Clinical decision-making strategies for rare somatic variants in cancer

Koopman, Bart

DOI:
10.33612/diss.213504207

General introduction

GENERAL INTRODUCTION

Cancer is a disease of abnormal cell growth and spread that ranks among the leading causes of death, with almost 10 million cancer-related deaths registered in 2020.[1] The past decades have seen the introduction of advanced molecular diagnostic techniques that enable an in-depth profiling of alterations in the DNA of a patient's tumor. Using this information, pathologists can reach a more appropriate diagnosis and oncologists can treat patients with drugs that specifically target the cancer's vulnerabilities. However, molecular testing results are increasingly difficult to interpret, which can influence the diagnosis or treatment decision. The aim of this thesis is to explore such complex results from routine molecular diagnostics and investigate strategies that can be used to improve decision-making, with special attention for the role of multidisciplinary Molecular Tumor Boards (MTBs). This general introduction will summarize background information about somatic variants in cancer, molecular profiling techniques, targeted therapies, and MTBs, and will outline the aim and scope of this thesis.

Somatic variants in cancer

Normal cells can transform into cancer cells (malignant transformation) by accumulating (epi)genetic changes in the cell's genome (DNA).[2] The human body has various built-in mechanisms by which changes in DNA ('variants') can be prevented, repaired or eliminated. If the variant nevertheless persists, it usually has no consequences. However, a variant can contribute to malignant transformation when it affects a gene that stimulates or suppresses processes such as cell growth, proliferation, cell survival, abnormal differentiation, and, ultimately, the ability to invade adjacent tissue or spread to other anatomical sites.[2] Genes that naturally stimulate these processes can contribute to malignant transformation when a variant up-regulates their function (oncogenes), whereas genes that naturally suppress these processes can contribute to malignant transformation when they are inactivated (tumor suppressor genes).[3] Variants that contribute to malignant transformation are called 'pathogenic' ('disease-causing') or 'oncogenic' ('cancer-causing') variants. A normal cell requires an accumulation of multiple pathogenic variants to transform into cancer. Such variants may be present in all cells including germ cells and thus inherited by next generations (germline variants) or acquired during the course of life in non-germ cells (somatic variants). There are various types of somatic variants involved in cancer development.[4] Currently, three types of somatic variants have the most clinical relevance: small-scale mutations, gene copy number alterations (CNAs), and chromosomal rearrangements (**Figure 1**). Small-scale mutations involve a limited amount of nucleotides within or around a gene, including substitutions, insertions or deletions of one or more nucleotide(s) with various effects on the resulting protein.[5] A CNA occurs when there is a gain or loss of a chromosomal region

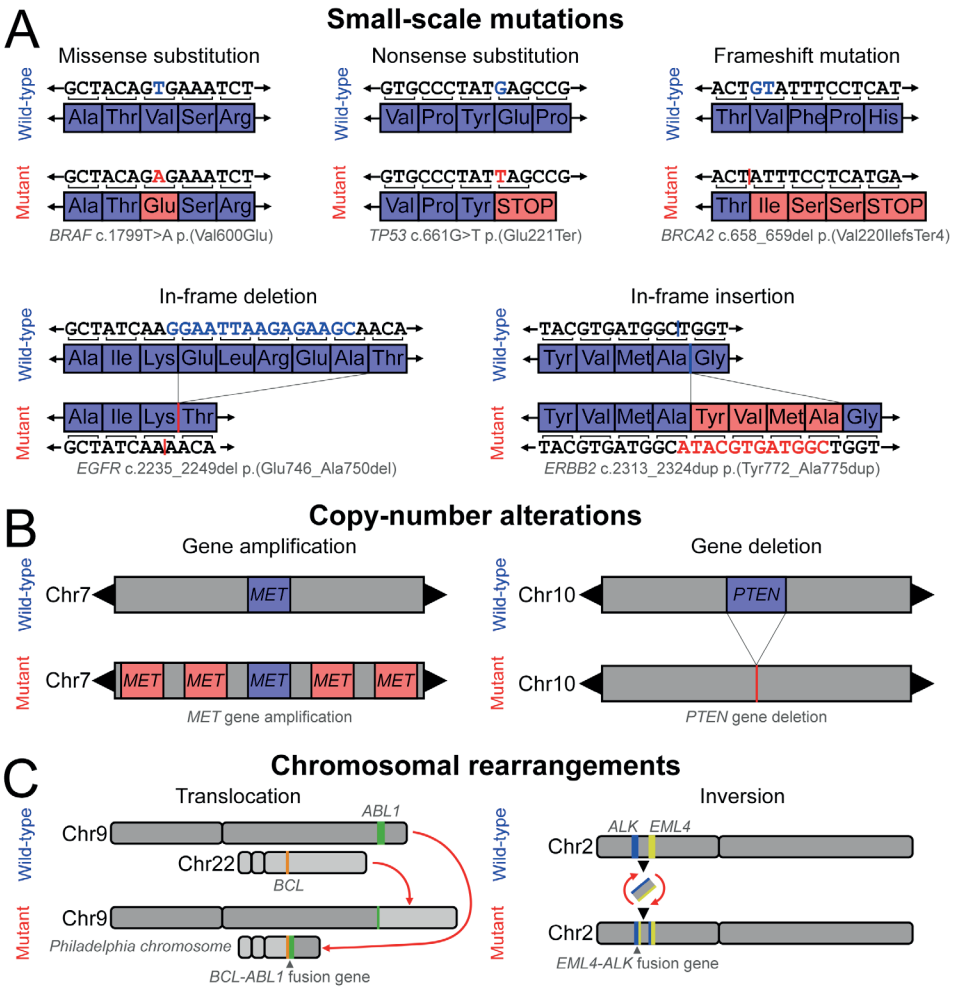

Figure 1. Examples of the most common clinically relevant somatic variants observed in cancer. Visualization of different types of somatic variants that are commonly detected in routine diagnostics of various types of cancer. **A,** Small-scale variants, which involve a limited number of nucleotides within or around a gene. **B,** Copy-number alterations, which is a gain or loss of a chromosomal region hosting one or more genes. **C,** Chromosomal rearrangements, which can result in (oncogenic) fusion genes. A common mechanism in hematological malignancies, the juxtaposition of a promotor or enhancer region of a gene to the full coding region of an oncogene, is not depicted. *Chr, chromosome.*

hosting one or more genes: CNAs with potential oncogenic effects include amplifications or deletions of such regions.[6,7] Chromosomal rearrangements, such as translocations or inversions, can result in (oncogenic) fusion genes or the juxtaposition of a promotor or enhancer region of a gene to the full coding region of an oncogene.[8,9] There are other types of somatic variants that can contribute to malignant transformation, but these are outside of the scope of this thesis.

Molecular markers

Somatic variants that serve as biological markers (biomarkers) of disease are called molecular markers.[10] In patients with cancer, molecular markers can have clinical relevance when they have diagnostic, prognostic and/or predictive consequences.[11] A diagnostic molecular marker supports or determines a diagnosis. For example, in soft tissue or bone lesions, distinct gene fusions are pathognomonic for the final diagnosis; the detected fusion genes are thus diagnostic molecular markers.[12] A prognostic molecular marker can be used as an indicator of prognosis irrespective of treatment. One example of a prognostic molecular marker is a deletion or deleterious mutation in tumor suppressor gene *POLE* in endometrial cancer, which is associated with improved survival as opposed to other molecular subclasses.[13] Furthermore, a predictive molecular marker predicts the tumor response to treatment. For instance, activating small-scale mutations in *EGFR* in non-small cell lung cancer (NSCLC) are associated with response to EGFR inhibitors.[14] As such, *EGFR* mutations can be considered predictive molecular markers for targeted therapy.

Targeted therapy

Therapies that target specific predictive molecular markers are collectively known as 'molecularly targeted therapy' or simply 'targeted therapy'.[15] This includes a variety of small molecule inhibitors, monoclonal antibodies and antibody-drug conjugates. Pioneering drugs in this class of cancer therapy were anti-HER2 monoclonal antibody trastuzumab (for HER2-overexpressing breast cancers),[16] and small molecule multi-kinase inhibitor imatinib (for chronic myeloid leukemias driven by the *BCR-ABL* fusion gene).[17] Trastuzumab and imatinib were approved for these diseases by the European Medicines Agency (EMA) in the early 2000s. They were followed by the introduction of erlotinib (2005) and gefitinib (2009), which are small molecule inhibitors targeting *EGFR* mutations in NSCLC.[14,18] The arsenal of clinically available, EMA-approved targeted therapies started rapidly expanding in 2012 with the approval of vemurafenib for *BRAF* p.(V600)-mutant melanoma and crizotinib for *ALK* fusion-positive NSCLC.[19,20] Over a span of ten years, the EMA approved over 35 (combinations of) drugs for a variety of molecular indications in a range of cancers; as of October 2021, the total amount of available drugs tallied 45 (**Table 1**).[21] In addition, numerous other targeted drugs are pending EMA approval (**Table 2**),[22] in clinical trials, or in preclinical development.

Table 1. EMA-approved targeted therapies for specific molecular variants (as of October 1, 2021)[21]

Molecular marker	Type of cancer	Targeted drug	Initial EMA approval date
ALK fusion	NSCLC	Crizotinib	14/11/2012
		Ceritinib	06/05/2015
		Alectinib	16/02/2017
		Brigatinib	22/11/2018
		Lorlatinib	06/05/2019
BCR-ABL fusion (Philadelphia chromosome)	CML	Imatinib	07/11/2001
		Dasatinib	20/11/2006
		Nilotinib	19/11/2007
		Bosutinib	27/03/2013
	ALL	Imatinib	07/11/2001
		Dasatinib	20/11/2006
		Ponatinib	01/07/2013
BRAF p.(V600) mutation	Colorectal cancer	Encorafenib/Cetuximab	30/04/2020
	Melanoma	Vemurafenib/Cobimetinib	17/02/2012
		Dabrafenib/Trametinib	26/08/2013
		Encorafenib/Binimetinib	19/09/2018
	NSCLC	Dabrafenib/Trametinib	23/02/2017
BRCA1 mutation *BRCA2* mutation	Breast cancer Ovarian cancer Pancreatic cancer Prostate cancer	Olaparib	16/12/2014
	Ovarian cancer	Rucaparib	23/05/2018
	Breast cancer	Talazoparib	20/06/2019
EGFR amplification	Squamous NSCLC	Necitumumab	15/02/2016
EGFR mutation (except exon 20 insertions)	NSCLC	Erlotinib	19/09/2005
		Gefitinib	24/06/2009
		Afatinib	25/09/2013
		Osimertinib	01/02/2016
		Dacomitinib	02/04/2019
ERBB2 amplification	Breast cancer	Trastuzumab	28/08/2000
		Lapatinib	10/06/2008
		Pertuzumab	04/03/2013
		Trastuzumab emtansine	15/11/2013
		Neratinib	31/08/2018
		Trastuzumab deruxtecan	18/01/2021
		Tucatinib	11/02/2021
	Gastric / GEJ cancer	Trastuzumab	17/12/2009
FGFR2 fusion	Cholangiocarcoma	Pemigatinib	26/03/2021
FIP1L1-PDGFRA fusion	HES / CEL	Imatinib	28/02/2005

Table 1. Continued

Molecular marker	Type of cancer	Targeted drug	Initial EMA approval date
FLT3 mutation	AML	Midostaurin Gilteritinib	18/09/2017 24/10/2019
KIT mutation	GIST	Imatinib	19/03/2009
MSI-H/dMMR	Colorectal cancer	Pembrolizumab Ipilimumab/Nivolumab	10/12/2020 20/05/2021
	Endometrial cancer	Dostarlimab	24/04/2021
NTRK1 fusion *NTRK2* fusion *NTRK3* fusion	Any solid tumor	Larotrectinib Entrectinib	19/09/2019 31/07/2020
PDGFRA p.(D842V)	GIST	Avapritinib	24/09/2020
PDGFRA fusion *PDGFRB* fusion	MDS / MPD	Imatinib	23/12/2005
PD-L1 expression	Various cancers	Nivolumab Pembrolizumab Atezolizumab Durvalumab	17/07/2015 17/07/2015 20/09/2017 21/09/2018
PIK3CA mutation	Breast cancer	Alpelisib	27/07/2020
RET fusion *RET* mutation	NSCLC Thyroid cancer	Selpercatinib	11/02/2021
RET mutation	MTC	Selpercatinib	11/02/2021
ROS1 fusion	NSCLC	Crizotinib Entrectinib	21/07/2016 31/07/2020

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CEL, chronic eosinophilic leukemia; CML; chronic myeloid leukemia; dMMR, mismatch repair deficient; EMA, European Medicines Agency; GEJ, gastro-esophageal junction; GIST, gastro-intestinal stromal tumor; HES, hypereosinophilic syndrome; MDS, myelodysplastic syndrome; MPD, myeloproliferative disease; MSI-H, microsatellite instability high; MTC, medullary thyroid cancer; NSCLC, non-small cell lung cancer.

Molecular pathology and molecular profiling techniques

The emergence of molecular markers with diagnostic, prognostic and/or predictive relevance has necessitated routine testing of patients' tumors for the presence of these markers. The discipline within pathology that focuses on the detection of molecular markers is molecular pathology, also known as molecular diagnostics.[23] Molecular pathology broadly comprises any technique used for the testing of nucleic acids (DNA, RNA) in tissue, cytology or plasma samples.[24] In pathology departments throughout the Netherlands, clinical scientists in molecular pathology (CSMP) are responsible for the implementation, interpretation and quality control of these techniques, and the reporting of their

results.[25] Some techniques, such as polymerase chain-reaction (PCR)-based methods and fluorescence in-situ hybridization (FISH), have been available for years.[26,27] However, novel, state-of-the-art DNA- and RNA-based molecular profiling techniques have quickly become feasible for implementation in molecular pathology laboratories due to the swift expansion of clinical indications that justify molecular diagnostic testing in routine oncology practice.[28] One technology that has especially revolutionized molecular pathology is next-generation sequencing (NGS).[29] This technology allows for parallel, targeted sequencing of multiple genes, covering broader genomic regions than only hotspot mutations and simultaneously profiling tumor tissue from multiple patients.[25,30]

Table 2. Targeted therapies pending EMA approval (as of October 1, 2021)[22]

Molecular marker	Type of cancer	Targeted drug
ALK fusion	NSCLC	Ensartinib
BCR-ABL fusion (Philadelphia chromosome)	CML	Asciminib
BRCA1 mutation	Ovarian cancer Fallopian tube cancer Primary peritoneal cancer	Veliparib
EGFR exon 20 insertions	NSCLC	Amivantamab
ERBB2 amplification	Breast cancer	Margetuximab Trastuzumab duocarmazine
	Gastric / GEJ cancer	Trastuzumab deruxtecan
FGFR2 fusion	Cholangiocarcoma	Infigratinib
FGFR2 fusion *FGFR2* mutation *FGFR3* fusion *FGFR3* mutation	Urothelial carcinoma	Erdafitinib
IDH1 mutation	Cholangiocarcinoma	Ivosidenib
KRAS p.(G12C)	NSCLC	Sotorasib
MET exon 14 skipping	NSCLC	Capmatinib Tepotinib
RET fusion	NSCLC	Pralsetinib
TP53 mutation	MDS	Eprenetapopt

CML; chronic myeloid leukemia; EMA, European Medicines Agency; GEJ, gastro-esophageal junction; MDS, myelodysplastic syndrome; NSCLC, non-small cell lung cancer.

Interpretation of 'rare' somatic variants in cancer

As a consequence of introducing extensive molecular profiling into routine practice, the interpretation of molecular results has become more challenging. CSMPs, pathologists and oncologists increasingly have to deal with (combinations of) somatic variants that they rarely encounter, or have not encountered before, and of which the clinical consequence is therefore uncertain. These variants have been received different designations in literature: terms that have been used in literature include 'unknown',[31] 'rare',[32] 'uncommon',[33] 'complex',[34] or 'compound'.[35] These terms are often interchangeable. To understand the variety of challenges that arise from 'rare' somatic variants, these terms require further explanation.

The vast majority of somatic variants are 'unknown' variants. A variant can be truly classified as 'unknown' when it has not been biologically or clinically characterized – in other words, when its pathogenicity has not been investigated. In genetics and pathology, these variants are termed 'variants of unknown or uncertain clinical significance' (VUS).[36] When a CSMP finds a variant that has not been encountered before, this does not mean it is always a VUS: cancers generally harbor thousands of somatic variants, most of which have no biological effect. Early studies have estimated that every cancer generally harbor more than 10,000 somatic variants, most of which are unique to that patient.[37] VUS are often unique; as a result, CSMPs often need to assess numerous variants that they have not encountered before in routine diagnostics. Their interpretation is dependent on consulting different (inter)national databases and published research that may elucidate the biological effect of a variant or the association between a variant and clinical outcome.

A 'rare' pathogenic somatic variant is a variant that has been (biologically or clinically) characterized as (likely) to induce cancer, but that occurs at a low frequency. This is a broad definition that can encompass many types of variants, depending on the criteria used. The Dutch guideline for the treatment of advanced NSCLC defines 'rare' as a frequency of a variant in <5% of the general population of NSCLC.[38] By this definition, however, *ROS1* fusions (0.8% prevalence),[39] for which the Dutch guideline recommends treatment with crizotinib,[38] would classify as 'rare', whereas *RET* fusions (prevalence 1.0%),[39] for which the guideline has no recommendation yet,[38] would not. In addition, variants that are rare in one type of cancer may be common in others: for example, fusions involving one of the *NTRK* genes are highly enriched in secretory carcinomas, congenital mesoblastic nephroma and infantile fibrosarcoma (>90% of cases), but rare in more common types of cancer such as NSCLC and colorectal cancer (<1% of cases).[40] Furthermore, many unique variants are 'rare', but can be grouped into a 'common' denominator. For example, deleterious mutations in tumor suppressor gene *TP53* occur in many types of cancer (38–50%),[41] but a multitude of different mutations have been described, of which many have an individual frequency of <1%. Thus, defining a somatic variant as 'rare' based on an arbitrary cut-off of prevalence

does not always reflect difficulty in interpretation; rather, whether or not a variant should be considered 'rare' is dependent on the cancer type it was discovered in and its potential clinical relevance.

The term 'uncommon' mutation is generally synonymous with 'rare', but it has been popularized to distinguish rare ('uncommon') *EGFR* mutations – such as exon 20 insertions, p.(G719)- and p.(L861)-mutations – from the 'common' *EGFR* exon 19 deletions and p.(L858R) mutations. A third term that is sometimes used to described individual rare variants is 'non-canonical', referring to the opposite of 'canonical', which is defined as a universally accepted standard. In other words, a 'non-canonical' variant would be a variant for which current standards (clinical guidelines) do not have an answer, which is again a broad definition as most guidelines do not provide in-depth definition of which specific variants are included in common denominators. For example, '*KRAS* exon 2 mutations' are a contra-indication for anti-EGFR treatment in colorectal cancer, but some somatic mutations in *KRAS* exon 2 – such as *KRAS* p.(E31K)[42] – are not pathogenic and therefore of no clinical relevance.

'Complex' and 'compound' are terms that usually denote the co-existence of multiple pathogenic somatic variants and/or VUS. This can include co-existence of multiple activating mutations in different genes, which occurs rarely, as driver mutations are often mutually exclusive,[43] or multiple mutations affecting one (allele of a) gene, such as compound *EGFR* mutations (G719X/S768I).[35] These categories of rare mutations are more frequently found in patients who relapse on targeted therapy. Cancers can develop resistance by acquiring additional mutations that bypass the inhibiting effects of a drug.[44] A well-known example is the secondary *EGFR* p.(T790M) mutation, which prevents the binding of EGFR inhibitors such as gefitinib, afatinib and erlotinib in NSCLC patients with activating *EGFR* mutations.[45] Third-generation EGFR inhibitor osimertinib reverts this resistance mechanism, as it can still bind despite the secondary p.(T790M) mutation.[46] A large variety of (rare) molecular mechanisms of resistance have been described for both *EGFR*-mutant and *ALK*-rearranged NSCLC patients,[47,48] some of which are actionable with other targeted drugs. This has justified sequential molecular diagnostic testing to treat patients with second- and third-line targeted drugs, but has further increased the complexity of interpretation of molecular testing results.

Altogether, these different categories of 'rare' somatic mutations can each represent uncertainties in interpretation of molecular testing results. CSMPs and pathologists increasingly have to deal with rare somatic variants of which they cannot always determine the clinical consequence. On the other hand, treating oncologists are faced with complex molecular results that they have not been trained to understand. These challenges have instigated the implementation of MTBs.

Molecular Tumor Boards (MTBs)

Rare somatic variants require a multidisciplinary approach to ensure the interpretation of molecular testing results is optimized for individual patients. To this effect, various academic centers around the world have established multidisciplinary MTBs,[49–59] in which representatives from molecular pathology and clinical oncology meet periodically to discuss difficult cases and provide a patient-tailored clinical recommendation. MTBs are complimentary to conventional, cancer type-specific multidisciplinary team (MDT) meetings. Conventional MDTs integrate clinical information, imaging, laboratory results and pathological assessment to determine the patient's disease stage and the subsequent appropriate guidelines-based choice of treatment in patients with a specific type of cancer. MDT meetings are thus predominantly a clinical discussion. In contrast, MTBs provide a clinical interpretation and integration of (rare) somatic variants into the clinical context of the patient, taking into account the technical aspects of molecular testing results, the biological rationale of pathogenicity and the evidence regarding actionability, often in a histology-agnostic manner.[50,51,53,54,56,57,60] In contrast to MDTs, discussions within an MTBs often go beyond the directive of current guidelines. Cases discussed in an MTB therefore require a structured review of (online) data- and knowledge bases and an elaborate discussion to prioritize targets within the MTB.

In the Netherlands, MTBs have been operating since 2014,[61] but unlike MDTs,[62] there is no quality directive on how an MTB should operate. Experts disagree on the type of patients who are eligible to be reviewed by an MTB, the cancer types that should be included in the scope of an MTB, and the health professionals that should participate in MTB meetings.[63,64] Not surprisingly, the MTBs that have been published thus far show major differences,[65,66] and recommendations provided by MTBs seem to vary among different institutions internationally.[67] Furthermore, there is no consensus on the most optimal approach for achieving a treatment recommendation. As MTBs are increasingly being incorporated into standard-of-care cancer diagnostics and therapy, there is a risk that patients receive different treatment recommendations when they are treated in different centers within the same healthcare system. Therefore, it is necessary to gain insight into the differences between existing MTBs in the Netherlands and to establish tools and strategies for decision-making within and outside MTBs.

AIM AND SCOPE OF THIS THESIS

The aim of this thesis is to investigate strategies that can be used for the clinical interpretation of challenging results from molecular pathology for patients with cancer. MTBs are a foremost resource for oncologists, pathologists and CSMPs to translate rare somatic variants into a diagnostic or treatment recommendation. Insight into the organizational infrastructure, methods and subsequent treatment outcomes of MTBs in the Netherlands is currently lacking. Therefore, the aim of **part I** is to investigate the methods of current MTBs associated with tertiary cancer referral centers in the Netherlands. **Chapter 2** will analyze the methods and subsequent adherence and treatment outcome resulting from MTB recommendations in a single tertiary cancer referral center. In **chapter 3**, the focus will be expanded to the rest of the Netherlands, comparing the organizational structure and differences in targeted therapy recommendations among MTBs hosted in all eight tertiary cancer referral centers in the Netherlands. In **chapter 4**, the core of MTB decision-making is further explored by investigating how rare variants are interpreted by CSMPs associated with these MTBs and which available resources were used to facilitate this assessment. In addition, the agreement among these experts in the interpretation of pathogenicity and actionability of challenging somatic variants was assessed.

Within and outside of the MTBs, oncologists, pathologists and CSMPs are faced with a variety of challenges at different levels of decision-making that require strategies tailored to each situation for an optimal diagnostic or treatment recommendation. This can range from choosing the appropriate testing method to the treatment outcome of patients with rare somatic variants that are treated with targeted therapy. The aim of **part II** was to explore these specific challenges and determine strategies that can be used to solve them. **Chapter 5** centers around establishing the appropriate testing method that can be used to detect fusions in *NTRK1–3*. **Chapter 6** will illustrate the diagnostic significance that can result from the detection of rare (predictive) somatic variants, based on a patient with lung adenocarcinoma harboring multiple such unusual variants. In **chapter 7**, the utility of applying a classification system for actionability will be investigated based on a landscape of clinically actionable, uncommon *EGFR* mutations in Dutch patients with NSCLC. **Chapter 8** will investigate the potential advantage of basing treatment decisions on *in vitro* and (limited) clinical evidence to predict the clinical tumor response of ALK inhibitors directed towards on-target resistance mutations in *ALK* fusion-positive NSCLC patients. **Chapter 9** focuses on the significance of off-target (bypass) mutations and the possibility to repurpose currently approved drugs to treat patients with such resistance mechanisms.

Finally, **chapter 10** summarizes the results of this thesis, provides a framework for the optimal decision-making strategy that can be applied when detecting a rare somatic variant in routine diagnostics, and highlights how recent advances in molecular pathology may affect these strategies.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, *et al*. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**(3): 209–249.
2. Croce CM. Oncogenes and cancer. *N Engl J Med* 2008; **358**(5): 502–511.
3. Klein G. Oncogenes and tumor suppressor genes. *Acta Oncol* 1988; **27**(4): 427–437.
4. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**(5): 646–674.
5. 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**(6): 703–713.
6. Beroukhim R, Mermel CH, Porter D, *et al*. The landscape of somatic copy-number alteration across human cancers. *Nature* 2010; **463**(7283): 899–905.
7. Albertson DG. Gene amplification in cancer. *Trends Genet* 2006; **22**(8): 447–455.
8. Soda M, Choi YL, Enomoto M, *et al*. Identification of the transforming *EML4–ALK* fusion gene in non-small-cell lung cancer. *Nature* 2007; **448**(7153): 561–566.
9. Snuderl M, Kolman OK, Chen YB, *et al*. B-cell lymphomas with concurrent *IGH-BCL2* and *MYC* rearrangements are aggressive neoplasms with clinical and pathologic features distinct from Burkitt lymphoma and diffuse large B-cell lymphoma. *Am J Surg Pathol* 2010; **34**(3): 327–340.
10. Sidransky D. Emerging molecular markers of cancer. *Nat Rev Cancer* 2002; **2**(3): 210–219.
11. Shaw A, Bradley MD, Elyan S, Kurian KM. Tumour biomarkers: diagnostic, prognostic, and predictive. *BMJ* 2015; **351**: h3449.
12. Miettinen M, Felisiak-Golabek A, Luiña Contreras A, *et al*. New fusion sarcomas: histopathology and clinical significance of selected entities. *Hum Pathol* 2019; **86**: 57–65.
13. Bosse T, Nout RA, McAlpine JN, *et al*. Molecular Classification of Grade 3 Endometrioid Endometrial Cancers Identifies Distinct Prognostic Subgroups. *Am J Surg Pathol* 2018; **42**(5): 561–568.
14. Sequist LV, Martins RG, Spigel D, *et al*. First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic *EGFR* mutations. *J Clin Oncol* 2008; **26**(15): 2442–2449.
15. Mendelsohn J. 45 – From Bench to Bedside with Targeted Therapies. In: *Mendelsohn J, Gray JW, Howley PM, et al. – The Molecular Basis of Cancer: Fourth edition*. London: Elsevier; 2014: 621–634.
16. Slamon DJ, Leyland-Jones B, Shak S, *et al*. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *New Engl J Med* 2001; **344**(11): 783–792.
17. Savage DG and Antman KH. Imatinib mesylate--a new oral targeted therapy. *New Engl J Med* 2002; **346**(9): 683–693.
18. Jackman DM, Yeap BY, Sequist L V, *et al*. Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients treated with gefitinib or erlotinib. *Clin Cancer Res* 2006; **12**(13): 3908–3914.

19. Chapman PB, Hauschild A, Robert C, *et al*. Improved survival with vemurafenib in melanoma with *BRAF* V600E mutation. *New Engl J Med* 2011; **364**(26): 2507–2516.
20. Kwak EL, Bang Y-J, Camidge DR, *et al*. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *New Engl J Med* 2010; **363**(18): 1693–1703.
21. European Medicines Agency: Medicines. European Medicines Agency. Accessed July 16, 2021. https://www.ema.europa.eu/en/medicines.
22. Horizonscan Geneesmiddelen: Overzicht van toekomstige innovatieve geneesmiddelen. Zorginstituut Nederland. 2021. Accessed July 16, 2021. https://www.horizonscangeneesmiddelen.nl/geneesmiddelen.
23. Harris TJR, McCormick F. The molecular pathology of cancer. *Nat Rev Clin Oncol* 2010; **7**(5): 251–265.
24. Warshawsky I. Chapter 1 Molecular Biology Basics for the Pathologist. In: *Cell and Tissue Based Molecular Pathology: First edition*. London: Elsevier, 2009: 3–9.
25. Deans ZC, Costa JL, Cree I, *et al*. Integration of next-generation sequencing in clinical diagnostic molecular pathology laboratories for analysis of solid tumours; an expert opinion on behalf of IQN Path ASBL. *Virchows Arch* 2017; **470**(1): 5–20.
26. Kluin P, Schuuring E. Molecular cytogenetics of lymphoma: where do we stand in 2010? *Histopathology* 2011; **58**(1): 128–144.
27. Thunnissen FBJM, Tilanus MGJ, Ligtenberg MJL, *et al*. Quality control in diagnostic molecular pathology in the Netherlands; proficiency testing for patient identification in tissue samples. *J Clin Pathol* 2004; **57**(7): 717–720.
28. Chakravarty D, Solit DB. Clinical cancer genomic profiling. *Nat Rev Genet* 2021; **22**(8): 483–501.
29. Mardis ER. Next-generation DNA sequencing methods. *Annu Rev Genomics Hum Genet* 2008; **9**: 387–402.
30. Garraway LA. Genomics-driven oncology: framework for an emerging paradigm. *J Clin Oncol* 2013; **31**(15): 1806–1814.
31. Bi J, Thiel KW, Litman JM, *et al*. Characterization of a *TP53* Somatic Variant of Unknown Function From an Ovarian Cancer Patient Using Organoid Culture and Computational Modeling. *Clin Obstet Gynecol* 2020; **63**(1): 109–119.
32. Menzer C, Menzies AM, Carlino MS, *et al*. Targeted Therapy in Advanced Melanoma With Rare *BRAF* Mutations. *J Clin Oncol* 2019; **37**(33): 3142–3151.
33. Brindel A, Althakfi W, Barritault M, *et al*. Uncommon *EGFR* mutations in lung adenocarcinomas: Clinical features and response to tyrosine kinase inhibitors. *Ann Oncol* 2018; **29**: viii747–viii748.
34. Simionato F, Calvetti L, Cosci M, Scarparo S, Aprile G. Case Report: A Metabolic Complete Response to Upfront Osimertinib in a Smoker Non-Small Cell Lung Cancer Patient Harbouring *EGFR* G719A/V769M Complex Mutation. *Onco Targets Ther* 2020; **13**: 12027–12031.
35. Hayashi T, Kohsaka S, Takamochi K, *et al*. Clinicopathological characteristics of lung adenocarcinoma with compound *EGFR* mutations. *Hum Pathol* 2020; **103**: 42–51.
36. Richards S, Aziz N, Bale S, *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College

of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; **17**(5): 405–423.
37. Tomlinson I, Sasieni P, Bodmer W. How many mutations in a cancer? *Am J Pathol* 2002; **160**(3): 755–758.
38. Niet-kleincellig longcarcinoom: Landelijke richtlijn. NVALT. 2020. Accessed July 8, 2021. https://richtlijnendatabase.nl/richtlijn/niet_kleincellig_longcarcinoom/startpagina_-_niet-kleincelling_longcarcinoom.html.
39. AACR Project GENIE Consortium. AACR Project GENIE: Powering Precision Medicine through an International Consortium. *Cancer Discov* 2017; **7**(8): 818–831.
40 Cocco E, Scaltriti M, Drilon A. *NTRK* fusion-positive cancers and TRK inhibitor therapy. *Nat Rev Clin Oncol* 2018; **15**(12): 731–747.
41. Olivier M, Hollstein M, Hainaut P. *TP53* mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harb Perspect Biol* 2010; **2**(1): a001008.
42. Janakiraman M, Vakiani E, Zeng Z, *et al*. Genomic and biological characterization of exon 4 *KRAS* mutations in human cancer. *Cancer Res* 2010; **70**(14): 5901–5911.
43. Roosan MR, Mambetsariev I, Pharaon R, *et al*. Evaluation of Somatic Mutations in Solid Metastatic Pan-Cancer Patients. *Cancers* 2021; **13**(11): 2776.
44. Housman G, Byler S, Heerboth S, *et al*. Drug resistance in cancer: an overview. *Cancers* 2014; **6**(3): 1769–1792.
45. Yun C-H, Mengwasser KE, Toms A V, *et al*. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proc Natl Acad Sci U S A* 2008; **105**(6): 2070–2075.
46. Mok TS, Wu Y-L, Papadimitrakopoulou VA. Osimertinib in *EGFR* T790M-Positive Lung Cancer. *N Engl J Med* 2017; **376**: 1993–1994.
47. Gainor JF, Dardaei L, Yoda S, *et al*. Molecular Mechanisms of Resistance to First- and Second-Generation ALK Inhibitors in *ALK*-Rearranged Lung Cancer. *Cancer Discov* 2016; **6**(10): 1118–1133.
48. onetti A, Sharma S, Minari R, Perego P, Giovannetti E, Tiseo M. Resistance mechanisms to osimertinib in *EGFR*-mutated non-small cell lung cancer. *Br J Cancer* 2019; **121**(9): 725–737.
49. Basse C, Morel C, Alt M, *et al*. Relevance of a molecular tumour board (MTB) for patients' enrolment in clinical trials: experience of the Institut Curie. *ESMO Open* 2018; **3**(3): e000339.
50. Bryce AH, Egan JB, Borad MJ, *et al*. Experience with precision genomics and tumor board, indicates frequent target identification, but barriers to delivery. *Oncotarget* 2017; **8**(16): 27145–27154.
51. Dalton WB, Forde PM, Kang H, *et al*. Personalized Medicine in the Oncology Clinic: Implementation and Outcomes of the Johns Hopkins Molecular Tumor Board. *JCO Precis Oncol* 2017; **1**: PO.16.00046.
52. Harada S, Arend R, Dai Q, *et al*. Implementation and utilization of the molecular tumor board to guide precision medicine. *Oncotarget* 2017; **8**(34): 57845–57854.
53. Kaderbhai CG, Boidot R, Beltjens F, *et al*. Use of dedicated gene panel sequencing using next generation sequencing to improve the personalized care of lung cancer. *Oncotarget* 2016; **7**(17): 24860–24870.

54. Moore DA, Kushnir M, Mak G, *et al*. Prospective analysis of 895 patients on a UK Genomics Review Board. *ESMO Open* 2019; **4**(2): e000469.
55. Réda M, Richard C, Bertaut A, *et al*. Implementation and use of whole exome sequencing for metastatic solid cancer. *EBioMedicine* 2020; **51**: 102624.
56. Rolfo C, Manca P, Salgado R, *et al*. Multidisciplinary molecular tumour board: a tool to improve clinical practice and selection accrual for clinical trials in patients with cancer. *ESMO Open* 2018; **3**(5): e000398.
57. Trédan O, Wang Q, Pissaloux D, *et al*. Molecular screening program to select molecular-based recommended therapies for metastatic cancer patients: analysis from the ProfiLER trial. *Ann Oncol* 2019; **30**(5): 757–765.
58. Trivedi H, Acharya D, Chamarthy U, *et al*. Implementation and Outcomes of a Molecular Tumor Board at Herbert-Herman Cancer Center, Sparrow Hospital. *Acat Med Acad* 2019; **48**(1): 105–115.
59. Varnier R, Le Saux O, Chabaud S, *et al*. Actionable molecular alterations in advanced gynaecologic malignancies: updated results from the ProfiLER programme. *Eur J Cancer* 2019; **118**: 156–165.
60. Knepper TC, Bell GC, Hicks JK, *et al*. Key Lessons Learned from Moffitt's Molecular Tumor Board: The Clinical Genomics Action Committee Experience. *Oncologist* 2017; **22**(2): 144–151.
61. Moleculaire Tumor Board Groningen. UMCG Pathologie en Medische Biologie. Accessed July 21, 2021. https://www.umcg.nl/NL/UMCG/Afdelingen/Pathologie/Professionals/moleculaire-diagnostiek/Moleculaire-Tumor-Board-Groningen.
62. Integraal Kankercentrum Nederland. Kwaliteitscriteria multidisciplinair overleg (mdo). 2016: 1–15.
63. van der Velden DL, van Herpen CML, van Laarhoven HWM, *et al*. Molecular Tumor Boards: current practice and future needs. *Ann Oncol* 2017; **28**(12): 3070–3075.
64. Willemsen AECAB, Krausz S, Ligtenberg MJL, *et al*. Molecular tumour boards and molecular diagnostics for patients with cancer in the Netherlands: experiences, challenges, and aspirations. *Br J Cancer* 2019; **121**(1): 34–36.
65. Luchini C, Lawlor RT, Milella M, Scarpa A. Molecular Tumor Boards in Clinical Practice. *Trends Cancer* 2020; **6**(9): 738–744.
66. Larson KL, Huang B, Weiss HL, *et al*. Clinical Outcomes of Molecular Tumor Boards: A Systematic Review. *JCO Precis Oncol* 2021; **5**: 1122–1132.
67. Rieke DT, Lamping M, Schuh M, *et al*. Comparison of Treatment Recommendations by Molecular Tumor Boards Worldwide. *JCO Precis Oncol* 2018; **2**: PO.18.00098.

PART ONE

Molecular Tumor Boards: organization, methods and outcomes