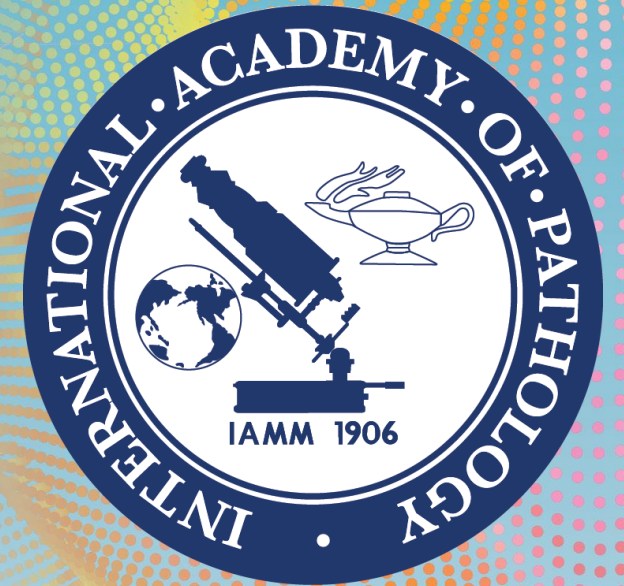


Whole Genome Sequencing as a Diagnostic Aid for Difficult Cases

Owen Prall

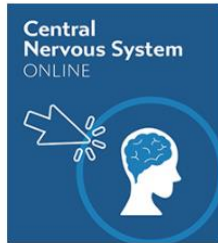
Peter MacCallum Cancer Centre, VIC (data in talk)
John Hunter Hospital, NSW (current)



Tumour diagnosis



Breast tumours (5th ed)



Central Nervous System Tumours update 2016



Digestive system tumours (5th ed)



Endocrine tumours (4th ed)



Eye tumours (4th ed)



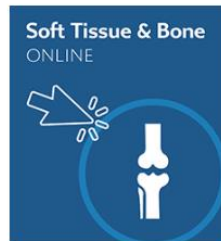
Female Genital Tumours (5th ed)



Head and neck tumours (4th ed)



Skin tumours (4th ed)



Soft Tissue and Bone Tumours (5th ed)



Thoracic tumours (Beta)



Tumours of Haematopoietic and Lymphoid Tissues 2017 (Beta)

Pattern recognition/specific features

Clinical, H&E, IHC, FISH, NGS

Difficulties:

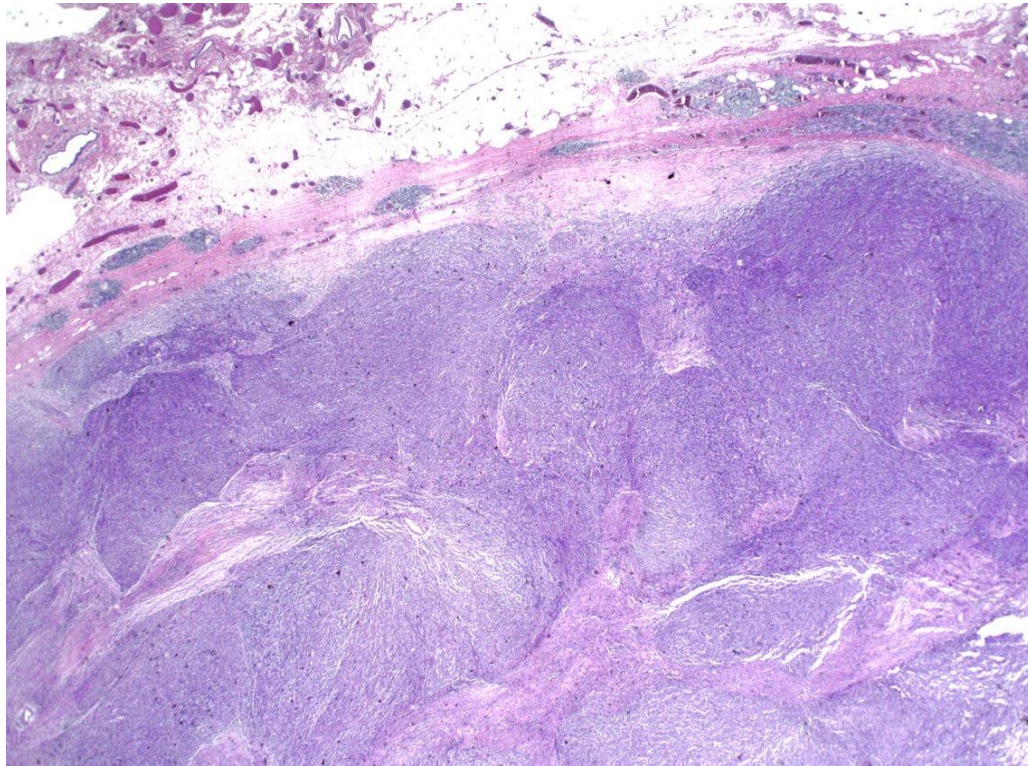
-undifferentiated/dedifferentiated

-rare

-no assay

31F, breast mass ?inflammatory leiomyosarcoma

Stephen Fox



Inflammatory leiomyosarcoma is a distinct tumor characterized by **near-haploidization**, few somatic mutations, and a primitive myogenic gene expression signature

Elsa Arbajian¹, Jan Köster², Fredrik Vult von Steyern³ and Fredrik Mertens¹

¹Division of Clinical Genetics, Department of Laboratory Medicine, Lund University, Lund, Sweden;

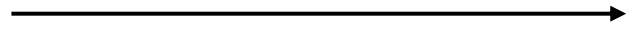
²Department of Pathology, Skåne University Hospital, Lund, Sweden and ³Department of Orthopedics and Clinical Sciences, Lund University and Skåne University Hospital, Lund, Sweden

Inflammatory leiomyosarcoma is a soft-tissue tumor resembling conventional leiomyosarcoma, but with a prominent intrinsic inflammatory component. Previous studies have suggested that inflammatory leiomyosarcoma differs genetically from leiomyosarcoma, but in-depth analyses are lacking. Here we provide a comprehensive picture of the genome and transcriptome of inflammatory leiomyosarcoma by combining cytogenetic, single-nucleotide polymorphism array, mRNA-sequencing, and whole-exome sequencing data. The results show that inflammatory leiomyosarcoma has a specific genetic profile characterized by near-haploidization with or without subsequent whole-genome doubling. **Consistently, both parental copies of chromosomes 5 and 22 are preserved.** Apart from **recurrent mutation of the *NF1* gene**, additional somatic events that could serve as driver mutations were not found at either the nucleotide or the genome level. Furthermore, no fusion transcripts were identified. Global gene expression profiling revealed particularly prominent differential expression of genes, including *ITGA7*, *MYF5*, *MYF6*, *MYOD1*, *MYOG*, and *PAX7*, involved in muscle development and function, providing strong argument for grouping inflammatory leiomyosarcoma with myogenic sarcomas, rather than with myofibroblastic lesions. Combined with previously published data, there are now 10 cases of inflammatory leiomyosarcoma with confirmed near-haploid genotype. These patients differ from leiomyosarcoma patients in being younger (median 41 years), showing a male predominance (9:1), and few relapses (1 of 8 informative patients). Thus, the clinical, morphological, and genetic data provide compelling support for inflammatory leiomyosarcoma being a distinct subtype of myogenic tumors.

Modern Pathology (2018) 31, 93–100; doi:10.1038/modpathol.2017.113; published online 8 September 2017

	Large panel (e.g. TSO500)	WGTS
tissue	FFPE, 40-100 ng, 20% purity	fresh, 250 ng, 30% purity
access	increasing	limited
cost, expertise	moderate	high
TAT	3-4 weeks	4-6 weeks
coverage	>1000x	100x
germline	no	yes
SNV, indels, CNV, RNA	restricted/targeted	comprehensive
TMB	yes	yes (more accurate)
MSI	yes	yes
signatures	limited (UV)	comprehensive (UV, smoking, HRD, MSI)
other	potentially higher sensitivity	purity/ploidy, complex rearrangements, LOH, RNA expression, DNA/RNA correlation, algorithms (HRDetect, CHORD, CUPPA)

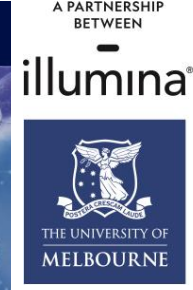
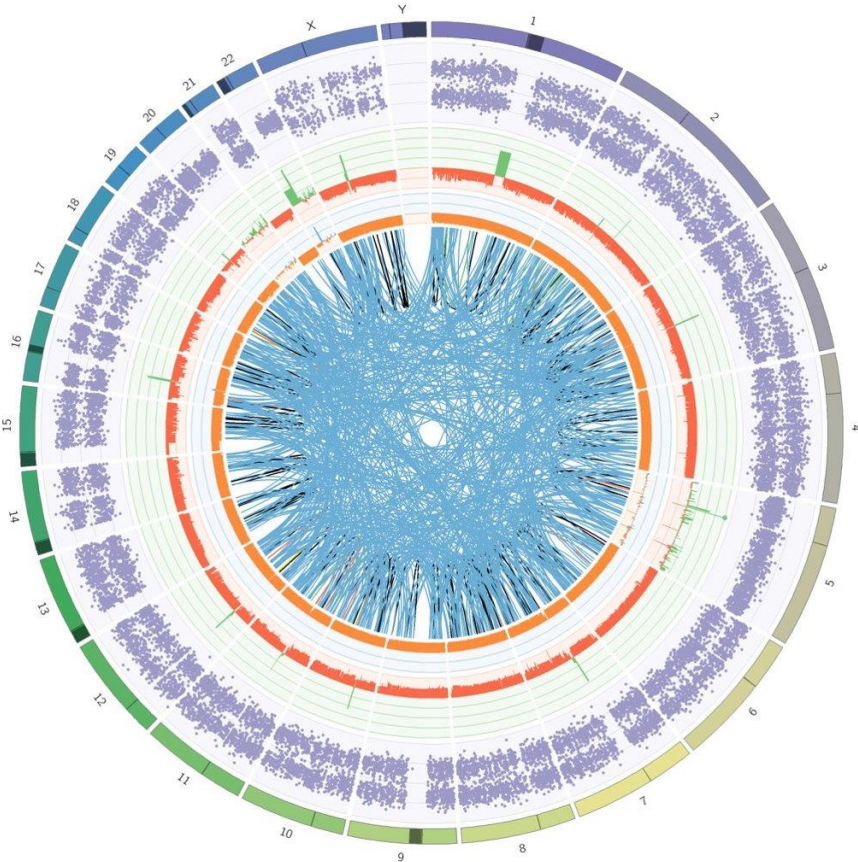
31F, 30mm breast mass



Whole Genome/Transcriptome Sequencing (WGTS)

Sean Grimmond

Joseph (Joep) Vissers



The Advanced Genomics Collaboration

WGTS findings:

near haploinsufficiency (except Chr. 5, 22): CN loss, LOH, B-allele

*NF1 p.K490**

*TP53 p.R213**

*Germline MUTYH Y101**

PATHOLOGIST-INITIATED WGTS

- pilot ~50 cases
- major clinical impact

- significant diagnostic dilemma
- ≥ 2 x histopathologists with subspecialty expertise
- (no CNS, paediatric, GYN, lymphoma)



Catherine Mitchell



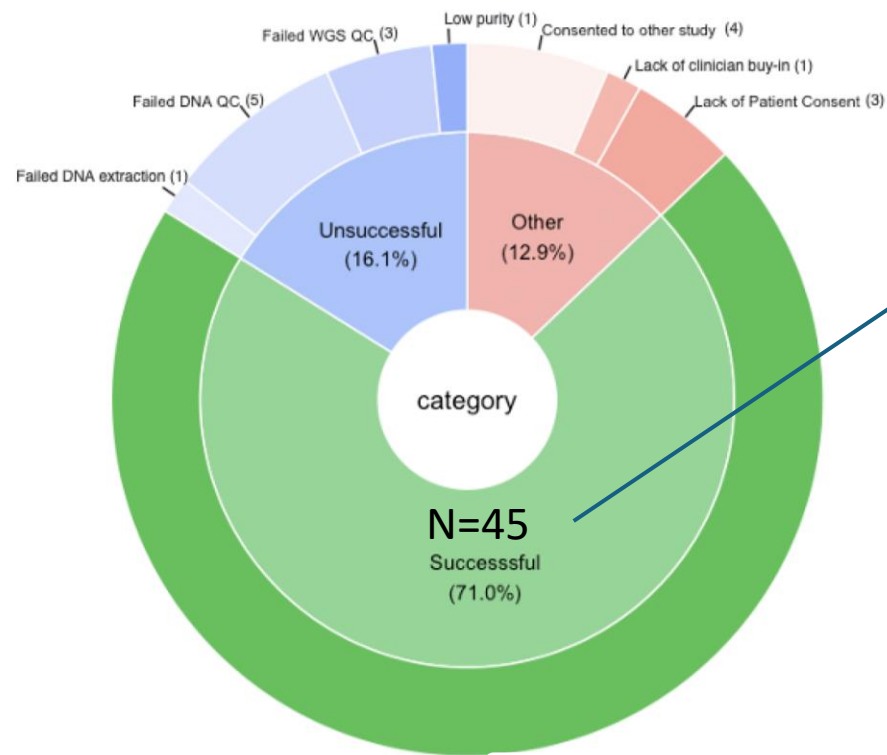
Joep Vissers



Wing-Yee Lo



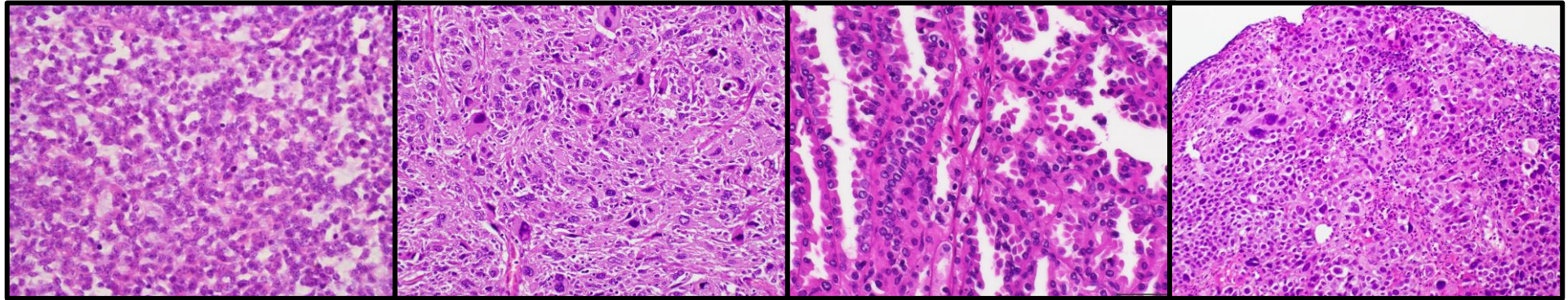
WGTS cohort



- mean 43.8 yrs (19-81)
- 25x F, 20x M
- 32x FFPE, 13x fresh
- 34x large/excisional specimens, 11x core/small Bx

- median 23 IHC/case
- FISH (EWSR1, FUS, SS18, MDM2, n=17)
- small panel DNA NGS (OPA, n=16)
- large panel (TSO500, n=3)
- RNA NGS (Illumina TruSight 507 targeted genes, n= 8)

Pre-WGTS categories



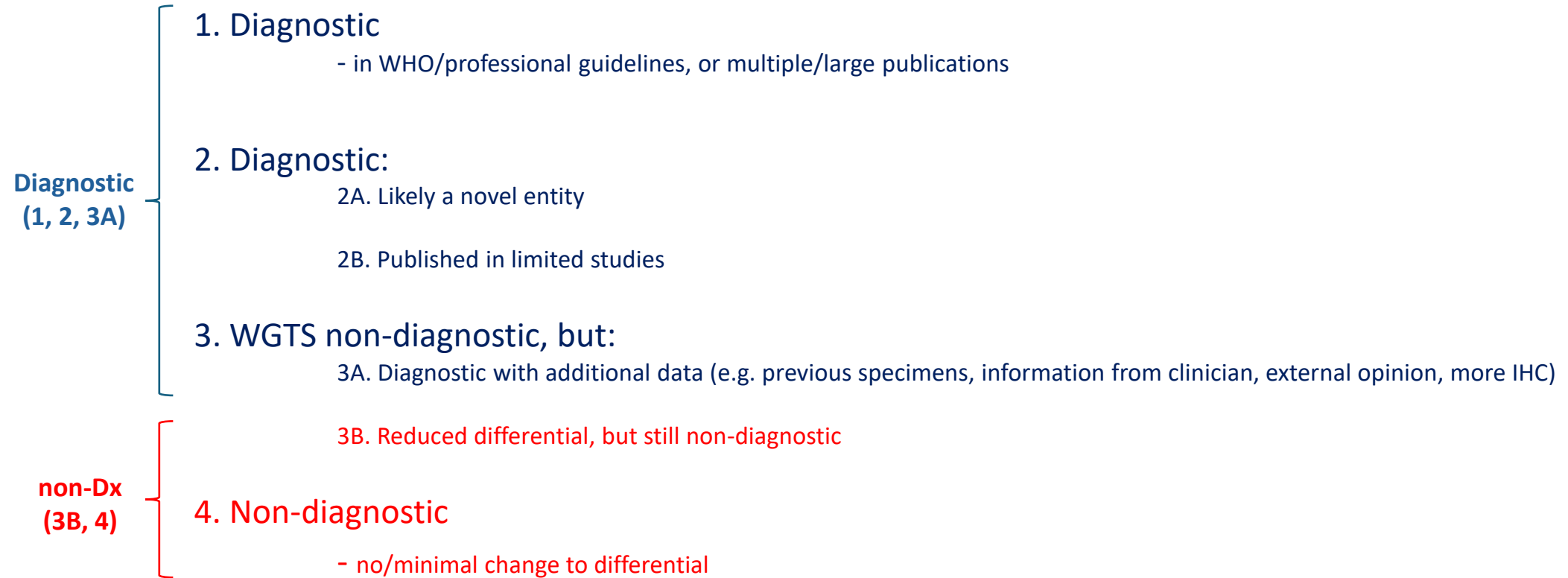
Monomorphic

Pleomorphic

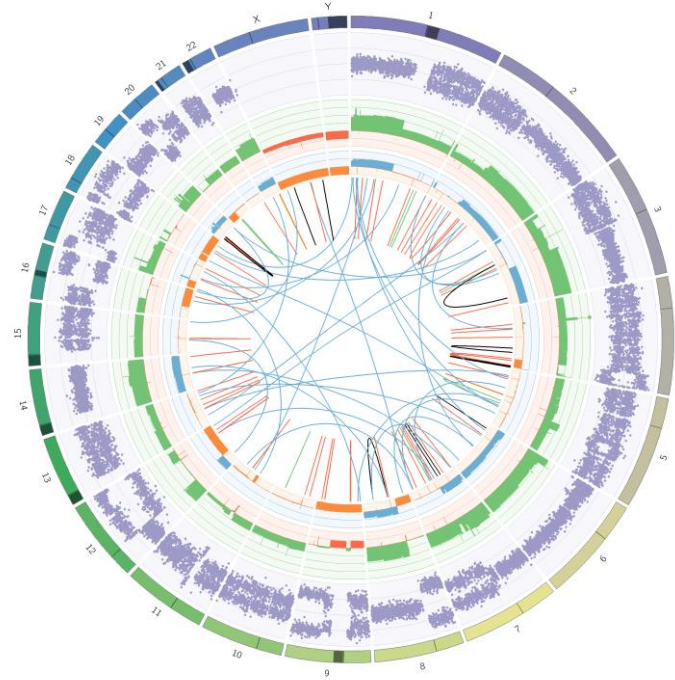
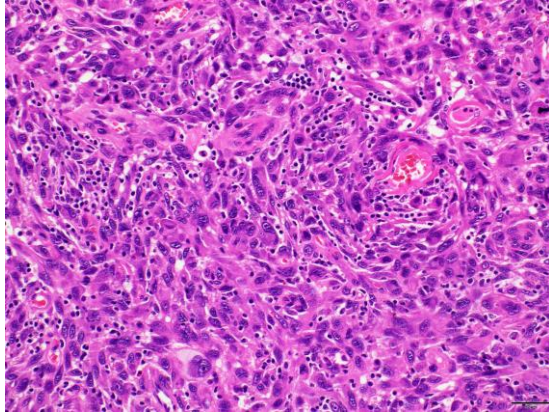
Renal cell neoplasm

SWI/SNF-deficient
INI1/SMARCB1 or SMARCA4 IHC

Post-WGTS categories



57M, solitary lung mass



WGTS findings:

TMB 88.9 muts/Mb

Signature 7 (UV)

NOTCH1 frameshift, LOH

TP53 hotspot, LOH

CDKN2A nonsense, LOH

TERT promoter hotspot

CUPPA – cutaneous origin, non-melanocytic

Pre-WGTS

Category – pleomorphic

#IHC - 43

Malignant - yes

NGS (OPA) - *TP53*

Lineages - 4

DDx – carcinoma, sarcoma, mesothelioma, melanoma

Post-WGTS

Category – 1

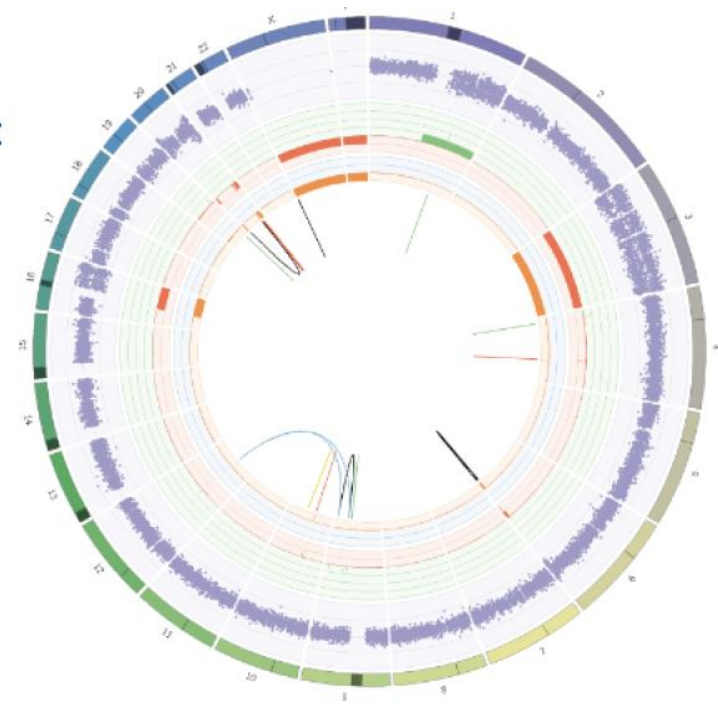
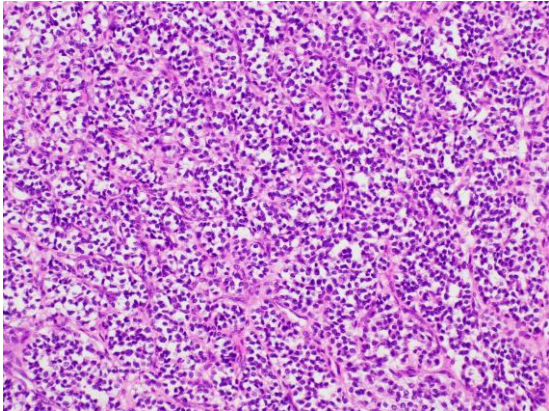
Malignant - yes

Lineages – 1

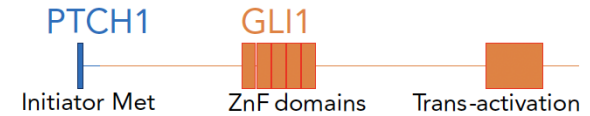
Other – Tier 1 therapeutic (ICI)

Dx – metastatic cutaneous SCC

42M, mesenteric mass



WGTS
PTCH1-GLI1 fusion



Pre-WGTS

Category – monomorphic
#IHC - 23
FISH – negative (*EWSR1*, *FUS*)
Malignant - yes
Lineages - 1

DDx - clear cell sarcoma-like tumour of GIT,
myoepithelial carcinoma (external opinion)

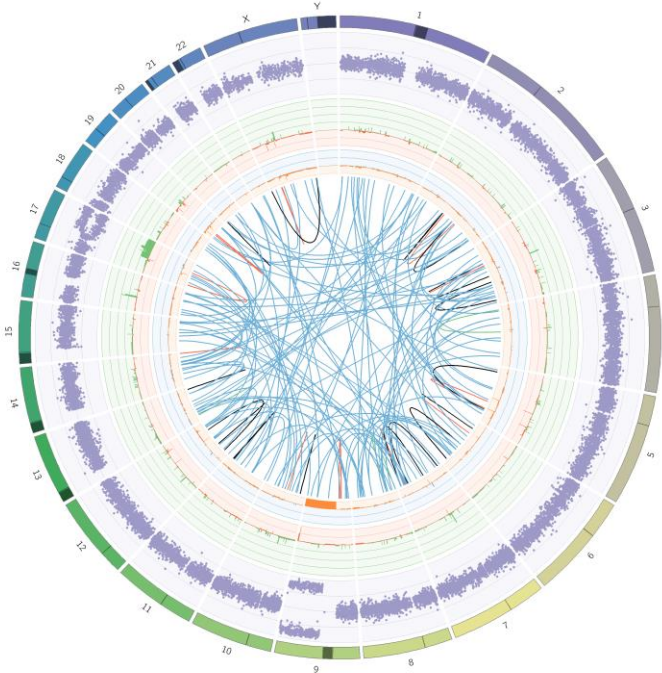
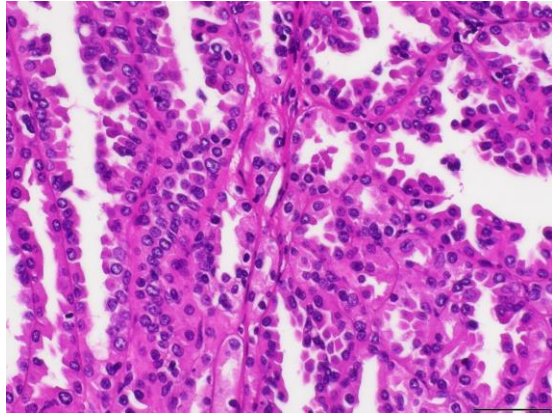


Post-WGTS

Category - 1
Malignant - yes
Lineages – 1

Dx – *GLI1*-activated epithelioid cell tumour

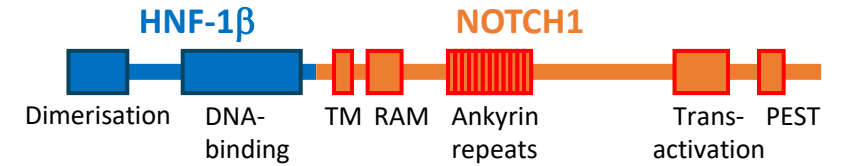
31F, renal mass



WGTS findings:

HNF1B-NOTCH1 fusion

Signature 3 (BRCA/HRD) but HR-proficient (CHORD, HRDetect)



Pre-WGTS

Category – renal neoplasm

#IHC - 17

Germline NGS – negative

Malignant - yes

Lineages - 1

DDx – papillary, MITF, FH/SDH-deficient, NOS



Post-WGTS

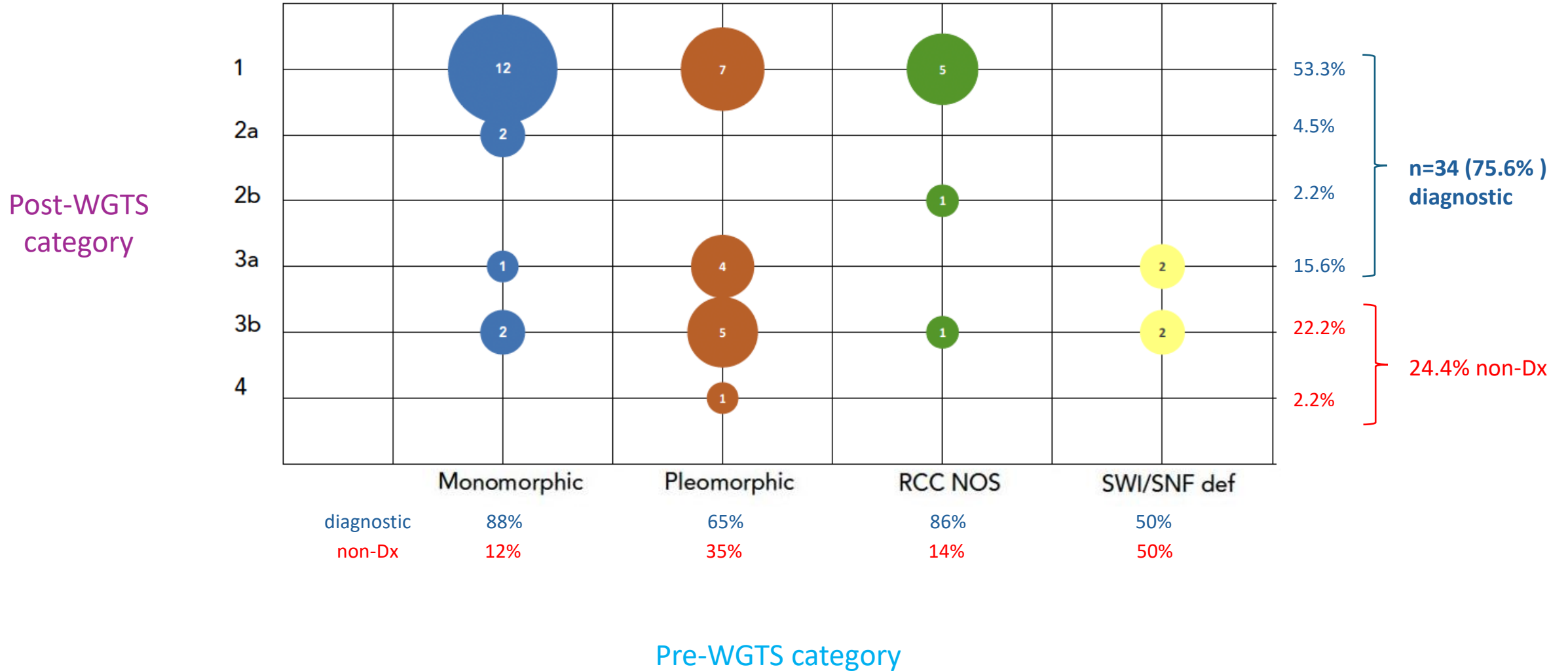
Category – 2A (1x case in TCGA RCC database)

Malignant - yes

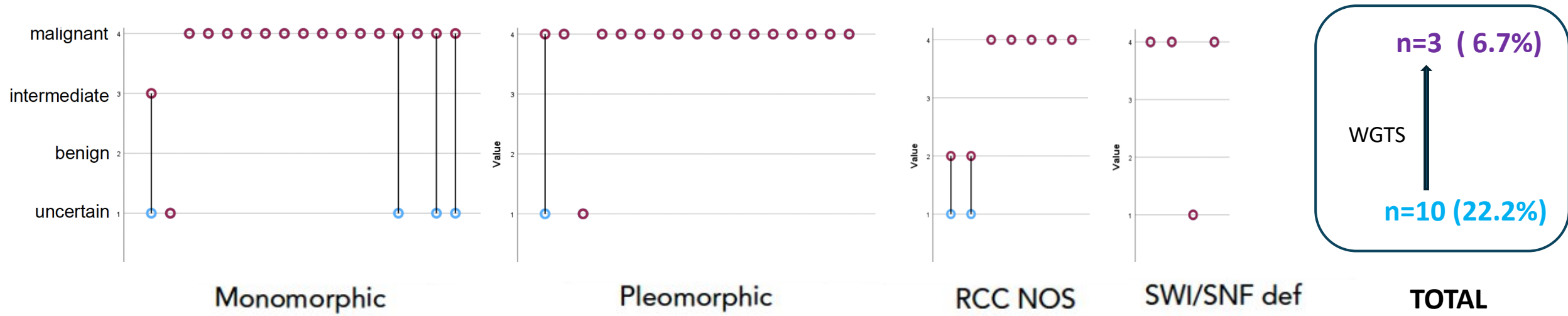
Lineages – 1

Dx – RCC NOS (*NOTCH1*-rearranged renal cell carcinoma ?)

Diagnostic yield post-WGTS

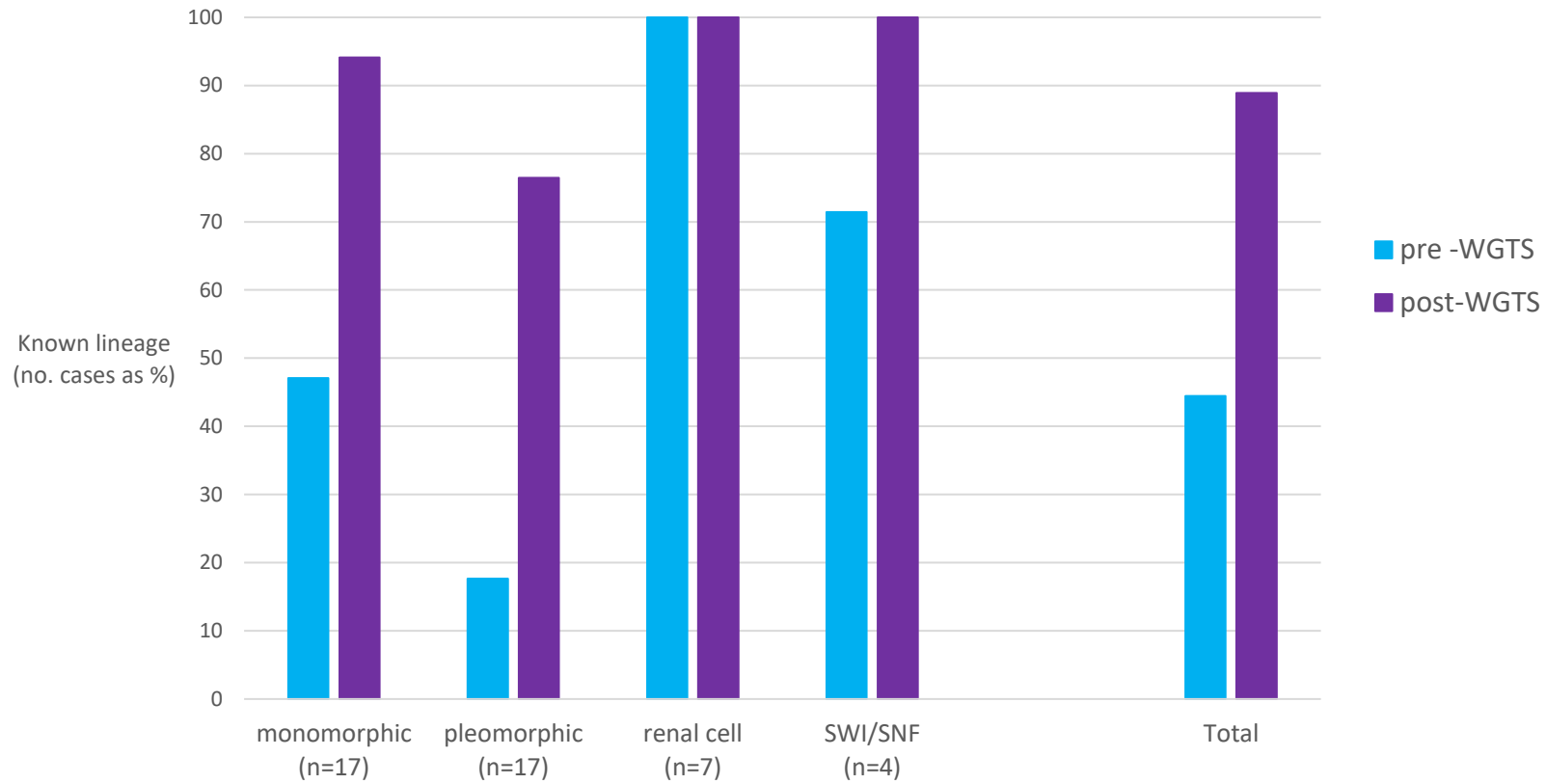


Uncertain malignant potential

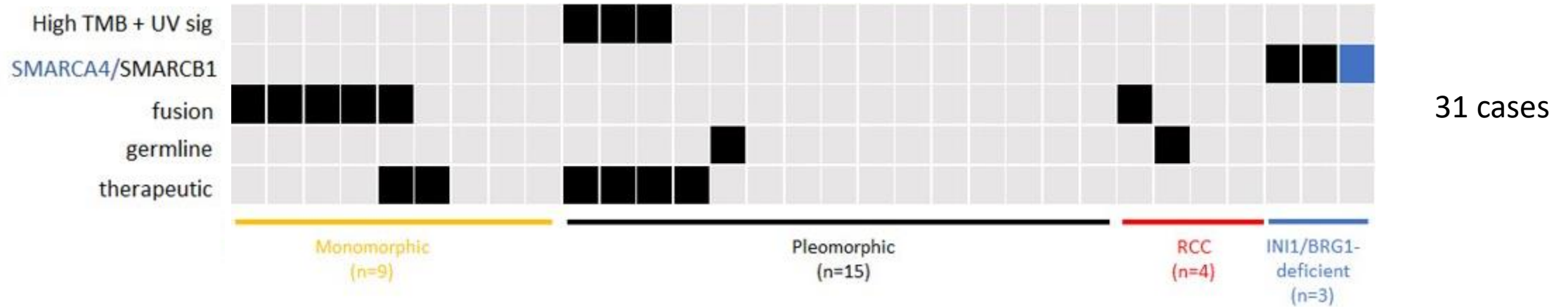


WGTS improves certainty of lineage

(carcinoma, sarcoma, melanoma, mesothelioma...)



Clinical benefit beyond diagnosis



3x pathogenic germline variants

- BRCA1 (TNBC)
- FH (RCC)
- MUTYH (inflammatory LMS)

5x Tier 1 therapeutic findings

- 1x HRD (TNBC)
- 3x high TMB (cSCC, INI1-deficient cSCC, Merkel cell carcinoma)
- 1x ROS1 fusion (spindle cell rhabdomyosarcoma)

Diagnostic cheaper, faster, more accessible assays ?

Assay	n	notes
IHC	1	FH-def RCC
FISH	1	CCND1-rearranged vascular-invasive renal oncocytoma
TSO500	8 (17.8%)	5/17 (29.4%) pleomorphic
Illumina TruSight RNAseq	8 (17.8%)	7/17 (41.1%) monomorphic
Only by WGTS	14 (31.1%)	41.2% of the 34 diagnostic cases

Diagnostic use of WGTS

- Essential for selected cases
- FFPE not necessarily a barrier (access, cost)
- other approaches:
 - newer IHC
 - expert opinion
 - TSO500 (pleomorphic)
 - Illumina TruSight RNAseq (monomorphic)

Thank you

Patients

Clinicians & pathologists who contributed cases



Catherine Mitchell

Stephen Fox



Joep Vissers

Sean Grimmond
Wing-Yee Lo

Sehrish Kanwal
Oliver Hofmann
Kym Pham



**The
Advanced
Genomics
Collaboration**