www.esmo.org

# ESMO HANDBOOK OF **CANCER GENETICS AND GENOMICS**

Edited by Angela George, David SP Tan, Marcia Hall and Tania Fleitas Kanonnikoff

**ESMO** Handbook Series

# ESMO HANDBOOK OF
# **CANCER GENETICS AND GENOMICS**

# ESMO HANDBOOK OF
# **CANCER GENETICS AND GENOMICS**

Edited by

Angela George
*The Royal Marsden Hospital and The Institute of Cancer Research, London, UK*

David SP Tan
*Department of Haematology-Oncology, National University Cancer Institute, National University Hospital Singapore; Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore; NUS Centre for Cancer Research (N2CR), Yong Loo Lin School of Medicine, National University of Singapore; Cancer Science Institute, National University of Singapore, Singapore, Republic of Singapore*

Marcia Hall
*Mount Vernon Cancer Centre, Northwood; Brunel University London, London, UK*

Tania Fleitas Kanonnikoff
*Medical Oncology Department, Hospital Clínico Universitario de Valencia–INCLIVA, Valencia, Spain*

ESMO Press

First published in 2025 by ESMO Press.



For orders, corporate sales, foreign rights, and reprint permissions, please contact:
ESMO Head Office
Licensing and Publications Sales Department
Via Ginevra 4
6900 Lugano
Switzerland
Tel: +41 (0)91 973 19 00
Email: publication_support@esmo.org
www.esmo.org

# Contents

# Editors


## Angela George

*The Royal Marsden Hospital and The Institute of Cancer Research, London, UK*


Dr Angela George is a Consultant Medical Oncologist and Consultant in Oncogenetics at The Royal Marsden Hospital, London, UK. She specialises in the systemic treatment of gynaecological cancers with a particular focus on the use of genomic information in treatment. She is also Clinical Director of Genomics at The Royal Marsden Hospital, and Medical Director of the North Thames Genomic Medicine Service, UK, providing genomic testing on a regional and national level. She led the implementation of the Mainstreaming Cancer Genetics programme, incorporating genetic testing into the routine care of patients with ovarian, breast and pancreatic cancers.

Dr George is involved in multiple national and international groups, including the European Society for Medical Oncology (ESMO) Precision Oncology Working Group and the British Society of Genetic Medicine. She sits on the Clinical Advisory Group for the National Health Service (NHS) England Genomics Unit. Dr George has authored multiple peer-reviewed publications and undertakes a variety of clinical and translational research.


## David SP Tan

*Department of Haematology-Oncology, National University Cancer Institute, National University Hospital Singapore; Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore; NUS Centre for Cancer Research (N2CR), Yong Loo Lin School of Medicine, National University of Singapore; Cancer Science Institute, National University of Singapore, Singapore, Republic of Singapore*


Dr Tan is a Senior Consultant Medical Oncologist at the National University Cancer Institute, Singapore and an Associate Professor in the Department of Medicine at the Yong Loo Lin School of Medicine, National University of Singapore and Principal Investigator at the Cancer Science Institute, National University of Singapore. His primary clinical and research interests are in gynaecological cancers, precision oncology and early-phase trials. He has authored over 100 peer-reviewed scientific papers and received several awards for his research.

Dr Tan is currently the Head of the Innovation Transfer Office at National University Health Systems (NUHS) and Director of the Research Transformation Office at NUHS. He was the founding President of the Gynecologic Cancer Group Singapore (GCGS), and inaugural President of the Asia-Pacific Gynecologic Oncology Trials Group (APGOT). He currently chairs the APGOT Protocol Review Committee. He is also the past chair of the Cervical Cancer Research Network (CCRN) of the Gynecologic Cancer InterGroup (GCIG) and is a member of the Executive Board of Directors of the GCIG.


## Marcia Hall

*Mount Vernon Cancer Centre, Northwood; Brunel University London, London, UK (retired)*


Marcia Hall has now retired but was Professor of Translational Oncology at Brunel University London, London, UK and a Consultant in Medical Oncology at Mount Vernon Cancer Centre, Northwood, UK. She has a background in managing patients with a wide range of cancer types (gastrointestinal, germ cell, breast) most recently focusing solely on the treatment of patients with gynaecological cancers.

Professor Hall's research focus was related to the identification of homologous repair deficiency from circulating (blood samples) factors, and she was the lead for research and development at Mount Vernon Cancer Centre between 2013 and 2021.

Nationally, she has sat on the UK National Cancer Research Institute gynaecological cancers subgroup and, internationally, was a member of the ESMO Educational Publications Working Group (2015–2023; Deputy Chair: 2018–2021).


## Tania Fleitas Kanonnikoff

*Medical Oncology Department, Hospital Clínico Universitario de Valencia–INCLIVA, Valencia, Spain*


Dr Tania Fleitas Kanonnikoff is a medical doctor from the Universidad Nacional de Asunción, Paraguay, a medical oncologist trained at Hospital La Fe, Valencia (2008), Spain and holds a PhD in Medicine from the Autonomous University of Barcelona (2011), Barcelona, Spain. She currently works at the Hospital Clínico Universitario de Valencia and the INCLIVA Biomedical Research Institute, specialising in oesophagogastric tumours. Dr Fleitas leads a multidisciplinary research group investigating tumour biology, the microenvironment, microbiome and resistance mechanisms in gastric cancer.

Her international experience includes a postdoctoral fellowship at Dana-Farber Cancer Institute (2016), Boston, MA, USA, where she deepened her expertise in gastric cancer immunoprofiling. She coordinates major international consortia, including LEGACy and AIDA (Artificially Intelligent Diagnostic Assistant), and contributes to European clinical guidelines and research project evaluations. Dr Fleitas is a Faculty Coordinator for ESMO (Gastrointestinal cancer, upper digestive) and is a member of the ESMO Educational Publications Task Force. She has published extensively in high-impact scientific journals.

# Editors' Declarations of Interest

**Dr George** has declared personal advisory board honoraria from AbbVie and AstraZeneca; personal speaker fees from GSK; institutional fees from Guardant Health for her role as a member of the steering group in relation to the Royal Marsden Hospital/Guardant partnership.

**Dr Tan** has declared personal advisory board honoraria from AstraZeneca, Bayer, MSD, Eisai, Roche, Genmab, GSK and Boerhinger Ingelheim; personal speaker honoraria from AstraZeneca, MSD, Roche, GSK, Eisai and Takeda, and steering committee honoraria from Daiichi Sankyo; institutional research grants from Bayer, Karyopharm Therapeutics, AstraZeneca and Roche; and institutional fees for his role as coordinating principal investigator from AstraZeneca and Bergen Bio, as a steering committee member from Daiichi Sankyo and as local principal investigator from Zeria Pharmaceutical Co Ltd, Bayer and Byondis. He holds stocks/shares in Asian Microbiome Library (AMILI).

He has declared receiving product samples for research studies at his institution from MSD, Eisai, AstraZeneca and Cyclacel Pharmaceuticals; and for a clinical trial from Daiichi Sankyo. He is a member of the Board of Directors for the Gynecologic Cancer Intergroup (GCIG), was President of the GyneCologic Cancer Group Singapore (GCGS) and ex-Chair of the Asia–Pacific Gynecologic Oncology Trials Group (APGOT).

**Professor Hall** has declared no conflicts of interest.

**Dr Fleitas Kanonnikoff** has declared personal advisory board honoraria from Amgen, AstraZeneca, BeiGene and MSD; and personal invited speaker honoraria from Amgen, Astellas, Bayer, Bristol Myers Squibb, Lilly, MSD, Roche and Servier.

She has declared non-remunerated roles as principal investigator for trials funded by Adaptimmune, Amgen, AstraZeneca, Beigene and Daiichi Sankyo.

# Contributors

**Alsina M.** Department of Medical Oncology, Hospital Universitario de Navarra (HUN), Pamplona; Translational Medical Oncology Unit, Navarrabiomed – Instituto de Investigación Sanitaria de Navarra (IdISNA), Pamplona, Spain

**Blanc-Durand F**. Medical Oncology Department, Institut Gustave Roussy, Villejuif, France

**Carausu M.** Breast Cancer Translational Research Laboratory J.-C. Heuson, Jules Bordet Institute, Université Libre de Bruxelles (ULB), Brussels, Belgium

**Cartwright E.** GI and Lymphoma Unit, The Royal Marsden Hospital, London, UK

**Castro E.** Medical Oncology Department, Hospital Universitario 12 de Octubre, Madrid, Spain

**Chan A.** Prince of Wales Hospital, Sha Tin, Hong Kong SAR, China

**Collet L.** Breast Cancer Translational Research Laboratory J.-C. Heuson, Jules Bordet Institute, Université Libre de Bruxelles (ULB), Brussels, Belgium; Department of Medical Oncology, Centre Léon Bérard, Lyon, France

**de Velasco G.** Medical Oncology Department, Hospital Universitario 12 de Octubre, Madrid, Spain

**Dienstmann R.** Oncology Data Science, Vall d'Hebron Institute of Oncology, Barcelona; University of Vic – Central University of Catalonia, Vic, Spain. Oncoclínicas Medicina de Precisão, Oncoclínicas & Co, São Paulo, Brazil

**George A.** The Royal Marsden Hospital and The Institute of Cancer Research, London, UK

**Haferlach T.** MLL Munich Leukemia Laboratory, Munich, Germany

**Harrold E.** Trinity St. James's Cancer Institute, Dublin, Ireland

**Hörst K.** MLL Munich Leukemia Laboratory, Munich, Germany

**Kee D.** Department of Medical Oncology, Austin Health, Heidelberg, VIC; Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

**Kühn C.** MLL Munich Leukemia Laboratory, Munich, Germany

**Lam J.** Prince of Wales Hospital, Sha Tin, Hong Kong SAR, China

**Maccari M.** Department of Surgery, Oncology and Gastroenterology, University of Padua, Padua; Medical Oncology 1, Veneto Institute of Oncology IOV-IRCCS, Padua, Italy

**Minniti G.** Department of Radiological Sciences, Oncology, and Anatomical Pathology, Sapienza University of Rome, Rome; IRCCS Neuromed, Pozzilli (IS), Italy

**Moreno-Perez I.** Medical Oncology Department, Hospital Universitario Clinico San Carlos, Madrid, Spain

**Pérez-Fidalgo J.A.** Medical Oncology Department, Hospital Clinico Universitario of Valencia, Valencia; INCLIVA, University of Valencia, Valencia, Spain

**Ray-Coquard I.** Department of Medical Oncology, Centre Léon Bérard, Lyon, France

**Ruiz-Vico M.** Medical Oncology Department, Hospital Universitario 12 de Octubre, Madrid; Biomedicine Research PhD Program, Universidad Complutense de Madrid, Madrid, Spain

**Sotiriou C.** Breast Cancer Translational Research Laboratory J.-C. Heuson, Jules Bordet Institute, Université Libre de Bruxelles (ULB), Brussels, Belgium

**Stadler Z.K.** Clinical Genetics Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

**Tan D.** National Cancer Centre Singapore (NCCS), Singapore, Republic of Singapore

**Tan D.S.P.** Department of Haematology-Oncology, National University Cancer Institute, National University Hospital Singapore; Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore; NUS Centre for Cancer Research (N2CR), Yong Loo Lin School of Medicine, National University of Singapore; Cancer Science Institute, National University of Singapore, Singapore, Republic of Singapore

**Tan W.-C.** National Cancer Centre Singapore (NCCS), Singapore, Republic of Singapore

# Reviewers

The editors and authors wish to thank the following reviewers.

**Judith Balmaña,** Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain

**Sarah Derks,** Amsterdam UMC, Amsterdam, Netherlands

**Andrew Furness,** The Royal Marsden Hospital, London, UK

**Emilie Le Rhun,** University Hospital Zurich, Zurich, Switzerland

**Darren Lim,** National Cancer Centre Singapore, Singapore, Republic of Singapore

**Herbert Loong**, The Chinese University of Hong Kong, Hong Kong SAR, China

**Caterina Marchiò,** Candiolo Cancer Institute, Turin, Italy

**Richard Rosenquist Brandell,** Karolinska Institutet, Stockholm, Sweden

**Joaquin Mateo,** Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain

**Shani Paluch-Shimon,** Hadassah Medical Center, Jerusalem, Israel

**Winette van der Graaf,** Netherlands Cancer Institute (NKI), Amsterdam, Netherlands

**Michelle Wilson,** Auckland City Hospital, Auckland, New Zealand

# Abbreviations

| | |
|---|---|
| **ADC** | Antibody–drug conjugate |
| **ADT** | Androgen deprivation therapy |
| **AK** | Actinic keratosis |
| **ALK** | Anaplastic lymphoma kinase |
| **ALL** | Acute lymphoblastic leukaemia |
| **AML** | Acute myeloid leukaemia |
| **AR** | Androgen receptor |
| **ARPi** | Androgen-receptor pathway inhibitor |
| **ATP** | Adenosine triphosphate |
| **BC** | Breast cancer |
| **BCC** | Basal cell carcinoma |
| **BCLC** | Breast Cancer Linkage Consortium |
| **BRRM** | Bilateral risk-reducing mastectomy |
| **BSO** | Bilateral salpingo-oophorectomy |
| **BTC** | Biliary tract cancer |
| **CC** | Cholangiocarcinoma |
| **CCND1** | Cyclin D1 |
| **ccRCC** | Clear-cell renal cell carcinoma |
| **CDK4/6** | Cyclin-dependent kinase 4/6 |
| **CGH** | Comparative genomic hybridisation |
| **CHIP** | Clonal haematopoiesis of indeterminate potential |
| **ChRCC** | Chromophobe renal cell carcinoma |
| **CI** | Confidence interval |
| **CIN** | Chromosomal instability |
| **CLDN18.2** | Claudin 18.2 |
| **CLL-IPI** | Chronic lymphocytic leukaemia International Prognostic Index |
| **CML** | Chronic myeloid lymphoma |
| **CMML** | Chronic myelomonocytic leukaemia |
| **CMS** | Consensus molecular subtype |
| **CNA** | Copy number alteration |

| | |
|---|---|
| **CNS** | Central nervous system |
| **CPS** | Combined positive score |
| **CRC** | Colorectal cancer |
| **CRPC** | Castration-resistant prostate cancer |
| **cSCC** | Cutaneous squamous-cell carcinoma |
| **CSG** | Cancer susceptibility gene |
| **ctDNA** | Circulating tumour DNA |
| **CUP** | Carcinoma of unknown primary |
| **DDR** | DNA damage response |
| **dMMR** | Mismatch repair deficient |
| **EBER** | Epstein–Barr virus-encoded small RNA |
| **EBV** | Epstein–Barr virus |
| **EC** | Endometrial carcinoma |
| **eCC** | Extrahepatic cholangiocarcinoma |
| **EGFR** | Epidermal growth factor receptor |
| **EMA** | European Medicines Agency |
| **ER** | Oestrogen receptor |
| **ERα** | Oestrogen receptor alpha |
| **ESS** | Endometrial stromal sarcoma |
| **ESCAT** | ESMO Scale for Clinical Actionability of molecular Targets |
| **ESMO** | European Society for Medical Oncology |
| **ESMO-MCBS** | ESMO-Magnitude of Clinical Benefit Scale |
| **ET** | Endocrine therapy |
| **FAP** | Familial adenomatous polyposis |
| **FDA** | Food and Drug Administration |
| **FFPE** | Formalin-fixed paraffin-embedded |
| **FGFR** | Fibroblast growth factor receptor |
| **FGFR2b** | Fibroblast growth factor receptor 2b |
| **FH** | Fumarate hydratase |
| **FIGO** | International Federation of Gynecology and Obstetrics |
| **FISH** | Fluorescent *in situ* hybridisation |
| **FLOT** | Fluorouracil, leucovorin, oxaliplatin, docetaxel |
| **g*APC* PV** | Germline *APC* pathogenic variant |
| **g*BRCA1/2*** | Germline *BRCA1/2* |
| **GEA** | Gastro-oesophageal adenocarcinoma |
| **GENIE** | Genomics Evidence Neoplasia Information Exchange |
| **GI** | Gastrointestinal |

| | |
|---|---|
| **GIS** | Genomic instability score |
| **GIST** | Gastrointestinal stromal tumour |
| **gPV** | Germline pathogenic variant |
| **GS** | Genomically stable |
| **HBOC** | Hereditary breast and ovarian cancer syndrome |
| **HCC** | Hepatocellular carcinoma |
| **HER** | Human epidermal growth factor receptor |
| **HGSC** | High-grade serous carcinoma |
| **HH** | Hedgehog (pathway) |
| **HLA** | Human leucocyte antigen |
| **HNSCC** | Head and neck squamous-cell carcinoma |
| **HPV** | Human papillomavirus |
| ***H.pylori*** | Helicobacter pylori |
| **HR** | Hazard ratio |
| **HRD** | Homologous recombination deficiency |
| **HRR** | Homologous recombination repair |
| **iCC** | Intrahepatic cholangiocarcinoma |
| **ICI** | Immune checkpoint inhibitor |
| **IGH** | Immunoglobulin heavy locus |
| **IHC** | Immunohistochemistry |
| **IPSS-M** | Molecular International Prognostic Scoring System |
| **ISH** | *in situ* hybridistation |
| **ITD** | Internal tandem duplications |
| **LFS** | Li–Fraumeni syndrome |
| **LGSC** | Low-grade serous carcinoma |
| **LS** | Lynch syndrome |
| **m7-FLIPI** | m7-Follicular Lymphoma International Prognostic Index |
| **MAPK** | Mitogen-activated protein kinase |
| **Mb** | Megabase |
| **MB** | Medulloblastoma |
| **MCC** | Merkel cell carcinoma |
| **MCPyV** | Merkel cell polyomavirus |
| **mCRC** | Metastatic colorectal cancer |
| **mCRPC** | Metastatic castration-resistant prostate cancer |
| **MDS** | Myelodysplastic neoplasms |
| **MHC** | Major histocompatibility complex |
| **MIBC** | Muscle-invasive bladder cancer |

| | |
|---|---|
| **MMR** | Mismatch repair |
| **MPN** | Myeloproliferative neoplasms |
| **MRD** | Measurable/minimal residual disease |
| **MRI** | Magnetic resonance imaging |
| **MSI** | Microsatellite instability |
| **MSI-H** | Microsatellite instability-high |
| **MSS** | Microsatellite stability |
| **MTB** | Molecular tumour board |
| **mTOR** | Mammalian target of rapamycin |
| **mut/Mb** | Mutation per megabase |
| **NCCN** | National Comprehensive Cancer Network |
| **NF1** | Neurofibromin 1 |
| **NF-κB** | Nuclear factor kappa B |
| **NGS** | Next-generation sequencing |
| **NMIBC** | Non-muscle-invasive bladder cancer |
| **NPC** | Nasopharyngeal carcinoma |
| **NSCLC** | Non-small-cell lung cancer |
| **NSMP** | No specific molecular profile |
| **NTRK** | Neurotrophic tyrosine receptor kinase |
| **OC** | Ovarian cancer |
| **OPC** | Oropharyngeal carcinoma |
| **ORR** | Overall response rate |
| **OS** | Overall survival |
| **OSCC** | Oesophageal squamous-cell carcinoma |
| **PARP** | Poly(ADP-ribose) polymerase |
| **PARPi** | Poly(ADP-ribose) polymerase inhibitor |
| **PC** | Prostate cancer |
| **PCR** | Polymerase chain reaction |
| **PDAC** | Pancreatic ductal adenocarcinoma |
| **PD-1** | Programmed cell death protein 1 |
| **PD-L1** | Programmed death-ligand 1 |
| **PEComa** | Perivascular epithelioid cell tumour |
| **PFS** | Progression-free survival |
| **PI3K** | Phosphatidylinositol-3-kinase |
| **pMMR** | Mismatch repair proficient |
| **POLE** | DNA polymerase ε |
| ***POLE*mut** | *POLE*-mutated |
| **PR** | Progesterone receptor |

| | |
|---|---|
| **pRCC** | Papillary renal cell cancer |
| **PREMM$_5$** | Prediction Model for gene Mutations |
| **PSA** | Prostate-specific antigen |
| **PTV** | Protein-truncating variant |
| **PV** | Pathogenic variant |
| **Rb** | Retinoblastoma protein |
| **RCC** | Renal cell carcinoma |
| **rPFS** | Radiographic progression-free survival |
| **RRSO** | Risk-reducing salpingo-oophorectomy |
| **SCC** | Squamous-cell carcinoma |
| **SCLC** | Small-cell lung cancer |
| **SDH** | Succinate dehydrogenase |
| **SDO** | Salpingectomy with delayed oophorectomy |
| **SERD** | Selective oestrogen-receptor degrader |
| **SHH** | Sonic Hedgehog |
| **SNP** | Single-nucleotide polymorphism |
| **STIC** | Serous tubular intraepithelial carcinoma |
| **STS** | Soft tissue sarcoma |
| **TAP** | Tumour area positivity |
| **TCGA** | The Cancer Genome Atlas |
| **TCR** | T-cell receptor |
| **T-DXd** | Trastuzumab deruxtecan |
| **TKD** | Tyrosine kinase domain |
| **TKI** | Tyrosine kinase inhibitor |
| **TMB** | Tumour mutational burden |
| **TMB-H** | Tumour mutational burden-high |
| **TNBC** | Triple-negative breast cancer |
| **TPS** | Tumour proportion score |
| **TRK** | Tropomyosin receptor kinase |
| **TSC** | Tuberous sclerosis complex |
| **TUBA** | Early Salpingectomy (TUbectomy) with Delayed Oophorectomy in gBRCA1/2 PV Carriers (study) |
| **UC** | Urothelial carcinoma |
| **UTUC** | Upper-tract urothelial carcinoma |
| **UV** | Ultraviolet |
| **VEGF** | Vascular endothelial growth factor |
| **VHL** | Von Hippel–Lindau syndrome |
| **VUS** | Variant of uncertain significance |

| | |
|---|---|
| **WES** | Whole exome sequencing |
| **WGS** | Whole genome sequencing |
| **WHO** | World Health Organization |
| **WHO CNS5** | WHO Classification of Tumours of the Central Nervous System, Fifth edition |
| **WISP** | Women Choosing Surgical Prevention (study) |
| **wt** | Wild type |

# Acknowledgements

We would like to express our heartfelt appreciation to the staff of ESMO for their tremendous support and assistance during the creation of this book. Their expertise and guidance in the field of oncology were essential to the book's success. Their advice and feedback helped us refine every chapter.

We would like to extend our sincere thanks to Claire Bramley, Aude Galli, Matthew Hillier and Nicki Peters for their unwavering assistance throughout the journey. It is with deep gratitude that we acknowledge their remarkable contribution. Thank you.

Dr Angela George, on behalf of all editors

# Preface

Throughout the last decade, we have seen an overwhelming increase in the use of genomic information to define diagnosis, inform prognosis, avoid excessive treatment toxicity and select the optimal targeted agent for treatment. It is now difficult to think of a tumour type where one of the above is not a routine part of patient care. In this approach, we have seen dramatic improvements in the outcome of patients, exemplified by the treatment of tumours such as non-small-cell lung cancer and high-grade serous ovarian cancer. However, this shift from 'one-treatment-fits-all' to a molecularly matched model has brought with it huge complexity and a need for oncology clinicians to rapidly increase their genomic literacy.

The *ESMO Handbook of Cancer Genetics and Genomics* is designed to help clinicians stay up to date with the relevant germline and somatic mutations in their area of practice. It provides real-world guidance on what is now essential for optimal patient care and gives insights into what may be coming in the future in a succinct and easily accessible format. Written by a multidisciplinary group of authors with expertise in oncology and haematology, with peer-reviewed chapters, this handbook is a comprehensive guide to ensure that all oncologists can navigate this new world of genomically-guided oncology with confidence.

Dr Angela George, on behalf of all editors

# 1 Introduction to Cancer Genomics in Clinical Practice: Testing, Reporting and Actionability

F. Blanc-Durand[1]

E. Cartwright[2]

A. George[3]

D.S.P. Tan[4]

[1]*Medical Oncology Department, Institut Gustave Roussy, Villejuif, France*
[2]*GI and Lymphoma Unit, The Royal Marsden Hospital, London, UK*
[3]*The Royal Marsden Hospital and The Institute of Cancer Research, London, UK*
[4]*Department of Haematology-Oncology, National University Cancer Institute, National University Hospital Singapore; Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore; NUS Centre for Cancer Research (N2CR), Yong Loo Lin School of Medicine, National University of Singapore; Cancer Science Institute, National University of Singapore; Singapore; Republic of Singapore*

Oncology is arguably the medical discipline that has benefited most from the advancements in genomic medicine. From unveiling the roots of complex oncogenic processes to identifying pivotal molecular targets driving cancer progression, cancer genomics has revolutionised the management of solid tumours and haematological malignancies, ushering in an unprecedented level of truly personalised medicine. Although the degree of benefit varies across tumour types, genomic medicine technologies – including next-generation sequencing (NGS), RNA sequencing, comparative genomic hybridisation (CGH) and advanced bioinformatic tools – have had a positive impact on virtually all malignancies. These approaches have contributed to refining prognosis,

identifying predictive biomarkers or uncovering targetable genomic alterations. Understanding the molecular foundations of carcinogenesis and clonal evolution, selecting appropriate molecular techniques, and implementing germline testing are now essential components of modern oncology. With the rapid integration of genomic testing into clinical practice, there is an urgent need to standardise practices to fully harness the potential of precision oncology and to recognise and address the recurrent pitfalls associated with these technologies.

## What and How to Test: Tumour Somatic Mutation, Circulating Tumour DNA or Germline?

### Carcinogenesis and Clonal Evolution

Since the mid-20th century, and most notably Knudson's work on retinoblastoma, cancer has been recognised as "a genetic disease of somatic cells" (Knudson et al, 1976). Carcinogenesis involves the transformation of a normal cell into a malignant tumour cell through the accumulation of multiple genetic alterations that confer a growth advantage, resistance to apoptosis and immune evasion, among other hallmarks of cancer. Based on this, a simplistic view of clonal evolution has been established in which a single progenitor cell acquires several mutations associated with a survival advantage, leading to its clonal expansion. Subsequently, multiple different subclones emerge, harbouring additional mutations responsible for clonal heterogeneity.

The key mutational drivers responsible for oncogenesis are classically categorised into oncogenes and tumour suppressor genes. Mutated or amplified oncogenes promote cell proliferation, whereas deleterious alterations in tumour suppressor genes remove the normal apoptosis signals. Additionally, alterations in DNA repair pathways, which are responsible for maintaining genome integrity under normal conditions, increase the amount of genetic damage and have been recognised as another oncogenic driver.

Understanding the molecular principles behind each cancer, and

particularly identifying the roots of carcinogenesis, has rapidly been recognised as essential for developing effective personalised therapeutic strategies.

### Molecular Techniques in Clinical Practice – Which Test to Use?

Tumour classification and biomarker assessment have traditionally relied on histopathology, immunohistochemistry (IHC) and cytogenetics to provide diagnostic and prognostic information. IHC is a cost-effective and widely accessible technique that enables the *in situ* visualisation of protein expression within preserved tissue architecture. It is routinely employed for tumour subtype classification and predictive biomarkers such as hormone receptors, human epidermal growth factor receptor 2 (HER2) expression and mismatch repair (MMR) protein status. Similarly, cytogenetic techniques – including karyotyping and *in situ* hybridisation (ISH) – offer direct insight into chromosomal alterations such as translocations and amplifications, which can serve as diagnostic hallmarks and therapeutic targets across various malignancies (e.g. *EWSR1* rearrangements in Ewing sarcoma). While IHC and cytogenetics provide critical phenotypic and structural information, the advent of high-throughput molecular techniques has further expanded our ability to characterise the underlying molecular drivers of tumorigenesis and significantly contributed to the development of targeted therapeutic strategies. These molecular assays are also typically compatible with formalin-fixed paraffin-embedded (FFPE) tumour tissue, though recent advances are expanding their application to minimally invasive samples, including blood and other body fluids.

**Polymerase chain reaction (PCR):** this technique amplifies a specific DNA sequence of interest and can be useful for detecting known mutations.

**Sanger sequencing:** this is a low-throughput technique but highly accurate method for obtaining the DNA sequence and identifying single-nucleotide variations as well as small insertions and deletions.

**NGS:** this high-throughput sequencing method can target specific regions (targeted NGS), the whole exome (WES) or the whole genome (WGS).

NGS can detect a wide array of genetic alterations including nucleotide variants, copy number alterations and gene translocations. NGS is the most-used technique for the comprehensive molecular profiling of cancer. In addition to identifying actionable mutations, large, targeted panels – typically encompassing at least 1 megabase (Mb) of genomic content – enable the assessment of complex biomarkers such as tumour mutational burden (TMB), mutational signatures and microsatellite instability (MSI).

**Comparative genomic hybridisation:** this technique compares the profile of tumour DNA with normal DNA to identify chromosomal gains and losses, making it particularly useful for detecting copy number alterations.

### Liquid Biopsies and Cancer Detection

Liquid biopsy is a minimally invasive procedure for collecting blood or cancer-related effusions, that enables molecular analyses and other applications. While not a substitute for tissue biopsy, liquid biopsies offer complementary information and present several advantages beyond patient convenience. They capture tumour genetic heterogeneity between primary and circulating tumour material and provide a real-time assessment of the molecular landscape, potentially identifying drivers of disease progression and resistance mechanisms. Key technologies for analysing liquid biopsies include digital PCR and NGS, which have demonstrated high sensitivity in detecting and quantifying circulating tumour DNA (ctDNA). In early-stage disease, the utility of liquid biopsy to assess minimal residual disease (MRD) following curative-intent treatment and to monitor for disease relapse is being assessed using tumour-informed and tumour-agnostic approaches. Tumour-informed assays monitor mutations detected in tissue requiring both tissue and plasma samples, while tumour-agnostic assays are plasma-only assays integrating genomic and epigenomic signatures to detect ctDNA. Liquid biopsy using a tumour-agnostic approach holds considerable promise for the implementation of molecularly matched therapies, particularly in cases where tissue biopsy is challenging.

In addition to molecular profiling and identification of targeted treatment options, further applications of liquid biopsy include development of multicancer early-detection tests using methylation changes to detect early-stage disease and the likely site of origin with implications for early diagnosis, when the chance of cure is greatest.

However, it is noteworthy that the sensitivity of ctDNA assays can be low in early-stage cancers or tumours with low shedding. Additionally, several pitfalls must be considered, including the detection of non-tumour-related alterations such as clonal haematopoiesis of indeterminate potential (CHIP) and incidental germline variants leading to false-positive results.

## Germline Testing and Genetic Counselling

Germline testing aims to identify inherited genetic alterations that may predispose individuals to cancer or hereditary syndromes. While it is routinely recommended for patients with young-onset disease or a personal or family history of inherited cancer risk, its implications extend far beyond the index case. Germline testing can inform preventive strategies not only for the affected individual but also for at-risk, asymptomatic relatives who may benefit from tailored surveillance or risk-reduction strategies. Given the potential psychological, ethical and medical implications, germline testing should be conducted with the informed consent of the patient and within the framework of a dedicated genetic-counselling unit to ensure appropriate interpretation and management.

The testing process involves three key steps:

1. **Counselling consultation:** this step involves discussing the implications of testing in terms of prevention, therapeutic strategy and the potential social impact, as well as obtaining the patient's informed consent

2. **Sample collection and testing:** usually blood or saliva is collected and tested for genetic alterations

3. **Discussion of findings:** the results are discussed with the patient, including the associated risks and management options. These options may include increased surveillance, prophylactic strategies and informing at-risk relatives.

Overall, genetic counselling is a pivotal component of germline testing, helping patients understand the benefits, limitations and potential outcomes of the test. With an increasing overlap between genomics and oncology, mainstreaming cancer genetics seeks to integrate genomic testing in oncology with the aim of accelerating germline testing and the implications of this into patient care.

## Making Sense of a Genomic Test Report

Depending on the context, clinicians have access to a variety of molecular tests, ranging from focused panels covering a few comprehensively arranged genes, to whole genome analysis. When small, targeted panels – typically covering up to 50 genes – are used, molecular testing focuses on the most prevalent and clinically validated actionable alterations, considerably simplifying interpretation. However, with the current shift to larger comprehensive panels, encompassing hundreds of genes (often exceeding 500), the likelihood of identifying unexpected alterations that may uncover actionable targets has increased considerably. Importantly, this may ultimately benefit patient care through additional approved or investigational personalised treatment strategies or enhanced tumour classification.

However, this also poses a risk for misinterpretation of molecular reports, potentially resulting in inappropriate clinical decisions or missed therapeutic opportunities for the patient. Several trials have determined that only around 40% of patients present at least one targetable alteration, although this number is likely to increase in the future. Therefore, regardless of the cancer origin and the type of molecular test performed, there should be a systematic standardised approach to interpreting the report.

Reading a test report is not necessarily straightforward: not all genes altered are actionable; not all alterations in actionable genes affect gene function and not all gene alterations are druggable. Furthermore, a druggable alteration does not carry the same clinical significance across different tumour types. It is therefore essential to assess these different elements before orienting the patient towards a molecularly matched strategy. To support this process, European Society for Medical Oncology (ESMO) recommendations on the clinical reporting of genomic test results emphasise the importance of structured reporting, clinical annotation and evidence-based actionability (Van De Haar et al, 2024).

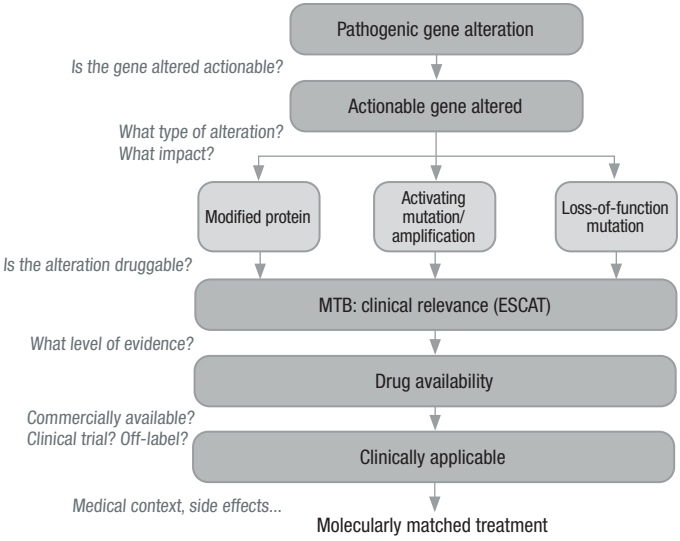


*Figure 1 Ideal Management of Mutation Test Report.*

Abbreviations: ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; ESMO, European Society for Medical Oncology; MTB, molecular tumour board.

Beyond genetic alterations affecting DNA sequence, several recently discovered molecular markers, such as MSI and homologous recombination deficiency (HRD), have demonstrated their importance in patient stratification and treatment decisions. These markers should also be considered when requesting molecular testing.

### Pathogenic Mutations Versus Variant of Uncertain Significance

A pathogenic mutation (or pathogenic variant) is defined as a change in the DNA sequence (e.g. mutation, amplification, deletion, translocation) that has been shown to cause or contribute to tumourigenesis. This modified sequence can alter the protein sequence, disrupt gene transcription or impair RNA processing, thereby participating in the oncogenic process. By contrast, a variant of uncertain significance (VUS) is a change in the DNA sequence for which the impact is unknown and possibly non-existent. Lastly, a benign mutation (or benign variant) has no impact on protein function or splicing and thus, should not be considered for therapeutic decision-making.

For an expert geneticist or molecular biologist, determining the pathogenicity of a somatic variant can be challenging and generally requires combining epidemiological datasets from single-nucleotide polymorphism (SNP) databases (Exome Sequencing Project, 1000 Genomes Project, Exome Aggregation Consortium) and disease variant databases (e.g. The Cancer Genome Atlas [TCGA], Genomics Evidence Neoplasia Information Exchange [GENIE]). A variant is likely to be pathogenic if it is absent from SNP databases, has been reported in several cancer datasets, is localised in a cluster hotspot, or if *in vitro* studies have established its damaging effect. Conversely, a benign mutation is generally detected with a minor allelic frequency of more than 5% in SNP databases (even more than 0.1% is highly suggestive) or if it has been established as non-damaging in *in vitro* studies. Importantly, many other criteria are taken into consideration and these are continuously evolving.

Consequently, detected variants are classified into five categories depending on their level of pathogenicity (Table 1). Management

decisions are primarily based on pathogenic variants (class 4 and 5), although VUS can also provide insight. However, the crucial challenge lies in identifying these pathogenic and likely-pathogenic variants and continuously updating them with the latest scientific data.

*Table 1 Pathogenicity Classification of Somatic Variants and Functional Consequences.*

| Pathogenicity classes | Functional consequences |
|---|---|
| Class 1 - Benign | Normal functionality, high-frequency variant |
| Class 2 – Likely benign | Likely neutral effect after familial or functional studies, lower-frequency variant |
| Class 3 – VUS | Rare variant with potential effect on gene function based on biological knowledge or bioinformatics tools |
| Class 4 – Likely pathogenic | Likely to be linked to altered gene functionality |
| Class 5 - Pathogenic | Variant certain to disrupt gene function |

Abbreviations: VUS, variant of uncertain significance.

Certain mutated genes are highly specific to particular tumour types (e.g. *APC* in colorectal cancer); therefore, cancer origin needs to be considered when assessing variant pathogenicity (Table 2). Moreover, molecular profiling can be utilised as a tool to predict cancer origin when the primary tumour is unknown. Similarly, when detecting a gene variant that has never or rarely been reported in a given tumour type, considerable caution should be exercised in its interpretation. Furthermore, such an unexpected variant should prompt practitioners to consider a possible misdiagnosis or even an occult malignancy, especially if the molecular profile was obtained from ctDNA.

*Table 2 Gene Alterations Highly Specific to Particular Cancer Types.*

| Gene alteration | Cancer type |
|---|---|
| *APC* mutation | Colorectal cancer<br>Gastric cancer |
| *BCR–ABL1* translocation | Chronic myeloid leukaemia |
| *EWSR1–FLI1* translocation | Ewing sarcoma |
| *KIT* mutation | GIST<br>Melanoma |
| *PDGFRA* mutation | GIST |
| *RET* mutation | Medullary thyroid carcinoma |
| *RET–PTC* translocation | Papillary thyroid carcinoma |

Abbreviation: GIST, gastrointestinal stromal tumour.

Finally, several pathogenic variants are associated with various molecular consequences, such as high TMB in cases of *POLE* mutation, MSI in cases of mutations in MMR genes, or high genomic instability score (GIS) in cases of *BRCA1/2* mutations, and suggestive of HRD. Therefore, a variant that is associated with its anticipated molecular effect is more likely to be pathogenic, whereas, in the absence of this phenotype, the variant is more likely to be benign. Additionally, in tumours with high TMB, the interpretation of individual variants requires particular caution, as many alterations may represent passenger mutations with limited to no contribution to tumourigenesis.

### Actionable Genes and 'Druggability'

An actionable gene (or molecular driver) is defined as a gene for which one or more pathogenic alterations have demonstrated clinical significance and can be targeted with personalised interventions. Typically, these genes play a critical role in the development, progression or maintenance of cancer, and their alterations can provide predictive or prognostic information (Table 3).

The list of actionable genes for a particular cancer type is limited and subject to practice guidelines, particularly for the most prevalent genes, concerning the diagnosis and management of these subpopulations *(e.g. EGFR*-mutated non-small-cell lung cancer, *ERBB2*-amplified breast cancer, *BRCA1/2*-mutated ovarian cancer).

However, the continuous discovery of novel, uncommon targets and the emergence of tumour-agnostic alterations, which could potentially provide alternative therapeutic options, have driven the use of large-panel sequencing. This has resulted in an increased number of actionable genes identified for each cancer type. Several studies have already demonstrated the clinical benefit of molecularly driven oncology on a tumour-specific basis, supporting the value of targeted treatments tailored to individual molecular profiles. The benefit of tumour-agnostic (basket) approaches has also been demonstrated but requires a higher threshold of efficacy for regulatory approval. Therefore, when interpretating a genomic profiling report, it is essential to consider the most relevant and validated

actionable genes for the specific cancer type, while remaining open to the potential utility of tumour-agnostic molecular targets such as *NTRK*, TMB-high and MSI.

*Table 3 List of Actionable Genes and the FDA-approved Molecularly Matched Drug.*

| Genes | Druggable gene alterations | FDA-approved therapeutics * |
|---|---|---|
| *AKT1* | Mutation | Capivasertib (2023) |
| *ALK* | Fusion | Crizotinib (2011), ceritinib (2014), alectinib (2015), brigatinib (2017), lorlatinib (2018), ensartinib (2024) |
| *BCR–ABL1* | Fusion | Imatinib (2001), dasatinib (2006), nilotinib (2007), ponatinib (2012) asciminib (2024) |
| *BRAF* | Mutation | Vemurafenib (2011), dabrafenib (2013), binimetinib + encorafenib (2023) |
| *BRCA1/2* | Mutation | Olaparib (2014), rucaparib (2016), niraparib (2017), talazoparib (2018) |
| *EGFR* | Mutation | Gefitinib (2003), erlotinib (2004), afatinib (2013), osimertinib (2015) dacomitinib (2018), lazertinib (2024), amivantamab (2024) |
| *ERBB2* | Mutation, amplification | Trastuzumab (1998), lapatinib (2007), pertuzumab (2012), T-DM1 (2013), neratinib (2017), T-DXd (2019), tucatinib (2020) zanidatamab (2024) |
| *ESR1* | Mutation | Elacestrant (2023) |
| *EZH2* | Mutation | Tazemetostat (2020) |
| *FGFR2* | Fusion, mutation | Erdafitinib (2019) , pemigatinib (2020) |
| *FLT3* | Mutation | Midostaurin (2017), gilteritinib (2018), quizartinib (2023) |
| *IDH1/2* | Mutation | Enasidenib (2017), ivosidenib (2018), vorasidenib (2024) |
| *KIT* | Mutation, fusion | Imatinib (2002), avapritinib (2020), ripretinib (2020) |
| *KMT2A* | Translocation | Revumenib (2024) |
| *KRAS* | Mutation | Sotorasib (2021), adagrasib (2022), avutometinib + defactinib (2025) |
| *MET* | Mutation, fusion | Cabozantinib (2012), capmatinib (2020), tepotinib (2021) |
| *NRG1* | Fusion | Zenocutuzumab (2024) |
| *NTRK* | Fusion | Larotrectinib (2018), entrectinib (2019) |
| *PDGFRA* | Mutation | Avapritinib (2020) |
| *PIK3CA* | Mutation | Idelalisib (2014), alpelisib (2019), capivasertib (2023), inavolisib (2024) |
| *PTEN* | Mutation | Capivasertib (2023) |
| *RET* | Mutation, fusion | Selpercatinib (2020), pralsetinib (2020) |
| *ROS1* | Fusion | Crizotinib (2016), entrectinib (2019), repotrectinib (2023) taletrectinib (2025) |

Abbreviations: FDA, U.S. Food & Drug Administration; T-DM1, trastuzumab emtansine; T-DXd, trastuzumab deruxtecan.

*FDA approvals until 11 June 2025. For current labelling information, please visit https://www.fda.gov/drugsatfda

Pathogenic gene alterations can be druggable in several different ways. First, a mutated gene can encode a new protein that can be specifically targeted, distinguishing it from the wild-type protein (e.g. *BCR–ABL1* fusion gene).

Second, a mutated oncogene may cause the activation of downstream pathways through a gain-of-function mutation or gene amplification (e.g. *KRAS, EGFR, PIK3CA* mutations, *ERBB2* amplification). These activated genes drive the activation or increased activity of the pathway, leading to the cancer's dependency on this pathway. In such cases, inhibitors can disrupt this oncogenic process (e.g. through tyrosine kinase inhibition) or exploit the overexpression of the protein to selectively target and kill cancer cells carrying this alteration.

Third, deleterious mutations in tumour suppressor genes can create novel molecular dependencies that can be targeted via a synthetic lethality approach (e.g. *BRCA1/2* mutations). Indeed, a deficient pathway results in a high dependency on compensatory biological mechanisms in cancer cells. This alternative cellular equilibrium can be disrupted by inhibitors, leading to fatal cellular damage.

As noted, certain genetic alterations are strongly correlated with specific molecular consequences that can be directly measured. Therefore, the detection of a genomic scar, even in the absence of a known molecular culprit, can represent a promising therapeutic target (e.g. high GIS in the absence of *BRCA1/2* mutation).

Finally, some genetic alterations are responsible for specific immune responses that can be subsequently targeted with immunotherapeutic agents. For example, deleterious mutations affecting the MMR pathway or DNA polymerase ε *(POLE)* lead to the accumulation of hundreds of somatic mutations. This accumulation generates high neoantigen exposure and promotes a strong immunogenic response that can be therapeutically exploited.

## Clinical Evidence (ESCAT Classification) and Molecular Tumour Boards

The immense variety of molecular alterations detected, the complexity of their molecular consequences, the exponential increase in molecularly matched drugs, and the broad spectrum of druggability, from experimental drugs with limited preclinical evidence to phase III-approved drugs, highlight the need for a standardised scale of actionability. This scale should be based on the level of clinical evidence associated with each alteration. Several ranking tools have been developed in recent years to standardise the interpretation of pathogenic variants. These tools aim to facilitate clinical decision-making by prioritising variants based on their predicted functional impact, oncogenic potential and relevance to existing targeted therapies.

The ESMO Scale of Clinical Actionability for molecular Targets (ESCAT) is a standardised, six-level, evidence-based classification system for molecular alterations (Table 4). This framework represents a valuable tool to help clinicians prioritise the use of targeted therapies and identify patients who are most likely to benefit from precision medicine approaches. However, it is important to note that the level of evidence is highly dynamic and subject to change over time, necessitating regular re-evaluation. Additionally, the ESCAT system does not assess the depth of clinical benefit of therapeutics. To address this, complementary tools such as the ESMO Magnitude of Clinical Benefit Scale (ESMO-MCBS) have been developed to quantify the therapeutic impact of treatments, integrating survival outcomes and quality of life.

*Table 4 Simplified Version of the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT).*

*Adapted from Mateo J, Chakravarty D, Dienstmann R, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Ann Oncol 2018; 29:1895–1902.*

| ESCAT level of evidence | Clinical significance |
|---|---|
| ESCAT I | There is a molecularly matched drug that demonstrated improved survival outcomes in clinical trials in this tumour type |
| ESCAT II | The molecularly matched drug has been associated with antitumour activity in prospective/retrospective reports in this tumour type, but the magnitude of benefit compared to standard therapeutic options is unknown |

*Table 4 Simplified Version of ESCAT.* (Continued)

| ESCAT level of evidence | Clinical significance |
|---|---|
| ESCAT III | There is a suspected molecularly matched drug based on the efficacy observed in another tumour type or in another similar molecular alteration |
| ESCAT IV | No clinical evidence of efficacy of the drug has been reported but there is *in vitro/in vivo* preclinical evidence of activity |
| ESCAT V | The molecularly matched drug does not prolong survival outcomes, however there is evidence supporting combinatory approaches |
| ESCAT X | Lack of evidence for actionability |

In the context of this complex interpretation of actionability, cancer centres have implemented molecular tumour boards (MTBs) to harmonise practices and expand the potential beneficiaries of molecular medicine. MTBs consist of multidisciplinary panels of expert clinicians (oncologists, radiologists and pathologists), biologists (geneticists, molecular biologists) and bioengineers (biostatisticians, bioinformaticians). The objective of MTBs is to review and interpret the molecular alteration(s) detected in each patient within the context of the tumour type, the anticipated functional effect of the variant, drug accessibility and patient's background, in order to make informed treatment recommendations. Although the clinical value for patients is still being investigated, it is anticipated that the role of MTBs will increase significantly in the near future.

## Limitations and Perspectives

Although the amount of molecular information provided to physicians keeps increasing, only a minority of patients derive significant benefit from it. While the proper identification of druggable variants is continually refined and updated through new tools and processes such as MTBs, drug accessibility remains a critical element in implementing precision medicine throughout the patient's journey. In the absence of a regulatory approved, molecularly matched drug, guiding a patient towards a personalised treatment often necessitates participation in clinical trials or the off-label use of drugs. Consequently, molecular medicine has blurred the traditional boundaries between research and classical care, frequently challenging our evidence-based decisions and presenting as many questions as opportunities.

Beyond identifying molecular drivers, the in-depth characterisation of tumours, accelerated by continuous advances in molecular biology techniques, has the potential to unravel the pivotal cellular processes that could influence treatment decisions. However, achieving this comprehensive understanding will likely require the integration of multiple omics datasets, which can probably be most effectively managed and interpreted by deep-learning frameworks. Consequently, the future of oncology will necessitate mastery of both novel molecular biology technologies and innovative approaches leveraging artificial intelligence.

In parallel, it is essential that regulatory authorities acknowledge the growing relevance of genomic biomarkers in guiding precision therapies and adapt their frameworks to support the dynamic integration of emerging molecular targets. This includes facilitating the cross-utilisation of established targeted therapies, as well as expediting the approval of novel agents when supported by robust biomarker-driven evidence. Such flexibility will be critical to ensure timely patient access to the most appropriate therapeutic options in an era of increasingly personalised cancer care.

## Further Reading


Knudson AG, Meadows AT, Nichols WW, Hill R. Chromosomal deletion and retinoblastoma. N Eng J Med 1976; 295:1120–1123.

Le Tourneau C, Delord JP, Gonçalves A, et al. SHIVA investigators. Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. Lancet Oncol 2015; 16:1324–1334.

Massard C, Michiels S, Ferté C, et al. High-throughput genomics and clinical outcome in hard-to-treat advanced cancers: results of the MOSCATO 01 trial. Cancer Discov 2017; 7:586–595.

Mateo J, Chakravarty D, Dienstmann R, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Ann Oncol 2018; 29:1895–1902.

O'Dwyer PJ, Gray RJ, Flaherty KT, et al. The NCI-MATCH trial: lessons for precision oncology. Nat Med 2023; 29:1349–1357.

Patch C, Middleton A. Genetic counselling in the era of genomic medicine. Br Med Bull 2018; 126:27–36.
Tsimberidou AM, Kahle M, Vo HH, et al. Molecular tumour boards — current and future considerations for precision oncology. Nat Rev Clin Oncol 2023; 20:843–863.
Van Allen EM, Wagle N, Levy MA. Clinical analysis and interpretation of cancer genome data. J Clin Oncol 2013; 31:1825–1833.
Van de Haar J, Roepman P, Andre F, Balmaña, et al. ESMO recommendations on clinical reporting of genomic test results for solid cancers. Ann Oncol 2024; 35: 954–967.
Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 2017; 23:703–713.

## Declaration of Interest:

**Dr Blanc-Durand** has declared personal advisory board fees from AstraZeneca; personal speaker remuneration from Eisai; and a travel grant from GSK. He is a member of the Board of Directors for Cureety; and received institutional research funding from AstraZeneca, Daiichi Sankyo and GSK.

**Dr Cartwright** has declared personal advisory board fees from Pfizer and financial support from Servier to attend an ESMO preceptorship.

**Dr George** – see page xii

**Dr D.S.P. Tan** – see page xii

# Cancer Predisposition Syndromes

2

E. Harrold[1]

Z.K. Stadler[2]

[1]*Trinity St. James's Cancer Institute, Dublin, Ireland*
[2]*Clinical Genetics Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA*

Cancer predisposition syndromes are caused by inherited germline genetic mutations conferring heightened susceptibility to specific cancers. The spectrum of these syndromes is defined by gene penetrance (and associated cancer risk) and the distinct histological, immunohistochemical and molecular features of the component tumours. Identification of cancer predisposition syndromes is an integral component of contemporary cancer care. It facilitates the implementation of individualised surveillance recommendations and cancer risk-reduction strategies, and enables the therapeutic exploitation of underlying germline mutations, as evidenced by the efficacy of poly(ADP-ribose) polymerase (PARP) inhibitors in patients with germline pathogenic variants (gPVs) in *BRCA* genes. Efficacy of these agents in patients with pathogenic variants (PVs) in other homologous recombination deficiency (HRD) genes is less clearly established, and as such not yet included in clinical guidelines.

## Diagnosis

Multigene panel testing has largely replaced single-gene or syndrome-based testing. Multigene testing has demonstrated that if classical criteria for testing were applied, a significant proportion (up to 50%) of patients harbouring gPVs would not be identified. Universal germline genetic testing, regardless of family history, is now recommended for multiple cancer types including ovarian, pancreatic and high-risk or metastatic prostate cancer. Multiple guidelines now recommend *BRCA1/2* testing

in **all** patients with breast cancer ≤65 years old, acknowledging that in patients 50–65 years old the majority of gPVs identified may be in moderate penetrance genes. Given increased awareness, affordability, access and actionability of germline genetic testing, a gradual transition towards both tumour testing for somatic genetic alterations as well as genetic testing of normal (germline) DNA in all patients with cancer is anticipated.

## *BRCA* and the Homologous Recombination Genes

Homologous recombination repair (HRR) represents the most precise and high-fidelity DNA damage repair system for the repair of DNA double-strand breaks. Functional defects in HRR are called HRD. The most well-known causes of HRD are loss-of-function mutations in genes involved in HRR including *BRCA1*, *BRCA2*, *PALB2* and *RAD51C/D*, as well as promoter hypermethylation of *BRCA1* and *RAD51C*.

### *BRCA1/BRCA2*

When *BRCA1* (located on chromosome 17q 21.31) and *BRCA2* (located on chromosome 13q 13.1) are functioning normally, they are critically involved in the process of DNA repair. If either is mutated, double-strand breaks in DNA cannot be repaired, resulting in genomic instability and subsequent oncogenic transformation of non-tumourigenic cells into tumour-initiating cells, which lack normal restrictions on cell growth. Germline *BRCA1/2* (g*BRCA1/2*) PVs can be inherited from either parent in an autosomal dominant fashion, with each child of a parent with a g*BRCA1/2* PV having a 50% chance of inheriting the PV. Approximately 1 in 381 people in the general population have a gPV in *BRCA1*, and 1 in 277 in *BRCA2*, with higher variant frequencies in certain ethnic populations (i.e. Ashkenazi Jewish, Icelandic or Finnish).

#### Breast Cancer and Ovarian Cancer Risk in *BRCA1/2* Mutation Carriers

While the pivotal Breast Cancer Linkage Consortium (BCLC) studies were the first to comprehensively estimate lifetime cancer risk in *BRCA1/2* mutation carriers, importantly noting the differential risk between g*BRCA1* and g*BRCA2* mutations, the ascertainment of individuals with a significant

family cancer history likely overestimated cancer risk. In more recent large meta-analyses, the most frequently quoted breast cancer risk estimates predict that women with a g*BRCA1* PV have a 55%–72% risk of developing breast cancer by 70–80 years of age, and women with a g*BRCA2* PV have a 45%–69% risk over the same period. 39%–44% of women with g*BRCA1* PVs and 11%–17% of women with g*BRCA2* PVs will develop ovarian cancer by 70–80 years of age. Breast and ovarian cancer risks according to the individual's age (ranging by 10-year intervals) have been reported by several studies and are especially useful for patient counselling and timing of risk-reducing surgeries.

Approximately 80% of breast cancers arising in g*BRCA1* PV carriers will be triple-negative (specifically basal subtype), however, breast cancers arising in g*BRCA2* PV carriers are more heterogeneous and inclusive of hormone-positive disease. Tubo-ovarian cancers arising in g*BRCA1/2* PV carriers are most commonly high-grade serous carcinomas. It has been established that these tumours predominantly start in the fimbrial end of the fallopian tube; the original observation in 1999 that these serous ovarian cancers had the histological appearance of fallopian tube epithelium and furthermore, that serous tumours of the ovary, fallopian tube and peritoneum exhibited similar biological and clinical behaviour, has been validated in subsequent studies. Detailed examination of the fallopian tube specimens of g*BRCA1/2* PV carriers undergoing risk-reducing salpingo-oophorectomy (RRSO) identified early precursor lesions, serous tubular intraepithelial carcinomas (STICs), which further corroborated this theory of origin. The use of PARP inhibitors in *BRCA*-associated breast and ovarian cancer is predicated on the synthetic lethality that occurs in the presence of a g*BRCA* mutation, and on the synergistic inhibition of the compensatory PARP-mediated repair that would otherwise function to maintain DNA repair.

Breast cancer screening in women with g*BRCA1/2* PV comprises annual magnetic resonance imaging (MRI) aged 25 to 39 years, and, from the age of 40 to 75 years, annual mammogram and breast MRI screening with and without contrast. Consideration of bilateral risk-reducing mastectomy (BRRM) is also recommended. Regarding ovarian cancer risk, for which

no reliable screening modality has been established, the recommendation for risk-reducing surgical procedures is based on age-related risk for ovarian/fallopian tube cancer. Among carriers of g*BRCA1*, RRSO is recommended between 35 and 40 years. Among carriers of g*BRCA2*, as ovarian cancer onset is an average of 8–10 years later than in g*BRCA1* PV carriers, it is reasonable to delay RRSO until age 40–45 years, unless the age at diagnosis of ovarian cancer in a family member warrants earlier consideration. The challenge with this approach is the iatrogenic induction of premature menopause and its associated complications. Integrating more contemporary understanding of the tubal origin of these high-grade serous ovarian cancers has led to interest in salpingectomy with delayed oophorectomy (SDO). Kwon et al. (2013) assessed the costs and benefits of three risk-reducing strategies in g*BRCA* PV carriers who had not yet had breast or ovarian cancer: (1) bilateral salpingo-oophorectomy (BSO) at age 40 years, (2) bilateral salpingectomy at age 40 years, and (3) bilateral salpingectomy at age 40 years followed by bilateral oophorectomy at age 50 years (SDO). The comparison found that BSO was associated with the lowest cost and highest life expectancy. However, when quality-of-life measures were included, bilateral salpingectomy followed by delayed oophorectomy yielded the highest quality-adjusted life expectancy. A number of prospective trials are investigating SDO, including the Women Choosing Surgical Prevention (WISP) study (NCT02760849), the Early Salpingectomy (TUbectomy) with Delayed Oophorectomy in g*BRCA1/2* PV Carriers (TUBA) study (NCT02321228), the PROTECTOR trial (UK: ISRCTN25173360), and the SOROCk study (NCT04251052). Long term follow-up will be required to determine whether this approach is comparable from the perspective of ovarian cancer risk reduction.

## HRD Cancer Predisposition Syndromes and RRSO

When considering RRSO, it is critical that counselling reflects the most accurate estimates of ovarian cancer risk. In the absence of reliable screening modalities for ovarian cancer, the benefit of RRSO is well established in women harbouring a gPV in *BRCA1/2* as well as *BRIP1* and *RAD51C/D*. However, for women with PVs in *PALB2* and *ATM,* RRSO

is particularly controversial, as more recent studies have consistently demonstrated that increased risk of ovarian cancer is dependent on family history; this risk should be considered on an individual basis rather than being driven by uniform recommendations for all carriers.

Other major cancer susceptibility genes (CSGs) associated with breast and/or ovarian cancer with high-risk surveillance and/or risk-reducing recommendations are listed in Table 1.

*Table 1 Non-BRCA HRD Genes and Respective Associated Cancer Risk.*

| Gene | Associated cancer risk | Surveillance and/or risk-reducing recommendation |
|---|---|---|
| *ATM* | Absolute breast cancer risk: 20%–30% | ■ Annual mammogram from age 40 onwards; consider breast MRI with and without contrast from age 30–35 onwards according to a personalised breast cancer risk assessment inclusive of other risk factors<br>■ Insufficient evidence to recommend routine RRM |
| | Absolute ovarian cancer risk: 2%–3% | Insufficient level of evidence to recommend routine RRSO |
| | Absolute pancreatic cancer risk: ~5%–10% | Screening for pancreatic cancer could be considered particularly if feasible in the context of a clinical trial and if family history of pancreatic cancer in FDR/SDR |
| *BARD1* | Absolute breast cancer risk: 17%–30% | ■ Annual mammogram and consider breast MRI with and without contrast starting at age 40, based on personalised cancer risk assessment inclusive of other risk factors<br>■ Insufficient evidence for RRM, manage based on family history |
| | No established association with ovarian cancer | N/A |
| *BRIP1* | Insufficient data to define absolute risk | Insufficient data to define, management based on family history |
| | Absolute ovarian cancer risk: 5%–15% | RRSO recommended from age 45–50 years |
| *CHEK2* | Absolute breast cancer risk: 23%–27% | ■ Annual mammogram from age 40 onwards; consider breast MRI with and without contrast from age 35–40 onwards, according to a personalised breast cancer risk assessment inclusive of other risk factors<br>■ Insufficient evidence to recommend routine RRM |
| | No established association with ovarian cancer | N/A |

*Table 1 Non-BRCA HRD Genes and Respective Associated Cancer Risk.* (Continued)

| Gene | Associated cancer risk | Surveillance and/or risk-reducing recommendation |
|---|---|---|
| *PALB2* | Absolute breast cancer risk: 32%–53% | ■ Annual mammogram and breast MRI with and without contrast from age 30<br>■ Reasonable to discuss RRM |
| | Absolute ovarian cancer risk: 3%–5% | Reasonable to consider RRSO from age 45–50 years |
| | Absolute pancreatic cancer risk: 2%–5% | Screening for pancreatic cancer could be considered, particularly if feasible in the context of a clinical trial and if family history of pancreatic cancer in FDR/SDR |
| *RAD51C & RAD51D* | Breast cancer risk: conflicting evidence; consistent association with triple-negative breast cancer | ■ Mammogram and consideration of breast MRI from age 40, based on personalised cancer risk assessment including other risk factors<br>■ RRM not currently recommended |
| | Absolute ovarian cancer risk:<br>■ *RAD51C*: 11%<br>■ *RAD51D*: 13% | RRSO recommended |

Abbreviations: FDR, first-degree relative; HRD, homologous recombination deficiency; MRI, magnetic resonance imaging; N/A, not applicable; RRM, risk-reducing mastectomy; RRSO, risk-reducing salpingo-oophorectomy; SDR, second-degree relative.

## Prostate and Pancreatic Cancer Risk in *BRCA1/2* Mutation Carriers

The initial BCLC studies suggested an association between g*BRCA1/2* PV and prostate and pancreatic adenocarcinomas (particularly for g*BRCA2*), and subsequent research confirmed these associations. Multiple more recent studies found the prevalence of a gPV in exocrine pancreatic neoplasms to range from 3.9% to 19.8%, based on the population assessed and the numbers of genes tested. In one of the largest and most heterogeneous cohorts, Hu et al. (2018) reported gPVs in six genes (*CDKN2A*, *BRCA1/2*, *TP53*, *MLH1*, *ATM*) among 5.5% of all patients with pancreatic cancer, and this included patients both with and without a family history of the disease. The degree of pancreatic cancer risk varies according to the gene implicated, with higher risks associated with gPVs in *CDKN2A*, *BRCA2* and *ATM*.

Appreciation of pancreatic cancer risk in carriers of gPVs, including *BRCA1/2*, has prompted debate on the value of pancreatic cancer screening, with a recent study demonstrating the potential to detect earlier-stage tumours, and potentially an improvement in survival. In September 2024 the updated National Comprehensive Cancer Network (NCCN) guidelines recommended consideration of pancreatic screening in higher-risk individuals with specific gPVs.

*BRCA*-associated pancreatic cancers are predominantly ductal adenocarcinomas, although pancreatic neuroendocrine tumours associated with g*BRCA2* PVs have been reported, and respond favourably to platinum-based chemotherapy in the first-line setting. Mirroring the efficacy of PARP inhibitors in other g*BRCA*-associated tumours, the POLO study of maintenance olaparib established the role of PARP-inhibitor maintenance therapy in the first-line setting for g*BRCA*-associated metastatic pancreatic cancer.

In addition to an increased lifetime risk of male breast cancer, men with a g*BRCA2* PV face up to a 19%–61% lifetime risk of developing prostate cancer. g*BRCA2*-associated prostate cancer tends to be more aggressive, occurs at a younger age, and is more likely to be metastatic at diagnosis. The use of PARP inhibitors in patients with metastatic castration-refractory prostate cancer harbouring g*BRCA2* represents an emerging effective treatment strategy.

## Gastric Cancer Risk in *BRCA1/2* Mutation Carriers

The possible association of g*BRCA1/2* with gastric cancer has been unresolved for some time. In a large cohort of over 5000 *BRCA*-families, the relative risk of gastric cancer was increased in both g*BRCA1* and g*BRCA2* PV carriers, 2.17 (95% confidence interval [CI]: 1.25–3.77) and 3.69 (95% CI: 2.4–5.67) fold, respectively (Li et al, 2022). However, this study had several limitations, such as the retrospective family-based nature, with self-reported cancer family history, which may be inaccurate and missing data regarding the age at diagnosis. Prospective studies with pathological confirmation have not shown

an association to date. At the time of publication these data are not robust enough to recommend routine surveillance gastroscopy in all g*BRCA* PV carriers. However, emerging evidence suggests a potential modification of g*BRCA*-associated gastric cancer risk by means of other environmental factors, such as *Helicobacter pylori (H. pylori)* infection, and screening and treatment for *H. pylori* in carriers of g*BRCA1/2* PVs would appear to be reasonable.

## Melanoma Risk in *BRCA1/2* Mutation Carriers

Several studies (Gumaste et al, 2015; Tuominen et al, 2016; Jonsson et al, 2019) suggested an increased melanoma risk associated with gPVs in *BRCA1/2*. However, in a larger study with integration of both somatic and germline sequencing, this association was not validated. There are no specific melanoma surveillance guidelines available at the time of publication.

## Li–Fraumeni Syndrome

Li–Fraumeni syndrome (LFS) is an autosomal dominant syndrome caused by gPVs in *TP53*. LFS confers an 80%–90% lifetime risk of cancer, with up to 21% of cancers occurring before age 15. Classical LFS-associated cancers include sarcoma, brain tumours, leukaemia, breast cancer and adrenocortical tumours. More recently, early-onset gastric cancer has been recognised as part of the spectrum of LFS-associated tumours.

Testing for suspected LFS is driven by clinical criteria. The classical criteria for LFS were proposed in 1988 and required a combination of an individual <45 years old with a sarcoma **and** a first-degree cancer-affected relative <45 years old **and** an additional first- or second-degree cancer-affected relative <45 years old, **or** a sarcoma at any age. The Chompret criteria were proposed in 2001 and modified in 2015. These criteria for testing broadly relate to a diagnosis of cancer(s) from the LFS spectrum (soft tissue sarcoma, osteosarcoma, central nervous system tumour, breast cancer, adrenocortical tumour), age of the patient

at diagnosis and/or number of first- or second-degree relatives with an LFS-spectrum cancer.

With the advent of precision oncology and increased somatic tumour testing, *TP53* PVs are some of the most common findings on tumour-only analysis. While most are somatic variants, germline testing in cancer-affected individuals with a *TP53* PV identified on tumour-only genomic testing should be considered, particularly if they meet any of the Chrompret criteria or have a personal history of any cancer before the age of 30.

Recommended surveillance includes annual whole-body MRI with diffusion weighting, upper and lower endoscopy every 2 to 5 years from 25 years old (or 5 years earlier than the earliest diagnosis of a relative with colorectal or gastric cancer) and, among female carriers, annual breast MRI starting from age 20–29, along with consideration of bilateral mastectomy. Prostate-specific antigen (PSA) screening should be carried out in male carriers from age 40. Annual skin surveillance is also recommended.

## Lynch Syndrome

Lynch syndrome (LS) is a pan-cancer predisposition syndrome associated with a high lifetime risk of both synchronous and metachronous cancers. Population prevalence is estimated to be 1 in 279, and it is significantly underdiagnosed. It is defined by inactivating gPVs in the DNA mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*) and terminal deletions in *EPCAM*, conferring elevated risks of synchronous and metachronous cancers. Inactivation of the second wild-type allele causes impaired genome maintenance and renders cells unable to repair certain classes of mutations occurring during DNA replication. Over time, this results in high tumour mutational burden (TMB) and enrichment of mutations in microsatellites, termed microsatellite instability (MSI). This high TMB, enriched for mutations encoding immunogenic proteins, sensitises these LS-associated MMR-deficient (dMMR) tumours to immune checkpoint blockade in both the early and metastatic settings.

The classical canonical tumours associated with LS are colorectal cancer (CRC) and endometrial cancer. Historically, testing for LS was based on clinical criteria, such as the Amsterdam II criteria and the Revised Bethesda guidelines, that take into consideration tumour type, age at diagnosis, family history and other clinical features. The application of these clinical criteria was not only cumbersome but also missed a significant proportion of LS patients. Universal immunohistochemical analysis for loss of the four MMR proteins is recommended as a screening mechanism for LS in patients with endometrial cancer and CRC, given the proportion of patients that are missed when applying clinical criteria. Additionally, this now has implications for treatment with immunotherapy. Since 2017, online evidence-based risk-prediction tools, such as the Prediction Model for gene Mutations ($PREMM_5$) can help identify individuals for LS testing. Additionally, it is increasingly recognised that MSI is associated with LS pan-cancer, with ~16% of cancer patients with an MSI-high tumour harbouring LS, inclusive of tumours not classically part of the LS spectrum. Focusing on non-canonical LS-associated tumours, cumulative incidences at 75 years for upper gastrointestinal cancers (gastric [non-diffuse], duodenal, bile duct or pancreatic) among patients with LS were 10%, 17% and 13% in *MLH1*, *MSH2* and *MSH6* PV carriers, respectively, per the Prospective Lynch Syndrome Database.

LS-associated cancer risks differ according to the underlying MMR variant, for example, patients with *MLH1*-associated LS have the greatest risk of CRC. As such, NCCN and ESMO (European Society for Medical Oncology) guidelines for cancer surveillance in patients with LS are now MMR gene-specific. In general, lower CRC risk is associated with *PMS2* and *MSH6*, leading to some guidelines recommending a delay in age of initiation of colonoscopy surveillance and reduced frequency. Extracolonic cancer risks seem to be most pronounced in *MSH2* carriers, but, unfortunately for many of the associated cancers, no efficacious surveillance currently exists.

## Familial Adenomatous Polyposis Syndrome

Familial adenomatous polyposis (FAP) is caused by gPVs in the *APC* gene (g*APC* PV). This autosomal dominant condition is characterised by the development of hundreds to thousands of adenomas in the colorectum. In its classical form, risk of CRC is clearly defined at 100% with the average age of diagnosis being 39 years. This CRC risk underpins the recommendation for prophylactic colectomy when a g*APC* PV is confirmed. Other associated risks include a more modest association with gastric cancer, and the otherwise rare cribriform-morular variant of papillary thyroid cancer. Non-malignant associations are listed in Table 2.

*Table 2 Polyposis Syndromes and Colorectal Cancer.*

| Gene | Function | CRC and polyp phenotype | Other cancers and associations |
|---|---|---|---|
| *Autosomal dominant CRC and polyposis syndromes* | | | |
| *Lynch syndrome (MLH1, MSH2, MSH6, PMS2)* | DNA MMR gene | Early-onset CRC | Endometrial, urothelial, ovarian, gastric, small bowel and pancreatic cancers, sebaceous neoplasms, skin cancers |
| *Familial adenomatous polyposis (APC)* | Tumour suppressor gene | Adenomatous polyposis, CRC | Gastric cancer, thyroid cancer, desmoids, osteomas, dental abnormalities CHRPE, benign cutaneous lesions |
| *POLE/POLD1* | Proofreading deficiency | Adenomatous polyposis, CRC | Endometrial cancer, possibly others |
| *Li–Fraumeni syndrome (TP53)* | Tumour suppressor gene | Early-onset CRC | Pan-cancer, early-onset soft tissue sarcoma, osteosarcoma, central nervous system tumours, breast cancer, adrenocortical cancer, gastric cancer |
| *GREM1* | Encodes for members of the BMP antagonist family<br>Tumour promotion/tumour suppressor function | Mixed polyposis, CRC | N/A |
| *Juvenile polyposis syndrome (BMPR1A/SMAD4)* | ■ *BMPR1A*: encodes BMP receptor 1A, which binds to ligands in the TGFβ pathway and activates SMAD proteins. Activated SMAD protein complex regulates cell growth and division<br>■ *SMAD4*: transcription factor, tumour suppressor | Hamartomatous juvenile polyposis, CRC | Gastric cancer and polyposis, especially in g*SMAD4* |

*Table 2 Polyposis Syndromes and Colorectal Cancer.* (Continued)

| Gene | Function | CRC and polyp phenotype | Other cancers and associations |
|---|---|---|---|
| *Autosomal recessive CRC and polyposis syndromes* | | | |
| *MUTYH-associated polyposis (biallelic MUTYH)* | Base-excision repair gene | Polyposis, CRC | Upper gastrointestinal polyposis, small bowel cancer<br>Less common: thyroid, ovarian and bladder cancer |
| *NTHL1 tumour syndrome (biallelic NTHL1)* | Base-excision repair gene | Polyposis, early-onset CRC | Endometrial cancer, cervical cancer, urothelial carcinoma of the bladder, meningiomas, unspecified brain tumours, basal cell carcinomas, head and neck squamous-cell carcinomas, haematological malignancies |
| *MBD4, biallelic* | Base-excision repair gene | Polyposis, CRC | Acute myeloid leukaemia, uveal melanoma |
| *MSH3, biallelic* | MMR gene | Polyposis, CRC | Unknown (gastric, brain) |
| *MLH3, biallelic* | MMR gene | Polyposis, CRC | Unknown |
| *CMMR-D (biallelic MLH1, MSH2, MSH6, PMS2)* | MMR gene (MSI-H) | Very early-onset CRC | Café-au-lait spots, brain tumours, leukaemia, lymphoma, small intestinal cancers, urothelial cancers, endometrial cancers |

Abbreviations: BMP, bone morphogenic protein; CHRPE, congenital hypertrophy of the retinal pigment epithelium; CRC, colorectal cancer; g, germline; MMR, mismatch repair; MSI-H, microsatellite instability-high; N/A, not applicable; TGFB, transforming growth factor beta.

As opposed to classic FAP, attenuated FAP is usually caused by mutations in the 5' or 3' end of the *APC* gene and has a highly variable phenotype with fewer colonic adenomas (<100), which tend to be proximally distributed and flat rather than polypoid. Management is individualised based on polyp burden.

In addition to LS and FAP, a variety of other CRC susceptibility genes have been described, with variable clinical phenotypes inclusive of both autosomal-dominant and autosomal-recessive modes of inheritance (Table 2).

## Identification of Potential Germline Mutations from Somatic Panels

With increasing use of tumour genomic analysis in patients with cancer, it is critical to accurately determine whether a PV is somatic or germline in origin. In 2019, the ESMO Precision Medicine Working Group published guidelines derived from analyses of paired sequencing data from 17 152 cancer samples, in which 1494 PVs were identified across 65 CSGs. The Working Group recommended germline-focused tumour analysis to be restricted to variants with an allele frequency >30% for single nucleotide variants, or >20% for small insertions/deletions. The recommendation for germline-focused tumour analysis in the 'off-tumour' context, defined as CSGs not previously demonstrated to confer predisposition to that specific tumour type, should be restricted to 'high-actionability CSGs'. These include *BRCA1/2*, *BRIP1*, *MLH1*, *MSH2*, *MSH2*, *MSH6*, *PALB2*, *PMS2*, *VHL*, *RAD51C/D*, *RET*, *SDHA*, *SDHAF2*, *SDHB*, *SDHC*, *TSC2*, *MUTYH*, *FLCN*, *FH*, *BAP1* and *POLE* in tumours arising at any age, and *RB1*, *APC*, *TP53* and *NF1* in tumours arising in individuals <30 years old. The guidelines recommended that germline-focused tumour analysis of 'standard actionability' CSGs should be restricted to the 'on-tumour' setting, and recessively acting 'high actionability-CSGs' (currently *MUTYH* alone) were recommended to be included for germline-focused tumour analysis, but reporting and germline follow-up testing should be undertaken only if two PVs are detected.

## Conclusion

Identification of a cancer predisposition syndrome has significant therapeutic relevance, it triggers cascade testing and instigation of appropriate screening schedules in other family members of patients with a gPV, thus allowing for targeted tumour interception.

## Further Reading

Amadou A, Achatz MIW, Hainaut P. Revisiting tumor patterns and penetrance in germline TP53 mutation carriers: temporal phases of Li–Fraumeni syndrome. Curr Opin Oncol 2018; 30:23–29.

Bedrosian I, Somerfield MR, Achatz MI, et al. Germline testing in patients with breast cancer: ASCO–Society of Surgical Oncology Guideline. J Clin Oncol 2024; 42:584–604.
Bougeard G, Renaux-Petel M, Flaman JM, et al. Revisiting Li-Fraumeni syndrome from TP53 mutation carriers. J Clin Oncol 2015; 33:2345–2352.
Gumaste PV, Penn LA, Cymerman RM, et al. Skin cancer risk in BRCA1/2 mutation carriers. Br J Dermatol 2015; 172:1498–1506.
Hu C, Hart SN, Polley EC, et al. Association between inherited germline mutations in cancer predisposition genes and risk of pancreatic cancer. JAMA 2018; 319:2401–2409.
Jakubowska A, Nej K, Huzarski T, et al. BRCA2 gene mutations in families with aggregations of breast and stomach cancers. Br J Cancer 2002; 87:888–891.
Jonsson P, Bandlamudi C, Cheng ML, et al. Tumour lineage shapes BRCA-mediated phenotypes. Nature 2019; 571:576–579.
Kuchenbaecker KB, Hopper JL, Barnes DR, et al. Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. JAMA 2017; 317:2402–2416.
Kwon JS, Tinker A, Pansegrau G, et al. Prophylactic salpingectomy and delayed oophorectomy as an alternative for BRCA mutation carriers. Obstet Gynecol 2013; 121:14–24.
Latham A, Srinivasan P, Kemel Y, et al. Microsatellite instability is associated with the presence of Lynch syndrome pan-cancer. J Clin Oncol 2019; 37:286–295.
Li S, Silvestri V, Goska L, et al. Cancer risks associated with *BRCA1* and *BRCA2* pathogenic variants. J Clin Oncol 2022; 40:1529–1541.
Lilyquist J, LaDuca H, Polley E, et al. Frequency of mutations in a large series of clinically ascertained ovarian cancer cases tested on multi-gene panels compared to reference controls. Gynecol Oncol 2017; 147:375–380.
Liu YL, Breen K, Catchings A, et al. Risk-reducing bilateral salpingo-oophorectomy for ovarian cancer: a review and clinical guide for hereditary predisposition genes. JCO Oncol Pract 2022; 18:201–209.
Lowry KP, Geuzinge HA, Stout NK, et al. Breast cancer screening strategies for women with ATM, CHEK2, and PALB2 pathogenic variants: a comparative modeling analysis. JAMA Oncol 2022; 8:587–596.
Mandelker D, Donoghue M, Talukdar S, et al. Germline-focussed analysis of tumour-only sequencing: recommendations from the ESMO Precision Medicine Working Group. Ann Oncol 2019; 30:1221–1231.
Mandelker D, Zhang L, Kemel Y, et al. Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal DNA vs guideline-based germline testing. JAMA 2017; 318:825–835.
Møller P, Seppälä TT, Bernstein I, et al. Cancer risk and survival in path_MMR

carriers by gene and gender up to 75 years of age: a report from the Prospective Lynch Syndrome Database. Gut 2018; 67:1306–1316.
NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Version 1.2026, July 10, 2025© 2025 National Comprehensive Cancer Network® (NCCN®), Genetic/Familial High-Risk Assessment: Breast, Ovarian, Pancreatic and Prostate Version 1.2026, July 10, 2025 — March 6, 2025 (last accessed 27 July 2025)
Nyberg T, Frost D, Barrowdale D, et al. Prostate cancer risks for male BRCA1 and BRCA2 mutation carriers: a prospective cohort study. Eur Urol 2020; 77:24–35.
Shindo K, Yu J, Suenaga M, et al. Deleterious germline mutations in patients with apparently sporadic pancreatic adenocarcinoma. J Clin Oncol 2017; 35:3382–3390.
Stadler ZK, Maio A, Chakravarty D, et al. Therapeutic implications of germline testing in patients with advanced cancers. J Clin Oncol 2021; 39:2698–2709.
Tischkowitz M, Balmaña J, Foulkes WD, et al. Management of individuals with germline variants in PALB2: a clinical practice resource of the American College of Medical Genetics and Genomics (ACMG). Genet Med 2021; 23:1416–1423.
Tuominen R, Engström PG, Helgadottir H, et al. The role of germline alterations in the DNA damage response genes BRIP1 and BRCA2 in melanoma susceptibility. Genes Chromosomes Cancer 2016; 55:601–611.
Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004; 96:261–268.
Vasen HF, Möslein G, Alonso A, et al. Guidelines for the clinical management of familial adenomatous polyposis (FAP). Gut 2008; 57:704–713.
Yang X, Leslie G, Doroszuk A, et al. Cancer risks associated with germline *PALB2* pathogenic variants: an international study of 524 families. J Clin Oncol 2020; 38:674–685.

## Declaration of Interest:

**Dr Stadler** has declared personal honoraria from JCO Precision Oncology and UptoDate in her role as Editor. She has also received institutional royalties from SOPHiA Genetics.

**Dr Harrold** has declared personal advisory board honoraria from Takeda. She has also received a personal educational grant from Novartis to attend the ESMO 2023 congress.

# Genomics in Lung Cancer and Head and Neck Cancer

3

D. Tan[1]
W.-C. Tan[1]
A. Chan[2]
J. Lam[2]
[1]*NCCS, National Cancer Centre Singapore, Singapore, Republic of Singapore*
[2]*Prince of Wales Hospital, Sha Tin, Hong Kong SAR, China*

The integration of genomics into oncology has transformed cancer diagnosis and treatment, including in lung cancer and head and neck cancer. Both cancer types exhibit significant molecular heterogeneity, necessitating tailored therapeutic approaches guided by genomic profiling.

Head and neck cancers, particularly head and neck squamous-cell carcinomas (HNSCCs), present a unique genomic landscape influenced by factors such as human papillomavirus (HPV) status. Compared with HPV-negative HNSCCs, HPV-positive HNSCCs are associated with better prognosis due to the action of HPV oncoproteins in the deregulation of cell-cycle control mechanisms. Nasopharyngeal carcinoma (NPC) has a distinct geographical and ethnic distribution compared with other types of head and neck cancers. While genomic analysis in head and neck cancer is gaining momentum, the identification of clinically actionable mutations is less established than for lung cancer. Furthermore, the integration of biomarkers such as tumour mutational burden (TMB) and HPV status into treatment decisions is increasingly recognised in head and neck oncology, reflecting a more nuanced approach to patient stratification.

In lung cancer, particularly non-small-cell lung cancer (NSCLC), the focus has predominantly been on identifying actionable oncogenic drivers, such as mutations in *EGFR*, *ALK* and *ROS1*. Routine molecular testing is now essential, as it guides the use of targeted therapies and

immunotherapies. Of note, the emergence of resistance mechanisms following targeted therapies necessitates ongoing molecular profiling to guide subsequent treatment strategies.

## Lung Cancer

Lung cancer comprises distinct disease subtypes, historically classified based on morphology, including NSCLC and small-cell lung cancer (SCLC). NSCLC is primarily classified into squamous and non-squamous (the majority being of adenocarcinoma histology), with the main implications being choice of cytotoxic chemotherapy and molecular testing. With the development of biomarker-directed systemic therapies, the diagnostic paradigm increasingly includes routine molecular testing and genomic profiling, especially for non-squamous NSCLC. For squamous-cell carcinoma (SCC) and SCLC, the role of next-generation sequencing (NGS) remains unclear, given the paucity of actionable targets.

The inherent range and heterogeneity in molecular alterations and testing platforms can often present challenges in interpreting biomarker data to guide treatment decisions. This section details some of the considerations and recommendations in the practice of precision oncology in lung cancer management.

### Key Molecular Features

Testing paradigms are most established in NSCLC, specifically in lung adenocarcinoma. The primary goal is to identify oncogenic drivers that are therapeutically tractable, with implications for frontline treatment options, especially *EGFR*, *ALK*, *ROS1*, *cMET* exon14 skipping mutations and *RET* alterations. As the pre-test probability for oncogenic drivers can vary, it is important to ascertain key clinicopathological features, such as smoking status, younger age (<50 years old) at diagnosis, and adenosquamous or sarcomatoid histological subtypes (specifically for *cMET* exon14 skipping mutations). Increasingly, programmed death-ligand 1 (PD-L1) expression, detected by immunohistochemistry (IHC), is used to predict response to immune checkpoint inhibitors (ICIs), especially in the absence of oncogenic drivers. In some tumours related to prior tobacco exposure, a high TMB may be observed, although it remains unclear if this is prognostic or predictive.

Another key feature is the emergence of therapeutic resistance after targeted therapies. While the mechanisms are not yet fully understood, common convergent concepts include on-target alterations that affect drug binding (e.g. T790M/C797X) with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), development of parallel bypass alterations (e.g *MET* amplification) and lineage transition (e.g. small-cell transformation). These features underscore the importance of post-resistance tissue biopsies and profiling, which European Society for Medical Oncology (ESMO) guidelines recommend be offered to patients with *EGFR*-mutated NSCLC who have progressed on osimertinib.

### Predictive and Personalised Strategies

In NSCLC, the identification of genomic alterations, found in 30%–70% of patients depending on geography and ethnicity, has significant implications for targeted therapeutics such as TKIs and antibody–drug conjugates (ADCs).

ESMO guidelines recommend mandatory testing for oncogenic drivers in non-squamous NSCLC, where targeted therapeutics are approved for routine use, with broader testing indicated to direct earlier access to drugs and clinical trials. Testing to inform personalised treatment with off-label use of therapeutics requires due consideration of the level of evidence for benefit, as defined in the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Emerging data also highlight the potential impact of co-existing alterations such as *TP53* and *STK11*, as well as TMB.

Targeted therapeutic options for NSCLC, based on individual genomic alterations, are detailed in Table 1.

In *EGFR*-mutated NSCLC, actionable resistance mechanisms with potentially targeted approaches include *MET* amplification (tepotinib, savolitinib, amivantamab–lazertinib) and histological small-cell transformation (etoposide-containing platinum-doublet chemotherapy).

Due to the inherent limitations of small biopsy specimens, molecular profiling using plasma circulating tumour DNA (ctDNA) is validated to guide therapy for advanced NSCLC as positive concordance with tissue sampling is generally high (reported sensitivity 80%; Leighl et al, 2019).

*Table 1 Actionable Alterations in NSCLC.*

Adapted from Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2024; 35:588–606.

| Gene | Alteration | ESCAT score | Therapeutic options |
|---|---|---|---|
| *EGFR* | Common mutations | IA | 1st generation EGFR-TKI (gefitinib, erlotinib)<br>2nd generation EGFR-TKI (afatinib, dacomitinib)<br>3rd generation EGFR-TKI (osimertinib, lazertinib)<br>EGFR–MET bispecific antibodies (amivantamab) |
| | Acquired p.T790M mutation | IA | 3rd generation EGFR-TKI (osimertinib) |
| | Exon 20 insertions | IA | EGFR-MET bispecific antibodies (amivantamab) |
| | Uncommon mutations (p.G719X, p.S768I, p.L861Q) | IA<br>IB | 2nd generation EGFR-TKI (afatinib)<br>3rd generation EGFR-TKI (osimertinib) |
| *ALK* | Fusions | IA | 1st generation ALK-TKI (crizotinib)<br>2nd generation ALK-TKI (ceritinib, alectinib, brigatinib, ensartinib)<br>3rd generation ALK-TKI (lorlatinib) |
| *ROS1* | Fusions | IB | Crizotinib, entrectinib, lorlatinib |
| *KRAS* | p.G12C | IA | Sotorasib, adagrasib |
| *RET* | Fusions | IA | Selpercatinib, pralsetinib |
| *MET* | Exon 14 skipping mutation | IB | Crizotinib, capmatinib, tepotinib, savolitinib |
| *BRAF* | p.V600E | IB | Dabrafenib + trametinib, encorafenib + binimetinib |
| *ERBB2* | Hotspot mutations | IIB | Pan-HER TKI<br>Trastuzumab deruxtecan |
| *NRG1* | Fusions | IIB | Anti-HER2/HER3 bispecific antibodies |

Abbreviations: ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; ESMO, European Society for Medical Oncology; HER, human epidermal growth factor receptor; MET, mesenchymal epithelial transition; NSCLC, non-small-cell lung cancer; TKI, tyrosine kinase inhibitor.

However, limitations in the sensitivity of plasma-based assays should be recognised and managed accordingly. Moreover, detecting fusions and copy number changes may be suboptimal in many ctDNA platforms. Non-informative test results should prompt reflex tissue testing,

particularly in patients with phenotypic characteristics suggestive of oncogene-driven disease.

The use of ctDNA assays to assess minimal residual disease (MRD) or suboptimal disease response, monitor for the development of resistance mechanisms before clinical progression, or as a screening tool in asymptomatic individuals remains under investigation.

## Recommendations for Genetic Testing

### *Metastatic disease*

Broad-panel NGS platforms are increasingly preferred as they can investigate multiple genes concurrently. Their widespread adoption has made the cost-effectiveness of upfront multigene testing comparable with sequential testing approaches in NSCLC. However, when prioritisation of molecular testing is necessary due to limited resources, sequential single-gene or hotspot-based panel approaches may be used to interrogate high-value biomarkers such as *EGFR*, *ALK* or *ROS1* alterations as a first pass. The role of NGS testing for squamous histology and SCLC is less clear since results are not clinically actionable.

In resource-limited settings it may be necessary to prioritise testing only in patients where the potential benefit is greatest. For example, phenotypic cohorts with higher incidence of oncogenic drivers, such as patients without history of tobacco exposure, or younger patients (<50 years old).

Aside from NGS, testing for alterations of significance may include more traditional methods of genomic sequencing, cytogenetic studies or IHC. In cases of inadequate tissue profiling, ctDNA may be used to identify actionable genomic alterations.

### *Early-stage disease*

Recent phase III trials have demonstrated the survival benefit of adjuvant targeted therapy in early-stage *EGFR*-mutated NSCLC (ADAURA trial, overall survival hazard ratio [HR] 0.49) and *ALK*-mutated NSCLC (ALINA trial, disease-free survival HR 0.24). When adjuvant therapy is indicated and available for the treatment of early-stage disease, molecular

profiling for *EGFR* and *ALK* is necessary. Data have also demonstrated the clinical benefit of perioperative ICIs, therefore comprehensive molecular profiling before treatment initiation is important to define personalised multimodal treatment strategies and to identify patients who may not benefit from this approach.

Molecular testing has an established role in the management of NSCLC in both the early and advanced settings. Given its biological and molecular heterogeneity, deeper genomic profiling is often essential to further understand the different disease subtypes at various stages of the treatment journey, so as to optimise patient outcomes through personalisation of therapy.

## Head and Neck Cancer

Head and neck cancer encompasses a heterogeneous group of malignancies that can be further classified based on their complex anatomical sites and histological characteristics. Among these, SCC is the predominant histological type. With the recognition of HPV as a significant risk factor for oropharyngeal carcinoma (OPC), it has become evident that HPV-associated HNSCCs exhibit distinct clinical and molecular features compared with their HPV-negative counterparts. Consequently, HPV-positive and HPV-negative HNSCCs are now considered separate entities. HPV-positive HNSCCs are generally associated with a better prognosis, which has led to clinical trials such as the NRG-HN005 trial aimed at evaluating various de-escalation strategies to optimise treatment for patients with HPV-positive OPC. Additionally, NPC constitutes a specific type of cancer originating in the nasopharynx, exhibiting a distinct geographical and ethnic distribution compared with other head and neck cancers. This nuanced understanding of the different types of head and neck cancers is crucial for the development of tailored therapeutic approaches and for improving patient outcomes.

### Key Molecular Features

#### *HPV-negative HNSCC*

HPV-negative HNSCC is marked by several early and frequent genetic alterations that play a critical role in its pathogenesis. Among these, the

loss of chromosomal regions 3p and 9p is prevalent, alongside mutations in the *TP53* gene, which occur in approximately 80% of cases. These mutations encompass a wide spectrum, including truncating mutations and well-characterised inactivating missense mutations. In addition to *TP53* mutations, inactivating alterations in the *CDKN2A* gene, which encodes the $p16^{INK4A}$ protein, are reported in about 50% of patients with HNSCC, but are uncommon in HPV-positive HNSCC. This protein disrupts the cyclin D–cyclin-dependent kinase 4 (CDK4) and cyclin D–cyclin dependent kinase 6 (CDK6) complexes, leading to impaired regulation of cell cycle arrest. A subset of HPV-negative HNSCCs exhibit very few copy number alterations (CNAs) and retain wild-type *TP53*. These are characterised as CNA-silent tumours and demonstrate a more favourable prognosis. Further research in this subgroup reveals activating mutations in *HRAS* and inactivating mutations in *CASP8*, which is more common in females with no history of smoking or alcohol consumption. Overall, the intricate landscape of genetic changes in HPV-negative HNSCC underscores the complexity of this malignancy and highlights the need for tailored therapeutic approaches.

### *HPV-positive HNSCC*

The oncogenic mechanisms of HPV are primarily driven by the deregulated expression of the E6 and E7 oncoproteins. E6 targets and inactivates the tumour suppressor p53, while E7 inactivates retinoblastoma protein (Rb), leading to significant disruption of cell-cycle regulation. These molecular events inhibit p53-mediated apoptotic responses, promoting cellular immortalisation and facilitating the accumulation of both epigenetic and genetic alterations that contribute to carcinogenesis. Of note, genes most frequently altered in HPV-negative HNSCC are largely unaffected in the HPV-positive fraction, including *TP53* and *CDKN2A*. This supports the notion that genetic and/or epigenetic alterations leading to inactivation of p53 and $p16^{INK4A}$–cyclin D1–Rb pathways are functionally equivalent to binding and inactivation of p53 and Rb by HPV E6 and E7. Molecular alterations in phosphatidylinositol-3-kinase (PI3K) pathway genes, particularly those involving activating mutations and amplifications of the oncogene *PIK3CA*, constitute the most common genetic changes in HPV-driven HNSCC.

### *Nasopharyngeal carcinoma*

NPC is particularly endemic in southern China and southeast Asia, where the undifferentiated subtype accounts for >90% of cases, with the other subtypes being keratinising SCC and non-keratinising SCC. The high incidence of NPC in these regions is attributed to a combination of genetic and environmental factors. Specific human leucocyte antigen (HLA) haplotypes and multiple germline variants in the major histocompatibility complex (MHC) region have been associated with increased genetic susceptibility to NPC, which may partially explain the geographical patterns in incidence. Additionally, certain high-risk variants within the Epstein–Barr virus (EBV) genome, such as *BALF2* and *EBER*, are implicated in the disease. The interplay between these unique genetic factors and EBV variants contributes to a heightened risk of persistent EBV infection and clonal expansion of EBV-infected epithelial cells. Early driver events in NPC include deletions in chromosomes 3p and 9p, leading to the inactivation of key tumour suppressor genes such as *CDKN2A* and *TGFBR2*, which facilitate persistent latent EBV infection (type II). The expression of various viral oncogenic proteins, including EBNA1, LMP-1 and LMP-2A, alongside non-coding RNAs such as EBV-encoded small RNAs (EBERs) and BART microRNAs, promotes global epigenetic changes in the host genome, ultimately triggering various cancer hallmarks. One predominant oncogenic driver in NPC is the dysregulation of the nuclear factor kappa B (NF-κB) signalling pathway. This complex interaction of genetic susceptibilities, viral factors and epigenetic modifications, underscores the multifaceted nature of NPC pathogenesis and highlights the need for comprehensive strategies in its diagnosis and treatment.

## Predictive Value and Personalised Strategies

With the advent of NGS technologies, the identification of potentially targetable genomic alterations in HNSCC has become more feasible. Although these actionable molecular alterations have shown promise in improving outcomes for other tumour types, their efficacy in HNSCC remains less clear. Consequently, molecular events that were unvalidated in terms of therapeutic efficacy in HNSCC must be approached with

caution, as there is a risk of worse outcomes when matched targeted therapies are applied without sufficient evidence. To assist clinicians in selecting appropriate patients for targeted treatment strategies, several tools have been proposed to rank actionable molecular alterations based on their levels of clinical evidence. One notable example is ESCAT, which provides a framework for assessing the clinical relevance of molecular targets. Additionally, an umbrella biomarker-driven study, EORTC-1559-HNCG, has been designed to evaluate the clinical efficacy of various targeted treatments in patients with recurrent or metastatic HNSCC, based on the molecular profiling of metastatic tumour biopsies.

Targetable genomic alterations in HPV-negative HNSCC include *EGFR*, *FGFR1–3*, *HER2*, *PIK3CA* and *HRAS*. Other potentially actionable cell-cycle genomic alterations are *TP53* mutation, cyclin D1 (*CCND1*) amplification and *CDKN2A* inactivation. In HPV-positive OPC, *PIK3CA* amplifications/mutations are found in about 50% of cases, whereas the other genomic alterations are rare. In NPC, the prevalence of actionable alterations is generally low. Given the prevalence of genomic alterations affecting cell-cycle control (such as *CDKN2A* inactivation and *CCND1* amplification), targets such as CDK4/6 and BCL3 warrant further evaluation.

### *EGFR target/ERBB family blockers*

Afatinib, an oral irreversible pan-human epidermal growth factor receptor (HER) TKI, demonstrated efficacy in patients with recurrent/metastatic HNSCC pre-treated with platinum, as illustrated in the LUX-Head & Neck 1 trial. Post-hoc analyses revealed that *EGFR* amplification and p16/ErbB3-negativity may be potential predictive biomarkers for long-term benefit from afatinib, though results were not conclusive due to the small sample size.

### *Tumour mutational burden*

ICIs, including nivolumab and pembrolizumab, have been shown to be effective in recurrent/metastatic HNSCC clinical trials (CheckMate141, KEYNOTE-040 and KEYNOTE-048). TMB, defined as the total

number of somatic variations per defined region of a tumour genome, has emerged as a tumour-agnostic biomarker for ICI response in patients with advanced cancer. A meta-analysis in 2024 confirmed the prognostic and predictive value of TMB among HNSCC patients. Research on combining the different biomarkers is ongoing. For NPC, TMB is usually <10 mutations per megabase and not effective in predicting treatment response to ICIs. In contrast, a dynamic change in plasma EBV DNA after commencement of immunotherapy, which reflects tumour burden, was shown to predict treatment response in the POLARIS-02 study.

### *Circulating EBV and HPV DNA analysis*

Circulating EBV DNA and HPV DNA provide prognostic information and facilitate monitoring of recurrence in endemic NPC and HPV-positive HNSCC, respectively. Ongoing trials are evaluating the use of circulating viral DNA biomarkers to personalise treatment. Concurrently, various methodologies for analyses of circulating EBV and HPV DNA have been developed, including polymerase chain reaction (PCR), digital PCR and NGS, improving the diagnostic performance for cancer detection.

## Recommendations for Genetic Testing

Identification of potential targets might be considered to develop targeted therapeutics in recurrent/metastatic HNSCC. At the time of publication, more data are awaited from biomarker-driven clinical trials in HNSCC, such as the EORTC-1559-HNCG trial (UPSTREAM). ADCs are an emerging area of interest in the treatment of HNSCC, though their use is still largely investigational. Assessment of expression levels of target proteins (such as EGFR and HER2) by IHC could potentially identify the patient subgroup that might benefit from the ADC therapy.

# Further Reading

## Lung Cancer


Aredo JV, Wakelee HA. Top advances of the year: perioperative therapy for lung cancer. Cancer 2024; 130:2897–2903.

de Bortoli T, Benary M, Horak P, et al. Tumour mutational burden and survival with molecularly matched therapy. Eur J Cancer 2023; 190:112925.

Hendriks LE, Cortiula F, Mariamidze E, et al. ESMO oncogene-addicted metastatic non-small-cell lung cancer Living Guidelines, v1.2 - January 2025.https://www.esmo.org/guidelines/living-guidelines/esmo-living-guideline-oncogene-addicted-metastatic-non-small-cell-lung-cancer

Hendriks LE, Kerr KM, Menis J, et al. Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol 2023; 34:339–357.

Leighl NB, Page RD, Raymond VM, et al. Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non-small cell lung cancer. Clin Cancer Res 2019; 25:4691–4700.

Mateo J, Chakravarty D, Dienstmann R, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Ann Oncol 2018; 29:1895–1902.

Pascual J, Attard G, Bidard FC, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2022; 33:750–768.

Tan AC, Lai GGY, Tan GS, et al. Utility of incorporating next-generation sequencing (NGS) in an Asian non-small cell lung cancer (NSCLC) population: incremental yield of actionable alterations and cost-effectiveness analysis. Lung Cancer 2020; 139:207–215.

Tan AC, Tan DSW. Targeted therapies for lung cancer patients with oncogenic driver molecular alterations. J Clin Oncol 2022; 40:611–625.

Tsuboi M, Herbst RS, John T, et al. Overall survival with osimertinib in resected *EGFR*-mutated NSCLC. N Engl J Med 2023; 389:137–147.

Wu YL, Dziadziuszko R, Ahn JS, et al; ALINA Investigators. Alectinib in resected *ALK*-positive non-small-cell lung cancer. N Engl J Med 2024; 390:1265–1276.

## Head and Neck Cancer


Burtness B, Harrington KJ, Greil R, et al; KEYNOTE-048 Investigators. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. Lancet 2019; 394:1915–1928.

Chan ATC, Lee VHF, Hong RL, et al. Pembrolizumab monotherapy versus chemotherapy in platinum-pretreated, recurrent or metastatic nasopharyngeal cancer (KEYNOTE-122): an open-label, randomized, phase III trial. Ann Oncol 2023; 34:251–261.

Ferris RL, Blumenschein G Jr, Fayette J, et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. N Engl J Med 2016; 375:1856–1867.

Galot R, Le Tourneau C, Guigay J, et al. Personalized biomarker-based treatment strategy for patients with squamous cell carcinoma of the head and neck: EORTC position and approach. Ann Oncol 2018; 29:2313–2327.

Machiels JP, Haddad RI, Fayette J, et al; LUX-H&N 1 investigators. Afatinib versus methotrexate as second-line treatment in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck progressing on or after platinum-based therapy (LUX-Head & Neck 1): an open-label, randomised phase 3 trial. Lancet Oncol 2015; 16:583–594.

Marret G, Bièche I, Dupain C, et al. Genomic alterations in head and neck squamous cell carcinoma: level of evidence according to ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT). JCO Precis Oncol 2021; 5:215–226.

Leemans CR, Snijders PJF, Brakenhoff RH. The molecular landscape of head and neck cancer. Nat Rev Cancer 2018; 18:269–282.

Rodrigo JP, Sánchez-Canteli M, Otero-Rosales M, et al. Tumor mutational burden predictability in head and neck squamous cell carcinoma patients treated with immunotherapy: systematic review and meta-analysis. J Transl Med 2024; 22:135.

Wong KCW, Hui EP, Lo KW, et al. Nasopharyngeal carcinoma: an evolving paradigm. Nat Rev Clin Oncol 2021; 18:679–695.

Yom SS, Harris J, Caudell JJ, et al. Interim futility results of NRG-HN005, a randomized, phase II/III non-inferiority trial for non-smoking p16+ oropharyngeal cancer patients. Int J Radiat Oncol Biol Phys 2024; 120, S2–S3.

## Declaration of Interest:

**Dr D Tan** has declared institutional advisory board honoraria from Amgen, AstraZeneca, Bayer, BeiGene, Boehringer Ingelheim, DKSH, Eisai, Genmab, GSK, Guardant Health, Merck, Pfizer, and Takeda; institutional research funding from ACM Biolabs, Amgen, AstraZeneca and Pfizer. He is also a steering committee member for Novartis.

He has declared a non-remunerated advisory role for Prism AI.

**Dr W-C Tan** has declared institutional invited speaker honoraria from Astellas, AstraZeneca, Bayer Pharmaceuticals, Ipsen, Merck and MSD; an institutional research grant from Ipsen, institutional funding for his work as local principal investigator for Bicycle Therapeutics and institutional funding for his role as a coordinating principal investigator from SingHealth Duke-NUS Academic Medicine.

**Prof Chan** has declared institutional research funding from Merck Sharp & Dohme (Asia) Ltd, MSD International GmbH, NRG Oncology, Foundation Inc, and Pfizer Corporation Hong Kong Limited. He has received personal congress travel and accommodation expenses from Roche.

**Dr Lam** has declared institutional royalty fees from GRAIL. He is a member of the Board of Directors of DRA.

# Genomics in Gastrointestinal Cancers

4

M. Alsina[1, 2]

R. Dienstmann[3-5]

[1]*Hospital Universitario de Navarra (HUN), Department of Medical Oncology, Pamplona, Spain*

[2]*Translational Medical Oncology Unit, Navarrabiomed – Instituto de Investigación Sanitaria de Navarra (IdISNA), Pamplona, Spain*

[3]*Oncology Data Science Group, Vall d'Hebron Institute of Oncology, Barcelona, Spain*

[4]*University of Vic – Central University of Catalonia, Vic, Spain*

[5]*Oncoclínicas Medicina de Precisão, Oncoclínicas & Co, São Paulo, Brazil*

Gastrointestinal (GI) tumours comprise a heterogeneous group of neoplasms that includes colorectal, gastro-oesophageal, hepatobiliary and pancreatic cancers. Colorectal cancer (CRC) is the most common cause of death from cancer (9.3% of all cases), after lung cancer, followed by hepatocellular carcinoma (7.8%). Gastric cancer is the fifth most common cause of cancer death globally (6.8%). Environmental changes have altered the incidence of some GI cancers, for example distal gastric cancers are less common because of improvements in food preservation but there is an increasing incidence of gastro-oesophageal cancers, linked to obesity and gastro-oesophageal reflux. There is also a worrying, and poorly understood, increase in the incidence of GI cancers amongst those aged under 50 years. Although anatomically distinct, GI tumours share some genomic features such as microsatellite instability (MSI) and chromosomal instability (CIN). There are also location-specific alterations that may represent targets for precision medicine.

This chapter presents a summary of molecular alterations with clinical relevance that are guiding targeted treatments in different gastrointestinal tumours.

# Oesophageal Squamous-Cell Carcinoma

Oesophageal squamous-cell carcinoma (OSCC) is the most prevalent type of oesophageal cancer globally. The Cancer Genome Atlas (TCGA) research network identified frequent cell-cycle dysregulations (mainly *CCND1* amplifications), *EGFR* amplifications/mutations, as well as *SOX2/p63* amplifications. So far, no distinct therapeutic options for these subtypes are available.

## Advanced Disease

OSCC appears to be moderately more sensitive to immune checkpoint inhibitors (ICIs) than oesophageal adenocarcinoma. Nevertheless, the benefit from immunotherapy is clearly increased in high programmed death-ligand 1 (PD-L1) tumours. First-line treatment with chemotherapy in combination with pembrolizumab or nivolumab is recommended, for patients with a PD-L1 combined positive score (CPS) ≥10 or a PD-L1 tumour proportion score (TPS) ≥1%, respectively. Combinations with tislelizumab have recently been approved for patients with PD-L1-positive tumours (tumour area positivity [TAP] score ≥5%), based on the RATIONALE-306 trial. Treatment with nivolumab plus ipilimumab without chemotherapy may be an option in fit patients with PD-L1-TPS ≥1% tumours and low tumour burden.

*Table 1 Recommendations for the Use of NGS for Patients with Squamous-Cell Carcinoma, ESCAT Levels I/II.*

Adapted from Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2024; 35:588–606.

| Gene/ signature | Alteration | Scoring method | Estimated prevalence | Early-stage disease | Advanced disease |
|---|---|---|---|---|---|
| PD-L1 | Expression (TPS, CPS or TAP) | TPS ≥1%<br>CPS ≥10<br>TAP ≥10% | 50%<br>40%<br>35% | N/A | First-line treatment with anti-PD-1 plus chemotherapy |

Abbreviations: CPS, combined positive score; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; ESMO, European Society for Medical Oncology; N/A, not applicable; NGS, next-generation sequencing; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TAP, tumour area positivity; TPS, tumour proportion score.

## Gastro-oesophageal Adenocarcinoma

Gastro-oesophageal adenocarcinomas (GEAs) comprise a heterogeneous group of tumours arising between the mid-oesophagus and distal stomach. The TCGA describes four molecular subtypes: Epstein–Barr virus (EBV), MSI, CIN and genomically stable (GS). MSI and EBV subtypes typically feature high immunogenicity, and MSI represents an established biomarker for immunotherapy. Moreover, the CIN subtype has been associated with a better response to chemotherapy, whereas the contrary happens with GS, which is normally enriched by diffuse histology. GEAs also display substantial intrinsic heterogeneity (spatial and temporal), as well as discordances between primary and paired metastatic lesions, suggesting an important role for clonal evolution.

### Early-Stage Disease

The small population with mismatch repair deficient (dMMR)/MSI-high (MSI-H) tumours require identification to consider participation in clinical trials evaluating neoadjuvant immunotherapy. Given the high response rates, organ preservation strategies are currently under investigation. The addition of trastuzumab to the perioperative FLOT schema (fluorouracil–leucovorin–oxaliplatin–docetaxel) may be considered for downstaging bulky HER2-positive tumours, based on phase II trials. Based on data from the phase III MATTERHORN trial, the addition of durvalumab to FLOT is expected to become the new standard of care; the benefit of immunotherapy was mainly observed in patients with PD-L1-positive GEAs (assessed by TAP score ≥1%).

### Advanced Disease

The targeted therapeutic approach for advanced GEA currently comprises four biomarkers; HER2, dMMR/MSI, PD-L1 and claudin 18.2 (CLDN18.2).

Trastuzumab with chemotherapy should be offered to patients with HER2-positive GEA (immunohistochemistry [IHC] expression 3+, or 2+ with gene amplification), and pembrolizumab/nivolumab may be added in the case of PD-L1 co-expression (CPS cut-off level ≥1). Trastuzumab deruxtecan represents the best approved option for second-line treatment, if HER2 positivity is maintained after progression on trastuzumab.

Patients with dMMR/MSI-H tumours benefit from the addition of an anti-programmed cell death protein 1 (PD-1) agent to the first-line chemotherapy regimen.

PD-L1 expression represents the second biomarker for immunotherapy indications in GEA. The complexity of CPS and TAP reporting methods and PD-L1 cut-off scores make standard guidelines for immunotherapy challenging. Pembrolizumab, nivolumab or tislelizumab in combination with first-line chemotherapy should be used for PD-L1-positive GEAs. Considering PD-L1 as a continuous variable, higher cut-off levels (CPS ≥5, TAP ≥5%) are associated with better responsiveness.

Zolbetuximab is recommended in patients with CLDN18.2-positive tumours.

Future data is expected to indicate the treatment of fibroblast growth factor receptor 2b (FGFR2b)-positive tumours with bemarituzumab or other anti-FGFR2b agents, with or without the addition of immunotherapy.

Testing multiple biomarkers is essential in metastatic GEA; however, it can be challenging. To avoid delays, upfront and parallel testing for key actionable IHC biomarkers with a short turnaround time is critical for timely therapeutic decisions. Multigene next-generation sequencing (NGS) is not recommended for GEAs, outside of a clinical trial (Table 2).

### Recommendations for Germline Testing

Up to 3% of patients with gastric cancer may harbour *CDH1* mutations. Germline testing is recommended if a hereditary syndrome is suspected.

*Table 2 Recommendations for the Use of NGS for Patients with Gastro-oesophageal Adenocarcinoma, ESCAT Levels I/II.*

Adapted from Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2024; 35:588–606.

| Gene/ signature | Alteration | Scoring method | Estimated prevalence | Early-stage disease | Advanced disease |
|---|---|---|---|---|---|
| MSI-H/dMMR | MSI-H/dMMR | IHC (PCR and NGS also accepted) | 5%–9% | Consider immunotherapy | First-line treatment with immunotherapy |
| *ERBB2* | Overexpression or amplification | IHC ± ISH | 12%–20% | Consider adding trastuzumab in bulky tumours | First-line treatment with trastuzumab and chemotherapy |
| PD-L1 | Expression (CPS or TAP) | IHC | 60% (CPS ≥5) 55% (TAP ≥5%) | Consider adding immunotherapy with perioperative chemotherapy | First-line treatment with anti-PD-1 antibodies and chemotherapy |
| CLDN18.2 | Expression | IHC | 40% | N/A | First-line treatment with zolbetuximab and chemotherapy |

Abbreviations: CLDN18.2, claudin 18.2; CPS, combined positive score; dMMR, mismatch repair deficient; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; ESMO, European Society for Medical Oncology; IHC, immunohistochemistry; ISH, *in situ* hybridisation; MSI-H, microsatellite instability-high; N/A, not applicable; NGS, next-generation sequencing; PCR, polymerase chain reaction; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TAP, tumour area positivity.

# Pancreatic Ductal Adenocarcinoma

Although advances in the molecular understanding of pancreatic ductal adenocarcinoma (PDAC) have been made in recent years, the therapeutic translation to precision oncology approaches has been modest. PDAC is driven by multiple oncogene mutations, with the most frequent being *KRAS* (>90% of cases). Alterations in genes specifically involved in the homologous recombination repair (HRR) pathway (such as *BRCA1/2*) are also relevant in patients with PDAC.

## Advanced Disease

Although *KRAS* mutation assessment is generally recommended, targeted therapies are only available for the small subset of patients with the G12C variant. Novel selective KRAS inhibitors for different variants are under investigation.

Germline *BRCA* testing is more relevant as patients with pathogenic mutations in *BRCA1/2* benefit from maintenance treatment with poly(ADP-ribose) polymerase (PARP) inhibitors, such as olaparib, after response or disease stabilisation with first-line platinum-based chemotherapy.

*KRAS*-wild type (wt) metastatic PDAC occurs in 10% of this population and therapeutic opportunities may exist. Broad NGS panels are recommended to screen for potentially actionable alterations, mainly fusion events. These include targeted approaches, both approved and in clinical trials, such as *NTRK*, *RET* and *NRG1* fusions (Table 3). Finally, dMMR/MSI-H PDAC is a very rare entity, and the efficacy of ICIs in patients with refractory disease is modest.

*Table 3 Recommendations for the Use of NGS for Patients with Pancreatic Ductal Adenocarcinoma, ESCAT Levels I/II.*

Adapted from Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2024; 35:588–606.

| Gene/ signature | Alteration | Scoring method | Estimated prevalence | Early-stage disease | Advanced disease |
|---|---|---|---|---|---|
| *BRCA1/2* | Germline pathogenic/ likely pathogenic variants | NGS | 4%–7% | N/A | Consider PARPis at maintenance after a first-line treatment with chemotherapy |
| *KRAS* | Mutation (any) | NGS | 90% | N/A | Clinical trial (pan-KRAS inhibitors) |
| *KRAS* | p.G12C | NGS | 1%–2% | N/A | Clinical trial (KRAS G12C TKIs) |
| *PALB2* | Germline pathogenic/ likely pathogenic variants | NGS | 3%–4% | N/A | Consider clinical trial with PARPis at maintenance after a first-line treatment with chemotherapy or in the refractory setting |
| *NRG1* | Fusions | NGS | 7% | N/A | Clinical trial (anti-HER2/HER3 bispecific antibody) |

Abbreviations: ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; ESMO, European Society for Medical Oncology; HER, human epidermal growth factor receptor; N/A, not applicable; NGS, next-generation sequencing; p, point; PARPi, poly(ADP-ribose) polymerase inhibitor; TKI, tyrosine kinase inhibitor.

### Recommendations for Germline Testing

Patients with proven *BRCA* mutation-related PDAC will need referral to the local genetic services to offer family testing.

## Biliary Tract Cancer

Biliary tract cancer (BTC) accounts for <1% of all cancers and includes three types: cholangiocarcinoma (CC), gallbladder and ampullary cancer. CC comprises two different entities, intrahepatic (iCC) and extrahepatic (eCC), each with distinct molecular features. Despite patients with BTC being grouped as a single entity in clinical trials, ~40% present with potentially targetable molecular alterations, such as *IDH1* mutations, *BRAF* V600E mutations or *FGFR2* fusions, which is changing the treatment approach for these patients, particularly those with iCC.

### Advanced Disease

Molecular profiling should be carried out before or during first-line therapy, as recommended by European Society for Medical Oncology (ESMO) guidelines. A broad NGS panel should cover *FGFR2* fusions, *IDH1*, *KRAS* and *BRAF* mutations as well as *HER2* amplification to test for actionable alterations, together with tumour-agnostic biomarkers such as *NTRK* and *RET* fusions.

First-line treatment with cisplatin–gemcitabine plus durvalumab or pembrolizumab is recommended, regardless of immune biomarker status. Any second-line treatment should be based on the results of genomic testing. There are certain molecular alterations that are associated with the anatomical location of the tumour, for example *FGFR2* fusions and *IDH* mutations occur almost exclusively in iCC, while *KRAS* mutation and *HER2* amplification are most common in eCC and gallbladder cancers.

Ivosidenib is the first approved therapy for patients with *IDH1*-mutated tumours, representing 10%–20% of the iCC population. *FGFR2* fusions or rearrangements occur in up to 14% of cases of iCC. Many reversible adenosine triphosphate (ATP)-binding kinase inhibitors have demonstrated clinical activity (e.g. infigratinib and pemigatinib), but

their efficacy is limited by development of acquired resistance mutations affecting amino acid residues in the kinase domain. Futibatinib, a next-generation, covalent-binding FGFR1–4 inhibitor, has shown antitumour activity in patients with FGFR-altered tumours, including those with acquired resistance mutations to ATP-competitive FGFR inhibitors.

*BRAF* V600E mutations are present in ~5% of CCs for which the combination of dabrafenib and trametinib is indicated.

HER2 pathway activation (protein overexpression or gene amplification/mutation) is seen in up to 20% of gallbladder tumours and 5%–10% of eCCs. Targeted treatment strategies should be considered in these patients (Table 4).

Although rare, patients with BTC may harbour pathogenic variants of homologous recombination genes, such as *BRIP1*, *ATM* and *BRCA*. Such patients, whose cancers may be more susceptible to treatment with DNA cross-linking agents (e.g. platinum compounds) should be considered for clinical trials of agents such as PARP inhibitors.

Prevalence of dMMR/MSI in BTC is <1%, but in these cases immunotherapy is recommended even in the refractory setting. *NTRK* and *RET* fusions are similarly rare drivers in BTC but matched targeted approaches can be considered here (Table 6).

*Table 4 Recommendations for the Use of NGS for Patients with Biliary Tract Cancer, ESCAT Levels I/II.*

Adapted from Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2024; 35:588–606.

| Gene/ signature | Alteration | Scoring method | Estimated prevalence | Early-stage disease | Advanced disease |
|---|---|---|---|---|---|
| *IDH1* | Mutations | NGS | 8%–18% iCC | N/A | Second-line treatment with IDH inhibitors (ivosidenib) |
| *FGFR2* | Fusions | NGS | 5%–15% iCC | N/A | Second-line treatment with pan-FGFR TKIs |

*Table 4 Recommendations for the Use of NGS for Patients with Biliary Tract Cancer, ESCAT Levels I/II. (Continued).*

| Gene/ signature | Alteration | Scoring method | Estimated prevalence | Early-stage disease | Advanced disease |
|---|---|---|---|---|---|
| *ERBB2* | Overexpression or amplification | IHC ± ISH or NGS | 10%–20% eCC and GBC | N/A | Clinical trial (anti-HER2 monoclonal antibodies (trastuzumab–pertuzumab; zanidatamab), anti-HER2 ADCs (trastuzumab deruxtecan) |
| *ERBB2* | Mutations | NGS | 3%–5% | N/A | Clinical trial (anti-HER2 monoclonal antibodies, pan-HER TKIs) |
| *BRAF* | Mutations (V600E) | NGS | 5% | N/A | Clinical trial (BRAF inhibitors + MEK inhibitors) |
| *KRAS* | Mutations (p.G12C) | NGS | <1% | N/A | Clinical trial (KRAS G12C TKIs) |
| *KIT* | Mutations/ insertions/ deletions/ indels | NGS | 85% | N/A | Clinical trial (KIT/PDGF TKIs) |
| *PDGFRA* | Mutations/ insertions/ deletions/ indels | NGS | 10%–15% | N/A | Clinical trial (KIT/PDGF TKIs) |

Abbreviations: ADC, antibody–drug conjugate; eCC, extrahepatic cholangiocarcinoma; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; ESMO, European Society for Medical Oncology; FGFR, fibroblast growth factor receptor; GBC, gallbladder carcinoma; HER, human epidermal growth factor receptor; iCC, intrahepatic cholangiocarcinoma; IHC, immunohistochemistry; ISH, *in situ* hybridisation; N/A, not applicable; NGS, next-generation sequencing; p, point; TKI, tyrosine kinase inhibitor.

### Recommendations for Germline Testing

Germline testing is recommended in the rare instance of hereditary syndromes being identified or suspected.

## Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and represents a major global healthcare challenge. Although viral hepatitis and alcohol remain important risk factors, non-alcoholic fatty liver disease is rapidly becoming the dominant cause of HCC. Knowledge of the genetic underpinnings of HCC is crucial to understanding its development and progression; the identification of

the genetic, somatic and epigenetic associated features may open new frontiers for risk assessment and early detection.

### Advanced Disease

Following the approval of new first- and second-line agents, as well as the establishment of ICI-based therapies as standard of care, the treatment landscape for intermediate and advanced-stage HCC is more diverse than ever. However, molecular profiling is not currently recommended since it has no direct impact on decision-making.

Currently, genomic testing is not recommended for advanced HCC outside of clinical trials.

## Colorectal Cancer

The incorporation of molecular markers and subtypes has somewhat improved the ability to determine prognosis in patients with CRC. For example, patients with recurrent metastatic MSI-H CRC (consensus molecular subtype 1 [CMS1]) now receive ICIs, leading to substantially better outcomes. Conversely, patients with CRC of a mesenchymal phenotype (CMS4) remain poorly responsive to standard chemotherapies. Also, patients with *KRAS*- and *BRAF* V600E- (i.e. Val600Glu) mutated tumours derive benefit from targeted therapies.

### Early-stage Disease

The subset of 10%–15% patients with stage II CRC with dMMR or MSI-H tumours are at a very low risk of disease recurrence, therefore adjuvant chemotherapy is not indicated. Patients with these tumours have exceptional responses to neoadjuvant ICIs in the early setting. The only other prognostic indicator, Immunoscore (a digital pathology immune-based assay that quantifies CD3- and CD8-positive T-cell infiltrates at the tumour centre and invasive margins) is a validated tool to identify patients.

As in many other malignancies, the role of circulating tumour DNA (ctDNA) in the detection of minimal residual disease (MRD) in CRC remains unproven although an exciting option for the future.

## Advanced Disease

In advanced disease, genomic features also guide treatment decisions. For metastatic CRC, approximately 5% of patients are characterised by dMMR/MSI-H tumours and therefore eligible for first-line treatment with ICIs, pembrolizumab or nivolumab–ipilimumab. Also, mutations in *POLE* and *POLD1* are linked to ultra-mutant genotypes, defining another very small subset of patients with metastatic CRC (mCRC, over 100 mutations/megabase) who respond well to ICIs. Although limited, there is some evidence that patients with *POLE/D1*-mutated mCRC have more favourable outcomes compared with MSI-H populations when treated with ICIs, in terms of tumour response and survival. Conversely, mCRC patients with microsatellite stability (MSS) and *POLE*-wt tumours represent a population with poor response to ICIs, even in the presence of high tumour mutational burden (TMB). Therefore, TMB per se is not a biomarker with clinical impact.

Besides the presence of a high mutational load, there are numerous other clinically relevant genomic biomarkers in mCRC to guide treatment decisions. The mutation of *RAS/BRAF* and amplification of *HER2* (*ERBB2*) are well-established predictive markers. The presence of these alterations all denote resistance to the anti-epidermal growth factor receptor (EGFR) agents (cetuximab and panitumumab). Only patients with *RAS*-wt and *BRAF*-wt tumours of the left colon should be considered for first-line treatment with anti-EGFR agents in combination with chemotherapy. For right-sided tumours, this strategy could be adopted only if downsizing is needed, given their poor outcomes. Currently, chemotherapy combinations with anti-vascular endothelial growth factor (VEGF) agents represent a better choice for disease control in right-sided tumours. RAS-expanded NGS testing identifies less common mutations which activate the RAS pathway, *HER2* amplification and gene fusions. Such testing potentially hyper-selects an ‘ultra wt’ population who may gain real benefit from anti-EGFR treatment, even eliminating the predictive value of many clinical features such as right- or left-sided tumour prognostic associations. Finally, new therapeutic options for the small subset of patients with *KRAS* G12C CRC may improve outcomes (Table 5).

*Table 5 Recommendations for the Use of NGS for Patients with Colorectal Cancer, ESCAT Levels I/II.*

Adapted from Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2024; 35:588–606.

| Gene/ signature | Alteration | Scoring method | Estimated prevalence | Early-stage disease | Advanced disease |
|---|---|---|---|---|---|
| MSI-H/dMMR | MSI-H/dMMR | IHC (PCR and NGS also accepted) | 4.5% | Omit adjuvant chemotherapy for stage II<br>Consider clinical trial with neoadjuvant immunotherapy | First-line with PD-1 checkpoint inhibitors |
| *KRAS, NRAS* | Mutations (exon 2, 3 and 4) | PCR or NGS | 53% | N/A | First-line with anti-EGFR monoclonal antibodies in combination with chemotherapy |
| *BRAF* | Mutations (V600E) | PCR or NGS | 8.5% | N/A | First-line with encorafenib (BRAF inhibitor) plus cetuximab (EGFR inhibitor) and chemotherapy<br>If not received in the first line, the combination of encorafenib and cetuximab should be considered in the second line |
| *KRAS* | Mutations (p.G12C) | PCR or NGS | 4% | N/A | Clinical trial (KRAS G12C TKIs + anti-EGFR monoclonal antibodies) |
| *ERBB2* | Overexpression or amplification | IHC, ISH or NGS | 2% | N/A | Clinical trial (anti-HER2 monoclonal antibodies ± anti-HER2 TKIs; anti-HER2 ADCs) |
| *POLE* | Mutations | NGS | <1% | N/A | Clinical trial (PD-1 checkpoint inhibitors) |
| cMET | Overexpression | IHC | 15% | N/A | Clinical trial (anti-MET antibodies) |

Abbreviations: ADC, antibody–drug conjugate; dMMR, mismatch repair deficient; EGFR, epidermal growth factor receptor; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; ESMO, European Society for Medical Oncology; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, *in situ* hybridisation; MSI-H, microsatellite instability-high; N/A, not applicable; NGS, next-generation sequencing; PCR, polymerase chain reaction; PD-1, programmed cell death protein 1; TKI, tyrosine kinase inhibitor.

Encorafenib (BRAF inhibitor) with cetuximab (anti-EGFR antibody) has been shown to be beneficial for patients with *BRAF* V600E mutations.

Although the presence of HER2 overexpression and/or amplification is a well-known predictor of resistance to anti-EGFR therapies in patients with *KRAS*-wt mCRC cancer, different drugs and combinations targeting HER2 are currently under investigation in the first-line setting. Anti-HER2 therapies such as trastuzumab plus pertuzumab, trastuzumab plus tucatinib or trastuzumab deruxtecan may be considered for patients with HER2-positive tumours (based on IHC overexpression or gene amplification) in the refractory setting. Table 5 summarises distinct molecular aberrations that guide therapeutic choice and inclusion criteria for clinical trials. Regarding tumour-agnostic indications, *NTRK1/2/3* and *RET* fusions are infrequent in CRC but enriched in the dMMR/MSI population (Table 6).

Rechallenge with anti-EGFR agents has shown promising outcomes in patients with *RAS*-wt tumours, particularly in cases where liquid biopsy shows no emergence of resistant clones, such as acquired alterations in *RAS*, *BRAF*, *HER2* or *MET* genes. Randomised interventional studies are needed to depict the role of ctDNA in monitoring emergent acquired mutations and related dynamic changes in treatment.

Finally, cMET overexpression has been described in CRC, thus representing a new potential target for targeting antibodies, such as ABBV-400, currently under investigation.

### Recommendations for Germline Testing

Lynch syndrome is the dominantly inherited CRC syndrome caused by the presence of a pathogenic MMR gene variant, representing up to 5% of cases. Germline testing is recommended for all patients with early-onset CRC, dMMR/MSI, or a familial history of CRC. However, one exception is loss of MLH1 expression with a *BRAF* V600E mutation, which is associated with MLH1 hypermethylation and is attributed to sporadic CRC.

*Table 6 Recommendations for the Use of NGS for Patients with Tumour-agnostic Genomic Alterations, ESCAT Levels I/II*

Adapted from Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2024; 35:588–606.

| Gene/ signature | Alteration | Scoring method | Estimated prevalence | Early-stage disease | Advanced disease |
|---|---|---|---|---|---|
| *NTRK1/2/3* | Fusions | NGS | N/A | N/A | Clinical trial (TRK inhibitors) |
| MSI-H/dMMR | MSI-H/dMMR | IHC (PCR and NGS also accepted) | N/A | N/A | Clinical trial (PD-1 checkpoint inhibitors) |
| *RET* | Fusions | NGS | N/A | N/A | Clinical trial (RET inhibitors) |
| *BRAF* | Mutations (V600E) | NGS | N/A | N/A | Clinical trial (BRAF inhibitors + MEK inhibitors) |
| *FGFR1/2/3* | Fusions Mutations | NGS | N/A | N/A | Clinical trial (Pan-FGFR TKIs) |
| TMB-H | Mutations | NGS | N/A | N/A | Clinical trial (PD-1/PD-L1 checkpoint inhibitors) |

Abbreviations: dMMR, mismatch repair deficient; ESCAT, ESMO scale for Clinical Actionability of molecular Targets; ESMO, European Society for Medical Oncology; FGFR, fibroblast growth factor receptor; IHC, immunohistochemistry; MSI-H, microsatellite instability-high; N/A, not applicable; NGS, next-generation sequencing; PCR, polymerase chain reaction; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TMB-H, tumour mutational burden-high; TKI, tyrosine kinase inhibitor; TRK, tropomyosin receptor kinase.

# Further Reading


Alsina M, Diez M, Tabernero J. Emerging biological drugs for the treatment of gastroesophageal adenocarcinoma. Expert Opin Emerg Drugs 2021; 26:385–400.

Bhamidipati D, Pellatt A, Subbiah V. Targeting all BRAF alterations: the (re)-search continues. JCO Precis Oncol 2024; 8:e2300670.

Chalabi M, Verschoor YL, Tan PB, et al. Neoadjuvant immunotherapy in locally advanced mismatch repair-deficient colon cancer. N Engl J Med 2024; 390:1949–1958.

Dumbrava EEI, Balaji K, Raghav K, et al. Targeting *ERBB2* (*HER2*) amplification identified by next-generation sequencing in patients with advanced or metastatic solid tumors beyond conventional indications. JCO Precis Oncol 2019; 3:PO.18.00345.

Eng C, Yoshino T, Ruíz-García E, et al. Colorectal cancer. Lancet 2024; 404:294–310.

Gouda MA, Subbiah V. Precision oncology for biliary tract tumors: it's written in blood! Ann Oncol 2022; 33:1209–1211.
Klempner SJ, Cowden ES, Cytryn SL, et al. PD-L1 immunohistochemistry in gastric cancer: comparison of combined positive score and tumor area positivity across 28-8, 22C3, and SP263 Assays. JCO Precis Oncol 2024; 8:e2400230.
Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2024; 35:588–606.
Napolitano S, Martini G, Ciardiello D, et al. Targeting the EGFR signalling pathway in metastatic colorectal cancer. Lancet Gastroenterol Hepatol 2024; 9:664–676.
Sahin IH, Saridogan T, Ayasun R, et al. Targeting KRAS oncogene for patients with colorectal cancer: a new step toward precision medicine. JCO Oncol Pract 2024; 20:1336–1347.
Shitara K, Muro K, Watanabe J, et al. Baseline ctDNA gene alterations as a biomarker of survival after panitumumab and chemotherapy in metastatic colorectal cancer. Nat Med 2024; 30:730–739.
Stenzinger A, Vogel A, Lehmann U, et al. Molecular profiling in cholangiocarcinoma: a practical guide to next-generation sequencing. Cancer Treat Rev 2024; 122:102649.
Subbiah V, Gouda MA, Ryll B, et al. The evolving landscape of tissue-agnostic therapies in precision oncology. CA Cancer J Clin 2024; 74:433–452.
Vogel A, Meyer T, Sapisochin G, et al. Hepatocellular carcinoma. Lancet 2022; 400:1345–1362.
Yoshino T, Hooda N, Younan D, et al. A meta-analysis of efficacy and safety data from head-to-head first-line trials of epidermal growth factor receptor inhibitors versus bevacizumab in adult patients with RAS wild-type metastatic colorectal cancer by sidedness. Eur J Cancer 2024; 202:113975.

## Declaration of Interest:

**Dr Alsina** has received personal advisory board honoraria from Amgen, AstraZeneca, BeiGene, Bristol Myers Squibb (BMS), Dragonfly Therapeutics, Jazz Pharmaceuticals, Merck Sharp & Dohme and Novartis; and personal speaker honoraria from Astellas, AstraZeneca, Beigene, BMS, Jazz Pharmaceuticals and Lilly. She holds leadership roles with the European Organisation for Research and Treatment of

Cancer Gastrointestinal Tract Cancer Group (EORTC GITCG) and the Digestive Tumour Treatment Group (TTD); and a non-remunerated role as principal investigator with Merck Serono. She is also a member of the ESMO Faculty.

**Dr Dientsmann** has declared personal speaker honoraria from Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, BMS, Gilead, GSK, Ipsen, Johnson & Johnson, Libbs, Lilly, Merck Sharp & Dohme, Pfizer, Roche, Sanofi, Servier and Takeda; and personal advisory board honoraria from Foundation Medicine and Pfizer. He has received institutional research grants from AstraZeneca, Daiichi Sankyo, GSK, Merck, Novartis and Pfizer. He is an employee of Oncoclinicas and holds stocks and shares in Trailing.

# Genomics in Urological Cancers

5

M. Ruiz-Vico[1,2]

I. Moreno-Perez[3]

G. de Velasco[1]

E. Castro[1]

[1]*Medical Oncology Department. Hospital Universitario 12 de Octubre, Madrid, Spain*

[2]*Biomedicine Research PhD Program, Universidad Complutense de Madrid, Madrid, Spain*

[3]*Medical Oncology Department. Hospital Universitario Clinico San Carlos, Madrid, Spain*

In the last decade, an improved understanding of the biology of genitourinary tumours has led to new drugs with a significant impact on patient outcomes. Although several prognostic factors have been identified to help patients and clinicians make treatment decisions, the number of biomarkers predictive of response is limited.

## Key Molecular Features in Tumour Types

### Prostate Cancer

Prostate cancer (PC) is a molecularly heterogeneous disease in which various subgroups with different prognoses and therapeutical vulnerabilities have been identified.

The most common genomic alterations in localised and metastatic PC involve fusions of the erythroblast transformation specific transcription factor family, particularly the *TMPRSS2–ERG* fusion (40%–50%). Notably, copy number alterations (CNAs) and other structural variants are more prevalent than point mutations, emphasising the significance of genomic rearrangements in PC biology and progression.

The Cancer Genome Atlas (TCGA) Research Network proposed a classification of localised PC into seven molecular subtypes: tumours with *ERG* (46%), *ETV1* (8%), *ETV4* (4%) and *FLI1* (1%) fusions, and *SPOP*- (11%), *FOXA1*- (3%) and *IDH1*- (1%) mutated PCs. *SPOP* mutations are the most frequent mutations in localised PC and are mutually exclusive with *ETS* fusions. This molecular classification can cluster 74% of the tumours analysed. The remaining tumours (26%) are enriched for mutations in *TP53*, *KDM6A* and *KMT2D*; deletions in chromosomes 6 and 16; and *MYC* and *CCND1* amplification.

Resistance to androgen deprivation therapy (ADT) leads to castration-resistant PC (CRPC). Androgen receptor (AR) signalling is the main driver of disease progression, with *AR* alterations (i.e. gene amplification, mutations, splice variants etc.) identified in >50% of cases. Beyond *AR*, ~30% of advanced tumours harbour genomic changes in other relevant pathways. Commonly enriched alterations in CRPC include *TP53* mutations (>40%), in PI3K pathway genes such as *PTEN* loss (~45%), DNA repair genes (25%, *BRCA1/2* mutations), *RB1* loss (~20%), Wnt pathway genes (~15%), epigenetic regulator genes (~20%), *CDK12* loss (5%-7%) and MAPK pathway genes (~5% of cases). Co-occurrence of these alterations is frequently observed.

Homologous recombination repair (HRR) is the DNA damage response (DDR) pathway most frequently impaired in PC. In the PROfound study, 28% of the samples analysed harboured at least one HRR alteration: *BRCA2* (8.7%), *CDK12* (6.3%), *ATM* (5.9%), *CHEK2* (1.2%) and *BRCA1* (1%). Co-occurring aberrations were found in 2.2% of cases. Alterations in the mismatch repair (MMR) pathway are usually identified in 3% of cases. DDR alterations are early events in the evolution of lethal PC.

A significant proportion of DDR alterations found in tumours have a germline origin, as is the case in almost half of *BRCA2* mutations. Overall, germline mutations in DDR genes are found in 3.5% of men with low-risk localised disease, 6.2% of those with very high-risk disease and up to 12% of patients with metastatic PC. Several studies have consistently reported inherited mutations in *BRCA2* as the most common DDR germline mutation at all stages of the disease.

However, despite the important advances in the field, genomics alone does not fully explain PC evolution and multiple studies are investigating the role of transcriptomic profiles and epigenetic changes in disease progression and treatment resistance.

### Urothelial Cancer

Most urothelial carcinomas (UCs) arise in the bladder, but 5%–10% of cases occur in the pelvis and ureter, which is referred to as upper-tract UC (UTUC). Mutational patterns in bladder cancer differ by tumour grade and stage. The most frequently mutated genes in non-muscle-invasive bladder cancer (NMIBC) are the *TERT* promoter (73%), *FGFR3* (49%), *KDM6A* (36%), *PIK3CA* (26%), *STAG2* (23%), *ARID1A* (21%) and *TP53* (21%). *FGFR3*, *STAG2* and *PIK3CA* mutations are enriched in low-grade tumours, while *TP53* mutations are enriched in high-grade tumours. *HRAS/KRAS* mutations (10%–15%) are evenly distributed by stage and grade.

The TCGA project reported a high tumour mutational burden (TMB, median 5.5 mutations/megabase [Mb]) and genomic alterations in 69% of the chemotherapy-naïve muscle-invasive bladder cancers (MIBC) analysed. The most frequently altered gene was *TP53* (38%), followed by *KDM2A* (28%), *KDM6A* (26%), *ARID1A* (25%) and *PIK3CA* (22%). In addition, 15%–25% of samples had *RB1* alterations and 10%–14% had *FGFR3* mutations. Alterations in different DDR pathways have been reported in 3%–12% of MIBCs. Six molecular subtypes of MIBC have been proposed: basal/squamous (35%), luminal papillary (24%), luminal unstable (15%), stroma-rich (15%), luminal non-specified (8%) and neuroendocrine-like (3%). These subtypes differ in their underlying oncogenic mechanism, infiltration by immune and stromal cells and histological and clinical characteristics, and present prognostic value.

In a series of 195 UTUCs, the most frequently altered genes were *FGFR3* (40%), *KMT2D* (37%), *KDM6A* (32%), *TP53* (26%)

and *ARID1A* (23%). *FGFR3, HRAS* and *STAG2* mutations were enriched in low-stage and low-risk UTUC tumours. Compared with bladder tumours, *FGFR3* and *HRAS* mutations were more frequent in UTUCs and *TP53, RB1* and *ERBB2* mutations were more prevalent in bladder cancers.

Germline pathogenic variants in highly actionable cancer-predisposition genes are predominantly found in DDR genes. Approximately 5% of patients with UC harbour a *BRCA1/2* or an MMR mutation, the latter enriched in UTUC.

### Renal Cancer

The TCGA project analysed 843 samples of renal cell carcinoma (RCC), including clear-cell RCC (ccRCC), papillary RCC (pRCC) and chromophobe RCC (ChRCC).

In ccRCC, CNAs were more frequently observed than in other RCC subtypes and often involved an arm or an entire chromosome. Loss of 3p (91%) resulted in CNA in genes that are also frequently mutated (*VHL, PBRM1, BAP1* and *SETD2*). Loss of 14q, associated with *HIF1A* loss, was noted in 45% of samples. Gains of 5q (67% of samples) and additional focal amplifications refined the region of interest to 60 genes in 5q35. Focal amplification also implicated *PRKCI* and the MDS1 and EVI1 complex locus *MECOM* at 3p26, the p53 regulator *MDM4* at 1q32, *MYC* at 8q24 and *JAK2* on 9p24. Focally-deleted regions included the tumour suppressor genes *CDKN2A* at 9p21 and *PTEN* at 10q23, putative tumour suppressor genes *NEGR1* at 1p31, *QKI* at 6q26 and *CADM2* at 3p12 and the genes that are frequently deleted in cancer, *PTPRD* at 9p23 and *NRXN3* at 14q24. The most frequently mutated genes were *VHL, PBRM1*, *SETD2*, *KDM5C*, *PTEN*, *BAP1*, *MTOR* and *TP53.*

pRCC type 1 and type 2 have been shown to be distinct types of kidney cancer characterised by specific genetic alterations. Type 1 tumours were associated with *MET* alterations, whereas type 2 tumours were characterised by *CDKN2A* silencing, *SETD2* mutations, *TFE3* fusions and increased expression of the NRF2-antioxidant response element

pathway. A CpG island methylator phenotype was observed in a distinct subset of type 2 tumours, characterised by *FH* mutations and poor survival.

Loss of one copy of the entire chromosome, for most or all of chromosomes 1, 2, 6, 10, 13 and 17, was seen in the majority of ChRCC cases (86%). Losses of chromosomes 3, 5, 8, 9, 11, 18 and 21 were also noted at significant frequencies (12%–58%). ChRCC displayed a low TMB (~0.4 mutations/Mb). The most frequently mutated genes were *TP53* (32%) and *PTEN* (9%), as no other genes were found to be mutated at a frequency higher than 5%, including mutations in cancer-relevant genes such as *MTOR*, *NRAS*, *TSC1* or *TSC2*.

In 2022, the World Health Organization (WHO) introduced a molecularly driven RCC subclassification with 11 subgroups. Molecularly defined renal tumours are those which show very heterogeneous morphological aspects and therefore cannot be diagnosed by morphology alone. Such tumours include previously described molecular subtypes (such as SDH-deficient RCC), as well as new entities including *SMARCB1*-deficient medullary RCC, *TFEB*-altered RCC, *ALK*-rearranged RCC and *ELOC*-mutated RCC, fumarate hydratase (FH)-deficient RCC and tumours with *TSC* mutations or mammalian target of rapamycin (mTOR) pathway activation. Although the clinical relevance of several of these molecular subtypes is still being defined, some (e.g. FH-deficient RCC) already have therapeutic and prognostic implications.

About 5%–10% of patients with RCC harbour germline mutations that result in several cancer-predisposition syndromes: BAP1 tumour-predisposition syndrome (*BAP1*), Birt–Hogg–Dubé syndrome (*FLCN*), hereditary leiomyomatosis and RCC syndrome (*FH*), hereditary papillary renal carcinoma (*MET*), hereditary paraganglioma/pheochromocytoma syndrome (*SDH* genes), tuberous sclerosis complex (*TSC*) and Von Hippel–Lindau syndrome (*VHL*).

## Predictive Value and Personalised Strategies

### Prostate Cancer

Germline and somatic alterations in *BRCA1* and *BRCA2* predict response to poly(ADP-ribose) polymerase inhibitors (PARPis). Less common HRR alterations may also sensitise tumours to PARP inhibition, but their predictive value has not been established. PROfound was the first phase III biomarker-based trial in metastatic CRPC (mCRPC) and demonstrated a significant radiographic progression-free survival (rPFS) and overall survival (OS) benefit from olaparib for patients included in cohort A from the PROfound trial (patients with *BRCA1*, *BRCA2* and *ATM* alterations) compared with a second AR pathway inhibitor (ARPi). The phase III TRITON3 trial, demonstrated a benefit in rPFS from rucaparib compared with docetaxel or a second ARPi for patients with *BRCA1/2* alterations but not for those with *ATM* mutations. Three phase III trials, PROpel, TALAPRO-2 and MAGNITUDE, have investigated the combination of an ARPi and a PARPi, demonstrating a hierarchical benefit aligned with biology: *BRCA1/2* alterations > HRR alterations > unselected patients > HRR-proficient tumours.

In 2024, The U.S. Food and Drug Administration (FDA) published the results of a pooled analysis of multiple trials of PARPis in mCRPC, confirming that the greatest benefit is seen in patients with *BRCA1/2* and *PALB2* alterations, while an apparent lack of benefit has been observed in patients with *CHEK2* or *ATM* mutations. In this analysis, patients with *CDK12* defects did not benefit from PARPi monotherapy, but rPFS and OS improvement was observed in patients who received the combination of a PARPi with an ARPi, compared with an ARPi alone.

Alterations in the PI3K pathway are mostly related to *PTEN* loss and are rarely due to *PIK3CA* or *AKT* mutations. In the phase III IPATential150 study, the combination of the AKT inhibitor ipatasertib with abiraterone resulted in improved rPFS in patients with *PTEN* loss and mutations in *PIK3CA* or *AKT*, but no OS advantage was noted. At the time of publication, there are no PIK3CA/AKT inhibitors approved for use in PC.

## Urothelial Cancer

The FGFR 1–4 inhibitor erdafitinib has been demonstrated to prolong time to disease progression and OS in patients with metastatic UC with *FGFR2/3* mutations and disease progression after one or two prior treatments, including anti-programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1), compared with clinician's choice of chemotherapy (docetaxel or vinflunine).

Identifying the patients who will respond to anti-PD-L1/PD-1 therapies remains a challenge. Patients whose tumours expressed high levels of PD-L1 responded better to atezolizumab monotherapy in the IMvigor130 study. However, PD-1/PD-L1 assessment has failed to consistently identify patients likely to respond to immune checkpoint inhibitors (ICIs) across trials where different methodologies and cut-offs were used. According to European Medicines Agency (EMA) approvals, PD-L1 immunohistochemistry is required to select patients with UC who are ineligible for cisplatin and considered for first-line ICI monotherapy (e.g. a combined positive score [CPS] ≥10 for pembrolizumab or a tumour proportion score [TPS] ≥5% for atezolizumab), as well as for treatment in the adjuvant setting with nivolumab. PD-L1 positivity is not required for platinum-refractory UC or for maintenance treatment with avelumab following chemotherapy, although greater benefit was noted in this population in the JAVELIN Bladder 100 study. ICIs may also be considered in microsatellite instability-high (MSI-H) and TMB-high (TMB-H) tumours, as well as in those with MMR defects.

A significant development in the treatment of advanced UC is the identification of nectin-4 as a biomarker and target for treatment with the antibody–drug conjugate enfortumab vedotin. In the EV-302 study, when given in combination with pembrolizumab, enfortumab vedotin demonstrated an improvement in OS in previously untreated locally advanced or metastatic UC. However, no preselection for biomarkers, including PD-L1 and nectin-4 expression, was performed in the study.

### Renal Cancer

The high prevalence of *VHL* mutations paved the way for therapies that target angiogenesis, such as vascular endothelial growth factor (VEGF) inhibitors. More recently, the HIF2α inhibitor belzutifan has demonstrated significant antitumour activity in tumours related to inherited *VHL* mutations (NCT03401788).

In patients with *TSC* mutations that result in a constitutive activation of mTOR signalling (including angiomyolipoma associated with TSC or lymphangioleiomyomatosis), significant responses to everolimus have been reported in the EXIST-2 trial.

In pRCC with *MET* alterations, the selective MET-tyrosine kinase inhibitor savolitinib has demonstrated antitumour activity but failed to improve patient outcomes compared with sunitinib in the SAVOIR trial.

## Recommendations for Genetic Testing

### Prostate Cancer

The European Society for Medical Oncology (ESMO) recommends somatic *BRCA1/2* testing (ESMO Scale for Clinical Actionability of molecular Targets [ESCAT] IA) to identify candidates for treatment with PARPis. *PALB2* alterations are still categorised as ESCAT IIB, due to their low prevalence in PC, resulting in limited information on survival outcomes. Other HRR alterations may be tested if PARPis are available for patients with these alterations. MSI and MMR defects should also be tested by next-generation sequencing (NGS) to identify candidates for treatment with ICIs, which can be found in 2%–3% of patients with metastatic PC.

ESMO and most scientific societies also recommend germline testing for all patients with metastatic PC. Panels should include high-risk genes such as *BRCA1*, *BRCA2*, MMR genes and *HOXB13* and may include other moderate-risk genes, such as *ATM*, *CHEK2* or *PALB2*. Germline testing is also recommended for patients of Ashkenazi ancestry with high-risk localised PC and for those with a personal or family history of cancer.

A practical approach to integrate the recommendations of tumour and germline testing in advanced PC is to perform tumour testing in all patients to identify candidates for PARPi therapy and follow with germline testing based on tumour testing results. If tumour-only sequencing identifies a mutation in highly actionable cancer-predisposition genes associated with PC risk (i.e. *BRCA1/2* or MMR genes), a potential germline origin of the variant should be investigated. Germline testing is also advised after negative tumour-only sequencing results if an inherited mutation is suspected based on personal or family history, as the tumour-only approach may miss some variants.

### Urothelial Carcinoma

ESMO recommends NGS testing for TMB, MSI and MMR status in patients with advanced UC, as these markers predict responsiveness to ICIs (ESCAT IC). The prevalence of MSI-H and MMR deficiency is ~3% in bladder cancer and 9% in UTUC. The assessment of *FGFR* fusions and mutations is also recommended (ESCAT IC).

Germline testing should be offered to patients with UC whose tumours are MSI-H or MMR-deficient; to those with family history of cancer that meet the Amsterdam/Bethesda criteria for the hereditary breast and ovarian cancer syndrome (HBOC) and those diagnosed at a young age.

### Renal Cancer

Germline testing should be offered to patients if they are either diagnosed aged ≤46 years, present with bilateral or multifocal tumours, have one or more first- or second-degree relatives with RCC, have related disorders associated with known predisposing conditions or have exhausted standard therapeutic options. Multigene panels should include *BAP1*, *FH*, *FLCN*, *MET*, *SDHA*, *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, *PTEN* and *VHL*. *TSC1/2* may be included based on personal or family history. Tumour-only sequencing should be followed by germline testing if a mutation in a gene related to a cancer-predisposition syndrome is identified (i.e. *FH*, *FLCN*, *TSC1/TSC2* and *SDH* genes) except for *VHL* mutations without

other clinical features as these events are frequent in RCC, but only 1.5% of cases have a germline origin.

## Further Reading


Bissler JJ, Kingswood JC, Radzikowska E, et al. Everolimus for angiomyolipoma associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): a multicentre, randomised, double-blind, placebo-controlled trial. Lancet 2013; 381:817–824.

Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature 2013; 499:43–49.

Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. Cell 2015; 163:1011–1025.

Cancer Genome Atlas Research Network; Linehan WM, Spellman PT, Ricketts CJ, et al. Comprehensive molecular characterization of papillary renal-cell carcinoma. N Engl J Med 2016; 374:135–145.

Carlo MI, Ravichandran V, Srinavasan P, et al. Cancer susceptibility mutations in patients with urothelial malignancies. J Clin Oncol 2020; 38:406–414.

Davis CF, Ricketts CJ, Wang M, et al. The somatic genomic landscape of chromophobe renal cell carcinoma. Cancer Cell 2014; 26:319–330.

Fallah J, Xu J, Weinstock C, et al. Efficacy of poly(ADP-ribose) polymerase inhibitors by individual genes in homologous recombination repair gene-mutated metastatic castration-resistant prostate cancer: a US Food and Drug Administration pooled analysis. J Clin Oncol 2024; 42:1687–1698.

Hussain M, Mateo J, Fizazi K, et al. Survival with olaparib in metastatic castration-resistant prostate cancer. N Engl J Med 2020; 383:2345–2357.

Kuzbari Z, Bandlamudi C, Loveday C, et al. Germline-focused analysis of tumour-detected variants in 49,264 cancer patients: ESMO Precision Medicine Working Group recommendations. Ann Oncol 2023; 34:215–227.

Jonasch E, Donskov F, Iliopoulos O, et al. Belzutifan for renal cell carcinoma in von Hippel–Lindau Disease. N Engl J Med 2021; 385:2036–2046.

Loriot Y, Matsubara N, Park SH, et al. Erdafitinib or chemotherapy in advanced or metastatic urothelial carcinoma. N Engl J Med 2023; 389:1961–1971.

Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2024; 35:588–606.

Parker C, Castro E, Fizazi K, et al. Prostate cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2020; 31:1119–1134.

Powles T, Albiges L, Bex A, et al. Renal cell carcinoma: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol 2024; 35:692–706.
Powles T, Bellmunt J, Comperat E, et al. ESMO Clinical Practice Guideline interim update on first-line therapy in advanced urothelial carcinoma. Ann Oncol 2024; 35:485–490.
Robertson AG, Kim J, Al-Ahmadie H, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. Cell 2017; 171:540–556. e25.
Robinson D, Van Allen EM, Wu YM, et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015; 161:1215–1228.
Terraf P, Pareja F, Brown DN, et al. Comprehensive assessment of germline pathogenic variant detection in tumor-only sequencing. Ann Oncol 2022; 33:426–433.
Tung N, Ricker C, Messersmith H, et al. Selection of germline genetic testing panels in patients with cancer: ASCO Guideline. J Clin Oncol 2024; 42:2599–2615.

## Declaration of Interest:

**Dr Ruiz-Vico** has received personal speaker honoraria from Astellas and Recordati; a personal research grant from the Spanish Society of Medical Oncology (SEOM); and travel grants from Johnson & Johnson and Merck. She has received an institutional research grant from Astellas Pharma Europe Ltd.

**Dr Moreno-Perez** has received personal speaker honoraria from Johnson & Johnson and Merck Sharp & Dohme; and congress registrations from Bayer and Merck.

**Dr de Velasco** has received personal advisory board honoraria from Arcus, Astellas, AstraZeneca, Bristol Myers Squibb (BMS), Eisai, Ipsen and Merck Sharpe & Dohme; and personal speaker honoraria from Astellas, Bayer, BMS, Ipsen, Janssen, Merck, Merck Sharp & Dohme, Novartis, Pfizer and Roche. He has received an institutional research grant from Roche.

**Dr Castro** has received personal advisory board honoraria from

AstraZeneca, Bayer, Daiichi Sankyo, Janssen, Lilly, Medscape, Merck Sharp & Dohme, Novartis and Pfizer; personal speaker honoraria from Astellas, AstraZeneca, Janssen, Medscape, PeerVoice and Pfizer; personal writing engagement honoraria from Pfizer; and steering committee honoraria from Janssen, Pfizer and Telix. She has received institutional research grants from Bayer, Janssen and Pfizer; and institutional funding for her work as local principal investigator for AstraZeneca, Janssen, MacroGenics, Merck Sharp & Dohme and Pfizer.

# Genomics in Gynaecological Cancers and Breast Cancers

6

L. Collet[1, 2]

M. Carausu[1]

C. Sotiriou[1]

I. Ray-Coquard[2]

[1]*Breast Cancer Translational Research Laboratory J.-C. Heuson, Jules Bordet Institute, Université Libre de Bruxelles (ULB), Brussels, Belgium*

[2]*Department of Medical Oncology, Centre Léon Bérard, Lyon, France*

Both gynaecological and breast cancers exhibit complex molecular alterations that drive tumourigenesis and guide personalised therapeutic approaches. Gynaecological cancers often display a high degree of genomic instability, with mutations in *TP53* or in genes involved in the homologous recombination DNA repair pathway and in the RAS–RAF–MAPK pathway playing dominant roles in tumourigenesis. Breast cancer, in contrast, is classified into several subtypes, based on hormone receptor and HER2 statuses, characterised by distinct genomic alterations.

## Gynaecological Cancers

### Epithelial Ovarian Cancers

High-grade serous carcinoma (HGSC) is the most frequent subtype, accounting for over 65% of all epithelial ovarian cancers. Other rare subtypes include endometrioid and clear-cell carcinoma, mucinous carcinoma, carcinosarcoma and low-grade serous carcinoma (LGSC), each associated with specific molecular alterations and outcomes.

#### *Key molecular features*

HGSC is characterised by a high genomic instability and few recurrent mutations, with *TP53* mutations present in more than 95% of cases. Additionally, about 50% of HGSC cases exhibit homologous

recombination deficiency (HRD), resulting in genomic scars including loss of heterozygosity, telomeric allelic imbalance and large-scale state transitions, detectable through HRD assays. While HRD can be the consequence of germline or somatic *BRCA1/2* mutations (20%–25%), *BRCA1* epigenetic silencing (~10%) and loss of function of other homologous recombination-related genes (e.g. *RAD51B/C*), some mechanisms are still unknown. Furthermore, *CCNE1* amplification, found in 15%–20% of HGSCs, is largely present in *BRCA1/2*-wild type and homologous recombination-proficient tumours. *BRCA1/2* mutations are also observed in 10% of endometrioid, clear-cell carcinomas and carcinosarcomas, and anecdotical cases of LGSCs. In addition, approximately 15% and 6% of endometrioid and clear-cell carcinomas exhibit mismatch repair deficiency (dMMR) or *POLE* mutations, respectively. Other recurrent mutations are observed in *PIK3CA* (40%–50%) and *ARIDA1* (35%–50%) genes. *ERBB2* amplification is found in 20% of mucinous carcinomas and 6% of carcinosarcomas. Mucinous carcinomas and LGSCs are also characterised by activating mutations in the RAS–RAF–MAPK pathway, with *KRAS*, *NRAS* and *BRAF* mutations occurring in over 50% of LGSC cases.

### *Genomic alterations and therapeutic strategy*

HRD and *BRCA1/2* alterations confer sensitivity to platinum-based chemotherapy and poly(ADP-ribose) polymerase inhibitors (PARPis). First-line maintenance therapy with olaparib, niraparib or rucaparib showed a 60%–70% reduction in progression risk for *BRCA*-mutated advanced ovarian cancers (OCs) (Table 1). In HRD tumours, rucaparib and niraparib demonstrated a 40%–50% reduction in progression risk. In 2019, the PAOLA-1 trial showed benefits in progression-free survival (PFS) and overall survival (OS), with the bevacizumab plus olaparib first-line maintenance combination in patients with *BRCA*-mutated or HRD tumours. Although patients who progress on PARPis have a poorer response to subsequent platinum-based chemotherapy and a worse prognosis, less than 30% of patients experienced progression with a PARPi plus bevacizumab combination in the PAOLA-1 trial, compared with over 50% with a PARPi alone; this suggests that bevacizumab may delay the development of PARPi resistance. Importantly, PARPis have also shown a PFS benefit in patients

with recurrent OC responding to platinum-based chemotherapy. However, the comparatively smaller magnitude of benefit observed in this setting supports the preferential use of PARPIs in first-line maintenance. Hence, HRD and *BRCA1/2* testing should be performed at the time of diagnosis, and prior to neoadjuvant chemotherapy in patients who are not suitable for primary debulking surgery. In contrast, *CCNE1* amplification and *BRCA1/2* reversion mutation confer resistance to PARPis and platinum-based chemotherapy. Early-phase trials have indicated that cell-cycle checkpoint-targeted therapies such as CDK2 and WEE1 inhibitors may be effective in *CCNE1*-amplified tumours.

Immune checkpoint inhibitors (ICIs) have demonstrated significant efficacy in dMMR tumours across cancer types, and should be considered for patients with relapsed dMMR OC. In 2024, the DESTINY-PanTumor02 phase II trial showed the efficacy of trastuzumab deruxtecan (T-DXd), an anti-HER2 (human epidermal growth factor receptor 2) antibody–drug conjugate (ADC), in HER2-overexpressing tumours, including OC. Finally, clinical trials support the use of MEK inhibitors, alone or in combination with FAK inhibitors, in LGSC with alterations in the RAS–RAF–MAPK pathway.

**Recommendations for genetic testing**

- Germline and/or somatic *BRCA1/2* mutation and HRD testing at diagnosis in all patients with high-grade, epithelial non-mucinous OC.
- To be considered: *BRCA1/2* reversion-mutation testing at relapse, especially if PARPis are still a treatment option.

If access to ICIs or targeted therapies or inclusion in a clinical trial are a possibility, the following tests should be considered:

- Mismatch repair (MMR) status testing, especially for endometrioid and clear-cell carcinoma
- *NRAS*, *KRAS* and *BRAF* mutation testing for LGSC
- HER2 overexpression, especially for clear-cell, mucinous OC and carcinosarcoma OC
- For diagnostic purposes, *FOXL2* (somatic) and *DICER1* (somatic and germline) testing should be performed for sex cord-stromal tumours.

*Table I ESCAT Testing Recommendations for Genomic Alterations in Gynaecological Cancers.*

| ESCAT | Alteration | Matched drug | Testing recommendation: detection | Setting for testing | Tumour type |
|---|---|---|---|---|---|
| IA | Somatic or germline *BRCA1/2* | PARPis ± bevacizumab (olaparib, niraparib, rucaparib, olaparib plus bevacizumab) | Recommended: tumour tissue | Advanced stage III/IV *BRCA1/2*-mutated ovarian cancers in first-line maintenance therapy<br>*In platinum-sensitive relapse if patient did not receive PARPi in first-line* | All epithelial non-mucinous ovarian cancers |
| | HRD | PARPis ± bevacizumab (olaparib, niraparib, rucaparib, olaparib plus bevacizumab) | Recommended: tumour tissue<br>Genomic instability test | Advanced stage III/IV HRD-mutated ovarian cancers in first-line maintenance therapy<br>*In platinum-sensitive relapse if patient did not receive PARPi in first-line* | All epithelial non-mucinous ovarian cancers |
| | MSI status | dMMR Chemotherapy + ICIs (pembrolizumab, dostarlimab, atezolizumab, durvalumab) | Recommended: tumour tissue<br>MMR IHC or MSI test | Advanced stage III/IV dMMR endometrial carcinoma in first-line | Endometrial carcinoma<br>*Recommended in early-stage endometrial carcinoma to guide adjuvant chemotherapy and radiotherapy decisions* |
| | | pMMR Chemotherapy + ICIs ± PARPis (pembrolizumab, dostarlimab + niraparib, durvalumab + olaparib) | | Advanced stage III/IV pMMR endometrial carcinoma in first-line | |
| IB | Somatic *TSC1/TSC2* mutations | mTOR inhibitors (nab-sirolimus, sirolimus, everolimus, temsirolimus) | Recommended: tumour tissue | Advanced PEComa with *TSC1/2* mutations | Uterine PEComa |

*Table I ESCAT Testing Recommendations for Genomic Alterations in Gynaecological Cancers.* (Continued)

| ESCAT | Alteration | Matched drug | Testing recommendation: detection | Setting for testing | Tumour type |
|---|---|---|---|---|---|
| IC | HER2 | HER2-targeted antibody–drug conjugates (trastuzumab deruxtecan) | Recommended: tumour tissue<br>ASCO/College of American Pathologists guidelines for scoring HER2 IHC in gastric cancer | Advanced ovarian, cervical and endometrial cancer with HER2 2+/3+ in recurrence after standard-of-care treatment<br>*Mainly observed in uterine and ovarian carcinosarcoma, serous uterine carcinoma and mucinous ovarian cancers* | Ovarian, cervical and endometrial cancer |
| | Microsatellite instability | ICIs | Recommended: tumour tissue<br>MMR, IHC or MSI testing | Advanced ovarian, cervical and endometrial cancer in recurrence after standard-of-care treatment<br>*Mainly observed in clear-cell and endometrioid ovarian cancers* | Ovarian and cervical cancer |
| | *NTRK* fusion | TRK inhibitors (entrectinib, larotrectinib) | Recommended: tumour tissue or blood<br>IHC, FISH, RNA or DNA sequencing | Advanced ovarian, cervical and endometrial cancer with *NTRK* fusion recurrence after standard-of-care treatment<br>*Mainly observed in uterine sarcoma, should be offered in first-line setting in uterine sarcoma with NTRK fusion* | Ovarian, cervical and endometrial cancer |
| IIA | N/A | N/A | N/A | N/A | N/A |
| IIB | Somatic *BRAF/KRAS/NRAS* mutations | MEK inhibitors (cobimetinib, trametinib) | Recommended: tumour tissue | Advanced stage III/IV low-grade serous ovarian cancer in recurrence after standard-of-care treatment | Ovarian cancer |
| | *CCNE1* amplification | WEE1 inhibitors (adavosertib) | Could be considered in certain situations: tumour tissue | Advanced platinum-resistant ovarian cancer | Ovarian cancer |
| | Somatic *KRAS* mutations | MEK ± FAK inhibitors (avutometinib ± defactinib) | Could be considered in certain situations: tumour tissue | Advanced stage III/IV low-grade serous ovarian cancer in recurrence after standard-of-care treatment | Ovarian cancer |

*Table I ESCAT Testing Recommendations for Genomic Alterations in Gynaecological Cancers.* (Continued)

| ESCAT | Alteration | Matched drug | Testing recommendation: detection | Setting for testing | Tumour type |
|---|---|---|---|---|---|
| IIIA | *Somatic alteration in PIK3CA, AKT1, KRAS, BRAF, ESR1* | N/A | N/A | Not recommended in clinical routine | N/A |
| IIIB | *Somatic alteration in PALB2, ATM, ATR, CHEK1/2, RAD51B/C/D, CDK12, MAP2K1, MAP3K1, MAPK1, HRAS, NRAS, PIK3CB, AKT2, TSC1/2* | N/A | N/A | | |
| IVA | *Somatic alteration in ARID1A/B, PALB2, ATM, ATR, CHEK1/2, TP53BP1, DNA-PK, FANC genes, CDH4, ERCC genes, POLL, MRE11, RAD51B/C/D, CHEK1/2, TP53, CCNE1, GAS6/AXL, ABCB1, ABCG2, AKT2, SLC7A11* | N/A | N/A | | |
| IVB | N/A | N/A | N/A | | |
| V | N/A | N/A | N/A | Not recommended in clinical routine | N/A |
| X | N/A | N/A | N/A | | |

Level of evidence: 1A, Randomised clinical trials with clinically meaningful improvement in survival; 1B, Non-randomised clinical trial with clinically meaningful benefit; 1C, Clinical trial with benefit across tumour types; 11A, Retrospective studies with clinically meaningful benefit; 11B, Prospective clinical trial with improved response to therapy but no data on survival; 111A, Clinical benefit in a different tumour type; 111B, Similar predicted functional impact as a Tier 1 abnormality in the same gene or pathways, but without supportive clinical data; 1VA, Preclinical evidence from *in vitro* or *in vivo* models; 1VB, Actionability predicted *in silico*; V, Antitumour activity expected in combination; X, No evidence for actionability.

Abbreviations: ASCO, American Society of Clinical Oncology; dMMR, mismatch repair deficiency; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; ESMO, European Society for Medical Oncology; FISH, fluorescent *in situ* hybridisation; HER2, human epidermal growth factor receptor 2; HRD, homologous recombination deficiency; ICI, immune checkpoint inhibitor; IHC, immunohistochemistry; mTOR, mammalian target of rapamycin; MMR, mismatch repair; MSI, microsatellite instability; N/A, not applicable; PARPi, poly(ADP-ribose) polymerase inhibitor; PEComa, perivascular epithelioid cell tumour; pMMR, mismatch repair proficiency; TRK, tropomyosin receptor kinase.

## Endometrial Cancers

Endometrial carcinoma (EC) is the fourth most frequent carcinoma in women in Europe, with a rapidly rising incidence. The most common histological subtype is endometrioid EC (80%), followed by serous carcinoma (10%) and carcinosarcoma (5%–10%). Uterine sarcomas are rare diseases represented by leiomyosarcoma (the most common subtype), endometrial stromal sarcoma (ESS), adenosarcoma, undifferentiated uterine sarcomas and tumours of uncertain malignant potential, including perivascular epithelioid cell tumours (PEComas) and *NTRK*-rearranged sarcomas.

### *Key molecular features*

In 2013, The Cancer Genome Atlas integrated multi-omics characterisation of EC, leading to the identification of four molecular subtypes: *TP53*-abnormal, *POLE*-mutated (*POLE*mut), dMMR and 'no specific molecular profile' (NSMP). To facilitate clinical application, a clear diagnostic algorithm has been developed (Léon-Castillo, 2023). *ERBB2* amplification occurs in 17%–33% of carcinosarcomas and serous carcinomas. Molecular alterations also guide the diagnostic and therapeutic strategies in uterine sarcoma. Low-grade ESSs are characterised by the *JAZF1–SUZ12* gene fusion in ≥70% of cases, and other less frequent rearrangements such as *JAZF1–PHF1* or *EPC1–PHF1*, while high-grade ESSs are mainly driven by *YWHAE–NTM2A/B* fusion and *BCOR* rearrangement. *TSC1/2* mutations are found in ≥80% of PEComas. *NTRK*-rearranged sarcomas are a distinct entity.

*Genomic alterations and therapeutic strategy*

While *TP53*-abnormal tumours have a poor prognosis, *POLE*mut tumours have an excellent outcome. NSMP and dMMR tumours have an intermediate prognosis. In 2025, the NSMP subgroup was further refined based on hormone receptor status and histological grade, with oestrogen receptor (ER)-negative tumours or grade III tumours being associated with poorer prognosis, regardless of ER status.

In early-stage disease, *TP53*-abnormal tumours as well as high-grade and/or ER-negative NSMP tumours with any myometrial invasion, are considered high risk for relapse and should be treated with adjuvant chemoradiotherapy. Conversely, International Federation of Gynecology and Obstetrics (FIGO) stage I or II *POLE*mut tumours are treated as low-risk disease, without the need for adjuvant therapy.

For advanced disease, ICIs combined with platinum-based chemotherapy showed a 60%–70% reduction in the risk of progression or death in dMMR EC. This has established ICIs as the standard of care in first-line advanced dMMR and *POLE*mut EC (Table 1). The RUBY Part 2 and DUO-E trials also demonstrated improved PFS with first-line chemoimmunotherapy followed by maintenance therapy combining ICIs and PARPis in MMR-proficient (pMMR) EC, establishing this as a standard therapeutic strategy. In *TP53*-wild type tumours, selinexor, a selective inhibitor of the nuclear export protein XPO1, suggested a PFS benefit in first-line maintenance therapy in the SIENDO trial; the XPORT-EC-042 phase III trial, dedicated to this *TP53*-wild type population, is ongoing at the time of publication.

HER2-targeted therapy should be considered in *ERBB2*-amplified tumours. Regarding rare cancers, patients with *TSC1/2*-mutated uterine PEComas may benefit from mammalian target of rapamycin (mTOR) inhibitors, which have shown an overall response rate (ORR) greater than 40%. Additionally, neurotrophic tyrosine receptor kinase (NTRK) inhibitors (larotrectinib or entrectinib) have demonstrated an ORR of 75% in *NTRK*-rearranged tumours.

**Recommendations for genetic testing**

- Molecular classification including MMR status, *POLE* and *TP53* mutations for early-stage and advanced-stage ECs.
- To be considered: testing for HER2 overexpression.
- Gene fusion panels including *JAZF1*- and *NTRK*-rearrangements are recommended for the diagnosis of uterine sarcoma.
- *TCS1/2* mutation testing is recommended in uterine PEComas.

## Cervical Carcinoma

Cervical cancer is rare in Europe but remains a major public health problem in low-income countries. Most cases (80%) are squamous-cell carcinomas and more than 90% are associated with human papillomavirus (HPV).

### *Key molecular features*

Genomic instability is prevalent, but recurrent mutations in *PIK3CA*, *PTEN*, *TP53* or *KRAS* have been reported. HER2 overexpression is also found in more than 20% of cervical cancers. In addition, gastric-type adenocarcinoma, a rare, aggressive and HPV-independent subtype of cervical cancer, can be associated with *STK11* mutations and Peutz–Jehgers syndrome.

### *Genomic alterations and therapeutic strategy*

The DESTINY-PanTumor02 phase II trial showed the efficacy of T-DXd in the cervical cancer cohort, with an ORR of 63% in HER2-overexpressing tumours. *STK11* mutations are associated with poor outcome and treatment resistance.

**Recommendation for Genetic Testing**

- Consider HER2 overexpression testing in the relapsed setting if access to HER2-targeted therapy is an option.
- *STK11* germline testing should be considered in gastric-like adenocarcinoma of the cervix.

# Breast Cancer

## Genomics in Breast Cancer

Breast cancer (BC) remains a significant global health challenge, being the most diagnosed cancer and the leading cause of cancer-related death among women worldwide, according to GLOBOCAN 2022. Based on histology and immunohistochemical markers, international guidelines classify BC into four subtypes for prognosis and clinical decisions: luminal A-like (30%–40%), luminal B-like (20%–30%), HER2-positive (12%–20%), and triple-negative BC (TNBC, ~15%). These subtypes correspond to the molecular intrinsic subtypes identified by gene expression profiling: luminal A, luminal B, HER2-enriched and basal-like, respectively.

## Key Molecular Features

Sporadic BC pathogenesis is driven by genomic alterations in pathways involving hormones (ER, progesterone receptor, androgen receptor), growth factors (*ERBB2*, fibroblast growth factor receptor 1 [FGFR1]), cell-cycle regulation (cyclin-dependent kinase 4/6 [CDK 4/6], *RB1*, *TP53*), PI3K–AKT–mTOR and RAS–RAF–MAPK. About 70% of BCs are linked to recurrent copy number alterations (CNAs), gene amplifications (*ERBB2*, *CCND1*) or deletions (*CDH1*, *CDKN2A/B*, *PTEN*). Tumours driven by recurrent mutations are mostly luminal. Key somatic mutations are found in genes such as *TP53*, *PIK3CA*, *MYC*, *CDH1*, *ERBB2*, *FGFR1*, *CCND1*, *RB1*, *AKT1* and *GATA3*. Actionable alterations are described in Table 2.

Different mutational landscapes characterise BC subtypes. Luminal A tumours harbour more frequent *PIK3CA* (45%) and *GATA3* (14%) mutations than luminal B tumours, while the latter has a higher frequency of *TP53* mutations (29%) and CNAs involving gains in 8q and 17q and losses in 13q and 16q. HER2-enriched BC is characterised by *ERBB2* amplification (~80%) as well as numerous genomic gains and losses, alongside mutations in the *TP53* (72%) and *PIK3CA* (39%) genes. TNBC has a higher mutational burden, with *TP53* mutations as the most frequent alterations (~80%), followed by mutations affecting

*PIK3CA*, *PTEN*, *RB1* and genes involved in homologous recombination DNA repair, leading to HRD. The mutational profile also varies between primary BC and metastases, which were found to be enriched with mutations in *ESR1*, *PTEN*, *CDH1*, *PIK3CA* and *RB1* (in particular, as a mechanism of resistance to CDK4/6 inhibitors), along with *MDM4* and *MYC* amplifications and *ARID1A* deletions.

*Table 2 ESCAT Testing Recommendations for Genomic Alterations in Breast Cancer.*

| ESCAT | Alteration | Matched drug | Testing recommendation: detection | Setting for testing |
|---|---|---|---|---|
| IA | *ERBB2* amplification | HER2-targeted therapy (monoclonal antibodies, TKIs, ADCs) | Recommended: tumour tissue | Early-stage or advanced breast cancer with HER2 IHC 2+ |
| | Germline *BRCA1/2* mutations | PARP inhibitors (olaparib, talazoparib) | Recommended: blood | Early-stage or advanced breast cancer in patients that meet eligibility criteria |
| | *PIK3CA* mutations | PI3K inhibitor (alpelisib) + ET (fulvestrant) | Recommended: tumour tissue | HR-positive/HER2-negative advanced breast cancer after progression on ET with CDK4/6 inhibitor |
| | | AKT inhibitor (capivasertib) + ET (fulvestrant) | | |
| | *ESR1* mutations | SERD (elacestrant) | Recommended: tumour tissue or blood | HR-positive/HER2-negative advanced breast cancer after progression on ET with CDK4/6 inhibitor |
| | *PTEN* mutations/ deletions or *AKT1* mutations | AKT inhibitor (capivasertib) + ET (fulvestrant) | Recommended: tumour tissue | HR-positive/HER2-negative advanced breast cancer after progression on ET with CDK4/6 inhibitor |
| IB | Non-randomised clinical trials with clinically meaningful benefit | N/A | N/A | N/A |

*Table 2 ESCAT Testing Recommendations for Genomic Alterations in Breast Cancer.* (Continued)

| ESCAT | Alteration | Matched drug | Testing recommendation: detection | Setting for testing |
|---|---|---|---|---|
| IC | Microsatellite instability | PD-1 checkpoint inhibitors (pembrolizumab) | May be considered if matched drug available: tumour tissue | Advanced breast cancer after progression on subtype-specific standard therapies |
| | *NTRK* fusions | TRK inhibitors (larotrectinib, entrectinib) | May be considered if matched drug available: tumour tissue or blood | Advanced breast cancer after progression on subtype-specific standard therapies |
| | High tumour mutational burden (≥10 mut/Mb) | PD-1 checkpoint inhibitors (pembrolizumab) | May be considered if matched drug available: tumour tissue or blood | Advanced breast cancer after progression on subtype-specific standard therapies |
| IIA | Retrospective studies with clinically meaningful benefit | N/A | N/A | N/A |
| IIB | *ERBB2* mutations | HER2-targeted TKI (neratinib) ± trastuzumab/ fulvestrant | Could be considered in certain situations: tumour tissue or blood | Option in HER2-negative advanced breast cancer after progression on subtype-specific standard therapies and matched drug available |
| | | HER2-targeted ADC (trastuzumab deruxtecan) | | |
| | Germline *PALB2* mutations | PARP inhibitor (olaparib) | Could be considered in certain situations: blood | Option in advanced breast cancer progressing on prior therapy and matched drug available |
| | Somatic *BRCA1/2* mutations | PARP inhibitor (olaparib) | Could be considered in certain situations: tumour tissue or blood | Option in advanced breast cancer progressing on prior therapy and matched drug available |

*Table 2 ESCAT Testing Recommendations for Genomic Alterations in Breast Cancer.* (Continued)

| ESCAT | Alteration | Matched drug | Testing recommendation: detection | Setting for testing |
|---|---|---|---|---|
| IIIA | *MDM2* amplification | N/A | Not recommended in clinical routine | N/A |
| IIIB | N/A | | | |
| IVA | Somatic alterations in *ARID1A/B, ATR/ATM/ PALB2, CDH1, IGF1R, INPP4B, MAP2K4/ MAP3K1, MT4, MYC, NF1, PIK3R1, RUNX1/ CBFB, SF3B1, TP53* | | | |
| IVB | N/A | | | |
| V | N/A | N/A | Not recommended in clinical routine | N/A |
| X | *CCND1, FGFR1* amplifications | | | |

Level of evidence: 1A, Randomised clinical trials with clinically meaningful improvement in survival; 1B, Non-randomised clinical trials with clinically meaningful benefit; 1C, Clinical trials with benefit across tumour types; 11A, Retrospective studies with clinically meaningful benefit; 11B, Prospective clinical trials with improved response to therapy but no data on survival; IIIA, Clinical benefit in a different tumour type; 111B, Similar predicted functional impact as a Tier I abnormality in the same gene or pathway, but without supportive clinical data; 1VA, Preclinical evidence from *in vitro* or *in vivo* models; 1VB, Actionability predicted *in silico*; V, Prospective studies with objective response, but without clinically meaningful benefit; X, No evidence for actionability.

*The ESCAT level (I or II) for *AKT1/PTEN* alterations is debated at the time of publication. ESCAT level I indicates targets (and matched drugs) ready for routine use; level II, investigational targets; levels III and IV, hypothetical targets; levels V and X, targets studied for combination development.

Abbreviations: ADC, antibody–drug conjugate; CDK4/6, cyclin-dependent kinase 4/6; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; ESMO, European Society for Medical Oncology; ET, endocrine therapy; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IHC, immunohistochemistry; mut/Mb, mutation per megabase; SERD, selective oestrogen-receptor degrader; N/A, not applicable; PARP, poly(ADP-ribose) polymerase; PD-1, programmed cell death protein 1; TKI, tyrosine kinase inhibitor; TRK, tropomyosin receptor kinase.

Hereditary BC constitutes approximately 10% of all cases, mainly driven by germline pathogenic variants (PVs) in genes involved in DNA repair via homologous recombination, such as *BRCA1/2* (the most frequently implicated), *PALB2*, *ATM*, *CHEK2* and *RAD51C/D*, leading to HRD. Around 3% of patients carry germline PVs in high-risk genes (with odds ratios >4) that significantly increase the risk of BC. These PVs are *BRCA1* (cumulative lifetime risk of 72%, particularly linked to TNBC), *BRCA2* (69%, more often linked to luminal B-like BC), *PALB2* (33%–58% risk), *CDH1* with an increased risk of lobular BC (39%–52%), as well as rarer syndromic genes associated with markedly elevated risks – *PTEN* (Cowden syndrome, 25%–85%), *STK11* (Peutz–Jeghers, 45%–50%) and *TP53* (Li–Fraumeni, 40%–79%). Another 3% have germline PVs in moderate-risk genes, which confer a 2–4-fold increase in odds ratio, and encompass *ATM* and *CHEK2*. The remaining 4% have unidentified genetic risk factors. Meanwhile, low-risk genes, such as *BARD1*, *RAD51C/D* (specifically associated with TNBC) and MMR genes, may also be involved in BC susceptibility. Importantly, cancer risk varies significantly by specific mutation within genes. Protein-truncating variants (PTVs) carry the strongest associations with BC. Pathogenic missense variants in *BRCA1*, *BRCA2* and *TP53* carry risks similar to PTVs, while rare missense variants in *CHEK2*, *ATM* and *TP53* are modestly associated with BC, with minimal clinical impact, underscoring the importance of variant pathogenicity in risk assessment.

### Clinical Relevance and Personalised Strategies

In 2018, the European Society for Medical Oncology (ESMO) introduced the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) to categorise DNA alterations by clinical utility. Table 2 summarises genomic alterations in BC by ESCAT level, their matched drugs, and testing recommendations, with the most clinically relevant discussed below.

Germline *BRCA1/2* PVs increase BC and OC risk (and may increase the risk for other malignancies), and risk-reducing strategies such as risk-reducing salpingo-oophorectomy (RRSO) and risk-reducing mastectomy should be offered to carriers of PVs. These mutations also predict response to platinum-based chemotherapy and to PARPis (olaparib, talazoparib), approved

for early and advanced stages based on phase III trials demonstrating improved PFS (OlympiAD and EMBRACA trials in advanced BC) or OS (OlympiA trial in early BC). While efficacy for other HRD-related germline mutations is not established, there is evidence for the clinical efficacy of PARPis in the presence of germline *PALB2* mutations, alongside somatic *BRCA1/2* mutations (found in 3% of patients) in the TBCRC 048 phase II study.

*ERBB2* amplification, a marker of poor prognosis, predicts benefit from anti-HER2 therapies, including monoclonal antibodies (trastuzumab, pertuzumab), tyrosine kinase inhibitors (lapatinib, neratinib, tucatinib) and ADCs (trastuzumab emtansine, T-DXd). Its predictive value is well-supported by randomised trials, improving survival in both early and advanced stages, and *ERBB2* amplification is routinely assessed via *in situ* hybridisation. *ERBB2* mutations, occurring in about 5% of BCs independent of amplification, activate HER2 signalling and influence sensitivity to HER2-targeted therapies. Patients with tumours harbouring these mutations had clinical responses to neratinib in the phase II SUMMIT trial, and to T-DXd in the phase II DESTINY-PanTumor01 basket study.

*PIK3CA* mutations, present in ~30% of BCs, are associated with ER positivity, older age and better disease-free survival. Based on the phase III SOLAR-1 trial, the PI3K inhibitor alpelisib is approved in combination with fulvestrant for *PIK3CA*-mutated advanced luminal BC, after progression in first-line with endocrine therapy (ET) and a CDK4/6 inhibitor. The CAPItello-291 trial also led to the approval of the AKT inhibitor capivasertib combined with fulvestrant, for patients with *PIK3CA/AKT1/PTEN* alterations, together with a tissue-based next-generation sequencing (NGS) assay as a companion diagnostic device (*PTEN* deletions are difficult to detect with blood-based assays). Both trials showed improved PFS but no OS benefit. NGS is recommended for the detection of these genomic alterations, although the ESCAT level (I or II) for *AKT1*/*PTEN* alterations is debated.

*ESR1* mutations activate ER alpha (ERα) independent of oestrogen, causing resistance to ET. These mutations, common in advanced luminal BC (30%–40%) after aromatase inhibitor therapy, are associated with worse survival. The U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved the selective ER degrader (SERD) elacestrant for *ESR1*-mutated advanced luminal BC after progression on first-line with ET and a CDK4/6 inhibitor, based on improved PFS in the phase III EMERALD trial. Routine testing for *ESR1* mutations is now recommended if the patient is eligible for elacestrant therapy. Testing recommendations for genomic alterations in BC are detailed in Table 2.

## Further Reading

### Gynaecological Cancers


Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature 2011; 474:609–615.

Gershenson DM, Miller A, Brady WE, et al. Trametinib versus standard of care in patients with recurrent low-grade serous ovarian cancer (GOG 281/LOGS): an international, randomised, open-label, multicentre, phase 2/3 trial. Lancet 2022; 399:541–553.

Ledermann JA, Matias-Guiu X, Amant F, et al. ESGO–ESMO–ESP consensus conference recommendations on ovarian cancer: pathology and molecular biology and early, advanced and recurrent disease. Ann Oncol 2024; 35:248–266.

Léon-Castillo A. Update in the molecular classification of endometrial carcinoma. Int J Gynecol Cancer 2023; 33:333–342.

Meric-Bernstam F, Makker V, Oaknin A, et al. Efficacy and safety of trastuzumab deruxtecan in patients with HER2-expressing solid tumors: primary results from the DESTINY-PanTumor02 phase ii trial. J Clin Oncol 2024; 42:47–58.

Mirza MR, Chase DM, Slomovitz BM, et al. Dostarlimab for primary advanced or recurrent endometrial cancer. N Engl J Med 2023; 388:2145–2158.

Patch AM, Christie EL, Etemadmoghadam D, et al. Whole-genome characterization of chemoresistant ovarian cancer. Nature 2015; 521:489–494.

Ray-Coquard I, Leary A, Pignata S, et al. Olaparib plus bevacizumab first-line maintenance in ovarian cancer: final overall survival results from the PAOLA-1/ENGOT-ov25 trial. Ann Oncol 2023; 34:681–692.

Ray-Coquard I, Pautier P, Pignata S, et al. Olaparib plus bevacizumab as first-line

maintenance in ovarian cancer. N Engl J Med 2019; 381:2416–2428.
Vergote I, Perez Fidalgo A, Valabrega G, et al. ENGOT-EN20/GOG-3083/XPORT-EC-042 - a phase III, randomized, placebo-controlled, double-blind, multicenter trial of selinexor in maintenance therapy after systemic therapy for patients with p53 wild-type, advanced, or recurrent endometrial carcinoma: rationale, methods, and trial design. Int J Gynecol Cancer 2024; 34:1283–1289.

## Breast Cancer

Aftimos P, Oliveira M, Irrthum A, et al. Genomic and transcriptomic analyses of breast cancer primaries and matched metastases in AURORA, the Breast International Group (BIG) Molecular Screening Initiative. Cancer Discov 2021; 11:2796–2811.
Breast Cancer Association Consortium; Dorling L, Carvalho S, Allen J, et al. Breast cancer risk genes — association analysis in more than 113,000 women. N Engl J Med 2021; 384:428–39.
Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012; 490:61–70.
Coates AS, Winer EP, Goldhirsch A, et al. Tailoring therapies—improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Ann Oncol 2015; 26:1533–1546.
Condorelli R, Mosele F, Verret B, et al. Genomic alterations in breast cancer: level of evidence for actionability according to ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Ann Oncol 2019; 30:365–373.
Gennari A, André F, Barrios CH, et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. Ann Oncol 2021; 32:1475–1495.
ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium. Pan-cancer analysis of whole genomes. Nature 2020 578:82–93.
Loibl S, André F, Bachelot T, et al. Early breast cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol 2024; 35:159–182.
Mateo J, Chakravarty D, Dienstmann R, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Ann Oncol 2018; 29:1895–902.
Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in

2024: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2024; 35:588–606.
NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Version 4.2025, April 17, 2025© 2025 National Comprehensive Cancer Network® (NCCN®), Breast Cancer Version 4.2025 – April 17, 2025 (last accessed 29 July 2025).
Nik-Zainal S, Davies H, Staaf J, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature 2016; 534:47–54.
Sessa C, Balmaña J, Bober SL, et al. Risk reduction and screening of cancer in hereditary breast-ovarian cancer syndromes: ESMO Clinical Practice Guideline. Ann Oncol 2023; 34:33–47.

## Declaration of Interest:

**Dr Collet** has received congress travel grants from AstraZeneca, GSK and PharmaMar and personal invited speaker honoraria via her institution from AstraZeneca and GSK.

**Dr Carausu** has declared no conflicts of interest.

**Professor Sotiriou** has declared institutional advisory board honoraria from Amgen, Astellas, Merck Sharp & Dohme (MSD) and Seattle Genetics; personal advisory board honoraria from Cepheid and Signatur Biosciences; personal speaker honoraria from Prime Oncology, and institutional speaker honoraria from Exact Sciences. He has received personal travel funding from Pfizer, and institutional travel funding from Roche. He also holds stocks/shares in Signatur Biosciences.

**Dr Ray-Coquard** has declared personal advisory board honoraria from AbbVie, Adaptimmune, Agenus, Amgen, AstraZeneca, Bristol Myers Squibb (BMS), Clovis, Daiichi Sankyo, Deciphera Pharmaceuticals, EQRx, Eisai, Genmab, GSK, ImmunoGen, Incyte, Loxo@Lilly, MacroGenics, Merck Serono, Mersana Therapeutics, MSD, Novartis, Oxnea, pharma&, PMV Pharma, Regeneron Pharmaceuticals, Roche, Seagen and Sutro Biopharma; she has received institutional funding for translational research from BMS and MSD. She has declared

her past role as president for GINECO (Groupe d'Investigateurs National des Etudes des Cancers Ovariens et du sein) and as principal investigator for the PAOLA1 trial.

# Genomics in Skin, Neurological and Rare Cancers

7

D. Kee[1]

J.A. Pérez-Fidalgo[2]

M. Maccari[3]

G. Minniti[4]

[1]*Department of Medical Oncology, Austin Health, Heidelberg; Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia*

[2] *Medical Oncology Department, Hospital Clinico Universitario of Valencia, Valencia; INCLIVA, University of Valencia, Valencia, Spain*

[3]*Department of Surgery, Oncology and Gastroenterology, University of Padua, Padua; Medical Oncology 1, Veneto Institute of Oncology IOV-IRCCS, Padua, Italy*

[4] *Department of Radiological Sciences, Oncology, and Anatomical Pathology, Sapienza University of Rome, Rome; IRCCS Neuromed, Pozzilli (IS), Italy*

This chapter highlights advances in cancer genome profiling for a variety of different adult and paediatric cancers, including skin, brain and rare cancers. The role of genetic alterations such as gene fusions, deletions or mutations in the diagnosis of a specific cancer and the selection of patients for targeted agents will be discussed.

## Skin Cancers

### Non-Melanoma Skin Cancers

Keratinocyte cancers, which include basal cell carcinoma (BCC) and squamous-cell carcinoma (SCC), are the most common skin cancers. Both BCC and SCC are primarily driven by ultraviolet (UV) radiation exposure, with associated characteristic genomic alterations.

#### *Basal cell carcinoma*

BCC is the most frequently diagnosed skin cancer in Europe, accounting

for up to 80% of non-melanoma skin cancers. It is locally aggressive but rarely metastasises. BCCs are strongly associated with chronic UV radiation exposure and exhibit the highest tumour mutational burden (TMB) of any human cancer, with a median TMB of 65 mutations per megabase (mut/Mb), and dominant UV-mutation signature.

Although most mutations identified in BCC are likely silent or passenger events, aberrant hedgehog (HH) pathway signalling is central to BCC pathogenesis. Genetic alterations that lead to activation of this pathway are near universal: most commonly somatic loss-of-function mutations in *PTCH1* (70%–75%) or activating mutations in *SMO* (10%–20%), followed by inactivating variants in *SUFU* (approximately 8%) or *PTCH2*. Germline mutations in *PTCH1* (and, less frequently, *PTCH2* and *SUFU*) are associated with hereditary basal cell naevus syndrome. The SMO inhibitors vismodegib and sonidegib are effective in treating BCC by inhibiting aberrant HH pathway activation. Beside the HH pathway, other common alterations in BCC involve the genes *TP53*, *LATS1/2*, *DPH3/OXNAD1*, *TERT* and *MYC*.

### *Squamous-cell carcinoma*

Cutaneous SCC (cSCC) is the second most common skin cancer and has a higher propensity for metastases than BCC. cSCC typically arises from premalignant actinic keratoses (AKs), which develop in chronically sun-exposed skin. Similar to BCC, cSCC has a high TMB, with a median of 35–50 mut/Mb. However, cSCC lacks a common molecular driver. Instead, its genomic landscape is characterised by the inactivation of numerous tumour suppressor pathways, making development of targeted therapies in cSCC challenging.

The most common recurrent genetic alterations identified in cSCC are UV-induced inactivating point mutations in *TP53* (50%–90%) and *NOTCH1* or *NOTCH2* (75%). These mutations are acquired early, being detectable in AKs and even in apparently normal surrounding skin. Progression to invasive SCC requires an accumulation of additional events such as the inactivation of *CDKN2A* and mutations in *HRAS*. These become more common in invasive disease and are associated with more aggressive behaviour.

### *Merkel cell carcinoma*

Merkel cell carcinoma (MCC) is a rare but aggressive neuroendocrine skin cancer. It primarily affects older adults (≥65 years) and presents as a fast-growing, painless nodule on sun-exposed skin. Similar to other small cell/neuroendocrine tumours, MCC commonly exhibits inactivation of *TP53* and *RB1*, along with disrupted NOTCH pathway signalling. Other frequently altered genes are *KMT2D*, *PTEN/PIK3CA*, *CDKN2A*, *ARID1A* and *FAT1*. Furthermore, MCCs segregate into two distinct molecular groups: TMB-high tumours associated with a UV-mutation signature, and TMB-low tumours associated with Merkel cell polyomavirus (MCPyV) DNA integration. Both processes lead to the high expression of neoantigens and derive similar benefit from immune checkpoint therapies.

### Melanoma

Melanomas constitute only 4% of all skin cancers but are responsible for 80% of skin cancer-related deaths. Most cutaneous melanomas are linked to UV radiation exposure and are prevalent in fair-skinned populations. However, melanomas also develop in non-sun-exposed areas such as acral, mucosal and uveal sites. These are less common but account for a higher proportion of melanomas diagnosed in non-fair-skinned populations.

The different melanoma subtypes have varied genomic profiles, but nearly all melanomas demonstrate aberrant activation of the mitogen-activated protein kinase (MAPK) pathway. This results from distinct genomic events that categorise melanomas into four molecular classes: *BRAF*-mutated, *RAS*-mutated, neurofibromin 1 (NF1)-inactivated and triple wild-type.

*Table 1 Melanoma Subtypes and Key Molecular Characteristics.*

| | Cutaneous melanoma | Acral melanoma | Mucosal melanoma | Uveal melanoma |
|---|---|---|---|---|
| *BRAF*-mutated | 42% | 22% | 9% | 0 |
| *RAS*-mutated | 28% | 20% | 20% | 0 |
| NF1-inactivated | 23% | 26% | 34% | 6% |
| Triple wild-type | 8% | 32% | 37% | 94% |
| Median TMB (mut/Mb) | 36.3 | 2.1 | 2.3 | 0.5 |

Abbreviations: mut/Mb, mutation per megabase; NF1, neurofibromin 1; TMB, tumour mutational burden.

### *BRAF-mutated melanomas*

*BRAF* mutations occur in 35%–50% of melanomas; of these, approximately 85% are *BRAF* V600E and 5% *BRAF* V600K variants. *BRAF* V600E variants are most frequent in melanomas arising in intermittently sun-damaged skin and in younger patients (≤50 years). Less common *BRAF* V600K or K601E variants (and *NRAS* variants) are associated with chronically sun-damaged skin and older patients. *BRAF* V600-mutated melanomas are sensitive to selective BRAF inhibitors (dabrafenib, vemurafenib and encorafenib) and MEK inhibitors (trametinib, cobimetinib and binimetinib) used together in combination therapy.

### *NRAS-mutated melanomas*

*NRAS* mutations are present in 20%–30% of melanomas. Therapeutic options specific to *NRAS*-mutated melanoma are limited. MEK inhibitors have modest activity, that has not led to improved overall survival compared with chemotherapy.

### *NF1-mutated melanomas*

NF1 is a GTPase-activating protein, which, when inactivated, contributes to MAPK pathway hyperactivation by reducing the cycling of activated

RAS back to its inactive state. NF1-inactivated melanomas are most strongly linked to chronic sun damage and older patients, and have a higher TMB compared to other subtypes. More than 80% of desmoplastic melanomas belong to this subgroup and are exceptionally responsive to immune checkpoint therapies.

### *Triple-wild-type melanomas*

Triple-wild-type melanomas comprise the most diverse clinicopathological and molecular subgroups. Common genomic drivers in this group include alterations in *KIT*, *GNAQ*, *GNA11* and *SF3B1*. Rare but clinically significant targetable fusions in *ALK* and *NTRK* can also be present. A significant proportion of acral and mucosal melanomas belong to this group and have a significantly lower TMB and responsiveness to immunotherapy than other melanomas. However, *KIT* mutations are most common in mucosal (17%) and acral (10%) melanomas, with those bearing *KIT* exon 11 and 13 mutations potentially benefiting from imatinib or other KIT inhibitors.

Somatic mutations in key melanoma oncogenes such as *BRAF*, *NRAS*, *GNAQ* and *GNA11* are acquired early in melanoma development. They are present in most benign naevi, with only a minority ever progressing to melanoma. Progression from dysplastic lesions to melanoma *in situ*, and eventually to invasive melanoma, is primarily driven by UV-related mutagenesis, reflected by the accumulation of UV-signature point mutations and additional key genomic alterations. The most common alterations include homozygous loss of *CDKN2A*, *TERT* promoter mutations, and loss of *TP53* and *PTEN*. Germline *CDKN2A*, *CDK4* and *BAP1* variants are commonly associated with familial types of melanoma. Uveal melanomas are a distinct subtype of melanocytic neoplasm, characterised by near universal activating variants in *GNAQ* or *GNA11*, and an exceptionally low TMB. While these primary mutations have limited therapeutic significance, changes in other genes such as *BAP1*, *EIF1AX*, *SF3B1* and chromosomal abnormalities in chromosomes 3, 6 and 8, are strongly correlated with metastatic risk and can inform surveillance strategies.

### Molecular Testing Recommendations for Skin Cancers

- Melanomas: assessment for *BRAF* V600 mutations. Extended panels should include *NRAS* and *KIT*, as these may allow access to targeted therapies. Inclusion of other genes: *NF1*, *GNAQ/GNA11*, *CDKN2A*, *TP53* and *TERT* can assist with diagnosis.
- BRAF immunohistochemistry is sensitive for *BRAF* V600E mutations but has a low sensitivity for other actionable *BRAF* V600 variants.
- Germline testing should be considered in individuals with multiple primary melanomas, melanoma diagnosed before age 45, or a family history of melanoma and/or related cancers such as pancreatic, breast or brain tumours.
- Molecular testing is not routinely required for BCC and SCC.
- Comprehensive genomic profiling may assist the diagnosis of SCC of unknown primary or undifferentiated tumours, through demonstration of features such as high TMB, a UV-mutation signature, and other characteristic genomic profiles.

## Brain Tumours

The fifth edition of the World Health Organization (WHO) Classification of Tumours of the Central Nervous System (WHO CNS5) recommends the identification of specific prognostic and predictive genomic alterations for accurate classification of both adult and paediatric brain tumours.

### Gliomas, Glioneuronal Tumours and Neuronal Tumours

Several DNA sequence alterations, such as *IDH1/2*, *H3-3A*, *ATRX*, *CDKN2A/B*, 1p/19q-codeletion, *TERT*, *EGFR*, and the combination of chromosome 7 gain and chromosome 10 loss, are crucial for diagnosing adult-type and paediatric-type diffuse gliomas.

#### *Adult-type diffuse gliomas*

- Astrocytoma, *IDH*-mutated (WHO grade 2–4): characterised by *IDH1/2* mutations, often combined with *ATRX* mutations. The presence of *CDKN2A/B* homozygous deletion classifies the tumour as WHO grade 4. *IDH* mutations are targets for the central nervous

system (CNS)-penetrant IDH1/2 inhibitor vorasidenib, which has shown significant prolongation of progression-free survival in grade 2 *IDH*-mutated gliomas.

- Oligodendroglioma, *IDH*-mutated, 1p/19q-codeleted (WHO grade 2–3): *IDH* mutations are early events in gliomagenesis and may occur along with 1p/19q codeletion and *TERT* promoter mutations.
- Glioblastoma, *IDH*-wild type (WHO grade 4): typically diagnosed based on microvascular proliferation and/or necrosis. However, *TERT* promoter mutations, *EGFR* amplification, and the combination of chromosome 7 gain and chromosome 10 loss are sufficient for diagnosis of molecular glioblastoma, even in the absence of classical histological features. *MGMT* promoter methylation status is a strong prognostic marker and may predict the benefit of temozolomide chemotherapy.

*Paediatric-type high-grade diffuse gliomas*

- Diffuse midline glioma, H3 K27-altered: characterised by a single amino acid substitution at codon K27 in the H3.3 histone protein.
- Diffuse hemispheric glioma, H3 G34-mutated: involves a single amino acid substitution at codon G34 in the H3.3 histone protein.
- Infant-type hemispheric glioma: high-grade glioma occurring in newborns and infants, often involving fusion genes such as *ALK*, *ROS1*, *NTRK1/2/3* or *MET*.

Other tumours, including paediatric-type diffuse low-grade and high-grade gliomas, circumscribed astrocytic gliomas, and glioneuronal and neuronal tumours, lack *IDH1/2* and histone mutations, but may harbour other alterations such as *PDGFRA*, *FGFR*, *NTRK*, *BRAF* V600E or *MYCN* amplification. Identifying these alterations is clinically relevant due to the availability of targeted therapies. Data published in 2022 show that treatment with dabrafenib (a BRAF inhibitor) and trametinib (a MEK inhibitor) was effective in 58 adults with recurrent high- or low-grade gliomas harbouring a *BRAF* V600E mutation (Wen et al, 2022). In 45 adults with recurrent high-grade glioma (31 with glioblastoma),

the overall response rate (ORR) was ≥30% with a median duration of response of 37 months. In the low-grade glioma cohort, 9 out of 13 patients achieved an objective response, with median duration of response not reached.

### *Ependymomas*

Ependymomas are classified based on their location: supratentorial, posterior fossa and spinal. Molecular subgrouping is recommended, with *ZFTA* fusion or *YAP1* fusion for supratentorial ependymomas and *MYCN* amplification for spinal ependymoma.

## Medulloblastomas and Meningiomas

Accurate classification of medulloblastomas (MBs) and meningiomas relies heavily on their genomic profiles.

### *Medulloblastomas*

MBs are categorised into three molecular groups: WNT-activated, Sonic Hedgehog (SHH)-activated, and non-WNT/non-SHH MBs. Large-scale methylation and transcriptome profiling have further identified four molecular subgroups within SHH MBs, and eight subgroups within non-WNT/non-SHH MBs. Commonly altered genes include:

- *APC*, *DDX3X*, *SMARCA4* and *CTNNB1* in WNT-activated MBs
- *TP53*, *PTCH1*, *SUFU* and *SMO* mutations, along with *MYCN* and *GLI2* amplifications in SHH-activated MBs
- *MYC* and *MYCN* amplifications, as well as *PRDM6* and *KDM6A* mutations in non-WNT/non-SHH MBs.

WNT-activated MBs generally have a very favourable prognosis, while non-WNT/non-SHH MBs require more aggressive multimodal therapy. SHH-driven MBs may benefit from targeted therapies, such as the SMO inhibitors sonidegib and glasdegib.

### *Meningiomas*

The presence of *TERT* promoter mutations and/or *CDKN2A/B* homozygous deletion have been added as criteria in the WHO CNS5 for diagnosing grade 3 meningioma, independent of histological appearance. Of note, recent findings suggest that the presence of chromosomal arm 1p deletion in combination with 22q deletion in WHO grade 1 CNS meningiomas should be considered as a criterion for the diagnosis of WHO grade 2 CNS meningioma. In addition, there is emerging evidence supporting a predictive role for a wide range of molecular alterations and markers, including *NF2*, *AKT1*, *SMO*, *SMARCE1*, *PIK3CA*, *CDKN2A/B*, *CDK4/6*, *TERT*, *TRAF7*, *BAP1*, *KLF4*, *ARID1/2*, *SUFU*, programmed death-ligand 1 (PD-L1), *SSTR2A*, progesterone receptor/oestrogen receptor (PR/ER), *mTOR*, *VEGF(R)* and *PDGFR*. Regarding the European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets (ESCAT) evidence-level criteria, no molecular target reached ESCAT tier I (ready for clinical use), with only mTOR pathway activation and *NF2* alterations reaching ESCAT tier II (investigational).

## Rare Cancers

### Soft Tissue Sarcoma

Soft tissue sarcomas (STSs) encompass over 80 different malignant entities occurring in various soft tissues. STSs are rare, with an estimated incidence of 4–5 per 100 000 people per year in Europe. The systemic management of STSs varies by subtype, with chemotherapy being the primary treatment. Genomic testing aids in diagnosing certain STSs and guides therapeutic decisions, particularly for tumours with characteristic gene fusions.

#### *Characteristic gene fusions in STS*

Gene fusions are a key diagnostic tool in multiple STSs. Some of the most frequently diagnosed STSs and their associated fusions are:

- Synovial sarcoma: characterised by the *SS18–SSX* fusion gene

- Myxoid liposarcoma: typically involves the *FUS–DDIT3* fusion
- Ewing sarcoma: defined by the *EWSR1–FLI1* fusion
- Alveolar rhabdomyosarcoma: usually involves the *PAX3–FOXO1* fusion
- Desmoplastic small-round-cell tumour: characterised by the *EWSR1–WT1* fusion.

### *Pan-tumour therapeutics in STS*

The following alterations are extremely rare in sarcomas:

- *NTRK* fusion-positive sarcomas: tropomyosin receptor kinase (TRK) inhibitors such as larotrectinib and entrectinib have shown promising results, with larotrectinib achieving a 75% ORR in *NTRK*-positive tumours (Drilon et al, 2018)
- *RET* fusion-positive tumours: selpercatinib has demonstrated efficacy, achieving a 43.9% ORR in *RET* fusion-positive tumours (LIBRETTO-001)
- *ALK* translocation: common in inflammatory myofibroblastic tumours. Anaplastic lymphoma kinase (ALK) inhibitors such as crizotinib have shown effectiveness, with a 50% ORR in *ALK*-positive cases (EORTC 90101 CREATE)
- Microsatellite instability-high (MSI-H)/mismatch repair deficient (dMMR) tumours: pembrolizumab is an option in selected patients with MSI-H/dMMR tumours (KEYNOTE-158).

### *Germline mutations in STS*

Patients with STS and a family history of early-onset or multiple sarcomas, breast cancer or adrenocortical carcinoma (and many other malignancies) should be considered for *TP53* germline testing, to rule out Li–Fraumeni syndrome. Notably, sarcomas can be part of many other hereditary syndromes (Connolly et al, 2025).

## Gastrointestinal Stromal Tumours

Gastrointestinal stromal tumours (GISTs) are mesenchymal neoplasms arising from the interstitial cells of Cajal in the gastrointestinal stroma. Molecular classification is crucial for prognosis and treatment decisions.

- *KIT* mutations: *KIT* exon 11 mutations are the most common (70%–75%) and highly sensitive to imatinib. *KIT* exon 9 mutations are less common (9%–20%) and less sensitive, with higher doses of imatinib (800 mg daily) recommended in advanced disease.
- *PDGFRA* mutations are uncommon (5%–10%). Although mutations analogous to those in *KIT* are reported, exon 18 D842V mutations are the most common (6%) and are resistant to imatinib; avapritinib is preferred in this subgroup.
- Wild-type GISTs: these tumours lack *KIT* or *PDGFRA*, *NF1* mutations and succinate dehydrogenase (SDH) deficiency and are insensitive to imatinib. SDH deficiency can be identified with immunohistochemistry and may require further assessment for hereditary implications.
- A subset of GISTs has *BRAF* V600 mutations and may respond to BRAF and MEK inhibitors.
- *NTRK* fusion-positive tumours can benefit from TRK inhibitors.

### Carcinoma of Unknown Primary

Carcinoma of unknown primary (CUP) is defined as a carcinoma where standard diagnostic work-up fails to identify the primary tumour. Approximately 50% of CUPs are well-to-moderately differentiated adenocarcinomas, the other half includes poorly differentiated adenocarcinomas, SCCs or undifferentiated neoplasms. Genomic testing can identify profiles suggesting a particular tumour type, including characteristic fusions or mutation signatures, and can detect potential therapeutic biomarkers, such as high TMB, *BRAF* V600E mutations, and *NTRK* or *RET* fusions.

### Neuroblastoma

Neuroblastoma is a tumour of the developing sympathetic nervous system, primarily occurring in childhood. The most relevant genomic alteration is *MYCN* amplification, associated with a poor prognosis. Hereditary neuroblastoma can be caused by germline mutations in *ALK* and *PHOX2B*. *ALK* mutations are present in 6%–17% of sporadic neuroblastomas and are potential targets for ALK inhibitors.

### Molecular Testing Recommendations in Rare Cancers

- STS: genomic testing is recommended in case of diagnostic challenges, and in patients fit for a targeted agent that is either available or can be given in the context of a clinical study. *NTRK* testing can be useful in certain cases.
- GIST: molecular analysis for *KIT*, *PDGFR* and *BRAF* are essential for guiding treatment.
- CUP: genomic testing is recommended to assist characterisation and identification of potential treatment targets, with *BRAF*, *NTRK* and *ALK* being key biomarkers.
- Neuroblastoma: *ALK* mutations are common and may be targeted by ALK inhibitors.
- Agnostic: *NTRK* and *RET* fusions should be analysed in rare cancers, given their role as predictive biomarkers for TRK and RET inhibitors, respectively. MSI-H/dMMR or TMB are also important predictive biomarkers.

## Further Reading

### Skin Cancers

Bonilla X, Parmentier L, King B, et al. Genomic analysis identifies new drivers and progression pathways in skin basal cell carcinoma. Nat Genet 2016; 48:398–406.

Brazel D, Kumar P, Doan H, et al. Genomic alterations and tumor mutation burden in Merkel cell carcinoma. JAMA Netw Open 2023; 6:e2249674.

Inman GJ, Wang J, Nagano A, et al. The genomic landscape of cutaneous SCC reveals drivers and a novel azathioprine associated mutational signature. Nat Commun 2018; 9:3667.

Newell F, Johansson PA, Wilmott JS, et al. Comparative genomics provides etiologic and biological insight into melanoma subtypes. Cancer Discov 2022; 12:2856–2879.

## Brain Tumours

Capper D, Reifenberger G, French PJ, et al. EANO guideline on rational molecular testing of gliomas, glioneuronal, and neuronal tumors in adults for targeted therapy selection. Neuro Oncol 2023; 25:813–826.

Landry AP, Wang JZ, Patil V, et al. Chromosome 1p loss and 1q gain for grading of meningioma. JAMA Oncol 2025; 11:644–649.

Li Y, Song Q, Day BW. Phase I and phase II sonidegib and vismodegib clinical trials for the treatment of paediatric and adult MB patients: a systemic review and meta-analysis. Acta Neuropathol Commun 2019; 7:123.

Louis DN, Perry A, Wesseling P, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. Neuro Oncol 2021; 23:1231–1251.

Mellinghoff IK, van den Bent MJ, Blumenthal DT, et al; INDIGO Trial Investigators. Vorasidenib in IDH1- or IDH2-mutant low-grade glioma. N Engl J Med 2023; 389:589–601.

Northcott PA, Buchhalter I, Morrissy AS, et al. The whole-genome landscape of medulloblastoma subtypes. Nature 2017; 547:311–317.

Rudà R, Capper D, Waldman AD, et al. EANO - EURACAN - SNO guidelines on circumscribed astrocytic gliomas, glioneuronal, and neuronal tumors. Neuro Oncol 2022; 24:2015–2034.

Sahm F, Aldape KD, Brastianos PK, et al. cIMPACT-NOW update 8: clarifications on molecular risk parameters and recommendations for WHO grading of meningiomas. Neuro Oncol 2025; 27:319–330.

Sahm F, Bertero L, Brandner S, et al. European Association of Neuro-Oncology guideline on molecular testing of meningiomas for targeted therapy selection. Neuro Oncol 2025; 27:869–883.

Weller M, van den Bent M, Preusser M, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. Nat Rev Clin Oncol 2021; 18:170–186.

Wen PY, Stein A, van den Bent M, et al. Dabrafenib plus trametinib in patients with $BRAF^{V600E}$-mutant low-grade and high-grade glioma (ROAR): a multicentre, open-label, single-arm, phase 2, basket trial. Lancet Oncol 2022; 23:53–64.

## Rare Cancers

Casali PG, Blay JY, Abecassis N, et al. Gastrointestinal stromal tumours: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2022; 33:20–33.

Connolly EA, Boye K, Bonvalot S, et al. Genetic predisposition in sarcomas: clinical implications and management. EClinicalMedicine 2025; 83:103203.
Drilon A, Laetsch TW, Kummar S, et al. Efficacy of larotrectinib in TRK fusion-positive cancers in adults and children. N Engl J Med 2018; 378:731–739.
Demetri GD, De Braud F, Drillon A, et al. Updated integrated analysis of the efficacy and safety of entrectinib in patients with NTRK fusion-positive solid tumors. Clin Cancer Res 2022; 28:1302–1312.
Gronchi A, Miah AB, Dei Tos AP. Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2021; 32:1348–1365.
Matthay KK, Maris JM, Schleiermacher G, et al. Neuroblastoma. Nat Rev Dis Primers 2016; 2:16078.
Maio M, Ascierto PA, Manzyuk L, et al. Pembrolizumab in microsatellite instability high or mismatch repair deficient cancers: updated analysis from the phase II KEYNOTE-158 study. Ann Oncol 2022; 33:929–938.
Salama AKS, Li S, Macrae ER, et al. Dabrafenib and trametinib in patients with tumors with $BRAF^{V600E}$ mutations: results of the NCI-MATCH trial subprotocol H. J Clin Oncol 2020; 38:3895–3904.
Schöffski P, Sufliarsky J, Gelderblom H, et al. Crizotinib in patients with advanced, inoperable inflammatory myofibroblastic tumours with and without anaplastic lymphoma kinase gene alterations (European Organisation for Research and Treatment of Cancer 90101 CREATE): a multicentre, single-drug, prospective, non-randomised phase 2 trial. Lancet Respir Med 2018; 6:431–441.
Subbiah V, Wolf J, Konda B, et al. Tumor-agnostic efficacy and safety of selpercatinib in patients with RET fusion-positive solid tumours other than lung or thyroid tumours (LIBRETTO-001): a phase 1/2, open-label, basket trial. Lancet Oncol 2022; 23:1261–1273.
WHO Classification of Tumours Editorial Board. Soft tissue and bone tumours. WHO classification of tumours series, 5th ed. Vol. 3. Lyon (France): International Agency for Research on Cancer; 2020.

## Declarations of Interest

**Dr Kee** has declared advisory board honoraria from Bristol Myers Squibb, ImCheck Therapeutics, Medison Pharma, Merck Sharp & Dohme; and personal speaker honoraria from Novartis. He holds a leadership role with the Australian Rare Cancer Portal and is a member

of the Board of Directors at Melanoma Research Victoria.

**Dr Perez Fidalgo** has declared personal advisory board honoraria from AbbVie, Abilify Pharma, AstraZeneca, Clovis, Daiichi Sankyo, GSK, Karyopharm, pharma&, PharmaMar, Regeneron, Roche and personal speaker honoraria from AstraZeneca, Clovis, Eisai, GSK, pharma& and PharmaMar. He has declared institutional fees for his role as a coordinating principal investigator from AstraZeneca and institutional research funding from GSK, Novartis and PharmaMar. He has declared being a non-remunerated project lead and coordinating principal investigator of trials for AstraZeneca, GSK and Novartis. He has taken leadership roles as a member of the executive committee for Grupo Español de Investigación en Sarcomas (GEIS) and El Grupo Español de Investigación en Cáncer ginecológicO (GEICO). He is a steering committee member for AbbVie, Artios Pharma and AstraZeneca. He has declared employment with the University of Valencia, Spain. He is a member of Early Drug Development Working Group for European Network of Gynaecological Oncological Trial Group (ENGOT) and a member of Adolescent and Young Adult working group of the Spanish Society of Medical Oncology (SEOM).

**Dr Maccari** has declared institutional research funding for her work as a sub-investigator on clinical trials sponsored by Carthera, Chimerix, Inc., Nerviano Medical Sciences Srl, Philogen S.p.A. and Servier; a non-remunerated role as an invited speaker for Bayer; and financial travel and accommodation support to attend the AIOM congress in 2023.

**Dr Minniti** has declared personal speaker honoraria from Accuray, BrainLab and Pfizer; and personal advisory board honoraria from AstraZeneca, Novocure and Servier. He has declared being a member of the board of directors for the European Association of Neuro-Oncology, and Brain Tumour Group Treasurer for the European Organisation for Research and Treatment of Cancer (EORTC).

# Genomics in Haematological Malignancies

8

K. Hörst
C. Kühn
T. Haferlach
*MLL Munich Leukemia Laboratory, Munich, Germany*

## Genetics Gain Relevance in Haematological Malignancies

In the realm of haematological malignancies, genomics is unveiling critical insights that are fundamentally shifting both diagnostic and therapeutic paradigms. Advancements in our understanding of their molecular pathogenesis are based on the increasing relevance of genetics in the diagnosis of these neoplasms. In haematological diagnostics, genetics include both molecular genetics and cytogenetics. While technologies such as next-generation sequencing (NGS) aim to identify specific sequence variants at the molecular level, cytogenetics with chromosome analysis and fluorescence *in situ* hybridisation (FISH) enables the detection of chromosomal abnormalities such as gains and losses, deletions, amplifications and translocations that occur at a larger, structural level. Together, these genetic alterations serve not only as diagnostic and prognostic markers but also as targets for tailored therapies.

## Key Molecular Features of Myeloid and Lymphoid Neoplasms

Haematological malignancies are broadly categorised into two main groups: myeloid and lymphoid neoplasms. Both represent a complex spectrum of entities, each characterised by distinct molecular features. Understanding the key molecular characteristics is crucial for the

accurate diagnosis and tailored treatment of these disorders.

## A Closer Look at the Complex Molecular Dynamics of Myeloid Malignancies

Myeloid neoplasms are characterised by the clonal proliferation of myeloid lineage cells and can be broadly categorised into myeloproliferative neoplasms (MPN), myelodysplastic neoplasms (MDS) and acute myeloid leukaemia (AML) (see also Table 1). The molecular pathogenesis of these disorders involves a variety of genetic alterations that affect different cellular pathways, including cytokine signalling, cell-cycle regulation and epigenetic modification.

1. ***BCR–ABL1* in chronic myeloid leukaemia (CML):** The Philadelphia chromosome, resulting from a translocation between chromosomes 9 and 22 (t[9;22][q34;q11]), produces the *BCR–ABL1* fusion gene, leading to a constitutively active tyrosine kinase that is central to the pathogenesis of CML.

2. ***JAK2, CALR* and *MPL* mutations in *BCR–ABL1*-negative MPN:** The discovery of the *JAK2* V617F mutation in a significant proportion of MPN was a seminal finding that highlighted the role of dysregulated JAK–STAT signalling in myeloproliferation. Subsequently, mutations in *CALR* and *MPL* were also identified in *JAK2*-negative MPN, further underscoring the centrality of aberrant cytokine signalling.

3. ***SF3B1, SRSF2* and *U2AF1* mutations in MDS:** Mutations in splicing factor genes such as *SF3B1*, *SRSF2* and *U2AF1* are common in MDS and are associated with specific disease phenotypes and prognoses. These mutations lead to aberrant RNA splicing and contribute to the pathogenesis of MDS through the dysregulation of gene expression.

4. ***FLT3*, *NPM1* and *CEBPA* in AML:** AML is characterised by a high degree of genetic heterogeneity. *FLT3* internal tandem duplications

*Table 1 Selected Myeloid Neoplasms with Molecular Features and Relevance (according to WHO 2024).*

Adapted from: WHO Classification of Tumours, 5th Edition, Volume 11. IARC, Lyon, France, 2024.

| Entity | Characteristic genetic changes | Clinical use (diagnostics/therapy) |
|---|---|---|
| Myeloid precursor lesions<br>Clonal haematopoiesis (CHIP)<br>Clonal cytopenia of undetermined significance (CCUS) | Driver gene mutations (e.g. *TET2*, *DNMT3A*, *ASXL1*, *JAK2*) | Detection of one or more somatic mutations is essential for diagnosis |
| Myeloproliferative neoplasms (MPN)<br>Chronic myeloid leukaemia (CML)<br>*BCR–ABL1*-negative MPN | <br>*BCR–ABL1* fusion<br>*JAK2* V617F, *CALR*, *MPL*, *CSF3R* | <br>Essential for diagnosis, target for TKI therapy<br>Respective driver mutation essential for diagnosis |
| Mastocytosis | *KIT* mutations (especially *KIT* D816)<br>*TET2*, *SRSF2*, *ASXL1*, *RUNX1*, *JAK2* | Minor criterion for diagnosis, *KIT* D816V as target for selective KIT inhibitors (e.g. avapritinib)<br>Additional mutations with often poor prognosis |
| Myelodysplastic neoplasms (MDS) | 3 subtypes with defining genetic abnormalities (low blasts and 5q del, low blasts and SF3B1, biallelic *TP53* inactivation) | Detection of genetic abnormality essential for diagnosis of each subtype<br>IPSS-M for risk stratification |
| MDS/MPN<br>Chronic myelomonocytic leukaemia (CMML) | *SF3B1* subtype<br>*TET2*, *SRSF2*, *ASXL1*, etc. | Essential for diagnosis<br>Desirable: recommended minimal gene set for mutation profiling |
| Acute myeloid leukaemia (AML) | 13 subtypes with defining genetic abnormalities *(PML–RARA*, *RUNX1–RUNX1T1*, *CBFB–MYH11*, *DEK–NUP214*, *RBM15–MRTFA*, *BCR–ABL1*, *KMT2A*, *MECOM*, *NUP98*, *NPM1*, *CEBPA*, myelodysplasia-related, others) | Genetic signature essential for diagnosis of each subtype<br>2022 ELN risk classification by genetics at initial diagnosis<br>FLT3 as target for FLT3 inhibitors |
| Secondary myeloid neoplasms<br>Myeloid neoplasms with germline predisposition | *RUNX1*, *GATA2*, *CEBPA*, *DDX41*, *TP53*, etc. | Specific germline predisposition and myeloid malignancy itself can influence prognosis and therapy response |
| Myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions | 7 subtypes with defining TK gene fusions *(PDGFRA*, *PDGFRB*, *FGFR1*, *JAK2*, *FLT3*, *ETV6–ABL1* and others) | Genetic signature essential for diagnosis of each subtype<br>Targets for TKI, such as FLT3 inhibitors |
| Acute leukaemia of mixed or ambiguous lineage | 3 subtypes with defining genetic abnormalities (*BCR–ABL1*, *KMT2A* rearrangement, other alterations) | Presence of specific genetic alterations essential for diagnosis |

Abbreviations: ELN, European LeukemiaNet; IPSS-M, Molecular International Prognostic scoring system; TK, tyrosine kinase; TKI, tyrosine kinase inhibitor; WHO, World Health Organization.

(ITDs) and mutations in *NPM1* and *CEBPA* are among the most common genetic alterations. These mutations not only have diagnostic and prognostic significance but also serve as targets for detection of measurable residual disease (MRD) or molecularly targeted therapies.

## Molecular Insights into Lymphoid Neoplasms

Lymphoid neoplasms, including various types of lymphoma and acute lymphoblastic leukaemia (ALL), arise from the clonal proliferation of lymphocytes and are characterised by diverse genetic abnormalities that affect lymphocyte development, survival and proliferation. A more detailed selection of lymphoid neoplasms is provided in Table 2.

*Table 2 Selected Lymphoid Neoplasms with Molecular Features and Relevance (according to WHO 2024).*

Adapted from: WHO Classification of Tumours, 5th Edition, Volume 11. IARC, Lyon, France, 2024.

| Entity | Characteristic genetic changes | Clinical use (diagnostics/therapy) |
|---|---|---|
| B-lymphoblastic leukaemia/ lymphoma | Various, depending on subtype (hyper/hypodiploidy, iAMP21, *BCR–ABL1* fusion, *KMT2A*, *ETV6–RUNX1*, *TCF3–PBX1*, IGH–*IL3*, *TCF–HLF*, etc.) | Genetic signature essential for diagnosis and risk stratification of each subtype |
| Mature B-cell neoplasms | | |
| Chronic lymphocytic leukaemia (CLL)/small lymphocytic lymphoma | del(11q), del(13)(q14), del(17p), trisomy 12, *TP53*, *IGHV* | Evaluation recommended for prognosis/prediction<br>CLL-IPI as risk score for prognosis |
| Hairy cell leukaemia | *BRAF* V600E | Confirmation of diagnosis, target for BRAF inhibitors |
| Splenic marginal zone lymphoma (SMZL) | del(7q), *KLF2*, *NOTCH2* | Not sufficient for diagnosis, exclusion of genes typically mutated in other CD5-negative lymphomas (e.g. *BRAF* V600E) |
| Lymphoplasmacytic lymphoma | *MYD88* p.L265P, *CXCR4* | Desirable diagnostic criterion |
| Follicular lymphoma | t(14;18)(q32;q21)/IGH–*BCL2* | *BCL2* or *BCL6* rearrangements and/ or a lack of *IRF4* rearrangements as desirable diagnostic criteria; m7-FLIPI as risk score for prognosis |
| Mantle cell lymphoma | t(11;14)(q13;q32) leading to IGH–*CCND1* rearrangement, *TP53* | Essential for diagnosis |

*Table 2 Selected Lymphoid Neoplasms with Molecular Features and Relevance (according to WHO 2024).* *(Continued)*

| Entity | Characteristic genetic changes | Clinical use (diagnostics/therapy) |
|---|---|---|
| Diffuse large B-cell lymphoma | Various, including *MYC*, *BCL2*, *BCL6* rearrangements | Desirable for diagnosis: reporting of isolated *MYC* or dual *MYC* and *BCL6* rearrangements (to exclude high-grade B-cell lymphomas [HGBCLs]); genetic testing, if relevant for clinical decision-making |
| Burkitt lymphoma | *MYC* translocation, usually t(8;14) (q24;q32) | Essential for diagnosis |
| Plasma cell myeloma/multiple myeloma | Various, often gains of chromosomes, also translocations involving the IGH locus, deletions of 17p | Revised International Staging System (R-ISS) for multiple myeloma includes cytogenetic risk factors for prognosis |
| T-lymphoblastic leukaemia/ lymphoma | TRB and/or TRG rearrangements | Not reliable for lineage assignment in acute leukaemia. Clonal TCR-rearranged sequences may be used to monitor residual disease after therapy |
| Mature T-cell and NK-cell neoplasms | | |
| T-prolymphocytic leukaemia | *TCL1* rearrangements (14q32 or Xq28)<br>Abnormalities in chromosomes 11, 8, 5, 12, 13 or 22, or complex karyotype | Essential for diagnosis<br>Minor diagnostic criteria (at least one required) |
| Primary cutaneous T-cell lymphoid proliferations and lymphomas | (Mono-)clonally rearranged TCR genes | No impact on prognosis in most subtypes; desirable for diagnosis of difficult cases of mycosis fungoides and selected cases of LyP; suggested as risk factor for development of LyP-associated lymphomas |
| Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) | Clonal TRB and/or TRG rearrangement | TCR rearrangement desirable for diagnosis |
| Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma (PCAETL) | Gain-of-function mutations in *JAK2*, *STAT3* and *STAT5B* | Activating mutations or fusions of *JAK2* pathway genes desirable |
| Intestinal T-cell and NK-cell lymphoid proliferations and lymphomas | Clonal TCR rearrangement (or somatic mutation) | Can assist in distinction from inflammatory disorder |
| Indolent NK-cell lymphoproliferative disorder (iNK-LPD) of the gastrointestinal tract | Absence of clonal TCR rearrangement | Relevant for differential diagnosis |
| Monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL) | MEITL: *JAK3/STAT5* mutations, *SETD2* inactivation | TCR clonality assays may support the diagnosis in equivocal cases; in problematic cases, demonstration of *JAK3/STAT5* mutations and/or *SETD2* inactivation are helpful in differentiating MEITL from EATL |

*Table 2 Selected Lymphoid Neoplasms with Molecular Features and Relevance (according to WHO 2024).* (Continued)

| Entity | Characteristic genetic changes | Clinical use (diagnostics/therapy) |
|---|---|---|
| Hepatosplenic T-cell lymphoma | Clonal TCR rearrangement, i(7q), trisomy 8 | Desirable for diagnosis |
| Nodal T-follicular helper cell lymphoma | Conal TCR gene rearrangement and/or *RHOA* p.G17V or *IDH2* p.R172 mutation, t(5;9)(q33;q22)/*ITK–SYK* | Desirable or essential for diagnosis, concurrent *TET2*, *DNMT3A*, *IDH2* mutations are associated with an adverse prognosis |
| EBV-positive T-cell and NK-cell lymphoma | Monoclonal TCR gene rearrangement | Supports a T-cell lineage but is negative in tumours of NK-cell lineage |

Abbreviations: CD, cluster of differentiation; CLL-IPI, Chronic Lymphocytic Leukaemia-International Prognostic Index; EATL, enteropathy-associated T-cell lymphoma, EBV, Epstein–Barr virus; IGH, immunoglobulin heavy; LyP, lymphomatoid papulosis; m7-FLIPI, m7-Follicular Lymphoma International Prognostic Index; MEITL, monomorphic epitheliotropic intestinal T-cell lymphoma; NK, natural killer; TCR, T-cell receptor; TRB, T-cell receptor beta; TRG, T-cell receptor gamma; WHO, World Health Organization.

1. **Immunoglobulin heavy locus (IGH) translocations in B-cell lymphomas:** Translocations involving the IGH and various oncogenes (e.g. *MYC*, *BCL2* and *BCL6*) are hallmarks of many B-cell lymphomas. These translocations lead to the overexpression of oncogenes and drive lymphomagenesis.
2. **T-cell receptor (TCR) rearrangements in T-cell lymphomas:** Similar to B-cell neoplasms, T-cell lymphomas often exhibit rearrangements of the TCR genes, leading to the aberrant expression of various genes that promote T-cell proliferation and survival.
3. ***RAS–RAF–MEK–ERK* pathway alterations in ALL:** Mutations in components of the *RAS–RAF–MEK–ERK* signalling pathway are common in ALL and contribute to the uncontrolled proliferation of lymphoblasts.

While the mutations exemplified for both myeloid and lymphoid neoplasms are somatic, germline mutations can also play a role in the predisposition to haematological malignancies. In the context of myeloid malignancies, several key germline mutations have been identified: genes frequently involved include *RUNX1*, *CEBPA* and *GATA2*, each playing a pivotal role in haematopoiesis and, when mutated, predisposing individuals to myeloid neoplasms. *RUNX1* mutations are often associated with familial platelet disorder with a predisposition to AML. Also, mutations in *CEBPA* are linked to a familial form of AML.

*GATA2* mutations predispose individuals to a variety of conditions, including AML, chronic myelomonocytic leukemia (CMML) and others, often presenting with a complex clinical picture that includes immunodeficiency and lymphoedema. *DDX41* stands out among these genes due to its unique functions in RNA metabolism and innate immunity. Germline mutations in *DDX41* are associated with a familial predisposition to myeloid malignancies, particularly AML and MDS.

In the lymphoid malignancies, germline mutations in genes such as *ATM*, *TP53* and *BTK* play crucial roles. *ATM* mutations are a hallmark of ataxia-telangiectasia, a condition that significantly increases the risk of developing lymphoid malignancies. *TP53* mutations are associated with Li–Fraumeni syndrome, leading to a heightened risk of various cancers, including lymphomas. In addition, X-linked agammaglobulinaemia, an immunodeficiency, is caused by *BTK* mutations. Of note, germline mutations in *IKZF1* and *PAX5* are linked to an increased risk of developing ALL.

## Navigating Haematological Malignancies: from Prediction to Personalisation

Not only in diagnostics, but also in risk stratification, genetic features are becoming increasingly important. For example, the Molecular International Prognostic Scoring System (IPSS-M) represents a significant advancement in the prognostication of MDS. It includes mutations in 31 genes alongside traditional cytogenetic abnormalities, reflecting the growing understanding of the molecular basis of MDS. Risk scores integrating genetic characteristics have also been developed for other entities, such as the chronic lymphocytic leukaemia International Prognostic Index (CLL-IPI) or the m7-Follicular Lymphoma International Prognostic Index (m7-FLIPI).

The ability to identify genetic signatures associated with specific haematological malignancies not only promises improved patient stratification for existing therapies but also the discovery of novel

therapeutic targets. Tyrosine kinase inhibitors (TKIs) targeting *BCR–ABL1* have revolutionised the treatment of CML. The discovery of the *BCR–ABL1* fusion gene has led to the development of TKIs, such as imatinib, which have dramatically improved the prognosis for CML patients. Resistance to TKI therapy, often due to mutations within the ABL1 kinase domain, remains a clinical challenge. Monitoring for these mutations is crucial for guiding treatment decisions.

*FLT3* mutations, including ITDs (*FLT3*-ITDs) and point mutations in the tyrosine kinase domain (*FLT3*-TKD), are among the most common genetic alterations in AML and are associated with a poor prognosis. This has led to the development and use of FLT3 inhibitors, exemplifying the role of molecular abnormalities in matching treatments. For example, midostaurin, a multikinase inhibitor with activity against FLT3, among other things, has been in use for years. In 2023, quizartinib was approved, the first drug specifically tailored to *FLT3*-ITD. Also, the presence of specific mutations can guide the use of targeted therapies, such as IDH1 and IDH2 inhibitors, further emphasising the shift towards personalised treatment strategies.

In addition to their diagnostic value in T-cell malignancies, gene panels facilitate the identification of actionable mutations that inform personalised treatment strategies. The expanding molecular understanding of T-cell malignancies has led to the development and clinical application of targeted therapies, including JAK and anaplastic lymphoma kinase inhibitors, hypomethylating agents and immunotherapies.

The predictive value of germline mutations needs to be considered as their identification is important for assessing familial risk and appropriate treatment strategies. For example, individuals with a familial predisposition to AML due to a *CEBPA* mutation generally have a more favourable prognosis when treated with conventional chemotherapy. In MDS and AML, deleterious germline variants in *DDX41* often lead to a later onset of disease and may be accompanied by a more indolent

course, though the prognosis can vary widely based on the presence of additional somatic mutations and disease characteristics.

## Recommendations for Genetic Testing

Genetic profiling enables and confirms a solid diagnosis of haematological malignancies, provides crucial prognostic information and guides treatment decisions. In diagnostics, the selected genes of a comprehensive gene panel should be analysed based on the clinical picture.

**Myeloid Gene Panels:**

- **Chromatin modifiers:** *ASXL1*, *ASXL2*, *EZH2*, *TET2*
- **Transcription factors:** *BCOR*, *BCORL1*, *CEBPA*, *CUX1*, *ETV6*, *GATA1*, *GATA2*, *RUNX1*, *ZEB2*
- **Kinase signalling:** *BRAF*, *CBL*, *CSF3R*, *FLT3*, *FLT3*-ITD, *JAK2*, *KIT*, *KRAS*, *MPL*, *NF1*, *NOTCH1*, *PDGFRA*, *PDGFRB*, *PTPN11*
- **Metabolism:** *CALR*, *IDH1*, *IDH2*, *TET2*
- **RNA splicing:** *SF1*, *SF3A1*, *SF3B1*, *SRSF2*, *U2AF1*, *U2AF2*, *ZRSR2*
- **DNA methylation:** *DNMT3A*, *TET2*
- **Other:** *ATRX*, *DDX41*, *ETNK1*, *FBXW7*, *IL6R*, *MYD88*, *NPM1*, *NRAS*, *PHF6*, *PIGA*, *PPM1D*, *PRPF8*, *PTEN*, *RAD21*, *SH2B3*, *SMC1A*, *SMC3*, *STAG2*, *SUZ12*, *TP53*, *UBA1*, *WT1*

**Lymphoid Gene Panels:**

- **Chromatin modifiers:** *ARID1A*, *CREBBP*, *EP300*, *EZH2*, *KMT2D*, *TET2*
- **Transcription factors:** *BCL6*, *CARD11*, *ETV6*, *FOXO1*, *IRF4*, *MYC*, *NOTCH1*, *NOTCH2*, *PAX5*, *RUNX1*, *ZEB2*
- **Kinase signalling:** *ATM*, *ATR*, *BTK*, *FYN*, *JAK1*, *JAK2*, *JAK3*, *KRAS*, *MAP2K1*, *PLCG1*, *PLCG2*, *STAT3*, *STAT5B*, *STAT6*
- **BCL2 family:** *BCL2*, *BCL10*, *BIRC3*
- **Other:** *BRAF*, *CCL22*, *CCND1*, *CD28*, *CD79B*, *CXCR4*, *DIS3*, *DNMT3A*, *EGR1*, *FLT3*, *ID3*, *IDH2*, *IKZF1*, *IL7R*, *KLF2*, *KLHL6*,

*MEF2B*, *MYD88*, *NRAS*, *PHF6*, *POT1*, *PTEN*, *RHOA*, *RPS15*, *SGK1*, *SOCS1*, *TNFAIP3*, *TP53*, *UBR5*, *VAV1*, *XPO1*

Genetic profiling should not be a one-time activity; monitoring for emerging mutations during treatment can provide insights into disease evolution and resistance mechanisms. In fact, MRD assessment has emerged as a critical tool for refining genetic risk stratification and therapy monitoring in haematological malignancies.

Overall, recommendations for genetic testing in haematological malignancies emphasise its role in enabling a precision medicine approach, tailoring treatment to the genetic landscape of the disease to optimise outcomes.

## Further Reading


Alaggio R, Amador C, Anagnostopoulos I, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Leukemia 2022; 36:1720–1748.

Arber DA, Orazi A, Hasserjian RP, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood 2022; 140:1200–1228.

Bannon SA, Routbort MJ, Montalban-Bravo G, et al. Next-generation sequencing of DDX41 in myeloid neoplasms leads to increased detection of germline alterations. Front Oncol 2021; 10:582213.

Bernard E, Tuechler H, Greenberg PL, et al. Molecular International Prognostic Scoring System for myelodysplastic syndromes. NEJM Evid 2022; 1:EVIDoa2200008.

Döhner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood 2022; 140:1345–1377.

Heuser M, Freeman SD, Ossenkoppele GJ, et al. 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. Blood 2021; 138:2753–2767.

Hochhaus A, Baccarani M, Silver RT, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia 2020; 34:966–984.

Huber S, Baer C, Hutter S, et al. Risk assessment according to IPSS-M is superior

to AML ELN risk classification in MDS/AML overlap patients defined by ICC. Leukemia 2023; 37:2138–2141.
Huber S, Haferlach T, Müller H, et al. MDS subclassification-do we still have to count blasts? Leukemia 2023; 37:942–945.
International CLL-IPI working group. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. Lancet Oncol 2016; 17:779–790.
Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: myeloid and histiocytic/dendritic neoplasms. Leukemia 2022; 36:1703–1719.
Pastore A, Jurinovic V, Kridel R, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. Lancet Oncol 2015; 16:1111–1122.
WHO Classification of Tumours Editorial Board. Haematolymphoid Tumours. WHO Classification of Tumours, 5th Edition, Volume 11. Lyon: International Agency for Research on Cancer, 2024. Available at: https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/Haematolymphoid-Tumours-2024 (date last accessed, 22 May 2025).

## Declaration of Interest:

**Dr Hörst** has declared employment with MLL Munich Leukemia Laboratory.

**Dr Kühn** has declared employment with MLL Munich Leukemia Laboratory.

**Professor Haferlach** has declared employment with MLL Munich Leukemia Laboratory. He has also declared his part-ownership in MLL Munich Leukemia Laboratory.

# Index

Note:
Abbreviations used in the index are listed on pages xvii–xxii.
References to figures are indicated by page numbers suffixed by 'f'.
References to tables are indicated by page numbers suffixed by 't'.

## B

## C

# D

## E

## F

## G

## H

## I

## J



## K

## L

## M

## N

## O

## P

## Q



## R

# S

# T

## U

## V



## W



## X



## Y



## Z

# ESMO HANDBOOK OF
# CANCER GENETICS AND GENOMICS

Edited by Angela George, David SP Tan, Marcia Hall and Tania Fleitas Kanonnikoff

Throughout the last decade, there has been an overwhelming increase in the use of genomic information to define diagnosis, inform prognosis, avoid excessive treatment toxicity and select the optimal targeted agent for treatment. The *ESMO Handbook of Cancer Genetics and Genomics* has been written to help clinicians stay up to date with the germline and somatic mutations relevant to their area of practice. It provides real-world guidance on what is now essential for optimal patient care and ensures that all oncologists can navigate this new world of genomically-guided oncology with confidence.

ESMO Press

**ESMO** Handbook Series
European Society for Medical Oncology
Via Ginevra 4, 6900 Lugano, Switzerland

www.esmo.org