Title: Targeting *ARHGEF4* in cancer

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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.