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Comparative study of intraoperative frozen section and scrape cytology with final histopathological diagnosis in ovarian masses (a study of 57 cases)

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Abstract

Background: Making a decision on the extent of surgery for ovarian tumors depends on intraoperative diagnosis. **Aims:** The aim of this study was to compare the intraoperative cytology and frozen section for the diagnosis of ovarian masses. **Materials and Methods**: 57 patients with ovarian masses who underwent surgery for ovarian neoplasms were evaluated by intraoperative scrape and Frozen Section techniques and compared to final histological diagnosis. Assessment of the overall accuracy of the intraoperative diagnosis was classified as concordant or discordant. **Results**: The sensitivity, specificity, positive predictive value, Negative predictive value and accuracy of scrape smear were 89.47%, 100%, 100%, 95% and 96.49% respectively. The sensitivity, specificity, positive predictive value, Negative predictive value and accuracy of Frozen section were 94.74%, 100%, 100%, 97.43% and 98.25% respectively. **Conclusion**: sensitivity and accuracy of frozen section in detecting malignancy is higher than scrape cytology, while specificity in both the methods are same. Frozen section is more accurate than smear preparations in the intraoperative assessment of ovarian tumors in this study. However, the cytology preparation were helpful in supporting the histological diagnoses, and in some cases, provided additional useful information. Thus, cytology has a complementary role to frozen section in intraoperative assessment of ovarian lesions. At the centers where the facilities of frozen section are not available, intraoperative scrape cytology is a useful tool for intraoperative diagnosis of tumor.

Keywords: Frozen section, Intraoperative assessment, Scrape Smear cytology, Ovarian masses

Introduction

Ovarian neoplasms are a heterogeneous group of benign and malignant tumors of epithelial, stromal and germ- cell origin[1].

The purpose of the intraoperative consultation is to provide the surgeon with immediate information concerning the nature of the lesion, to assess the adequacy of the sample, or to evaluate margins[2].

Frozen section is routinely used by the surgical pathology laboratories for intra-operative diagnosis[7] that aids in the categorization of tumors as benign, borderline and malignant, and thus guides in tailoring the extent of surgical therapy, particularly in patients requiring fertility preservation. Accuracy of frozen section has been quoted by several studies to be fairly high, and this ranges from 73% to 98% in determining the status of malignancy[3].

Intra-operative cytology has high accuracy rates, excellent preservation of cellular details, and the possibility of identifying focal, macroscopically undetectable neoplastic lesion in large tissue fragments. Scrape smear cytology is a modification of imprint cytology in which cells are harvested by scraping the cut surface of the specimen[4]. It is an economical, simple and quick method of intra-operative diagnosis with acceptable sensitivity and high specificity, and does not alter the utility of the specimen for subsequent histopathology examination.

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It can be used as an adjunct to frozen section and its routine utilization in ovarian lesions could aid in expanding the knowledge of cytology of ovarian neoplasms[5].

Histopathology of a paraffin section remains the ultimate gold standard in tissue diagnosis[6].

Material & methods

The study was cross sectional study conducted in the Department of Pathology, at tertiary care center in the duration of two years from October 2017 to October 2019, which was approved by Institutional Ethics Committee for human research (IECHR). A total of 57 cases were studied. Data for retrospective study was obtained from departmental records, tissue block and slides. Data for prospective study was obtained from ovarian masses for frozen section received in histopathology section with proforma designed for each patient.

The unfixed fresh specimen of the tumor was immediately delivered to Pathology Department with all the clinical details of the patient for evaluation. The scrape smear (SS) cytology and Frozen section(FS) both the techniques were evaluated independently.Gross examination of the tumor removed was done by inspection, palpation, The specimen was then cut with a sharp knife into two halves. The cut surface was wiped off the excess blood, if present, with the help of a filter paper. Again, reinspection and repalpation of the tumor was done. The most appropriate area thought to be representative of lesion was chosen. The area was scraped with a sharp scalpel or the end of a glass slide, depending upon the type of tissue. A semifluid drop thus obtained was spread over a glass slide. One to four slides per case were taken from different representative areas. The slides were labeled and immediately put into 95% ethyl alcohol and stained with hematoxylin-eosin, then frozen sections was obtained from the same areas of the scraping. Two to five sections were taken depending on the size and heterogeneity of the lesions. Sections of 4–5 microns were taken and rapid hematoxylin and eosin staining was done. The specimens were then fixed in formalin. Sections were taken from the

same area from where scrapings were taken. Paraffin blocks of the sections were processed in the routine way and sections were stained with hematoxylin and eosin (H and E).

All the sections studied microscopically under low and high power and categorized according to the status of neoplasm into benign, borderline and malignant tumor.

The diagnosis obtained by intraoperative scrape and frozen section based on cellularity and cell morphology were compared with final histopathological diagnosis in terms of diagnostic sensitivity, to differentiate between benign and malignant lesions.

Each technique was evaluated independently and the results were recorded separately. Both the techniques (SS and FS) were combined to make final intraoperative impression.

The cases were regarded as concordant or discordant as follows.

Concordant

The cases were classified as concordant if the category of tumor classification was correct regarding its nature that is benign/malignant as intraoperative surgical management of tumor did not change with further subtyping. In addition, the cysts/masses which were given nonspecific and benign cytological diagnosis on SS and FS and were later recorded as benign on histopathology(infective, hemorrhagic) were included under miscellaneous category and not regarded as discordant.

Discordant

The cases where incorrect intraoperative assessment affected the intraoperative surgical management.

The tumors were graded histologically according to World Health Organization classification.

Sensitivity, specificity, and diagnostic accuracy were calculated using descriptive statistics.

Observations and results

The present study included retrospective and prospective intraoperative evaluation of 57 cases of ovarian masses.

According to the histopathological diagnosis, the most common benign lesion in the study was Mucinous cystadenoma(11 cases, 19.30%), followed by Serous cystadenoma(8 cases, 14.04%) and Mature teratoma(8 cases,14.04%).

In this study most common malignant lesion was Serous cystadenocarcinoma(9 cases, 15.79%) followed by Granulosa cell tumor(5 cases, 8.78%).

According to this study, among fifty- seven cases, maximum cases were found in 41-50 years of age group(13 cases), followed by 51-60 years of age group (12 cases).

Distribution according to category of tumor most common lesion was Benign surface epithelial tumors(19 cases, 33.34%), followed by malignant surface epithelial tumor(11 cases, 19.30%). The 3rd common lesion was Benign germ cell tumors (majority was teratoma, 8 cases, 14.04%).

Table: 1 Association between different types of ovarian masses and clinical data, macroscopic and intraoperative picture, and laterality of ovarian masses

	Benign		Borderline		Malignant		Chi square	P value
	No.	%	No.	%	No.	%		
Clinical picture	20	35.09	00	0	37	64.91	13.15	0.0014
Macroscopic and intraoperative picture	38	66.67	1	1.75	18	31.58		
Laterality								
Unilateral	33	57.90	00	0	10	17.54	9.58	0.0083
Bilateral	5	8.77	1	1.75	8	14.03		

There was statistically significant difference in the studied patients as regard the clinical data, macroscopic and intraoperative picture **(p value: 0.0014)**, also there was statistically significant association as regard the laterality of the ovarian masses **(pvalue: 0.0083).**

In the present study Histopathology (paraffin section) was the gold standard method for diagnosis. Out of total 57 cases 38(66.67%) cases were diagnosed as Benign ovarian tumors, 1 case(1.75%) as Borderline tumors and 18(31.58%) cases as Malignant ovarian tumors.

In scrape smear cytological diagnosis out of 57 cases 40(70.18%) and 17(29.82%) cases were diagnosed as benign tumors and malignant tumors respectively with two false negative cases. On frozen section study 39(68.42%) cases diagnose as benign tumors, 2(3.51%) cases diagnosed as borderline tumors and 16(28.07%) cases diagnosed as malignant tumors with one false negative case. There was no any false positive case in both of the methods.

Table: 2 Comparison of results between both methods

| Characteristics	Frozen section	Scrape cytology
Sensitivity	94.74%	89.47%
Specificity	100%	100%
False positive	0%	0%
False negative	5.26%	10.53%
Positive predictive value	100%	100%
Negative predictive value	97.43%	95%
Diagnostic accuracy	98.25%	96.49%

For the purpose of statistical calculation, all the borderline malignancies were included in the malignant group. (Malignancy = Borderline + Malignant lesions)

In present study sensitivity and accuracy of frozen section in detecting malignancy is higher than scrape cytology, While specificity in both the methods are same.

kappa value of scrape cytology and frozen section were 0.919 and 0.96 respectively, which shows almost perfect agreement between scrape cytology and paraffin section and almost perfect agreement between frozen section and paraffin section.
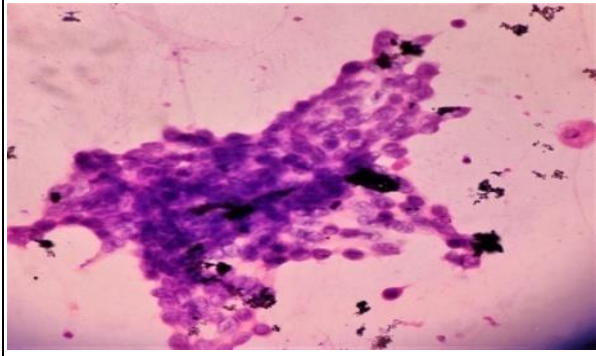
Fig.1 : Serous cystadenoma with monolayered sheets of cuboidal cells with uniform nucleus (H& E ,40x)
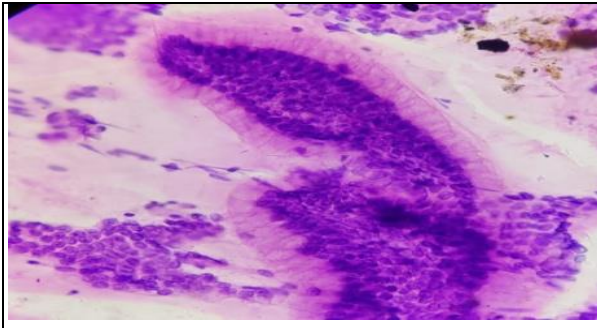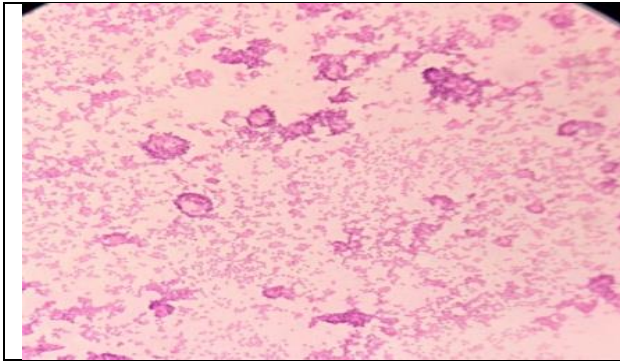

Fig. 2: Scrape cytology of mucinous cystadenoma-columnar cells in monolayered sheets with honey-comb appearance and peripheral palisading
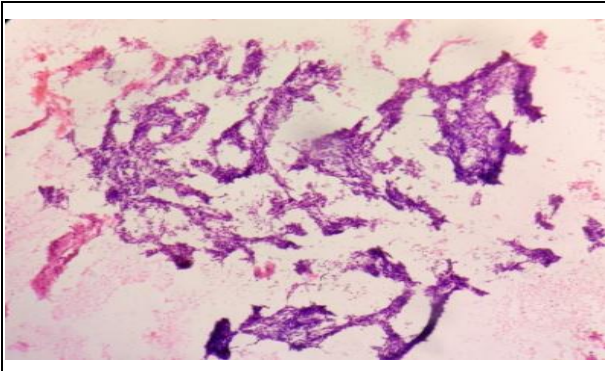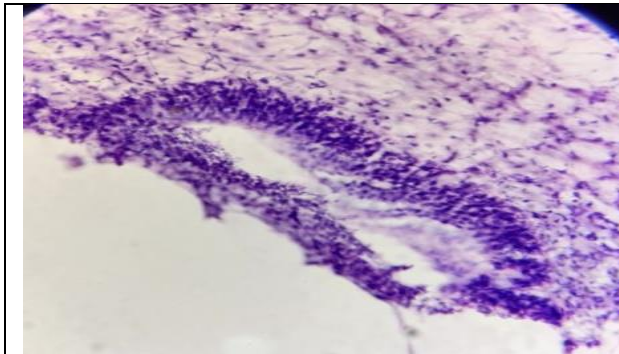

**Fig. 3 : scanner view of papillary serous carcinoma**
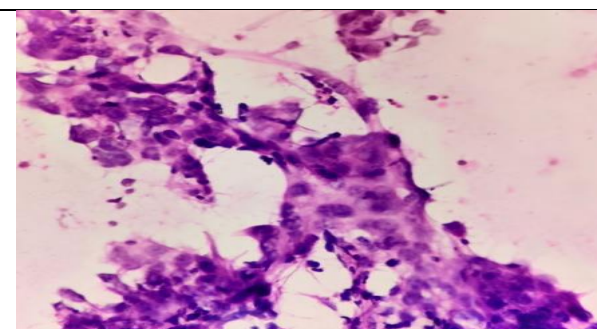
Figure 4: Scrape cytology of Serous cystadenocarcinoma (H&E section, 40x) pleomorphic, highly malignant cells with atypical mitosis

Fig.5: Scrape cytology of granulose cell tumor showing cells in aggregates and microfollicular pattern.
The cells have a scanty cytoplasm and nucleus with grooves and nucleoli.

Fig.6: Scrape cytology shows immature neuroepithelial element with cellular stroma (H&E, 40X)
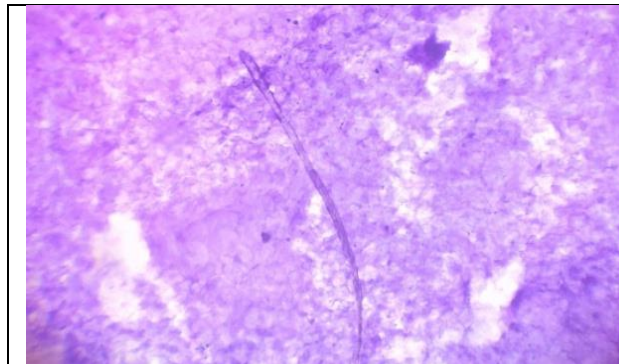
Fig.7: Scrape cytology shows anucleated squames and hair

Discussion

Ovarian masses are frequently being subjected to Scrape smears (SS) and Frozen Section (FS) examination so as to determine the extent of surgery required, and complete resection along with removal of draining lymph nodes can be done in a single operation. Also in cases which are benign, the extensive surgical resection can thus be avoided. Cytomorphological examination of SS alone along with appropriate clinical details can play a crucial role in resource-limited setting where FS is not possible as part of intraoperative consultation.

Traditionally, intraoperative pathological assessment was based on frozen sections. In 1927, Dudgeon and Patrick[7] introduced cytology as a method of intraoperative pathological evaluation. They were first to describe imprint smears of fresh tissuein rapid microscopic diagnosis of tumors. In ovarian lesions, Intraoperative cytology (IOC) has been reported to have comparable diagnostic accuracy to frozen sections[8].

Scrape cytology is a modification of imprint cytology and its diagnostic accuracy is better than imprint cytology[9]. Scraping of the cut surface prior to smearing facilitates the harvesting of cells. Hence, scrape cytology could be preferred over touch preparation/imprint cytology as the former technique would yield much more material than the latter[10].

Different authors have reported several advantages of IOC such as: (1) rapidity of preparation with preserved accuracy; (2) simple and inexpensive method; (3) preservation of cellular details with no problems of freezing artefact; (4) no tissue loss; (5) possible identification of focal neoplastic lesions or variable elements in large tissue fragments; (6) can be done even when only limited tissue is available; and (7) minimal contamination with safe handling. In addition, adipose and necrotic tissues are easier to examine on SS as these are difficult to embed in FS[9,11,12,13].

Over a period of 24 months, the collected data was analysed and compared with other studies to determine usefulness of both the methods.

Table: 3 Comparative diagnostic Accuracy values in Frozen section.

Studies	Number of cases studied	Diagnostic accuracy
Rose et al.[14]	383	92.7%
Wang et al.[15]	299	96%
Maheswari et al.[16]	217	91.2%
Sukumaran et al.[17]	233	91.8%
Subbian et al.[3]	135	84.25%
Jena and Burela[18]	49	89.7%
Swati Bhardwaj et al.[19]	30	100%
Present study	**57**	**98.25%**

Table: 4 Comparative statistical values of Frozen-histological correlation

	Sensitivity	Specificity	Ppv	Npv	Accuracy
Amany M. Abdelghany et al.[1]	90%	100%	100%	93.8%	96%
El
ElahehOuladsahebmadarek et al.[20]	94.94%	90.62%	94.91%	90.62%	92.68%
Dr.P .Supriya et al.[21]	87.5%	90.9%	77.8%	95.2%	90%
Bohara et al.[22]	85.18%	96.15%	95.83%	86.21%	90.56%
Present study	**94.74%**	**100%**	**100%**	**97.43%**	**98.25%**

Table: 5 Comparative statistical values of cyto-histological correlation

	Sensitivity	Specificity	Ppv	Npv	Accuracy
ElahehOuladsahebmadarek et al.[20]	94.91	89.06	88.88	95	94.91
Bohara et al.[22]	88.88	96	96	88.88	92.31
Present study	**89.47**	**100**	**100**	**95**	**96.49**

Table: 6 Comparative diagnostic Accuracy values in scrape cytology

Studies	Number of cases studied	Diagnostic accuracy
Dudgeon, et al.[7]	200	95.5%
Pickren, et al.[23]	1819	97.4%
Mavec[24]	100	93.0%
Suen, et al.[25]	108	96.3%
Shidham, et al.[9]	249	98.4%
Kontozoglou, et al.[26]	215	99.1%
Kolte and satarkar[4]	75	97.3%
Present study	**57**	**96.49%**

Michael et al. performed a comparative study of intraoperative cytology and frozen sections in 63 cases and reported that cytology was slightly better than frozen sections[11]. In contrast, in the present study, the histological diagnosis of frozen sections was slightly better than that of cytology. This difference may have been due to the different methods used to obtain cytological materials. Michael et al. used a combination of imprint cytology (40 cases), fine-needle aspiration cytology (38 cases), and scrapes (5 cases)[11]. FNAC and scrapes are superior to imprints[11] and this combination of methods may have led to their conclusion that cytology was better than frozen sections.

Stewart et al[27]. and shihoazami et al[28]. in their study found that FS are more sensitive compared with cytology diagnosis, like similar results in our study.

We observed that specificity and PPV in diagnosing benign/malignant lesion on SS were 100% and 100%, respectively; however sensitivity of FS and SS were 94.74% and 89.47% respectively. The reason for a lower sensitivity value in this study was probably due to inability of accurately diagnosing borderline/malignant serous tumors for the below-said reasons. However, specificity was similar to other studies based on SS/FS diagnosis alone and thus confirm the overall reliability of a malignant intraoperative diagnosis.

The diagnosis which frequently missed on FS and SS is that of primary mucinous adenocarcinoma as described in various studies in literature[27,10,29,30] similar to the present study. This has been mainly based on the sampling error that is due to multiplicity of heterogeneous areas especially in mucinous tumors and also to the fact that these are large in size and only routine paraffin embedding enables sampling from a number of areas. Hence, multiple smears

from heterogeneous areas on gross should be sampled which is only possible through study of SS.

A jain gupta[31] et al in their study there was a case of mucinous cystadenocarcinoma was wrongly diagnosed as mucinous cystadenoma. We also found the same result in our study as discordance between mucinous tumors. As the reason was large size of the tumor and containing benign as well as malignant components and excess of suppurative inflammation. Representative areas were not taken for frozen section. In scrape cytology there were presence of only dense inflammatory infiltration which were obscuring cellular morphological details of cells which gave false negative result in our study.

There was a statistically significant difference in the studied patients as regard the clinical data, macroscopic and intraoperative picture (p value<0.05). The reason was radiological findings as complex ovarian cysts and in some patients raised CA 125 levels raised clinical suspicion of malignancy.

Bohara, et al[22] in their study they found maximum number of cases were mucinous tumors while in other studies serous group of epithelial tumors were the major group[23,24] as in our study we also found majority was serous group of epithelial tumors.

One case of sertoli leydig cell tumor was not adequately diagnosed by scrape smears and frozen section both, because of lack of representative area. Limited sections for frozen section were not helpful in this case. Multiple paraffin sections from different areaswere helpful to make correct diagnosis.

The pitfalls of scrape cytology lie in determining borderline cases, as it cannot determine invasion. One case of borderline tumor was wrongly diagnosed as benign tumor on scrape cytology as lack of representative area and atypical nuclear features. The same case was correctly diagnosed by frozen section.

One case of low grade serous carcinoma was not exactly diagnosed by frozen section. While in scrape cytology smears micropapillary pattern, atypical nuclear features and psammomabodies gave the clue towards malignant serous lesion. So, adequately diagnosed by scrape cytology.

All the benign serous tumors (n=8) were predominantly cystic filled with pale straw colored fluid and prominent veins on the surface. Scrape smears comprised papillaroid clusters as well as monomorphic sheets of epithelial cells with small dark bland nuclei and moderate to abundant cytoplasm. In majority of the cases background was clean and 3 cases showed macrophages in the background.

There were 9 cases of serous adenocarcinoma, all of which were solid cystic or predominantly solid. The scrape smears were richly cellular showing cells with a high nucleocytoplasmic ratio present in papillary clusters as well as dispersed singly. Background showed necrosis.

There were two cases of mucinous cystadenocarcinoma. Among them one was presented with scrcoma like mural nodules. One case misdiagnosed because of suppurative inflammation. Cells from representative area was not taken while scraping.

There were 8 cases of Mature cystic teratoma, all of which were cystic and majority of them containg sebum like material and hair or tooth. Smears showed anucleatesquames, amorphous dirty material, hair follicle and adipose tissue. The findings in case of mature cystic teratoma were similar to Ganjeiet al[29].

All the stromal tumors were solid in consistency. In 5 cases of granulosa cell tumor, IOC smears showed high cellularity with cells in groups and monolayered sheets. The nuclei were round to oval with granular evenly dispersed chromatin and small to inconspicuous nuclei. Three of the five cases showed an occasional acinar structure with amorphous reddish violet bodies (Call-Exner bodies). The cytological features were similar to those found by Ali et al.[30] in their study of FNAC of 10 cases of adult granulose cell tumor.

In cases of fibroma, cellularity was scanty to moderate, scrape smears showed occasional fragment of tightly packed benign appearing spindle-shaped cells and few scattered oval to spindle-shaped cells in the background.One case of Immature teratoma was adequately diagnosed by both of the methods, showed numerous neuroepithelial elements merging with cellular stroma.Intraoperatively, a definitive diagnosis in benign lesions is not always possible by SS unlike FS as observed in this study and by Stewart et al.[27]; and amitajain gupta[31], this does not actually hamper intraoperative management.

There were no false-positive diagnosis of carcinoma using either the cytology or the frozen section techniques. After slide review of the discordant cases, it appeared that the false negative diagnosis using both the cytology and frozen section preparations were because of sampling error.

In a meta-analysis of 18 studies comparing frozen section diagnosis of ovarian pathology with the final histopathology showed that its sensitivity to detect benign and malignant lesions varies from 65 to 97% and 71 to 100%, respectively, and specificity from 97 to 100 and 98.3 to 100%, respectively[32].

Alvarez Santin C et al. in their study they found that the diagnostic accuracy of Intraoperative cytology has been reported to be as high as 97%, sensitivity 93%, specificity 98%, PPV 91%, and NPV 98%[33]. In our study, the sensitivity, specificity, PPV, NPV, and accuracy of 89.47%, 100%, 100%, 95% and 96.49%, respectively, were recorded. This was comparable to frozen section diagnosis with a sensitivity, specificity, PPV, NPV, and accuracy of 94.74%, 100%, 100%, 97.43%, and 98.25%, respectively.

Conclusion

Finally, we conclude that frozen section proved to be more accurate than smear preparations in the intraoperative assessment of ovarian tumors in this study. However the cytology preparations were helpful in supporting the histological diagnosis, and in some cases, provided additional useful information.

Thus, cytology has a complementary role to frozen section in the intraoperative assessment of the ovarian lesion. It is simple, rapid, inexpensive and reliable technique requiring about 5-10 minutes. It also does not need costly infrastructure or technical expertise and does not have the risk of instrument contamination. At centers where the facilities of frozen section are not available, intraoperative scrape cytology is a useful tool for intraoperative diagnosis of tumor.

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