MONITORING OF TUMOUR GROWTH AND ITS DRUG-INDUCED REGRESSION BY LASER AND ULTRASOUND REFLECTOMETRY

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ABSTRACT

The techniques of He–Ne laser reflectometry which is sensitive to colour, composition and blood flow through tissues and, ultrasound backscattering amplitude (BSA) measurements to detect the structural changes taking place within the narrow region of the tissues have been used to monitor the growth and drug-induced regression of fibrosarcoma in albino rats. The changes in laser reflectance and the BSA appear earlier than any measurable size of the tumour in tissues. During its growth the reflectance decreases, whereas the BSA shows variation depending on the internal structure of tissues. After the introduction of the anti-tumour drug, these parameters show respective changes and attain their values similar to those for control rats.

INTRODUCTION

THERE has been an increasing interest in the development of non-invasive techniques for the monitoring of tumours during their induction, growth process and effect of various chemotherapeutic agents. The measurement of the size of tumour by calipers, although a standard technique[1], can provide information only after the tumour has grown to a measurable size. The application of diagnostic ultrasound has been based on the measurement of backscattering amplitude of these tissues[2] which has been applied to the imaging of advanced growth of tumours.

The advancement of laser technology has provided valuable tools for medical diagnosis. The absorption and scattering of laser radiation depend on the colour, composition and blood flow through the tissues. Based on this principle various techniques, such as laser trans-illumination (detection of changes in skin tissues for dermatological applications), have been developed[3,4], but as such these techniques have limited applications.

Recently, we have applied the integration sphere technique to monitor the tissues located at various regions of human body[5]. As the size of the sphere is large, this technique could not be applied to monitor the tissues of complex configurations. To do so we have further modified this by optical fibres[6] and have applied for the monitoring of the healthy and diseased tissues under *in vitro* conditions[7] and tumour growth in rats[8].

During the passage of laser radiations in the medium, depending on the size, concentration and oxygen contents of cells, multiple interactions take place. Due to diffusion characteristics of the radiations in such a medium an area much larger than the beam diameter is formed[9,10] which further increases with tissue thickness. Thus the measurement of laser reflectance from such tissues provides an integrated information of tissue composition. On the other hand, ultrasonic beam from a focussed transducer can travel greater depth, being scattered only at the interface regions in the tissues is attributed to the change in the acoustical impedance (product of the local density and ultrasound velocity in the medium). The backscattered pulse-echo sequences are received with respect to time by the same transducer and can be gated for a particular depth so that the specific information on the tissue changes within the gated region can be obtained[11]. The combination of these techniques can thus provide information on the overall changes of the tissue structure and the specific variation at any location within the tumour region. To the best of our knowledge such studies have not been reported so far. The aim of our work is to apply both the laser and ultrasonic reflectometry to *in vivo* tissues to provide complementary information on the growth and drug-induced regression of fibrosarcoma in experimental rats.

METHOD

(*a*) *Preparation of the specimen tumours:*

The experimental white albino rats were divided into two groups, the first group was used as control

($n = 5$) and the second group ($n = 10$) was injected with the fibrosarcoma homogenate (suspended in EBSS) of 2 to 5×10^5 cells into the thigh subcutaneously. The transplanted tumour became palpable in 3–5 days and thereafter grew steadily. This group was further divided into two subgroups. In one subgroup no antitumour drug was given while in the second subgroup on the eleventh day the first dose of Daunomycin (0.2 mg/dose/rat) and on the thirteenth day, the second dose of the same drug were injected.

(*b*) *Clinical aspects of the specimen tumour:*

The transplanted tumour steadily grew up to the end of the third week. The animals eventually died in about 4 weeks. The histopathology of the well-developed fibrosarcoma showed that its growth is associated with its specific characteristics such as interwoven bundles of immature fibroblasts with minimal amount of collagen. The tumour was highly cellular with nuclei elongated and hyperchromatic. Gross examination showed that the tumour was mildly encapsulated, and was hard and nodular.

(*c*) *Laser and ultrasound measurements:*

Laser radiation from a 2.0 mW He–Ne laser was directly transmitted by an optical fibre of active diameter 1.5 mm and length 610 mm to a non-random type bifurcated light guide (Y-guide) of active diameter 5 mm at its common end. The output end of this guide was connected to another fibre of active diameter 3 mm and length 470 mm. The output was detected by SGD–1000A photodiode-amplifier and finally read on a digital voltmeter. The schematic of this technique is shown in figure 1. This instrument was calibrated with the $MgCO_3$ coated aluminium plate which reflects 100 %. Thereafter all the measurements with respect to this reflectance were carried out.

Ultrasonic backscattering amplitude from a tissue volume (30 mm^3) located subcutaneously up to a depth of about 6 mm was monitored by a wideband pulse-echo focussed transducer[12]. The transducer (diameter 12 mm) operated at frequency 7.5 MHz and had a focal length 100 mm. This was placed above the tumour by a water-delay coupling tube such that the tumour was in the farfield and focal region of the transducer. This procedure eliminated multiple reflections from the skin and the uncertainty due to near field, and was least sensitive to small angular changes. The ultrasonic echos from the tumour were displayed in the high frequency oscilloscope so that the range gate could be set to select echos from a particular region of the tumour which could be analyzed in a slowly sweeping spectrum analyzer. The schematic of ultrasonic instrumentation is shown in figure 2. For each rat, to estimate the mean backscattering amplitude (BSA) at a frequency of 6 MHz, corresponding to large signal-to-noise ratio of transducer, 5 spectra were recorded and averaged. The range gate (6μsec duration; tissue depth 4.5 mm below skin thickness of 2 mm) and the IF filter bandwidth for spectral analysis were such that these provided a good dynamic range using the well-known criterion[13] for detecting the varying echo amplitudes from the tumour, due to its varying constitutents of solid, necrotic or cystic nature.

(*d*) *Data acquisition and analysis:*

Prior to each measurement, the hair above the tumour region was removed and the animals were anaesthetized by exposing to ether vapour. Thereafter the animals were gently placed on the table and observations carried out.

Prior to each measurement by laser reflectometry the instrument was calibrated and the observations from the control and experimental rats were then recorded. From these observations the averaged values of reflectance for each category of animals were obtained.

For ultrasonic measurement, the transducer was gently placed on the tumour region by water coupling and ultrasonic spectra for each group were obtained. An example of such spectra for control and tumour animals on eighth day is shown in figure 3. From the observations made on rats of each group, the average

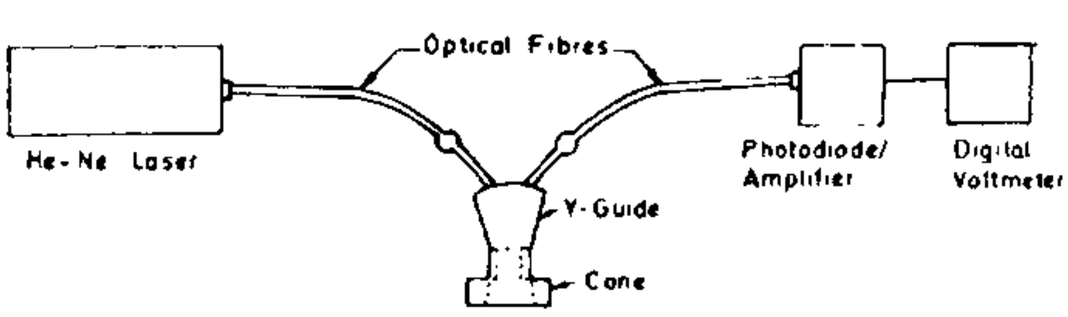

Figure 1. Schematic of He–Ne laser reflectometer.

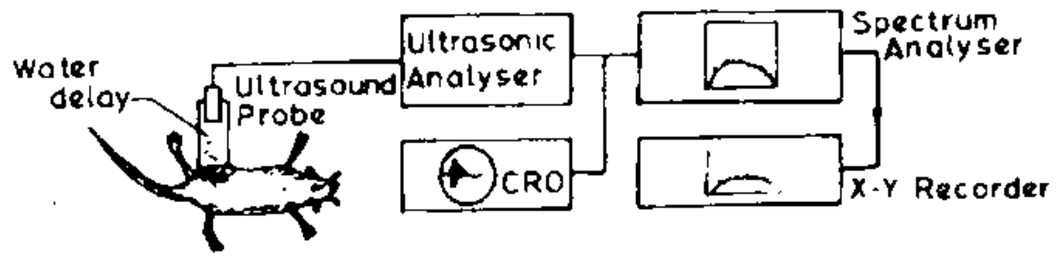

Figure 2. Schematic of ultrasonic instrument to measure backscattering amplitude.

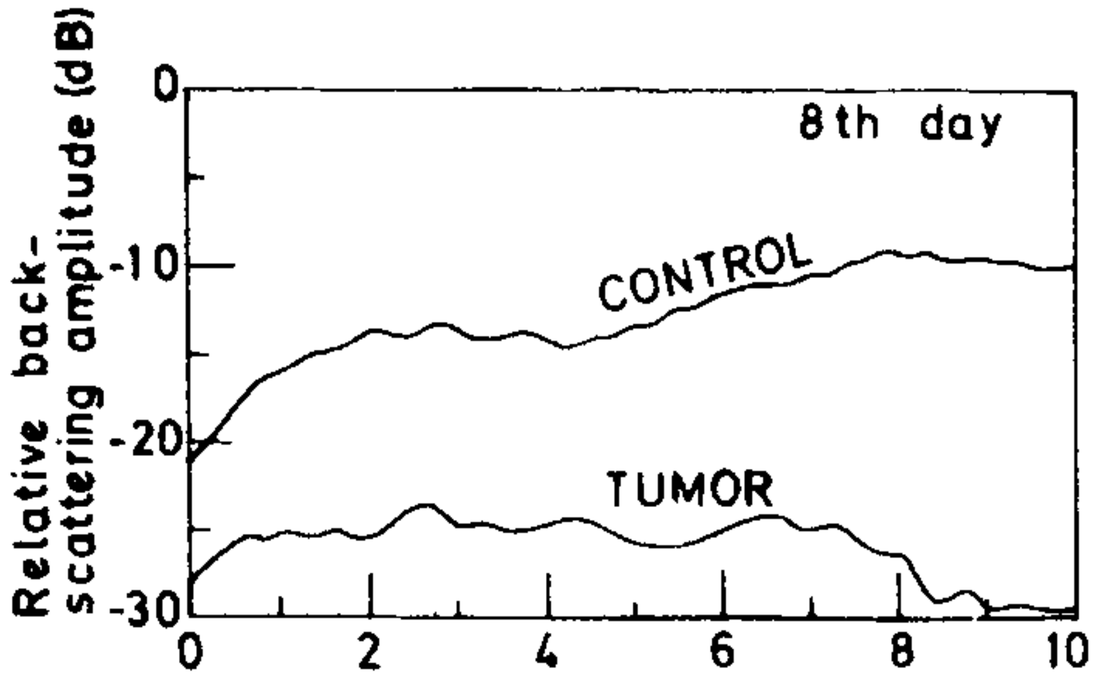

Figure 3. Variation of relative backscattering amplitude of tumour and control tissues at various frequencies on eighth day.

values of the corresponding BSA on various days were obtained.

The size of the tumour was measured regularly by vernier calipers and from this the mass of the tumour was determined by formula $T = LW^2/2$, where L is the maximum diameter and W is the minimum diameter.

RESULTS AND DISCUSSION

In normal adult tissues, cell division and cell death are equally balanced. As far as tumour cells are concerned, this control seems to be set at a different level. In these regions the tissue composition and associated blood flow due to formation of new capillaries are changed[14]. Its rate of growth, chemical and morphological properties are different from that of normal tissues and are easily distinguishable when they occur in soft tissues. While the doubling time of the tumour cells may be long, and growth fraction small, these cells still continue to grow as there is inequality between the number of cells that are dividing and those that are being lost as end cells. Despite the growth of new capillaries the blood supply cannot keep pace with the growth of tissues and because of its inadequate supply of nutrients and insufficient rate of elimination of toxic substances, the tissue necrosis in the central region takes place[1]. The presence of blood or its decomposed products produce a brownish colour in tumours[15] which may lead to the change in the overall characteristics of these tissues.

To measure the tumour growth and its drug-induced regression, laser and ultrasonic backscattering provide important information on the various

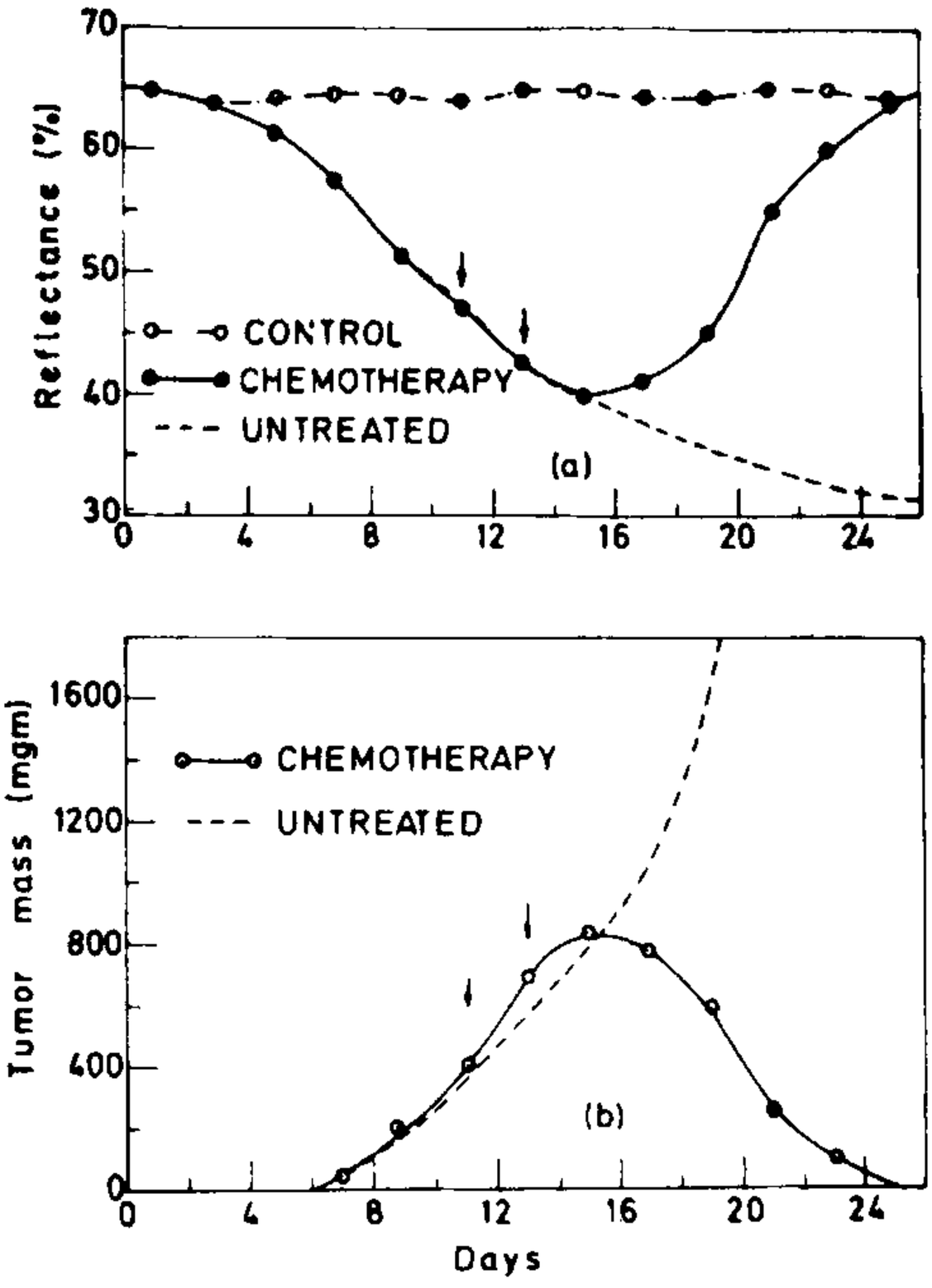

Figure 4. Change in tumour reflectance (**a**) and size (**b**) versus duration (days) before and after the administration of chemotherapy drug.

phases of the growth of fibrosarcoma in tissues. The absorptance and reflectance of such tissues depend on their composition at various stages of its development. Increased blood flow in tumour leads to increased absorption and thus reflectance reduces. Further growth of such tissues and inadequate blood supply tend to produce structural changes in the tumour composition and thus contributing to the further reduction of the reflectance. These reflectance changes are shown in figure 4a. The untreated tumour continues to grow in rats and the reflectance reduces to its minimum as the tumour attains its largest size before the death. In rats injected with Daunomycin (the drug which directly inhibits protein synthesis and blocks DNA function) after a lapse of two days the recovery phase of the tumour appears to begin which is completed in 10 days (average value) as determined by the reflectance value which is same as that of the control. These measurements are furthar substantiated by the tumour mass variation during this

process (figure 4b) but its initial phase or growth which is associated with the neo-vascularization could not be detected by this method. In untreated tumour the reflectance tends to decrease while its mass increases till the death of rats.

These measurements show that the changes in laser reflectance are associated with the growth and regression of tumour but this could not be related with the structural changes taking place at a particular site in the tumour. For this purpose ultrasonic backscattering technique has been employed which can selectively detect these changes. The measured average BSA for control and tumour tissues on eighth day (figure 3) show that there is significant difference of this parameter of these tissues. Furthermore the BSA shows a frequency-dependent phenomenon. Compared to control, tumour tissues show a decrease in BSA as the frequency increases. The increased attenuation at high frequencies indicating the presence of inhomogeneities in tissues. The BSA values determined on various days at frequency 6 MHz are shown in figure 5. In the initial phase of the tumour the BSA increases and reaches a maximum around the sixth day, which could be due to the neovascular changes in tumour. Around the 8th day this parameter shows a minimum which could be due to liquefaction, possibly induced by tissue necrosis. As the tumour mass further increases in size, the BSA increases due to increasing heterogeneity of the tumour growth; in the case of untreated tumour the BSA reaches an ultimate value higher than that of control possibly due to its structural stabilization[1]. In the drug-treated rats the BSA values tend to become close to that of control.

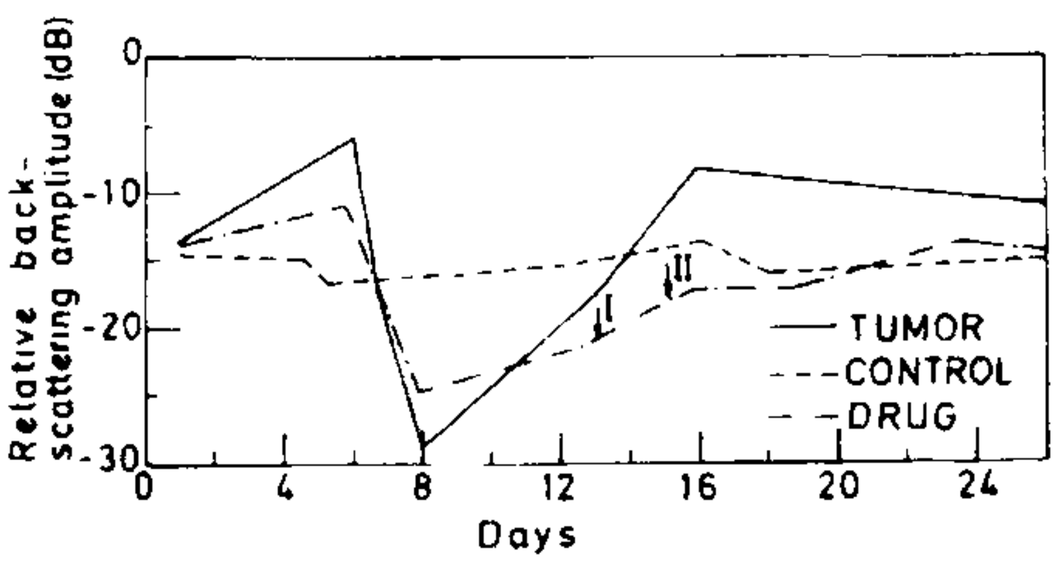

Figure 5. Comparison of the relative backscattering amplitude at various time intervals in control, tumour and drug-administered rats at frequency 6 MHz.

The ultrasonic attenuation which depends on the tissue structure increases with increase of inhomogeneities[16]. Similarly, the corresponding changes in laser reflectance have been observed, which are also associated with the structural changes in the tissue composition. The decreased reflectance could be attributed to the increased vascularization and associated changes in the tissue structure. The well-developed tumour shows a maximum decrease which has further been substantiated by our *in vitro* observations[7]. Similarly, during the final phase the BSA decreases compared to that on the 16th day. This could be attributed to the hypervascularity of tumours which is more echogenic due to the presence of more interfacial structures like blood vessels, fascial planes, etc[17].

A comparative study of the changes in various parameters during the growth and drug-induced regression of tumour is shown in table 1. As it has been pointed out earlier the variations of ultrasonic BSA and laser reflectance depend on different mechanisms. Accordingly the corresponding changes at various time intervals in these parameters have been observed.

Table 1 *Comparative analysis of the changes in measured parameters in drug-injected rates and their possible mechanisms*

Duration (days)	Decrease in reflectance %	Change in BSA %	Mass (mg)	Possible mechanisms (as suggested in ref. 1, 14 and 20)
4	7.6	+32.1	—	Primary neoplasm
8	18.46	−96.4	105.5	Neovascular changes associated with liquefaction
12	30.7	−42.8	520.8	Tumour growth
16	38.46	−28.5	810.7	Tumour stabilization
20	18.5	−21.4	400.2	Onset of drug-induced regression
24	0	+ 7.2	51.5	Tissue composition recovers to normal except some local tissue changes

The laser reflectance continues to decrease, whereas, the BSA indicates the variation corresponding to the specific changes taking place in the medium. There are two important mechanisms detected by these techniques: (a) the initial variation of these parameters while there is no measurable change in tissue size, indicating the change in the internal structure; (b) the recovery of laser reflectance to normal control value indicating the optical normality attained by the tissues while the BSA and mass of tumour still show variation and thus indicating the presence of inhomogeneities in tissue structure. This shows that the observations of these methods complement each other to detect the structural changes within the tissues. Our BSA observations agree with the various changes detected with microscopic and ultrasonic techniques for tumour structures[18,19].

While laser method provides integrated information on tissues changes, the BSA is limited to a small structure of 30 mm^3 below the skin. For better correlation of the observation the ultrasonic data should be recorded by scanning the complete tissue structure by an array of ultrasonic transducers operating as transmitter and receiver. The microscopic and histopathological details at various levels of tumour growth may further help in correlating this data. This forms the aim of our ongoing research which will be reported shortly.

CONCLUSIONS

The laser light reflectance is very sensitive to tissue structural changes and detects even before these could be of appreciable size to be measured by calipers and provides integrated data of the tissue growth. Further details of tissue growth have been characterized by the ultrasonic backscattering amplitude which provides information on the micro-level changes taking place within the tissue structure. The combination of these techniques could be an ideal method to monitor the various induced changes during the growth and regression of the tumours.

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