Gemcitabine- and Fluropyrimidine- based Chemotherapy Reduces Myeloid Derived Suppressor Cells in Pancreatic and Esophagogastric Cancer

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Introduction

Myeloid derived suppressor cell (MDSC) and T regulatory cell (Treg) accumulation is proposed as an important mechanism of tumor immune evasion in murine models and cancer patients. MDSCs represent a heterogeneous population of cells, arisen from myeloid progenitor cells which have failed to terminally differentiate into mature granulocytes and macrophages. In humans, MDSC have been defined as cells that express CD33 and CD11b, but lack the expression of the MHC class II molecule HLA-DR and lack markers of mature myeloid and lymphoid cells (lineage negative)[1].

Pre-clinical data demonstrates that Gemcitabine[2] and Fluropyrimidine treatment leads to substantial falls in MDSCs in murine cancer models. In a study of commonly used cytotoxics, Gemcitabine and 5FU were the only agents which reduced murine MDSC levels[3]. In humans, Gemcitabine has previously been shown to reduce Tregs. In 10 patients, Tregs were reduced at day 1 and day 2 following treatment, compared to baseline[4]. We are aware of only one clinical study specifically addressing the effect of chemotherapy on MDSCs levels[1]. In 6 patients where the chemotherapy was unspecified, MDSC levels tracked changes in tumor volume.

We analyzed MDSC and Treg percentages in Pancreatic and Esophagogastric cancer patients pre- and post- Gemcitabine and Fluropyrimidine/Platinum based chemotherapy. In addition, we analyzed whether such changes were associated with response to treatment, by correlation with changes in tumor volume.

Materials and Methods

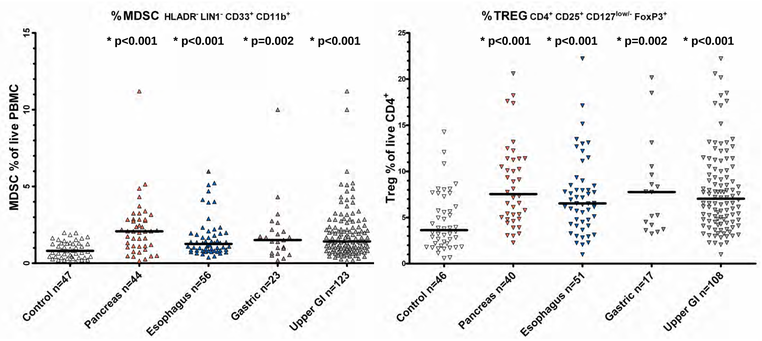
Patients: Venous blood was collected from Pancreatic and Esophagogastric cancer patients, prior to and following treatment with Gemcitabine or Fluropyrimidine/Platinum based chemotherapy. PBMC were isolated and stored for subsequent flow-cytometric batch-analysis.

Immunophenotypic analysis of cells: PBMC were recovered and aliquoted for MDSC and Treg analysis. The LIVE/DEAD Cell stain Kit (Invitrogen, UK) was used to differentiate viable and dead cells. For MDSC phenotyping, anti-HLA DR-APC Cy7, anti-Lin1(CD3,14,16,19,20,56)-FITC, anti-CD33-PE Cy5 and anti-CD11b-PE Cy7 monoclonal antibodies were used (BD Biosciences, Europe). For Treg phenotyping, anti-CD4-PE Cy7, anti-CD25-APC Cy7 and anti-CD127-PE cell surface stains were performed (BD Biosciences, Europe), before permeabilization and staining for intra-nuclear FoxP3, including an isotype control (eBioscience, Europe). Samples were analyzed using a MACSQuant flow cytometer with MACSQuantify software.

Assessment of Tumor Volume Change: Tumor volume pre- and post- chemotherapy was quantified by a radiologist blinded to study intent and assay results, via CT measurement of tumor volume using the RECIST v1.1 criteria of measurable target lesions.

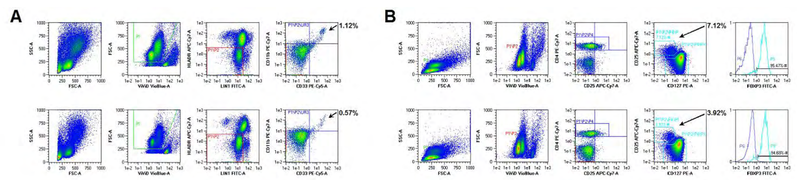
Statistical Methods: MDSC and Treg percentages were compared in pre- and post- chemotherapy samples using the non-parametric Wilcoxon signed rank test for paired samples.

High MDSCs and Tregs in Upper GI Cancer Patients vs. Controls



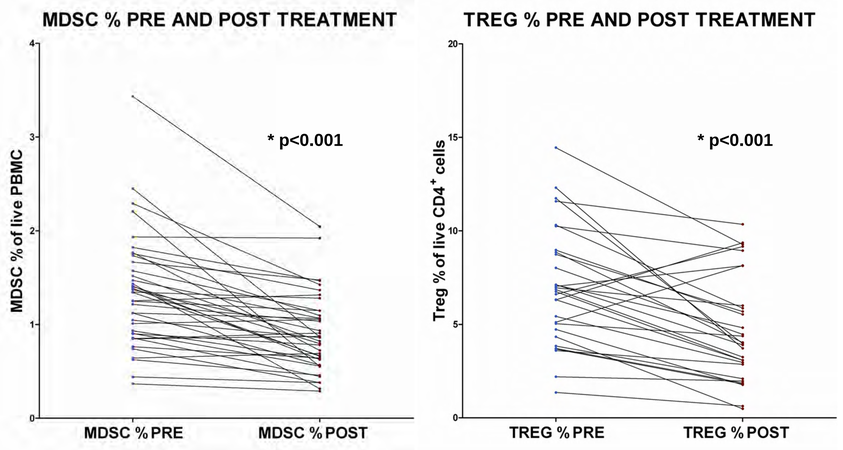
- Previously, we have reported a statistically significant difference in MDSC and Treg % in Pancreatic and Esophagogastric cancer patients vs. Controls
- No Control donor had an MDSC % >2.0% (n=47)
- In a multivariate analysis, MDSC % was statistically associated with survival (p=0.007, Hazard Ratio 1.22, 95% Confidence Interval 1.06-1.41)

Representative FACS plots of MDSC and Treg Pre- and Post- Treatment



FACS plots of a gastric cancer patient pre- *(above)* and post- *(below)* 5-FU based chemotherapy
A, HLADR$^-$ Lin1$^{low/-}$ CD33$^+$ CD11b$^+$ MDSCs B, CD4$^+$ CD25$^+$ CD127$^{low/-}$ FoxP3$^+$ Tregs

MDSC and Treg % Pre- and Post- Chemotherapy



- MDSC and Treg levels were statistically significantly reduced following chemotherapy
- Change in MDSC % was associated with Tumor Volume % change (Spearman r=0.45, p=0.005)

Pancreatic Cancer Patients: Tumor Volume and MDSC % Change

Tumour Volume % Change	MDSC % Change From Baseline	MDSC Value Pre-Treatment	MDSC Value Post-Treatment
- 48.7	- 50.3	1.3	0.7
- 43.5	- 27.0	1.6	1.1
- 36.7	+ 4.2	1.0	1.1
- 27.7	- 21.4	0.4	0.3
- 22.4	- 31.9	1.4	0.9
- 21.4	- 55.6	1.4	0.6
- 20.0	- 27.6	1.5	1.1
- 18.9	- 47.9	0.9	0.4
- 17.4	- 43.3	1.4	0.8
- 16.9	- 8.2	1.3	1.1
- 9.4	- 38.5	0.9	0.6
- 2.2	- 11.5	1.7	1.5
- 0.6	- 40.5	**3.4**	2.0
0.0	- 4.7	1.3	1.3
+ 2.4	- 13.1	0.9	0.8
+ 20.0	+ 7.7	0.6	0.7

Acknowledgments

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Esophagogastric Cancer Patients: Tumor Volume and MDSC % Change

Tumour Volume % Change	MDSC % Change From Baseline	MDSC Value Pre-Treatment	MDSC Value Post-Treatment
- 80.0	- 17.7	1.0	0.9
- 61.4	- 22.2	1.8	1.4
- 60.0	- 59.0	1.8	0.7
- 57.4	- 45.8	1.5	0.8
- 49.1	- 14.8	1.2	1.0
- 45.7	- 49.4	1.1	0.6
- 32.4	- 52.3	1.4	0.7
- 30.0	- 78.0	1.4	0.3
- 29.3	- 64.6	**2.5**	0.9
- 27.4	- 19.5	1.8	1.5
- 21.1	- 48.6	0.7	0.4
- 1.1	- 13.9	0.4	0.4
0.0	- 75.0	**2.2**	0.6
0.0	- 26.7	0.6	0.5
+ 8.5	- 0.7	1.9	1.9
+ 13.2	+ 6.8	0.9	0.9
+ 18.5	- 15.8	0.8	0.6
+ 20.0	- 36.9	1.7	1.1
+ 20.7	- 32.1	0.9	0.6
+ 25.0	- 37.8	**2.3**	1.4
+ 37.3	+ 5.0	1.3	1.3
+ 50.0	+ 3.1	0.8	0.9
+ 63.6	+ 5.1	1.1	1.1

Results

- 16 Pancreatic cancer patients receiving Gemcitabine based chemotherapy, and 23 Esophagogastric cancer patients receiving Fluropyrimidine/Platinum chemotherapy were analyzed
- There was a statistically significant reduction in pre- and post- treatment MDSC % ($p<0.001$)
- There was also a statistically significant reduction in Treg % ($p<0.001$)
- Of the 21 pancreatic and esophagogastric cancer patients having a >10% decrease in Tumor Volume, 20 had a fall in MDSCs
- Of these, 60% of patients' MDSCs fell by >30%
- In the remaining 18 patients with a ≤10% reduction in Tumor Volume, there was still a significant reduction in MDSC % ($p=0.002$)
- 7 patients had a reduction in Tumor Volume of 0-10%, 4 of which had a >25% fall in MDSCs
- 11 patients had an increase in Tumor Volume, none of which had a >8% increase in MDSCs
- 3 of these 11 patients had a >30% fall in MDSC %
- In all patients with initial MDSC % above the upper limit of normal (>2.0%), Gemcitabine or Fluropyrimidine treatment reduced the MDSC % to within normal range, independent of response

Conclusions

We have demonstrated that Gemcitabine and Fluoropyrimidine based chemotherapy cause a significant reduction in both MDSC and Treg numbers. This reduction is seen even in patients who fail to show an objective response to treatment. All patients with an initial MDSC level above the normal range showed a fall in MDSC level to within the normal range as a result of chemotherapy. These findings require validation in further datasets; we are performing further analyses to determine the duration of this effect following chemotherapy, and also to establish the functional impact of chemotherapy on these cells.

References

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