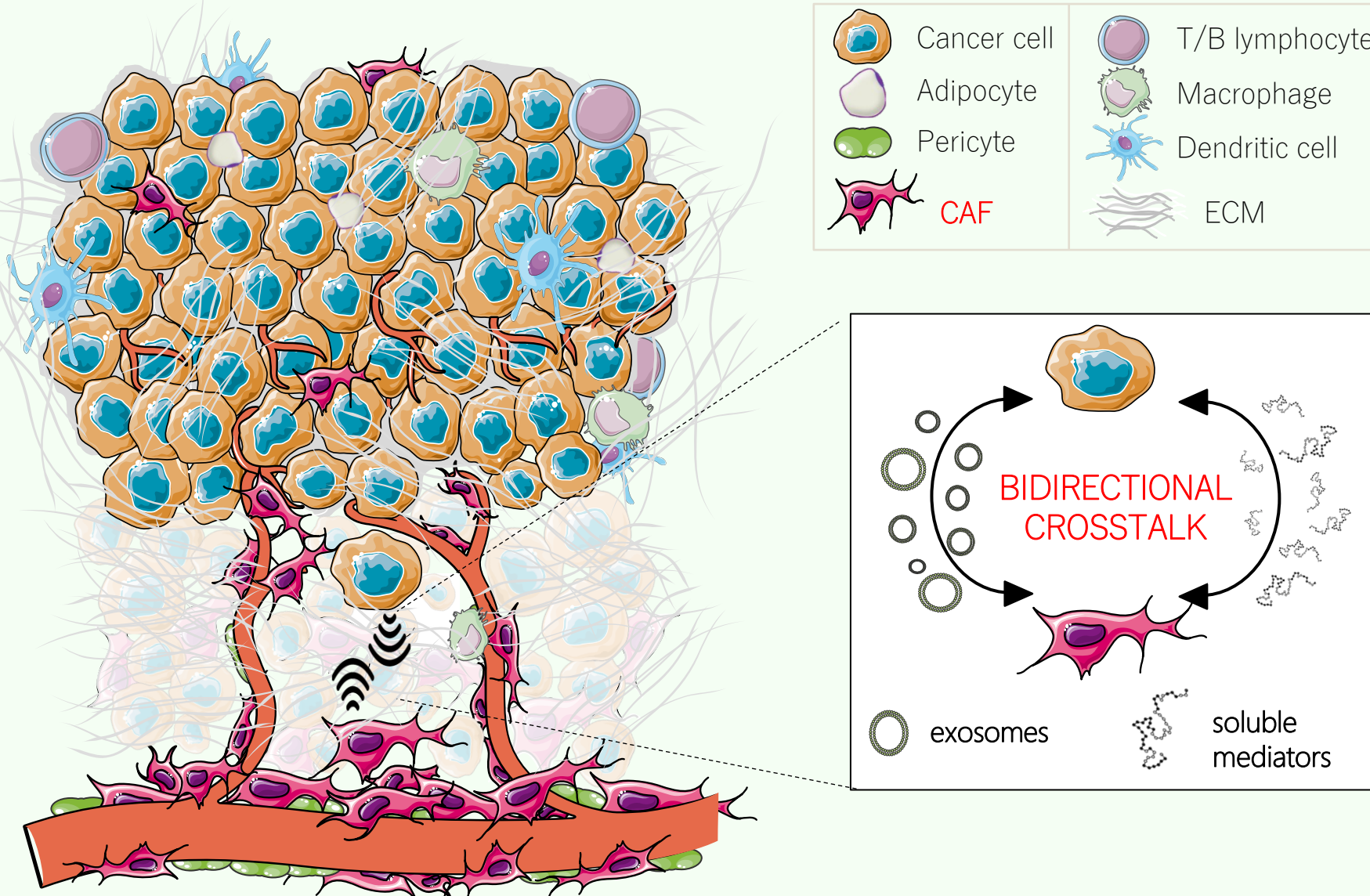


PROJECT DESIGN

1) Background: Tumor microenvironment (TME), an interacting network of malignant and non-malignant cells, plays key role affecting tumor progression and resistance^{1,2}. Cancer-associated fibroblasts (CAFs) represent a pivotal player in TME modulation, promoting a bidirectional crosstalk with neighboring tumor cells^{3,4}. Targeting the tumor stroma should improve cancer treatments significantly and CAFs emerge as a new appealing target for the development of innovative therapies.

TUMOR MICROENVIRONMENT

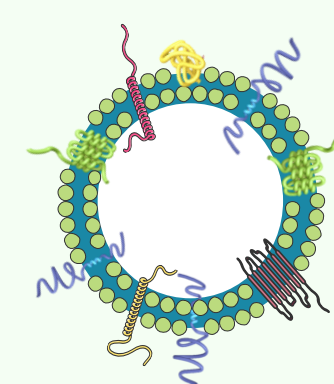


2) Aim: We propose a novel strategy for targeting TME to involve CAFs in eradicating malignant cells and re-establishing a normal microenvironment. This could be accomplished using CAFs as a "depot" for the production of cytotoxic tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), which induces apoptosis selectively in cancer cells.

3) Strategy: Cancer cell membrane-derived nanoparticles (CCMNPs) are exploited as CAF-targeted vectors of two alternative plasmids:

- Extracellular domain of TRAIL (a114–281) in fusion with a secretory signal sequence (plasmid 1)
- Full-length transmembrane TRAIL (plasmid 2)

CAFs transfection will lead to a high rate local production of recombinant secretable-TRAIL (s-TRAIL) or TRAIL-armed exosomes (ex-TRAIL). These will be released in the extracellular milieu as soluble factors (strategy 1) or by means of plasma membrane budding (strategy 2) respectively. Once in extracellular milieu, both s-TRAIL and ex-TRAIL will be directed towards cancer cells, reflecting the natural fate of paracrine signals during the crosstalk process.

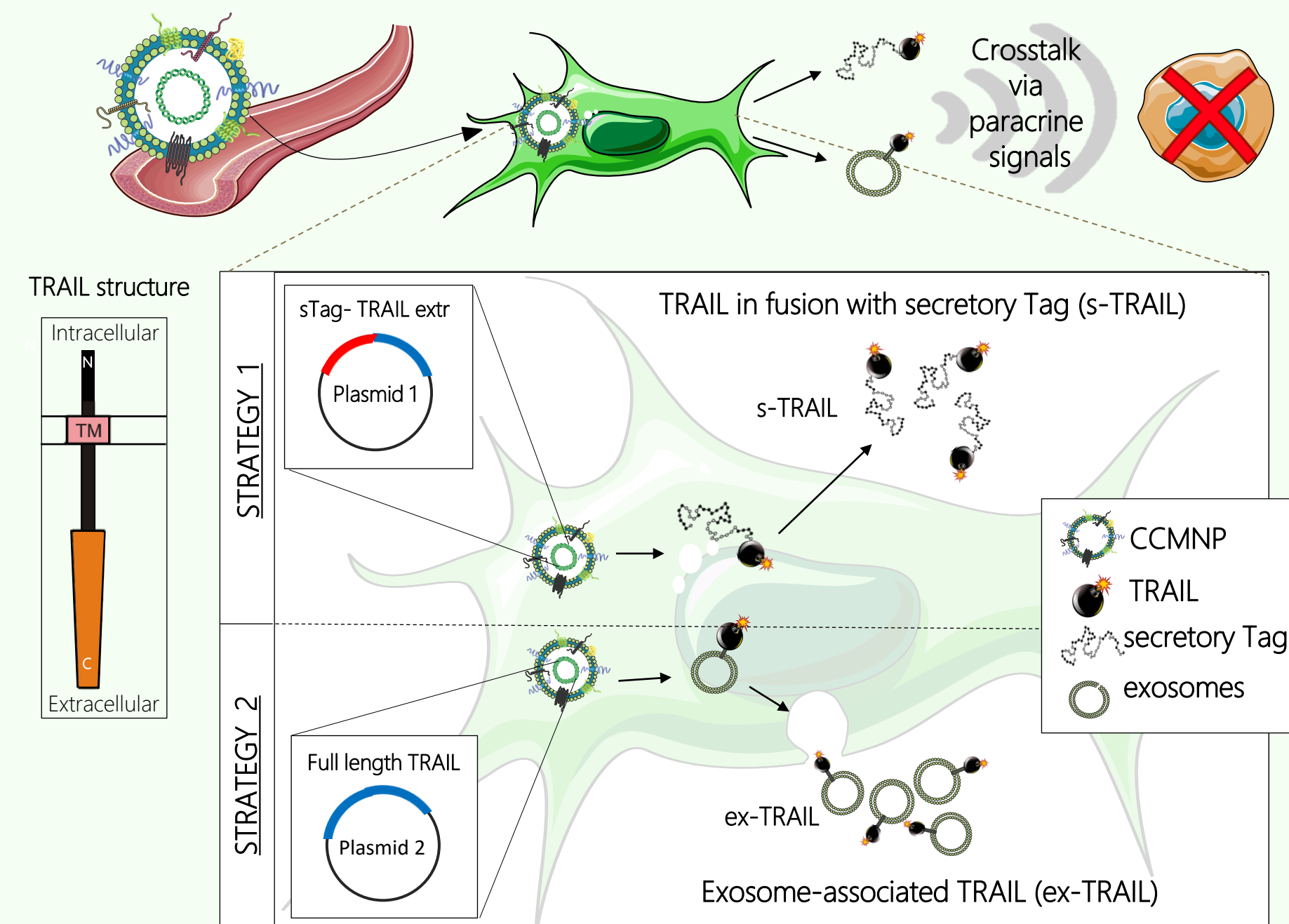


BIOMIMETIC CCMNPs

Biomimetic CCMNPs, obtained by decomposition of the tumor cells membrane, offer advantages as gene therapy vectors:

- Immune-escaping capability
- Spontaneous tropism for TME⁷

REPROGRAM CAFs IDENTITY INVOLVING THEM IN THE FIGHT AGAINST CANCER CELLS



4) Research plan

WP1: Plasmid construction and transfection tests

WP2: Synthesis and characterization of CCMNPs

WP3: Activity experiments in 2D and 3D models

References: 1) Roma-Rodrigues C, et al. (2019). *Int J Mol Sci*, 20, 840; 2) Chen and Song (2018). *Nature*, 18, 99–115 3) Balkwill FR, et al. (2012). *J Cell Sci*, 125, 5591–6; 4) Fiori ME, et al. (2019). *Mol Cancer*, 18, 70; 5) Lang J, et al. (2019). *ACS Nano*, 13, 12357–12371; 6) Miao L, et al. (2016). *ACS Nano*, 10, 9243–9258; 7) Li B, et al. (2018). *Nanomedicine*, 13, 16.

PRELIMINARY EXPERIMENTS

WP1: PLASMID CONSTRUCTION AND TRANSFECTION TESTS

Plasmid, encoding the full-length TRAIL in fusion with GFP at N terminal, has been selected for preliminary transfection studies. Hela cells were transfected with TRAIL plasmid using Lipofectamine. TRAIL-induced death in cancer cells was detected at 24 h post transfection. Plasmid encoding GFP was used as negative control.

