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Multiplexed *in vitro* models of primary human B cell activation

White Paper

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Introduction

B cells are a class of lymphocyte that are important in the adaptive immune response, offering protection against pathogens[1]. They secrete antibodies and are also professional antigen-presenting cells. B cell pathology is central to many autoimmune diseases such as multiple sclerosis, rheumatoid arthritis and type-1 diabetes. Additionally, a number of cancers are caused by B cell malignancy. Therefore, B cells are important drug discovery targets; for example, the anti-CD20 antibody rituximab is clinically effective against many B cell related pathologies[1,2]. Robust assay models for B cell function are crucial to further exploit this therapeutic potential.

At Domainex, we have established *in vitro* assay systems to multiplex several B cell maturation readouts using primary human B cells[3]. Activation with the TLR9 agonist, ODN2006, causes increased proliferation and secretion of the maturation markers; IL-6, IL-8 and IgG. Pharmacological inhibition of this effect has been clearly demonstrated with a known phosphoinositide 3-kinase (PI3K) inhibitor.

Methodology

Cryopreserved human peripheral B cells (StemCell Technologies) were revived and seeded in polyornithine-coated 96 well plates. Cells were pre-treated with test compound before stimulation with the TLR9 agonist, ODN2006. Cell confluency was measured kinetically using IncuCyte S3 imaging and supernatants were sampled for quantification of IL-6, IL-8 and IgG by AlphaLISA in parallel at varied timepoints.

Example Data

B cell proliferation was measured as confluency changes using InuCyte kinetic imaging (Figure 1). Compared to unstimulated cells, ODN2006 caused an increase in confluency over 8 days. This proliferation effect was inhibited completely by dasatinib.

Similarly, IL-6, IL-8 and IgG secretion were markedly increased by TLR9 stimulation, an effect that was strongly inhibited by dasatinib (Figure 2). Therefore, TLR9-mediated activation of primary B cells can be both quantified and pharmacologically inhibited with a small molecule drug.

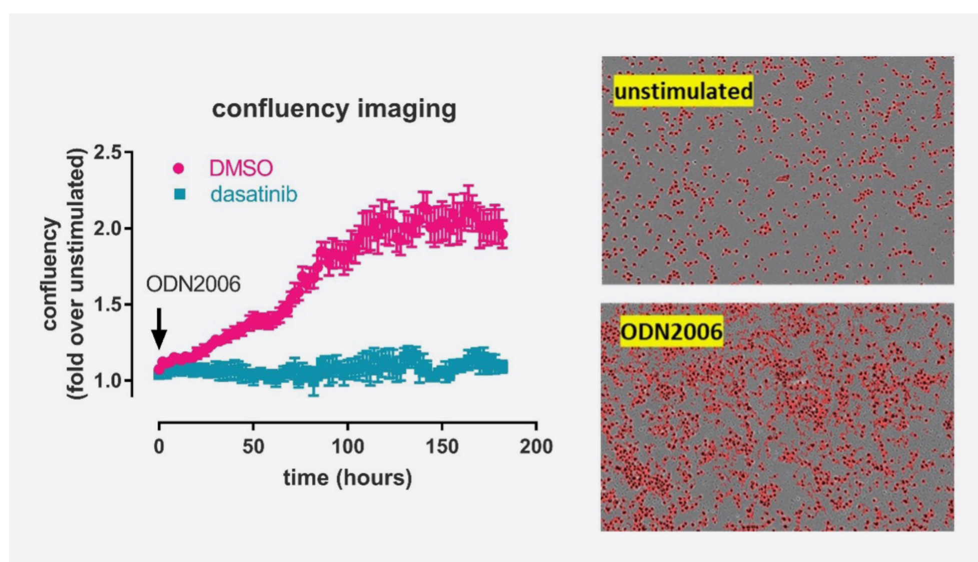

Figure 1: Lefthand panel: Kinetic confluency changes in B cells after ODN2006 stimulation (single dose at T=0). Righthand panel: Cell images with confluence mask superimposed in red after 180 hours stimulation with ODN2006

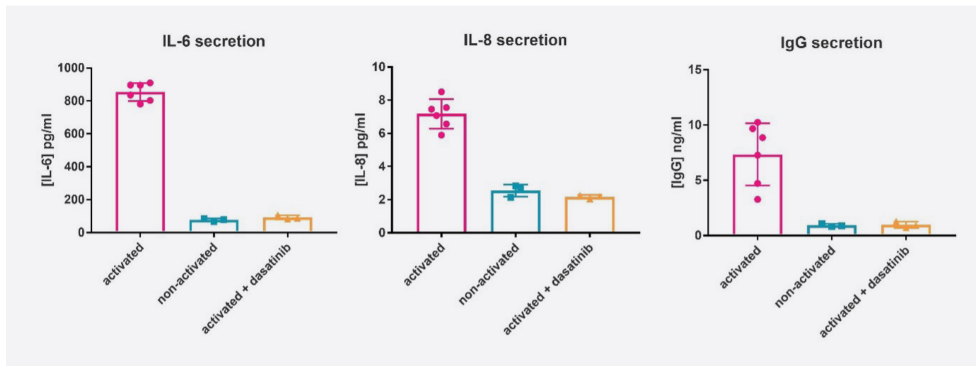

Figure 2: AlphaLISA quantification of IL-6, IL-8 and IgG secretion from B cells

Concentration-response analysis of the PI3K gamma selective inhibitor, IPI-549, showed potent and near complete inhibition in all four readouts (Figure 3) with IC_{50} values between 50 and 500 nM. This is consistent with the known role of this kinase in B cell biology[4].

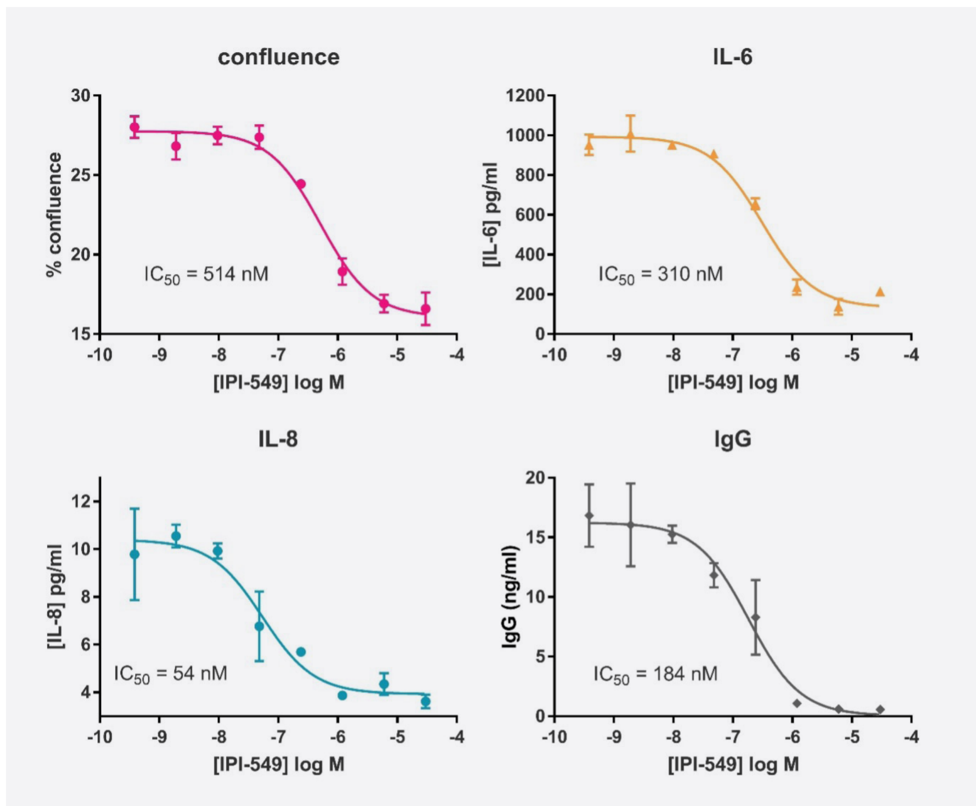

Figure 3: Concentration-response curves of IPI-549 against the four B cell activation readouts (with IC_{50} values shown)

References

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