Therapeutic monitoring of CDK4/6is using DBS: an LC-MS/MS method to overcome the haematocrit effect

Posocco B.[1], Orleni M.[1], Cecchin El.[1], Gagno S.[1], Montico M.[2], Peruzzi E.[1], Roncato R.[1,4], Gerratana L.[3], Corsetti S.[3], Puglisi F.[3,4], Toffoli G.[1], Cecchin Er.[1]

[1] Experimental and Clinical Pharmacology Unit-CRO Aviano, National Cancer Institute, IRCCS, Aviano, Italy

[2] Clinical Trial Office, Scientific Direction-CRO Aviano, National Cancer Institute, IRCCS, Aviano, Italy

[3] Department of Medical Oncology-CRO Aviano, National Cancer Institute, IRCCS, Aviano, Italy

[4] Department of Medicine, University of Udine, Udine, Italy

Background: Anticancer CDK4/6 inhibitors (CDK4/6is) are oral drugs chronically administered and exhibit interindividual variability in plasma exposure, with the coefficient of variation of C_{min} ranging from 40 to 95%. Data on the exposure-efficacy relationship are still controversial for ribociclib and palbociclib, while a relationship with progression-free survival and tumour shrinkage has been reported for abemaciclib. In addition, significant exposure-toxicity relationships have been reported for all CDK4/6is, mostly related to neutropenia. The clinical application of TDM for CDK4/6is is still exploratory but could become a useful tool to optimise cancer therapy. To facilitate the translation of this application into practise, we have developed an LC-MS/MS method for the quantification of abemaciclib (and its metabolites M2 and M20), palbociclib, ribociclib and letrozole (which are usually administered in combination with CDKis) in dried blood spot.

Methods: Blood samples were collected using HemaXis DB10 from patients participating in a clinical trial (CRO 2022-14) ongoing at the National Cancer Institute. A 10 µL-spot was extracted with ultrapure water followed by protein precipitation. A reversed-phase XBridge BEH C18 column was used, with methanol as organic mobile phase and pyrrolidine-pyrrolidinium formate buffer (pH 11.3) as aqueous mobile phase. A triple quadrupole mass spectrometer was used for detection.

Results: The haematocrit (Hct) effect was minimized thanks to the specific sample preparation and the use of a calibration curve with Hct of 36% in the range of 22-55%. To date, a total of 26 patient samples have been collected. The method proved to be precise (CV% ≤8%) and accurate (acc% between 90-107%). Preliminary clinical validation results showed a good prediction of plasma concentration based on DBS values.

Conclusions: The analytical validation showed that the developed method is precise, accurate and reproducible and is suitable for routine use thanks to the ease of sample collection and processing. The possibility to quantify CDK4/6is and letrozole in DBS samples will be defined once clinical validation is completed. The use of DBS to monitor CDK4/6is exposure in clinical practise together with patient pharmacogenetic profiling and drug-drug interactions analysis will ultimately improve the cancer patients management through more precise and personalised treatment.

Key Words: abemaciclib, ribociclib, palbociclib, cyclin-dependent kinase inhibitors, therapeutic drug monitoring, mass spectrometry