

*Three-in-one bullets: Small molecule inhibitors of the beta-common receptor*

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Cytokines of the beta-common ( $\beta$ c) family, namely granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin (IL)-3 and IL-5, are regulators of myelopoiesis that also play non-hematopoietic roles in multiple biological systems in health and disease [1]. These cytokines bind to specific heterodimeric receptors consisting of a cytokine-specific  $\alpha$ -subunit and the  $\beta$ c subunit, which is common to all three cytokines. Aberrant signaling in the  $\beta$ c cytokine signaling pathway has been linked to hematological malignances and certain solid tumours [1]. The shared use of the  $\beta$ c subunit by GM-CSF, IL-3 and IL-5 and the fact that it has a common site (site 2) used by all 3 cytokines makes it an attractive target to simultaneously disrupt the interaction of the three cytokines with their receptors and potentially impact disease.

We have used the crystal structure of the  $\beta$ c subunit, solved in complex with GM-CSF and the GM-CSF receptor  $\alpha$ -subunit [2, 3], to identify small molecule inhibitors of  $\beta$ c. Site 2, the major interaction interface between the cytokine and the  $\beta$ c subunit, was investigated as a target for a virtual screen of a library of five million commercially available compounds. 52 compounds were chosen for initial testing, of which only 42 were soluble enough to be run in a surface plasmon resonance (SPR) assay against immobilized  $\beta$ c homodimeric protein. The SPR assay identified 11 compounds as binders of the  $\beta$ c protein. The binding affinities of the three strongest binders were determined in SPR kinetic assays. As the putative binding site for the small molecules is partially occluded by lattice contacts in our  $\beta$ c crystals, our crystallographic approach has focused upon obtaining crystals of the individual  $\beta$ c domain (D4) that harbors most of the important site 2 residues, together with the compounds. Cell-based assays are also being simultaneously carried out to investigate their functional effects. The most promising small molecule hits will be further developed using structure-based drug design principles and may provide the basis for future drug development.

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