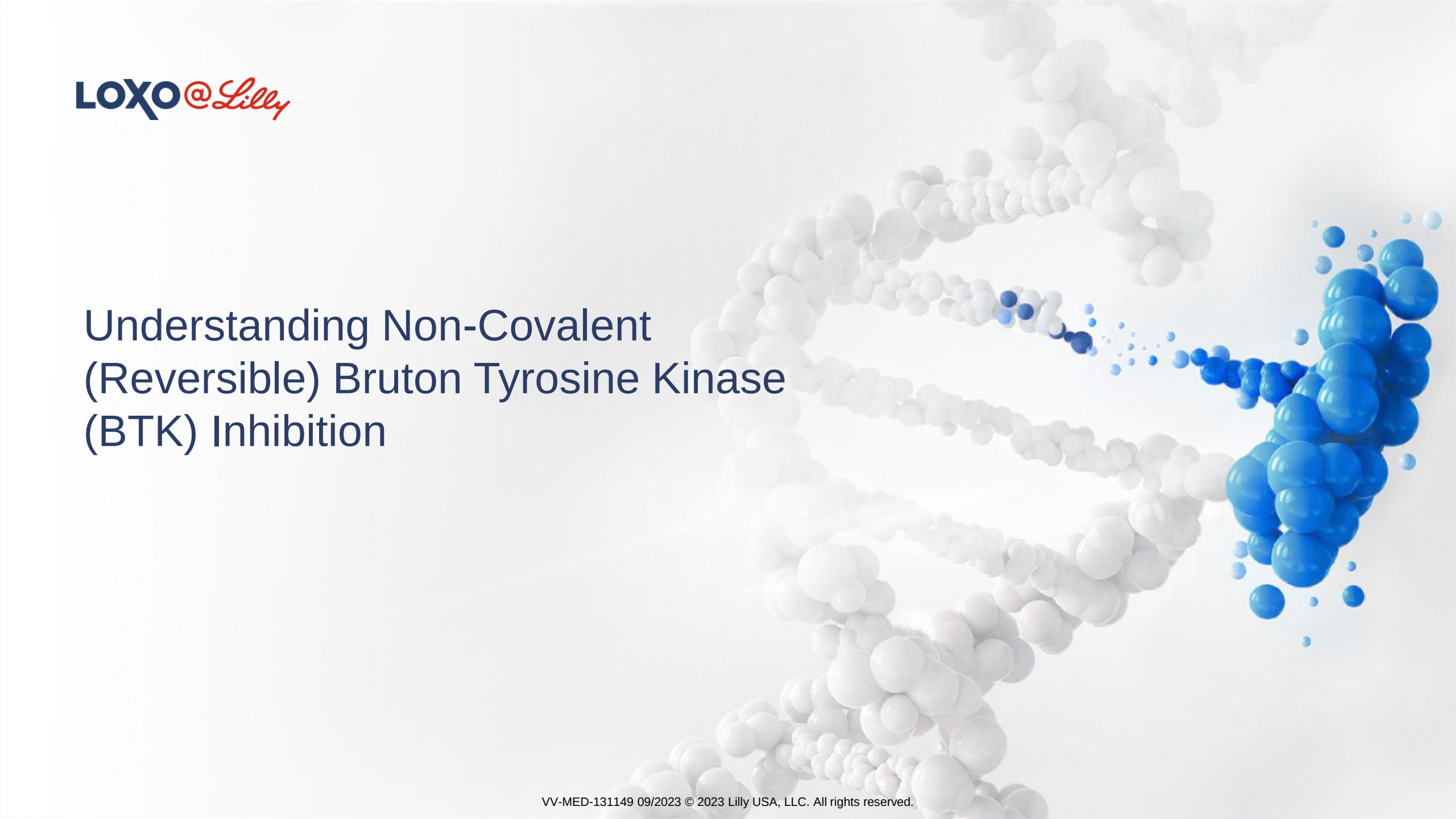
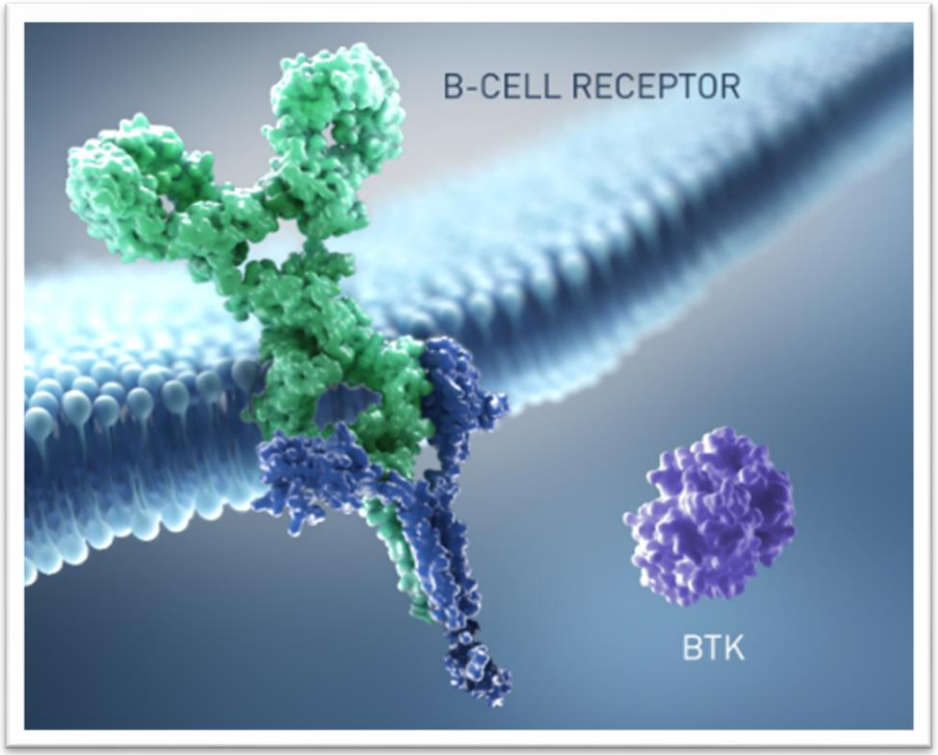
Understanding Non-Covalent (Reversible) Bruton Tyrosine Kinase (BTK) Inhibition



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.

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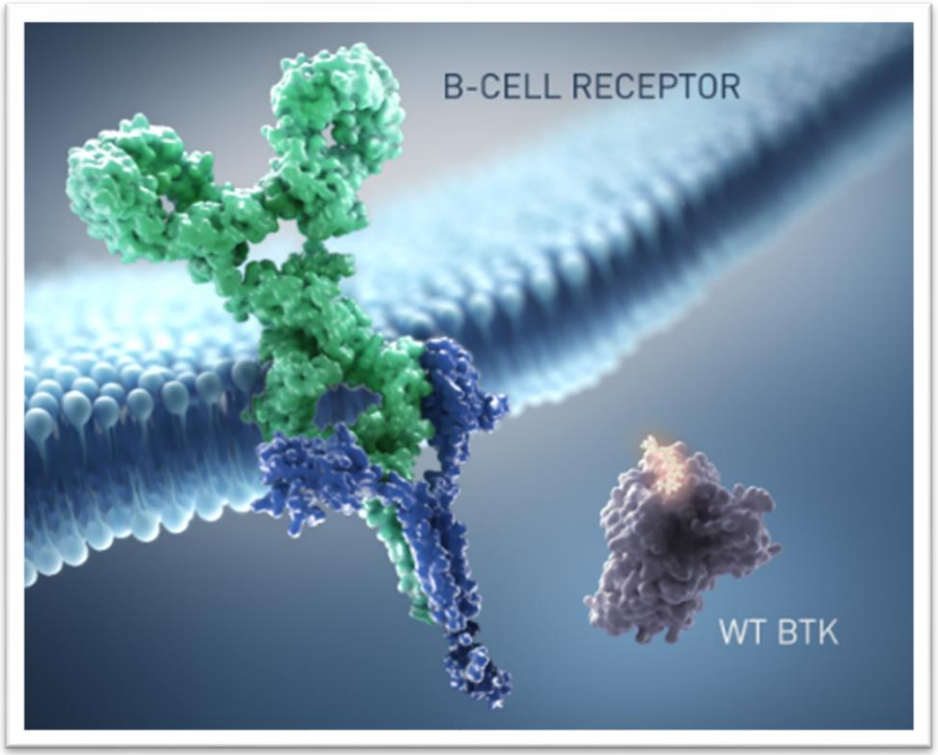
Inhibition of BTK Blocks BCR Signaling[1-3]

- 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.

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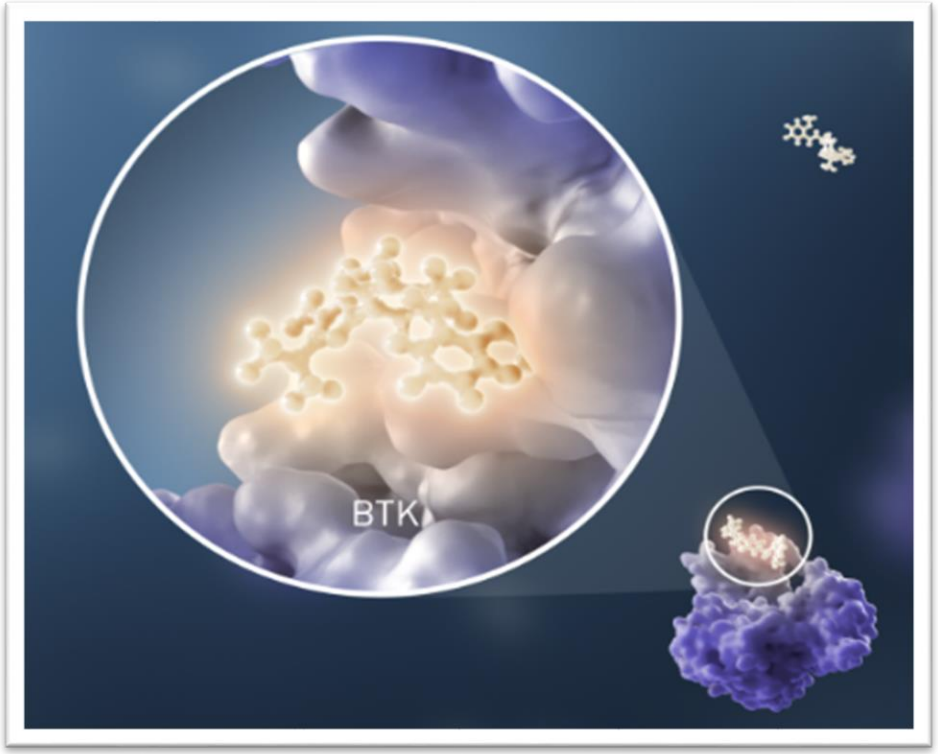
Covalent and Non-covalent (Reversible) BTK Inhibition Differ in their Modes of Binding[1-4]

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 domain[1-4]
  - Blocks the ATP-binding site, inhibits autophosphorylation of BTK, and results in irreversible inhibition of kinase activity[3-6]



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.

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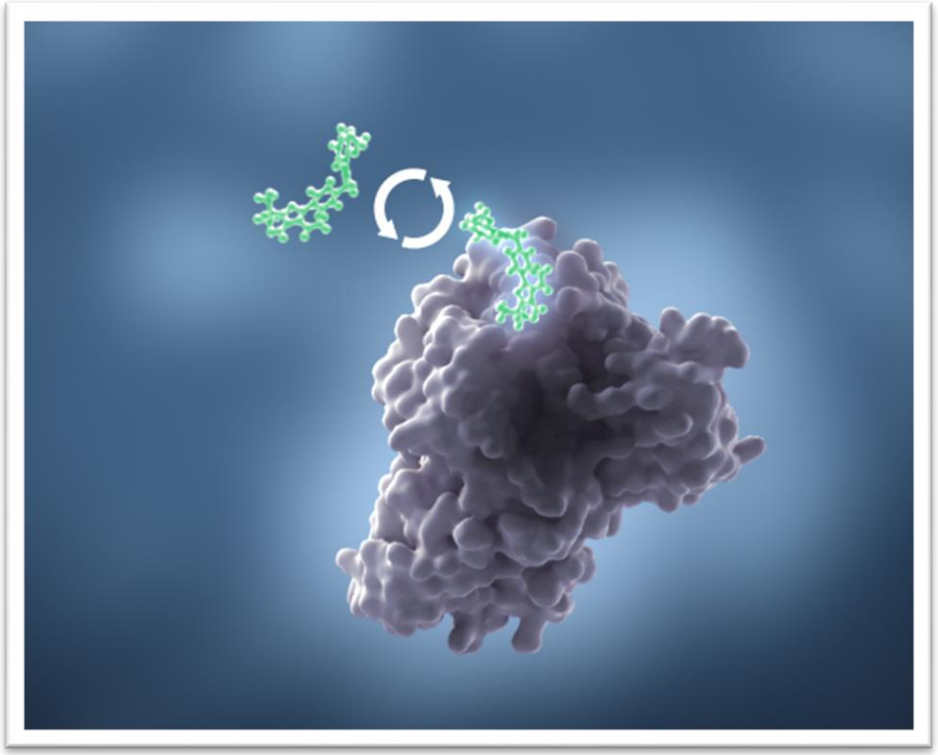
Covalent and Non-covalent (Reversible) BTK Inhibition Differ in their Modes of Binding[1-3]

- 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 C481[4]
- Blockade of BTK with non-covalent inhibition provides a different way to regulate BCR signaling[1-4]



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; 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.

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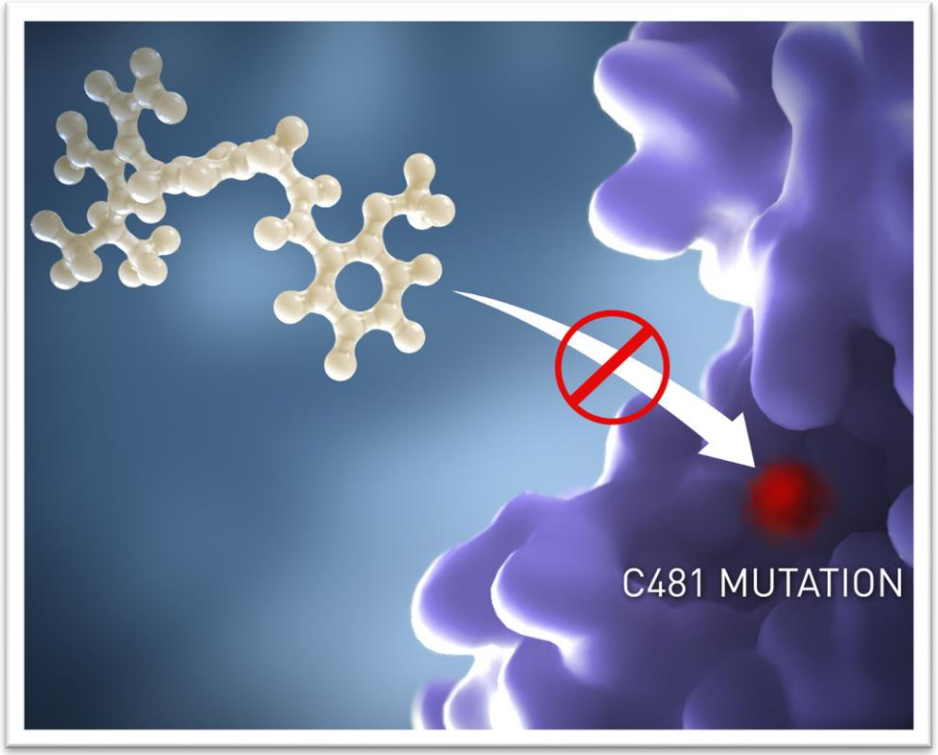
BTK Mutations Can Diminish Covalent Inhibition Against BTK Blockade[1-3]

- 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 signaling[1-3]
- 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.

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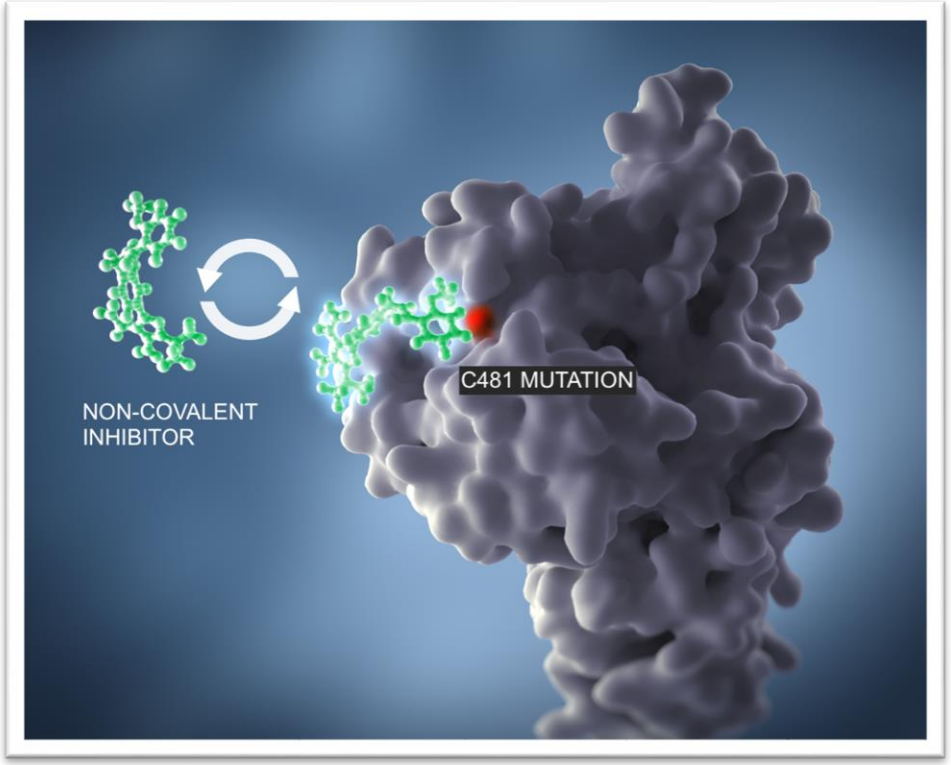
Non-covalent (Reversible) Inhibition Acts in a Different Way to Inhibit BTK, Regardless of Common Acquired Resistance Mutations[1-3]

- Preclinical research has demonstrated that non-covalent BTK inhibition does not require binding to C481[1-3]
- As a result, BTK blockade with non-covalent inhibition may be possible for patients with C481 mutations[1-3]
- A binding mechanism without dependence on C481 could provide an alternative option with potential to reestablish BTK inhibition and restore blockade of B-cell signaling[1-3]



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 pathway[1-3]

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.

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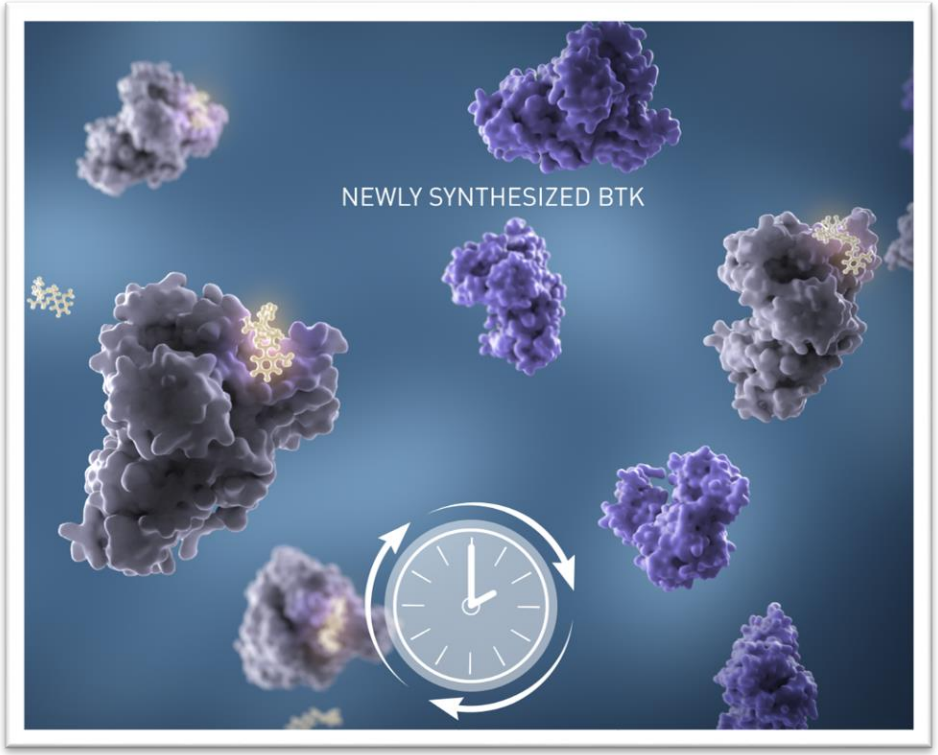
Consistently High Target Coverage can be Challenging due to BTK Resynthesis[1,2]

- 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 resistance[2-5]
- 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.

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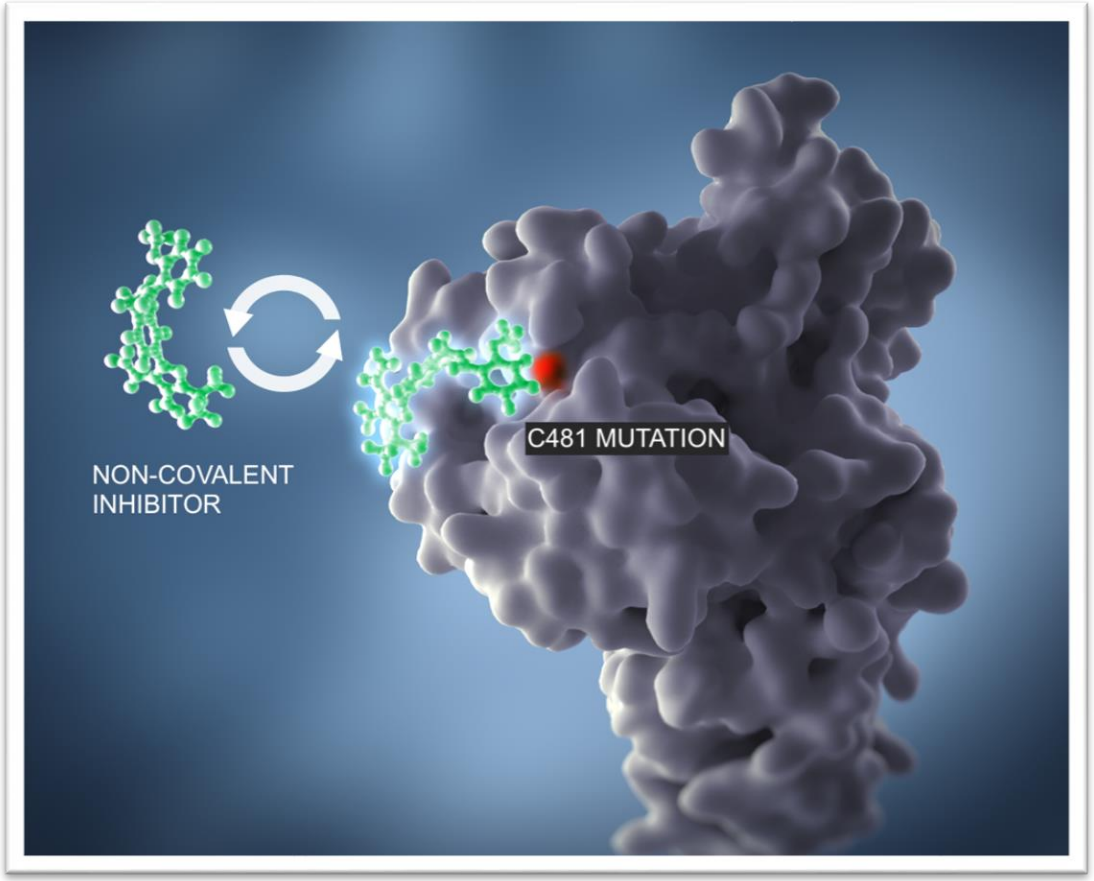
BTK Inhibition may be Possible, Even After BTK Pathway Blockade is No Longer Achievable with Covalent Inhibition[1]

- Non-covalent BTK inhibition is an important area of research[2]
  - Binding to C481 is not required so activity may be unaffected in C481-mutated disease[2]
- 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 needed[1-3]



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