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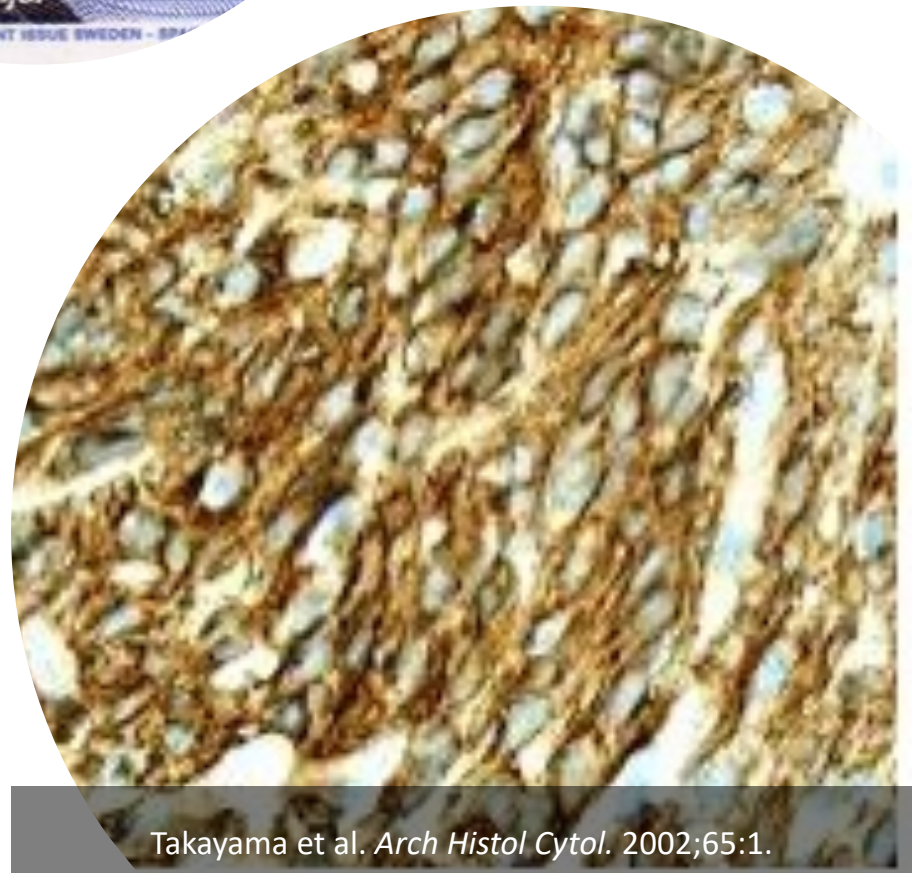
GIST, Osteosarcoma, Soft Tissue Sarcoma

Gastrointestinal Stromal Tumor (GIST)

- Arise from pacemaker cells of the gut originally described by Cajal
  - Intercalated between intramural neurons and smooth muscle cells
  - Generate electrical slow waves
- **KIT-positive** fibroblast-like cells
- 95% of reported cases of GIST are positive for KIT (CD117)

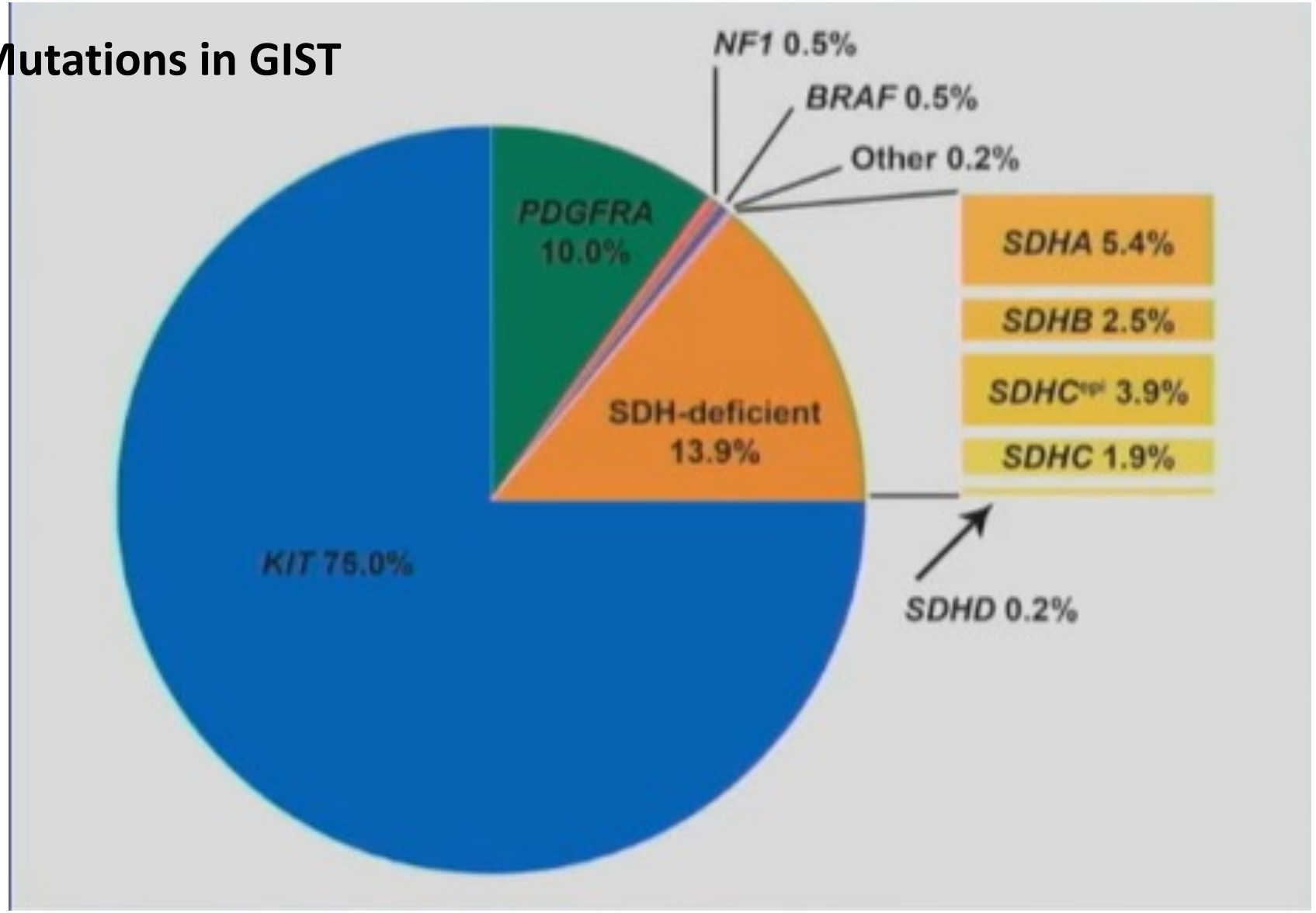


Takayama et al. *Arch Histol Cytol.* 2002;65:1.

GIST

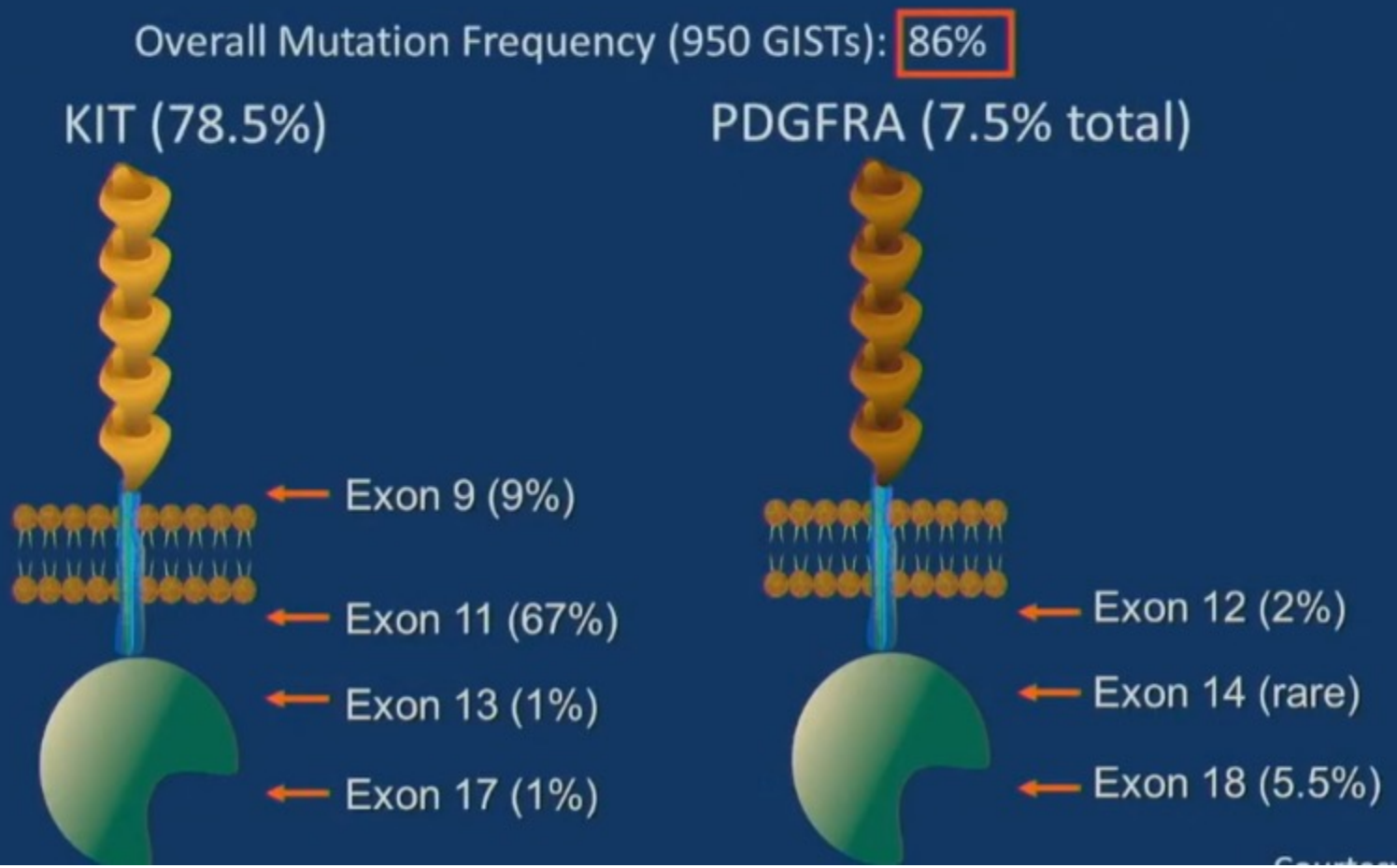
- Sporadic
- Genetic syndromes
  - Germ line KIT/PDGFRA
  - Carney-Stratakis Syndrome
    - Germ line SDH subunit mutations, autosomal dominant
    - GIST and paraganglioma
  - Carney triad
    - Nonhereditary, SDHC promoter hypermethylation
    - GIST+ paraganglioma + Pulmonary Chondroma
  - Neurofibromatosis
    - Germline NF-1 mutations
    - Autosomal dominant

Primary Mutations in GIST



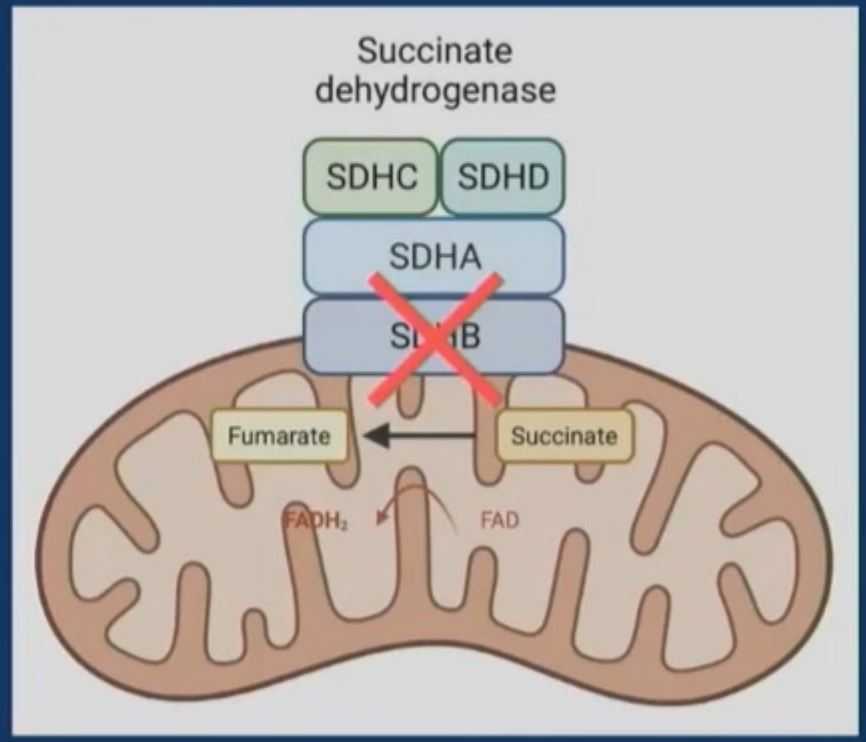
KIT and PDGFRA gain-of-function mutations are primary drivers of oncogenic signal in GIST

Overall Mutation Frequency (950 GISTs): 86%



SDH-deficient GISTs have distinct biologic and morphologic features

- Loss-of-function mutations of *SDHA*, *SDHB*, *SDHC*, or *SDHD* in ~80% of cases
- *SDHC* promoter methylation (*SDHC*-"epimutated") in ~20% of cases
- → Inactivation of SDH complex causes global epigenetic changes
- Loss of function of <u>any</u> subunit leads to SDHB loss by IHC → useful diagnostic marker



Settas N, *Mol Cell Endocrinol*. 2018

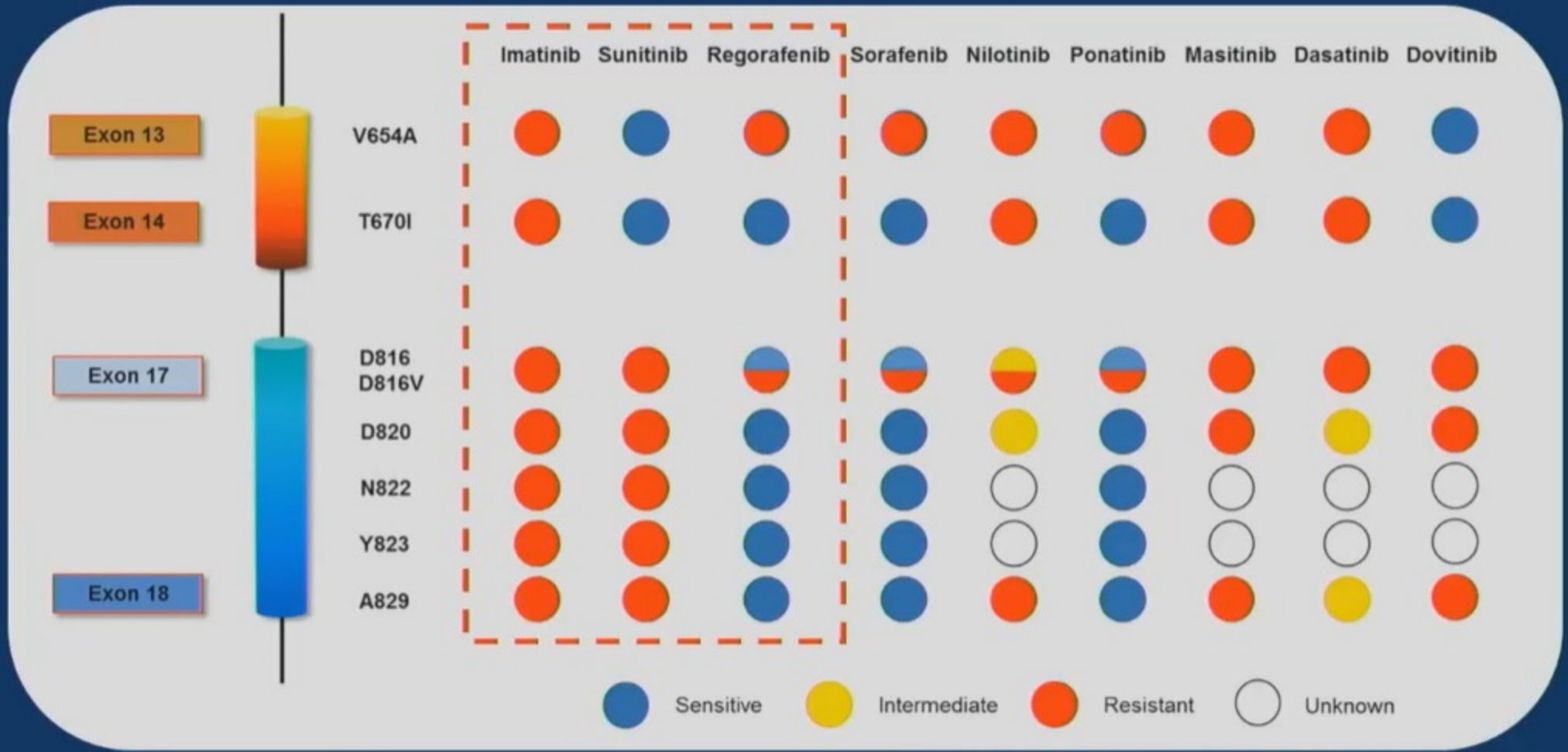
GIST recurrence after Primary resection

Recurrence dependent on # mitoses, size of tumor, anatomic location

Mitotic Index (per 50 HPFs)	Tumor Parameter		Risk of Progressive Disease (%)		
	Size (cm)	Gastric (%)	Duodenum	Jejunum/Ileum	Rectum
≤ 5	≤ 2	0 (none)	0 (none)	0 (none)	0 (none)
≤ 5	> 2 to ≤ 5	1.9 (very low)	4.3 (low)	8.3 (low)	8.5 (low)
≤ 5	> 5 to ≤ 10	3.6 (low)	24 (moderate)	Insufficient data	Insufficient data
≤ 5	> 10	10 (moderate)	52 (high)	34 (high)	57 (high)
> 5	≤ 2	None*	High*	Insufficient data	54 (high)
> 5	> 2 to ≤ 5	16 (moderate)	73 (high)	50 (high)	52 (high)
> 5	> 5 to ≤ 10	55 (high)	85 (high)	Insufficient data	Insufficient data
> 5	> 10	86 (high)	90 (high)	86 (high)	71 (high)

Abbreviation: HPF, high-power field.
*Small numbers of cases.

KIT / PDGFRA secondary genotype predicts response to TKIs



Heinrich, *JCO* 2008; Serrano, *BJC* 2019; Serrano, *Oncotarget* 2019

Metastatic GIST

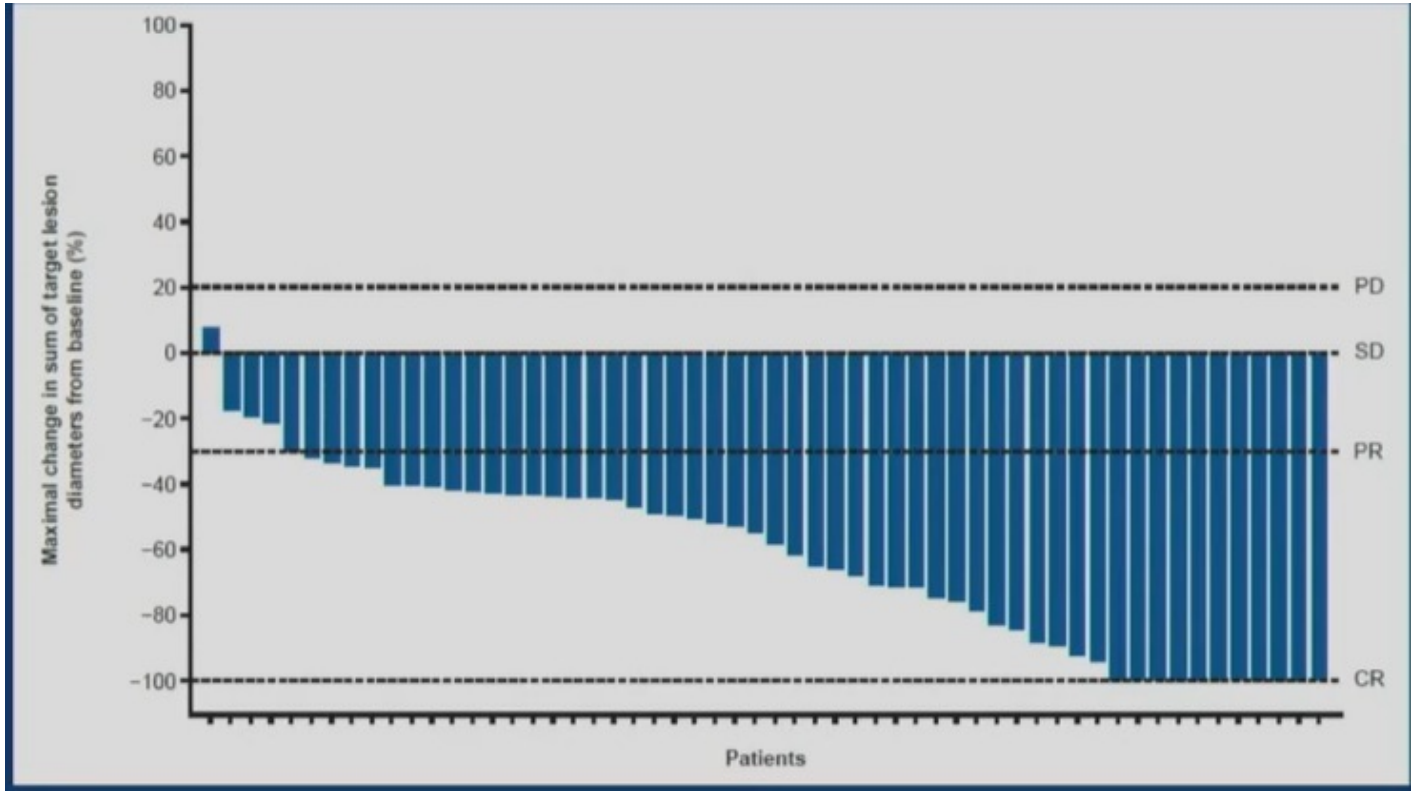
	Imatinib (n=147)	Sunitinib (n=207)	Regorafenib (n=133)
ORR	68.1%	6.8%	4.5%
$SD_{12\ weeks}$	15.6%	53%	48.1%
TTP/PFS	24 mo	5.6 mo	4.8 mo

Imatinib resistant GIST

- Ripretinib
  - Binds to activation loop and switch pocket
  - Phase III INVICTUS trial:
  - Ripretinib v placebo 2:1, Crossover allowed
  - 129 pts: heavily pretreated: ≥ 3 L
  - mPFS: 6.3 v 1 m
  - ORR 9.4%
  - OS 15.1 v 11.6 (crossover) v 1.5m
  - Grade 3 AE rare, Grade 1-2 Alopecia 49%, HFS 21%

Imatinib Resistant GIST: Avapritinib

- PDGFRA exon 18 D842V
- Phase I Navigator trial
  - N=56
  - ORR **91%**
  - DCR ≥ 16 weeks, 100%
  - mPFS 34 m
- Adverse effect
  - Cognitive effects 40%
    - Memory impairment
    - Confusional state
    - Psychosis



GIST Summary

- Mutational analysis should be performed on all patients with GIST to guide appropriate primary therapy and to assess resistance mechanism at progression

- GIST progression is associated with accumulating mutations
  - Avapritinib has significant activity against PDGFRA D842V mutation

- wt-KIT associated with SDH mutations, no standard therapy
  - some activity of VEGF inhibitors

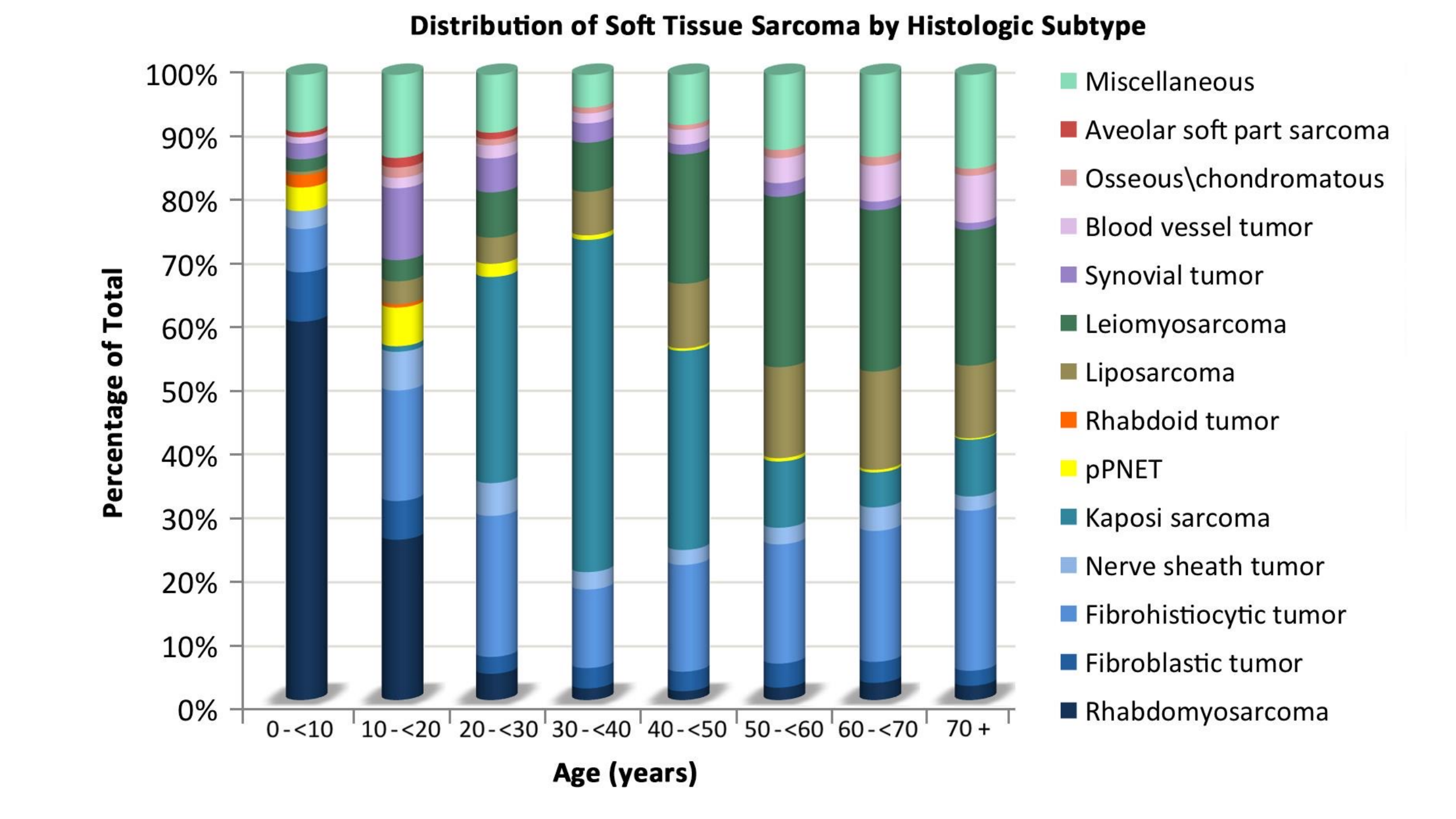
SOFT TISSUE SARCOMA

Soft Tissue Sarcoma 2022 ACS estimates

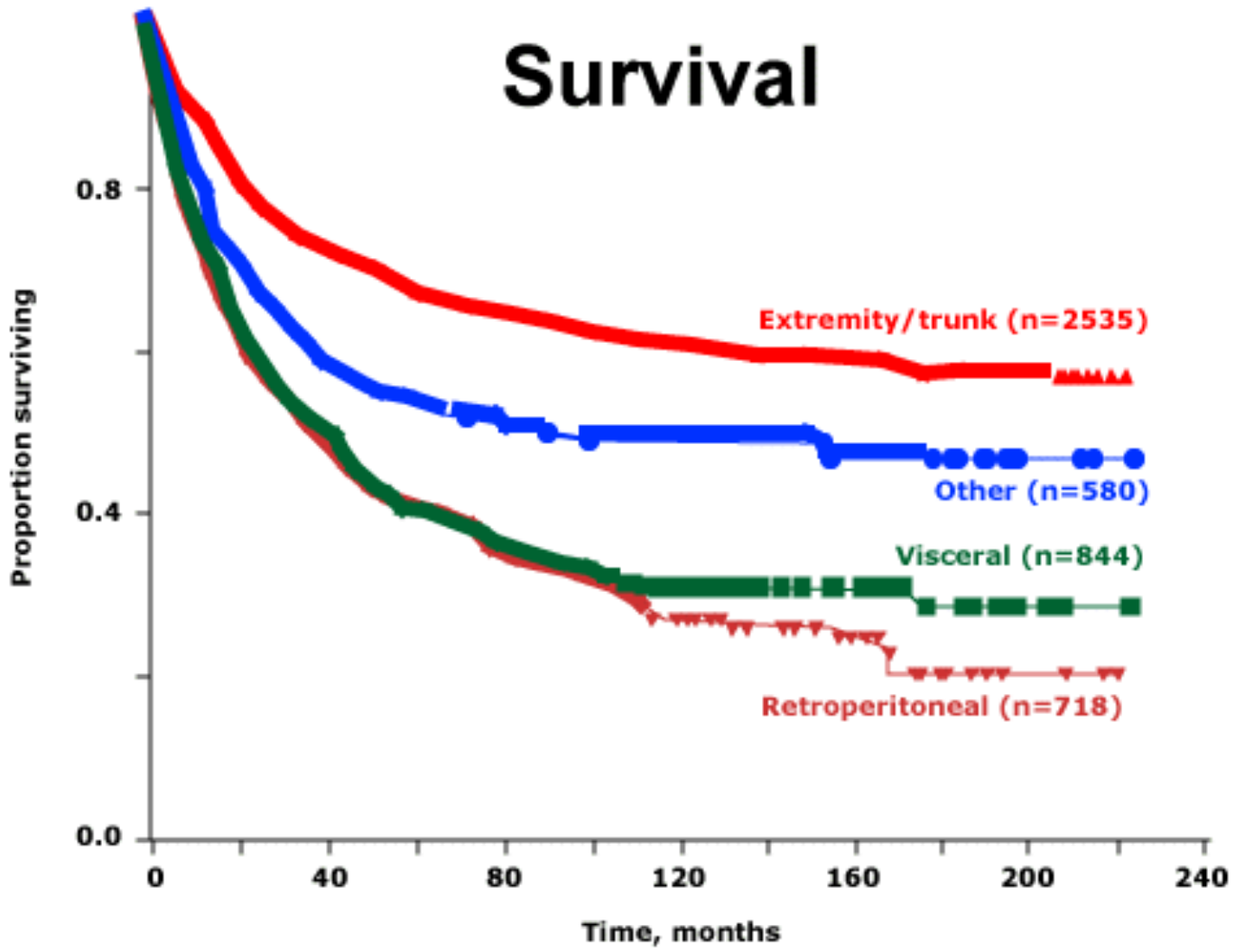
Incidence	13,190
Annual Deaths	5,130
5 yr survival	65%

Pitfalls in Sarcoma Treatment and Research

- Extensive variation in histologic subtypes (n > 50)
  - Descriptor “soft tissue sarcoma” is not a single entity
  - Specific molecular alterations may identify unique tumor types
  - Discordant diagnoses among pathologists at different centers problematic (≈30%)
  - Similar molecular alterations may occur with different morphologic appearance (e.g., NTRK fusion)
- Incidence across the age spectrum from childhood to late adulthood
  - Distinct histologies occur in various age ranges
- Tumors of similar histology may occur in disparate locations complicating surgical access and local control


Distribution of Soft Tissue Sarcoma by Histologic Subtype
Percentage of Total
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0 - <10
10 - <20
20 - <30
30 - <40
40 - <50
50 - <60
60 - <70
70 +
Age (years)
Miscellaneous
Aveolar soft part sarcoma
Osseous\chondromatous
Blood vessel tumor
Synovial tumor
Leiomyosarcoma
Liposarcoma
Rhabdoid tumor
pPNET
Kaposi sarcoma
Nerve sheath tumor
Fibrohistiocytic tumor
Fibroblastic tumor
Rhabdomyosarcoma

MSKCC
1982-2001



Survival in primary soft tissue sarcoma according to primary site

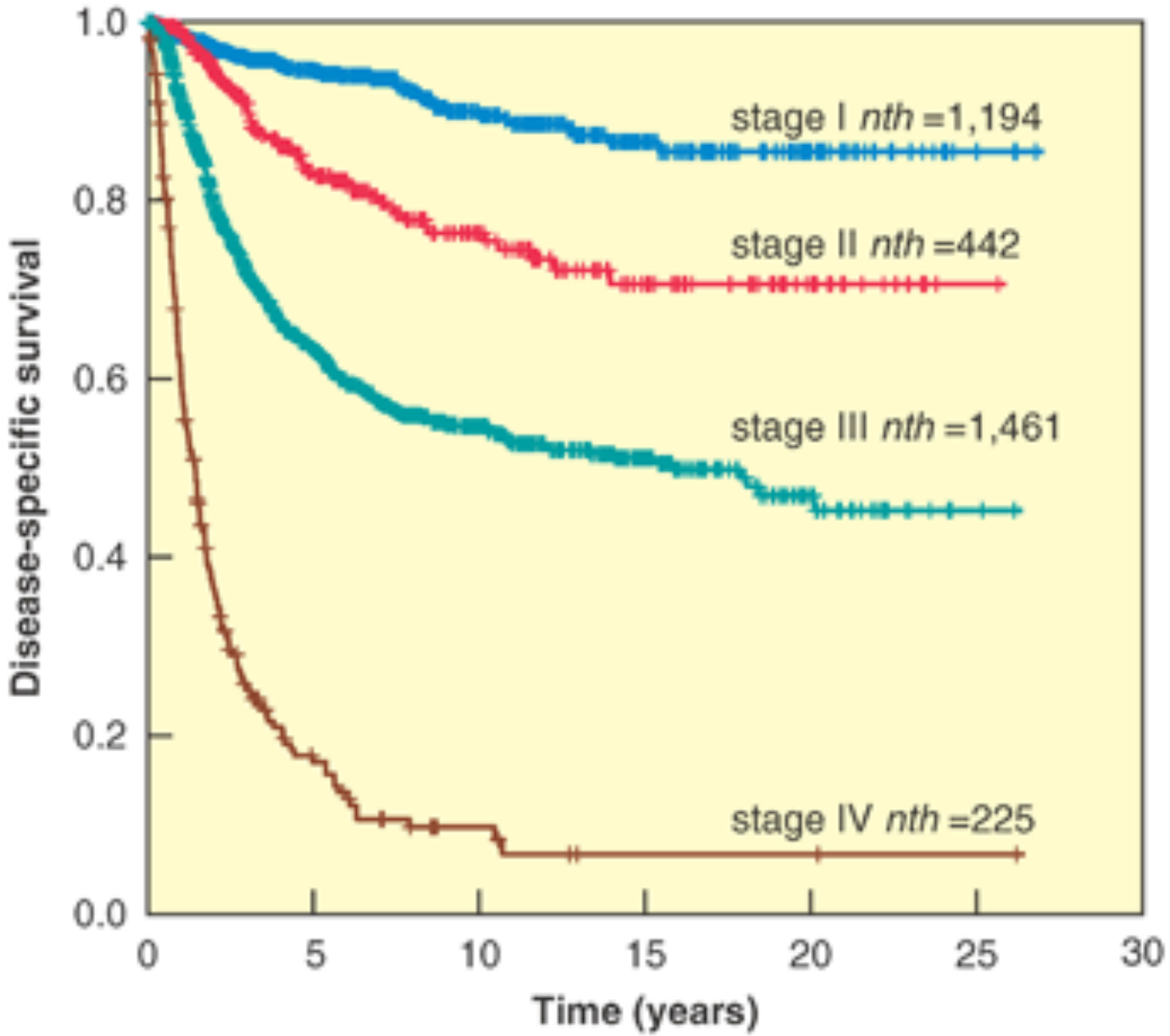


Figure 90.3 Disease-specific survival for patients with extremity soft tissue sarcoma according to the 2010 American Joint Committee on Cancer staging system. The data are for 3,322 patients seen at Memorial Sloan Kettering Cancer Center from 1982 through 2013.

Genomic alterations in STS

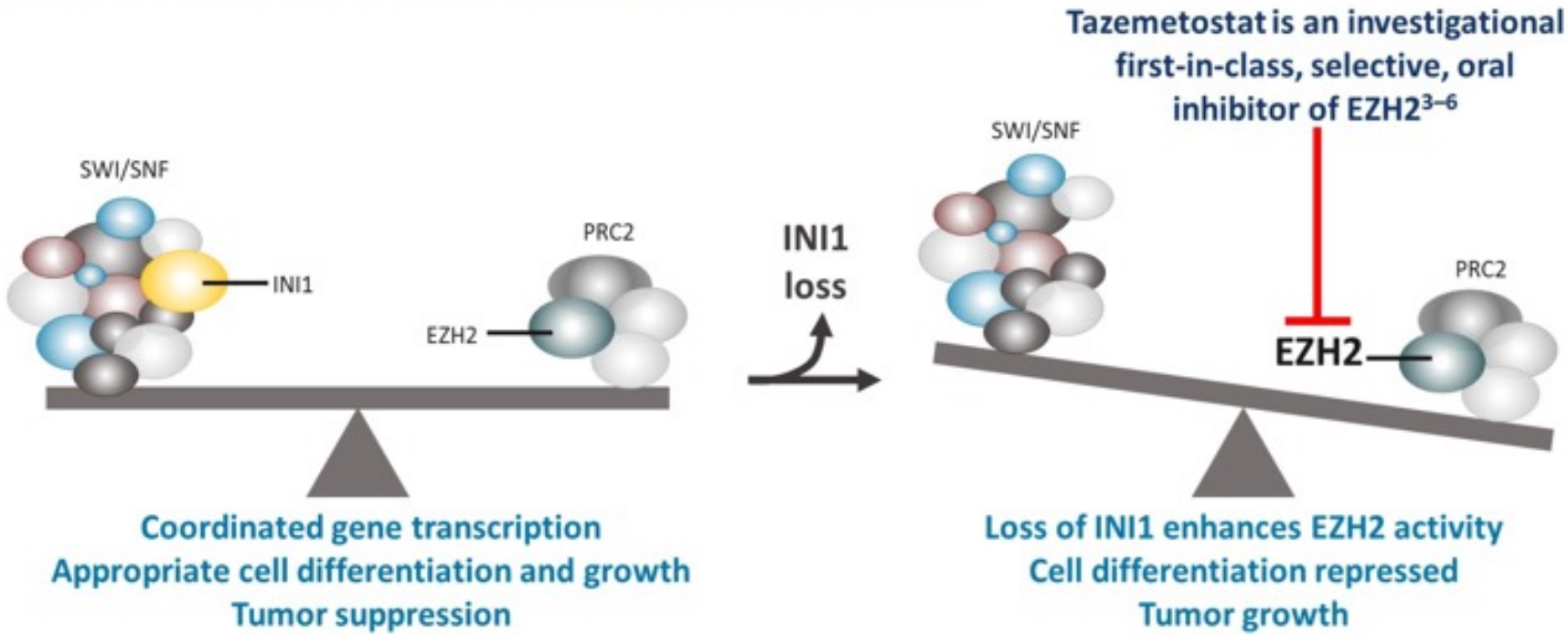
- Single reciprocal translocations
  - Ewing's sarcoma (EWS-FLI-1): transcription factor
  - Synovial sarcoma t(X;18), chromatin remodeling protein
  - Round cell liposarcoma (TLS-CHOP, EWSR1-CHOP [DDIT3])
- Specific oncogenic mutations or recurrent amplification
  - WD/DD Liposarcoma: CDK4/6 amplification
  - Epithelioid sarcoma – INI-1 deficient
- Complex karyotypic abnormalities
  - Leiomyosarcoma, undifferentiated pleomorphic sarcoma (UPS), angiosarcoma

Actionable mutations in Sarcoma

Disease	Actionable Genetic Alteration	Rx
ASPS	ASPSL-TFE-3	Cediranib
Alveolar Rhabdomyosarcoma	PAX3-FHKR (FOXO1A)	Temsirolimus + Cixutumumab
Dedifferentiated Liposarcoma	CDK4 amplification, MDM2	Palbociclib
DFSP	COL1A1-PDGFB	Imatinib
Epithelioid sarcoma	INI-1 deficient	**Tazemetostat**
Fibromatosis	CTNNB1 or APC	Imatinib, sorafenib
GIST	C-kit PDGFRA D842V Mutation	Imatinib **Avapritinib**
Infantile Myofibroblastic Tumor	TPM3-ALK	Crizotinib, Ceritinib
NTRK fusion sarcomas	NTRK	**Larotrectinib**
PEComa	LOH TSC1	**mTOR inhibitor** (sirolimus, everolimus)
Solitary fibrous tumor (SFT)	NAB2-STAT6	VEGF TKI, sunitinib
Tenosynovial Giant Cell tumor	COL6A3-CSF1	**Pexidartinib**

BACKGROUND

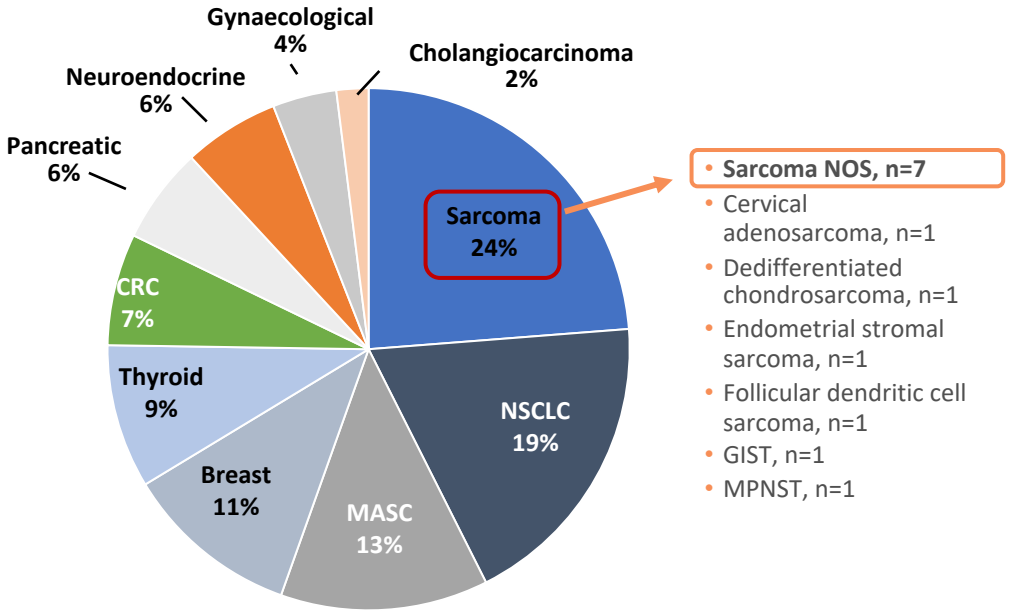
LOSS OF INI1 CREATES AN ONCOGENIC DEPENDENCY ON ENHANCER OF ZESTE HOMOLOG 2 (EZH2)



	Histology	#pts	PR	SD	CBR	
Tazemetostat 800 mg po BID	Epithelioid sarcoma > 90% INI-1(-)	62	9 (15%); 6 1L (24%)	35	71%	selective inhibitor of EZH2 Phase II: 51% pts with tumor regression, mPFS 23.7 wks: no prior Rx 42.1 wks; (c/w doxo 12wks) DOR NR (34.1wks –NE)

NTRK gene fusions in Sarcoma

- *NTRK* genes (NTRK1, NTRK2 or NTRK3) encode the TRK receptors (TRKA , TRKB and TRKC, respectively)[1,2]
- Around ¼ of adult *NTRK* gene fusions positive tumours are sarcomas[2]



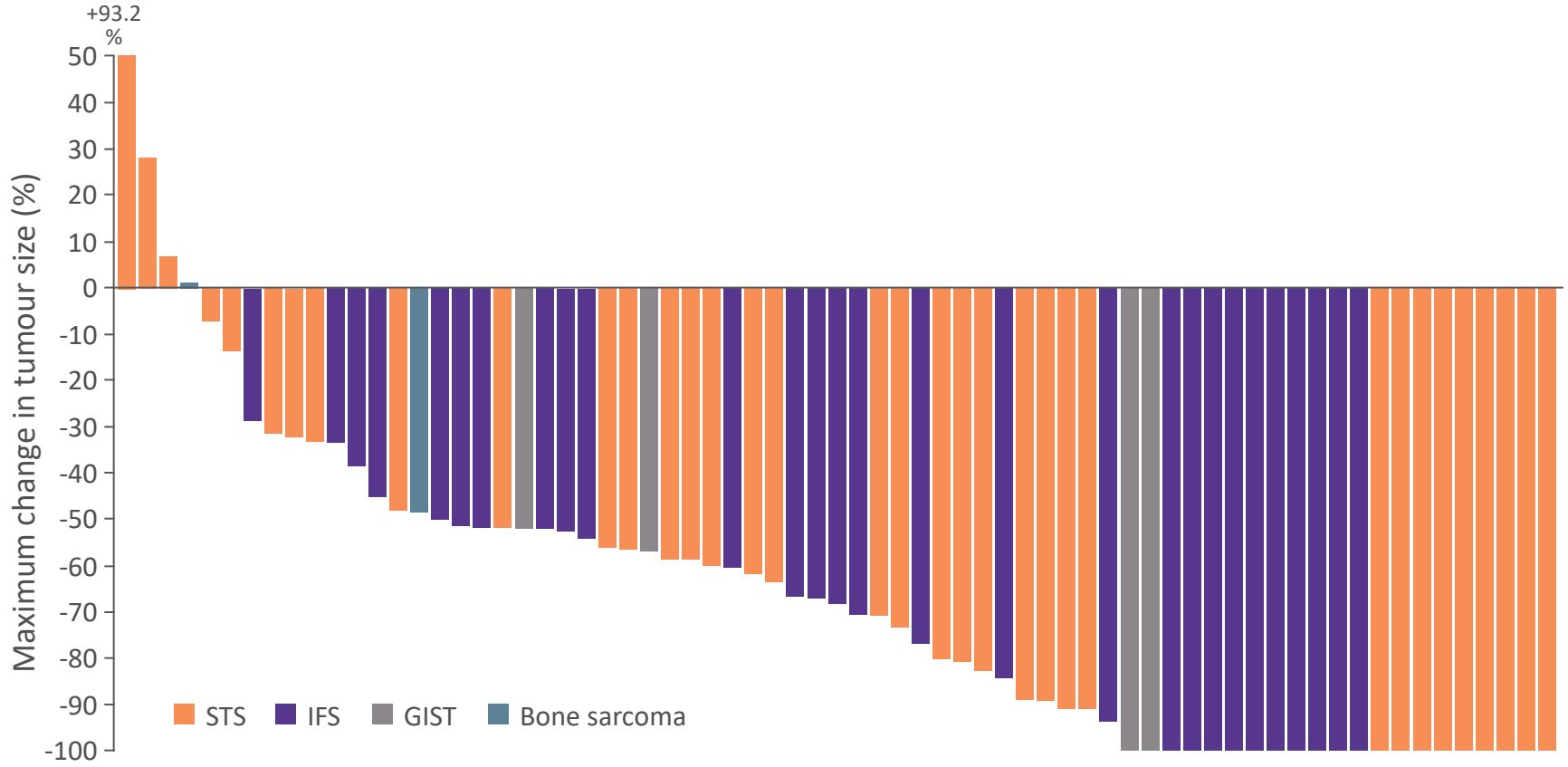
- Low incidence in sarcomas < 5%

CRC, colorectal cancer; GIST, gastrointestinal stromal tumour; NOS, not otherwise specified; MASC, mammary analogue secretory carcinoma; MPNST, malignant peripheral nerve sheath tumor, NSCLS, i lung cancer; *NTRK*, neurotrophic tropomyosin-related kinase; TRK, tropomyosin receptor kinase

1. Cocco, et al. Nat Rev Clin Oncol 2018;15:731–47; 2. NTRK CONNECT BluePrint. Available from: https://ntrkconnect.info/ntrk-gene-fusions-trk-inhibitors-and-testing-approaches-blueprint/. Accessed March 2020; 3. Doebele, et al. Lancet Oncology 21:271-282;

TRK-INHIBITION PROVIDES ROBUST RESPONSES IN PATIENTS WITH *NTRK* GENE FUSION-POSITIVE SARCOMA

EFFICACY OF LAROTRECTINIB IN SARCOMAS HARBOURING TRK FUSIONS: BEST CHANGE IN TARGET LESIONS



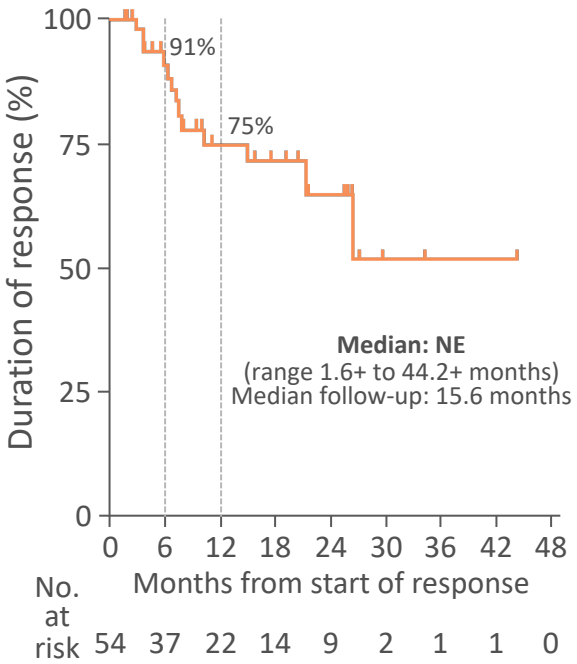
Data cut-off: Feb 19, 2019

IFS, infantile fibrosarcoma; *NTRK*, neurotrophic tropomyosin receptor kinase; STS, soft tissue sarcoma; TRK, tropomyosin receptor kinase; GIST, gastrointestinal stromal tumour

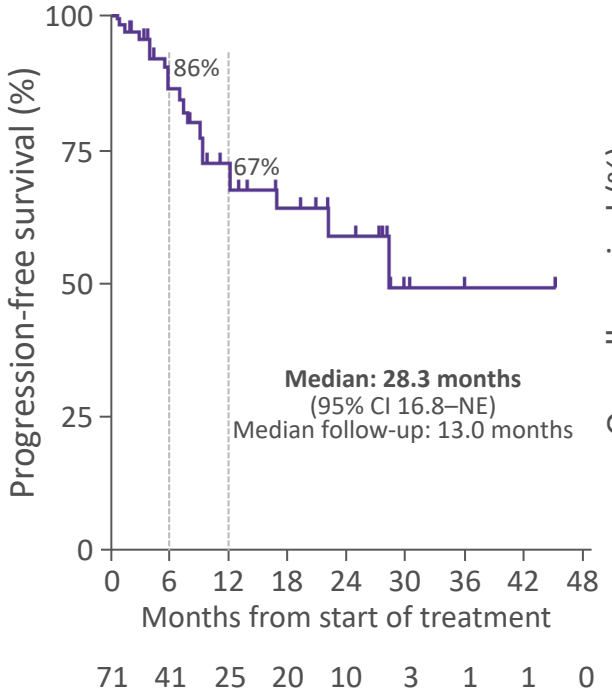
Demetri GD, et al. CTOS 2019. Abstract #3254588

TRK-INHIBITION PROVIDES DURABLE RESPONSES IN PATIENTS WITH *NTRK* GENE FUSION-POSITIVE SARCOMA

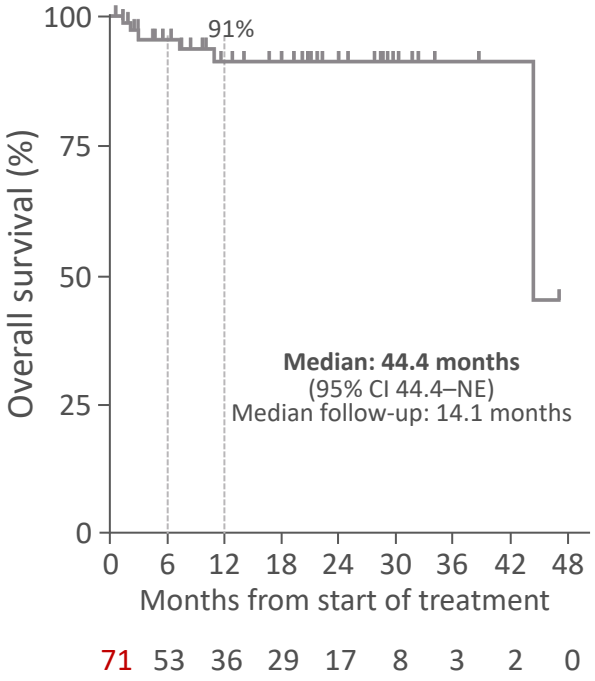
DURATION OF RESPONSE



PROGRESSION-FREE SURVIVAL



OVERALL SURVIVAL



Data cut-off: Feb 19, 2019

NE, not estimable; NTRK, neurotrophic tropomyosin receptor kinase; TRK, tropomyosin receptor kinase

Demetri GD, et al. CTOS 2019. Abstract #3254588

New Agents

	Histology	# Pts	CR/PR	SD	Comments
Nab-sirolimus IV qw 2/3	PEComa	31	1/11 (39%) 5 > 1 y	52%	25: mutations TSC2 (9) Responses 8/9 mPFS 10.6 m; mOS 40.8 m
Abemaciclib 200 mg po BID	DDLPS	30 50% = 1L	1 PR 3 (>10% ↓)		PFS @ 12 wks: 76% (c/w 40%) mPFS 30.4w AEs gr 3/4: cytopenias, diarrhea
Palbociclib 125 mg/d x 3 wks	LMS, other (77%)	22	0	11 (50%)	Heavily pretreated SFT, LMS best response, 30% 6 mo PFS Neutropenia, thrombocytopenia Correlation between mPFS/OS and expression of ↑CDK4, ↓CDKN2A

APROMISS Trial

- Phase III trial of AL3818 (anlotinib/catequentinib) v DTIC
- Multi-targeted TKI: VEGFR, C-kit, PDGF β, FGFR1/2/3
- Second line therapy
- Randomized 2:1
- 79 pts
- mPFS: 2.89 v 1.64 mos (p = 0.0015)
-

PFS@	4m	6m	12m
TKI (%)	48.1	42.3	26.9
DTIC (%)	14.8	11.1	3.7

Epithelioid Hemangioendothelioma (EHE)

- Translocation of WWTR1 (TAZ) and CAMTA1 results in fusion protein → activation of MAP kinase pathway
- Tremetinib (MEK inhibitor) 2 mg po daily
- No. Patients: 42 F>M
- TAZ-CAMTA1
  - Positive 27; Negative 7
- AE (#pts): rash (35) fatigue (22), alopecia (12), edema (11) GI, anemia (14), hypoalbuminemia (11)
- 0 CR, 3 PR (TAZ-CAMTA1 negative), SD 40% > 6 months
- mPFS 8.2 m, OS 15 m
- QOL: decreased pain at 4 weeks

Immunotherapy and STS

Agent	ORR (%)	mPFS (m)	RR by subtype	
Ipilimumab	0	1.9	SS	Maki, 2013
Pembrolizumab	**18**	4.5	**UPS 23%** (2 CR), LPS 10%	Burgess, 2019
Atezolizumab	**42**	NR	**ASPS**	Coyne, 2018
Nivolumab	0	1.8	Uterine LMS	Ben-Ami, 2017
Nivolumab Nivolumab +Ipilimumab	5 **16**	1.7 4.1	ASPS, LMS **UPS 28.6**, LPS 14.3	D'Angelo, 2018
Durvalumab/Tremelimumab	**14.3**	2.8	**ASPS 50%,** chordoma 20%, **AS/UPS 20%**	Somaiah, 2020

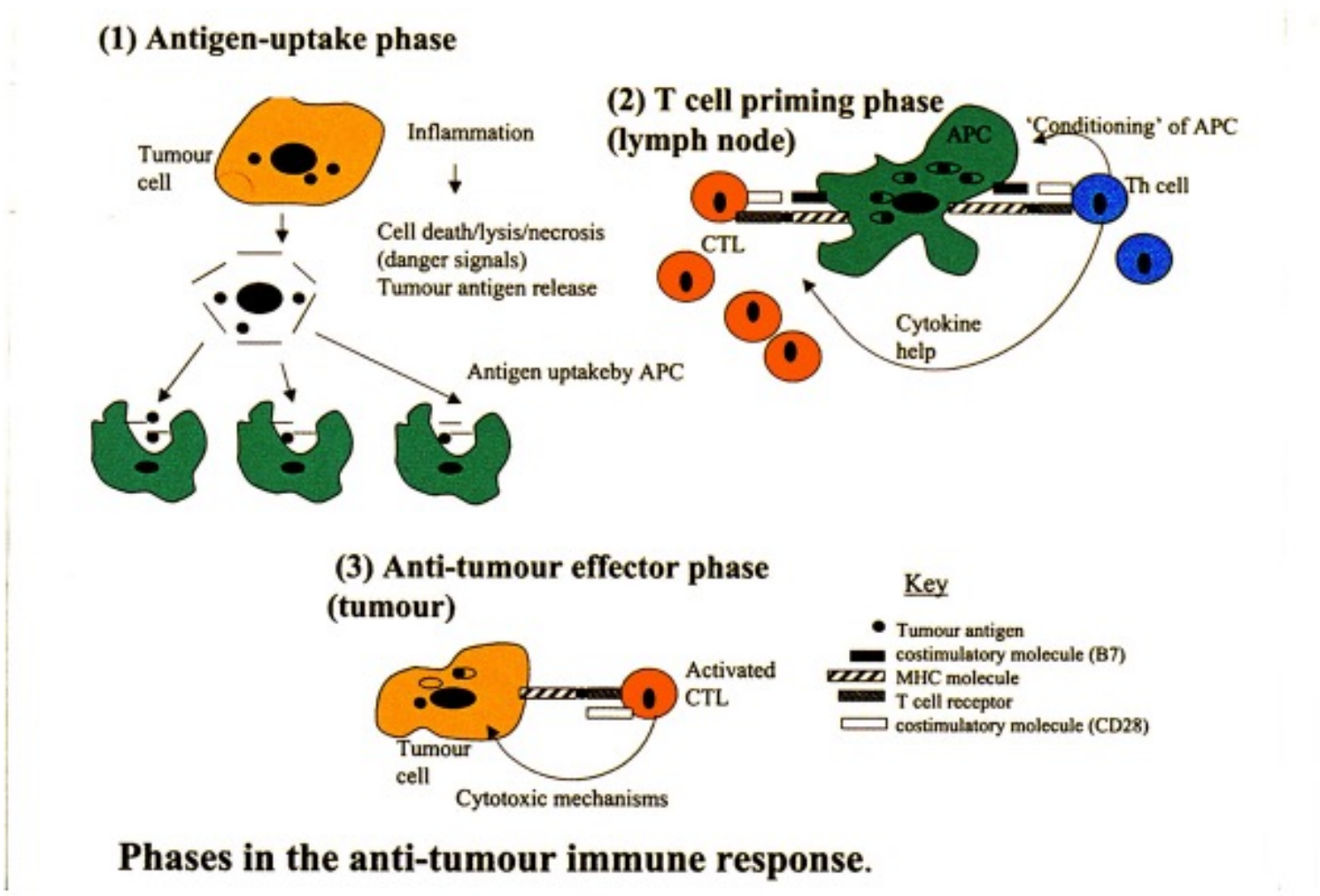
IO/chemo combinations

Agent	ORR (%)	mPFS (m)	RR by subtype	
Pembrolizumab	**18**	4.5	**UPS 23%** (2 CR), LPS 10%	Burgess, 2019
Pembrolizumab + Doxorubicin	**22**	7.8	**UPS 66%,** LPS 40%, LMS 30%	Pollack, 2019
Pembrolizumab + Cyclophosphamide	2	1.4	SFT	Toulmonde, 2018
Pembrolizumab + Eribulin	**5.3**	2.8	LMS	Nathenson, 2020
Pembrolizumab + Axitinib	**25**	4.7	**ASPS 54.5**; non-ASPS 9.5	Wilky, 2019
Nivolumab	5	1.7	ASPS, LMS	D'Angelo, 2018
Nivolumab + Sunitinib	9.3	5.9	AS, ESMC, SS, ASPS	Martin-Broto, 2019
Nivolumab + Ipilimumab + Trabectedin	**22**	NR	Multiple	Chawla, 2019

Predictors of Response to CPI

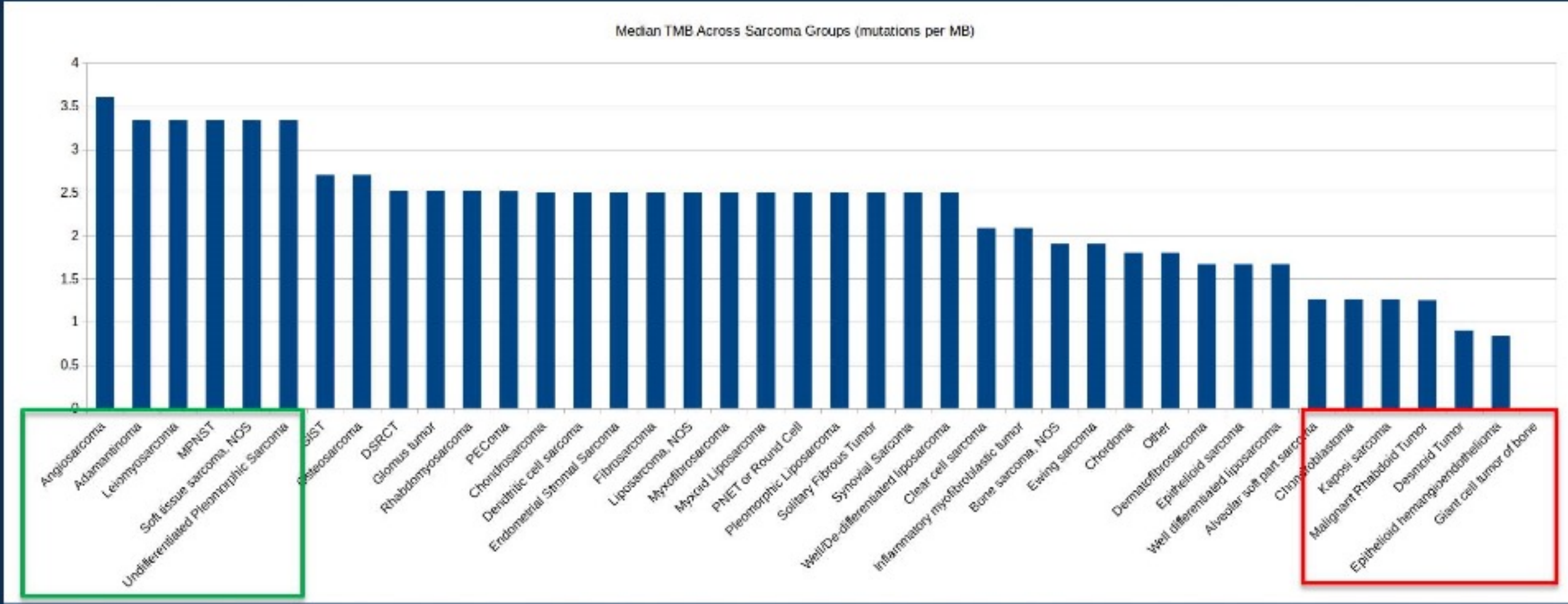
- Tumor Mutational Burden (TMB) > 10 mutations/MB
  - Surrogate for neoantigens
  - In general sarcomas have low TMB

- Microsatellite status – High
  - Sarcomas are microsatellite stable (MSS)



Phases in the anti-tumour immune response.

Tumor Mutational Burden for each Sarcoma Sub-types



Median TMB = 2.5 (0 – 328)

Gounder, ASCO 2017, FoundationOne analysis

Soft Tissue Sarcoma
Histology specific response to immunotherapy

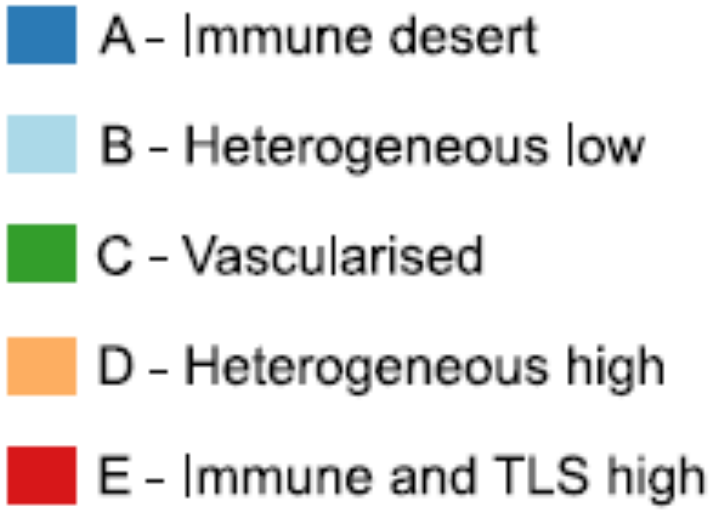
Histology	Drugs	Response Rate	Comment
Angiosarcoma	Anti-CTLA4, Pembrolizumab, Axitinib + Pembrolizumab	71%	High TMB in Head and Neck 1^{o}
ASPS	Atezolizumab Pembrolizumab + axitinib	42% 55%	MSH/MLH inactivation in 18-27% Noncanonical MMR pathways aberrant in 70%
UPS	Pembrolizumab Nivolumab + Ipilimumab	23% 29%	
DDLPS	Pembrolizumab Nivolumab + ipilimumab	10% 14%	
Uterine LMS	Nivolumab	0%	

Tawbi et al. Lancet Oncology 2018, Chen et al. ASCO 2020, Coyne et al. CTOS 2018, Florou et al. JITC 2019, Wilky et al. Lancet Oncology 2019, Ben-Ami Cancer 2017

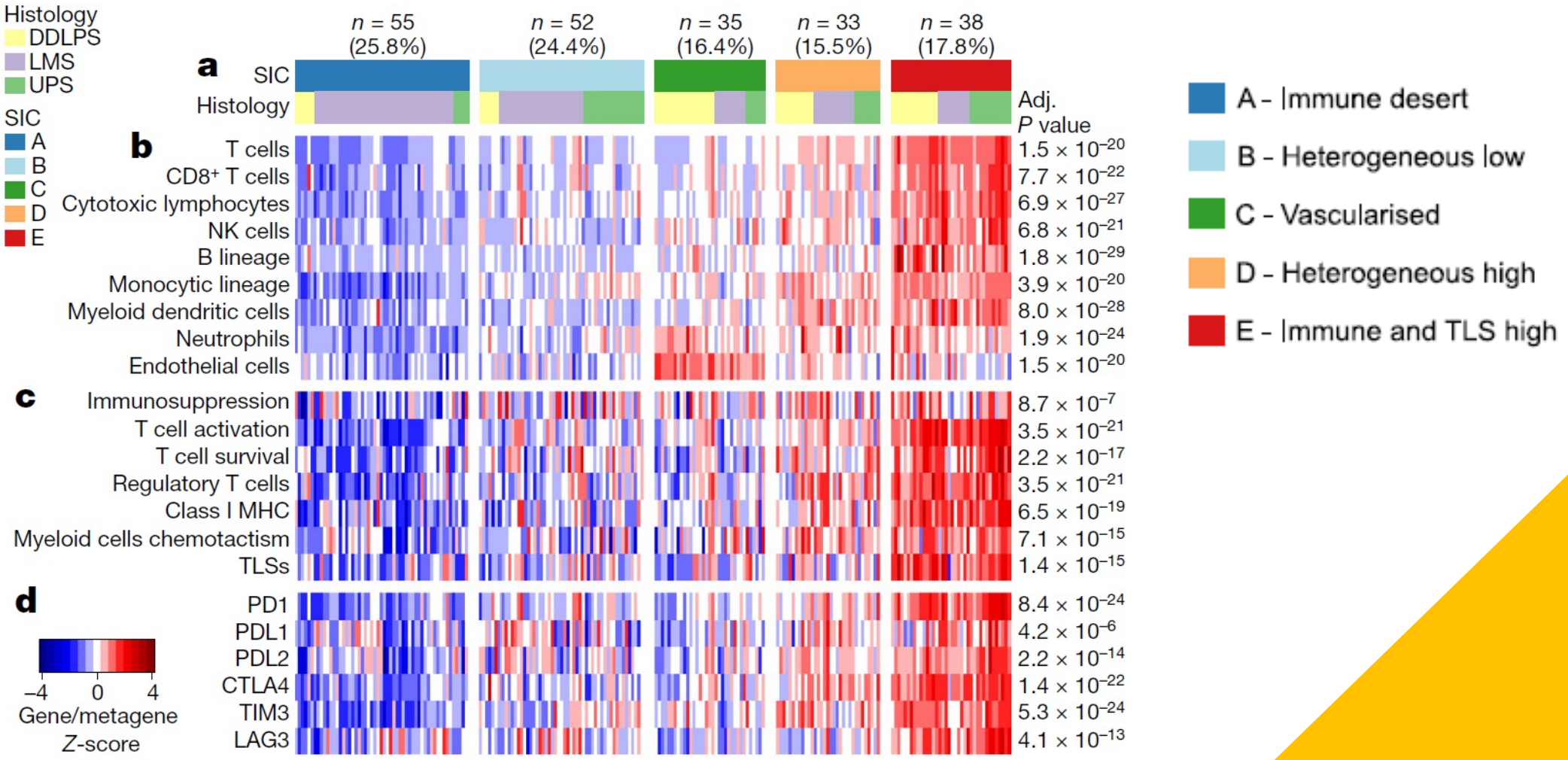
Sarcoma Immune Classification (SIC)

Petitprez, Nature, January 2020

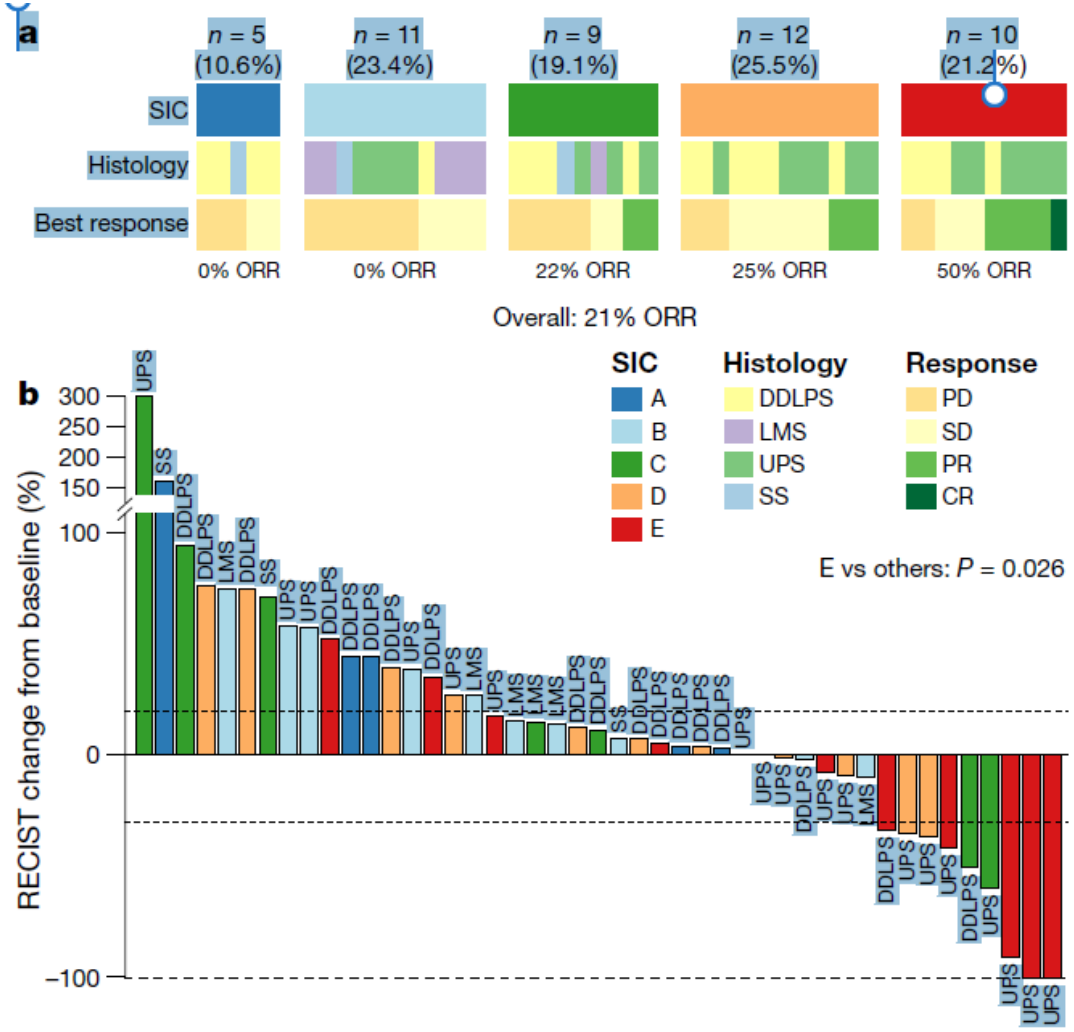
- Gene expression profiling of 4 independent cohorts
  - Composition of tumor microenvironment (TME) by MCP counter
    - e.g., T cells, NK cells, dendritic cells, endothelial cells, B cells
  - Functional orientation of immune TME incl tertiary lymphoid structures (TLS)
  - Expression of genes related to immune checkpoints
  - Association of SIC profile with histology



Sarcoma Immune Classification (SIC)/Sarc028/Pembro



Petitprez, et all Nature, 2020, 577:556



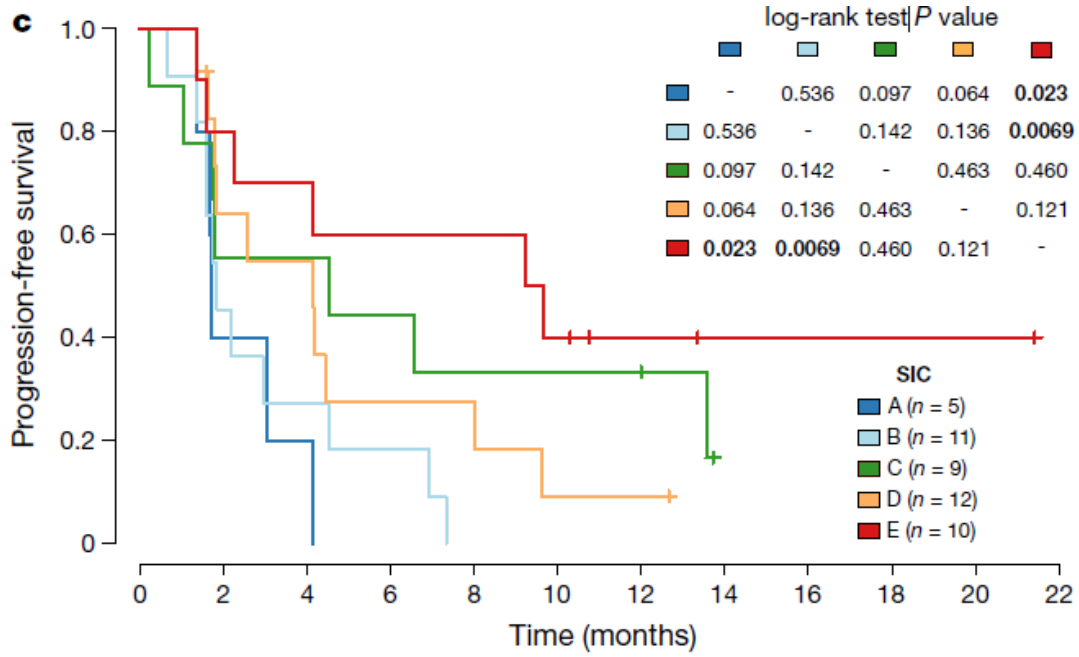
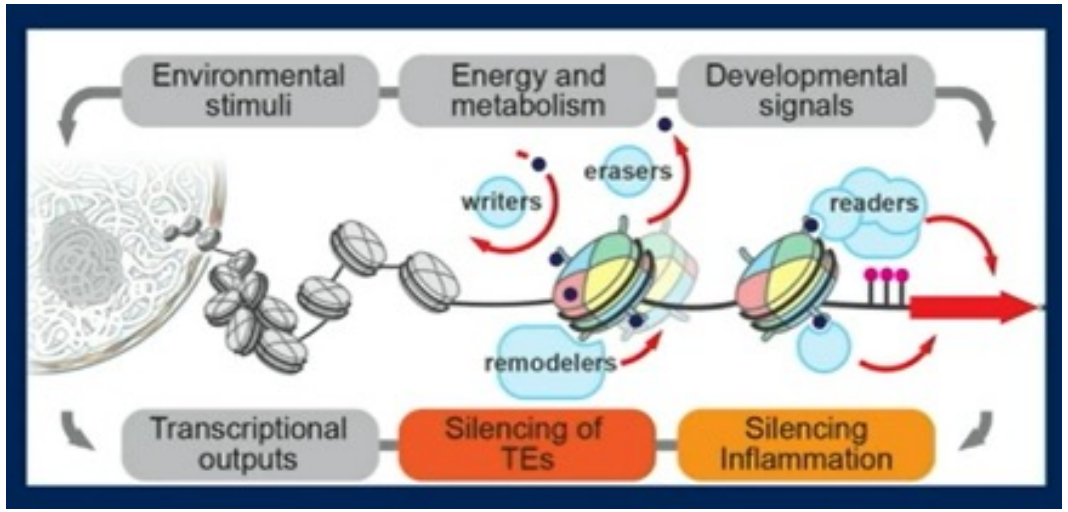


Fig. 4 | SICs are strongly associated with STS response to PD1 blockade

Tumor Immunogenicity

- Immune “hot” baseline profile of sarcomas associated with improved ORR to CPI
- Epigenetic states control inflammatory pathways by silencing transposable elements (TE) which are repetitive sequences, resembling viral sequences, representing a large part of genome



- De-repression of TEs linked to anti-tumor immunogenicity through antiviral inflammatory response
- IKZF1, transcription factor which interacts with chromatin modifying complexes
- Increased IKZF1, correlates with increased inflammatory signaling with improved ORR in patients treated with CPI
- Immune “hot” phenotype is associated with increased expression of TEs and IKZF1
- Induction of TE de-repression and IKZF1 through epigenetic alterations may promote CPI response

Nacev, ASCO, 2022

SPEARHEAD 1 Trial
“CAR-T cells” for sarcoma

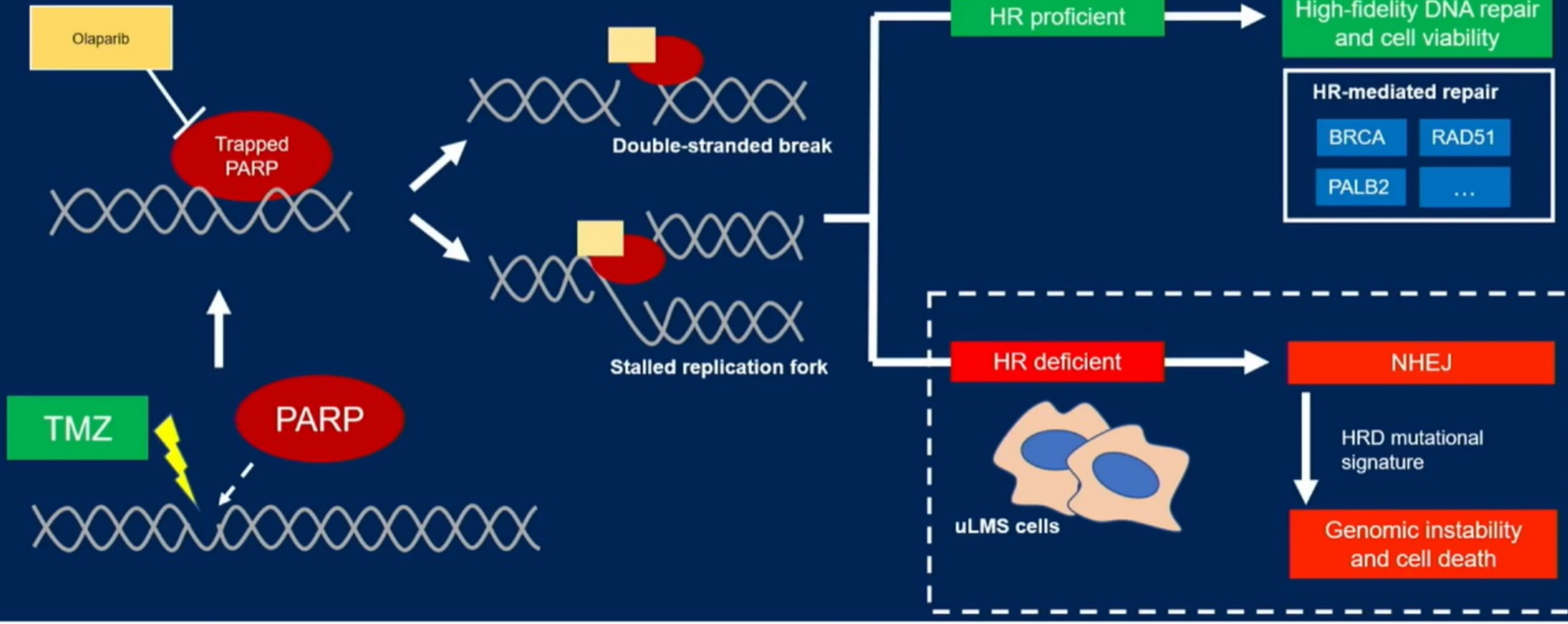
- Sarcomas expressing MAGE-A4 in patients with HLA-A*02
- Leukapheresis collection of autologous T cells for processing: SPEAR (specific peptide enhanced affinity receptor) cells
- Afamitresgene autoleucel (Afami-cel)
- At least one prior Rx with doxo or IFOS
- 37 pts (screened > 300)
  - 32 Synovial Sarcoma
  - 5 MRC LPS
- Pretreatment with leucodepleting drug

- Response (n =25)
  - ORR 39%
    - CR 2 (SS)
  - Disease control rate (DCR) 85%
  - MDR NR, durable
- Adverse effects (> 30% pts)
  - Cytokine release syndrome 22/37
    - 95% ≤ Grade 2
  - Neutropenia
  - Lymphopenia
  - Nausea
  - Fatigue
  - Pyrexia
  - Anemia
  - Cytopenia
    - ≥ G3 at 4 weeks in 6 pts

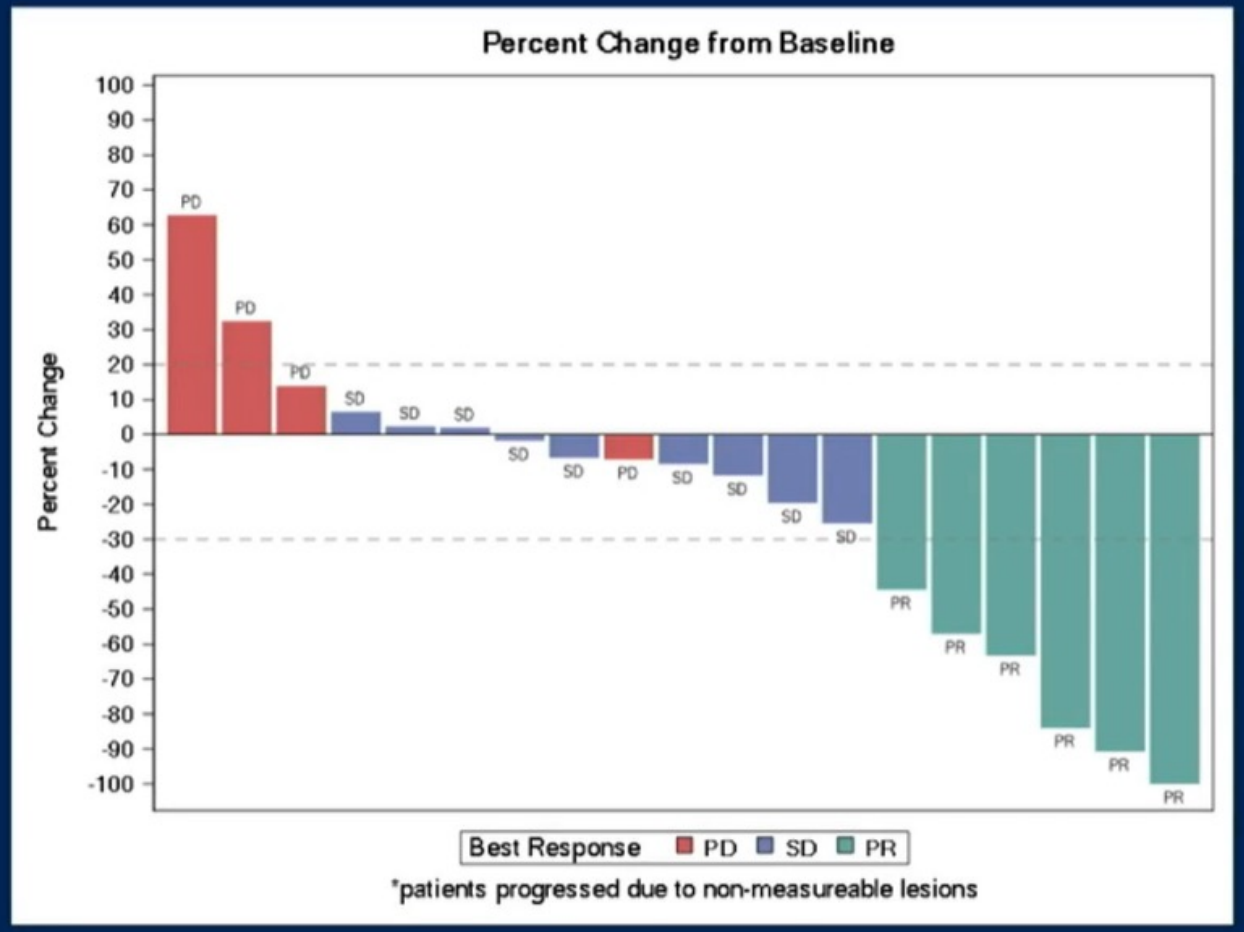
Uterine leiomyosarcoma (uLMS)
NCI 10250

- Demonstrates high levels of replicative stress and homologous recombination (HR) DNA repair defects
- Genomic alterations in HR genes observed in 18-25%
- Temozolomide 75 mg/m2/d po + Olaparib 200 mg po BID x7 q 21days
- Concept:
  - Temozolomide induces single stranded DNA breaks which are repaired by PARP
  - PARP inhibition results in replication arrest or double stranded breaks
  - HR proficient cells →repair
  - HR deficient cells → genomic instability and cell death

HR deficiency sensitizes tumors to PARP inhibition



Updated Clinical Results From NCI 10250



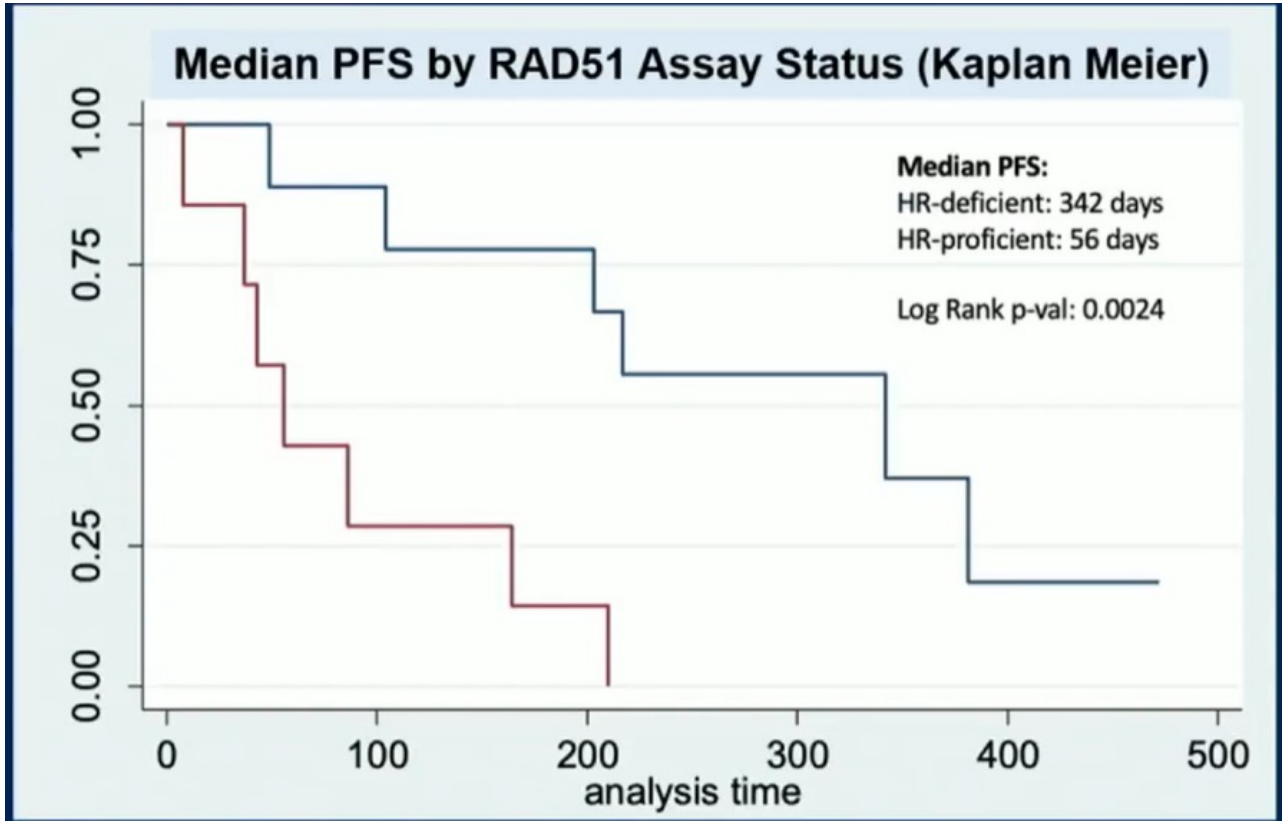
	n	%
Complete Response	0/22	**0%**
Partial Response	6/22	**27%**
Stable Disease	9/22	**41%**
Progressive Disease	4/22	**18%**
Not Evaluated	3/22	**14%**
ORR Within First 12 mo	5/22	**23%**
Updated ORR	6/22	**27%**
Updated Clinical Benefit Rate (CR + PR + SD)	15/22	**68%**

Data cutoff (clinical data): April 2022

Duration of response median 12 m, 4 pts on Rx > 2 years

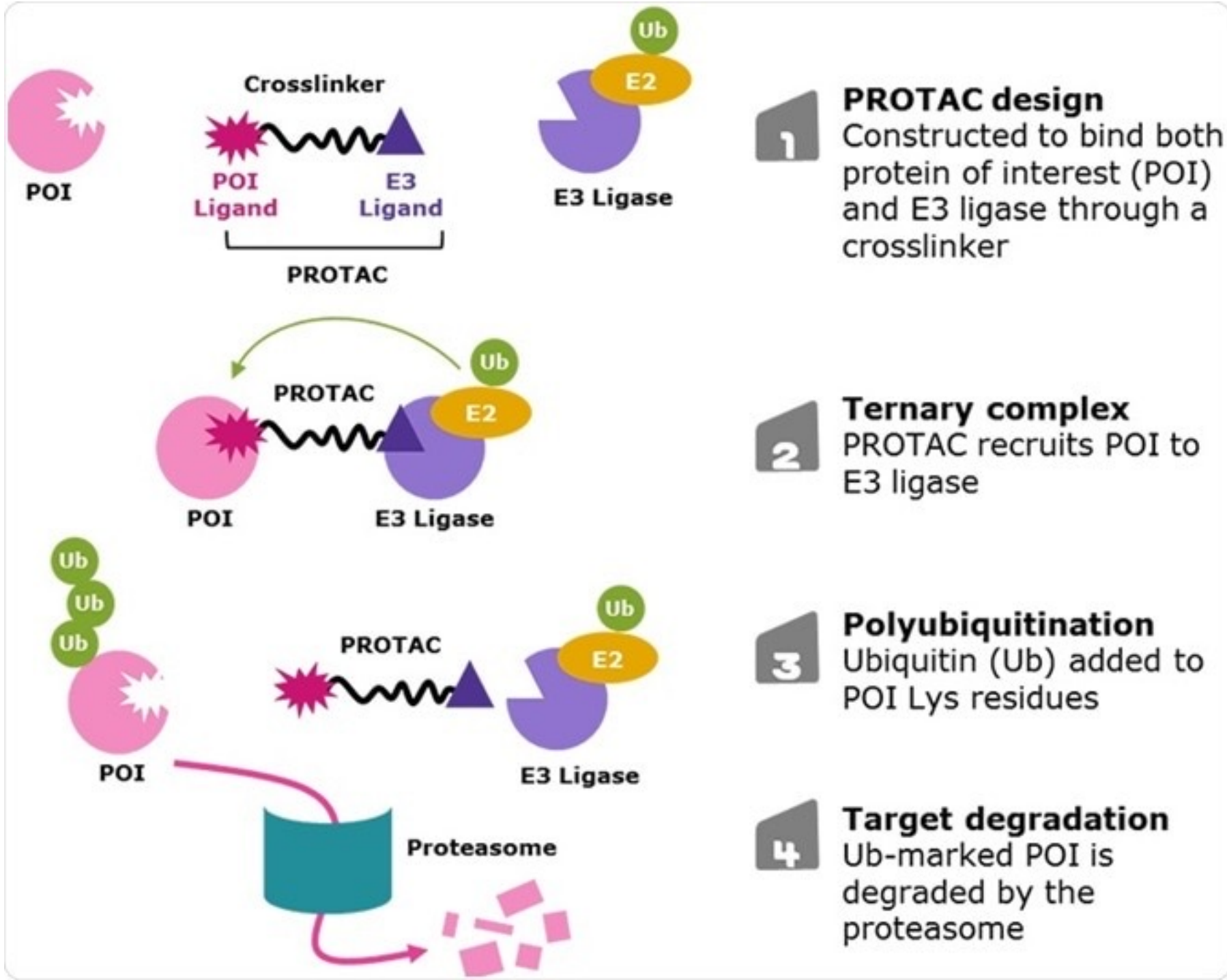
Uterine Sarcoma
Olaparib and Temozolomide

- RAD51 essential role in HR; can be assessed by IHC
- Absence of RAD51 foci implies HR deficiency
- Key biomarker; absence associated with improved PFS
- Detected 5 patients as HR deficient without corresponding genomic alteration on WES; ATRX mutations did NOT predict HR deficient.
- RAD51 functional assay demonstrated “BRCAness” = Homologous recombination repair defect (HRD) in absence of BRCA1/2 mutation



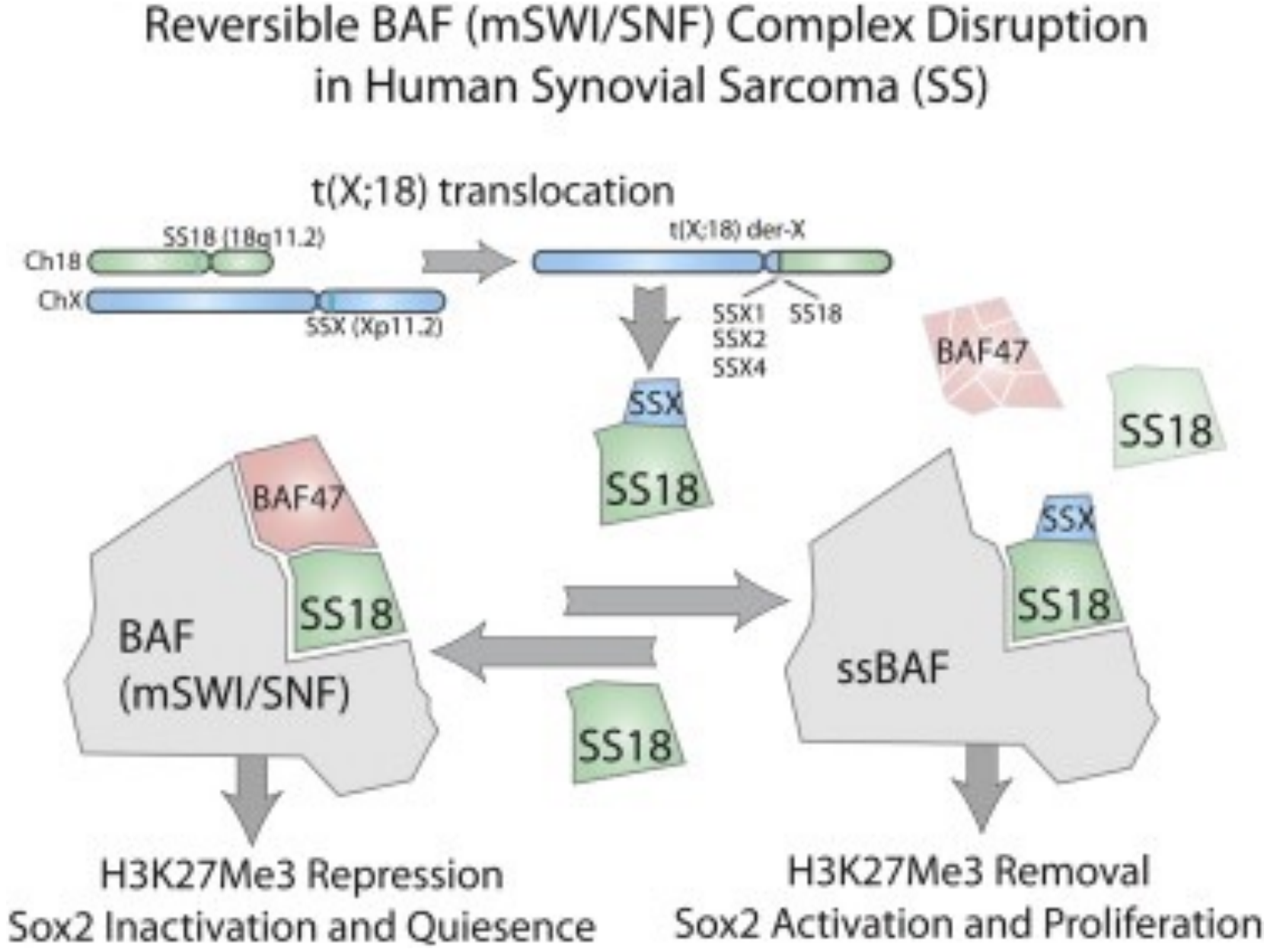
Targeted Protein Degradation (TPD)

- Strategy to target proteins with
  - active sites which are broad
  - shallow pockets that are difficult to bridge with small molecules
  - ‘smooth’ surfaces that offer few sites for a small molecule to bind
- Class of agents defined as proteolysis-targeting chimera (PROTAC) protein degraders
  - Heterobifunctional small molecules consisting of two ligands joined by a linker: one ligand recruits and binds a protein of interest (POI) while the other recruits and binds an E3 ubiquitin ligase, examples c-myc, kras
  - Molecular glues, promote ubiquitylation of a POI by enhancing a protein–protein interaction (PPI) between a ligase and a potential substrate


Crosslinker
POI
POI Ligand
E3 Ligand
PROTAC
Ub
E2
E3 Ligase
1
PROTAC design
Constructed to bind both protein of interest (POI) and E3 ligase through a crosslinker
PROTAC
Ub
E2
POI
E3 Ligase
2
Ternary complex
PROTAC recruits POI to E3 ligase
Ub
Ub
Ub
POI
PROTAC
Ub
E2
E3 Ligase
3
Polyubiquitination
Ubiquitin (Ub) added to POI Lys residues
Proteasome
4
Target degradation
Ub-marked POI is degraded by the proteasome

Phase I FHD-609

- Protein degrader
- Target: BRD9 component SWI-SNF complex



Summary

- Soft tissue sarcomas represent a heterogenous population of diseases with varying genetic alterations; Genomic profiling may aid in clinical diagnosis and is critical to identify targets for effective therapy
- Only a modest percentage of sarcoma types are responsive to immunotherapy. SPEAR cell technology is intriguing but at present applies to a minority population
- Attempts to identify immune “hot” phenotypes with biomarkers and to enhance immunogenicity by epigenetic alterations being studied
- Biomarkers, which identify “BRCAness” such as RAD51 foci functional assay by IHC, identify HR deficient states providing clues to response to PARP inhibitors
- At present most effective targeted therapies for sarcomas involve inhibition of tyrosine kinases with small molecules
- Emerging protein degradation technology offers possibility to target sarcomas with defined genetic alterations which at present are considered undruggable