1163 Sensitive monitoring of sequential targeted therapy resistance in relapsed/therapy refractory chronic lymphocytic leukemia

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INTRODUCTION

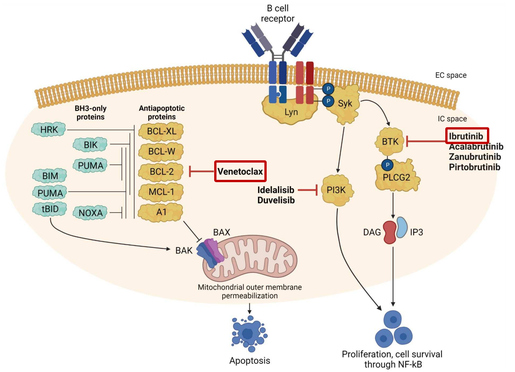


Figure 1. Small molecule drugs and their respective targets in chronic lymphocytic leukemia.

- Small molecule drugs, such as the BTK inhibitor **ibrutinib** and the BCL2 inhibitor **venetoclax** have revolutionized the therapeutic landscape of therapy refractory and relapsed (R/R) chronic lymphocytic leukemia (CLL).
- Despite the **remarkable response rates**, a subset of CLL patients receiving targeted therapies experience **disease progression**.
- **Secondary ibrutinib resistance** is characterized by the presence of the ***BTK* p.Cys481Ser resistance mutation** in 80-100% of the cases.
- **Secondary venetoclax resistance** is associated with harboring pathogenic variants of the **antiapoptotic *BCL2* gene** in approximately half of the cases.

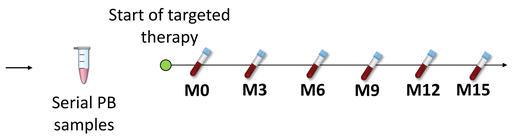
OBJECTIVES

- To develop a **droplet digital polymerase chain reaction (ddPCR)**-based method for the detection of the **most frequent resistance mutations** conferring secondary resistance to **ibrutinib** or **venetoclax** therapy
- To dissect **the temporal heterogeneity** observed in R/R CLL in the context of targeted therapy by the molecular monitoring of the ***BTK* p.Cys481Ser** and ***BCL2* p.Gly101Val & p.Asp103Tyr** mutations
- To aid the **standard-of-care monitoring** of the efficacy of ibrutinib and venetoclax therapies in CLL

MATERIALS & METHODS I.

Patients with R/R CLL:

Ibrutinib: 83 patients
Venetoclax: 67 patients



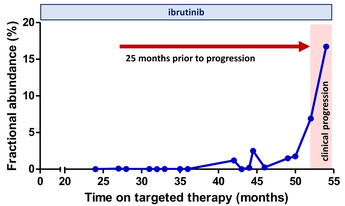
MATERIALS AND METHODS II.

- **Custom designed** ddPCR assays for ***BTK* p.Cys481Ser, *BCL2* p.Gly101Val and p.Asp103Tyr**
- Fractional abundance (**FA**) was calculated as follows:

$$FA = \frac{mutant\ DNA\ molecules}{mutant{+}wild\ type\ DNA\ molecules}\ x\ 100$$

RESULTS I.

Ibrutinib-treated cohort:



- ***BTK* p.Cys481Ser** was detected in **72.7% (32/44)** of patients experiencing disease progression
- First detection of *BTK* p.Cys481Ser predated the first clinical signs of relapse with a **median 8 months** (0-28 months)

Figure 2. In the serial peripheral blood samples of Patient #13, first detection of *BTK* p.Cys481Ser predated the first signs of clinical progression by more than two years.

Venetoclax-treated cohort:

- **43,5% (10/23)** of the patients experience disease progession **harbored *BCL2* p.Gly101Val** and/or **p.Asp103Tyr**
- Repertoire of ***BCL2* mutations**

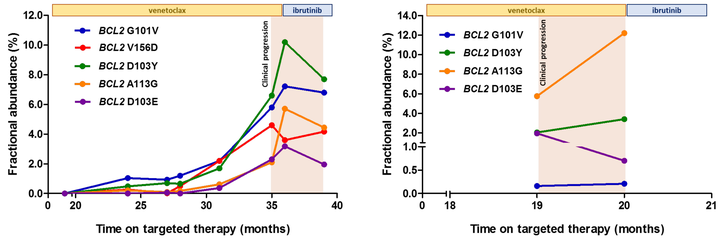
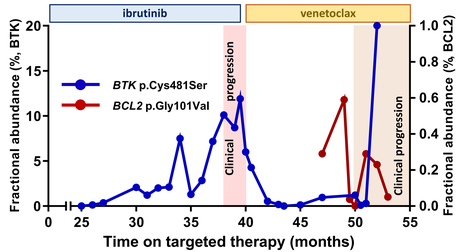


Figure 3. Patients #31 and #40 harbored multiple *BCL2* mutations simultaneously as detected by droplet digital PCR.

RESULTS II.

Sequential resistance to ibrutinib & venetoclax:



- **11 cases** with **sequential resistance** to ibrutinib & venetoclax
- **3 patients** carried both ***BTK* p.Cys481Ser & *BCL2* mutations**

Figure 5. Patient #3 harbored *BTK* p.Cys481Ser first detected approximately 14 months prior to the first clinical signs of secondary ibrutinib resistance. In the serial samples obtained during subsequent venetoclax therapy *BCL2* p.Gly101Val was also detected predating secondary venetoclax resistance.

CONCLUSIONS

- **Sensitive ddPCR-based methods** are optimal for the **early detection** of an impending relapse as well as for the identification of underlying molecular mechanisms in **up to two-thirds of R/R CLL patients** treated with targeted agents experiencing relapse or disease progression.
- Molecular monitoring of **sequential resistance to ibrutinib and venetoclax by ddPCR** is a feasible method able to capture the temporal heterogeneity observed in R/R CLL.
- Our results imply the selected gene mutations as **promising potential molecular biomarkers** for use in the standard-of-care diagnostic work-up of targeted therapy resistance in CLL.

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