to elucidate associations with therapeutic responses or prognosis, and functional analysis of these candidate genes in CML cell lines is required to confirm these findings.

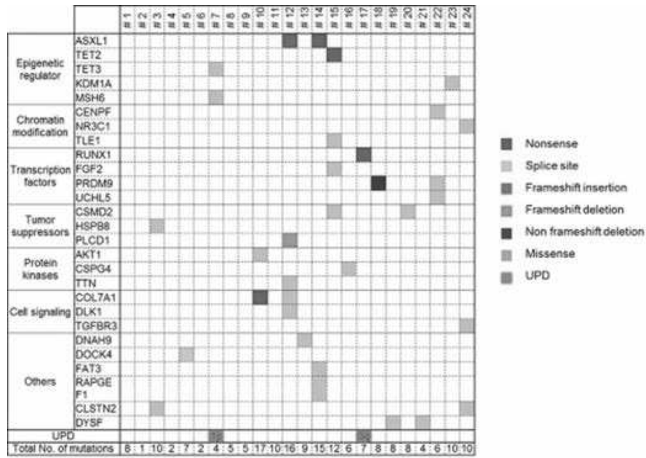

Figure 1.

P601

ROCK AS THERAPEUTICAL TARGET FOR MORGANA LOW CML

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Background: Atypical chronic myeloid leukemia (aCML) is an haematological neoplasm characterized by a median overall survival of 12.4 months. The standard treatments for aCML patients are chemotherapeutic drugs. However, these treatments result inefficient in inducing remission from the pathology. Recently we demonstrated that the haploinsufficiency of Morgana, an Hsp90 co-chaperone, *in vivo* is sufficient to induce a lethal and transplantable CML-like myeloid neoplasm characterized by non recurrent cytogenetic abnormalities in the bone marrow. Morgana is able to bind to and inhibit Rho-kinases, which are emerging as key oncogenic players in haematological disorders
Aims: Identify fundamental altered pathways in aCML to uncover the biological basis of the disease and find new therapeutical targets.
Methods: The bone marrow of morgana heterozygous mice and chronic myeloid leukemia patients has been analyzed extensively by flow cytometry and immunoistochemestry. Murine and human CML bone marrow cells and *in vitro* cellular models (K562 and THP-1 cells) have been tested for sensitivity to the ROCK inhibitor Fasudil.
Results: We demonstrated that diseased morgana heterozygous mice show ROCK hyperactivation in the bone marrow and that inhibition of these kinases results in apoptosis of morgana^{low} bone marrow leukemic cells without affecting normal cells survival. Moreover, in THP-1 cells Morgana downregulation enhances ROCK activity promoting cell proliferation while ROCK inhibition significantly reduces the proliferation of these cells. Interestingly, we found that the Morgana-ROCK pathway is altered in the 16% of Philadelphia-positive CML patients where ROCK hyperactivation, cooperating with BCR-ABL signalling, leads to imatinib resistance. In this context, treatment with a ROCK inhibitor restores the efficacy of imatinib to induce apoptosis. In addition, we found Morgana downregulation and ROCK hyperactivation in the bone marrow of all aCML patients we tested.
Summary/Conclusions: Taken together these results point out Morgana as an oncosuppressor and ROCK as potential therapeutical target for Morgana^{low} CML patients.

P602

A NOVEL C-TERMINAL HSP90 INHIBITOR WITH THERAPEUTIC EFFECT IN IMATINIB RESISTANT CML

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Background: The introduction of specific BCR-ABL tyrosine kinase (TK) inhibitors, especially imatinib mesylate (Gleevec), revolutionized the clinical treatment of Chronic Myeloid Leukemia (CML). However, in many cases stable remission cannot be sustained through several escaping mechanisms, such as mutations in the ABL-kinase (*e.g.* T315I & M351T). These insights raise an urgent need to develop alternative treatment strategies. An attractive approach is targeting Heat Shock Protein 90 (Hsp90), which acts as a molecular chaperone and facilitates the folding of several oncogenic proteins including, BCR-ABL.FDA approved Hsp90 inhibitors are available and show anti tumor activity but to the best of our knowledge they all target the N terminal domain of Hsp90 and initiate heat shock response (HSR) with severe side effects (Wang & McAlpine, 2015). Thus C-terminal Hsp90 inhibition can be an attractive approach in targeting Imatinib resistant CML with low toxicity. We have identified hotspots in the C-terminal domain of Hsp90 (Ciglia *et al.*, 2014) and designed several non-peptidic inhibitors, which target Hsp90's C-terminal dimerization.
Aims: Generation and characterization of novel compounds targeting C terminal dimerization of Hsp90 *in vitro* and *in vivo*.
Methods: The specificity of selected inhibitor (DDK88) to Hsp90 was determined by Hsp90 dependent luciferase refolding assay followed by efficacy experiments using imatinib sensitive and resistant myeloid leukemic cell lines *in vitro* as well as in *in vivo* Xenograft model.
Results: In the present study, we have extensively characterized a novel and promising therapeutic compound (DDK88) for patients with Imatinib resistant CML *in vitro* and *in vivo*. DDK88 exhibits anti-proliferative and cytotoxic activity in several human myeloid leukemic cell line models (*e.g.* K562 - 5.72±0.31µM, KCL-22 - 2.74±0.52µM) and induces cell cycle arrest, early differentiation and inhibits colony formation. DDK88 disrupts Hsp90's chaperone activity to BCR-ABL protein. Hence *in vitro* application of DDK88 revealed down regulation of BCR-ABL protein expression and its downstream signaling network including, STAT5a, CRKL, AKT and mTOR proteins. Moreover, imatinib resistant CML cell lines are equally sensitive to DDK88 (*e.g.* K-562r - 6.24±0.52 µM, KCL-22r - 2.86±0.63µM) as compared to imatinib sensitive cells. In the same way, DDK88 inhibits proliferation and BCR-ABL kinase activity of 3 clinically relevant imatinib-resistant (~10 µM) BCR-ABL mutant (T315I, M351T & E255K - ~3µM) cell lines. Notably, unlike clinical inhibitors targeting N-terminal (*e.g.* AUY922), DDK88 does not induce HSR, evaluated by protein expression of HSF-1, Hsp70, Hsp40 and Hsp27. DDK88 has a therapeutic window as its inhibitory effects on healthy cord blood (CB) derived mononuclear cells (MNCs) and CD34+ cells were significantly less potent as compared to the human leukemic cell lines. Furthermore, *in-vivo* proof of concept studies demonstrate the efficacy of DDK88 at 0.5 mg/kg in a K-562-Luciferase Xenograft tumor model. DDK88 reduced tumor burden with respect to tumor weight (DDK88 0.2±0.01g *vs* vehicle 1.26±0.44g (p=0.04)). Immunoblot analysis of tumor samples derived from DDK88 treated mice revealed the absence of HSR as well as downregulation of BCR-ABL kinase activity and its associated downstream signaling pathways.
Summary/Conclusions: This study provides *in vitro* and *in vivo* characterization of a novel anti-Hsp90 compound, which is specific against its C-terminus. Hence further improvement and testing of DDK88 in pre-clinical studies can be a promising strategy to target imatinib resistant CML and to avoid HSR.

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P603

RESISTANCE IN CHRONIC MYELOID LEUKEMIA: THERAPEUTIC TARGETING OF ESCAPE VIA CSF2RB

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Background: Treatment of chronic myelogenous leukemia (CML) with BCR-ABL tyrosine kinase inhibitors (TKI) achieves high rates of molecular response. However, BCR-ABL-positive leukemic stem and progenitor cells persist implying the need for lifelong treatment. Bone marrow stroma plays an important role in inhibiting apoptosis. Cytokines such as interleukin 3 (IL-3) and granulocyte/macrophage-colony stimulating factor (GM-CSF) mediate BCR-ABL-independent survival of progenitor cells via common receptor subunit CSF2RB. Disruption of the CSF2RB axis by the Janus kinase1/2-inhibitor ruxolitinib overcomes cytokine-mediated resistance *in vitro*. We previously demonstrated upregulation of CSF2RB in BCR-ABL-transformed cells as potential resistance mechanism, and now provide an indepth molecular and functional analysis.