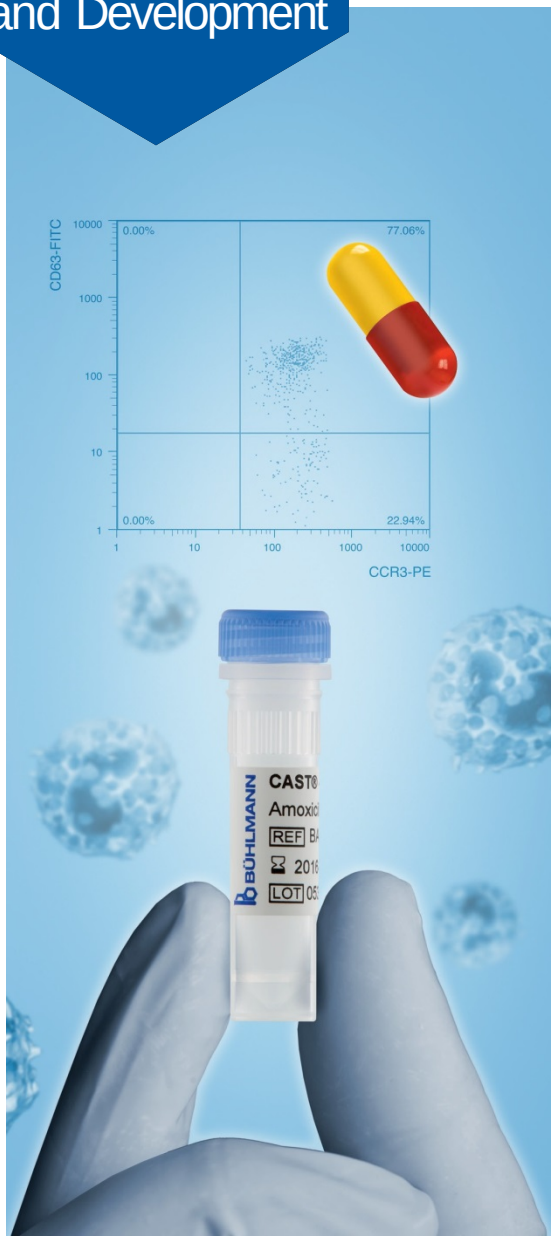
Basophil Activation

The Biomarker for Drug Potency and Efficacy

Flow CAST®
the new bioassay
in Drug Discovery
and Development



Flow CAST®: basophil activation as functional readout for drugs targeting immune cell signaling pathways

- Typical targets: PI3Kδ, BTK, SYK
- Bioactivity discrimination between PI3Kδ and PI3Kγ
- Scalable up to 384-well plate format

Evaluating Basophil Activation Status with Flow CAST® as a Surrogate Marker for Activation of other Immune Cells

Flow CAST® Basophil Activation Test can be effectively used to measure whole blood activity of cell signaling inhibitory drugs

The signaling pathways involved in basophil activation have been implicated in allergic responses, autoimmune disorders, and oncology.

Immune cells share several key kinases such as PI3Kδ, SYK, BTK, that are critical in their specific activation pathways (Figure 1).

Aberrant activation of these pathways promotes diseases like carcinogenesis and tumor angiogenesis and autoimmune diseases.

Targeting the uncontrolled activation of these pathways is the focus of several pharmaceutical companies with small molecule inhibitors, currently at different stages of development.

The development of robust tests to assess bioactivity of these compounds is fundamental for the success of these projects.

Basophils are a functional cell system in whole blood

Monitoring basophil activation in whole blood via flow cytometry is an accurate method for measuring a pharmacodynamic response to small molecule inhibitors against components of these signaling cascades.

Why basophils?

Basophils are key effector cells in allergy.

The clinical impact of basophil activation is due to the unique ability of blood basophil granulocytes to degranulate upon binding of an allergen to its specific IgE bound on membrane high affinity IgE-receptor (FcεRI).

The degranulation induced by the FcεRI stimulation can be easily measured by flow cytometry quantifying the expression of cell surface activation markers such as CD63.

As for B cells, T cells and other immune cells, PI3Kδ and PI3Kγ, SYK, BTK are key components in basophil activation signaling pathways.

Inhibition of the activity of these kinases in basophils results in the inhibition of degranulation and expression of CD63 on cell surface.

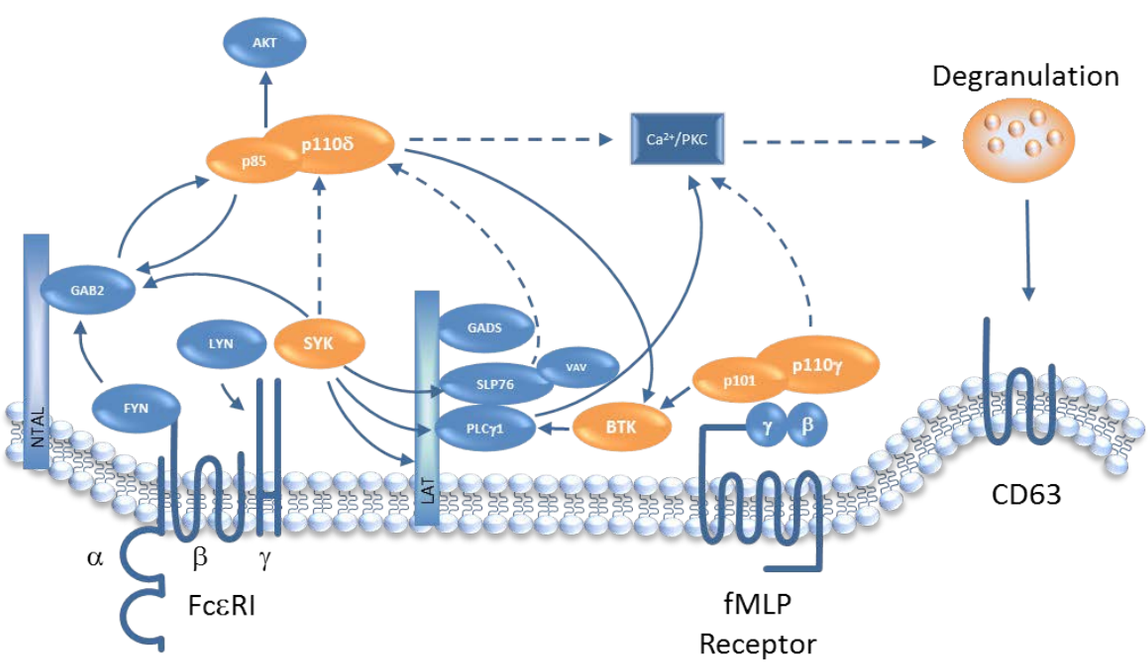


Figure 1

How Flow CAST® Works

Flow CAST® assay

Flow CAST® is routinely used for the determination of basophil activation in whole blood samples by flow cytometry. The unique marker combination - CCR3 and CD63 - combines the simple gating properties for basophil detection by CCR3 with the robust application of the proven activation marker CD63.

Flow CAST® includes two specific activators of basophils. The anti-FcεRI is a highly specific monoclonal antibody binding to high affinity IgE receptor. It activates the immune signaling pathway in basophils.

The fMLP activates the basophils in a non-immunological way, binding its specific G-protein-coupled receptor.

Specific inhibition of Basophil Activation

Compounds developed to block the aberrant activation of the immune cell signaling pathway can be easily assessed for their bioactivity effect on basophil activation. Their efficacy is determined as inhibition of the percentage of CD63 positive basophils (Figure 2).

Dose response curves allow for the determination of the dose required for 50% inhibition (IC50) by different compounds (Figure 3).

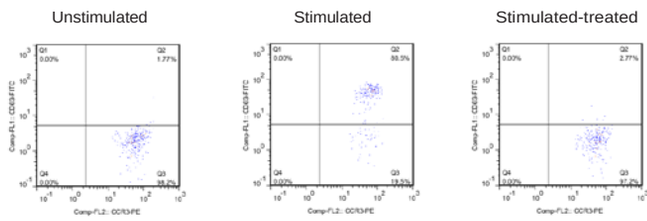
The specificity of drug candidates for a selected pathway can be assessed using different basophil activators.

SYK and BTK are mainly involved in the FcεRI pathway. Specific inhibitors of these kinases are expected to block the activation induced by the anti-FcεRI Ab but not by fMLP.

Basophils express two isoforms of PI3Ks, PI3Kδ mainly involved in the FcεRI pathway, and PI3Kγ, that, as in all other leukocytes, is crucial in the signaling cascade of G-protein coupled receptors, like the fMLP receptor.

Flow CAST®, with the two activators, gives the opportunity to determine the selective bioactivity of drugs targeting PI3K between the delta and the gamma subunit. The IC50 of PI3Kδ is determined in basophils activated by anti-FcεRI Ab and the IC50 of PI3Kγ is determined in basophils activated by fMLP (Figure 4).

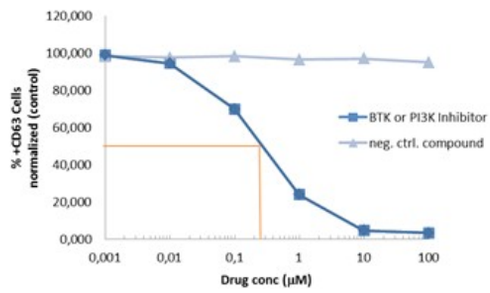
Basophil Activation in whole blood measured by Flow Cytometry



Activation inhibited by drug treatement

Figure 2

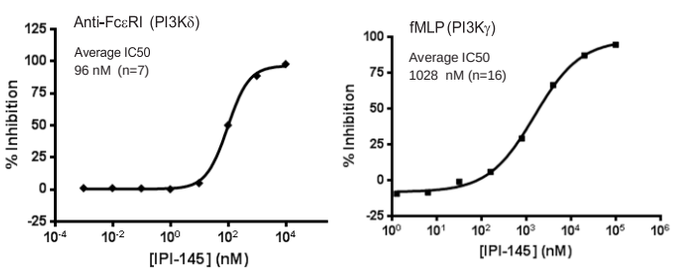
Treatment with a typical kinase inhibitor prevents activation of the peripheral blood basophils



Effect of a PI3K, BTK or SYK inhibitor at different doses on the basophils activated by the anti-FcεRI antibody. Example of a typical result.

Figure 3

Check the specificity and selectivity of your drugs activating basophils via distinct specific pathways



Typical result: IPI-145 Inhibits Anti-FcεR1 (PI3K-δ) and fMLP (PI3K-γ) induced Basophil activation in whole blood. Adapted from Winkler et al., Chemistry and Biology 20, 1364-1374, Nov 2013

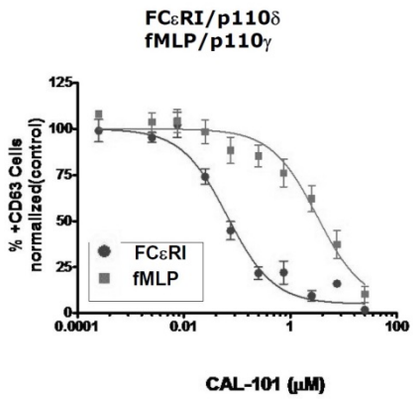
Figure 4

Flow CAST®: Basophil activation in the hands of kinase Drug Discovery and Development

Several pharmaceutical companies have utilized basophil activation as a surrogate marker during their drug development process (Figure 5).

Company	Compound	Target
Gilead Sciences, Inc.	CAL-101, Idelalisib*	PI3kδ
Pharmacyclics Janssen	PCI-32765, Ibrutinib	BTK
Infinity Pharmaceuticals	IPI-145, Duvelisib*	PI3kδ
TG Therapeutics	TGR-1202	PI3kδ
Pathway Therapeutics	PWT-143	PI3kδ

*referenced Flow CAST® as basophil activation test utilized in their studies according to our knowledge
Please ask for citation list at www.buhlmannlabs.ch



Anti-FcεRI and fMLP-induced basophil activation in PBMCs measuring CD63 upregulation using Flow CAST®. Data are presented as the percent of CD63 positive basophils normalized to DMSO vehicle control. Data are mean ± SD and are representative of one to four independent experiments. Adapted from BLOOD, 13 JANUARY 2011, VOLUME 117, NUMBER 2

Figure 5

Flow CAST®: Your partner in high throughput screening

Field Study: Gilead has adapted a robust whole blood activation assay (Flow CAST®) to run on an automated 384-well platform.

Historically, flow cytometric analysis of whole blood has been low-throughput, with the highest throughput being limited to 96-well plate formats. This is because smaller well sizes are prohibitive for effective red blood cell lysis. As basophils are a rare population, a minimum amount of whole blood per well is required to yield the signal needed for a robust assay.

Miniaturization was achieved through multiple rounds of red blood cell lysis and washing in deep well 384-well plates. This miniaturization allows for increased throughput of compound testing in lead optimization efforts and reduce the FTE resources required.

	96-well	384-well
Throughput (# compounds/week)	15	200
Compound plate format	Single Point	Duplicate Points
FTE	2	1
Projects Supported	2	3+

Development of a Robust Automated 384-well Whole Blood Flow Cytometry Assay
S Wise, B Steiner, K Huynh, L Lad, N Pagratis
Gilead Sciences, Inc.
SLAS 2014

BÜHLMANN

BÜHLMANN Laboratories AG
Baselstrasse 55
CH – 4124 Schönenbuch/Basel
Switzerland

BUHLMANN Diagnostics Corp.
USA / Canada
info@buhlmannlabs.com
www.buhlmannlabs.com Phone
844 300 9799

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Flow CAST®	FK-CCR	100 tests
CAST® ELISA	EK-CAST	192 wells
	EK-CAST5	480 wells

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