Comparison of *ERBB2* and *MDM2* gene amplification analysis by next-generation sequencing copy number variation and fluorescence in situ hybridization in solid tumors

Scott Kuan-Wen Wang, MD/Ph.D,[1] , Anthony Caruso Ph.D,[1], Yong Shi Ph.D,[1], Laila Mnayer Ph.D,[1], Jonathan Earle MD[1]
[1]Department of Pathology and Laboratory Medicine, Hartford Hospital, Hartford, Connecticut

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Introduction

Copy number variations (CNV) in cancer result in gains or losses of DNA and may be oncogenic[1]. Next generation sequencing (NGS) tests detect multiple CNVs in massively parallel fashion. However, different NGS methodologies may have variations in detection capabilities, cutoffs and assay linearity[2,3]. Here, we evaluate the relationship between *ERBB2* and *MDM2* copy numbers assessed by NGS and results obtained by clinical FISH testing in a community hospital setting.

Method

Hospital archives were searched for specimens in which results of both *ERBB2* or *MDM2* FISH tests and somatic targeted NGS testing were available. Raw CNV Fold changes (FC) were electronically extracted from the NGS database, for patients who also had FISH results. FISH results were manually extracted from the medical records. NGS CNV FC results were compared to *ERBB2*/*CEP17* and *MDM2*/*CEP12* ratios reported by FISH, respectively.

FISH and NGS CNV results were labeled positive for ratio/FC ≥2 and negative for ratio/FC<2, and compared in these categories. Additionally, *MDM2*/*CEP12* FISH ratios and *MDM2* NGS CNV FC were compared as continuous variables, using linear regression analysis.

Result

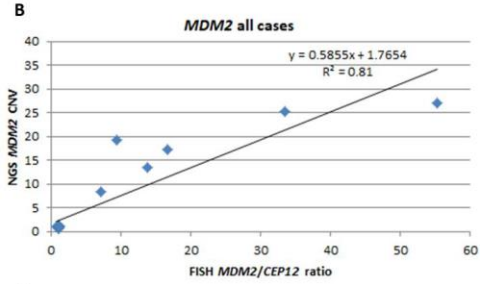
A total of 23 cases of *ERBB2* and 22 cases of *MDM2* tests were compared, from January 2020 to June 2023.

Result (cont'd)

A

		ERBB2/*CEP17* FISH ratio	
	Case#	<2	>2
ERBB2 NGS CNV FC	<2	19	3
	>2	0	1

B



C

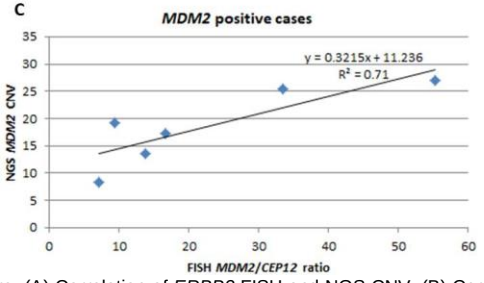


Figure. (A) Correlation of *ERBB2* FISH and NGS CNV. (B) Correlation of *MDM2* FISH and NGS CNV in all *MDM2* cases. (C) Correlation of *MDM2* FISH and NGS CNV in *MDM2* positive cases.

Among the *ERBB2* FISH cases, 19 were negative, while 4 were positive. The *ERBB2*/*CEP17* FISH ratios ranged from 0.75 to 1.74 (mean=1.20) in negative cases and from 2.58 to 4.23 (mean=3.29) in positive cases. The NGS CNV FC ranged from 0.91 to 1.99 (mean=1.48) in negative cases and from 1.30 to 4.13 (mean=2.17) in positive cases. *ERBB2* NGS CNV showed low sensitivity (25%) for detection of *ERBB2* copy gain, with high specificity (100%).

Result (cont'd)

Among the *MDM2* FISH cases, 16 were negative, while 6 were positive. The *MDM2*/*CEP12* FISH ratios ranged from 0.71 to1.22 (mean=1.03) in negative cases and from 7.15 to 55.29 (mean=20.40) in positive cases. The NGS CNV FC ranged from 0.71 to 1.30 (mean=1.06) in negative cases and from 8.36 to 27.07 (mean=18.50) in positive cases. The correlation (R^2) between FISH and NGS CNV was 0.81 for all cases and 0.71 for positive cases. *MDM2* NGS CNV showed high sensitivity and specificity (both 100%) for detection of MDM2 copy gain.

Conclusion

There are strong correlations between CNV assessments by clinical targeted NGS testing and FISH for *ERBB2* and *MDM2*, in a real-world community hospital setting. Future directions include assessment of additional cases, additional genes evaluated by FISH, as well as copy number assessments by clinical array comparative hybridization testing. These data will aid in refining the NGS pipelines and thresholds for positivity, however, orthogonal testing methodologies, such as FISH are likely to remain indispensable for borderline cases.

References

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I do not have any relevant financial relationships to disclose.