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3D1, a novel anti-nodal monoclonal antibody to target melanoma

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Nodal ligands are potent morphogens, belonging to the TGF-β superfamily. In humans, nodal is rarely expressed during late development and adulthood. Nodal physiological expression is typically observed only in embryonic tissues and embryonic stem (ES) cells. Aberrant re-activation of nodal expression in adults is associated with a number of tumours such as metastatic melanoma as well as breast, colon, prostate and ovarian carcinomas. Typically, nodal can trigger the Smad2/3 intracellular pathways by binding the type I (ALK 4/7) and type II (ActRIIB) activin-like serine-threonine kinase receptors, also in the presence of the co-receptor Cripto-1. In addition, like other ligands of the TGF-β superfamily, nodal can also activate MAPK cascade which, in a BRAF mutation-dependent manner, converges on ERK and PI3K-AKT pathway. Nodal signaling plays a relevant role in the aggressive progression of metastatic melanoma; indeed its inhibition blocks the tumorigenic capacity and the plasticity of aggressive human melanoma cells. In this context, targeting and inhibition of nodal signaling represents an attractive and alternative strategy to block melanoma progression and other cancers. With the aim to produce antibodies able to recognize Nodal and to block it's signaling by preventing also its association with Cripto-1, we have generated a set of monoclonal antibodies targeting one of the CBR (Cripto-Binding-Region) sites which encompass residues around Glu49 and Glu50 of Nodal. Using a subtractive ELISA screening we have selected a potential neutralizing monoclonal antibody, named 3D1, which recognizes the nodal E49 and E50 hot-spot residues. 3D1 mAb strongly associates with full-length rh Nodal (KD 1.4 nM) and recognizes the endogenous protein in a panel of human melanoma cell lines by western blot and FACS analyses. Melanoma cells treated *in vitro* with 3D1 show a significant reduction of nodal protein expression level and of its downstream signaling molecules P-Smad2 and P-ERK. 3D1 treatment blocks cell proliferation reducing P-H3 and Cyclin-B1 with a concomitant increase of p27 and prevents the anchorage-independent growth and vasculogenic network formation, too. Nude mice xenografts models, both subcutaneous orthotropic and lung colonization models, treated with 3D1 show anti-tumor effects in terms of reduced tumor volume and lung tumor burden, respectively. Collectively these data, suggest that 3D1 is a promising diagnostic reagent for detection of nodal and a novel bio-therapeutic agent for targeting nodal expressing cancers.

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i-bodies: Novel single domain antibodies against GPCRs and Ion channels in the treatment of fibrosis

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i-bodies are small, stable, human scaffolds engineered from information gained from the shark single domain antibodies. The presence of a long CDR3 enables better access to complex proteins such as GPCRs and ion channels. We have screened this phage displayed i-body library on GPCRs and ion channels expressed in different formats. We have obtained a panel of high affinity single domain antibodies specific for the chemokine receptor CXCR4. CXCR4 is known to be up-regulated in a number of cancers and recently has been implicated as a central player and a therapeutic target in fibrosis. Although all i-bodies bind with high affinity each of i-bodies have different functional profiles with respect to modulation of cAMP, calcium efflux, inhibition of β-arrestin signaling and blocking of HIV infection. When two lead i-bodies were injected intraperitoneally they were found to completely block SDF-1-induced leukocyte recruitment in an air pouch model of inflammation in mice. Importantly, unlike most other CXCR4 antagonists, they did not mobilize stem cells from the bone marrow. Thus these i-bodies would be ideal for long-term anti-fibrosis therapy. Indeed we have shown that i-bodies are able to block the recruitment of fibrocytes into the lungs of mice with bleomycin induced pulmonary fibrosis and that the anti CXCR4 i-bodies have anti-inflammatory and anti fibrotic effects in several different animal models. Moreover we suggest that i-body provide a unique resource for obtaining human antibody single domains to currently intractable membrane proteins.

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