



Algorithm for pathologists.

Tumor cell cluster is defined as a cluster of five or more tumor cells. Additional recommendations: Pathologists should ensure that biopsy or resection specimens used for HER2 testing are rapidly placed in fixative, ideally within 1 hour (cold ischemic time), and are fixed in 10% neutral buffered formalin for 6 to 72 hours. Routine histology processing and HER2 testing should be performed according to analytically validated protocols. Pathologists should identify areas of invasive adenocarcinoma and also mark areas with strongest intensity of HER2 expression by immunohistochemistry (IHC) in the gastroesophageal adenocarcinoma (GEA) specimen for subsequent scoring when in situ hybridization (ISH) is required.

General interpretation and correlation of HER2 ISH and IHC scores for gastric/GEJ carcinoma

Subasinghe et al 2019

ISH	ISH signals account	Interpretation
HER2 signals	<4 Signals/nucleus in the tumor cells	HER2 not amplified; IHC is expected to be negative or equivocal
	4-6 Signals/nucleus	Correlate with the HER2/CEP17 ratio and IHC*
	>6 Signals/nucleus	HER2 amplified; IHC expected to be 3+ with a few IHC2+ cases
HER2/CEP17 ratio	<2.0	Not amplified
	≥2.0	HER2 amplified†

CEP17, Centromeric probe 17; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

* Polysomy or extra copies of both HER2 and CEP17 may be responsible.

† Absolute HER2 signal count is preferentially considered than the ratio in some situations

TABLE 1. Summary of alternative definitions of HER2 positivity

Study	IHC		ISH	
			Mean gene CN	HER2/CEP17 ratio
U.S. Food and Drug Administration/EMA/Asia-Pacific ¹⁶	+3 alone		–	–
	2+	and	–	≥2
ToGA study ¹²	3+ alone		–	≥2 alone
Gomez-Martin et al ¹⁵	3+ alone		9.4	4.7
American Society of Clinical Oncology/College of American Pathologists ²³	3+ alone			
	2+	And, either	>6 or	>2
Australian ²²	3+		>6	>2
	2+	and		

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