Diagnostic challenges of today's lung cancer pathology: personalizing therapy by immunohistochemical and molecular biomarkers

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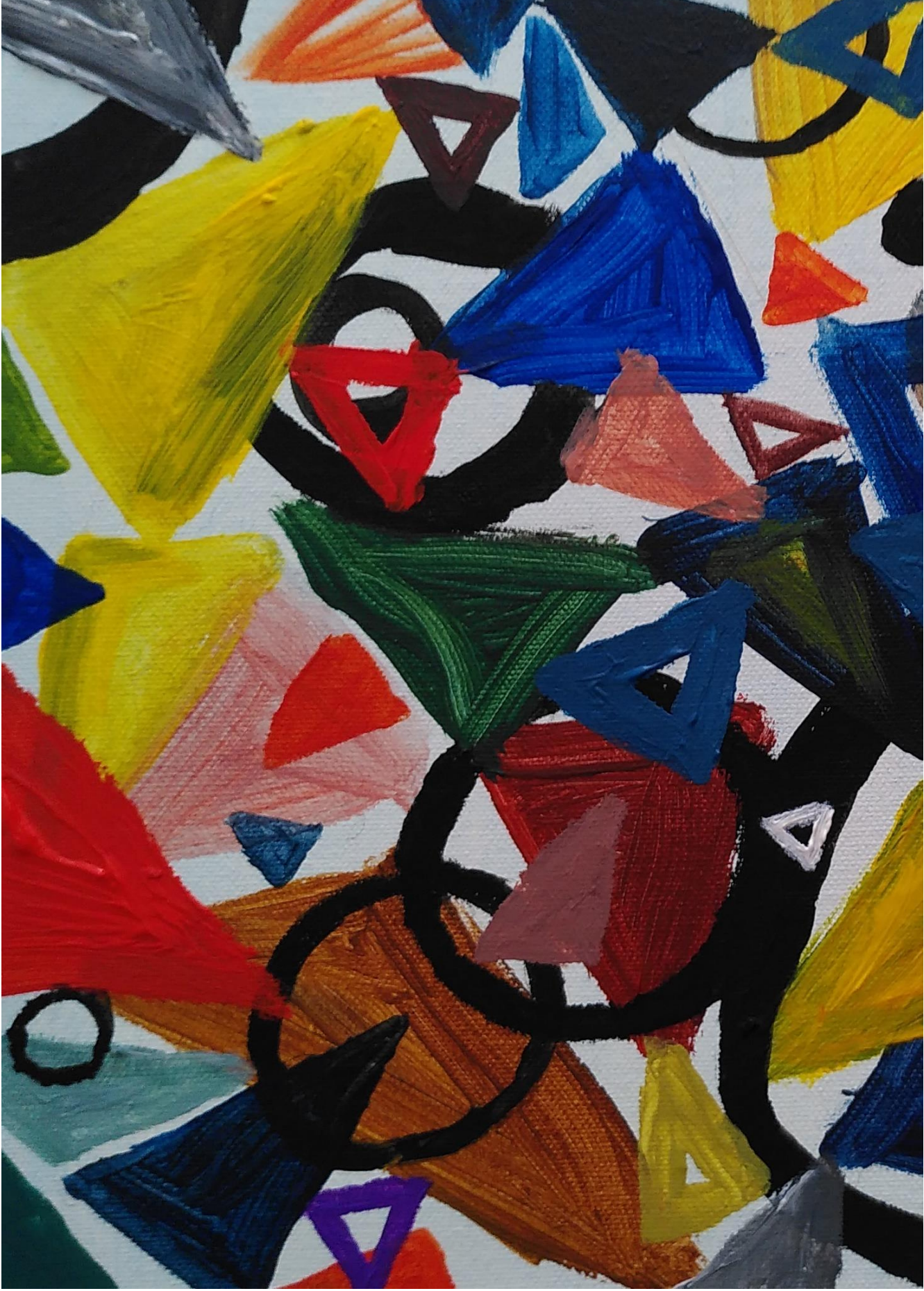


CHAPTER 7

DISCUSSION

Chapter 7: Discussion, summary and future challenges

7.1 A typical NSCLC patient journey

In the Introduction, a case was described, in which a patient presents with an asymptomatic pulmonary node. (Figure 1) What we can draw from this case is that lung cancer, even in relatively low stages, can have a detrimental and malignant course. Pathologists are prominently involved in the diagnostics and management of NSCLC, as they provide the crucial information in the key treatment decision-making moments.

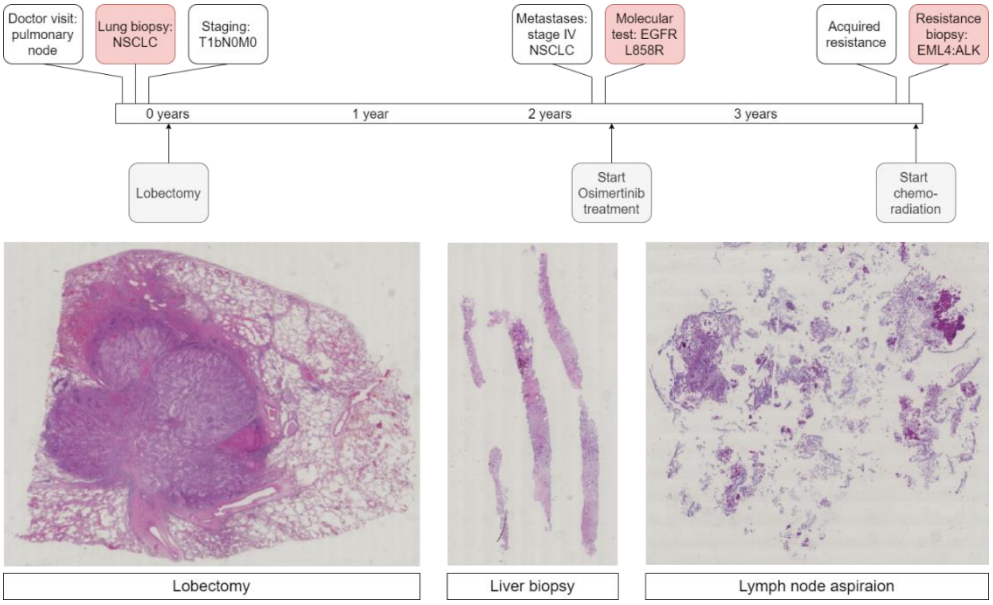


***Figure 1**: Key decision making moments for pathologists in the NSCLC patient journey.*

7.2 The optimal diagnostic work-up at key decision-making moments in NSCLC: a pathologists' dilemma

If one thing has become clear from both this thesis and the literature on NSCLC, it's that NSCLC is an incredibly heterogeneous, many-faced and deadly disease. No two lung tumors are exactly the same, which is no small feat, as the volume of NSCLC patients is so enormous. A wide variance exists with regard to age, smoking history, tumor grade, TNM-stage, oncogenic driver

mutation, co-mutations, PD-L1 score, anti-tumor immune response, resistance mechanisms, metastatic behavior, treatment response and prognosis. This makes personalizing the diagnosis and management of lung cancer crucial – but also highly complex.

In this thesis, we investigated three key decision-making moments for pathologists in NSCLC: early stage diagnosis (1), late stage diagnosis (2) and acquired *EGFR* TKI resistance (3). We provide a rationale for molecular and immunohistochemical testing sequences at each instance, while taking into account the challenges: tissue scarcity, time constraints, costs of testing and comprehensiveness. Below, we address each of these key decision-making moments, including the specific challenges that a modern day pathologist needs to balance carefully.

7.2.1 Key decision making moment 1: Early stage diagnosis

The workup for early stage NSCLC initially did not typically include NGS or IHC. However, with (neo-)adjuvant immunotherapy and adjuvant Osimertinib around the corner, that limited workup is about to change. Additionally, the number of early stage patients will rise in the years to come, due to the imminent implementation of targeted population screening for NSCLC, following data from the NELSON-trial. [1]

With the transition of targeted and immunotherapeutic therapies to the (neo-)adjuvant setting, early stage NSCLC diagnostics will thus become more similar to the treatment-naïve stage IV workup. While this transition to (neo-)adjuvant treatment is ongoing, it's important to take note of how frequent targets occur in early stage tumors, and how these patients and tumors differ from the late stage variants. In **Chapter 2**, we identified *EGFR* mutations in 13% of tumors in stage IIIA or lower, whereas this was 9% in stage IIIB and IV. Especially the earliest stages (stage 0 and stage IA) were enriched for *EGFR* mutations (27% and 18% respectively).

Additionally, as illustrated in **Chapter 2,** there are substantial differences between early stage *EGFR*-mutated and late stage *EGFR*-mutated cancers, including type of *EGFR* mutation, co-mutations, growth pattern and smoking history. This underlines the complex heterogeneity of lung adenocarcinoma, and is an argument in favor of developing comprehensive multi-factorial risk assessment tools instead of current 'one size fits all' protocols.

7.2.2 Key decision making moment 2: Late stage diagnosis

In the treatment-naïve stage IV setting, patients need to be screened for PD-L1 expression and targetable mutations. However, with the growing list of targetable mutations, multiple challenges arise: choosing the correct molecular panel, the role of immunohistochemistry for fusion detection, and PD-L1 expression scoring. Each is discussed separately below.

7.2.2.1 Molecular diagnostics in stage IV NSCLC

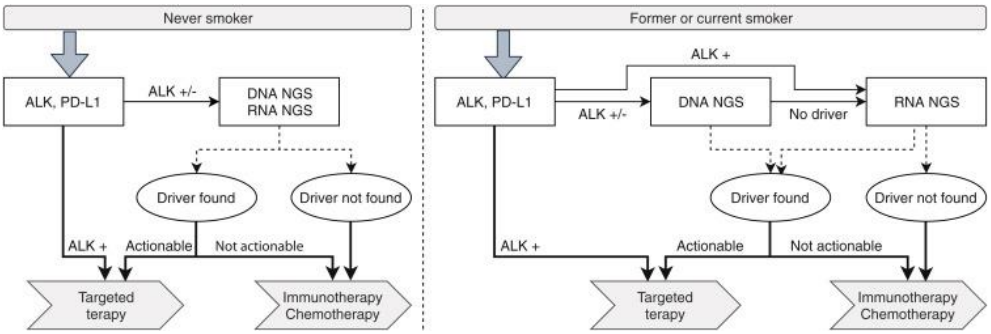
All relevant targets need to be covered by the molecular workup. This seems simple, but in the past years, the number of actionable targets has risen constantly, and will continue to do so. A small targeted panel which only includes targetable alterations, will need to be adjusted with every new target, and is therefore not future-proof. In addition, there are many targets for which an experimental TKI is available via clinical trials, compassionate use or early access programs. Those targets are not officially 'actionable', but finding them can still be worthwhile for individual patients. In addition, selected co-mutations (such as *TP53* and *STK11* – as discussed in **Chapter 2** and **Chapter 6**) are relevant to identify as well, as they can be indicative of the malignant potential and therapy resistance.

The molecular screening in stage IV should thus at least cover (1) the eight currently targetable targets: *EGFR*, *BRAF*, *HER2*, *ALK*, *ROS1*, *RET*, *NTRK* and *MET*; (2) targets for which clinical trials exist, such as *KRAS* G12C and *NRG1*; (3) clinically relevant co-mutations, such as *STK11* and *TP53*. In practice, this means that each specimen should be tested for point mutations, deletions, insertions, amplifications, exon skipping and fusions. Most of these alterations can be detected with small, targeted DNA NGS panels, but for exon skipping and fusion detection, broad DNA NGS panels (such as WGS) or targeted RNA NGS is required.

A major issue with choosing the optimal molecular diagnostics sequence in stage IV is tissue scarcity. In 30% of the treatment-naïve stage IV patients, as discussed in **Chapter 3**, the entire workup needs to be performed on a (small) cytology specimen, often acquired via endoscopic lymph node fine needle aspiration, with a limited number of tumor cells. With broader panels, a higher DNA input is required, which can thus be challenging in these small biopsies and cytology. Taking a new biopsy, with risk of co-morbidity, causes substantial

diagnostic delay, and must be prevented as much as possible. Based on the **Chapter 3** data, we therefore recommend performing targeted DNA NGS first, followed by RNA NGS when no driver is identified. In never-smokers, fusions are far more prevalent (32% of cases, **Chapter 3**) compared to current and former smokers (4% of cases). Therefore, RNA NGS is more relevant for never-smokers, and it should be performed immediately, not only after DNA NGS is driver-negative. (Figure 2) Both of these workups proved relatively tissue-efficient, while covering all required targets.

Figure 2*: Stage IV NSCLC workup, separate for never-smokers and smokers. (Chapter 3)*



7.2.2.2 Immunohistochemistry for fusion detection

An exception to the multi-target approach in the molecular stage IV workup is *ALK*. Since *ALK* immunohistochemistry is highly sensitive and specific for *ALK* fusions, fast pre-screening with *ALK* IHC is defensible, and sometimes reduces the turnaround time with several days.

The same exception that can be made for *ALK* immunohistochemistry is not applicable to *NTRK* and *ROS1*. Whereas *ALK* IHC is highly sensitive and specific, *ROS1* IHC has problematic false-positivity (as demonstrated in **Chapter 3**) and *NTRK* IHC – as described in **Chapter 5** – false-negativity and false-positivity. A sequential approach, with pan-TRK immunohistochemistry first and confirming positive cases with RNA NGS, would result in missing 18% of actionable *NTRK* fusions.

7.2.2.3 PD-L1 immunohistochemistry

Being the companion biomarker for immunotherapy, the immunohistochemical PD-L1 expression score is mandated in all stage IV

NSCLC workups. However, pathologists should recognize the flaws that this biomarker intrinsically harbors. There is substantial inter- and intraobserver variance around the cutoff point, and PD-L1 expression does not predict response to immunotherapy perfectly – some PD-L1-high patients fail to respond and vice versa. Unfortunately, the search for alternative, more reliable biomarkers has not yet been successful.

Part of the current scientific effort is directed at improving PD-L1 as a biomarker, by reducing interobserver variability and overcoming human scoring bias. In **Chapter 4**, it was demonstrated that automated deep learning algorithms can be reliable, and potentially valuable as a scoring assistant in difficult cases around the 50% cutoff point. As inter- and intraobserver variance is an issue for pathologists in several tasks (Ki67, nuclear grade, Gleason score, ER-expression, etc.), automated, computer-mediated scoring, comparable to PD-L1 scoring as described in **Chapter 4**, could very well be implemented more widely in the near future.

7.2.3 Key decision making moment 3: Acquired TKI resistance

With the recent introduction of TKIs into routine NSCLC treatment regimens, pathologists were confronted with a new problem: how to find the resistance mechanism in acquired resistance biopsies? Resistance biopsies – like stage IV biopsies – generally don't contain a an abundance of tumor cells, but need to be tested for a wide range of targets. Known resistance mechanisms include: small cell transformation, squamous transformation, *EGFR*, *HER2*, *MET*, *KRAS*, *BRAF*, PIK3CA, *ALK*, *RET*, FGFR, *ROS1*, *NTRK* and *MET*. The landscape of genomic alterations after TKI resistance thus bears some similarity to the treatment-naïve workup. An important difference however is the clonal heterogeneity in resistance biopsies, which leads to non-mutual exclusivity of resistance mechanisms and impaired amplification detection.

Whereas oncogenic driver mutations such as *BRAF* and *EGFR* are mutually exclusive in treatment-naïve tumors, resistance mechanisms co-occur in resistance biopsies, in at least 7% of cases. A sequential approach, with RNA NGS only when no driver is identified in DNA NGS, as recommended in the treatment-naïve setting, is therefore not comprehensive in resistance biopsies, as resistance mechanisms may co-occur. However, it must be noted that co-occurrence of fusions and exon skipping events with other resistance

mechanisms (except *EGFR* amplification and *PIK3CA*) is rare, so omitting RNA NGS in selected cases could be defensible in case of logistical, tissue-quantity or financial constraints.

Due to clonal heterogeneity, amplification detection with DNA NGS is impaired, as DNA NGS is heavily dependent on the tumor cell percentage for copy number analysis. When not all tumor cells harbor the amplification or the tumor cell percentage is low, the copy number can be underestimated, leading to the missing of amplifications. *MET* and *HER2* amplifications are among the most frequently occurring and (experimentally) targetable resistance mechanisms, so missing those amplifications is not optimal. Our data in **Chapter 6** shows that up to 30% of *HER2* and *MET* amplifications are missed by DNA NGS in the acquired resistance setting. It's therefore important to use additional *MET* and *HER2* testing in resistance biopsies, with *MET* ISH and *HER2* IHC or ISH.

The complete *EGFR* TKI workup therefore includes: morphologic examination, DNA NGS, RNA NGS, *HER2* ISH of IHC and *MET* ISH. (Figure 3)

7.3 Future challenges

Although the recommendations throughout this thesis are helpful for choosing the optimal workup in the current NSCLC landscape at key decision-making moments, there is still room for improvement in the treatment and management of future NSCLC patients. Even with the combined research effort of the past decades, most NSCLC patients still die and we still have insufficient knowledge on the biologic mechanisms underlying disease behavior. There are important scientific lacunae that we will need to cover in the coming years, including a different approach to patient risk stratification, improving molecular methods and prevention.

7.3.1 Improved risk stratification

Currently, up to 50% of patients who undergo 'curative' surgical resection die of lung cancer, including the patient in our case at the beginning of this Chapter. This is likely due to the presence of micro-metastases at the time of surgery, which are not detected during routine staging. Current risk assessment in the clinic is based solely on TNM-stage, which is shown to be a relatively poor predictor. A large number of potential biomarkers for

metastasis after surgery have been described already, for example histological differentiation, pleural invasion and specific mutations. [2] However, none of these biomarkers provide a perfect prognostication, and the search for novel and more integrated biomarkers is still ongoing.

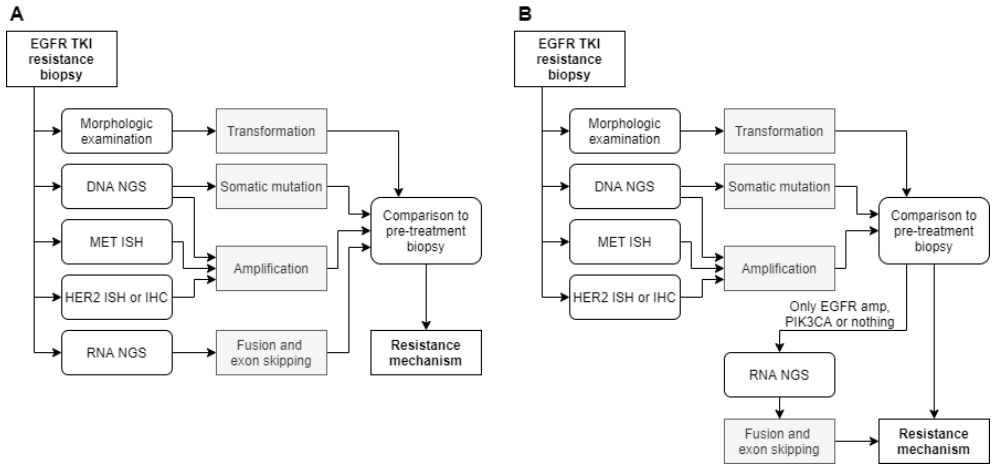


***Figure 3**: Recommended workup for EGFR TKI resistance biopsy testing. (Chapter 6) A: Recommended, comprehensive workup. B: Alternative workup in case of logistical, tissue-quantity or financial constraints.*

Another main challenge that will hopefully be solved in the near future is the selection of patients for immunotherapy. Although some patients respond excellent to immunotherapy, other patients respond barely, or only for a short amount of time. PD-L1 and tumor mutational burden (TMB) are established biomarkers for response, but imperfect ones – some patients with a low PD-L1 and TMB respond remarkably well, and vice versa. Additionally, there is substantial variability between pathologists in PD-L1 assessment and between laboratories in the TMB assessment. An urgent need for novel, better biomarkers for immunotherapy response therefore exists. In recent years, several promising biomarkers have emerged, for example CD8+ tumor infiltrating T-cells, or the presence of tertiary lymphoid structures, [3, 4] but real treatment implications for NSCLC patients are still far away.

In the TKI-treated NSCLC patients, specifically Osimertinib, there is a wide variety in progression-free survival between patients. If we would know in advance what the expected time to resistance would be, treatment and follow-up regimens could be specifically tailored to suit individual patients. This could

potentially reduce the burden of regular screening, give rise to novel treatment innovations, and improve quality of care.

What all these risk stratification problems all have in common is their complex, multi-factorial nature. If we can draw any conclusions from the past decades of cancer research, it's that lung cancer is an incredibly complex, many-faced, capricious disease. Current-day biomarkers such as TNM attempt to simplify all of these biological factors into one biomarker. This approach, although ambitious and hopeful, neglects the incredible complexity and heterogeneity of the biochemical processes that make up the tumor behavior. Any single biomarker is thus by definition a poor representation and it's naïve to expect an accurate response prediction from it.

As illustrated by the problems discussed above, there is an urgent clinical need for more comprehensive, multi-factor biomarkers and prediction models. These problems – prediction problems with a large number of potential risk factors – are difficult to solve with plain statistics, but ideal for machine learning. While humans have great difficulty to comprehend 'big data', deep learning models are well-suited for it. In the past several years, there has been an almost exponential increase in the number of biomedical studies utilizing artificial intelligence (AI). There is some hope that this line of research will unlock the problem of risk stratification in NSCLC.

However, although AI is a promising tool, its place in the routine Pathology diagnostics is still only beginning to be established. Although some laboratories are now using fast-throughput scanners for a large portion of the diagnostic load, routine computer-aided diagnostics is still a distant dot on the horizon. The current digital infrastructure in virtually all laboratories is not able to accommodate AI-models yet, which will need to change in years to come. Additionally, the digitalization of laboratories will need to be paralleled with an increase in pathologist's digital awareness. In order to assess the benefit of AI-models, one needs to understand how AI-models work and be aware of the pitfalls. Currently however, AI has no place in the curriculum of pathologists-in-training.

Another challenge in the field of digital pathology and machine learning is domain adaptation. (Figure 4) It's well known that the performance of AI-models is often domain-specific, and models don't generalize well to other

laboratories. Small differences in cutting technique, scanner settings or staining methods (domain differences) can have substantial consequences for model performance. This will become an issue in long-term model use as well, as most laboratories purchase new laboratory equipment every few years. Data scientists need to come up with easy-to-implement domain adaptation models, and should work together with pathologists to determine a standardized, periodic quality assessment protocol for AI-tools.

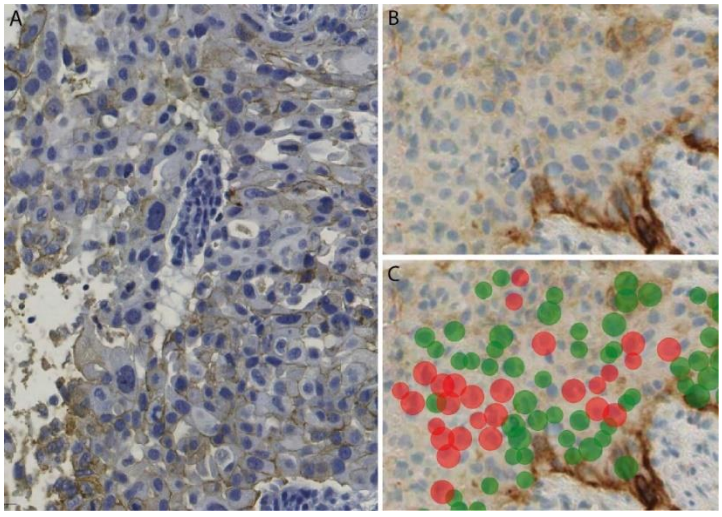


***Figure 4**: Domain adaptation example. A: PD-L1 slide from LUMC, using 22C3 antibody. B: PD-L1 slide from Erasmus MC, using SP263 antibody and a different immunostainer. C: Predictions from LUMC-trained PD-L1 algorithm on Erasmus MC slide, failing to correctly detect most cells due to domain differences.*

7.3.2 Towards whole genome sequencing for all?

In the near future, whole genome sequencing (WGS) and liquid biopsy will be used more often. Whereas WGS is now inefficient for lung cancer biopsies and cytology due to tissue scarcity, the techniques involving WGS are becoming more tissue-efficient. Tissue scarcity is therefore unlikely to remain a limitation for long. In addition, WGS is becoming less expensive each year, which promotes the availability worldwide. Eventually, we will perform WGS on more often, regardless TNM stage.

In addition, liquid biopsy is now used only in selected cases. Although liquid biopsy still has some problematic limitations, such as the inability to detect

fusions, exon skipping and copy number alterations, it could be useful in selected cases, such as the TKI resistance setting. In **Chapter 6**, we demonstrated that *EGFR* TKI resistance cases have substantial intratumor genomic heterogeneity. In the 43-49% of resistance cases, no mechanism is identified, which could be caused by sampling error and potentially solved by liquid biopsy. For patients with acquired resistance to Osimertinib, liquid biopsy could thus become a meaningful addition.

7.3.3 Smoking eradication

Although future research will undoubtedly improve lung cancer diagnostics and mortality by using the most novel, cutting-edge techniques, their combined benefits are insignificant compared to what we would win when tobacco would be eradicated. Smoking is the main cause of lung cancer, and up to 90% of lung adenocarcinomas occur in former or current smokers. (**Chapter 3**) However, as discussed in **Chapter 1**, smoking prevalence is only slowly decreasing, and still rising in some countries.

The first anti-tobacco campaign originated from 1604, when King James I of England argumented that smoking was "A custome lothsome to the eye, hatefull to the Nose, harmefull to the braine, dangerous to the Lungs, and in the blacke stinking fume thereof, neerest resembling the horrible Stigian smoke of the pit that is bottomelesse". [5]

Statistical evidence for the detrimental effects of smoking was first reported to the public much later, in 1950, when epidemiologists Doll and Hill indisputably demonstrated a causal relation between smoking and lung cancer, first with their study in the London oncology wards, and later with their British Doctors Study. In their initial study, they proved that smoking was 25 times more prevalent in lung cancer patients compared to patients in the non-oncology ward. [6] They followed up their study with a prospective questionnaire study amongst British doctors, and demonstrated a much higher lung cancer related death rate in heavy smoking doctors. [7] The link between second-hand smoke and lung cancer was demonstrated in the 1980s, [8, 9] and the harmful effects of third-hand smoke are currently becoming more clear. [10, 11]

Since the poetic allegations of King James I and the thorough epidemiologic research of Doll and Hill, smoking prevention measures have increased in both quantity and quality. Currently, there are multiple evidence-based smoking

cessation interventions for individuals, including nicotine replacement, pharmacological treatment with buproprion and varenicline and behavioral interventions. These measures combined yield a 24% success rate 1 year after smoking cessation attempt, which is much better than the 3-5% when patients try to stop themselves, but still disappointingly low. [12]

Currently, there are many evidence-based population-level public health interventions, including: heavy taxes on tobacco products, [13] advertisement bans, [14] increasing the minimum age for legal access, [15, 16] reducing tobacco retailer density, [17] prohibiting smoking in public [18, 19] and awareness campaigns. [20] The main aim of these interventions is to reduce the number of people – especially children and young adults – who initiate smoking.

Historically, all (plans for) smoking prevention interventions are met with strong opposition from the tobacco industry, by means of misinformation and manipulation. When Doll and Hill first published about the causal relation between lung cancer and tobacco smoking in 1950, [6] their findings – although epidemiologically sound – were disputed by the tobacco industry, who fabricated contradictory studies and flooded the media with enlisted doctors claiming that Doll and Hills' research was controversial and lacked proof. [21] It wasn't until 1954, after Doll and Hill had repeated their study prospectively, on an even larger scale and with the same results, [7] that the link was finally acknowledged. Jeffrey Wigand, former vice-president of research and development at Brown & Williamson and one of the most influential whistleblowers in history, exposed that 'big tobacco' had, in fact, known about the detrimental health effects of smoking for decennia and was actively working towards making smoking even more addictive. He received several death threats and lawsuits.

In the Netherlands and the European Union, there is still evidence, today, that the tobacco industry influences politicians to delay or adjust plans for smoking prevention, [22] and routinely bypasses advertisement bans, for example via the use of social media influencers. [23]

However, it's still possible for decades-long traditions to change. New Zealand was recently internationally commended for announcing a comprehensive package of smoking prevention interventions at once. Their plan is to

completely eradicate smoking, in order to make their current 14-year-olds unable to ever buy tobacco products in their lifetime, and the first of a truly smoke-free generation. However promising, New Zealand is still the only country to make such far-reaching policy changes. Other countries – including the Netherlands – sluggishly struggle politically with the tobacco lobby, the idea of limiting people's 'free choice' to smoke, and losing substantial income from tobacco taxes. Although a complete ban on smoking reduces healthcare costs substantially in the long run, it's a painful financial choice in the short term.

Bradford Hill, confronted with the limited acknowledgement following their first paper in 1950, argumented to Richard Doll that "the researchers' job is to report, not campaign", but this viewpoint has shifted significantly since the 1950s. CanMEDS roles 'Maatschappelijk handelen' and 'Gezondheidsbevorderaar' are now included in the Dutch medical curriculum as important capacities of a modern doctor, with an emphasis on prevention, [24] and doctors have become increasingly active in the media, backing anti-tobacco activists. This is a crucial development in the long run, as the best treatment for cancer patients is obviously to keep them from becoming sick in the first place.

7.4 Conclusion

At the end of this thesis, we are a small step closer to optimizing and personalizing the diagnosis and treatment of NSCLC, by providing a rationale for each of the three key decision-making moments in NSCLC management. For lung cancer pathologists however, the journey toward precision medicine is far from over. The discovery of novel treatments, interventions and biomarkers are following each other up more rapidly than ever before, by which the scientific beast that is our collective academic knowledge has slowly begun to move NSCLC towards the categories of 'preventable', 'curable' and 'manageable'. The lung cancer pathologist thus has a crucial and central role to play in the next decades: navigating new diagnostic challenges, learning to work with novel and unexpected innovations, and working together more intensively than ever before with molecular biologists, clinicians and radiologists. These new circumstances might ask more of lung cancer pathologists than ever before in history, but their effort – although enormous –

could just make it possible to provide a brighter future for the cancer patients of tomorrow.

References

1. de Koning HJ, Van der Aalst CM, De Jong PA, et al., *Reduced Lung-Cancer Mortality with Volume CT Screening in a Randomized Trial.* N Engl J Med, 2020. **382**(6): p. 503-513.
2. Uramoto H and Tanaka F, *Recurrence after surgery in patients with NSCLC.* Transl Lung Cancer Res, 2014. **3**(4): p. 242-249.
3. Bai R, Lv Z, Xu D, et al., *Predictive biomarkers for cancer immunotherapy with immune checkpoint inhibitors.* Biomark Res, 2020. **8**: p. 34.
4. Giraldo NA, Sanchez-Salas R, Peske JD, et al., *The clinical role of the TME in solid cancer.* Br J Cancer, 2019. **120**(1): p. 45-53.
5. King James I of England, *A Counterblaste to Tobacco*. 1604.
6. Doll R and Hill AB, *Smoking and carcinoma of the lung; preliminary report.* Br Med J, 1950. **2**(4682): p. 739-748.
7. Doll R and Hill AB, *The mortality of doctors in relation to their smoking habits; a preliminary report.* Br Med J, 1954. **1**(4877): p. 1451-1455.
8. Hirayama T, *Non-smoking wives of heavy smokers have a higher risk of lung cancer: a study from Japan.* Br Med J (Clin Res Ed), 1981. **282**(6259): p. 183-5.
9. Trichopoulos D, Kalandidi A, Sparros L, et al., *Lung cancer and passive smoking.* Int J Cancer, 1981. **27**(1): p. 1-4.
10. Sleiman M, Gundel LA, Pankow JF, et al., *Formation of carcinogens indoors by surface-mediated reactions of nicotine with nitrous acid, leading to potential thirdhand smoke hazards.* Proc Natl Acad Sci U S A, 2010. **107**(15): p. 6576-81.
11. Jacob P 3rd, Beonwitz NL, Destaillats H, et al., *Thirdhand Smoke: New Evidence, Challenges, and Future Directions.* Chem Res Toxicol, 2017. **30**(1): p. 270-294.
12. Laniado-Laborín R, *Smoking cessation intervention: an evidence-based approach.* Postgrad Med, 2010. **122**(2): p. 74-82.
13. Bader P, Boisclair D and Ferrence R, *Effects of tobacco taxation and pricing on smoking behavior in high risk populations: a knowledge synthesis.* Int J Environ Res Public Health, 2011. **8**(11): p. 4118-4139.
14. Saffer H and Chaloupka F, *The effect of tobacco advertising bans on tobacco consumption.* J Health Econ, 2000. **19**(6): p. 1117-37.
15. Millett C, Lee JT, Gibbons DC, et al., *Increasing the age for the legal purchase of tobacco in England: impacts on socio-economic disparities in youth smoking.* Thorax, 2011. **66**(10): p. 862-5.
16. *Public Health Implications of Raising the Minimum Age of Legal Access to Tobacco Products*, Bonnie RJ, Stratton K and Kwan LY. Editors. 2015, National Academies Press (US), Washington (DC).
17. Lee JGL, Kong AY, Sewell KB, et al., *Associations of tobacco retailer density and proximity with adult tobacco use behaviours and health outcomes: a meta-analysis.* Tob Control, 2021: p. tobaccocontrol-2021-056717.
18. Lemstra M, Neudorf C and Opondo J, *Implications of a public smoking ban.* Can J Public Health, 2008. **99**(1): p. 62-5.
19. Catalano MA and DB Gilleskie, *Impacts of local public smoking bans on smoking behaviors and tobacco smoke exposure.* Health Econ, 2021. **30**(8): p. 1719-1744.

20. Farrelly MC, Davis KC, Duke J, et al., *Sustaining 'truth': changes in youth tobacco attitudes and smoking intentions after 3 years of a national antismoking campaign.* Health Educ Res, 2009. **24**(1): p. 42-8.
21. Gardner MN and Brandt AM, *"The doctors' choice is America's choice": the physician in US cigarette advertisements, 1930-1953.* Am J Public Health, 2006. **96**(2): p. 222-232.
22. *Marlboro-fabrikant is slimmer dan de concurrentie (en de politiek)*, in *Follow The Money*. 2020: https://www.ftm.nl/artikelen/philip-morris-concurrentie-politiek.
23. Kaplan S, *Big Tobacco's Global Reach on Social Media*, in *The New York Times*. 2018.
24. Scheele F, Teunissen P, Van Luijk S, et al., *Introducing competency-based postgraduate medical education in the Netherlands.* Med Teach, 2008. **30**(3): p. 248-53.