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ORIGINAL ARTICLE

Maintaining a methods database to optimize solid tumor tissue culture: Review of a 15-year database from a single institution

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Abstract

Chromosome analysis of solid tumors provides valuable information for diagnosis and patient management, yet successfully culturing solid tumors can be challenging. The Children's Mercy (CM) Cytogenetics laboratory has compiled a database of 1371 non-lymphoma solid tumors cultured since 2002. Analysis of the tumor culture data found a culture success rate of 91.6%. Abnormal karyotypes were identified in 47.0% of these tumors. A quality improvement project reviewed the database for methods, cell culture success, yield of clonally abnormal karyotypes, culture failure, tumor diagnostic category, and other. This review revealed processes that could be optimized with minor changes to methods in a subset of tumors. Three tumor/method pair examples are provided including adrenal cortical carcinomas (ACCs), choroid plexus tumors (CPTs), and neuroblastoma. The successful culture of tumors as defined by capture of clonally abnormal cells is dependent upon several factors including culture medium, monolayer versus suspension culture, length of time in culture, method of disaggregation and other. The database serves as a quality assurance tool that enables continuous improvement in culture success rate and abnormal yield. It is also an educational resource for laboratory technologists, residents and fellows. Using the database to track methods and results ensures consistency in routine tumor processing, facilitates oversight to optimize methods for quality, and improves results for patient care.

Keywords Cancer, Chromosome, Solid tumor culture, Database, Quality improvement.

Introduction

The Children's Mercy (CM) cytogenetics laboratory has consistently performed genetic analysis of all new and recurrent pediatric solid tumors, in addition to adult solid tumors from outside institutions. Pathologists select representative samples from fresh resected tumor, place them in transport medium and send them to the Cytogenetics laboratory where they are processed as quickly as possible. Beginning in 2002, a database was initiated to record all aspects of tumor sample processing. The purpose of the database was and is to facilitate teaching and as a reference for culture methods that were/are used to successfully culture different tumor types. The cytogenetics laboratory technologists use and maintain the CM database.

As an aspect of quality assurance, the data were reviewed for overall success and failure rates of tumor culture as recommended in the American College of Medical Genetics (ACMG) Standards and Guidelines for Clinical Genetics Laboratories performing chromosome studies for solid tumor abnormalities [1]. For a Cytogenetics Fellow Continuous Quality and Practice Improvement (CQPI) project, the pediatric data collected after mid-2008 were examined and presented as a poster at the Cancer Genomics Consortium annual meeting [2].

Herein we describe the 2002–2017 CM experience and the benefits of maintaining a tumor culture database. This

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paper encompasses all tumor culture data from 1281 pediatric tumors and 90 non-pediatric tumors collected between January 2002 through September 2017. We have included as Supplemental files, our tumor culture protocol, tumor harvest protocol, solid tumor media protocols, transport media protocol, the Excel database template, and an algorithm for determining which culture type is best for culture initiation at the time of sample receipt.

Solid tumors as described in this paper include all histologically diagnosed solid tumors that were sent to the lab, excluding lymphomas. Tumors may arise from any tissue type in the body. Tumors are classified by the World Health Organization (WHO) in book publications http://publications.iarc.fr/Book-And-Report-Series/Who-Iarc-Classification-Of-Tumours that are used by pathologists when making diagnoses. For the purposes of laboratory cell culture, we use a simplified algorithm to lump tumors into 'small round cell tumors' that are characterized by lack of adhesion between the cells like bone marrow cells and 'monolayer tumors' that are characterized by cell to cell adhesion like skin cells. Using this format, most tumors fall into one or the other category, which is then used to select which medium to use and whether the cells should be cultured in suspension or in monolayer. The laboratory goal is to provide the tumor cells an environment conducive to their growth. The tumor cells that grow in monolayer culture need attachment, thus petri dish or flask cultures work best. The 'small round tumor' cells grow in suspension and will not attach to plastic or glass if they are plated as a monolayer culture. Some tumors may grow either in suspension or monolayer, often depending on their level of differentiation; the more undifferentiated or immature cell types may do better in suspension. For this reason, if possible, the lab will initiate both suspension and monolayer cultures for those tumor types.

The cytogenetics laboratory uses this simplified algorithm to help the technologists in sample processing. The cytogenetics laboratory is dependent on the pathologist to select the sample. The fresh tumor, received directly from the surgical theatre, is viewed by the pathologist who judges by gross examination what piece to send for cytogenetic studies. There is no estimation of tumor burden and the composition of the piece given to cytogenetics cannot be truly characterized. If the diagnosis is not known, the pathologist will usually provide a differential diagnosis. The pathologist tries to select tumor tissue leaving out normal tissue and necrotic tissue. In the cytogenetics lab, the sample is processed using the information provided on the requisition. During the culture period, the pathologist may communicate updates about the working diagnosis or may just issue the final diagnosis once the histopathology studies are complete.

Materials and methods

Data from the entire database were reviewed to determine overall success of tumor culture. Successful culture was defined as obtaining sufficient analyzable metaphase cells for chromosome analysis. Initial and final tumor diagnoses were reviewed to accurately reflect the pathological diagnosis and categorize each tumor as a malignant, benign, or uncertain (borderline) process. True culture success was defined as capture and analysis of abnormal clonal or normal cell populations that appropriately represented the neoplastic or the benign process, respectively.

Culture details of individual tumor types were analyzed to determine which methods led to better results, e.g., if both suspension and monolayer cultures were initiated, did one method type consistently provide the clonal karyotype. This led to observations that changed the protocol for these tumors: neuroblastoma, a common mesenchymal childhood tumor, adrenal cortical carcinoma, a rare pediatric epithelial tumor, and choroid plexus tumors, rare brain tumors. Comparisons were made where different culture conditions were used during processing for the same tumor sample.

Description of the database

An Excel spreadsheet with fields for recording case identifier, collection date, processing date, reason for referral or diagnosis, tumor location or site of origin, institution of origin, karyotype result, mechanical disaggregation method used, use of Collagenase, specific cultures initiated, Colcemid strength and times used for harvest, culture(s) that yield metaphase cells, harvest time(s) that yield metaphase cells, and a field for 'other' comments. Fields for yes/no (true/false) are used to record same day receipt, tissue culture failure, suspension culture, monolayer culture, abnormal result.

Details of each culture recorded include the type of culture initiated (suspension vs monolayer on coverslips or in flasks), culture medium (supplemented RPMI for suspension cultures and supplemented alpha MEM for monolayer cultures), culture times (including direct, overnight, number of days in culture before harvest), and Colcemid exposure time (overnight, minutes, hours, days). Results of the culture methods are recorded (presence/absence of metaphase cells, which culture(s) yielded the metaphase cells, and which culture(s) yielded the abnormal metaphase cells). Tissue culture failures are recorded, as are causes for the failure (including sample size, age of the sample at receipt in lab, necrosis as determined by pathology, wrong diagnosis at intake resulting in non-optimal cultures initiated, and disaggregation method used). A comment field is used as needed to record any other pertinent observations. Karyotype results are recorded. Abnormal and normal results are correlated with the pathology diagnosis to determine clinically successful cultures.

Results

All tumors

Between January 2002 and September 2017, 1371 non-lymphoma solid tumor samples were cultured (1281 pediatric and 90 adult tumors). The overall tissue culture success rate, as defined by the capture of metaphase cells for chromosome analysis, was 91.6% (1256/1371) with pediatric and adult culture success rates of 90% and 87%, respectively. Clonal chromosome abnormalities were identified in 47% (589/1256) of the successfully cultured tumors, with pediatric and adult detection rates of clonal abnormalities in 45.7% and 65.4%, respectively. The overall abnormal detection rate for the pediatric solid tumors was 42.5% over the entire period of

Table 1 Pediatric Solid Tumor Results 2008–2017.

	Malignant		Benign		Uncertain	
Abnormal karyotype	332	57.0%	15	8.2%	13	17.3%
Normal karyotype	213	36.6%	151	82.5%	56	74.7%
Tissue culture failure	37	6.4%	17	9.3%	6	8.0%
Total	582		183		75	

2002–2017. The tissue culture failure rate for pediatric solid tumors was 8% over that time-frame.

Upon review of the surgical pathology report for tumor details and final diagnoses, the tumors were divided into malignant, benign and uncertain categories according to the pathology report. Correlation of these tumor categories with the detection of clonal abnormalities in the successfully cultured tumors from 2008 to 2017 is shown in Table 1. True malignant tumors represented 69.3% (582/840) of all pediatric cases during that time. The remaining cases were benign (183/840, 21.8%) or of uncertain (75/840, 8.9%) histologic type. Benign tumors included adenomas or other non-malignant processes. The uncertain category included tumors that had borderline histology, tumor types with an uncertain natural history, tumors that may recur locally, adenomas with uncertain activity, tumors that may act in a benign or aggressive manner, and maturing cell types.

Brain tumors accounted for 31.3% (263/840) of all pediatric non-lymphoma solid tumors received between 2008 and 2017. An abnormal karyotype was identified in 55.5% (146/263), a normal karyotype in 38.4% (101/263), and tissue culture failure of 6.1% (16/263) of the cases. All but three of the 263 brain tumor cases had a malignant diagnosis.

These data show how often the malignant pathology diagnosis correlated with the finding of a clonal karyotype. In this way, we can see how often we found the clonal karyotype versus a normal karyotype in a malignant process. We can also see how often a clonal karyotype was found in a benign or histologically uncertain process.

Causes of tissue culture failure

Tumor culture failures could be attributed to (1) unknown diagnosis, wrong working diagnosis or insufficient information at the time of sample intake such that culture type selection was non-optimal, e.g., monolayer versus suspension culture, (2) delayed transit of sample to lab which may reduce cell viability, (3) non-optimal sample, e.g., insufficient amount of sample, no tumor in the sample, or necrosis within the sample as noted on the final pathology report, and (4) technologists not using the methods database optimally, e.g., not taking the time to check the database before initiating cultures. During this study project, it was observed that regularly scheduled review of the data could further optimize methods for specific tumor types.

CQPI reveals tumor types with warranted process changes

Pediatric tumor data collected after mid-2008 were reviewed for the Fellow Continuous Quality and Practice Improvement (CQPI) project. Analysis indicated that yield of clonal abnormalities might be improved in specific tumor types if protocols were adjusted. Here we describe the process changes instituted after review for 3 specific tumors: adrenal cortical carcinomas (ACC), choroid plexus tumors (CPT), and neuroblastomas (NB).

Adrenal cortical carcinoma (ACC)

Adrenal cortical carcinoma is an aggressive epithelial cancer that arises from the cortex of the adrenal gland. Because ACC is an epithelial tumor, monolayer cultures are always initiated. ACC is a rare tumor, with a bimodal age distribution, with pediatric cases primarily found in children under 5 years of age. ACC often metastasize and have an overall 5-year survival rate of approximately 20–35% [3,4]. Karyotypically, ACC are usually complex with a high number of structural aberrations [5]. The rarity of ACC makes it unlikely that the technologist initiating cultures has prior experience with the tumor type. Details from previous successfully cultured ACC can be used as a guide or reference with these rare tumor types.

Seven adrenocortical carcinomas were cultured. Six (86%) tumors showed complex clonal abnormalities. Review of the data showed that all tumors were initiated as monolayer cultures after mechanical disaggregation and 6 of the 7 tumors were also enzymatically digested using collagenase. The one monolayer tumor culture processed without collagenase yield only normal metaphase cells. Suspension cultures, which were also initiated for 3 of the tumors, yielded only normal metaphase cells. The enzymatically digested tumor cultures grew best allowing for harvest 6–7 days sooner than the cultures without collagenase exposure.

This review made it clear that for this tumor type, enzymatic digestion is critical to growing the tumor cells and capturing the abnormal clone. The collagenase releases the tumor cells from the surrounding stroma, which promotes faster growth, and timely harvest prevents overgrowth of normal cells. The protocol was altered to state that all ACC are processed with both mechanical and enzymatic digestion. Monolayer cultures are always initiated on coverslips with flask culture back-up. Suspension cultures are no longer initiated, thus saving time and resources.

Choroid plexus tumors (CPT)

Choroid plexus tumors are rare brain tumors that arise from the choroid plexus that lines the ventricles. CPTs are epithelial tumors and grow best in monolayer culture. CPT usually occur in children under two years of age [6,7]. CPT includes benign Grade I choroid plexus papillomas, Grade II atypical choroid plexus tumors, and Grade III choroid plexus carcinomas. The Grade III tumors are karyotypically distinct from Grade I or II tumors. While Grade I and II tumors are commonly hyperdiploid without structural abnormalities, Grade III carcinomas are often low-hypodiploid with approximately 32 chromosomes and may have complex structural abnormalities [8–10].

Review of methods used for 18 choroid plexus tumors found disaggregation methods had significant impact on

Table 2 Choroid plexus tumors - detection of clonal abnormalities.

Carcinoma (Grade III) ($N=7$)	Disaggregation method	Abnormal clone detected (number of tumors)	Normal cells only detected (number of tumors)
	Mechanical only	5	0
	Mechanical + Collagenase	1	1
Papilloma (Grades I, II) ($N=11$)	Disaggregation method	Abnormal clone detected (number of tumors)	Normal cells only detected (number of tumors)
	Mechanical only	4	1
	Mechanical + Collagenase	2	4

clonal metaphase cell yield in this tumor type. Abnormal karyotypes were obtained in 12 of the 18 (67%) CPT, 9 using mechanical only disaggregation. Three tumors were exposed to collagenase after mechanical disaggregation. Five of the 6 (83%) karyotypically normal tumors were exposed to collagenase after mechanical disaggregation.

Six of seven (86%) Grade III CP carcinomas yielded the abnormal clone; 5 of the 6 (83%) using only mechanical disaggregation and one using mechanical plus collagenase. One tumor processed using mechanical plus collagenase yielded only normal cells. Six of eleven (55%) Grade I or II CP papillomas (CPP) yielded an abnormal clone; 4 of the 6 (67%) using mechanical only disaggregation and two using mechanical plus collagenase. Of the 5 CPP with a normal karyotype, 4 (80%) were exposed to collagenase after mechanical disaggregation (Table 2).

Our data showed that CPTs processed with collagenase often did not yield the abnormal clone. The protocol for CPT was altered to ensure these tumors always have cultures established from tumor processed only with mechanical disaggregation. When sufficient tumor is available, some of the fresh tumor is exposed to collagenase with additional cultures initiated. Cultures are grown as coverslip monolayer cultures with a back-up flask culture established when sufficient tumor is received.

Neuroblastoma

Neuroblastoma (NB) is a malignant pediatric cancer derived from immature cells of the sympathetic nervous system. The cells of a NB tumor may be very immature (undifferentiated) or may show various levels of maturation. The pathologist cannot tell the cytogenetic technologist the stage of cellular maturation at the time the sample is sent. Thus, both suspension and monolayer cultures are initiated if sufficient tumor is received. If sample size is small, monolayer cultures are initiated. The cultures are reviewed each day for cell growth, cell attachment, and/or formation of cell clusters floating in the medium. If tumor cell clusters form and grow, the culture is harvested as a suspension culture.

NB is the most common solid tumor outside the central nervous system, is genetically heterogeneous, and accounts for approximately 15% of all pediatric cancer deaths [11]. Diploid and near-diploid tumors with approximately 46 chromosomes are prognostically less favorable than near-triploid tumors with approximately 69 chromosomes [12]. Ploidy as well as segmental chromosome aberrations and *MYCN* amplification impact outcome [13]. These genomic factors in addition to tumor stage and differentiation and patient age are used to stratify patients for therapeutic intervention. Thus is it critical that the genetics of these tumors be optimally assessed.

From our database of 59 NB tumors cultured, 51 (86%) were karyotyped and 34 of those karyotyped (67%) were abnormal. There were 9 (18%) hyperdiploid tumors, 25 (49%) near-diploid / near-tetraploid tumors, and 17 (33%) karyotypically normal tumors. This percentage of hyperdiploid karyotypes is significantly lower than expected, given that studies report a ratio of hyperdiploid to near-diploid of ~2:1 [14,15]. This suggests the culture methods used were suboptimal for capture of clonally abnormal metaphase cells, particularly from hyperdiploid NB.

Review of the methods used for NB tumors showed that while both suspension and monolayer cultures were initiated in most tumors, the abnormal cell population was most often harvested from tumors that had been exposed to collagenase, were cultured in flasks, harvested as a suspension culture, and grown in culture for 3–7 days. Monolayer cultures from coverslips most often yielded normal metaphase cells. Same day receipt of sample with immediate processing is an important factor. Of the samples that failed to grow, 75% were not received the date of sample collection.

Examination of cell harvests using different concentrations of Colcemid, exposed for different lengths of time, e.g., overnight, 24, 48, 72, 96, or more hours, has shown the abnormal clone is most often captured from longer cultures. Specifically, NB cells are now consistently grown in flask suspension cultures with cell cluster growth reviewed daily. The lab protocol was altered to reflect these changes and all NB have one or more suspension cultures harvested between days 3 and 7 in culture.

Discussion

The American College of Medical Genetics (ACMG) publishes Standards and Guidelines for Clinical Genetics Laboratories with updates approximately every five years. The most recent guidelines document for Chromosome Studies for Solid Tumor Abnormalities was published in 2016 [1]. It states, 'For quality assurance, the laboratory should monitor the number and types of tumors received, the percentage of tumors with abnormal results, the cell culture success rate, and the success rate for FISH studies'.

The CM lab experience with maintaining a tumor database provides a number of benefits for the lab staff and results in a higher yield of clinically useful genetic information for patient management. The database provides a ready source of tumor specific tissue culture information for processing. Even when the technologist has no experience with a tumor type,

the information stored in the database provides sufficient information to assist in the choice of appropriate cultures for initiation and for the choice of optimal harvest parameters, thereby leading to a greater likelihood of success. This resource reduces or eliminates the inter-technologist variability. New technologists or fellows are trained in tissue culture by an experienced technologist until they pass competency tests, after which there is supervision available if needed.

CM technologists use the database as a reference when initiating and harvesting cultures and for teaching new personnel how to approach different tumor types, e.g., culture type (suspension versus monolayer), culture vessel (coverslip, flask), medium type (supplemented RPMI, supplemented alpha-MEM), method of disaggregation (mechanical and/or enzymatic), harvest method and times, Colcemid strength and time. Technologists recognize the importance of intake information that may include diagnosis and/or tumor histology, and specific culture factors that can impact tumor tissue culture success, such as same day initiation of tumors into culture. Delays in transit and inadequate information accompanying the tumor impacts optimal processing and correlates with higher culture failure rates.

The database is a quality assurance / quality improvement resource. The accessibility to the data collected by technical staff assures there are reliable culture processes. A periodic review of the employed methodologies for recurrently seen tumors can save resources, e.g., supplies, reagents and technical time, and maintain a high level of quality and reliability of tumor culture. Review of recorded data for specific tumor types can reveal a culture parameter, harvest time, or method of disaggregation that contributes to the consistent success or failure for a particular tumor type. This reflects the differences in the cell types encountered with tumors. Once recognized, the methodology for that tumor type can be modified to improve results.

Limitations of the study

This study was from one point in time. The database, comprised mainly of pediatric tumors, many quite rare, is limited by the number of processed samples that were available for comparison of methods. All tumors were not processed by the same technologist, which introduces some variability, though the goal of maintaining the database and technologist training is to reduce that variation. The tumor burden and true composition of each sample is an unknown at the time sample is processed. Variable sample size and time to receipt after collection can limit the culture options and affect tumor growth.

This study highlighted that regular review of the data would be beneficial and could further improve tumor processing. And that while the lab has maintained this database for more than 15 years, we have not utilized it to its full potential. It is apparent, though, that the work of maintaining this database as a living document has been worth the time and effort by the staff as evidenced by the overall high rate of this laboratory's solid tumor culture success.

Summary

The primary goal of tumor tissue culture is to obtain metaphase cells for chromosome analysis to assess the clonal aberrations specific to a tumor. This methodology remains a powerful tool even in the age of microarray, sequencing and bioinformatics. The successful chromosome analysis of tumor tissue provides a whole genome assessment, which can reveal diagnostic reciprocal translocations, a pattern of aberrations which is diagnostic of a specific tumor diagnosis, as well as identify subclonal evolutionary populations.

For clinical laboratories that receive solid tumor samples for cell culture and karyotyping, maintaining a record of the methods used for tumor cell growth and harvest provides a great resource for teaching and learning. It also provides a quality assurance tool, which contributes to high success rates. Periodic review of the data should be done to assure the best approaches are being used. The review should be an overall assessment of successes and failures as well as specific tumors to reveal how specific tumor types respond to culture conditions. With this practice, clinical laboratories can expect to improve the yield of clinically useful genetic information for management of patients who have solid tumors.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.01.006.

References

[1] Cooley LD, Morton CC, Sanger WG, Saxe DF, Mikhail FM. Section E6.5-6.8 of the ACMG technical standards and guidelines: chromosome studies of lymph node and solid tumor-acquired chromosomal abnormalities. Genet Med 2016;18(6):643–8.
[2] Smith SC, Warren LM, Cooley LD. Tumor culture for chromosome analysis: children's mercy cytogenetics laboratory 15-year database of methods. Abstract. Cancer Genet 2016;209(6):294.
[3] Kerkhofs TM, Verhoeven RH, Van der Zwan JM, Dieleman J, Kerstens MN, Links TP, Van de Poll-Franse LV, Haak HR. Adrenocortical carcinoma: a population-based study on incidence and survival in the Netherlands since 1993. Eur J Cancer 2013;49(11):2579–86.
[4] McAteer JP, Huaco JA, Gow KW. Predictors of survival in pediatric adrenocortical carcinoma: a Surveillance, Epidemiology, and End Results (SEER) program study. J Pediatr Surg 2013;48(5):1025–31.
[5] Dohna M, Reincke M, Mincheva A, Allolio B, Solinas-Toldo S, Lichter P. Adrenocortical carcinoma is characterized by a high frequency of chromosomal gains and high-level amplifications. Genes Chrom Cancer 2000;28(2):145–52.
[6] Lam S, Lin Y, Cherian J, Qadri U, Harris DA, Melkonian S, Jea A. Choroid plexus tumors in children: a population-based study. Pediatr Neurosurg 2013;49(6):331–8.
[7] Ogiwara H, Dipatri AJ, Alden TD, Bowman RM, Tomita T. Choroid plexus tumors in pediatric patients. Br J Neurosurg 2012;26(1):32–7.
[8] Kamaly-Asl ID, Shams N, Taylor MD. Genetics of choroid plexus tumors. Neurosurg Focus 2006;20(1):E10.
[9] Roland B, Pinto A. Hyperdiploid karyotype in a choroid plexus papilloma. Cancer Genet Cytogenet 1996;90(2):130–1.
[10] Ruland V, Hartung S, Kordes U, Wolff JE, Paulus W, Hasselblatt M. Choroid plexus carcinomas are characterized by complex chromosomal alterations related to patient age and prognosis. Genes Chrom Cancer 2014;53(5):373–80.

[11] Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. Nat Rev Cancer 2003;3(3):203–16.
[12] Kaneko Y, Kanda N, Maseki N, Sakurai M, Tsuchida Y, Takeda T, Okabe I, Sakurai M. Different karyotypic patterns in early and advanced stage neuroblastomas. Cancer Res 1987;47(1): 311–318.
[13] Bogen D, Brunner C, Walder D, Ziegler A, Abbasi R, Ladenstein RL, Noguera R, Martinsson T, Amann G, Schilling FH, Ussowicz M, Benesch M, Ambros PF, Ambros IM. The genetic tumor background is an important determinant for heterogeneous MYCN-amplified neuroblastoma. Int J Cancer 2016;139(1):153–63.
[14] Tomioka N, Kobayashi H, Kageyama H, Ohira M, Nakamura Y, Sasaki F, Todo S, Nakagawara A, Kaneko Y. Chromosomes that show partial loss or gain in near-diploid tumors coincide with chromosomes that show whole loss or gain in near-triploid tumors: evidence suggesting the involvement of the same genes in the tumorigenesis of high- and low-risk neuroblastomas. Genes Chrom Cancer 2003;36:139–50.
[15] Ladenstein R, Ambros IM, Pötschger U, Amann G, Urban C, Fink FM, Schmitt K, Jones R, Slociak M, Schilling F, Ritter J, Berthold F, Gadner H, Ambros PF. Prognostic significance of DNA Di-tetraploidy in neuroblastoma. Med Pediatr Oncol 2001;36:83–92.