Prognostic significance and microenvironment influence of cMYC in osteosarcoma

MIB Agents FACTOR Conference
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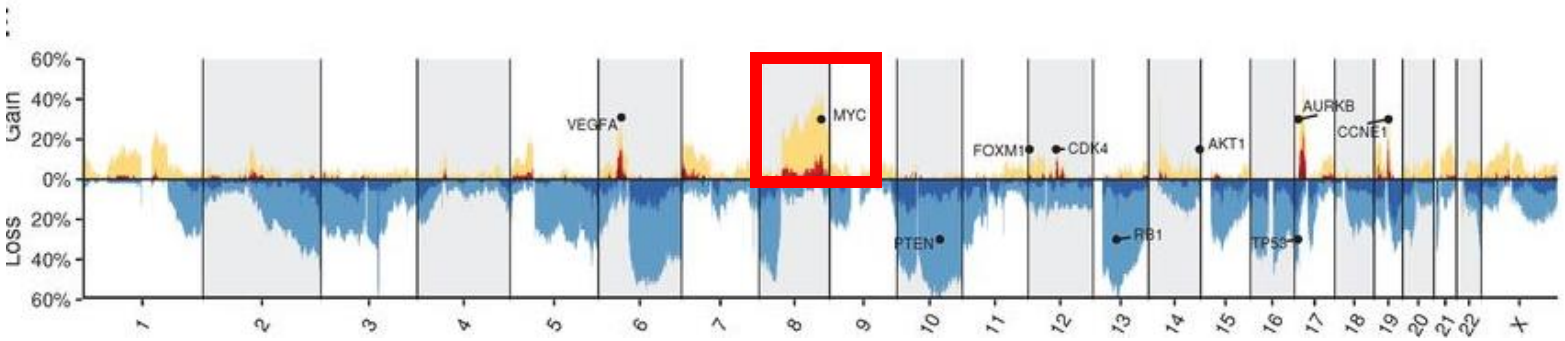
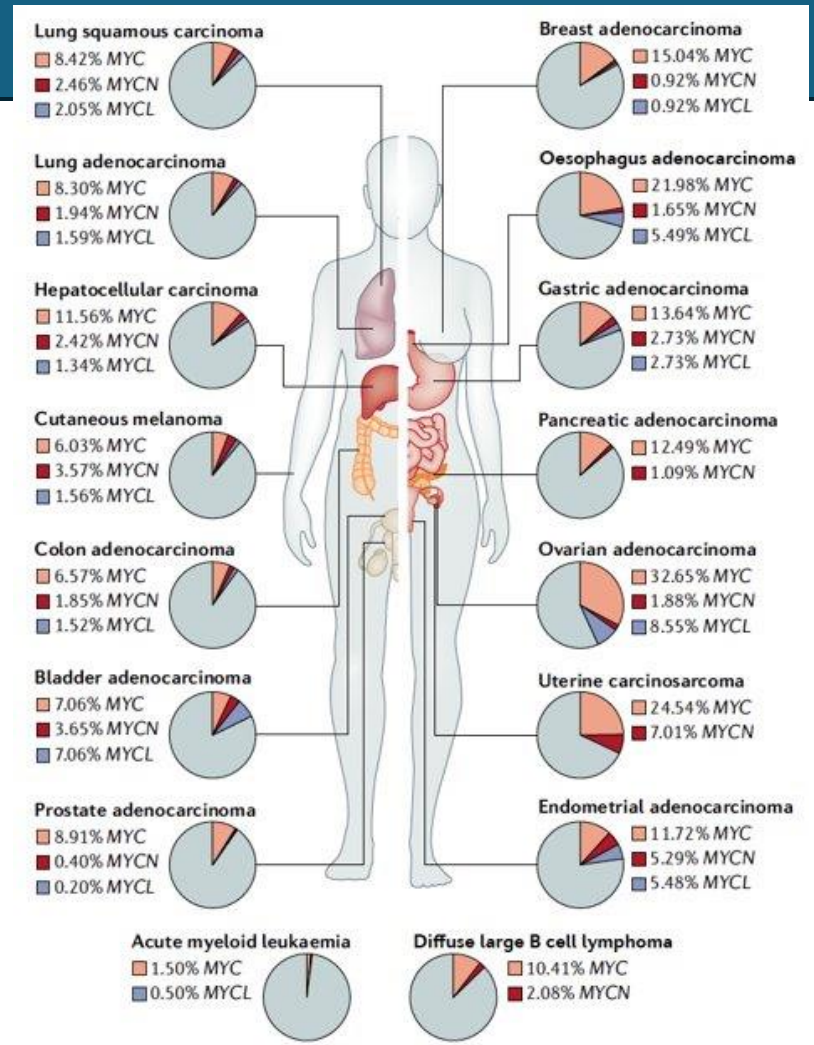
c-MYC plays a significant role in cancer development and progression

- Dysregulation of c-MYC, through overexpression or amplification, is a common feature in many human cancers and is associated with poor prognosis.

- cMYC gain occurs in a subset of patients with osteosarcoma.





Sayles et al. Cancer Discov (2019)

Dhanasekaran et al, Nature Reviews Clinical Oncology (2022)

Mounting evidence cMYC is poor prognostic factor in osteosarcoma

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Clinical Targeted Next-Generation Panel Sequencing Reveals *MYC* Amplification Is a Poor Prognostic Factor in Osteosarcoma

Authors: Amanda E. Marinoff, MD, Liam F. Spurr, BS, Christina Fong, MD, Yvonne Y. Li, MD, Suzanne J. Forrest, MD, Abigail Ward, BS, Duong Doan, BA, Laura Corson, PhD, Audrey Mauguen, PhD, Navin Pinto, MD, Luke Maese, DO, Susan Colace, MD, Margaret E. Macy, MD, AeRang Kim, MD, Amit J. Sabnis, MD, Mark A. Applebaum, MD, Theodore W. Laetsch, MD, Julia Glade-Bender, MD, Daniel A. Weiser, MD, Megan Anderson, MD, Brian D. Crompton, MD, Paul Meyers, MD, Ahmet Zehir, PhD, Laura MacConaill, PhD, Neal Lindeman, MD, Jonathan A. Nowak, MD, Marc Ladanyi, MD, Alanna J. Church, MD, Andrew D. Cherniack, PhD, Neerav Shukla, MD, and Katherine A. Janeway, MD SHOW FEWER | AUTHORS INFO & AFFILIATIONS

Prognostic Value of the G2 Expression Signature and MYC Overexpression in Childhood High-Grade Osteosarcoma

Authors: Roelof van Ewijk, MD, PhD, Laura S. Hiemcke-Jiwa, MD, PhD, Jayne Y. Hehir-Kwa, PhD, Nathalie Gaspar, MD, PhD, Lianne M. Haveman, MD, PhD, Uta E. Flucke, MD, PhD, Rana Dandis, PhD, Marc van Tuil, BSc, Claudia Y. Janda, PhD, Michiel A.J. van de Sande, MD, PhD, Lizz van der Heijden, MD, PhD, Marco J. Koudijs, PhD, Johannes H.M. Merks, MD, PhD, Bastiaan B.J. Tops, PhD, Antonin Marchais, PhD, and Lennart A. Kester, PhD SHOW FEWER | AUTHORS INFO & AFFILIATIONS

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Journal of Clinical Oncology®
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Meeting Abstract: 2025 ASCO Annual Meeting I

FREE ACCESS | Pediatric Oncology | May 28, 2025

MYC amplification and protein expression as prognostic markers in pediatric and young adult osteosarcoma.

Authors: Matthew Nagy, Olivia Puopolo, Erin Alston, Sreekar Challa, Evelina Ceca, Yvonne Y. Li, Andrew D. Cherniack, Lorena Lazo de la Vega, Alanna J. Church, and Katherine A. Janeway | AUTHORS INFO & AFFILIATIONS

Meeting Abstract: 2025 ASCO Annual Meeting I

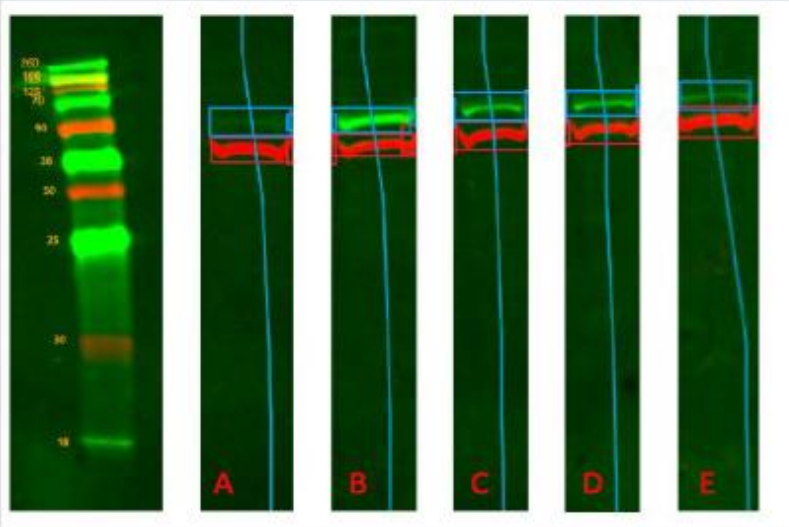
FREE ACCESS | Pediatric Oncology | May 28, 2025

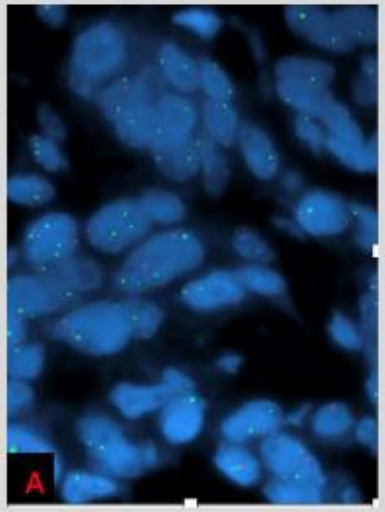
MYC amplification as a prognostic biomarker in osteosarcoma: A report from the Children's Oncology Group.

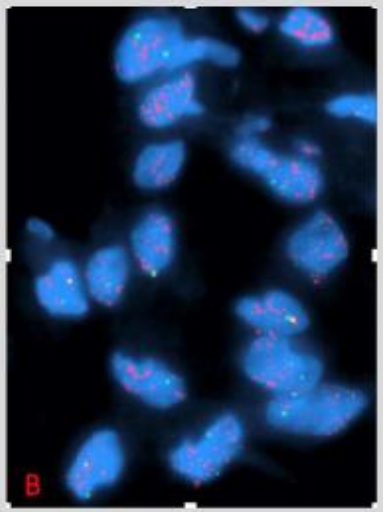
Authors: Sarah Whittle, Allen Buxton, Kevin Fisher, Mark D. Krailo, Dolores H. López-Terrada, Donald Williams Parsons, Damon R. Reed, Natalie DelRocco, and Katherine A. Janeway | AUTHORS INFO & AFFILIATIONS

Publication: Journal of Clinical Oncology • Volume 43, Number 16_suppl • https://doi.org/10.1200/JCO.2025.43.16_suppl.10045

cMYC amplification and protein expression in OS cell lines



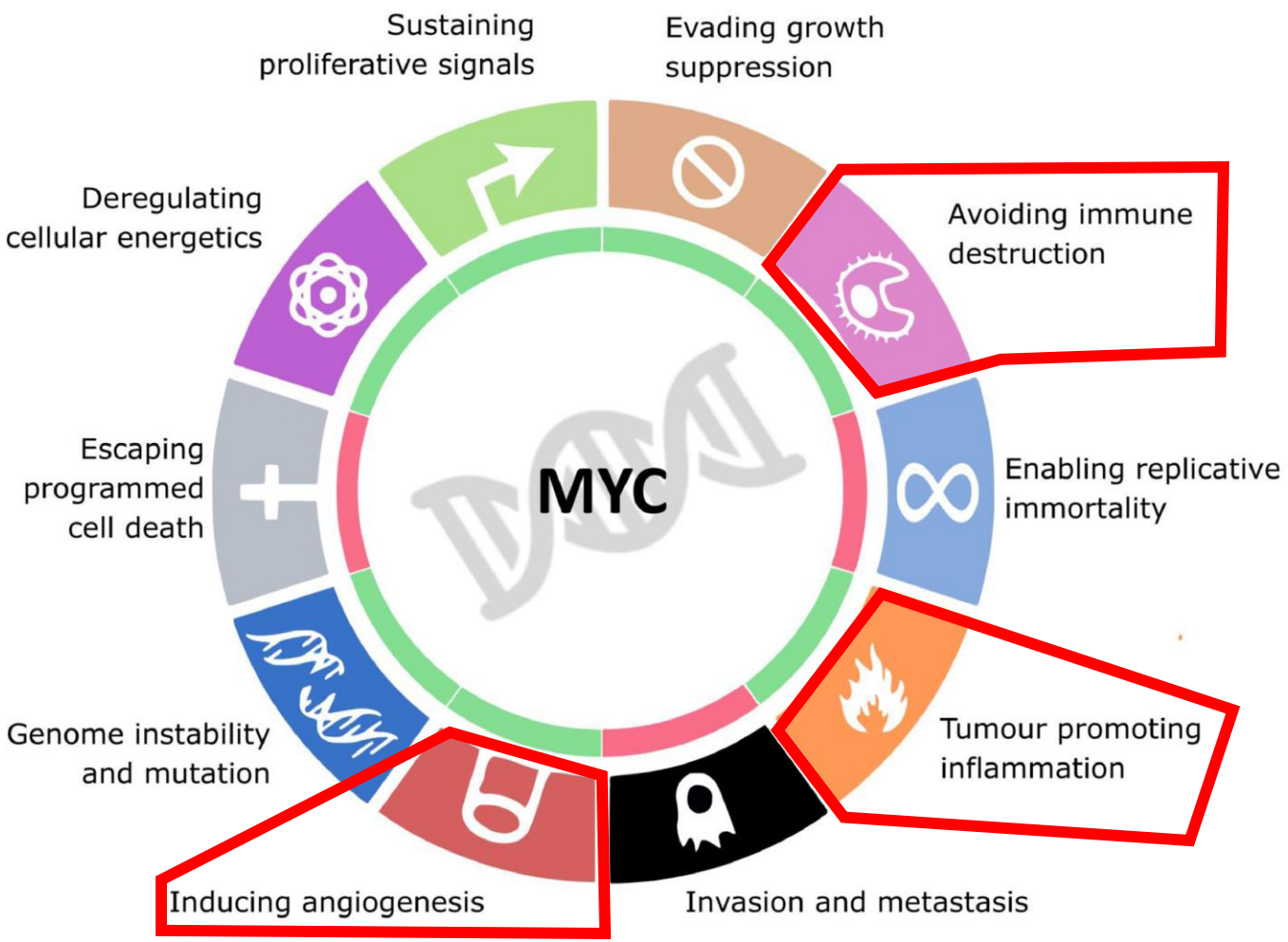




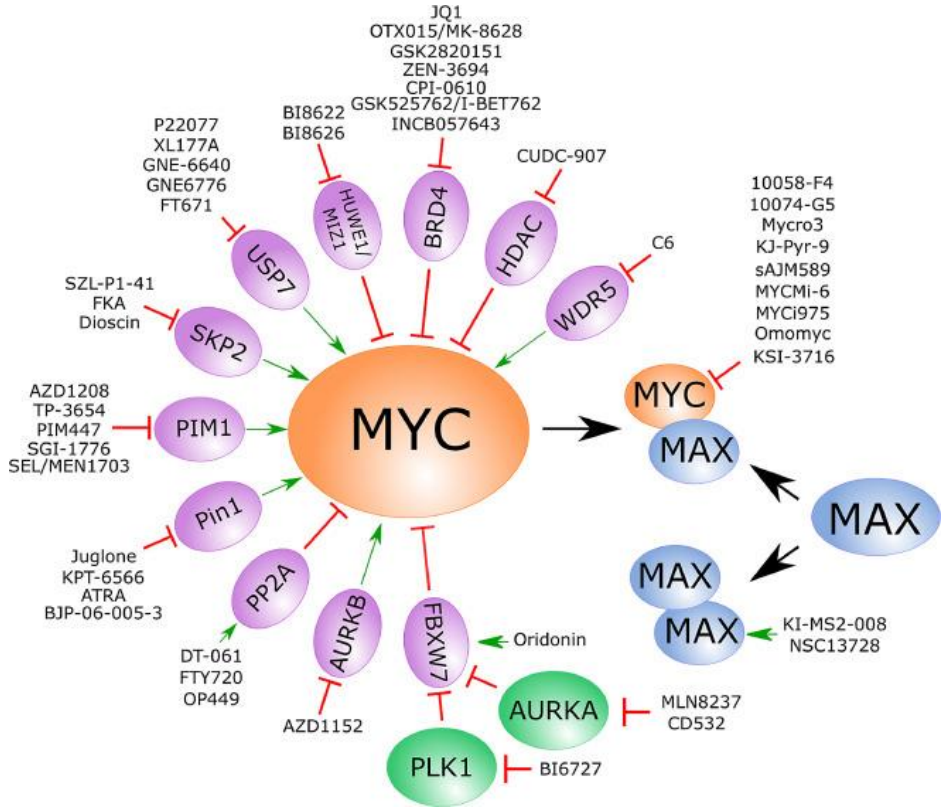
Cell Line	cMYC Signal	B-Actin Signal	cMYC Protein Expression	cMYC Amplification Status (cMYC/CEP8 ratio)
A) SJSA-1	8090	846000	LOW	NEG (0.6)
B) 143B	97100	652000	HIGH	NEG (0.8)
C) U2OS	41500	645000	HIGH	LOW (2.1)
D) MG-63	35900	667000	HIGH	HIGH (4.8)
E) Saos-2	19500	835000	LOW	LOW (2.7)

Approaches to therapeutically exploit cMYC

Potential Therapeutic Effects of MYC Targets



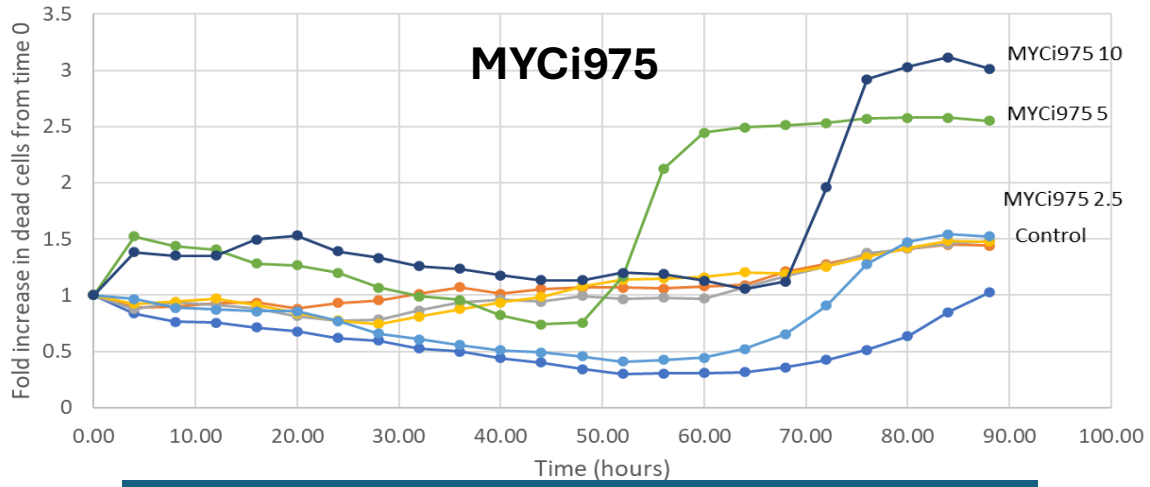
Direct & Indirect Approaches



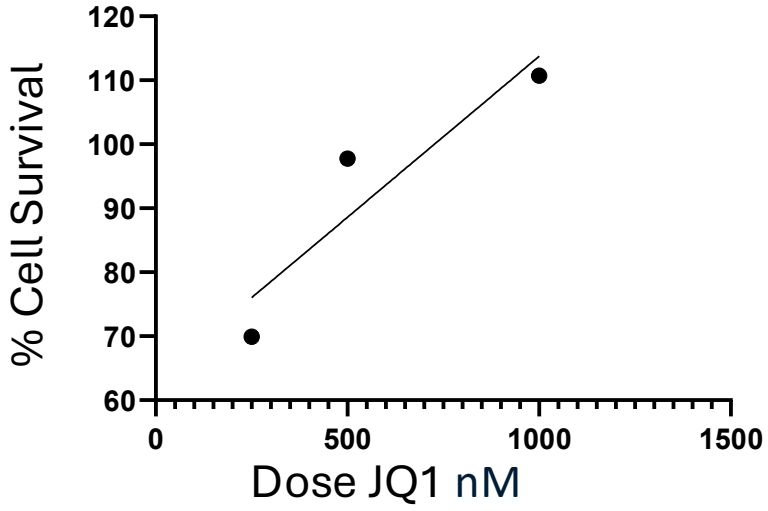
Llombart & Mansour eBioMedicine (2022)

Targeting cMYC protein kills 143B OS cells

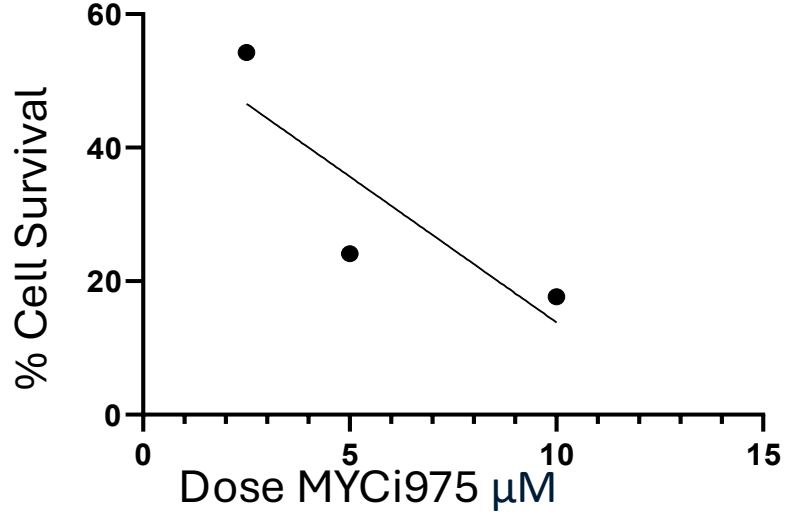
	cMYC Protein Expression Status (Western Blot)	cMYC Gene Amplification Status (FISH)
U2OS	HIGH	LOW
Saos-2	LOW	LOW
143B	HIGH	NEGATIVE
MG63	HIGH	HIGH
SJSA-1	LOW	LOW



Targeting transcription regulation



Targeting protein interaction



This ongoing project aims to:

1) Evaluate cMYC status by IHC expression and FISH detected amplification in OS patient specimens

2) Describe the tumor immune microenvironment landscape of cMYC dysregulated OS patient specimens

Patient cohorts and methods

Children's Hospital Colorado cohort

- Patients identified with diagnosis of osteosarcoma between January 1, 2000 and November 1, 2021
- 80 of those patients have archival tissue at CHCO

Oregon Health and Science University cohort

- Patients identified with diagnosis of osteosarcoma between January 1, 2000 and October 1, 2019
- 75 of those patients have archival tissue at OHSU

FISH (Zytovision Probes)

- Non-Amplified: MYC/C8 <2
- Low-Level Amplification: MYC/C8 2-9
- High-Level Amplification: MYC/C8 >9

cMYC expression (IF & IHC)

- H-score (nuclear vs whole cell)
- Digital pathology with pathologist review

Multiplexed IF analysis

Digital pathology with pathologist review

Panel 1: CD19, CD4, CD31, PD-1, FOXP3, CD8

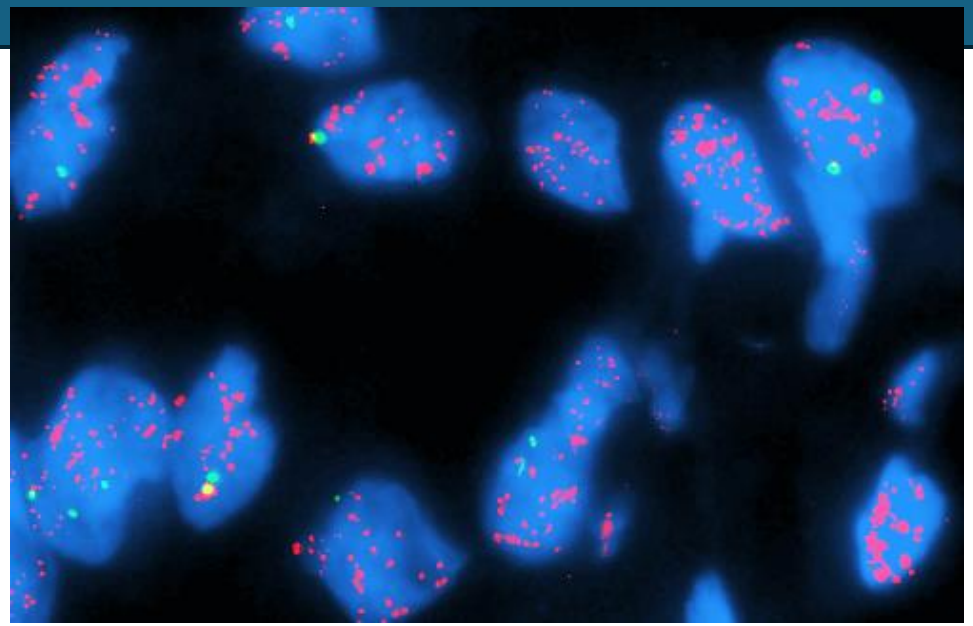
Panel 2: PLD1, CD163, CD206, CD45, CD68

Clinical Characteristics by Cohort

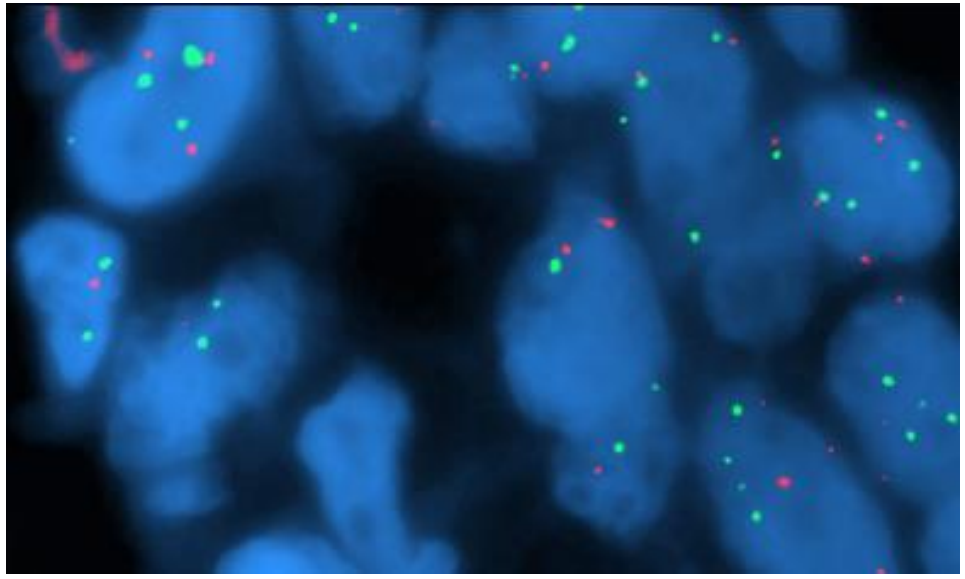
Characteristic	OHSU No. (%)	CHCO No. (%)
Patients	75	53
Sex		
Male	50 (66%)	19 (36%)
Female	25 (33%)	34 (64%)
Age		
<18 years	48 (64%)	51 (96%)
>18 years	37 (36%)	2 (4%)
Metastatic at diagnosis		
Yes	9 (12%)	11 (21%)
No	66 (88%)	42 (79%)
Tumor Necrosis		
>90% (Good)	26 (35%)	20 (43%)
<90% (Poor)	49 (65%)	27 (57%)
Vital status		
Deceased	32 (43%)	14 (26%)
Alive	43 (57%)	39 (74%)

FISH Assay for cMYC Amplification

cMYC amplified



cMYC wild-type



Samples Tested	N= 75
% Positive	20% (N = 15)
% Negative	69% (N=52)
% Failure	11% (N = 8)

Results: Positive for high level *MYC* amplification

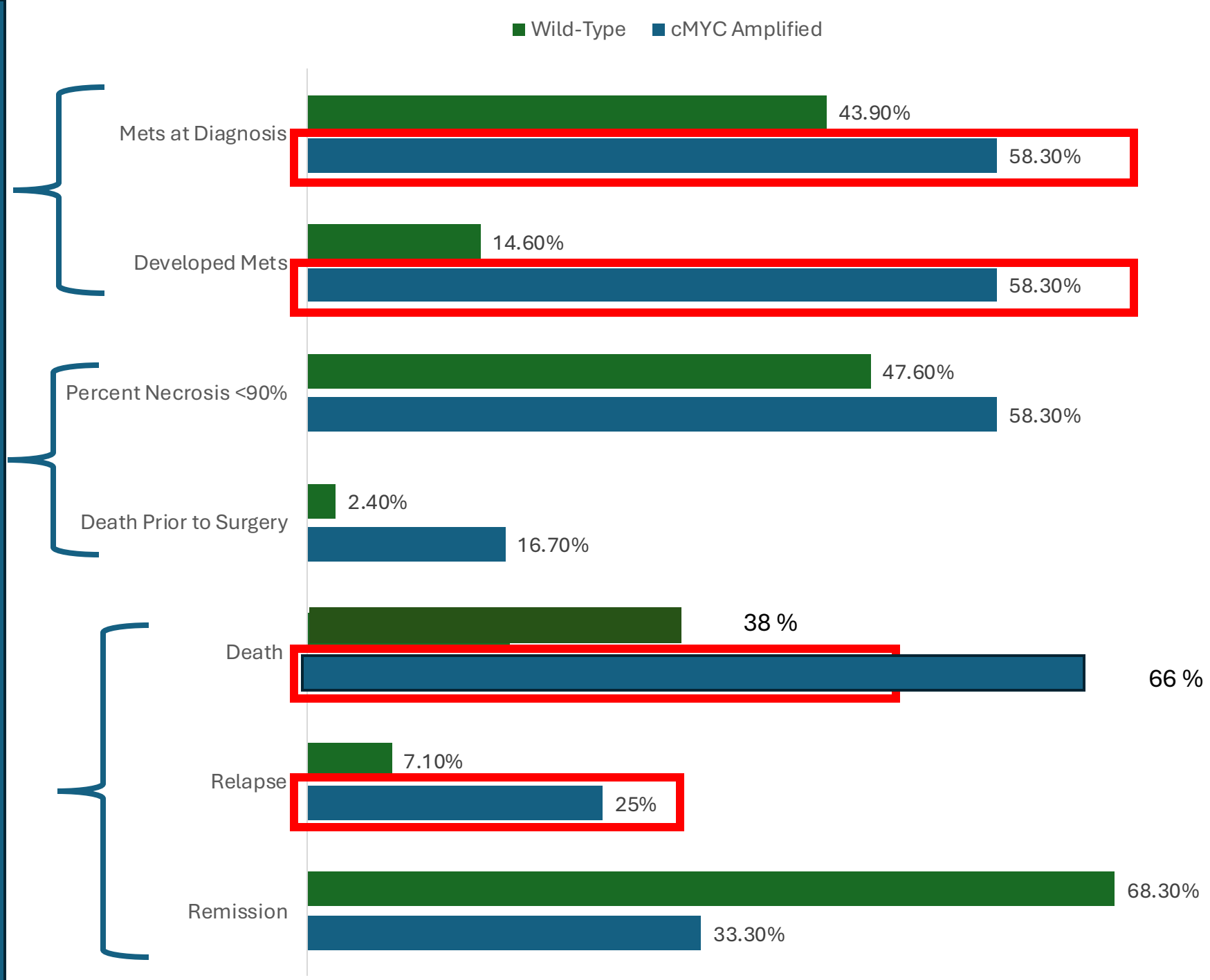
MYC gene (8q24) mean copy per cell: 18.1
CEP8 (8p11.1-q11.1) mean copy per cell: 1.6
MYC/CEP8 ratio: 11.2
Number of cells scored: 55

CHCO Clinical Data Analysis

	cMYC Amplified Tumors N = 12	cMYC wild-type Tumors N = 41
Sample Type		
Initial Biopsy – No Chemo	10 (83.3%)	27 (65.8%)
Biopsy after Chemo	2 (16.7%)	11 (26.8%)
Resection after Chemo	0	3 (7.3%)
Mean Age	12.8 years	12.3 years
Gender		
Male	5 (41.6%)	14 (34.1%)
Female	7 (58.3%)	27 (65.8%)
Treatment Received		
Chemotherapy Only	0 (0%)	1 (2.4%)
Chemotherapy & Surgery	9 (75%)	35 (85.3%)
Chemotherapy, Surgery & Radiation	1 (8.3%)	4 (9.8%)
Chemotherapy & Radiation	2 (16.7%)	0
Chemotherapy Received		
MAP	5 (41.6%)	19 (46.3%)
MAP + IE	4 (33.3%)	9 (21.9%)
MAP + Other	0	4 (9.8%)
MAP + IE + Other	3 (25.0%)	9 (21.9%)

cMYC amplification is associated with increased rates of relapse and death

Samples Tested	N=78
% Positive	23% (N=12)
% Negative	54% (N=42)
% Failure	32% (N=24)

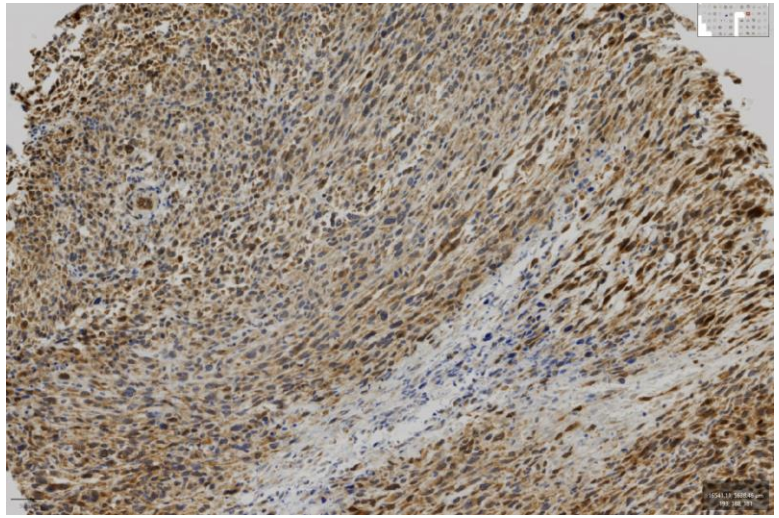


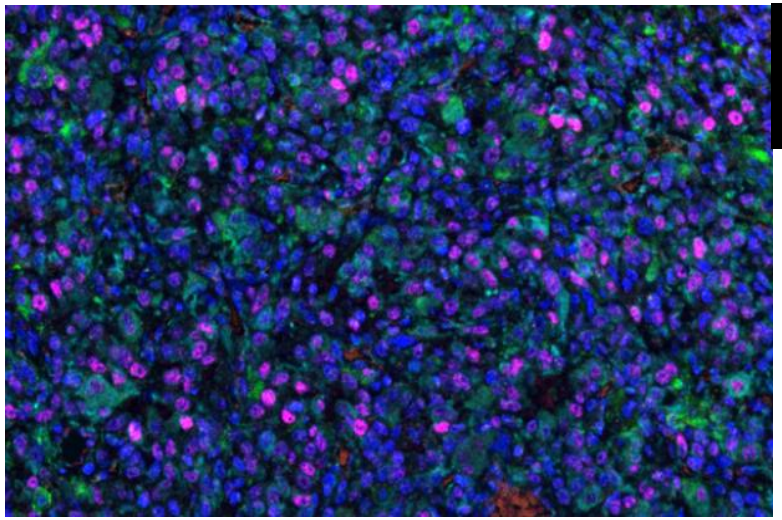
IHC/IF cMYC Analysis is ongoing

cMYC Immunohistochemistry

cMYC Immunofluorescence

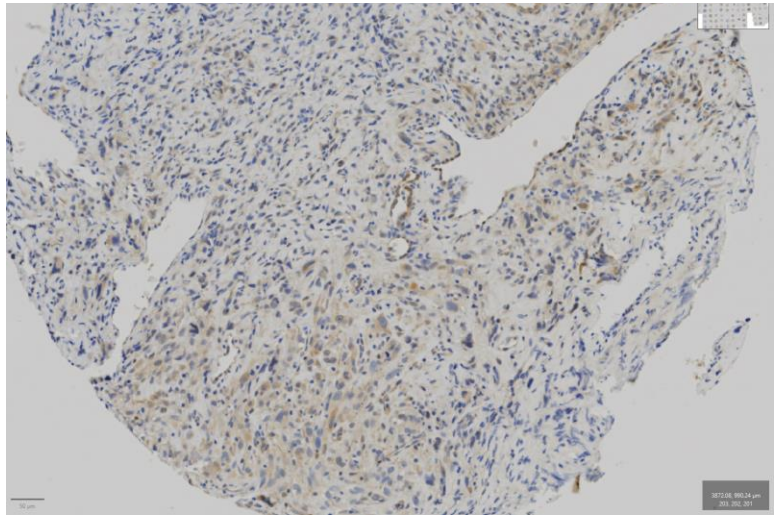
Positive

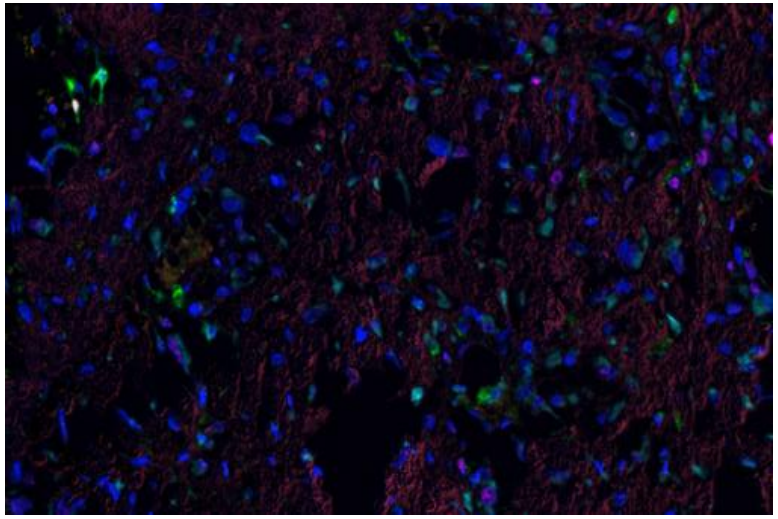




cMYC
DAPI
Vimentin

Negative

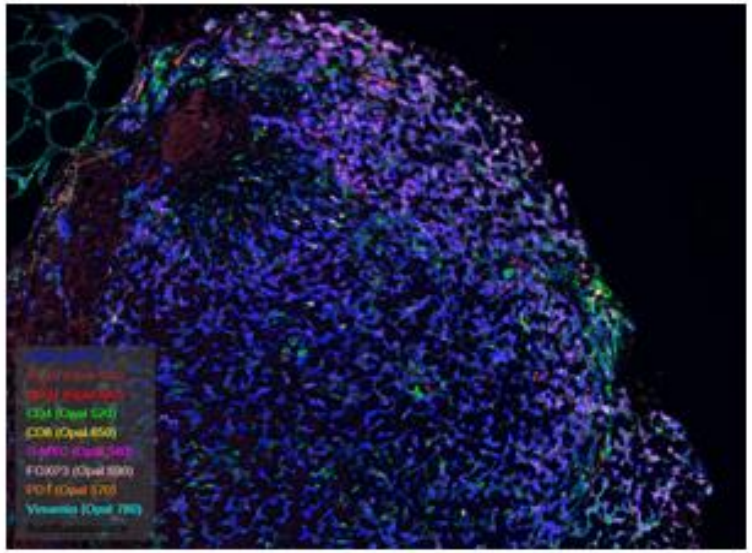




Discordant amplification vs protein expression status

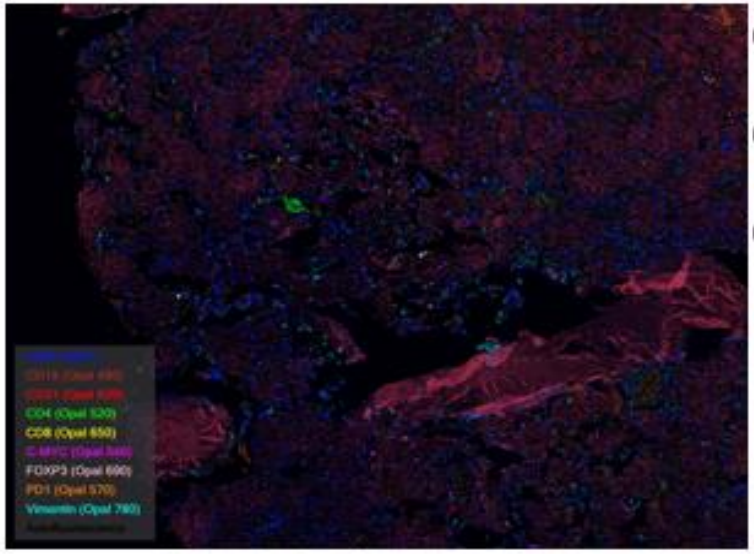
Identifier	FISH Amplification	Protein Expression	Stage	%Necrosis	Outcome
R22-00054 K1	NEG	POS	Metastatic	95% tumor necrosis	Alive
R22-00054 Q1*	NEG	NEG	Localized	>99% tumor necrosis	Relapsed, Alive
R22-00054 R1*	POS	POS	Metastatic	60% tumor necrosis	Death
R22-00055 I1*	NEG	NEG	Localized	50% tumor necrosis	Death
R22-00055 S1	POS	POS	Metastatic	N/A	Death
R22-00055 U1	POS	POS	Localized	99% necrosis	Alive
R22-00062 T1	POS	NEG	Localized	30% tumor necrosis	Alive

cMYC Expression/Amplification & Immune infiltrate



R22-00055 S1				
FISH	**IHC**	**Sample Type**	**Stage**	**Outcome**
POS	POS	Diagnosis	Metastatic	Death

Total Number Cells = 3130						
cMYC+	CD19+	CD31+	CD4+	CD8+	FOXP3+	PDL1+
1059 (33.8%)	0 (0%)	45 (1.4%)	55 (1.7%)	8 (0.2%)	204 (6.5%)	1220 (38.9%)



R22-00062 T1				
FISH	**IHC**	**Sample Type**	**Stage**	**Outcome**
POS	NEG	Diagnosis	Non-metastatic	Alive

Total Number Cells = 4795						
cMYC+	CD19+	CD31+	CD4+	CD8+	FOXP3+	PDL1+
307 (6.4%)	0 (0%)	20 (0.4%)	48 (1.0%)	2 (0.04%)	27 (0.5%)	120 (2.5%)

Next steps

- Complete cMYC IF analysis from CCHO and HCI
  - Evaluate correlation with patient outcome
  - Multivariate analysis with known prognostic factors
  - Evaluate the relationship between copy number and protein expression (spearman correlation)
  - Combine
- Complete tumor immune microenvironment analysis
- Probe what biology could drive differences in cMYC status based on assessment level & methodology → protein level, gene expression, DNA copy number
- Evaluate a prospectively gathered and uniformly treated cohort

Thank you

Patients and Families

University of Colorado

- Wilky Lab
- HMSIR Core

Colorado State University

- Regan Lab

Oregon Health and Science University

- Lara Davis

University of Utah/HCI

- BMP Core

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- HHOW (Dietz)

SFA SARCOMA FOUNDATION of AMERICA

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