ImmunoTools IT-Box-Cy55M-Award 2013

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Blocking the MIF/CD74 axis using anti-CD74 Nanobodies.

Blocking the MIF/CD74 interaction by directly targeting CD74 via Nanobodies (i.e. camelid-derived single-domain antibody-fragments (~15kDa) which are a new class of molecular tracers that are routinely identified with nanomolar affinity for their target and are easily tailored for molecular imaging and drug delivery applications) forms the basis of my PhD project. Furthermore, in contrast to conventional antibodies, Nanobodies feature a very fast biodistribution profile due to their small size and physicochemical properties.

Multiple clinical studies have indicated that macrophage migration inhibitory factor (MIF), an ubiquitously expressed pleiotropic immune-regulatory pro-inflammatory cytokine that controls metabolic and inflammatory processes, has the ability to sustain inflammatory responses in the face of endogenous or exogenous glucocorticoids and promotes cell recruitment. It can be considered as a biomarker for inflammation-associated diseases such as autoimmune diseases, metabolic disorders, systemic infections as well as sepsis and cancer.

Regarding its mechanism of action, the MIF signal transduction is initiated following binding to CD74 (an evolutionary conserved type II transmembrane glycoprotein, also known as the invariant chain of the MHC-II complex or Ii, which is ubiquitously expressed, although predominant on antigen presenting cells, and upregulated upon stimulation of the cells with antigen). This induces its phosphorylation and recruitment of CD44, which then activates SRC family non-receptor tyrosine kinases, leading to ERK1/2 phosphorylation and NF-κB activation. This ultimately leads to the production of Cyclin D1 (i.e. crucial in cell proliferation and the cell cycle) and ETS/AP1 (involved in the gene expression of TLR4, CAMs (cell surface adhesion molecules) and inflammatory molecules including but not limited to TNF, IFN-γ, IL-2, IL-6, IL-8 and MIF). In addition, the MIF/CD74 interaction leads to the down regulation of p53, which in combination with MIF's anti-oxidative properties inhibits apoptosis. Notably, this receptor complex may be associated with the CXCR2 or CXCR4 chemokine receptors, which also bind MIF and mediate cell recruitment.

Much of our read-out is based on the induction of pro-inflammatory cytokines ie. TNF, IFN-γ, IL-6 and chemotaxisis.

The potential of the anti-CD74 Nbs to inhibit these MIF-mediated effects will be evaluated using different in vitro assays (cell cultures/proliferation assays/chemotaxis assays), whereby we will scrutinize different Tumor models. Hence, the IT-Box CY55M would be in first instance of great benefit to stimulate cell cultures (ie PECs, BM cells, spleen cells, etc.) and subsequently evaluate if the MIF/CD74 is activated and/or if the antagonists can interfere in this activation. The results obatained from these in vitro assays would allow us to gage the effectivity of our Nanobodies prior to establishing in vivo models.

Much of the success of our Nanobodies are gaged via cytokine read-outs via ELISA and cell proliferation assays. Using the named cytokines/chemokines to estabolish concentration-dependant titrations will allow assessing more accurately the amout of cytokine being produced by the cells upon stimulation and consequently define the amount of Nanobody necessary (or whether the Nanobodies are capable) to reduce cytokine output to accceptable levels.

ImmunoTools IT-Box-Cy55M for Amanda Sparkes

includes 55 recombinant mouse cytokines

rm EGF, rm Eotaxin / CCL11, rm FGF-a / FGF-1, rm FGF-b / FGF-2, rm FGF-8, rm Flt3L / CD135, rm G-CSF, rm GM-CSF, rm GRO-a / CXCL1, rm GRO-b / CXCL2, rm IFNgamma, rm IL-1alpha, rm IL-1beta, rm IL-2, rmIL-3, rm IL-4, rm IL-5, rm IL-6, rm IL-7, rm IL-9, rm IL-10, rm IL-11, rm IL-13, rm IL-15, rm IL-16, rm IL-17A, rm IL-17C, rm IL-17F, rm IL-19, rm IL-20, rm IL-21, rm IL-22, rm IL-25 / IL-17E, rm IL-27, rm IL-31, rm IL-33, rm IP-10 / CXCL10, rm LIF, rm MCP1 / CCL2, rm M-CSF, rm MIP-1α/ CCL3, rm MIP-1β / CCL4, rm MIP3α / CCL20, rm MIP3β / CCL19, rm NGF-beta, rm PDGF-AA, rm PDGF-BB, rm RANTES / CCL5, rm sCD40L / CD154, rm SCF, rm SDF-1α / CXCL12a, rm SDF-1β / CXCL12b, rm TNFα, rm TPO, rm VEGF DETAILS