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1. ΔNp63 defines bipotent precursors in murine pancreas development

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ΔNp63 is suggested to be the driver of the basal-like molecular subtype of PDAC and is recently reported to be present in the ductal epithelium in healthy and chronic pancreatitis patients, however a role for ΔNp63 in pancreas development has not been considered.

ΔNp63 expression was characterized in wild-type (CD-1) mouse embryonic pancreas by a combination of immunohistochemistry and multiplex immunofluorescence. Tamoxifen (4-OHT) administration at several embryonic time points in Tp63-CreERT; ROSA26-mT-mG mice enabled fate tracing of ΔNp63$^+$ cells during pancreatic development. For KO analysis we used ΔNp63 knock-in mice in which the ΔNp63-specific exon is replaced by GFP.

A transient expression of ΔNp63 in the embryonic pancreas starts from as early as E10.5 and lasts until E16.5. At E12.5, ΔNp63 shows a regionalized expression pattern, being enriched at the distal tips of the epithelial branches, the niche of the multipotent pancreatic progenitors, where the ΔNp63$^+$ cells represent a subset (10 ± 1 %). Moreover, single-cell sequencing analysis shows a downregulation of ΔNp63 in the trunk compartment upon tip-trunk segregation. Lineage-tracing experiments show that ΔNp63$^+$ cells are initially maintaining the multipotent progenitor pool but later become bipotent and restricted to generate (centro-) acinar cells and ductal cells. ΔNp63 knockout pancreata display hypoplasia and show a defect in the ventral and the caudal part of the of the splenic lobe.

In conclusion, this study is the first to identify ΔNp63 as a marker distinguishing multipotent from bipotent primed pancreatic progenitors, providing new insights into pancreatic morphogenesis, particularly in exocrine cell differentiation.

See also poster number 1

2. Unveiling B7-H3 as novel target for CAR T-cell therapy in Multiple Myeloma

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Background

Both anti-BCMA CAR T-cell therapy and bispecific antibodies targeting BCMA have demonstrated high efficacy in treating Multiple Myeloma (MM). However, due to the intense targeting of BCMA, antigen loss occurs in up to one-third of patients, leading to treatment resistance. This highlights the need for alternative therapeutic targets. In this study, we propose B7-H3 (CD276) as a novel antigen for CAR T-cell therapy in MM.

Methods

B7-H3 expression was evaluated in both MM cell lines and the CD138-positive fraction of bone marrow aspirates from MM patients. Western blot and flow cytometry were used to assess B7-H3 protein levels. In vitro cytotoxicity was measured in MM cell lines by luciferase monitoring, with BCMA-specific CAR T cells serving as a benchmark. Secreted cytokines, (IFN-γ, IL-2, and TNF-α) were quantified using ELISA. Primary killing assays were conducted using bone marrow aspirates from MM patients. The in vivo efficacy was tested in two xenograft mouse models.

Results

B7-H3 was detected in the total cell lysates of 3/5 MM cell lines tested by western blot. Flow cytometry of CD138-positive MM cells from bone marrow aspirates showed B7-H3-positive MM cells in 13/20 patients, ranging from 49.04%-99%. Primary T cells were lentivirally transduced to express BCMA- or B7-H3-specific CARs.

These CAR T cells exhibited potent cytotoxicity against B7-H3-positive MM cell lines, which correlated with the secretion of high levels of IFN-γ, IL-2, and TNF-α. Both BCMA- and B7-H3 CAR T cells demonstrated comparable cytotoxicity against primary MM patient samples. In vivo, B7-H3-specific CAR T cells significantly reduced MM cell burden in the bone marrow and extended survival in xenograft mouse models compared to control T cells.

Conclusion

Our findings show that B7-H3 is expressed in approximately two-thirds of MM patients and that B7-H3-specific CAR T cells are highly effective in eliminating B7-H3-positive MM cells. These results provides a strong rationale for clinical translation.

See also poster number 2

3. Overcoming the Hypoxia Challenge: Hydrogen Peroxide as Radiosensitizer for Solid Tumors

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Objective

The hypoxic microenvironment is the primary factor causing radioresistance in solid tumors. The established radiosensitizer, hydrogen peroxide (H_2O_2) has limited clinical use due to systemic toxicity. To address this issue, intratumoral injection of Kochi Oxydol for Unresectable Carcinomas (KORTUC) has been developed, which combines H_2O_2 with sodium hyaluronate (SH). This project investigates the underlying effects of H_2O_2 and KORTUC.

Materials/Methods

CT26 and 4T1 were exposed to H_2O_2/KORTUC and the radiomodulatory properties were evaluated under hypoxia in 2D and 3D models. Effects on ROS, DNA damage, apoptosis and ferroptosis were evaluated. Oxygen consumption rate, mitochondrial complex activity and oxygen pressure were assessed. The effects on tumor growth and hypoxia were assessed in tumor bearing mice in combination with irradiation.

Results

While SH alone exhibited radioprotective properties, the combination of SH and H_2O_2 in KORTUC sensitized hypoxic CT26 and 4T1 cells, resulting in an enhancement ratio of 2.6 and 2.5. A dose-dependent reduction in oxygen consumption rate (OCR) was observed, correlating with the temporary inhibition of mitochondrial complexes I and II activity.

Injection of KORTUC resulted in a significant surge of O_2 levels under hypoxia. Elevations in superoxide, DNA damage and an accumulative increase in the levels of apoptosis and ferroptosis could be detected when combining KORTUC with radiation. KORTUC radiosensitized CT26 tumors, resulting in a growth delay of 14 days and a reduction in poorly oxygenated regions within the tumor.

Conclusion

The radiosensitizing effects of KORTUC can be attributed to the reoxygenation of hypoxic cells and the inhibition of cellular respiration through the blockade of mitochondrial complexes I and II. Our *in vivo* results indicate that KORTUC enables the use of higher concentrations of H_2O_2 without inducing toxic effects. Gaining a deeper understanding of the underlying pathways of KORTUC is essential for the development of combination therapies for cancer patients.

See also poster number 3

4. Conflict or integration of palliative care in oncological phase I trials: A qualitative study

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Introduction

People with cancer who are out of treatment can participate in experimental phase 1 trials. Participants rarely benefit and often have a limited life expectancy. While evidence shows that palliative care improves quality of care for participants and their carers, little is known about its role and integration, or potential conflict, in phase 1 trials.

Objectives

This study aimed to provide insight into: (1) How quality of life is incorporated into the care for participants in phase 1 trial, (2) The benefits of integrating palliative care into phase 1 studies, and (3) What barriers exist to such integration.

Methodology

We conducted semi-structured face-to-face interviews with: 15 phase 1 trial participants, 4 family carers, 12 phase 1 staff members, 6 referring oncologists, 5 palliative care providers, and 4 general practitioners. We recruited participants in the University Hospitals of Ghent, Antwerp, and Brussels, applying convenience sampling. Two primary researchers coded the transcripts collaboratively, applying qualitative content analysis.

Results

Palliative care is offered reactively, not proactively. Healthcare providers agree that integrating it into the trial process would be beneficial. Barriers include limited time, lack of continuity of care, and negative associations with palliative care terminology. Trial participants acknowledge the potential benefits but feel it is not needed for them yet.

Conclusion

Palliative care is not currently integrated into the care of cancer patients in phase 1 oncology trials in Belgium. Healthcare providers believe this integration would be beneficial, but emphasize the need to address participants' negative associations with palliative care to ensure its effectiveness.

See also poster number 4

5. Ferroptosis is a pivotal player in radiotherapy-induced cell death of colorectal cancer cells.

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Background & purpose: Ferroptosis, an iron-dependent type of regulated cell death (RCD), has recently been associated with radiotherapy (RT) efficacy. However, the impact of ferroptosis inducers (FINs) on various colorectal cancer (CRC) cell lines varies tremendously. Therefore, this study aims to elucidate the importance of ferroptosis in RT-induced RCD, comparing it with two established forms: apoptosis and necroptosis.

Materials & methods: Human CRC cell lines (DLD-1, HT29, HCT116), and a murine CRC cell line (CT26) were included. RT-induced RCD was assessed by flow cytometric staining. To determine the precise percentage of RCD post-radiation, colony formation assay (CFA) was employed following treatment with cell death inhibitors: ferrostatin-1 (ferroptosis inhibitor), Z-VAD-FMK (apoptosis inhibitor) or necrostatin-1 (necroptosis inhibitor). Additionally, the impact of hypoxia (1% O_2) and fractionated RT on RCD percentages was analysed using CFA. *In vitro* results were confirmed in 3D- spheroid models and validated in a CT26 tumor model.

Results: Irradiation significantly elevated the levels of apoptosis, necroptosis and ferroptosis, irrespective of the oxygen concentration. Inhibition of ferroptosis reduced cell death to a similar degree as inhibition of apoptosis and necroptosis. These findings were confirmed in 3D models. Hypoxic conditions and fractionated RT decreased overall RCD. *In vivo* experiments affirmed the pivotal role of ferroptosis, showing it to be similarly involved as necroptosis and superior to apoptosis in RT-induced RCD of the CT26 tumor model.

Conclusion: Ferroptosis is equally involved in RT-induced RCD in CRC cells compared to apoptosis and necroptosis. However, under hypoxic conditions and following fractionation the importance of ferroptosis decreases. Despite this, the reduction was less pronounced than that observed for apoptosis and necroptosis, suggesting that ferroptosis is an ideal type of RCD to trigger in a clinical setting. Overall, this study highlights the potential of FINs as effective clinical radiosensitizers.

See also poster number 5

6. Spatial transcriptomic and single cell sequencing data reveals PTGES as a conserved potential therapeutic target for basal cells in pancreatic cancer.

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Pancreatic Ductal Adenocarcinoma (PDAC) is characterized by a poor prognosis, partly attributable to tumor cell plasticity. Interpatient tumor cell heterogeneity manifests as a spectrum of transcriptomic subtypes, going from classical to basal-like subtype, the latter being the most aggressive and therapy-resistant. This spectrum is even recapitulated within a single patient down to the level of a single tumor duct, as our group recently reported. We also discovered basal cells in the ductal epithelium of the normal human pancreas, which share strong phenotypic similarities with tumor cells of the basal-like subtype, such as expression of ΔNp63 and KRT5 (Keratin 5). Our study aimed to identify conserved basal cell targets that are amenable to therapy.

We performed spatial transcriptomics on healthy pancreas, chronic pancreatitis and PDAC samples utilizing GeoMx®, CosMx® (Nanostring) and Resolve Molecular Cartography. Computational analysis of these datasets revealed predominant Prostaglandin E Synthase (PTGES) expression in basal cells. This enzyme plays a pivotal role in prostaglandin E2 synthesis, impacting tumor formation and growth. Additionally, bulk RNA-sequencing indicated a significantly higher expression of PTGES in basal-like PDAC. Analysis of publicly available single-cell datasets confirmed PTGES expression in tumor cells exhibiting basal cell characteristics in PDAC. Collectively, these findings suggest an association between PTGES and a basal identity across healthy pancreas, PDAC, and the cancer-predisposing condition of pancreatitis.

In conclusion, PTGES was unveiled as a basal-cell unique gene, offering a promising avenue for further exploration in the pursuit of effective stratified therapies for PDAC.

See also poster number 6

7. AXL acts as a negative regulator of cancer immunity in Acute Myeloid Leukemia

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Rationale

Receptor tyrosine kinase AXL is expressed in many cancer types and has been associated with therapy resistance. While its prognostic and therapeutic value in Acute Myeloid Leukemia (AML) has been shown, its function in the immune microenvironment (IME) is yet to be determined. In this study, we evaluated the effect of AXL-specific single domain antibodies (sdAbs) on tumor progression and the IME *ex vivo* and *in vivo*.

Methods

AXL expression was determined in naive and AXL$^+$ C1498 mice. The effect of AXL-targeting was evaluated by treatment of C1498-bearing mice with sdAb20-Fc or R3B23-Fc (control) for 10 days or until end-stage. Using bone marrow samples of AML patients (n=9), we determined the effect of sdAb20-Fc on immune cells and tumor cells *ex vivo*, as single agent therapy and in combination with immune checkpoint inhibitors (ICIs). All samples were analyzed using multi-parameter flow cytometry.

Results

AXL expression was mostly found on macrophages and dendritic cells (DCs) of naive and AML-bearing mice. While AXL targeting *in vivo* had no effect on tumor load, sdAb20-Fc did induce a significant decrease in CD8$^+$ T cells, accompanied by a significant increase in PD-1 expression on CD4$^+$ and CD8$^+$ T-cells.

The percentage of DCs remained unaffected, however we did also observe an increase in PD-1 expression. Based on these findings, sdAb20-Fc was combined with ICIs targeting PD-1 and PD-L1, as well as the novel identified checkpoint Galectin-9. SdAb20-Fc only acted synergistically in combination with anti-Galectin-9 therapy, as observed by direct effects on tumor cells of AML patient samples.

Conclusions

Despite its therapeutic value in AML, targeting of AXL results in negative regulation of the cancer immunity, particularly by reducing the $CD8^+$ T cells. As AXL directly influences immune checkpoint expression, its combination with ICIs such as Galectin-9 therapy might be considered for further therapeutic applications.

See also poster number 7

8. The display of an anti-CS1 nanobody by small extracellular vesicles does not improve disease targeting in multiple myeloma

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Introduction: Multiple myeloma (MM) is an incurable plasma cell cancer predominantly residing in the bone marrow (BM). Due to inevitable refractory disease, novel therapeutic options are needed. Small extracellular vesicles (sEVs) are promising therapeutic cargo delivery vehicles due to their high biocompatibility and ability to traverse biological barriers. This study aims to engineer HEK293-derived sEVs to display a nanobody (Nb) targeting CS1, a well-established surface marker of MM cells, to increase sEV specificity to MM-associated organs and MM cells.

Methods: HEK293 cells were stably transfected with Nb-SDC1CTF fusion proteins, linked with the juxtamembranal domain of CD4 to prevent membrane cleavage. Western blot and confocal microscopy confirmed sEVs sorting and correct cell membrane topology. Control sEVs without Nb and with an irrelevant Nb were included. Binding of anti-CS1 Nb-displaying sEVs to CS1 was evaluated by incubating sEVs with soluble CS1 and analyzing size-exclusion chromatography (SEC) fractions for co-elution of CS1 with sEVs by western blot. To evaluate the effect of the anti-CS1 Nb on sEV biodistribution, DiR-labeled sEVs were intravenously injected in 5T33MM mice. After 24h, organs were imaged with a Fluobeam800 camera. MM cell-specificity was determined by measuring DiR fluorescence by flow cytometry.

Results: Cleavage-resistant constructs were expressed with the correct topology and were highly enriched in sEVs. Both human and murine anti-CS1 Nb-displaying sEVs showed binding to soluble forms of CS1. Mononuclear cells isolated from spleen, spine and legs showed no enhanced sEV specificity and/or selectivity towards MM cells. Interestingly, in myeloma-bearing mice, anti-CS1 Nb display increased sEV accumulation in liver and lungs.

Conclusion: Anti-CS1 Nbs were successfully displayed on the sEV surface. While these EVs efficiently bind soluble forms of CS1, they do not improve MM targeting in *vivo*. Further work will explore suborgan distribution of sEVs expand the flow cytometric panel to include immune cell populations.

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See also poster number 8

9. Targeting DNMT3B in multiple myeloma: a novel epigenetic-based targeting approach that displays potent anti-myeloma activity and enhances sensitivity to standard of care agents and anti-CD38 monoclonal antibodies

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Objective

In about half of the MM patients, genetic defects and/or abnormal expression are observed in epigenetic modifiers (epiplayers) at the time of diagnosis and this further increases at relapse, indicating an important role for epiplayers in MM cell drug resistance (DR). However, so far, only for two epiplayers, MMSET and EZH2, a clear role in MM cell DR has been formally established. Using RNASeq data from the MMRF CoMMpass study, we found that the epiplayer DNMT3B is significantly increased in the relapsed setting, suggesting a role in MM relapse. Here, we explored the role of DNMT3B in MM cell biology and drug response.

Results

We found that high DNMT3B levels are linked with a more aggressive phenotype, as evidenced by increased DNMT3B levels in HMCL compared to healthy bone marrow plasma cells (BMPC) and primary myeloma cells and increased levels in patients with aberrations in TP53, including del17, or belonging to the proliferation molecular subgroup; two groups linked with a bad prognosis. In line, high DNMT3B levels also correlated with a poor overall survival in newly diagnosed and relapsed MM patients. Targeting DNMT3B either by genetic inhibition (DNMT3B knockdown; KD) or by the DNMT3B selective inhibitor Nanaomycin A (NA) resulted in impaired MM cell growth, survival and clonogenicity. Furthermore, primary human MM cells were found to be much more sensitive to NA treatment compared to human BMSC. Gene set enrichment analysis following RNA-Seq revealed that mainly pathways involved in epigenetic regulation, cell cycle & apoptosis and stemness & maturation are significantly deregulated upon DNMT3B KD. Lastly, NA (re)sensitized MM cells to the standard of care agents Bortezomib or Melphalan and the immunotherapies daratumumab and isatuximab.

Conclusion

Together, our findings provide first evidence that DNMT3B could be a novel promising epigenetic target to overcome or delay relapse in MM.

See also poster number 9

10. Recommended Physiotherapy Modalities for Oncology Patients with Palliative Needs and Its Influence on Patient-Reported Outcome Measures: A Systematic Review

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Background

This review aims to explore the role of physiotherapy in early and traditional palliative care (PC) for oncology patients, focusing on its impact on six patient-reported outcomes (PROMs), namely fatigue, pain, cachexia, quality of life (QoL), physical functioning (PHF), and psychosocial functioning (PSF). The purpose is to assess the effectiveness of various physiotherapy interventions and identify gaps in the current research to understand their potential benefits in PC better.

Methods

A systematic literature search was conducted across PubMed, Embase, and Web of Science, concluding on December 21, 2023. Two independent reviewers screened the articles for inclusion. The Cochrane Risk of Bias Tool 2 was employed to assess the risk of bias, while the GRADE approach was used to evaluate the certainty of the evidence.

Results

Nine randomized controlled trials (RCTs) were included, with most showing a high risk of bias, particularly in outcome measurement and missing data. Cognitive behavioral therapy (CBT) was the only intervention that significantly reduced fatigue, enhanced PHF, and improved QoL and emotional functioning.

Graded exercise therapy (GET) did not yield significant results. Combined interventions, such as education with problem-solving or nutritional counseling with physical activity, showed no significant effects. Massage significantly improved QoL and reduced pain, while physical application therapies were effective in pain reduction. Mindful breathing exercises (MBE) improved QoL but had a non-significant impact on appetite. The overall certainty of the evidence was low.

Conclusions

Physiotherapy can positively influence PROMs in oncology PC; however, the low quality and high risk of bias in existing studies highlight the need for more rigorous research to confirm these findings and guide clinical practice.

See also poster number 10

11. Capturing Experiences In The Daily Lives of People Living With Advanced Cancer: an Observational Experience Sampling Methods Study

Authors: Joran Geeraerts (End-of-Life Care Research Group VUB & UGent), Lara Pivodic, Kim De Nooijer, Lise Rosquin, Mark De Ridder, Geert Crombez, Eline Naert, Lieve Van den Block

Background: People with advanced cancer often experience symptoms or distress during their daily lives which traditional patient-reported outcome measures fail to capture. Experience sampling methods, which prompt patients to complete self-reports multiple times per day, could uncover patients' experiences with high ecological validity. However, the methods' feasibility and participation burden are unknown.

Objective: To assess the feasibility and acceptability of experience sampling methods in people with advanced breast or lung cancer and to explore patient-experienced symptoms, concerns, wellbeing, and their fluctuations in daily life.

Methods: We conducted an observational experience sampling methods study with people with advanced breast or advanced lung cancer. Using a smartphone, patients were asked to complete 10 assessments per day for 6 days, and a baseline and follow-up questionnaire to assess participants' study experiences.

Results: Forty out of 80 contacted patients (50%) participated. Patients completed 1943 out of 2400 scheduled assessments (81%); one person dropped out. Results revealed considerable variability of symptoms, concerns, and wellbeing over time (mean ICC=0.55, SD=0.11), such as for feeling energized and global wellbeing. Patients indicated positive study experiences, finding the study not tiring (M=1.5, SD=1.3; 1="Completely disagree", 7="Completely agree") and willingness to participate again (M=5.6, SD=2).

Conclusion: Experience sampling methods are feasible and acceptable for people with advanced cancer and can capture fluctuations in symptoms, concerns, and wellbeing in their daily lives. These findings support further research in this and other oncology populations, potentially leading to important breakthroughs in how to optimally support patients in their daily lives.

See also poster number 11

12. Ductal adenocarcinoma and adenosquamous carcinoma of the pancreas differentially recapitulate a newly defined basal-luminal classification of the healthy tissue.

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Pancreatic ductal adenocarcinoma (PDAC) exists in a classical and basal-like/squamous subtype, the latter drawing upon similarities with squamous tumours in other organs and adenosquamous carcinoma of the pancreas (ASCP). While epithelial tumours often recapitulate cell states of the tissue of origin, in the pancreas such relationship has not been established.

Building on our identification of ΔNp63$^+$ KRT5$^+$ cells with basal features in the human pancreatic duct, we employed spatial transcriptomics (NanoString GeoMx®, Resolve Molecular Cartography® and NanoString CosMx®) on human samples to decipher normal cell states and relate these to cancer.

Comparison between KRT5$^+$ and KRT5$^-$ cells in healthy human pancreas and chronic pancreatitis revealed that KRT5$^+$ cells harbour a signature akin to basal and suprabasal cells in stratified epithelia, with differential expression of *TP63* and *MUC4*. Single-cell spatial analysis revealed that human pancreatic ducts contain a pancreatic basal cell (PBC) and four luminal populations (LUM A-D). Within KRT5$^+$ cells, ΔNp63$^+$ PBCs can be distinguished from suprabasal MUC4$^+$ LUM-B cells that differ from neighbouring LUM-A cells. LUM-C cells were confined to intercalated/collecting ducts while LUM-D cells belong to ductal glands. Selected mucins can distinguish the respective cell types.

Prognostic values and congruency with bulk and single-cell transcriptomic signatures demonstrated that LUM-A and LUM-C profiles are associated with classical PDAC, whereas LUM-B and PBC are associated with basal-like PDAC and lowered survival. In contrast to (basal-like) PDAC, ASCP displayed a prominent spatial unmixing of LUM-B cells and PBCs with functional differences; the former residing in the centre of the tumour nests while the latter proliferating at the outer rim. The PBCs in ASCP also exhibit a more aggressive phenotype in pathway analysis.

In conclusion, pancreatic tumours mirror a newly unveiled luminal-basal organization of the human pancreatic duct, providing for the first time a tumour nomenclature that draws upon native tissue logics.

See also poster number 12

13. Intratumoral (IT) administration of autologous myeloid dendritic cells (myDC) with the immunologic adjuvant AS01$_B$ plus ipilimumab (IPI) and IV nivolumab (NIVO) in patients with refractory advanced melanoma: a phase Ib clinical trial

Authors

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Background

Advanced melanoma patients (pts), who progress on immune checkpoint blockade (ICB) or targeted therapy (for *BRAF*^{mut}) have a poor outcome. Conventional CD1c (BDCA-1)$^+$ and CD141 (BDCA-3)$^+$ myDC in the tumor microenvironment are crucial for eliciting antitumor immune responses and the effectiveness of PD-1/CTLA-4 ICB. Following a single IT injection of autologous myDC with (repeated Q2w thereafter) the synthetic saponin-based immune adjuvant AS01$_B$ and IPI plus IV NIVO, 3 responses were obtained in 8 refractory melanoma pts (J. Tijtgat et al. JITC 2024).

Methods

In a second cohort of this phase Ib trial the safety of a weekly dose-intensified regimen of IT 10 mg IPI and 50 µg AS01$_B$, plus 10 mg NIVO IV Q2w was investigated. As previously, autologous blood myDC isolated by leukapheresis, were injected into the same lesion (day 2). Baseline and on-treatment biopsies were collected.

Results

Six pts (3 M, med age 55y [35-60]) with unresectable stage IIIC (n=2), IV-M1a (n=3), and -M1c (n=1) received the planned study treatment with a median of 5 IT and 6 IV injections. Two pts obtained a complete response (CR), and 1 pt a stable disease (disease control rate 50%). A pathological CR was documented in 3 out of 10 injected

lesions (in 2 pts with "overall" 1 CR, and 1 SD). mPFS was 35w, mOS was not reached. Treatment related adverse events (TRAE) included transient grade 1/2 injection site reactions and constitutional symptoms. There were no grade 4-5 TRAE. Multiplex IHC analysis of tumor biopsies has been done.

Conclusion

The combination of IT CD1c (BDCA-1)$^+$/CD141 (BDCA-3)$^+$ DC-injection, with weekly IT IPI and AS01$_B$, plus low-dose IV NIVO is tolerable and demonstrated clinically meaningful anti-tumor activity in refractory advanced melanoma. The weekly administration regimen is considered the maximum tolerated treatment intensity. The trial continues as a randomized phase II trial.

See also poster number 13

14. Rethinking Chemotherapy Dosing: The Impact of Body Composition, Physical Activity, and Pharmacokinetics on Dose-Limiting Toxicities in Breast Cancer Patients.

Background

Breast cancer is one of the most prevalent cancers among women, with paclitaxel (PTX) being a key chemotherapeutic agent. Despite its efficacy, PTX is linked to dose-limiting toxicities (DLTs), including chemotherapy-induced peripheral neuropathy and neutropenia. This can negatively impact patients' health-related quality of life (HRQoL) and treatment adherence/tolerance/efficacy. Preliminary evidence suggests that body composition and physical activity (PA) may impact DLTs, but comprehensive research exploring these correlations is lacking.

Purpose

The primary objective is to identify specific body composition parameters (e.g., muscle mass, fat mass) and PA levels that affect the occurrence and severity of DLTs in breast cancer patients treated with PTX. Using these factors, a pharmacokinetic-pharmacodynamic (PK-PD) model will be developed to predict how different doses and patient-specific characteristics influence prognostic and toxic outcomes.

Methods

This prospective observational cohort study will recruit 40 female breast cancer patients receiving weekly PTX (12 cycles) at UZ Brussel. Body composition will be assessed using Dual X-ray Absorptiometry (DXA) and Bioelectrical Impedance Analysis (BIA) at baseline, cycle 6, and cycle 12. PA will be monitored using the actigraph wGT3X-BT for seven days following cycles 1, 6, 9, and 12. PTX pharmacokinetics will be assessed through dried blood spot (DBS) and blood samples at four time points. DLTs will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Population PK-PD models will be developed using Monolix.

Results

Recruitment is ongoing (clinicaltrial.gov ID NCT06387901), with preliminary results (N=10) expected in April 2025. These findings will explore how body composition and physical activity modulate DLT risk in PTX-treated patients. Implications: The results could influence physiotherapy interventions targeting muscle mass, fat, and PA in breast cancer patients.

This research contributes to the understanding of exercise-pharmacology in cancer, providing new insights into how PA and body composition interact with chemotherapy pharmacokinetics.

Len De Nys, Anita Barzegarfallah, Amy de Haar-Holleman, Katrien Lankmans, Stéphanie Wuyts, Steurbaut Stephane, Davind Beckwée, Provyn Steven, Elke Gasthuys, Sofie Vande Casteele, An Vermeulen, Jan Van Bocxlaer, Nele Adriaenssens

Affiliations: VUB, UZB, UGent.

This study has received ethical approval from the Commission of Medical Ethics of the Universitair Ziekenhuis Brussel (UZ Brussel), Brussels, Belgium

See also poster number 14

15. mRNA-based chimeric antigen receptor (CAR)-T cell therapy: an *in vitro* validation

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Background: Glioblastoma (GBM) is a lethal brain cancer for which more effective treatments are urgently needed. In this context, chimeric antigen receptor (CAR)-T cell therapy has gained traction, demonstrated by the 30 registered clinical trials (clinicaltrials.gov) and industrial interest. Most CAR-T cells are produced with viral platforms, while nonviral platforms such as mRNA are gaining interest due to (1) the ease and low cost of mRNA production, (2) the ability to transfect T cells at high efficiency without prior activation and (3) the safety profile of mRNA engineered cells. Therefore, we investigated *in vitro* transcribed mRNA as a nonviral delivery method for CARs targeting B7-H3, an antigen highly expressed on immunotherapy refractory GBM cells.

Materials and methods: Human T cells were isolated from leukapheresis and frozen until further use. Following thawing, T cells were electroporated with mRNA encoding a CAR targeting B7-H3 or the idiotype of 5T2 multiple myeloma cells (5T2Id, negative control). Each CAR-T product was co-cultured with B7-H3$^+$ LN229 cells. After 3 or 6 days, IFN-γ secretion was determined by ELISA and flow cytometry analysis was performed to study T-cell activation and tumor cell killing. Moreover, tumor cell killing was assessed in real-time by co-incubating CAR-T cells with GFP$^+$ B7-H3$^+$ LN229 cells for 3 days.

Results: mRNA-based CAR-T cells manufacturing proved to be time- and cost-efficient, showing high CAR expression levels over a 5-day culture. CAR-T cells targeting B7-H3 but not 5T2Id demonstrated remarkable killing capacity, evidenced by a significant decrease in tumor cells coinciding with significant production of IFN-γ and upregulation of CD134 and PD-1 activation markers.

Conclusion: We showed that mRNA-based CAR-T cells can efficiently target tumor cells demonstrating the suitability of mRNA as a reliable, time- and cost-efficient platform for CAR-T engineering. This encourages further use of mRNA in CAR-T research and potentially clinical practice.

See also poster number 15

16. Dissecting the biology and applicability in vaccine research of dendritic cells differentiated from monocytes isolated via counterflow centrifugation

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Background

Human dendritic cells (DCs) are extensively used in vaccine research. For practical reasons, mainly *ex vivo* monocyte-derived DCs are used. However, the DC-manufacturing process impacts DC-characteristics and as a result conclusions of *ex vivo* studies, prompting in-depth characterization of DCs to understand their relevance.

Methods

We optimized counterflow centrifugation on the Rotea™ device to enrich monocytes and differentiate them to DCs. We studied monocyte and DC heterogeneity using transcriptome and multicolor flow cytometry analysis. We further studied the DC-phenotype using transcriptome and flow cytometry analysis at baseline and following transfection with lipid nanoparticles (LNPs) that were empty or that encapsulated mRNA encoding the antigen MAGE-A1. Finally, T-cell activation was studied to assess the mRNA-transfected DCs' T-cell stimulatory capacity.

Results

We showed that monocytes ($CD14^+$) represented the majority of the leukocytes in the product enriched by counterflow centrifugation, though a considerable fraction of lymphoid cells was still present. As a result, DC purity following differentiation was moderate. To improve DC purity, lymphoid cells were further depleted prior to differentiation. The DCs mainly displayed conventional DC-characteristics (Lin^-HLA-$DR^+CD45RA^+CD123^-CD11c^+$), though a minor population displayed plasmacytoid DC-characteristics (Lin^-HLA-$DR^+CD45RA^+CD123^+CD5^-AXL^-$). Based on the expression of co-stimulatory and co-inhibitory markers, DCs were considered immature. We showed efficient transfection of these DCs with mRNA-LNPs. Compared to DCs exposed to empty LNPs, DCs transfected with mRNA-LNPs showed a more mature phenotype and were able to stimulate T cells.

Conclusions

We show that monocyte-derived DCs, which are widely used in vaccine research, represent a heterogeneous cell population that display an immature phenotype. We show that these immature DCs can be efficiently transfected with mRNA-LNPs, resulting in their activation and potential to activate T cells, implying that these DCs serve as a good *in vitro* model to study the potential of next-generation nanoparticle-based vaccines.

See also poster number 16

17. Spatial co-localization of macrophages and basal cells in the pancreas

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Tissues employ conserved cellular plasticity to adapt and maintain homeostasis, which is crucial for resilience against disease-related challenges. Whilst plasticity of the pancreatic acinar cells has been widely studied, the duct cell compartment has been neglected. Our lab discovered pancreatic basal cells (PBCs) within this ductal compartment in both healthy and in diseased context. The prevalence of PBCs increases in pancreatic ductal adenocarcinoma, the most prevalent and most deadly pancreatic cancer, as well as in chronic pancreatitis (CP), which is a known risk factor for developing this cancer. These cells could play a role in cancer development, transitioning into different cell states in response to environmental stressors, much like basal cells in other organs. Indeed, in human airway, macrophages stimulate the differentiation of basal cells and aid in the restoration of the epithelial barrier after injury. We hypothesize a similar process occurs in the pancreas.

We use spatial transcriptomics (STX) and immunohistochemistry to study the basal cell (KRT5+) niche with specific attention to the macrophages (CD163+) in chronic pancreatitis.

Using STX, we demonstrate that macrophages are located in proximity to the basal cells and infiltrate the basal layer of pancreatic ducts. At the protein level we confirm increased macrophage presence in KRT5+ ducts compared to KRT5- ducts. We also found a positive correlation between KRT5 and CD163 in KRT5+ ducts.

The pattern of macrophage accumulation around KRT5+ cells in CP may indicate a role for these immune cells in modulating the basal layer. We are now working to further characterize these macrophages and their relationship with basal cells using STX and computational analysis in R, with the aim of replicating these findings in vitro.

See also poster number 17

18. Chenggong Tu [1,2] : See poster number 18

19. H Satilmis[1] : See poster number 19

20. Arthur Esprit[1] : See poster number 20

21. I. Dirven[1] : See poster number 21

22. Vincent De Man [1] : See poster number 22

23. Fien Meeus [1,2] : See poster number 23

24. Jonathan Baldan[1]: See poster number 24