# Structural and Mechanistic Insights into Cabotegravir (CAB) Resistance in HIV-1 Integrase

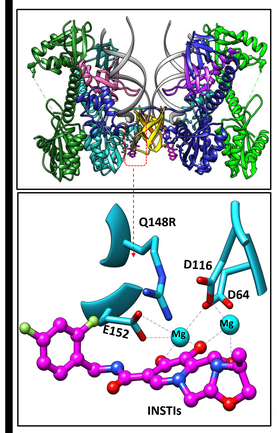
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## Project Goals

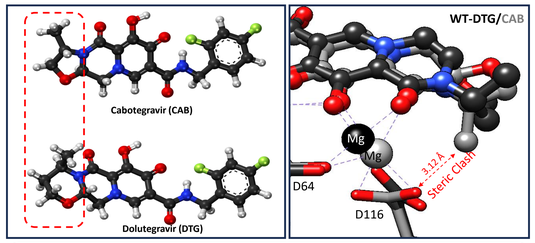
**Introduction**: HIV-1 integration into the host genome is a crucial step in viral replication, mediated by Integrase (IN). Integrase strand transfer inhibitors (INSTIs), such as Dolutegravir (DTG) and Cabotegravir (CAB), target the active site of IN. However, drug resistance mutations (DRMs) can compromise the efficacy of these inhibitors.

**Active Site**




- The active site contains a catalytic triad of $Mg^{2+}$ ions, which are crucial for DNA cleavage and strand transfer.
- Key residues such as Asp64 (D), Asp116 (D), Glu152 (E) coordinate with $Mg^{2+}$ and forms DDE-Mg network to facilitate catalysis.
- Antiretroviral drugs or IN strand transfer inhibitors (INSTIs) chelate the $Mg^{2+}$ ions, preventing the enzyme from integrating viral DNA into the host genome
- DRMs at or near active site affects the drug binding.

**INSTIs (CAB vs. DTG)**




- CAB and DTG are naphthyridine-based second-generation INSTIs.
- The only structural difference is the left-ring attachment to the naphthyridine core.
- Strong van der Waals interaction causes steric clash with D116 in CAB may lead to D116 reorientation.

## Methodology

1. **Virology EC50 Measurements (Smith et al. NCI/CCR/NIH)**
2. **High resolution Cryo-EM Structures (Tao Jing, Salk Institute for Biological Studies)**
3. **Computational analysis** involving high resolution Molecular Dynamics (MD), Free energy (FEP) changes, Hybrid quantum mechanics and molecular mechanics (QM/MM)

### Virology EC50 Measurements

| System | CAB | DTG |
|---|---|---|
| WT | 1.8±0.5 | 2.6±0.3 |
| E138K | 2.6±0.5 | 1.8±0.5 |
| G140A | 2.3±0.4 | 1.5±0.3 |
| Q148R | 8.0±1.4 | 1.7±0.1 |
| E138K/G140A | 0.9±0.3 | 0.9±0.1 |
| E138K/Q148R | 19.9±5.7 | 5.3±0.9 |
| G140A/Q148R | 62.2±5.9 | 11.4±1.5 |
| **E138K/G140A/Q148R** | **688.4±26.5** | **81.3±4.8** |

### High resolution Cryo-EM Structures

All computational studies are based on four experimental structures resolved by **Tao Jing**.

| Structures | Resolution (Å) | PDB ID |
|---|---|---|
| WT-DTG* | 2.80 | 8FN7 |
| E138K/G140A/Q148R-DTG* | 2.50 | 8FNO |
| WT-CAB | 2.95 | New |
| E138K/G140A/Q148R-CAB | 2.43 | New |

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- CAB and DTG both remains potent in presence of single mutations
- DTG remains potent against all combination of double mutations.
- CAB losses its potency significantly due to triple mutation (E138K/G140A/Q148R) compared to WT and DTG.

## Questions

1. **Why does CAB significantly lose potency against E138K/G140A/Q148R mutations?**
2. **Why is DTG more potent than CAB against E138K/G140A/Q148R mutants?**

## Acknowledgements

**Group Members**

- Dmitry Lyumkis (PI)
- Tao Jing
- Avik Biswas
- Jocelyn Olvera
- All other members

**Collaborators**

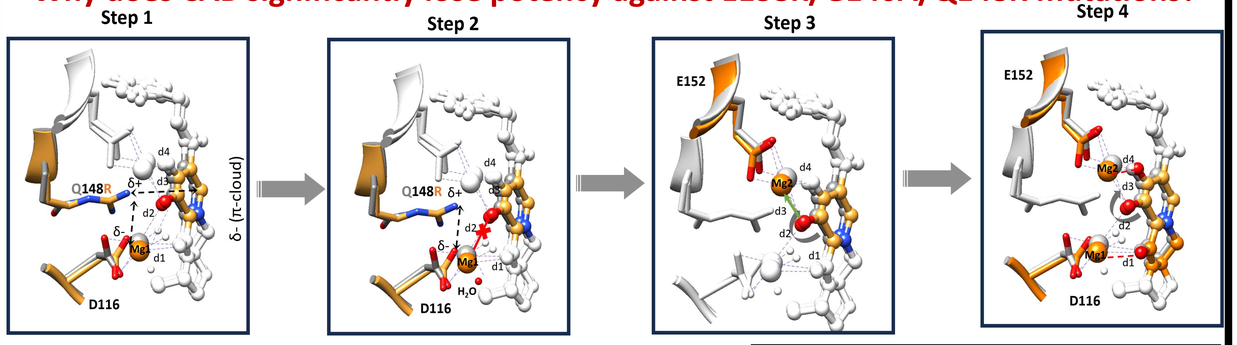
- Min Li and Robert Craigie (NIDDK/NIH)
- Steven J. Smith and Terrence R. Burke Jr. (NCI/CCR/NIH)
- Eric O. Freed (NCI/CCR/NIH)
- Stephen H. Hughes (NCI/CCR/NIH)
- Ronald M. Levy (Temple University)

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- Pioneer Postdoctoral Fellowship (Tao Jing)
- Schmidt AI Postdoctoral Fellowship (Avik Biswas)
- Timken Sturgis Foundation (Jocelyn Olvera)

## Key Findings

### Why does CAB significantly lose potency against E138K/G140A/Q148R mutations?



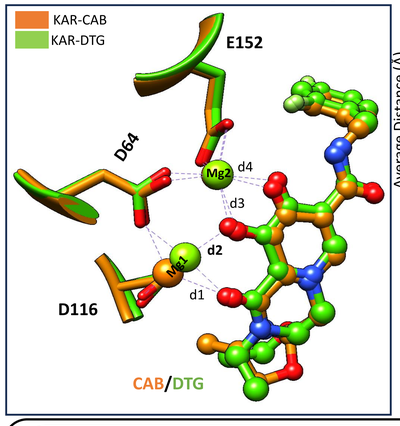

**CAB Drug Resistance Mechanism**

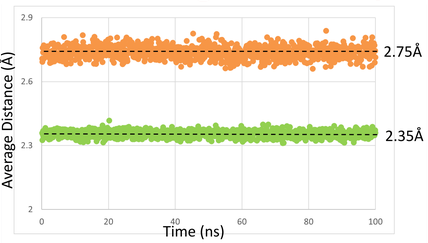
- **Step1**: 148R interaction with D116 help to repositioned the D116-Mg and get the steric relief.
- **Step 2**: This reposition disconnect Mg-O (d2) interaction and changes Mg1 co-ordinations.
- **Step 3**: The disruption of Mg-O(d2) leads CAB shift in such a way that the O-Mg2 (d3) bond distance decreases.
- **Step 4**: The CAB shift also leads to increase in Mg-O distance (d1 and d4).

| Distance | Mg-CAB Avg. Distance (Å) WT | KAR |
|---|---|---|
| Mg1-O(d1) | 2.57 | 2.73 |
| **Mg1-O(d2)** | 2.73 | 3.48 |
| **Mg2-O(d3)** | 2.69 | 2.46 |
| **Mg2-O(d4)** | 1.71 | 2.32 |

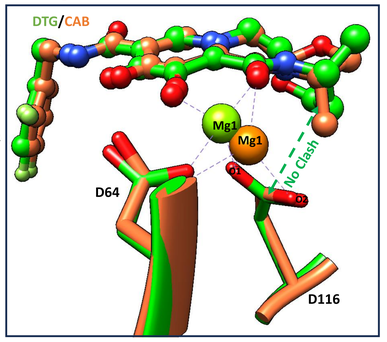
- A shift of CAB (<~0.33> Å) due to KAR mutations compared to WT.
- Maximum increase in distance is observed in d2 (<~0.75> Å).

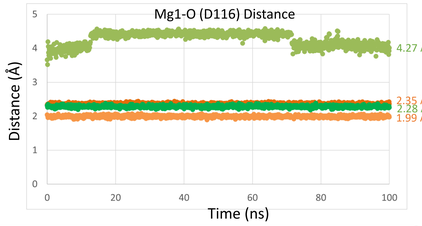
### Why is DTG more potent than CAB against E138K/G140A/Q148R mutants?




| Distance | Mg-CAB Avg. Distance (Å) DTG | CAB |
|---|---|---|
| Mg1-O(d1) | 2.24 | 2.73 |
| **Mg1-O(d2)** | **2.42** | **3.58** |
| Mg2-O(d3) | 2.37 | 2.46 |
| Mg2-O(d4) | 2.38 | 2.32 |




- KAR Mutation shifts CAB (<~0.40> Å).
- No Steric clash in KAR-DTG with D116 like CAB
- D116 has 1 coordination with Mg1 in KAR-DTG which makes D116 more flexible.
- Q48R mutation has less effect on the Mg1-D116: No sever reposition of Mg1 or DTG.

## Summary

- Virological EC50 shows that CAB loses its potency significantly (FC: ~380) in presence of triple mutation E138K/G140A/Q148K.
- Strong van der Waals interaction leads to steric clash between D116 and CAB.
- Steric clash may lead to reorientation of D116 in CAB and decrease the fexibality of the D116 sidechain.
- 148R mutation has sever impact on the Mg1 coordination and its soundings in CAB which lead to displacement of CAB from its binding pocket.
- No steric clashes in DTG allow a more flexible D116 side chain, compensating for the Q148R mutation's impact in the active site, leading to only a mild DTG displacement.