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STATE-OF THE-ART IN THE MANAGEMENT OF CANCER

CANCER OF THE CENTRAL NERVOUS SYSTEM — PART I

EPIDEMIOLOGY, ETIOLOGY, DIAGNOSIS AND PROGNOSIS

MEETING COVERAGE

CANCER OF THE CENTRAL NERVOUS SYSTEM

FROM THE AMERICAN ASSOCIATION OF CANCER RESEARCH CONFERENCE
JUNE 7-11, 1997, SAN DIEGO, CA

A REPORT ON MOLECULAR MEDICINE OF CANCER, A GENETIC DISEASE

FROM THE NINTH ANNUAL USHA MAHAJANI SYMPOSIUM AT THE SALK INSTITUTE, SEPTEMBER 5, 1997

STATE-OF THE-ART IN THE MANAGEMENT OF CANCER

CANCER OF THE CENTRAL NERVOUS SYSTEM — PART I EPIDEMIOLOGY, ETIOLOGY, DIAGNOSIS AND PROGNOSIS

Although relatively rare, primary tumors of the central nervous system (CNS) are the second most common cancers in those aged 0 to 34, the fourth most common cause of cancer death in males aged 35 to 54 and the leading cause of cancer-related mortality in individuals between the ages of 34 to 45 years (CA: A Cancer J for Clinicians 1996; 46:8-9,19). Also, the brain is a frequent site of metastasis for many common primary tumors, complicating treatment, negatively affecting quality of life and hastening death.

WORLDWIDE EPIDEMIOLOGY OF PRIMARY CNS TUMORS

Incidence and mortality rates associated with primary cancer of the CNS in selected world regions are shown in Exhibits 1 and 2. The Scandinavian countries and Israel report the highest incidence rates of primary brain cancer and Asian countries the lowest. However, worldwide statistics of primary CNS cancers are difficult to compare because, in many countries, limited diagnostic capabilities fail to distinguish primary from metastatic tumors.

There are numerous types of benign and primary malignant brain tumors (Exhibit 3). Several of these are extremely rare and some occur mostly in children. The most common malignant tumors are gliomas which have a very poor prognosis.

USA Epidemiology

The Central Brain Tumor Registry estimates that 40% of reported brain tumors in the USA are benign. Similarly, the American Brain Tumor Association reports that, in the USA, benign and malignant brain tumors occur in 10.9 per 100,000 population and that 4.8 per 100,000 population are successfully treated. Primary malignant brain tumors comprise 1.3% of all cancers diagnosed in the USA (CA: A Cancer J for Clinicians 1996; 46:8-9,19). These tumors are diagnosed in 17,600 Americans annually and result in 13,200 deaths (2.4% of all cancer deaths in the USA). Primary malignant tumors of the CNS are most commonly found in the brain (Exhibit 4). In the USA, the majority of primary malignant brain tumors are either glioblastomas or astrocytomas (Exhibit 5).

Since 1973, incidence of primary brain cancer has risen by 25% (Exhibit 6) and mortality by 16%. This represents a 1.2% annual increase in brain cancer incidence. Most of the increase is attributable to improvements in diagnostic methods in the elderly, and not necessarily to an increase in number of cases (NIH, Cancer Rates and Risks, 1996 May:114).

Gender appears to have some effect on the CNS cancer incidence. Because brain cancer is rare and its potential causes are many, there are no demonstrated gender consistencies and no single confirmed etiologies are noted in epidemiologic studies. In the USA, however, incidence and mortality of CNS cancer is 7.7 and 5.5 cases per 100,000 in males, respectively, and 5.5 and 4.4 cases per 100,000 in females, respectively (CA: A Cancer J for Clinicians, 1996, 46: 8-9, 19). In 1997, of the 13,200 deaths in the USA attributable to cancer of the CNS, 7,200 occurred in males and 6,000 in females. Interestingly, gliomas are more common in males than females, and the reverse is true for meningiomas (Exhibit 7). This suggests a possible hormonal influence in type of tumor development in one or both of these populations.

Race may also be a factor in CNS tumors which are, in general, more common in whites than blacks (Exhibit 6) and least common in Asians. However, these data may be skewed by fewer confirmed or reported cases diagnosed in affected black populations. The majority of brain cancers in whites are gliomas and, in blacks, meningiomas (NCI: SEER Cancer Statistics Review, July 1996).

Age also plays a role in CNS cancer as it is more commonly encountered in children and young adults than other maligancies. Incidence of primary brain tumors in children under the age of 15 is between 3.3 and 5.1 per 100,000 (NCI: SEER Cancer Statistics Review, July 1996; Central Brain Tumor Registry of the United States, 1996 Annual Report). Approximately 3,059 children are diagnosed with a primary brain tumor in the USA each year. Primary CNS cancer accounts for approximately one-fifth of all cancers in children under age 15. However, 60% of all children with CNS tumors survive into adulthood (Cohen M and Duffner P, Brain Tumors in Children, Second Edition, Raven Press, New York, 1994:3). In children under the age of 10, CNS cancer is the second most commonly occurring cancer after acute leukemias. Brain tumors are more common in children age 7 and younger than in older children, and tend to be more common in boys than girls (Exhibit 8). In children, predominant CNS tumor types are astrocytic tumors and primitive neuroectodermal tumors such as medulloblastoma or neuroblastoma (Exhibit 9). In contrast, adult CNS tumors are glioblastomas, astrocytomas, or meningiomas (Exhibit 5).

EPIDEMIOLOGY OF SECONDARY CNS TUMORS

CNS tumors may also be secondary malignancies occurring in patients who have survived after having been diagnosed with primary cancers of other organs. Such tumors are becoming more commonplace as more patients survive a first bout with cancer. An exploratory study was conducted using data from the Surveillance, Epidemiology, and End Results (SEER) Program to estimate the relative risk (RR) of developing a second primary brain tumor following other cancers. Elevated RR of

brain tumors such as astrocytoma and glioblastoma multiforme were detected after bladder cancer in both men and women, after sarcoma in men, and after colorectal and endometrial cancer in women. The highest RR observed in this study was for CNS lymphoma following any first primary malignancy in men (Ahsan H, etal, J Clinical Oncology, 1995 Dec, 13(12):2931-5).

Often a differential diagnosis between metastatic spread from a prior malignancy and a new "secondary" neoplasm is not performed, particularly in the case of brain lesions, because of their poor prognosis that discourages clinicians from ordering a diagnostic workup. Secondary cancers of the CNS are devastating late complications of cancer. Approximately 80% of secondary brain neoplasms are supratentorial and have a very poor prognosis. However, in some cases, differential diagnosis could change the therapeutic management and the prognosis associated with the malignancy. For instance, about 8% of primary central nervous system lymphomas (PCNSL) occur as second malignancies. PCNSL are rare neoplasms of B cell origin that constitute less than 1% of non-Hodgkin's lymphoma (NHL) cases. Because prognosis and standard management of brain metastases and PCNSL are almost completely different, it is important to distinguish one from the other; while treatment of brain metastases is often palliative, the goal of PCNSL treatment is curative (Reni M, etal, J Neuro-Oncology, 1997 Apr, 32(2):135-42).

Exhibit 1
Estimated Worldwide Incidence of Primary Brain and Nervous System Cancer in 1997

Country	Male		Female		Total	
	(#)	Rate*	(#)	Rate*	(#)	Rate*
Denmark	255	9.8	248	9.3	503	9.5
France	1,624	5.7	1,319	4.4	2,943	5.0
Germany	2,064	5.0	1,540	3.6	3,604	4.3
Greece	314	6.0	230	4.3	544	5.1
Ireland	113	6.4	89	5.0	203	5.7
Italy	1,962	7.0	1,475	5.0	3,437	6.0
Luxembourg	11	5.5	9	4.4	21	4.9
Netherlands	372	4.8	284	3.6	657	4.2
Portugal	336	7.1	313	6.1	649	6.6
Spain	1,249	6.5	981	4.9	2,230	5.7
UK, England & Wales	1,609	5.6	1,165	3.9	2,774	4.7
UK, Scotland	152	6.1	116	4.4	268	5.2
EEC Total	10,061	5.9	7,772	4.4	17,834	5.1
Austria	235	6.0	196	5.0	431	5.4
Finland	163	6.5	133	5.3	295	5.8
Iceland	11	8.4	12	9.0	24	8.7
Malta	11	6.0	10	5.2	21	5.5
Norway	214	9.8	188	8.6	401	9.1
Sweden	473	10.7	469	10.6	942	10.5
Switzerland	218	6.1	150	4.2	369	5.1
Non-EEC Total	1,326	7.8	1,157	6.6	2,483	7.2
Bulgaria	225	5.3	199	4.5	423	4.9
Czech Republic	291	5.8	212	4.0	503	4.9
Hungary	270	5.7	250	4.8	520	5.2
Poland	791	4.2	795	4.0	1,586	4.1
Romania	407	3.9	296	2.7	703	3.3
Slovakia	139	5.3	119	4.3	258	4.8
Slovenia	35	3.7	25	2.5	60	3.1
Eastern Europe excluding USSR	2,157	4.6	1,895	3.8	4,053	4.2
EUROPE Total**	13,545	5.8	10,825	4.4	24,370	5.1
Armenia	34	2.0	23	1.3	57	1.6
Belarus	147	3.0	111	2.0	258	2.5
Estonia	33	4.9	28	3.6	61	4.2
Kazakhstan	171	2.1	131	1.5	302	1.8
Kyrgyzstan	29	1.3	26	1.1	54	1.2
Latvia	45	4.0	41	3.1	86	3.5
Lithuania	19	1.1	19	1.0	38	1.0
Russia	2,214	3.2	1,733	2.2	3,948	2.7
Tajikistan	6	0.2	3	0.1	9	0.1
Ukraine	682	2.9	679	2.5	1,361	2.7
Uzbekistan	35	0.3	36	0.3	72	0.3
Former USSR Total	3,416	2.7	2,830	2.0	6,246	2.3

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EPIDEMIOLOGY OF METASTATIC CNS CANCER

Approximately 228,910 cases of primary malignancies of the CNS are diagnosed in North America, Europe and Japan (Exhibit 10). In the USA, in 1995, of 108,000 diagnosed brain or spinal cord tumors, approximately 28,500 were primary malignant and benign tumors and 80,000 were metastatic tumors (Central Brain Tumor Registry of the United States 1996; Laws E and Kamat T, CA: A Cancer J for Clinicians 1993; 43:263,265).

Although it is extremely rare that cancer of the nervous system will metastasize to other parts of the body, many other primary tumors, such as those of the breast, lung, kidney and colorectum, as well as melanoma, commonly metastasize to the brain. Because both lung and breast cancer impact large populations worldwide, brain cancer metastases are a common complication with devastating effects in those with advanced stages of the disease.

China	28,344	4.5	23,669	4.0	52,013	4.3
Hong Kong	125	3.8	103	3.3	228	3.6
India	11,995	2.4	7,953	1.7	19,948	2.1
Israel	272	9.9	278	10.0	551	10.0
Japan	2,156	3.5	2,629	4.1	4,785	3.8
Kuwait	50	4.2	31	3.5	81	3.9
Singapore	36	2.1	31	1.8	67	1.9
Thailand	617	2.1	602	2.0	1,218	2.0
Asia & Other Total	43,595	3.5	35,296	3.0	78,891	3.3
Argentina	972	5.5	725	4.0	1,697	4.7
Australia	469	5.1	462	5.0	931	5.0
Brazil	5,577	6.9	3,739	4.5	9,316	5.7
Costa Rica	64	3.6	33	1.9	98	2.8
Cuba	242	4.4	198	3.6	440	4.0
Paraguay	131	4.6	84	3.0	215	3.8
Philippines	833	2.2	574	1.5	1,407	1.8
New Zealand	109	6.1	99	5.5	207	5.8
Oceania, South America	8,398	5.3	5,188	3.2	13,586	4.3
Canada	1,200	8.4	970	6.6	2,170	7.5
United States	10,100	7.7	7,500	5.5	17,600	6.6
North America Total	11,300	7.8	8,470	5.6	19,770	6.7
TRIAD (N. America, Japan & Europe)**	27,001	6.1	21,924	4.8	48,925	5.4

**Number of cases per 100,000 population*

***Excluding the Former USSR*

Note: Brain and nervous system cancer includes ICD-9 Code 191

Source: Parkin DM, etal, Cancer Incidence in Five Continents, Vol.VI. IARC Scientific Publication 120; WHO, 1995 World Health Statistics Annual Report; CA: A Cancer Journal for Clinicians 1997;47(1):8-9

Incidence of metastatic brain tumors has been increasing. This is largely attributable to improvements in treating systemic cancer, allowing patients living longer and, therefore, increasing the opportunity for malignant cells to spread to the brain.

Neoplastic Meningitis

Neoplastic meningitis (NM; also known as meningeal carcinomatosis, malignant meningitis, or leptomeningeal metastases) occurs when other cancers metastasize to the meninges, the soft tissue surrounding the brain and spinal cord, and is a complication of many tumors. It is estimated to occur in approximately 8% of all cancer cases and is more common in small cell lung cancer and NHL, as well as in breast cancer and leukemia. Progression of malignant meningitis is associated with devastating neurological symptoms including loss of control of bodily functions such as vision, swallowing, bowel and urinary control, and may lead to paralysis.

Investigators at Johns Hopkins University (Baltimore, MD) confirmed in a retrospective study that NM is also relatively common in patients undergoing surgical resection of an isolated cerebellar metastasis (ICM). Of the 66 patients identified between January 1991 and June 1993, 55 underwent a surgical resection of a supratentorial metastasis and 11 (6 females and 5 males) underwent a surgical resection of an isolated cerebellar metastasis. Patients with cerebellar metastases ranged in age from 23 to 74 years (median age of 49 years) at the time of diagnosis, had stable systemic disease and an excellent performance status. However, 4 of the 11 patients (36%) developed unequivocal NM at 1, 3, 6, and 7 months following surgical resection and all died within 1 month from the diagnosis of NM. Among the other 55 patients, only one (2%) developed NM. Therefore, NM following surgical resection of an ICM may be common and potentially fatal. It is suggested that prophylactic intrathecal chemotherapy administered perioperatively may prevent this complication (Norris LK, etal, J Neuro-Oncology, 1997 May, 32(3):215-23).

NM is particularly difficult to treat and typical survival outlook for patients diagnosed with this condition is only

two to four months. Incidence estimates of NM vary, but at least 9,000 patients are diagnosed with the condition in the USA every year. However, incidence may be higher because NM is usually encountered in cancer patients with advanced disease and may not be adequately diagnosed.

ETIOLOGY OF PRIMARY TUMORS OF THE CNS

Etiology of primary CNS cancer remains obscure. However, epidemiologic studies have associated CNS cancer with genetic and environmental factors.

Hereditary/Familial Factors

Although rare, genetically inherited syndromes such as neurofibromatosis, the Li-Fraumeni family cancer syndrome, tuberous sclerosis and Turcot's syndrome, are linked with an excessive risk for developing brain cancer at a young age. A chromosome mutation that has been linked to the increased development of retinoblastoma and brain cancer, has been identified in multiple generations of a family.

Also, although family history of any cancer is not an important risk factor for adult glioma, a family history of primary brain cancer may be significant. First-degree familial medical histories of 462 adults newly diagnosed with glioma in the San Francisco Bay Area between August 1991 and March 1994 were compared with 443 matched controls. Cases and controls had equivalent personal histories of cancers and seizures, except of brain cancer and most nervous system conditions, but differed significantly regarding histories

Exhibit 2
Estimated Worldwide Mortality of Primary Brain and Nervous System Cancer in 1997

Country	Male		Female		Total	
	(#)	Rate*	(#)	Rate*	(#)	Rate*
Denmark	186	7.2	186	7.0	372	7.1
France	1,185	4.2	989	3.3	2,175	3.7
Germany	1,507	3.7	1,155	2.7	2,662	3.2
Greece	229	4.4	173	3.2	402	3.8
Ireland	83	4.7	67	3.8	150	4.2
Italy	1,432	5.1	1,107	3.8	2,539	4.4
Luxembourg	8	4.0	7	3.3	15	3.7
Netherlands	272	3.5	213	2.7	485	3.1
Portugal	246	5.2	235	4.6	480	4.9
Spain	912	4.7	736	3.7	1,648	4.2
UK, England & Wales	1,175	4.1	874	2.9	2,049	3.5
UK, Scotland	111	4.5	87	3.3	198	3.9
EEC Total	7,345	4.3	5,829	3.3	13,174	3.8
Austria	172	4.4	147	3.6	319	4.0
Finland	119	4.7	99	3.8	218	4.3
Iceland	8	6.1	9	6.8	18	6.5
Malta	8	4.4	7	3.8	16	4.1
Norway	156	7.2	141	6.3	297	6.7
Sweden	346	7.8	352	7.8	697	7.8
Switzerland	159	4.5	113	3.1	272	3.8
Non-EEC Total	968	5.7	868	5.0	1,836	5.3
Bulgaria	164	3.9	149	3.4	313	3.6
Czech Republic	212	4.2	159	3.0	371	3.6
Hungary	197	4.2	187	3.6	384	3.9
Poland	577	3.1	596	3.0	1,173	3.0
Romania	297	2.8	222	2.0	519	2.4
Slovakia	102	3.9	89	3.2	191	3.5
Slovenia	26	2.7	19	1.9	44	2.3
Eastern Europe excluding USSR	1,575	3.4	1,421	2.9	2,996	3.1
EUROPE Total**	9,888	4.2	8,119	3.3	18,006	3.8
Armenia	25	1.5	17	1.0	42	1.2
Belarus	108	2.2	83	1.5	191	1.8
Estonia	24	3.6	21	2.7	45	3.1
Kazakhstan	125	1.5	98	1.1	223	1.3
Kyrgyzstan	21	0.9	19	0.8	40	0.9
Latvia	33	2.9	31	2.3	63	2.6
Lithuania	14	0.8	14	0.8	28	0.8
Russia	1,616	2.3	1,300	1.7	2,916	2.0
Tajikistan	4	0.1	2	0.1	7	0.1
Ukraine	498	2.1	509	1.9	1,007	2.0
Uzbekistan	26	0.2	27	0.2	53	0.2
Former USSR Total	2,493	1.9	2,122	1.5	4,616	1.7

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of epilepsy, chickenpox, and shingles. Four cases (<1%) and no controls had known genetic disorders (3 had neurofibromatosis and 1 had tuberous sclerosis). Results led to the hypothesis that a common exposure, such as a viral infection, may play a more dominant role than genetic predisposition in the etiology of adult glioma (Wrensch M, etal, Am J Epidemiology, 1997 Apr 1, 145(7):581-93).

China	20,691	3.3	17,752	3.0	38,443	3.1
Hong Kong	91	2.8	77	2.5	169	2.6
India	8,757	1.8	5,965	1.3	14,721	1.5
Israel	199	7.2	209	7.5	408	7.4
Japan	1,574	2.6	1,972	3.1	3,545	2.8
Kuwait	36	3.1	23	2.6	59	2.9
Singapore	26	1.5	23	1.4	50	1.4
Thailand	450	1.5	451	1.5	901	1.5
Asia & Other Total	31,824	2.6	26,472	2.3	58,296	2.4
Argentina	710	4.0	544	3.0	1,253	3.5
Australia	342	3.7	347	3.8	689	3.7
Brazil	4,071	5.0	2,804	3.4	6,876	4.2
Costa Rica	47	2.6	25	1.4	72	2.0
Cuba	177	3.2	148	2.7	325	3.0
Paraguay	96	3.4	63	2.3	159	2.8
Philippines	608	1.6	430	1.1	1,038	1.4
New Zealand	79	4.5	74	4.1	153	4.3
Oceania, South America	5,420	3.4	4,435	2.8	9,855	3.1
Canada	770	5.4	610	4.1	1,380	4.7
United States	7,200	5.5	6,000	4.4	13,200	4.9
North America Total	7,970	5.5	6,610	4.4	14,580	4.9
TRIAD (N. America, Japan & Europe)**	19,432	4.4	16,700	3.6	36,132	4.0

**Number of cases per 100,000 population*

***Excluding the Former USSR*

Note: Brain and nervous system cancer includes ICD-9 Code 191

Source: Parkin DM, etal, Cancer Incidence in Five Continents, Vol.VI. IARC Scientific Publication 120; WHO, 1995 World Health Statistics Annual Report; CA: A Cancer Journal for Clinicians 1997;47(1):8-9

Environmental Factors

Exposure to one or several environmental factors have been linked to a higher incidence of brain cancer, such as electromagnetic fields and N-nitroso chemical compounds found in certain medications, cosmetics, lotions, tobacco and alcohol as well as in some industrial environments. Neurotoxins are also found in manufacturing of synthetic rubber, polyvinyl chloride, petroleum-based chemicals, pharmaceuticals, nuclear fuels and weapons, in the refining of crude oil, and in agriculture and consist of acrylonitrile, vinyl chloride, formaldehyde, lubricating oils, N-nitroso compounds, phenols, polycyclic aromatic hydrocarbons, organic solvents, and pesticides. Low-frequency electromagnetic fields (50-60 Hz) emitted by electric power lines and household appliances are also suspected environmental risk factors. There is less evidence that microwave frequencies (800-900 MHz) are hazardous (National Radiologic Protection Board, Vol 3; Chilton: NRPB, 1992). Studies have shown that high doses of ionizing radiation emitted from radiotherapy (not X-rays), pose an exceptionally high risk for nervous system cancers (Ron, etal, NEJM, 319:1033-1039, 1988).

Other Factors

Preclinical research has established that animals are most susceptible to chemical and viral neurocarcinogenesis in utero or during early postnatal development (Druckrey H, Xenobiotica; 3:271-303, 1973). In rats, the Avian sarcoma produces glial tumors and the Rous sarcoma causes gliosarcomas; in monkeys, the human polyoma virus (JC virus) has been implicated in glial neoplasms (Devida, Principles of Oncology, 5th Edition, 1997). After research verified the viral origin of CNS neoplasms in dogs, rats and monkeys, epidemiologic studies confirmed the link between an increase incidence in brain cancer and individuals exposed to farm animals or pets with viruses (Choi E, etal, Am J Epidemiology; 91:467-485,1970; Gold, etal, Am J Epidemiology; 109:309-319, 1979). Also, a high incidence of Epstein-Barr viral infection has been observed in humans with primary CNS lymphoma (NEJM 1983; 309:745). Variation in exposure or biologic response to common viral infections may also play a greater role in the etiology of adult glioma than family history (Wrensch M, etal, Am J Epidemiology, 1997 Apr 1, 145(7):581-93).

Other etiologies currently being postulated include severe head trauma and loud noise, possibly predisposing for the onset of acoustic neuroma (Schoenberg BS, Nervous System, 1982). Ringworm of the scalp, frequently diagnosed in Israeli children, when treated with radio-

therapy, predisposed many for the development of brain cancer (Ron, etal, Am J Epidemiology; 127:713-725, 1988).

MOLECULAR GENETICS AND MOLECULAR BIOLOGY OF BRAIN TUMORS

Understanding of the molecular basis of brain tumor development is expanding, providing new tools for diagnosis and therapeutic intervention. Numerous molecular markers have been linked to brain tumors (Exhibit 11) but their role in CNS malignancies has not been fully elucidated and their current utility in clinical medicine is negligible.

Gene Factors

Efforts directed toward explaining genetic events associated with brain tumors have identified gene alterations which occur commonly in these neoplasms. Although the majority of genes associated with these tumors have yet to be identified, as additional gene alterations become apparent, a better understanding of their role in the development of these neoplasms may lead to effective therapeutic interventions.

Also, CNS tumors exhibit a different molecular profile, depending on tumor type. It is, therefore, difficult to generalize genetic abnormalities by broad tumor type because significant variations exist within tumor subtypes. Gliomas, the most common primary tumor of the adult CNS, have been associated with a variety of genetic abnormalities, such as loss of heterozygosity (LOH) for chromosome 17p, mutation of the p53 gene, overexpression of the platelet-derived growth factor-α receptor (PDGF-αr), allelic losses of chromosomes 22q, 13q, and 19q, deletion of the interferon-α and β and CDKN2 loci on chromosome 9p, amplification and rearrangement of the epidermal growth factor receptor (EGFr) gene, and monosomy of chromosome 10 (Furnari FB, etal, Pediatric Neurosurgery, 1996, 24(1):41-9).

Chromosomal/gene abnormalities such as deletions/mutations in growth regulatory genes (tumor suppressor genes and oncogenes) have provided insights into the etiology of CNS neoplasms.

Studies using DNA markers that detect restriction fragment length polymorphisms have shown that loci on chromosomes 10 and 17p are lost frequently in tumor DNA obtained from malignant astrocytoma patients, suggesting that tumor suppressor genes important in astrocytoma tumorigenesis may be present on two different chromosomes. The most frequently-cited abnormality is loss of genetic information from chromosome 17p. This mutation occurs in astrocytomas, anaplastic astrocytomas and glioblastomas.

In one study of astrocytomas, LOH was detected on every autosome except on chromosome 21. Many tumors showed LOH for multiple chromosomes, and the number of chromosomes involved correlated with tumor histopathology. A high-resolution restriction fragment length polymorphism study of chromosome 10 loci showed that loss of broad regions of chromosome 10 was a common event, particularly in glioblastoma multiforme (Fults D, etal, Cancer Res, 1990 Sep 15, 50(18):5784-9). It is interesting to note that molecular genetic evidence suggests that tumor suppressor genes reside in a distinct region on chromosome 10 (Karlbom AE, etal, Human Genetics 1993;92:169-174). The ras suppressor gene (RSU1) has been localized to region 10p13 and has been shown to suppress *in vitro* and *in vivo* growth of cultured glioma cells (Tsuda T, etal, Oncogene 1995 Jul 20; 11(2):397-403). LOH on chromosome 19q and on chromosome 22 is associated with oligodendrogliomas (Reifenberger J, etal, American Journal of Pathology, 1994 Nov; 145(5): 1175-1190) and ependymomas (James CD and Olson JJ, Current Opinion in Oncology 1996; 8(3):188-195), respectively. Mutations of chromosome 3p gene are seen in von Hippel-Lindau (VHL) syndrome and appear to be specifically associated with hemangioblastoma, an uncommon CNS neoplasm (Kanno H, etal, Cancer Research, 1994 Sep; 54(18):4845-4847).

Alterations also exist in the expression of p53. The p53 tumor suppressor gene is frequently associated with the loss of one allele in malignant gliomas although a large number of malignant gliomas do not have a mutation in the p53 gene (Collins VP, Seminars in Cancer Biology, 1993 Feb; 4(1):27-32). For instance, although p53 gene mutations are present in more than two-thirds of secondary glioblastomas they are rarely found in primary glioblastomas, suggesting the presence of different genetic pathways (Watanabe, etal, Brain Pathology 1996; 6:217-24). Also p53 is deleted, mutated or both in at least one-third of sporadic malignant astrocytomas (Louis DN, Journal of Neuropathology and Experimental Neurology, 1994 Jan; 53(1):11-21). Loss of wild type p53 fostered a growth disadvantage on primary cortical astrocytes and facilitated their *in vitro* transformation (Bogler, etal, Cancer Research, 1995 Jul; 55(13):2746-2751).

Among 22 patients with primary WHO Grade II gliomas that, on recurrence, progressed to WHO Grades III or IV, mutations of the p53 gene (exons 5 to 8) were detected in 12 (10 of 13 astrocytomas, 1 of 7 oligodendrogliomas, 1 of 2 oligoastrocytomas). In each of these cases identical p53 mutations were present in the respective malignant recurrences. In all instances in which the p53 mutation was associated with p53 protein accumulation (10 of 12 cases), the percentage of p53 immunopositive tumor cells had increased from the primary to the recurrent tumor. These findings demonstrate that p53 is mutated in a high fraction of low-grade astrocytomas with progression to anaplastic astrocytomas and glioblastomas and that progression in such cases is frequently associated with an increase in the fraction of p53 immunopositive tumor cells (Reifenberger J, etal, J Neuropathology and Experimental Neurology, 1996 Jul, 55(7):822-31).

However, p53 may not be as significant in other types of human brain tumors. For instance, p53 deletions and mutations have been infrequently detected in medulloblas-

Exhibit 3
Types of Benign and Primary Malignant Tumors of the CNS

Types of Tumors	Description	Epidemiology (USA)
1 **GLIOMAS**	Tumors that arise from the glial or neuroepithelial (supportive) tissue of the brain; in some cases primary brain tumors of the CNS such as medulloblastoma, glioblastoma, and ependymoma (Levin B, Cecil Textbook of Medicine, 19th Edition, 1995; p. 716) and hereditary adenomatous polyposis may be associated with Turcot's syndrome, an autosomal recessive genetic disorder	The most prevalent primary brain tumor type
1.1 Astrocytic Tumors	Tumors that arise from astrocyte cells; part of the neuroepithelium of the brain	Account for about 50% of adult brain and spinal cord tumors and 19.5% of pediatric tumors
1.1.1 *Astrocytomas*	Includes diffuse, protoplasmic, and fibrillary astrocytomas	
1.1.2 *Pilocytic astrocytomas*	Slow growing, non-infiltrating, Grade I tumors; may form cysts or become enclosed in a cyst, and have the potential to become very large	Usually occur in children
1.1.2.1 *Optic tract glioma*	Frequently involve the optic chiasm and may also affect the optic nerve; the most common optic tract glioma is the pilocytic astrocytoma; anaplastic astrocytoma and glioblastoma multiforme (both malignant tumors) may also occur in this location. Symptoms include visual loss, rapid eye movements, esotropia, developmental delay, and an abnormally thin body; sometimes associated with neurofibromatosis (von Recklinghausen's Disease), a genetic disease	Often occur in children <age 10, especially those with neurofibromatosis
1.1.2.2 *Acoustic neuroma*	A slow growing, benign tumor of the vestibulocochlear, acoustic, or 8th cranial nerve; located in the angle between the cerebellum and the pons, in the posterior fossa; bilateral tumors are rare, tend to be familial and almost always associated with neurofibromatosis II. The malignant form of this tumor, malignant peripheral nerve sheath tumor (MPNST), is extremely rare	Accounts for <5% of primary brain tumors; typically develops in middle-aged adults and is twice as likely to occur in females than males
1.1.2.3 *Cerebellar astrocytoma*	A low grade, localized, cystic tumor	More common in children than adults
1.1.3 *Subependymal giant cell astrocytoma*	A slow growing, rarely metastatic, non-infiltrating, Grade I tumor; may form cysts or become enclosed in a cyst; may become very large; characterized by similar pathogenesis and method of treatment as pilocytic astrocytomas	Found in 5-7% of children diagnosed with tuberous sclerosis (Bourneville's disease), a hereditary disorder associated with seizures, skin nodules of the face, and mental retardation
1.1.4 *Low-grade astrocytoma*	Grade II, slow growing, infiltrating tumors, including protoplasmic (fibrillary) astrocytomas, and some pleomorphic xanthoastrocytomas	
1.1.5 *Anaplastic astrocytoma*	Grade III, malignant, infiltrating tumors, including gemistocytic tumors, and some pleomorphic xanthoastrocytomas	Accounts for 4.7% of childhood brain tumors
1.1.6 *Glioblastoma multiforme*	Grade IV, highly malignant, rapidly growing (can double its size every 10-11 days) astrocytoma that contains areas of dead tumor cells (necrosis); the most mixed-cell tumor of all brain tumors, making it one of the most difficult to treat, it develops rapidly de novo (primary glioblastoma) or through progression from low-grade or anaplastic astrocytoma (secondary glioblastoma); initial symptoms include headaches, seizures, memory loss, and behavioral changes caused by increased intracranial pressure	Represents approximately 30% of all primary brain tumors and approximately 50% of astrocytomas; most common in older adults, and in men; represents 4.9% of childhood brain tumors
1.1.6.1 *Gliomatosis cerebri*	Similar to glioblastoma multiforme, with cells more scattered and widespread, and no necrotic center; the diffuse nature of gliomatosis causes enlargement of the cerebrum, cerebellum or brain stem	

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1.2 Ependymoma	Arises from the ependymal cells that line the ventricles and central canal of the spinal cord; about 10% of these tumors spread via the cerebrospinal fluid; the two types of benign ependymomas include myxopapillary ependymoma, found in the spine, and subependymoma, found in the 4th ventricle	Represent 6% of all primary brain tumors, and 8.3% of all childhood brain tumors
1.2.1 *Papillary ependymoma*	An extremely rare, Grade II tumor; located in the cerebellopontine angle	
1.2.2 *Anaplastic ependymoma*	The Grade III, malignant form of the papillary ependymoma	
1.2.3 *PNET (primitive neuroectodermal tumor)*	A rare Grade IV ependymoblastoma tumor and type of neuroblastoma; usual location is in the cerebral hemispheres; diagnosed by CT or MRI scans, often showing surrounding edema and calcification	Most common in children
1.3 Oligodendroglioma	A slow-growing tumor arising from oligodendroglia (a type of supportive brain tissue), often containing both oligodendrocytes and astrocytes, as well as large amounts of calcification; commonly located in one of the cerebral lobes. Necrosis may be associated with aggressive lesions. Approximately 80% of patients present with a long history of seizures. Anaplastic oligodendrogliomas often recur and metastasize within the CNS despite surgical resection	Represents about 4% of all primary brain tumors; occurs most frequently in middle-aged individuals
1.4 Mixed gliomas (oligo-astrocytomas)	A tumor with a high proportion of more than one cell type, commonly containing both astrocytes and oligodendrocytes, and sometimes ependymal cells	
1.5 Medulloblastoma	A fast-growing, invasive tumor which is always located in the cerebellum and frequently metastasizes to other parts of the CNS via cerebral spinal fluid	Represents 15-20% of pediatric brain tumors; in addition, 30% of these tumors occur in young adults
1.6 Brain Stem Glioma	Any tumor which originates in or on the midbrain, pons, or medulla oblongata; the tumor may be an astrocytoma, ganglioglioma, or ependymoma	Represents 10-20% of all brain tumors in children
1.6.1 *Diffuse*	Diffuse with rapid onset of symptoms	Represents about 60-70% of brain stem tumors
1.6.2 *Focal*	May be solid or cystic, can occur in any area of the brain stem; usually associated with a gradual onset of symptoms	
1.6.3 *Exophytic*	Arises outside of the brain stem, but often grows into the fourth ventricle, causing symptoms associated with increased intracranial pressure due to blockage of the flow of fluid from the ventricle	
1.6.4 *Cervicomedullary spinal cord*	Arises in the medulla oblongata and extends into the cervical spinal cord	
2 **GERM CELL TUMORS**	Germ cell tumors that arise in the pineal or suprasellar regions of the brain; include germinoma, teratoma, choriocarcinoma, and the more aggressive embryonal carcinoma and yolk sac (endodermal sinus) tumors; mixed germ cell tumors also exist. Because these tumors have a tendency to spread via the CSF, diagnosis includes evaluation of the entire brain and spinal cord and, therefore, they are the only brain tumors that may be diagnosed by tumor markers found in the CSF, including AFP, PLAP and HCG	Represent approximately 5.1% of childhood brain tumors
2.1 Pineal region tumors	Located in the rear portion of the third ventricle	Represents <1% of all primary brain tumors, but 3% to 8% of childhood brain tumors
2.1.1 *Germinomas*	The most common tumor of the pineal region, representing over 1/3 of tumors in this area of the brain	Commonly occur in teen-agers
2.1.2 *Teratomas*	A rare tumor of infancy and childhood; often contains calcification and cysts and is usually found near the third ventricle in the midline	Represents 18-20% of all germ cell tumors; the most common brain tumor in newborns
2.1.3 *Pineoblastoma*	The malignant form of the pineocytoma is a slow growing, low grade tumor which causes headache, nausea and vomiting, lethargy, and double vision attributable to obstructive hydrocephalus	

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3 **NEURONAL TUMORS**		
3.1 Gangliocytoma (ganglioneuroma)	Gangliocytomas are tumors of mature ganglion cells affecting both mature nerves and supportive cells; these are small, slow growing tumors with distinct margins, thus metastasis and malignancy are rare. The most common site is the temporal lobe of the cerebral hemispheres, although they may also occur in the spine	Represent only 0.4% of all primary brain tumors; occurs most frequently in children and young adults
3.2 Neuroblastoma	Rare tumors (annual USA incidence is estimated at 500), commonly located outside the brain; the intracranial form is malignant and may metastasize throughout the CNS causing seizures, focal deficits, and increased intracranial pressure	85% of intracranial neuroblastomas also occur in children (referred to as PNET)
3.2.1 *Opsoclonus-myoclonus (OM)*	OM is a rare neurologic syndrome associated with oscillations affecting eyes and limbs; in children it occurs as a parainfectious process or a paraneoplastic syndrome in conjunction with neuroblastoma and in adults it is a complication of lung, breast, or gynecologic cancer	Presence of serum IgM and IgG to several neural antigens and improvement of symptoms with immunosuppressive therapy, point to an immune mechanism (Connolly AM, etal, J Pediatrics, 1997 Jun, 130(6): 878-84)
4 **CYSTIC BRAIN TUMORS**	Cysts are tumor-like masses that are filled with fluid	
4.1 Arachnoid cyst	An enlarged, fluid-filled area of the subarachnoid space in the area of the Sylvian fissure, cerebellopontine angle, cisterna magna or suprasellar region	
4.2 Colloid cyst of the 3rd ventricle	Usually attached to the roof of the 3rd ventricle and the choroid plexus, resulting in increased intracranial pressure; metastatic forms are unknown	Usually occurs in adults
4.3 Dermoid cyst	More common in the spine than in the brain; specifically in the lower end of the spine in persons ages 10-20; metastatic forms are unknown	Frequently occurs in children <10 years of age
4.4 Epidermoid cyst	More common than the dermoid cyst; occurs more frequently in the brain than in the spine, specifically in the cerebellopontine angle and pituitary area, and is usually benign	Most common in middle-aged adults
5 **TUMORS OF THE SELLAR REGION**		
5.1 Pituitary adenomas	Benign, slow growing tumors of the pituitary gland; classified as secreting or non-secreting; the majority are secreting and their treatment is determined by the hormone secreted. These tumors often invade the optic chiasm, causing visual loss and headache	Most commonly occur in young or middle-aged adults; represent about 10% of all intracranial tumors
5.2 Craniopharyngioma	A benign tumor that arises from the remnants of an embryonic structure, presents near the pituitary gland and often involves the third ventricle, optic nerve, and pituitary gland; malignancy and metastasis are unknown, however, the tumor grows by expansion, causing increased intracranial pressure attributable to obstruction of the foramen of Monro	Represents 2-3% of all primary brain tumors, and 5-10% of childhood brain tumors
5.2.1 *Adamantinomatous ("ordinary") craniopharyngioma*	A more cystic tumor than the papillary craniopharyngioma	Usually occurs in children
5.2.2 *Papillary craniopharyngioma*	A more solid tumor than the adamantinomatous craniopharyngioma	Occurs in adults
6 **CNS LYMPHOMAS**	Commonly located in the cerebral hemispheres; multiple tumors may be present and metastasis is common. Symptoms associated with these tumors include increased intracranial pressure, confusion, lethargy, memory loss, muscle weakness in one area of the body, and seizures	Affects people with a healthy immune system as well as immunocompromised hosts (organ transplant recipients or HIV+ patients)
7 **LOCAL EXTENSIONS FROM REGIONAL TUMORS**		
7.1 Chondroma	A rare, slow growing, benign tumor that usually originates at the base of the skull, primarily in the parasellar area or over the cerebral convexities. This tumor is made of cartilage that is formed by the meninges, is usually attached to the dura, and may present as single or multiple tumors	

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7.1.1 Chondrosarcoma	This extremely rare malignant form of the chondroma, arises from bone and is also composed of cartilage; it is locally invasive, but is slow-growing and rarely metastasizes	Common in adults >40 years of age
7.2 Chordoma	Originating at the base of the skull or the end of the spine, this tumor is benign and extradural, but usually invades adjacent bone	Represents only 0.2% of all primary CNS tumors; most often found in people ages 21-40
8 TUMORS OF THE MENINGES		
8.1 Meningiomas	Usually benign tumors originating in the meninges of the brain and spinal cord; the most frequent sites are over the surface of the brain, attached to the sagittal sinus (the major vein draining blood from the brain), at the base of the brain attached to the dura mater of the sphenoid sinus, the olfactory grooves, the region of the sella, or in the spine. Benign meningiomas are slow growing with distinct borders, and may become quite large before symptoms become obvious	Represent 15-20% of all primary brain tumors, and occur more often in middle-aged adults and in women
8.1.1 *Atypical meningiomas*	Not clearly malignant, but have an increased tendency to recur and are faster growing than typical meningiomas	
8.1.2 *Anaplastic meningiomas and hemangiopericytomas*	Malignant tumors which tend to invade adjacent brain tissue; occur in the same locations as meningiomas	
8.2 Hemangioblastoma	A slow-growing, benign, tumor-like mass that arises from blood vessels and is often cystic; single or multiple tumors may exist. Lindau disease and von Hippel-Lindau disease are hereditary forms of this tumor. The most common site is the cerebellum, which usually causes increased intracranial pressure and cerebellar dysfunction	Represents about 2% of all primary brain tumors; frequently presents in the 35-to-45 years-of-age group
9 UNCLASSIFIED TUMORS		
9.1 Dysembryoplastic neuroepithelial tumor	A low-grade, slow-growing tumor, usually diagnosed following a long history of uncontrollable partial complex seizures; this tumor is always supratentorial, generally in the temporal or frontal lobe of the cerebrum	Commonly occurs in individuals <20 years of age
9.2 Glomus jugulare	A very rare, slow growing, benign tumor located in the jugular foramen at the base of the skull, that widely invades the temporal bone. Symptoms include hoarseness, swallowing difficulties, hearing loss, a ringing in the ear, dizziness and/or blackouts. CT- or MRI-based diagnosis is confirmed with cerebral angiography because of this tumor's large blood supply	The most common tumor of the middle ear; presents most often in women aged 50-59 years
9.3 Lipomas	A rare, benign tumor most commonly located in the corpus callosum, composed of yellow fat deposits; may cause no symptoms	
9.4 Pseudotumor cerebri	Benign intracranial hypertension, literally a "false brain tumor" of unknown etiology that results in symptoms associated with increased intracranial pressure	Most common in women aged 20-50 years
9.5 Toxoplasmosis	A generalized infection of the central nervous system caused by the parasite, *Toxoplasma gondi*, found in the intestines of cats and in uncooked meats; it is diagnosed by a blood test (IgM-IFA) and CT or MRI scan	Immunocompromised individuals are at a high risk

Note: Classification specifications follow the World Health Organization Histology Groupings for Primary Brain Tumors

toma, suggesting that inactivation of another tumor suppressor gene or genes located on 17p is associated with tumorigenesis. Deletion of 17p is strongly associated with a negative prognosis in medulloblastoma (Cogen PH and McDonald JD, J Neuro-Oncology, 1996 Jul, 29(1):103-12).

Mutations of the p53 gene are rare in most childhood brain tumors. Loss of chromosome arm 17p DNA sequences is common in sporadic pediatric ependymomas but, in contrast to ependymomas in adults, deletion of chromosome arm 22q sequences is rare (von Haken MS, etal, Genes, Chromosomes and Cancer, 1996 Sep, 17(1):37-44).

Microsatellite instability, manifested by the presence of additional alleles or shifts of electrophoretic mobility at simple sequence tandem repeat loci and demonstrated in hereditary and sporadic tumors, is infrequent in many types of human brain tumors. Lower levels of microsatellite instability observed in brain tumors may be indicative of other mechanisms of genetic instability (Zhu J, etal, Oncogene, 1996 Apr 4, 12(7):1417-23).

Exhibit 4
Estimated Annual Incidence of Primary Brain Tumors by Site and Gender in the USA

Tumor Site	ICDO	Male		Female		Total	
		(#)	(%)	(#)	(%)	(#)	(%)
Brain	C71.0-C71.9	6,663	66.0	3,783	50.4	10,446	59.4
Supratentorial	C71.0-C71.4	2,833	42.5	1,232	32.6	4,065	38.9
Ventricles	C71.5	137	2.1	52	1.4	189	1.8
Infratentorial	C71.6-C71.7	493	7.4	211	5.6	704	6.7
Meninges, spinal cord, cranial nerves, other CNS	C70.0-C70.9, C72.0-C72.9	2,281	22.6	2,875	38.3	5,157	29.3
Pituitary	C75.1-C75.2	1,086	10.8	812	10.8	1,898	10.8
Pineal	C75.3	70	0.7	29	0.4	99	0.6
Total		10,100	100.0	7,500	100.0	17,600	100.0

Sources: Central Brain Tumor Registry of the United States. 1996 Annual Report; CA:A Cancer Journal for Clinicians 1997; 47(1):8-9; Laws E and Kamat T, CA:A Cancer Journal for Clinicians 1993; 43:263

Gene amplification, in addition to multiple chromosomal abnormalities, constitutes another genetic aberration that may potentially be involved in CNS neoplasms.

The epidermal growth factor receptor (EGFr) is a 170 kd membrane receptor encompassing 3 domains, an extracellular binding domain, a transmembrane region and an intracellular tyrosine kinase domain. The gene for EGFr, located on chromosome 7, is amplified and overexpressed in 40%-50% of highly-malignant CNS neoplasms (Collins VP. Seminars in Cancer Biology, 1993 Feb; 4(1):27-32). In 50% of cases having EGFr amplification, the genes undergo intragene deletion rearrangement that results in the overexpression of a protein lacking a portion of the extracellular domain of the wild type receptor (James CD and Olson JJ, Current Opinion in Oncology 1996 May; 8(3):188-195). The mutant receptor has been shown to be a constitutively activated tyrosine kinase (Nishikawa R, etal, Proc Natl Acad Sci, 1994 Aug; 91(16):7727-7731) that stimulates cell proliferation (Ekstrand AJ, etal, Oncogene, 1994 Aug; 9(8):2313-2320) and enhances tumorigenicity of human glioma cells in nude mice.

Among 22 patients with primary WHO Grade II gliomas that, on recurrence, progressed to WHO Grades III or IV, none of the primary low-grade and none of the recurrent high-grade tumors (7 anaplastic astrocytomas, 10 anaplastic oligodendrogliomas, 4 anaplastic oligoastrocytomas, and 5 glioblastomas) showed evidence of EGFr gene amplification. The general absence of EGFr amplification supports the hypothesis that the significance of p53 mutation and EGFR amplification may be different in glioblastomas that developed by progression from low-grade astrocytomas (secondary glioblastomas) compared to glioblastomas that developed rapidly in a de novo manner without a history of previous low-grade primary glioblastomas (Reifenberger J, etal, J Neuropathology and Experimental Neurology, 1996 Jul, 55(7):822-31).

It is now known that the activity of wild type p53 is regulated by a cellular protein termed MDM2 (murine double minute 2). This protein binds to and functionally inactivates p53 (Dekker N, etal, Nature, 1993 Apr; 362(6423): 852-855). Amplification of MDM2 gene has also been demonstrated in a subset of human malignant gliomas (Reifenberger G, etal, Cancer Research, 1993 Jun; 53(12):2736-2739). Yet another cellular mechanism for subverting p53 mediated growth regulation is the expression of the apoptosis inhibitor bcl-2. Alderson, etal, (Cancer Research 1995; 55:999-1001) has described human gliomas with wild type p53 that express bcl-2.

Fas/APO-1 (CD95) is also implicated as a mediator of apoptosis. Fas is frequently expressed in malignant gliomas (Tachibana O, etal, (1995) Cancer Res 55: 5528-5530). Immunoreactivity to Fas was detected in 11% of low-grade astrocytomas (WHO Grade II), 18% of anaplastic astrocytomas (WHO Grade III) and in 87% of glioblastomas (WHO Grade IV). In glioblastomas, Fas expression was almost exclusively observed in glioma cells surrounding foci of necrosis. Accumulation of glioma cells undergoing apoptosis in perinecrotic areas suggests that Fas-mediated apoptosis may play a role in the pathogenesis of necrosis which constitutes a histologic hallmark of glioblastoma multiforme (Tachibana O, etal, Acta Neuropathologica, 1996 Nov, 92(5):431-4).

Amplification of the MYCN gene is strongly associated with advanced stages of disease and a poor prognosis. However, not all patients with neuroblastomas with MYCN amplification have poor outcomes. Actually, a highly significant reduction was observed in the mean disease-free interval from 24.4 ± 4.7 months for cases with co-amplification of MYCN and DDX1, compared with 9.2 ± 1 months for those showing amplification of MYCN alone. DDX1, a gene encoding a DEAD [Asp(D)-Glu(E)-Ala(A)-Asp(D)] box protein recently mapped to chromosome 2p24, is frequently co-amplified with MYCN. In one

Exhibit 5
Estimated Breakdown of Primary and Metastatic Adult Brain Tumors by Site in the USA

Primary Extra-axial Tumors	(%)	Primary Intra-axial Tumors	(%)	Metastatic Tumors	(%)
Meningioma	80	Glioblastoma	47	Lung	37
Acoustic neuroma	10	Anaplastic astrocytoma	20	Breast	19
Pituitary adenoma	7	Astrocytoma	15	Melanoma	16
Other	3	Oligodendroglioma	5	Colorectal	9
		Lymphoma	2	Kidney	8
		Other	7	Other	11

Note: Brain and nervous system cancer includes ICD-9 Code 191

Sources: Vick NA, Section 13: Intracranial Tumors and States of Altered Intracranial Pressure, Cecil Textbook of Medicine 19th Edition, WB Saunders Co, 1992, p 2213

study, DDX1 was found to be amplified along with MYCN in 67% of cell lines and in 38% of MYCN amplified tumors. Co-amplification of DDX1 and MYCN was found more frequently in Grade IV or IVS tumors than lower grade (I-III) tumors. These observations indicate that the MYCN amplicon is of varied size and/or position relative to the MYCN gene, with DDX1 representing at least one other gene frequently co-amplified with MYCN (George RE, etal, Oncogene, 1996 Apr 4, 12(7):1583-7). Co-amplification of the DDX1 gene with the MYCN gene is a consequence of the segregation of continuous DNA stretches spanning both loci during the amplification process (Noguchi T, etal, Genes, Chromosomes and Cancer, 1996 Feb, 15(2):129-33), and the high frequency of DDX1 co-amplification is attributable to its proximity to MYCN (Amler LC, etal, Genes, Chromosomes and Cancer, 1996 Feb, 15(2):134-7; Kuroda H, etal, Oncogene, 1996 Oct 3, 13(7):1561-5).

Dysregulation of the cell cycle also plays a role in primary CNS cancer. A protein encoded by the p16 gene (located on chromosome 9p) acts as a negative regulator of cell growth and proliferation by binding to CDK-4 and preventing it from forming an active complex with cyclin D proteins (Serrano M, etal, Nature, 1993 Dec; 366(6456):704-707). The primary target of activated CDK-4 is the retinoblastoma (Rb) protein; activated CDK-4 phosphorylates the Rb protein. When the Rb protein is hypophosphorylated or inactive it arrests cells in the G1 phase of the cell cycle. When, on the other hand, the protein is phosphorylated or active, cellular proliferation occurs (Weinberg RA, Cell, 1995 May; 81(3):323-330). Schmidt, etal (Cancer Research 1994; 54,6353-6358), describes CDK-4 gene amplification in gliomas with intact p16 genes. Overexpression of CDK-4 occurs in as much as 15% of malignant gliomas while inactivation of p16 expression occurs in about 50% of malignant gliomas. In addition, in glial tumors and in cell lines where the expression of p16 or CDK-4 is normal (James CD and Olson JJ, Current Opinion in Oncology, 1996 May; 8(3):188-195), loss of retinoblastoma expression has been noted.

Growth Factors

As mentioned previously, EGFr is overexpressed in gliomas. Although EGF amplification has not been identified in human gliomas, EGF is known to act as a mitogen in glial cells. Transforming growth factors (TGF)α and β are EGF-like molecules; TGFα shares a 50% homology with EGF and binds to its receptor. Numerous tumors, including high-grade malignant gliomas secrete this factor. It is speculated that the α subunit of TGF acts to stimulate autocrine growth pathways. When TGFα was transfected into NIH3T3 cells, the cells' proliferative capacity increased but they were not transformed (Finzi E, etal, Proc Natl Acad Sci, 1987 Jun; 84(11):3733-3737). TGFβ has been shown to inhibit certain immune reactions which may affect tumor growth. Two forms of the β subunit exist in human gliomas. Type 2 β subunit is related to the polypeptide described as glioblastoma cell-derived T cell suppressor factor. Glioma cells secrete this factor which reduces the cytotoxic properties of tumor infiltrating lymphocytes (Bodmer S, etal, Journal of Immunology, 1989 Nov; 143(10):3222-3229). The β subunit of TGF has also been found to depress the activity of natural killer cells. It is postulated that the secretion of TGFβ in gliomas could allow tumors to grow out of control by inhibiting cells that would potentially kill these neoplasms.

DIAGNOSIS

Symptoms associated with brain tumors occur as a result of either increased intracranial pressure or focal cerebral dysfunction. Early symptoms due to increased intracranial pressure include nausea, vomiting, headache, mental slowness, papilledema, and loss of retinal venous pulsations. Late symptoms can include uncal or cerebellar-foramen magnum herniation. Symptoms associated with metastatic tumors include headaches, focal weakness, seizures, loss of sensation or difficulties with gait. Lethargy, emotional lability or personality changes are occasionally present. Often only minor cognitive signs are present.

Symptoms unique to specific regions in the cerebral hemisphere are as follows (Fine 1995 Clinical Review of Neuro-Oncology DFCI).

- tumors in the frontal lobe cause personality changes, weakness, dysphasia, seizures, frontal release signs and urgency of micturition
- tumors in the parietal lobe often manifest as neglect of contralateral side, receptive dysphasia, sensory extinction, disturbances of vibration and sensation, and seizures
- temporal lobe lesions can cause hemiparesis, homonymous hemanopia, memory problems and an irritable personality; temporal lobe seizures are often noted.
- cranial nerve palsies are often a result of brain stem lesions.
- cerebellar lesions lead to ataxia of the limbs or trunk, nystagmus and signs of increased intracranial pressure

Diagnosis of primary brain tumors includes a basic neurological examination in addition to blood tests and neuroimaging. When dealing with the possibility of brain lesions that are metastases of other primary tumors in patients with systemic cancer, it is important to rule out other space-occupying lesions because the morbidity associated with diagnosis and treatment is high. Because most brain lesions are hypodense or isodense, differential diagnosis includes infections, vascular lesions, primary brain tumors and other conditions such as multiple sclerosis, radiation necrosis and progressive multifocal leukoencephalopathy.

The mainstay of a diagnostic workup in primary brain tumors is neuroimaging. If a suspicious lesion is detected by a scanning procedure, a closed or open biopsy is performed. Other diagnostic procedures include evoked-potential tests and audiometry (used for detecting an acoustic neuroma), endocrine evaluations (used, along with scans, to diagnose a pituitary or hypothalamic tumor), and/or perimetry which measures the size of visual fields (used in diagnosing a tumor in the area of the optic chiasm, such as a pituitary tumor).

Neuroimaging

Neuroimaging techniques for brain cancer are improving thanks to a renewed broad scientific interest in human brain function. In the cancer area, the Human Brain Project, launched in 1993, led by the NIMH and co-sponsored by 16 federal organizations including the NIH, National Science Foundation, Department of Defense, NASA and the Department of Energy, has been coordinating information obtained from the use of sophisticated imaging technologies to ultimately better understand brain function and development.

MRI and CT are the major neuroimaging techniques. CT scanning is the most common test for intracranial lesions while MRI is useful in detecting spinal cord lesions of the posterior fossa. However, neither imaging technique has a high specificity. Small tumors, tumors adjacent to bone, brainstem tumors and low-grade tumors may be better visualized by MRI than CT. CT scanning is more effective in identifying calcification or bony erosion, and for some tumors, CT is more specific.

Although there is no absolute advantage of one type of imaging device over the other, CT is widely available and less expensive but MRI offers the greater potential. For instance, the Human Brain Project, equipped with more than 1,000 MRI scanners, relies exclusively on MRI to create the largest pediatric-brain data set in the world. Because building the database requires regular scans of healthy children, imaging devices requiring ionizing radiation, including CT, SPECT and PET, are ruled out. In addition, researchers point out that these technologies have not made the advances in image quality that MRI has. MRI is able to produce both detailed structural (morphometric) images and vibrant functional images by using new adaptations such as echo-planar and perfusion-diffusion imaging. Morphometric brain scans can reveal areas of edema in patients with brain tumors. Perfusion MRI and MR spectroscopy are used to reveal tumor histology and assess response to treatment. These tests have the ability to distinguish necrotic tissue from active tumor, a common problem in monitoring response to high doses of radiation (which causes necrosis) in patients with brainstem gliomas. In conjunction with non-imaging MR applications such as spectroscopy, which assesses chemical and cellular brain activity and presents it quantitatively, MRI is the diagnostic tool of choice.

Because many of the CNS tumors occur in children, developers have tried to design systems that meet pediatric needs. MRI now exhibits rapid-scanning capability, a significant bonus when imaging unsedated, fidgety children. Exam time is reduced from an hour to just a few minutes, making the procedure very tolerable for children as well as adults. With the high-field MRI, developed by Siemens Medical Systems (Iselin, NJ), imaging sequences are so fast that they can freeze motion and, therefore, prevent any movement from affecting image quality.

CT scanning may be the only alternative for brain tumor patients who cannot tolerate MRI scanning. Use of spiral, or helical, CT, such as Siemens Systems' Somatom Plus spiral CT (cleared by the FDA in early 1990), has improved on axial CT scanning. In spiral CT, the gantry spins at rates as fast as one full rotation in less than a second while the couch proceeds at a constant velocity through the scanning field of the rotating X-ray tube. Unlike conventional axial CT scanning, in which one revolution produces one slice, spiral CT yields multiple images with each revolution and allows the operator to alter some processing parameters after the scan, producing volumetric images that allow "re-slicing" of the data

according to a different set of parameters without having to re-scan the patient.

Toshiba America Medical Systems (TAMS; Tustin, CA) is developing the kidCT, a CT unit tailored for neonatal and pediatric markets. The device offers 50% lower dose, dedicated positioning and lower table height. In 1997, Toshiba also introduced a continuous imaging CT, Aspire CT, that displays images in real time. Also in 1997, Picker International (Highland Heights, OH) announced its new PQ 6000, "the only CT scanner to offer true massively parallel processing." Shimadzu (Kyoto, Japan) premiered a new spiral CT system, the SCT-7000T, claiming to be the fastest CT on the market with a reconstruction time as fast as two seconds and completing a brain study in less than 30 seconds.

Myelography and MRI with gadolinium enhancement can visualize disease in the meninges and provide differential diagnosis of neoplastic and non-neoplastic meningitis. Two distinct enhancement patterns are characterized, dura-arachnoid enhancement and pia-subarachnoid space enhancement. The dura-arachnoid pattern consists of curvilinear enhancement overlying the brain and immediately deep to the inner table of the calvaria, as well as along the falx and tentorium. Pial enhancement closely follows the brain surface into sulci and outlines the basal cisterns (Meltzer CC, etal, Radiology, 1996 Nov, 201(2):297-308).

Additional diagnostic tools include angiograms and nuclear tests such as PET scanning. The angiogram is useful before surgery to determine blood supply to a mass. For example, in a large meningioma an angiogram is often helpful in cutting off blood by vessel embolization, making the surgical procedure easier. It can also differentiate tumors from vascular lesions. PET scanning is the best available test for differentiating radiation necrosis from viable tumor. It can also provide information regarding the biological aggressiveness of tumors. However, it is important to note that a definitive diagnosis is based on tissue biopsy currently performed using sterotactic techniques.

Sterotactic Techniques for Tissue Sampling

Sterotactic techniques have allowed nearly all lesions, regardless of location, to be sampled. Sterotaxis is a technique whereby the surgeon's biopsy needle is guided by coordinates generated by a computer. These coordinates are based on MRI and CT images of the targeted lesion relative to points of reference on a sterotactic frame attached to the patient's head. The mortality rate from a stertotactic procedure is estimated to be 1% as opposed to an open biopsy where the mortality rate can range from 5%-27%. A positive tissue diagnosis is obtained 88% of the time with an open biopsy versus 96% with sterotactic biopsy (J Neurology 1986;64:872-878).

Manufacturers are constantly improving CT-guided interventional procedures. Picker introduced epi-Tools that is designed to streamline CT-guided biopsy. Elscint (Hackensack, NJ) introduced a CTscope package which displays real-time axial images during interventional procedures and a LaserGuide option that uses a laser system to enhance accuracy and speed when performing biopsies under CT.

Scintigraphy

Scintigraphy, using OctreoScan 111, a somatostatin analog (pentetreotide), labeled with In-111, provided by Mallinckrodt Medical BV (Petten, Holland), was evaluated as a clinical tool for tissue characterization and introduced for the *in vivo* visualization in somatostatin receptor (SR)-positive tissues. High densities of SR were present within meningiomas, high-grade astrocytoma and craniopharyngioma. Differentiation of low- and high-grade astrocytomas could not be successfully achieved because both grades showed intense radioactivity uptake, even though high-grade tumors lack SR. The latter might be due to the damaged blood-brain barrier and the poor radioactivity washout observed in high-grade astrocytomas. SR scintigraphy in non-neuroendocrine malignancies does not seem to be reliable for an initial tumor staging but rather is more suitable for a tissue characterization and is extremely useful for monitoring changes of SR expression after treatment (Limouris GS, etal, Hybridoma, 1997 Feb, 16(1):133-7).

In Vitro Tests

CSF examination is the most reliable way of making a definitive diagnosis. The CSF is examined for the presence of known tumor markers. Unfortunately, there are no definitive tumor markers for most primary brain tumors. Molecular markers identified in certain germ cell tumors include alphafetoprotein (AFP), human chorionic gonadotropin (HCG) and placental alkaline phosphatase (PLAP). Carcinoembryonic antigen (CEA) is a marker for a tumor of the arachnoid and/or pia mater membranes of the meninges (a leptomeningeal tumor). These are usually metastatic tumors. Therefore, at this time, if tumor markers are present, they are only helpful in the diagnosis and follow-up evaluation of germ cell and metastatic brain tumors only.

TUMOR STAGING

Results from MRI, CT, PET and SPECT scans or CSF tests may be used to determine tumor stage and help decide on treatment. Brain tumors are classified on the basis of tumor cell type and histologic grade. For some tumors, however, location and metastatic spread within the cerebrospinal fluid are also used in classification. Although metastases outside the central nervous system are extremely rare in primary brain tumors, "drop metastases" do occur in some cases of medulloblastomas and ependymomas. Benign tumors are rarely staged. Lymphomas and metastatic tumors are the most commonly staged brain tumors. The following approach, based on the TNM system, is used for staging:

T (primary tumor size/extent)	N (regional lymph node involvement)	M (distant metastasis)
T0 (primary tumor not present)	N0 (no lymph node involvement)	M0 (no distant metastasis)
T1, T2, T3, T4 (the higher the number, the larger the size or extent of the tumor)	N1, N2, N3 (the higher the number, the more lymph node involvement)	M1 (distant metastasis present)

Another staging system, the WHO grading system for brain tumors, uses a scale of I to IV:

Tumor Grade	Description
I	Benign, well-contained tumors that grow slowly and may usually be surgically removed to effect a cure
II	Low-grade, relatively slow-growing tumors that can infiltrate adjacent normal tissue and may recur, sometimes as a higher grade lesion
III	Mid-grade, medium-growth, malignant tumors that incorporate actively proliferating cells and infiltrate adjacent normal brain tissue; these tumors tend to recur, often as a higher grade
IV	High-grade, rapidly-growing, malignant tumors that contain necrotic tissue; Grade IV tumors are the most malignant; they reproduce rapidly, have a bizarre appearance when viewed under the microscope and infiltrate widely; these tumors induce the formation of new blood vessels so they can maintain their rapid growth and have areas of dead cells (necrosis) in their center
IVS	Special subcategory of Grade IV tumors

PROGNOSIS

Generally, primary CNS and brain tumors have a poor prognosis. One of the reasons for this is commonly occurring local metastases. Malignant gliomas, for example, frequently escape their main site of development. After surgical removal of the primary tumor some 70-80% of recurrent tumors are found less than 2 cm from the original tumor site, while 6-12% are located within 4 cm or more. These secondary tumors represent metastatic growth and not regrowth of the original tumor left behind during surgery. Brain tumors that represent metastasis from other primary cancers are mostly supratentorial and are nearly uniformly fatal.

Five-year or longer survival associated with primary brain tumors is estimated at only about 25%-29% (Exhibits 12 and 13). Often, the site of the tumor is as critical as its stage; even benign tumors may be deadly when inoperable because their location. According to SEER statistics, based on staging, localized and regionally-spread and distant tumors have similar survival rates (Exhibit 14). These results may be artifactual because most tumors included in the statistics are unstaged and few of the staged ones are advanced. Five-year survival rates and population estimates in the Triad are based on USA data (Exhibit 15).

MEETING COVERAGE

CANCER OF THE CENTRAL NERVOUS SYSTEM

FROM THE AMERICAN ASSOCIATION OF CANCER RESEARCH CONFERENCE JUNE 7-11, 1997, SAN DIEGO, CA

Management of cancers of the CNS is fraught with unique problems. Because of the existence of the blood-brain barrier, systemic chemotherapy is not always effective. Neurosurgery is the cornerstone of brain tumor management and yet, it is often very difficult to resect tumors from normal brain tissue, and many tumors are considered inoperable because of the likelihood of brain damage from the surgery itself. Furthermore, tumor regrowth after surgery is common. Innovative therapies are being designed to target therapeutic agents to brain tumors, and to inhibit tumor cell metastases following surgery.

TARGETING REGIONAL METASTASES OF BRAIN TUMORS

Mark L. Rosenblum of Henry Ford Hospital (Detroit, MI) discussed mechanisms of tumor invasion in the CNS and some of the inhibitors which are being developed that target regional metastases of primary brain tumors. Invasion of tumor cells beyond the site of primary tumor involves an interaction with the extracellular matrix (ECM). The cell must attach by way of cell surface receptor interactions with specific ECM components, degrade the ECM in order to clear a path for invasion, and then move into new territory. All of these mechanisms can be the targets of innovative therapies designed to abort brain tumor metastasis.

A tumor cell can leave the primary tumor and move to new sites by six basic mechanisms:

- The simplest mechanism is random tumor cell movement, which contributes to the local invasive process and may be stimulated by cell motility factors or cytoskeletal motility mechanisms.
- Another process contributing to tumor cell invasion is bulk CSF flow, a process which is aberrant in tumors because there is edema in cells surrounding the tumor and increased interstitial pressure within the tumor itself. This combination tends to push some of the tumor cells into the peritumor region.
- A third mechanism of invasion is mediated by proteases surrounding the tumor which degrade the ECM, allowing tumor cells to move through it.
- A fourth mechanism is integrin-mediated migration through basement membranes. Brain tumors can stimulate the production of ECM components such as laminin by neighboring normal brain cells, which essentially prepares a path for tumor cell migration.

Increased expression of CD44 and brain-enhanced hyaluronic acid binding protein (BEHABP) may occur.

- Other mechanisms include the production of extracellular molecules, such as tenasin or hyaluronic acid, that will be more permissive for movement of the cell through the ECM. Finally, there may be production of chemoattractant molecules from distant sites which promote tumor cell movement to these areas.

Exhibit 6
Incidence Rate of Brain and Nervous System Cancer by Race and Gender Over Two Decades in the USA

Gender	Race	Year of Diagnosis		Trends	
		1973-1974 Rate**	1990-1991 Rate**	% Change	Annual % change over 19 years
Males	Blacks	3.5	4.4	22.8	1.3
	Whites	6.4	7.9	24.0	1.2*
	All Races	6.0	7.4	23.0	1.1*
Females	Blacks	2.8	3.1	11.1	1.6
	Whites	4.4	5.7	29.4	1.4*
	All Races	4.2	5.3	27.7	1.3*
Both Sexes	Blacks	3.1	3.6	14.9	1.2
	Whites	5.3	6.7	26.1	1.3*
	All Races	5.0	6.3	24.6	1.2*

**Estimated annual percent change over the 19 year interval is statistically significant (P<0.05)*

***Number of cases per 100,000 population*

Note: Brain and nervous system cancer includes ICD-9 Code 191

Sources: NIH, SEER Cancer Statistics Review 1995:104-111; CA: A Cancer Journal for Clinicians 1997;47(1)8-9

Anti-invasive therapies are not considered cures in themselves, but as adjunct approaches in multimodality regimens. In a tumor model which recognizes that there are two distinct populations of tumor cells, pure primary tumor and invading tumor cells, surgery and radiation therapy are first-line defenses against the primary tumor. Adjunct therapies such as antiproliferative and anti-invasion therapies represent secondary defenses to protect against the development of metastatic tumors. In addition, new techniques must be developed to overcome the delivery restrictions imposed by the blood-brain barrier.

Targeting Protease Production

An obvious target for therapy is protease production by the tumor cell. The most important of these are the matrix metalloproteases (MMPs) and the cysteine and serine proteases. These enzymes degrade components of the ECM such as integrins and neural cell adhesion molecules (NCAM). Simultaneously, the cell also produces inhibitors of the proteases, and it is the balance between the production of proteases and their inhibitors that is important in the invasion process as well as the development of new blood vessels that nourish the tumor. Protease inhibitors are being developed by Prototek (San Francisco, CA) and Khepri Pharmaceuticals (Alameda, CA) that was acquired by Arris Pharmaceutical (now Axys Pharmaceutical; San Francisco, CA) in November 1995.

Peptidyl methyl ketone, a novel protease inhibitor in development that is an irreversible inhibitor of cathepsin B, which degrades plasminogen to form plasmin and is elevated in malignant gliomas, can be taken orally and is non-toxic.

Maramistat, under development by British Biotech (Oxford, UK and King of Prussia, PA), is an inhibitor of MMP which acts by chelating Zn at the active site, and is further along in development. In preclinical trials it was effective in human ovarian and small cell lung carcinoma models. In ongoing phase I/II trials it has had some effect in reducing tumor markers but has shown some musculoskeletal toxicity which limits the tolerated dose. Phase III clinical trials for its effect on malignant gliomas are planned.

Carboxy-amido-triazole (CAI) (NSC609974) is a metastasis inhibitor that targets a pertussin toxin-sensitive G protein. At 2-20 uM it inhibits Matrigel invasion by glioma cells by 60%, with no effect on cell viability. In phase I clinical trials, being conducted by the NCI, the drug stabilized disease in colorectal, pancreatic, renal cell and ovarian cancers, but there was some toxicity in the form of peripheral neuropathy; in very rare cases, there was cognitive dysfunction. Clinical trials for systemic cancer are underway and trials for malignant glioma are planned.

MECHANISMS OF DRUG-INDUCED APOPTOSIS

Normal cells undergo apoptosis in response to a number of external and internal signals. By contrast, tumor cells have often lost the ability to respond to those signals, and they continue to grow in spite of the occurrence of events that normally initiate cell death. Nevertheless, tumor cells grown *in vitro* can be induced to undergo apoptosis, indicating that a biochemical pathway leading to cell death is still intact. It should, therefore, be possible to kill cancer cells by identifying and triggering the appro-

priate activation signals of the apoptotic pathway *in vivo*.

Although many genes have been identified which are involved in this pathway, the multiple regulatory controls often limit the effectiveness of targeting a single gene. In order to study the specific defects in the apoptosis pathway in cancer cells by focusing more on cell biology than on the specific genes involved, David Hockenbery of Fred Hutchinson Cancer Center (Seattle, WA) examined the mechanism of apoptosis in two systems, colorectal cancer cells (C205) which undergo terminal differentiation followed by apoptosis, and the induction of apoptosis by aphidicolin treatment.

Exhibit 7
Estimated Incidence of Primary Brain Tumors by Major Histology Groups and by Gender in the USA

Histology	Male		Female		Total	
	(#)	(%)	(#)	(%)	(#)	(%)
Tumors of neuroepithelial tissue	5,734	56.8	3,118	41.6	8,852	50.3
Tumors of cranial and spinal nerves	701	6.9	555	7.4	1,256	7.1
Tumors of the meninges	1,728	17.1	2,624	35.0	4,352	24.7
Lymphomas and hemopoietic neoplasms	471	4.7	174	2.3	645	3.7
Germ cell tumors	91	0.9	15	0.2	106	0.6
Cysts and tumor-like lesions	11	0.1	8	0.1	19	0.1
Tumors of the sellar region	1,065	10.5	799	10.7	1,863	10.6
Local extensions from regional tumors	18	0.2	18	0.2	36	0.2
Unclassified tumors	281	2.8	189	2.5	471	2.7
Total	10,100	100.0	7,500	100.0	17,600	100.0

Note: Brain and nervous system cancer includes ICD-9 Code 191

Sources: Central Brain Tumor Registry of the Uninted States. 1996 Annual Report; CA:A Cancer Journal for Clinicians 1997; 47(1):8-9; NIH, SEER Cancer Statistics Review 1995:104-111

Induction of apoptosis in C205 colorectal cancer cells with the general tyrosine kinase inhibitor herbimycin A, a naturally occurring benzoquinoid ansamycin antibiotic, results in a massive proliferation of mitochondria. In spite of the increase in mitochondrial mass there was no change in function, which suggested that the new mitochondria were nonfunctional. At the ultrastructure level the new organelles were not coated with rough endoplasmic reticulum, which is characteristic of normal mitochondria, and they began to degenerate with time. To determine whether the mitochondrial proliferation was involved in apoptosis or was simply a marker for the process, subfractions of treated cells were prepared and assayed for their abilities to induce cell death. The assay measured the degree of nuclear degeneration induced when isolated nuclei were mixed with cell extracts. This approach located the apoptotic inducer in the heavy membrane fraction of herbimycin-treated C205 cells, and suggested that the mitochondria were directly contributing to apoptotic potential.

A mitochondrial protein which is known to be involved in the apoptotic pathway is bcl-2. This anti-apoptosis protein, which is often overexpressed in many types of tumors, is found on the outer mitochondrial membrane at contact sites where proteins are imported and exported. To determine whether bcl-2 might be involved in mitochondrial proliferation, human breast cancer cells were genetically altered to overexpress the protein. In these cells, as in the C205 cells, herbimycin treatment normally causes mitochondrial proliferation. When bcl-2 was overexpressed, however, the change in mitochondrial mass no longer occurred. This suggested that one of the functions of bcl-2 might be to inhibit mitochondrial proliferation, and provides evidence for the possible utility of bcl-2 in anti-cancer gene therapy.

Many therapeutic modalities kill cells via apoptosis, including hormones, cytokines, chemotherapeutics, irradiation and hyperthermia. Monitoring apoptotic cell death of cancer cells during treatment would be of benefit in determining the effectiveness of a particular therapy for individual patients, but there are few techniques for examining the early activation of tumor killing. Detection of mitochondrial proliferation might be one such test. To determine whether mitochondrial proliferation is a general indicator of apoptosis, several types of cells were treated with a drug which activates the cell death pathway.

Aphidicolin induces apoptosis by inhibiting DNA polymerase. The cells stop dividing but the cell volume increases, and they then begin to die. When Chinese hamster ovary (CHO) cells were treated with aphidicolin, they responded with an increase in mitochondrial mass without function and began to undergo apoptosis, just as in the C205 colon cancer cell model. By contrast, HeLa cells were unresponsive; they did not die after treatment, and there was no mitochondrial proliferation. In other cell lines treated with aphidicolin there was a good correlation between the ratio between mitochondrial mass and

function and the induction of apoptosis. Mitochondrial proliferation seems to be a good early marker for apoptosis and decreased clonogenic survival during chemotherapy.

Apoptosis may also be induced by oncogenes, which often exert conflicting signals, activating growth under one set of conditions or cell death under others. One of the best models of that type of conflicting signal is the effect of the myc oncogene. Under normal growth conditions, myc induces cell proliferation following mitogenic stimulation; however in low serum or the presence of cytostatic drugs, expression of myc induces the apoptotic pathway.

To examine the effect of myc-induced apoptosis on mitochondrial proliferation, fibroblast cells were transfected with an inducible myc gene and assayed for mean mitochondrial mass under various growth conditions. Serum deprivation alone resulted in a two-fold increase in mitochondrial mass; however when cells were grown in low serum and the expression of myc was induced, there was an even greater increase in mitochondrial mass. Thus, mitchondrial proliferation is not just a side effect of treatment with cytostatic drugs, but also occurs by oncogenic activation of the apoptotic pathway.

To account for his observations, Dr. Hockenbery proposed that mitochondrial replication can be dissociated from nuclear division after treatment with drugs which induce cytostasis. Mitochondrial replication occurs in the G0 to G1 phase of the cell cycle. Apoptosis is induced when cells get stuck in G1. It is possible that when cells are trying to divide, but can't, they undergo some kind of chaos reaction. There is a subsequent dissociation of nuclear replication events from the growth of cytoplasmic components, and this commits the cells to the apoptotic pathway. Under these conditions, mitochondria uncouple the oxidative phosphorylation process, oxygen-free radicals are produced, and cell death ensues.

It is presently unclear how mitochondrial function is modulated. The most likely possibility is that mitochondrial-associated proteins, such as bcl-2 or Bax, are influencing their function. For the development of cancer therapeutic agents it will be important to determine what is different about the mitochondrial growth pathway in cancer cells and how it can be manipulated to restore the apoptotic response.

TELOMERASE IN CANCERS OF THE NERVOUS SYSTEM

Normal cells have a limited life span in culture because of the existence of a cellular "clock." Disruption of that clock may be a critical step in the development of cancer, which results in the immortalization of the cell. Indeed, mechanisms responsible for normal aging may protect against cancer. The clock is associated with the loss of telomerase activity with aging. In contrast to the cells of normal adult tissues, almost all tumor cells have significant levels of telomerase activity which protects them from the normal aging process. Jerry W. Shay of the University of Texas Southwestern Medical Center (Dallas, TX) discussed the role of telomerase in cancer and described a new assay for telomerase activity that can be used as a prognostic marker for cancer cells.

To conceptualize the involvement of telomerase in development of immortalization in cancer, one can consider that there are two independent mechanisms that must be overcome for cells to divide. These are transition points leading to morality states M1 and M2. Getting stuck in M1 is equivalent to replicative senescence, or growth arrest. This condition can be induced by factors such as T antigen or other oncogenes which inactivate p53 and retinoblastoma (Rb) protein and results in a block in the G1 to S phase transition. Antisense oligonucleotides to p53 and Rb, for example, can extend the cell's life span by abrogating the proteins, but they do not immortalize the cells. In the transition to M2, senescent cells go through a crises induced by prolonged telomere shortening, and in rare instances some cells are able to spontaneously overcome this crisis by activating telomerase and stabilizing the length of their telomeres. At this point, M2, they are immortalized and can continue to proliferate.

Exhibit 8
Estimated Incidence of Pediatric Primary Brain Tumors by Gender and Age Group in the USA

Age Group	Males		Females		Total	
(Years)	(#)	Rate*	(#)	Rate*	(#)	Rate*
0 to 4	499	5.1	420	4.5	919	4.8
5 to 9	452	4.5	364	3.8	816	4.2
10 to 14	410	4.2	306	3.3	716	3.8
15 to 19	361	3.7	247	2.7	608	3.2
Total 0 to 19	1,721	4.4	1,338	3.6	3,059	4.0

** Age-specific rates per 100,000 population*

Note: Brain and nervous system cancer includes ICD-9 Code 191

Sources: Central Brain Tumor Registry of the Uninted States. 1996 Annual Report; CA: A Cancer Journal for Clinicians 1997; 47(1):8-9; NIH, SEER Cancer Statistics Review 1995:104-111

Telomeres shorten with age. In reproductive tissues, telomeres are long, averaging 20 TTAGGG repeats. In adult tissues, the length shortens to 4-10 repeats, on average, and there is a progressive erosion of telomere length with age. A rare genetic disease which results in premature aging called Hutchinson-Gilford Syndrome is associated with accelerated telomere shortening, and cells removed from these patients and cultured *in vitro* undergo senescence early. Experimentally lengthening the telomeres of these cells can extend their lifetime.

The reason telomeres shorten is related to the problem of end replication that is peculiar to linear pieces of nucleic acid. Since polymerases synthesize nucleic acids in a 5' to 3' direction, the leading strand synthesis is continuous while the lagging strand must be synthesized in a

discontinuous fashion. In DNA this is accomplished with RNA primers and the synthesis of short Okazaki fragments. The RNA primers are then replaced with deoxynucleotides and the fragments are ligated together. The end of the DNA in the lagging strand would tend to remain uncopied, resulting in a progressive shortening of the chromosome with each succeeding round of replication. To enable the complete replication of linear DNA, hexameric sequences are located at the ends of the DNA, at the telomeres; these sequences are specifically copied by the enzyme telomerase.

Telomerase contains a functional RNA moiety which is complementary to and base-pairs with the telomeric sequences, and serves as a primer for the reverse transcriptase activity of telomerase. So long as telomerase is present, telomeres generally maintain their length. But as cells age, telomerase is no longer produced and end replication ceases. This results in a gradual shortening of the ends of chromosomes over time. Eventually, gene segments lying near the ends of chromosomes become deleted and the cell may lose essential functions. This normally triggers the apoptotic pathway. Reactivation of telomerase can stabilize the telomere length and allow cells to proliferate continuously, a condition of immortalization in cancer development.

The classic assay for telomerase has been the TRAP assay, which is a polymerase chain reaction (PCR)-based assay that depends on the presence of telomerase to produce a ladder of newly synthesized fragments consisting of various lengths of telomere repeats. In the absence of telomerase, no ladder of labeled DNA fragments appears. This assay depends on preserving the cells' telomerase activity and therefore requires fresh tissue or cells such as those found in pathological fluids, fine needle aspirates, washes and brushes. In a retrospective study of meningioma, 100% of the malignant tumors, 92% of the atypical tumors and 17% of the ordinary tumors were found to have telomerase activity by the TRAP assay. The significance of the presence of telomerase activity in cancer cells was revealed when 5 out of 30 of the ordinary tumors with telomerase activity had recurrent tumor growth, whereas in the 25 out of 30 tumors lacking telomerase activity there were no recurrences. This suggests that the TRAP assay has diagnostic utility and that it is capable of predicting the probability of tumor recurrence.

In a study of neuroblastomas in 100 children by TRAP assay, 94 neuroblastomas were associated with telomerase activity, while none of the ganglioneuromas or normal adrenal glands had telomerase activity. Patients with tumors containing low telomerase activity survived while high activity was associated with a poor prognosis. Interestingly, telomerase activity was absent in neuroblastoma Grade IVS, even though Grades I through IV were associated with increasing telomerase activity. However, unlike other grades, Grade IVS has an 80% rate of remission after tumor reduction. In spite of the fact that one-third of the weight of a newborn with this condition can be tumor, the tumor cells do not contain telomerase activity or short telomeres. Thus, the presence of telomerase is not necessary for cancer, but a lack of telomerase is associated with a better prognosis.

According to the telomere hypothesis, developing cells express telomerase activity during embryogenesis and the activity persists in male germinal cells and "renewal" tissues, such as skin, intestinal epithelia, spleen and bone marrow. In these tissues, however, activity is only 5-10% that of germline activity and not is sufficient to prevent senescence. Differentiation represses telomerase activity and it is progressively lost in most tissues. *In vitro* expression of telomerase can be induced by agents which acti-

Exhibit 9
Estimated Incidence of Pediatric (Ages 0-19) Primary Brain Tumors by Histology Group and Gender in the USA

Histology	Male		Female		Total	
	(#)	(%)	(#)	(%)	(#)	(%)
Anaplastic astrocytoma	81	4.7	64	4.8	145	4.7
Glioblastoma	78	4.5	70	5.3	149	4.9
Pilocytic astrocytoma	333	19.3	255	19.0	587	19.2
Ependymoma/ anaplastic ependymoma	149	8.7	104	7.8	253	8.3
Astrocytoma	316	18.3	282	21.1	598	19.5
Malignant glioma	129	7.5	148	11.0	277	9.1
Benign glial, neuronal & mixed	92	5.3	64	4.8	155	5.1
Embryonal/ medulloblastoma	346	20.1	235	17.5	581	19.0
Germ cell	122	7.1	34	2.5	155	5.1
Craniopharyngioma	75	4.3	84	6.3	159	5.2
Total	1,721	100.0	1,338	100.0	3,059	100.0

Note: Brain and nervous system cancer includes ICD-9 Code 191

Sources: Central Brain Tumor Registry of the Uninted States. 1996 Annual Report; CA:A Cancer Journal for Clinicians 1997; 47(1):8-9; NIH, SEER Cancer Statistics Review 1995:104-111

Exhibit 10
Estimated Incidence of Metastatic Brain Cancer by Primary Tumor in Selected World Regions in 1997

	North America		Europe*		Japan		Triad*	
	(#)	(%)	(#)	(%)	(#)	(%)	(#)	(%)
Colorectal	8,300	9.1	15,750	12.2	2,795	14.3	26,845	11.7
Kidney	7,444	8.2	10,442	8.1	1,308	6.7	19,193	8.4
Melanoma	14,148	15.5	9,434	7.3	226	1.2	23,808	10.4
Lung	33,818	37.1	48,580	37.7	10,614	54.1	89,372	39.0
Breast	17,175	18.9	31,270	24.3	2,187	11.2	43,595	19.0
Other	10,221	11.2	13,402	10.4	2,474	12.6	26,096	11.4
Total	91,105	100.0	128,878	100.0	19,604	100.0	228,910	100.0

**Excluding the former USSR*

Sources: Central Brain Tumor Registry of the Uninted States. 1996 Annual Report; CA:A Cancer Journal for Clinicians 1997; 47(1):8-9; NIH, SEER Cancer Statistics Review 1995:104-111

vate differentiation, such as dimethyl sulfoxide (DMSO) or all trans retinoic acid (ATRA).

Telomerase activity, encountered in most malignant cells, is a potential target for cancer therapy. Some of the approaches to inhibiting this enzyme include targeting the RNA moiety with anti-sense oligonucleotides or peptide nucleic acids (PNA) or identifying direct inhibitors of the enzyme. The PNAs have 2-amino glycine backbones and form stable hybrids with RNA that are not hydrolyzed by proteases or nucleases. One 13-mer PNA has been found to be effective in preclinical trials. Natural inhibitors of telomerase may exist and there is some evidence that the human chromosome 3 encodes such a protein. When chromosome 3 was reintroduced into a renal cell carcinoma cell line, telomerase activity was inhibited, there was a dramatic decrease in telomere length, and the cells gradually quit proliferating, although they didn't die. This suggested that there may be tumor suppressors which are actually telomerase inhibitors, and that these might provide new approaches to cancer therapy.

In an effort to develop a test for telomerase which could be done on archival paraffin-embedded and formalin-fixed tissue, the human telomerase RNA (hTR) assay was developed. This test involves *in situ* hybridization to detect the RNA component of telomerase. In a study of pancreatic tumors, which are generally difficult to diagnose early, telomerase activity was detected in 98% of malignant tumors and in none of benign tumors. When pancreatic brushings from a series of patients were tested, 100% of pancreatic carcinomas were telomerase-positive, while none of the benign conditions were associated with telomerase activity. The hTR *in situ* assay is also adaptable to cells in tissue culture and should be a useful diagnostic tool.

HERPES VECTORS FOR BRAIN TUMOR THERAPY

Xandra Breakefield of Massachusetts General Hospital (Boston, MA) described the use of herpes simplex virus (HSV) vectors for gene therapy of brain tumors. One of the advantages of HSV is that it is highly infectious. The virus enters the cell by fusion and delivers genes to the nucleus, a more efficient process than the endocytotic route that adenovirus takes. Because it is a large DNA virus it also has an enormous recombinant gene carrying capacity. About 30 kilobases of its genome can be engineered to contain the therapeutic genes.

One problem in using viral gene therapy vectors to treat brain tumors is that it is necessary to selectively disrupt the blood-brain barrier. Solutions to this problem include use of bradykinin or osmotic shock. When HSV is delivered intravascularly by itself, it doesn't get into brain tissue. However, when HSV and bradykinin are administered together, the virus gets into the tumor but not into normal brain. When mannitol is used to provide an osmotic shock, virus does get into normal brain as well as tumor.

A strategy for enhancing anti-tumor immunity using HSV gene therapy vectors is to immunize with irradiated tumor cells that have been infected *ex vivo* with HSV vectors carrying the GM-CSF gene. In preclinical trials, an HSV amplicon vector carrying GM-CSF was delivered to glioblastoma GL261 mouse tumor cells by *ex vivo* infection, and the cells were irradiated and injected subcutaneously as a vaccine. When vaccination was done prior to intracranial implantation of the tumor, mice survived longer than untreated controls. When the vaccine was delivered to mice which were already bearing tumor, survival was also improved, but less dramatically than mice vaccinated prior to implantation. Vaccination resulted in an enhanced accumulation of eosinophils and lymphocytes to the tumor and better tumor rejection.

The standard approach to brain tumor therapy that is envisioned is to first remove the bulk of the primary tumor surgically. The new foci of tumor growth are next treated by giving prodrug and directly injecting vector. Any migratory cells can then be killed by an immune response stimulated by vaccination with irradiated cells infected with the GM-CSF gene therapy vector.

Exhibit 11
Molecular Markers for Brain Tumors and Cancer of the Central Nervous System

Marker	Description	Comments ❑ References
17p deletion (other than p53)	Inactivation of a tumor suppressor gene or genes located on 17p, other than p53, is associated with medulloblastoma tumorigenesis and a negative prognosis	Cogen PH and McDonald JD, Journal of Neuro-Oncology, 1996 Jul, 29(1):103-12
Alpha-fetoprotein (AFP)	Molecular marker for metastatic germ cell tumors	International Germ Cell Cancer Collaborative Group, Journal of Clinical Oncology, 1997 Feb, 15(2):594-603
C4-2	Gene that is expressed 3-4 fold higher in normal brain tissue as compared to glioblastoma multiforme; exhibits strong homology to cAMP-regulated phospho-protein-16 (ARPP-16)	C4-2 may serve as a potential tumor suppressor gene (Boynton AL, etal, AACR97, Abs. 3592:535)
Cathepsin B	Degrades plasminogen to form plasmin; elevated in malignant gliomas	
CD44	Hyaluronan receptor (cell adhesion molecule) on human MYCN amplified neuroblastoma cells	Lack of CD44 has been shown to be strongly associated with MYCN amplification (Gross N, etal, AACR97, Abs. 1938:288)
Cyclin-dependent kinase 4 with co-amplification of sarcoma amplified sequence (CDK4/SAS)	12q13-q15 chromosomal segment amplicons	15% of anaplastic astrocytomas and glioblastomas show amplification and overexpression of one or more genes from 12q13-q15 (Reifenberger G, etal, Cancer Res 1994, 54:4299-4303; Reifenberger G, etal, Cancer Res, 1996 Nov 15, 56(22):5141-5)
Cyclin-dependent kinase N2 (CDKN2) loci deletion	Deleted in human gliomas and malignant astrocytomas; may result from methylation and chromatin condensation; located on chromosome 9p or p16	Homozygous CDKN2/p16 deletions are more common in high-grade astrocytomas than Rb mutations or CDK4 amplification (stronger deleterious effect on cell cycle control) (Ono Y, etal, J Neuropathology and Experimental Neurology, 1996 Oct, 55(10): 1026-31; Furnari FB, etal, Pediatric Neurosurgery, 1996, 24(1):41-9; Costello JF, etal, Cancer Research, 1996 May 15, 56(10):2405-10)
DDX1 [Asp(D)-Glu-(E)-Ala(A)-Asp(D) or D-E-A-D box gene]	RNA helicase gene encoding a D-E-A-D box protein and mapped to chromosome 2p24; has been shown to be co-amplified with the MYCN gene (because of its proximity) in a proportion of neuroblastomas	George RE, etal, AACR97, Abs. 3790:565; Kuroda H, etal, Oncogene, 1996 Oct 3, 13(7):1561-5
Deleted in colorectal cancer (DCC) protein	A candidate tumor suppressor gene; neural cell adhesion molecule (NCAM) family protein	Absence or loss of DCC expression is associated with neuroblastoma dissemination (Reyes-Mugica M, etal, AACR97, Abs.1960:292; Ekstrand BC, etal, Oncogene, 1995 Dec 7, 11(11):2393-402)
Developmentally regulated EPH-related tyrosine kinase gene (DRT)	A developmentally regulated human protein-tyrosine kinase gene of the EPH family (EPHT3); encodes a receptor type protein-tyrosine kinase	Results suggest that there is auto activation of the DRT receptor kinase in neuroblastoma cells co-expressing LERK ligands, contributing to pathogenesis of neuroblastoma (Ikegaki N, etal, AACR97, Abs. 2971:444; Ikegaki N, etal, Human Molecular Genetics, 1995 Nov, 4(11):2033-45)
Epidermal factor receptor (EGFr) gene Type III	Double minute chromosomes representing an amplified gene that codes for a growth factor receptor; found in 40% of glioblastomas	EGFr amplification is different in secondary glioblastomas (progress from low-grade astrocytomas) (Reifenberger J, etal, J Neuropathology and Experimental Neurology, 1996 Jul,55(7):822-31; Collins VP, Seminars in Cancer Biology, 1993 Feb, 4(1):27-32; Moscatello DK, etal, Cancer Research, 1997 Apr 15, 57(8):1419-24)

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ERCC1 and ERCC2 on chromosome 19	Involved in DNA repair	Dabholkar MD, etal, Cancer Research, 1995 Mar; 55(6): 1261-1266
Fas/Apo-1 (CD95)	Membrane receptor that triggers apoptosis upon ligation with the anti-Fas IgM antibody CH11; may be a mechanism of cell death in perinecrotic cells of glioblastoma multiforme	Data show that human neuroblastoma expresses low levels of surface Fas and does not undergo apoptosis with anti-Fas antibody(Kotoula V, etal, AACR97, Abs. 1316:196; Tachibana O, etal, Acta Neuropathologica, 1996 Nov, 92(5):431-4)
Genes growth-arrest DNA damage 153 (GADD153), rap1B, α-2 macroglobulin receptor (A2mr), and interferon γ gene (IFNG)	Co-amplified in some tumors but not overexpressed consistently; also located on chromosome 12q13-q15	Reifenberger G, etal, Cancer Research, 1996 Nov 15, 56(22):5141-5
Glial fibrillary acidic protein (GFAP)	Gliotic marker	Chiang CS, etal, Int'l J Radiation Biology, 1997 Jul, 72(1):45-53
Human brain gene (HuD) mapped to 1p34 and protein	Neuronal-specific RNA-binding protein	Results suggest that HuD plays a critical role in sustaining high steady-state levels of MYCN mRNA and protein (Lazarova DL, etal, AACR97, Abs. 3789:564)
Human chorionic gonadotropin (HCG)	Molecular marker for metastatic germ cell tumors	International Germ Cell Cancer Collaborative Group, J Clinical Oncology, 1997 Feb, 15(2):594-603
Loss of heterozygosity at chromosome 10 (LOH 10)	Frequently associated with glioblastoma multiforme; there is a close association of LOH 10 and most malignant histologic stage of glioma and glioblastoma; arises as the clonal expansion of an earlier staged precursor	LOH for loci on chromosome 10 was observed in 28 of 29 tumors histologically classified as glioblastoma (malignancy Grade IV) whereas no such losses were observed in any of 22 lower grade gliomas (James CD, etal, Cancer Res 1988 Oct 1 48:19 5546-51)
Ras suppressor gene (RSU1) localized to region 10p13	Suppresses *in vitro* and *in vivo* growth of cultured glioma cells	Tsuda T, etal, Oncogene, 1995, 11 (2):597-403
PTEN located on human chromosome 10q23	A putative protein tyrosine phosphatase gene mutated in human brain cancer, PTEN has a protein tyrosine phosphatase domain and extensive homology to tensin, a protein that interacts with actin filaments at focal adhesions; these homologies suggest that PTEN may suppress tumor cell growth by antagonizing protein tyrosine kinases and may regulate tumor cell invasion and metastasis through interactions at focal adhesions	Detected in 31% (13/42) of glioblastoma cell lines and 17% (3/18) of primary glioblastomas (Li J, etal, Science, 1997 Mar 28, 275(5308):1943-7)
Loss of heterozygosity at chromosome 15 (LOH 15)	A novel putative suppressor gene found in 70% (21/29) of breast cancer cases with brain metastases; mapped to 15q14	Wick W, etal, Oncogene, 1996 Mar 7, 12(5):973-8
MAGE/BAGE	Genes that code for distinct antigens and give rise to immunogenic peptides recognized by autologous cytolytic T lymphocytes	Chen RL, etal, AACR97, Abs. 789:118; Fujie T, etal, Annals of Oncology, 1997 Apr, 8(4):369-72
Murine double minute 2 (MDM2)	Amplification targets located at 12q13-q15 found in human malignant gliomas	Reifenberger G, etal, Cancer Research, 1996 Nov 15, 56(22):5141-5
	Tumor cells immunoreactive to MDM2 were found in 52% of primary but in only 11% of secondary glioblastomas; MDM2 amplification occurred in 7% of primary but in none of secondary glioblastomas and only one out of 15 primary glioblastomas overexpressing MDM2 contained a p53 mutation	MDM2 overexpression with or without gene amplification may constitute a molecular mechanism of escape from p53-regulated growth control in the evolution of primary glioblastomas that typically lack p53 mutations (Biernat W, etal, J Neuropathology and Experimental Neurology, 1997 Feb, 56(2):180-5)
MHC class I, TAP-1, -2, LMP-2, and -7	Antigen presentation/processing genes	Chen RL, etal, AACR97, Abs. 789:118
MTB-Zf	Protein with 2 zinc-finger domains; expressed in primary brain tumors	May function as a transcription factor for the human heme-oxygenase-1 gene, and play a role in cell growth of differentiation (Muraosa Y, etal, European J Biochem, 1996 Feb 1, 235 (3):471-9)

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Multi-drug resistance 1 (MDR1) gene	Overexpressed in neuroblastoma cells	Fulda S, etal, Anti-Cancer Drugs, 1997 Jan, 8(1):34-41
MYCN	Amplification of MYCN oncogene found in 25% of primary tumors; mapped to 2p24	Kenyon RM, etal, AACR97, Abs. 2774:414
	Amplification in the IMR32 neuroblastoma cell line is thought to play a role in cell proliferation, differentiation, and neoplastic transformation	Bonvini P and Neckers L, AACR97, Abs. 3788:564; Bao J and Zervos AS, Oncogene, 1996 May 16, 12(10):2171-6
Neurofibromatosis 2 (NF2) gene	Tumor suppressor gene located on chromosome arm 22q; mutations were observed in 17 of 57 meningiomas and in 30 of 89 schwannomas but not in 17 ependymomas, 70 gliomas, 24 pheochromocytomas, 15 neuroblastomas or 6 medulloblastomas; all meningiomas and 1/2 of schwannomas with identified NF2 mutations demonstrated chromosome 22 allelic losses	Gutmann D, etal, AACR97, Abs. 1696:252; von Haken MS Genes, etal, Chromosomes and Cancer, 1996 Sep, 17(1):37-44; Mérel P, etal, Genes Chromosome Cancer 1995; 13(3):211-216
O^6-methylguanine-DNA methyltransferase (MGMT)	A DNA repair protein that removes alkyl adducts from DNA and may be important in tumor resistance to alkylating agents; MGMT expression in brain is developmentally regulated and mer status during the first trimester is a risk factor for neurocarcinogenesis in humans	Silber JR, etal, AACR97, Abs. 1220:182; Belanich M, etal, Cancer Chemotherapy and Pharmacology, 1996, 37(6):547-55
Osteopontin (OPN)	OPN is thought to be functionally involved in tumorigenesis; its exact role is unknown	Elucidation of regulatory mechanisms for OPN induction in glioma cells may facilitate rational design of novel therapeutics (Tucker MA, etal, AACR97, Abs. 1028:153)
p16 (MTS-1), p15 (MTS-2), CDK4 and cyslin D2	Multiple mechanisms of cell cycle deregulation in neuroblastoma; MTS-2 deletion is mapped to the 9p21 region; both p16 and p15 were deleted in 80% of primary malignant lymphomas of the brain but no no mutations of p16 were detected in any of the cases	Results suggest the presence of multiple mechanisms for deregulating the G1-S phase cell cycle transition in neuroblastoma (Omura-Minamisawa M, etal, AACR97, Abs. 1826:272); there is frequent and differential overexpression of p15 and p16 in neuroblastoma (Diccianni MB, etal, AACR97, Abs. 3317:495) and primary brain lymphoma (Kumanishi T, etal, Japanese J Cancer Research, 1996 Jul, 87(7):691-5)
p53 located on chromosome 17p	The most frequent genetic mutation in all adult tumor types; located on chromosome 17p	von Haken MS Genes, etal, Chromosomes and Cancer, 1996 Sep, 17(1):37-44
	Expression of immunoreactive p53 protein is detected in cerebral neuronal tumor; Li-Fraumeni syndrome fibroblast cultures lacking normal p53 function exhibit impaired nucleotide excision repair but resistance to ultraviolet light cytotoxicity	Ho YS, etal, AACR97, Abs. 2178:325
Platelet-derived growth factor receptor antagonist (PDGFra)	Double minute chromosomes representing an amplified gene that codes for a growth factor receptor; found in a small percentage of glioblastoma cases	Collins VP, Seminars in Cancer Biology, 1993 Feb, 4(1):27-32
Plasmin, its activator urokinase-type plasminogen activator (uPA), the receptor uPAr (CD87), and plasminogen activator inhibitor-1 (PAI-1) and PAI-2	Linked to cancer invasion and metastasis	Increase of uPA, uPAr, and/or PAI-1 is associated with tumor progression and with shortened disease-free and/or overall survival in patients with malignant solid tumors including brain cancer (Schmitt M, etal, Thrombosis and Haemostasis, 1997 Jul, 78(1):285-96)
Retinoid acid receptor γ1 (RARγ1)	RARgγ1 controls the expression of RARβ2 in neuroblastoma cells	Loss of expression of RARβ may have important pathogenic consequences during the development of human neuroblastoma (Ferrari N, etal, AACR97, Abs. 987:147)

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Retinoblastoma (rb) gene	Tumor suppressor gene found in brain metastases	Shiseki M, etal, Cancer Research, 1994 Nov 1, 54(21):5643-8
RE1-silencing transcription factor (REST)	Represses transcription of neuronal genes in non-neuronal cells	In human neuroblastoma cells, REST appears to be regulated at the level of the stem cell (Ross RA, etal, AACR97, Abs. 1029:154)
RET	Overexpression of MED2B-type mutant RET proto-oncogene in human neuroblastoma cells activates Jnk1 kinase, alters cell adhesion *in vitro*, and promotes metastasis *in vivo*	Marshall GM, etal, AACR97, Abs. 963:144
Telomerase	Neuroblastomas are associated with telomerase activity; patients with tumors containing low telomerase activity survive while high activity is associated with a poor prognosis; presence of telomerase is not necessary for cancer, but a lack of telomerase is associated with a better prognosis	See meeting coverage article in this issue
Tuberous sclerosis 2 (Tsc-2)	Tumor suppressor gene	Tsc-2 gene plays a critical role in brain development which is consistent with its playing a causal role in the development of brain lesions observed in most individuals afflicted with tuberous sclerosis (Rennebeck G, etal, AACR97, Abs. 1874:279)
von Hippel-Lindau gene (VHL)	Allele losses and mutation of VHL gene are involved in development of sporadic form of cerebellar hemangioblastomas	Lee JY, etal, AACR97, Abs. 3600:536
zf5-3	cDNA clone localized on chromosome 19	Data implicate zf5-3 as a potential mediator of neuroblastoma and neuronal differentiation (Dimitroulakos J, etal, AACR97, Abs. 1042:155)

Basic HSV Gene Therapy Vector

In the basic HSV gene therapy vector the inessential viral gene infected cell protein (ICP) 6 is replaced with a marker gene for lac Z. Since the virus carries a thymidine kinase (tk) gene, it can be used in combination with the pro-drug ganciclovir, which is activated by tk. To make the virus safer, the gamma 34.5 gene for neurovirulence is also knocked out. It is this HSV vector that is closest to clinical trials for human brain tumors. The virus will only replicate in dividing cells because it requires a cellular enzyme, ribonucleotide reductase, which is up-regulated when cells are dividing. This vector has been tested in preclinical trials using a rat gliocytoma model. In this system, tumor cells are injected into the brain and the vector is administered by direct injection intracranially. Some of the tumor is killed by virus propagation. The infection spreads a bit and then immunity kicks in. In experiments with pre-immune animals there is less spread of the virus, but still some primary infection.

When the vector is delivered together with ganciclovir the infected cells convert the drug to its active phosphorylated form, which acts by blocking DNA replication. There is a bystander effect due to spread of the activated drug through cell-cell contact. Long term survival of rats treated with vector and ganciclovir is improved from 100% death rates in the controls to 50% death rates in the treated animals. Tumor cell killing occurs by three mechanisms, direct killing by the virus, ganciclovir toxicity, and an anti-tumor immune response.

Improved HSV Recombinant Vector

An improved HSV recombinant vector has been developed which contains the cytochrome P450 gene instead of lac Z in the ICP6 replacement site. Expression of P450 sensitizes brain tumors to cyclophosphamide. Normal brain cells are unaffected by the drug because they lack the enzyme which converts it to its active DNA-cleaving metabolite. While cyclophosphamide can get into the brain, it normally remains in its inactive form. In cells expressing vector-associated P450, cyclophosphamide is activated and there is an enhanced bystander effect because the P450 gene product can be transferred to cells surrounding the infected cells through the extracellular space without cell-cell contact. In combination with ganciclovir as a second chemotherapeutic agent, there is a synergistic interaction between the two drugs and survival is even greater than with one drug alone.

HSV-1 Amplicon

Another gene therapy approach is the HSV-1 amplicon, which promotes cell-killing and also stably transforms cells to allow a lengthened period of transgene expression. The vector is a plasmid with the non-coding

elements of HSV, including the DNA origin of replication and a packaging element, and it can enter both dividing and non-dividing cells. Infection requires co-infection with an HSV helper virus which enables it to replicate. Both HSV and the amplicon virus are released but the amplicons are much more abundant because their multiplication is amplified 10-fold over that of the helper virus.

To enable the amplicons to genetically transform cells, the adenovirus-associated virus (AAV) insertion terminal repeat (ITR) sequences are added in a position which flanks the gene to be inserted in the host chromosome. The AAV ITRs integrate into a specific site on human chromosome 19. The transgene allows expression over a period of as long as 15 days, whereas expression from the normal vector generally only lasts about 10 days. Transduction of human hepatic cells with this vector results in stable transformation of approximately 6% of the cells.

Helper Virus-free Packaging System

Another gene therapy vector type is a helper virus-free packaging system. This vector is a set of HSV cosmids that contains the HSV genome in 5 pieces. All but one of the cosmids contain mutated packaging signals along with the HSV genes necessary for replication. The cosmid with normal packaging signals contains the therapeutic gene but no replication genes, and it is the only amplicon that is finally packaged. The advantage of this vector is that high titers on the order of 108 virions can be generated and there is no toxicity. This system results in dramatic extension of transgene expression in both dividing and non-dividing cells because there is replication amplification and integration of the transgene. There is also a potential for targeting using this system with a tissue-specific promoter.

FARNESYL TRANSFERASE INHIBITORS AND ANTI-RAS THERAPY

The ras proto-oncogene is involved in controlling a cell's decision to grow or to differentiate. Mutations in ras are associated with 10-50% of all human cancers, and anti-cancer therapies targeting these oncoproteins have focused on its biochemical activation by farnesylation. Farnesyl is a metabolite of farnesyl diphosphate that inhibits HMG-CoA reductase. It has been used under the name lovastatin (Mevacor; Merck) to treat high blood cholesterol conditions. In order to be active ras must be associated with the plasma membrane. Farnesyl diphosphate activates ras by converting it to ras-farnesyl, which can become inserted into membranes in an active form. This farnesylation (addition of a 15 carbon moiety) of ras is carried out by the enzyme farnesyl protein transferase (FPTase), which is essential in man, but not in yeast. In man, mutant ras is associated with cancer. In yeast, FPTase genetic knock-outs inhibit phenotypes associated with mutant ras. Thus, inhibitors of FPTase are likely to be useful as anti-cancer agents, and J. Gibbs of Merck Research Laboratories (West Point, PA) reported on the development and therapeutic activity of these agents.

Many FPTase inhibitors (FTI) have been developed, and some of them are prodrugs. All are esterified forms that can penetrate the membrane, and the prodrugs are activated by an intracellular enzyme that cleaves off the ester moiety. These drugs have good anti-tumor activity in culture and in animal models such as ras transgenic mice with mammary tumors (Oncomice). There is also biochemical specificity *in vitro*.

The three well-known ras proteins are Harvey ras, containing the sequence CVLS; Kirsten ras (CVIM); and N-ras (CVVM). Harvey and Kirsten ras proteins are both good substrates for FPTase, while N-ras, which is the most common mutated form of ras in human tumors, is not a good substrate. When tested in a soft agar assay, sensitivity to FTI did not correlate with the differential sensitivity of ras mutations to FPTase. This suggested that the effect of the inhibitor might be indirect and not directly due to farnesylation of ras. Other candidate substrates were also identified. At least a dozen labeled spots disappeared after FTI treatment, indicating that the inhibitor blocks farnesylation of other proteins as well.

Exhibit 12
Five-Year Survival Trends of Brain and Nervous System Cancer Cases in the USA

Year of Diagnosis	Males (%)	Females (%)	Total (%)
1960-1963	16.0	21.0	18.0
1970-1973	18.0	22.0	20.0
1974-1976	20.4	24.8	22.3
1977-1979	23.2	26.2	24.5
1980-1982	24.3	26.2	25.1
1983-1985	25.0	28.0	26.4
1986-1991	28.1	29.9	28.9

Note: Brain and nervous system cancer includes ICD-9 Code 191
Source: NIH, SEER Cancer Statistics Review 1995:104-111

Compared with doxorubicin treatment, the FTI inhibitor was more effective in prolonging survival of tumor-bearing animals, and it was less toxic; however some large tumors did not respond to treatment. Histologic changes included a decrease in cellularity and an increase in stromal mitotic index of the tumor. Treatment with FTI did not completely eliminate the tumor because there was regrowth after treatment stopped. Therapies using FTI may, therefore, require continuous treatment, but lack of toxicity makes long-term exposure to the drug possible. There were no histologic changes in cells in which farnesylated proteins are critical for function, such as retina, skeletal muscle and rapidly dividing tissues. Since there was no tumor necrosis or influx of immune cells, tumor rejection was presumed to be by apoptosis. This was confirmed by histologic section and staining for apoptotic cells.

Newer FTI compounds are being developed which are smaller and don't require a prodrug strategy. A few issues remain to be addressed in using this approach. Is the drug active against tumors with multiple genetic alterations? Is there activity against "real" human tumor? Will cells develop resistance to the drug, and will safety issues limit its usefulness?

Exhibit 13
Estimated Incidence, Two- and Five-Year Relative Survival for Malignant Brain Tumors by Histology Group

Histology Group	Incidence		Two-Year Survival		Five-Year Survival	
	(#)	(%)	(#)	(%)	(#)	(%)
Glioblastoma	7,362	41.8	648	8.8	250	3.4
Astrocytoma	4,613	26.2	2,085	45.2	1,596	34.6
Pilocytic astrocytoma	337	1.9	303	90.0	292	86.5
Diffuse astrocytoma	371	2.1	245	66.1	183	49.2
Anaplastic astrocytoma	1,042	5.9	467	44.8	309	29.7
Oligodendroglioma	660	3.7	506	76.7	399	60.4
Anaplastic oligodendroglioma	56	0.3	33	59.2	20	34.9
Ependymoma/ anaplastic ependymoma	544	3.1	410	75.3	337	62.0
Malignant glioma	1,380	7.8	453	32.8	359	26.0
Mixed glioma	338	1.9	248	73.5	187	55.4
Embryonal/ medulloblastoma	650	3.7	445	68.4	331	50.9
Neuroepithelial	153	0.9	78	51.4	64	42.2
Malignant neuronal, glial & mixed	94	0.5	59	62.8	46	48.5
Total brain and CNS tumors	17,600	100	5,981	34.0	4,373	24.8

Note: Brain and nervous system cancer includes ICD-9 Code 191

Sources: Central Brain Tumor Registry of the Uninted States. 1996 Annual Report; CA:A Cancer Journal for Clinicians 1997; 47(1):8-9; NIH, SEER Cancer Statistics Review 1995:104-111

TARGETING P53-DEFICIENT CELLS WITH ADENOVIRUS MUTANTS

Frank McCormack of UCSF Cancer Center discussed a strategy to treat tumors with p53 defects. Overall, 75% of all human tumors have some kind of defect in p53. The percentages of each type of tumor which contain p53 mutations include colon (65%), cervical (95%), head and neck (65%), hepatic (60%), non-small cell lung (50%), small cell lung (80%) and ovarian (60%) cancer and glioblastoma (20-60%). In most cases, the gene is missing; in others the wild type p53 gene is disrupted or its expression is affected by another mutation such as overexpression of the p53 inhibitor MDM2. Tumors with p53 defects are difficult to treat because they are associated with increased rates of relapse and decreased survival. Thus, there is a need for new therapeutic approaches.

One new approach is the use of an adenovirus vector derived from the mutant virus, developed by Arnold Berk at UCLA, called dl 1520. The virus was acquired by Onyx Pharmaceuticals (Richmond, CA) and renamed ONYX-015. Normally, when wild type adenovirus infects a cell it produces p55, a product of the viral E1B gene which is an inhibitor of the cellular tumor suppressor p53. The p55 viral protein binds p53 directly, and this somehow allows the virus to replicate efficiently and kill the cell. It is not clear whether this is the result of an inhibition of p53-mediated arrest of the cell cycle or of interfering with the apoptotic pathway. The mutant virus ONYX-015 lacks the p55 gene and can't inhibit p53. In the presence of active p53, the mutant virus can't replicate efficiently and the cells survive. Only when it infects p53 deficient cells does ONYX-015 replicate sufficiently to kill the cell. This simple strategy is a potential way of killing only those cells lacking p53, and in many cases it does so regardless of the presence of other oncogenic mutations.

Recent studies have shown ONYX-015 to be effective in killing a variety of cancer cell types in tissue culture, including one breast cancer cell line, 6 cervical cancer cell lines, 7 colon cancer cell lines and pancreatic and bone cancer cells lines. It also causes tumor rejection in nude mice. Tumor necroses as the virus spreads from the inner to outer regions, with outer cells dying last. Nude mice bearing human head and neck carcinoma survive longer when treated with ONYX-015 than untreated controls, and when virus treatment is combined with cisplatin chemotherapy, the rate of survival is even greater. When virus or chemotherapy treatment alone were compared, virus treatment resulted in better survival rates.

In a C33A mouse carcinoma model, the virus was delivered by tail vein injection. The fact that the tumor becomes infected by this route indicates that the virus can get out of the bloodstream and find and infect tumor cells. The virus is able to spread through solid tumors and can cause complete regression in 60% of treated tumors. Safety was monitored in cotton rats, which support adenovirus infection. At 1011 PFU/kg body weight, there was

no toxicity when ONYX-015 was administered as frequently as daily for 14 days.

The agent is now in phase I clinical trials. In one trial of head and neck, pancreatic and cervical carcinoma, the virus was administered by direct intratumoral injection. At the time this data was presented, some of the head and neck cancers were showing signs of necrosis. In another trial, regional therapy is being attempted for cancers associated with malignant ascites and malignant plural effusions, and is being administered by direct liver perfusion for hepatic cancer. In a third trial, the agent is being delivered intravenously for systemic and liver cancer. In all cases some necrosis of the tumor has been seen, even at low doses, and there is evidence of viral replication. Phase II clinical trials were scheduled to have begun in July, 1997.

One of the as yet unresolved issues regarding ONYX-015 include whether the virus is too immunogenic. Do tumor cells present antigen efficiently? Are infected cells killed by T cells? How much neutralizing antibody is raised and how efficient are antibodies at blocking virus spread within a tumor? Does immune suppression increase the potency of the treatment? A second issue is whether the virus is too toxic. It does replicate in normal cells and some normal cells may have no functional p53, which could lead to non-specific toxicity. So far, however, none has been seen. Finally, perhaps the virus isn't sufficiently potent. Can it replicate efficiently at the center of the tumor and does the presence of normal cells prevent viral spread?

A REPORT ON MOLECULAR MEDICINE OF CANCER, A GENETIC DISEASE

FROM THE NINTH ANNUAL USHA MAHAJANI SYMPOSIUM AT THE SALK INSTITUTE, SEPTEMBER 5, 1997

METHYLATION AND EPIGENETIC INSTABILITY IN THE DEVELOPMENT OF CANCER

Stephen B. Baylin, of the Comprehensive Cancer Center at Johns Hopkins University School of Medicine (Baltimore, MD) described the role of methylation in the development of cancer. Aberrant methylation as a mechanism of tumorigenesis has been documented in a few tumor types, but it is presently not well understood. Only a single DNA methyltransferase has been identified and there is evidence that there are trans-acting factors involved as well.

Imprinted Genes and Childhood Tumors

Methylation has a well-established role in a few childhood tumors associated with genetic imprinting. Imprinted genes are those from which only one allele is actively transcribed because the second allele was inactivated early in embryogenesis. Wilm's tumor and rhabdomyosarcomas are associated with imprinted genes on chromosome 11p15 and neuroblastoma is associated with deletion of an imprinted region on chromosome 1p36.3. The imprinted regions associated with these tumors either contain tumor suppressor genes or have an effect on their regulation. Genetic alterations of imprinted genes include deletions of maternal alleles or translocations in the region of imprinted gene clusters which disturb their methylation and expression patterns.

Methylation of Normal Genes

Methylation of many normal genes occurs during early embryogenesis. DNA methyltransferase functions in a nonspecific manner to methylate CpG islands in newly replicated DNA. Genes which are essential for "household" functions which must remain active, are protected from methylation, but those which are expressed in a tissue-specific or developmental stage-specific manner, become methylated and are only demethylated under appropriate conditions. Protection is presumably provided by trans-acting DNA-binding proteins which allow access to the chromosomal regions only under certain conditions. Locus-specific demethylation is mediated by other trans-acting factors, at least some of which are transcription factors.

Hypermethylation

By contrast to methylation of individual genes, hypermethylation occurs from a center and spreads out over a large chromosomal region which may cover several hundred kilobases. These centers are the sites of imprinted genes and often include genes whose RNA transcripts lack a coding region and are not translated. Hypermethylation decreases promoter activity. It takes multiple rounds of DNA replication to establish sufficient density of methylation for complete transcriptional silencing. Hypermethylation of promoter regions also occurs during aging. Some of these types of changes are highly conserved.

Aberrant methylation is sometimes one of the multiple steps which occur during tumor progression. A cell may first become heterologous for a degree of loss for a particular tumor suppressor gene. Clonal expansion of that aberrant cell is then driven by interactive molecular events, some of which are genetic, such as mutations, and some epigenetic, such as methylation. In colorectal cancer cells, promoter methylation occurs more often when mismatch repair genes are inactive.

Mismatch repair genes include the MLH1 and MSH2 genes associated with hereditary non-polyposis colon cancer (HNPCC). Both are considered "household" genes because they perform the important function of repairing DNA nucleotide mismatches, and when they are inactivated mutations accumulate rapidly. The MLH1 gene itself is hypermethylated in a few tumors with the mismatch phenotype. Two-thirds to three-quarters of the sporadically mutated tumors have a hypermethylated MLH1 gene, while the MSH2 gene has never been found

Exhibit 14
Five-Year Survival Rates for Brain and Nervous System Cancer in the USA in 1997

	Males			Females			Total		
Stage	**Incidence**	**5-year Survivors**		**Incidence**	**5-year Survivors**		**Incidence**	**5-year Survivors**	
	(#)	**(#)**	**(%)**	**(#)**	**(#)**	**(%)**	**(#)**	**(#)**	**(%)**
Localized	2,424	768	31.7	1,800	632	35.1	4,224	1,400	33.1
Regional + Distant	909	300	33.0	750	265	35.3	1,584	565	35.7
Unstaged	6,767	1,746	25.8	5,025	1,352	26.9	11,792	3,098	26.3
Total	10,100	2,815	27.9	7,500	2,248	30.0	17,600	5,063	28.8

Note: Brain and nervous system cancer includes ICD-9 Code 191

Sources: NIH, SEER Cancer Statistics Review 1995:104-111; CA:A Cancer Journal for Clinicians 1997; 47(1):8-9

to be hypermethylated. There appears to be a tendency to hypermethylate that precedes and catches up with the MHL1 gene in the process that leads to genetic instability.

Epimutations occur when genes are silenced by hypermethylation. Epimutations play a major role in cancer progression that may involve local and central changes in DNA methylation levels or its regulation. It may be possible to develop cancer therapeutics that target this type of tumorigenic change by reducing the level of methyltransferase.

Loss of a protective mechanism that cleaves out hypermethylated regions or one which blocks hypermethylation is another possible step in cancer progression. More research needs to be done to identify the factors that are involved in the regulation of DNA methylation in order to develop strategies to prevent the progression of epigenetic changes leading to the development of cancer.

CHROMOSOMAL TRANSLOCATIONS IN CANCER

Carlo Croce, of the Kimmel Cancer Center at Jefferson Medical College (Philadelphia, PA) described cancers which result from chromosomal translocations. In general, this type of tumorigenic change is an initial event in tumor progression; in far fewer cases, it is involved in tumor progression.

Bcl-2

An example of a type of cancer with chromosomal translocations is follicular lymphoma, involving a chromosome 14;18 translocation which affects the bcl-2 gene resulting in its overexpression. Bcl-2 overexpression which occurs in many types of cancers, inhibits apoptosis. It is presumed to account for the ability of many cancers to resist chemotherapy, which works by activating the normal cell death pathway.

However, most early prostate cancers are bcl-2 deficient, suggesting that bcl-2 overexpression is not the only mechanism operating to make early cancer cells resistant to chemotherapy. As prostate cancer progresses, the bcl-2 gene apparently becomes reactivated because some 75% of metastatic prostate tumors overexpress bcl-2, which probably serves as a late chemotherapy-resistance factor.

Phosphorylation of bcl-2 on one of its 19 serine residues (ser-70) promotes apoptosis. Inhibition of phosphatase by okadaic acid increases the degree of phosphorylation of bcl-2 serine-70 and stimulates apoptosis, and so does treatment with the microtubule stabilizers Taxol and Taxotere. When the status of bcl-2 was monitored during chemotherapy with doxorubicin, cisplatin, 5-fluorouracil, vincristine and vinblastine, only the latter two, which are microtubule stabilizers, were associated with bcl-2 phosphorylation. This implies that damage to the microtubule assembly-disassembly process induces bcl-2 phosphorylation which normally occurs only in the G1 phase of the cell cycle.

Another cancer which involves a chromosomal translocation is chronic lymphocytic leukemia. In this case, there is overexpression of bcl-2, but it is unphosphorylated. Cells become stuck in G0 and fail to divide, thus becoming immortalized. Phosphorylation of bcl-2 in this case can't be induced by okadaic acid or microtubule-damaging drugs. This suggests that bcl-2 phosphorylation is critical in activating the apoptotic pathway. To test this idea, wild type bcl-2 gene was mutated so that serine-70 could not be phosphorylated, and the mutant DNA was introduced into CAP cells, which are bcl-2 negative. These cells became protected from apoptosis. Furthermore, when cells are treated with dolostatin, a drug which induces microtubule polymerization, bcl-2 becomes phosphorylated and this, in turn, induces apoptosis.

Genes Involved in the Pre-initiation of Cancer

Understanding the role of translocations in cancer progression will likely be most useful to the development of innovative diagnostics and therapeutics that are directed at genes involved in pre-initiation of cancer. A new approach that has to be attempted is to try to target pre-malignant cells before they have a chance to divide a sufficient number of times to accumulate more mutations.

Most common malignancies are associated with some alteration in chromosome locus 3p. A genetic rearrangement which inactivates a tumor suppressor gene that occurs in acute lymphocytic leukemia involves LOH on chromosome 3.

A rare genetic alteration was identified in colorectal cancer, which involves a 3:18 translocation, LOH at 3p, and a small homozygous deletion. Exon trapping experiments with such cells led to the identification of a new tumor suppressor gene, FHIT (fragile histidine triad). This gene spans the FRA3B fragile site at 3p14.2. and encodes an enzyme (AP3A) which is involved in dinucleotide oligophosphate metabolism. There is a loss or aberrant expression of FHIT in many tumors, often as a result of a deletion within the gene which gives rise to a loss of functional mutation. FHIT is mutated in 50% to 80% of cancers. The FHIT gene is over one megabase in size, has five exons, with exon 5 being the first coding region, and its transcript is 1.1 kilobases. The FRA3B/FHIT locus has been sequenced and the breakpoint regions in 22 cancers have been identified. Most are at precisely the same nucleotide site, in each case near the line 1 element which is over one kilobase downstream of the 5' end of the gene. This suggests that the line 1 element is involved in self-fusion. Point mutations that knock out the enzyme activity of AP3A do not inhibit the gene's ability to repress malignancy, thus the role of the FHIT gene in the development of cancer is not yet understood.

EMERGING CYTOGENETIC TECHNOLOGIES TO DETECT QUANTITATIVE CHROMOSOME CHANGES

Difficulty in attempting to screen for genetic alterations that may be involved in cancer is the sheer size of the human genome, which contains 100,000 genes. Daniel Pinkel, of the Cancer Center at UC San Francisco School of Medicine reported on new techniques of enhanced cytogenetics for cancer diagnosis. Enhanced cytogenetics allows one to see the whole genome at one time and focus on small regions of 10,000 bases. Genome-scale DNA hybridization for cytogenetic analysis is done by multicolor chromosome studies using metaphase chromosomes and comparative genome hybridization (CGH) in a microarray setup. The technique involves labeling hybridization probe DNA for different chromosomes with biotin-labeled uridine and coupling with flourescein-labeled avidin, rabbit anti-DNA AAF, and rhodamine-labeled goat anti-rabbit. Metaphase chromsomes are stained by fluorescence *in situ* hybridization to a mixture of labeled probes derived from normal and tumor cells. Normal DNA is labeled with one color; tumor DNA with another. There are five color combinations and each individual chromosome can be identified by its characteristic color pattern. Differences between the DNA from the two sources register as differential signal intensities when the mean fluorescence intensity across the chromosome is measured. There is a higher color intensity for over-represented regions or normal DNA, which indicates a deletion or loss of the corresponding region in the tumor DNA genome. Although the resolution is poor, the technique provides a genetic region of focus for further sequence analysis. Hybridization by CGH in microarrays using cosmid targets improves the resolution by 200-fold.

BRCA1 AND BRCA2 GENES IN BREAST AND OVARIAN CANCER

Mary Claire King, of the University of Washington (Seattle, WA), described the latest studies on the BRCA1 and 2 genes in breast cancer. One controversy in the field is the location of the BRCA1 protein. Based on immunofluorescence studies using a single monoclonal antibody, Dr. King's laboratory found it in the nucleus. The nuclear pattern in resting cells is punctate, and in the mitotic cell the protein lies along the chromosomes. With other antibodies there is a perinuclear stain and nuclear dots, which was interpreted to possibly represent an end view of a crosswise section of a tubule. In other laboratories evidence localizes BRCA1 protein primarily to the cytoplasm.

The mechanism of loss of expression is not known, but may be shut-off by methylation because the gene has several CpG islands at the 5' end. There are also interspersed repeats with high numbers of Alu sequences. With Alu sequences accounting for 41% of the gene, the BRCA1 gene has the third highest Alu content of all genes in the database. High Alu-containing genes have aberrant mutations, recombination events arising from breakpoints, loop-outs and repair. Loss of expression is also associated with a genomic alteration found at the middle of exon 3, 1400 bps from the 5' end, which is simultaneously an inversion, duplication and deletion.

Mutations Vary by Geography

An analysis of BRCA1 gene sequences in women around the world who have had breast cancer, identified five major mutations which are found in clusters in different world regions. There is an AG deletion at nucleotide 185 that is the most common BRCA1 mutation in the USA and Britain, found primarily among those of Jewish descent. A single nucleotide deletion at 2594 is the only mutation found in Sweden and Denmark. At nucleotide 2803, there is an AA deletion that is the most frequent mutation in Holland and Belgium, but it is found nowhere else in the world. A deletion at nucleotide 418 is the second most common BRCA1 mutation in France and third most common mutation in the USA. An insertion at nucleotide 5382 is the most common mutation in France, is the second most common mutation in the USA, and the only mutation found in Italy.

Actionable Cancer-propensity Genes

One of the concerns about the value of identifying individuals with cancer-propensity genes is the lack of intervention options. One of the few approaches to attempt to intervene in the development of breast cancer

Exhibit 15
Incidence and Five-Year Survival of Brain and Nervous System Cancer in Selected World Regions by Stage at Diagnosis

	North America		Europe*		Former USSR		Triad*		
	Incidence (#)	Five-Year Survivors (#)	Incidence (#)	Five-Year Survivors (#)	Incidence (#)	Five-Year Survivors (#)	Incidence (#)	Five-Year Survivors (#)	Total (%)
Localized	4,745	1,575	5,849	1,942	1,499	498	11,742	3,898	33.2
Regional + Distant	1,779	608	2,193	749	562	192	4,403	1,504	34.2
Unstaged	13,246	3,484	16,328	4,294	4,185	1,101	32,780	8,621	26.3
Total	19,770	5,667	24,370	6,985	6,246	1,790	48,925	14,024	28.7

**Excluding the former USSR*

Note: Brain and nervous system cancer includes ICD-9 Code 191

Sources: NIH, SEER Cancer Statistics Review 1995:104-111; CA:A Cancer Journal for Clinicians 1997; 47(1):8-9; Parkin DM, etal, Cancer Incidence in Five Continents, Vol. VI. IARC Scientific Publication 120.

in women with a genetic predilection is prophylactic removal of breast tissue. To determine the efficacy of this option, women with and without a family history of breast cancer who underwent prophylactic mastectomies were followed for 17 years to determine how many went on to develop cancer. Among those having the procedure, fewer than 10% of the statistically expected cases of breast cancer occurred over the trial period whether or not there was a family history. Thus, the risk of developing breast cancer can be reduced by this approach, even in the case of cancer-prone family history.

Sporadic Cancers

While there is no question that BRCA1 and 2 are involved in familial cases of breast cancer, it is not yet clear whether the genes are also involved in sporadic cases. The types of mutations found in hereditary cases do not occur sporadically. While the biology of spontaneous and inherited tumors does not differ that much, there are several phenotypes associated with different mutations. Some mutations lie in the genes of functional partners in BRCA1 gene expression rather than in the BRCA1 gene itself.

Loss of expression of BRCA1 occurs in sporadic tumors. Half of breast cancers lose their ability to express this gene, while in one-quarter there is only partial loss. Loss of BRCA1 expression also occurs in ovarian cancer, and may be an indicator of disease stage. When BRCA1 expression was analyzed in ovarian tissues of varying degrees of malignancy, a correlation was found. While there was full expression in epithelial cells of the ovary, in benign cysts one-third failed to express BRCA1, one-third expressed reduced levels and another third expressed normal levels. In borderline ovarian carcinoma 40% showed partial expression and only 10% expressed normal levels of BRCA1. In frank carcinoma the numbers dropped to 10% for partial expression and 5% normal levels. Therefore, BRCA1 levels should be a useful indicator in diagnosing and monitoring progression of ovarian cancer.

Gene Therapy

Vectors have been designed to deliver the wild type BRCA1 gene in a retroviral vector, and clinical trials are now underway in breast and ovarian cancer. In some cases there has been a complete inhibition of tumor growth without any adverse effects on lung, colon or fibroblast growth. The original vector was inadvertently constructed with a stop codon at nucleotide 1835. Surprisingly, it was clinically efficacious for treatment of ovarian but not breast cancer. The genetic construct was a splice variant of exon 5-6 that contained a 22 bp deletion of a portion of exon 5, resulting in a stop codon, followed by a start codon in another reading frame which resulted in an inframe correction. So the protein product is a functional deletion with an N-terminus that is shortened by 8 kDa. Its properties are virtually identical to wild type BRCA1 protein.

When cells in culture are transfected with the BRCA1 gene, the culture medium from the growth of these cells is effective in inhibiting growth of ovarian cancer cells. The conditioned medium from cells transfected with wild type BRCA1, inhibits ovarian cancer cell growth, while that of a mutant BRCA 1 does not. It is unclear whether this effect is attributable to BRCA1 itself, or to something BRCA1 stimulates that is excreted into the medium. The protein does have a region of homology to secreted proteins. If it is a secreted protein, it is unlikely to be a tumor suppressor because it would be capable of affecting neighboring cells.

A phase I trial of BRCA1 gene therapy has recently been completed in which the LXSN-BRCA1 vector was delivered intraperitoneally by a small shunt. Twelve women with late stage ovarian cancer were recruited, most of whom had a family history of breast or ovarian cancer. The objective was to determine whether there was uptake and expression of the vector in malignant ovarian cancer cells and to determine the most efficacious vector dose. Vector expression was seen in 5-10% of the peritoneal cells. The tumors of four of the women pro-

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gressed, temporarily stabilized in seven and regressed in one, although this is not expected to be permanent. In a second look laparotomy and two resurgeries, there was no evidence of small tumor lesions, but there seemed to be little effect on the larger lesions. In the next planned trial women will be recruited with earlier-stage disease in the hope that this therapy will be effective for small early tumors.

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