



Flow CAST[®]: Testing Potency and Efficacy of Inhibitors of PI3K δ, PI3Kγ, BTK and SYK Activity

Michele Romano, PhD Product Manager

Flow CAST[®] is for Research Use Only. Not for use in diagnostic procedures.



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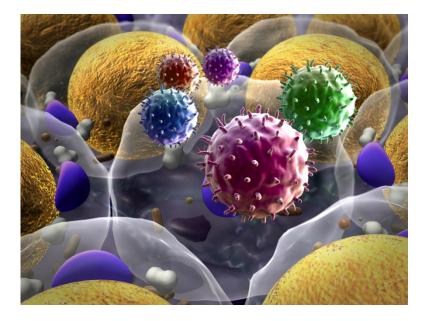


Focused Development: Targeting Immune Cell Signaling Pathway in Oncology

PI3K and BTK signaling may contribute to excess cell survival and proliferation, which can lead to variety of malignancies. As a result, PI3K and BTK are targets for many of the small molecule inhibitors currently in development.

Phosphatidylinositol 3-kinase (PI3K) and Bruton's tyrosine kinase (BTK) play an integral role in a number of cellular functions which ultimately lead to survival and growth of normal cells. Aberrant activation of this pathway promotes carcinogenesis and tumor angiogenesis. Research has shown that ~1/3 of human cancers develop as a result of aberrant PI3K signaling.

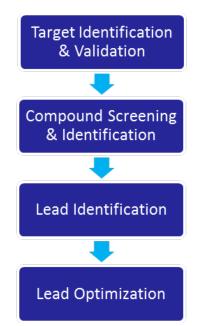
Inhibitors of the PI3K/BTK pathway are a large focus for several different pharmaceutical companies with >10 small molecule PI3K inhibitors currently at different stages of development.



Akinleye et al. Journal of Hematology & Oncology 2013, 6:88



Testing and Optimizing Kinase Inhibitors in Drug Development



In vitro biochemical assays:

Cheap and fast

Easy of use

potential high throughput

Lacks predictability

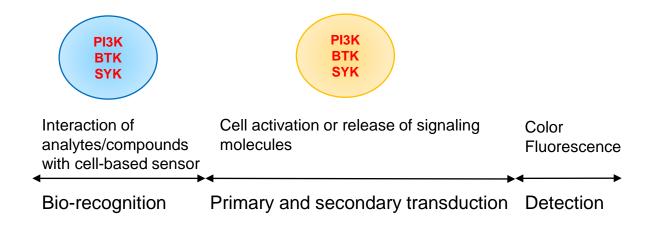
Poorly correlated with in vivo results

Does not provide physiological conditions

Kinase enzymatic assay for BTK, PI3K, SYK

Cell-based assay with transfected cell line

The Challenge of a Cell Based Sensor for Kinase Inhibitors



Target based High throughput Improved predictability Correlation with *in vivo* results

Speed up project Exclude compounds Save time and money

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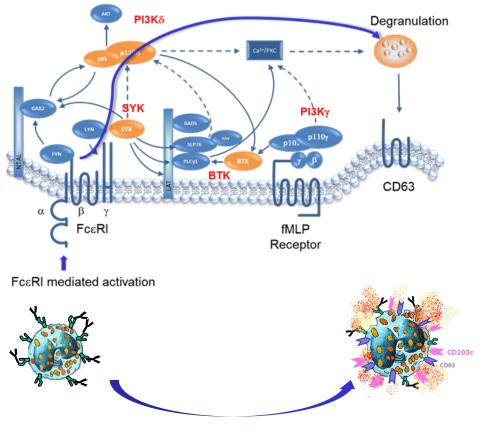
The Role of Immune Cell Signaling Kinases in Basophils

PI3K, BTK and SYK signaling pathways involved in basophil activation have been implicated in allergic responses, autoimmune disorders, and oncology.

The main function of basophils is to degranulate upon specific signals. Typically, basophil degranulation is activated by complex of allergen bound to specific IgE via the high affinity receptor for IgE.

Basophil degranulation and activation can be easily measured in flow cytometry by quantifying the expression of cell surface activation markers such as CD63. Allergic responses, autoimmune disorders and oncology are all comprised of signaling pathways that are also involved in modifying the activation status of basophils.

Several key kinases that are molecular targets in drug discovery, such as PI3K γ and PI3K δ , SYK, BTK are main components in the basophil activation signaling pathways.



Basophil Activation

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Basophils as a Surrogate Marker of Drug Potency and Efficacy

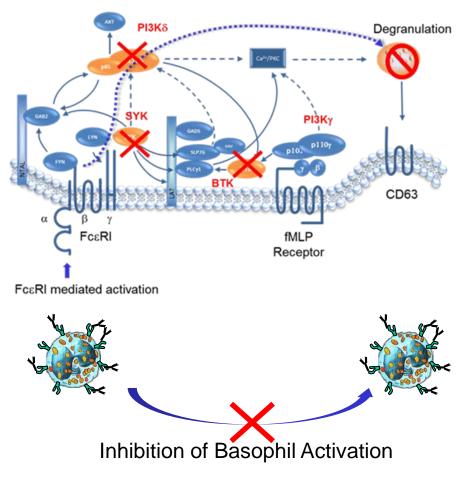
Quickly evaluate potency and efficacy of inhibitors of immune cell signaling pathway using inhibition of basophil degranulation as a surrogate read out.

Basophils, your cell based biosensor.

Basophils, the circulating immune cells of allergy, are perfect surrogate markers in human whole blood to check for the bioactivity of inhibitors of immune cells kinases.

Monitoring basophil activation in whole blood via flow cytometry is an accurate method for measuring pharmacodynamic responses to small molecule inhibitors against components of these signaling cascades. Alterations in the expression of cell surface basophil activation markers such as CD63 can be measured by flow cytometry.

These surrogate markers allow for a high throughput assessment of compound efficacy resulting in a faster failure of ineffective compounds.



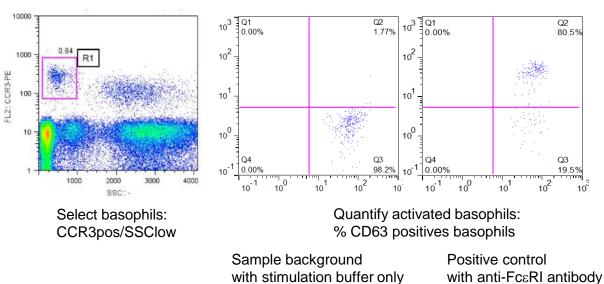
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A Validated Research Tool for Measuring Basophil Activation Status

Flow CAST[®] determines basophil activation in whole blood samples via flow cytometry.

BÜHLMANN 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 in one.

Activators: The **anti FcεRI** antibody is a highly specific monoclonal antibody which binds to the IgE receptor. The second control, tripeptide **fMLP**, activates the basophils in a non-immunological way.

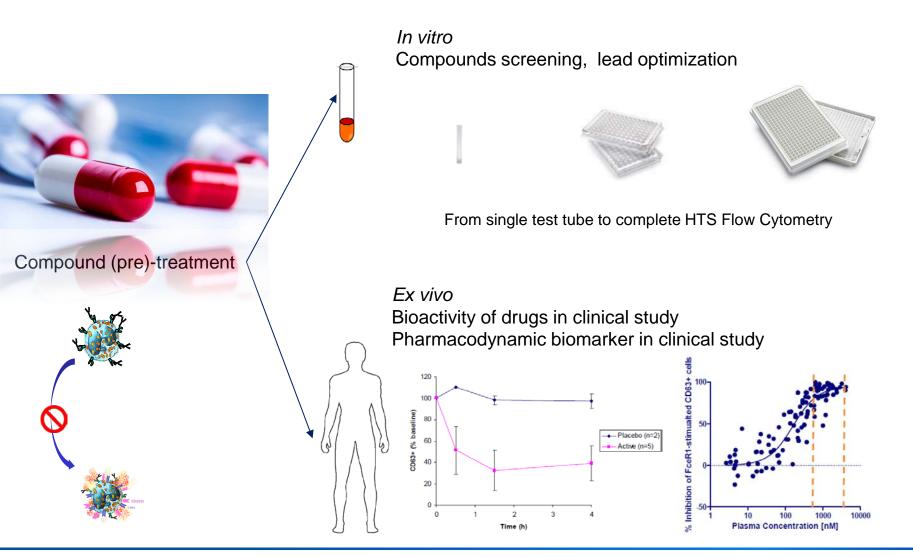


Simplified Procedure

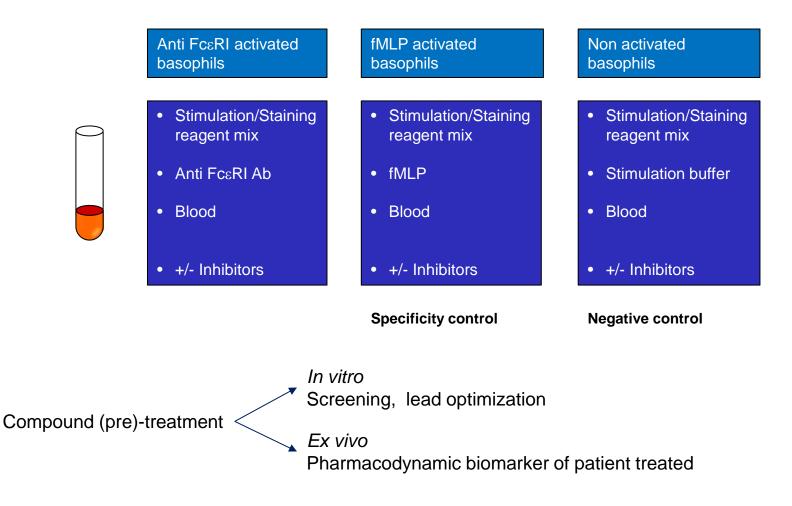
- Step 1: Collect blood with EDTA as anticoagulant
- Step 2: Stimulate and stain cells
- Step 3: Acquire on flow cytometer
- Step 4: Quantify the number of activated basophils (CCR3^{pos/}SSC^{low}/CD63^{pos})



Flow CAST[®] Support in Drug Development



Suggested Protocol for Inhibition Studies





Flow CAST[®] Adaptation to High Throughput Screening

Field Study: Gilead has adapted Flow CAST[®], a robust whole blood activation assay, 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 a 96-well plate format. 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 384well plates. This miniaturization allows for increased throughput of compound testing in lead optimization efforts and reduces the FTE resources required.



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

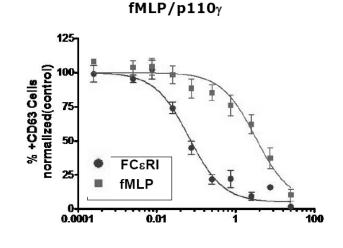


Flow CAST[®] for HTS of PI3K, BTK and SYK Inhibitors

Several pharmaceutical companies have successfully utilized basophil activation as a surrogate marker during their drug development process.

Company	Compound	Target
Gilead Sciences, Inc.	CAL-101, Idelalisib*	PI3kδ
Pharmacyclics Janssen	PCI-32765, Ibrutinib	ВТК
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



FCεRI/p110δ

CAL-101 (µM)

Anti-FccRI 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.

Blood, 13 January 2011, Volume 117, Number 2



Flow CAST[®]: Fast Results, Multiple Applications

The BÜHLMANN Flow CAST[®] assay offers an easy, fast, versatile tool for analyzing basophil activation status via flow cytometry. With the inclusion of two controls, the Flow CAST[®] assay can provide insight into drug potency and therapeutic efficacy for compounds in development.

- Validated test format
- Requires only 50 μL whole blood
- Robust, specific, sensitive, reproducible
- Results in 30 minutes
- Scalable up to 384 wells

BDC/BÜHLMANN Laboratories Experience in

- Evaluation and validation of customer specific protocol
- Implementation of the assay in ex vivo / in vitro testing workflow
- Data evaluation
- Technical and scientific support

For more information, contact

Michele Romano, PhD:

mr@buhlmannlabs.ch

Collin Shaw:

cms@buhlmannlabs.com



About BÜHLMANN/BUHLMANN Diagnostics Corp

BÜHLMANN Laboratories AG is a fully independent, medium sized and family owned Swiss company founded in 1976. They are the manufacturer of the Flow CAST[®] assay as well as many other products sold to the life science and clinical industries.

BUHLMANN Diagnostics Corp (BDC) is a North American affiliate of BÜHLMANN Laboratories AG. Located in a state-of-the-art facility in Southern New Hampshire, the BDC team collectively represents over 100 years of experience in serving both clinical and basic science research laboratories. BDC is the sole source for BÜHLMANN Products in the US and Canada.



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