between cases and controls ($p < 0.0001$) that reflects about a two-fold higher relative risk of sarcomas for patients with the homozygous mutated genotype with respect to the other genotypes (OR = 2.199, 95% CI: 1.507–3.209) (two-sided Fisher's Exact Test). The frequencies in blood donors were 43.7% for the homozygous wild type TT (213 individuals), 45.0% for the heterozygous TG (219 individuals) and 11.3% for the homozygous polymorphic variant GG (55 individuals). Considering the STS patients, the GG genotype was associated with an OR of 1.882 (95% CI: 1.150–3.082, $p = 0.0132$), while for osteosarcomas, GG patients have about a 2.5-fold risk (OR = 2.448, 95% CI: 1.595–3.759, $p < 0.0001$).
Conclusion: This data highlights that patients with the GG MDM2 309T>G polymorphism have a higher risk of developing sarcomas.

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Analysis of the mTOR signaling pathway versus proliferative response of tumor cells to the mTOR inhibitor RAD001 (everolimus)

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The mammalian "target of rapamycin" (mTOR) is an intracellular protein kinase controlling cell growth and proliferation, regulated by nutrient/energy availability and the PI3K/AKT pathway. Two mTOR complexes exist: a rapamycin-sensitive complex defined by interaction with raptor (regulatory-associated protein of mTOR) and a rapamycin-insensitive complex defined by interaction with rictor (rapamycin-insensitive companion of mTOR). Both complexes contain GbL, an essential subunit required for optimal mTOR activity.
RAD001 (everolimus), an oral mTOR inhibitor currently in Phase 2 clinical trials in oncology, shows antiproliferative activity in vitro in a wide range of tumor cell lines. Although most lines are sensitive to RAD001 treatment (IC50s for antiproliferative effects in the low/sub nM range; eg. BT474 breast: IC50 = 0.55±0.12 nM), a small number of lines are indifferent (IC50s in the >100 nM – uM range; eg. KB31 epidermoid: IC50 = 1778±800 nM). The reason why RAD001 is effective against some transformed cells but not others remains unclear, as does the role of mTOR-raptor vs. mTOR–rictor signaling in the cellular response to RAD001. Confirming previous data, we demonstrated in a mixed panel of cell lines ($n = 21$), representing lung, breast and colon cancer as well as glioblastoma, an association between higher phospho-(serine 473) AKT levels and the antiproliferative response to RAD001 in vitro ($r = -0.701$, $p < 0.001$; Spearman's Rank correlation). For raptor and rictor protein levels, however, no such correlation has been observed (Raptor: $r = -0.0989$, $p = 0.687$; Rictor: $r = -0.00589$, $p = 0.98$; Spearman's Rank correlation). Molecular analysis has shown that RAD001 treatment can induce AKT phosphorylation in a subset of tumor lines/models as well as in patient tumors. Interestingly, induction of pospho-AKT in vitro following mTOR inhibition does not predict the anti-proliferative response to RAD001. For example, BT474 and KB31 both exhibit induction of AKT phosphorylation, but with quite different sensitivities towards RAD001. As mTOR-rictor was recently shown to directly phosphorylate AKT on serine 473, a role for this mTOR complex in the cellular response to mTOR inhibition is feasible. We are currently investigating this question using siRNA approaches, overexpression of mTOR complex components and specific inhibitors.

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Epidermal Growth Factor Receptor (EGFR) Expression and Mutational Analysis in Synovial Sarcomas and Malignant Peripheral Nerve Sheath Tumors

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Background: Synovial sarcomas (SnSrc) and malignant peripheral nerve sheath tumors (MPNST) are rare mesenchymal tumors of adolescence and young adulthood. SnSrcs are characterized by either a monophasic or biphasic morphology and a distinct chromosomal translocation t(X;18), which has prognostic significance. MPNSTs are characterized histologically by a monophasic spindle cell histology. Previous work from our laboratory has demonstrated that SnSrcs express the receptor tyrosine kinases (RTKs) EGFR and Her2/neu. This present study extends the work to examine the expression of EGFR in MPNST, the role of the activated EGFR/MAPK pathway in both tumors and the characterization of potential targets of EGFR tyrosine kinase domain.
Methods: A tissue microarray was constructed containing 48 cases of SnSrc and 32 cases of MPNST. Immunohistochemistry (IHC) stains for EGFR, EGFRvIII, activated EGFR (Y1221-P-EGFR) and activated MAPK (T202/T204-P-p44/42 MAPK) were performed. Tumor DNA was extracted from fresh and formalin fixed paraffin-embedded tissue blocks. Exons 17−21 of the EGFR tyrosine kinase domain, as well as, Exon 2, containing codons 12/13 of KRAS, were amplified by PCR and sequenced.
Results: Immunohistochemistry (IHC) demonstrated that the EGFR is expressed in a majority of SnSrcs and MPNSTs. EFGRvIII immunoreactivity was negative. IHC was weakly positive for activated EGFR and MAPK. Sequence analysis of the EGFR genomic DNA did not demonstrate mutations in exons 17−21. However, a presumptive 20 base pair insertion was seen in Exon 18 in 7 of 48 samples of SnSrc (14.5%) and 8 of 32 MPNSTs (25%) respectively. The functional significance of this insertion is not known. Correlation of the 20 bp insertions to clinical outcome is presently being determined. KRAS mutations in codons 12/13 were not identified in either tumor type.
Conclusions: Expression of EGFR in SnSrc and MPNSTs with an intact EGFR/MAPK pathway has been hypothesized to contribute to the malignant potential of these tumors. Our study reveals the relative absence of known activating mutations in EGFR in both tumor types, which suggests that trials of small molecule inhibitors would be of little clinical benefit. A recent Phase II trial of erlotinib in 38 MPNST patients failed to detect a clinical response. A clinical study of treatment with cetuximab is ongoing and may help elucidate whether blockade of EGFR with antibodies is likely to be more active.

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Bone marrow stromal cells modulate cytotoxicity of 8-amino-adenosine in myeloma cell co-culture studies.

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Background: We have previously demonstrated that 8-amino-adenosine (8-NH_2-Ado) is rapidly taken up by human multiple myeloma (MM) cell lines and converted to 8-NH_2-ATP causing cytotoxicity, loss of endogenous ATP and loss of phosphorylation of key signaling kinases. However, myeloma cell growth *in vivo* is supported by the cytokine rich marrow microenvironment and this may modulate the cytotoxicity of 8-NH_2-Ado. To test this, we have co-cultured the MM cell line MM.1S with two well characterized human bone marrow stromal cell lines (Hs5 and Hs27A) and evaluated the cytotoxicity and uptake of 8-NH_2-Ado.
Materials and Methods: We have optimized the use of Hs5 and Hs27A cells in co-culture with MM.1S cells to provide a reproducible system for *ex vivo* drug screening. Cytotoxicity of 8-NH_2-Ado was evaluated by flow cytometric Annexin V staining on bone marrow stromal and MM cells separated by anti-CD38 staining. HPLC was used to quantify intracellular 8-NH_2-ATP and ATP. Alteration in signaling pathway kinases and phosphorylation were evaluated by immunoblotting.
Results: We have determined a dose-dependent specificity of 8-NH_2-Ado induced cytotoxicity towards the MM.1S cell line. The stromal cells undergo significantly less apoptosis than the MM.1S cells when treated with 3 micro molar 8-NH_2-Ado for 24 hrs, a dose that induces more than 85% apoptosis in the co-cultured MM.1S cells. To determine the basis of the selective cytotoxicity towards the MM.1S cells, we measured the uptake of 8-NH_2-Ado and conversion to 8-NH_2-ATP in both cell types. Interestingly, at the 3 micro molar concentration of 8-NH_2-Ado, stromal cells take up drug and generate 8-NH_2-ATP associated with a loss of intracellular ATP. To investigate the differential 8-NH_2-Ado-induced cytotoxicity we evaluated various signaling pathways associated with apoptosis and note a differential regulation of phospho-Akt and phospho-p38.
Conclusions: The purine nucleoside analogue, 8-NH_2-Ado is preferentially cytotoxic towards the MM.1S cells but not the bone marrow stromal cells in an *ex vivo* co-culture system that approximates the cytokine rich bone marrow milieu. This differential sensitivity is not accounted for by a differential uptake or conversion of 8-NH_2-Ado rather through selective expression and regulation of pathways associated with apoptosis in the MM.1S cell line. These results provide insight into the specific cellular targets associated with the therapeutic efficacy of 8-NH_2-Ado.