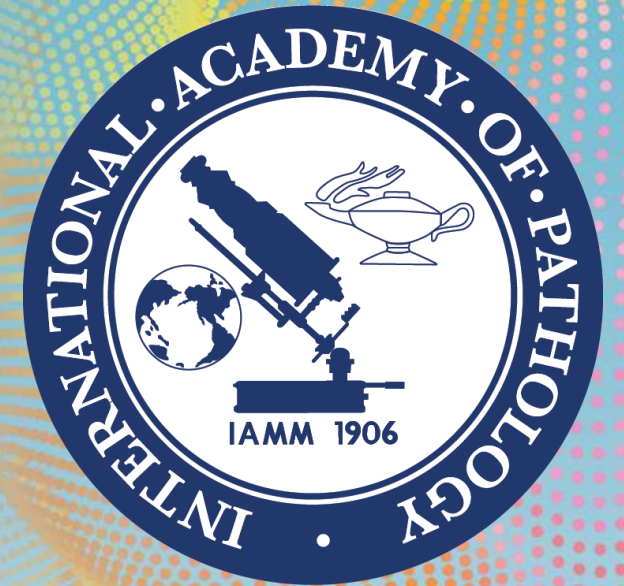


Molecular risk stratification in uveal melanoma: Utility of TCGA classification for clinical decision making

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Disclosure of Relevant Financial Relationships

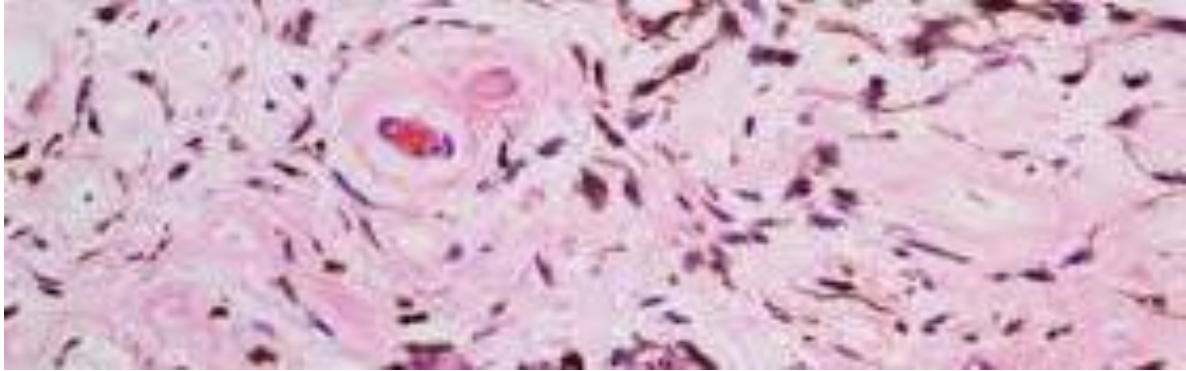
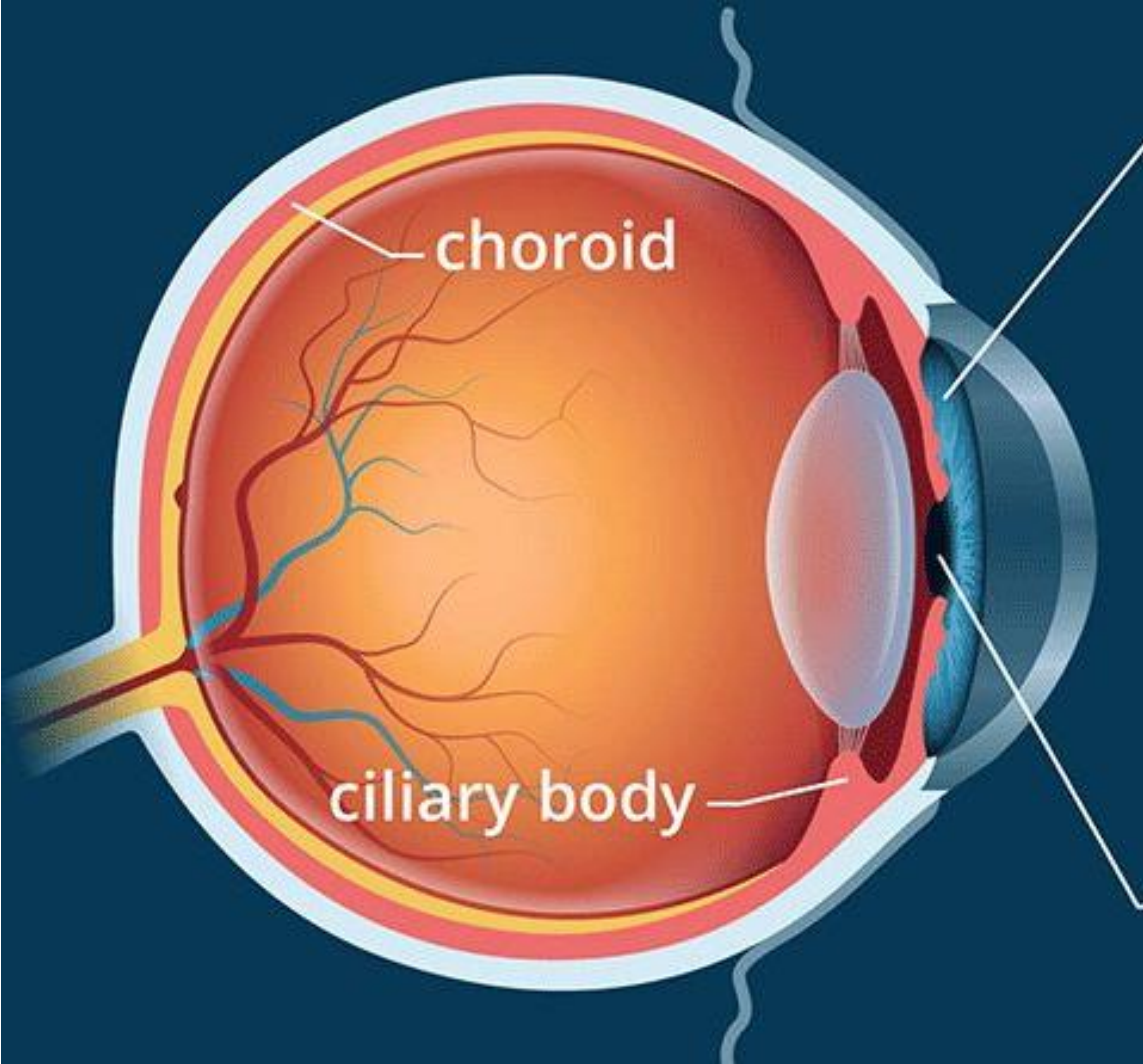
Incoming Ophthalmic Pathology Fellow, Sydpath- St. Vincent's hospital, Sydney

Consultant histopathologist, NM Medical, Mumbai

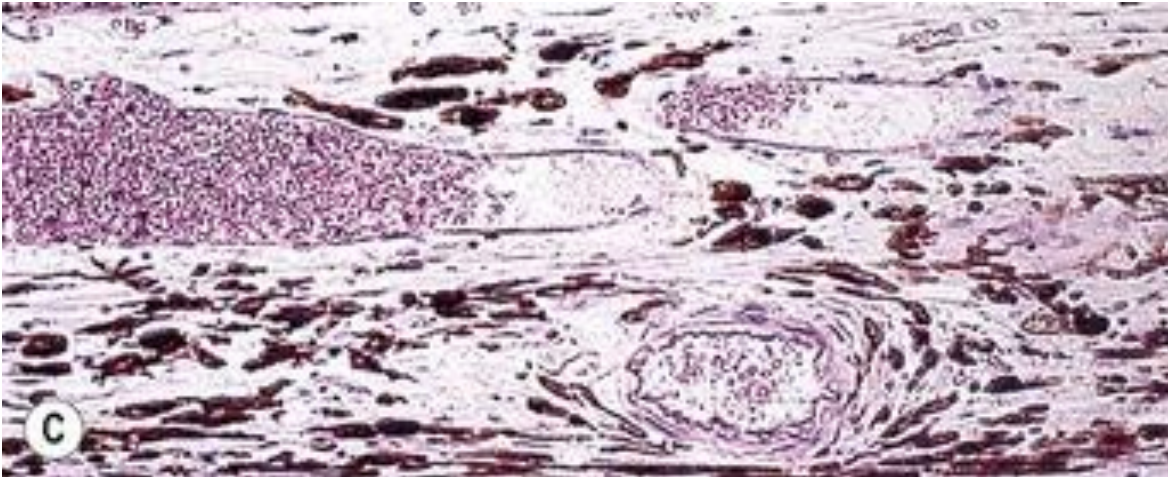
No financial disclosures

No conflicts of interests

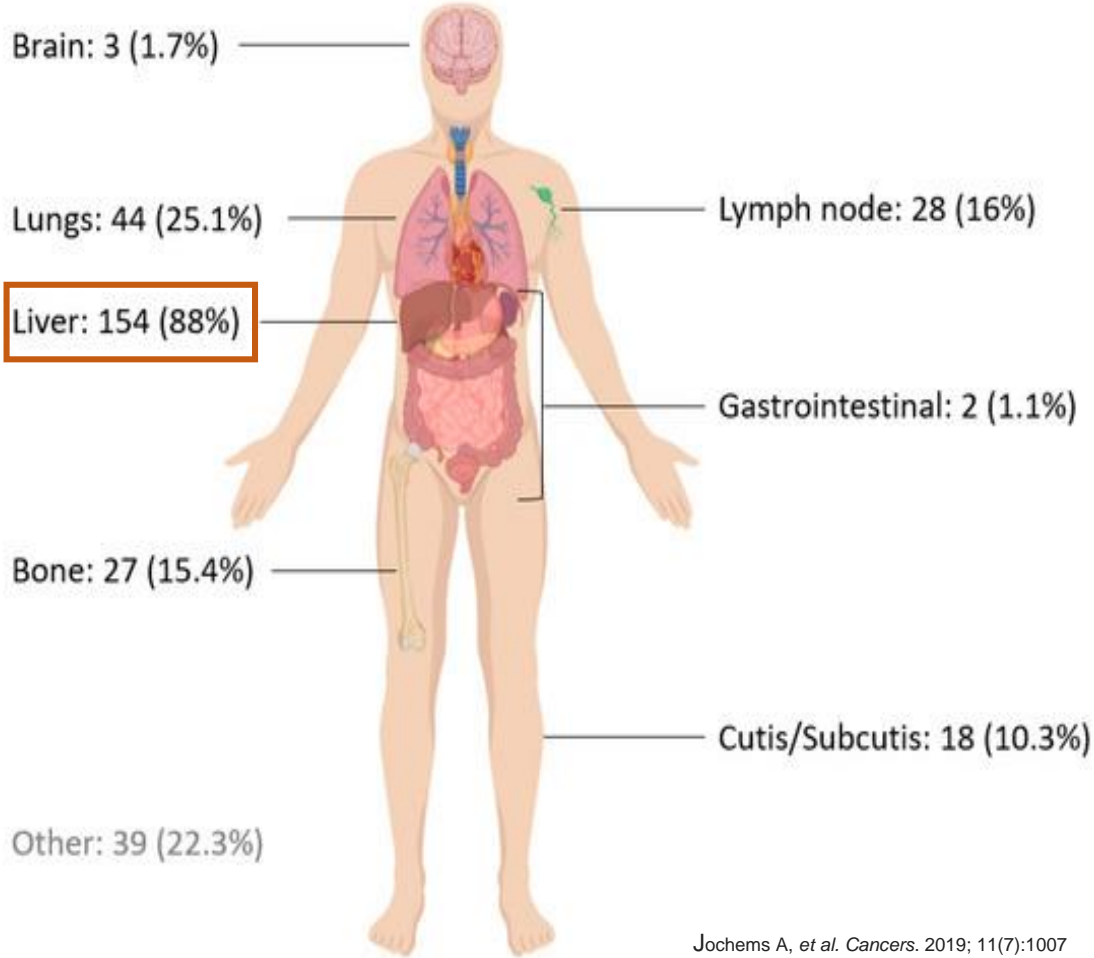
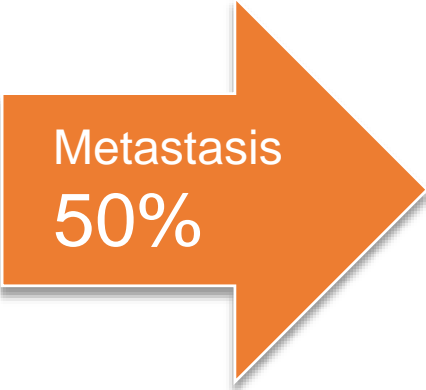
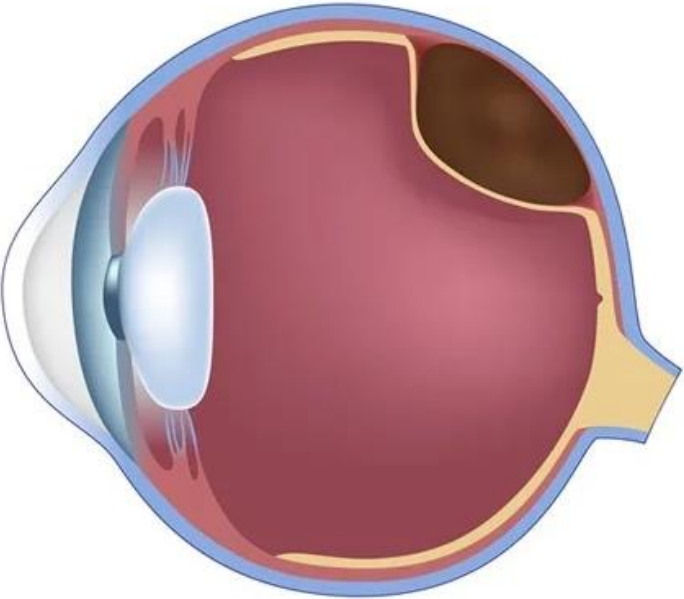
Uvea: Iris, Ciliary body & Choroid



- Vascularised pigmented stroma
- Neural crest derived
- Resident melanocytes



Uveal melanoma: Natural History



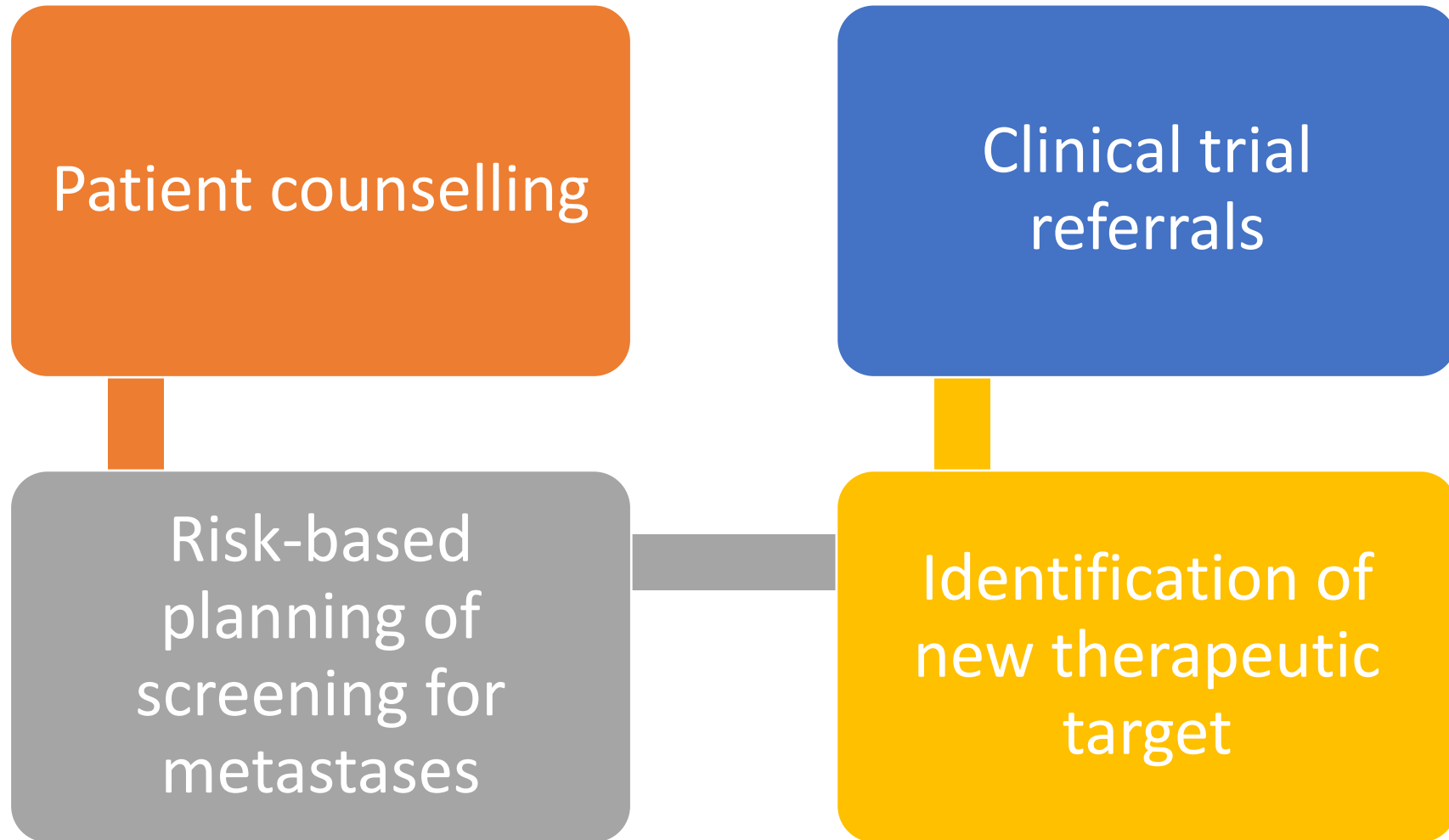
Incidence: 8-10 per million each year

Primary treatment:

- Brachytherapy
- Enucleation

Jochems A, et al. *Cancers*. 2019; 11(7):1007

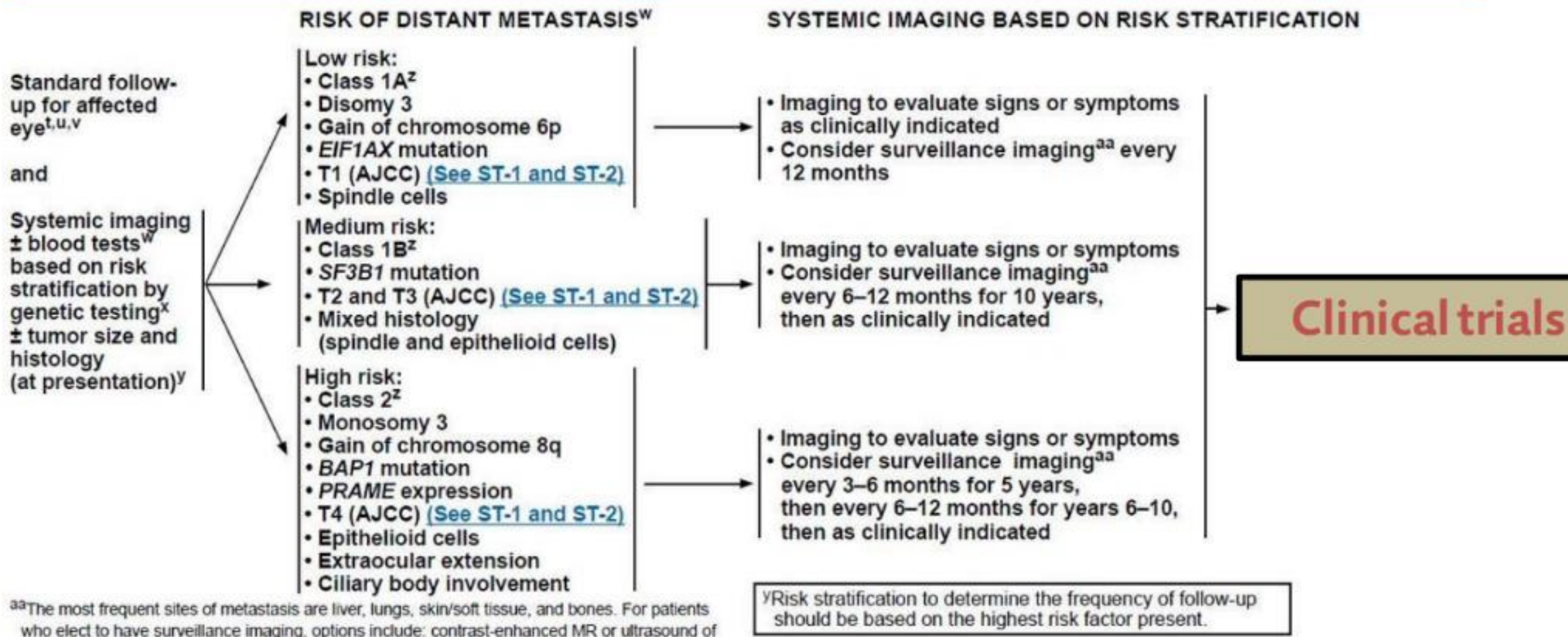
Why accurate prognostication matters



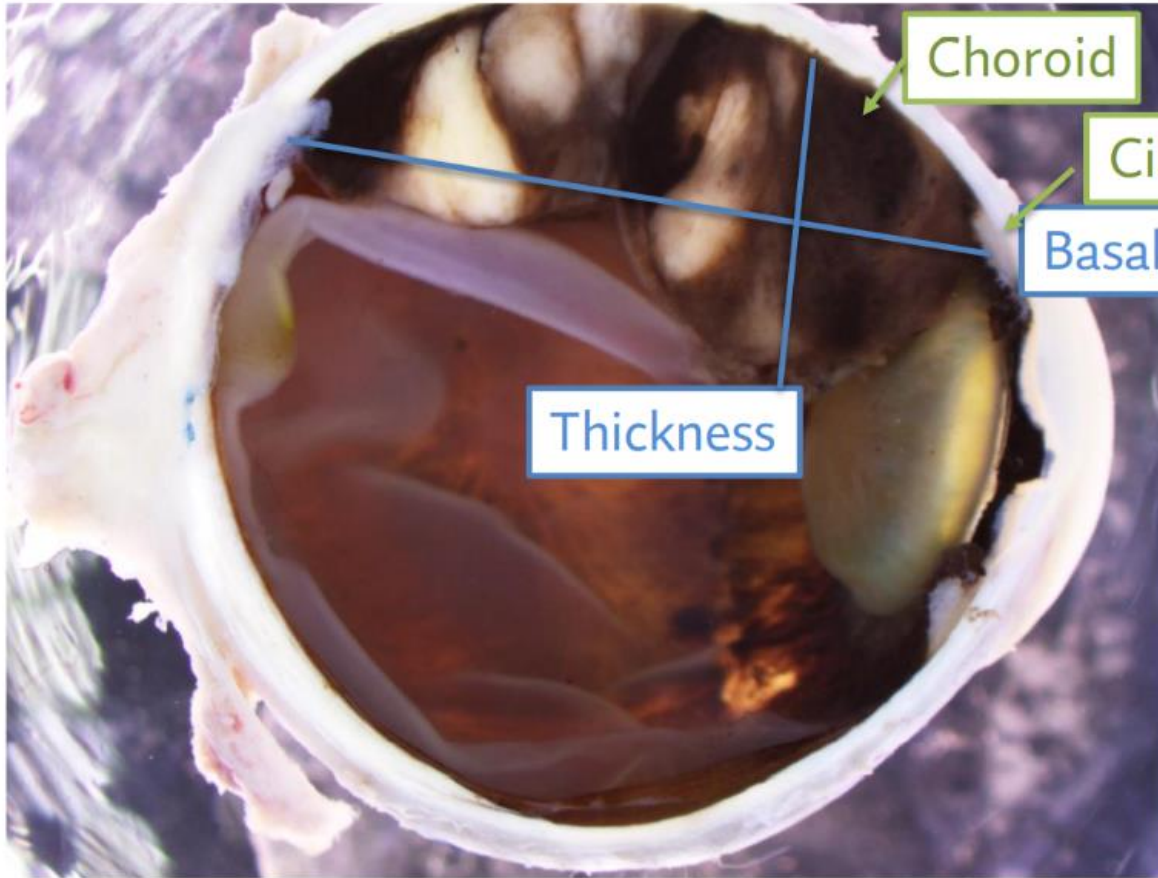


NCCN Guidelines Version 1.2020

Uveal Melanoma

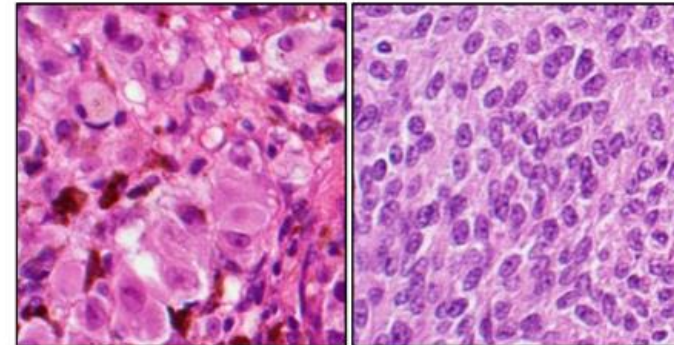


Risk factors for metastasis



- AJCC pT stage

Grade (cell type)



Epithelioid (G3)

Spindled (G1)

Mixed (G2)

Clinical

Tumour basal diameter

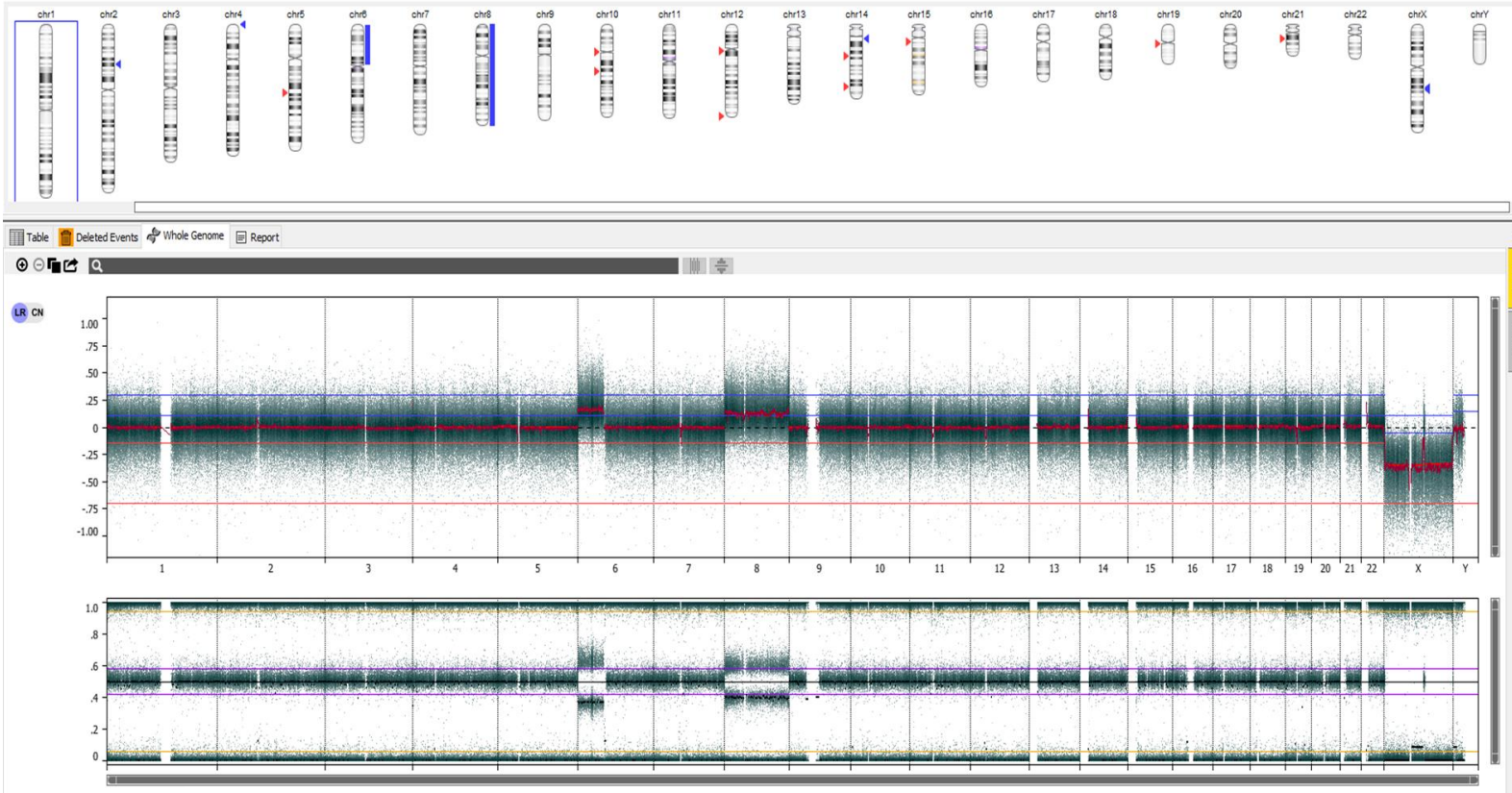
- Tumour thickness
- Ciliary body involvement
- Extraocular extension

Histopath:

- Epithelioid cell type
- Vascular lakes
- ECM loops
- High mitotic count

Predicting metastasis: Molecular karyotyping

Chromosome microarray

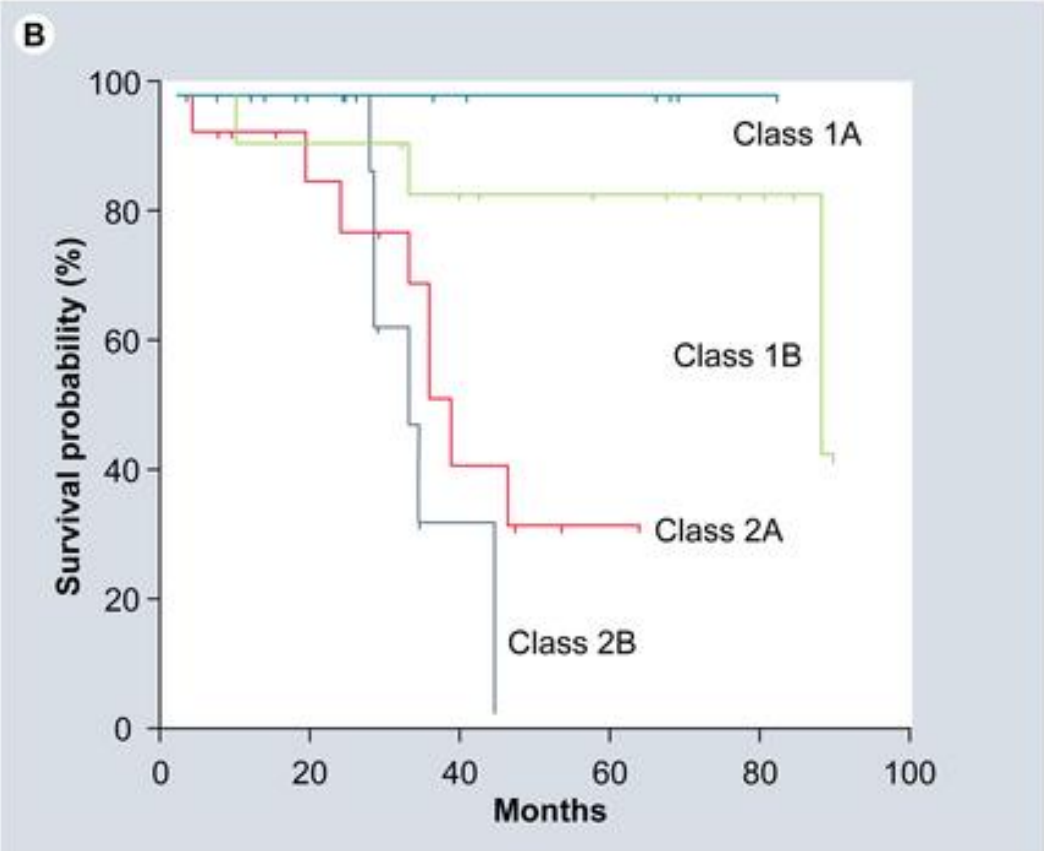
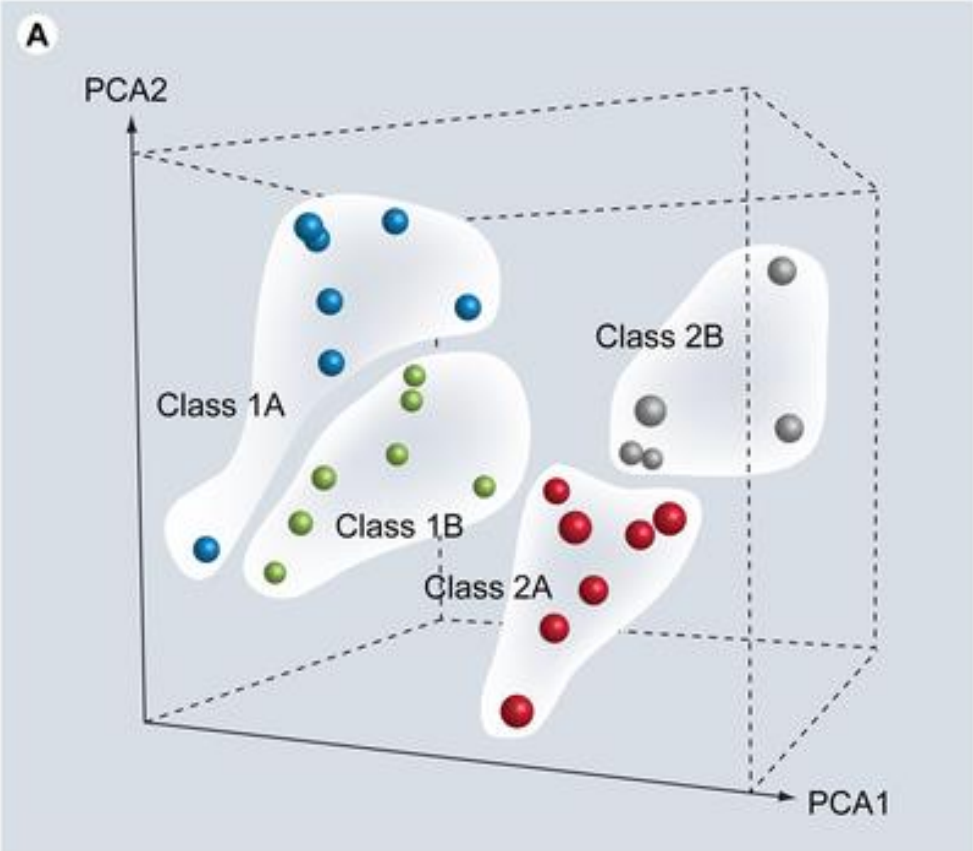


Poor prognosis (high risk of metastasis)

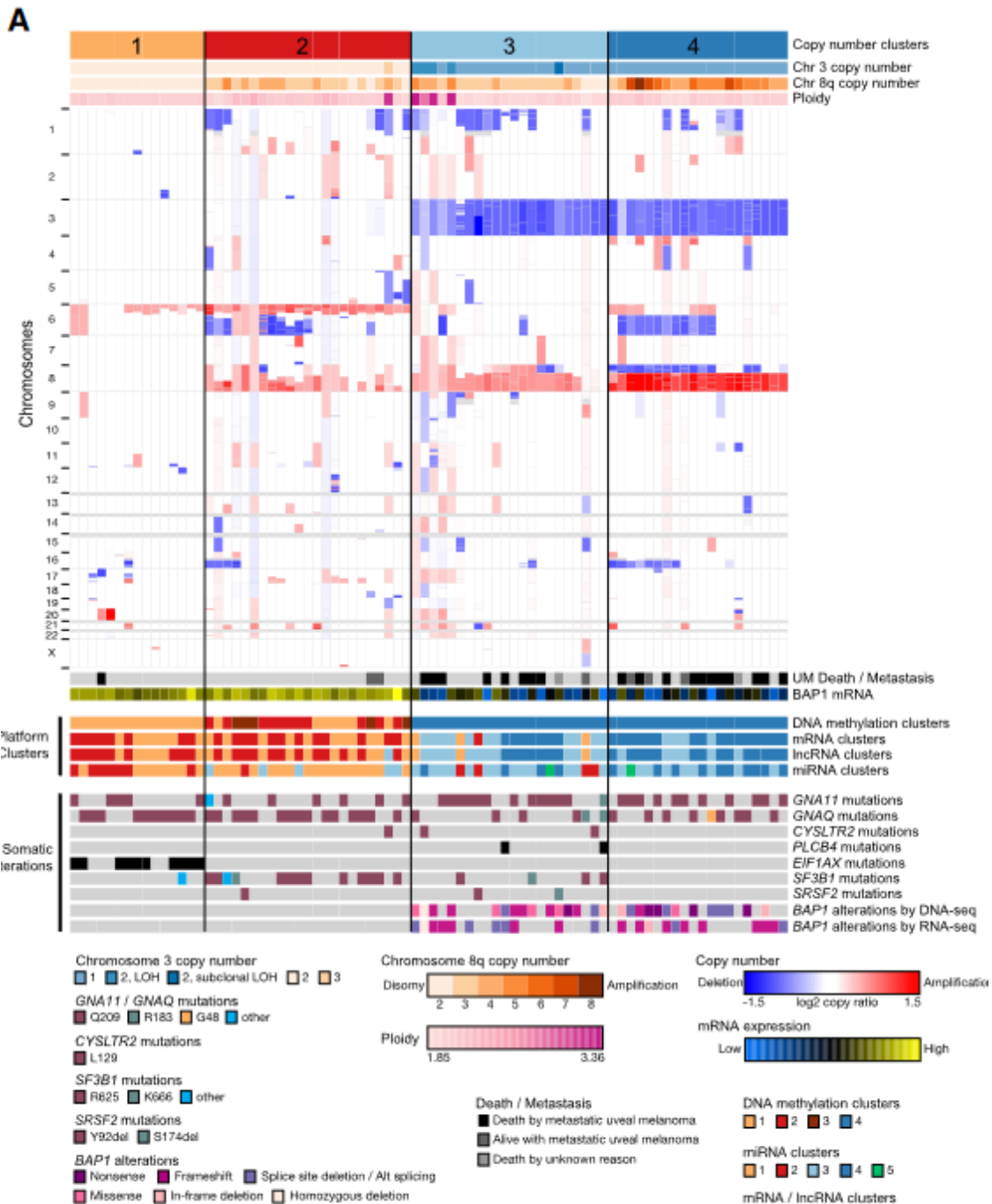
Monsomy 3
Gain 6p Loss 6q
Gain 8q

BAP1
SF3B1

Predicting metastasis: Gene expression profiling



Gene expression profiling (GEP): mRNA
Class 1: better prognosis
Class 2: poor prognosis



Rare Tumor Project of The Cancer Genome Atlas (TCGA)

In 2017, Robertson et al published the results of the YCGA project
Comprehensive, integrated, multiplatform molecular analysis of 80 primary UM

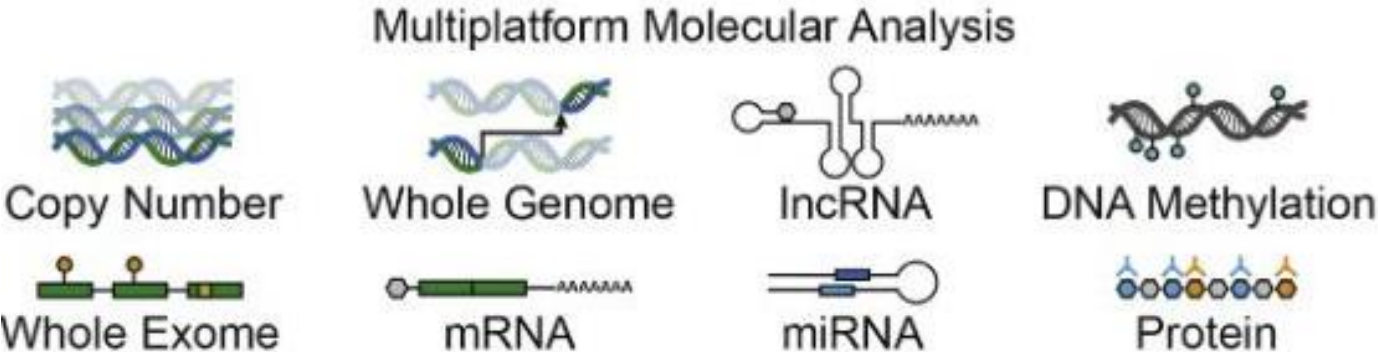
Mutations, genomic copy number alterations, transcriptomic and methylation profiles and associated clinicopathologic data were analyzed for all

Four molecularly-distinct groups of uveal melanoma were identified

Genomic landscape of Primary UM

The Cancer Genome Atlas (TCGA) Data Cancer cell vol. 32,2 (2017): 204 -220

TCGA classification



Four Molecularly Distinct Subsets

	Disomy 3 (D3)				Monosomy 3 (M3)			
Copy Number	1	2	3	4	1	2	3	4
Gene Alterations	<i>EIF1AX</i>	<i>SF3B1</i>	<i>BAP1</i> -aberrant					
DNA Methylation	1	2/3	4					
mRNA	1	2	1	2	3	4	3	4
lncRNA	1	2	1	2	3	4	3	4
Metastatic Risk	High							

Simplified TCGA

Editorial

Table 1. Overview of Types of Uveal Melanoma and Corresponding Chromosome Aberrations

	A	B	C	D
mRNA class	1	1	2	2
Chromosome aberrations	Infrequent	Infrequent	Frequent	Frequent
Chromosome 3	Disomy 3	Disomy 3	Monosomy 3	Monosomy 3
Chromosome 6	Extra 6p	Extra 6p		
Chromosome 8	Normal 8q	Partial extra 8q	Extra 8q	Extra 8q (multiple)
Inflammation	None	None	Some	Much
Prognosis	Favorable	Late metastases	Unfavorable	Unfavorable

Jager et al 2018; simplified and suggested the use of The A, B, C, and D classification to avoid confusion between the numerical classification used in both the Cancer Genome Atlas (TCGA) and the commercially available test (GEP classification).

Validation of TCGA

Genetic features and outcome of uveal melanoma in 658 patients based on The Cancer Genome Atlas (TCGA) Classification of A, B, C, & D

	The Cancer Genome Atlas (TCGA) Class			
	A	B	C	D
Mutational profile				
Chromosome 3	Disomy 3	Disomy 3	Monosomy 3	Monosomy 3
Chromosome 8	Disomy 8q	8q gain	8q gain	8q gains (multiple)
Prognosis per TCGA[19,20]				
Estimated outcome	Favorable	Late metastases	Unfavorable	Unfavorable

Shields et al; 2019

Aims of our study

- Determine the clinical utility of the TCGA classification in day to day clinical practice
 - Robertson et al TCGA
 - Simplified TCGA
 - Modified TCGA
- BAP 1 IHC correlation with chromosomal alterations

Cohort characteristics

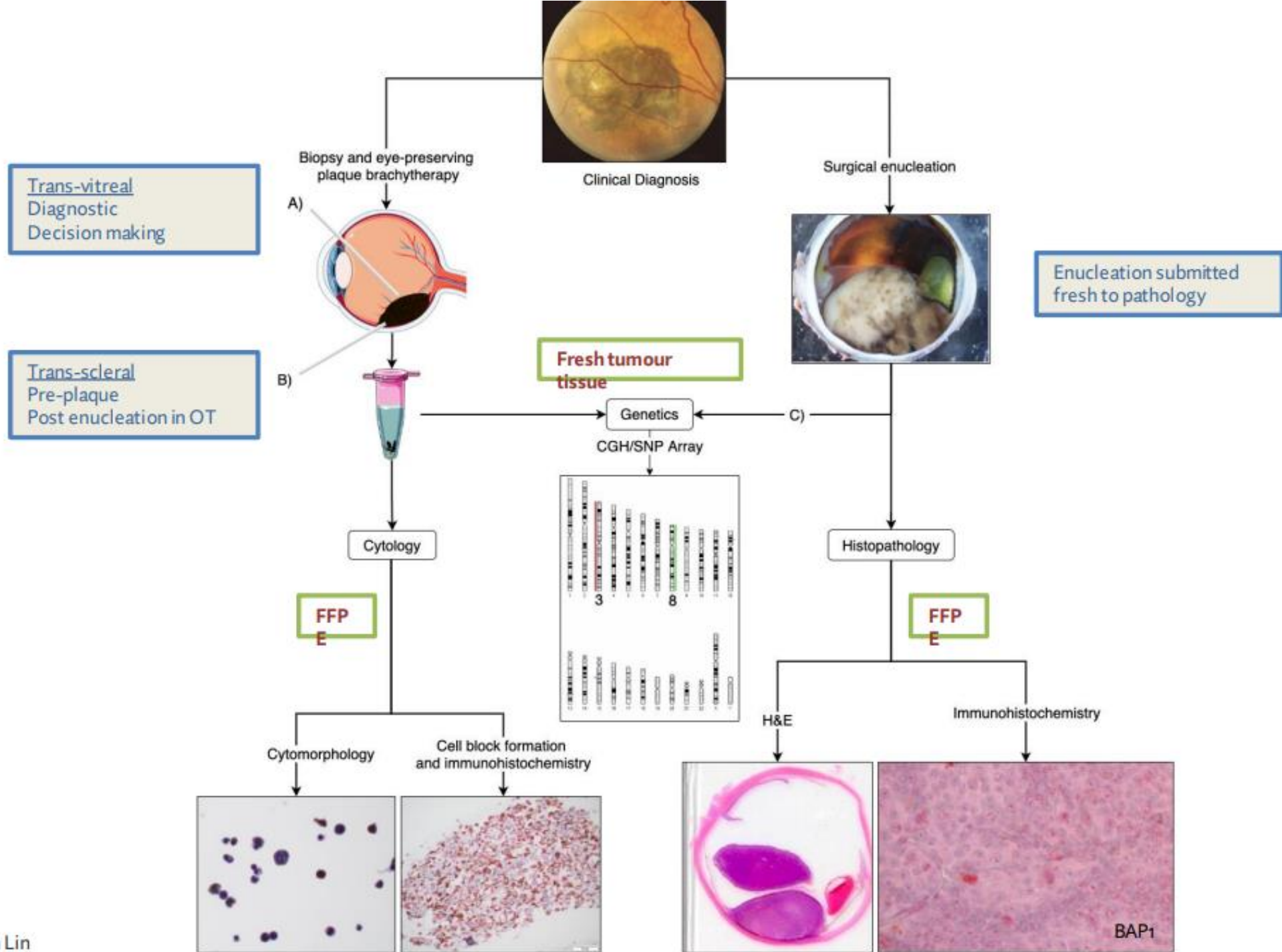
Retrospective case study (5 years: 2017-2022)

- Age(years): Mean: 62; range- 17 to 94
- Sex: Female: 98; Male: 116
- Patients from both Sydney and Melbourne
- Total cases: 214
- HPE analysis: 214
- BAP 1 IHC: 154 resulted

Genetic testing

- SNP array: 134
- Missing info: 80 (37.38%)
- Insufficient DNA/cells for SNP array: 43 (53.75%)
- No abnormality detected: 14 (17.5%)
- Not performed: 23(28.75%)

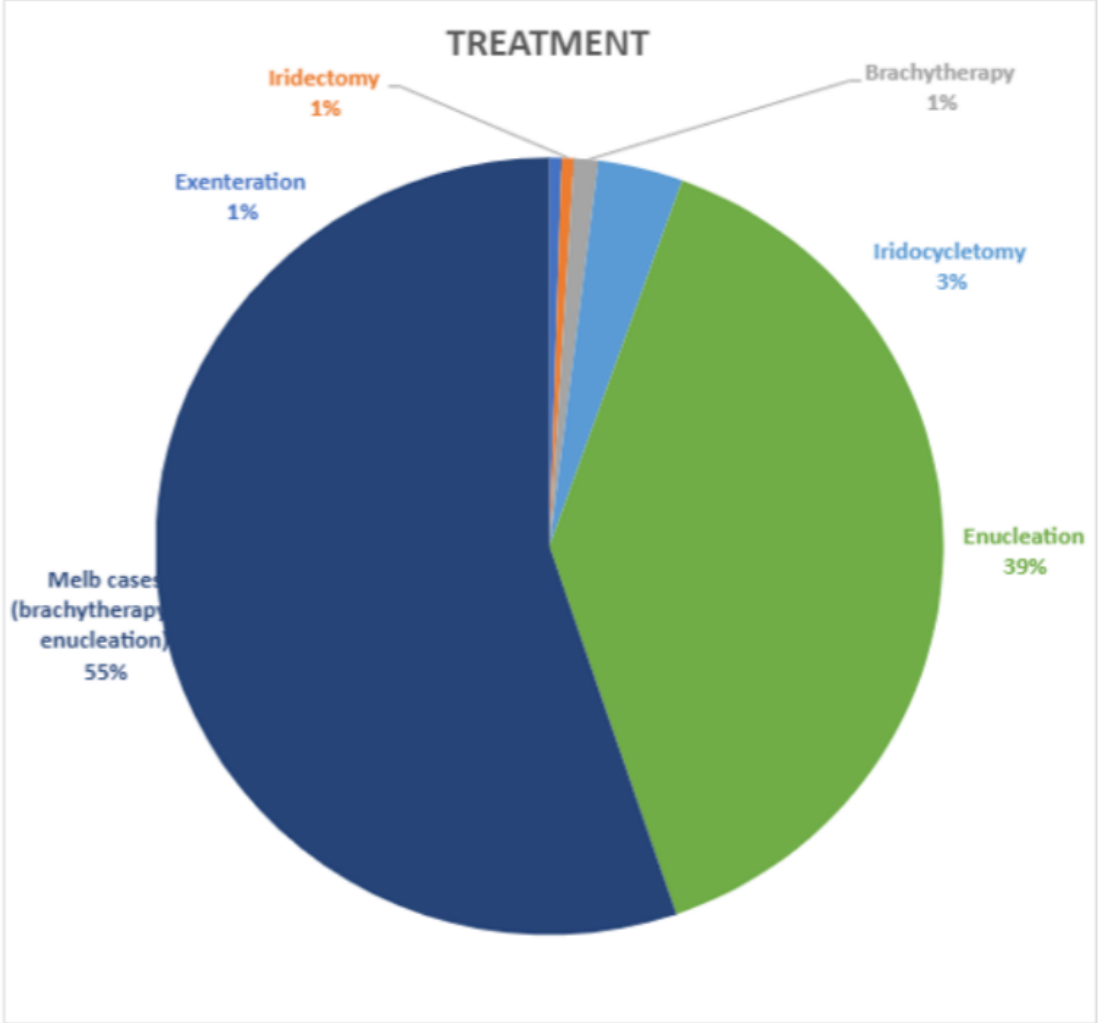
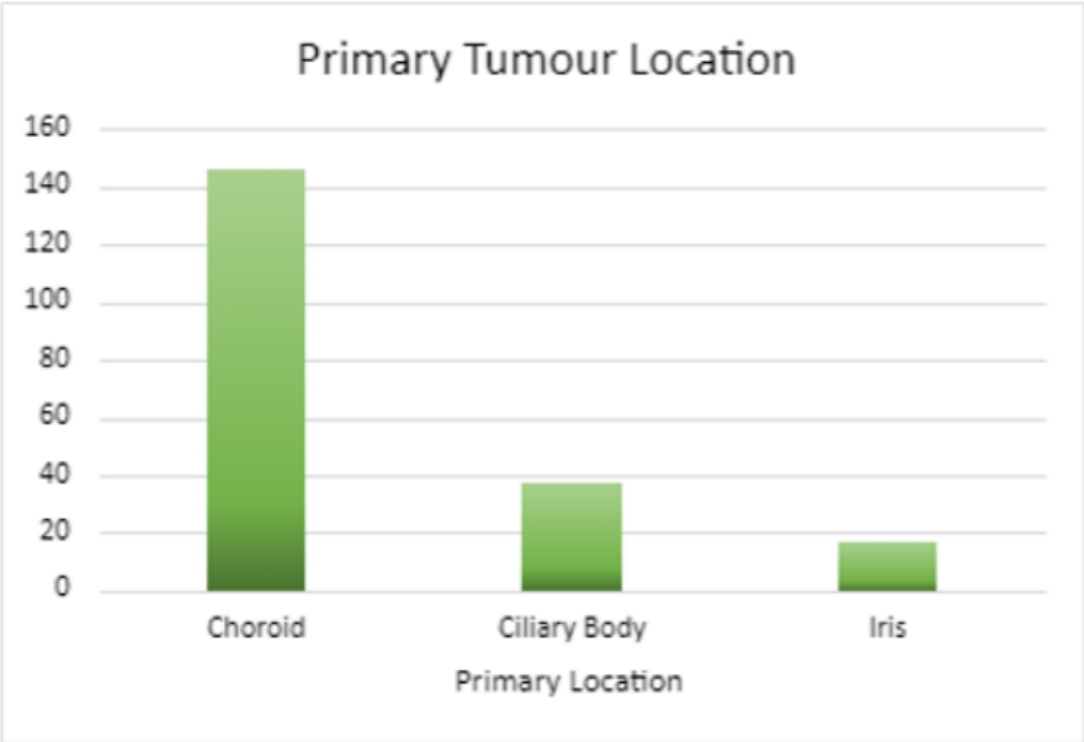
Multi step processing of UM samples at Sydpath



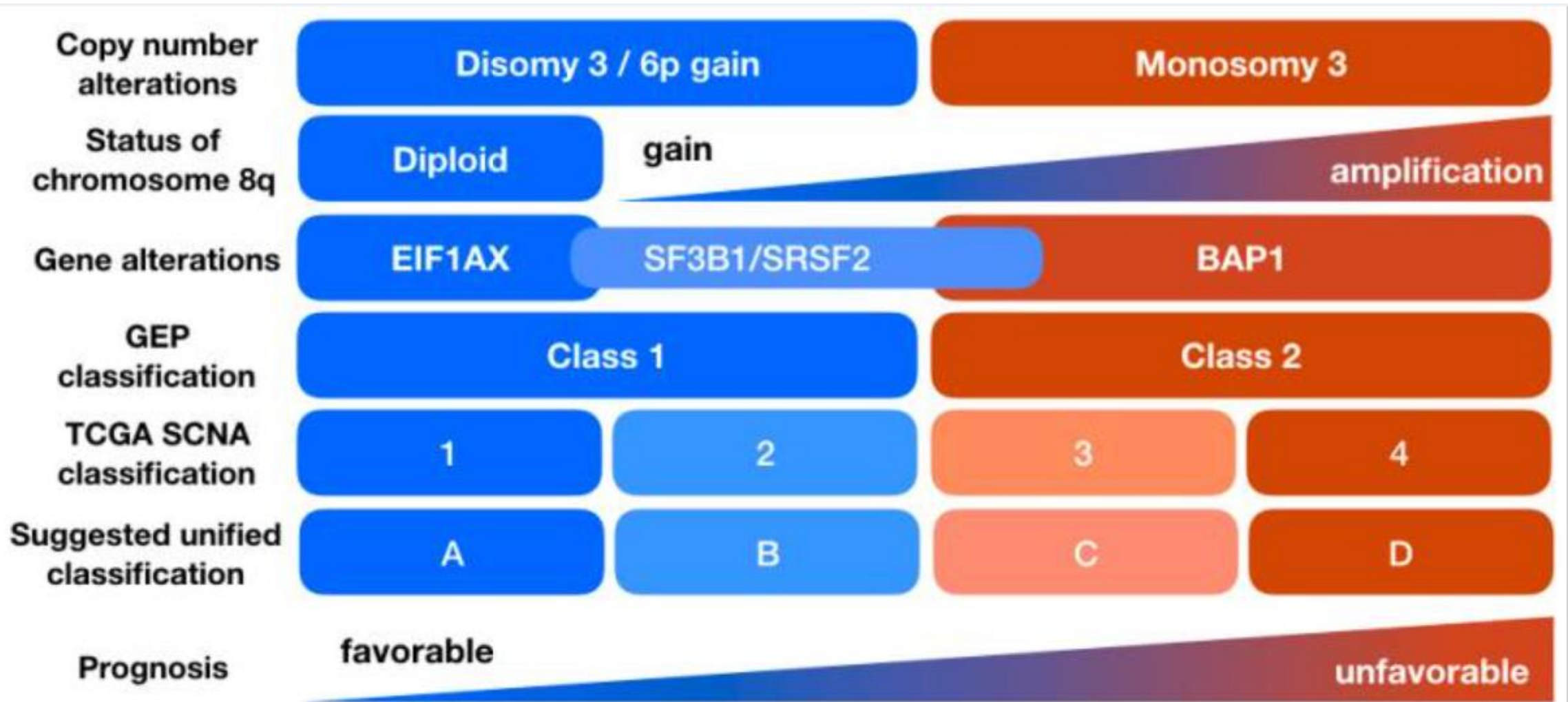
Methods

- BAP1 status by immunohistochemistry (retained or aberrant)
- Comparative Genomic Hybridisation:
 - Thermo Fisher Cytoscan 750 (pre 2020)
 - Illumina 850 (post 2020)

Site of primary tumour; Sample type

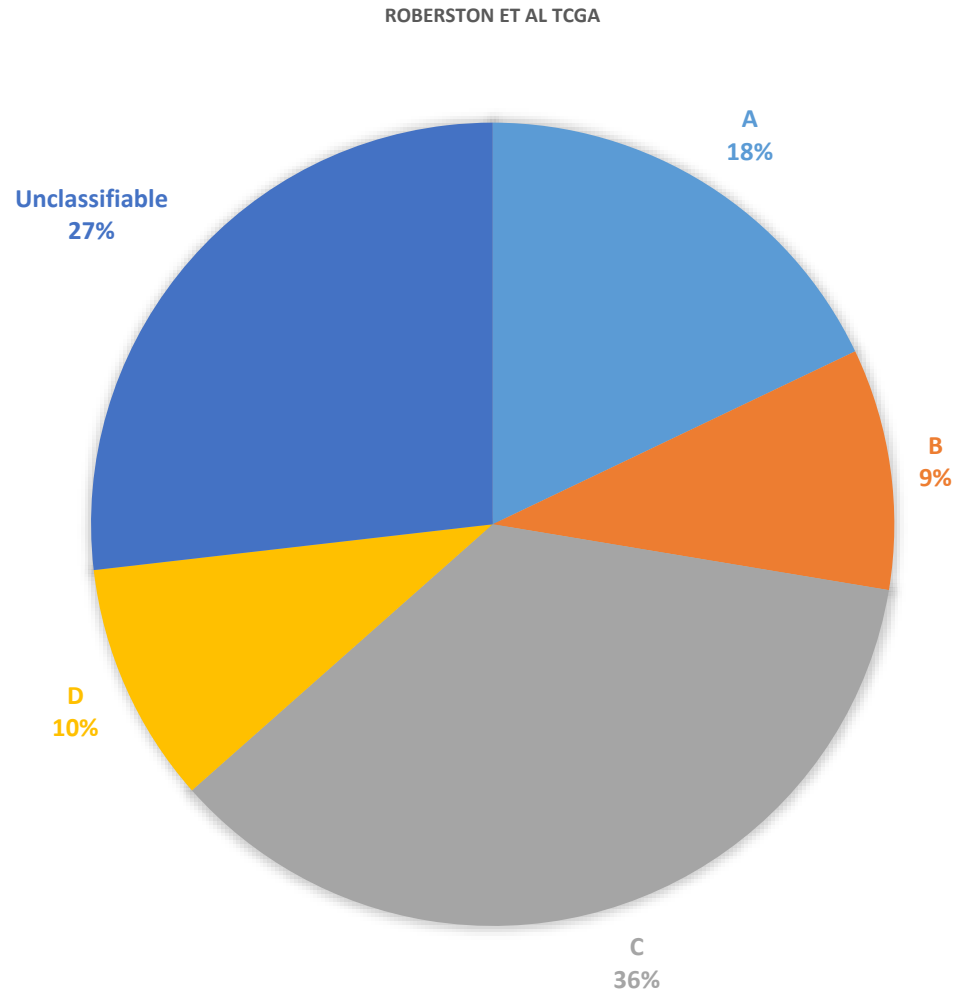


Overview of UM subtypes



"Molecular Characteristics of Uveal Melanoma: Insights from the Cancer Genome Atlas (TCGA) Project" by Bakhoun et al., 2019

TCGA classification

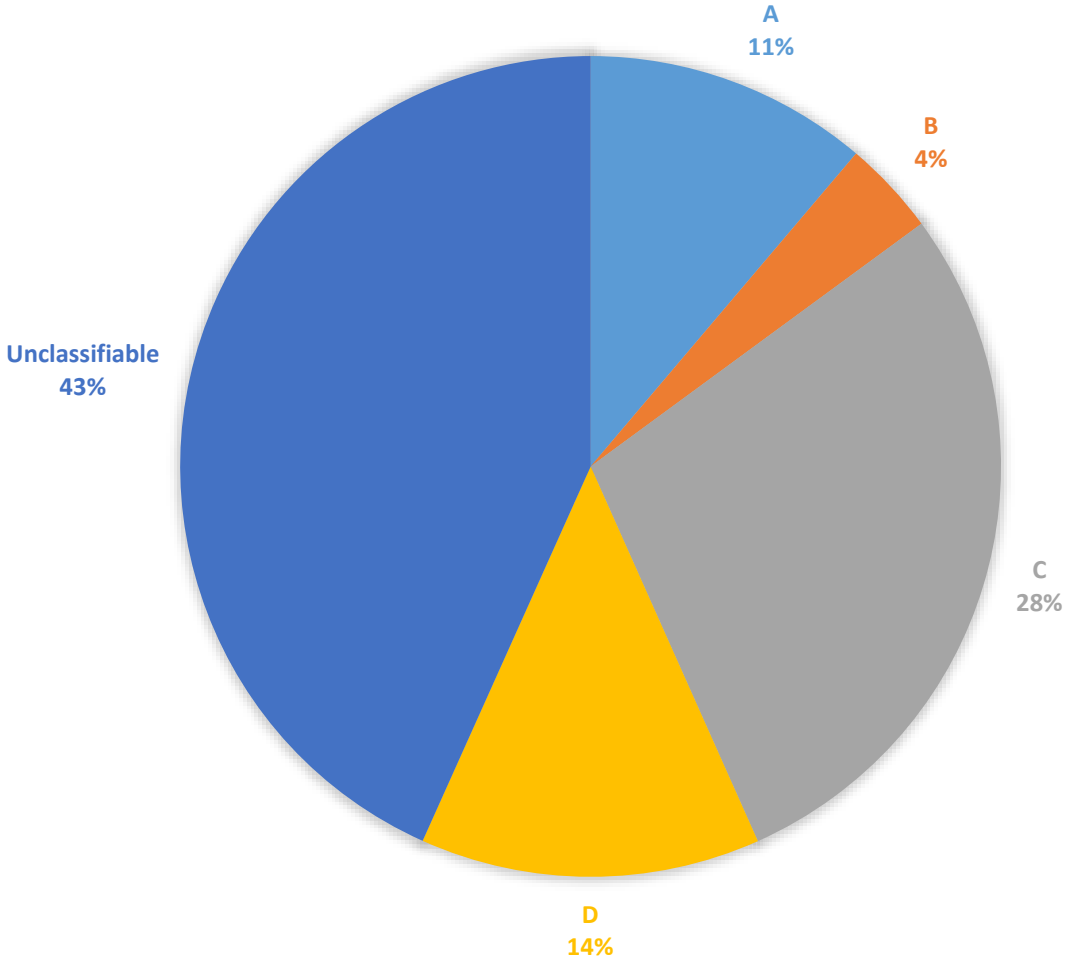


Unclassifiable features:

- D3+D8 with multiple chromosomal aberrations
- Homodisomy
- D3+ D8 with BAP1 aberrant
- D3 with aberrant BAP1
- M3 with retained BAP1
- D3 with multiple 8p gains
- Loss 3p with 8q gain
- Gain 3p with 8q gain
- D3 + D8 without 6p gain
- M3 with loss 8p, gain 6p, loss 6q

Simplified TCGA; Jager et al

JAGER ET AL

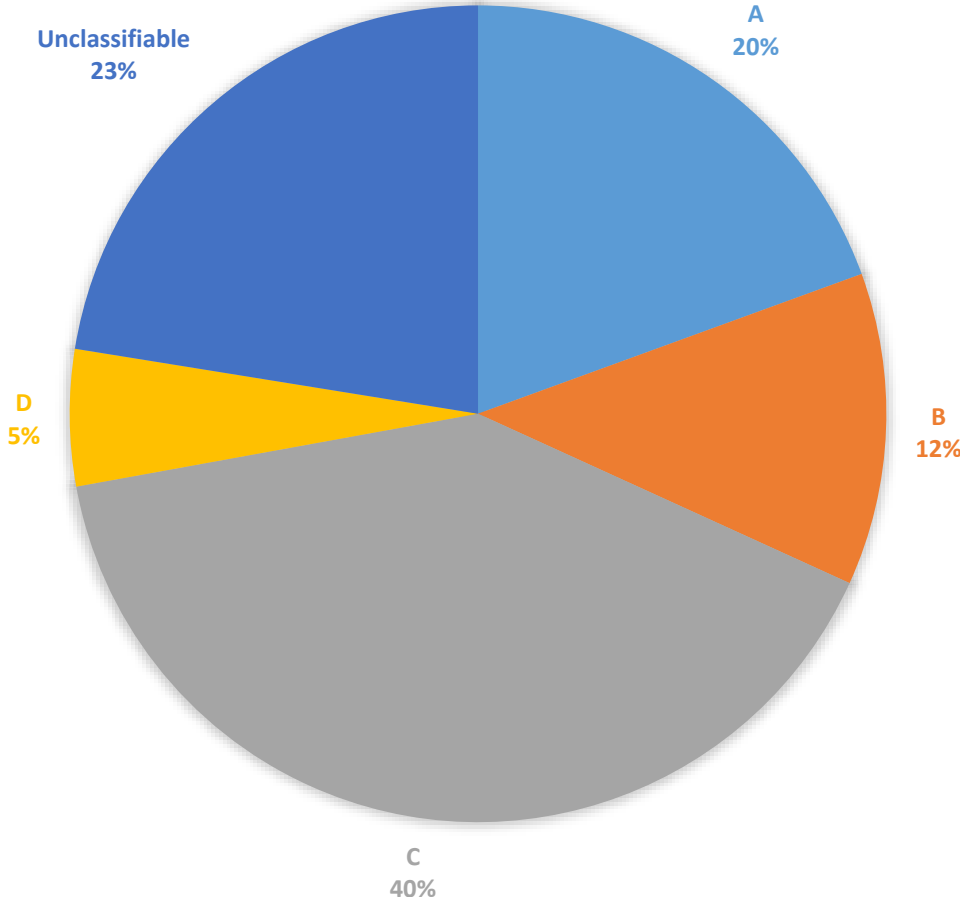


Unclassifiable features:

- D3 with multiple whole 8q gains (majority)
- Gain 8p
- D3 with 6p gain + Loss 6q
- Subclonal M3
- Partial M3
- Segmental loss 3p
- Subclonal loss 3q
- Gain 3p alone
- M3 alone
- M3 with partial 8q gains

Chromosomal change in chr. 3 and 8 alone; Shields et al

SHIELDS ET AL

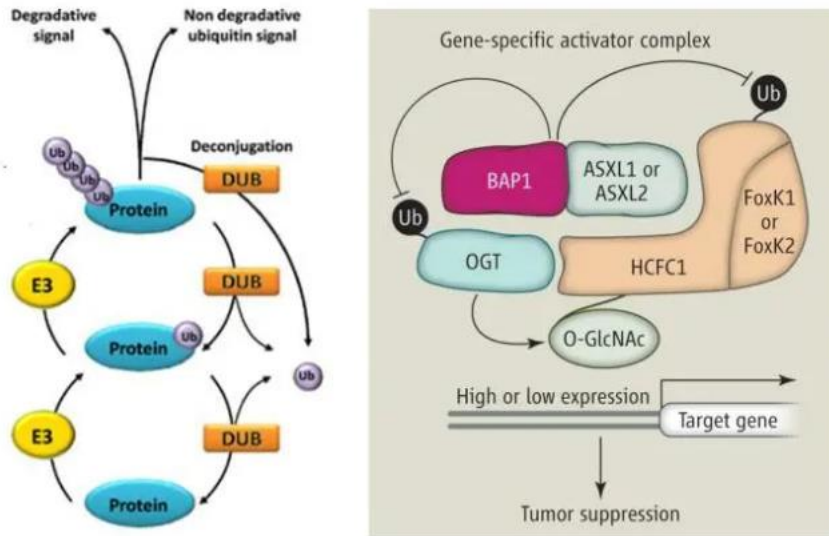


Unclassifiable features:

- Subclonal M3
- Partial M3
- Loss 3p alone
- Loss 3q alone
- Segmental loss 3p
- Subclonal loss 3q
- M3 alone
- M3 with loss 8p
- M3 with D8

BAP1 mutation in UM

BAP1 is a de-ubiquitinating enzyme (DUB) found in the nucleus



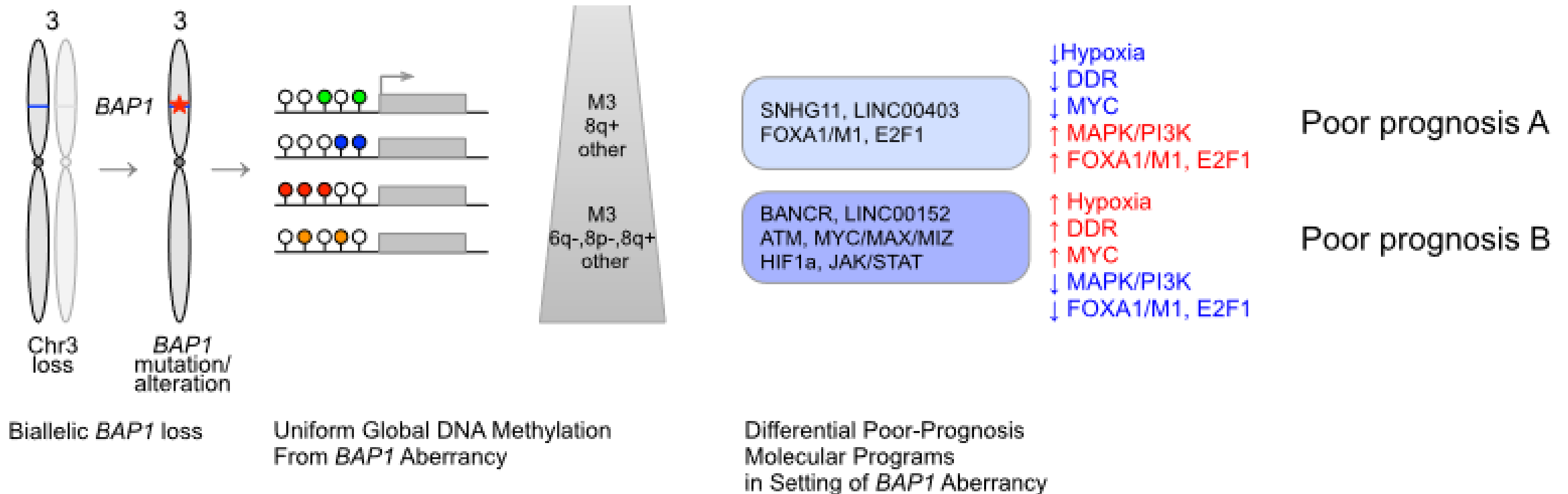
- Somatic BAP 1 mutations- poor prognosis UM
- Loss of BAP 1 gene expression can be documented well with IHC

Melanocytic phenotype loss

Loss of differentiation

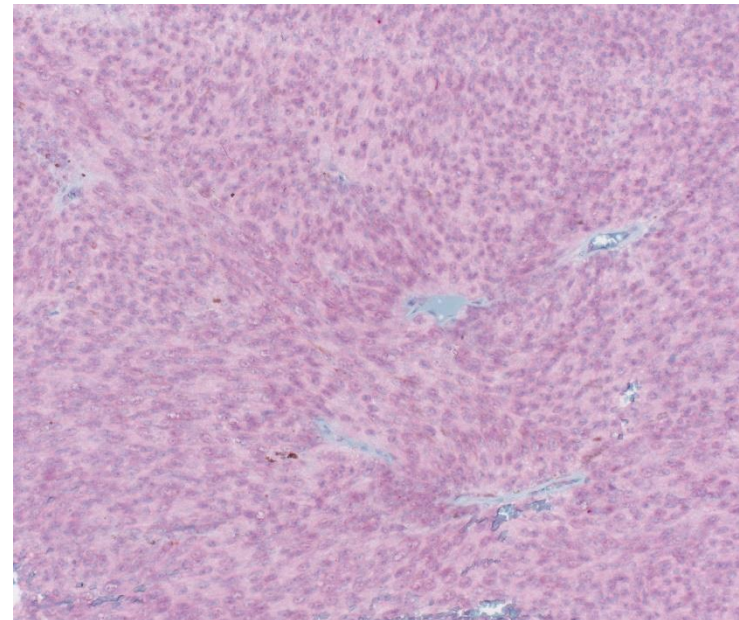
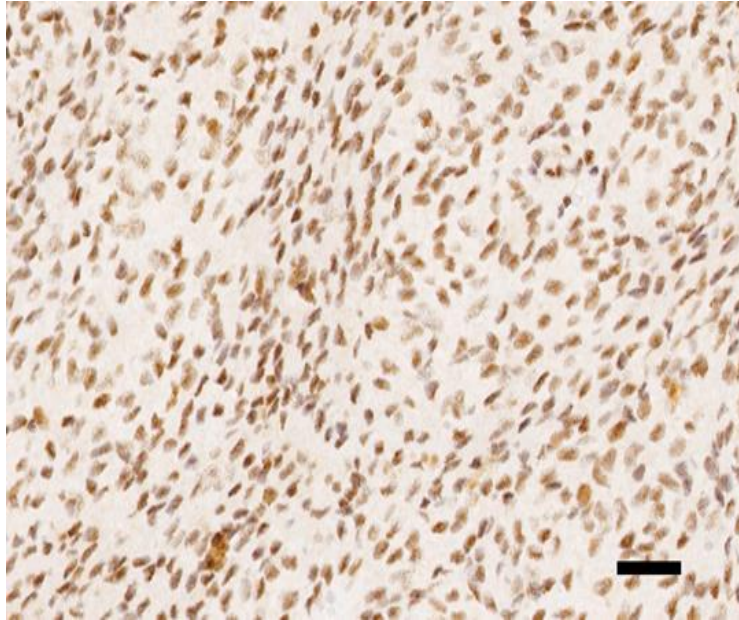
BAP1 germline mutations: 20% of familial cases of UM (Rai et al. 2017).

BAP1 mutations in TCGA



Robertson et al studied BAP1 mutations through DNA and RNA sequencing
 BAP1 mRNA expression: significantly higher in SCNA clusters 1 and 2 (D3) than in SCNA clusters 3 and 4 (M3)
 Identified BAP1 alterations in 85% of M3-UM
 BAP1 IHC was not done

BAP 1 status by IHC



BAP 1	Aberrant	Retained
M3	54 (85%)	6 (9.5%)
D3	6 (11.7%)	36 (70.5%)
Total	63	42

Of 214 in cohort, BAP1 IHC was conclusively resulted in 154 (72%)

Discordant BAP 1 : 24%

Clinical Utility: Current TCGA limitations

- Applying original – too complex
- Unclassifiable:
 - TCGA : 27%
 - Simplified TCGA: 43%
 - Modified TCGA: 23%
- Clinical prognostication in unclassifiable remains unanswered
- Group D variability: (10%/14%/5%): High variability- Risk stratification remains challenging
- BAP 1 IHC useful and practical, not included
- Discordant BAP1 IHC – difficult clinical decision making
- All modalities not available in all centres, not feasible in all specimens

Conclusion

- Genetics of UM is a rapidly advancing field
- Large panel NGS - new variants identified, clinical significance evolving
- Further refinement of prognostication is necessary

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