The evolving role of liquid biopsy in lung cancer

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Lung cancer is the single biggest cause of cancer-related death in Ireland. The majority of lung cancer cases (80-85%) are Non-Small Cell Lung Cancer (NSCLC). These are divided into adenocarcinoma, squamous cell carcinoma and less common histopathological subtypes. A proportion of these cancers, predominantly adenocarcinoma, have single identifiable mutations in cancer-causing genes (oncogenes) that drive the cancer's growth and progression. These "driver mutations" are outlined in Figure 1 and can be found in approximately two thirds of NSCLC adenocarcinoma. Many of these mutations can now be specifically targeted with inhibitory compounds by a method fashionably called "precision" or "personalized" oncology. These novel treatments for NSCLC with targetable driver mutations have revolutionized patient care over the past decade. For mutations in genes such as EGFR, ALK and ROS1, using targeted treatments in preference to chemotherapy has resulted in patients living longer, and with improved quality of life (QoL). Other mutations, such as KRAS, have proven more challenging to inhibit, but recently the KRAS G12C inhibitor, Sotorasib (Amgen) has been approved in this space by the FDA.

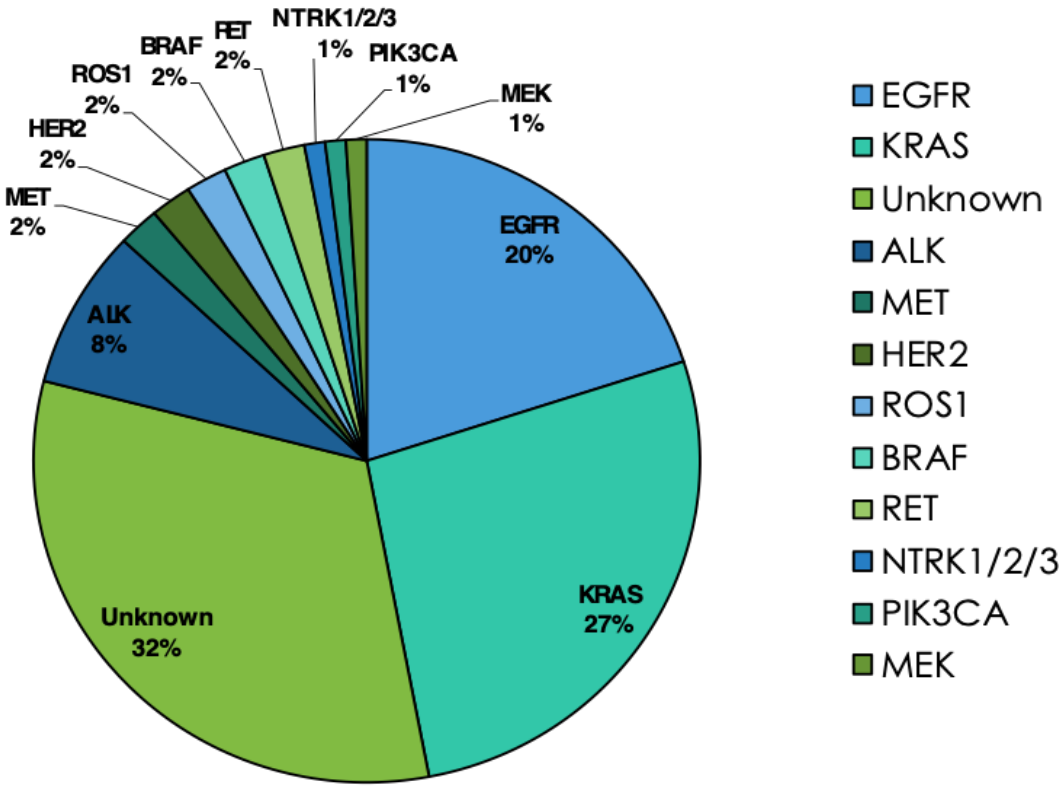
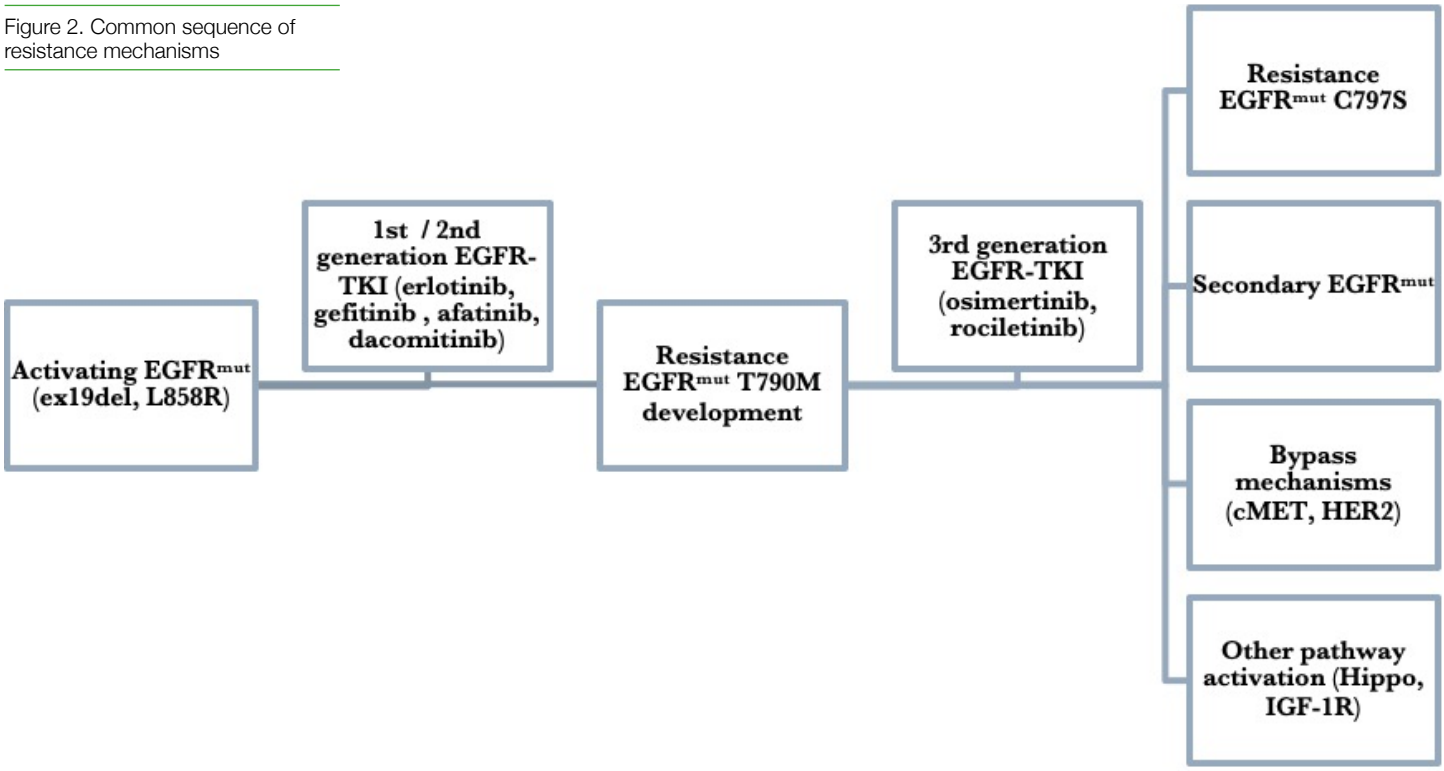


Figure 1: The breakdown of oncogenic mutations in Non-Small cell Lung Cancer (adenocarcinoma)

Although targeted therapies have had success in a variety of subtypes of NSCLC, tumours inevitably become resistant to these agents. Resistance can develop over months to years after treatment initiation. There are a wide variety of different resistance mechanisms. For instance, new secondary mutations of the same original mutation (T790M in EGFR-mutant ($EGFR^{mut}$) NSCLC), development of new different mutations (cMET, HER2) and dedifferentiation into another cancer subtype such as small cell differentiation (Figure 2). These mechanisms can terminate the effectiveness of the original targeted agent and lead to cancer progression. As a result, early

Figure 2. Common sequence of resistance mechanisms



identification of these resistance mechanisms is crucial in planning changes in treatment.

There are currently 10 NCCP-approved targeted agents for driver mutated NSCLC in Ireland (Table 1) and this is expected to expand in the coming years. Five in EGFR^{mut} NSCLC, five in ALK translocated NSCLC and one in ROS1 rearranged NSCLC. The appropriate sequencing of these targeted approaches for the same mutation should maximize patient's overall survival (OS) and QOL, at least from a bioplausibility point of view. Furthermore, detection of the specific resistance mutations that develop can help guide the choice of next compound.

The best treatment option for patients with driver mutations therefore requires a deep and repeated understanding of the molecular environment. This can be difficult to achieve with traditional tissue biopsy of lung cancer. Limitations include the not insignificant risk of bleeding or pneumothorax and the phenomenon of "tumour heterogeneity". This latter term describes the concept that the biopsied tumour itself might not be fully representative of metastatic disease elsewhere and indeed the totality of the microenvironment within the tumour itself. Liquid biopsy is an emerging tool in oncology that has rapidly expanding clinical utility, as its cost reduces and its sensitivity and specificity improves with modern technologies. Liquid biopsies can detect circulating tumour cells (CTC) or circulating tumour DNA (ctDNA) in peripheral blood samples and assess these DNA fragments for mutations. DNA mutations circulating in the blood are more representative of the whole tumour biology and can reveal different mutations from different tumour sites, thus lessening tumour heterogeneity challenges. Liquid biopsy is also less invasive than tumour biopsy techniques and can be done repeatedly throughout the disease course with relative ease. Liquid biopsy therefore offers a convenient, fast, and precise approach of identifying targetable oncogene mutations and resistance mechanisms in addition to and at times instead of the traditional methods in NSCLC.

The most common techniques for ctDNA analysis include amplification refractory mutation system (ARMS), droplet digital polymerase chain reaction (ddPCR) and next generation sequencing (NGS) based methods. Over the past decade, there have been significant improvements in detection techniques as well as a reduction in cost and turnaround time of these methods. ARMS is based upon qualitative PCR (qPCR) that uses specific probes to identify specific mutational variations. The commonly used cobas-ARMS to detect various EGFR^{mut} has 100% specificity, however up to 30% false negative rate in plasma samples. Thus, tumour biopsy still remains superior in EGFR^{mut} detection and liquid biopsy cannot yet replace tumour analysis. ALK fusions and rearrangements, KRAS variants and BRAF mutations are easily and rapidly identified through ARMS and ddPCR gene analysis methods, for example the Idylla™ platform (Biocartis Inc). In addition, actionable mutations sought out in NSCLC (Figure 1) can also be identified through NGS techniques. Whereas ddPCR and ARMS can only focus upon single-cell gene analysis or limited multi-gene panels, NGS can allow the entire tumour genome to be sequenced at once or preselected gene panels ranging from 10 to thousands of variants. Many biotechnology companies now offer NGS of blood ctDNA including FoundationOne Liquid CDx (Foundation Medicine) and Guardant360® Liquid Biopsy Assay (Guardant Health). It is expected that over the coming years many more platforms will come to market for NGS of liquid biopsy samples with reducing expense and faster turnaround. These ctDNA NGS analyses have excellent concordance with tumour biopsy NGS and in addition, can report tumour mutational burden (TMB) and microsatellite instability (MSI). These latter two measurements aim to clinically predict cancers that may be sensitive to immunotherapy agents. TMB is a measure of the number of gene mutations per megabase of DNA, the higher the result, the more genomically unstable the cancer may be. "High-TMB" patients are thought to be more "immunogenic". Thus, more likely to respond to reactivation of the immune system through immune checkpoint inhibitors, such as pembrolizumab (Keytruda®, MSD) and nivolumab (Opdivo®, BMS). MSI, in a similar fashion, reflects abnormalities of tumour DNA repair mechanisms and so again "MSI-high" tumours are more likely to have multiple tumour mutations that may enhance response to immunotherapy agents.

In NSCLC, ctDNA gene mutation(s) levels can change over time and in response to treatments. Liquid biopsy analyses show increasing levels of EGFRmut in response to targeted therapies as tumour cells die, undergo apoptosis and are shed into the bloodstream. This is then followed by reducing

Gene + variant	Approved drugs
EGFR mutation (all)	Erlotinib (Tarceva®, Roche) Gefitinib (Iressa®, AstraZeneca) Dacomitinib (Vizimpro®, Pfizer) Afatinib (Giotrif®, Boehringer Ingelheim)
EGFR T790M only	Osimertinib (Tagrisso®, AstraZeneca)
ALK translocation	Crizotinib (Xalkori®, Pfizer) Alectinib (Alcensa®, Roche) Brigatinib (Alunbrig®, Takeda) Ceritinib (Zykadia®, Novartis) Lorlatinib (Lorbrena®, Pfizer)
ROS1 translocation	Crizotinib (Xalkori®, Pfizer)

Table 1. Irish NCCP reimbursed targeted therapies for NSCLC

levels over the following weeks.[1,2] Tracking changes in ctDNA EGFR mutation levels can determine a patient's disease state and capture dynamic changes during targeted treatment in addition to the early identification of emerging resistance mutations. All this highlights the importance of tracking EGFR mutations throughout the disease course as an adjunct to clinical decision making and treatment planning.[3] A similar story can be seen in other targetable mutations. Crizotinib-resistant ALK rearrangements and point mutations can be detected by ctDNA liquid biopsy methods and lead to changes in patient treatment.[4,5]

Ireland has only recently seen approval of osimertinib (Tagrisso®, AstraZeneca) on 1st July 2020 based on AURA trial results. This is despite Food and Drug Administration (FDA) approval five years prior, in November 2015, and European Medicines Agency (EMA) approval in February 2016 in the same indication for T790M EGFR mutations. The FDA and EMA now approve its use as a first line treatment, but this is not yet accessible for Irish patients, on account of delays with drug reimbursement. With the approval of osimertinib, the need to perform regular liquid biopsy and tumour tissue (where feasible) is increasingly important. However, there is a growing cost to these targeted therapies. Osimertinib is the subject of confidential price negotiations with the HSE, but costs £4,722.30 monthly in the UK. With patients remaining on this agent for a year and beyond, the burgeoning individual cost, given the finite resources available in the HSE, needs to be carefully considered. Utilizing liquid biopsy in everyday practice for identification of biomarkers to predict tumour response and patient toxicity is critical to optimise the care of the individual patient. The cost of liquid biopsy is reducing all the time, and pairing these identified biomarkers with matched targeted treatments could lead to cost savings over time, by filtering out those who will not benefit from expensive anti-cancer compounds and carefully selecting those most likely to gain meaningful tumour responses to these therapies. This is likely to become increasingly important in the years to come as more and more expensive targeted agents are developed and used more frequently at earlier stages of lung cancer disease course, in the adjuvant, and even neoadjuvant, setting.

The analysis of liquid biopsy for NSCLC is currently practiced in an ad hoc and infrequent manner by most centres. In some hospitals, only the T790M mutation in sought for access to osimertinib. However, to obtain a deeper molecular understanding of oncogenic mutations identified in NSCLC and the evolution of these mutations over time, more structured assessment is key. The Thoracic Oncology team in Cork, with Dr Dearbhaile Collins as Principal Investigator, in collaboration with Professor Louise Burke and Dr Michael Bennett, is initiating a structured and regular mutational assessment protocol for NSCLC patients. All mutations and rearrangements in EGFR, ALK, KRAS, ROS1, HER2, BRAF, MET and NTRK identified will be tracked repeatedly over time. This will permit temporal evolution analysis of oncogenic driver mutations and biomarkers of NSCLC patients in serum and tumour tissue and embed this frequent assessment in standard cancer care. It is due to begin enrolling patients from the South and South West hospital group by the end of 2020. This strategy will also allow observations of mutational evolution under targeted treatment pressures, identifying resistance mutations prior to clinical and radiographic evidence of disease progression. We aim to correlate treatment modalities and changes in ctDNA with patient outcomes in real world practice. It will also enable our patients to access expanded access anticancer drug programmes and further clinical trial opportunities that otherwise may not be available.

Liquid biopsy in NSCLC needs to be integrated into patients care as standard. Lung cancers that are driven by the various detectable mutations outlined in Figure 1 evolve and change over time and in response to the treatments they are exposed to. Identifying and tracking these mutations using minimally invasive blood draws rather than challenging tumour biopsies allows a deeper understanding of the cancers evolution as well as minimizes the problem of tumour heterogeneity, both within the tumour itself and also between the different sites of disease. Liquid biopsy is becoming more cost-effective and time-efficient, making it suitable to aid real-time, individual patient treatment decisions. Using regular structured assessments of plasma for oncogene driver mutations, like that commencing in the Cork region, will allow prognostication and determination of the most appropriate treatment strategies for patients.

References on Request