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

White Paper

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Introduction

T cells are a class of lymphocyte that are important in the adaptive immune response, providing protection against pathogens. T cell receptors (TCRs), on the surface of T cells, recognise antigenic peptides displayed by antigen presenting cells, which, coupled with other costimulatory mechanisms, activate the T cell. CD4+ T cells, otherwise known as helper T cells, secrete cytokines which activate other immune cell subsets, whilst CD8+ T cells, otherwise known as cytotoxic T Cells, directly kill infected cells. Due to their overarching role in immune activation, the T cell compartment is associated with a range of autoimmune diseases such as rheumatoid arthritis, highlighting the need for T cells as drug targets.

At Domainex, we have established *in vitro* assay systems to multiplex several T cell activation readouts, using primary human CD4+ T cells. Stimulation with IL-2 alongside antibodies against CD3 and CD28 caused increased proliferation, cell clustering and secretion of the pro-inflammatory cytokine IFNγ. Pharmacological modulation of this effect was clearly demonstrated with a known phosphoinositide 3-kinase (PI3K) inhibitor.

Methods

Cryopreserved human peripheral blood CD4+ T cells (StemCell Technologies) were revived and seeded in polyornithine-coated 96 well plates. Cells were pre-treated with test compound before stimulation with human anti-CD3/CD28 T cell activator mix plus recombinant human IL-2. Cell confluency and clumping were measured kinetically using IncuCyte S3 imaging and supernatants were sampled in parallel for the quantification of IFNγ secretion by AlphaLISA at varied timepoints.

Example Data

Activation with anti-CD3/CD28 plus IL-2 caused a marked proliferation of T cells over five days, as measured using a cell imaging confluency mask (Figure 1). Using the same set of cells, an alternative analysis mask quantified the formation of cell clusters with an area > 5000 μm^2 (Figure 2). Cluster formation began after 40 hours and reached its maximum with complete confluence after five days, whilst an increase in proliferation was detected after 100 hours. The tryptophan metabolite L-kynurenine delayed the effect of stimulation of CD4+ T cell proliferation and clustering (Figures 1 and 2), consistent with its reported inhibitory role[1].

T cell activation also caused a large increase in IFNγ secretion that started 24 hours after stimulation and peaked at 72 hours (Figure 3).

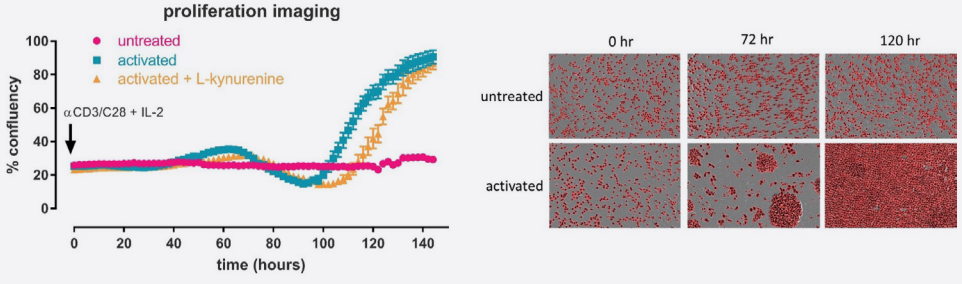

Figure 1: Left panel: Kinetic proliferation profiling of T cells after anti-CD3/CD28 + IL-2 activation (single doses at T=0) compared to control untreated cells. Right panel: Cell images with confluence mask superimposed in red at 0, 72, and 120 hours post activation.

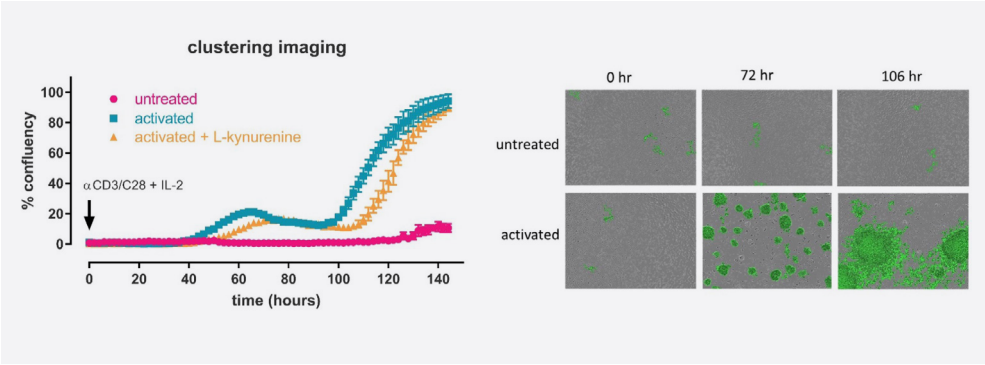

Figure 2: Left panel: Kinetic clustering profiling of T cells after anti-CD3/CD28 + IL-2 activation (single doses at T=0) compared to control untreated cells. Right panel: Cell images with clustering mask superimposed in green at 0, 72, and 106 hours post activation.

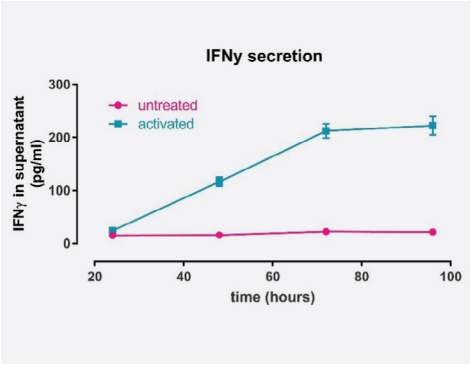

Figure 3: IFNγ secretion profile from T cells after ± activation with anti-CD3/CD28 + IL-2 at T=0.

The PI3K gamma selective inhibitor IPI-549 showed complete inhibition of proliferation, clustering and IFNγ secretion with an IC_{50} value between 1 and 4 μM (Figure 4). This is consistent with the known role of this kinase in T cell biology[2].

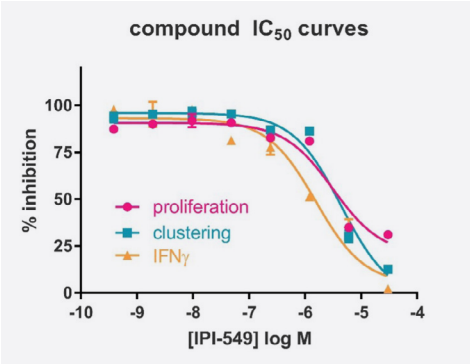

Figure 4: Concentration-response curves of IPI-549 in the three T cell activation readouts (IC50 = 3121 nM, 4406 nM, and 1550 nM for proliferation, clustering and IFNγ secretion, respectively). Readings were taken at day 3 for IFNγ and day 5 for proliferation and clustering.

References

1. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: Mediation of suppression by tryptophan metabolites. Peter Terness,Thomas M. Bauer, Lars Röse, Christoph Dufter, Andrea Watzlik, Helmut Simon and Gerhard Opelz. Journal of Experimental Medicine, 2002, 196, 447–457.

2. Phosphoinositide 3-kinase γ participates in T cell receptor-induced T cell activation. Isabela Alcázar, Miriam Marqués, Amit Kumar, Emilio Hirsch, Matthias Wymann, Ana C. Carrera and Domingo F. Barber. Journal of Experimental Medicine, 2007, 204, 2977–2987.

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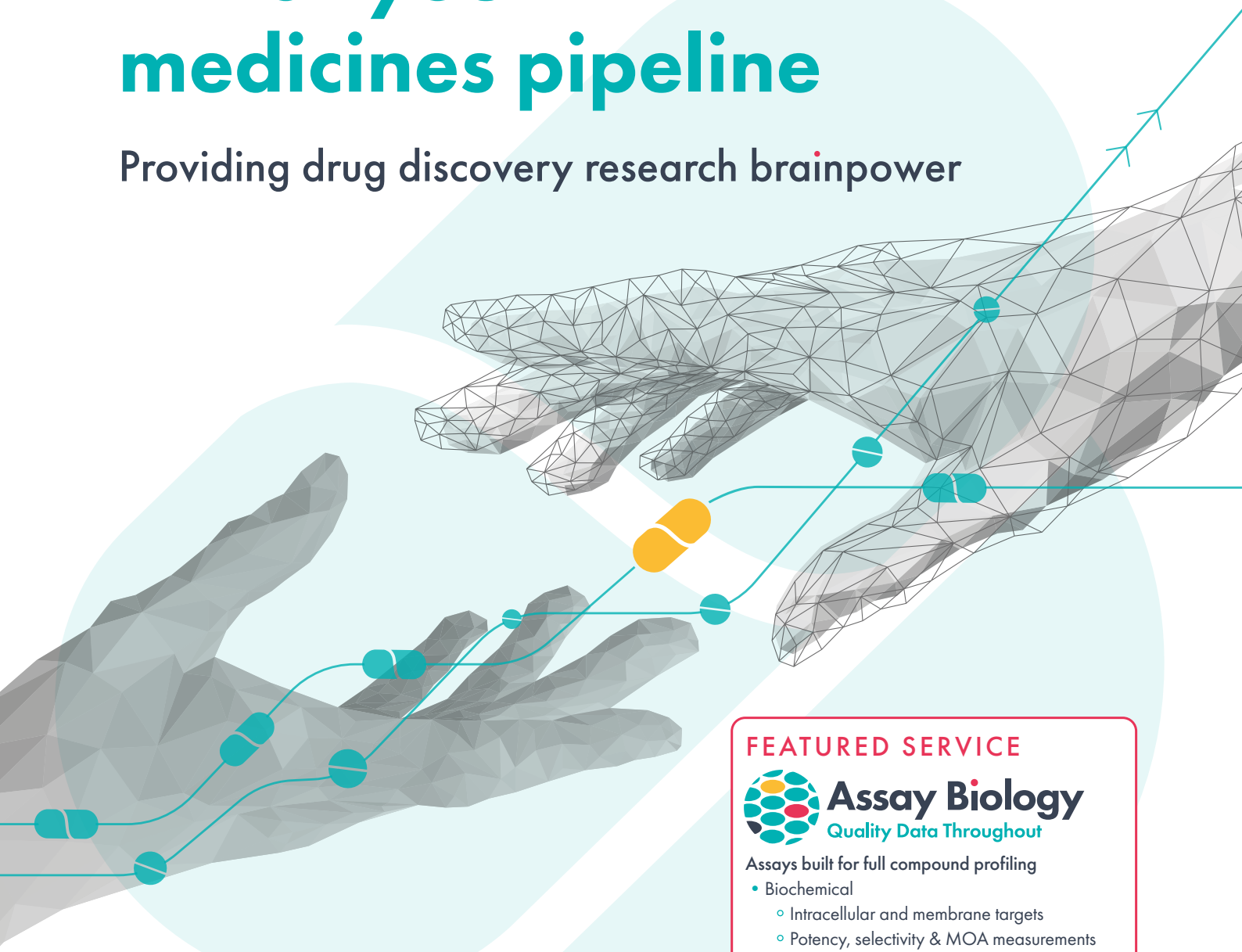

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