Title: Targeting ARHGEF4 in cancer

Mangolini M1, Gasparoli L1, Virely C1, Edwards D1,2, Bartram J2, Goulden N2, Ancliff P2, de Boer J1, Williams O1

1Developmental Biology and Cancer Section Programme, UCL Great Ormond Street Institute of Child Health 2Great Ormond Street Hospital, London, United Kingdom

Introduction: The chromosomal translocation t(12;21)(p13;q22) gives rise to a fusion gene encoding the chimeric transcription factor TEL-AML1 (also known as ETV6/RUNX1). This fusion gene is the single most common genetic abnormality in paediatric B-cell precursor acute lymphoblastic leukaemia (BCP-ALL). Despite a good initial treatment response, up to 20% of these patients relapse. All relapses retain the TEL-AML1 fusion gene and therefore would presumably remain sensitive to targeted therapies. Data from our lab has shown that the Rho Guanine Nucleotide Exchange Factor 4 gene (ARHGEF4 also known as ASEF1) is overexpressed specifically in t(12;21) ALL. ARHGEF4 was first identified through its ability to interact with the tumour suppressor gene product adenomatous polyposis coli (APC). This gene encodes a RAC1/CDC42 specific guanine nucleotide exchange factor and is responsible for the GDP/GTP exchange by accelerating the very slow intrinsic GDP dissociation, thereby initiating Rho signalling cascades. Rho GTPases are a family of small GTP-binding proteins that function as binary molecular switches and are involved in several important cellular functions such as gene transcription, survival, adhesion and cytoskeleton reorganisation.

Methods: Lentiviral-mediated shRNA knockdown of ARHGEF4 in ALL cell lines was confirmed by qRT-PCR. The functional effects of this knockdown were analysed using colony forming ability and apoptosis assays. G-LISA activation kits were used to define which Rho GTPases are activated by ARHGEF4 in the REH TEL-AML1 cell line. The role of ARHGEF4 in leukaemia progression in vivo was assessed using xenograft models.

Results: We previously determined that in normal tissue, ARHGEF4 is mainly expressed in foetal brain, prostate, salivary gland, testis and whole brain. By analysing gene expression datasets of childhood acute lymphoblastic leukaemia and
different solid cancers, we found that the *ARHGEF4* expression level is elevated in t(12;21) leukaemia and in squamous lung cell carcinomas. We validated these observations using patient derived xenografts of ALL samples. shRNA mediated silencing of *ARHGEF4* induced apoptosis, inhibited colony formation of TEL-AML1 cell lines. Furthermore, *ARHGEF4* silencing also inhibited proliferation of squamous lung carcinoma cell lines. Moreover, silencing *ARHGEF4* in TEL-AML1 ALL and lung cancer cell lines significantly impaired disease progression *in vivo* in xenografted NSG recipients, resulting in prolonged disease latency.

**Conclusion:** Our data support the hypothesis that ARHGEF4 plays a crucial role in the survival and proliferation of TEL-AML1 BCP-ALL and squamous lung cell carcinomas. Targeting ARHGEF4 and downstream pathways could represent a new strategy for future therapies.