Impact of cyclophosphamide treatment on myeloid cells in a context of tumor development

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Aim.

•Investigate the implication of Tumor Associated Macrophages (TAM) in cyclophosphamide induced tumor remodeling and relapse.

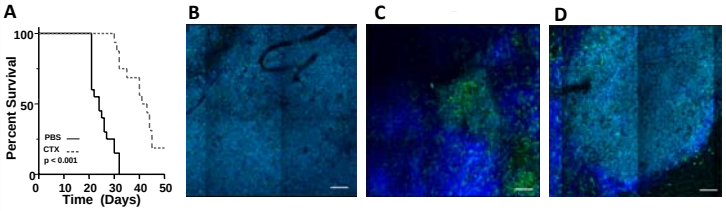
Model.

•Cyclophosphamide (CTX) is a myelo-ablative alkylating agent with immunomodulatory activities.

•CFP-EL4 are traceable tumor cells with kidney tropism.

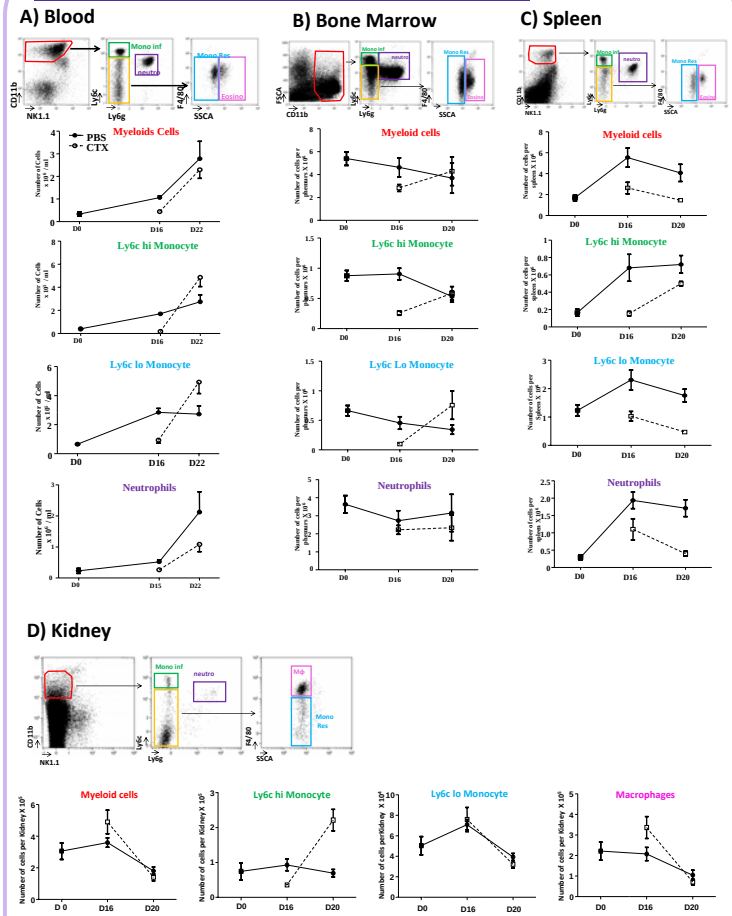
•CSF1R-CFP-Tg / *CX$_3$CR1*$^{+/GFP}$ - Knock In mice allow the differentiation TAM subset in vivo.

1) Tumor destruction and relapse after chemotherapy.



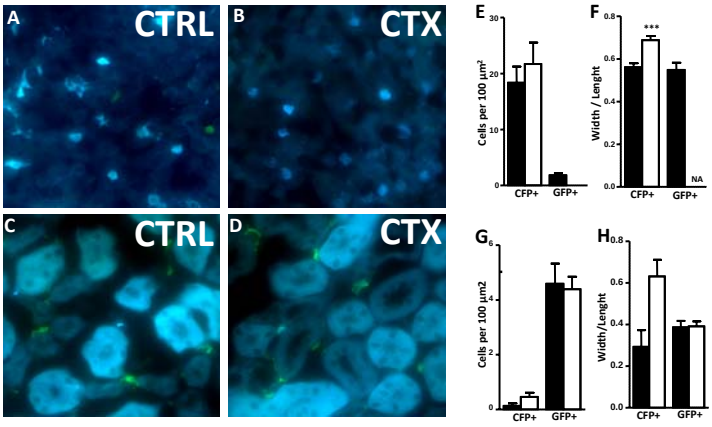
A) Treatment with cyclosphophamide of mice that received i.v. injection of EL4 increase significantely mice mean survival. Representative TPLSM image of EL4-mCFP tumor cells orthotopically injected in the kidney, B) before CTX treatment, C) 5 days after treatment and D) 10 days post treatment. Within 5 days we observed a decreased of the tumor mass followed by a relapse after 10 days. (Collagen fibers are in Blue (SHG) and TAM are in green (CX3CR1$^{gfp/+}$))

2) Impact of CTX on Myeloid cell distribution.



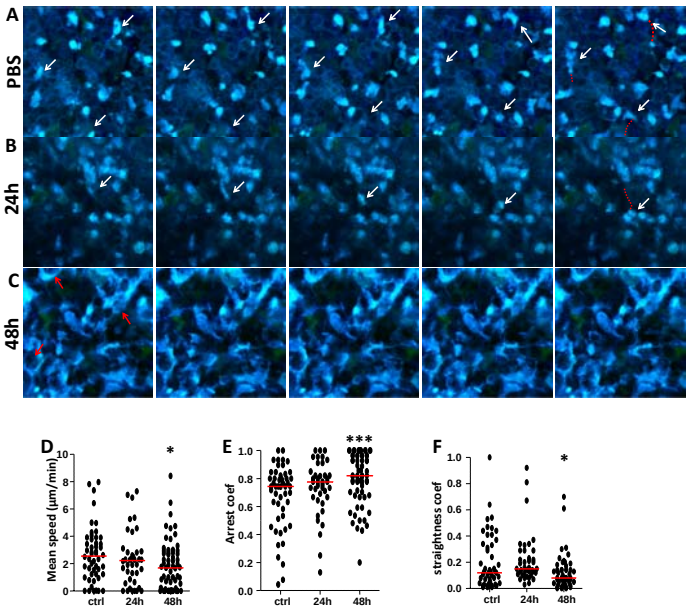
Single CTX treatment in mice inoculated with EL4 result in a decreased myeloid cell number in A) the Blood, B) the Bonne Marrow (BM) and C) the Spleen. This is followed by a rebound of all the myeloid populations in the blood and the BM. D) We observed an increased infiltration in the kidney by myeloid cells, mostly MΦ/DC, after CTX treatment, supporting their role in tumor destruction and/or relapse.

3) Cyclophosphamide induces *CSF1-R+* TAM activation.



When injected orthotopically in the kidney, EL4 cells constitute solid tumor. A) the tumor is infiltrated by numerous CSF1R+CFP+ cells with dentritic-like or more circular shapes and few CX3CR1$^{+/GFP}$xCSF1R-CFP- cells. C) Inversely, kidney healthy parenchyma is mostly infiltrated by dendritic-like CX3CR1$^{+/GFP}$-CSF1R-CFP- cells and few CSF1R+ CFP+ cells. B) CTX treatment did not significantly increase E) the number of CFP+ cells in the tumor, F) although CFP+ cells display a more circular shape suggesting modificated activation status. D) The treatment do not affect the dendritic like CX3CR1$^{+/GFP}$xCSF1R-CFP- cells from the healthy area G) and H).

4) CTX affects TAM behaviors.



Using in vivo imaging we studied tumor infiltrating myeloid cell behaviors before or after CTX treatment. A) Before treatment most of the CFP+ cells display very low mobility. D) Few cells with highest displacement properties migrate through the tumor, continuously making contact with tumor cells. B) 24 hours after CTX treatment, we observed D) a decrease of the speed mean and E) an increase of the arrest coefficient of the CFP+ cells. C) This trend is confirmed at 48h post treatment. At this stage, many CFP+ cells display a very outspread morphology, high protrusive with low displacement activities. Based on morphology, numerous CFP+ cells have undertaken phagocytosis (↖).

CONCLUSION.

•In vivo imaging of CSF1R-CFP/ *CX$_3$CR1*$^{+/GFP}$ Knock In mice allow fine characterization of myeloid cell behaviors in the tumor with or without cyclophosphamide treatment.

•CSF1R+CFP+ cells contribute to tumor remodelling after chemotherapy

•Contribution of the CSF1R+CFP+ cells to tumor destruction and relapse after cyclophosphamide treatment need to be investigated further.