●788 CONVENTIONAL VERSUS SPECIAL NEUROBLASTOMA (NB): FAVORABLE PROGNOSIS CORRELATES WITH STROMAL DIFFERENTIATION. Stephen Qualman*, Chisa Aoyama*, Hiroyuki Shimada*, Helen Chan†, Charles Smith†, Jane Chatten#. (Sponsored by William Zipf). *Dept. Path., Children's Hospital, Columbus, OH; Depts. †Ped. Hem./Onc. and ‡Path., The Hospital for Sick Children, Toronto; #Dept. Path., The Children's Hospital of Philadelphia.

Special NBs (with opsomyoclonus or OMC; IV-S) have an excellent prognosis (85% 2 year survival) vs. conventional NBs (29% 2 y.s.) in our patient pool; this was inexplicable by standard prognostic factors alone. We studied tumor biopsies prior to therapy from 46 special (12 Stage II-OMC, 34 IV-S) and 48 conventional (12 Stage II, 36 Stage III or IV) NBs to detect discerning tumor-associated antigens via immunoperoxidase technique. A unique and variable pattern of tumor stromal differentiation was identified in all NBs, consisting of the relative occurrence of S100 protein-positive (Schwann-type) versus ferritin (FER) positive satellite cells around tumor vessels. In any NB, the presence of any S100 cells vs. only FER cells significantly improved survival (S100-94% 2 y.s.; FER-29% 2 y.s.) ($p<0.001$). With decrease in FER in NBs, a significant ($p=0.03$) curvilinear increase in tumor stromal lymphoid aggregates occurred. Special NBs contained twice as many S100 cells ($p<0.002$), one-half as many FER cells ($p<0.001$) and 4 times as many lymphoid aggregates ($p<0.005$) as conventional NBs. Stromal perivascular satellite cell differentiation (Schwann-type or S100 vs. FER) is a new and significant prognostic factor in both special and all NBs; a favorable differentiation pattern (↑S100/↓FER cells) may be linked to enhanced immune response.

789 THROMBOCYTOPENIA IN CHILDREN WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION. Sreedhar P. Rao, Scott T. Miller, Senih Fikrig, John Moohr, Audrey K. Brown. Department of Pediatrics, SUNY-Health Science Center at Brooklyn, New York.

Thrombocytopenia was found in five (12.5%) of 40 children with evidence of HIV infection. Age of the patients at time of diagnosis of thrombocytopenia ranged from two months to two years; platelet counts were less than 50,000/cmm in all and less than 10,000/cmm in three. Two of five patients had thrombocytopenia at the time of diagnosis of HIV infection while three others developed it during the course of their illness. Three of five patients manifested bleeding (petechiae, echymosis, and epistaxis). Bone marrow examination revealed increased numbers of megakaryocytes in all. Increased levels of platelet associated antibody (PAIgG) were found in 2/2 patients tested. Four patients received intravenous gamma globulin (IVGG) in a dose of 2-3 g/kg over a 3-5 day period. Only one patient had a significant increase in platelet count (from 32,000 to 350,000 /cmm) two days after a five day course of IVGG therapy, but the count returned to 40,000/cmm in two weeks. Two patients whose platelet count did not improve after IVGG received prednisone and in one there was a moderate but transient increase in the platelet count. We conclude that immune thrombocytopenia occurs in children with HIV infection and that the rate of response to IVGG appears to be lower than that reported in HIV associated ITP in adults or non-HIV associated ITP in children.

●790 DECREASED TUMOR CYTOLYSIS OF NEUTROPHILS (PMN) FROM PATIENTS WITH CHRONIC GRANULOMATOUS DISEASE (CGD). Robert L. Roberts, Bonnie J. Ank, and E. Richard Stiehm. UCLA School of Medicine, Los Angeles, CA.

Human PMN are capable of lysing tumor cells after activation by stimuli such as phorbol myristate acetate (PMA). We compared PMN from normals and CGD patients for their ability to mediate tumor lysis and inhibit lysis by normal mononuclear cells (MNC). The CGD patients (whose PMN lack the ability to generate reactive oxygen intermediates, ROI) include 2 males (X-linked) and 3 females (autosomal). Human PMN were isolated on discontinuous Percoll gradients, and cytotoxicity was measured by ^{51}Cr release from the K562 cell line. PMN were activated by exposure to PMA (100ng/ml) at 4°C. In an 18 hr assay, normal PMN lysed 33.1±3.9% (n=17) of the target cells and CGD PMN lysed 10.8 ± 2.5% (12 experiments). Decreasing the effector:target ratio to 25:1 did not alter the lytic ability of normal PMN (31.1±4.5%,n=3) but did decrease the lysis by CGD PMN (6.4 ± 1.7%, $p<.025$). Lysis by both normal and CGD PMN could be almost totally blocked by cytochalasin B (5ug/ml), indicating a role for the cytoskeleton. Preliminary studies indicate that lysis by CGD PMN (but not normal PMN) is enhanced by both tumor necrosis factor and interferon-gamma. The addition of normal PMN to normal MNC decreased natural killer (NK) lysis from 68.8 ± 3.5% (MNC alone) to 41.4 ± 6.7% (MNC + PMN, $p<.001$). By contrast, CGD PMN did not decrease MNC-mediated lysis (68.2 ± 4.5%). Thus, tumor cytolysis by PMN appears to be dependent on both oxidative and non-oxidative mechanisms, whereas inhibition of NK cytotoxicity requires the generation of ROI.

791 INTRACRANIAL EXTENSION IN NON-PARAMENINGEAL HEAD AND NECK RHABDOMYOSARCOMA. A REPORT FROM THE INTERGROUP RHABDOMYOSARCOMA STUDY (IRS). Frederick B. Ruymann, Mohan S. Beltangady, William A. Newton, R. Beverly Raney, Seth J. Silberman, Melvin Tefft, Jorge A. Ortega, Harold M. Maurer for the IRS Committee of the CCSG and POG. The Ohio State University School of Medicine, Children's Hospital, Department of Pediatrics, Columbus, Ohio.

Intracranial extension of rhabdomyosarcoma (RMS) has been identified as a major cause of failure in parameningeal sites. Fifteen of 214 (7%) patients with nonparameningeal RMS had intracranial extension threatened or proven at diagnosis. The primary sites included: orbit (8), neck (2), cheek (2), parotid (1), oropharynx (1), and temporalis (1). Computerized tomographic scans in 13 cases were helpful in detecting the intracranial extension and defining the anatomical extent of the primary. One or more patterns of CNS extension were seen in the 15 patients: parameningeal extension (8), bony erosion of the orbit (7), infraorbital fissure involvement (4), brain metastases (3), meningeal metastases (2), cavernous sinus extension (2) and invasion of the optic nerve (1). Five of 6 cases receiving intrathecal chemotherapy and cranial radiotherapy according to guidelines for parameningeal sites survived. Metastases to the meninges and brain carried the gravest prognosis with no survivors. In the remaining patients 10/11 are alive at a median of 11 months from diagnosis. Careful evaluation of all patients with head and neck RMS may reveal threatened or proven intracranial extension. In these cases management according to guidelines for parameningeal head and neck sites is recommended.

●792 PLATELET 12-HYDROXY-5,8,10, HEPTADECATRIENOIC ACID (HHT) INCREASES PROSTACYCLIN PRODUCTION BY AN EFFECT ON VASCULAR CYCLOOXYGENASE. P. David Sadowitz, Yamaja, B.N. Setty, Marie J.Stuart, SUNY, Health Science Ctr., Dept. Pediatrics, Syracuse NY

No definite function has been attributed to HHT, a plt. arachidonic acid (AA) product produced in equivalent amounts to the plt. proaggregatory metabolite thromboxane (Tx.) We report that HHT stimulates prostacyclin production by endothelial cells, and have identified the mechanism for this effect. In human umbilical venous endothelial cells, HHT (0.5 and 1μM) stimulated prostacyclin (RIA for $6KPGF_{1\alpha}$) by 32 ±10% (ISE) and 42±13% ($p<0.05$ and 0.01). Similar changes were observed when the effect of HHT on exogenous ^{14}C-AA metabolism in fetal bovine aortic endothelial cells (FBAECs) was studied. $6KPGF_{1\alpha}$ was stimulated by 25±9% and 30±6% at HHT concs. of 0.5 and 1μM ($p<0.05$ and 0.01). While prelabelling experiments with ^{14}C-AA revcaled that HHT (1μM) had no effect on AA release from FBAEC membrane lipids (6481±232cpm/well control vs 6928±339 in HHT treated cells), kinetic analyses revealed that HHT affected vascular cyclooxygenase. HHT (1μM) increased Vmax in test microsomes (706±49pmol/mg/min) when compared to controls (524±47; $p<0.02$). No effect on Km was observed (6.16±0.64μM control vs 7.19±1.02 in test microsomes). The effect of HHT on plt. AA metabolism was next evaluated. Preincubation of washed plts. with HHT (1μM) did not enhance thrombin (0.2U/ml) induced plt TXB_2 (2.27±1.34pmol/10^6 plts. control vs 2.28±1.62 in HHT treated plts). No effect was also seen when AA (20μM) was the agonist. Further studies in plts prelabeled with ^{14}C-AA did not reveal an effect of HHT on plt. AA release. HHT stimulates vascular prostacyclin production without a concomitant effect on plt. AA metabolism. HHT may be an important local modulator of plt. plug formation.

793 DIHYDROFOLATE REDUCTASE FROM METHOTREXATE-RESISTANT HUMAN OSTEOSARCOMA CELLS. Judith K. Sato, Theodore J. DeFrank, Ann Yee, University of Southern California, Childrens Hospital of Los Angeles, Department of Pediatrics, Los Angeles, California.

Dihydrofolate reductase from human osteosarcoma cell lines resistant to Methotrexate (MTX) were examined. MTX resistance in TE85 osteosarcoma sublines was produced by exposing cells to gradually increasing concentrations of MTX. Cloned sublines of MTX-resistant cells are maintained in media containing 10^{-8} (TE85/R8), 10^{-7} (TE85/R7), or 10^{-6}M MTX (TE85/R6). Partial purification of human dihydrofolate reductase from both sensitive and resistant osteosarcoma cell lines was achieved using folate-sepharose affinity chromatography. Although the TE85/R6 cell line was 120-fold more resistant to MTX than the MTX-sensitive cells, the amplification of enzyme activity was only 3.3-fold. Monoclonal antibodies raised against dihydrofolate reductase from a MTX-resistant subline of L1210 murine leukemia cells (Proc. Am. Assoc. Cancer Res. 26:229, 1985) cross-reacted with reductases from human cells. Immunostaining with these antibodies was used to rapidly visualize elevated amounts of dihydrofolate reductase in MTX-resistant osteosarcoma cells. Further delineation of the exact mechanisms(s) accounting for the MTX resistance in these human osteosarcoma cells is currently under investigation.