

# Elucidating the allelic imbalance of MYC and therapeutic response in pancreatic ductal adenocarcinoma via patient derived organoids

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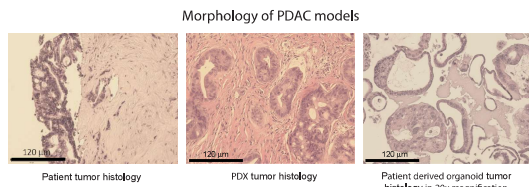
Abstract #: A036



## Abstract

### Background:

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal cancer with limited precision-based treatment options. KRAS is one of the most frequently mutated proto-oncogenes in PDAC and drives tumor progression through downstream MAPK/ERK signaling. MYC, a critical downstream effector, enhances cell proliferation and tumor maintenance in PDAC. With MYC as an attractive therapeutic target, predicting and overcoming mechanisms of acquired resistance remains a challenge. One mechanism of acquired resistance involves alterations in MYC gene copy number, leading to allelic imbalance and genomic instability. Although efforts have been made to understand the role of MYC amplification in PDAC, the extent to which MYC copy number heterogeneity impacts targeted therapy response in PDAC tumors remains unclear. Patient-derived organoids (PDOs) provide a clinically relevant model to analyze MYC allelic imbalance and determine whether MYC copy number variations influence treatment response.



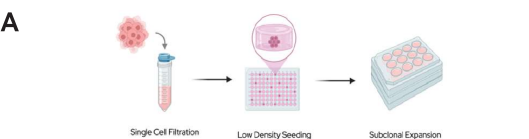
**Figure 1. Morphology of PDAC models.** Histology images of patient tumor, PDX tumor, and patient derived organoid tumor in 20x magnification.

### Results:

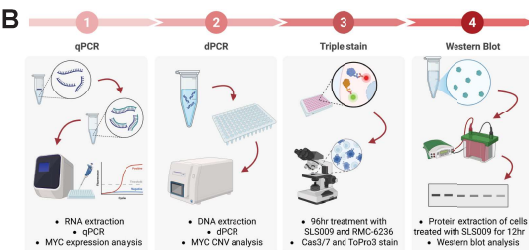
Eleven single cell derived subclones (D-P) were successfully established from a patient-derived MYC amplified PDAC organoid line. Digital PCR was performed on the parent PDAC culture, the established subclones, and a normal liver diploid control cell line. The parent culture showed an average copy number of 35 copies, while the subclones showed averages between 14-17 copies. These findings suggest MYC copy number heterogeneity between the parent population and its generated subclones. The parent culture (MYC-H) and an established subclone (MYC-L) were analyzed for treatment response to SLS009, RMC6236, and in combination. MYC-L had significantly increased Caspase-3/7 and ToPro (p<0.0001) compared to the MYC-H for both SLS009 and the combination treatments. There was no significant difference in Caspase-3/7 and ToPro for RMC6236 between MYC-H and MYC-L.

## Methods

Organoid cultures were seeded at low density to establish single cell derived subclones, which were expanded into distinct organoid lines.

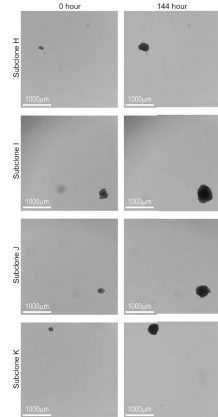


DNA from each subclone was analyzed using the QIAGEN QIAcuity dPCR kit to assess MYC copy numbers present within each clone. Subclones were then seeded on a 96 well plate and treated with the CDK9 inhibitor SLS009, the pan-RAS inhibitor RMC6236, or the combination to evaluate the influence of MYC copy number on therapeutic response. Following treatment, apoptosis and necrosis was assessed using the markers Caspase-3/7 and ToPro3.

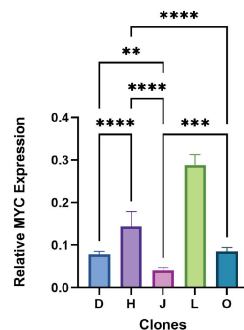


**Figure 2. (A)** Schematic showing organoid subclonal preparation from whole organoid to single cell. **(B)** Workflow of methods for key experiments using the confirmed subclonal organoids.

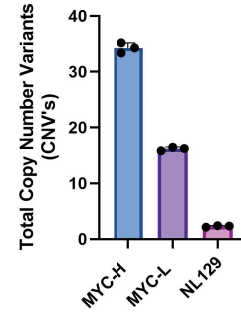
## Results: Decreased Copy Number of MYC in Subclonal Populations Increases Sensitivity to Pan-RASi Daraxonasib & CDK9i SLS009.



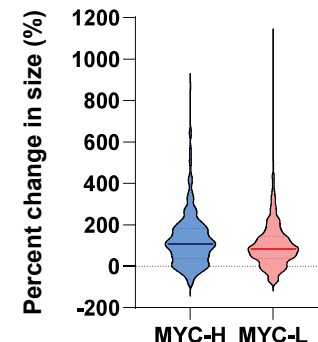
**Figure 3. Images demonstrating presence and growth of clones H, I, J, and K.** Clones were imaged at 0hrs and 144hrs after single cell seeding via brightfield imaging.



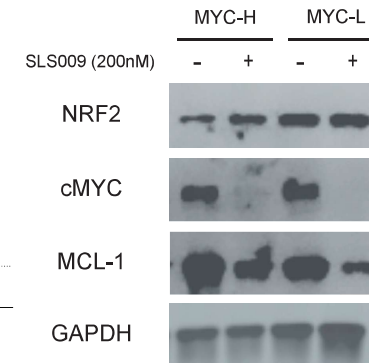
**Figure 4. Relative MYC Expression of initial single clones D,H,I,J,L,O quantified through qPCR.** Significance for clone L was omitted but it was significant to every other clone. Images demonstrating presence and growth of clones H, I, J, and K. Clones were imaged at 0hrs and 144hrs after single cell seeding via brightfield imaging.



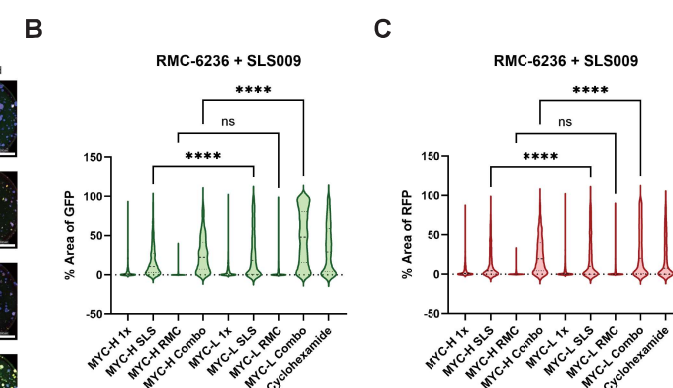
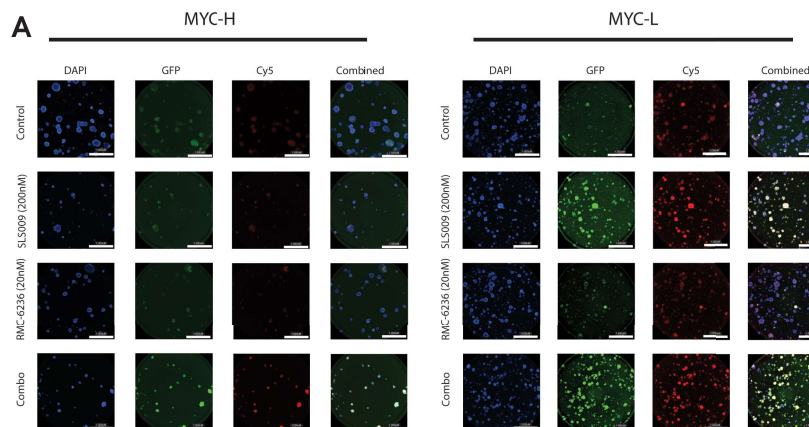
**Figure 5. Total copy number variants of MYC-L, MYC-H, and internal control NL129 quantified through QIAcuity dPCR.** The average copy number of MYC for MYC-H, MYC-L and NL129 is 35 copies, 16 copies and 2 copies, respectively.



**Figure 6. Percent change in size for MYC-H and MYC-L in 1x media.** Change in size was assessed from 0hr-144hr.



**Figure 7. Western blot analysis of pancreatic patient derived organoids treated with SLS009.** Patient derived organoids from MYC-H and MYC-L were treated with SLS009 (200nM) for 12 hours.



**Figure 8. Therapeutic response (A)** Violin plot displaying percent change in area of GFP for MYC-L and MYC-H in 1x feeding media, SLS009, RMC-6236, combination, and cycloheximide positive control **(B)** Violin plot displaying percent change in area of RFP for MYC-L and MYC-H in 1x feeding media, SLS009, RMC-6236, combination and cycloheximide positive control **(C)** Representative images of MYC-L and MYC-H triple stain at 72 hours with DAPI, GFP, Cy5 and overall when treated with SLS009, RMC-6236, and combination.

## Results

- Eleven single cell derived subclones were established from a MYC amplified PDO line. Subsequent treatment studies include the parent culture (MYC-H) and a selected subclone, Clone D (MYC-L).
- MYC-L had significantly increased apoptosis (28.68% v. 17.88% in parent culture, p<0.0001) and necrosis (22.50% v. 14.51% in parent culture p<0.0001) when treated with SLS009
- MYC-L had increased apoptosis (47.97% v. 26.59% in parent culture, p<0.0001) and necrosis (30.22% v. 24.82% in parent culture, p<0.0001) when treated with the combination
- There was no significant difference in apoptosis and necrosis between MYC-H and MYC-L when treated with RMC-6236
- Percent change in size was significantly decreased for MYC-L relative to MYC-H when treated with RMC-6236, SLS009 and in combination (p<0.0001)

## Conclusions

Expansion of parent PDAC organoids can have increased MYC copy numbers compared to derived subclones, suggesting MYC copy number can have intratumoral heterogeneity through extended passage. Furthermore, the results suggest that a greater MYC copy number may impact sensitivity to RMC-6236. MYC-H conferred significantly reduced apoptosis and necrosis when compared to MYC-L, across shared molecular profiles. Ongoing work is comparing across varied degrees of MYC copy number, validating background molecular heterogeneity, and investigating novel combination therapies to disrupt transcription.

## References

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