Characterization of patient-derived tumor-associated macrophages and setup of an *in vitro* model

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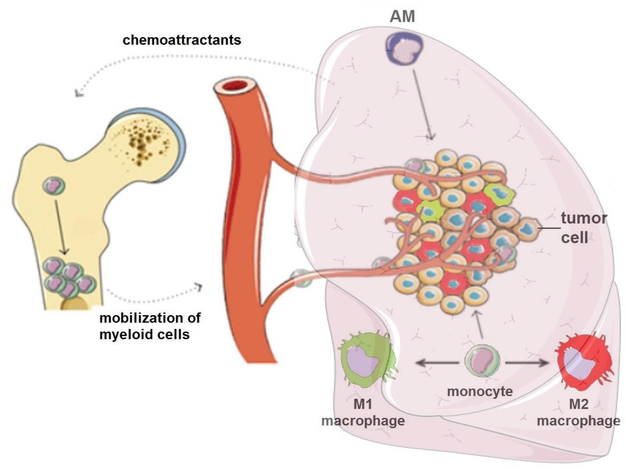
Introduction

Lung cancer represents the most common cause of cancer-related death worldwide.
Macrophages, developing out of infiltrating monocytes, have been shown to play a complex role in tumor progression. Depending on the tumor microenvironment, tumor-associated macrophages (TAM) can either be polarized to resemble a classical pro-inflammatory, tumor preventing (M1 macrophages) or an alternatively-activated, anti-inflammatory, and tumor promoting subtype (M2 macrophages). The dominance of the M2 subtype often correlates with a poor clinical prognosis [1].
Tissue resident macrophages in the lung, such as alveolar macrophages (AM), are believed to be of central importance in the immune response against infections and tumors [2].
Understanding the macrophage polarization processes might contribute to important therapeutic advances, where TAM can be re-edjucated to a tumor-suppressive phenotype and elicit a potent anti-tumor immune response.

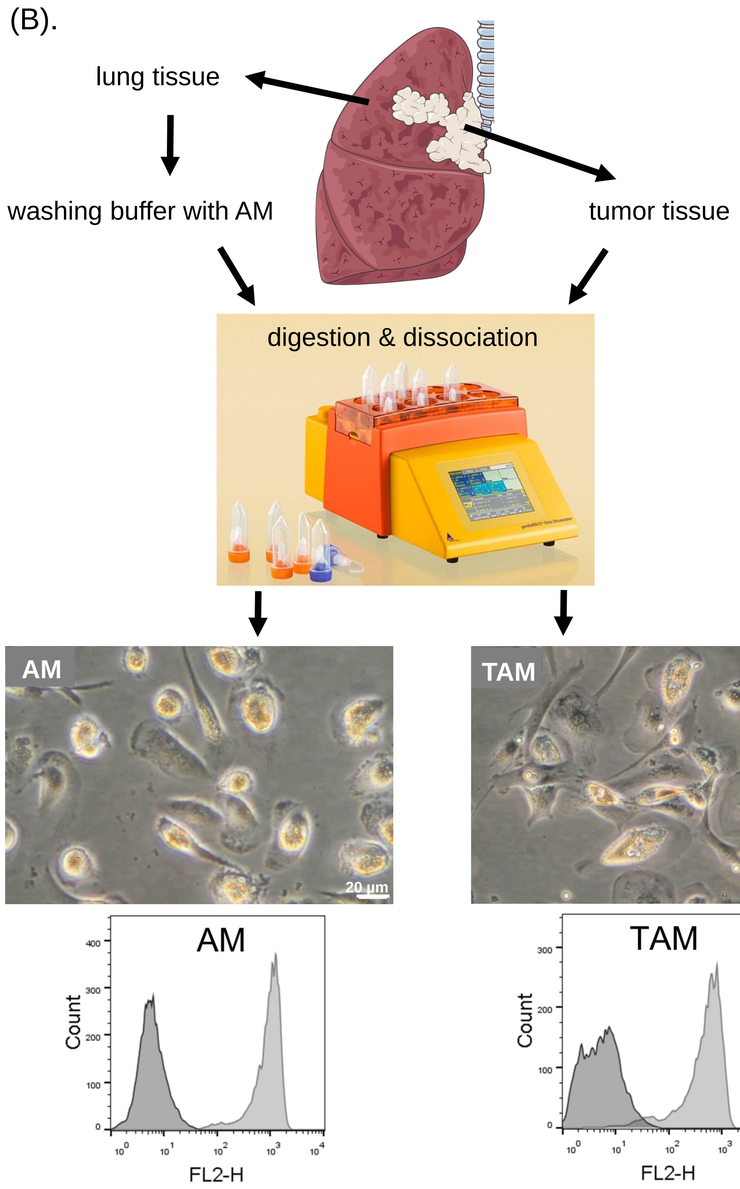


Aim

1. Characterize patient-derived TAM from lung tumor tissue.
2. Establish an *in vitro* model for polarized lung TAM.

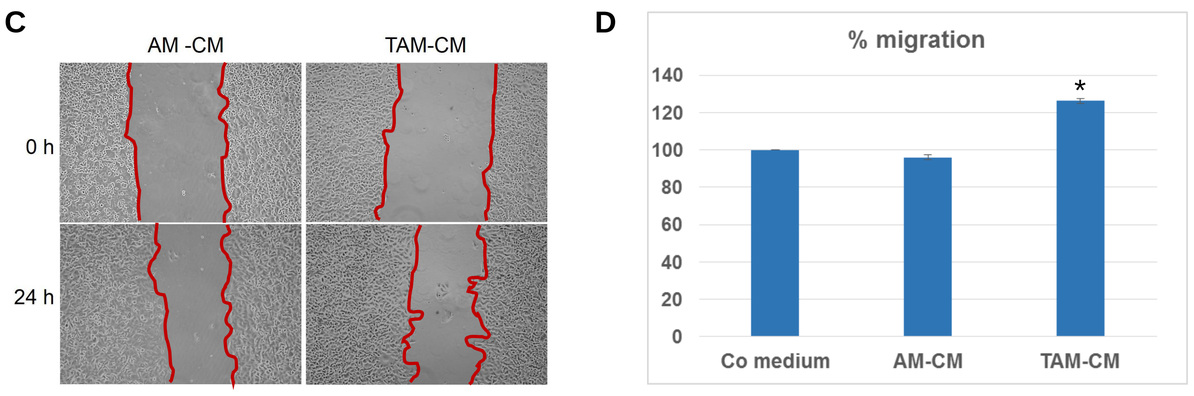
Isolation of *in vivo* differentiated TAM

AM and TAM were isolated from normal human lung tissue and tumor tissue. After mechanical dissociation and enzymatic digestion, a single-cell suspension was obtained and seeded into a culture flask for further experiments.
Microscopy showed that AM are round, large cells whereas TAM are heterogeneous in size and shape (A). CD68, a marker specific for macrophages, was detected in over 95% of the cells contained in AM and TAM suspension, confirming the purity of the preparation (B).



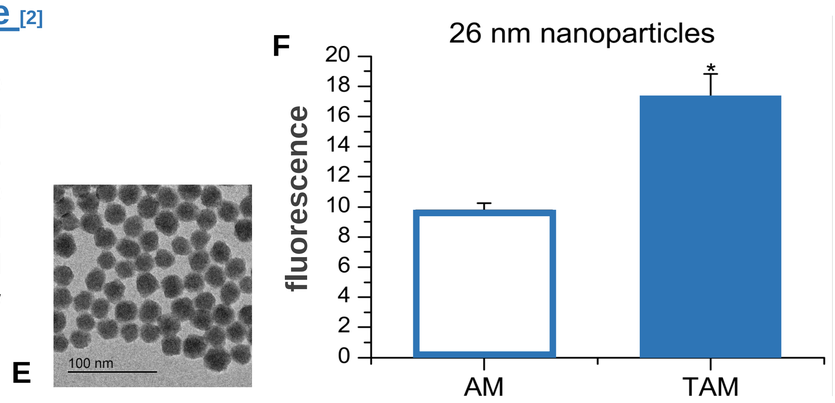
Cell migration assay

Cell migration assay allows to test for invasive and metastasis potential of tumor cells such as A549 upon incubation with AM-conditioned medium (AM-CM) and TAM-CM. Representative images immediately after scratching (0 h) and after 24 h are shown (C). Cell migration with TAM-CM is enhanced compared to AM-CM (D).



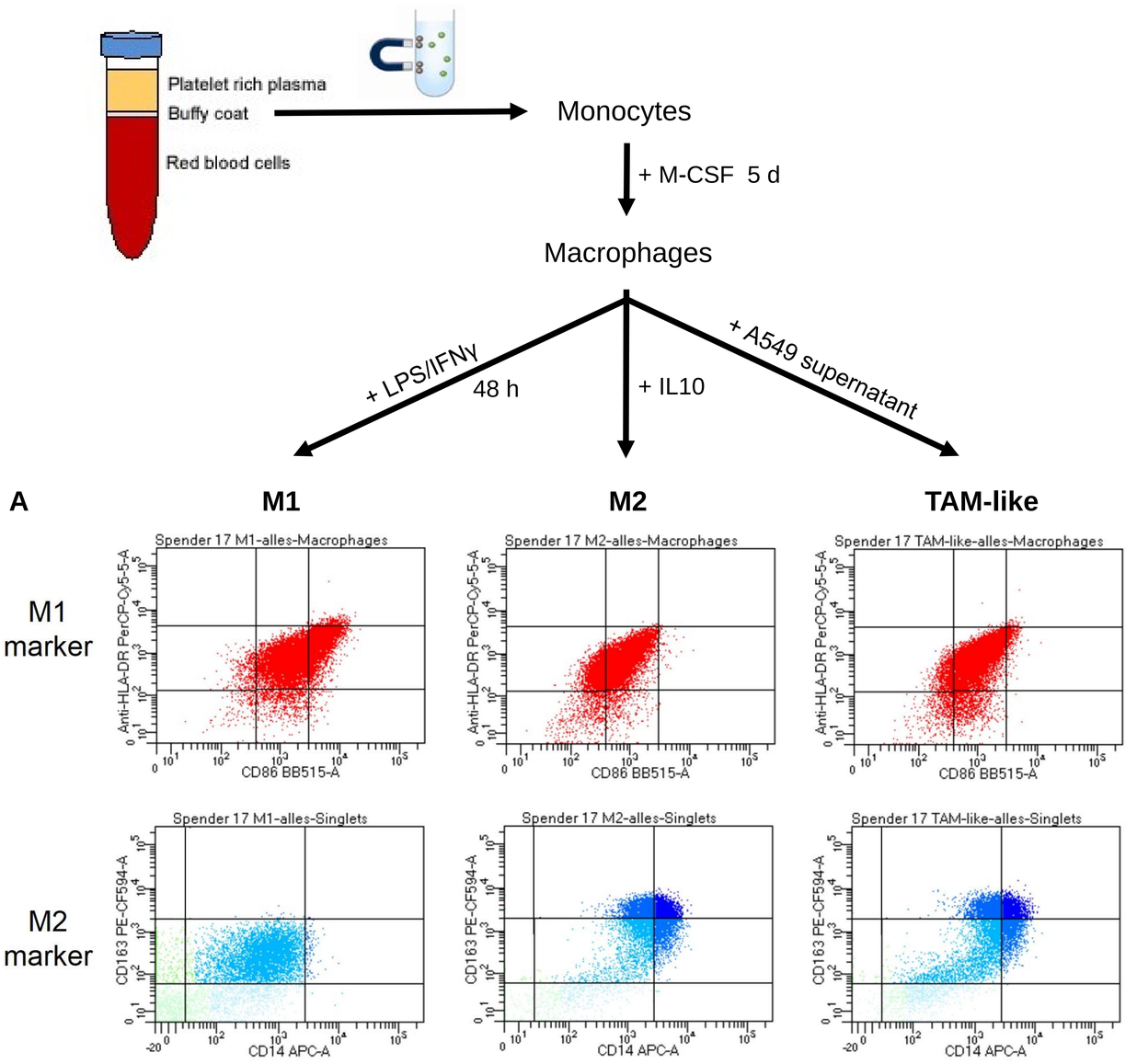
Nanoparticle uptake [2]

AM and TAM were incubated with 26 nm silica nanoparticles (E). The particle uptake was enhanced in TAM when compared to AM as assessed by flow cytometry (F).



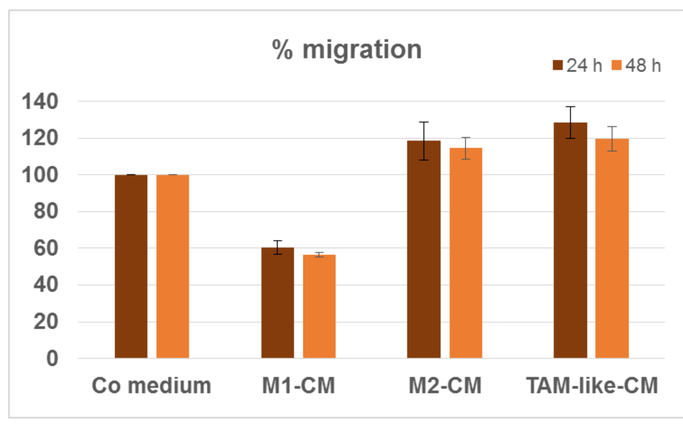
In vitro TAM model

Human monocytes were obtained from *buffy coats* after magnetic bead isolation. The cells were differentiated into macrophages with M-CSF for 5 days. After polarization for 48 h with either LPS/IFNγ (M1), IL10 (M2), or A549 tumor cell supernatant (TAM-like), cells were analysed by FACS for M1 (CD86, HLA-DR) and M2 markers (CD14, CD163) (A). Expressing more M2 than M1 markers, TAM-like cells seem to adopt the M2 phenotype.



Cell migration assay

Effects of M1-conditioned medium (M1-CM), M2-CM and TAM-like-CM on A549 lung cancer cell migration after 24 h and 48 h (B). Cell migration with M1-CM is decreased whereas tumor cell migration is enhanced with M2- and TAM-like-CM compared to control.



Summary and conclusion

In order to study TAM from human lung, we established an *in vitro* model with A549 cancer cells. The TAM-like cells in our model seem to represent the M2 phenotype, as indicated by FACS analysis and cell migration assay. Corresponding to our *in vitro* data, primary patient-derived TAM also enhance the migration of cancer cells and therefore seem to be polarized in M2 direction.
Interestingly, TAM showed an increased nanoparticle uptake capacity compared to AM, indicating them as potential target for chemotherapeutic nanoparticles.

Outlook

Characterize and compare AM and TAM by:

- surface marker (FACS)
- gene expression (RT-PCR)
- MMP activity (zymography)
- exosome release

References
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[3] Hoppstädter J; Seif M; **Dembek A**; Cavelius C; Huwer H; Kraegeloh A; Kiemer AK. **M2 polarization enhances silica nanoparticle uptake by macrophages.** Front Pharmacol. 2015 Mar 23;6:55.