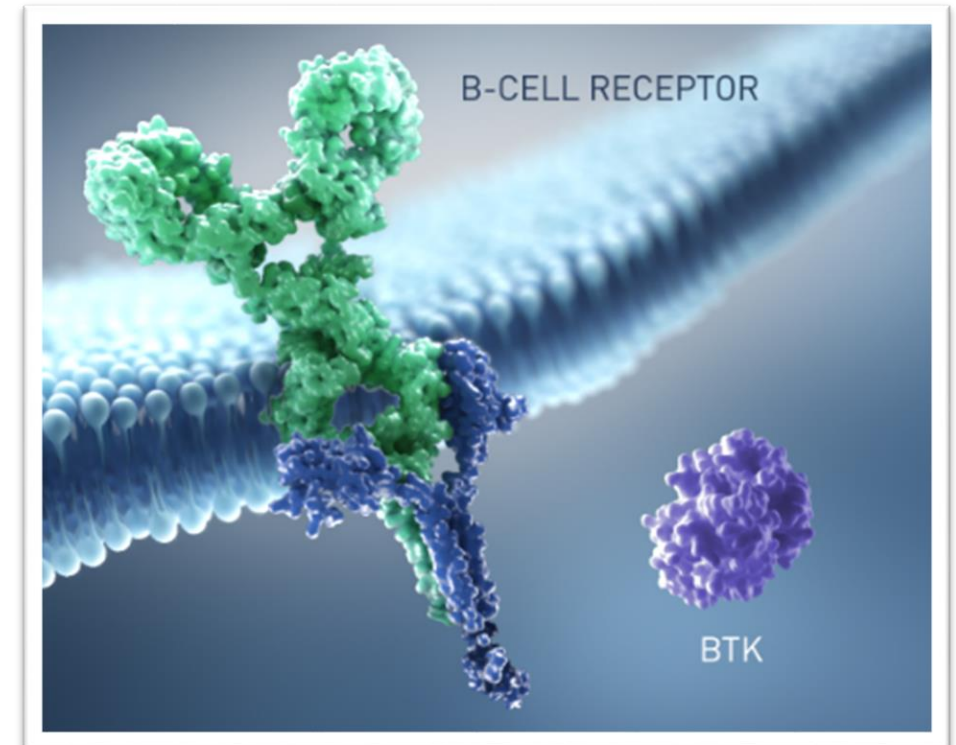


Understanding Non-Covalent (Reversible) Bruton Tyrosine Kinase (BTK) Inhibition



Activation of Bruton Tyrosine Kinase (BTK) Drives Proliferation of Malignant B-Cells^{1,2}

- Bruton tyrosine kinase (BTK) is a key component of B-cell development and survival acting via the B-cell receptor (BCR) signaling pathway^{1,3,4}
- Activation of the BTK pathway plays an important role in the pathophysiology of many B-cell lymphomas^{1,2,4}



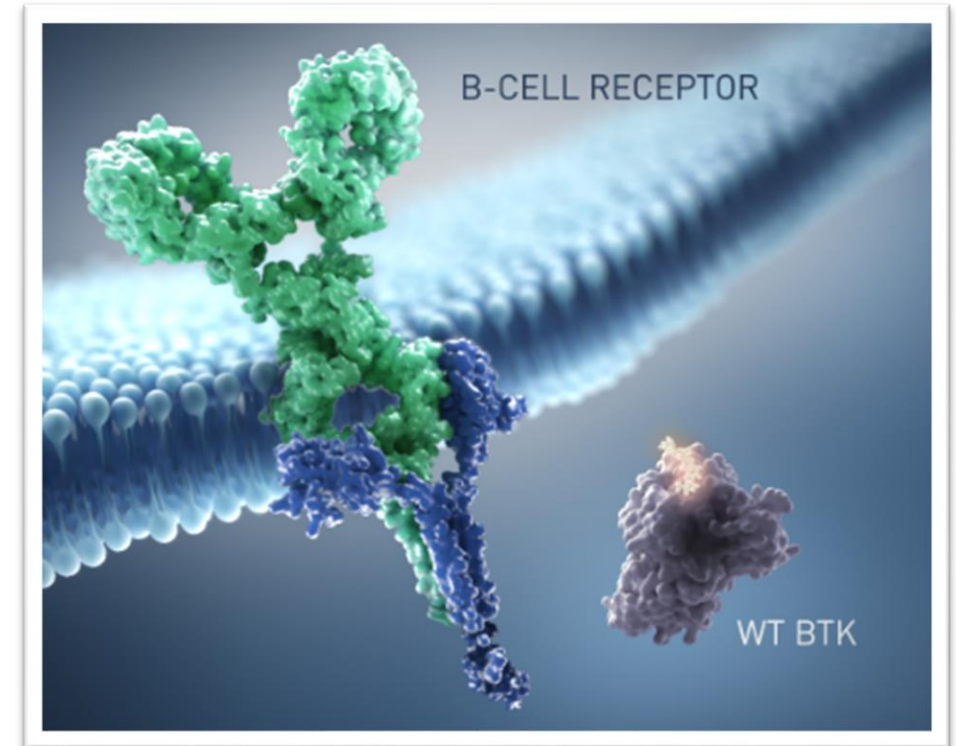
BTK is a key component of B-cell development and survival acting via the BCR signaling pathway (shown here is a schematic representation of BCR [green and blue] and BTK [purple])^{3,5,6}

BCR=B-cell receptor; BTK=Bruton tyrosine kinase.

1. Hendriks RW, et al. *Nat Rev Cancer*. 2014;14(4):219-232; 2. Pal Singh S, et al. *Mol Cancer*. 2018;17(1):57; 3. Estupiñán HY, et al. *Front Cell Dev Biol*. 2021;9:630942; 4. Gu D, et al. *J Hematol Oncol*. 2021;14(1):40; 5. Treanor B, et al. *Immunology*. 2012;136(1):21-27; 6. Friess MD, et al. *Front Immunol*. 2018;9:2947.

Inhibition of BTK Blocks BCR Signaling¹⁻³

- Inhibition of BTK disrupts ATP binding, blocking downstream enzyme phosphorylation, autophosphorylation, and activation^{1,2}
 - Blockade of BCR signaling reduces B-cell survival, proliferation, and migration^{2,3}



Inhibition of BTK blocks BCR signaling and reduces B-cell survival, proliferation, and migration (shown here is a schematic representation of BCR [green and blue], BTK [gray], and BTK inhibitor [yellow gold])¹⁻⁴

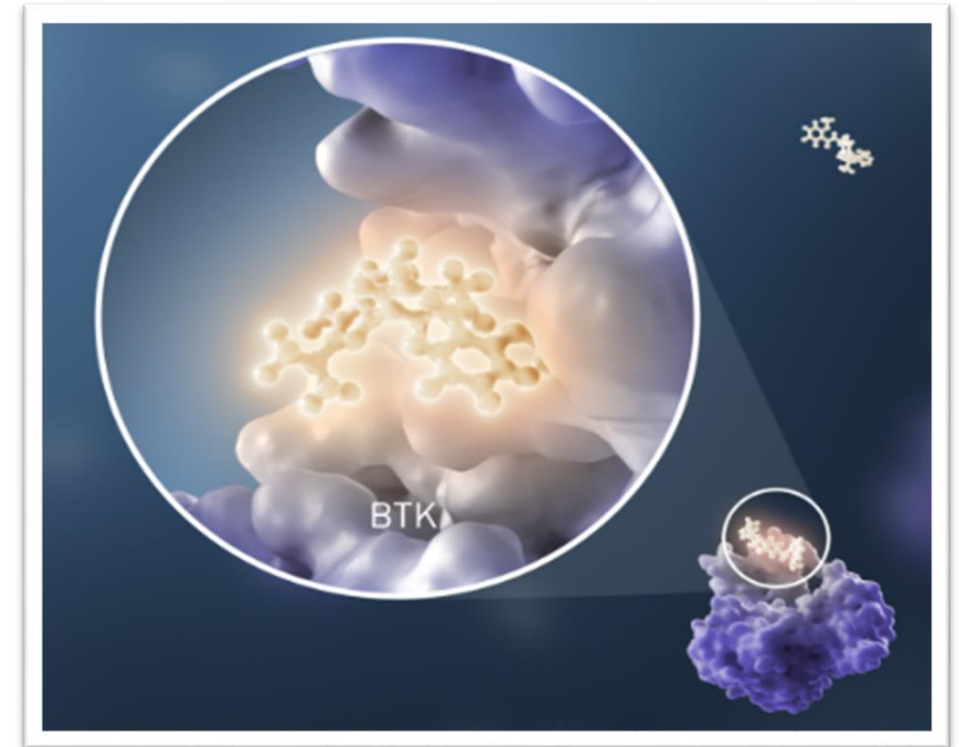
ATP=adenosine triphosphate; BCR=B-cell receptor; BTK=Bruton tyrosine kinase; WT=wild-type.

1. Estupiñán HY, et al. *Front Cell Dev Biol.* 2021;9:630942; 2. Gu D, et al. *J Hematol Oncol.* 2021;14(1):40; 3. Hendriks RW, et al. *Nat Rev Cancer.* 2014;14(4):219-232; 4. Treanor B, et al. *Immunology.* 2012;136(1):21-27.

Covalent and Non-covalent (Reversible) BTK Inhibition Differ in their Modes of Binding¹⁻⁴

BTK inhibition is primarily classified into two types^{1,2}:

- The first type is **covalent inhibition**:
 - Characterized by an irreversible covalent bond with the cysteine 481 (C481) residue present in the ATP-binding site of the BTK kinase domain¹⁻⁴
 - Blocks the ATP-binding site, inhibits autophosphorylation of BTK, and results in irreversible inhibition of kinase activity³⁻⁶



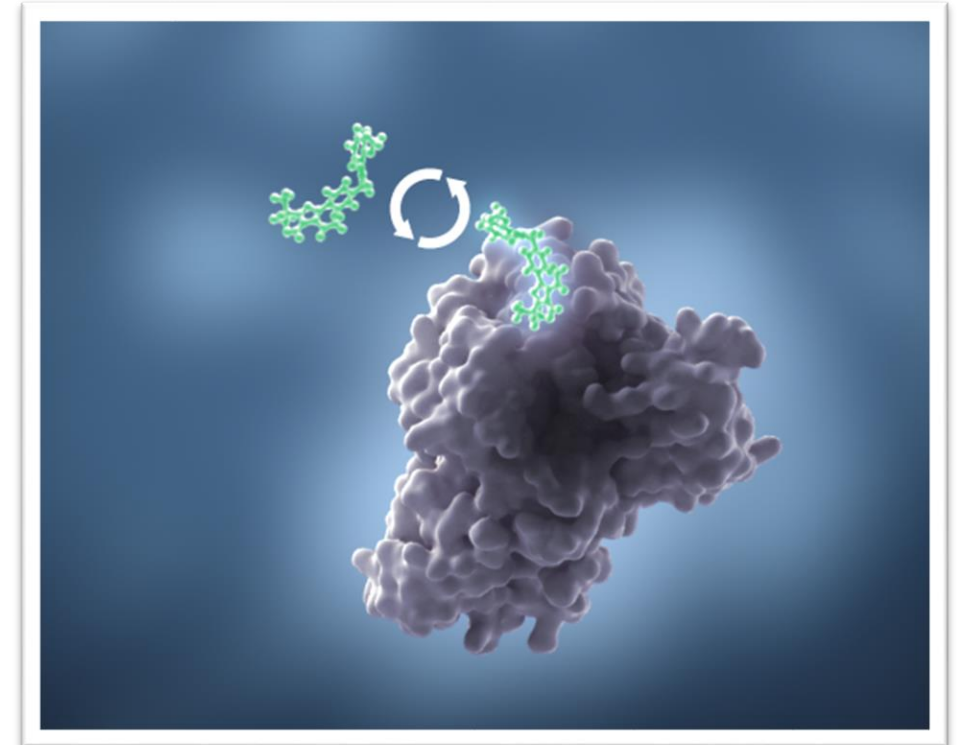
Covalent (irreversible) inhibition includes formation of a permanent bond with the target (shown here is a schematic representation of covalent inhibition [yellow gold])³⁻⁵

ATP=adenosine triphosphate; BTK=Bruton tyrosine kinase; C481=cysteine 481.

1. Tasso B, et al. *Molecules*. 2021;26(23):7411; 2. Brullo C, et al. *Int J Mol Sci*. 2021;22(14):7641; 3. Gu D, et al. *J Hematol Oncol*. 2021;14(1):40; 4. Tambaro FP, et al. *J Exp Pharmacol*. 2021;13:923-935; 5. Aljoundi A, et al. *Protein J*. 2020;39(2):97-105; 6. Gomez E., et al. *Blood*. 2023;142(1):62-72.

Covalent and Non-covalent (Reversible) BTK Inhibition Differ in their Modes of Binding¹⁻³

- The second type is **non-covalent (reversible) inhibition**¹⁻⁴:
 - Characterized by non-covalent binding interactions to BTK, in which kinase activity is inhibited by blocking ATP binding^{3,4}
 - Does not require binding to BTK C481⁴
- Blockade of BTK with non-covalent inhibition provides a different way to regulate BCR signaling¹⁻⁴



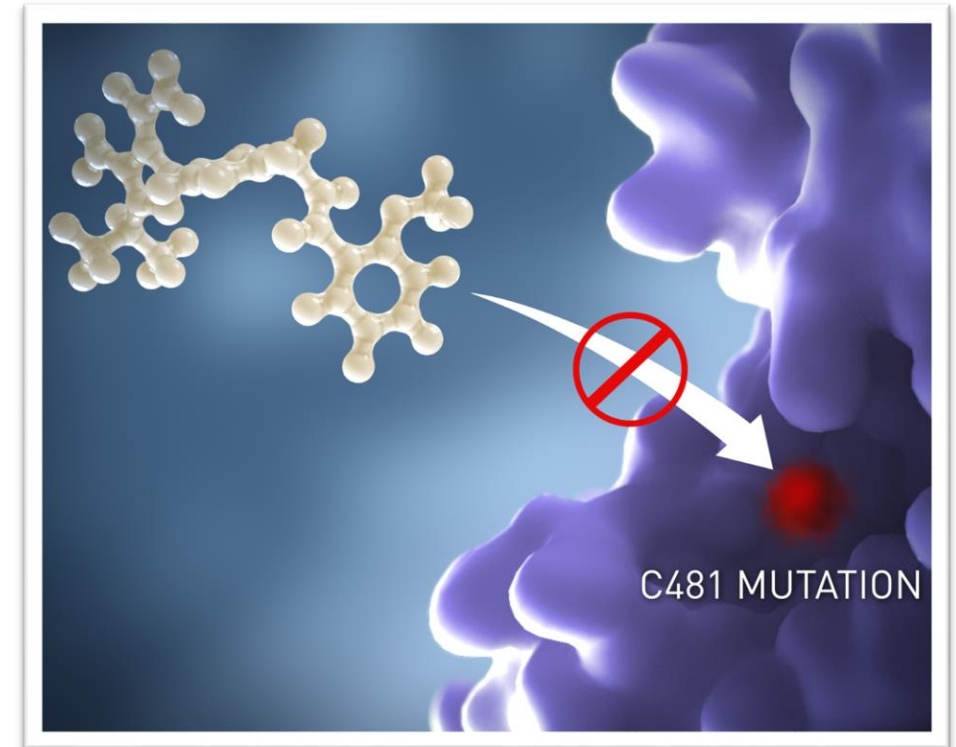
Non-covalent inhibition involves interacting with the target constantly through binding, unbinding, and rebinding (shown here is a schematic representation of non-covalent inhibition)³⁻⁵

ATP=adenosine triphosphate; BCR=B cell receptor; BTK=Bruton tyrosine kinase.

1. Tasso B, et al. *Molecules*. 2021;26(23):7411; 2. Brullo C, et al. *Int J Mol Sci*. 2021;22(14):7641; 3. Tambaro FP, et al. *J Exp Pharmacol*. 2021;13:923-935; 4. Gu D, et al. *J Hematol Oncol*. 2021;14(1):40; 5. Aljoundi A, et al. *Protein J*. 2020;39(2):97-105.

BTK Mutations Can Diminish Covalent Inhibition Against BTK Blockade¹⁻³

- The cysteine 481 residue is pivotal for BTK binding with covalent inhibition. Hence, BTK C481 mutations can greatly diminish the ability of covalent inhibition to block BTK and downstream signaling¹⁻³
- If B-cell signaling restarts and disease progresses, retreatment with covalent BTK inhibition may not be recommended^{4,5}
- Therefore, BTK blockade that appears diminished to further covalent inhibition highlights a potential unmet need^{4,5}



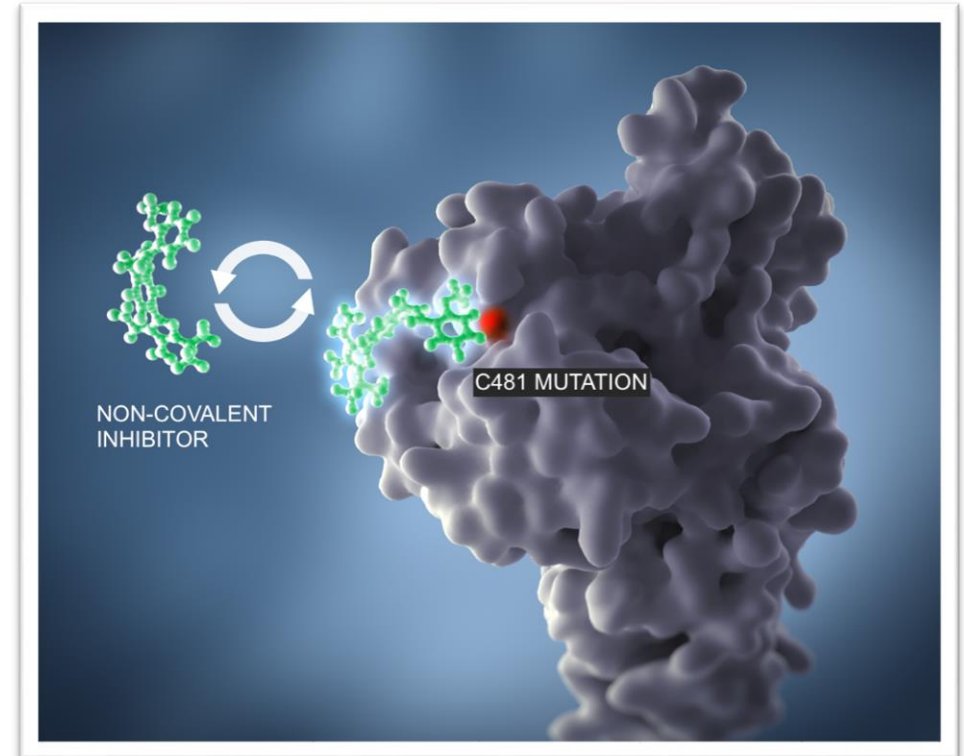
BTK C481 mutations [labelled] can diminish covalent inhibition of BTK (schematic representation of C481-mutated BTK that greatly diminishes the potential for covalent binding and inhibition at the ATP-binding site)¹⁻³

ATP=adenosine triphosphate; BTK=Bruton tyrosine kinase; C481=cysteine 481.

1. Tasso B, et al. *Molecules*. 2021;26(23):7411; 2. Brullo C, et al. *Int J Mol Sci*. 2021;22(14):7641; 3. Gu D, et al. *J Hematol Oncol*. 2021;14(1):40; 4. Cheah CY, et al. *Ann Oncol*. 2015;26(6):1775-1779; 5. McCulloch R et al. *Hematol Oncol Clin N Am*. 2020;34(5):923-939.

Non-covalent (Reversible) Inhibition Acts in a Different Way to Inhibit BTK, Regardless of Common Acquired Resistance Mutations¹⁻³

- Preclinical research has demonstrated that non-covalent BTK inhibition does not require binding to C481¹⁻³
- As a result, BTK blockade with non-covalent inhibition may be possible for patients with C481 mutations¹⁻³
- A binding mechanism without dependence on C481 could provide an alternative option with potential to reestablish BTK inhibition and restore blockade of B-cell signaling¹⁻³



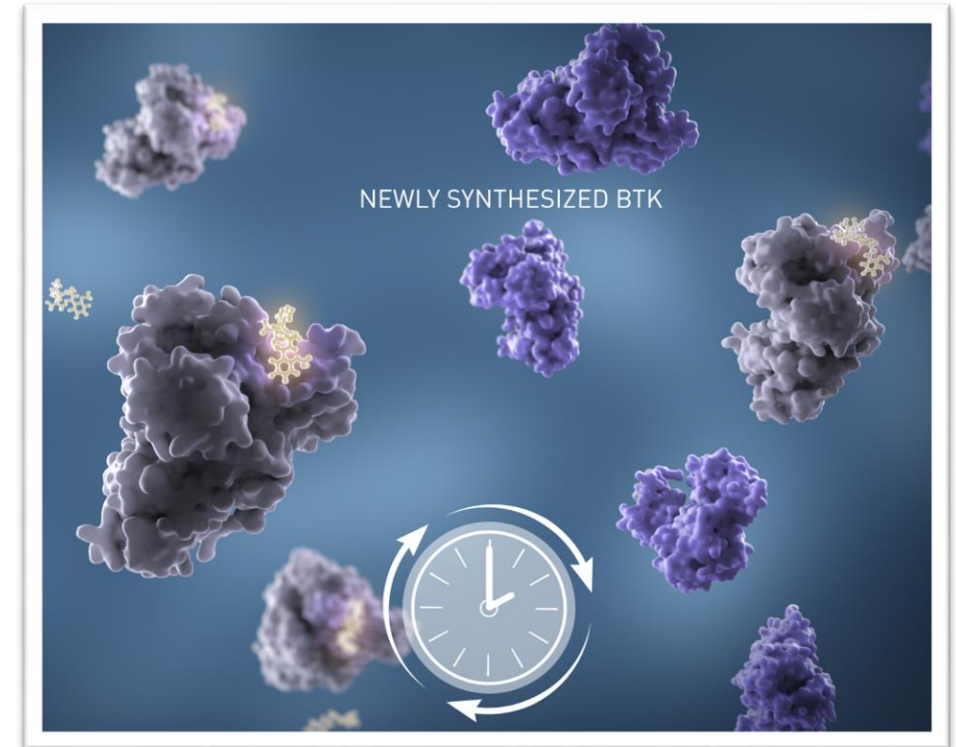
A C481-independent mechanism of binding and non-covalent inhibition has the potential to re-establish BTK binding and subsequent blockade of the B-cell signaling pathway¹⁻³

BTK=Bruton tyrosine kinase; C481=cysteine 481.

1. Tasso B, et al. *Molecules*. 2021;26(23):7411; 2. Brullo C, et al. *Int J Mol Sci*. 2021;22(14):7641; 3. Gu D, et al. *J Hematol Oncol*. 2021;14(1):40.

Consistently High Target Coverage can be Challenging due to BTK Resynthesis^{1,2}

- Over the course of the day, new BTK protein is synthesized by B-cells and continues to require BTK inhibition to inactivate it^{3,4}
 - Different tumors have different rates of BTK resynthesis. High proliferating tumors have higher rates of BTK turnover, and being able to continually inactivate BTK can be challenging^{2,3}
- As drug levels decline, newly synthesized BTK may escape inhibition and result in reactivation of the BCR pathway and potential drug resistance²⁻⁵
- A longer compound half-life may allow concentrations to remain elevated and continue to block signaling over time, regardless of BTK resynthesis rate^{1,2,6}



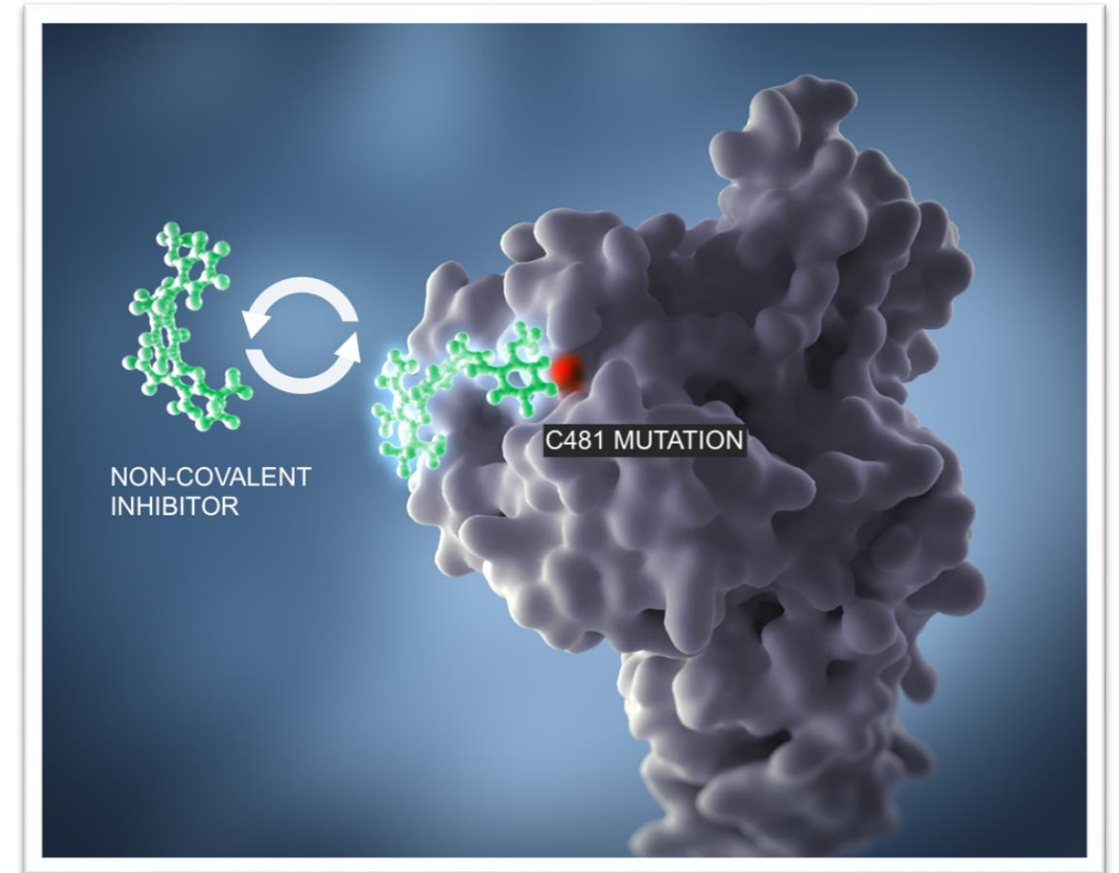
Over the course of the day, new BTK protein is synthesized by the B cells and continues to require BTK inhibition to inactivate it (schematic representation)³⁻⁵

BCR=B-cell receptor; BTK=Bruton tyrosine kinase.

1. Mato AR, et al. *Lancet*. 2021;397:892-901; 2. Gu D, et al. *J Hematol Oncol*. 2021;14(1):40; 3. Alsadhan et al. *Clin Cancer Res*. 2020;26(12):2800-2809; 4. Thompson PA. *Blood*. 2020;136(1):4-6; 5. Sun C. *Blood*. 2020; 136(1)93-105; 6. Mato AR, et al. *Lancet*. 2021;397(Suppl 4):892-901.

BTK Inhibition may be Possible, Even After BTK Pathway Blockade is No Longer Achievable with Covalent Inhibition¹

- Non-covalent BTK inhibition is an important area of research²
 - Binding to C481 is not required so activity may be unaffected in C481-mutated disease²
- Pharmacological properties, including long half-life and high oral bioavailability, that enable sustained BTK inhibition and consistent target coverage regardless of BTK turnover rate are needed¹⁻³



BTK=Bruton tyrosine kinase; C481=cysteine 481.

1. Mato AR, et al. Lancet. 2021;397:892-901; 2. Gu D, et al. J Hematol Oncol. 2021;14(1):40; 3. Thompson PA. Blood. 2020;136(1):4-6.