Multiple myeloma (MM) is a difficult disease to cure with median survival rate of around 7 years. In a recently published study, we confirmed the presence of residual proteasome inhibitor (PI)-resistant sub-population in drug-naive MM tumors. We hypothesize that this pre-existing resistant subclone may give rise to emerging resistance in course of PI treatment. In the current study, we used single cell transcriptomics to identify tumor subclones within human myeloma cell lines (HMCLs) based on a 48-gene model of predictive genetic signature for baseline PI response. Our results demonstrated the presence of pre-existing subclones of cells within untreated myeloma cells with a characteristic genetic signature profile distinct from the pre-treatment overall profile. This may help identify the presence and extent of intra-tumor heterogeneity in MM and may define residual pre-existing subclones resistant to PI therapies.

**OBJECTIVE**

Does GEP profile help in identifying pre-existing drug resistant clonal subpopulations within sensitive population?

To perform automated single-cell capture & cDNA synthesis from cellular RNA in individual cells and single-Cell qRT-PCR-based gene expression analysis of human myeloma tumor prior to exposure to PI treatment to identify resistant pre-existing subclones using a GEP-based model (48-gene panel of baseline responsiveness to PIs).

**METHODS**

- Automated single-cell capture and cDNA synthesis from cellular RNA were performed using Fluidigm’s C1™ Single-Cell Auto Prep System.
- The cDNA was harvested and transferred to BioMark HD System for single-cell targeted high-throughput qPCR-based gene expression analysis of a gene-panel using Fluidigm DELTAgene assays.
- *Stessman et al identified a 23-gene expression signature of baseline responsiveness to PI that was successful in stratifying good versus poor outcomes in human MM subjects from MM TT3 trial and APEX MM clinical trials (both using PI therapy).*
- *Shaughnessy *et al identified a 17-gene model from newly diagnosed MM patients on TT3 trial that could discriminate between low and high-risk myeloma with accuracies of over 95%.*

**RESULTS**

**U266 Parental vs U266 PI-resistant cells**

- Statistical analysis was performed using a combination of Fluidigm’s Singulair Analysis Toolset and the R Statistical analysis package.

**CONCLUSIONS**

- Multiple subclones of cells are present within untreated myeloma cells with a characteristic genetic signature profile distinct from the pre-treatment overall profile.
- PI-resistant subpopulations represent a pre-existing subset of PI-sensitive cell lines based on GEP profile that may give rise to emerging resistance in course of treatment with PIs.
- Similar findings have also been observed using multicolor flow cytometry. Immunophenotypic profiling of human myeloma cell lines (HMCLs) demonstrated unique signatures representing sub-clonal populations.

**FUTURE PERSPECTIVES**

- Our work will help identify the presence and extent of intratumor heterogeneity in MM and may define residual pre-existing subclones resistant to PI therapies.
- This will establish the use of microfluidics technology-based single-cell approaches as novel strategy to detect therapy-resistant subclones within bulk tumor populations from patient samples.

**REFERENCES**

- *Shaughnessy* *et al.* *Zhao, F.; Turra, B.; et al.* A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. Blood. 2007;109(6):2270-2281.

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