this effect was attenuated by LY294002 or PTEN overexpression. Blocking N-myc expression with siMYCN significantly reduced VEGF secretion in high N-myc expressing NB cell lines. Further, stimulation of PI3K with IGF-1 led to attenuation of VEGF secretion by siMYCN in low N-myc expressing cells. The inhibition of VEGF secretion with MYCN-knockdown was further enhanced when combined with rapamycin. **Conclusions:** PI3K-mediated angiogenesis is regulated by MYCN-dependent and -independent pathways in human NB. Our results, for the first time, show that N-myc plays an important role in the PI3K-mediated VEGF regulation in NB cells. Targeting *MYCN*, as a novel effector of PI3K-mediated angiogenesis, has significant potential for the treatment of highly vascularized, malignant NB. Pediatrics/Developmental Biology II: Development, Regeneration, & Cancer

168. CRANIAL OSTEOGENESIS AND SUTURE MORPHOLOGY IN XENOPUS LAEVIS: A UNIQUE MODEL SYSTEM FOR CRANIOFACIAL DEVELOPMENT. Bethany J. Slater[1], Karen J. Liu[2], Matthew D. Kwan[1], Natalina Quarto[1], Micheal T. Longaker[1]; [1]Stanford University School of Medicine, Palo Alto, CA; [2]King's College London, London, United Kingdom

Introduction: Proper ossification of the cranial bones and subsequent fusion of the cranial sutures provides protection for the central nervous system while allowing for expansion of the brain throughout childhood. Deviation from the normal complex process of craniofacial development results in various congenital malformations. In this study, we investigated the South African clawed frog, Xenopus laevis, as an alternative, simplified model system for studying cranial osteogenesis and suture patterning. The Xenopus head undergoes dramatic remodeling during metamorphosis; as a result, tadpole morphology differs greatly from the adult bony skull. These alterations have not been well described because of the extended larval period in Xenopus. Xenopus is a useful model system because the embryos can be obtained in large numbers and develop externally allowing for ex utero examination. Thus, the purpose of this study is to develop Xenopus as a unique and feasible experimental model for studying craniofacial development. **Methods:** To investigate Xenopus calvarial morphology, we examined late larval, metamorphosing, and post-metamorphosis froglet stages in intact and sectioned animals. Whole-mount analysis of the tadpoles was performed by Alcian blue staining to evaluate chondrogenesis and Alizarin red staining to assess osteogenesis. Using micro-computed tomography (μCT) scanning, we tracked the development of the frontoparietal bone and the surrounding bones for gross morphological changes. Histological analysis was then performed on skull sections to determine the suture morphology. Finally, we set out to determine the mechanisms underlying the large-scale cranial remodelling during metamorphosis. Thus, we analyzed cell death (by TUNEL staining), proliferation (by phospho-histone H3 staining), and tissue turnover (by zymography) during the period of remodeling of the skull roof. **Results:** Cranial osteogenesis occurs between Nieuwkoop and Faber Stage 52 and Stage 66 (just before metamorphosis). The ossification of the frontoparietal bone, the main constituent of the skull vault, initiates from lateral ossification centers, proceeding from posterior to anterior and ectocranially to endocranially. Histological analyses revealed posterior overlapping sutures; however, unlike mammals, no midline cranial sutures were apparent during cranial ossification. During metamorphosis, dramatic shape changes occur in the Xenopus head. We found that the larval head structures appeared largely cartilaginous while in the froglet, ossified bone makes up the vast majority of the head. This study demonstrated that tissue turnover during metamorphosis could be accounted for by abundant collagenase-1 (MMP-1) activity rather than from cell death by apoptosis. **Conclusions:** Our studies demonstrate the feasibility of tracking cranial osteogenesis in Xenopus and provide the groundwork for future molecular analyses. These findings establish a novel model system which can be employed for future manipulations to further elucidate cranial osteogenesis and suture biology and potentially lead to innovative, targeted treatments for craniofacial abnormalities. The ultimate translational aim of these studies is the understanding of molecular mechanisms underlying craniofacial malformations such as craniosynostosis, the premature fusion of one or more cranial sutures.

TRANSPLANT/IMMUNOLOGY II: CLINICAL/ MECHANISMS OF TRANSPLANT IMMUNOLOGY

169. PERI-TRANSPLANT B CELL DEPLETION IN MONKEYS ATTENUATES ALLOANTIBODY PRODUCTION AND CARDIAC ALLOGRAFT VASCULOPATHY (CAV). Shahrooz S. Kelishadi, Tianshu Zhang, Tiffany Stoddard, Christopher J. Avon, Mitch Higuchi, Emily Welty, Bao N. Nguyen, Stuart Mitchell, Agnes M. Azimzadeh, Richard N. Pierson III; University of Maryland Baltimore School of Medicine and Baltimore VAMC, Baltimore, MD

Introduction: To test whether the depletion of CD20+ B-cells at the time of engraftment alters the prevalence of anti-donor alloantibody or severity of CAV in the context of therapeutic immunosuppression with cyclosporine (CsA) or high-dose CD154 inhibition. **Methods:** Forty-one MLR-mismatched heterotopic cardiac cynomolgus allograft recipients were treated with high intensity anti-CD154 monotherapy (αCD154; n=17, 6 with ATG induction) or αCD154 with additional anti-CD20 therapy (rituximab 20mg/kg q wk for 4 weeks: αCD154 +αCD20; n=16, 11 with ATG). Eleven other animals received therapeutic CsA (target trough >500ng/ml), three of which received additional αCD20. Acute rejection was treated with steroids; graft survival was censored at 90 days. Anti-donor alloantibody was deemed positive if present (by flow cytometry) around time of explant. **Results:** 12 animals died with beating grafts, mainly with ATG-associated lung pathology, and are excluded from the survival analysis. Graft survival with αCD154 + αCD20 (median >90d) and proportion of grafts surviving to 90 days (6/7) was significantly increased relative to αCD154 (median 43d, $IQR_{(25-75)}$35-82d, $p<0.007$; 3/15 >90d). With therapeutic αCD154 (trough level >100 μg/ml until graft explant), 12/13 (92%) developed anti-donor antibody, while the αCD154 + αCD20 group had 3/7 (43%) ($p=0.03$); only the three animals with alloantibody had late (after day 60) CD154 levels below 100 μg/ml, and also exhibited CAV. Average CAV score at graft explant for the αCD154 group was 2.44 (+/− 0.44) vs. 0.68 (+/− 0.73) for the therapeutic αCD154+αCD20 group ($p<0.0005$); the three animals in the αCD154+αCD20 group with CD154 levels below target and with alloantibody elaboration also had higher CAV scores (range 1-2). Preliminary CAV scoring suggests that added αCD20 inhibited CAV (scores ranging 0.0-0.1) relative to therapeutic CsA alone (median 2.1; range 1.5-2.4); alloantibody analysis is in progress for these groups. **Conclusions:** Using αCD20 is associated with significant attenuation of CAV when used with either "therapeutic" αCD154 or CsA. Our findings demonstrate for the first time that αCD20 reduces the severity of CAV in conjunction with both conventional immunosuppression and costimulation pathway blockade, by mechanisms preventing alloantibody production and others that remain to be defined. Anti-CD154 and anti-CD20 antibodies were gifts from Biogen Idec Pharmaceuticals. ATG was a gift from Genzyme and Dialysis Clinics Inc.

170. NATIONWIDE VOLUME AND MORTALITY IN CIRRHOTIC PATIENTS AFTER ELECTIVE SURGERY. Nicholas Csikesz, Jennifer F. Tseng, Shimul A. Shah; University of Massachusetts, Worcester, MA

Despite a potential doubling of patients with hepatitis C and cirrhosis in the next decade, the outcomes after elective surgery in patients