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Blood 142 (2023) 5781–5783

The 65th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

The Impact of the Peptide Drug Conjugate Melflufen on the Myeloma Tumour Microenvironment

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Introduction

Melphalan flufenamide (melflufen), is a peptide-drug conjugate that delivers alkylating agents in tumors upon cleavage by aminopeptidases, and recently approved by the EMA for the treatment of relapsed/refractory multiple myeloma (MM). Although melflufen's MoA on tumor cells had been extensively studied, little is known about the drug impact on the tumor microenvironment. By applying single cell, multi-parametric, flow cytometry-based analysis to bone marrow mononuclear cells (BM-MNC) from MM patient samples treated *ex vivo*, we investigated the effect of melflufen on immune cell populations to assess for potential immune modulatory effects and to identify potential immune-based biomarkers of response.

Methods

Viably frozen BM-MNC from 26 samples of patients diagnosed with MM were used for multi-parametric flow cytometry-based drug sensitivity and resistance testing evaluation to melflufen after written informed consent following approved protocols in compliance with the Declaration of Helsinki. Drug sensitivity was assessed using five different concentrations of melflufen ranging between 0.5-5000 nM and cells incubated with drug or DMSO control for 72 hrs incubation. For analysis, we used antibody panels allowing us to identify and phenotype T and B lymphocytes, NK, progenitor, and malignant plasma cells (PC). Based on drug sensitivity scores (DSS) of gated PCs from drug treated and controls samples, we divided the samples into two groups - high sensitivity (HS, Q3 of PC-DSS) and low sensitivity (LS, Q1 of PC-DSS). Samples with no PCs detected (n=11) were excluded from PC subset analysis.

Results

The MM samples were divided based on the sensitivity of the PC population to melflufen with DSS > 21.25 defining a highly sensitive group and DSS < 15.9 defining a less sensitive group. We found that HS samples have higher abundance of mature NK and iNKT cells, although significance was low, likely due to the small cohort size. In addition, we discovered that effector memory (EM) and terminal effector (TE) T cells have higher sensitivity to melflufen, compared to naïve and central memory (CM) phenotypes (Fig. 1). More mature NK cells (Stage 5 and 6) also showed higher sensitivity than NK cells of earlier stages of maturation. Importantly, we observed that in many of the tested samples, progenitor-like CD34+CD38- cells either were not sensitive to the cytotoxic effect of the drug, or even proliferated in higher drug concentrations (Fig. 2). Interestingly, HS samples had significantly higher baseline abundance of this cell population ($p<0.05$). At the same time, response profiles of healthy B and malignant PCs were similar. Finally, in our tests, PCs in samples with trisomy 11 abnormality showed lower sensitivity to melflufen ($p<0.05$).

Conclusion

In a previous study, we found that effector-like cells could be associated with better response to melflufen. In this study, we observed the same trends and confirm our earlier findings demonstrating an association of NK and NKT cells with higher sensitivity to melflufen. Moreover, we identified differential response of CD4, CD8 and NK cell populations comprising the tumor microenvironment to melflufen suggesting that in addition to direct tumor cell killing, melflufen may have modulating effects on different tumor associated immune cell populations. Finally, we observed proliferation of CD34+CD38- progenitor cells, which might be relevant for lymphocyte proliferation and function, and additionally supporting potential modulating effect. Further analyses are being done to validate these findings.

Disclosures Svensson Gelius: *Oncopeptides AB:* Current Employment. **Acs:** *Oncopeptides AB:* Current Employment. **Heckman:** *WNTResearch:* Research Funding; *Kronos Bio:* Research Funding; *Novartis:* Research Funding; *Autolus:* Consultancy; *Amgen:* Honoraria; *Oncopeptides:* Research Funding; *Zentalis Pharmaceuticals:* Research Funding.

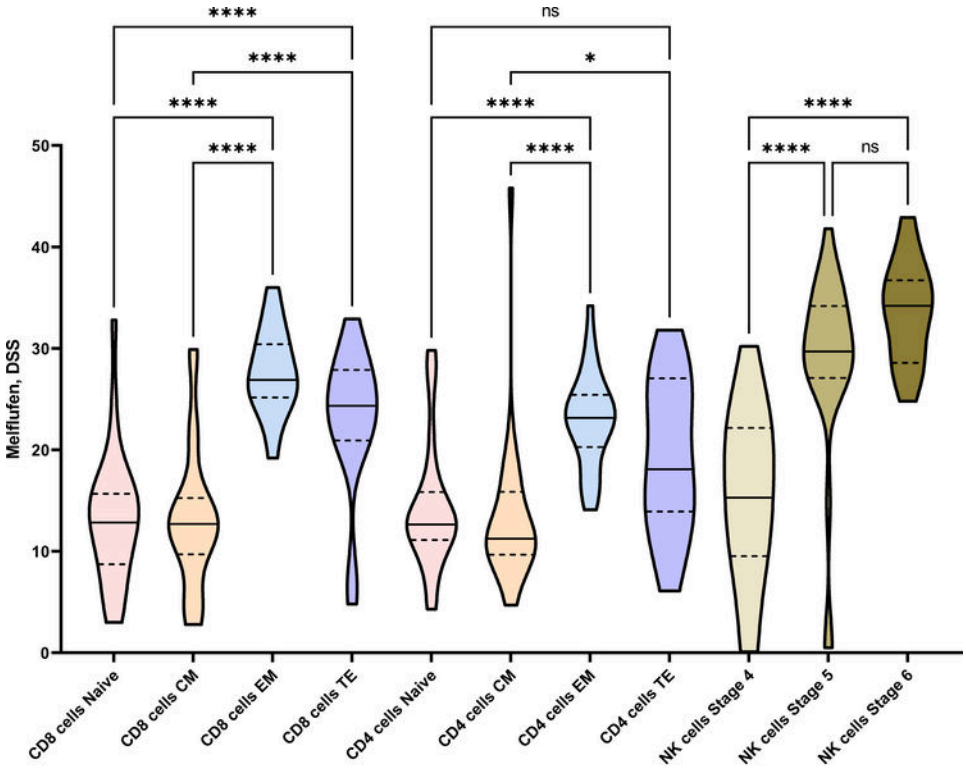

Figure 1. Differential response to melflufen of T and NK cells populations.

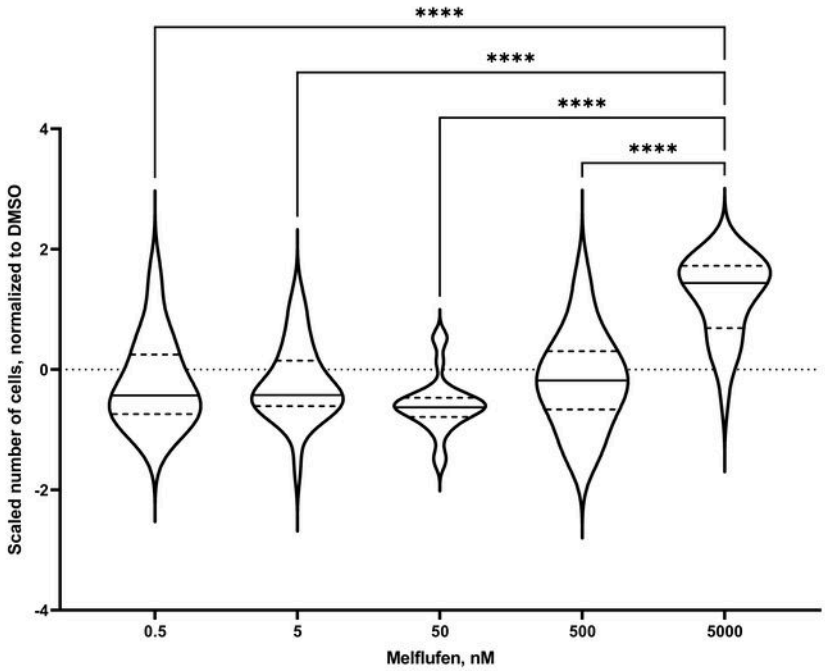

Figure 2. CD34+CD38- population response to melflufen.

Figure 1

https://doi.org/10.1182/blood-2023-188603