Unveiling of *BRAF* V600E Mutant Cells Sensitivity to Proteasome Inhibitors Using an Isogenic Cell Based Screening

V. Boscaro[1], D. Zecchin[2,3], A. Bardelli[2,3,4], F. Di Nicolantonio [2,3], M. Gallicchio[1]

[1]Dip. di Scienza e Tecnologia del Farmaco, Università di Torino, Italy
[2]Dept. of Oncology, Università di Torino, Italy
[3]IRCC Institute for Cancer Research and Treatment at Candiolo, Italy
[4]FIRC Institute of Molecular Oncology (IFOM), Milano, Italy

It is well known that cell models represent a good experimental tool to evaluate the correlation between genetic mutations and pharmacological response (Di Nicolantonio et al., 2008). We have previously created a panel of isogenic cell lines, using hTERT-HME1 epithelial cells, in which *PTEN* or *RB1* was silenced in combination with the targeted knock-in of cancer associated mutations in *EGFR, KRAS, BRAF* or *PIK3CA* oncogenes (Zecchin et al., 2013). We exploited this panel of cells to identify pharmacogenomics relationships by screening a library of 43 compounds. These include molecules targeting tyrosine kinase receptors (RTKs) or their effectors; compounds that do not target members of the RTK signaling pathways but are employed as anti-cancer therapies; and drugs in clinical use aside from cancer therapy but that have been shown to have anti-proliferative activity.
As expected, HER family inhibitors affected cell proliferations of hTERT-HME1 in which the *EGFR* E746-A750 allele was knocked in. Concomitant inactivation of *PTEN* partially rescued this phenotype. Interestingly, this experimental approach allowed us to discover that the *BRAF* V600E mutant cells had increased sensitivity to proteasome inhibitors, such as bortezomib and carlfizomib. In order to understand the relationship between *BRAF* mutated cell lines and proteasome inhibitors we measured the amount of ubiquitinated proteins following proteasome inhibitors treatment. We found that *BRAF* mutant cells accumulated more ubiquitinated protein with respect to the wild type counterpart. Moreover, treatment of hTERT-HME1 *BRAF* V600E with clinically relevant concentrations of bortezomib resulted in increased p21 levels and PARP cleavage. These data suggest that proteasome inhibitors decrease cell proliferation and induce an apoptotic response in *BRAF* V600E mutated cells probably through an accumulation in ubiquitinated proteins. These results were confirmed in a second *BRAF V600E* isogenic model, LIM1215, as well as in a panel of colorectal cancer cell lines, harboring or not *BRAF* V600E mutation. Notably, we found that cell sensitivity to proteasome inhibitors was independent of the expression of *PTEN* or *RB1*. These results support the efficacy of isogenic cell based screening approaches to unravel the relationship between multiple mutations and pharmacological response. Of translational relevance, we have demonstrated that *BRAF* V600E mutant cells are sensitive to proteasome inhibitors, suggesting that the proteasome could be a target for the treatment of BRAF mutant tumors.

Di Nicolantonio et al. (2008). *Proc Natl Acad Sci USA*. 105, 20864–20869.
Zecchin et al., (2013). *Hum Mutat*. 34, 330-337