# Genetic testing in solid tumours

Eric Lee, Oct 2019

# Cancer

- Genetic disease
- Driver mutations
- Passenger mutations

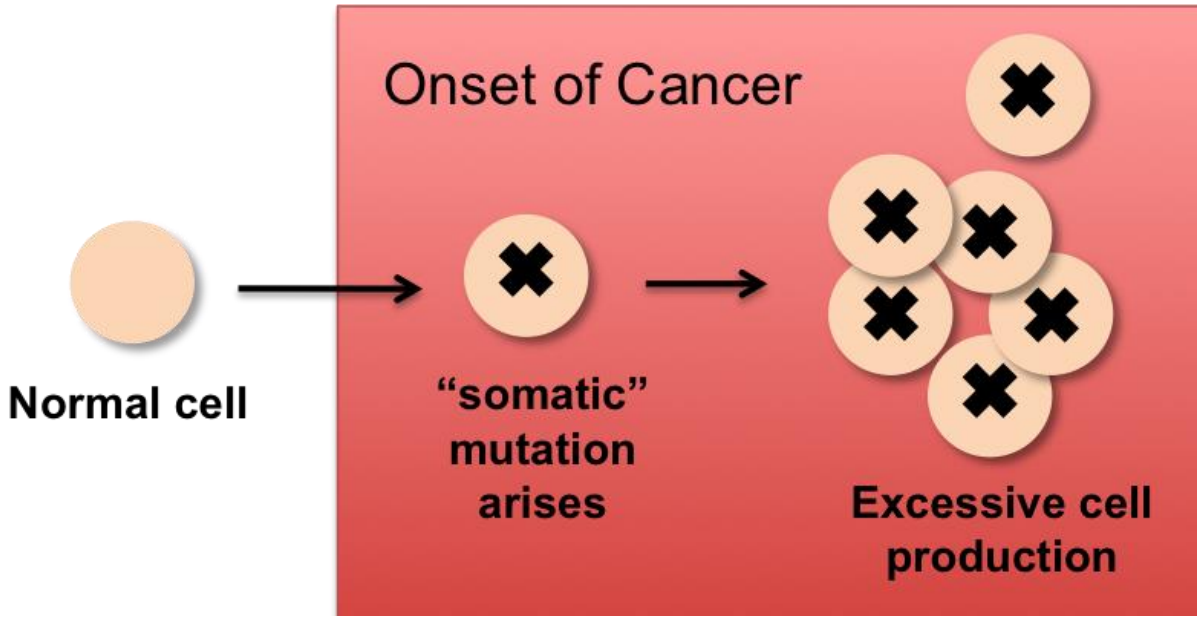



Image source: https://blog.23andme.com/health-traits/the-molecular-barcode-of-cancer-targeting-treatment-to-patient/

# Why test tumours for genetic mutations?

- Diagnosis/classification
- Prognosis
- Therapy
- Screen for germline mutations

Findings often have multiple implications

Examples for diagnosis/classification

- IDH1/2, 1p/19q co-deletion, MGMT hypermethylation in diffuse gliomas
- BCR-ABL1 translocation in CML
- JAK2 V617F mutation in MPN
- GNAS mutation in differentiating between fibrous dysplasia and osteosarcoma

Overlapping clinical/laboratory features

## Examples for prognosis

- IDH1/2, 1p/19q co-deletion, MGMT hypermethylation in diffuse gliomas
- POLE mutations in ultramutated endometrial cancer
- 13q-, T12, 17p-, 11q- in CLL

May influence therapeutic decisions (drug, dose, duration)

# Examples for therapy

- KRAS/NRAS mutations and anti-EGFR monoclonal antibodies in metastatic colorectal cancer
- BRAF V600 mutations and BRAF inhibitors in melanoma
- EGFR mutations and EGFR tyrosine kinase inhibitors (TKI) in non-small cell lung cancer
- ALK or ROS1 rearrangements and tyrosine kinase inhibitors in NSCLC
- EGFR T790M mutation and second-line EGFR TKI in NSCLC

NSCLC

- **73337**: EGFR gene status
- **73341**: FISH testing for ALK, if ALK IHC+ and EGFR-
- **73344**: FISH testing for ROS1, if ROS1 IHC+ and ALK IHC- and EGFR-
- **73351**: EGFR T790M mutation status

# NSCLC

| Target | Implications | PBS funding |
|---|---|---|
| KRAS activating mutation | Lack of response to EGFR TKI | N/A |
| BRAF V600 | May respond to BRAF inhibitors | No |
| HER2/ERBB2 exon 20 insertion | May respond to anti-HER2 therapy | No |
| MET exon 14 skipping | May respond to crizotinib | No |
| RET fusion | May respond to RET inhibitors | No |

Melanoma

- **73336**: BRAF V600 mutation status in unresectable stage III or stage IV metastatic cutaneous melanoma

Colorectal

- **73338**: KRAS/NRAS exons 2, 3, 4 in metastatic colorectal cancer

# Melanoma

| Target | Implications | PBS funding |
|---|---|---|
| BRAF non-V600 | Lack of response to BRAF inhibitors | N/A |
| NRAS activating mutation | Other mutations unlikely | N/A |
| KIT activating mutation | May respond to KIT inhibitors | No |

# Examples for germline implications

- MLH1 promoter hypermethylation and BRAF V600E in context of MLH1/PMS2 loss on immunohistochemistry in colorectal cancer
- BRCA1/2 mutations in ovarian cancer
- Many other genes where somatic mutations could also be germline: TP53, PTEN, SDHB/C/D

# Germline vs. somatic mutations: similarities

- Broad range of mutations
  - Small/large, balanced/unbalanced, genetic/epigenetic
- Can be gain- or loss-of-function
- Testing methods
  - Massively parallel sequencing (NGS), Sanger sequencing, real time PCR, droplet digital PCR, pyrosequencing, fragment analysis, FISH, methylation-specific PCR

# Germline vs. somatic mutations: differences

| | Germline | Somatic |
|---|---|---|
| Mutant allele frequency | 0, 50, 100% | Can be low level (surrounding tissue, heterogenity) |
| Test specimen | Whole blood | Commonly FFPE |
| Pre-test checks | - | Confirm presence of tumour |
| Change over time | Does not change | Does change |

# Pre-analytical considerations

- What is the clinical indication?
- Primary or metastatic?
- Is there enough tissue?
- Does the tissue contain tumour?
- Is there enough tumour?
- Enrich by micro- or macrodissection?

# Analytical considerations

- Testing platform?
- Required turnaround time?
- Limit of detection?
- Quality metrics?
- Real or artefact?
- Germline or somatic?

# Analytical considerations

Limit of detection affected by tumour %

Monoallelic change in half of tumour cells in 50% samples = detected at 25%

Monoallelic change in half of tumour cells in 10% sample = detected at 5%

# Analytical considerations – low level mutations

- Deamination?
- PCR artefact?
- Recurrent?
- Tumour type?
- Clinical context and utility?
- Orthogonal confirmation?

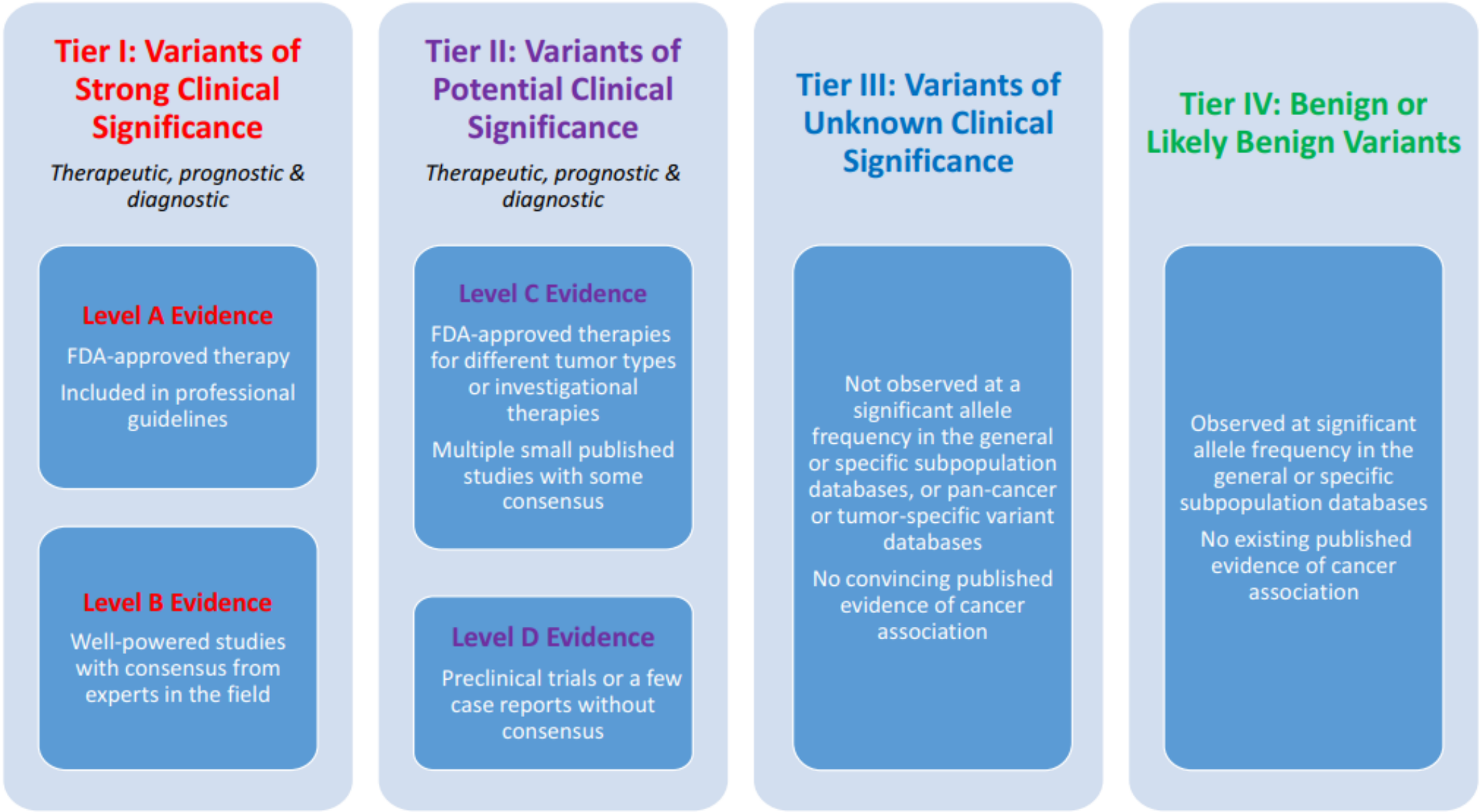
# Post-analytical considerations

- Variant classification
- Reporting requirements

# Post-analytical considerations

## Variant classification

- Recurrent or novel
- FDA/TGA approval
- Professional guidelines (e.g. WHO, NCCN)
- Databases
- Literature (tumour type, therapy, response)
- Predicted functional consequence




Joint consensus recommendations of the AMP, ASCO, and CAP

# Post-analytical considerations

## Reporting requirements (NPAAC MPS)

- Therapeutic significance must be stated
- Tumour %
- Variant allele %
- Germline incidental finding policy

Tumour-specific testing

- NSCLC
- Cutaneous melanoma
- Colorectal cancer

Tumour-agnostic testing

- Tumour mutational burden (TMB)
- Microsatellite instability (MSI)
- NTRK fusions
- Genes involved in homologous recombination

# Tumour mutational burden (TMB)

- High rate of somatic mutation in some tumours
- Higher load of neoantigens and more likely to be recognised by immune system
- Such tumours may be more likely to respond to immune checkpoint inhibitors
- Rate of protein-changing single nucleotide variants per million base pairs
- Ongoing area of research: threshold, panel size, relationship to PD-L1/MSI-H/TCR

# Microsatellite instability (MSI)

- High frequency of microsatellite mutations due to inactivation of MMR system
- Due to MLH1 hypermethylation or germline mutation in MMR gene
- MSI-H has been found in up to 24 types of cancers, or 4% of all adult cancers
- Many MSI-H tumours sensitive to immune checkpoint inhibitors
- Pembrolizumab approved by FDA for treatment of patients with unresectable/metastatic MSI-H or dMMR solid tumours

# Genes involved homologous recombination

- Homologous recombination is crucial for DNA double-strand break repair
- PARP is also involved in DNA strand break repair
- Tumours with mutations in HR genes (such as BRCA1/2) are more sensitive to PARP inhibitors
- Various PARP inhibitor therapies approved by FDA for germline BRCA1/2-mutated breast, ovarian, fallopian tube, primary peritoneal, and some pancreatic cancers

# NTRK fusions

- Oncogenic fusions involving NTRK1/2/3 tyrosine kinase genes
- Common in rare cancer types, rare in common cancer types
- Larotrectinib (TRK inhibitor) approved by FDA for treatment of metastatic or unresectable solid tumors that have an NTRK fusion without a known acquired resistance mutation
- Testing available by IHC, FISH, or NGS

Circulating tumour DNA (ctDNA)

'Liquid biopsy'

Release of tumour DNA into bloodstream

Potential advantages

- Non-invasive, less procedural risk
- Testing option in medically unfit patients or tumours that cannot be resected/biopsied
- Could use for serial testing for early detection of cancer/recurrence
- Potentially better represents all tumour DNA

# Limitations

- Follow-up tumour tissue testing may be required if no mutations detected on ctDNA
- May detect alterations unrelated to lesion of interest (e.g. CHIP)
- May require confirmation on tumour tissue to access PBS-funded therapy
- Little evidence of clinical validity and clinical utility for cancer screening, early stage cancer, or monitoring therapy

# Summary

- Many pre-analytical, analytical, post-analytical considerations unique to solid tumour testing
- Current clinical testing dominated by testing of tumour-specific markers on FFPE
- Emerging tumour-agnostic markers and circulating tumour DNA tests