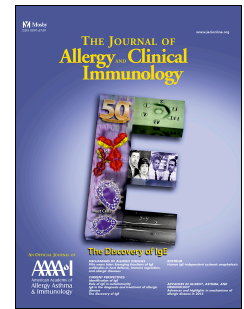


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Ibrutinib, a BTK inhibitor used for treatment of lymphoproliferative disorders, eliminates both
aeroallergen skin test and basophil activation test reactivity

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CAPSULE SUMMARY: Ibrutinib, a Bruton's tyrosine kinase (BTK) inhibitor, was shown to eliminate skin test reactivity *in vivo* and IgE-dependent basophil activation testing *ex vivo*. Blockade of the BTK pathway may represent a novel therapeutic strategy for the effective reduction of allergic reactivity.

KEY WORDS: Bruton's tyrosine kinase (BTK), basophil, mast cell, FcεRI, allergy, ibrutinib, acalabrutinib

TO THE EDITOR:

Approximately 15 million people, including 8% of children, in the United States have food allergy and are at risk of having life-threatening anaphylactic reactions¹. There is an unmet need for the prevention of such reactions. Tyrosine kinases, including Bruton's tyrosine kinase (BTK), have been shown to be critical for allergen reactivity by transducing FcεRI crosslinking signals into cellular activation and mediator release from mast cells and basophils². Pharmacyclics, Inc. together with Janssen Pharmaceuticals recently received FDA approval of ibrutinib (Imbruvica®, PCI-32765) as a selective, irreversible BTK inhibitor for the treatment of mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL) and Waldenstrom's macroglobulinemia (WM). Studies have demonstrated broad and durable activity, with excellent overall tolerability. For example, ibrutinib treatment has been shown to cause a transient increase in blood lymphocytes at the same time as a reduction in lymph node size is typically noted. Neutropenia has also been noted as a side effect in clinical trials with a frequency ranging from 15-22% of patients³⁻⁵. Regarding effects on allergic cell reactivity, Advanti et al. reported inhibition of IgE-dependent basophil activation in subjects receiving ibrutinib as determined *ex vivo* using the basophil activation test (BAT)⁶. *In vitro* studies done by MacGlashan et al. showed that ibrutinib, tested at clinically relevant concentrations, completely blocked FcεRI-dependent basophil release of histamine and leukotrienes, secretion of IL-4, and the BAT⁷. Furthermore, multiple studies have shown defects in FcεRI-dependent mast cell degranulation and cytokine production in patients with X-linked agammaglobulinemia (Xid), a disease caused by BTK deficiency, or in BTK null mice^{2, 8}. These data suggest that BTK signaling plays a critical role in basophil and mast cell IgE-dependent activation, and therefore, its safe and effective pharmacologic inhibition could result in profound anti-allergic effects.

We hypothesized that treatment with ibrutinib would attenuate allergen reactivity by interfering with IgE-dependent basophil and mast cell activation. In order to evaluate the effect of BTK inhibition on allergen reactivity in humans, we initiated a pilot study in patients who were prescribed ibrutinib as standard of care for treatment of underlying lymphoid malignancy. We performed skin testing and the BAT immediately before and during prescribed ibrutinib treatment. Using a Northwestern University IRB-approved protocol, we screened a total of 28 out of 35 patients prior to start of commercial ibrutinib treatment for treatment of MCL, CLL or WM over a period of approximately 18 months for the presence of aeroallergen sensitivity by brief questionnaire (see online repository), BAT, and aeroallergen skin testing using the Multi-Test® II device (Lincoln Diagnostics, Inc, Decatur, IL) employing eight skin test materials (saline, histamine, cat, dust mite mix, ragweed mix, mold mix, tree mix, and grass mix from ALK-Abello). We enrolled two allergic patients, both of whom were initiating treatment with ibrutinib 420 mg daily for CLL. Subject 1 was on daily intranasal fluticasone and remained on the same dosing regimen throughout the entire study. Subject 2 was not taking any anti-allergy medication. Per the IRB-approved protocol, subjects underwent repeat skin testing to the same 6 aeroallergen panel and BAT after 7 days and again after ≥ 30 days of ibrutinib treatment. At each time point, serum was collected and total and specific IgE levels were measured by Immucap®.

As shown in Figure 1, the enrolled subjects initially had a positive skin test to either cat allergen (Subject #1, Figure 1b) or ragweed (Subject #2, Figure 1b) in addition to a positive BAT (Figure 1c). Each of the two subjects was only skin test positive to a single aeroallergen, and neither became skin test positive to a new aeroallergen during the study. One week after starting ibrutinib, there was an almost complete loss of skin test reactivity as assessed by wheal and flare size, and basophil activation as measured by the BAT. In contrast, but as expected, skin test responses to histamine, and BAT responses to a non-IgE-dependent stimulus formyl-methionyl-leucyl-phenylalanine (fMLP), were unaffected (Figures 1b and c). These reduced IgE-dependent responses were sustained at 1-2 months of ibrutinib

treatment (Figure 1b and c), although one of the two subjects showed a decreased fMLP BAT response at 1-2 months. Loss of skin test reactivity and BAT responses were not due to changes in IgE levels because they were not altered during the study (Figure 1a), although these allergen-specific and total levels were below 0.35 kU/L. Whether these low levels were due, at least in part, to prior treatments received for their CLL is unknown.

In order to evaluate whether other BTK inhibitors could also inhibit basophil activation, we performed *in vitro* BAT studies to compare effects of acalabrutinib with that of ibrutinib. As shown in Figure 2, when added *in vitro* to whole blood of normal donors (using a separate IRB-approved protocol), these drugs, as expected, had no effect on fMLP-induced BAT because signaling via fMLP does not depend on tyrosine kinases. By contrast, both ibrutinib and acalabrutinib completely blocked BAT responses, with an IC_{50} of approximately 40 nM for ibrutinib and 105 nM for acalabrutinib. These IC_{50} s were higher than those previously reported^{7,9}, likely due to the fact that unlike prior studies, our experiments were performed with impure cells in whole blood in the presence of albumin, red blood cells, and other substances capable of binding to drug and thus lowering effective drug concentrations. Regardless, both ibrutinib and acalabrutinib inhibit IgE-dependent, but not IgE-independent, basophil activation at concentrations that are readily achieved with current clinical dosing regimens.

These results suggest that through inhibition of IgE-dependent basophil and mast cell activation, BTK antagonists may be able to block allergic reactions including food allergy. Although its ability to inhibit BAT *in vitro* and *ex vivo*, as well as passive cutaneous anaphylaxis in mice, has been reported⁸, to our knowledge, this is the first report of a pharmacological agent having true mast cell stabilizing activity capable of markedly inhibiting mast cell activation as measured by skin test reactivity. There are currently no treatments to prevent anaphylaxis, so agents such as BTK inhibitors have the potential to fill this void. It remains to be determined whether ibrutinib treatment will be similarly efficacious in suppressing skin test responses and BAT in poly-allergic subjects, and in subjects with higher levels of

allergen-specific IgE. However, a separate IRB-approved protocol is now in place that will allow us to expand our preliminary results to allergic subjects without lymphoproliferative disorders, to confirm the inhibitory mechanisms involved, to explore the duration of such inhibitory effects, and to determine if this strategy could be used in the management and treatment of allergic diseases including food allergy.

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FIGURE LEGENDS

Figure 1. Ibrutinib inhibits basophil and mast cell activation in two subjects prescribed ibrutinib for CLL treatment.

Demographics, BTK inhibitor clinical indication, ibrutinib dose, and IgE levels are listed for each subject (panel A). Skin test responses to cat allergen (subject 1) and ragweed (subject 2) before and during ibrutinib treatment (420 mg daily) in two patients being treated for CLL (panel B). Basophil activation responses to anti-IgE (72 and 89% positive respectively prior to starting ibrutinib) or fMLP (12 and 16% positive respectively prior to starting ibrutinib) are shown for these same two subjects (panel C).

Figure 2. Ibrutinib and acalabrutinib inhibit basophil activation *in vitro* as measured by the basophil activation test in whole blood samples obtained from healthy volunteers.

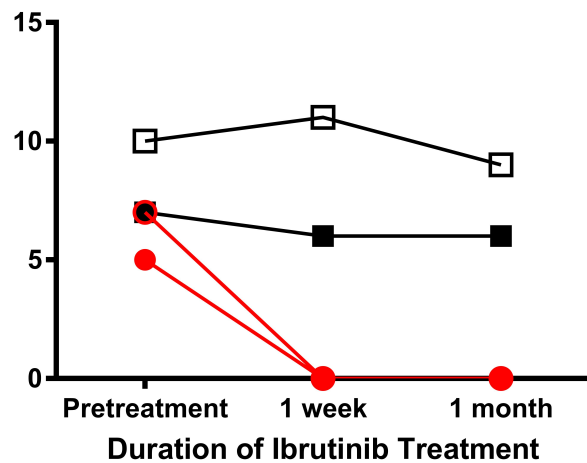
Whole blood samples were obtained from healthy volunteers and preincubated with the indicated concentrations of drug for 30 minutes, then BAT was performed with the addition of anti-IgE or fMLP. Levels of anti-IgE and fMLP BAT from which the values were normalized averaged $62 \pm 9\%$ and $26 \pm 1\%$ positive. Values represent mean $\pm$ SEM, N=3-4.

Figure 1A.

	Subject 1	Subject 2
Age (years)	75	49
Sex	Male	Male
Diagnosis	CLL	CLL
Ibrutinib Daily Dosage (mg)	420	420
Skin Test Reactivity	cat	ragweed
Total IgE pretreatment	2.2 kU/L	7.7 kU/L
Total IgE at 1 week	2.7 kU/L	4.9 kU/L
Total IgE at 1-2 months	3.3 kU/L	8.5 kU/L
Specific IgE for cat or ragweed pretreatment	<0.35 kU/L	0.27 kU/L
Specific IgE for cat or ragweed at 1 week	<0.35 kU/L	0.24 kU/L
Specific IgE for cat or ragweed at 1-2 months	<0.35 kU/L	0.31 kU/L

Skin Test Wheal/Flare Diameter (mm)

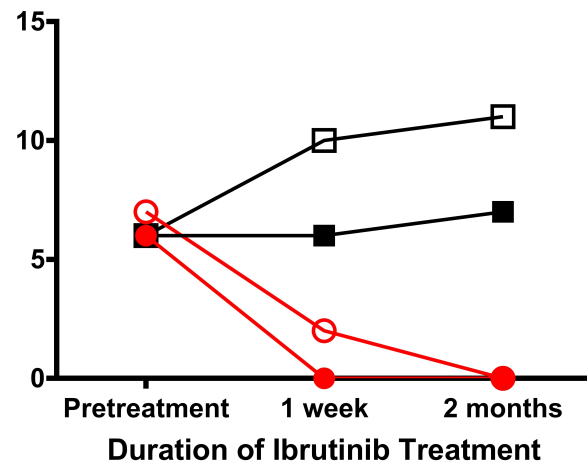
Subject 1



- Skin Test Wheal Diameter for Histamine Control
- ◻ Skin Test Flare Diameter for Histamine
- Skin Test Wheal Diameter for Cat Allergen
- Skin Test Flare Diameter for Cat Allergen

Skin Test Wheal/Flare Diameter (mm)

Subject 2



- Skin Test Wheal Diameter for Histamine Control
- ◻ Skin Test Flare Diameter for Histamine Control
- Skin Test Wheal Diameter for Ragweed Allergen
- Skin Test Flare Diameter for Ragweed Allergen

