combination therapy was observed on tumor growth, mice survival, and frequency and sites of metastatic lesions.

Results

IHC results show that tumor tissue is enriched with Flower Win, whereas Flower Lose isoforms are present in the stromal tissue surrounding the tumors (Fig-1b). Results show a significant reduction in volume of TN PDX observed over a period of 45 days ($p<0.0001$). Further the KM curves reveal that the mice survival (over 120 days) was significantly longer in the Anti-Flower Ab + TAC compared with TAC alone ($P<0.0001$) and with controls ($P<0.0001$). Lastly, combination therapy resulted in a significant reduction in metastatic frequency to kidney, liver, lung, brain, axillary and inguinal lymph nodes ($P<0.0001$).

Conclusions

This preclinical study demonstrated a significant improvement of survival, metastatic events and tumor regression of triple negative patient-derived tumors in mice treated with monoclonal anti-Flower ABs, compared with TAC alone. Preliminary data reveals comparable results with colon and pancreatic PDX tumor models.

Acknowledgements

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Ethics Approval

All animal studies performed in this research are approved by Champalimaud Institutional Review Board and the Portuguese Gov Ethical board (DGAV), the approval number is 0421/000/000.

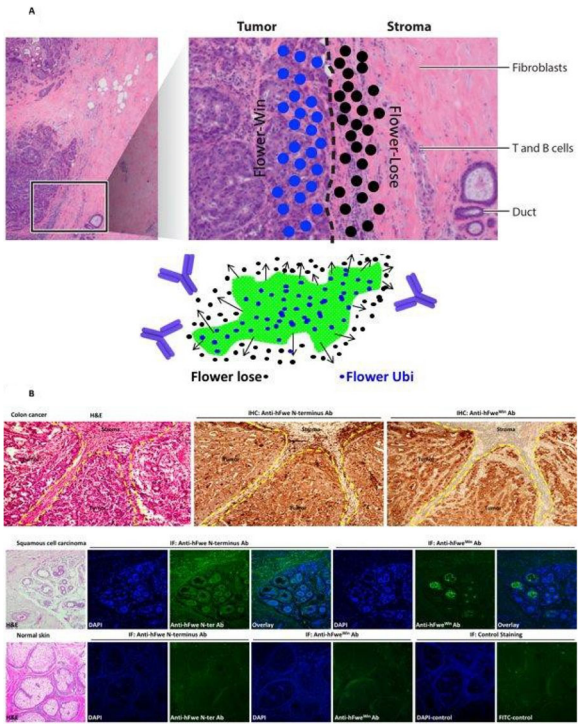

Fig. 1 (abstract P236). See text for description.

P237

CAR-modified killer MSC targeting GD2-positive Ewing sarcoma

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Background

Ewing sarcoma (ES) is an aggressive mesenchymal-derived tumor representing the second most common malignancy in children and young adults. Despite a marked improvement in the prognosis of patients, the mortality caused by metastases and recurrent disease remains high calling for novel strategies to sustain remission and improve outcome. New avenues of research have been opened by using the pro-apoptotic agent TNF-related apoptosis inducing ligand (TRAIL) and cells carrying TRAIL close to tumor have shown to increase its bioavailability. Mesenchymal stromal cells (MSC) have been under investigation as vehicles for the delivery of anti- tumor agents. We previously demonstrated that MSC expressing TRAIL can induce apoptosis in a variety of sarcomas exerting also a relevant antitumor activity against in vivo models of ES. However, while the interaction between TRAIL and receptors is clear, more obscure is the manner in which MSC can selectively target tumors. As metastases are a great challenge in ES patients, dedicated strategies to drive MSC targeting and persistence to metastatic sites, particularly involving lungs in ES, shall be required. Here, in an effort to maximize the therapeutic profile of MSC TRAIL, minimize off-target effects and accounting for metastatic disease, we originally developed a strategy where TRAIL is delivered by MSC that are also modified by an anti-GD2 chimeric antigen receptor (CAR) to target GD2-positive ES cells.

Methods
The anti-GD2 CAR was expressed in MSC by viral transduction together with TRAIL. The anti-tumor activity of these functionalized MSC was in vitro assessed targeting GD2-positive or negative ES lines. The enhanced binding ability of functionalized MSC to GD2-positive ES cells and the specificity of interaction was investigated, as well, both in vitro and in vivo.
Results
The functionalized MSC expressed high levels of both TRAIL and CAR preserving a robust anti-tumor activity against ES lines. Most importantly, the functionalized MSC killing was further reinforced by an enhanced targeting thanks to improved cell-to-cell interactions. Based on in vitro findings, we started to assess the anti-GD2 CAR potential in an in vivo lung metastases ES model to better elucidate the advantage conferred by the CAR on MSC- based delivery.
Conclusions
Our results suggest that the CAR here described might be a powerful tool to redirect MSC carrying TRAIL against GD2-expressing tumors. This targeting strategy holds the promise to combine site-specific and prolonged retention of MSC in ES metastases, thereby providing a more effective delivery of TRAIL against this still incurable cancer even after metastatic dissemination.

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Ethics Approval
The study was approved by the Ministry of Health (Italy), approval number 1278/2015-PR.

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Regulation of in vivo anti-tumor activity of adoptively transferred CAR-T cells using FDA approved small molecule drugs

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Background
Adoptive cell therapy with chimeric antigen receptor (CAR)-modified T cells has demonstrated clinical efficacy in the treatment of B cell malignancies and multiple myeloma. More widespread application of CAR-T cell therapy has been restricted by concerns about safety and observations of limited efficacy. Uncontrolled expansion of CD19- targeting CAR-T cells has caused severe CRS-related toxicity in many patients. Tumor antigen expression on healthy tissues can lead to on-target/off-tumor toxicity. Furthermore, prolonged antigen-dependent or independent CAR signaling may contribute to functional exhaustion, limiting efficacy. Adoptive transfer of T cells expressing a reversible, titratable, regulated CAR would support exogenous control of the level, activity, and timing of CAR expression for sustained anti-tumor efficacy and a more favorable safety profile. To exert control over CAR-T cells, we used destabilizing domain (DD) technology, fusing CAR to small protein domains which are unstable and degraded intracellularly but are reversibly stabilized by small molecule ligand binding, enabling exogenous control.
Methods
Using structure-guided engineering and mutagenesis screens, we identified mutations that destabilize human phosphodiesterase 5 (PDE5) protein and restabilize in the presence of FDA approved PDE5 inhibitors. To provide exogenous control over CAR-T cells, we fused destabilizing mutant-containing PDE5 domains (PDE5-DDs) to CD19-CAR (CD19-CAR-PDE5-DD), transduced human T cells with DD-modified CAR, and evaluated ligand dose-responsive CAR expression and activity. To evaluate anti-tumor activity in vivo, we implanted NSG mice with CD19+luciferase +Nalm6 cells and transplanted unmodified T cells or CD19-CAR-PDE5-DD T cells. We orally dosed mice with ligand or vehicle and monitored tumor growth by bioluminescent imaging to track tumor progression. We applied Kaplan-Meier analysis and the log-rank test to compare survival curves and median survival time.
Results
Human T cells transduced with CD19-CAR-PDE5-DD and treated with PDE5 ligands showed ligand-dependent CAR expression and activity in vitro. CD19 tumor-bearing mice treated with CD19-CAR-PDE5-DD T cells and stabilizing ligand showed a dose-dependent delay in tumor progression relative to ligand treated unmodified T cell recipients. Tumor growth inhibition was significant across ligand doses for CD19-CAR-PDE5-DD T cell recipients relative to unmodified T cell recipients. A significant survival advantage was observed in mice treated with CD19-CAR-PDE5-DD T cells relative to mice treated with unmodified T cells ($P<0.005$).
Conclusions
PPDE5-regulated CD19-CAR-T cell activity supported robust anti-tumor efficacy and increased survival in vivo.
These preclinical studies demonstrate the potential to reversibly stabilize a DD-regulated CAR in vivo using FDA approved small molecules, toward enhancing safety and efficacy of CAR-T therapies for the treatment of cancer

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Novel solid tumor gene therapy approach mediated by engineered Bifidobacterium longum generates efficient tumor-derived IL-12 expression and results in local and systemic anti-tumor immunity

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Background
Intravenous infusion of the probiotic obligate anaerobe, Bifidobacterium longum, selectively colonizes solid tumor tissues in preclinical models. Seeking to therapeutically exploit this observation, bifidobacteria was engineered to selectively shuttle plasmid DNA (pDNA) into solid tumor tissues resulting in membranous expression of therapeutically relevant transgenes such as interleukin-12 (IL-12).
Methods
An engineered, multi-domain fusion protein expressed locally by tumor-colonizing bacteria coordinates the binding and secretion of therapeutic pDNA molecules into the tumor microenvironment, mediating efficient genetic transfection of cancer cells, ultimately achieving tumor-derived expression of therapeutic genes. Membrane-bound IL-12 tumor expression was achieved in syngeneic mouse tumor models of colorectal cancer (CT-26) and melanoma (B16F10).
Results
In both models, B.longum engineered to deliver the IL-12 gene administered intravenously was well-tolerated and selectively colonized tumors. Robust and increasing expression of IL-12 was demonstrated within tumor tissues. Bacteria and/or transgene expression was undetectable in a comprehensive assessment of normal tissues. As predicted, IL-12 expression by the tumor resulted in the activation of both adaptive and innate immune responses. Increased pro-inflammatory cytokine levels and elevated tumor CD8+/Treg ratios were associated with the recognition of cancer specific antigens. B.longum mediated IL-12 gene delivery significantly inhibited tumor growth and combination therapy with anti-PD1 and anti-CTLA4 synergistically improved these outcomes.
Conclusions
Taken together, selective bacterial colonization of solid tumors employing intravenously-infused, genetically- engineered B.longum achieves robust and enduring tumoral expression of therapeutically relevant genes such as IL-12. A first-in-human clinical trial is planned for 2019.