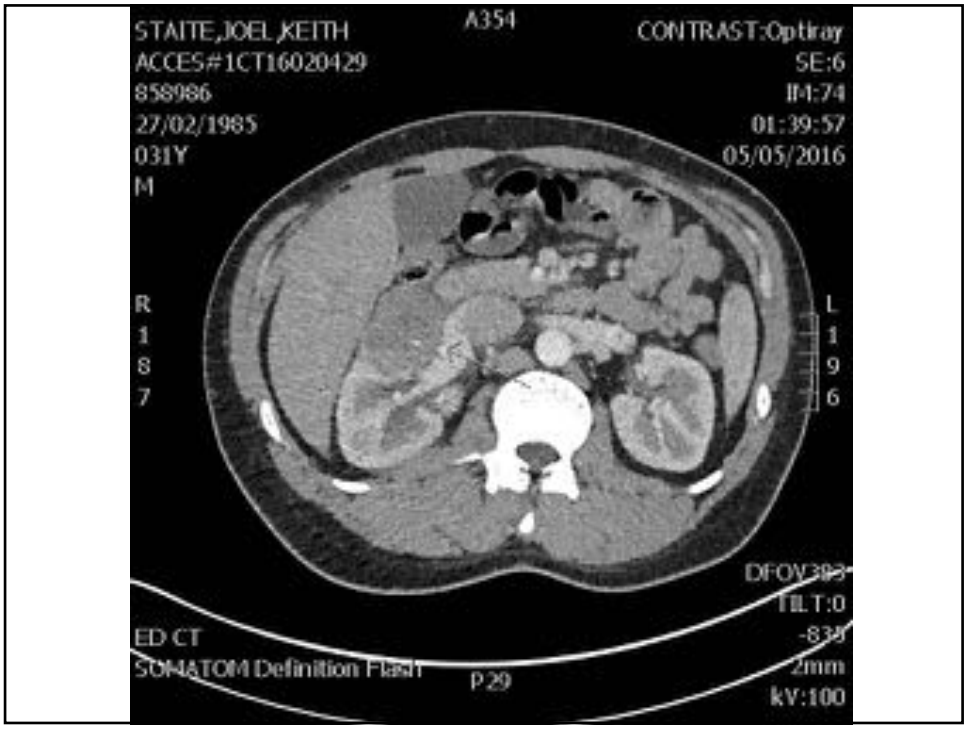
Queensland Molecular Tumour Board

8th May 2019
Room 2004, TRI, Princess Alexandra Hospital,
Woolloongabba, QLD

JS UR858986

- Summary: 34 year old male with metastatic collecting duct carcinoma
  - June 2016 – Laparoscopic radical nephrectomy
    - T3aN0R0
  - April 2017 – CT abdomen and pelvis
    - Two new right renal bed soft tissue nodules, interval regional lymph node enlargement and pelvic free fluid suspicious for disease recurrence with peritoneal metastasis
  - June 2017 – Open retroperitoneal lymph node dissection
    - 2 lymph node metastatic deposits removed
  - Feb 2018 – Admission
    - Peritoneal metastatic disease, weight loss and malignant ascites
  - March 2018 – Clinical Trial
    - Enrolled to UNISON trial
    - Current treatment Ipilumab and Nivolumab
  - May 2019
    - Complete remission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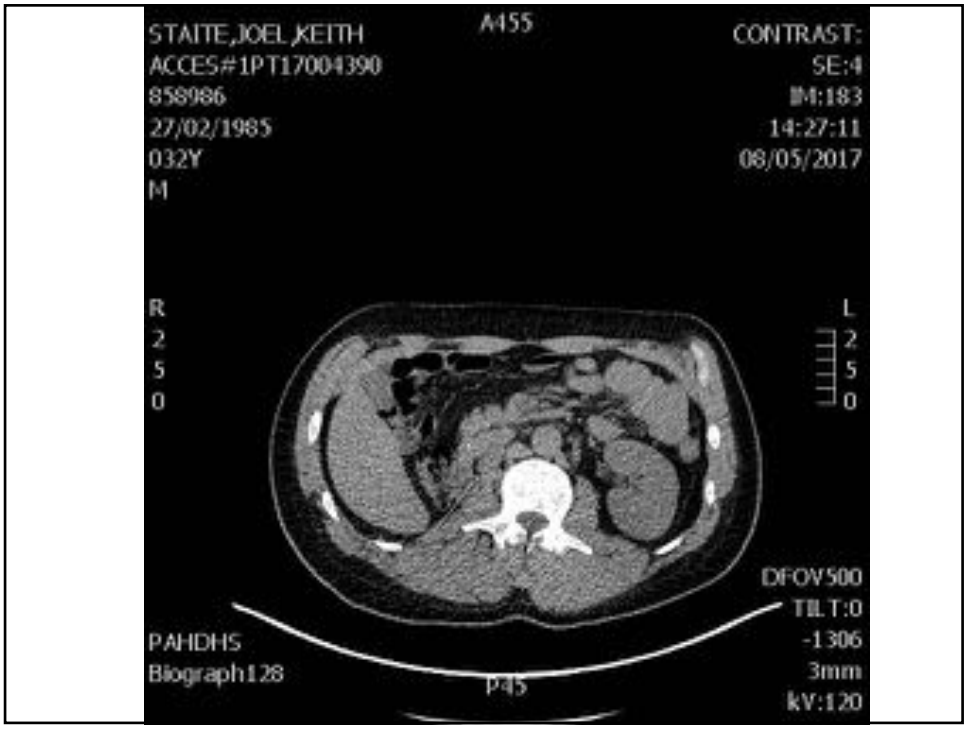
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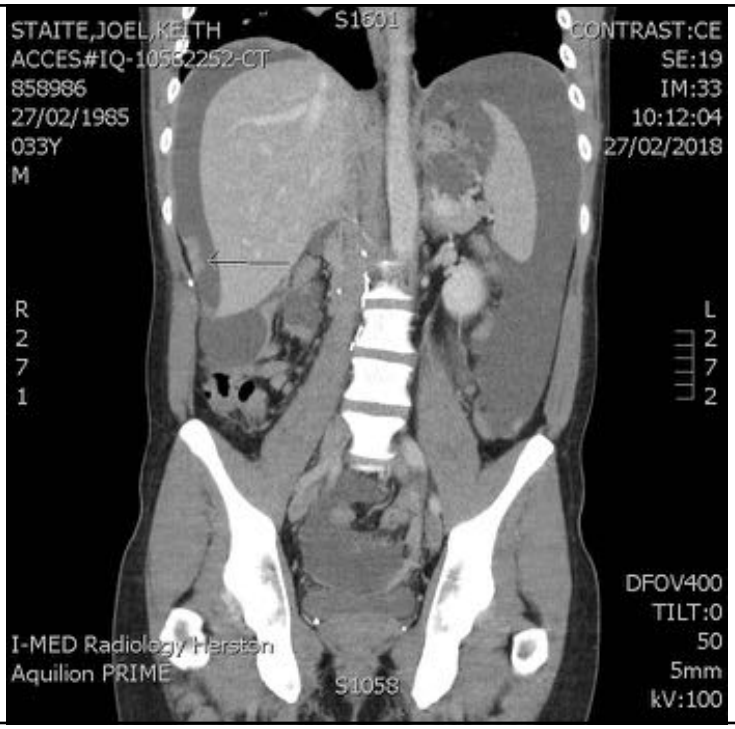
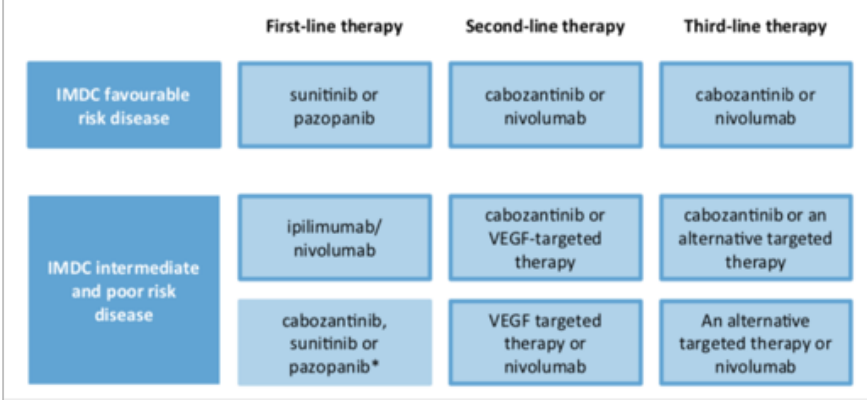


Renal cell carcinoma treatments

Clear Cell RCC – 70%

Figure 7.1: Updated European Association of Urology Guidelines recommendations for the treatment of first-line clear-cell metastatic renal cancer.



Non-Clear Cell RCC – 30%

- Outcome of these patients with targeted therapy is poorer than for ccRCC
- Targetted therapies
  - Temsirolimus
  - Everolimus
  - Sorafenib
  - Sunitinib

Trial	Treatment	Randomized?	Number Enrolled	Histology Type	Overall Response Rate	Progression-Free Survival	Overall Survival
ESPN	Sunitinib vs. everolimus	Yes	68 patients	All non-clear cell	9% vs. 3%	6.1 vs. 4.1 months	16.2 vs. 14.9 months
ASPEN	Sunitinib vs. everolimus	Yes	108 patients	All non-clear cell	18% vs. 9%	8.3 vs. 5.6 months	31.5 vs. 13.2 months
RECORD-3	Sunitinib vs. everolimus	Yes	66 patients	All non-clear cell	N/A	7.2 vs 5.1 months	N/A
SUPAP	Sunitinib	No	61 patients	Papillary	13% (type I) and 11% (type II)	6.6 months (type I) and 5.5 months (type II)	17.8 months (type I) and 12.4 months (type II)

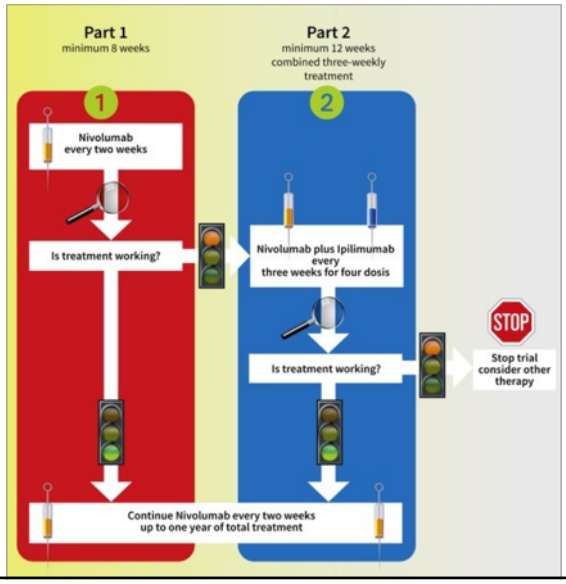
Systemic treatment options

- **For collecting duct carcinoma, due to its rarity and aggressiveness, there are no standard treatments
- Immune checkpoint inhibitors

	IMDC intermediate and poor risk			ITT population (secondary endpoint)		
	IPI/NIVO	sunitinib	HR	IPI/NIVO	sunitinib	HR
n	425	422		550	546	
RR 95% CI	42 (37-47)	27 (22-31)		39 35-43	32 28-36	
PFS 99.1 CI	11.6 (8.5-15.5)	8.4 (7.0-10.8)	0.82 (0.64-1.05)	12.4 (9.9-16.5)	12.3 (9.8-15.2)	0.98 (0.79-1.23")
OS 99.8 CI	NR (28.2-NR)	26.0 (22-NR)	0.63 (0.44-0.82	NE (NE-NE)	32.9 (NE-NE)	0.68 (0.49-0.95)

CI = confidence interval; HR = hazard ratio; IPI = ipilimumab; IMDC = International Metastatic Renal Cell Carcinoma Database Consortium; ITT = intention to treat; n = number of patients; NE = neutral effect; NIVO = nivolumab; NR = not reported; OS = overall survival; PFS = progression-free survival; RR = relative risk.

UNISON trial



Summary of Molecular Findings

Tumour Burden:	6.9 Mutations/Mbp
Tumour Purity Estimate:	[illegible]-61%

The samples fail QC for the following reasons:

- Percentage of reportable bases with < 60X coverage is greater than 10.0% (is 12.9%)

Somatic Mutations Summary

There were 2 reportable variants found in this sample.

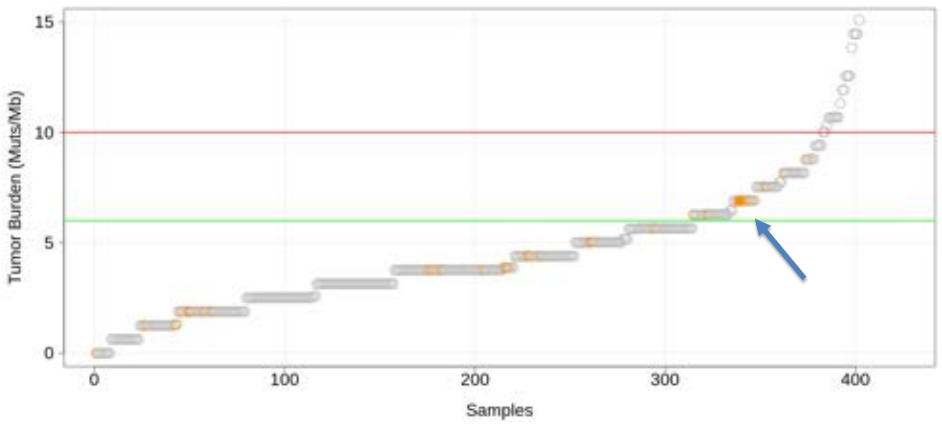
Gene	Mutation	Consequence	Variant Allele Frequency	
TSC1	NP_000359.1:p.Ser1043del	inframe_deletion	4.5%	B
PTEN	NP_001291646.2:p.Cys65_Ala66delinsSer	inframe_deletion	4.6%	C

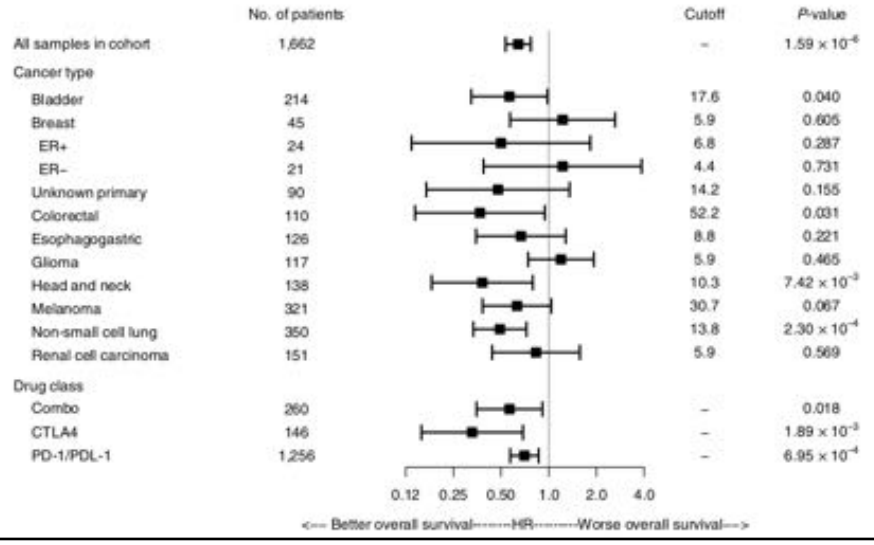
Somatic CNV Summary

There were 2 reportable CNVs found in this sample.

Gene	CNV Type	Copy Number	Start	Stop	Length	Whole/Partial	
SMARCB1	LOSS	0	24,105,492	24,717,489	611,998	Whole	D
NF2	LOSS	0	29,693,815	30,185,173	491,359	Whole	D

Tumour Molecular Burden





Other cases reported

Mizutani et. al to nivolumab in metastatic collecting duct carcinoma expressing PD-L1: A case report Mol. And Clin. Oncology 2017

67 yr male

- temsirolimus for recurrence of the lung and lymph node metastases for 30m
- Nivolumab complete response of the lung metastasis, stabilized the lymph node
- PBRM1 mutation (Miao et. al. Science 2018 ccRCC biomarker p=0.012)

Yasuoka et al. Nivolumab therapy for metastatic collecting duct carcinoma after nephrectomy: A case Medicine 2018

73 yr male

- Gemcitabine progressed liver, adrenal mets
- Nivolumab 2 courses partial response
- 5 courses with no progression

No genomic profiling but PD-L1 response reported

Variant curation

CHIYAN LAU 8 MAY 2019
PATHOLOGY QUEENSLAND

Genomic Variants

- DNA sequence deviation from a "reference sequence"
- GRCh37/hg19
- GRCh38/hg38
- NG_xxxxxx
- NM_xxxxxx
- ENSGxxxxxx
- ENSTxxxxxx

Types of variants

- Single nucleotide variants (SNVs)
- Insertions/deletions (indels)
- Copy number changes/variants (CNCs/CNVs) – larger deletions/duplications (e.g. whole exon, multiexonic, multigene); amplifications
- Structural variants (SVs) – translocations, inversions, fusions

Variant effect

- Some have no effect on protein sequence/structure (e.g. deep intronic variants, synonymous coding variants)
- Others result in amino acid substitutions (missense), or protein truncation or loss (nonsense, frameshift)
- Other effects: in-frame deletion/insertion of sequences, aberrant splicing, etc.

Variant – pathogenicity

- Not all deviations will cause disease
- Population studies/databases e.g. ExAC/gnomAD, EVS, DGV – large number of germline variants in general population which are tolerated
- Missense mutations may or may not affect protein function depending on biochemical difference between amino acids, location in functional domain/catalytic site, or effect on protein folding/stability, phosphorylation sites, etc.
- In the past, no standardization – some rely heavily on conservation, some on in silico, etc. Highly variable classification between labs.

Best practice guidelines

- Richards et al. 2015 – ACMG/AMP (germline/constitutional)
- Codifies:
  - Type of evidence – support pathogenic/benign
  - Weighting
  - Amount of evidence to support Classification
  - Caveats

Germline variant curation

- Pathogenic: PVS, PS, PM, PP
- Benign: BA, BS, BP
- 5 classes of variants: Pathogenic (C5), Likely pathogenic (C4), Variant of uncertain significance (C3), Likely benign (C2), Benign (C1)
- Examples:
- PVS1 - Nonsense/frameshift in gene where LOF is disease mechanism
- PP3 – multiple in silico algorithms consistently predict damaging
- Segregation (or lack of) with disease in pedigrees – depends on number of informative individuals
- PM2 – absent in population databases
- BA1/BS1 – frequency in population too high for disease
- Other evidence types: de novo (parents tested), functional studies/functional domain, co-inheritance with known pathogenic variant, specificity for patient phenotype

Rules for combining evidence

- (1 PVS + 1 PS) OR (1 PVS + 2 PM) OR (2 PS) etc = pathogenic
- (1 PS + 1 PM) OR (1 PS + 2 PP) OR (3 PM) etc = likely pathogenic
- (1 BA) OR (2 BS) = benign
- (1 BS + 1 BP) OR (2 BP) = likely benign
- Conflicting, or insufficient = VUS

- Now quite widely adopted internationally in clinical diagnostic setting for germline Mendelian (rare) disorders

Somatic (cancer) variant curation

- Questions:
- Is this gene important in this cancer type?
- Is this variant likely to disrupt the normal function of this gene?
- Is the direction of disruption consistent with pathogenesis (e.g. tumour suppressor vs oncogene)?
- Is there known clinical utility?

- Richards et al. ACMG germline guidelines not really designed for somatic, and many criteria do NOT work in somatic setting
- In somatic setting, focus is less on "disease causation", and more on impact on clinical care

AMP/ASCO/CAP (Li et al. 2017)

- Designed for somatic setting
- Effort for standardization of curation but less widely adopted than germline guidelines
- Therapeutic, prognostic, diagnostic significance
- Gives weighting for quality of evidence (Levels A to D)
- 4 Tier classification of variants
- Overlaps but differs from classification systems used by various somatic variant databases (which all differ from each other)

Criteria for evidence

- Level A – approved therapy, or professional guidelines, for the same specific tumour type
- Level B – well powered studies, with consensus from experts, for same tumour type
- Level C – approved therapy or professional guidelines, for a DIFFERENT tumour type; multiple small studies
- Level D – preclinical studies, case reports, small studies. Plausible significance

- In silico prediction – for reference only
- Population database frequencies
- Signaling pathways

Finding the evidence

- Multiple information sources:
- Literature – pubmed, google scholar
- Somatic variant databases: COSMIC, CiVIC, MyCancerGenome, OncoKB, cBioPortal, etc.
- NCCN, ELN, EviQ guidelines
- TGA, FDA, EMA
- In silico predictors: SIFT, PolyPhen, Provean, CADD, etc.
- Protein domain structure, missense constraint – Decipher, Uniprot
- Population databases - gnomAD

Classification of somatic variants

- Tier 1 – Strong clinical significance (Level A or B)
- Tier 2 – Potential clinical significance (Level C or D)
- Tier 3 – unknown clinical significance
- Tier 4 – benign or likely benign

- Takes into account availability of approved targeted therapy
- Classification likely to change with time
- Often fairly subjective

Pathology Queensland process

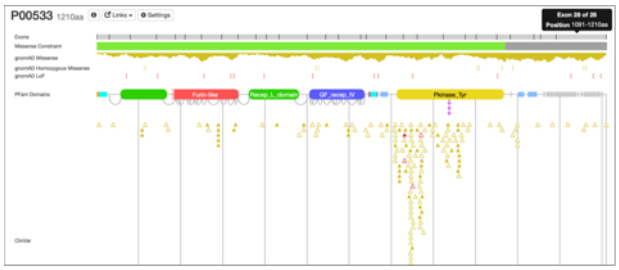
- Take into account some elements of ACMG germline criteria which can be applied (for reference)
- Search of literature and multiple somatic variant databases for previous reports, management guidelines
- Discussion for ambiguous cases – molecular genetic scientist, haematologists, anatomical pathologists, genetic pathologist
- Clinical reporting

An example

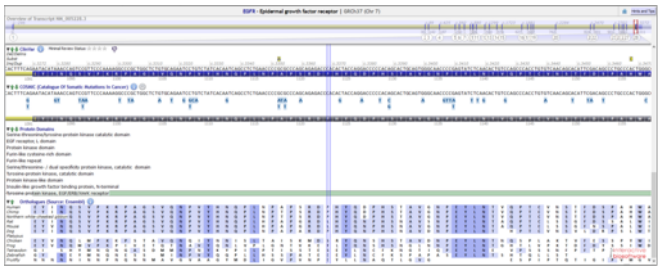
- 79 yo male
- R intermediate bronchus tumour – squamous cell Ca
- WES lung panel (14 genes) – EGFR:c.3368C>T p.(Pro1123Leu)
- Exon 28 of 28
- ? Significance
- ? Classification

EGFR

- Oncogene
- Cell signaling pathways – cell proliferation, differentiation,
- Activating variants in tyrosine kinase domain in NSCLC sensitive to TKI – Tier 1
- Is this missense change similar?



Exon 28 – not in functional domain



Proline 1123 not well conserved – leucine in rat and mouse

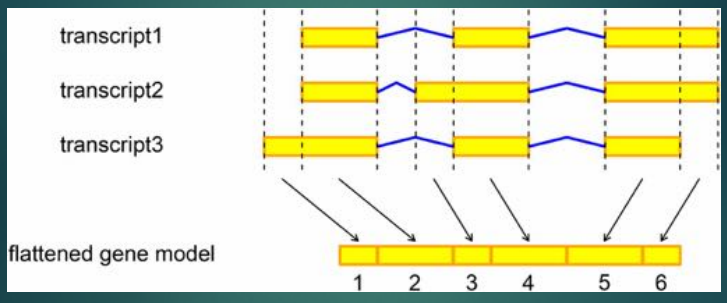
Other info

- Not reported in COSMIC or Clinvar or Civic or other databases
- In silico algorithms predict tolerated
- In gnomAD but low allele frequency in population (0.001%)

- Tier 3 – unknown significance

Some issues with NGS variant curation

Genes with multiple transcripts/isoforms



Xu Zhang, and Wei Zhang Genetics 2016;203:985-995

Variant – nomenclature

- Precisely describe what and where the change (deviation) is
- HGVS
- Often overlooked, but fundamentally important for interpretation, knowledge sharing
- Genomic – chr2:g.1234567G>A
- cDNA (transcript) – NM_002234:c.454C>T or NM_1002345:c.234C>T
- Protein – NP_203456:p.(Leu152Arg) or NP_034567:p.(Leu78Arg)

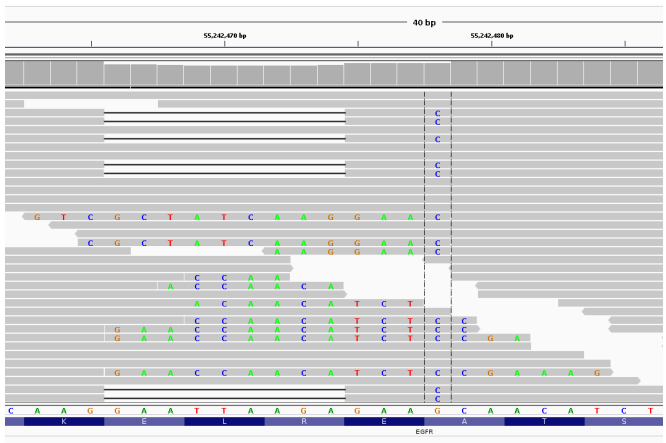
Variant calling with NGS

- Bioinformatics – often problems with indels:
- pipelines usually left-align for conformance with VCF specifications, but molecular genetics community uses HGVS standard which is right-aligned
- Sometimes calls a single change as two separate variants
- Requires local realignment and/or manual visualisation of BAM files

An example

- 55 yo female
- Lung adenocarcinoma
- WES analysed for 14 gene lung panel
- 2 EGFR variants detected by bioinformatics pipeline:
- 1. NM_005228.3:c.2239_2247del NP_005219.2:p.Leu747_Glu749del
- 2. NM_005228.3:c.2248G>C NP_005219.2:p.Ala750Pro

- In-frame deletion + missense variant, both in exon 19



- The two variants are phased together (in cis)
- Deletion left-aligned by pipeline
- Taken together, and adjusting to right alignment manually:
- c.2236_2248delinsC
- p.Leu746_Ala750delinsPro

- Well-described activating exon 19 deletion, with increased sensitivity to TKIs (Tier 1)

Key take-home messages

- Variant curation is not completely standardized even in germline setting - Somatic curation is even less standardized
- AMP/ASCO/CAP guidelines
- Just because a variant is in a gene associated with a particular disease/cancer type does not mean it is a clinically meaningful pathogenic variant
- No single data source/database provides all information
- Transcript/protein isoform reference sequences used are important