

## CD117 and PDGFR- $\alpha$ Immunostaining in GISTs

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**Abstract:** GISTs, the most common mesenchymal neoplasms of the GIT are generally characterized by activating KIT/PDGFR- $\alpha$  mutations; a small subset also shown to harbor molecular abnormalities like BRAF and SDHB mutations. Immunohistochemically though GISTs are often CD117 positive, CD117 is significantly less expressed in PDGFR- $\alpha$  mutant GISTs and a minority of PDGFR- $\alpha$  mutant GISTs are CD117 negative.

**Aim:** To analyse the clinicopathological features of 36 cases of GISTs and evaluate the immunohistochemical expression of the markers - CD117, PDGFR- $\alpha$ , CD34, SMA and S100.

**Materials and methods:** The clinicopathological features of the cases analysed and immunohistochemistry done on tissue sections. The correlation between the expression of markers and clinicopathological parameters observed with particular reference to CD117 and PDGFR- $\alpha$ .

**Statistical analysis:** Done using the SPSS software version 11.5. Difference in probability (P) values of  $\leq 0.05$  considered significant.

**Results:** CD117 was positive in 88.9% of cases, PDGFR- $\alpha$  in 72.2% of cases; 61.1% showed positivity for both CD117 and PDGFR- $\alpha$ . The four CD117 negative cases (11.1%) showed PDGFR- $\alpha$  positivity and the ten PDGFR- $\alpha$  negative cases (27.8%) showed CD117 positivity. The expression of the markers showed no significant difference as to the tumor location, cell type and risk category. The number of mitosis did not correlate with the tumor size and found to be statistically significant.

**Conclusion:** CD117 and PDGFR- $\alpha$  immunostaining done with caution in GISTs would have a definite role in the diagnosis, treatment with better targeted drugs and improving the prognosis of high risk patients.

**Keywords:** Gastrointestinal stromal tumors, CD117, PDGFR- $\alpha$ , clinicopathological features, immunohistochemistry.

### I. Introduction

Gastrointestinal stromal tumors [GISTs], the most common mesenchymal neoplasms of the gastrointestinal tract constitute approximately 2% of all GI neoplasms<sup>[4,12]</sup> They constitute a distinctive group of mesenchymal neoplasms that arise from the Interstitial cells of Cajal, the gastrointestinal pacemaker cells.<sup>[1]</sup>

GISTs may occur anywhere along the GIT, the most common locations being the stomach and the jejunum. They may also occur in extra-intestinal locations such as the mesentery, omentum and retroperitoneum where they are referred to as EGIST [Extra intestinal gastro intestinal stromal tumors].<sup>[3,4,20]</sup>

GISTs are characterized by activating KIT mutations in 70% to 85% of cases. The remaining 15% to 30% either harbor activating mutations of PDGFR- $\alpha$  (5 to 15%)<sup>[5]</sup> or BRAF (1% to 3%)<sup>[6]</sup> or are considered wild-type<sup>[20]</sup>, when no mutations of KIT, PDGFR- $\alpha$  and BRAF are found (4% to 6%). KIT, PDGFR- $\alpha$  and BRAF mutations have been shown to be mutually exclusive.<sup>[9]</sup>

Immunohistochemically, though GISTs are often CD117 positive, CD117 is significantly less expressed in PDGFR- $\alpha$  mutant GISTs and a minority of PDGFR- $\alpha$  mutant GISTs are CD117 negative. PDGFR- $\alpha$  immunorexpression is significantly high in these cases.<sup>[10]</sup> With the development of targeted tyrosine kinase inhibitor therapy for GISTs, CD117 and PDGFR- $\alpha$  immunorexpression in these tumors might have significant role in predicting prognosis and prescribing targeted therapies.

### II. Aim Of The Study

To analyse the clinical and histopathological features of GISTs in a group of 36 cases. Also to analyse the expression of a panel of immunohistochemical markers in GISTs with particular reference to CD117 and PDGFR- $\alpha$  which might serve as an indicator for targeted therapies.

### III. Materials And Methods

Tissue samples of 36 selected cases of GISTs collected from the medical records in the Institute of Pathology, Madras Medical College (from January 2005 to October 2010) were included in the study.

The formalin fixed paraffin embedded tissue sections of these cases were reviewed and histopathological diagnosis was confirmed with reference to tumor location, clinical and histopathological features. The study was approved by the Hospital Ethical Committee.

The following clinical and pathological data were analysed:

- Age/sex of the patient
- Clinical presentation
- Location of the tumor
- Tumor size
- Histological type
- Mitotic index in 50 HPF
- Risk categorization based on NIH consensus guidelines (tumor size and mitotic rate).

Selected representative tissue blocks of each case was evaluated for immunohistochemical expression of a panel of markers - CD117(BioGenex), CD34(BioGenex), SMA(BioGenex), S100(BioGenex) and PDGFR- $\alpha$ (Allied Scientific Products). IHC analysis was done using the Polymer-HRP Detection System of BioGenex, San Ramon, USA. CD117 and PDGFR- $\alpha$  evaluated with internal control of mast cells for CD117 and myenteric plexus cells for PDGFR- $\alpha$ . The IHC results were assessed in a semiquantitative manner: intensity of staining (strong, moderate, weak, none) and fraction of positive cells (3+, >75% cells; 2+, 10-75% cells; 1+, <10% cells; and negative, none).

The correlation between the expression of the markers and risk category, tumor size and mitotic rate were statistically analysed using the SPSS software version 11.5. A difference in probability (P) values of  $\leq 0.05$  was considered significant.

#### IV. Results

The study comprising 36 cases included 18 males and 18 females showing no gender predilection. The age distribution ranged from 19 yrs to 79 yrs with peak incidence in 41-60 yrs age group(28/36 cases). The most common clinical presentation was abdominal pain or discomfort (21/36 cases) followed by abdominal mass, anaemia and gastrointestinal bleeding.

All were primary tumors, the common locations being stomach(33.33%), small intestine(30.56%) and mesentery(25%) in decreasing order of frequency. The tumor size ranged from 2 to 28cm with a median size of 10cm. 25% of tumors were less than 5cm and 75% were more than 5cm, with almost equal distribution of cases in 6-10cm (36.1%) and more than 10cm (38.9%) categories. Grossly most tumors were nodular exophytic growth in the bowel wall with areas of hemorrhage, necrosis and cystic degeneration seen in high grade tumors. Histologically, most tumors were of spindle cell type (88.9%) and a minority had epithelioid(2.8%) and mixed(8.3%) cytomorphology. The mitotic activity ranged from 2-30/50HPF with more than half of the tumors showing  $\leq 5/50$ HPF.

Risk categorization with NIH consensus guidelines showed that majority of tumors(61%) fell into the high risk category. Majority of the small bowel and mesenteric GISTs(65%) were high grade tumors.

Immunohistochemical analysis done showed that CD117 was positive in 88.9% (32/36) of cases, PDGFR- $\alpha$  in 72.2% (26/36), CD34 in 63.8% (23/36), SMA in 58.3% (21/36) and S100 in 22.2% (8/36) of cases. The CD117 and PDGFR- $\alpha$  immunopattern observed in the tumors is shown in Table.1.

**Table 1**

CD117/PDGFR- $\alpha$ expression	No. of cases	Percentage
+/+	22	61.11
+/-	10	27.77
-/+	4	11.11

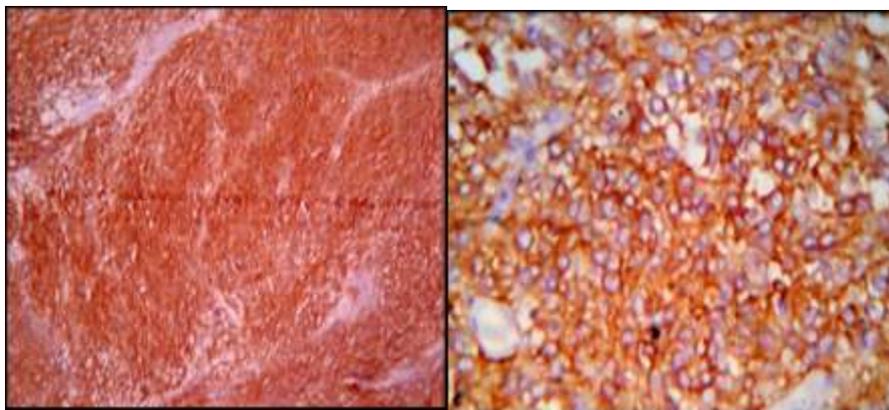
Of the 36 cases, 22 cases(61.1%) were positive for both CD117 and PDGFR- $\alpha$ . The four CD117 negative cases(11.1%) were positive for PDGFR- $\alpha$  and the ten PDGFR- $\alpha$  negative cases(27.8%) showed CD117 positivity.

Of the 32 CD117 positive cases in the study, 26 cases showed moderate to strong cytoplasmic and/or membranous staining in >75% of tumor cells. Only 3 cases showed <20% CD117 positive tumor cells and paranuclear dot-like immunostaining seen in some tumors (Fig.1,2,3). Of the 26 PDGFR- $\alpha$  positive cases, 23 cases showed cytoplasmic and/or membranous staining in >75% tumor cells; 3 cases showed <20% PDGFR- $\alpha$  positive tumor cells and paranuclear dot-like immunostaining in some tumors (Fig.4,5,6,7).

No significant difference was noted in the expression of CD117 and PDGFR- $\alpha$  as to the tumor cell type, location of the tumor and risk category (Table .2). One epithelioid GIST included in the study showed both CD117 and PDGFR- $\alpha$  positivity.

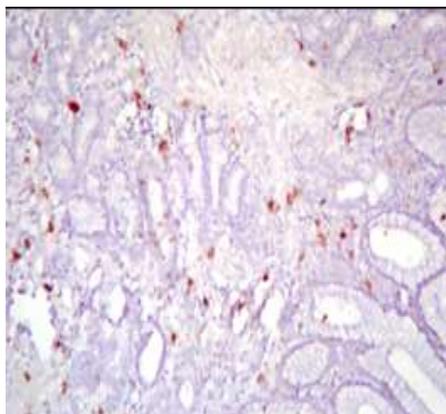
**Table 2**

S.No.	Histological parameters	Total no. of cases	CD117 positive No.(%)	PDGFR- $\alpha$ positive No.(%)
<b>Location of tumor</b>				
	Stomach	12	12(100%)	7(58.3%)
	Oesophagus	01	01(100%)	01(100%)
	Small intestine	11	09(81.8%)	09(81.8%)
	Colon	01	01(100%)	01(100%)
	Mesentery	09	08(88.9%)	06(66.6%)
	Retroperitoneum	02	02(100%)	02(100%)
<b>Cytomorphology</b>				
	Spindle cell type	32	29(90.6%)	22(68.8%)
	Epithelioid type	01	01(100%)	01(100%)
	Mixed cell type	03	02(66.6%)	03(100%)
<b>NIH risk category</b>				
	Very low	0	0	0
	Low	08	07	07
	Intermediate	06	05	04
	High	22	20	15

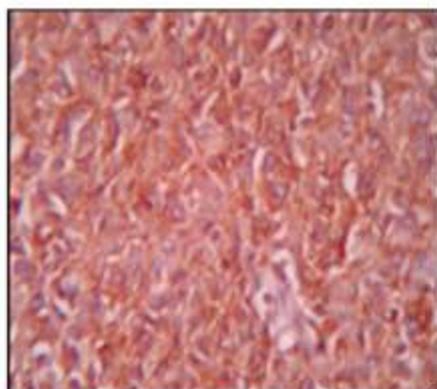


**Fig. 1. CD117 - strong cytoplasmic and membranous positivity in a gastric GIST (100x).**

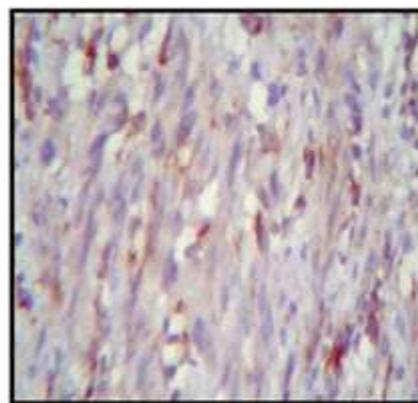
**Fig. 2. CD117-cytoplasmic positivity in epithelioid GIST with perinuclear accentuation in some cells (400x).**



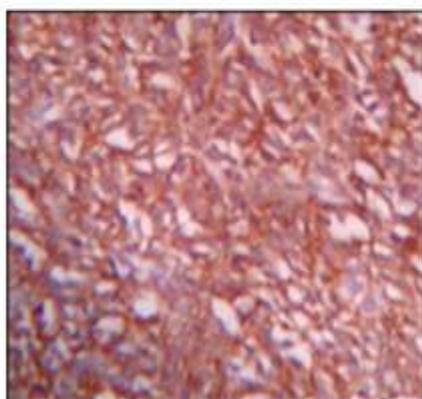
**Fig. 3. CD117 positive mast cells in the lamina propria served as internal control (100x).**



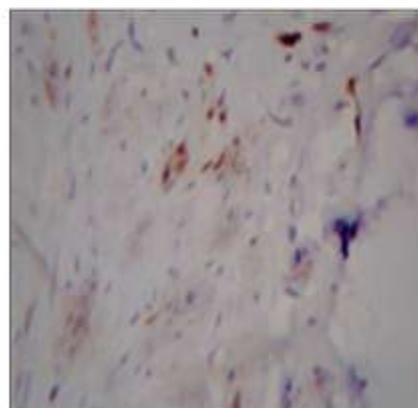
**Fig. 4. PDGFR- $\alpha$ : epithelioid GIST showing strong cytoplasmic positivity (400x).**



**Fig. 5. PDGFR- $\alpha$ : paranuclear dot-like positivity in some tumor cells(400x).**



**Fig. 6. PDGFR- $\alpha$  cytoplasmic and membranous positivity in tumor cells (400x).**



**Fig. 7. PDGFR- $\alpha$ : positivity of myenteric nerve plexus cells served as internal control (400x).**

Statistical analysis showed no significant association between the expression of antibodies and the risk categories. The number of mitosis did not correlate with the size of the tumor and was found to be statistically significant (Pearson Chi-Square test,  $P=0.019$ ; Fisher's Exact test,  $P=0.029$ ).

## V. DISCUSSION

GISTs, the most common mesenchymal tumors of the GIT are refractory to radiotherapy and conventional chemotherapy. They are generally CD117 positive, and display activating KIT/ PDGFR- $\alpha$  mutations. Occasionally they may harbor mutations in other genes, including BRAF and SDHB. GISTs have gained significant interest recently due to the good response seen with newer tyrosine kinase inhibitor therapies.

The current study evaluated the immunohistochemical expression of a panel of antibodies including CD117 and PDGFR- $\alpha$  in 36 cases of GISTs. Of the 36 GISTs studied, there was no gender preference and most of the patients in both sexes were beyond 40 years; the proportion of patients less than 40 years was only 5% in accordance with literature.<sup>[1,2,11]</sup>

The majority of GISTs were in stomach (33.33%) closely followed by those in small intestine (30.56%) and mesentery (25%). Morphologically, majority were spindle cell tumors (88.9%), and less frequently epithelioid (2.8%), and mixed cell tumors (8.3%) which correlates with literature.

The number of mitosis did not correlate with the tumor size, which showed statistical significance (Pearson Chi-Square test,  $P=0.019$ ; Fisher's Exact test,  $P=0.029$ ). Thus the tumor size per se is not a reliable predictor of biological behavior.<sup>[1,12]</sup> The tumor size and mitotic activity are the key parameters in assessing the biologic potential of GISTs and considered the most important prognostic factors.<sup>[4,1,12]</sup> The NIH consensus guidelines (Fletcher et al) also employs the two criteria to assess the risk category in GISTs at any anatomical site.

As in literature<sup>[1]</sup>, high grade GISTs were noted to be more common in men. Also, majority of the small bowel GISTs (63.63%) were high grade tumors. This is in accordance with Miettinen's statement<sup>[1]</sup> that small intestinal GISTs tend to be larger and more advanced when diagnosed.

As per literature<sup>[3,4]</sup>, GISTs are 90% to 95% CD117 positive, 60% to 70% CD34 positive, 30% to 40% SMA positive and 5 to 10% S100 positive. The immunoreactivity observed in the present study was 88.9% CD117 positive, 63.8% CD34 positive, 58.3% SMA positive, 22.2% S100 positive.

In accordance with literature<sup>[1]</sup>, the CD117 positivity observed was typically global, moderate to strong cytoplasmic and/or membranous immunostaining. 81.3% of CD117 positive tumors showed moderate to strong immunoreactivity in more than 75% of tumor cells and no tumor had less than 10% CD117 +ve tumor cells. Also Eva Wardelmann et al<sup>[14]</sup> in his study showed that 82.9% of GISTs expressed CD117 reactivity in more than 75% of tumor cells and no tumor had less than 10% CD117 positive tumor cells. The paranuclear dot-like (Golgi-zone) pattern was seen in some tumors; this was said to correlate with CD117 mutated cases. However Francesca Miselli et al<sup>[13]</sup>, in their study stated that the dot-like decoration was more frequent with PDGFR- $\alpha$ staining in PDGFR- $\alpha$ mutated cases and seen less commonly with CD117 immunostaining in CD117 mutated cases.

CD34, another sensitive though less specific marker for GIST was expressed in 75% of gastric GISTs and 63.6% of small intestinal GISTs. Also Miettinen and JersyLasota had stated that small intestinal GISTs show less CD34 positivity (50%) compared to gastric GISTs(80-85%); and the oesophageal and rectal GISTs show the highest frequency of CD34 positivity in their studies<sup>[1,3]</sup>. One case of oesophageal GIST included in the study showed CD34 positivity. Although none of the markers studied showed statistically significant correlation with risk category, CD34 was expressed in most low grade GISTs(7/8 cases) compared to that in high grade GISTs(12/22 cases) in this study. Also G Rossi et al<sup>[18]</sup> in their studies observed that CD34 was more frequent in low risk tumors. This is in agreement with previous results by Sarlomo-Rikala et al<sup>[15]</sup>, who reported a higher incidence of CD34 negativity in GISTs with malignant clinical behaviour. Moreover a similar phenomenon was also described by Goldblum<sup>[16]</sup> in dermatofibrosarcomaprotuberans, another CD34 positive soft tissue tumor in which CD34 immunoreactivity disappeared when fibrosarcomatous overgrowth occurred within the same lesion. These findings support the idea that loss of CD34 might be associated with tumor progression and with acquisition of a more aggressive behaviour, although further studies are needed to confirm this hypothesis definitely.

SMA was expressed in 72.7% of small intestinal GISTs and 58.3% of gastric GISTs in the study. This is in agreement with Miettinen's observation of more frequent SMA expression in small intestinal than gastric tumors<sup>[23]</sup>.

S100 reported to be rare (5 to 10%) in GISTs was observed in 22.2% of tumors. Also Eva Wardelmann et al<sup>[20]</sup>, KyoungMee Kim et al<sup>[21]</sup>, Ting Jung Wie et al<sup>[22]</sup> have observed S100 positivity in 19.4%, 20.5% and 24% of GISTs in their studies, respectively. However, the S100 positivity was in less than 10% of tumor cells in the present study in agreement with that by Eva Wardelmann et al<sup>[20]</sup>

PDGFR- $\alpha$  was expressed in 72% of tumors; 88.5% showed cytoplasmic and/or membranous staining in >75% tumor cells and 11.5% showed <20% PDGFR- $\alpha$ positive tumor cells and paranuclear dot-like immunostaining in some tumors. The pattern of CD117 & PDGFR- $\alpha$  expression observed in the study compared with some standard studies is noted in Table.3.

Table 3

Studies	No. of cases	CD117 positive cases (%)	PDGFR- $\alpha$ positive cases(%)	CD117/ PDGFR- $\alpha$ Immunoreactivity		
				+/+ (%)	-/+ (%)	+/- (%)
Francesca Miselli et al <sup>[13]</sup>	180	90	97	87	10	2.7
Peterson et al <sup>[17]</sup>	39	97.4	89	87	2.5	7.6
Claudia Mucciarini et al <sup>[24]</sup>	124	88.7	11.3	0	11.3	88.7
G Rossi et al <sup>[18]</sup>	125	93.6	6.4	0	6.4	93.6
Present study	36	88.9	72	61.11	11.11	27.77

The findings observed in the present study: coexpression of CD117 and PDGFR- $\alpha$  in 61% of tumors and PDGFR- $\alpha$  positivity in all the CD117 negative tumors(11.1%) are in agreement with the observations by Francesca Miselli et al<sup>[13]</sup> and Peterson et al<sup>[17]</sup>

Francesca Miselli et al<sup>[13]</sup> in his study involving 180 cases of GISTs showed 97% PDGFR- $\alpha$  positivity with CD117/PDGFR- $\alpha$  coexpression in 87% of cases and PDGFR- $\alpha$  positivity in all the CD117 negative cases(10%). Peterson et al<sup>[17]</sup> in his study involving 39 cases of GISTs showed similar results with 89% PDGFR- $\alpha$  positivity with 87% CD117/PDGFR- $\alpha$  coexpression and all the CD117 negative cases (2.5%) showing PDGFR- $\alpha$  positivity.

G Rossi et al<sup>[18]</sup> and Claudia Mucciarini et al<sup>[24]</sup> showed mutually exclusive expression of CD117 and PDGFR- $\alpha$  in GISTs in their studies. G Rossi et al<sup>[18]</sup> in their study had included a subset of gastrointestinal mesenchymal tumors (15 intraabdominal desmoids, 12 leiomyomas, 8 leiomyosarcomas, 3 schwannomas, 2 solitary fibrous tumors and one each case of inflammatory pseudotumor and fibroid polyp) in their immunohistochemical analysis and noted that all of them were negative for PDGFR- $\alpha$  except a small subset of desmoids (four) which were positive for PDGFR- $\alpha$ .

Eva Wardelmann et al<sup>[14]</sup> had also done mutational analysis of the study cases and observed that CD117 expression was lower in PDGFR- $\alpha$  mutant GISTs than in tumors carrying KIT mutations and vice versa.

Pauls et al<sup>[19]</sup> also reported that the immunohistochemical staining patterns of CD117 and PDGFR- $\alpha$  may predict the mutation type (prior to performing mutational analysis). In their study they found that most GISTs stained for both CD117 and PDGFR- $\alpha$ . However the staining intensity strongly correlated with the mutation type. GISTs with KIT mutations would stain strongly with CD117 and weakly with PDGFR- $\alpha$  and vice versa. Also the dot-like immunostaining of CD117 and PDGFR- $\alpha$  was found to be associated very frequently with mutations of the respective gene.

Francesca Miselli et al<sup>[13]</sup> also performed mutational analysis of the study cases; he observed that CD117 and PDGFR- $\alpha$  coexpression characterized most of the KIT mutated GISTs and wild type CD117/PDGFR- $\alpha$  GISTs; the -/+ (CD117/PDGFR- $\alpha$ ) immunophenotype group segregated exclusively with PDGFR- $\alpha$  mutated cases.

In summary, the present study and studies done elsewhere show that most GISTs were immunoreactive for both CD117 and PDGFR- $\alpha$  and the CD117 negative GISTs often expressed PDGFR- $\alpha$ . PDGFR- $\alpha$ , though a less sensitive and specific marker than CD117, when done in combination with CD117 would have definite clinical significance. The staining intensity, the pattern of staining of either marker and the CD117/PDGFR- $\alpha$  expression pattern would have therapeutic and prognostic implications.

## VI. Conclusion

The development of newer second generation and third generation TKIs for inhibition of CD117 and PDGFR- $\alpha$  has revolutionized the treatment of GISTs. Though complete surgical resection is the primary modality of treatment in localized nonmetastasized GISTs, the targeted therapy with TKIs has a significant role in high grade GISTs and those with metastasis.

GISTs are routinely diagnosed based on clinical and histopathological features, in conjunction with immunohistochemical analysis of CD117, when needed. However approximately 5% of GISTs are CD117 negative and their diagnosis would be supported by immunohistochemical analysis of DOG1, (a new sensitive and specific marker under research) and PDGFR- $\alpha$  (a less sensitive and specific marker under research). PDGFR- $\alpha$  though less sensitive and specific than DOG1, is involved in the tumorigenesis and hence would have definite therapeutic and prognostic implications. Though mutational analysis is the definite method to detect the activating mutations in CD117 and PDGFR- $\alpha$  genes, it is more expensive and time consuming. Hence the combined immunodetection of CD117 and PDGFR- $\alpha$  done with definite IHC protocol and controls would serve as an alternative indicator for targeted therapy in the absence of mutational analysis.

There are only a few literature focusing on the relationship of CD117 and PDGFR- $\alpha$  immunoeexpression with that of clinicopathological parameters, risk groups and mutations in GISTs. The studies done so far show varying results and no conclusive opinion has been arrived. With continuing research on the tumorigenesis, molecular profile, biological behavior and treatment in GISTs, we believe that combined immunoevaluation of GISTs with CD117 and PDGFR- $\alpha$  done with caution would have a definite role in the diagnosis, treatment with better targeted drugs and improving the prognosis in high risk patients.

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