KerrisdaleCapital

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Principia Biopharma Inc. (PRNB)

Setting the Record Straight on BTK Inhibition

In our original report on Principia Biopharma, we emphasized that BTK inhibitors have failed over and over in autoimmune diseases, which we attribute to a basic problem with their mechanism of action: their main effect is to impair the production of *new* B-cell lineages, leaving the previously established B-cell populations that cause autoimmunity largely unscathed. Alas, Sanofi – the French pharma firm that licensed Principia's BTK inhibitor SAR442168 for use in multiple sclerosis – doesn't seem to have gotten the memo. During Sanofi's recent earnings call, its global head of R&D rattled off what he views as the drug's key virtues, all the while perpetuating several myths and misconceptions. Here we respond point to point to Sanofi's paean to SAR442168, explaining why we remain unimpressed.

Sanofi statement	**Kerrisdale response**
"[W]e know from human genetics that BTK is required for B cells to function."	Humans with no BTK from birth can still produce small numbers of functional, normal B cells. Compensatory pathways (especially co-stimulation by T cells) can circumvent BTK.
"With an oral drug like '168, we can shut off but not kill the B cells. We think that modulating B cells is preferable. So we do not kill the B cells. We just put them to sleep for a while."	The notion that BTK inhibition is just a kinder, gentler equivalent to killing B cells is belied by the pattern of BTK inhibitors failing in autoimmune diseases where B-cell depletion succeeds.
"[Modulating instead of killing B cells] could potentially be highly important in clinical practice if a patient develops an infection while on treatment, and you need their antibody response to bounce back quickly."	Diminished immune-repertoire diversity caused by long-term BTK inhibition can't "bounce back quickly."
"'168 has an advantage among more advanced BTK inhibitors because its crosses the blood–brain barrier."	Other BTK inhibitors, including ibrutinib and evobrutinib, also cross the blood–brain barrier. Moreover, the reported concentration of '168 in the cerebrospinal fluid is so low that, based on Principia's own data, it would have little effect.
"Growing evidence suggests that these microglia cells are responsible for the persistent inflammation in the brains of MS patients"	Microglia clean up cellular debris and facilitate healing and remyelination; "quieting" them might harm patients.
"By inhibiting BTK, we can quiet the microglia"	BTK inhibition *in vivo* has little effect on microglia and similar cells.
"'168 appears to be an exceptionally selective BTK inhibitor."	'168 appears to be less selective than evobrutinib. *Contra* Sanofi, it's not special.

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BTK Is Not Required for B Cells to Function

Though Sanofi breezily asserts that "BTK is required for B cells to function," it's been clear for at least 22 years that that isn't true. People born with X-linked agammaglobulinemia (XLA) have defects in the gene coding for BTK and generally do not produce measurable amounts of the protein; as a result, almost no B cells successfully develop and make it out of their bone marrow. However, the key word is "almost"; the process of weeding out new B cells with defective B-cell–receptor (BCR) signaling (caused, for instance, by missing BTK) is "leaky," and some B cells manage to escape. In a study published in 1998, researchers managed to isolate and analyze these "leaky," BTK-less B cells, and found, to their surprise, that they behaved normally:

> If stimulated with anti-CD40 and IL-4, XLA B cells proliferated normally and produced significant amounts of IgE [a type of antibody]. … In addition, three of the five XLA patients studied were immunized with bacteriophage ΦX174 and produced low but detectable levels of antiphage-specific [antibodies]. Similarly, X-linked immunodeficiency mice, which carry a missense mutation in Btk, produced substantial amounts of antiphage [antibodies]. These results indicate that CD40 signaling is intact in B cells lacking demonstrable Btk, and that leaky B cells in XLA patients can proliferate, undergo isotype switching, and differentiate into specific [antibody]-producing cells.

Thus, B cells containing no BTK at all managed to proliferate and produce antibodies in response to receptor engagement, not just *in vitro* but also in live mice and humans injected with a harmless virus.

But how is this possible when BTK appears to be a key intermediary in the BCR signaling pathway? While there are likely several explanations, including enzymes upstream of BTK managing to route around it and directly phosphorylate its downstream targets, we believe the main factor is CD40. CD40 is a receptor found on B cells that allows them to be "co-stimulated" by T cells, which express a corresponding ligand called CD40L. For most B-cell–mediated immune responses *in vivo,* including both primary and secondary/recall responses, binding to a cognate antigen is not enough; T-cell "help" is also required, which means that a T cell recognizing part of the same antigen must give the B cell an extra boost via CD40/CD40L binding. This requirement for two near-simultaneous activation signals (BCR engagement plus T-cell help) mitigates against hasty and overaggressive B-cell reactions. B cells in turn elicit T-cell help by internalizing their cognate antigen and presenting peptides derived from it on their surface, ready for matching T cells to detect.

For our purposes, what matters about this system is that, even if the BCR pathway is impaired by the absence or inhibition of BTK, co-stimulation by T cells via CD40/CD40L is strong enough to overcome the impairment and allow for normal B-cell function. And *in vivo,* this T-cell help is readily available: after all, the whole evolutionary "reason" for concentrating B and T cells together in lymph nodes is to maximize the likelihood that the right B and T cells can find each other when they're needed. In many *in vitro* studies purporting to show the necessity of BTK for

B-cell function, there is no proxy for T-cell co-stimulation, and, sure enough, B cells do poorly. In real life, though, matters are different, and problems with BTK can be overcome.

This phenomenon was further documented in a 2003 study of BTK-less mice whose title straightforwardly declared that "CD40 Engagement Eliminated the Need for Bruton's Tyrosine Kinase in B Cell Receptor Signaling":

> Btk has been reported to be required for NF-κB activation and cellular proliferation resulting from BCR engagement. Our results demonstrate that this requirement is malleable, as we found that CD40 engagement provides the means to circumvent the block in BCR signaling produced by Btk mutation.

A 2005 follow-up confirmed the same pattern in healthy mice:

> We previously demonstrated that an alternate pathway for B cell signaling exists in *xid* B cells – we showed that this pathway is established by CD40L treatment and circumvents the need for Btk in BCR-induced NF-κB activation. The present work greatly expands on these initial observations, most importantly by showing that the alternate pathway is not idiosyncratic to *xid* B cells but exists in normal B cells as well.

More recently, a detailed analysis of secondary immune responses in mice showed similar results. Mice were injected under the skin with a model antigen to generate memory B cells in the skin-draining lymph nodes, then re-exposed to the antigen. Within the lymph nodes, memory B cells rapidly detected the antigen and, after interacting with nearby T cells, differentiated into antibody-producing plasma cells and proliferated. Giving the mice the BTK inhibitor ibrutinib before and during the secondary exposure to the antigen had no statistically significant effect on the number of plasma cells generated in the lymph nodes. Once again, T-cell help overcame problems with BTK and allowed for normal B-cell activity; functional BTK was not required.[1] But this in turn implies that BTK inhibitors won't have any material benefit in autoimmune disease – as the clinical data indeed suggest.

[1] Other, subtler effects on B cells may result from BTK inhibition, but not ones that are beneficial for autoimmunity. For instance, some B-cell responses, especially to polysaccharide antigens associated with certain types of bacteria, can be T-cell– independent, and BTK inhibitors may impair these, perhaps contributing to the susceptibility of patients on BTK inhibitors to opportunistic bacterial infections. Also, BTK does seem important for the germinal-center reactions that refine B-cell receptors and improve their binding affinity – but in patients with established autoimmunity, B cells have already been through one or more rounds of somatic hypermutation in germinal centers, so BTK inhibition is unlikely to have any benefit but may also contribute to infection susceptibility.

"Modulating" B Cells Is Not an Effective Substitute for Killing Them

In Sanofi's telling, the use of BTK inhibitors as an alternative to B-cell depletion sounds like a no-brainer: "we can shut off but not kill the B cells…We just put them to sleep for a while." Indeed, similar thinking seems to have motivated many pharmaceutical firms to pursue BTK inhibitors in diseases already effectively treated by B-cell depletion (like Principia itself in pemphigus). If killing B cells works, then surely shutting them off but letting them survive should work just as well, and perhaps more safely.

The problem is that, in autoimmune disease, this approach has generally failed. In the table below, we summarize the pattern of comparative effectiveness: while anti-CD20 antibodies that cause B-cell death frequently succeed, BTK inhibitors in the same conditions keep failing. (See our original report for more detailed discussions of the failed BTK inhibitors.)

Disease	Anti-CD20 antibodies	BTK inhibitors
rheumatoid arthritis	success (rituximab approved in 2006)	• spebrutinib: failure • acalabrutinib: failure • poseltinib: failure • fenebrutinib: mixed results • evobrutinib: failure • branebrutinib: failure
lupus	success (obinutuzumab in a recent Phase 2, rituximab in real-world off-label use (Sarsour et al. 2019))	fenebrutinib: failure
chronic spontaneous urticaria	possible success (2018 *n*=1 study: "8-month remission of refractory CSU following the use of rituximab"; cites four other published cases of rituximab use, of which three were "successful")	fenebrutinib: failure (a December 2019 update on ClinicalTrials.gov, still visible in Google's cache, noted that the Phase 2 trial was "Terminated (After interim analysis, the totality of data did not meet sponsor's pre-specified criteria to continue clinical development of fenebrutinib for CSU.)")
Sjögren's syndrome	"Although two large RCTs did not meet their primary endpoint, several beneficial clinical effects of treatment [with rituximab] have been shown" (Verstappen et al. 2017); continued off-label use (Sarsour et al. 2019)	tirabrutinib: failure

granulomatosis with polyangiitis	success (rituximab approved in 2011)	likely failure (in a recent *in vitro* study, cell cultures derived from patients with the disease and treated with a BTK inhibitor continued to produce pathological autoantibodies, and the difference in autoantibody production between BTK-inhibited and untreated cell cultures was not statistically significant)

Source: Kerrisdale analysis

If BTK inhibitors simply "shut off" B cells as Sanofi says, then they should replicate the success of B-cell–depleting anti-CD20 antibodies in autoimmune disease – but clearly they don't. Instead, as we explained above, BTK inhibitors have a far more modest effect on established B cells; they're no substitute for true depletion.

Immune Systems Diminished by BTK Inhibition Can't "Bounce Back Quickly"

Sanofi proposes that "modulating" B cells with a BTK inhibitor rather than killing them with an anti-CD20 antibody is safer because "if a patient develops an infection while on treatment, and you need their antibody response to bounce back quickly," you can just stop giving them the drug. However, this blithe statement betrays a misunderstanding of the long-term effects of BTK inhibition. By interfering with B-cell–receptor signaling in a context (development within the bone marrow) in which it typically cannot be bypassed via T-cell help, BTK inhibition blocks the creation of new B-cell lineages, leaving patients more and more reliant on pre-existing memory B cells.

Accordingly, data from patients treated with the BTK inhibitor spebrutinib for just four weeks showed that the average number of transitional B cells (those that have just migrated out of the bone marrow and into the peripheral blood) declined by 51%. Eventually, as pre-existing populations of so-called mature-naïve (or "virgin") B cells die off and new transitional B cells do not replace them, the overall mature-naïve population shrinks, as seen in the evobrutinib Phase 2 trial in MS, during which the average number of mature-naïve B cells among patients on 75 mg of evobrutinib declined ~30% over 48 weeks (Montalban et al. 2019, Table 2). Similar results have been reported with long-term ibrutinib treatment: researchers observed "impaired replenishment of the normal B cell pool with naïve B cells," leading eventually to "declining numbers of B cells in ibrutinib treated patients" and suggesting that "ibrutinib treated patients may have impaired responses toward neoantigens, and consequently responses towards vaccines may be dampened."

Imagine, then, a salient case: infection with a novel virus like COVID-19. Healthy people constantly generate new B cells whose receptors are specified by genes that are randomly reshuffled with each new cell, enabling them to bind and respond to unique antigens. Usually these new receptors prove to be irrelevant and the cells die off; new lineages then arise in their place, and the cycle continues. With a highly diverse, constantly evolving repertoire of B-cell receptors (and the antibodies they give rise to), people will often get "lucky" and turn out to have a B cell with a receptor equipped to handle a never-before-seen virus like COVID-19. But long-term BTK inhibition sharply reduces the population of new, unique B cells that might end up helping, leaving patients unusually vulnerable. Even if patients stop taking BTK inhibitors after being infected, as Sanofi suggests, their immune systems can't simply "bounce back"; it will take time to replenish their repertoires, and, because the process of generating unique new B-cell receptors is completely random, there's no reason to expect that the right kind of B cell will miraculously appear just when it is needed most. As we explained in our original report, this specific form of immunosuppression likely contributes to the high rate of serious opportunistic infection associated with BTK inhibitors.

The "Brain-Penetrant BTK Inhibitor" Isn't Very Brain-Penetrant

Sanofi and Principia repeatedly emphasize that '168 is "brain-penetrant"; the phrase is even included in the headline of Sanofi's press release about '168's Phase 2 trial results. Sanofi's R&D chief said explicitly during the last earnings call that "'168 has an advantage among more advanced BTK inhibitors because it crosses the blood–brain barrier. Remember that we established the CNS [central nervous system] exposure in Phase 1 with receptor occupancy in CNS pharmacology studies."

But what did Sanofi and Principia actually establish? In the Phase 1 data presented in 2019, four human subjects took 120 mg of '168 (the highest dose tested); two hours later, the average (technically, geometric mean) concentration of the drug in their cerebrospinal fluid (CSF) was 1.87 nanograms per milliliter. But is that a lot or a little? Digging around in the World Health Organization's most recent list of proposed generic drug names, we located the chemical formula for '168 – set to be officially renamed "tolebrutinib"[2] – and could thus calculate its molecular weight. With this value in hand (455.5 grams per mole), we can convert the CSF concentration of 1.87 ng/mL into more meaningful molar units: it equates to 4.1 nM. But here's the problem: **even according to Principia's own data, that concentration is too low to have much effect in real life**. For example, Principia has reported that the IC_{50} of '168 with respect to reducing the activation of B cells in whole blood is 10 nM, while the IC_{50} with respect to reducing the production of TNFα by microglia is a whopping 157 nM. We would quibble with the relevance of these stylized *in vitro* tests to actual multiple sclerosis in humans, but the main

[2] We confirmed that tolebrutinib is the same compound as '168 because its listed CAS (Chemical Abstract Service) number, 1971920-73-6, is the same one used in EU Clinical Trials Register entries for the '168 Phase 2 trial.

point is this: while Sanofi and Principia crow about hitting a 4nM concentration in the CSF, the concentrations that they elsewhere say are necessary to achieve significant effects are much higher – almost 40x higher in the case of microglia. In short, all this talk of '168 being "brain-penetrant" rings hollow. The drug does make its way into the cerebrospinal fluid, but not in large amounts, especially if the goal is to modulate microglia.

Nor is it clear that '168 "has an advantage among more advanced BTK inhibitors" when it comes to entering the central nervous system. It is well documented that the first-generation BTK inhibitor ibrutinib crosses the blood–brain barrier robustly in mice (Goldwirt et al. 2018) and humans (Dunleavy et al. 2015, Bernard et al. 2015). As for evobrutinib, the BTK inhibitor from Merck KGaA that is also being tested in multiple sclerosis, while we lack data for humans, we do have results in mice showing that the drug crosses the blood–brain barrier, as assessed not merely in the CSF but directly in the brain. In Merck's words, "while BTK occupancy in the brain was initially low [it] reached high levels at the end of the experiment" after repeat dosing, ultimately averaging ~80%. Indeed, looking at drug concentrations within the brain (as opposed to BTK occupancy), evobrutinib reached ~4.5 ng/mL two hours after the first dose, higher than what was seen with '168 in human CSF, both in absolute and molar terms (10 nM for evobrutinib).[3] By the end of the experiment, after repeat dosing, the concentration of evobrutinib in the brain increased to 5 to 12 ng/mL (12 to 29 nM), depending on the dose consumed – again, substantially higher than '168's much ballyhooed 1.87 ng/mL in human CSF. Though it remains uncertain whether these mouse data will hold up in humans, it is reasonable to expect that they will; after all, for both ibrutinib and '168, the ability to cross the blood–brain barrier in mice was replicated in humans.[4] Thus'168's "advantage" in brain penetration, as touted by Sanofi and Principia, is likely an illusion.

We also dispute Sanofi's claim in its February 6th press release that '168 "may be the first B-cell-targeted MS therapy that not only inhibits the peripheral immune system, but also crosses the blood-brain barrier to suppress immune cells that have migrated into the brain." The most effective[5] existing MS treatment, the anti-CD20 antibody ocrelizumab, is a B-cell–targeted therapy, as are its older cousin rituximab and its likely future competitor ofatumumab. These molecules, which cause the death of mature B cells, are large and do not easily cross the blood–brain barrier.

However, this impermeability is not absolute. Just last week, a group of researchers funded by Novartis presented mouse data showing that anti-CD20 antibodies, whether injected intravenously or subcutaneously, achieved "significant uptake" throughout the central nervous system, including in the spinal cord, cerebellum, and frontal cortex. In humans, a Phase 2 study of intravenous rituximab in primary CNS lymphoma showed that rituximab "was detected in the CSF of all patients in whom it was assayed," though only at concentrations that "fell between

[3] Evobrutinib's molecular weight is 429.5 g/mol, slightly lower than '168's 455.5, so equal concentrations of the two drugs in ng/mL terms correspond to higher evobrutinib doses in more relevant molar terms.
[4] For '168, mouse data for BTK occupancy in brain and spinal cord were disclosed in 2017, though only at the high dose of 5 mg/kg (higher than then 120mg total dose tested for CSF penetration in the Phase 1 trial).
[5] See McCool et al. 2019.

1% and 0.1% of serum levels." In five out of seven patients, the peak concentration exceeded 1,000 ng/mL. Other research has shown that even at concentrations that are orders of magnitude lower, ranging from 1 to 82 ng/mL depending on the details of the assay, rituximab can achieve most of its maximum cell-killing potential. In other words, while it's true that anti-CD20 antibodies don't penetrate the central nervous system very effectively, enough molecules make it through that they could plausibly make a dent in the local population of infiltrating B cells, perhaps contributing to the efficacy of these treatments in MS. '168's ability to achieve a 1.87 ng/mL concentration in the CSF hardly makes it look special in this context. (Since there is strong "evidence of bidirectional trafficking of distinct B cell clones (both into and out of the CNS)" in multiple sclerosis (Li et al. 2018), it's unclear how much the ability to penetrate the CNS itself actually matters for treatment efficacy, but we will grant the point for the sake of argument.)

In sum, what Sanofi calls a "brain-penetrant BTK inhibitor" doesn't appear to be very "brain-penetrant" at all, and there is little reason to see it as uniquely potent in this regard relative to other B-cell–targeted therapies in MS.

Microglia Are Not a Good Target for MS Therapy

Microglia are the resident immune cells of the brain, closely resembling macrophages and acting to consume and dispose of foreign matter and cellular debris. Though Sanofi points to "growing evidence" that these cells "are responsible for the persistent inflammation in the brains of MS patients," this concept remains speculative, controversial, and, we believe, dangerous. In the wake of autoimmune attacks on the central nervous system (primarily conducted by B cells, antibodies, T cells, and the complement system), microglia take action to eliminate damaged cells, bits of myelin, and other rubbish, thereby clearing the way for healing and remyelination. Whether they also "misbehave" and exacerbate disease is unproven; indeed, it's possible they act as both "good guys" and "bad guys," at times doing harm and at times repairing it. In light of this uncertainty, treatment aimed at simply shutting down microglia seems at least as likely to hurt patients as to help them. In the unusually impassioned words of a recent study:

> Whether microglia have a role in cortical damage is extremely important for the MS community. If this concept is accepted, development of therapies to reduce microglial activation could have devastating consequences. Microglia are in the brain to protect it and they do so through several mechanisms. Phagocytic removal of debris is fundamental to slowing the progression of MS and other neurodegenerative diseases. Many brains in our MS autopsy cohort have lost over 30% of their volume and include significant cortical thinning. Amazingly, little cellular debris can be detected in these brains, which is indicative of the efficiency of microglia in removing cellular debris. Inhibition of microglial activation and suppression of debris removal would accelerate, not ameliorate, disease progression.

> In summary, we conclude that there is no convincing evidence supporting the concept that microglia are responsible for the cortical damage in MS. Microglia respond to cortical damage, rather than causing it.

Perhaps fortunately for patients, though, BTK inhibition is unlikely to have the broad effects on microglia that Sanofi and Principia claim.

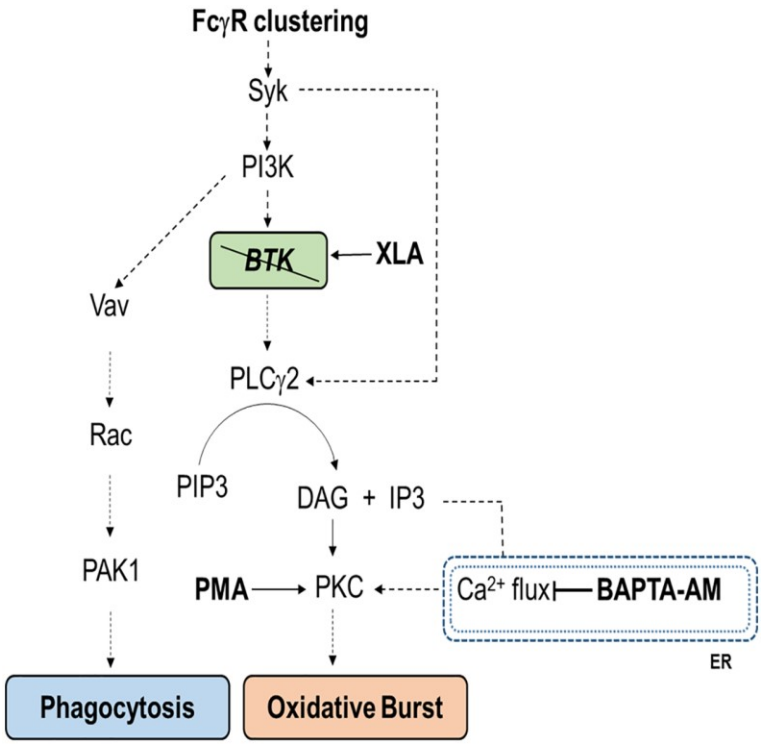
BTK Inhibition Doesn't "Quiet the Microglia"

BTK plays its most important role in B cells – particularly in the development of new B-cell lineages in the bone marrow – but it's true that the protein is present in many types of immune cells, typically acting as an intermediary in a receptor-driven signaling pathway (just one link in a long chain). Nonetheless, it's striking that, in humans with genetic mutations that prevent the production of BTK, the only glaring deficiency is in B cells and the antibodies they give rise to; BTK's other functions seem to matter much less. In the words of one group of researchers, "Interestingly, although Btk expression is abundant in neutrophils as well as many other cells of hematopoietic lineage, patients maintained on sufficient Ig therapy [*i.e. intravenous infusions of antibodies derived from healthy donors*] are generally healthy, suggesting that Btk is either dispensable outside the B-cell compartment, and/or that compensatory kinases maintain normal functions in other cells…[BTK-deficient] patients maintained on adequate Ig-replacement therapy do not have infections suggestive of innate immune defects."

Nonetheless, Principia has pointed out that microglia express BTK and has contended that the protein "is required for FcγR signaling"; according to *in vitro* data presented by the company, its BTK inhibitor '168 "inhibits microglial FcγR activation through durable occupancy of BTK." FcγR is a class of receptors that enable a variety of immune cell types, including microglia, to bind to antibodies as a prelude to ingesting whatever the antibodies are attached to (phagocytosis) or taking other defensive actions, like secreting cytokines. Indeed, one of the mechanisms by which anti-CD20 therapies actually result in B-cell death is via antibody-dependent cellular phagocytosis (ADCP) mediated by FcγR: phagocytic cells use their Fcγ receptors to detect the anti-CD20 antibodies attached to the surface of B cells, then consume the cells. In MS, Principia and Sanofi's theory seems to be that inhibiting BTK will, by means of blocking FcγR signaling, prevent microglia from consuming myelin and other components of the central nervous system that may be tagged by autoantibodies.

But direct investigation of monocytes (phagocytes closely related to microglia) derived from human patients with XLA (the condition caused by mutations leading to the absence of BTK) has shown that "BTK is not required for migration, phagocytosis and the production of reactive oxygen species (ROS) following engagement of FC gamma receptors"; XLA monocytes, despite being totally deficient in BTK, behave normally. The researchers concluded that this BTK-independence resulted from two factors: first, the ordinary pathway for FcγR-mediated *phagocytosis* doesn't run through BTK; and second, though the ordinary pathway for other FcγR-mediated effects does involve BTK, enzymes upstream of BTK can route around it to

achieve the same downstream results. The concept is illustrated in the following diagram from Cavaliere et al. 2017, showing how, in the absence of BTK, the kinase Syk is capable of directly phosphorylating PLCγ2 (which BTK would normally be responsible for):



Source: Cavaliere et al. 2017

Confirming these findings, Ren et al. showed that the BTK inhibitor ibrutinib "did not affect monocyte FcγR-mediated phagocytosis, even at concentrations higher than those achieved physiologically." While these authors did find that ibrutinib "suppressed FcγR-mediated cytokine production" (possibly through off-target effects on kinases other than BTK, as discussed further below), this suppression was readily overcome by exposing the monocytes to the cytokine interferon gamma or co-culturing them with natural-killer cells – in essence, putting them in a more physiologically realistic milieu rather than leaving them isolated in a Petri dish. Similarly, researchers who assessed macrophages' consumption of B cells tagged with anti-CD20 antibodies found that BTK inhibition with acalabrutinib (a more selective drug than ibrutinib) had little to no effect.

Perhaps the most convincing evidence that BTK inhibition doesn't materially impact FcγR-mediated phagocytosis *in vivo* comes from clinical trials of ibrutinib added to rituximab. If BTK were really "required for FcγR signaling," as Principia has said, then inhibiting BTK with ibrutinib would stop macrophages from ingesting B cells tagged by rituximab; their Fcγ receptors might bind to rituximab, but the resulting signal would fizzle out, preventing downstream effects like

phagocytosis.[6] As a result, rituximab would attach to B cells but not lead to their death, rendering it impotent. Indeed, years ago, some researchers raised these exact concerns about potential antagonism between BTK inhibition and B-cell depletion. In reality, though, adding ibrutinib to rituximab in the B-cell malignancy known as Waldenström's macroglobulinemia did not impair its B-cell–killing impact; quite to the contrary, it led to sharply lower antibody levels and a much higher rate of progression-free survival. This outcome makes perfect sense under our analytical framework: in a disease characterized by overproduction and aberrant proliferation of B cells, periodically killing off most of those cells should help, but continuously blocking *new* B-cell production via BTK inhibition should be even better. On the other hand, if BTK truly were crucial to FcγR-mediated phagocytosis, then inhibiting it would also inhibit the B-cell depletion caused by rituximab – a theory that has now been falsified in the clinic.

Overall, then, research involving both genetic BTK deficiency and pharmaceutical BTK inhibition has shown that, *in vivo,* Fcγ receptors like those found in microglia can continue to function independent of BTK; the notion advanced by Principia that BTK inhibition can block "the microglial FcγR pathway in antibody-mediated demyelination" doesn't stand up to scrutiny.

While most of the analysis of the importance of BTK to FcγR signaling has involved phagocytes other than microglia, direct examination of microglia has likewise shown that, under BTK inhibition, microglia largely keep operating as usual. In one recent study, microglia treated *in vitro* with a BTK inhibitor and set loose on fragments of synapses continued to consume them readily, with only a modest difference observed between the BTK-inhibited microglia and their untreated peers. BTK-inhibited microglia also continued to migrate normally in response to perceived injury and continued to produce the same amounts of various cytokines in response to stimulation with the bacterial molecule LPS. These cells are hardly "quiet."

Subjecting Microglia to BTK Inhibition May Make Patients Specifically Vulnerable to Fungal Infections of the Brain

The one area in which BTK inhibition did "quiet" the microglia in the study just discussed has no relevance to autoimmunity or multiple sclerosis but may explain some of the alarming real-world dangers of BTK inhibition. Microglia treated with a BTK inhibitor, though unscathed along most dimensions, *were* significantly less likely to consume one target in particular: zymosan, a substance made from yeast cell walls. Zymosan phagocytosis is primarily mediated not by FcγR but by a different, fungus-specific receptor called Dectin-1, and BTK plays an important role in the Dectin-1 signaling pathway. Similarly, another study from a different group of researchers suggested that ibrutinib reduces the ability of human macrophages to combat the fungus *Aspergillus fumigatus,* an organism that is ubiquitous but generally harmless.

[6] As VanDerMeid et al. 2018 notes, "the primary *in vivo* mechanism of cellular cytotoxicity of circulating CD20 mAb-opsonized cells in mice is ADCP by fixed macrophages in the liver." Based on several lines of evidence, the authors "propose that ADCP is [also] likely to be the major cytotoxic mechanism for CD20 mAbs in humans," though other mechanisms exist.

In the clinic, invasive fungal infections, especially those caused by *Aspergillus fumigatus,* are a well documented side effect of BTK inhibition with ibrutinib, often occurring in patients who "lacked classical clinical risk factors for fungal infection" and frequently resulting in death. (Similar cases have also occurred with the newer, more selective BTK inhibitor acalabrutinib, suggesting that BTK inhibition, not other off-target effects of ibrutinib, are to blame.) In one retrospective survey of 33 cases of invasive fungal infections in patients receiving ibrutinib, "[i]nvasive aspergillosis…was overrepresented (27/33) and **was associated with a cerebral localization in 40% of the cases**. Remarkably, most cases of invasive fungal infections occurred with a median of 3 months after starting ibrutinib." Other studies have reported similar cases of fungal infection of the brain during ibrutinib treatment, concluding that "[c]entral nervous system mycoses [i.e. fungal infections] should be considered as a potential complication of ibrutinib." Indeed, a recent review of many published cases of invasive fungal infections associated with ibrutinib, including 11 that infiltrated the central nervous system, went so far as to issue a "call for action," urging drug developers to do a better job of assessing the risk of fungal infection preclinically.

The *in vitro* research showing that BTK inhibition impairs fungus detection by the Dectin-1 receptor, including within microglia, aligns perfectly with the clinical experience of alarming rates of fungal infection, especially within the brain, in patients on BTK inhibitors. Thus, while a great deal of evidence shows that inhibited or absent BTK doesn't *broadly* suppress the activities of cells like microglia, we believe that it may specifically weaken their ability to fend off fungi, making patients vulnerable to potentially devastating infections without any positive impact on unrelated conditions like multiple sclerosis.

Relative to the Competition, Principia's Drug Is Not "Exceptionally Selective"

Sanofi management characterized SAR442168's selectivity as its "final point of differentiation," one that is "hugely important":

> '168 appears to be an exceptionally selective BTK inhibitor. And as a consequence, we did not see in Phase 1 the off-target safety issues experienced by some competitor molecules.

To be fair, '168 likely *is* more selective than the first-generation BTK inhibitor ibrutinib, which binds not only to BTK but to several other enzymes with similar structures, potentially causing side effects like rash and diarrhea and inspiring the push for less promiscuous second-generation inhibitors like zanubrutinib and acalabrutinib.

However, '168 isn't really competing with ibrutinib. Instead, it's chasing Merck KGaA's evobrutinib, which has already entered Phase 3 trials for multiple sclerosis – and '168 appears

to be *less* selective than evobrutinib. In 2017, Principia presented data showing that, when the drug "was cross-screened for its ability to inhibit the enzymatic activity of 250 protein kinases at a concentration of 1μM," it caused >90% inhibition of only 12 out of those 250 kinases. Meanwhile, when Merck researchers subjected evobrutinib to the same test (but with 267 non-BTK kinases rather than 250), they found that evobrutinib caused >90% inhibition of just **one** non-BTK kinase (and exactly 90% inhibition for a second) (Haselmayer et al. 2019). In other words, while '168 materially inhibits at least 12 other kinases besides BTK, evobrutinib has similar off-target effects on just one or two other kinases. If Sanofi is correct that more selective BTK inhibitors can expect fewer "off-target safety issues," then the safety advantage belongs to Merck – not to Sanofi and Principia.

Conclusion

We'll all have to wait for the Phase 3 results expected in 2024 and 2025 before we know for certain whether Sanofi and Principia's BTK inhibitor works in multiple sclerosis. In the meantime, though, the companies' numerous errors and misstatements should cast doubt on the trustworthiness of the reasoning that got them to this point. It certainly wouldn't be the first time that Big Pharma has blundered.

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