THIS BLUEPRINT HAS BEEN DEVELOPED UNDER THE GUIDANCE OF A STEERING COMMITTEE WHICH INCLUDED THE FOLLOWING MEMBERS:

- *Dr. Judith Balmaña - Vall d'Hebron Institute of Oncology, Barcelona, Spain*
- *Prof. Simon Boulton - Francis Crick Institute, London, UK*
- *Prof. Charlie Gourley - University of Edinburgh Cancer Research Centre, Edinburgh, UK*
- *Prof. Jonathan Ledermann - UCL Cancer Institute, London, UK*
- *Prof. Sibylle Loibl - German Breast Group, Neu-Isenburg, Germany*
- *Dr. Mark J. O'Connor - AstraZeneca, Cambridge, UK*
- *Prof. Eric Pujade-Lauraine - Université Paris Descartes - Hôpitaux Universitaires Paris Centre site Hôtel-Dieu, Paris, France*
- *Dr. Violeta Serra - Vall d'Hebron Institute of Oncology, Barcelona, Spain*

DDR BLUEPRINT

Document purpose

To provide a brief introduction and a reference guide to key aspects of the importance of DNA Damage Response (DDR) and its therapeutic potential in cancer. This BluePrint is intended for the broader professional oncology community, including oncologists, surgeons, radiation oncologists, research nurses etc. - involved in treating patients with cancer. It is recommended to use this BluePrint together with the accompanying BluePrint on PARP inhibition.

DDR – sensing, signalling and repairing DNA damage

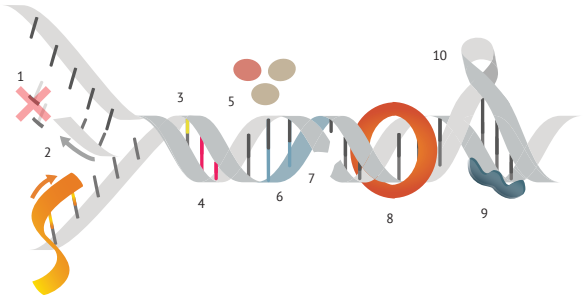
DNA damage can be sustained due to **endogenous factors** (spontaneous or enzymatic reactions, chemical modifications, replication errors, replication stress) **or exogenous factors** (UV radiation, ionizing radiation, genotoxic chemicals).

Cells have evolved a number of repair pathways to overcome this; they are collectively referred to as **DNA Damage Response**.

DNA Repair Mechanisms: the main DNA repair pathways, the type of DNA lesions they repair and their function

DNA repair mechanism and type of lesion	Repair function
Base Excision Repair (BER) ⇨ Single strand breaks	• Spontaneous hydrolytic decay products • Oxidative and alkylating modifications to bases or the sugar phosphate backbone
Nucleotide Excision Repair (NER) ⇨ Bulky adducts	• Removes bulky DNA lesions formed by UV light, environmental mutagens and some cancer chemotherapeutic adducts (e.g. platinum compounds)
Mismatch Repair (MMR) ⇨ Mispaired bases	• Base-base mismatches and insertion/deletion mispairs generated during DNA replication and recombination or caused by oxidative DNA damage
Homologous Recombination Repair (HRR) ⇨ Double-strand breaks (DSB) ⇨ Inter/intra-strand cross links	• Repairs DSBs using as a template the undamaged homologous sequence provided by the "sister chromatid" (hence mostly error-free) • Involved in the recovery of stalled or broken replication forks during DNA replication.
Non-Homologous End Joining (NHEJ) ⇨ Double-strand breaks	• Direct re-ligation of broken DNA molecules (without the requirement for a homologous template)

1 Limitation of essential replication factors
2 Conflicts between replication and transcription
3 Misincorporation of ribonucleotides
4 DNA lesions
5 Oncogene-induced replication stress
6 Common fragile sites
7 Nicks, gaps, and ssDNA
8 Topoisomerase arrest
9 Chromatin inaccessibility
10 Unusual DNA structures



Replication stress represents a type of DNA damage that occurs during DNA replication. It is characterised by **generation of aberrant replication fork structures** containing single-stranded DNA and can manifest in a number of forms. Common to all these different forms is the potential to slow down or stall DNA polymerase progression and result in DNA polymerization being uncoupled from the helicase that is unwinding the DNA. If unrepaired, replication stress can lead to premature mitosis, and increased mutagenesis. This ultimately results in genome instability and is a hallmark of pre-cancerous and cancerous cells.

Replication Stress Response involves activation of DDR pathways that **prevent the collapse of the replication fork** and the subsequent generation of cytotoxic DNA Double Strand Breaks.

DDR plays an important role in health and disease

There are approx. 450 human DDR genes coding for proteins with diverse roles in various physiological processes including **immune receptor diversity, production of gametes for sexual reproduction, telomere homeostasis and aging.**

Deregulation of DDR is involved in the aetiology of many diseases such as: **genetic disorders** (e.g. Fragile X syndromes, Friedrich's ataxia, spinocerebellar ataxia, Leigh syndrome, myoclonic epilepsy, Leber's hereditary optic neuropathy), **neurodegenerative disorders** (e.g. Alzheimer's, Huntington's and Parkinson's), **immune pathologies** (e.g. B- and T-cell immune deficiency), **infertility, cardiovascular disease, metabolic syndrome** and **cancer**.

DDR is often dysregulated in cancer

Several DNA repair and DDR signalling proteins are associated with familial cancer predisposition syndromes due to inactivating **germline mutations**. Moreover, **somatic mutations** in different DDR pathway genes have also been described in **10-35%** of sporadic cancers (see table below).

Examples of DDR genes involved in cancer

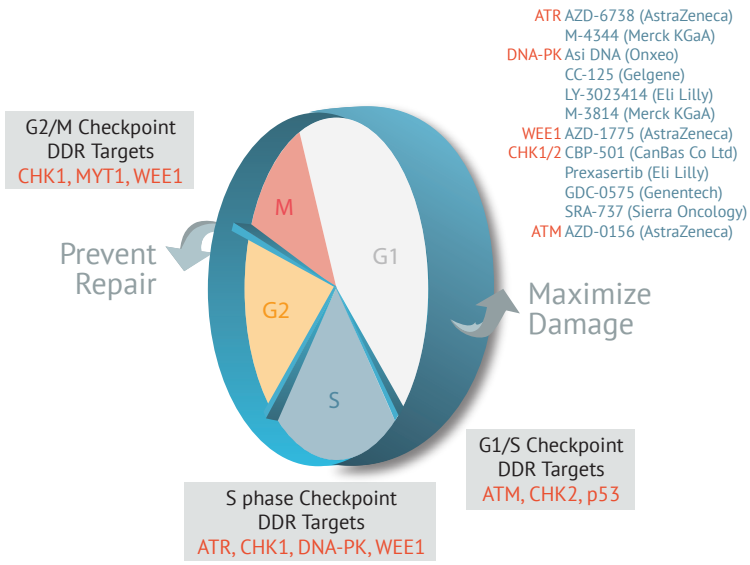
DNA repair mechanism	Gene examples	Cancer predisposing syndrome
BER	*OGG1*	Renal, breast and lung cancer
	XRCC1	Non-small cell lung cancer
NER	*ERCC1*	Lung and skin cancer, and glioma
	XP	Xeroderma pigmentosum predisposing to skin cancer. Also increased risk of bladder and lung cancer
MMR	*MSH2, MLH1*	Lynch syndrome predisposing to colorectal cancer as well as endometrial, ovarian, stomach, small intestine, hepatobiliary tract, upper urinary tract, brain and skin cancer
HRR	*BRCA1, BRCA2*	Increased risk of breast, ovarian, prostate, pancreatic, as well as gastrointestinal and haematological cancer, and melanoma
	FANC	Group of proteins associated with Fanconi anaemia predisposing to squamous cell carcinomas and acute myeloid leukaemia (e.g. FANCA, FANCB)
NHEJ	*KU70*	Breast, colorectal and lung cancer
	KU80	Lung cancer
Cell cycle checkpoints	*ATM*	Ataxia-telangiectasia predisposing to leukaemia, breast and pancreatic cancer
	ATR	Leukaemia, lymphoma, gastric and endometrial cancer

DDR deregulation may lead to cancer through various mechanisms:

- **Dysregulation of one or more DDR pathways** driving genetic instability; e.g. when in HRR-deficient cells DSBs are inappropriately repaired by NHEJ or BRCA1 or ATM is lost, resulting in translocations and copy number changes, and dependency on remaining DDR pathways
- **Increased levels of replication stress** through, for example, overexpression of oncogenes such as cyclin E, c-MYC, K-Ras, resulting directly or indirectly in slowed or stalled DNA polymerase progression and subsequently DNA polymerization being uncoupled from the helicase that is unwinding the DNA. Finally, this may lead to increased mutagenesis by 1) Break-induced replication (BIR), or 2) Polymerase switching to error-prone translesion synthesis (TLS) polymerases
- **Increased levels of endogenous damage**, by, for example, the generation of ROS which bypasses cell cycle checkpoints
- Because of any combination of the above, and/or sustained by the **application of anticancer therapies** (e.g. radiation or chemotherapy)

DDR can be exploited in cancer therapy

DDR integrates regulation of cell cycle progression and DNA repair allowing time for repair thus preventing permanent DNA damage. There are **three checkpoints** where the cell cycle may arrest in response to DNA damage and various proteins are part of the checkpoint transduction pathways (see figure below).



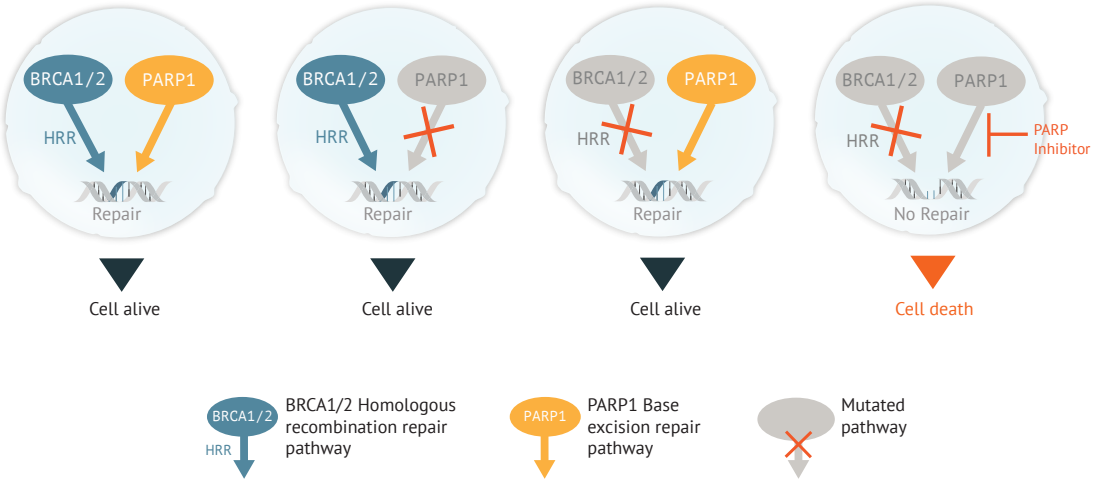
Cell Cycle and DDR targeting
The three key cell cycle checkpoints and associated proteins are being targeted by small molecule inhibitors in clinical trials (top, right-hand box). Cancer cells have increased susceptibility to S-phase-induced DNA damage that in turn may lead to:
1. replication catastrophe or apoptosis (unsustained levels of S-phase DNA damage), **or**
2. mitotic catastrophe (DSBs carried into mitosis)

Hence, **DDR-targeting agents** could be used, on one hand, to maximize DNA damage in G1 and S-phase and on the other, to prevent repair in G2, thus ensuring the maximum amount of DNA damage is taken into mitosis.

Synthetic lethality is a validated concept in cancer therapy

In cases where one DNA repair pathway is defective, cells are usually able to repair DNA damage by switching to an alternative repair mechanism. This is how mutated cells are able to survive. However, cells are unable to survive in situations where one mechanism is defective (e.g. through BRCA1 or BRCA2 mutation) and another is also blocked by pharmacological inhibition (PARP1 inhibition). This is referred to as **synthetic lethality**.

Synthetic lethality:



The concept of synthetic lethality has been exploited in the clinic and led to the development of a new class of cancer therapy; PARP inhibitors (see accompanying PARPi BluePrint). The same concept can be applied to other molecules in the DDR pathway. Several such products targeting DDR are currently in clinical development (see Appendix I).

Biomarkers and patient selection – beyond BRCA testing

There are several biomarkers that may be used to identify patients more likely to respond to DDR agents. These include:

- **Approved companion diagnostics** based on detecting germline or somatic BRCA1/BRCA2 mutations, as currently applied in the treatment of ovarian cancer
- **Patient selection assays** that provide evidence of biomarker activity
  - PARPi detection of DNA genomic scars based on LOH (loss of heterozygosity), LST (large-scale state transitions) and TAI (telomeric allelic imbalance)
  - Non-BRCA1/2 HRR proteins, such as RAD51 foci formation by immunofluorescence, for PARPi
  - Lack of checkpoint proteins such as ATM-deficiency by IHC, for ATRi
  - Oncogenes known to increase replication stress (cyclin E and c-MYC overexpression) or surrogate markers of replication stress: phosphorylated RPA foci and pan-nuclear H2AX phosphorylation (by immunofluorescence/ immunohistochemistry)

DDR is a rapidly emerging and promising area for future anticancer therapy

Efficacy of DDR inhibitors may increase when combined with other DNA damaging agents. The hope is that DDR inhibitors will find further use in clinical practice, either in addition to or even replacing chemotherapy. Combinations of multiple DDR inhibitors or combining DDR inhibitors with antiangiogenic agents or immune checkpoint inhibitors pose interesting novel strategies.

References (Reviews)

- *Brown JS, Kaye SB, Yap TA. PARP inhibitors: the race is on. Br J Cancer. 2016;114(7):713-5.*
- *Brown JS, O'Carrigan B, Jackson SP, Yap TA. Targeting DNA Repair in Cancer: Beyond PARP Inhibitors. Cancer Discov. 2017;7(1):20-37.*
- *Curtin NJ. DNA repair dysregulation from cancer driver to therapeutic target. Nat Rev Cancer. 2012;12(12):801-17.*
- *Dobbelstein M, Sørensen CS. Exploiting replicative stress to treat cancer. Nat Rev Drug Discov. 2015;14(6):405-23.*
- *Friedberg EC. A brief history of the DNA repair field. Cell Res. 2008;18(1):3-7.*
- *Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009;461(7267):1071-8.*
- *Jeggo PA, Pearl LH, Carr AM. DNA repair, genome stability and cancer: a historical perspective. Nat Rev Cancer. 2016;16(1):35-42.*
- *Kaelin WG Jr. The concept of synthetic lethality in the context of anticancer therapy. Nat Rev Cancer. 2005;5(9):689-98.*
- *Nijman SM. Synthetic lethality: general principles, utility and detection using genetic screens in human cells. FEBS Lett. 2011;585(1):1-6.*
- *O'Connor MJ. Targeting the DNA Damage Response in Cancer. Mol Cell. 2015;60(4):547-60.*
- *Pearl LH, Schierz AC, Ward SE, Al-Lazikani B, Pearl FM. Therapeutic opportunities within the DNA damage response. Nat Rev Cancer. 2015;15(3):166-80.*
- *Roos WP, Thomas AD, Kaina B. DNA damage and the balance between survival and death in cancer biology. Nat Rev Cancer. 2016;16(1):20-33.*
- *Zeman MK, Cimprich KA. Causes and consequences of replication stress. Nat Cell Biol. 2014;16(1):2-9.*

Glossary

Cell Cycle

- The process of cell division. It is divided into three stages: interphase, the mitotic (M) phase and cytokinesis
- Interphase consists of G1 phase (cell growth), followed by S phase (DNA synthesis) and then by G2 phase (cell growth)

DNA Damage

- An alteration in the chemical structure of DNA that occurs either directly or indirectly to the nucleotides in the genome. It is caused by exogenous and endogenous sources
- Examples of endogenous DNA damage: base hydrolysis (loss), deamination (spontaneous or enzymatic conversions), reactive oxygen and nitrogen species, alkylation or histone demethylation due to for example, formaldehyde production (chemical modifications), DNA mismatch, insertion, deletion (replication errors) or replication stress

DNA Damage Tolerance Mechanisms

- Mechanisms promoting the bypass of single-stranded DNA lesions encountered by DNA polymerases during DNA replication
- Two mechanisms: translesion synthesis and error-free template switching

DDR Associated Pathways

- Chromatin remodelling: patterns of dynamic changes in the structure of chromatin generated by chemical modifications of histones and controlling whether the DNA wrapped around the histones is transcribed or not
- Telomere maintenance: activation of this mechanism is indispensable for the immortalization of cells and is required for most cancer cells to maintain their telomeres
- Checkpoint factors: control mechanisms that ensure proper division of the cell, each controlling entry into the next cell cycle phase
- Ubiquitin response: ubiquitination is the process by which a ubiquitin moiety is added to a substrate protein mediating its degradation via the proteasome or altering its localization or biological behaviour. Ubiquitylation plays a critical role in regulating DDR and other pathways
- p53 pathway: activated in response to a number of cellular stresses including DNA damage, which triggers cell-cycle arrest, apoptosis or DNA repair
- Chromosome segregation: occurs during mitosis and is the process by which the mitotic spindle separates the duplicated chromosomes into the daughter cells

Mitotic Catastrophe
- A mechanism of delayed mitosis-linked cell death that is unrelated to programmed cell death/apoptosis
- Consists of a sequence of events resulting from premature or inappropriate entry of cells into mitosis that can be caused by chemical or physical stresses

Other DNA Repair Mechanisms
- Direct lesion reversal
- Fanconi anaemia pathway
- DNA damage tolerance mechanisms
- Interstrand crosslink
- DNA-protein crosslink

Replication Fork: partial separation of the DNA double helix that forms a Y-shaped structure and allows access to polymerases for DNA replication

Replication Stress: DNA replication is the most vulnerable cellular process that can lead to genome instability. It involves:
- Break-induced replication (BIR): also known as recombination-dependent replication or break-copy duplication and contributes to the repair of broken replication forks and allows telomere lengthening in the absence of telomerase (mutagenic)
- Polymerase switching to error-prone TLS polymerases, which employ specialized polymerases to traverse the damage (error-prone)

Abbreviations

ATM	Ataxia telangiectasia mutated kinase	**LOH**	Loss of heterozygosity
ATR	Ataxia-and Rad-related kinase	**LST**	Large-scale state transitions
ATRi	Ataxia-and Rad-related kinase inhibitor	**MLH1**	MutL homologue 1
BER	Base excision repair	**MMR**	Mismatch repair
BIR	Break-induced replication	**MSH2**	MutS protein homolog 2
BRCA1/2	Breast cancer 1/2 susceptibility protein	**NER**	Nucleotide excision repair
Chk1/2	Checkpoint kinase 1/2	**NHEJ**	Non-homologous end joining
c-MYC	Myc proto-oncogene	**NSCLC**	Non-small cell lung cancer
Chk2	Checkpoint kinase 2	**OGG1**	8-Oxoguanine glycosylase
DDR	DNA damage response	**PARP**	Poly(ADP-ribose) polymerase
DNA	Deoxyribonucleic acid	**PARPi**	Poly(ADP-ribose) polymerase inhibitor
DNA-PK	DNA-dependent protein kinase	**RAD51**	DNA repair protein
DSBs	Double strand breaks	**ROS**	Reactive Oxygen Species
ERCC1	ERCC excision repair 1	**RPA**	Replication protein A
FANC	Fanconi anemia complementation groups	**SCLC**	Small cell lung cancer
G1 (phase)	Gap/ growth phase 1	**ssDNA**	Single-stranded DNA
G2 (phase)	Gap/ growth phase 2	**TAI**	Telomeric allelic imbalance
H2AX	Variant of histone H2	**TLS**	Translesion synthesis
HRR	Homologous recombination repair	**UV**	Ultraviolet
IHC	Immunohistochemistry	**WEE1**	WEE1 G2 checkpoint kinase
K-Ras	Ras proto-oncogene	**XP**	Xeroderma pigmentosum
		XRCC1	X-ray repair cross-complementing protein 1

Appendix I: DDR inhibitors currently approved or in clinical development

DDR Target	Compound Name	Company Name	Highest Development Stage	Indication
PARP1/2	Olaparib*	AstraZeneca	Marketed	Ovarian cancer
	Rucaparib	Clovis Oncology	Marketed	Ovarian cancer
	Niraparib	Tesaro	Marketed	Ovarian cancer
	Veliparib	AbbVie	Phase III	Ovarian cancer, Breast cancer, Non-Small Cell Lung Cancer
	Talazoparib	Pfizer	Phase III	Ovarian cancer, Breast cancer, Non-Small Cell Lung Cancer
	Several other compounds are in Phase III or earlier – please refer to PARPi BluePrint paper			
Chk1/2	CBP-501	CanBas Co Ltd	Phase II	Non-Small Cell Lung Cancer
	Prexasertib	Eli Lilly and Company	Phase II	Small Cell Lung Cancer (SCLC), Ovarian Cancer, Triple Negative Breast Cancer, Metastatic Castrate Resistant Prostate Cancer
	GDC-0575	Genentech	Phase I	Solid tumours
	SRA-737	Sierra Oncology Inc	Phase I	Solid tumours
WEE1	AZD-1775	AstraZeneca	Phase II	SCLC, Squamous Cell Lung Cancer, Ovarian Cancer, Triple Negative Breast Cancer, Advanced Acute Myeloid Leukaemia or Myelodysplastic Syndrome, Gastric Cancer, Head and Neck Cancer, Pancreatic Cancer
ATR	AZD-6738	AstraZeneca	Phase I	Various solid malignancies
	M-4344	Merck KGaA	Phase I	Various solid malignancies
DNA-PK	CC-115	Celgene Corp	Phase II	Glioblastoma
	LY-3023414	Eli Lilly and Company	Phase II	SCLC, Endometrial Cancer, Prostate Cancer, Pancreatic Cancer, Lymphoma
	AsiDNA	Onxeo SA	Phase I	Various solid malignancies
	M-3814	Merck KGaA	Phase I	Various solid malignancies
ATM	AstraZeneca	AZD-0156	Phase I	Various solid malignancies
MTH1	Karonudib	Bayer AG	Phase I	Solid tumours

** Following completion of this DDR BluePrint document, on January 12th 2018, AstraZeneca and Merck announced that the US Food and Drug Administration (FDA) approved LYNPARZA® (olaparib), for use in patients with deleterious or suspected deleterious germline BRCA-mutated (gBRCAm), human epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer who have been previously treated with chemotherapy in the neoadjuvant, adjuvant or metastatic setting. Patients with hormone receptor positive (HR+) breast cancer should have been treated with a prior endocrine therapy or be considered inappropriate for endocrine therapy. Patients are selected for therapy based on an FDA-approved companion diagnostic for LYNPARZA.*