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Comprehensive genomic profiling for lung, breast and unknown primary cancers

Making Education Easy

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Abbreviations used in this review

ALK = anaplastic lymphoma kinase
CUP = carcinoma of unknown primary
EGFR = epidermal growth factor receptor
ER = estrogen receptor
FISH = fluorescence in situ hybridization
IHC = immunohistochemistry
MSI = microsatellite instability
NGS = next-generation sequencing
NSCLC = non-small cell lung cancer
PD-L1 = programmed cell death ligand 1
PFS = progression-free survival
SNP = single nucleotide polymorphisms
TMB = tumour mutational burden

This review is a summary of a presentation on comprehensive genomic profiling for lung, breast and unknown primary cancers given by Professor Jeffrey Ross in Wellington, in November 2018.

Barriers to precision medicine

Barriers to precision medicine in cancer include clinical, technological and access challenges. The cancer genome is complex with hundreds of genes and millions of alterations driving tumour growth, and every patient's tumour genomic profile is unique. In each patient there are in excess of 10,000 genomic alterations, but only 5-10 are biologically relevant and as few as 1-2 are clinically relevant.

Current testing panels have limitations in that most of the 'tumour type' and 'hot spot' panels only look at frequently altered genes and only at commonly altered areas of the gene. Real world samples also carry limitations, such as low tumour purity (frequently seen in metastatic/recurrent/post-treatment samples) and the fact that less invasive sampling results in smaller available specimen size for analysis. Furthermore, lack of access to therapies is an ongoing issue for many patients, with few approved targeted therapies in many tumour types, difficulties in accessing and enrolling in clinical trials, and difficulties in accessing off-label therapies.

'**Precision medicine** is a medical model that proposes the customisation of healthcare, with tailoring of medical decisions, practices, treatments or products to the individual patient.'

Genomic alterations driving tumour growth

Four types of genomic alterations drive tumour growth: base substitutions, insertions and deletions (indels), copy number alterations, and rearrangements. Base substitutions (e.g. the *EGFR* L858R mutation in lung cancer and the *BRAF* V600E mutation in melanoma) are the easiest type of genomic alteration to find, followed by rearrangements. Copy number alterations are more difficult to identify, especially those with lower or borderline amplification. Professor Ross believes that the borderline amplifications with copy numbers of 5 or 6 are less likely to respond to targeted therapy and therefore may not be as important to identify. Copy count is important; the more copies of the gene the higher likelihood that it is an oncogene-addicted cancer. Indels (e.g. the *EGFR* exon 19 deletion in lung cancer) are the most difficult alterations to identify, especially in dilute samples. Professor Ross' group use Foundation Medicine's hybrid capture-based Illumina Hi Seq technology to identify such indels. He believes that the cost and time associated with using this system is well offset by the lower rate of false-negative results.

Foundation Medicine tests

Foundation Medicine's tests for detecting cancer-related genes are summarised in **Table 1.** The assay also identifies TMB and MSI status.

Table 1. Foundation Medicine tests

	FOUNDATIONONE® CDx [1]	FOUNDATIONONE® LIQUID [2]	FOUNDATIONONE® HEME [3]
Target tumour types	All solid tumours	Liquid biopsy (ctDNA) - All solid tumours	Haematologic malignancies, sarcomas*
Specimen†	FFPE tissue	Peripheral whole blood	FFPE tissue, bone marrow aspirate, or peripheral whole blood
Number of genes includes	324 (DNA)	70 (DNA)	406 (DNA) 265 (RNA)
Cancer immunotherapy biomarkers	MSI and TMB	MSI TMB in late 2019	MSI and TMB
Companion diagnostic	FDA-approved CDx for 17 targeted therapies		

* Soft tissue and bone; † For full details, refer to specimen instructions at www.foundationmedicine.com.
ctDNA: circulating tumour DNA; FFPE: formalin-fixed paraffin-embedded tissue; IHC: immunohistochemistry; MSI: microsatellite instability; TMB: tumor mutational burden. 1. Foundation Medicine, Inc. (2018) FoundationOne® CDx Technical Specifications; 2. Foundation Medicine, Inc. (2018) FoundationOne® Liquid Technical Specifications; 3. Foundation Medicine, Inc. (2017) FoundationOne® Heme Technical Specifications and Test Overview.

FoundationOne® Companion Diagnostic (CDx) for solid tumours is a single assay that uses a comprehensive genomic profiling approach utilising hybrid capture-based next-generation sequencing (NGS) to identify a patient's individual molecular alterations and match them with relevant targeted therapies and clinical trials.[1,2] FoundationOne® interrogates the entire coding region of relevant cancer genes and selected introns, and finds genomic alterations missed by current diagnostic approaches.[1-7]

FoundationOne® CDx detects CDx-associated alterations across five common cancers (NSCLC, colorectal cancer, melanoma, breast cancer, ovarian cancer). Approximately 34% of patients exhibit such alterations. Genomic findings and their targeted therapies are outlined in **Table 2**. The test has a sensitivity of 95-99% across alteration types, and a high specificity (positive predictive value >99%).[1] The sample must have an area of ≥25 mm^2, a surface of ≥1 mm^3, and a tumour content of ≥20%. The turnaround time for this diagnostic is 10-12 days.

Table 2. FoundationOne® CDx for solid tumours detects genetic alterations across five common cancers

Indication	Genomic Finding	Therapy
Non-Small Cell Lung Cancer	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	Giotrif® (afatinib), Iressa® (gefitinib) or Tarceva® (erlotinib)
	EGFR exon 20 T790M alterations	Tagrisso® (osimertinib)
	ALK rearrangements	Alecensa® (alectinib), Xalkori® (crizotinib) or Zykadia® (ceritinib*)
	BRAF V600E	Tafinlar® (dabrafenib) + Mekinist® (trametinib)
Melanoma	*BRAF* V600E	Tafinlar® (dabrafenib) or Zelboraf® (vemurafenib)
	BRAF V600E and V600K	Mekinist® (trametinib) or Cotellic® (cobimetinib) + Zelboraf® (vemurafenib)
Colorectal Cancer	*KRAS* wildtype (absence of mutations in codons 12 & 13)	Erbitux® (cetuximab)
	KRAS and *NRAS* wildtype (absence of mutations in exons 2, 3 and 4)	Vectibix® (panitumumab*)
Breast Cancer	*ERBB2* (HER2) amplification	Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumab emtansine) or Perjeta® (pertuzumab)
Ovarian Cancer	*BRCA1/2* alterations	Rubraca® (rucaparib*)

*Not registered in NZ
N.b. Some agents may not be registered for some of the indications in this table.
ALK = anaplastic lymphoma kinase; EGFR = epidermal growth factor receptor

Since 2011, Foundation Medicine has co-authored over 400 publications reporting on assay and biomarker validations, and the clinical validity and utility of comprehensive genomic profiling. The articles include case reports and review articles, and cover almost all tumour types, including some rare types.

Comprehensive genomic profiling in NSCLC

In NSCLC, FoundationOne® CDx can detect all four classes of genomic alterations (see **Table 2**):

- Base substitutions (e.g. *EGFR* L858R, *MET* exon 14 splice site mutations)
- Insertions/deletions (e.g. *EGFR* exon 19 deletions, *EGFR/ERBB2* exon 20 insertions)
- Copy number alterations (e.g. *ERBB2* [HER2] amplification, *RB1* loss)
- Rearrangements/fusions (e.g. *EML4-ALK. AGK-BRAF, FGFR3-TACC3*)

In addition, the test also detects microsatellite instability (MSI) and tumour mutational burden (TMB) and has the option for supplemental PD-L1 IHC. If PD-L1 expression is detected, first-line therapy may consist of nivolumab, pembrolizumab or atezolizumab.

Professor Ross explained that some gene fusions (especially *ALK* alterations such as *EML4-ALK*) in NSCLC are particularly treatable, and that a report showing this alteration indicates a potentially favourable outcome for the patient. He added that alterations in *ALK* and *EGFR* are missed in 17-35% of cases by other genomic testing methods (e.g. FISH).[5] Furthermore, 17% of *EGFR* exon 19 deletions missed by standard testing are detected by comprehensive genomic profiling.[3]

The options for the molecular management of advanced lung adenocarcinoma are also increasing and there is society support for molecular profiling. For example, the US National Comprehensive Cancer Network (NCCN) strongly advise the use of broader molecular profiling with the goal of identifying rare driver mutations for which effective drugs may already be available, and to appropriately counsel patients regarding the availability of clinical trials.[8] The NCCN advocates for broad molecular profiling as a key component for the improvement of care in patients with NSCLC.[8]

Calculating the tumour mutational burden

TMB is used to predict the neoantigen load. Calculating the TMB requires a sensitive, hybrid capture-based Illumina Hi Seq approach with high coverage depth. This test requires a significant amount of DNA to be sequenced for accurate calculation. The TMB is reported as the number of mutations per megabase of DNA sequenced (mut/Mb). Germline variants, SNPs and copy number alterations are excluded.

Both TMB and neoantigen load predict sensitivity to PD-1 blockade with immune checkpoint inhibitors in NSCLC. A study by Rizvi et al. published in 2015 revealed that higher nonsynonymous mutational burden in NSCLC tumours was associated with improved objective response, durable clinical benefit and progression-free survival (PFS).[9] Peters et al., suggest that the accuracy of predicting first-line response to immune checkpoint inhibitors in NSCLC is further improved by combining TMB status with PD-L1 expression level, as demonstrated in the nivolumab-treated arm of the CheckMate 026 trial.[10] In that trial, patients with a high TMB (≥243 mutations) exhibited an improved median PFS and objective response rate with nivolumab compared with chemotherapy (PFS 9.7 vs 5.8 months [HR 0.62; 95% CI 0.38-1.00] and 46.8% vs 28.3%, respectively).[10]

Emerging predictive biomarkers

Emerging predictive biomarkers of immune checkpoint inhibitor response in NSCLC include markers of efficacy (such as *BRAF* mutation and *MET* Exon 14 splice site mutation), markers of resistance (*STK11* [*LKB1*]), and markers of hyper-progression (*MDM2* amplification).

Liquid biopsy

Candidates for liquid biopsy (collection of circulating tumour DNA from a blood sample) assay include patients in whom traditional biopsy is inaccessible or impractical, or insufficient, and patients who have relapsed on targeted therapies and in whom repeat biopsy cannot be performed.

In some cases, a tumour may not be advanced enough to detect targetable driver mutations in the blood. Professor Ross explained that the main role of liquid biopsy so far has been in tracking resistance mutations and such mutations have been identified approximately 70% of the time by this technique when they are known to be present in the tumour tissue.[11,12] He pointed out that a lot of lung cancer liquid biopsies are undertaken following failure of *EGFR* and *ALK* treatment, in order to identify resistance mutations and drive subsequent therapy.

Comprehensive genomic profiling in relapsed/ refractory/metastatic breast cancer

Breast carcinomas are commonly classified into four subtypes based on hormone receptor expression: basal, luminal A, luminal B, and HER2 overexpressed. Using comprehensive genomic profiling it is now possible to identify targetable genomic alterations and redefine breast carcinoma classification into therapeutically relevant subtypes.[13]

Professor Ross and colleagues extracted DNA from formalin-fixed paraffin embedded sections from 8654 consecutive breast cancer cases and undertook comprehensive genomic profiling on hybridisation-captured, adaptor ligation-based libraries for up to 315 cancer-related genes.[13] They discovered that several distinct pathways are altered in breast cancer and that these pathways are treatable by FDA approved agents for oncology indications (**Table 3**). Their analysis revealed that 80.4% of breast cancer tumours harbour a genomic alteration in ≥1 pathway and that 31.2% harbour alterations in just one pathway.

The utility of comprehensive genomic profiling in relapsed *CDH1*-mutated invasive lobular carcinoma has also been demonstrated. Actionable genomic alterations have been reported in 86% of cases and a high incidence of *ERBB2* alterations.[14] These actionable alterations informed treatment decisions for these patients.

Liquid biopsy

In breast cancer, there is a high positive rate of detection of mutations in the blood. Professor Ross explained that in approximately 80% of relapsed breast cancer cases a blood sample could provide an insight into the genomic profile of the cancer. Furthermore, *ESR1* mutations are detected in the blood twice as often as they are detected in a patient's tumour biopsy samples.

Comprehensive genomic profiling in cancer of unknown primary origin

The incidence of cancer presenting as a metastatic carcinoma of unknown primary (CUP) site has varied between 2% and 9% of all cancer diagnoses. Approximately two-thirds of CUP cases are adenocarcinomas with mucin production, tubule formation and immunohistochemistry findings that confirm an adenocarcinoma, but not a specific primary site of origin of the tumour. The remaining one-third of CUP tumours are a mix of non-adenocarcinoma CUP including squamous cell carcinomas, neuroendocrine carcinomas and large cell undifferentiated carcinomas.

Professor Ross pointed out that these patients are usually treated with untargeted cytotoxic chemotherapy and generally have a very poor prognosis. In this group of patients there may be huge potential benefit for cancer genome sequencing and subsequent personalised precision therapy. In 2015 Professor Ross co-authored a paper in which he described the analysis of 200 cases of CUP using comprehensive genomic profiling. From these 200 cases, 841 alterations in 121 genes were identified (401 base substitutions, 217 gene amplifications, 140 short indels, 66 gene homozygous deletions and 17 gene rearrangements).[15] Furthermore, 96% of cases harboured at least one alteration, for a mean of 4.21 alterations per CUP. At least one potentially clinically relevant genomic alteration that could guide decisions for targeted treatment was identified in 169 (85%) CUP cases (90% adenocarcinoma CUP and 75% non-adenocarcinoma CUP). Potential treatment options for the genomic alterations identified by comprehensive genomic profiling in the two forms of CUP are shown in **Table 4.**

Table 4. Clinically relevant genomic alterations in adenocarcinoma CUP and non-adenocarcinoma CUP associated with targeted therapies[15]

Genomic Alteration	ACUP	Non-ACUP	Total CUP	Associated Targeted Therapies
EGFR substitution	6	0	6	Erlotinib, Afatinib, Gefitinib, Osimertinib
ERBB2 amplification	4	2	6	Trastuzumab, Lapatinib, Pertuzimab, Trastuzumab-DM1, Afatinib
BRAF substitution	8	3	11	Vemurafenib, Dabrafenib, Trametinib, Cobimetinib
ALK substitution	0	2	2	Crizotinib, Ceritinib, Alectinib, Lorlatinib
RET fusion/substitution	1	0	1	Cabozantinib
MET amplification	2	3	5	Crizotinib Agents in late stage trials
ERBB2 substitution	8	1	9	Lapatinib, Afatinib, Neratinib
Totals	29	11	40	

ALK = anaplastic lymphoma kinase; CUP = carcinoma of unknown primary; EGFR = epidermal growth factor receptor

CUP sequencing trials are now underway. The CUPISCO trial (ClinicalTrials.gov Identifier: NCT03498521) is designed to study whether the personalised/precision approach to CUP using comprehensive genomic profiling and subsequent targeted therapies will yield significantly improved clinical benefit versus platinum-based chemotherapy. It is hoped that this trial will provide evidence of enhanced disease control and increased overall survival, thereby fulfilling an unmet medical need for patients with this aggressive form of cancer.

Table 3. Potentially targetable pathways in breast cancer identified by comprehensive genomic profiling[13]

8654 Breast Carcinomas	ERBB pathway	Hormone therapy resistant (*ESR1* mutated)	HRD	Immuno-oncology sensitive	PI3K/AKT/ mTOR pathway	FGFR pathway	CDK pathway	Other targetable kinases
Total Cases	1294	796	1266	419	4375	2650	2685	424
% Total Cases	15%	9%	15%	5%	51%	31%	31%	5%
Unique Cases	274	109	309	48	1442	226	231	58
% Unique Cases	3%	1%	4%	<1%	17%	3%	3%	<1%
Relevant Therapies	Trastuzumab Pertuzumab Afatinib Lapatinib Neratinib*	Fulvestrant * Tamoxifen	Olaparib, PARP inhibitors	Pembrolizumab, Nivolumab, Atezolizumab, Ipilumumab	Everolimus, Temsirolimus*	Pazopanib, Ponatinb*	Palbociclib	Sorafenib, Regorafenib*, Dabrafenib, Vemurafenib, Crizotinib, Cabozantinib*, Sunitinib

*Not registered in NZ

CDK = cyclin-dependant kinase; HRD = homologous recombination deficiency; PARP = poly ADP-ribose polymerase

Take-home messages:

- Barriers to precision medicine in cancer include clinical, technological and access challenges
- Four types of genomic alterations drive tumour growth: base substitutions, insertions and deletions (indels), copy number alterations, and rearrangements
- FoundationOne® CDx for solid tumours is a comprehensive genomic profiling approach to identify a patient's individual molecular alterations and match them with relevant targeted therapies and clinical trials
- FoundationOne® CDx also detects MSI and TMB
- Liquid biopsy analysis is useful for tracking resistance mutations
- In approximately 80% of relapsed breast cancer cases a blood sample could provide an insight into the genomic profile of the cancer
- There is huge potential benefit for cancer genome sequencing in CUP and subsequent personalised precision therapy.

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