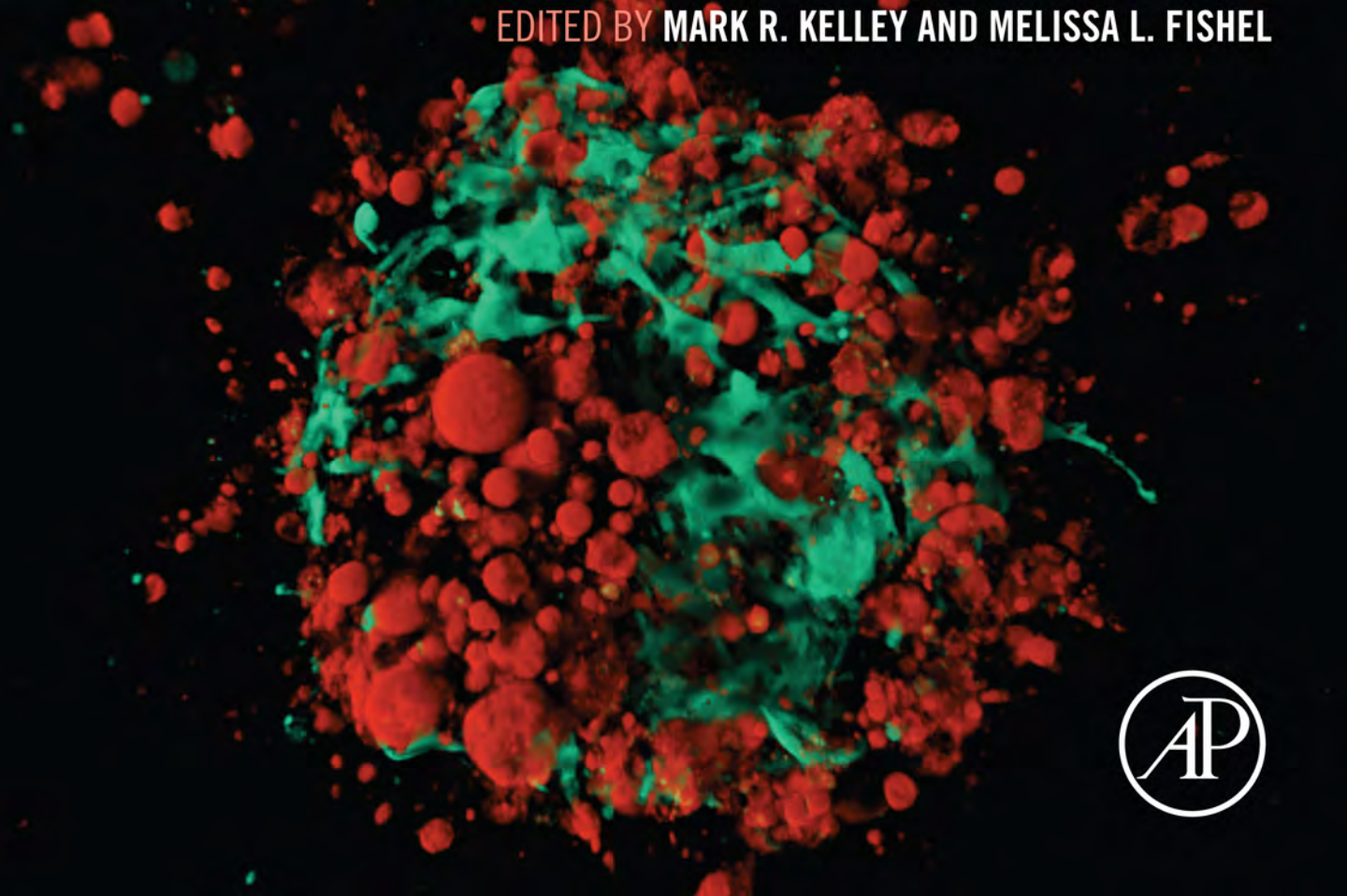
SECOND EDITION

DNA REPAIR IN CANCER THERAPY

MOLECULAR TARGETS AND CLINICAL APPLICATIONS

EDITED BY MARK R. KELLEY AND MELISSA L. FISHEL



DNA Repair in Cancer Therapy

Page left intentionally blank

DNA Repair in Cancer Therapy

Molecular Targets and Clinical Applications

Second Edition

Edited by

Mark R. Kelley, PhD

Departments of Pediatrics, Biochemistry & Molecular Biology, and Pharmacology & Toxicology; Herman B Wells Center for Pediatric Research; Basic Science Research, Indiana University Simon Cancer Center; Pediatric Molecular Oncology and Experimental Therapeutics Program; Chemical Biology and Drug Discovery Initiative; Center for Pancreatic Cancer Research, Indiana University School of Medicine, Indianapolis, IN, United States

Melissa L. Fishel, PhD

Herman B Wells Center for Pediatric Research, Department of Pharmacology & Toxicology, IU Simon Cancer Center, Indiana University School of Medicine, Indianapolis, IN, United States

ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1800, San Diego, CA 92101-4495, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-0-12-803582-5

Publisher: Mica Haley
Acquisition Editor: Rafael Teixeira
Editorial Project Manager: Lisa Eppich
Production Project Manager: Lucía Pérez
Designer: Matthew Limbert

Typeset by Thomson Digital

Contents

List of Contributors

Moulay A. Alaoui-Jamali
Departments of Medicine and Oncology, Faculty of Medicine, Lady Davis Institute for Medical Research and Segal Cancer Centre, McGill University, Montreal, Canada

Anthony J. Berdis
Department of Chemistry, and Center for Gene Regulation in Health and Disease, Cleveland State University; Case Comprehensive Cancer Center Red5 Pharmaceuticals, LLC, Cleveland, OH, United States

Krikor Bijian
Departments of Medicine and Oncology, Faculty of Medicine, Lady Davis Institute for Medical Research and Segal Cancer Centre, McGill University, Montreal, Canada

Ranjit S. Bindra
Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT, United States

Kerstin Borgmann
Laboratory of Radiobiology & Experimental Radiooncology, University Medical Center Hamburg, Hamburg, Germany

Jung-Suk Choi
Department of Chemistry, and Center for Gene Regulation in Health and Disease, Cleveland State University,Cleveland, OH, United States

Markus Christmann
Institute of Toxicology, University Medical Center, Mainz, Germany

Manolo D'Arcangelo
Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom

Anvesh Dasari
Department of Chemistry, and Center for Gene Regulation in Health and Disease, Cleveland State University,Cleveland, OH, United States

Andrew J. Deans
Genome Stability Unit, St. Vincent's Institute of Medical Research, Fitzroy; Department of Medicine (St Vincent's), University of Melbourne, Parkville, VIC, Australia

Ekkehard Dikomey
Laboratory of Radiobiology & Experimental Radiooncology, University Medical Center Hamburg, Hamburg, Germany

Yvette Drew
Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom

Melissa L. Fishel
Herman B Wells Center for Pediatric Research, Department of Pharmacology & Toxicology, IU Simon Cancer Center, Indiana University School of Medicine, Indianapolis, IN, United States

Navnath S. Gavande
Indiana University School of Medicine, Indianapolis, IN, United States

Sabrina Köcher
Laboratory of Radiobiology & Experimental Radiooncology, University Medical Center Hamburg, Hamburg, Germany

Bernd Kaina
Institute of Toxicology, University Medical Center, Mainz, Germany

Mark R. Kelley
Departments of Pediatrics, Biochemistry & Molecular Biology, and Pharmacology & Toxicology; Herman B Wells Center for Pediatric Research; Basic Science Research, Indiana University Simon Cancer Center; Pediatric Molecular Oncology and Experimental Therapeutics Program; Chemical Biology and Drug Discovery Initiative and Center for Pancreatic Cancer Research, Indiana University School of Medicine, Indianapolis, IN, United States

Malte Kriegs
Laboratory of Radiobiology & Experimental Radiooncology, University Medical Center Hamburg, Hamburg, Germany

Wael Mansour
Laboratory of Radiobiology & Experimental Radiooncology, University Medical Center Hamburg, Hamburg, Germany

Sarah A. Martin
Centre for Molecular Oncology, Barts Cancer Institute, Queen Mary University of London, London, United Kingdom

Ivana Murfuni
Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Julienne J. O'Rourke
Genome Stability Unit, St. Vincent's Institute of Medical Research, Fitzroy; Department of Medicine (St Vincent's), University of Melbourne, Parkville, VIC, Australia

Ann-Christin Parplys
Laboratory of Radiobiology & Experimental Radiooncology, University Medical Center Hamburg, Hamburg, Germany

Katherine S. Pawelczak
NERx Biosciences, Indianapolis, IN, United States

Ruth Plummer
Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom

Mattia Poletto
CRUK & MRC Oxford Institute for Radiation Oncology, Department of Oncology, University of Oxford, Oxford, United Kingdom

Simon N. Powell
Department of Radiation Oncology and Molecular Biology Program, Memorial Sloan Kettering Cancer Center, NY, United States

Ulrich Rass
Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Thorsten Rieckmann
Laboratory of Radiobiology & Experimental Radiooncology, University Medical Center Hamburg; Department of Otorhinolaryngology and Head and Neck Surgery, University Medical Center Hamburg, Hamburg, Germany

Kai Rothkamm
Laboratory of Radiobiology & Experimental Radiooncology, University Medical Center Hamburg, Hamburg, Germany

Jeremy Setton
Department of Radiation Oncology and Molecular Biology Program, Memorial Sloan Kettering Cancer Center, NY, United States

Behzad Shariati
Department of Pharmacology & Toxicology, Indiana University School of Medicine, Indianapolis, IN, United States

Gianluca Tell
Laboratory of Molecular Biology and DNA repair, Department of Medical and Biological Sciences, University of Udine, Udine, Italy

John J. Turchi
Departments of Medicine, and Biochemistry & Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, United States

Pamela S. VanderVere-Carozza
Indiana University School of Medicine, Indianapolis, IN, United States

Carlo Vascotto
Laboratory of Molecular Biology and DNA repair, Department of Medical and Biological Sciences, University of Udine, Udine, Italy

Michael R. Vasko
Department of Pharmacology & Toxicology; Department of Anesthesiology, Indiana University School of Medicine, Indianapolis, IN, United States

Sabrina D. Wurzba
Departments of Medicine and Oncology, Faculty of Medicine, Lady Davis Institute for Medical Research and Segal Cancer Centre, McGill University, Montreal, Canada

Noah Zanville
Department of Community and Healthy Systems, Indiana University School of Nursing, Indianapolis, IN, United States

Page left intentionally blank

Preface

The field of DNA repair is enjoying a remarkable time of interest with the recent Nobel Prize in Chemistry awarded to pioneering scientists in "Mechanistic Studies in DNA Repair": Thomas Lindahl, Paul Modrich, and Aziz Sancar. In addition, the use of specific DNA repair inhibitors in cancer clinical trials is rapidly expanding, and so is the development of additional molecules that are either being tested or are rapidly moving through the preclinical developmental stage. With precision medicine aiding in our selection of patient and proper chemotherapeutic agent, scientists and clinicians are gathering a better understanding of the DNA repair deficiencies that a tumor possesses and attacking its so-called Achilles Heel. However, this recent focus has not decreased the basic science interest in pursuing research in the still-unknown mechanisms involving various DNA repair pathways. Particularly interesting is the continuing discovery of interactions between the various pathways that will afford opportunities for future translational and clinical efforts. We have tried to include the most current information possible in the contents, however, as the field is rapidly accelerating we acknowledge that some information will not be current by publication time, particularly the information concerning specific inhibitor molecules and clinical or preclinical successes and failures. In spite of this, we anticipate that this book will be a strong reference for those who want to delve into DNA repair and understand pathways, their basic mechanisms, the relevance of DNA repair to human cancer. This background information will not be eclipsed by future discoveries, but serve as the foundation for future studies. With this being the second edition, we are impressed that we already had so much to update in the field of DNA repair and translational cancer research. This is a testimony to the hard work of the contributors' and scientists in this exciting and expanding field. In the end, the real purpose of this book is to try and give an overview of where those who study DNA repair stand in understanding and development agents to fight against cancer. This area has been highly underappreciated and is finally gaining the recognition it so richly deserves.

Page left intentionally blank

Acknowledgments

We would like to thank all of the members of the scientific community, particularly the scientists who have worked in the area of DNA repair for decades to bring us to this point in time when a large amount of the fruits of their labor is now being implemented in human cancer diagnoses and treatments. Also, thanks to all our experts who have contributed chapters in this book as such undertakings are time consuming on already hectic schedules. The gorgeous cover art with the tumor spheroid in coculture with cancer-associated fibroblasts was generated with the help of Dr Malgorzata Kamocka in the Indiana Center for Biological Microscopy. Her eye for detail and imaging expertise allowed us to capture the spheroids resulting in a wonderful cover for this edition of the book. Additional gratitude goes out to our laboratory and staff, and special thanks go to Lana Christian who helped us significantly on the first chapter and the general overview of the field. This project was also spearheaded and supported by the excellent people at Elsevier, particularly Lisa Eppich, as well as their devoted staff to bring this book to fruition in a timely manner.

Support for Mark Kelley on this effort came from the Herman B Wells Center for Pediatric Research, Department of Pediatrics at Indiana University School of Medicine, Indiana University Simon Cancer Center, Jeff Gordon Research Foundation and the Riley Children's Foundation through the Betty and Earl Herr Chair in Pediatric Oncology Research. This support was instrumental in the completion of this project. I would like to personally thank my wife, Sue, who has always supported me and my career for over the 36+ years of our marriage, and my children Jennifer and Scott. I would also like to thank Melissa Fishel for her help on not only this project, but on our continued collaboration in the various areas of DNA repair and cancer studies.

Support for Melissa Fishel on this effort came from the Herman B Wells Center for Pediatric Research, Indiana University Simon Cancer Center and the Riley Children's Foundation. I am grateful to work alongside many colleagues who care so much about basic and translational research and inspire me to keep moving forward. This project was more enjoyable due to working with Mark Kelley as he continues to challenge and motivate me in my career, through multiple collaborations and a lasting friendship. I also must acknowledge my husband, Nick and children, Lanie and Micah for their endless understanding of my passion for science and cancer research and my faith that fuels my life.

Page left intentionally blank

CHAPTER

1 OVERVIEW OF DNA REPAIR PATHWAYS, CURRENT TARGETS, AND CLINICAL TRIALS BENCH TO CLINIC

M.R. Kelley*, M.L. Fishel**

**Departments of Pediatrics, Biochemistry & Molecular Biology, and Pharmacology & Toxicology; Herman B Wells Center for Pediatric Research; Basic Science Research, Indiana University Simon Cancer Center; Pediatric Molecular Oncology and Experimental Therapeutics Program; Chemical Biology and Drug Discovery Initiative and Center for Pancreatic Cancer Research, Indiana University School of Medicine, Indianapolis, IN, United States; **Herman B Wells Center for Pediatric Research, Department of Pharmacology & Toxicology, IU Simon Cancer Center, Indiana University School of Medicine, Indianapolis, IN, United States*

CHAPTER OUTLINE

DNA Repair in Cancer Therapy. http://dx.doi.org/10.1016/B978-0-12-803582-5.00001-2

INTRODUCTION

It can be argued that genomic integrity is the fulcrum upon which cellular survival and homeostasis rest. In healthy cells, these two cellular "prime directives" stay balanced, and the information encoded in DNA guides that balance. However, cancer tips the scales, creating an incessant drive for survival and proliferation [1]. In the process, cancer cells ignore or override signals to repair DNA damage—or repair it poorly. This furthers their mutagenesis, aggressiveness, and resistance to treatments [2,3].

To understand and thwart this process, we need to examine DNA and the intricate safety net that protects it from endogenous and exogenous insults. This protective system must patrol nearly two meters of human genomic material that are precisely folded and condensed to fit inside a micrometer-scale cell nucleus. Despite that compactness, mechanisms ensure DNA's accessibility for accurate, timely replication, transcription, translation, and damage repair [4].

In human cells that are dividing, the entire genome is replicated every few hours [5]. To protect the genome, every cell in the body employs distinct but interrelated systems to detect and eliminate DNA damage so that faithful, accurate, and timely replication of the genome can continue [6]. Yet, we know that does not always happen—and the relationship between DNA damage and cancer is undeniable [7].

Discoveries in the first half of the 20th century demonstrated that DNA could be altered as well as damaged. However, the notion of DNA *repair* did not become a fixture in the lexicon of molecular and cellular biology until the 1960s [8]. Conceptualizing how DNA repair pathways could be targeted to kill cancer cells came many steps and many discoveries later.

Evidence that defective DNA repair predisposed a person to cancer was first discovered the late 1960s [9,10]. In the 1960s and 1970s, the main DNA pathways were discovered [8]. In the mid-1970s, Radman published the first extensive work on DNA damage tolerance [8,11]. In the strictest sense, Radman's work paved the way for identifying high- versus low-fidelity polymerases—but conceptually it became a springboard for many future discoveries regarding mutagenic transformation and cell-cycle checkpoints.

Despite that body of work, early forays into developing anticancer agents did not conceive of manipulating a DNA repair pathway—or a protein within a pathway—to kill cancer cells. Multiple models of how researchers thought cancer behaved and should be treated came and went before the molecular biology of DNA repair gained widespread attention outside of academia. In those early stages of developing chemotherapeutics, the majority of drugs damaged DNA directly (structurally) [12,13]. Many

of those agents (such as cisplatin, doxorubicin, temozolomide, cyclophosphamide) continue to occupy prominent roles in cancer treatment today.

However, researchers saw that such induced damage could be repaired. As the intricacies of each pathway were uncovered and the effects of cumulative mutations were understood more thoroughly, alterations in DNA repair mechanisms were identified as early events in tumorigenic transformation [2]. From that, the field of DNA repair inhibition emerged—with its early focus on enhancing the efficacy of DNA-damaging agents by the rational pairing of a DNA repair inhibitor with a direct DNA-damaging agent.

Exploiting the tumor's repair capacity created new classes of drugs that inhibit a particular pathway or a critical protein within a pathway. This line of drug development continues to yield rewards as well as uncover unexpected challenges. Homeostasis is maintained, in part, by ensuring that processes do not depend solely on any single protein or pathway—otherwise, genomic integrity could be disrupted too easily by environmental or mutational events [14]. Thus, in the quest to find more effective cancer treatments, basic and translational research continues to seek ways to work with (or work around) nature's innate fail-safe methods.

Therein lies the importance of understanding the DNA damage response (DDR) encompasses the DNA repair pathways as well as cell cycle checkpoints. These checkpoints, the cell's Quality Assurance (QA) and Quality Control (QC) managers, normally halt the cell cycle when DNA is damaged, allowing time for repairs that proceed on the basis of the type and extent of damage sustained, as well as when the damage occurs in the cell cycle. In counterpoint, cancer hijacks those systems to ensure tumor survival [1].

Even when cancer appears to be eradicated, a new primary cancer or a late recurrence of the first cancer can arise. This may be inherent in the cancer itself or caused by the anticancer treatments given. Indeed, today approximately 19% of cancer diagnoses have a previous history of primary malignancy [6,15]. Knowing more about how pathways function normally versus during carcinogenesis gives researchers much-needed ammunition to exploit those differing characteristics for therapeutic benefit. That is the focus of this book.

The path to new drug discovery starts with basic research—and, for DNA repair inhibition, that starting point is accurate molecular mapping of DNA repair pathways. The value of such efforts was recognized in 2015 when Nobel Prizes for Chemistry were awarded to Drs Tomas Lindahl, Paul Modrich, and Aziz Sancar for their characterization of three DNA repair pathways (base excision repair, mismatch repair, and nucleotide excision repair, respectively) [16].

DNA damage is a hallmark of cancers [17]; damaging it further to induce tumor cell death is the goal of anticancer agents. However, targeting the DNA repair machinery still can pose problems of causing collateral damage in healthy cells [18] unless DDR mechanisms are fully elucidated. This is a growing area of research in cancer survivorship and quality of life.

Researchers are coming closer to an answer in the continuing quest of how to selectively kill cancer cells—not only by providing the right treatment but also by identifying the best therapeutic window for it. Intuitively, the most attractive targets for repair inhibition would appear to be (1) a rate-limiting step in a pathway, (2) a protein unique to a pathway, (3) a pathway exhibiting altered responses or activities due to the cancer, (4) differential expression of pathways, or (5) aberrant circumstances that create a selective window for treatment. As subsequent chapters explain, the reality of developing effective DNA repair inhibitors is not that straightforward.

DDR status also has emerging value in identifying subpopulations of tumors and stratifying patients diagnostically, prognostically, and therapeutically. This is an exciting prospect. However,

it faces numerous hurdles, including (1) accurate identification of exploitable DNA repair defects, (2) development of efficacious druggable targets with more selective activity and more favorable side effect profiles, (3) differentiating between the person's normal DNA repair processes and the tumor's DNA repair capabilities, and (4) cells' normal inclination to tackle any type of DNA damage—naturally occurring or therapeutically induced—with equal determination and even creativity [3,19–21].

Still, if key processes in the continuum of cancer transformation and progression can be interrupted, then it should be possible to stop cancer in its tracks. Deficiencies in a particular DNA repair pathway can lead to increased levels of other DNA repair proteins, either in the same pathway or a different one. Compensating for a deficiency is paramount to efficient DNA repair, and by extension, cancer survival [3]. Capitalizing on cancer-cell deficiencies to turn them against themselves is the conceptual framework for synthetic lethality—a new approach being pursued in the war against cancer.

This introductory chapter lays the foundation for understanding the DDR and how it is being exploited therapeutically. Sections encapsulate each major repair pathway, describe general principles behind therapeutic inhibitors, and summarize those already in use versus in development. Portions of this chapter glance backward to explain how past research has enabled current accomplishments; other portions look forward to future possibilities in this field. This sets the stage for subsequent chapters that explain each aspect of DNA damage control and repair in detail, along with specifics regarding each pathway and its inhibitors in development or clinical use.

OVERVIEW OF DNA REPAIR PATHWAYS

With one exception, all the DNA repair pathways follow five steps: Recognition, Recruitment, Removal, Reconstruction, and Reinstatement. The Recognition step detects and locates the damage, and, as needed, unwinds the surrounding chromatin. In the Reinforcement/Recruitment step, proteins stabilize the damaged site, attract other proteins to help with the repair, and provide a scaffold upon which those proteins can attach and work. The Removal step rids the DNA strand of the damaged bases and prepares the site so it can receive undamaged base(s). The Reconstruction step inserts new nucleotide(s) to fill the gap. Finally, the Reinstatement step restores the correct configuration of the DNA helix by performing "cleanup" chores, which include various combinations of removing flaps, sealing nicks, and ligating ends. Fig. 1.1 shows the most common categories of proteins that perform each step.

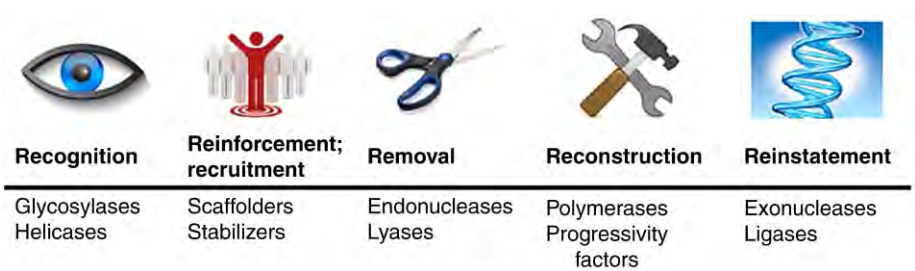


FIGURE 1.1 Overview of Proteins Engaged in Each Step of DNA Repair

Table 1.1 Summary of Cell Cycle Phase When Each DNA Repair Pathway is Most Active

Activity	G0	G1	S	G2	M
Cellular description	**Resting** No mitotic-related activity	**Growth** In size, protein supplies, organelles	**Synthesis** Cell duplicates its DNA and centrosomes	**Preparation** More growth; genome proofreading to ensure readiness for mitosis	**Mitosis** Cell division; the genome is halved
Checkpoint activity for cell cycle progression (kinase + its activator)		*Early in G1*: CDK4 and 6 + Cyclin D *Late in G1*: CDK2 + Cyclin E; p53	CDK2 + Cyclin A; p53	p53	CDK1 + Cyclin B
When DNA repair pathways are most active	NHEJ	NHEJ	LP-BER MMR TC-NER HR	HR	
	DR, SP-BER, and GG-NER are equally active in all cell cycle phases. NHEJ may also be active in early S phase. BER also plays a key role in DNA repair in postmitotic (neuronal) cells.				

Key: CDK, *cyclin-dependent kinase;* DR, *direct repair;* GG-NER, *global genome nucleotide excision repair;* HR, *homologous recombination;* LP-BER, *long-patch base excision repair;* MMR, *mismatch repair,* NHEJ, *nonhomologous end joining;* SP-BER, *short-patch base excision repair;* TC-NER, *transcription-coupled nucleotide excision repair.*

Some repair pathways are more active in certain parts of the cell cycle than others [6,22] (see Table 1.1). Under normal circumstances, many repair pathways are most active during or immediately after the cell doubles its DNA—but before the DNA divides. If repairs were not made at that phase, then the risk of mutation would be high. Other pathways work continuously to offset DNA damage that occurs during normal cellular metabolism and other endogenous processes [19,22–29]. Knowing how and when the pathways normally function (Table 1.1) helps researchers discover the differences in how cancer aberrantly performs DNA repair.

A short, simplified description of each DNA repair pathway follows. For more details, refer to the individual chapter for each pathway.

DIRECT REPAIR

Elegant in its deceptive simplicity, the Direct Repair (DR) pathway the only single-step mode of DNA repair. It is also the only pathway that does not involve excising bases or distorting DNA's phosphodiester backbone to perform repairs [30]. DR's sole job is to remove an alkyl group from certain oxygen positions of damaged guanine and thymine bases. The fastest, most commonly made repair [31] occurs

in response to normal cellular metabolism and environmental carcinogens that methylate guanine and thymine bases. In response, the DNA repair protein O^6-methylguanine DNA methyltransferase (also called MGMT or AGT) transfers one methyl group (an alkyl adduct) from the O^6 position of guanine to the MGMT molecule in a stoichiometric reaction. It is a suicide mission [32]; the transfer inactivates MGMT, which is then degraded. Thus, cells must continually manufacture more MGMT to perform this crucial function.

When MGMT production is impaired, it triggers wide-ranging effects on other repair pathways and cellular survival responses [31]. For example, if alkyl adducts persist during replication, thymine mispairings occur, leading to erroneous G:C-to-A:T transitions or strand breaks—all necessitating lengthier repairs by other DNA repair pathways [30]. Chapter 2 provides more details regarding MGMT's importance.

BASE EXCISION REPAIR

The base excision repair (BER) pathway corrects the most prevalent forms of DNA damage; that is, those occurring from oxidation, alkylation, deamination, and ionizing radiation (IR) [20]. In healthy cells, most single-strand breaks (SSBs) come from endogenous reactive oxygen species (ROS), and BER is the first line of defense to repair them [6]. Although these types of lesions produce subtle damage involving relatively few bases and no helix distortion, the damage, left unchecked, would cause base mispairings. If the mispairings were transcribed, they would lead to mutations [20].

One of BER's hallmarks is its 11 damage-sensing glycosylases. Some of them are so substrate-specific that their use can depend on which base the lesion is paired with [23]. All of them remove the damaged nucleoside(s) by cleaving its *N*-glycosidic bond, leaving an abasic (AP) site [33].

Although the AP site provides a substrate for the next step, it is more cytotoxic and mutagenic than the removed lesion [34,35]. To ensure prompt, accurate repairs, most glycosylases remain bound to the site until additional enzymes bind and execute the next repair step [36]. That "baton handoff" feature appears to exist for all BER steps; it ensures that the harmful intermediates generated throughout BER are never left "unattended" [37–39].

The Removal step (preparing the AP site for repair) falls to a unique enzyme called APE1/Ref1, or AP endonuclease/Redox Factor 1 (often shortened to APE1). APE1 processes the loose ends that remain after the lesion is excised, creating special termini to accept the new base(s) and a single-strand break (SSB)—another toxic intermediate [20,38]. APE1 also recruits additional proteins to the site to complete the repair.

As the dual name of this protein implies, APE1/Ref1 has other functions. Employing a complex thiol/sulfide exchange, the protein's unique reduction/oxidation (redox) signaling properties help maintain transcription factors in their reduced, active form [20]. These and other APE1 functions are discussed in detail in Chapter 3.

Throughout BER repairs, a scaffolding protein (XRCC1) stabilizes the damaged area, attracts other proteins required for the repair, provides a foundation for protein anchoring, and coordinates sequential binding and release [36,40]. XRCC1's action also pries the helix open to give other proteins ready access to the damage [38,41].

After endonuclease activity is complete, BER diverges into two subpathways—depending on the type and extent of damage sustained, the kind of AP site generated in the first step, the type of termini that APE1 produces, and the cell cycle phase in progress when the damage occurs [20,39] (see Table 1.2).

Table 1.2 Overview of Steps in DNA Repair Pathways

Pathway	Recognition	Reinforcement; Recruitment	Removal	Reconstruction	Reinstatement
General progression	(glycosylases/ helicases)	(scaffolding proteins/ stabilizers)	(endo- or exonucleases/lyases)	(polymerases/ progressivity factors)	(Nucleases/ligases)
	> > >	> > >	> > > >	> > >	> > >
DR	(a single protein, MGMT, in a stoichiometric reaction)				
BER					
Short-patch	Damage-specific glycosylase, PARP1	XRCC1 / PARP	APE1	Pol β	Ligase III
Long-patch				FEN1 / PCNA	Ligase I
MMR	MSH2-MSH3 or MSH2-MSH6	RPA, RFC	Exo1	MLH1-PMS1 Pol δ / PCNA MLH1-PMS2	Ligase I
NER					
global genome	XPC + Rad23B, XPA	RPA, XPA	TFIIH (includes helicases XPB, XPD) XPF+ ERCC1, XPG	Pol δ or Pol ε / RFC, PCNA	XPG / Ligase I
Transcription Coupled	CSA, CSB				
NHEJ	MRN complex Ku70 + Ku80	XRCC4 + XLF	DNA-PK (Ku + DNA-PKcs; and, as needed: artemis, PNKP, WRN, APLF)	Usually Pol μ or Pol λ	Ligase IV
HR	MRN complex, BRCA1	RPA, γH2AX, Rad52, BRCA2	MRN complex, BRCA1	Polymerases / Rad51 filament, XRCC2, XRCC3, Rad54, BLM, WRN, artemis, others	TOPOIIIα, resolvase/ Ligase I

Notes: Some proteins serve functions during multiple repair steps. Some proteins in double-strand repair work in both the HRR and NHEJ pathways. A darker color within a row indicates a subpathway or other coupling of repair proteins on the basis of the type of damage to be repaired.

In both subpathways, a polymerase synthesizes one or more new bases and a ligase completes the repair by sealing the nick in the single-stranded DNA [42,43]. The way the bases are added, including which polymerase and ligase are used, varies according to the subpathway chosen [3].

As its name implies, short-patch (SP) BER takes less time to perform its task, which is repair of normal, single-base AP sites. Long-patch (LP) BER preferentially repairs oxidized and reduced AP sites, replacing sequences of two to eight nucleotides in a lengthier process. Conveniently, if SP-BER activity produces a site on which its corresponding polymerase cannot work, repairs switch to LP-BER [23].

The more extensive damage that LP-BER attends to requires additional proteins for repairs [20,39]. The Reconstruction step of LP-BER includes a sliding clamp that monitors the polymerase's progress and prevents it from dissociating prematurely (PCNA, or proliferating cell nuclear antigen), and an additional stabilizer that also inserts the newly synthesized nucleotides (replication factor-C, or RFC). In the final Reinstatement activity, flap endonuclease 1 (FEN1) cuts off the string of old, damaged nucleotides [3] (see Table 1.2).

A special BER protein called PARP1 [poly-(ADP ribose) polymerase 1] not only detects damage but also determines whether it is too extensive to repair. It plays a role in decondensing the chromatin around the damage site and also influences other pathways [20,44]. Those functions are discussed more fully later in this chapter and in detail in Chapter 3.

MISMATCH REPAIR

During DNA replication, proofreading polymerases may fail to detect errors. When that occurs, mismatch repair (MMR) steps in as an immediate postreplicative repair mechanism, enhancing replication fidelity by several orders of magnitude [24]. In ways that are still being elucidated, MMR's damage sensors can discriminate between the parental DNA strand and the newly synthesized strand—and mark the correct segment for removal [24]. Then, using the parental strand as a template, MMR corrects the base sequence on the new daughter strand [19,45].

MMR corrects single-base mismatches (A:G, T:C) and misaligned short nucleotide repeats, such as small insertion/deletion loops. These errors may be part of normal DNA replication or a consequence of exposure to agents that cause base modifications, including endogenous reactive species and exogenous alkylating agents [45–47]. However, if those errors are not corrected by the end of S phase, frameshift mutations and microsatellite instabilities will occur [24,48].

In the Recognition step, one of two damage recognition complexes (MSH2:MSH6 or MSH2:MSH3) form, the type of mismatch needing repair [49]. MSH2:MSH6 recognizes single-base substitutions and the smallest insertion/deletion loops; MSH2:MSH3 recognizes any insertion/deletion loop involving up to 10 nucleotides [32,45].

After the MSH complex identifies the type of mismatch, it recruits another complex, comprising MutL homolog 1 (MLH1) and one of its binding partners, PMS1 or PMS2 (postmeiotic-segregation increased protein). Interestingly, the MSH and MLH complexes form a sliding clamp that moves until it encounters a single-strand DNA gap [45].

In the meantime, a stabilizing protein (RPA, or Replication Protein A) functions as flagger and traffic manager at the damage site. RPA enables another stabilizing protein (RFC) and a progressivity factor (PCNA) to bind to and protect the site. Collectively, this cluster of proteins functions to attract the next complex's attention. When the MutL complex encounters that cluster at the single-strand gap,

definitive identification on the daughter strand is confirmed, allowing a DNA exonuclease (Exo1) to enter the DNA structure. Guided by the MLH:MSH complex, Exo1 removes the damaged area plus a margin beyond it. The MLH:MSH complex remains bound until excision is completed. Then a DNA polymerase (Pol δ) synthesizes DNA in place of the excised sequence, while the progressivity factor PCNA slides along the new sequence to check the work in progress and keep Pol δ on task. Finally, Ligase I joins the new DNA to the existing daughter strand [45].

Because MMR corrects errors on only the new, daughter strand, one can see inherent problems if the damage is on the parent (template) strand. That situation can lead to double strand breaks (DSBs) [19].

Loss of MMR activity leads to a mutator phenotype [50] and affects many processes, including DDR and cell cycle checkpoints [49]. Thus, counterintuitively, MMR functionality is needed to enhance chemotoxicity—and loss of MMR function causes or increases chemoresistance [47,48]. For this reason, development of direct MMR inhibitors is problematic [21]. However, MMR status in tumors its therapeutic modulation can be exploited in other ways (described in Chapter 6).

NUCLEOTIDE EXCISION REPAIR

Nucleotide excision repair (NER) repairs helix-distorting, bulky lesions and large adducts when only one of the two DNA strands is affected [20]. UV radiation and chemical mutagens including platinating agents cause such damage. The former crosslinks adjacent pyrimidine bases; [51] the latter crosslinks purine bases and creates intrastrand adducts [20]. All such damage blocks DNA replication and transcription.

Because NER works on many kinds of structurally unrelated types of damage, it is both highly versatile and flexible [51,52]. Accordingly, it utilizes more than 30 proteins in multistep "cut-and-patch" processing [17,52,53]. But, in contrast to the variety of lesions it detects and repairs, only two damage-recognition complexes discern the location and type of damage [52].

To perform its diverse work, NER employs two subpathways: global genome repair (GG-NER) and transcription coupled repair (TC-NER). The subpathway names hint at their distinct temporal roles during the cell cycle. TC-NER acts upon lesions that block an active transcription site—that stall an RNA polymerase in its elongating activity [25]. In contrast, GG-NER operates during all phases of the cell cycle, repairing damage on both transcribed and nontranscribed strands of active genes [26]. The speed of GG-NER varies widely according to the type of lesion being repaired [17].

In the simplest terms, NER: (1) recognizes the damage, (2) assembles a repair complex and unwinds a section of DNA on both strands, (3) performs dual incision damage excision activities, (4) synthesizes new nucleotides using the undamaged DNA strand, then (5) ligates the repaired section of DNA. DNA incision and repair is restricted to the damaged strand so that the complementary undamaged strand can later serve as a template for the "patch" process [52].

NER employs two different damage-sensing complexes, which define the subpathways. After the lesion is detected, a large repair complex forms. Although decades of study have been spent mapping how NER works [16], more is still being learned regarding which proteins serve as recruiters for others and the order in which the proteins arrive or depart. However, all must be assembled before any alteration is made to DNA's phosphodiester backbone [26].

A nine-unit complex called transcription factor IIH (TFIIH) executes the first phase of repair. This complex includes two helicases (XPB and XPD) and other proteins (XPA and RPA) that open and

stabilize the helix as well as direct traffic. In activities that blur the Recognition, Recruitment, and Removal steps, NER's damage complex and first repair complex remain attached to the DNA while two different endonucleases (XPG and XPF, the latter acting in conjunction with ERCC1) perform precision cutting functions. Each incises one side of the damaged strand, several nucleotides away from the damage. Depending on whether the damage occurs on the 5′ or 3′ side, a differing number of nucleotides are removed around the lesion [26,52].

After that initial repair step, RPA coordinates the assembly and workings of a second repair complex. Synthesis of new nucleotides rests heavily on Replication factor C (RFC). As it binds to the excision gap, RFC mediates the entry and activity of PCNA, a sliding clamp that binds to DNA polymerases δ and ε, checking their progress and keeping them from dissociating before they finish their reconstruction activities. Finally, Ligase I attaches the newly synthesized repair patches to the preexisting DNA [26,52,54].

Many genes are involved in NER; their expression and NER's repair capacity can be modulated by oxidative stress [17]. Studies of people with genetic deficiencies in NER provided early clues as to why they were highly prone to developing various cancers. Thus, many proteins in this repair pathway bear names that start with "XP" or "CS," alluding to those genetic conditions (xeroderma pigmentosum and Cockayne syndrome, respectively). Decreased NER functioning is an important modulator of disease [55], as seen in the dramatic drop in NER activity in breast cancer [56]. But innate or acquired NER deficiency renders cells sensitive to platinating agents—as seen in the 95% cure rate of testicular cancer treated with cisplatin [26].

DOUBLE-STRANDED BREAK REPAIR

Double-strand breaks (DSBs) are the most serious, toxic, difficult-to-repair forms of DNA damage. If not repaired properly, DSBs can lead to mutations, deletions, translocations, and genome amplifications [1,2,57]. In healthy cells, transient DSBs can form when topoisomerases uncoil DNA for transcription. Normally, spontaneous DSBs are rare, approximately 10 per day [58]. The most common kinds of breaks due to other causes are (1) breaks in replication forks when polymerases stall at the site of unrepaired base lesions, and (2) breaks in both DNA strands at or near the same point on the double helix [27,59]. Anticancer treatments can induce multiple kinds of double-stranded DNA damage simultaneously, including base damage, base loss, nonstandard chemistry of the 3′ and 5′ ends (unligatable termini), and/or intra- and interstrand breaks within one or two helix turns [60].

DSB repair faces many challenges such as loss of information content and physical integrity on both strands, and highly diverse broken ends [61]. To repair such damage, human cells employ two main pathways: nonhomologous end joining (NHEJ) and homologous recombination repair (HRR). The choice of pathway depends the cell cycle phase in progress, the complexity of repairs required, and whether the damaged DNA ends are "blunt" (easy to rejoin) or "dirty" (nonligatable) [27,28,49,58,61,62]. Checkpoints evaluate the end processing required, which partially dictates how DSBs should be repaired [28,61]. That decision is crucial in the recombination process.

NHEJ can operate during any cell cycle phase but is most active in G0 and G1 (before DNA replication), whereas HRR activity occurs during S and G2 phases (after replication) (see Table 1.1). NHEJ is faster, is used more frequently on DSBs, and is more simplistic than HRR; however NHEJ is more prone to error. The longer, more meticulous template-based activity of HRR ensures the highest-fidelity repair [19,27,28,32,59].

Interestingly, the first responder to a DSB is a damage sensor complex common to both NHEJ and HRR [63]. Called MRN (short for Mre11-Rad50-Nbs1), its flexible configuration appears to determine not only which pathway is chosen but also its divergent functions on the basis of the pathway. In NHEJ, it serves as a DNA tether; in HRR, it also performs nucleolytic surgery [58,63] (see Table 1.2). Our emerging understanding of MRN's functionality is only one of many mysteries of DSB repair that more than 40 years of research have tried to uncover [63]. Much more continues to be learned regarding HHR, NHEJ, and their respective subpathways (see Fig. 1.2). The following paragraphs summarize the main HR and NHEJ pathways.

Nonhomologous End Joining

Nonhomologous end joining (NHEJ) rejoins DSB ends with minimal processing. Because it does not search for or use a large segment of DNA as a template for determining which bases were present before the damage occurred, repairs proceed quickly—with the potential for (1) loss of nucleotides from either side of the DSB junction or (2) alteration of base pair sequences at the breakpoint [17,27,32]. Thus, NHEJ paradoxically can contribute to genome protection as well as mutation—despite NHEJ being favored for DSB repair the vast majority of the time [64].

Why give preference to an error-prone pathway when so much is at stake? In the human genome, approximately 40% of all DNA is repetitive information. DNA's condensed chromatin structure makes

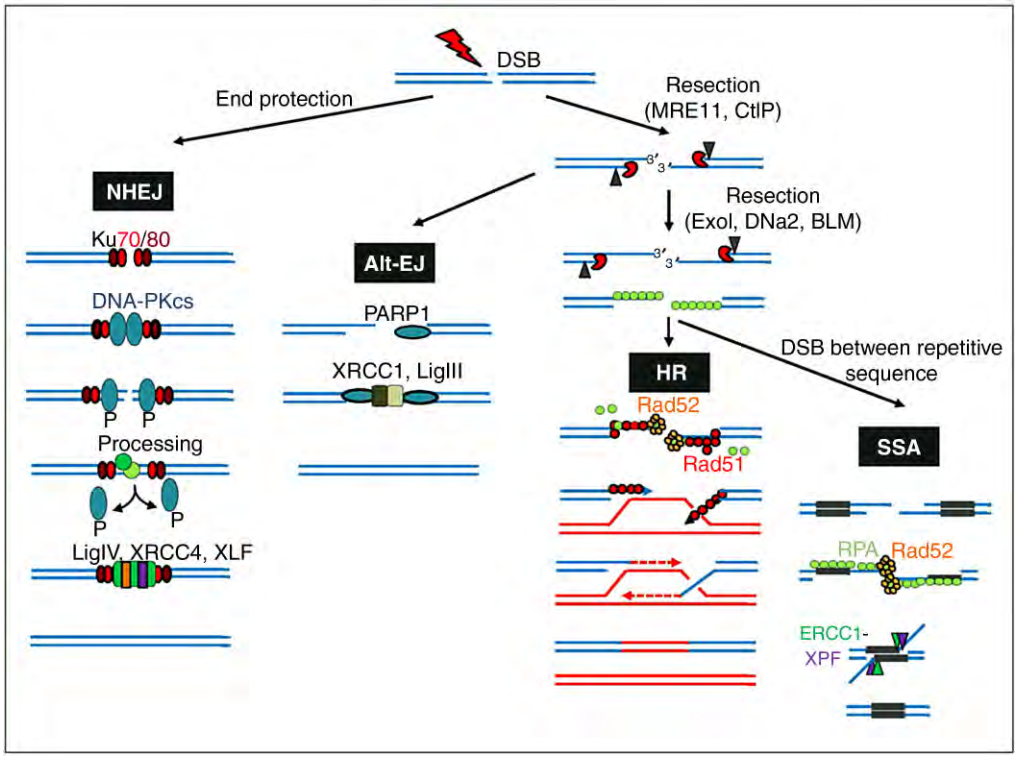


FIGURE 1.2 Overview of Double-Strand DNA Repair

a homology search in G2-M phase challenging [65] as well as ill-advised [61]. Only in late S, G2, and M phases when DNA is replicating is a sister chromatid positioned optimally for a repair pathway to read an extended area of homology. Without that availability, it would be too easy to choose inappropriate homology partners for the repair—which could lead to chromosomal translocations [28,61].

Additionally, the "classical" version of NHEJ usually does a more faithful job than it gets credit for [28,66,67]. Repair of "clean" breaks often does not result in any information loss or chromosomal rearrangements, but repair of "dirty" breaks (eg, due to irradiation) can result in loss of genetic information [66].

In simple terms, NHEJ: (1) detects, aligns, protects, and tethers the DSB ends together; (2) minimally processes the damage, removing unligatable end groups, (3) fills and (4) seals the break [66]. Much, but not all of the rejoining occurs at areas of microhomology (one to four nucleotides) [61,65], and that difference is now considered an NHEJ subpathway. Other subpathways exist as well (which are covered in Chapter 9).

Perhaps the most urgent challenge of NHEJ is to bring the two ends into physical proximity of each other and protect them from nucleolytic attack [61]. The Ku heterodimer, a damage sensor, initiates that protection by forming a ring around the ends and recruiting other proteins for end processing [27,28,68,69]. The rate limiter of the repair is DNA-dependent protein kinase catalytic subunit (DNA-PKcs) [19]. When it binds to Ku, it becomes DNA-PK, a multitasking shield, docking port, and "tool belt" for various kinds of DNA end processing enzymes [27,68,69]. How NHEJ picks the correct proteins for the task is still unknown; [70] but, collectively, they exhibit great mechanistic flexibility for acting on a wide range of DNA end structures [61]. Ku contributes lyase activity to that effort [58,69]. Another complex (XRCC4 + Ligase IV + XLF) creates a filament to bridge the ends and promote stable, efficient ligation after end processing is complete [19,69]. Incredibly, Ligase IV has the flexibility to ligate across gaps and join incompatible DNA ends if needed [70].

But NHEJ's repair is not merely a means of aligning, cleaning, and ligating the ends. Gaps may still exist. In activity that remains unclear, NHEJ employs polymerases that can circumvent the need for a continuous template. In contrast to other polymerases, they have (1) an additional domain that can act downstream of short gaps, (2) distinct preferences for substrate structures, and (3) differential dependency upon needing/using a few to no paired bases as a template [71]. One could say that they make their own instructions as much as they take "traditional" instructions for Resynthesis.

That and many aspects of NHEJ remain a mystery, including whether its steps are sequential, iterative, or flexible according to the complexity of the damage [61,69,70]. Variations on NHEJ repair are still being characterized; the most highly studied form is V(D)J recombination, which occurs only in T and B cells and is essential for fostering lymphocyte diversity [72].

Homologous Recombination Repair

Homologous recombination repair (HRR) is a complex template-directed repair. It works during the most critical point of cell replication—after DNA is copied but before it divides. With the two chromatids still held together by a cohesion complex, HRR takes advantage of having a full copy of DNA present and proximally accessible. This enables HRR to find a large area of homology (usually on a sister chromatid) and use it as a template to reconstruct the damaged DNA strand [27,28,59].

HRR's role in maintaining genomic stability cannot be overstated. Its timely intervention is crucial in restarting replication forks [19,32]. Otherwise, gene mutations and the potential loss of bases equal that of an entire chromatin arm could occur [27].

In the earliest step of HRR, MRN activates checkpoint kinases that arrest the cell cycle and recruit additional DNA repair proteins [63,73]. Through end resection, MRN also forms the single-stranded DNA (ssDNA) at the DSB end [58] that is required for initiating recombination. Additionally, this action commits the repair to HR, preventing NHEJ intervention [58,62]. The ssDNA extends beyond the original breakpoint, a crucial feature for enabling Rad51 to attach to the ssDNA's 3′ end to search for an area of homology on the sister chromatid [19,60]. HRR has several subpathways (discussed in Chapter 8), but all of them share the same initial steps in processing the DSB to this 3′ overhanging tail. Collectively, the early steps to create the overhang are called presynapsis [60].

RPA coats and protects that exposed stretch of DNA so the Rad51 filament can form on the ssDNA and can search for DNA sequences similar to that on the 3′ overhang [74]. When Rad51 finds that area of homology—its template for resynthesis—it invades the homologous sequence, displacing part of its strand (a process called synapsis) [60]. Like loosening a thread in a garment, Rad51 pulls on part of the DNA strand, creating a DNA heteroplex called a D-loop.

In an ATP-dependent reaction, Rad51 oversees the pairing and exchange of homologous DNA sequences within the sister chromatid [75]. Rad51 slides the D-loop along as it reads the selected area of homology. Rad52 contributes a unique activity in annealing, or pairing, complementary single strands of DNA bound to RPA [60]. In the canonical version of HRR, the overhang progressively extends as new nucleotides are generated beyond the original breakpoint.

Numerous proteins help protect the ends from nuclease activity, facilitate strand invasion and filament migration, and contribute to the extensive synthesis of more than 50 new nucleotides [71]. The components of the MRN complex also lend a hand. For example, Nbs1 recruits and funnels other repair proteins to the site, while helping maintain the DNA damage checkpoint. Rad50 serves as a tether; MRE11 possesses both exo- and endonuclease functions [76].

During Resynthesis, as the D-loop is pushed along the border between DNA's heteroduplex and homoduplex, an X-shaped structure develops, called a Holliday junction. Resolution of this junction (postsynapsis) occurs in various ways [77], enabling the final steps of strand migration and Reinstatement to be executed as planned. At the conclusion of HRR, potential flaps are removed, nicks are sealed, and ends are ligated [27].

Many tumor suppressors participate in HRR activity, including BRCA1, BRCA2, and ATM [19]. Overviews of their importance in DNA repair modulation follow in this chapter and are discussed at length in Chapter 8. However, the underpinnings of DNA repair inhibition lie within the simplest repair pathway: DR—and its sole repair protein, MGMT.

MGMT INHIBITION: FIRST FORAY INTO DNA REPAIR INHIBITION

The study of MGMT is a study in "firsts." It was the first DNA repair gene studied at length [18], and it was the target of the earliest attempt to develop a DNA repair inhibitor [30]. Close to 25 years of studying MGMT has made it the most widely studied DNA repair pathway [31].

MGMT's restricted, apparently "simple" mechanism of action fostered not only its study but also paved the way for many discoveries regarding many aspects of DNA repair in general (dysregulation, crosstalk, malignant transformation)—and the notion that repair pathways can be therapeutically modulated [18,30,31,78]. Examination of MGMT continues to guide translational research of repair inhibitors overall.

In the 1970s, nitrosureas were introduced as a chemotherapeutic for glioblastoma and other malignant gliomas [78]. Alkylating DNA at various positions on guanine (primarily N^7, but also O^6 and O^4), nitrosureas subsequently cause single- or double-strand damage (the latter by crosslinking) [18]. Although nitrosureas were initially touted as being highly selective for gliomas [79], scientists quickly learned that something could reverse the DNA damage that they inflicted. That "something" was O^6-methylguanine-DNA methyltransferase (MGMT), a DNA repair protein that removes alkyl groups in a single-step "suicide" reaction [31]. MGMT's discovery in the late 1970s ushered in the era of researching DNA repair pathways as potential clinical targets [8].

The first true discovery of a DNA-repair-inhibiting agent came when researchers found that using a decoy target for MGMT could be a method of inhibition [30]. The idea of using a pseudosubstrate to deplete MGMT evolved, and, in 1990, O^6-BG (O^6-benzylguanine) was identified as a potent MGMT inhibitor. Its journey from bench to bedside heralded another "first:" it was the first anticancer agent developed on the basis of eliciting a target *effect* rather than determining a maximally tolerated dose [80].

Although altering the methylation status of MGMT fell short of expectations in chemosensitizing tumors to alkylating agents [78], it still had clinical merit and continued to be studied. In a less intuitive application of MGMT modulation, its induced overexpression in bone marrow stem cells was found to protect the marrow against myelosuppression of healthy cells. Thus, MGMT was also the first molecule targeted as a myeloprotective agent [18].

Despite MGMT being upregulated in many cancers, MGMT's inhibition sensitized both tumor cells and healthy cells to alkylating agents, resulting in obligatory dosage reductions and suboptimal treatment results. So scientists once again faced the question of how to selectively kill tumors while sparing normal cells. That question remains today.

Still, studies of MGMT continue to reveal more information about DNA repair pathways. Although MGMT is not critical for survival, it is an important linchpin in DNA repair [30]. For example, MGMT repairs only one lesion that comprises a very small portion of all DNA methyl adducts, but MGMT's activity (or lack thereof) can redirect how other pathways repair tumor cells' DNA [20,31,32] (see Table 1.3) and induce apoptosis. Low levels of MGMT contribute to cancer initiation and progression [31], which is one reason why MGMT inhibitors are used in concert with other anticancer agents.

Although MGMT inhibition has a reduced status as a therapeutic, MGMT status has become a mainstay as a biomarker in clinical use. Both are detailed in Chapter 2.

Table 1.3 How MGMT's Activity in Direct Repair Influences Other DNA Repair Pathways

Pathway Compensation	Mechanism
DR → MMR	When MGMT is unsuccessful in removing O^6-methylguanine in DR, the MMR pathway can correct O^6-meG mispairs.
DR → MMR → HRR or NHEJ	When MGMT repairs guanine/thymine mismatches postreplicatively, MMR attempts to repair the damage; but DSBs are created that are repaired by either HRR or NHEJ.
DR → BER	BER repairs mismatch pairs and other alkylation adducts that MGMT does not repair.
DR → NER	When MGMT cannot repair larger adducts at guanine's O^6 position, NER repairs them.

Abbreviations: BER, *base excision repair;* DR, *direct repair;* DSBs, *double-strand breaks;* HRR, *homologous recombination repair;* MMR, *mismatch repair;* NER, *nucleotide excision repair;* NHEJ, *nonhomologous end joining.*

PARP: THE ARCHETYPICAL INHIBITOR

PARP is a superfamily of proteins that are abundant in cell nuclei. Three members of that family have roles in DNA repair, with PARP1 dominating that activity [81,82]. PARP1 is most widely known as a "molecular nick sensor" [83]. Like MGMT, PARP is not required for survival; but it is important for maintaining genetic stability. Also like MGMT, PARP's study has led to many "firsts."

PARP was first described in 1963 [57], but it took 40 more years for it to enter clinical trials in the fight against cancer. As part of the enzymatic machinery of BER, PARP1's job is to sense SSBs, assess the extent of their damage, decide whether the damage can or should be repaired, then approve repairs or trigger apoptosis (see Fig. 1.3). PARP1 "flags" its approval by binding to the damage site and undergoing a conformational change, which recruits proteins to relax the chromatin, scaffold the damage, and repair the site [81,84,85].

In 2003, the first PARP inhibitor (PARPi) entered clinical trials [30,84] much like MGMT inhibitors did: as a chemosensitizer, but without regard to tumor selection for its DNA repair function [57,86]. And, like trials with MGMT inhibitors, a PARPi was first used in combination with temozolomide (TMZ). Trials of such nonselective combination therapy continued until 2005, when two seminal papers published in *Nature* provided proof of concept regarding the efficacy of using PARP inhibitors as single agents to treat BRCA-deficient cell lines from germline breast cancers [87]. That research birthed the concept of "treating a weakness" [57].

Fast-forwarding a decade, the FDA approved the first PARP inhibitor (olaparib) [88] late in 2014—not for breast cancer as one might suppose, but for previously treated ovarian cancer [81,89]. Today, six PARPi compounds are in Phase 2 and 3 clinical trials for breast cancer [81].

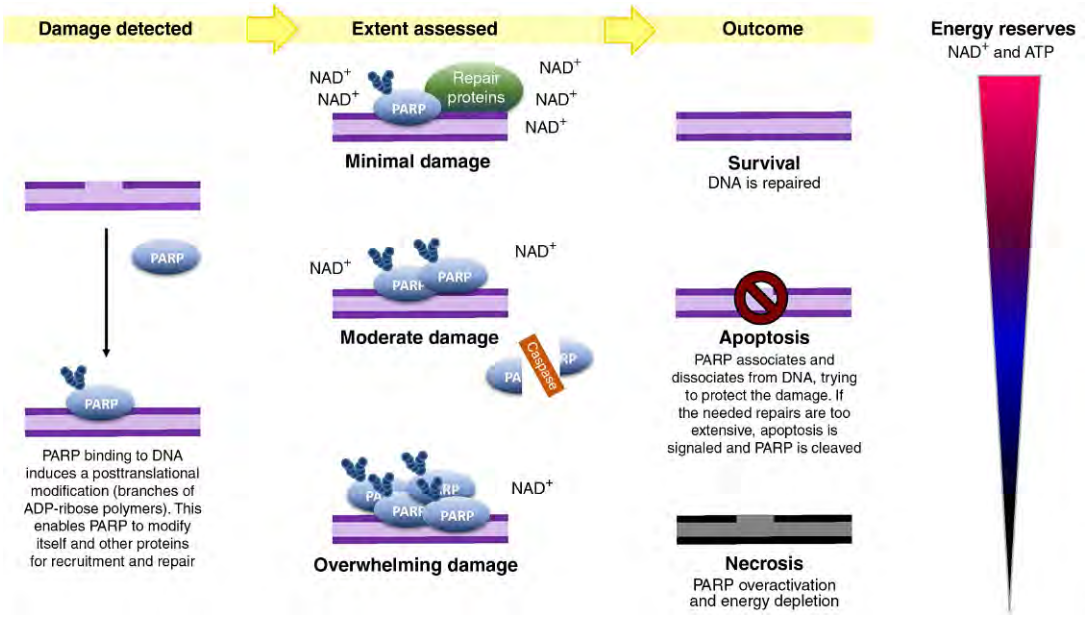


FIGURE 1.3 PARP's Assessment of Extent of DNA Damage and Cell Fate

PARP's clinical efficacy on BRCA-deficient tumors remains one of the most exciting recent developments in clinical oncology [30,84,86,90], but we are still in the early stages of tapping its therapeutic potential [91]. One reason for that is PARP's interconnectedness with other DNA repair pathways. Another reason is that we are still figuring out how PARP works [82].

In brief, PARP inhibition stalls BER, which causes unrepaired SSBs to accumulate. When that unrepaired damage is replicated, the lesions collapse the DNA replication fork; then DSBs to accumulate. Thus, DNA repair normally conducted in BER is forced into a DSB repair pathway. Cells that are missing both alleles of BRCA 1 or BRCA2 have no HRR functionality, which leaves repairs in the hands of NHEJ. Its limited ability to repair extensive DSB damage leads to tumor cell death [1,85,89].

However, the cause and effect of PARP inhibition is not quite as straightforward as that. To more fully understand PARP inhibition, we must account for two additional twists: PARP's normal interactions with other DNA repair pathways, and something called PARP trapping.

PARP works both inside and outside of BER. PARP activates XRCC1 in the HRR pathway [1] and is involved in a regulatory feedback loop with BRCA1 [85]. At the same time, PARP appears to inhibit the NHEJ pathway by inactivating DNA-PKcs and ATM's checkpoint activity [1]. Although PARP's overall contribution to the classical NHEJ pathway is still uncertain, collectively, PARP's actions can affect which DSB repair pathway is selected [85,92].

PARP TRAPPING

Until recently, PARP inhibitors were assumed to work solely by inhibiting PARP 1/2's catalytic activity because they were designed to competitively bind in the enzymatic substrate conserved site. "Trapping" PARP where it parks on DNA prevents recruitment of repair complexes and denies PARP's dissociation from DNA. However, research is finding there is more to it than meets the eye. Additional cytotoxic effects occur—ones that cannot be explained away by the differing sizes of various PARPis, their differences in catalytic inhibition, or any differential off-target activity. Something else is causing very large magnitudes of difference in trapping "strength" [93].

In models still being tested, research suggests that PARPis may also induce an allosteric change in PARP1 and PARP2 that stabilizes their associations with DNA. Thus, current data support a dual mechanism of cell killing by PARPis: via direct inhibition as well as a (reversible) conformational change that alters the protein's dynamics. This latter mechanism has been called PARP "poisoning" [93]. Learning more about PARP trapping may lead to more efficacious therapeutic drug combinations that cooperate with each other [85].

PARP inhibition is a stunning example of "treating a weakness" [27]. Normal cells can live without PARP1. Even though SSBs would still accumulate, the HRR pathway would repair them when they become DSBs during replication. Thus, a PARP deficiency by itself is not lethal; neither is a BRCA deficiency (although BRCA-deficient cells have no HRR functionality). But adding a PARPi to a BRCA deficiency becomes lethal (discussed in the next section of this chapter).

BROADENING APPLICATIONS

As research uncovers more types of cancer that share clinicopathological features with BRCA-mutated cancers [89], the potential for using PARP inhibitors with other tumor types continues to broaden [86]. The general principle remains the same: either to overwhelm DNA repair capacity or abrogate other

pathways that are essential for cancer survival. Tantalizing possibilities exist for enhancing or inducing "BRCA-ness" to create synthetic lethality [90,92]. For example, evidence exists that ATM loss, silencing of XRCC1, and other alterations in various tumors can compromise HRR and induce sporadic "BRCA-ness" [85]. The careful combination of PARP inhibition with specified tumor mutations is an early and clear example of precision medicine.

As those possibilities are researched, new treatment combinations hope to help historically difficult-to-treat tumors, including treatment-resistant cancers, advanced cancers, and those with few treatment options [84]. At the time of writing, well over 150 clinical trials were in progress for using PARP inhibitors as monotherapy or combination therapy to treat a wide variety of tumors [81,85,89,94]. Even maintenance treatment with olaparib has been investigated for high-risk populations, but the benefits of that are unproven [89].

Applications of PARP inhibition are also extending beyond oncology. Many inflammatory diseases are PARP mediated; in most cases, PARP inhibition suppresses inflammation [44]. Mounting evidence exists that PARPis have cardioprotective properties, blunting the body against ischemia-reperfusion injury (eg, after stroke, myocardial infarction, circulatory shock) [85,95,96], as well as chronic and acute inflammation (eg, arthritis, asthma, diabetes, autoimmune pathologies, septic shock) [44,85]. Preclinical investigations and early clinical trials are in progress using PARPis for such conditions [85].

PARP's role in inflammation alludes to its involvement in transcriptional regulation and many other biological functions pertinent to cancer [44,85,89,97]. Those discussions are beyond the scope of this section. For further details on PARP's many functions, see the 2015 reviews by Bai, Rodriguez, and Feng, as well as Chapter 4 of this book.

RESISTANCE TO PARP INHIBITORS

Although therapeutic PARP inhibition is an éclat of efficacious anticancer treatment, tumors can acquire resistance to PARP inhibitors. This can happen in several ways [81,82,89,91,98]:

- Acquisition of secondary BRCA mutations (such as reverse mutations) can restore normal BRCA function.
- Partial loss of 53BP1 (a tumor suppressor protein) partially restores HHR function.
- Upregulation of Rad51 activity or inhibition of Ku80 attenuates NHEJ activity.
- Activation/overexpression of P-glycoprotein (P-gp), a membrane transport protein causes increased drug efflux, reducing the oral availability and brain penetration of a PARPi.
- Epigenetic silencing or increased turnover of PARP causes PARPi resistance.

Counterintuitively, PARP needs to be intact for a PARP inhibitor to work. Partial or complete loss of PARP function renders cells resistant to PARP inhibition. However, the level of PARP expression in tumor cells should not be used as the sole predictor of tumor response. Differences in trapping strength and other variables must be taken into consideration [93].

We have much to learn from continuing study of PARP. Which of its functions are critical for clinical response? How can we identify molecular markers that may predict PARPi sensitivity? Does PARP differentially control other disease processes through transcriptional regulation? How do other members of the PARP family contribute to cancer pathophysiology [85]? Only time will tell.

SYNTHETIC LETHALITY: TARGETING A CELL THAT IS ALREADY GENETICALLY UNSTABLE

A hallmark of homeostasis is that its processes do not (or should not) depend on any single component. To that end, cells employ robust, sometimes redundant or overlapping systems to maintain genomic integrity in the face of diverse genetic and environmental challenges [14]. However, cancer is a disease of errors. Unless it is stopped, cancer's mutagenicity inexorably increases, progressively accumulating genetic alterations, including point mutations, insertion mutations, gene amplification or inactivation, chromosomal translocations, local DNA rearrangements, and allelic haplodeficiencies [1,2,57] (see Fig. 1.4). All such changes alter the functionality and regulation of homeostatic pathways, including the machinery of DNA repair.

If neoplastic cells develop characteristics that normal cells do not, then cancers should manifest a genetic "signature" that can be exploited to develop tumor-specific treatments. Synthetic lethality is one of the most advanced strategies that use such signatures to add to the arsenal of anticancer treatments [1,19].

Synthetic lethality is an interaction of two co-occurring genetic or epigenetic events that results in cellular death [14]. Independently, neither defect is lethal on its own. However, the first defect forces the cells to depend on an alternative means for carrying out the functions lost by that defect. When the right kind of second defect co-occurs with the first, their combined presence becomes deadly to cells [1,14,94,99]. Cells that contain a normal copy of either gene should not be affected [2,57].

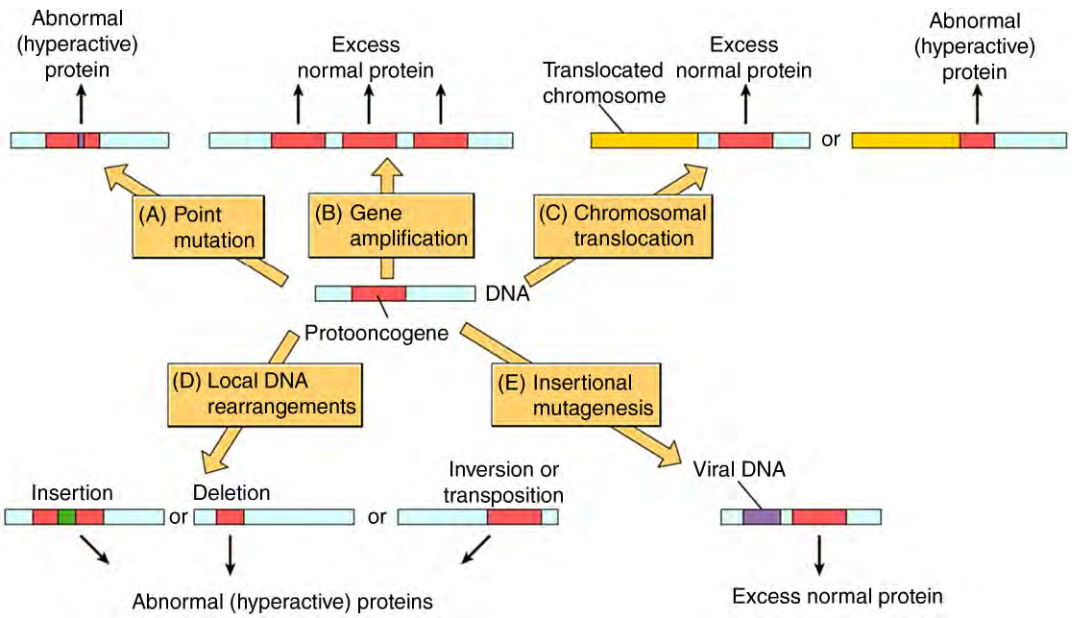


FIGURE 1.4 How Alterations to DNA Produce Abnormal Proteins

Reprinted with permission from Pearson Education.

In cancer treatment, one defect can arise from mutagenesis; the other can be induced or exploited by therapeutic inhibition. Finding the right tumor-specific target involved in that alternative pathway and developing an inhibitor of that codependent process is the ultimate goal in researching synthetic lethal (SL) pairs.

The idea of synthetic lethality is not new; Dobzhansky first described it in 1946. However, it took another 51 years before Hartwell et al. suggested that the concept could be applied to cancer therapeutics [57]. Excitement built to think that targeting a weakness unique to cancer could potentially solve the conundrum of killing cancer cells while sparing normal cells [2,17].

Synthetic lethalities offer many potential advantages as cancer treatments: (1) greater tumor selectivity, (2) higher therapeutic index, (3) lower dosing requirements, (4) identification of targets previously considered not "druggable," and (5) increased understanding of strategic sequencing or timing of drug administration [2,82].

In cancer cells, aberrations in DNA damage recognition and repair are common. Identifying those defects can serve as both biomarkers as well as starting points for finding SL pairs. (Similar efforts are underway to identify SL pairs in other pathways crucial for cancer survival [1]). As research identifies more about abnormal molecules and aberrant cellular signaling in cancer versus normal cells, more targets are being identified as potential candidates for SL combinations [82]. The latest screening methods to identify SL pairs are discussed at the end of this chapter in Section "Future Perspectives."

Despite the impressive success that PARP inhibitors have on tumors lacking alternative functional components of the HHR pathway, we do not yet know the exact mechanism of action of PARP inhibition or the extent to which it affects PARP's other functions. This hints at inherent challenges in finding and therapeutically harnessing equally or more complex SL pairs. Indeed, research is revealing that combinations are less predictable than imagined.

Even though a DNA repair pathway may contain a cancer-related defect, its SL pair may not be found strictly within the realm of other DNA repair pathways. It may be found in another cancer survival pathway or even in a pathway that is not "classically" related to tumor viability or drug resistance [82]. In essence, research may need to do more than look for two "train tracks"—it may need to jump the tracks to identify seemingly unrelated SL pairs. For example, a DNA polymerase inhibitor (gemcitabine) combined with a mitotic inhibitor (nab-paclitaxel) is in clinical use for treating metastatic pancreatic cancer [100]. More examples follow.

APE1/Ref-1's redox function maintains many transcription factors in their active, reduced state. Some of those factors are widely recognized as tumorigenic regulators. One of them is STAT3, a transcription factor that contributes to cell survival, tumor angiogenesis and metastasis in pancreatic ductal adenocarcinoma (PDAC). Recently STAT3's transcription activity was shown to be under redox control, but the mechanism for that was unknown. *Ex vivo* studies revealed that dual targeting with a STAT3 blockade and an APE1 redox inhibitor synergistically inhibited proliferation and viability in human PDAC cells [101].

Another example is HMOX-1, a candidate protumorigenic gene product that contributes to radio- and chemotherapy resistance. Small-molecule inhibitors of HMOX-1 are in preclinical development. NRF2 (nuclear factor erythroid-related factor 2) signaling regulates oxidative stress and induces HMOX-1 expression. Recent discoveries showed that inhibition of APE1's redox activity activates NRF2, which led to cell studies of combined APE1 and HMOX-1 blockade. Under hypoxic conditions, the combination synergistically kills PDAC cells [102].

Another SL pair between two different cancer survival pathways is APE1 redox inhibition combined with inhibition of VEGF activity (using bevacizumab) [103]. In advanced 3D cell cultures, gemcitabine coupled with an APE1 redox inhibitor appears to stop the crosstalk between tumor cells and fibroblasts, sensitizing the tumor to gemcitabine-induced cell killing [104]. More speculatively, a DNA repair inhibitor coupled with a P-gp inhibitor (which limits cellular efflux) could increase cytotoxicity [105]. Perhaps an inhibitor of cellular adhesion would work well with DNA inhibition [106].

TIMING AND CONTEXT

To truly deliver personalized medicine, we need to pinpoint four variables: (1) the tumor's phenotype, (2) the pathway(s) that can be exploited therapeutically to kill it, and (3) the best timing (therapeutic window) and (4) best mode of delivery that will yield the greatest benefit. Variable (1) will lead to variable (2), and variable (2) includes SL pairing. Variable (3) takes into consideration sequential versus concurrent treatments, staggered versus continuous treatment rounds, whether to treat at presentation versus during relapse, and what context will enhance treatment the most. Variable (4) involves drug carriers and the tumor microenvironment, which are summarized in the last section of this chapter.

A good example of optimized timing is with triple-negative breast cancer, another difficult-to-treat cancer. Time-staggered treatment with an epidermal growth factor receptor inhibitor (EGFRi) is proving to be more efficacious than co-administering it with traditional DNA-damaging chemotherapy. For reasons still being discovered, the staggered timing "rewires" the tumor's cellular network to reactivate components of the extrinsic apoptotic pathway. This potentiates the effects of subsequent DNA-damaging therapy [82].

CHALLENGES WITH SYNTHETIC LETHALITY

As with any other anticancer treatment, the SL approach comes with a theoretical risk of second cancers and secondary acquired resistance. Carcinogenesis is fraught with genetic heterogeneity, which increases the need for developing truly personalized medicine. Two other concerns are specific to SL. The cost to run library screens to find potential pairs is high. Also, misleading "'off-target" effects can confound detection of clinically relevant results [82]. The Section "Future Perspectives" of this chapter discusses the plethora of data to sort/interpret, and the challenges inherent in discerning what is relevant. Few clear-cut patterns of deficiencies or mutagenic changes exist outside of those seen in certain familial cancers, which complicates the discernment of overlapping but slightly different functionalities of paralogous proteins [2]. Additionally, today's technology platforms may not be able to detect all potential interacting genes—although the latest designs get us closer. Finally, all anticancer treatments, even targeted ones, still cause some collateral damage [82].

CONCLUSIONS

Ironically, an innate deficiency in DNA repair creates an undesirable cancer risk—but it can be exploited to create a synthetic lethality [75]. SL strategies move us further away from dose-intense, cytotoxic, nontargeted therapies toward highly selective, personalized therapy. Although methylation of MGMT's promoter fell short of being an SL agent, PARP inhibition is a dramatic glimpse into the potential power of synthetic lethality, an intense topic of research. Undoubtedly, many SL pairs are still waiting to be discovered.

DNA DAMAGE CHECKPOINTS

Checkpoints are the cell cycle's master switches, driving orderly progression through each cell phase [107]. During times of genotoxic stress, checkpoints pause the cell cycle to allow repairs before DNA damage can become permanent through replication and mitosis [19,74,108]. Collectively, checkpoint pathways and DNA repair pathways constitute the DDR [74,109,110]. This section introduces how checkpoints normally work and what goes awry when they are dysfunctional.

Genomic insults and replication stress can trigger signaling transduction processes known as the DNA damage checkpoint. The overall functions of checkpoint signaling are to (1) determine how to cope with the stressor and (2) coordinate cell cycle activity during and after that critical decision. In doing so, checkpoint signaling affects cell cycle progression as well as DNA replication and DNA repair mechanisms [49,111,112].

If DNA damage is minimal, checkpoint signaling may not be activated [49]. But when it is activated, the many proteins involved collectively function like a quality control manager, precisely monitoring DNA status throughout the process. Multiple layers of tightly regulated decision-making processes determine whether the cell will undergo DNA repair, become senescent, or be destroyed through apoptosis. Checkpoints also play a role in selecting the most appropriate method of DNA repair to employ—and allotting sufficient time for that repair [113,114].

The checkpoint response includes an arsenal of strategies that can be deployed depending on what goes astray and when [28,49,61,107,113,114]. Checkpoints may:

- prevent cell cycle progression
- segregate damaged chromosomes
- prevent generation of secondary lesions
- modify transcription
- direct lesions to the most appropriate repair pathway
- decide cell fate overall

Interestingly, checkpoint decisions for senescence and apoptosis can actually help prevent tumorigenesis during early stages of genetic instability [109,113]. It is notable that markers of senescence and DDR signaling are present in early-stage premalignant lesions but are lost during carcinogenic progression [109,115].

MAJOR PLAYERS IN CHECKPOINT SIGNALING

Activation of checkpoint signaling is not due to the DNA lesion itself but by DNA repair proteins involved in damage recognition and initial lesion processing [113]. Looking back to the Recognition and Reinforcement/Recruitment steps of various DNA repair pathways, one can see that those steps create a unique "common structure"—a single-strand DNA region—that triggers checkpoint signaling (Fig. 1.5) [49,74,114].

Checkpoint signaling is a highly regulated, collaborative effort converging from six sources:

- sensors
- proximal transducer kinases
- distal transducer kinases
- mediators
- effectors
- cell cycle engines

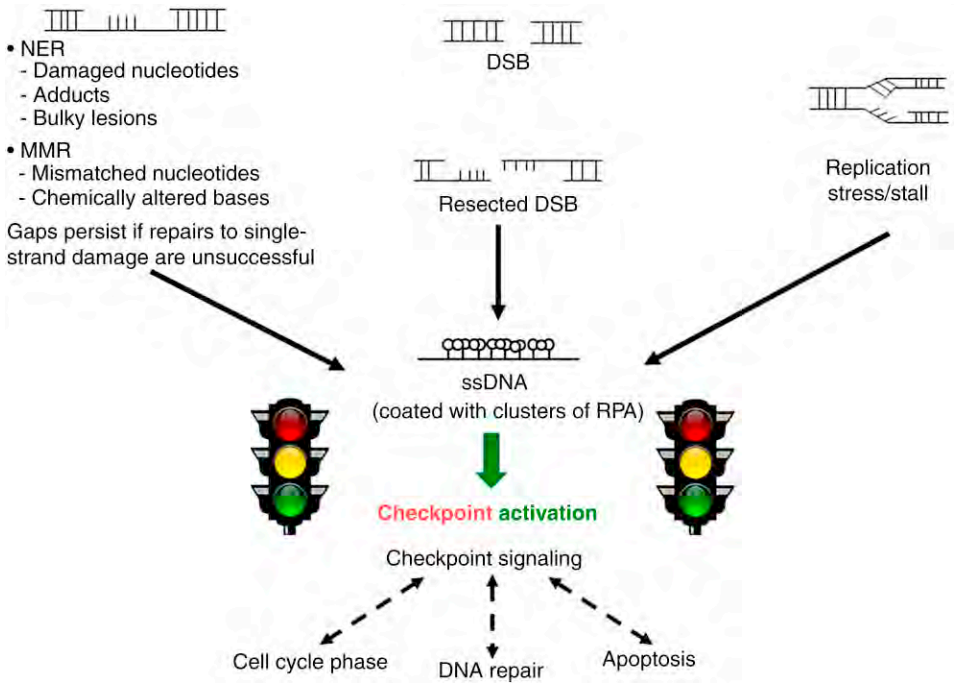


FIGURE 1.5 Sources of Checkpoint Activation: Coated ssDNA

Sensors recognize structural abnormalities of damaged DNA or chromatin. Proximal (apical, or initiating) transducer kinases function like sensors but require activation from additional proteins to transduce the damage signals. Mediators coordinate signaling and assess the temporal and spatial progression of the DDR. When activated, both the proximal and distal transducer kinases phosphorylate multiple effectors that, in turn, alter the activities of the cell cycle engines called CDK-cyclin complexes—thus triggering temporal cell cycle arrest [49,74,113] (see Fig. 1.6).

The apical (initiating) kinases of the DDR cascade are proteins named ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3-related). They represent two distinct (but sometimes overlapping) kinase signaling cascades: the ATM-Chk2 and ATR-Chk1 pathways [113].

THE TWO CHECKPOINT SIGNALING PATHWAYS

The ATM pathway is activated by DNA double-strand breaks (DSBs) that occur outside of S phase. The ATR pathway responds to single-strand breaks (ssDNA) [108,110,115]. Simplistically, ATR activation is most commonly associated with stalled or collapsed replication forks during S phase, but ATR is also triggered by other DNA lesions that expose ssDNA, including resected ends of DSBs and ssDNA gaps generated during DNA repair [74,108,113,115] (see Fig. 1.5).

As described earlier in this chapter, DSB repair by HRR requires ends processing to yield a 3′ ssDNA segment, which RPA coats. That serves as the substrate for the Rad51 complex and also activates

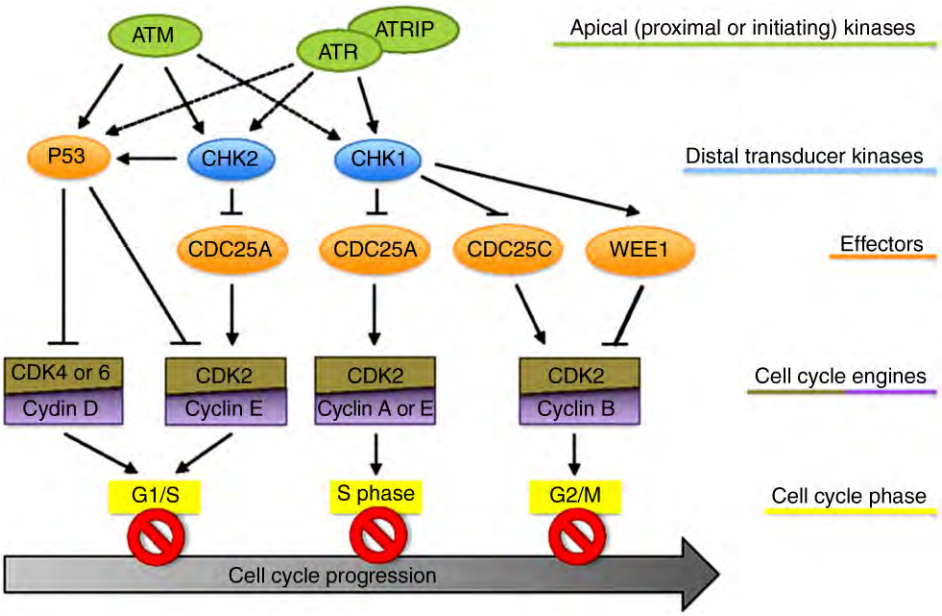


FIGURE 1.6 Checkpoint Pathways

the ATR checkpoint-signaling pathway; both are early steps in committing the repair to HRR [116]. Similarly, ssDNA is generated when a replication fork stalls. A helicase keeps unwinding DNA for a few hundred base pairs directly downstream of the fork, which uncouples the leading and lagging strand polymerases, exposing ssDNA. RPA coats the exposed ssDNA, which activates the ATM checkpoint signaling pathway [108,110,115].

Throughout the signaling cascade, complex phosphorylation events and other posttranslational modifications (PTMs) function as "on" and "off" switches at precise times, activating, amplifying, or silencing kinase signaling. Timely initiation and removal of the signal sources are equally important. Just as DDR activation through PTMs causes cell cycle arrest and influences DNA repair, removal of protein modifications or degradation of signaling kinases is essential for silencing the checkpoint and allowing the cell to "recover," that is, reenter the correct cell cycle phase [117]. Table 1.4 summarizes each step and function of checkpoint signaling, along with examples of the main checkpoint kinases involved.

As noted in Table 1.4, cellular responses to DNA damage are coordinated primarily by two distinct kinase signaling cascades: the ATM-Chk2 and ATR-Chk1 pathways. Both proximal signal transducers—ATM and ATR—are serine/threonine protein kinases (members of the PI3K family). Working in conjunction with mediators that accumulate at the damage site, ATM and ATR phosphorylate multiple substrates, including the distal signal transducers, Chk1 or Chk2. That sets off a phosphorylation cascade promoting the activation, translocation, or stabilization of effectors as needed. The inhibitory

Table 1.4 Main Sources and Types of Checkpoint Signaling

Signal Originator	Function	Examples
Sensor	• Constantly scans DNA for breaks, distortions, and chromosomal abnormalities	MRN complex, PARP, RPA-coated ssDNA (possibly DNA-PKcs)
Proximal transducer kinase	• Phosphorylates substrates to "boost the signal" calling for a cellular response • Cooperates with mediators and distal transducer kinases	ATM, ATR
Mediator	• Accumulates at damage sites to help recruit repair proteins • Tracks progress during damage response • Coordinates phosphorylation of ATM's and ATR's substrates	*ATM mediators:* MDC1, 53BP1, BRCA1 *ATR mediators:* TopBP1, claspin
Distal transducer kinase	• Amplifies damage signals from sensors and ATM or ATR • Spreads the signal by phosphorylating effectors that can halt the cell cycle • Regulates origin firing during replication stress	*ATM pathway:* Chk2 *ATR pathway:* Chk1
Effector	• Signals cell cycle engines to direct their activities	*ATM pathway:* p53 *ATR pathway:* CDC25 proteins Note: Secondary effectors are not included in this list.
Cell cycle engine	• Halts/resumes cell cycle progression and transcription OR induces senescence or apoptosis	CKD–cyclin complexes

function of those effectors or their downstream targets alters the expression of the cyclin-dependent kinase complexes (CDKs + cyclins) [19,74,113,117].

CDKs are protein kinases that require interaction with other proteins (here, cyclins) to induce a conformational change, exposing the CDK's binding cleft so it can exert its kinase abilities [29,74,113,115]. Appropriate levels of CDK-cyclin complexes apply the brakes to the cell cycle phase in progress.

NORMAL, LOW-STRESS CHECKPOINT ACTIVITY

During a normal cell cycle, the ATR-Chk1 pathway guards and initiates S phase progression, whereas ATM-Chk2 monitors and allows M phase [113,118]. CDK 1 and 2 protein levels remain fairly constant throughout all cell phases, although their activity changes when a companion cyclin binds to it [119]. In contrast, the synthesis, activity, and destruction of cyclins dramatically rise and fall in a cyclical fashion [111,112,120] (see Fig. 1.7).

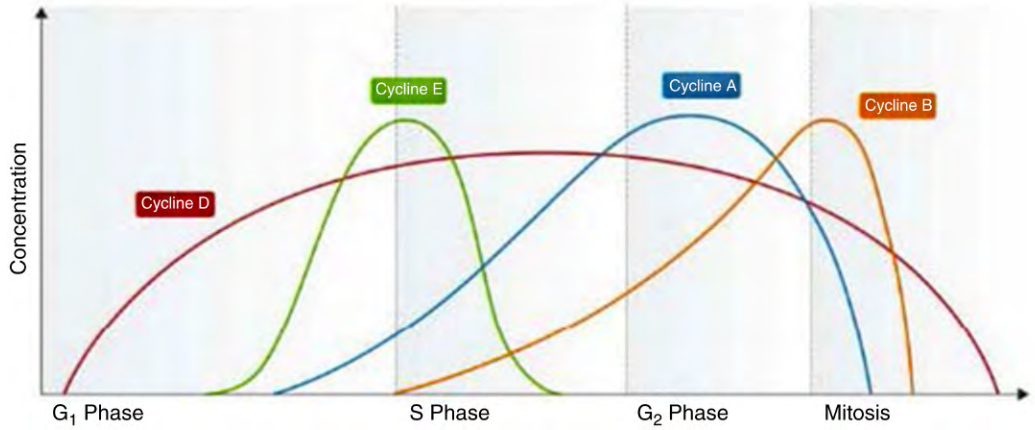


FIGURE 1.7 Rise and Fall of Cyclin Activity According to Cell Cycle Phase

Various CDKs are most active during particular cell phases [6,29] (see Table 1.2 and Fig. 1.6). Pauses are a normal part of every cell cycle; however, reentry after those pauses differs when DNA damage is present [117].

ROLES BEYOND CHECKPOINT SIGNALING

Checkpoint signaling performs functions complementary to the DDR [49,114]. CDKs' phosphorylation abilities also modify transcription, cell differentiation, apoptosis, and DNA repair activities [29]. As an example regarding the latter, checkpoint-dependent phosphorylation of BRCA1 seems to affect whether the HRR or NHEJ pathway is used to repair DSBs [49]. For a comprehensive discussion of additional checkpoint functions, see Lim's 2013 review.

CHANGING MODELS OF CHECKPOINT SIGNALING

Much remains to be learned about checkpoint signaling. ATM and ATR's distinct but overlapping divisions of labor and crosstalk are still being elucidated. The same is true of the distal transducer kinases (Chk1 and Chk2). For example, Chk1 regulates both the S and G2/M checkpoints via downstream effectors. Interestingly, Chk2 can do the same, through p53 as well as other effectors that Chk1 influences [112]. This hints that Chk 1 and Chk2's division of labor may not be as compartmentalized as previously thought.

The classic model of checkpoint signaling depicts the presence of DSBs as triggering Chk2 to arrest the cell cycle by acting upon p53 at the G1/S and G2/M transitions. This is particularly crucial because DNA damage during S phase gives rise to intermediates that are not found in other phases of the cell cycle under normal conditions [108]. In contrast, replication stress triggers Chk1 to signal Cdc25A to arrest the cell cycle at G2 [110,115]. Although Chk1 and 2 share homology and some overlapping functionality, they are not functionally interchangeable [74].

In similar fashion, both pathways' downstream targets (Cdk–cyclin complexes) were thought to be distinct and specific to various cell cycle stages and could be activated only very selectively. Emerging evidence indicates that Cdk1 and Cdk2 may share some cyclin-binding partners and that p21 and p27

may target/inhibit all the different Cdk1/2-cyclin pairing combinations. To confirm that, researchers need to further characterize the differences and functional relations between Cdk1 and Cdk2 [118], as well as their peak activation levels throughout the cell cycle [119]. The complexities of the two pathways' functions are discussed in detail in Benada's excellent 2015 review.

WHAT HAPPENS WHEN CHECKPOINTS DERAIL

Checkpoint abnormalities are a hallmark of tumor progression and neoplastic transformation [19]. Tumoral loss of control mechanisms for DNA replication cause increased cellular stress and loss of checkpoint "braking" mechanisms that are selective to cancer cells [108]. The aberrant functioning varies by the kinase involved.

ATR and Chk1 appear to be mutated only rarely in cancers; the opposite is true for ATM and Chk2. ATM mutations predispose people to genomic instability and cancer because of ATM's downstream effects on BRCA1/2 and HRR [115]. Even people who are heterozygous for ATM or Chk2 mutations have a higher incidence of cancer, likely due to the mutational allele interacting with environmental or occupational risk factors. Thus, people can live with impaired or even total loss of ATM-Chk2 signaling—but at the cost of high risk of mutagenesis. In contrast, loss of ATR or Chk1 function seems essential for the survival of many, but not all types of cells [115].

p53 is the most commonly mutated gene in all solid cancers; loss of p53 expression influences both Chk1 and Chk2 [74,111]. Cancer cells deficient in p53 lack an effective way to activate the G1 checkpoint, so maintaining the G2/M checkpoint fully depends on checkpoint kinases. Additionally, low or absent p53 signaling abrogates the inhibitory influence of its primary effectors, p21 and p27. Those natural checkpoint inhibitors (CKIs) normally would cause the CDK-cyclin complexes to pause the cell cycle when DNA damage is sensed. However, in the absence of that signaling, the CDK-cyclin complexes essentially have a nonstop "green light" to continue the cell cycle and enter mitosis regardless of what replication stress or DNA damage is present [74].

Overexpression of cyclins, the regulatory subunits of CDK–cyclin complexes, is common in cancers [111]. In contrast, mutations in CDKs are rare; but other dysfunctional proteins can cause CDKs to be overexpressed in cancers [120].

Other aberrations can derail checkpoints. Activation of oncogenes such as Ras, Myc, and Cyclin E abnormally increases CDK activity, DNA replication origin firing, and replication stress [74]. In addition, chemotherapy and IR activate cell cycle checkpoints [112]. The next section of this chapter discusses how further disruption [17,112] of an already-dysfunctional DDR is being exploited therapeutically.

TIMING IS EVERYTHING

With checkpoints, timing is everything—in normal cells as well as how CKIs are being developed. Their effectiveness hinges in no small part on timing. Here are a few brief examples [108]:

- Slowly proliferating cells can evade the effects of drugs intended to induce greater replicative stress by simply pausing their cell cycle during G1 phase.
- CDK inhibitors decrease the efficacy of nucleoside analogs unless the latter are administered first so the DNA can incorporate them.
- The rate of DNA synthesis is virtually unchanged despite cells having defective DDR mediators (such as ATM, Chk 1 or 2).

Many other unexpected challenges have arisen in the development of CKIs, but equally as many exciting possibilities exist, such as SL pairing that capitalizes on loss of p53 expression and its effect on HRR.

Clinically and therapeutically, checkpoint timing poses a dual question: how to stop the cell cycle engine if it already has poorly functioning brakes, and where is the best place to stop it. Strategies for therapeutic checkpoint inhibition are overviewed in Section "Inhibitors in Development" of this chapter.

INHIBITORS IN DEVELOPMENT

As subsequent chapters will explain in more detail, some small-molecule DNA repair inhibitors can work as standalone therapy; but many others have gained notoriety as combination therapies because of their ability to sensitize tumors to the cytotoxic effects of other DNA-damaging agents or potentiate the therapeutic efficacy of agents that directly damage DNA. Ideally, smaller amounts of drugs would be needed (and thus, milder side effects experienced) if a DNA repair inhibitor could synergize the damage caused by another cytotoxic agent. That, in fact, does occur some of the time. However, in a substantial number of cases, drug doses still need to be decreased due to unacceptable side effects. This historically has been true when a DNA repair inhibitor has been administered in combination with traditional chemotherapy, such as an alkylating agent or an antimetabolite [30]. As research moves further in the direction of targeted therapy, it hopes to avoid that excessive collateral damage with combinations of targeted therapies (repair inhibitors, immune inhibitors, growth signal inhibitors, chromatin remodeling inhibitors, and so on).

Although it makes sense to target enzymes unique to a pathway or those that are a rate-limiting step within a pathway, at least four major hurdles lie in the way of progress.

1. Because of the importance of maintaining our genetic code, repair pathways can back each other up. Redundancy helps ensure genomic fidelity [20,31,32].
2. Significant crosstalk occurs between pathways. This is true not only among DNA repair pathways but also between DNA repair and other pathways critical for cancer survival. This alludes to casting a wider net in finding ways to inhibit DNA repair. Overlaying our knowledge of cancer's broader characteristics, such as replication stress, increased ROS, and cancer stem cell fate, may uncover new approaches to inhibiting DNA repair and creating SL pairs [82,108]. This may be seen most dramatically in the development of CKIs—which are following three broad strategies: a CKI + a DNA repair inhibitor, a CKI + an inhibitor of oncogene signaling, or a CKI + an exploitable mutation (such as an HRR defect) [19,74,82,107].
3. Inhibition of enzymatic function does not always produce a radio- or chemosensitive phenotype. About half the time, the opposite occurs. For example, inhibition of BER generally increases the effectiveness of traditional chemotherapeutics; however, the opposite occurs when MMR or NHEJ function is lost [21,30].
4. Although it is tantalizing to inhibit multiple pathways at once, practical issues regarding untenable toxicities exist [30].

Thus, the continuing work of basic science discoveries is crucial in uncovering more mysteries of how the pathways work—finding the right combination of "switches" to kill cancer cells.

A number of DNA repair inhibitors are in clinical use and more are in development [18,26,30,48,121–135]. The compounds and their mechanisms of action are discussed at length in their respective chapters and are summarized in Table 1.5.

Table 1.5 Summary of DNA Repair Inhibitors in Development and in Use

Pathway	DDR Activities	When Repairs Made (before, during, after replication)	Targets for Inhibition	Rationales for Inhibition (i)		Developmental Stage/Comments
				Pros	Cons	
DR	Removes alkyl groups by direct transfer	Before	MGMT	Unique to this pathway Saturable reaction	Pathway redundancies can repair damage from MGMT inhibition. Inhibition does not work if MMR is deficient	O^6-benzylguanine (O^6-BG) was the 1st one developed as a chemosensitizer; still in use today
BER	Repairs nonbulky lesions produced by alkylation, oxidation or deamination	Before	APE1	Unique to BER No other protein can perform its varied functions. A rate-limiter in repairs Overexpressed in many cancers	Difficulties isolating and therapeutically manipulating APE1's endonuclease vs redox activities	Selective APE1 inhibitors: *Of endonuclease activity:* Preclinical testing: CRT0044876, AR03, Compound 3 *Of redox activity:* • Phase 1 trials: APX3330 • Cell studies: APX3330 analogs
			PARP	Unique to BER No other protein performs its functions.	Differences in strength/efficacy of various PARPis Secondary mutations can cause resistance to PARPis	PARPis are in development and use: • FDA-approved: olaparib • Phase 2, 3 trials: olaparib, veliparib, talazoparib, niraparib, rucaparib • Phase 1 trials: CEP9722, BGB-290, E7449 and analogs • Phase 0 trials: JPI-289
			FEN1	Overexpressed in many cancers	Involved in multiple pathways	FEN1 inhibitors tested in cell studies only to date
			Pol β	Rate-limiter in repairs Overexpressed in many cancers	Difficult to develop an inhibitor that affects DNA repair but not replication	Many compounds have been identified, but few are specific or potent enough to be clinically useful. Inhibiting lyase activity may be more effective than inhibiting Pol β

MMR	Repairs single-base mismatches and misaligned short nucleotide repeats, small insertion/deletion loops	During and after	MSH1 MSH2	Unique to MMR Bypassing its damage-sensing ability could induce lethal cell damage.	Lack of MMR functionality confers a highly mutational phenotype and resistance to antimetabolites and topoisomerase inhibitors.	In purified protein studies: • MSH1 deficiency + Pol γ inhibition is SL • MSH2 deficiency + Pol β is SL Methotrexate is in Phase 2 trials as an MSH2 inhibitor. Restoration of MMR function can sensitize tumors to various anticancer drugs.
			MLH1	Often epigenetically silenced in cancer	Difficulties in developing a reactivator specific to MLH1	
NER	Repairs large adducts and helix-distorting lesions to one DNA strand	During (TC-NER) Before or after (GG-NER)	RPA	Essential to NER Mutations are linked to carcinogenesis	RPA inhibition creates increased reliance on the ATR/Chk1 pathway.	Cell studies: HAMNO
			XPA	Unique to NER May be a rate-limiting factor	NER inhibition overall is problematic because of these proteins' large binding surfaces; however, "hot pockets" within those surfaces may be druggable	Phase 1, 2 trials: UCN-01 (7-hydroxystaurosporine)
			XPF/ERCC1	Unique to NER Mutations are linked to carcinogenesis		In silico results only
HRR	Extensive DSB repair using a sister chromatid as a template	During S and G2 phases	c-Abl	Inhibits Rad51 activity	Participates in many other cellular processes Cross-reacts with DNA-PK in the NHEJ pathway	Imatinib (an Abl inhibitor) has been clinically available since 2001
			PARP1	See details in BER		PARPis are in development and use (see aforementioned)

(*Continued*)

Table 1.5 Summary of DNA Repair Inhibitors in Development and in Use (*cont.*)

Pathway	DDR Activities	When Repairs Made (before, during, after replication)	Targets for Inhibition	Rationales for Inhibition (i)		Developmental Stage/Comments
				Pros	Cons	
			HSP90	Upregulated in many cancers Intrinsically tumor-specific; essential role in oncogene addiction Coordinates spatial and temporal order of protein interactions required to create HRR complexes	Difficult to produce Although inhibition blocks multiple pathways critical to cancer survival, that increases its chances of off-target effects.	HSP90is in development: • Preclinical: geldanamycin, radicicol • Phase 1, 2 trials: 17-DMAG (alvespimycin), PU-H71, ATI13387, AUY922, Debio 0932, IPI-504, SNX-5422, MPC-3100, KW-2478, XL888 • Phase 1, 2, 3 trials: ganetispib, 17-AAG
			Proteasomes	Affect HRR in several ways, especially its early steps Prolonged inhibition can halt ubiquitination reactions Especially helpful in treating hematologic malignancies	Affect other pathways in ways not fully understood: • Modifies PCNA function in MMR • Decreases NER functionality • Suppresses NHEJ	Proteasome inhibitors in development: • Phase 1: marizomib (NPI-0052), carfilzomib (PR-171), CEP-18770 • Phase 1, 2: bortezomib (PS341), ixazomib (UARK 2014-14), oprozomib (ONX 0912)
HRR			BRCA2		Activity appears to be specific to HRR In presence of BRCA2, PTEN, or ATM deficiency, this inhibitor exhibits SL activity. Synergistic with IR, etoposide, and PARPi	Cell studies and mouse models: YU238259

HRR			ATR ATM CHK1 CHK1/2 WEE1			ATR – 2 inhibitors in Phase 1/2 (AZD6738, VX-970) ATM – 1 inhibitor in Phase 1 (AZD0156) CHK1 – 3 inhibitors in Phase 1/2 (MK-8776, GDC-0575, LY2603618) CHK1/2 – 1 inhibitor in Phase 1/2 (LY2606368) WEE1 – 1 inhibitor in Phase 1/2 (AZD1775)
NHEJ	Simple repair/ rejoining of DSB ends regard-less of sequence homology	Before or after	DNA-PKcs	Unique to NHEJ Essential for NHEJ activity	NHEJ inhibition increases DNA damage tolerance, chemoresis-tance, and mutations.	Cell studies: NU7026, NU7441
			PNKP	Unique to NHEJ		Cell studies: A12B4C3
			Ligase IV	A rate-limiting step; cannot work if XRCC4 is absent		Cell studies and mouse models: SCR7
			S-phase cells			Mibefradil: NCT02202993 Radio- and chemosensitizer in upfront GBM
Indirect methods of DSB repair inhibition • MEK1/2 inhibitors, which target the Raf-MEK-MAPK pathway, inhibit DSB repair when *KRAS* mutations are present • In some cases, an ATR inhibitor can turn an HHR or NHEJ defect into a synthetic lethality						
Polymerases	Link one nucleotide at a time to the end of an exist-ing DNA "primer" chain	During		Some are unique to pathways or substrates	Difficult to isolate re-pair inhibition without affecting polymerases involved in DNA rep-lication	>60 potential inhibitors have been identified, but they lack the specificity and potency to be clinically useful

(*Continued*)

Table 1.5 Summary of DNA Repair Inhibitors in Development and in Use (*cont.*)

Pathway	DDR Activities	When Repairs Made (before, during, after replication)	Targets for Inhibition	Rationales for Inhibition (i)		Developmental Stage/Comments
				Pros	Cons	
Checkpoints	Arrest the cell cycle to allow time for DNA repairs	During	ATM ATR Chk1 Chk2 Others	All are unique to this pathway Many cancers seem to be highly dependent on one CDK Pathway redundancy can enable simultaneous inhibition of several CDKs	Timing of delivery is very crucial CKIs typically block more than one kinase	Chk-1 selective, Chk1/2, ATR/Chk1 inhibitors and combination inhibitors are in various stages of development (examples: a Chk1 + a Top1 inhibitor, Chk1 + a microtubule inhibitor) SL pairs being tested include: • CDK1/2 + selective PI3Kis • CDK1 + MYCi • CDK6 + VHLi (a HIF-1α regulator) • CDK4 + K-Ras • CDK5/12 + PARP

Key: *"i" at the end of a protein name, inhibitor.* **Proteins mentioned**: APE1, *AP endonuclease/Redox Factor 1;* ATM, *ataxia telangiectasia mutated;* ATR, *ataxia telangiectasia and Rad3-related;* BER, *base excision repair;* CDK, *cyclin-dependent kinase;* Chk, *checkpoint;* DNA-PKcs, *DNA-dependent protein kinase catalytic subunit;* DR, *direct repair;* ERCC1, *excision repair cross-complementation group 1;* FEN1, *flap endonuclease 1;* HR, *homologous recombination;* HSP90, *heat shock protein 90;* MGMT, *methylguanine methyltransferase;* MLH, *MutL homolog;* MMR, *mismatch repair;* MSH, *MutS protein homolog;* NER, *nucleotide excision repair;* NHEJ, *nonhomologous end joining;* PARP, *poly-(ADP ribose) polymerase 1;* PNKP, *polynucleotide kinase/phosphatase;* Pol, *polymerase;* RPA, *replication protein A;* SL, *synthetic lethality;* Top, *topoisomerase;* XP *(A or F), xeroderma pigmentosum (group A or F).*

Sources: *This information comes from clinicaltrials.gov, the other chapters in this textbook, and references noted in the paragraph that introduces this table. For further details, see the respective chapters of this textbook.*

FUTURE PERSPECTIVES

The goal of any anticancer regimen is to deliver the right treatment, in the right amount, at the right time, to the right target (and with sufficient penetration). That is the essence of precision medicine [136]. Many advances in technology have brought us closer to that ideal. Techniques for DNA sequencing are many times faster today than ever before [137], as is X-ray crystallography for elucidating three-dimensional structures of proteins [137,138]. DNA "encyclopedias" such as GENCODE7 [139] and the REPAIRtoire database of DNA repair pathways [140] are available and continually augmented. Ever-expanding biologic databases have helped identify biomarkers, surrogate endpoints, and patient characteristics. Genomics, proteomics, metabolomics, and diverse cellular assays are helping to more accurately classify cancers and stratify patients [141]. Burgeoning compound libraries can be analyzed faster and at a tenth of the cost than they could 10 years ago, thanks to high-throughput screening (HTS) [137] and the development of computational tools to analyze such massive sets of such data [141].

Still, the rate of new cancer drug approval hovers around 5%, and most metastatic cancers remain incurable. Although the cost of new-drug development has grown steadily over the past 50 years, R&D "efficiency" (the number of drugs brought to market per billion US dollars spent on R&D) has steadily declined [137,142]. This has tightened the purse strings of research funding sources.

Yet multiple "grand challenges" have been issued around the globe—by governments, private funding agencies, academic centers, and others—to tackle pressing problems that research can solve. The importance of basic research is underscored in many of these challenges. For example, in October 2015, Cancer Research UK in London announced it was dedicating at least £100 million (US$153 million) over 5 years to fund research teams to map the cellular composition of a tumor, identify carcinogens' molecular "fingerprints" in tumors, and three other cancer-related challenges [143]. In January 2015, the president of the United States launched a Precision Medicine Initiative—with a near-term focus on cancers and a longer-reaching aim for a wide range of conditions [141].

To tackle those initiatives with confidence, we must ask some difficult questions. Why do so many anticancer treatments—even targeted treatments—fail due to unacceptable toxicities or lack of therapeutic efficacy if the targets and concepts are based on sound science? Is technology on track but behind in its "evolution" for overcoming the woes [106,142] that halt so many Phase 2 and 3 clinical trials? Or is something fundamentally missing in the continuum from basic science to preclinical and clinical stages of development? What "deficiencies" exist in understanding disease processes, drugs' mechanisms of action, or other factors? What do we have now, how can we use it more effectively, and what do we need to obtain to break this apparent bottleneck?

One thing is certain: the days of doing "more of the same" are over.

This section summarizes the current state of DNA repair and cancer drug research overall, as well as current and emerging technologies. Additionally, this section offers forward-thinking suggestions for future directions.

WHAT WE KNOW

Now, more than ever before, we have more knowledge about DNA repair pathways. This achievement of basic science recently received international recognition when the 2015 Nobel Prize in Chemistry was awarded to three scientists who have spent years mapping the molecular workings of three DNA

repair pathways. Drs. Tomas Lindahl, Aziz Sancar, and Paul Modrich were recognized for their work on the BER, NER, and MMR pathways, respectively [16].

Our knowledge about the repair pathways has enabled us to identify many proteins that are dysregulated in cancers. This has yielded both therapeutic targets as well as biomarkers. But targets that look promising in screens or preclinical studies often do not pan out. Even established drugs behave differently than expected in some people. So what can basic science do to help ensure greater success in this path?

WHAT WE NEED TO KNOW: THE ART AND SCIENCE OF "BETTER"

The current drug-development path typically starts with human cancer cell lines being cultured "in a dish" and tested, then grown and tested in animal models, while being exposed to potential drug candidates. Numerous iterations of in vitro and ex vivo studies follow, utilizing immortalized cell lines of human cancers to varying extents. After years of basic and preclinical testing, candidate drugs move into years of clinical trials. Although stunning successes have resulted from this approach, its shortcomings need to be addressed. The rest of this chapter describes how research is rising to that challenge.

Better Screening Techniques

The earliest stages of identifying a potential therapeutic target historically involved phenotypic screening; that is, the use of cell lines or an animal model of a disease state to monitor a single parameter (such as production of a protein) to interrogate a potential agent [144]. To speed that process and screen more potential compounds, HTS evolved, leveraging automation and microtiter plates of 96–1536 wells to rapidly test target classes of potential biological modulators (eg, kinases, nuclear receptors). From potentially hundreds of thousands of compounds, "active hits" are identified, and then verified through further study.

Today's average pharmaceutical library exceeds one million compounds, with equally large needs to test those compounds [145]. Technology has responded, with the greatest advances in HTS in the last 20 years being in simplification, miniaturization, and increased reliability. Although pharmaceutical companies start with the dedicated goal of creating a new drug, basic science begins with a biological phenomenon such as DNA replication and looks for insights into the biology of the process. The ultimate goal may be the same, but the approaches and priorities of such "chemical genomics" differ. For example, in basic science, HTS uncovered tumor suppressor properties of p53 and other significant findings related to DNA repair [146].

Although HTS has value, increasing concerns have been raised about the quality of hits being fairly poor in terms of clinical relevancy, efficacy, and toxicity [144]. In other words, many discrepancies exist between in vitro and in vivo results [147]. Cells grown in a plastic dish do not mimic the environment they come from [106]; also, biological interactions cannot be measured readily in a whole-well assay [145].

High-content screening (HCS) endeavors to solve those problems. Utilizing an array of imaging and other microscopy assays, researchers can measure complex phenotypic outcomes that are more closely linked to disease states. The multiparametric measurements that HCS is capable of can also perform certain aspects of pharmacokinetics and pharmacodynamics during primary screens [144]. HCS, by definition, requires long assay development times, requires specialized expertise to perform,

and has a lower throughput than HTS. Data handling systems are overcoming the challenges inherent in sorting complex cell phenotypes at high throughput. As with any system, human intervention must correctly interpret and ultimately classify the findings.

Better in Vitro Models: 3D Cell Cultures

In revisiting the toxicities/lack of efficacy problems that surface in initially promising drug candidates, an increasing body of evidence indicates one key reason for this: preclinical models do not adequately recapitulate the complexity or heterogeneity of a tumor. In other words, research needs better preclinical models that bridge the gap between preclinical and clinical settings [106,137,147–153].

Indeed, a tumor is not simply a set of neoplastic cells. It is a complex, multicellular system with a necrotic core, quiescent cells, and proliferating perimeter cells [147]. Moreover, the malignant cells establish bidirectional interactions with each other, as well as with neighboring nonmalignant cells [106,151]. This interplay between the tumor, stroma, and extracellular matrix comprises the tumor microenvironment—and researchers are learning that modulating it is a key to more efficacious drug development, drug delivery, and optimal treatment selection for patients [151–154].

Interactions in the tumor microenvironment can:

- amplify the transcriptional output of signaling cascades constitutively activated by mutations [106]
- change gene expression/biomarkers selectively [152,155]
- alter tumor phenotypes in the absence of genetic lesions [106]
- attenuate immunity-related antitumor responses [106]
- increase or decrease the effectiveness of synthetic lethalities [106]

To enable researchers to discover more of these interactions, cell-culturing techniques are evolving accordingly. In the past, monolayer (2D) cell cultures were the most popular platform for early stage testing of potential antineoplastic compounds. However, cells lose many of their in vivo characteristics when grown in a monolayer in serum-containing medium [147]. They "flatten," losing their shape, polarity, and some of their biomarkers. Although 2D cell culture grow much faster than cells do in vivo [156], such cultures cannot support the mix of cells present in the tumor microenvironment or recapitulate their effects on cell dynamics—including altered gene expression, aberrant cell–cell signaling, and dysfunctional tissue reorganization [147,155]. Similarly, the effect of immune response on treatment efficacy cannot be observed [152].

In contrast, 3D cultures can coax cells to self-assemble as tumor spheroids, which recreate many in vivo characteristics of the tumor and its microenvironment [148,155]. Grown in hanging drops, spinner flasks, NASA rotary cell culture systems, or nonadhesive cell suspensions, spheroid cultures can demonstrate pathophysiological gradients such as a necrotic core, varying gradients, localized growth kinetics [147,150,156], and even ductal architecture (in pancreatic tissue) [157]. When co-cultured with endothelial cells and stromal cells such as fibroblasts and/or immunologic cell types (eg, tumor-associated macrophages), the spheroid cultures can help researchers distinguish *in vitro* how each type of cell contributes to treatment efficacy [147,152,158]. For example, results to date show that fibroblasts and immunologic cells have a profound impact on tumor cells' responsiveness to anticancer drugs [152].

Preparing 3D spheroid cultures from human tumors eliminates some of the problems cited with mouse models, including interspecies differences and loss of tumor heterogeneity. 3D cultures can reveal much about the differences in normal versus tumor tissues, especially differences in permeability

and how drugs accumulate in the tissues [151]. 3D cultures can recapitulate the hypoxic conditions most solid tumors exhibit in patients. Functional drug testing with 3D cultures could potentially simplify patient stratification [152]. More broadly, 3D model systems also have applications for tissue regeneration, wound healing, stem cell differentiation, and more [147,155].

As with all technologies, 3D cultures are imperfect. Some types of cells naturally seem to grow more compactly as spheres [148]; whether that accurately reflects in vivo biomechanics is unknown. Thus, techniques are evolving to make the spheroids more uniformly shaped [147,148]. Technical matters of cell sorting and scalability are still being optimized. A complex deconstruction/purification process precedes reconstruction and culture growth (Fig. 1.8). The degree of culture complexity required to yield incrementally useful information remains unknown. As more parameters are controlled simultaneously, data analyses become exponentially more complicated. To date, 3D cultures cannot be used for HTS, although nanoimprinting to create nanoculture plates is closing that gap [150].

Researchers cannot fully recapitulate the entire tumor microenvironment yet, but they are getting closer. 3D cultures utilizing biocompatible scaffolds (biomimetics) and additional cell types are in development (see Fig. 1.8). This should provide unprecedented looks into the physical, chemical, and mechanical signaling that occur in the tumor microenvironment [159]. However, with complexity come increased cost, time, and dilemmas of how to control more variables. Even batch-to-batch variations in components such as collagen can alter results and affect reproducibility [160]. Finally, difficulties in sample recruiting and tumor acquisition could limit widespread use of 3D spheroids for such applications.

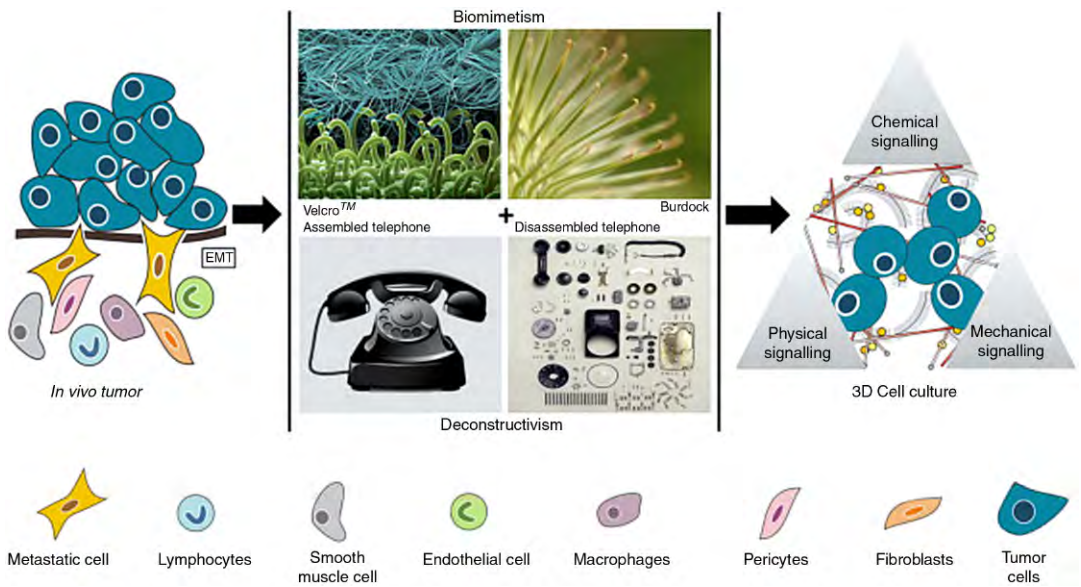


FIGURE 1.8 Deconstructing and Reconstructing 3D Cell Cultures

Reprinted from Adv Drug Deliv Rev 79–80: 40–49, © 2014, Alemany-Ribes M and Semino CE, Bioengineering 3D Environments for Cancer Models, with permission from Elsevier.

However, the tumor tissue spheroid model in all its iterations (including the ones that follow) moves us closer to accurately recreating and directly studying the tumor microenvironment. The use of 3D cultures shows great promise in late-stage preclinical testing and as a supplement to in vivo animal experiments. As such, 3D spheroids can serve as an important bridge between preclinical and clinical testing [147,152,155,159,160].

3D Cultures + Microfluidics = Tumor on a Chip

Advancements in microtechnology, including microfabrication, microfluidics, and microarrays have made the concept of a "lab on a chip" (LOAC) a reality [161,162]. Although the applications are not yet widespread, in research circles they are proving themselves as next-generation point-of-testing tools. Taking that a step further and into the realm of oncology research, a variation of "lab on a chip" that employs microfluidics and 3D spheroid cultures is being developed to enable assays of human tumors for characteristics including proliferation, migration, and cytotoxicity [147,163].

One of many reasons to develop a "tumor on a chip" device is to improve drug efficacy. Despite the intentions of all targeted therapies, they still need to reach and infiltrate their target. But that is not the case nearly often enough. To combat that problem, numerous technologies have been developed to help ensure better drug delivery, including: receptor-conjugated drugs; ligand-targeted therapies; drugs encased in collagenase, liposomes or micelles; drugs attached to dendrimers (nonimmunogenic, nanoscaled polymers), various nanoparticles, and so on [164]. Although the delivery method varies, all have the common goals of achieving greater tumor penetration with uniform drug delivery. However, both problems persist, for at least several reasons: overexpression of drug efflux systems, altered cell permeability due to irregular, misshapen endothelial cells around tumors, and the higher pressure of interstitial fluid inside tumors than in surrounding healthy tissue. In short, the tumor has multiple ways to thwart the desired killing effects of anticancer therapeutics [151,163,165]. As researchers become better at recapitulating the tumor microenvironment, those barriers are starting to fall.

Tumor-on-a-chip technology provides a unique way to observe alterations to efflux, permeability, and interstitial pressure [151]. Microfluidic channels within the device contain human tumor cells, endothelial cells, and other components that mimic the tumor microenvironment. Other channels can introduce drugs or other components (such as growth factors) in varying combinations. Now being dubbed T-MOC (tumor-microenvironment-on-chip) [163], such miniaturized devices are enabling researchers probe how tumors respond to stimuli as well as how they respond to anticancer drugs.

Early analyses of such efforts have already been revealing. Studies are showing that microenvironment- induced drug resistance is not universal across all cancers for a given therapeutic (or even in cultures from healthy donors) [106,161]. Drug distribution within a tumor is extremely heterogeneous; tumor regions within 100 mm of each other can have vastly different local drug concentrations [149].

Better Mouse Models

Despite the advantages of human cancer tissue spheroid models over mouse models, the xenograft mouse model, both subcutaneous and orthotopic, remains the gold standard for oncological drug testing. Essentially two types of mouse models exist: those that use immunocompromised mice, and those that use immunocompetent mice.

Xenografts in Athymic Nude or NOD/SCID Mice

Immunocompromised mice enable human tumors to be implanted subcutaneously or orthotopically (into a target organ) without danger of the mouse rejecting the foreign tissue. For this purpose, athymic

nude mice or NOD/SCID mice are used. However, human response to tumor invasion includes an immune component, which, by design, is lacking in these mice. That lack has led some to question the applicability of results derived from that model. To partially overcome that limitation, human immune cells are now added, creating a patient-derived xenograft (PDX) model. However, full restoration of the human immune system is not possible [166].

Genetically Engineered Mouse Model

A different approach to a mouse model is the use of immunocompetent mice that possess genetic alterations. In a genetically engineered mouse model (GEMM), one or more genes that are putatively involved in malignant transformation are deleted, mutated, or overexpressed; then the effects are studied over time. This model offers many advantages, including a realistic tumor microenvironment, the ability to user various mouse strains from different genetic backgrounds, and the ability to follow a tumor from its earliest time points on. However, the number of genes that can be targeted are limited. As such, a GEMM cannot reproduce the complexity or heterogeneity of many cancers, including extensive aneuploidy and loss- or gain-of-function features. And, because the tumor generated is a mouse tumor—not a human tumor—limited correlations can be made between mouse response and anticipated human response [106,166].

Metastatic Mouse Models

Although orthotopic tumors are harder to track and follow than subcutaneously implanted tumors; the former more accurately recapitulates the tumor microenvironment. Taking that concept a step further, preclinical mouse models now are endeavoring to recapitulate tumor metastasis [167]. Briefly, three examples using GEMMs follow.

In the first model, GEMMs are implants with a primary human tumor; half are treated to prevent metastases; all are observed for metastatic growth. In the second model, a GEMM is implanted with a primary tumor, which is then transplanted subcutaneously or orthotopically into secondary recipients at the same primary site. In a third model, called an orthometaststic xenograft model, a GEMM is implanted with a primary tumor at a primary site; then primary tumor cells are removed from the GEMM and transplanted into other mice at secondary sites to which the primary tumor characteristically metastasizes. In all cases, treated versus untreated control groups are compared. The premise is that these methods will provide clinically relevant information about metastatic disease at the preclinical stage [106].

Mouse Hospital

In a very new, novel effort to predict treatment response, Clohessy has employed the concept of a coclinical trial and mouse hospital. With the intent to use it during early clinical studies, the goal is twofold: (1) to decrease the time and cost required to achieve a study response by administering equivalent protocols in parallel for mice and humans, and (2) to help stratify patients to see who benefits the most from a specific treatment regimen. The result should be rapid evaluation and real-time data integration between the two [153].

A plethora of technical issues surround the successful execution of this prototype. As nearly as is technologically possible, the mice need to reflect the genetic alterations identified in the patient. The mice are literally kept in a “hospital,” and Clohessy describes the requirements of that controlled environment in detail. Parallel drug administration, laboratory tests, and follow-up are performed simultaneously on both mice and humans for correlative purposes (see Fig. 1.9). This coclinical paradigm can facilitate real-time adjustment to clinical trial parameters, helping to establish which patient populations will be best served [153].

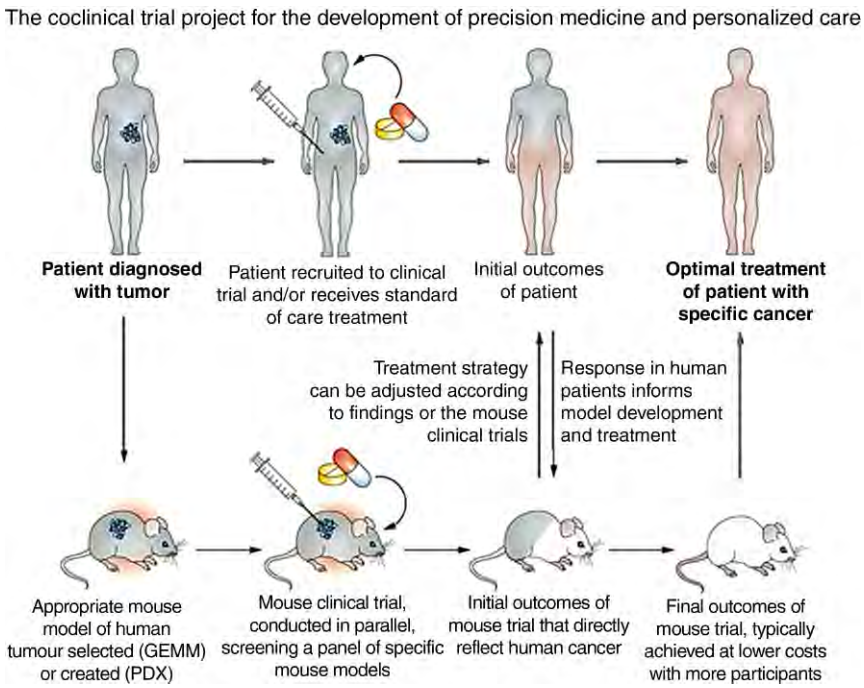


FIGURE 1.9 Coclinical Trial With a Mouse Hospital

Reprinted by permission from Macmillan Publishers Ltd; Clohessy JG, Pandolfi PP. Mouse hospital and co-clinical trial project–from bench to bedside. Nat Rev Clin Oncol 2015;12(8):491–498, © 2015.

The ambitious model also has value in identifying subpopulations of patients without clinical trial criteria needing to be overly restrictive. Novel subgroups of tumors could be uncovered; patient subpopulations could be identified by early response to standard-of-care versus experimental therapies, and better correlation of prognostic factors and overall survival could be gleaned from fully aligned coclinical trials [153].

Although experts are divided on how faithfully any mouse model can reflect human physiology and pathobiology [153,168], all of them have important roles to play in furthering our understanding of cancer development and treatment. It cannot be overlooked that xenograft models previously contributed to therapeutic successes including the use of bortezomib and melphalan for treating multiple myeloma, trastuzumab for treating HER2/neu-overexpressing breast cancers, and bevacizumab for curbing metastatic growth by blocking VEGF-A [166]. Richmond suggested that orthotopic human tumor xenografts are better suited for predicting drug response in human tumors, whereas GEMMs are most useful for determining the role of specific genes in tumor development and progression [166]. Such distinctions will likely blur as mouse models become increasingly sophisticated.

The common goal of all new mouse models is to more faithfully recreate tumors and their surrounding environments so that we can come closer to testing exactly how tumors behave in vivo. However, all mouse models have limitations. One way in which that is being addressed is through greater use of biomarkers.

Biomarkers, Surrogate Endpoints, and Companion Diagnostics

Technology continues to provide us with ample amounts of data regarding genes that are dysregulated in cancer. The challenge is to determine what the data mean—and which portions matter with respect to tumorigenic transformation and drug development [1]. In other words, data acquisition can be a double-edged sword when hunting for clinically relevant biomarkers. Obtaining the most useful samples to test for biomarkers is an additional challenge [19].

In basic science, biomarkers can guide early-stage small-molecule drug development [169]. Most of those biomarkers represent mutations of activated oncogenes, and indeed a number of small-molecule inhibitors have been developed as a result.

Although biomarkers can provide diagnostic, prognostic, or pharmacologic information to inform patient care [170], the ones that get the most attention are those associated with drug sensitivity or resistance [169]. Examples of biomarkers that indicate low response rates to targeted therapies include T3151 mutations in leukemia (they do not respond to BCR-ABL kinase inhibitors), tumors with p53 mutations (they do not respond to MDM2 inhibitors), KRAS mutations (which convey resistance to anti-EGFR therapies), and abnormalities in BRAF or RAS (which convey resistance to BRAF inhibitors). Conversely, examples of biomarkers that indicate sensitivity to certain drugs include EGFR mutations that render non-small-cell lung cancers sensitive to erlotinib and HER2 overexpression in breast cancer that is amenable to trastuzamab [171]. A test for PARP functionality now serves as an indicator of utility for treating advanced ovarian cancer [88]. Such biomarkers that have positive predictive value for treatments are called companion diagnostics, or CDxs (“theranostics” in academic terms) [169].

Companion Diagnostics

The success of a companion diagnostic lies in the strength of its biomarker hypothesis—that the presence of a particular protein is truly indicative of a patient population/condition that will benefit from the treatment in question. Biomarker hypotheses are often deduced during the basic science and preclinical phases of drug development [169]. Without a comprehensive understanding of the disease’s molecular pathology and the drug’s mechanisms of action [169,170], a CDx could cause more harm than good.

Insurers, regulators, and caregivers laud the promise that companion diagnostics hold for improving the predictability of treatment success (and, by extension, the oncology drug development process). However, to avoid false test results, a CDx must demonstrate a high degree of analytical and clinical validity—which, to some extent, is comparable to the safety and efficacy documentation that is required in submitting a new drug for approval [169]. A false negative could deprive a patient of a much-needed treatment; a false positive could lead to a potentially harmful and/or unnecessary treatment [169,172]. Because many biologic characteristics of cancer are not isolated to one cancer type (eg, *HER2* amplification is a large factor in gastric cancer as well as breast cancer), it underscores that what matters most in determining drug response is not the tumor’s origin in the body but rather the molecular pathways that drive the cancer [169].

A 2015 metaanalysis regarding outcomes from using companion diagnostics shows the value and limitations of this approach. CDxs are associated with modest improvements in efficacy of targeted anticancer drugs, less risk of discontinuing therapy, and longer progression-free survival compared to people who received drugs without CDxs. However, CDxs did not appear to influence the magnitude of benefit in overall survival and there was no difference in risk of toxic death between the two groups (OR = 1.40 vs 1.27, $p = 0.69$) [173].

Thus, although excitement for CDx-guided drugs is high and CDxs represent real progress within oncology, we must avoid slipping into a "one biomarker, one drug" mindset. That echoes of the earliest models of cancer research—an "infectious disease" mentality where we mistakenly thought we could match a drug with a disease, the response would be linearly dose-dependent, and that would be the end of it [2]. Although now we know that is the exception rather than the rule, we must remind ourselves that such a mindset with CDx-guided drugs is not a sustainable paradigm. At some point in disease progression, resistance will develop for all those drugs [169]. And tumor progression is driven by altered gene regulation that is not always clearly defined by somatic mutations [136]. Cancer cells can overcome genotoxic effects in at least three ways: (1) by reversal of a genetic or epigenetic effect, (2) through emergence of a compensatory mechanism, or (3) via development of a tolerance mechanism [12]. Because cancer is an accumulation of errors, it would be naïve to think that only one type of error would exist or that we could always detect it that readily.

Although CDx is a growing field, we already need to think beyond the present paradigm of a treatment decision based on a single biomarker and look forward to a more multimodal approach that will integrate multiple biomarkers and multiple targeted cancer drugs being used simultaneously to block more than one signal pathway. This will call for multiplex assays such as gene expression arrays or next-generation sequencing (NGS) [169,174]—and we need to plan today for their widespread use tomorrow.

Next-Generation Sequencing

NGS-based diagnostics are particularly suited to the complex, heterogeneous genetic composition of tumors. Tests are already available as single-gene or multigene assays and multitranscript panels, and some panels are being used in clinical trial assays to guide patients to the most appropriate experimental treatment. Despite this, the FDA has not yet approved an NGS CDx. Many challenges exist for the adoption of such tests—including the tests' long turnaround times (up to two weeks), availability of a sufficient DNA sample, quality control standardization, how to handle variants of unknown significance, and the current regulatory climate [136].

However, NGS is already conspicuous in a national trial that supports the US Precision Medicine Initiative [141]. In August 2015, the National Cancer Institute opened the NCI-MATCH (*M*olecular *A*nalysis for *T*herapy *CH*oice) trial (www.cancer.gov/nci-match), which utilizes NGS to match a patient's tumor DNA with a drug that targets mutations specific to the tumor, regardless of the tumor's origin [175] (see Fig. 1.10). Participants are adults whose cancer has not responded or is no longer responding to treatment. NCI-MATCH's broad-based NGS screening, the large number of treatment arms, flexible treatment options, and follow-up data should shed new light on the effectiveness of treating cancers according to their molecular abnormalities.

Liquid Biopsies

To overcome problems inherent in obtaining core tumor samples and making these exotic technologies scalable and readily available, significant progress is being made in utilizing peripheral blood to isolate tumor cell DNA for testing. Also known as "liquid biopsies," it currently remains in the realm of research. The rationale for using blood samples is that an estimated one million cells are shed per gram of tumor per day into the peripheral circulation [176]. Although those cells have left the tumor microenvironment, they retain many characteristics that are difficult to recapitulate, as this section has already discussed. Ongoing studies are attempting to determine whether abnormal proteins and the DNA repair response in peripheral blood reflect that of the tumor mass [46,177–182].

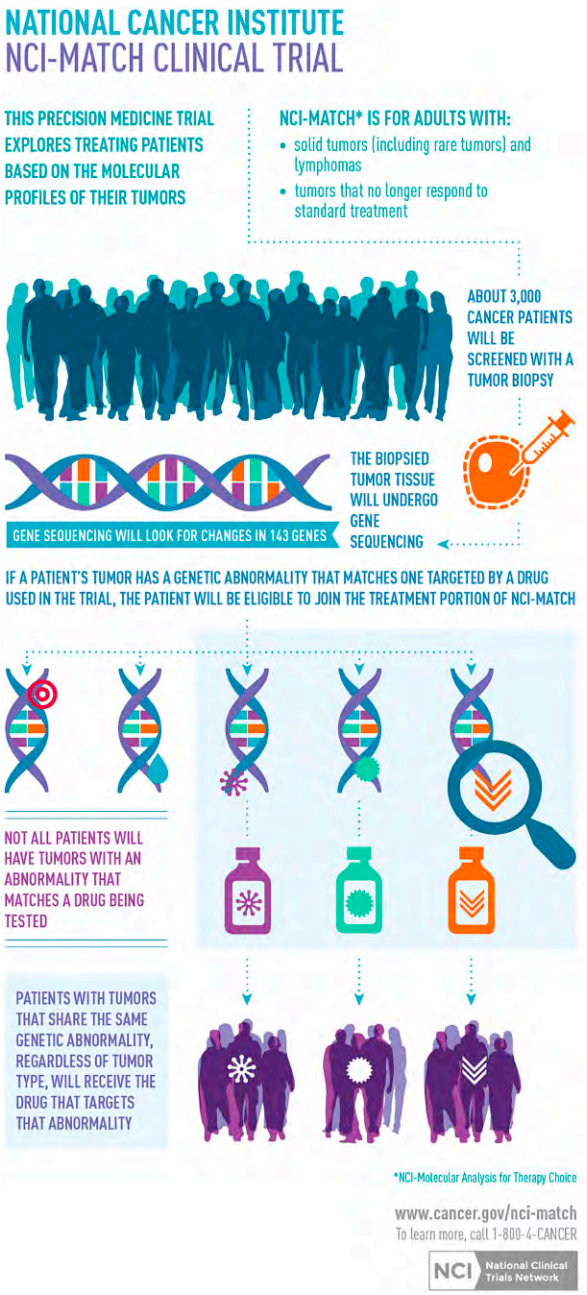


FIGURE 1.10 Infographic of the NCI-MATCH Trial

Reprinted with permission from the National Cancer Institute.

If liquid biopsies do accurately reflect tumor activity, then perfecting their use could shed light on parameters that currently cannot be measured directly. For example, replicative stress is an important contributor to DNA damage and cancer overall. To date, tests can measure only downstream consequences of replicative stress; hence, biomarkers of apoptosis or proliferation are surrogates of replicative stress. Furthermore, they usually require tumor biopsies (as opposed to a peripheral blood sample). Although direct biomarkers of replicative stress exist (eg, ssDNA and phosphorylated signaling intermediates), they are very transient and difficult to preserve even in biopsies. Unstable metabolites, such as ROS or intermediates of nucleotide metabolism are even more difficult to isolate and measure [108]. Liquid biopsies potentially could capture and measure those parameters.

As both functional and genetic biomarkers become more reliable and specific, they will be better able to detect all forms of alterations to DNA repair [46,108,183]. They also will be able to stratify patients predictively for response to irradiation [183] and targeted drugs that disrupt signaling pathways [108].

Synthetic Lethalities

Finding synthetic lethalities combines techniques used for finding biomarkers and screening for drug candidates. Despite all we know about DNA repair pathways, SL pairs have been harder to find than expected. This may be due to many reasons:

- *Limitations in funding*: The cost to run library screens is high [82].
- *Limitations of our knowledge*: We need to continue to learn more about pathway crosstalk, posttranslational modifications, and other effects on gene expression. For example, the tumor microenvironment can either help or hurt synthetic lethalities. Under hypoxic conditions, solid tumors are more sensitive to PARP inhibitors. A lower pH in the tumor microenvironment can decrease drug efflux in certain cancers [106]. We also need to learn more about threshold dosing required to induce adverse effects, as well as account for other cellular processes that can influence therapeutic response [168].
- *Limitations of technology*: Running the same screen using three different SL screening methods can yield three different results. The lack of overlap is troubling; more confirmatory results should be appearing. Their absence may be due to differences in technique, technology, methodology ("forward" versus "reverse" screening), cell lines used, computational algorithms for interpreting the data, and the chosen starting point for the search [14,132]. Additionally, misleading "off-target" effects can confound detection of clinically relevant results [82].
- *Limitations of vision*: Sir James Black, who shared a 1988 Nobel Prize in Physiology/Medicine [184] for discoveries of important principles for drug treatment, admonished researchers to practice the art of "obliquity"—that is, the art of looking for one thing and finding something else. Undiscovered SL pairs are undoubtedly lurking in combinations that researchers have not thought to consider. SL pairs for DNA repair inhibition do not need to stem solely from DNA repair processes. On the basis of observations of convergent pathways, pairing DNA repair inhibition with inhibition of another cancer survival pathway is underway. For example, it is well established that tumor hypoxia usually contributes to treatment resistance [185]. APE1 redox inhibition, coupled with carbonic anhydrase 9/12 (CA9/12) inhibition has very recently been shown to block HIF1-α signaling and slow the growth of pancreatic tumor cells even under extreme hypoxia [186].

As screening technology evolves, off-target effects in SL screening should decrease. Such effects may be due to variable, incomplete, or nonspecific mRNA knockdown, which somewhat limits the degree of control that can be exerted over gene expression [187]. Also, the current RNAi platform designs may not be able to detect all potential interacting genes. However, the advent of CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 technology is helping overcome such hurdles [82].

CRISPR Technology

CRISPR technology is currently the most advanced way to change endogenous gene expression level and protein function. Capable of greater knockdown efficiency than RNA interference, CRISPR can also be used to target noncoding RNAs. Although studies to date using CRISPR have employed forward screening, anticipations are high that reverse genetic screening will become equally as available and possibly surpass the utility of HCS [187,188].

Crystal-Ball Gazing

So what does all this mean for the future landscape of oncology R&D?

Although traditional chemotherapy is still a mainstay in the arsenal of anticancer treatments, we are moving away from that and toward greater use of targeted therapeutics, including targeted small-molecule inhibitors of DNA repair pathways and other pathways critical for cancer survival. That trend will continue.

Targeted therapies represent a giant step in the direction of precision medicine—person-centered, multifaceted diagnosis and treatment based on patterns of genomic, epigenomic, exposure, and other data from both clinical and research realms. The tall task of precision medicine alludes to massive amounts of data gathering and sharing, interdisciplinary and interagency partnerships (as proposed in the 2011 NIH report, *Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease* [189]).

But what are the best sources and most reliable methods for generating such data and ensuring their clinical relevancy? What do future charges and future prospects hold for DNA repair research and cancer research overall?

Bench to bedside, we need to address seven buckets of needs:

- molecular mapping
- biomarkers
- tumor models
- technology
- clinical trials
- treatments
- mindset

Molecular mapping:

- Continue to more fully characterize DNA repair pathways on the molecular level.
- Discover more about how other cancer survival pathways influence DNA repair pathways.
- Recognize that protein expression levels may not equate to DNA repair ability, or lack thereof. Look additionally at functional repair assays, such as measurements of the ability of proteins to localize into foci [190] (which now can be captured with time-lapse imaging) [191].
- Phenotype tumors more accurately and completely.

- Initiate large-scale efforts to collect patient tumor cells in both early- and late-stage disease to determine differences in their molecular profiles (as well as preclinical responses to treatments) [47,106].
- Use new molecular information to screen for biomarkers and druggable targets.

Biomarkers [136,172]:

- Determine the best way to assess potential biomarkers (in terms of testing, scoring, quantifying cutoff points).
- Discover more reliable biomarkers and develop companion diagnostics where they are needed.
- Ensure that biomarkers are reliable, easily detected by existing laboratory technology, and readily available.

Tumor models [106,141,153,159,160,167]:

- Create more reliable models for preclinical testing.
- Develop additional tumor cell line models and animal models that more accurately portray in vivo conditions.
- Develop more diverse types of tumor models: ones that show tumor progression, focus on one aspect of tumor pathobiology, or truly replicate metastases.
- Find ways to maintain tumor identities long-term in complex cultures (such as 3D cultures, scaffolded cultures).

Technology [141,147,159]:

- Improve both the technology and analytics for gene profiling to more accurately determine what changes in gene expression are clinically relevant.
- Employ advanced techniques to improve drug candidate screening techniques and preclinical testing for drug efficacy.
- Standardize technologies and validation processes to help ensure greater chance of accurate, clinically relevant results.
- Find ways to make preclinical and clinical testing practical, affordable, and scalable.
- Overcome current limitations of HTS with 3D cultures to speed testing on them (such as employing techniques of cell patterning).
- Build a cancer “knowledge network” to store and share molecular and medical data with scientists, clinicians, and other stakeholders.

Clinical trials:

- Create new paradigms for clinical trials that identify but do not exclude subpopulations and provide ways to test and treat them within the scope of the trial [141].
- Genetic criteria can be a double-edged sword—one that we do not yet know how to wield fully. An unnecessarily narrow patient population may hinder both enrollment and the ability to validate effective treatments [153].
- Create clinical trials with novel designs (such as the mouse hospital) to validate whether such approaches will improve the track record of drug development [141].
- Consider how to restructure clinical trials and their endpoints for highly heterogeneous cancers: those that have relapsed, progressed, metastasized, or are late stage [153].

- Utilize novel techniques such as implantable devices to fast track both monotherapy and combination therapy trials of inhibitors [149]. Researchers must demonstrate the safety of both [121].

Treatments:

- Enable more widespread use of pretreatment genotyping, to help ensure better treatment response [192].
- Incorporate emerging knowledge to minimize toxicities, maximize drug effectiveness, and optimize the timing [13,30] and administration of DDR inhibitors—as monotherapy, combination therapy, or sequential therapy. This is predicated on utilizing new knowledge about cell cycle perturbations, molecular patient profiles, influences of the tumor microenvironment, stage of cancer involved, and that stage's threshold of DNA damage tolerance [6,30].
- Consider broader use of DNA repair inhibitors as minimally toxic agents in treating premalignant or early neoplastic lesions, as tumor inactivation of DNA damage signaling and DNA repair is often a relatively early event during carcinogenesis [12].
- Create more effective carriers to ensure targeted, deep-penetrating drug delivery.

Mindset:

- Look obliquely for solutions, such as SL pairs that target DNA repair pathways as well as nonobvious partner pathways.
- Turn apparent "failures" into successes. For example, developing an MGMT inhibitor to block competent MGMT function was not nearly as successful as exploiting MGMT defects [121].
- Look for the unexpected.

Perhaps a good watchword in DNA repair and research is, "Never say 'can't;' always say 'how.'" For example, protein–protein interactions (PPIs) were previously thought to not be druggable because they have large, flat binding sites. However, researchers are now finding that within those large sites are multiple smaller "hot pockets" that induce conformational changes [193,194]. Learning how to exploit those to disrupt PPIs could spawn a variety of PPI inhibitors—as well as provide new knowledge about inhibition processes in general. More broadly speaking, the relevance of the interactome network in modulating the functions of each DDR protein is still emerging [195] and is opening new avenues for translational research.

Conclusions

Collins' commentary to the president's Precision Medicine Initiative noted that we need to "Encourage creative approaches to precision medicine, test them rigorously, and ultimately use them to build the evidence base needed to guide clinical practice … Pursuing research advances will enable better assessment of disease risk, understanding of disease mechanisms, and prediction of optimal therapy for cancer—and, ultimately, many more diseases" [141]. The initiative speaks to the need for creative, out-of-the-box approaches in detecting, measuring, and analyzing a dizzying array of biomedical information. And, as Collins duly noted, "Ambitious programs like this cannot be fully planned; they need to evolve—through the individual and collaborative efforts of researchers" [141].

Although all the improvements outlined in this chapter will not perfectly simulate the clinical setting, they will go far in bridging the current gap between preclinical and clinical testing [106], expanding our knowledge and our ability to exploit it therapeutically.

ACKNOWLEDGMENTS

Financial support for this work was provided by the National Institutes of Health, R21NS091667 to MRK and the National Cancer Institute CA167291 to MRK and MLF. Additional financial support was provided by the Earl and Betty Herr Professor in Pediatric Oncology Research, Hyundai Hope on Wheels, Jeff Gordon Children's Foundation and the Riley Children's Foundation to MRK. Also, special thanks to Lana Christian of CreateWrite, Inc., for her writing and editing assistance.

REFERENCES

[1] Kamal A, Shaik TB, Malik MS. Embracing synthetic lethality of novel anticancer therapies. Expert Opin Drug Discov 2015;10(10):1–14.
[2] Kaelin WGJ. Synthetic lethality: a framework for the development of wiser cancer therapeutics. Genome Med 2009;1(99).
[3] Bapat A, Fishel ML, Kelley MR. Going ape as an approach to cancer therapeutics. Antioxid Redox Signal 2009;11(3):651–68.
[4] Fraser J, Williamson I, Bickmore WA, Dostie J. An overview of genome organization and how we got there: from FISH to Hi-C. Microbiol Mol Biol Rev 2015;79(3):347–72.
[5] de Saro FJL. Regulation of interactions with sliding clamps during DNA replication and repair. Curr Genomics 2009;10(3):206–15.
[6] Khanna A. DNA damage in cancer therapeutics: a boon or a curse? Cancer Res 2015;75(11):2133–8.
[7] Helleday T. DNA repair as treatment target. Eur J Cancer 2011;47(Suppl 3):S333–5.
[8] Friedberg EC. A brief history of the DNA repair field. Cell Res 2008;18(1):3–7.
[9] Cleaver JE. Defective repair replication of DNA in xeroderma pigmentosum. Nature 1968;218(5142):652–6.
[10] Setlow RB, Regan JD, German J, Carrier WL. Evidence that xeroderma pigmentosum cells do not perform the first step in the repair of ultraviolet damage to their DNA. Proc Natl Acad Sci USA 1969;64(3):1035–41.
[11] Friedberg EC. Suffering in silence: the tolerance of DNA damage. Nat Rev Mol Cell Biol 2005;6(12):943–53.
[12] Helleday T, Petermann E, Lundin C, Hodgson B, Sharma RA. DNA repair pathways as targets for cancer therapy. Nat Rev Cancer 2008;8(3):193–204.
[13] Gullotta F, De Marinis E, Ascenzi P, di Masi A. Targeting the DNA double strand breaks repair for cancer therapy. Curr Med Chem 2010;17(19):2017–48.
[14] Nijman SM. Synthetic lethality: general principles, utility and detection using genetic screens in human cells. FEBS Lett 2011;585(1):1–6.
[15] Morton LM, Onel K, Curtis RE, Hungate EA, Armstrong GT. The rising incidence of second cancers: patterns of occurrence and identification of risk factors for children and adults. Am Soc Clin Oncol Educ Book 2014;e57–67.
[16] The Nobel Prize in Chemistry. http://nobelprize.org; 2015.
[17] Hoeijmakers JH. DNA damage, aging, and cancer. N Engl J Med 2009;361(15):1475–85.
[18] Gerson SL. MGMT: its role in cancer aetiology and cancer therapeutics. Nat Rev Cancer. 2004;4(4):296–307.
[19] Curtin NJ. DNA repair dysregulation from cancer driver to therapeutic target. Nat Rev Cancer 2012;12(12):801–17.
[20] Luo M, He H, Kelley MR, Georgiadis MM. Redox regulation of DNA repair: implications for human health and cancer therapeutic development. Antioxid Redox Signal 2010;12(11):1247–69.
[21] Kelley MR, Fishel ML. DNA repair proteins as molecular targets for cancer therapeutics. Anticancer Agents Med Chem 2008;8(4):417–25.
[22] Mjelle R, Hegre SA, Aas PA, et al. Cell cycle regulation of human DNA repair and chromatin remodeling genes. DNA Repair 2015;30:53–67.

[23] Robertson AB, Klungland A, Rognes T, Leiros I. DNA repair in mammalian cells: base excision repair: the long and short of it. Cell Mol Life Sci 2009;66(6):981–93.
[24] Guarne A, Charbonnier JB. Insights from a decade of biophysical studies on MutL: roles in strand discrimination and mismatch removal. Prog Biophys Mol Biol 2015;117(2–3):149–56.
[25] Hanawalt PC. Subpathways of nucleotide excision repair and their regulation. Oncogene 2002;21(58): 8949–56.
[26] Shuck SC, Short EA, Turchi JJ. Eukaryotic nucleotide excision repair, from understanding mechanisms to influencing biology. Cell Res 2008;18(1):64–72.
[27] Helleday T, Lo J, van Gent DC, Engelward BP. DNA double-strand break repair: from mechanistic understanding to cancer treatment. DNA Repair 2007;6(7):923–35.
[28] Deriano L, Roth DB. Modernizing the nonhomologous end-joining repertoire: alternative and classical NHEJ share the stage. Annu Rev Genet 2013;47:433–55.
[29] Lim S, Kaldis P. Cdks, cyclins and CKIs: roles beyond cell cycle regulation. Development 2013;140(15): 3079–93.
[30] Plummer R. Perspective on the pipeline of drugs being developed with modulation of DNA damage as a target. Clin Cancer Res 2010;16(18):4527–31.
[31] Kaina B, Christmann M, Naumann S, Roos WP. MGMT: key node in the battle against genotoxicity, carcinogenicity and apoptosis induced by alkylating agents. DNA Repair 2007;6(8):1079–99.
[32] Fleck O, Nielsen O. DNA repair. J Cell Sci 2004;117(Pt 4):515–7.
[33] Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem 2004;73:39–85.
[34] Lindahl T. Instability and decay of the primary structure of DNA. Nature 1993;362(6422):709–15.
[35] Guillet M, Boiteux S. Endogenous DNA abasic sites cause cell death in the absence of Apn1, Apn2 and Rad1/Rad10 in *Saccharomyces cerevisiae*. EMBO J 2002;21(11):2833–41.
[36] Vidal AE, Boiteux S, Hickson ID, Radicella JP. XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein-protein interactions. EMBO J 2001;20(22):6530–9.
[37] Williams RS, Kunkel TA. FEN nucleases: bind, bend, fray, cut. Cell. 2011;145(2):171–2.
[38] Hegde ML, Hazra TK, Mitra S. Functions of disordered regions in mammalian early base excision repair proteins. Cell Mol Life Sci 2010;67(21):3573–87.
[39] Fortini P, Dogliotti E. Base damage and single-strand break repair: mechanisms and functional significance of short- and long-patch repair subpathways. DNA Repair 2007;6(4):398–409.
[40] Marsin S, Vidal AE, Sossou M, et al. Role of XRCC1 in the coordination and stimulation of oxidative DNA damage repair initiated by the DNA glycosylase hOGG1. J Biol Chem 2003;278(45):44068–74.
[41] Sobol RW. CHIPping away at base excision repair. Mol Cell 2008;29(4):413–5.
[42] Frosina G, Fortini P, Rossi O, et al. Two pathways for base excision repair in mammalian cells. J Biol Chem 1996;271(16):9573–8.
[43] Evans AR, Limp-Foster M, Kelley MR. Going APE over ref-1. Mutat Res 2000;461(2):83–108.
[44] Bai P. Biology of poly(ADP-ribose) polymerases: the factotums of cell maintenance. Mol Cell 2015;58(6):947–58.
[45] Martin SA, Lord CJ, Ashworth A. Therapeutic targeting of the DNA mismatch repair pathway. Clin Cancer Res 2010;16(21):5107–13.
[46] Reed AM, Fishel ML, Kelley MR. Small-molecule inhibitors of proteins involved in base excision repair potentiate the anti-tumorigenic effect of existing chemotherapeutics and irradiation. Future Oncol 2009;5(5):713–26.
[47] Rowe BP, Glazer PM. Emergence of rationally designed therapeutic strategies for breast cancer targeting DNA repair mechanisms. Breast Cancer Res 2010;12(203):1–11.
[48] Kinsella TJ. Coordination of DNA mismatch repair and base excision repair processing of chemotherapy and radiation damage for targeting resistant cancers. Clin Cancer Res 2009;15(6):1853–9.

[49] Lazzaro F, Giannattasio M, Puddu F, et al. Checkpoint mechanisms at the intersection between DNA damage and repair. DNA Repair 2009;8(9):1055–67.
[50] Li GM. Mechanisms and functions of DNA mismatch repair. Cell Res 2008;18(1):85–98.
[51] Costa RM, Chigancas V, Galhardo Rda S, Carvalho H, Menck CF. The eukaryotic nucleotide excision repair pathway. Biochimie 2003;85(11):1083–99.
[52] Dip R, Camenisch U, Naegeli H. Mechanisms of DNA damage recognition and strand discrimination in human nucleotide excision repair. DNA Repair 2004;3(11):1409–23.
[53] Laine JP, Mocquet V, Egly JM. TFIIH enzymatic activities in transcription and nucleotide excision repair. Methods Enzymol 2006;408:246–63.
[54] Spivak G. Nucleotide excision repair in humans. DNA Repair 2015;36:13–8.
[55] Latimer JJ, Majekwana VJ, Pabon-Padin YR, Pimpley MR, Grant SG. Regulation and disregulation of mammalian nucleotide excision repair: a pathway to nongermline breast carcinogenesis. Photochem Photobiol 2015;91(2):493–500.
[56] Latimer JJ, Johnson JM, Kelly CM, et al. Nucleotide excision repair deficiency is intrinsic in sporadic stage I breast cancer. Proc Natl Acad Sci USA 2010;107(50):21725–30.
[57] Underhill C, Toulmonde M, Bonnefoi H. A review of PARP inhibitors: from bench to bedside. Ann Oncol 2011;22(2):268–79.
[58] Aparicio T, Baer R, Gautier J. DNA double-strand break repair pathway choice and cancer. DNA Repair 2014;19:169–75.
[59] Helleday T. Homologous recombination in cancer development, treatment and development of drug resistance. Carcinogenesis 2010;31(6):955–60.
[60] Li X, Heyer WD. Homologous recombination in DNA repair and DNA damage tolerance. Cell Res 2008;18(1):99–113.
[61] Lieber MR, Ma Y, Pannicke U, Schwarz K. Mechanism and regulation of human non-homologous DNA end-joining. Nat Rev Mol Cell Biol 2003;4(9):712–20.
[62] Cejka P. DNA end resection: nucleases team up with the right partners to initiate homologous recombination. J Biol Chem 2015;290(38):22931–8.
[63] Lafrance-Vanasse J, Williams GJ, Tainer JA. Envisioning the dynamics and flexibility of Mre11-Rad50-Nbs1 complex to decipher its roles in DNA replication and repair. Prog Biophys Mol Biol 2015;117(2-3):182–93.
[64] Kakarougkas A, Jeggo PA. DNA DSB repair pathway choice: an orchestrated handover mechanism. Br J Radiol 2014;87(1035):20130685.
[65] Srivastava M, Raghavan SC. DNA double-strand break repair inhibitors as cancer therapeutics. Chem Biol 2015;22(1):17–29.
[66] Daley JM, Gaines WA, Kwon Y, Sung P. Regulation of DNA pairing in homologous recombination. Cold Spring Harb Perspect Biol 2014;6(11). a017954.
[67] Betermier M, Bertrand P, Lopez BS. Is non-homologous end-joining really an inherently error-prone process? PLoS Genet 2014;10(1):e1004086.
[68] Grundy GJ, Moulding HA, Caldecott KW, Rulten SL. One ring to bring them all—the role of Ku in mammalian non-homologous end joining. DNA Repair 2014;17:30–8.
[69] Davis AJ, Chen DJ. DNA double strand break repair via non-homologous end-joining. Transl Cancer Res 2013;2(3):130–43.
[70] Davis AJ, Chen BP, Chen DJ. DNA-PK: a dynamic enzyme in a versatile DSB repair pathway. DNA Repair 2014;17:21–9.
[71] Ramsden DA, Asagoshi K. DNA polymerases in nonhomologous end joining: are there any benefits to standing out from the crowd? Environ Mol Mutagen 2012;53(9):741–51.
[72] Mansilla-Soto J, Cortes P. VDJ recombination: artemis and its in vivo role in hairpin opening. J Exp Med 2003;197(5):543–7.

[73] van den Bosch M, Bree RT, Lowndes NF. The MRN complex: coordinating and mediating the response to broken chromosomes. EMBO Rep 2003;4(9):844–9.
[74] Benada J, Macurek L. Targeting the checkpoint to kill cancer cells. Biomolecules 2015;5(3):1912–37.
[75] Ward A, Khanna KK, Wiegmans AP. Targeting homologous recombination, new pre-clinical and clinical therapeutic combinations inhibiting RAD51. Cancer Treat Rev 2015;41(1):35–45.
[76] Hopfner KP. DNA double-strand breaks come into focus. Cell 2009;139(1):25–7.
[77] West SC, Blanco MG, Chan YW, Matos J, Sarbajna S, Wyatt HD. Resolution of recombination intermediates: mechanisms and regulation [published online ahead of print September 14, 2015]. Cold Spring Harb Symp Quant Biol 2015; pii: 027649.
[78] Park CK, Park SH, Lee SH, et al. Methylation status of the MGMT gene promoter fails to predict the clinical outcome of glioblastoma patients treated with ACNU plus cisplatin. Neuropathology 2009;29(4):443–9.
[79] Connors TA, Hare JR. Proceedings: mechanism of action of tumour inhibitory nitrosoureas. Br J Cancer 1975;31(2):264.
[80] Rabik CA, Njoku MC, Dolan ME. Inactivation of O^6-alkylguanine DNA alkyltransferase as a means to enhance chemotherapy. Cancer Treat Rev 2006;32(4):261–76.
[81] Livraghi L, Garber JE. PARP inhibitors in the management of breast cancer: current data and future prospects. BMC Med 2015;13:188.
[82] McLornan DP, List A, Mufti GJ. Applying synthetic lethality for the selective targeting of cancer. N Engl J Med 2014;371(18):1725–35.
[83] Petrucco S, Sensing DNA. damage by PARP-like fingers. Nucleic Acids Res 2003;31(23):6689–99.
[84] Drew Y, Plummer R. The emerging potential of poly(ADP-ribose) polymerase inhibitors in the treatment of breast cancer. Curr Opin Obstet Gynecol 2010;22(1):67–71.
[85] Feng FY, de Bono JS, Rubin MA, Knudsen KE. Chromatin to clinic: the molecular rationale for PARP1 inhibitor function. Mol Cell 2015;58(6):925–34.
[86] Helleday T. Putting poly (ADP-ribose) polymerase and other DNA repair inhibitors into clinical practice. Curr Opin Oncol 2013;25(6):609–14.
[87] Bryant HE, Schultz N, Thomas HD, et al. Specific tumor-killing of BRCA-deficient tumours with inhibitors of poly (ADP-ribose) polymerase. Nature 2005;434(14):913–7.
[88] FDA approves Lynparza to treat advanced ovarian cancer. (FDA website). http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm427554.htm; 2014.
[89] Sonnenblick A, de Azambuja E, Azim HA Jr, Piccart M. An update on PARP inhibitors–moving to the adjuvant setting. Nat Rev Clin Oncol 2015;12(1):27–41.
[90] Wicha MS. Development of 'synthetic lethal' strategies to target BRCA1-deficient breast cancer. Breast Cancer Res 2009;11(5):108.
[91] Bose CK, Basu N. PARP inhibitors and more. J Turk Ger Gynecol Assoc 2015;16(2):107–10.
[92] Lord CJ, Ashworth A. Mechanisms of resistance to therapies targeting BRCA-mutant cancers. Nat Med 2013;19(11):1381–8.
[93] Shen Y, Aoyagi-Scharber M, Wang B. Trapping poly(ADP-ribose) polymerase. J Pharmacol Exp Ther 2015;353(3):446–57.
[94] Anwar M, Aslam HM, Anwar S. PARP inhibitors. Hered Cancer Clin Pract 2015;13(1):4.
[95] Curtin NJ, Szabo C. Therapeutic applications of PARP inhibitors: anticancer therapy and beyond. Mol Aspects Med 2013;34(6):1217–56.
[96] Morrow DA, Brickman CM, Murphy SA, et al. A randomized, placebo-controlled trial to evaluate the tolerability, safety, pharmacokinetics, and pharmacodynamics of a potent inhibitor of poly(ADP-ribose) polymerase (INO-1001) in patients with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention: results of the TIMI 37 trial. J Thromb Thrombolysis 2009;27(4):359–64.
[97] Rodriguez MI, Majuelos-Melguizo J, Marti Martin-Consuegra JM, Ruiz de Almodovar M, Lopez-Rivas A, Javier Oliver F. Deciphering the insights of poly(ADP-ribosylation) in tumor progression. Med Res Rev 2015;35(4):678–97.

[98] Bouwman P, Jonkers J. Molecular pathways: how can BRCA-mutated tumors become resistant to PARP inhibitors? Clin Cancer Res 2014;20(3):540–7.
[99] Meyskens FL Jr, Gerner EW. Back to the future: mechanism-based, mutation-specific combination chemoprevention with a synthetic lethality approach. Cancer Prev Res 2011;4(5):628–32.
[100] Von Hoff DD, Ervin T, Arena FP, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med 2013;369(18):1691–703.
[101] Cardoso AA, Jiang Y, Luo M, et al. APE1/Ref-1 regulates STAT3 transcriptional activity and APE1/Ref-1–STAT3 dual-targeting effectively inhibits pancreatic cancer cell survival. PLoS One 2012;7(10):e47462.
[102] Fishel ML, Wu X, Devlin CM, et al. Apurinic/apyrimidinic endonuclease/redox factor-1 (APE1/Ref-1) redox function negatively regulates NRF2. J Biol Chem 2015;290(5):3057–68.
[103] Li Y, Liu X, Zhou T, et al. Suppression of choroidal neovascularization through inhibition of APE1/Ref-1 redox activity. Invest Ophthalmol Vis Sci 2014;55(7):4461–9.
[104] Fishel ML, Cheng H, Shahda S, Kelley MR. APX3330 Drug development for clinical trials targeting APE1/Ref-1 in pancreatic cancer. Herman B Wells Institute for Pediatric Research: Indiana; Indiana University School of Medicine: Indiana; 2015.
[105] Ferreira RJ, Ferreira M-JU, dos Santos DJVA. Reversing cancer multidrug resistance: insights into the efflux by ABC transports from in silico studies. WIREs Comput Mol Sci 2015;5:27–55.
[106] McMillin DW, Negri JM, Mitsiades CS. The role of tumour-stromal interactions in modifying drug response: challenges and opportunities. Nat Rev Drug Discov 2013;12(3):217–28.
[107] McNeely S, Beckmann R, Bence Lin AK. CHEK again: revisiting the development of CHK1 inhibitors for cancer therapy. Pharmacol Ther 2014;142(1):1–10.
[108] Dobbelstein M, Sorensen CS. Exploiting replicative stress to treat cancer. Nat Rev Drug Discov 2015;14(6):405–23.
[109] Weber AM, Ryan AJ. ATM and ATR as therapeutic targets in cancer. Pharmacol Ther 2015;149:124–38.
[110] Branzei D, Foiani M. The checkpoint response to replication stress. DNA Repair 2009;8(9):1038–46.
[111] Wesierska-Gadek J, Maurer M, Zulehner N, Komina O. Whether to target single or multiple CDKs for therapy? That is the question. J Cell Physiol 2011;226(2):341–9.
[112] Tse AN, Carvajal R, Schwartz GK. Targeting checkpoint kinase 1 in cancer therapeutics. Clin Cancer Res 2007;13(7):1955–60.
[113] Zannini L, Delia D, Buscemi G. CHK2 kinase in the DNA damage response and beyond. J Mol Cell Biol 2014;6(6):442–57.
[114] Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. Mol Cell 2010;40(2):179–204.
[115] Smith J, Tho LM, Xu N, Gillespie DA. The ATM-Chk2 and ATR-Chk1 pathways in DNA damage signaling and cancer. Adv Cancer Res 2010;108:73–112.
[116] Mimitou EP, Symington LS. DNA end resection—unraveling the tail. DNA Repair 2011;10(3):344–8.
[117] Shaltiel IA, Krenning L, Bruinsma W, Medema RH. The same, only different—DNA damage checkpoints and their reversal throughout the cell cycle. J Cell Sci 2015;128(4):607–20.
[118] Bashir T, Pagano M. Cdk1: the dominant sibling of Cdk2. Nat Cell Biol 2005;7(8):779–81.
[119] Xu N, Libertini S, Black EJ, et al. Cdk-mediated phosphorylation of Chk1 is required for efficient activation and full checkpoint proficiency in response to DNA damage. Oncogene 2012;31(9):1086–94.
[120] Vermeulen K, Van Bockstaele DR, Berneman ZN. The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. Cell Prolif 2003;36(3):131–49.
[121] Maxmen A. Beyond PARP inhibitors: agents in pipelines target DNA repair mechanisms. J Natl Cancer Inst 2010;102(15):1110–1.
[122] Rai G, Vyjayanti VN, Dorjsuren D, et al. Synthesis, biological evaluation, and structure-activity relationships of a novel class of apurinic/apyrimidinic endonuclease 1 inhibitors. J Med Chem 2012;55(7):3101–12.
[123] Kelley MR, Logsdon D, Fishel ML. Targeting DNA repair pathways for cancer treatment: what's new? Future Oncol 2014;10(7):1215–37.

[124] Neher TM, Shuck SC, Liu JY, Zhang JT, Turchi JJ. Identification of novel small molecule inhibitors of the XPA protein using in silico based screening. ACS Chem Biol 2010;5(10):953–65.
[125] McNeil EM, Astell KR, Ritchie AM, et al. Inhibition of the ERCC1-XPF structure-specific endonuclease to overcome cancer chemoresistance. DNA Repair 2015;31:19–28.
[126] Glanzer JG, Liu S, Wang L, Mosel A, Peng A, Oakley GG. RPA inhibition increases replication stress and suppresses tumor growth. Cancer Res 2014;74(18):5165–72.
[127] Usanova S, Piee-Staffa A, Sied U, et al. Cisplatin sensitivity of testis tumour cells is due to deficiency in interstrand-crosslink repair and low ERCC1-XPF expression. Mol Cancer 2010;9:248.
[128] Khandelwal A, Crowley VM, Blagg BS. Natural product inspired N-terminal Hsp90 inhibitors: from bench to bedside? Med Res Rev 2015;36(1):92–118.
[129] Stachelek GC, Peterson-Roth E, Liu Y, et al. YU238259 is a novel inhibitor of homology-dependent DNA repair that exhibits synthetic lethality and radiosensitization in repair-deficient tumors. Mol Cancer Res 2015;13(10):1389–97.
[130] Middleton FK, Patterson MJ, Elstob CJ, et al. Common cancer-associated imbalances in the DNA damage response confer sensitivity to single agent ATR inhibition. Oncotarget 2015;6(32):32396–409.
[131] Estrada-Bernal A, Chatterjee M, Haque SJ, et al. MEK inhibitor GSK1120212-mediated radiosensitization of pancreatic cancer cells involves inhibition of DNA double-strand break repair pathways. Cell Cycle 2015;14(23):3713–24.
[132] Vymetalova L, Krystof V. Potential clinical uses of CDK inhibitors: lessons from synthetic lethality screens. Med Res Rev 2015;35(6):1156–74.
[133] Selvaraju K, Mazurkiewicz M, Wang X, Gullbo J, Linder S, D'Arcy P. Inhibition of proteasome deubiquitinase activity: a strategy to overcome resistance to conventional proteasome inhibitors? Drug Resist Updat 2015;21-22:20–9.
[134] Kocab AJ, Duckett CS. Inhibitor of apoptosis proteins as intracellular signaling intermediates. FEBS J 2015;283(2):221–31.
[135] Tamura K. Development of cell-cycle checkpoint therapy for solid tumors. JPN J Clin Oncol 2015;45(12):1097–102.
[136] Pant S, Weiner R, Marton MJ. Navigating the rapids: the development of regulated next-generation sequencing-based clinical trial assays and companion diagnostics. Front Oncol 2014;4:78.
[137] Scannell JW, Blanckley A, Boldon H, Warrington B. Diagnosing the decline in pharmaceutical R&D efficiency. Nat Rev Drug Discov 2012;11(3):191–200.
[138] Shi Y. A glimpse of structural biology through X-ray crystallography. Cell 2014;159(5):995–1014.
[139] Harrow J, Frankish A, Gonzalez JM, et al. GENCODE: the reference human genome annotation for The ENCODE Project. Genome Res 2012;22(9):1760–74.
[140] Milanowska K, Krwawicz J, Papaj G, et al. REPAIRtoire—a database of DNA repair pathways. Nucleic Acids Res 2011;39:D788–92.
[141] Collins FS, Varmus H. A new initiative on precision medicine. N Engl J Med 2015;372(9):793–5.
[142] Doak BC, Over B, Giordanetto F, Kihlberg J. Oral druggable space beyond the rule of 5: insights from drugs and clinical candidates. Chem Biol 2014;21(9):1115–42.
[143] Ledford H. World's largest cancer charity lays out field's grand challenges. Nature 2015;526:2.
[144] Singh DK, Krishna S, Chandra S, Shameem M, Deshmukh AL, Banerjee D. Human DNA ligases: a comprehensive new look for cancer therapy. Med Res Rev 2014;34(3):567–95.
[145] Janzen WP. Screening technologies for small molecule discovery: the state of the art. Chem Biol 2014;21(9):1162–70.
[146] Dove A. High-throughput screening goes to school. Nature Methods 2007;4(6):2.
[147] Patel NR, Aryasomayajula B, Abouzeid AH, Torchilin VP. Cancer cell spheroids for screening of chemotherapeutics and drug-delivery systems. Ther Deliv 2015;6(4):509–20.

[148] Singh M, Close DA, Mukundan S, Johnston PA, Sant S. Production of uniform 3D microtumors in hydrogel microwell arrays for measurement of viability, morphology, and signaling pathway activation. Assay Drug Dev Technol 2015;13(9):570–83.
[149] Jonas O, Landry HM, Fuller JE, et al. An implantable microdevice to perform high-throughput in vivo drug sensitivity testing in tumors. Sci Transl Med 2015;7(284). 284ra257.
[150] Yoshii Y, Furukawa T, Waki A, et al. High-throughput screening with nanoimprinting 3D culture for efficient drug development by mimicking the tumor environment. Biomaterials 2015;51:278–89.
[151] Sethi P, Jyoti A, Swindell EP, et al. 3D tumor tissue analogs and their orthotopic implants for understanding tumor-targeting of microenvironment-responsive nanosized chemotherapy and radiation. Nanomedicine 2015;11(8):2013–23.
[152] Hoffmann OI, Ilmberger C, Magosch S, Joka M, Jauch KW, Mayer B. Impact of the spheroid model complexity on drug response. J Biotechnol 2015;205:14–23.
[153] Clohessy JG, Pandolfi PP. Mouse hospital and co-clinical trial project—from bench to bedside. Nat Rev Clin Oncol 2015;12(8):491–8.
[154] Chaudhuri PK, Ebrahimi Warkiani M, Jing T, Kenry, Lim CT. Microfluidics for research and applications in oncology. Analyst 2015;141(2):504–24.
[155] Yamada KM, Cukierman E. Modeling tissue morphogenesis and cancer in 3D. Cell 2007;130(4):601–10.
[156] Hsiao AY, Torisawa YS, Tung YC, et al. Microfluidic system for formation of PC-3 prostate cancer co-culture spheroids. Biomaterials 2009;30(16):3020–7.
[157] Boj SF, Hwang CI, Baker LA, et al. Organoid models of human and mouse ductal pancreatic cancer. Cell 2015;160(1-2):324–38.
[158] Devi KS, Mishra D, Roy B, Ghosh SK, Maiti TK. Assessing the immunomodulatory role of heteroglycan in a tumor spheroid and macrophage co-culture model system. Carbohydr Polym 2015;127:1–10.
[159] Alemany-Ribes M, Semino CE. Bioengineering 3D environments for cancer models. Adv Drug Deliv Rev 2014;79–80:40–9.
[160] Sung KE, Beebe DJ. Microfluidic 3D models of cancer. Adv Drug Deliv Rev 2014;79–80:68–78.
[161] Sarkar S, Cohen N, Sabhachandani P, Konry T. Phenotypic drug profiling in droplet microfluidics for better targeting of drug-resistant tumors. Lab Chip 2015;15(23):4441–50.
[162] Myung JH, Hong S. Microfluidic devices to enrich and isolate circulating tumor cells. Lab Chip 2015;15(24):4500–11.
[163] Kwak B, Ozcelikkale A, Shin CS, Park K, Han B. Simulation of complex transport of nanoparticles around a tumor using tumor-microenvironment-on-chip. J Control Release 2014;194:157–67.
[164] Aziz K, Nowsheen S, Georgakilas AG. Nanotechnology in cancer therapy: targeting the inhibition of key DNA repair pathways. Curr Mol Med 2010;10(7):626–39.
[165] Albanese A, Lam AK, Sykes EA, Rocheleau JV, Chan WCW. Tumour-on-a-chip provides an optical window into nanoparticle tissue transport. Nat Commun 2013;4:2718.
[166] Richmond A, Su Y. Mouse xenograft models vs GEM models for human cancer therapeutics. Dis Model Mech 2008;1(2–3):78–82.
[167] Unger C, Kramer N, Walzl A, Scherzer M, Hengstschlager M, Dolznig H. Modeling human carcinomas: physiologically relevant 3D models to improve anti-cancer drug development. Adv Drug Deliv Rev 2014;79-80:50–67.
[168] Guerard M, Baum M, Bitsch A, et al. Assessment of mechanisms driving non-linear dose-response relationships in genotoxicity testing. Mutat Res Rev Mutat Res 2015;763:181–201.
[169] Olsen D, Jorgensen JT. Companion diagnostics for targeted cancer drugs—clinical and regulatory aspects. Front Oncol 2014;4:105.
[170] Zhao X, Modur V, Carayannopoulos LN, Laterza OF. Biomarkers in pharmaceutical research. Clin Chem 2015;61(11):1343–53.

[171] List of cleared or approved companion diagnostic devices (in vitro and imaging tools). http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm; 2015.
[172] Trusheim MR, Berndt ER. The clinical benefits, ethics, and economics of stratified medicine and companion diagnostics. Drug Discov Today 2015;20(12):1439–50.
[173] Ocana A, Ethier JL, Diez-Gonzalez L, et al. Influence of companion diagnostics on efficacy and safety of targeted anti-cancer drugs: systematic review and meta-analyses. Oncotarget 2015;6(37):39538–49.
[174] Dietel M, Johrens K, Laffert MV, et al. A 2015 update on predictive molecular pathology and its role in targeted cancer therapy: a review focusing on clinical relevance. Cancer Gene Ther 2015;22(9):417–30.
[175] NCI-molecular analysis for therapy choice (NCI-MATCH) trial. http://www.cancer.gov/nci-match; 2015.
[176] Chandrasekaran S, Marshall JR, Messing JA, Hsu JW, King MR. TRAIL-mediated apoptosis in breast cancer cells cultured as 3D spheroids. PLoS One 2014;9(10):e111487.
[177] Marsit CJ, Koestler DC, Christensen BC, Karagas MR, Houseman EA, Kelsey KT. DNA methylation array analysis identifies profiles of blood-derived DNA methylation associated with bladder cancer. J Clin Oncol 2011;29(9):1133–9.
[178] Karampini E, McCaughan F. Circulating DNA in solid organ cancers-analysis and clinical application. QJM 2016;109(4):223–7.
[179] Alix-Panabieres C, Pantel K. Liquid biopsy in cancer patients: advances in capturing viable CTCs for functional studies using the EPISPOT assay. Expert Rev Mol Diagn 2015;15(11):1411–7.
[180] Ravelli A, Reuben JM, Lanza F, et al. Breast cancer circulating biomarkers: advantages, drawbacks, and new insights. Tumour Biol 2015;36(9):6653–65.
[181] Gasparri ML, Savone D, Besharat RA, et al. Circulating tumor cells as trigger to hematogenous spreads and potential biomarkers to predict the prognosis in ovarian cancer. Tumour Biol 2015; [epub ahead of print].
[182] Best MG, Sol N, Kooi I, et al. RNA-Seq of tumor-educated platelets enables blood-based pan-cancer, multiclass, and molecular pathway cancer diagnostics. Cancer Cell 2015;28(5):666–76.
[183] Willers H, Gheorghiu L, Liu Q, et al. DNA damage response assessments in human tumor samples provide functional biomarkers of radiosensitivity. Semin Radiat Oncol 2015;25(4):237–50.
[184] The Nobel Prize in Physiology or Medicine 1988. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1988/black-bio.html; 2015.
[185] Wilson WR, Hay MP. Targeting hypoxia in cancer therapy. Nat Rev Cancer 2011;11(6):393–410.
[186] Logsdon DP, Cheng H, Luo M, et al. Targeting APE1/Ref-1 results in inhibition of hypoxia signaling genes (Abstract B158). Paper presented at AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics, Boston, MA; November 7, 2015.
[187] Agrotis A, Ketteler R. A new age in functional genomics using CRISPR/Cas9 in arrayed library screening. Front Genet 2015;6:300.
[188] Dow LE. Modeling disease in vivo with CRISPR/Cas9. Trends Mol Med 2015;21(10):609–21.
[189] Toward precision medicine: building a knowledge network for biomedical research and a new taxonomy of disease. Washington DC: National Academy of Sciences; 2011.
[190] Birkelbach M, Ferraiolo N, Gheorghiu L, et al. Detection of impaired homologous recombination repair in NSCLC cells and tissues. J Thorac Oncol 2013;8(3):279–86.
[191] Krotz D. Time-lapse analysis offers new look at how cells repair DNA damage, http://newscenter.lbl.gov/2015/09/01/time-lapse-dna-damage/; 2015.
[192] de Bono JS, Ashworth A. Translating cancer research into targeted therapeutics. Nature 2010;467(7315):543–9.
[193] Arkin MR, Tang Y, Wells JA. Small-molecule inhibitors of protein-protein interactions: progressing toward the reality. Chem Biol 2014;21(9):1102–14.
[194] Schorpp K, Rothenaigner I, Salmina E, et al. Identification of small-molecule frequent hitters from alpha screen high-throughput screens. J Biomol Screen 2014;19(5):715–26.
[195] Rolland T, Tasan M, Charloteaux B, et al. A proteome-scale map of the human interactome network. Cell 2014;159(5):1212–26.