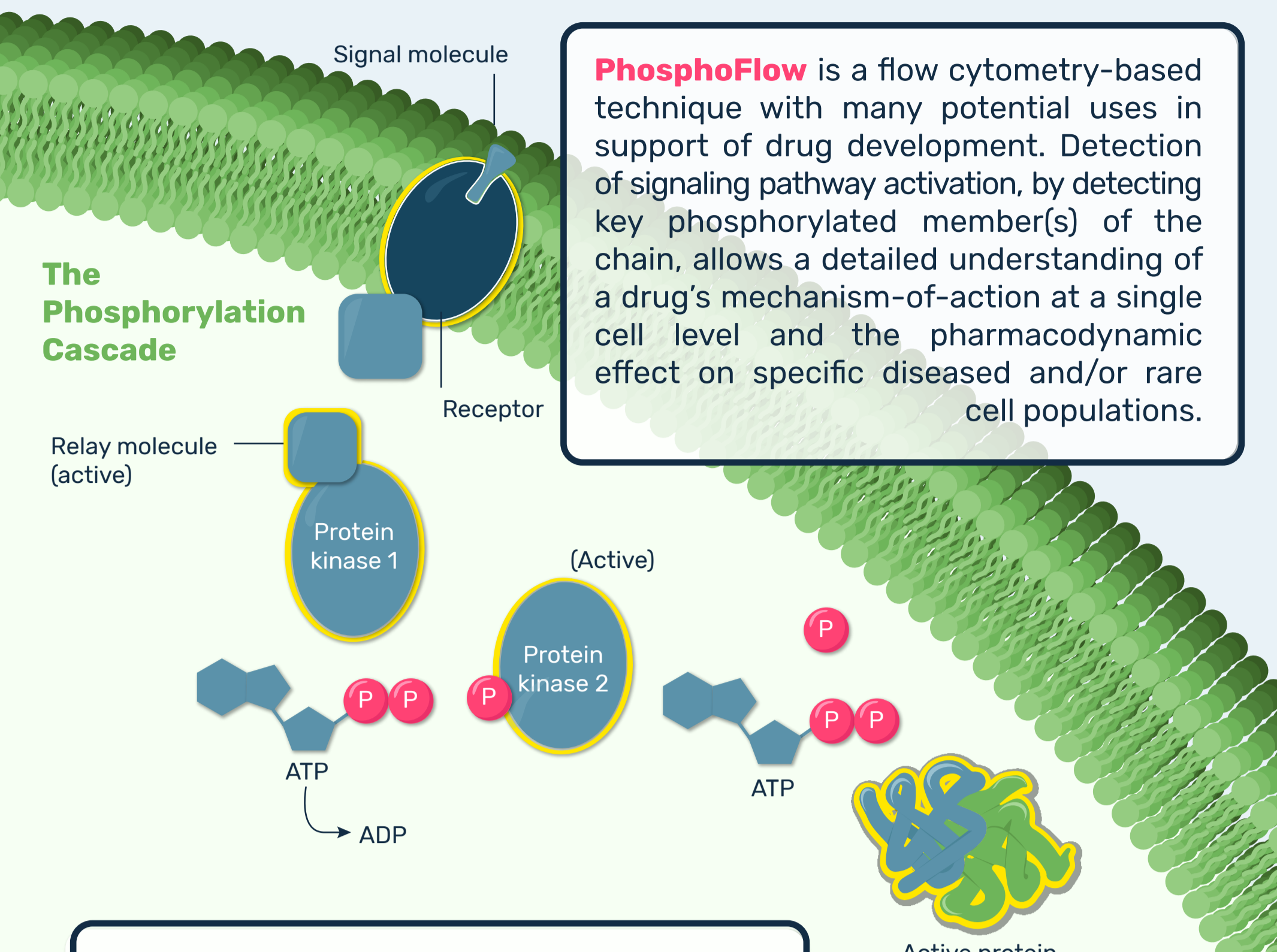


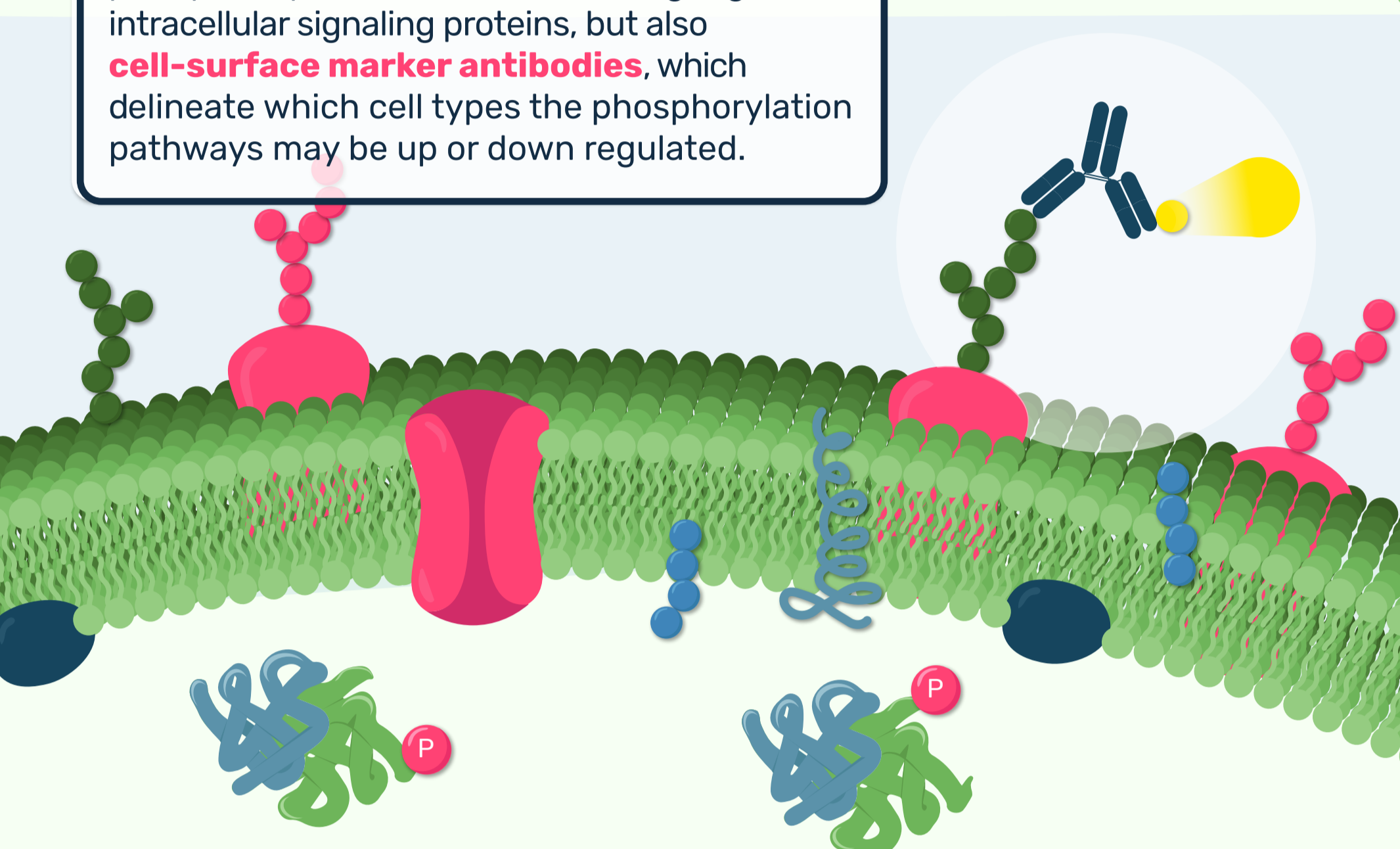
The use and applications of PhosphoFlow

What is PhosphoFlow?



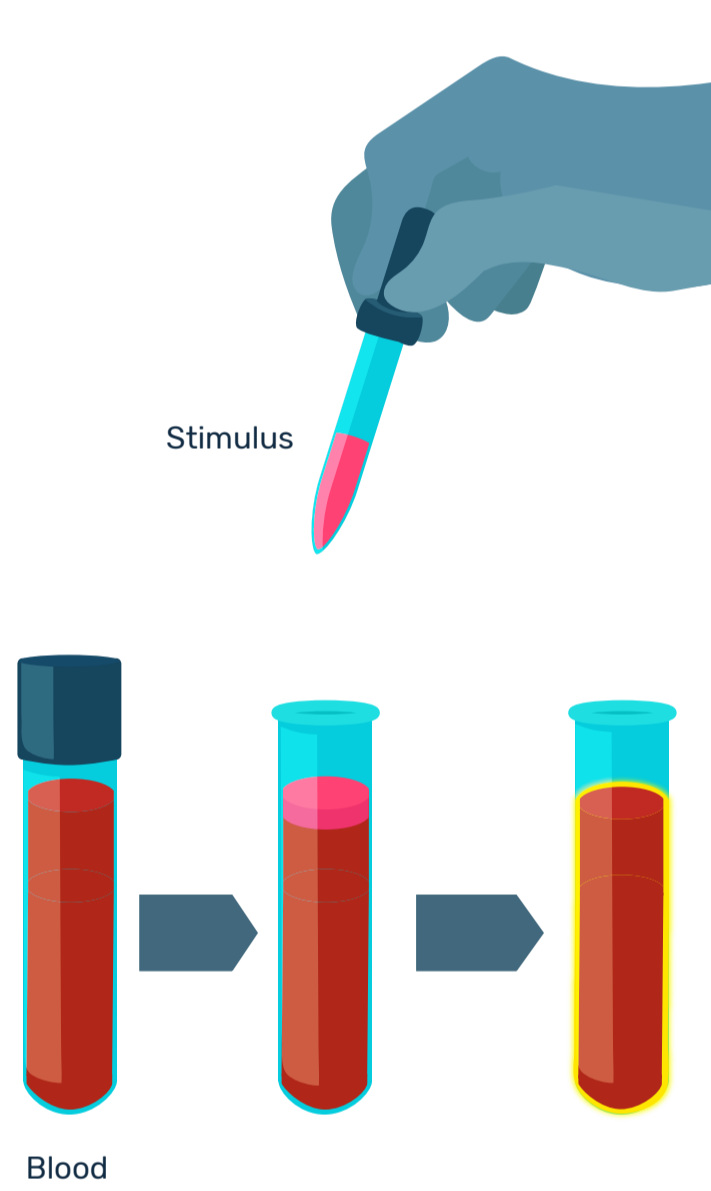
PhosphoFlow is a flow cytometry-based technique with many potential uses in support of drug development. Detection of signaling pathway activation, by detecting key phosphorylated member(s) of the chain, allows a detailed understanding of a drug's mechanism-of-action at a single cell level and the pharmacodynamic effect on specific diseased and/or rare cell populations.

Using this technique, we are able to generate multi-factorial datasets, by using not only phospho-specific antibodies to highlight intracellular signaling proteins, but also **cell-surface marker antibodies**, which delineate which cell types the phosphorylation pathways may be up or down regulated.



The applications of PhosphoFlow

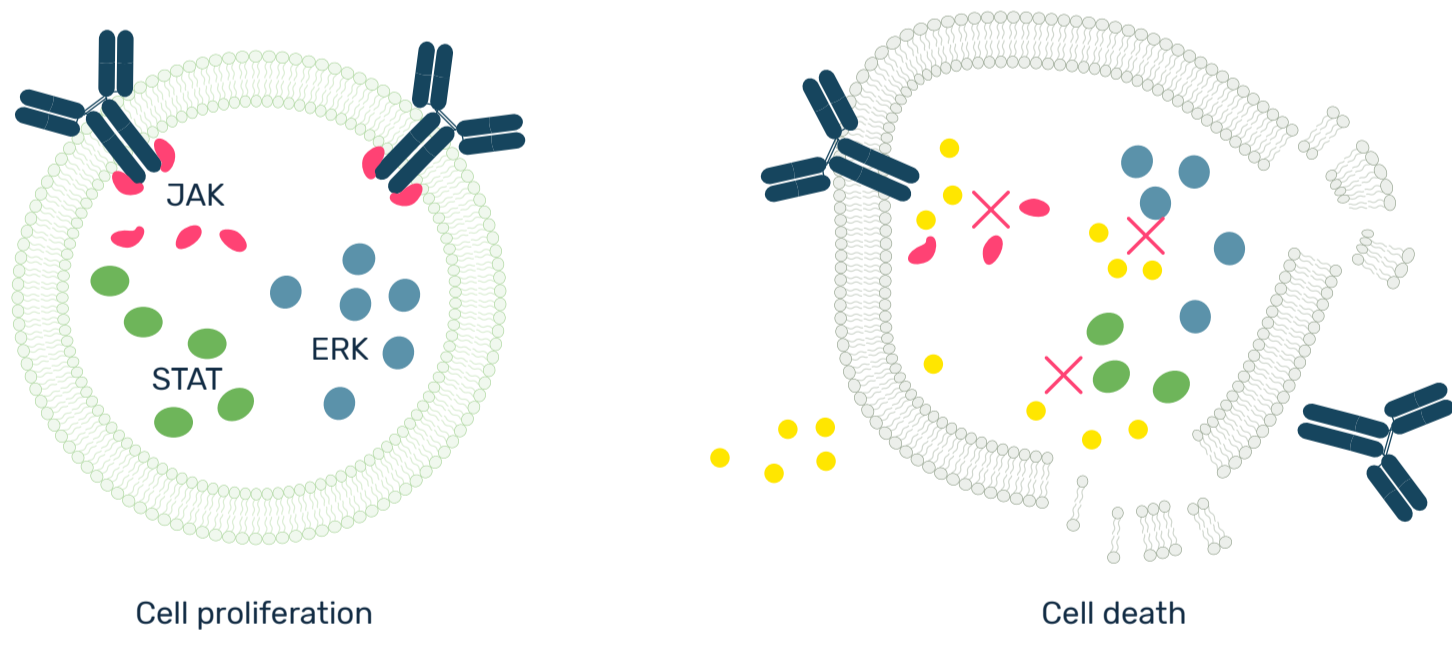
Early phases



- Functional detection of activated lymphocytes & other leukocyte subsets.
- To evaluate pathological conditions in cells.
- As a screening tool to identify drugs & genes effecting key intracellular signaling pathways.
- As a tool to monitor immune responses in humans.

In its simplest form, PhosphoFlow can be performed on *ex vivo* activated whole blood, which is an easy source compared to unstimulated controls to investigate drug action or target biology.

Clinical phases



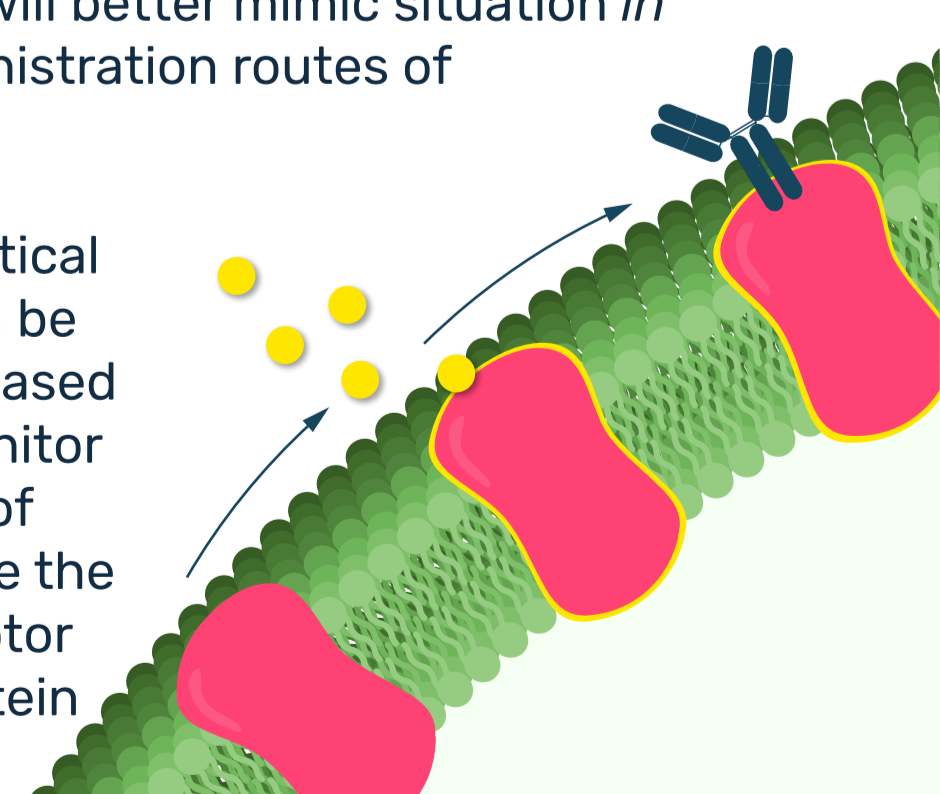
In clinical phases, for example, in oncology, the progression of a number of Leukaemia's and solid tumors are governed by aberrant signaling pathways with constitutively active (phosphorylated), members (ex. JAK/STAT, ERK etc.) and these can be monitored as an indication of drug efficacy.

Clinical trials

In clinical trials buccal mucosa and skin biopsies are used as a source of healthy epithelium to test anticancer agents. These can prove problematic as access to material and pharmacodynamics effect depends on the pathway being active.

This limitation can be easily overcome in the whole blood samples. Myriad stimulatory substances are known to activate/hit specific pathways, which will better mimic situation *in vivo* due to common administration routes of pharmaceuticals.

Furthermore, in a bioanalytical context, PhosphoFlow can be used as a functional cell-based bioassay to effectively monitor the neutralising potential of anti-drug antibodies where the drug target maybe a receptor upstream of phospho-protein cascades.



Summary

In essence, PhosphoFlow is multiplex biomarker analysis in a cell-based environment providing information on a functional and biological level. Multiple phospho-specific targets can be used to discern the activation of multiple, simultaneous pathways, all in a statistically robust manner and obtained from a relatively small sample of whole blood.

This infographic has been created as part of a Bioanalysis Zone feature in association with LGC.