

mTOR SIGNALLING IN ACUTE LYMPHOBLASTIC LEUKEMIA

Linda J. Bendall¹ Philip Saunders¹, Roman Crazzolaro¹, John Hewson, Adam Cisterne¹, Rana Baraz¹, Marilyn Thien¹ and Ken F. Bradstock²

¹Westmead Institute for Cancer Research, Westmead Millennium Institute, Westmead. NSW. 2145, Australia

²Department of Haematology, Westmead Hospital, Westmead. NSW. 2145, Australia

Acute lymphoblastic leukemia is the most common childhood cancer and despite good responses to chemotherapy-based protocols, relapsed acute lymphoblastic leukemia is still more common than most other childhood malignancies and the outcome following relapse remains poor. Therefore the development of more potent but less toxic drugs for the treatment of ALL is imperative. We previously demonstrated that signalling through PI-3K is important for ALL cell proliferation and survival. mTOR is a known target of PI-3K and since inhibitors of mTOR are available and currently used in the clinic, we investigated the effects of the mammalian target of rapamycin (mTOR) inhibitor, RAD001 (Everolimus), both *in vitro* and in a NOD/SCID model of human childhood B cell progenitor ALL. *In vitro*, inhibition of mTOR signaling by RAD001 resulted in cell cycle arrest at low doses, and depolarization of mitochondrial membranes producing cell death through a largely caspase independent mechanism at higher concentrations. When combined with DNA damaging agents high dose RAD001 enhanced caspase dependent cell death. This was associated with inhibition of the induction of p53 and p21 by DNA damaging agents, and increased expression of c-jun and PUMA. In a NOD/SCID model of human ALL RAD001 treatment of established disease increased the median survival of mice from 21.3 days to 42.3 days ($p < 0.02$). RAD001 together with vincristine significantly increased survival compared to either treatment alone ($p < 0.02$). In keeping with *in vitro* data RAD001 induced a cell cycle arrest in the G_{0/1} phase with associated dephosphorylation of the retinoblastoma protein, and reduced cyclin dependent kinase 4 and 6 levels. Ultrastructure analysis demonstrated the presence of autophagy and limited apoptosis in cells of RAD001 treated animals. In contrast cleaved PARP suggested apoptosis in cells from animals treated with vincristine or the combination of RAD001 and vincristine, but not in those receiving RAD001 alone. In conclusion, we have demonstrated the activity of RAD001 both *in vitro* and in an *in vivo* leukemia model supporting further clinical development of mTOR inhibitors for the treatment of patients with ALL.