Evidence for Unified Assessment Criteria of HER2 IHC in Colorectal Carcinoma

Mark G. Evans[1], Harris B. Krause[1], Andrew Elliott[1], Emil Lou[2], Hassan Ghani[1], Rhonda K. Yantiss[3], Monica T. Garcia-Buitrago[3], Jinru Shia[4], Rona Yaeger[4], Matthew J. Oberley[1], David A. Bryant[1], Jaclyn F. Hechtman[1]

[1]Caris Life Sciences, Phoenix, AZ, [2]University of Minnesota Masonic Cancer Center, Minneapolis, MN, [3]University of Miami Miller School of Medicine/Jackson Health System, Miami, FL, [4]Memorial Sloan Kettering Cancer Center, New York, NY

Introduction

- HER2 expression is an important biomarker for the management of RAS wild-type metastatic colorectal carcinoma.
- Immunohistochemistry (IHC) with reflex *in situ* hybridization (ISH) is accepted as a standard method of assessment, yet there are currently two sets of criteria used to interpret results:
- HERACLES criteria: ISH confirmation when IHC staining is 3+ in 10-49% of cells.
- My Pathway criteria: Do not require ISH confirmation when IHC staining is 3+ in 10-49% of cells.
- We aimed to assess the prevalence of HER2 3+ heterogeneity and its association with HER2 copy number amplification (CNA).

Materials and Methods

- Paraffin-embedded tumor samples underwent DNA (592-gene or whole exome) and RNA (whole transcriptome) sequencing, utilizing the Agilent SureSelect Human All Exon V7 bait panel (Santa Clara, CA) and Illumina NovaSeq technology (San Diego, CA).
- HER2 (4B5) expression was tested by IHC.
- A subset of tumors were tested for HER2 amplification via ISH and/or via NGS (amplified, CNA ≥6 copies).
- X2/Fisher-Exact tests were applied where appropriate, with p-values adjusted for multiple comparisons (p< 0.05).

Results

	HER2 pos	HER2 pos het	HER2 eq-ishp	HER2 low	HER2 neg	Statistic	p-value
Count (N)	166	28	72	1401	11541		
Median Age [range] (N)	59 [22 - >89] (166)	61 [22 - 85] (28)	65 [31 - 82] (72)	62 [19 - >89] (1401)	63 [14 - >89] (11541)	Kruskal-Wallis	0.028
Female	40.4% (67/166)	42.9% (12/28)	45.8% (33/72)	46.8% (656/1401)	45.4% (5239/11541)	chi-square	0.577
Male	59.6% (99/166)	57.1% (16/28)	54.2% (39/72)	53.2% (745/1401)	54.6% (6302/11541)	chi-square	0.577

Table 1. Summary of Patient Characteristics

Of 13,796 CRC with HER2 IHC, 93.9% were negative for HER2 overexpression (intensity <2 or <10% tumor cell expression); 4.8% (656/13605) were equivocal (2, ≥10%). Only, 1.4% of tumors were either positive or heterogeneously positive for HER2 overexpression (3+, ≥10%).
Abbreviations: pos - IHC positive; Het+ - IHC heterogenous; eq-ishp - IHC equivocal/ISH positive; low - IHC low (1+ intensity in ≥10% or 2+ staining in ≥10% but ISH negative); neg - IHC negative.

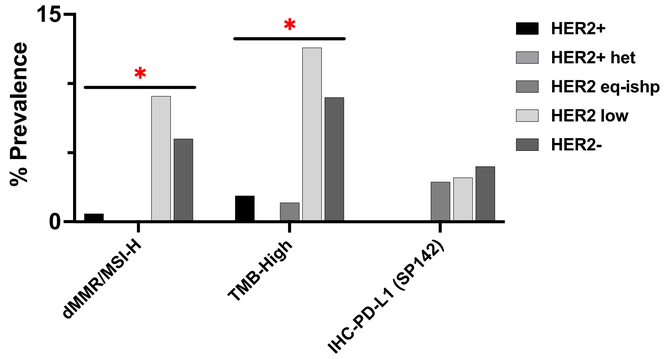


Table 2. Additional Genomic Features

Microsatellite instability in 0.5%, 11.6%, & 6.2% of HER2 pos, eq, & neg cases
TMB high (≥10 Muts/Mb) was mostly detected in the neg and eq tumors.
Only 1.6% of HER2 pos cases showed a mutational burden ≥10 Muts/Mb.
Abbreviations: dMMR - mismatch repair protein deficient by IHC; MSI-H - microsatellite instability; TMB - tumor mutational burden.

Features	Positive (HER2 pos)	Negative (HER2 pos)	Percentage (HER2 pos)	Positive (HER2 pos het)	Negative (HER2 pos het)	Percentage (HER2 pos het)	Positive (HER2 eq-ishp)	Negative (HER2 eq-ishp)	Percentage (HER2 eq-ishp)	Positive (HER2 low)	Negative (HER2 low)	Percentage (HER2 low)	Positive (HER2 neg)	Negative (HER2 neg)	Percentage (HER2 neg)	Statistic	q-value
CNA-ERBB2	153	6	96.2	21	7	75.0	29.0	40	42.0	2	1337	0.15	7	10824	0.06	Fisher's Exact	0.0125
CISH-Her2 CISH	67	0	100.0	21	1	95.5	69.0	0	100.0	0	214	0	—	—	—	chi-square	<0.001

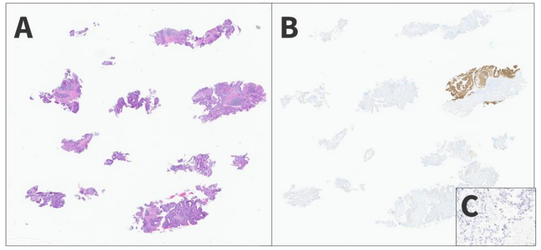
Table 3. HER2 IHC Status, Heterogeneity, and Correlation with ISH and Gene Amplification Studies

Of HER2 overexpressing tumors, 13.1% (25/191) had heterogenous HER2 overexpression (HER2/Het+, 3+ staining of 10-49% of cells).
Twenty cases were HER2/Het+ and had ISH testing. Of these, 100% (20/20) demonstrated amplification via ISH.
Ninety-six percent (153/159) of HER2+ CRC (3+, ≥50%) also showed *ERBB2* amplification by NGS, whereas 80% (20/25) of HER2/Het+ CRC demonstrated *ERBB2* amplification by NGS (p<0.001).

HER2 IHC/ISH

Figure 1. Example of HER2/Het+ CRC

Hematoxylin/eosin staining (**A**, 40x magnification) and HER2 IHC (**B**, 40x magnification) display 3+ staining in 15% of neoplastic cells. HER2 ISH confirms *ERBB2* amplification (inset **C**, 200x magnification).



Conclusions

- CRCs that were HER2/Het+ were invariably ISH positive, while NGS was not as sensitive for HER2 amplification in this subgroup. Our results suggest that ISH is likely unnecessary for CRC with 3+ HER2 overexpression in 10-49% of tumor cells, and that NGS has suboptimal sensitivity for this cohort.

References

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Contact

Jaclyn Hechtman, MD (jhechtman@carisls.com)

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