# **Eosinophils inhibit malignant pleural mesothelioma** response to chemotherapy

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- Malignant pleural mesothelioma (MPM) is accompanied by an inflammation characterized by immune cells infiltration such as macrophages, monocytes, lymphocytes, neutrophils and eosinophils.
- MPM is one of the cancers in which eosinophilia has a strong association. Cases of MPM with eosinophilic pleural effusions, tissue eosinophilia (TATE) and peripheral blood eosinophilia have been described.

• What is the link between eosinophilia and response to treatment and/or survival of MPM patients?







Results

**Figure 1 :** Blood eosinophilia inhibits MPM patients response to platin salt and pemetrexed chemotherapy.

T-cell

NK cel

B-cell

**MDSCs** 

Eosinophi

Mesothelioma cell

Exhausted T-cell

(A) Mean survival (in months) of patients with and without blood eosinophilia ( $\geq$  300 /  $\mu$ l of blood) before cisplatin and pemetrexed treatment. Bars represent means +/- SD. Normality of population were assessed by Shapiro-Wilk and means were compared with a Mann-Withney test. (B) Kaplan-Meier curve of patients with and without blood eosinophilia. Statistical significance was calculated by Log-Rank (Mantel-Cox) test and Hazard ratios are reported on the graphs. (C) Immunohistochemistry of MPM tumors was performed for hematoxylin and eosin, and CCR3.





# Figure 3: Valproate-differentiated EOL-1 inhibit MPM response to cisplatin and pemetrexed in the spheroid model.

(A) Experimental model for relevance of eosinophil supernatant in spheroids. M14K cells were cultivated in a 96wells coated with DMEM-Agarose 1.5% in presence or absence of differentiated EOL-1 supernatant for 72 hours



SN Diff-EOL1 +

# Figure 2: Valproate-differentiated EOL-1 supernatant inhibits MPM response to cisplatin and pemetrexed.

(A) Experimental model for EOL1 differentiation. EOL-1 cells were differentiated into eosinophils with valproate 2mM for 8 days. (B) Differentiated EOL1 were labelled for CCR3 and actin, stained with DAPI and analyzed by confocal microscopy (magnification 40x). (C) Eosinophil peroxidase activity of progenitors and differentiated cells stimulated or not with IL-5 100 ng/ml was monitored by a colorimetric assay and analyzed with a spectrophotometer. (D) Protocol evaluating response to chemotherapy in presence of supernatant from differentiated cells (SN Diff-EOL1). MPM cells were then cultivated in presence or absence (mock) of the supernatant at 25% v/v for 48 hours. Cells were treated with 10 μM cisplatin and 10 μM pemetrexed (C+P) for 48 hours. (E) Annexin V/PI staining plots were collected. (F, J) Apoptotic rates of M14K and ZL34 cells were analyzed. (G, K) Cell cycle profiles were collected by flow cytometry after cell permeabilization and propidium iodide staining. (H) Proportion of M14K cells with fragmented DNA (i.e., Sub-G1). (L) Proportion of ZL34 in S phase. (I, M) Proportion of M14K and ZL34 cells with DNA double-strands breaks were analyzed after staining with anti-γH2AX antibody. Data are expressed as mean +/- SD, each dot representing an independent test. Normality was checked with a Shapiro-Wilk test and means were compared by t-test (C) or one-way ANOVA followed by Tukey's multiple comparison test.

#### response to chemotherapy.

(A) M14K cells and CFSE-labelled differentiated EOL-1 were monitored by time-lapse microscopy using an IncuCyte S3 Live-Cell imaging system (Essen Bioscience) equipped with an environmental chamber maintained at 37°C in a humidified 5% CO<sub>2</sub> atmosphere. (F) Differentiated EOL-1 stimulated or not with IL-5 100 ng/ml were labelled for CD63 and degranulation was monitored by IncuCyte S3 Live-Cell imaging system (Essen Bioscience) equipped with an environmental chamber maintained at 37°C in a humidified 5% CO2 atmosphere. (B) Differentiated EOL-1 supernatant was treated either with N-ethlymaleimide (NEA;  $10^{-7}$  M) or with thiogalactoside (TDG; 49  $\mu$ M) for 4 hours at 37° C in a humidified 5% CO<sub>2</sub> atmosphere. Supernatant was then added to M14K cells for 48 hours before treatment with cisplatin and pemetrexed for 48 hours. (C) Apoptotic rates of M14K cells were determined by flow cytometry after staining with annexin V-FITC and propidium iodide. Bars represent mean +/- SD from at least 4 independent experiments performed in duplicates. Normality of populations was verified with a Shapiro-Wilk test and means were compared by one-way ANOVA followed by Tukey's multiple comparison test.



# Conclusion

Eosinophils inhibit malignant pleural mesothelioma response to standard chemotherapy (cisplatin + pemetrexed) in patients and in cell culture.

Eosinophils inhibit MPM response through an interaction with MPM cells that involves galectin-10 and -3.

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