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POSTER ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Extracellular Vesicles Secreted from Daratumumab Resistant Cells Promote Resistance and Proliferation of Daratumumab Sensitive Cells, Possibly through the Transfer of miRNA Cargo

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Multiple Myeloma (MM) is the second most common hematologic malignancy and remains incurable. Daratumumab (dara) is a potent anti-CD38 monoclonal antibody used for MM treatment, but responses are heterogeneous, and resistance is inevitable. There is hence an urgent need for identification of biomarkers to predict responses and in-depth characterisation of mechanisms of resistance.

We have previously demonstrated that the exosome biogenesis pathway plays a significant role in the survival of dara resistant (dara R) cell lines. Here, we highlight that the enriched expression of exosome pathway proteins reported in dara R cell lines is not detected in Lenalidomide or Bortezomib-resistant cell lines, or in other monoclonal antibody resistant cell lines such as anti-CD20 (Rituximab)-resistant cell lines, thus indicating that this mechanism is unique to daratumumab resistance. Coculture of dara sensitive (dara S) cell lines with dara R cell lines separated by a cell-impermeable transwell insert promoted cell proliferation and reduced response to dara treatment in dara S cell lines thereby suggesting a potential role for extracellular vesicles (EVs) secreted by dara R cells in mediating these responses. In this study, we seek to investigate the role of EVs secreted by dara R cells, in particular, that of EV-derived miRNAs in the tumour microenvironment which can help predict and mediate dara resistance.

We have originally described the establishment of three dara R cell lines in Natural Killer T-cell Lymphoma (NKTL), T-cell acute lymphoblastic leukaemia (T-ALL) and MM via long-term exposure to increasing concentrations of dara. To assess the specific role of EVs in mediating resistance, we isolated and purified EVs from the media of dara R cell lines and added it to the culture of dara S cells. To confirm uptake of dara R EVs by sensitive cell lines, EVs isolated from resistant cell lines were stained with PKH26 and characterised by binding to CD81 and CD63 beads via flow cytometry. Uptake of dara R EVs by dara S cell lines was confirmed through flow cytometry and confocal microscopy. We observed that uptake of dara R EVs directly promote proliferation and reduced cytotoxic response to dara treatment in dara S cells. Next, we assessed the effect of inhibiting dara R EV secretion by applying Neticonazole and Ketoconazole, prior to coculture of resistant and sensitive cell lines. Interestingly, coculturing azole-treated resistant cell lines with sensitive cell lines mitigated the increase in proliferation and dara resistance.

Exosomal miRNAs are known to play a pivotal role in regulating cellular pathways driving cancer cell resistance and survival. To evaluate the potential role of EV-derived miRNA cargo, we first extracted miRNAs from the EVs purified from dara R and dara S cell lines and then performed an miRNA profiling screen with the PanoramiR panel (MiRXES) which targets 376 miRNAs expertly curated from miRBase 22, HMDD 3.2 and The Cancer Genome Atlas. We discovered that EVs derived from resistant cell lines expressed higher levels of proliferative miRNAs, miR-155 and miR-181a. These findings were validated through qPCR, confirming the increased expression of these miRNAs in both resistant cell lines and EVs derived from resistant cell lines. We also observed a concomitant downregulation of mir-155 target genes such as BACH1, JARID2 and SMAD5 in the resistant cell lines.

This positive observation led us to extend the study into the clinic. Extracellular vesicles were isolated from the plasma fraction of bone marrow samples obtained from 17 patients with MM prior to treatment with dara based combinations either in the upfront or relapsed setting. miRNA extraction was performed and samples evaluated by miRNA sequencing. We found that

the EVs from patients with a progression free survival (PFS) of less than 2 years after dara based therapy (dara R patients) had an enrichment of mir-155 and mir-181a as compared to those with a PFS longer than 2 years. These data align with our findings in the cell lines and suggest that these miRNAs may be utilised as potential biomarkers for daratumumab resistance. We are currently elucidating the biological basis for dara resistance mediated by these miRNAs. A deeper understanding of the mechanisms involved may pave the way for clinical evaluation of EV- directed therapeutics to overcome dara resistance.

Disclosures De Mel: *Pfizer:* Other: advisory board.

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