. In the same vein, the 2002 concept of Irwin Weinstein [3], called "Oncogene Addiction," argued that cancers harboring multiple genetic abnormalities are dependent on (or "addicted" to) one or only a few mutated genes. Hence, the reversal of just one or a few of these abnormalities might inhibit cancer cells. The excessive reliance of tumor cells on specific intracellular pathways is well exemplified by the dependency of chronic myeloid leukemia on BCR-ABL, an oncogenic fusion protein [4]. Yet another class of dependency is the so-called "non-oncogene addiction" [5], which is exemplified by the reliance of a fraction of colorectal tumors on autocrine loops involving the epidermal growth factor receptor (EGFR) and two of its ligand growth factors [6].

The harvest of the new era of molecular targeted therapy of cancer is truly impressive. This started in 1997, when three pioneer drugs were approved, namely imatinib, a kinase blocker specific to BCR-ABL, trastuzumab, a monoclonal antibody (mAb) to HER2 (a kin of EGFR), and rituximab, a mAb specific to CD20. Remarkably, the first wave of drugs was followed by an avalanche, such that the first two decades of the new millennium have witnessed the approval of more than 60 new cancer drugs, primarily protein kinase inhibitors (PKIs) and recombinant mAbs. This fruitage already dwarfs the preceding era of chemotherapeutic drugs and is bound to dominate, in the near future, some clinical indications of oncology.

In spite of the optimistic scenario, the wide occurrence of patient's resistance to the new drugs casts long shadows. In most cases, only a fraction of patients responds to molecular targeted therapies. This "primary" or "de novo" resistance laid the

foundation for a new discipline that searches for biological indicators, or biomarkers, potentially able to predict who are the patients that will benefit from a particular targeted therapy [7]. Similarly, "secondary" or "acquired" resistance characterizes patients who initially respond but become resistant while under treatment. Importantly, as more molecular targeted drugs are entering routine use in oncology wards around the world, we learn that resistance to the new drugs is one of the main barriers to further progress. From a biological perspective, acquired resistance may reflect, on the one hand, the remarkable robustness and adaptability shared by all living systems [8], and, on the other hand, the surprisingly large intra- and intertumoral genetic heterogeneity [9].

The objective of this volume, entitled *Resistance to Anti-Cancer Therapeutics Targeting Receptor Tyrosine Kinases and Downstream Pathways,* is to review the dynamic field of resistance to molecular targeted anti-cancer therapies. Because many of the new drugs block the action of trans-membrane receptors for growth factors, such as EGFR, or the signaling pathways such receptors commonly activate, for example the RAS-to-ERK pathway, we decided to focus on drugs targeting receptor tyrosine kinases (RTKs) and the oncogenic biochemical reactions they stimulate. In general, two classes of mechanisms might confer resistance. The first class refers to alterations occurring at the level of drug or host (patient), rather than within the malignant tissue. For example, pharmacokinetics effects that deplete a drug prevent uptake or metabolize it into less active fragments. The other class of mechanisms brings together all adaptive alterations taking place in the cancerous tissue. This group of molecular and cellular mechanisms represents tumor adaptation while under drug treatment [10, 11]. For example, Darwinian microevolution instigated by a molecular targeted drug may newly generate or expose pre-existing clones of cancer cells harboring novel, drug-resistant mutants [12], or amplify the target to quench the drug in question. Other mechanisms that enable evasion involve activation of alternative pathways of cell survival, which use either a parallel (bypass) track or an active target downstream of the blocked molecular target [11].

The first half of the volume concentrates on drugs targeting the RTK molecules themselves, whereas the other part deals with resistance to inhibitors of the corresponding downstream signaling pathways. A chapter contributed by **Livio Trusolino and Simonetta Leto** opens the volume and highlights the case of colorectal tumors treated with anti-EGFR mAbs, i.e., cetuximab or panitumumab. Importantly, *KRAS* point mutations and gene copy number gains are responsible not only for primary but also for acquired resistance in approximately 50% of patients who relapse while on mAb treatment. Other evasion mechanisms include activation of compensatory pathways (e.g., BRAF, PI3K, HER2, and MET), but mutations activating EGFR or preventing cetuximab binding appear to be quite rare. Notably, Trusolino and Leto conclude that the very same alterations that account for intrinsic refractoriness also foster progressively diminished response in the course of treatment. The second chapter, by **Maicol Mancini and Yosef Yarden**, deals with resistance of lung cancer to ATP-mimicking molecules, PKIs like erlotinib and crizotinib, which are respectively specific to mutant forms of EGFR or ALK fusion proteins. Remarkably, resistance to these and other first-generation PKIs evolves in

patients within 10–24 months. Unlike resistance of colorectal cancer to anti-EGFR mAbs, the major mechanism conferring resistance of lung tumors to erlotinib entails second-site mutations. Other mechanisms of acquired resistance that have been confirmed in clinical specimens include increased expression of the compensatory RTKs MET and AXL, *EGFR* amplification, mutations in the *PIK3CA* gene, or a pronounced epithelial-to-mesenchymal transition. As exemplified by Mancini and Yarden, once resolved, mechanisms conferring resistance to PKIs may pave the way for next-generation drugs, or they may identify combination therapies simultaneously inhibiting the primary and alternative routes to oncogenesis.

The introduction and clinical approval of trastuzumab, a mAb specific to HER2, has significantly improved survival of patients with HER2-positive ($HER2^+$) metastatic breast cancer. However, as with other therapeutic antibodies, resistance to trastuzumab significantly shortens clinical application or necessitates alternative treatments, such as the addition of a second anti-HER2 antibody, called pertuzumab. **Jennifer Hsu and Mien-Chie Hung** discuss three molecular mechanisms potentially underlying resistance: (i) Upregulation of downstream signaling, such as the PI3K/AKT pathway, due to mutations in the gene encoding PI3K and/or inactivation or loss of PTEN, which antagonizes PI3K and negatively regulates AKT activities. (ii) Hindrance of trastuzumab binding to HER2 by means of either ectodomain shedding and generation of a constitutively active, truncated form of HER2, or through alternative translation initiation of *HER2* mRNA. An alternative mechanism involves binding of the cell surface glycoprotein mucin-4 (MUC4) to the extracellular domain of HER2, which can mask the trastuzumab-binding site on HER2. And (iii) Overexpression of other RTKs, such as EGFR, EPH-A2, the insulin-like growth factor receptor 1 (IGF-1R) and its ligands, or MET and its ligand, the hepatocyte growth factor (HGF). Resistance to experimental drugs targeting MET is described in a chapter contributed by **Simona Corso and Silvia Giordano.** HGF-MET signaling plays an important role in tumor progression, in particular during the late stages of invasive growth and metastasis. Nevertheless, Corso and Giordano critically discuss the status of MET targeting in highly metastatic tumors. Despite compelling evidence obtained in preclinical studies, which demonstrated that MET targeting in the respective MET-addicted tumors could be of therapeutic value, so far the results obtained in clinical trials employing MET-targeted drugs (e.g., PKIs and mAbs to MET or to HGF) have been disappointing. Among the reasons they list are questionable selection of patients, relatively rare and heterogeneous amplification of the *MET* gene, the need to differentiate between ligand-dependent and mutational activation of MET, as well as the choice of the type of PKIs and the frequent compensatory signaling by EGFR and the HER family members.

Following ligand binding and receptor dimerization, RTKs undergo catalytic activation that culminates in trans-phosphorylation of cytoplasmic proteins, as well as evokes transcriptional responses in the nucleus [13]. Simultaneous firing of several linear cascades is typical to active forms of RTKs, but mainly two such cascades are frequently activated in tumors due to mutations within critical components. These are the RAS-RAF-MEK-ERK cascade and the PI3K-AKT-mTOR

pathway. After reviewing the mutational status of the RAS-to-ERK pathway in tumors, **Galia Maik-Rachline, Izel Cohen, and Rony Seger** focus on clinically approved inhibitors of BRAF (e.g., vemurafenib) and MEK (e.g., dabrafenib), which are used to treat patients with metastatic melanoma. As with other PKIs, intrinsic resistance limits drug application, although the targeted mutation in BRAF is present. Interestingly, this may involve, among other factors, large secretome changes, which establish a tumor microenvironment that supports expansion of drug-resistant cancer cell clones but exhibits susceptibility to combination therapies. Resistance often emerges also in patients who initially respond to BRAF inhibitors. Mechanisms underlying the emergence of secondary resistance are surprisingly varied: expression of drug-resistant isoforms of the target, alterations of downstream components that reactivate the ERK cascade, and induction of upstream components (or other signaling pathways) that bypass the pharmacological effect. In analogy to the ERK pathway, hyperactivation of the PI3K cascade is frequent in human tumors, as reviewed by **Pau Castel and Maurizio Scaltriti**. Although this provided strong rationale to develop inhibitors targeting many different components of the pathway, the responses observed in patients treated with such inhibitors have been, in general, short lived and anecdotal. In the last few years, however, large clinical studies have demonstrated that specific compounds (e.g., AKT catalytic inhibitors and specific PI3Kalpha inhibitors) can elicit strong antitumor activities if administered to patients selected on the basis of specific activating mutations. Nevertheless, as described by Castel and Scaltriti, intrinsic and acquired resistance to inhibitors of the pathway currently limit the activity of these agents, but combinatorial strategies may delay emergence of drug resistance.

Inhibition of apoptosis is oncogenic and characterizes a broad range of tumor types, whereas promotion of cell cycle arrest is tumor suppressive [14]. Individual BCL-2 (B-cell lymphoma 2) family members couple apoptosis regulation and cell cycle control, while serving as a signaling nexus among kinase cascade-driven growth/survival signals. **Konstantinos Floros, Anthony Faber, and Hisashi Harada** open their chapter by reviewing genomic alterations, such as *BCL-2* translocations, which lead to a gain-of-function anti-apoptotic signal. They later describe venclexta (venetoclax), the first drug targeting BCL-2, which has been approved for the treatment of patients with chronic lymphocytic leukemia (CLL). Two other, yet experimental drugs, are also described and the authors provide a critical discussion of mechanisms of resistance, as well as pharmacological strategies that overcome resistance. For example, a combination of venetoclax and ibrutinib, a Bruton Tyrosine Kinase (BTK) inhibitor, which is also approved for CLL. Like the BCL-2 inhibitor's field, the arena of drugs able to halt the cell cycles is relatively young and only a few drugs have been approved. Normally, cell cycle entry and progression are tightly regulated by growth factors, but deregulation of the cell cycle is a common characteristic of cancer cells. Progression through the cell cycle is critically controlled by multiple signaling pathways (e.g., ERK, PI3K, and integrin signaling), which activate enzymes known as cyclin-dependent kinases (CDKs); hence small molecule CDK inhibitors are being developed as potential cancer therapeutics. The first pharmacological CDK4/6 inhibitors, Palbociclib and Ribociclib, have been approved for the treatment of women with hormone receptor

positive, HER2-negative advanced breast cancer. **Wolf Ruprecht Wiedemeyer** describes frequent genomic alterations targeting the CDK-cyclin-RB-E2F axis in cancer and later critically reviews emerging mechanisms of response and resistance to CDK4/6 inhibitors. In addition, Wiedemeyer discusses drug combinations, such as palbociclib and inhibitors of the RTK/PI3K/AKT/mTOR pathway, and the yet unknown clinical potential of CDK1/2 inhibitors.

The last chapter, by **Nili Dahan, Ksenia Magidey, and Yuval Shaked**, deals with resistance to clinically approved anti-angiogenic agents, such as bevacizumab, an anti-VEGF (vascular endothelial growth factor) antibody, and PKIs able to inhibit angiogenesis (e.g., sorafenib and sunitinib). The authors refer to the unexpected complexity of clinical application of such agents and the frequently observed resistance to therapy, thought to underlay the generally modest gains in long-term survival. Given that anti-angiogenic agents target the tumor-supporting vascular system, rather than the malignant tissue, and the cellular heterogeneity of the microenvironment, resistance likely reflects both tumor- and host-mediated mechanisms. Because the tumor microenvironment is genetically stable and highly dynamic, resistance to anti-angiogenesis agents involves no overt genetic aberrations but rather activation of alternative mechanisms that sustain tumor vascularization, including a plethora of cytokines and growth factors secreted by bone marrow-derived cells and tumor-associated macrophages. Understanding these mechanisms is key to developing strategies able to overcome therapy resistance and improve clinical outcome of patients treated with anti-angiogenesis drugs.

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About the Series Editor

Benjamin Bonavida, Ph.D. is currently Distinguished Research Professor at the University of California, Los Angeles (UCLA). His research career, thus far, has focused on basic immunochemistry and cancer immunobiology. His research investigations have ranged from the mechanisms of cell-mediated killing, sensitization of resistant tumor cells to chemo-/immunotherapy, characterization of resistant factors in cancer cells, cell-signaling pathways mediated by therapeutic anticancer antibodies, and characterization of a dysregulated NF-κB/Snail/YY1/RKIP/PTEN loop in many cancers that regulates cell survival, proliferation, invasion, metastasis, and resistance. He has also investigated the role of nitric oxide in cancer and its potential antitumor activity. Many of the above studies are centered on the clinical challenging features of cancer patients' failure to respond to both conventional and targeted therapies. The development and activity of various targeting agents, their modes of action, and resistance are highlighted in many refereed publications.

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About the Guest Editors

Moshe Elkabets received a B.Sc. in medical laboratory, and both a Master and a Ph.D. in tumor immunology from Ben-Gurion University of the Negev, Beer-Sheva, Israel. For postdoctoral training, he first studied at the laboratory of Dr. Sandra McAllister (Brigham's and Women Hospital, Harvard Medical School) and later at the laboratory of Dr. Jose Baselga (Massachusetts General Hospital and Memorial Sloan Kettering Cancer Center). Since 2015, Dr. Elkabets leads a research laboratory in the Department of Microbiology, Immunology and Genetics at Ben-Gurion University of the Negev. His laboratory is interested in cancer therapeutics, primarily resistance to molecular targeted therapies (e.g., PI3K and EGFR inhibitors) and immunotherapies. Dr. Elkabets' research is currently supported by the Israel Science Foundation (ISF), Israel Cancer Association (ICA), Israel National Center for Personalized Medicine (INCPM), an Alon Fellowship, and the Teva Founders Prize.

Yosef Yarden is the Harold and Zelda Goldenberg Professor of Molecular Cell Biology. He currently serves as Director of the Dwek Institute for Cancer Therapy Research and Head of the Department of Biological Regulation of the Weizmann Institute of Science. He received a B.Sc. in biological and geological sciences from the Hebrew University of Jerusalem (1980), and a Ph.D. in molecular biology from the Weizmann Institute of Science (1985). His postdoctoral training was undertaken at Genentech, Inc., in San Francisco and at the Massachusetts Institute of Technology. In 1988, he joined the Weizmann Institute of Science's faculty. At the Institute, he has served as Dean of the Faculty of

Biology (1997–1999), Vice President for Academic Affairs (1999–2001), Director of the MD Moross Institute for Cancer Research (1999–2001), and Dean of the Feinberg Graduate School (2001–2007). Dr. Yarden's research attempts to resolve the transcriptional and other molecular mechanisms underlying growth factor's action, as well as the pharmacological opportunities offered by such understanding.