Phase 0 study of the effect of microdose melphalan on gene expression in mononuclear cells from peripheral blood in patients with multiple myeloma

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Purpose: The purpose of the study was to identify specific genes that might be up- or downregulated in the mononuclear cells (MNC) in the peripheral blood in multiple myeloma patients who receive a microdose of melphalan.

Primary Outcome Measures: Change from baseline in gene expression at 15, 30, 60, 120 minutes upon microdose drug exposure. The primary outcome measure was determination of differential and significantly expressed genes across time for successive samples from each individual patient. The analysis was based on global gene expression profiling and differentially expressed genes were identified using pairwise comparisons of samples means by two sample t-tests and corrections for multiple testing.

Patients and methods: Six patients with newly diagnosed multiple myeloma were included (4 males, 2 females). The patients received standard induction therapy with 4 series of VCD (bortezomin, cyclophosphamide and dexamethasone). The study treatment consisted of a microdose of iv melphalan 2 mg/sqm (1% of standard dose) given two hours prior to planned standard dose iv melphalan (200 mg/sqm).

Report: A microdose of 2 mg/sqm iv melphalan (1% of standard dose) was given two hours prior to planned standard dose melphalan. This study treatment did not cause any adverse events. Five ml of peripheral blood was collected in EDTA tubes prior to microdose and at 15, 30, 60 and 120 minutes post-microdose injection. MNCs were for each timepoint immediately purified by density centrifugation in Ficoll-Pacque Plus and split into fractions for flow cytometry analysis and RNA extraction for gene expression profiling. Flow cytometric analysis were performed on 100.000 MNC stained with the EuroFlow LST panel resulting in no detectable changes in cellular composition during the microdose time time. Global GEP was performed on total RNA extracted by a combination of Trizol and Qiagen protocols as previously described [1]. Labeling and hybridization of cDNA to Human Exon 1.0 ST (Exon) Arrays (Affymetrix) was performed and Array data .CEL-files from the Exon array were generated by the Affymetrix GeneChip Command Console Software (AGCC). Data were RMA normalized and combat corrected to remove patient specific effects. A variance filter was used to select the most variable genes over all experimental settings. The data were first analyzed by PCA plots. Detection of the genes with significant change over time was conducted by linear models, where significance was determined on the basis of un-adjusted p-values and p-values adjusted for multiple testing. The analysis on the significant genes included unsupervised clustering and it was assessed whether clustering based on time or patient occurred. Detection of patterns across time by inspecting the PCA trajectories over time of all the significant genes, in both 2 and 3-dimensions were conducted. Finally, detection of change in patterns of gene expression over time by applying self-organizing maps (SOMS) was pursued. By unsupervised clustering, data did not cluster by patients nor time.

Results: No genes showed significant changes during the microdosis time period, when analyzed using multiple test correction. However, the genes showing most significance using un-adjusted p-values showed a small but systematic changes in a three dimensional PCA plot illustrating that the total composition of MNCs experience changes in immediate response to melphalan and that the effect is gradually lost after 120 minutes post microdose injection. However, one should be cautious interpreting these results, due to the pre-selection of significant genes. There were no clear patterns using SOMs. Finally, an analysis restricted to REGS genes and genes commonly associated with sleep patterns were conducted[2]. None of these showed significant changes over time and did not cluster the data patient or time wise.

References:

1. Dybkær, K. *et al.* A diffuse large B-cell lymphoma classification system that associates normal B-cell subset phenotypes with prognosis. *J. Clin. Oncol.* **33,** 1379–1388 (2015).
2. Falgreen, S. *et al.* Generation of cell line derived gene expression signatures for response to cyclophosphamide, doxorubicin, or vincristine validated in diffuse large B-cell lymphoma chohorts. *Prep.* (2013).