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Neuroblastoma Cell Lines

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Historical Introduction

Neuroblastoma accounts for approximately 9% of all childhood cancers, occurring once out of 8,000 live births. This results in an annual incidence of approximately 1 in 10^5 children less than 15 years of age world-wide (1). The median age at diagnosis is approximately 22 months with over one-third diagnosed at less than 1 year of age and over 88% diagnosed by the age of 5. Some studies indicate a bimodal age distribution with one peak at approximately 1 year and the second between 2 and 4 years (2).

In children under 5 years of age, neuroblastoma usually presents in the abdominal region involving the sympathetic ganglia of the paraspinal region or the adrenal gland. In infants under a year of age there is a higher incidence of tumors in the thoracic region. In Stage I and II neuroblastoma where tumor is confined to the originating organ or surrounding tissue, the prognosis is quite favorable. However in Stage III and IV NB where tumor extends beyond the midline, is metastatic or involves bony lesions the prognosis for patients is quite poor. It is possible that early stage disease (Stage I,II, IVS) is a distinct entity from late stage (Stage III, IV) disease because their response rate to therapy and their molecular genetic and biologic characteristics are distinct.

Of all human tumors Neuroblastomas have one of the highest rates of spontaneous tumor regression. This is primarily due to an unusual presentation of neuroblastoma called Stage IVS that occurs in infants under a year of age who present with widely disseminated disease that typically resolves with minimal therapy. Stage IVS tumors have intrigued scientists studying neuroblastoma who feel that an understanding of this disease entity will provide clues to the more devastating presentations of neuroblastoma.

A report of familial NB which affected 4 of 5 siblings lead Knudson and Meadows to formulate a "Mutation Model for Neuroblastoma" in 1976 (3). This model proposed that all NB derive from a single cell that is transformed from a normal cell by two mutations; one that may arise prezygotically in the germline and the second that arises in a somatic cell of the target tissue. This "two-hit" model of tumor development was hypothesized to describe the genetics of retinoblastoma tumors (4) and subsequently was verified with the identification of *Rb*, the retinoblastoma tumor suppressor gene. While the probability of tumor occurrence would be high in familial or germline cases of neuroblastoma, the actual incidence is quite low. This low incidence may be due to the fact that the elaboration of both mutations causes an embryonic lethal condition or that there is a failure to detect affected parents because they have occult tumors that spontaneously regress or benign tumor forms such as ganglioneuromas. (5) (6). The majority of neuroblastoma arise sporadically and as such germline mutations would not occur and both mutations would arise somatically. Neuroblastomas occur early in life and the age associated incidence suggests that the target cell may differentiate or does not persist into adult life. For sporadic neuroblastomas to occur, both mutations must occur in a cell before it fully differentiates.

Neuroblastoma Cell Culture

Initially the short term <u>in vitro</u> culture of neuroblastoma tumors was used as a tool for diagnosis by Murray and Stout (7) who found that explants of

tumors grown in plasma-clot cultures readily elaborated axons. The long term culture of neuroblastoma tumors and the differentiated properties these cells express in culture have fascinated investigators (8) who study these cultures in order to understand the propensity of neuroblastoma cells to differentiate in vivo and in vitro.

Tumor tissue can be obtained from primary tumor samples obtained from surgical resections, fine needle and bone marrow aspirates and occasionally from peripheral blood. Typically samples of solid tumor are placed in culture media (Dulbecco's Modified Eagles Medium[DMEM] +10mM HEPES buffer or RPMI-1640) containing from 3-15% fetal calf serum, 100 IU/ml penicillin and 100ug/ml streptomycin. Tumor tissue is minced with scissors or a scalpel and may be filtered through a fine wire mesh under sterile conditions (TD; tissue dispersion) and cultured in complete media at 37ºC in a humidified atmosphere containing 5% CO_2. Frequently fibroblasts grow out of these preparations, however, if the tumor sample is cultured on extracellular matrix components such as laminin or collagen the growth of fibroblasts may be reduced. Bone marrow samples should be aspirated into a preservative free-heparinized syringe. Techniques for the isolation of the tumor cells from marrow cells have varied from direct culture (C) to density gradient centrifugation (DG). In the direct culture method, bone marrow is diluted into media and red blood cells are washed off during in vitro culture. Red blood cells may be separated from tumor cells by placing the diluted bone marrow sample on a ficoll-hypaque gradient (density 1.077g/l) and centrifuging for 30 min at 500xg at room temperature. The interface layer containing the tumor cells and normal bone marrow cells is collected and washed several times before its cultured in complete media.

Cultures should be observed several times a week over the first few weeks and the media replenished every few days. Many neuroblastoma tumor cells will attach to the culture dish, however tumor spheroids may remain in suspension thus the spent media may contain non-adherant tumor cells and should be centrifuged and the pelleted cells re-cultured in a separate flask. After washing detached cells in media, cells may be resuspended in fresh media in multiple or larger tissue culture flasks. The use of antibiotics minimizes the possibility of microbial contamination during the initial phase of culture, however long-term use in established cultures can lead to occult contamination with mycoplasm.

Primary neuroblastoma cells in culture may be small, rounded or tear shaped cells with a relatively immature appearance although cells frequently elaborate long neurite-like processes. NB cells may grow in cell aggregates that are only loosely substrate adherent. The morphological appearance of established neuroblastoma cell lines varies (see Biological features) with cells having a neuroblastic, intermediate and substrate-adherant or flat morphology (9, 10). Cultures may be homogeneous or heterogeneous for these cell types.

Reports of the adaptation of neuroblastoma cell lines to serum-free conditions in the absence of additional growth factors have been infrequent. The studies of El-Badry et al (11) revealed that the SK-N-AS cell line adapted to serum-free conditions because it constitutively expressed IGF-2, whereas other NB cell lines required IGF-2 to grow in serum-free conditions. Table 1 lists the clinical features from which over 100 neuroblastoma cell lines have been derived.

Cytogenetics

The most widely characterized cytogenetic alterations in neuroblastoma tumors include the loss or rearrangement of the distal portion of the short arm of chromosome 1 (1p31-term) (12) (13) and amplification of the N-myc gene (14). These cytogenetic features are most commonly found in advanced stage tumors and most neuroblastoma cell lines are derived from advanced stage tumors (Table 2 and 2A).

Alterations and deletions of chromosome 1 are found in many neural crest tumors such as melanoma, neuroepitheliomas, pheochromocytoma, and other tumors including breast cancer, Wilm's tumors and colon carcinoma. This implies that several putative tumor suppressor genes are located on chromosome 1p. In Neuroblastoma, chromosome 1p deletions are the most consistent nonrandom genetic alteration and results in chromosome 1p monosomy (12). The deletion is variable in neuroblastoma but consistently encompassing 1p36.1 to 1pter. At least two putative suppressor genes have been proposed to reside in this region as deletions can be grouped into those whose deletion encompasses 1p36.3 and/or 1p36.1(15, 16). Recently a candidate tumor suppressor gene p73 has been identified that maps to 1p36.33 and is deleted in the cell line (SK-N-AS) that contains the smallest 1p interstitial deletion described to date. The p73 gene is a compelling candidate NB tumor suppressor gene as it has structural and functional homologies to p53, a common tumor suppressor gene that is not typically altered in NB tumors or cell lines. Transfection of p73 into the SK-N-AS cell line suppresses its tumorigenic phenotype. Many NB cell lines such as CHP-212, SMS-KAN and SK-N-BE(2) are monoallelic for p73 and express very low levels of p73 mRNA and no protein for p73. It is not known if the failure to express p73 is due to imprinting of the remaining p73 allele. Some neuroblastomas such as IMR-32 however do express p73 (17).

MYCN maps to 2p23-23 and is the common gene contained in the double minute chromosomes (DMs) and homogeneous staining regions (HSRs) that characterize a subset of NB tumors that have a particularly poor prognosis(18). The size of the amplicon varies and other genes in found in the amplicon include ornithine decarboxylase, and DDX, a DEAD box protein encoding gene (19). The relationship between DMs and HSRs is not well understood, although there exist in the CHP126 cell line two populations of cells, some which contain HSRs and some that contain a variable number of DMs. This lead to the hypothesis that DMs were derived from HSRs and were different manifestations of the same underlying genetic alteration (20). The variable number of DMs in tumor cells results from the varying distribution of DMs during cytokinesis. A recent report suggests that only 1 of the possibly 2 tumor suppressor genes localized to 1p is associated with amplification of N-myc (21).

A recent study using comparative genomic hybridization identified that chromosome 17 gain occurred in a high percentage of cases, in Stage 1 and 2 and even 4S suggesting that alterations in this region may be one of the early events involved in NB tumorigenesis (22). Chromosome 17 has tumor suppressor activity in NB cells (23) and mutations and amplification of nm23, a gene cells on chromosome 17q21 that has a metastases suppressing ability on some tumor cell types are detected in poor prognosis patients (24).

Frequently rearrangements on chromosomes 11, 14 and 17 have been noted; 12 of 37 (32%) informative cases contain LOH involving 11q13-11q23 or 11p and 6 of 27 (22%) informative cases contain LOH on 14q (25) while in a

sample of 35 NB cell lines 23% had alterations of 17q (26). Typically these genetic alterations have been detected in advance stage neuroblastomas and derived cell lines and rarely in tumors from low stage disease.

Alterations or mutations in many noted tumor suppressor genes such as p53 (27), RB, and p16, p18, p27 (CDKN2)(28) and RET (29) have rarely been identified. Occasionally deletions in NF1 have been noted in neuroblastoma cell lines (3/17 analyzed)(30) The prevalence of LOH or mutations in NF1 has not been thoroughly analyzed although there is a recent report of a neuroblastoma patient with a homozygous deletion in NF1(31) and translocations and alterations of the region near NF1 on 17q have been described (32)

Although the oncogenic form of N-ras was initially isolated from the SK-N-SH neuroblastoma cell line, mutated N-ras genes are not commonly detected in human neuroblastoma tumors (1/15)(33) and mutations in N-ras, Ki-ras and H-ras were not detected in another series (0/24). Furthermore, it was noted that earlier passages and some sublines of SK-N-SH didn't have a mutated N-ras gene. Although a mutation at codon 59 in N-ras was detected in a subline of SK-N-SH, it was different from the codon 61 mutation detected in the original transforming N-ras. This suggests that the mutation in N-ras was not present in the primary tumor and was probably acquired during <u>in vitro</u> culture (34).

Histopathology

The cells from which neuroblastomas are thought to arise are the postganglionic sympathetic neuroblasts of the embryonal neural crest. The neural crest is a transitory structure that arises during the closure of the neural tube. Neural crest cells migrate ventrally and laterally to contribute to a variety of tissues including the peripheral nervous system, medullary cells of the adrenal gland, calcitonin producing cells of thyroid, pigmented cells, and mesectodermal derivatives. Growth and differentiation specific peptides encountered by migratory neural crest cells are thought to influence their development and lineage specific differentiation.

The histopathologic appearance of neuroblastoma represents a spectrum from sheets of monomorphic undifferentiated, small, round blue cells to nests of neuroblasts surrounded by fibrillar bundles to differentiated ganglionic cells. These histologic subtypes correspond to neuroblastoma, ganglioneuroblastoma and ganglioneuroma, respectively. Neuroblastoma is a small, round blue cell tumor characterized by the presence of neuritic processes, neuropil and/or Homer-Wright rosettes which are neuroblasts surrounded by eosinophilic neuropil. Immunohistochemical stains for neurofilaments, synaptophysin and neuron-specific enolase have been utilized to distinguish it from other small round blue cell tumors of childhood. Ganglioneuroblastoma is a heterogeneous group in which tumors contain the spectrum of immature to fully differentiated cells and some stromal component. Ganglioneuroma is composed of mature ganglion cells, neuropil and Schwann cells. Recent evidence indicates that the Schwannian component of these tumors represents an infiltrate of normal cells rather than the differentiation of the tumor cells into Schwannian cells (35). Shimada and colleagues have developed a classification system based on histopathologic features and age which has prognostic utility (36). In this system the presence or absence of Schwann cell stroma; the degree of differentiation and the mitosis-karyorrhexis index (MKI) were considered in addition to age at

diagnosis. Favorable histology associated with a good prognosis is: stroma rich without a nodular pattern in any age group; or stroma poor histology in ages 1.5-5years of age with a MKI < 100; and in ages <1.5 with a MKI of <200. Histology associated with an unfavorable prognosis is: stroma rich with a nodular pattern in any age group; stroma poor over the age of 5; or histology with differentiation(1.5-5 years) or with MKI>100 (1.5-5years) or MKI>200 (<1.5years). A simplified histopathologic study by Joshi et al (37) found that calcification and low mitotic rate (<10 mitosis/10 high powered fields) predicted a favorable outlook independent of age and stage. Current efforts are underway to develop a unified international neuroblastoma classification system.

Table 3 highlights the pathologic characteristics of a number of neuroblastoma cell lines. The pattern of immunoreactivity was utilized for a time to distinguish neuroblastoma from other small round blue cell tumors. Neuroblastomas and cell lines typically stain poorly for Class I Major Histocompatibility antigens, are usually positive for neuron-specific enolase and a monoclonal anibody named HSAN1.2 ((38). Neuroblastomas form tumors in nude mice although the number of tumor cells required for tumor formation is high (typically 10^8 cells) and the latency for tumor formation is long(several months) (39). A neuroblastoma tumor model in SCID (severe combined immunodeficient disease) mice has been developed (40). Both the SCID and nude mice models study the biologic behavior of tumors in a complex system although the severely impaired immune systems of these animals obviates studies involved with the immune regulation of neuroblastoma cell growth. Other animal models include a neuroblastoma model developed in transgenic animals using the tyrosine hydroxylase promoter to selectively express N-myc in neural cells during development (41) and a transgenic mouse model using the middle T antigen of polyoma virus (42). Although it is too early to determine if the biology of these tumors is similar to the human neuroblastomas, the utility of these transgenic models is the presence of an intact immune system.

Biologic Features

Several lines of evidence suggest that neuroblastoma cell lines, like their neural crest cell antecedents, express neuronal and/or neuroendocrine properties. One of the hallmarks of NB cells in culture is their spontaneous or induced elaboration of neuritic processes. Other neuronal properties include; the synthesis of neurotransmitter biosynthetic enzymes; expression of neurofilaments; opioid, muscarinic and neurotrophin receptors expression; dense core granules presumed sites of catecholamine storage; immunoreactivity to neuron specific enolase. Neuroblastoma cell lines tend to have a adrenergic phenotype producing relatively high levels of tyrosine hydroxylase and dopamine-ß-hydroxylase while peripheral neuroepitheliomas or Ewing's cell lines tend to produce choline aceltyltransferase (43). There are few lineage specific markers discriminating neuronal from neuroendocrine cell types.

Many established neuroblastoma cell lines contain at least 3 morphological variants that contribute to the heterogeneity in these cell lines; neuroblastic (N), flat or substrate adherant (S) and intermediate (I) cell types (10, 44, 45). Morphologic subtypes from a number of neuroblastoma cell lines have been cloned (Table 4) and also can be distinguished by characteristic biochemical markers. Some clonal populations have the capacity to spontaneously

interconvert or transdifferentiate from one morphologic type to another. It is thought that this heterogeneity may reflect their derivation from multi-potent neural crest precursors. N-type and I-type cells express neurofilament proteins while I-type and S-type cells are more strongly positive for vimentin than N-type cells. S-type cells synthesize collagen and fibronectin similar to Schwannian cells. Most S-type cells do not synthesize readily detected levels of tyrosine hydroxlyase or dopamine-ß-hydroxylase although some produce tyrosinase. A study of cell surface antigen expression indicated that the S-type cells shared antigenic characteristics more in common with a fibroblast like meningeal cell rather than a Schwannian cell ((46, 47). Neural crest cells can give rise to ectomesenchyme, including skeletal and connective tissues of the head and face which also includes meninges. These features have led to a model in which N-type cells are proposed to resemble embryonic sympathoblasts, S-type cells resemble Schwannian, glial or melanocytic progenitor cells or ectomesenchymal derivatives and the I-type cells have an intermediate phenotype and the potential to differentiate to N- or S-type cells (45).

An interesting characteristic of neuroblastoma tumors is that even in advance stage disease the primary tumors are sensitive to chemotherapy although clinical progression inevitably occurs. A number of investigators have isolated neuroblastoma cell line pairs from primary tumor tissues prior to treatment and from samples taken at relapse after intensive chemotherapy or radiotherapy. These include pairs such as SK-N-BE(1) and SK-N-BE(2) (48); SMS-KCN and SMS-KCNR(49); SMS-KAN and SMS-KANR(49); CLB-BerLud1 and CLB-BerLud2(50); and NB69 and NB69(2). Many of these cell lines have been studied to examine tumor cell genetics and biology in the setting of progressive disease. For example, increased expression of N-myc was detected in cell lines from patients with progressive disease (51). However, it has been difficult to distinguish potential treatment related changes from innate tumor cell heterogeneity. A number of NB cell lines have been cultured in cytotoxic drugs to generate drug-resistant cell lines <u>in vitro</u> (52). These drug resistant cell lines have been valuable in studying mechanisms of drug-resistance yet it is unclear how these <u>in vitro</u> generated cell lines reflect the pathologic processes that occur in patients with progressive disease.

A number of cell lines have also been developed from tumors located at different sites; KP-N-SI(LA) from lymph node and KP-N-SI(FA) from bone(53); CHP126 from a primary retroperitoneal tumor and CHP126(BL) from the peripheral blood post therapy(54); CLB-Ge1 from bone marrow and CLB-Ge2 from lymph node; CLB-Ma1 from primary tumor tissue and CLB-Ma2 from bone marrow (50) and NB69(2) lung, NB69(2) liver and NB69(2)sternum (55). In the case of NB69(2) the cell lines from the metastatic sites after therapy essentially contained identical karyotypes and cytogenetic alterations that were both common and distinct from the cytogenetic alterations observed in the cell line derived from the primary tumor ((55). In another study, using a panel of nine monclonal antibodies the immunophenotype of the parental and 2 cell lines from different metastatic sites was stable and relatively uniform although growth rates differed (53).

The ability of many neuroblastoma cell lines to differentiate in response to a variety of biologic response modifiers has lead to the use of neuroblastoma cell lines as model systems to study neuronal and neuroendocrine cell development

as well as regulation of catecholamine biosynthesis. Oncologists have searched for agents that induced terminal differentiation which may have less or different toxicities from conventional cytotoxic agents. Table 5 lists a number of agents that induce reduce cell growth and induce differentiation or even apoptosis in selected neuroblastoma cell lines. Phenylacetate and retinoids (all-trans-retinoic acid, 13-cis retinoic acid and 9-cis retinoic acid) are or have been in Phase I trials as single agents or in combination with Interferonα (all trans-retinoic acid).

Retinoids induce marked neuronal differentiation and arrest of cell growth in some neuroblastoma cell lines (i.e.. LA-N-5, LA-N-1, SMS-KCNR) (56, 57) but have little effect on others (i.e. SK-N-AS, SH-EP)(58). Neuroblastoma cells constitutively express RARα; RARγ RARβ and RXRs although the levels of RARβ are reduced compared to RARα and RARγ(59). Treatment with nanomolar concentrations of retinoids is sufficient to increase RARß expression and induce neurite extension, however micromolar concentrations are required to significantly inhibit cell growth under standard culture conditions (60). Using receptor selective agonists evidence indicates that both RAR and RXR are required for maximal effects on growth and differentiation. Differentiation includes elaboration of extensive neuritic processes that are ultrastructurally and electrophysiologically similar to normal neurons, decreases in a number of proto-oncogenes N-myc(61), c-myb(61), B-myb genes and increases in TrkB, and RET receptors and their protein kinase activity. The increase in TrkB and RET kinase activity have been shown to induce neurites in NB cells (62) (63).

The ability of many of the biologic response modifiers to induce of apoptosis or cell death in NB cell lines has only been recently appreciated. While a prominent effect of retinoids is to induce neuronal differentiation, RA has been shown to induce apoptosis in a subset of cells by inducing tissue transglutaminase in SK-N-BE2(C) cells (64, 65). HPR, fennretamide has been shown to induce apoptosis in neuroblastoma cells(66). Interferonγ has been shown in a number of cell lines to induce cell death in a subpopulation of cells within 24-36 hours after treatment although the mechanism is unknown (67).

A number of genetically modified Neuroblastoma cell lines have been generated (partially listed in Table 6) that are valuable tools with which investigators can probe the role of particular genes that may be important in the biology of neuroblastoma. The amplification of N-myc has been proposed to be a progression related event as it occurs in only a subset of NB tumors. By constitutively expressing a vector containing anti-sense N-myc and thus decreasing cellular N-myc levels, the growth (68) as well as the invasiveness of NB cells decreased(69). A limitation of genetically modified cell lines constitutively expressing the gene of interest is clonal heterogeneity, which may be problematic depending on the biologic or biochemical trait being assessed. The use of regulated expression vector systems or retroviral systems with a high transfection efficiency enables assessment without the potentially confounding issue of clonal variability. Using a tet-regulated N-myc expression vector, it was recently shown that increased N-myc expression increases cell growth by decreasing the time cells transit through the cell cycle (70), thus confirming studies that indicated a role for N-myc in cell growth. The use of genetically modified cells has also shown that activation of TrkA (71), TrkB (62) or RET (63)

signal transduction paths are capable of inducing differentiation in neuroblastoma cells.

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TABLE 1: Clinical Features

	CELL LINE	PATIENT yr.mo/sex	PRIMARY SITE	METASTATIC SITE	ORIGIN	TREAT	CULTURE	STAGE	REF.
1	IMR32	1.1/M	abdom.	unk.	abdom.	none	TD	unk	(72)
2	SK-N-SH	4/F	thorax	BM	BM	+	C	4	(9)
3	SK-N-BE(1)	1.8/M	unk.	BM	BM	none	C	4	(73)
4	SK-N-BE(2)	2.2/M	unk.	BM	BM	+	C	4	(48)
5	SMS-KAN	3/F	pelvic	BM, LN	pelvic	none	TD	4	(49)
6	SMS-KANR	3.8/F	pelvic	BM	BM	+	DG	4	"
7	SMS-KCN	0.11/M	adrenal	LN, bone, BM	adrenal	none	TD	4	"
8	SMS-KCNR	1.2/M	adrenal	BM	BM	+	DG	4	"
9	SMS-MSN	5/M	adrenal	BM	BM	none	DG	4	"
10	SMS-SAN	3/F	adrenal	BM	BM	none	DG	4	"
11	SMS-LHN	2/M	femur mass	BM, bone	femur	+	TD	4	(74)
12	HTLA230	0.11/M	unk	BM	BM	unk	NM	4	(75)
13	SJNB-1(EB)	2.6/M	adrenal	LN, mediast.	adrenal	+	TD	4	(76)
14	SJNB-2	4/F	unk.	BM	BM	none	DG		"
15	SJNB-3	0.10/F	adrenal	BM,bone	BM	unk.	DG	3	"
16	SJNB-4(SD)	1/M	adrenal	BM, bone	BM	+	DG	4	"
17	SJNB-5	2/F	adrenal	BM, bone	BM	unk.	DG	3	"
18	SJNB-6	unk/M	unk.	BM	BM	unk.	DG	4	"
19	SJNB-7	0.07/M	adrenal	testicle	adrenal	unk.	TD	3	(76)
20	SJNB-9	5/F	adrenal	BM	BM	none	TD	3	"
21	SJNB-10	2/M	adrenal	liver	liver	unk.	TD	3	"
22	SJNB-12	0.10/F	kidney	LN	abdomen	unk	TD	3	"
23	SJNB-13	1/M	adrenal	pleural, LN	LN	unk.	TD	3	"
24	SJNB-14	1/F	adrenal	BM, bone	BM	unk.	DG	3	"
25	SJNB-16	5/F	unk.	BM, optic nerve, bone	BM	unk.	DG	3	"
26	SJNB-17	1/F	adrenal	LN,BM bone	BM	unk.	DG	3	"
27	KP-N-RT-BM	1.2/F	adrenal	BM, bone	BM	unk.	DG	4	(53)
28	KP-N-RT-LN	1.2/F	adrenal	BM,LN, bone	LN	unk	TD	4	"
29	KPNRT-BMV	1.2/F	adrenal	BM, bone	BM	unk.	DG	4	(77)
30	KP-N-SI(LA)	5/M	adrenal	LN, bone	LN	unk.	TD	4	(78)
31	KP-N-SI(FA)	5/M	adrenal	LN, bone	bone	unk.	TD	4	(79)
32	KP-N-YN	2/M	adrenal	LN	LN	unk.	TD	3	(78)
33	KP-N-AY	2.6/F	adrenal	LN, BM	BM	none	DG	4	(80)
34	KP-N-AYR	2.6/F	adrenal	LN , BM	BM	+	DG	4	"
35	GI-ME-N	2/F	adrenal	LN, BM	BM	+	DG	4	(81)
36	GI-LI-N	1.11/F	adrenal	BM	P.B.	unk.	DG	4	(82)
37	GI-LA-N	2.3/M	adrenal	LN	LN	+	TD	3	(83)
38	GI-CA-N	0.9/F	adrenal	BM	BM		DG	4	(82)
39	IGR-N-835	2/F	adrenal	BM, bone	adrenal	+	TD	4	(84)
40	IGR-N-91	8/M	adrenal	BM, bone	BM	+	DG	4	(85)
41	CA-2-E	1/M	abdomen	BM, bone	BM	+	DG	4	(86)
42	WSN	2.9/F	abdomen	-	abdomen	-	TD	3	(87)
43	MSN	1.2/F	abdomen	BM	BM	-	DG	4	"
44	ACN	3.3/M	abdomen	BM, bone	BM	+	DG	4	(88)
45	POG-1382.2	3.5/F	retroperit.	unk.	retroper.	+	TD	3	P. Houghton, pc
46	POG-1771	2.10/M	adrenal	unk.	adrenal		TD	4	"
47	POG-1643	1.7/M	retroperit.	unk.	retroper.	none	TD	4	"
48	POG-1691	1.9/M	retroperit.	unk.	retroper.	+	TD	4	"

49	RN-GA	1.8/F	adrenal	LN	adrenal	unk.	TD	3	(89)
50	NBL-S	3.6/M	adrenal	none	adrenal	none	TD	3	(90)
51	NBL-W	0.6/M	adrenal	liver	adrenal	none	TD	4S	(91)
52	NUB-7	0.7/M	adrenal	BM-LN	LN		TD	4S/4	(92)
53	CLB-Pe	0.6/F	adrenal	none	adrenal	+	DG	1	(50)
54	CLB-Ge1	1.6/M	retroperit	LN, bone	BM	unk.	DG	4	"
55	CLB-Ge2	1.6/M	retroperit	LN, bone	LN	unk.	TD	4	"
56	CLB-Be	1.6/M	adrenal	LN,BM	LN	+	TD	3	"
57	CLB-Tr	1.3/F	adrenal	liver, bone	BM	+	DG	4	"
58	CLB-Ma1	0.9/F	abdomen	BM	abdomen	none	TD	4	"
59	CLB-Ma2	0.9/F	abdomen	BM	BM	none	DG	4	"
60	CLB-Es	1/M	adrenal	LN, bone	BM	none	DG	4	"
61	CLB-Bac	3.2/M	adrenal	BM, liver, bone	adrenal	+	TD	4	"
62	CLB-BerLud1	5.5/M	adrenal	BM,bone	BM	none	DG	4	"
63	CLB-BerLud2	5.5/M	adrenal	BM,bone	BM	+	DG	4	"
64	CLB-Br	3.7/F	retroperit	BM,LN,bone	BM	none	DG	4	"
65	CLB-Ca	11.4/F	adrenal	BM,bone	BM	+	DG	4	"
66	CLB-Ba	2.3/M	adrenal	BM,LN,bone	BM	none	DG	4	"
67	CLB-Ga	4/M	retroperit	BM,LN,bone mediastinum	BM	-	DG	4	"
68	CLB-Re	4.6/M	adrenal	BM, bone, LN	BM	+	DG	4	"
69	LA-N-1	2/M	unk.	BM	BM	+	TC	4	(93)
70	LA-N-2	3/F	abdominal	unk.	abdominal	-	TD	4	"
71	LA-N-5	0.4/M	unk.	BM	BM	unk	DG	unk.	R. Seeger, pc
72	LAN-6	5.8/M	adrenal	BM,bone	BM	+	DG	4	(74)
73	CHP-126	1.2/F	retroperit.	-	retroper.	none	TD	3/4	(94)
74	CHP-126B1	1.9/F	"-	-	PB	+	plasma	unk	(54)
75	CHP-134	1.1/M	adrenal	LN	LN	+	TD	4	(94)
76	NGP	2.6/M	unk.	BM, lung	lung	+	TD	unk	(95)
77	NMB-7	0.10/F	unk.	BM	BM	+	DG	unk	"
78	NB9	1.10/M	adrenal		adrenal	none	TD	4	"
79	NB16	2.11/F	unk	BM	BM	none	DG	4	"
80	NB19	1/F	unk	BM	BM	+	DG	4	"
81	NB56	2/M	adrenal	BM pleural effusion	adrenal	+	TD	4	"
82	NB69(CHP270)	1.4/M	adrenal	ascites	adrenal	-	TD	3	"
83	NB69(2)	2.3/M	adrenal	lung liver sternum	lung liver sternum	+	TD	3	"
84	NB76	3/M	adrenal	ascites	ascites	-	TD	3	"
85	NLF	3/M	abdomen	no	abdomen	none	TD	3	(14)
86	CHP-166	2.6/M	unk	BM	BM	-	DG	unk	(26)
87	CHP234	3/F	unk	BM	BM	none	DG	4	(96)
88	CHP212	1.8/M	kidney mass	-	kidney mass	none	TD	unk	(94)
89	NAP	unk /F	unk.	BM	BM	+	DG	unk	(26)
90	NJB	2.9/F	unk.	LN	LN	unk.	TD	unk -	(95)
91	NLB	2.3/F	abdomen	-	abdomen	unk.	TD	unk	"
92	SJ-N-KP	5/?	unk	BM	BM	unk	DG	unk	(97)
93	SJ-N-CG(NCG)	0.6/M	unk.	BM	unk.	unk.	DG	4	"
94	VA-N-BR	6/M	abdomen	serosal, liver, pelvic	serosal	+	TD	unk	(98)
95	MMH	3/M	unk.	BM	BM	+	DG	unk	(26)
96	SK-N-AS	8/F	adrenal	BM	BM	+	C	4	L. Helson, pc
97	CHP 901	2/M	unk.	BM	BM	unk.	DG	4	A. Evans, pc
98	NB-19	0.10/M	adrenal	liver, BM	BM	unk.	DG	4	(26)

99	CHP-903	3/F	adrenal	BM	BM	unk	DG	4	A. Evans, pc
100	PER 106	1.5/M	suprarenal	BM	BM	none	DG	4	(99)
101	PER 107	2.6/M	suprarenal	BM	BM	none	DG	4	"
102	PER 108	2.9/M	suprarenal	BM	BM	+	DG	4	"
103	STA-NB-1.1	3.6/M	adrenal	-	adrenal	unk	TD	3	P. Ambros, pc
104	STA-NB-1.2	3.9/M	adrenal	-	adrenal	none	TD	3	(132)
105	STA-NB-2	1.6/M	adrenal	BM	adrenal	none	TD	4	"
106	STA-NB-3	1.8/F	adrenal	-	adrenal	none	TD	2	"
107	STA-NB-4	1.5/M	adrenal	BM	adrenal	none	TD	4	"
108	STA-NB-5	0.6/M	adrenal	BM, liver, LN	adrenal	none	TD	4S/4	"
109	STA-NB-6	2.1/N	retroperitoneum	-	retroperit-oneum	none	TD	3	"
110	STA-NB-7	1.7/M	adrenal	-	adrenal	none	TD	3	"
111	STA-NB-8	2.3/F	adrenal	BM, bone	BM	none	DG	4	"
112	STA-NB-9	0.3/F	adrenal	BM, liver	liver	none	TD	4	"
113	STA-NB-11	-/M	adrenal	BM	adrenal	+	TD	4	"

BM=bone marrow;TD=tissue dispersion;C=direct culture;DG=density gradient;LN=lymph node;PB=peripheral blood; NM=nude mouse;unk= unknown, pc=personal communication

Table 2: Common Chromosomal Alterations in Neuroblastoma Cell Lines

	CELL LINE	1p alteration		N-myc amplification			N-myc single copy	CHROMOSOME 17	REF
		1pdel	t(1p)		HSR	DM			
1	IMR32	+	-	+		+			(13, 97)
2	SK-N-SH	-	-	-			+		(26, 97)
3	SK-N-BE(1)	+	-	+		+			(97)
4	SK-N-BE(2)	+	+	+	+			t(3;17)(p21;q21)	(26)
5	SMS-KAN	+		+		+		del(17),t(17;18)	(49)
6	SMS-KANR	+		+		+			"
7	SMS-KCN	+		+		+		t(17;20)(q21;q13)	"
8	SMS-KCNR	+		+		+		t(17;20)(q21;q13)	"
9	SMS-MSN	-		+		+		i(17q)	"
10	SMS-SAN		+	+		+		t(11;17)(q24;q21)	"
11	SMS-LHN	-		-			+		(74)
12	HTLA-230	unk		+		+			
13	NB-1(EB)	+	+	-			+		(76)
14	NB-2	?	+	+		+		t(1;17)(p32;q21)	"
15	NB-3	-		-			+		"
16	NB-4(SD)	+	+	+	+				"
17	NB-5	+	+	+		+			"
18	NB-6	+		+					"
19	NB-7	+		+	+				"
20	NB-9	-		-			+		"
21	NB-10	+		+		+			"
22	NB-12	+		+		+			"
23	NB-13	+	+	+	+			t(1;17)(p31;q21)	"
24	NB-14	?		+	+				"
25	NB-16	+	+	-			+		"
26	NB-17	+		-			+		"
27	KP-N-RT	+		+	+	+			(53)
28	KP-N-SI(LA)	-	+	-			+		(78)
29	KP-N-SI(FA)	-	+	-			+		(79)
30	KP-N-YN	+		+	+	+			(78)
31	KP-N-AY	+		+		+		-17; der(17)	(80)
32	KP-N-AYR	+		+		+		i(17q)	"
33	GI-ME-N	+		-			+		(82)
34	GI-LI-N	+			+				"
35	GI-CA-N	-		-			+		"
36	IGR-N-835	-	+	-			+	t(11;17)(p11;q11)	(84)
37	IGR-N-91	+		+	+	+			"
38	CA-2-E	?		+					(86)
39	WSN	+		+		+			(87)
40	MSN	+		+	+	+			"
41	ACN	-		-			+	c-myc amplification	(88)
42	POG 1382.2	+		+					P. Houghton, pc
43	POG 1771	-	-	+				der(17) t(17;?)(p11;?)	"

44	POG 1643	+		+						"
45	POG 1691	-		+	+					"
46	RN-GA	+					+			(89)
47	NBL-S	-		-			+			(90)
48	NBL-W	+		+	+					(91)
49	NUB-7	+		+	+			17q$^+$		(92)
50	CLB-Pe	-		+	+					(50)
51	CLB-Ge1	+		+						"
52	CLB-Ge2	+		+						"
53	CLB-Be	+		+	+					"
54	CLB-Tr	+		+						"
55	CLB-Ma1	+		+	-	+				"
56	CLB-Ma2	+		+						"
57	CLB-Es	+		+						"
58	CLB-Bac	+		+						"
59	CLB-Ber Lud1	+		+						"
60	CLB-Ber Lud2	+		+						"
61	CLB-Br	+		+						"
62	CLB-Ca	+		+						"
63	CLB-Ba	+		+						"
64	CLB-Ga	+		-			+			"
65	CLB-Re	+		+						"
66	LA-N-1	+		+	+					(97)
67	LA-N-2	+		+		+				"
68	LA-N-5	+		+						
70	LAN-6	-		-			+			(74)
71	CHP-126	-	+	+	+	+				(13, 97)
72	CHP-134	+	+	+	+	-				"
73	NGP	+	+	+	+					(97)
74	NMB	+	+	+	+					(13)
75	NB9	+		+		+				"
76	NB16	-	+	+		+		17q$^+$		"
77	NB19	-	-	+	+					"
78	NB56	-	-	+	+					"
79	NB69	-	+	-	-	-	+			"
80	NB69(2)		+	-	-	-	+			"
81	NB76		+	+	+					"
82	CHP-166	+		+		+				(26)
83	NAP	-	-	+	+	+		+17		(26, 97)
84	SJ-N-CG(NCG)	+		+	+					(97)
85	MMH	+		+		+				(13)
86	SK-N-AS	+	-	-			+			(100)
87	VA-N-BR	+					+?			(98)
88	CHP-903	-	-	+						K. Kisselbach, pc
89	NLF	+		+	+					(97)
90	STA-NB-1.1	+		+						(132)
91	STA-NB-1,2	+		+						"
92	STA-NB-2	+		-			+	t(1;17)		"
	STA-NB-3	+		+						"

94	STA-NB-4	+	+				“
95	STA-NB-5	+	+				“
96	STA-NB-6	+	-		+	t(1;17)	“
97	STA-NB-7	+	+				“
98	STA-NB-8	+	+			t(1;17)	“
99	STA-NB-9	+	+			t(1;17)	“
100	STA-NB-11	+	+				

Table 2A: SUMMARY OF CELL LINES WITH SPECIFIC GENETIC ALTERATIONS

1p alteration			N-myc amplified		N-myc single copy	Chrom.17 alteration
1pdel	1pdel	t(1p)	HSR	DM		
IMR32	CLB-Ma(2)	SK-N-BE(2)	SK-N-BE(2)	IMR32	SK-N-SH	SMS-KAN
SK-N-BE(1)	CLB-Es	SMS-SAN	LAN-1	SK-N-BE(1)	NB-17	SMS-KCNR
SMS-KAN	CLB-Bac	NB-16	NB-19	SMS-KAN	NB-69	NB-16
SMS-KNCR	CLB-Ber-Lud1	NB-69	NB-56	SMS-KCNR	NB-1(EB)	SMS-KCN
LAN-1	CLB-Ber-Lud2	NB-76	SMS-KANR	SMS-MSN	NB-9	NB-15
LAN-2	CLB-Br	NB-1(EB)	NB-4 (SD)	NB-9	NB-16	KP-N-AY
NB-9	CLB-Ca	NB-2	NB-6	NB-16	KP-N-SI	KP-N-AYR
SMS-KCN	CLB-Ba	NB-5	NB-7	NB-76	KP-N-SI(FA)	KPNRT-BMVC6
SMS-KANR	CLB-Ga	NB-7	NB-8	SMS-KCN	RN-GA	KP-N-RT
NB-4(SD)	CLB-Re	NB-13	NB-14	SMS-SAN	GI-ME-N	IGR-N-835
NB-5	SMS-LHN	NB-14	NB-19	NB-2	GI-CA-N	POG-1771
NB-6	NB 9	NB-16	KP-N-RT	NB-3	IGR-N-835	NUB-7
NB-7	CHP-166	KP-N-SI	KPNRT-BMV-C6	NB-4	ACN	CLB-Ga
NB-8	MMH	KP-N-SI(FA)	GI-LI-N	NB-5	NBL-S	CLB-Ba
NB-9	NMB	KP-N-SI(LA)	CA-2-E*	NB-6	SMS-LHN	MMH
NB-12	SK-N-AS	KP-N-AYR	POG-1691	NB-9	LAN-6	SMS-MSN
NB-13	CLB-Ge1	NB-69(2)	NBL-W	NB-12	SK-N-AS	SK-N-BE(2)
NB-15	CLB-Ge2	NB-4(SD)	NUB-7	NB-15	CLB-G2	STA-NB-2
NB-16	CLB-Be	NBL-S	CLB-Be	HTLA-230	NB-3	STA-NB-6
NB-17	CLB-Tr	CLB-Ca	CLB-Pe	KP-N-RT	STA-NB-6	STA-NB-8
KP-N-RT	CLB-Ma(1)	CLB-Ga	CHP-126	WSN	STA-NB-2	STA-NB-9
KP-N-YN	NLF	CHP-126	CHP-134	MMH		
KP-N-AY	SJ-N-KP(NKP)	CHP-134	NGP	NMB		
RN-GA	SJ-N-CG(NCG)	NGP	NMB	CHP-126		
KPNAY-BMVC6	VA-N-Br		IGR-N-91	CHP-166		
GI-ME-N	NB-19		NB-13*	CLB-Ma(1)		
GI-LI-N			POG-1382.2*	CLB-Ma(2)*		
IGRN-91			POG-1771*	CLB-Es*		
WSN			POG-1643*	CLB-Bac*		
POG-1382.2			CLB-B2	CLB-Ber-Lud1*		
POG-1643			CLB-Ge2*	CLB-Ber-Lud2*		
NBL-W			CLB-Ge1*	CLB-Br*		
NUB-7			SJ-N-CG(NCG)	CLB-Ca*		
			CLB-Tr*	CLB-Re*		
			CHP-903	KP-N-AY		
			NLF	KP-N-AYR		
			STA-NB-9*	SJ-N-KP(NKP)		
			STA-NB-1.1*	LA-N-2		
			STA-NB-4*	STA-NB-7*		
			STA-NB-5*	STA-NB-11*		
			STA-NB-1.2*	STA-NB-3*		

* unkown if amplification is carried on DMs or HSRs

TABLE 3: PATHOLOGY

Cell line	Original Tumor	Xenograft
IGR-N-91-cultured on ECM; both epithelial and neuroblastic cells seem	tumor from BM; mIBG positive	immature NB metastases to LN, kidney
IGR-N-835-floating clusters of small round cells; anchored polygonal cells with few neurites	abdominal tumor tissue; typical immature NB; after chemotherapy	immature NB
HTLA-230- round to bi-polar morphology some with processes 2-3x cell soma	Unfavorable; stroma-poor histology; high MKI,	stroma poor histology; high mitosis-karyorrhexis index hemorragic EM-few processes rare granules
RN-GA-cells were mainly flat with few neurites, biopolar rectangular cells.	Undifferentiated neuroblastoma; epethlial-like tumor cell clusters EM- neural filaments & dense core granules.	Typical undifferentiated NB, poorly vascularized
GI-ME-N-extremely substrate adherant with an irregular polygonal shape and few long neuritic processes	undifferentiated, small round cell NB	short latency in nude mice; grew as undifferentiated, small, round cell NB
VA-N-BR-spindle shape with uni-or bi-polar cytoplasmic extensions	primitive NB, occasional fibrovascular stroma-; positive immunostain for neurofilament & chromogranin, vimentin and desmin	dense pattern of irregularly nucleated cells with moderate neovascularization; stains for desmin and vimentin but not neurofilament or chromogranin
SK-N-SH-round, tear shaped cells with "epithelial-like" or flat cells, dense core granules by EM	not available	tumors in cheek pounch of hamsters are small cells, compactly arranged with many mitotic figures.
SMS-KAN-loosely adherant, teardrop shaped cells that grow in clumpts with neuritic processe; epithelial-like cell component present	undifferentiated NB with poor pseudorossette formation; EM showed neurosecretory granules	NA
SMS-KANR- smaller cells with few neurites; no epithelial cell component	tumor cells from BM; post chemotherapy	NA
SMS-MSN- loosely adherant polygonal cells which after crisis had few neurites	tumor from BM; positive for cathecholamine fluorescence; EM revealed neurosecretory granules	NA
SMS-KCN- teardrop shaped cells with few neurites; epithelial cell component present	undifferentiated NB; EM showed neurosecretory granules	NA

SMS-KCNR- teardrop to polygonal shaped cells with few neurites; rare epithelial cells	tumor cells from BM after chemotherapy	NA
SMS-SAN-small round to teardrop shaped cells with some neurites	undifferentiated small round blue cell tumor originally diagnosed as embryonal rhabdomyosarcoma; tumor from BM had catecholamine fluorescence; EM- neurosecretory granules	NA
NBL-S- small tear-drop cells, some with short delicate neurite-like processes, weakly substrate adherant , aggregate growth	undifferentiated neuroblastoma with little stroma	NA
NBL-W- small tear-drop neuroblastic cells and substrate-adherant flat cells	undifferentiated neuroblastoma, favorable histology: stroma poor with a MKI=120	NA
PER-106 - suspension culture with large aggregates and few substate adherant cells **PER-107, PER-108**- derived after chemotherapy, adherant PER-107 has small round cells with few neuritic processes PER-108 have epithelial shape	unfavorable and stroma poor histology with a high mitosis-karyorrhexis index EM showed occasional neurosecretory granules, neuritic processes with microtubules	NA
KP-N-RT-small round cells loosely substrate adherant with few neuritic processes.	undifferentiated neuroblastoma; EM showed few dense core granules	NA
NUB-7- predominantly grows in tightly adherant colony formation; heterogeneous mophology of isolated cells predominantly I-type	nests of neuroblasts surrounded by fibrovascular stroma, numerous Homer-Wright rosettes, isolated areas with ganglioneuroblastoma	NA

Table 4: NB Cell Variants– Biologic Characteristics

Parental Cell line	Subline	Type	Enzymes	Colony Formation	Ref.
SKNSH	SH-SY5Y	N	TH, DβH	+	(10)
	SH-EP	S	–	–	"
	SH-EPI	S	Tyr	NT	"
	SH-EPIE	S	–	NT	"
	SH-IN	I	TH, DβH	+	(10)
LA-N-I	LAI-15n	N	TH	+	(10)
	LAI-19n	N	TH	+	"
	LAI-21n	N	TH	+	"
	LAI-5s	S	Tyr	–	"
	LAI-6s	S	ND	–	"
	LAI-22n/i	N/I	DβH	NT	"
SK-N-BE(2)	BE(2)-M17	N	TH	NT	(10)
	BE(2)-M17V	N	–	NT	"
	BE(2)-M17F	S	–	NT	"
	BE(2)-M17M	I	–	NT	"
	BE(2)-7S	S	Tyr	NT	(10)
	BE(2)-C	I	DβH, TH	+	"
SK-N-BE(1)	SK-N-BE(1)n	N	–	NT	(10)
	SK-N-BE(1)s	S	–	NT	
SMS-KCN	KCN-62n	N	TH	NT	(10)
	KCN-65n	N	TH	NT	"
	KCN-71n	N	TH	NT	"
	KCN-83n	N	TH	NT	"
	KCN-9s	S	Tyr	NT	"
NAP	NAP(H)n	N	TH	NT	(10)
	NAP(H)s	S	ND	NT	"
NBL-W	NBL-W-N	N	TH, DβH	NT	(91)
	NBL-w-S	S	ND	NT	"
RT-BMV	RT-BMV-I	N	NT	NT	(77)
	RT-BMV-C6	I	NT	NT	"

N=neuronal subtype; S=substrate adherent; flat subtype; I= intermediate subtype; TH=tyrosine hydroxylase; DBH=dopamine-β-hydroxylase; tyr=tyrosinase; ND=not dectected; NT=not tested;-=negative

Table 5: Effect of Differentiation Agents on Selected Neuroblastoma Cell Lines

Cell line	Treatment	Cell types	Ref
SMS-KCNR	INFγ	N, A	(104)
	RA	N, S	(57)
	BrdU	S	(105)
	cAMP	N, A	(106)
LA-N-5	RA	N	(56)
	cAMP	N	(107)
	VIP	N	(108)
	Vit D	N	(109)
	Phenylacetate	N	(110)
	INFγ	N, A	(111, 112)
	Ara-C	N	(112, 113)
	TPA	Nb	(112)
	TNF	N	(112)
SK-N-SH/SH-SY5Y	RA, +BDNF	N, NE	(60)
	TPA	N	(114)
	NGF	N	(62, 115)
	cAMP	N	(116)
	Herbimycin A	N	(117)
GI-ME-N	INFγ	N, A	(118)
	RA	N	(66)
	Ara-C	N	(113)
	HPR	A	(66)
LA-N-1	RA	N	(66)
	INFγ	N	(118)
	Ara-C	Nb	(113)
	HPR	A	(66)

INFγ= interferon gamma; RA=retinoic acid; Ara-C= cytosine arabinoside; BDNF=brain derived neurotrophic factor; HPR=hydroxyphenylretinamide; TPA=phorbol esters; VIP= vasactive intestinal peptide; BrdU= bromodeoxyuridine; cAMP;5'-cyclic adenosine monophosphate; Vit D=vitamin D; TNF=tumor necrosis factor; N=neuronal; A=apoptotic; Nb=neuroblastic; NE=neuroendocrine; S=Schwannian

Table 6.

Examples of Genetically Modified Neuroblastoma Cell Lines

Cell Line	Parental Line	Genetic Modification	Ref
IMR5-BCL2	IMR5(subclone IMR32)	Bcl2; neo	(119)
NBASI-5	NBL-S	antisense N-myc; neo	(68)
NBS-1	"	sense N-myc; neo	"
NBS-2	"	" "	"
AS-14.2N$^+$	SK-N-AS	N-myc; neo	(120)
AS-11.4N$^+$	"	N-myc; neo	"
15N-TrkB	"	TrkB; neo	(62)
411	SMS-KCNR	N-myc; neo	(61)
512	"	N-myc; neo	"
422	"	N-myc; neo	"
1810	HTLA230	TrkA; neo	(121)
cl.4M	SK-N-BE	MAX; neo	(122)
cl.6M	"	MAX; neo	"
cl.3N	"	N-myc; neo	"
cl.9N	"	N-myc; neo	"
SK-N-AS:γIGN	SK-N-AS	IFNg; neo	(123)
LA-N-6:γIGN	LA-N-6	IFNγ; neo	"
LA-N-5:γIFN	LA-N-5	IFNγ; neo	"
LA-N-1:γIFN	LA-N-1	IFNγ; neo	"
SK-N-FI:γIFN	SK-N-FI	IFNγ; neo	"
TET-2N	SH-E-P	tet regulated N-myc; hygro.	(70)
TET-2	"	tet transactivator; neo	"
TET-21N	"	tet regulated N-myc; hygro.	"
TET-21	"	tet transactivator; neo	"
SH-400;310;907 SH-802;803	SK-N-SH	N-myc;neo	(124)
15NA-P4;E1;D1	LAI-15N	TrkA; neo	(125)
tTA sense tTA-antisense	SK-N-BE(2)	tissue transglutaminase; neo	(65)
RET/PTC1 RET/PTC3 RET/MEN2A RET/MEN2B	SK-N-BE(2)	activated RET genes; neo	(63)
B-myb	LA-N-5	B-myb; neo	(126)
B-myb antisense	"	antisense B-myb	"
SY5Y-TrkA	SH-SY5Y	TrkA; neo	(71)
LAN5-TrkA	LA-N-5	TrkA; neo	"
SKMYC2 SKMYC6	SK-N-SH	N-myc	(127)
SKMYAS	SK-N-SH	antisense N-myc	"
SH400;310;907 SH-802;803	SK-N-SH	N-myc;neo	(124)

neo=neomycin resistant; tet=tetracyline regulated; hygr=hygromycin resistant