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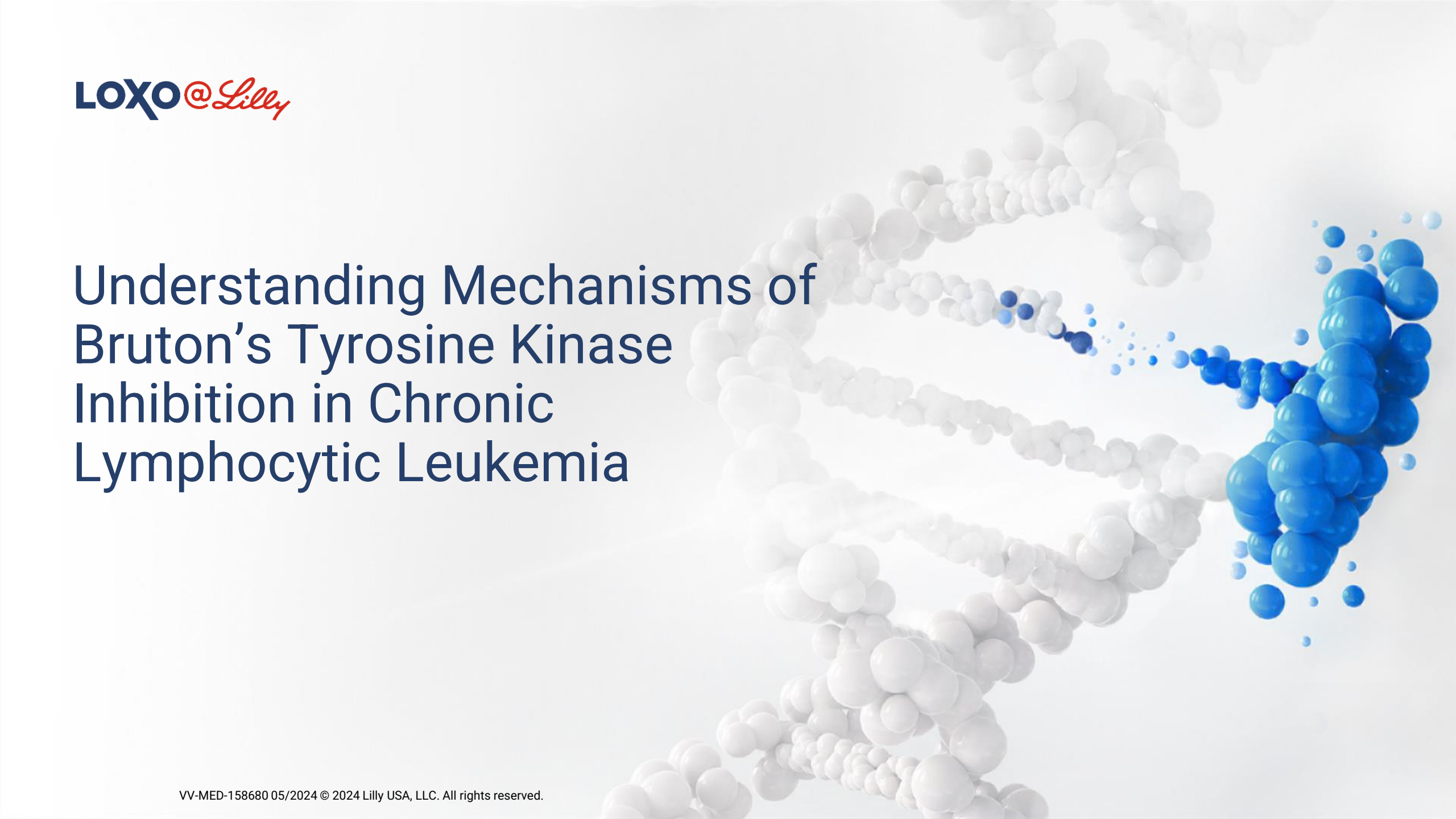
Understanding Mechanisms of Bruton's Tyrosine Kinase Inhibition in Chronic Lymphocytic Leukemia



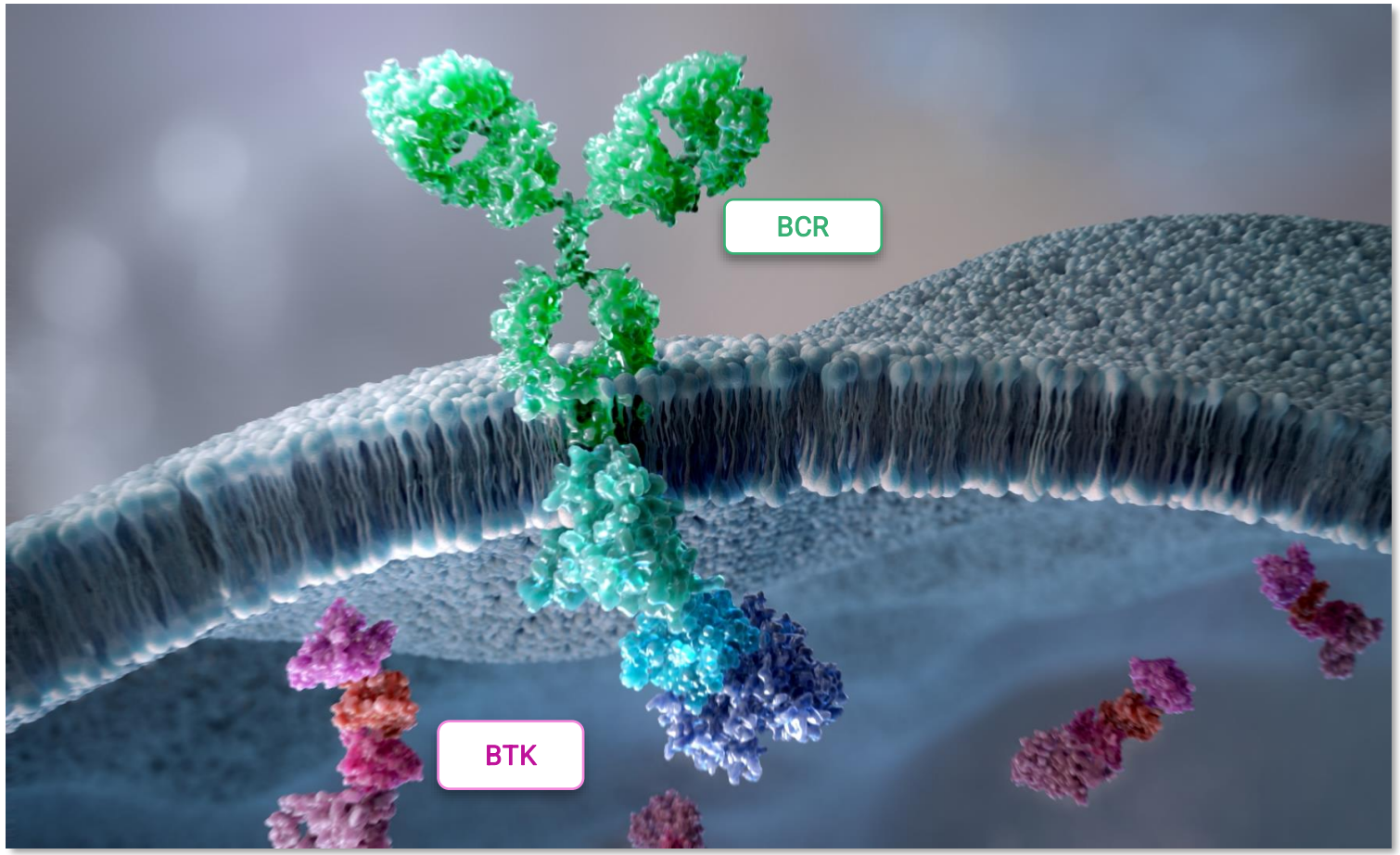
Overactive BTK Signaling Can Drive Malignant B-cells in CLL[1-3]

BTK is a key mediator of normal B-cell development[1-4]

- BTK is activated downstream of cell-surface BCRs[2-4]
- BTK is crucial for the proliferation, differentiation, and survival of peripheral B-cells[2,3]

Aberrant BTK signaling can drive the initiation of CLL and survival of malignant cells[1-3]

- It has been shown BTK is[1]
  - Overexpressed in CLL B-cells
  - Constitutively phosphorylated in some CLL samples
- BTK signaling may also support malignant cell migration to proliferative centers in lymph nodes[1]



A schematic representation of BTK (magenta) and a BCR (green) on the surface of a B-cell.

BCR, B-cell receptor; BTK, Bruton's tyrosine kinase; CLL, chronic lymphocytic leukemia.

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.

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The Central Role of BTK in BCR Signaling Makes it an Important Therapeutic Target in CLL[1-3]

Inhibition of BTK activity can lead to CLL cell death[1-3]

- Binding of inhibitory agents to the BTK kinase domain results in inhibition of BTK and leads to[1,2]
  - Blockage of BTK catalytic activity
  - Suppression of downstream BCR signaling
  - Impairment of malignant B-cell survival

A schematic representation of BTK (magenta) and a molecule with BTK inhibitory properties (dark gray).

BCR, B-cell receptor; BTK, Bruton's tyrosine kinase; CLL, chronic lymphocytic leukemia.

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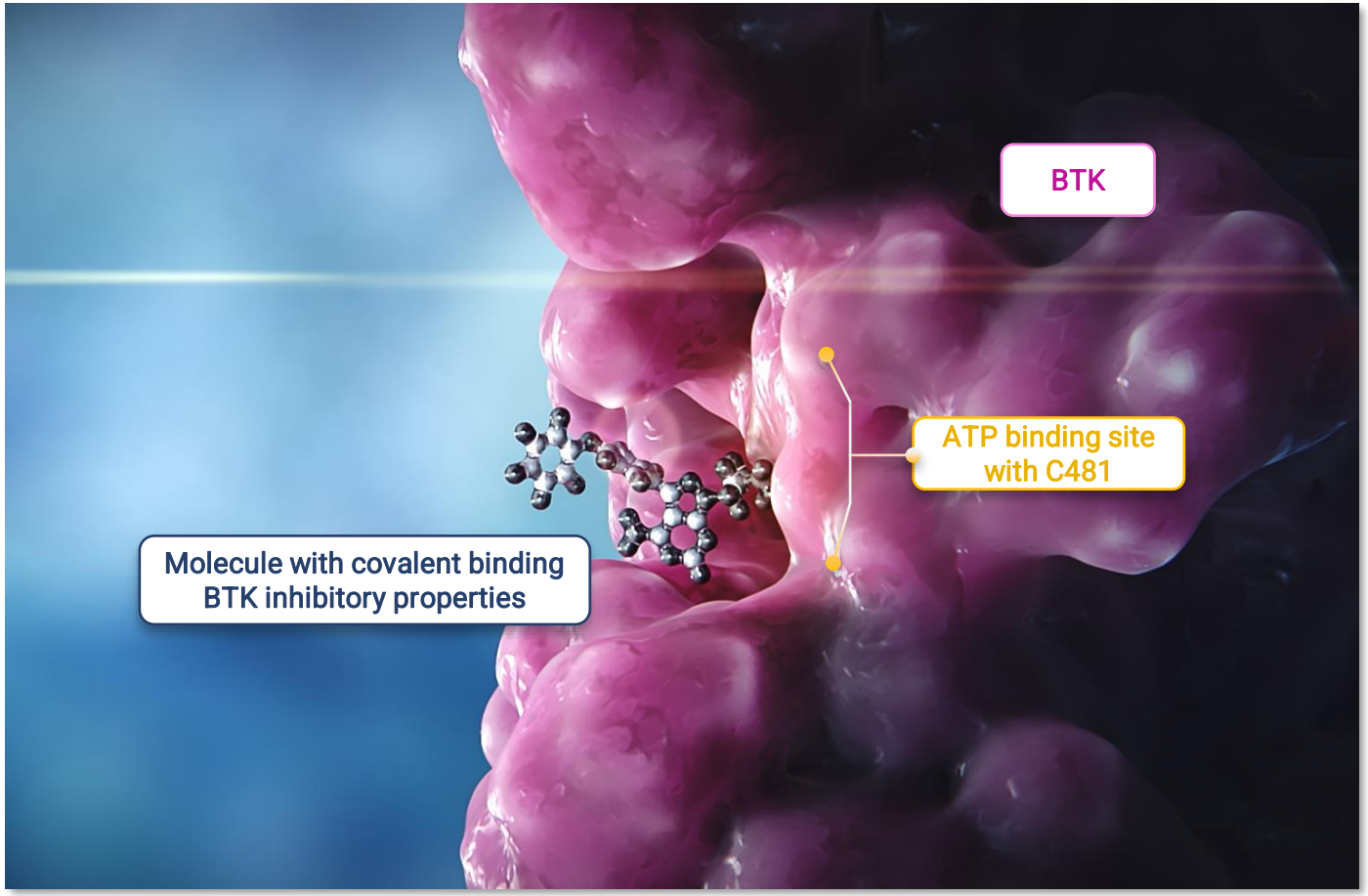
Covalent Binding in the BTK Kinase Domain Results in Irreversible BTK Inhibition[1-6]

The BTK kinase domain contains a C481 residue that is key to covalent (irreversible) BTK inhibition[1-6]

- The C481 residue is present in the ATP-binding site of the BTK kinase domain[2-6]
- Binding the C481 residue blocks BTK autophosphorylation and activation[1,2]
  - This results in suppression of downstream BTK activity in CLL cells[1,2]



A schematic representation of BTK (magenta) and a molecule with covalent binding BTK inhibitory properties (dark gray) at the BTK ATP binding site.

ATP, adenosine triphosphate; BTK, Bruton's tyrosine kinase; C481, cysteine 481; CLL, chronic lymphocytic leukemia.

1. Pal Singh S, et al. *Mol Cancer*. 2018;17(1):57. 2. Gu D, et al. *J Hematol Oncol*. 2021;14(1):40. 3. Estupiñán HY, et al. *Front Cell Dev Biol*. 2021;9:630942.
4. Tasso B, et al. *Molecules*. 2021;26(23):7411. 5. Sun C, et al. *Blood*. 2020;136(1):93-105. 6. Tambaro FP, et al. *J Exp Pharmacol*. 2021;13:923-935.

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Non-covalent Binding of BTK Results in Reversible BTK Inhibition[1,2]

Non-covalent (reversible) binding and inhibition of BTK is independent of the C481 residue[1,2]

- Non-covalent interactions also block ATP binding, which inhibits BTK kinase activity[2]
- Non-covalent inhibition involves interacting with the target constantly through binding, unbinding, and rebinding (e.g., hydrogen bonding, hydrophobic interactions, etc.)[3]
  - This interaction does not require binding to the BTK C481 residue[2]

A molecule with non-covalent binding BTK inhibitory properties (pink).

ATP, adenosine triphosphate; BTK, Bruton's tyrosine kinase; C481, cysteine 481.

1. Brullo C, et al. *Int J Mol Sci*. 2021;22(14):7641. 2. Tambaro FP, et al. *J Exp Pharmacol*. 2021;13:923-935. 3. Aljoundi A, et al. *Protein J*. 2020;39(2):97-105.

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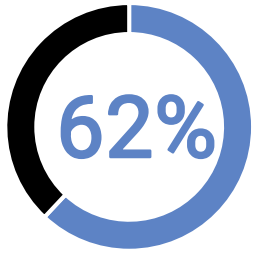
Resistance Mutations Can Diminish the Efficacy of Covalent BTK Inhibition in CLL[1-3]

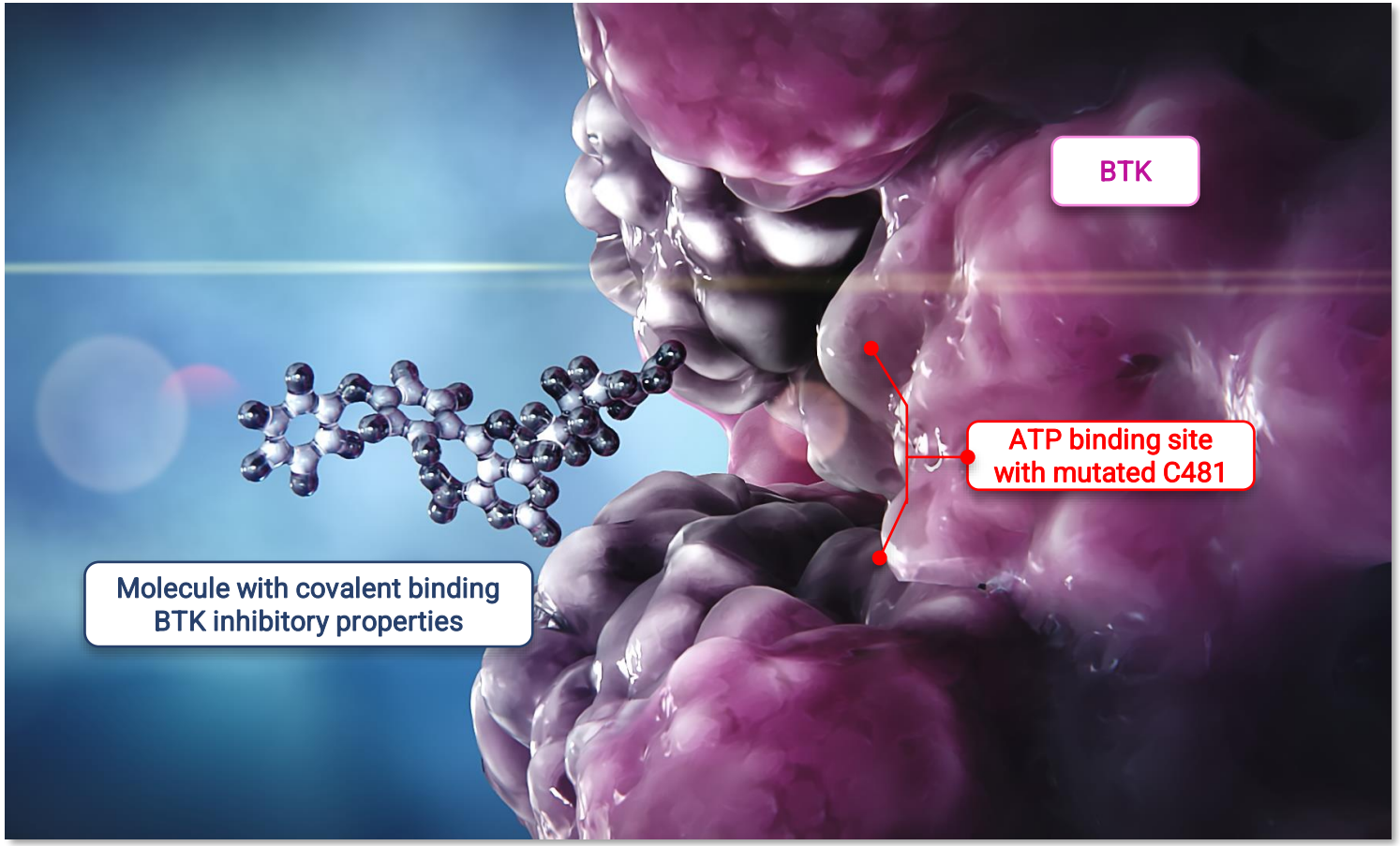
Mutations in C481 can greatly diminish the inhibition of BTK through covalent binding[1,2]

- Mutations in the C481 residue of BTK are a major mechanism of acquired resistance to covalent inhibition of BTK in CLL[1-3]



of patients with CLL treated with a molecule that covalently inhibits BTK were found to have C481 mutations at the time of disease progression or RT[3]

- Because of the dependence of covalent BTK inhibition on C481, therapeutic interventions in CLL that rely on covalent BTK inhibition are not recommended for patients with C481 mutations[1,4]



A schematic representation of BTK (magenta) and a molecule with covalent binding BTK inhibitory properties (dark gray) at the BTK ATP binding site harboring a C481 mutation.

BTK, Bruton's tyrosine kinase; C481, cysteine 481; CLL, chronic lymphocytic leukemia; RT, Richter transformation.

1. Gu D, et al. *J Hematol Oncol.* 2021;14(1):40. 2. Brullo C, et al. *Int J Mol Sci.* 2021;22(14):7641. 3. Kangal-Shamanna R, et al. *Cancer.* 2019;125(4):559-574. 4. Shadman M. *JAMA.* 2023;329(11):918-932.

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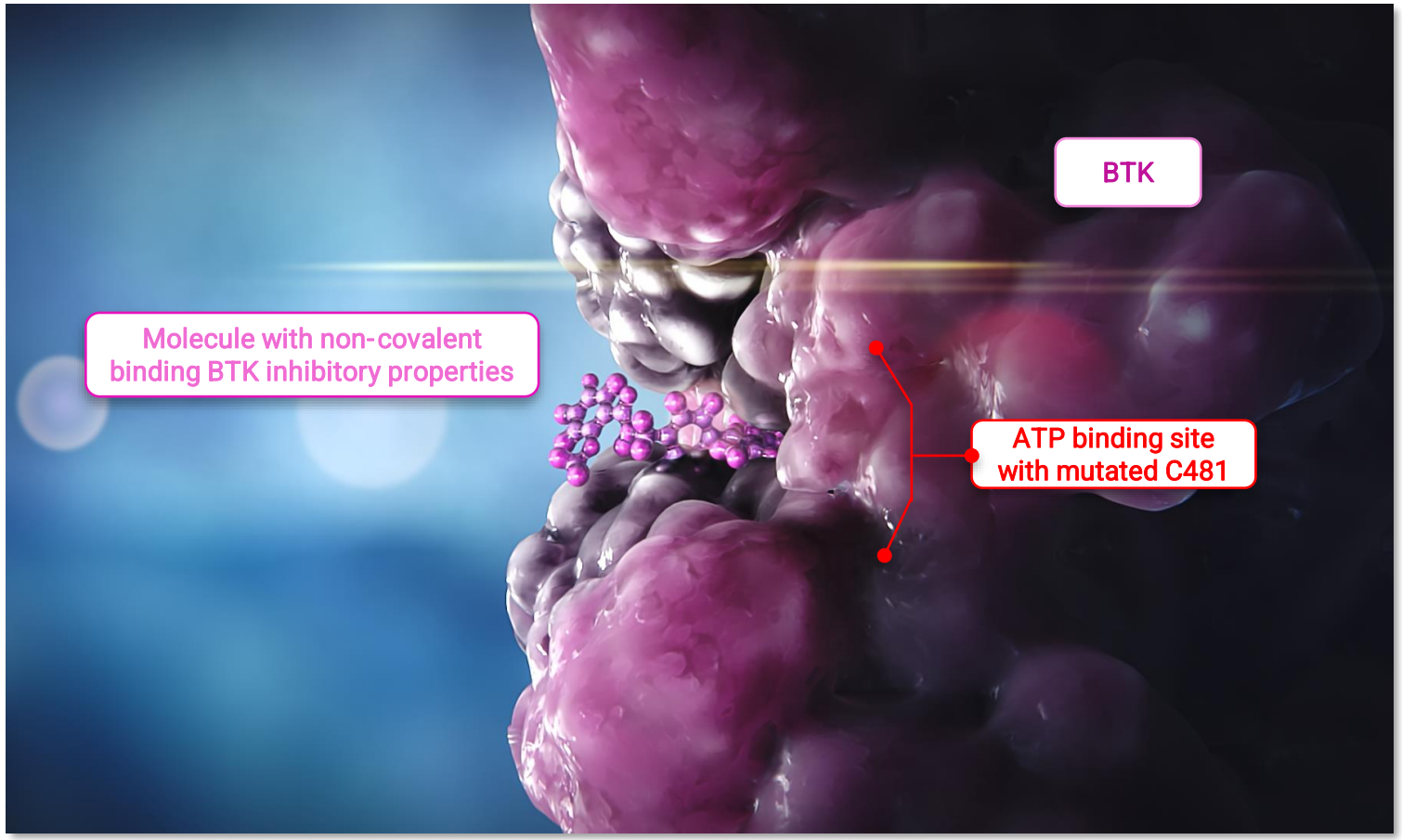
Non-covalent BTK Inhibition Can Overcome C481 Resistance Mutations to Provide Continued BTK Inhibition[1-4]

BTK proteins with C481 mutations remain susceptible to non-covalent inhibition[1-3]

- Non-covalent inhibition of BTK does not require binding to C481[1,3,4]
  - As a result, BTK blockade with non-covalent inhibition remains possible for patients with BTK harboring C481 mutations[1,3,4]

In patients with CLL who have developed resistance to prior covalent BTK inhibition, a binding mechanism that avoids dependence on C481 could provide an alternative option for CLL treatment with the potential to reestablish BTK inhibition and restore blockade of B-cell signaling[1,3,4]



A schematic representation of BTK (magenta) and a molecule with non-covalent binding BTK inhibitory properties (pink) at the BTK ATP binding site harboring a C481 mutation.

BTK, Bruton's tyrosine kinase; C481, cysteine 481; CLL, chronic lymphocytic leukemia.

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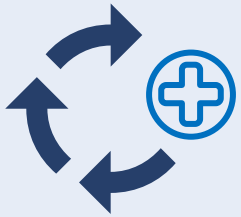
Key Takeaways[1-7]

- Inhibition of the BTK protein can suppress downstream BCR signaling and is a key therapeutic target in CLL[1-3]
- The BTK protein can be inhibited by covalent (irreversible) and non-covalent (reversible) binding[4-6]
- Mutations in the C481 residue of the BTK ATP binding site can diminish covalent inhibition of BTK[2,5]
- Non-covalent binding can circumvent the effects of C481 mutations and continue BTK inhibition[2,4,7]



The different effects of C481 mutations on covalent and non-covalent BTK inhibition can help inform optimal sequencing of CLL treatments[6,7]

What are important pharmacologic properties to extend BTK inhibition?

- Long half-life[6]
- High oral bioavailability[5]
- High selectivity for the BTK protein[6]

Mechanisms of acquired resistance to newer BTK inhibitors, both covalent and noncovalent, are still being investigated. More data are needed to better inform appropriate treatment sequencing and understand patterns of potential cross resistance[7]

BTK, Bruton's tyrosine kinase; C481, cysteine 481; CLL, chronic lymphocytic leukemia.

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