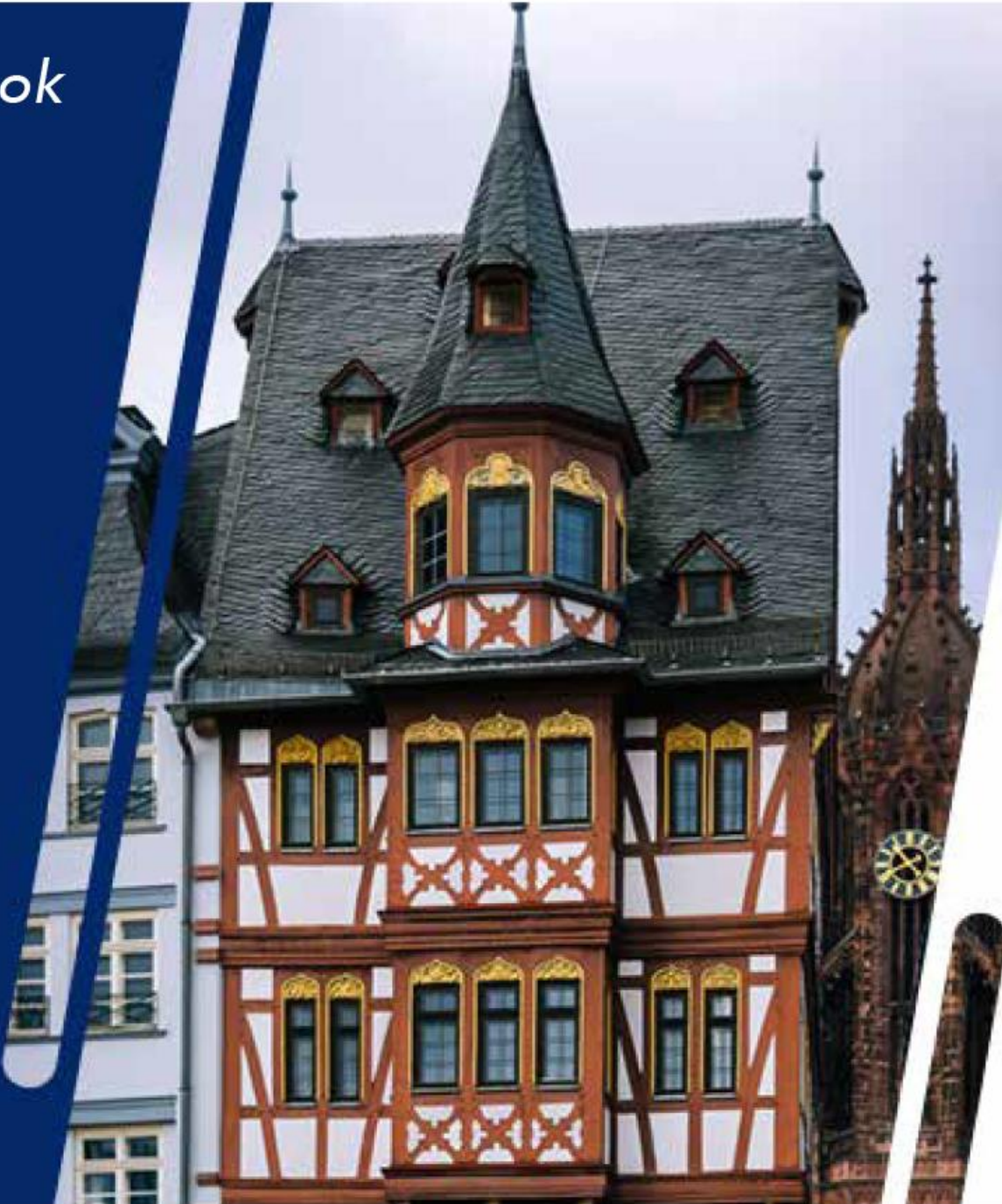# Immuno Oncology 2023

The 4ᵗʰ Immuno-Oncology
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Abstract Book

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Biologic markers in Immuno-Oncology

Cytomorphology of Melanoma with Special Emphasis on Immune Checkpoint Proteins CTLA-4, PD-1 and PD-L1

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Background: Development of therapeutic anti- PD-1/PD-L1/CTLA-4 monoclonal antibodies leading to the reactivation of specific antitumor immune response has emerged as a promising strategy for immunotherapy of cancers including melanoma.

Objectives: Given the complexity of the tumor microenvironment and the dynamic interaction between tumor and immune cells, PD-1/PD-L1/CTLA-4 regulatory pathways in tumors need a better understanding.

Methods: To address this issue, we performed an immunophenotyping of CTLA4/PD1/PD-L1-positive cells in melanoma employing multiple immunofluorescent immunolabeling. For immunolabeling, we used primary antibodies to PD-1, PD-L1, CTLA-4 and a panel of CD antibodies raised against diverse cell types (CD1a, CD3, CD8 and CD68).

Results: We found that CTLA-4 and PD-L1 were not expressed in malignant cells in melanoma but were expressed in the tumor microenvironment including tumor-associated inflammation cells such as macrophages and dendritic cells. Moreover, we found that CTLA-4 expression was not limited to T-lymphocytes.

Conclusion: Detection of CTLA-4 in various cells other than T-lymphocytes suggests the participation of this molecule not only in the well-known classical scheme for regulating T-lymphocyte activity. The expansion of the existing ideas about the role of CTLA-4 may create new approaches for improving the quality of diagnosis and implies broader effects of CTLA-4 on immune regulation.

Biologic markers in Immuno-Oncology

Integration of RNA in situ hybridization and sequential immunofluorescence for same-slide fully automated multiomics analysis of the tumor microenvironment

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Spatial biology has transformed our understanding of the tumor microenvironment (TME) by enabling the study of tissue composition and intercellular interactions at a single-cell level while preserving spatial context. Combining the detection of spatial markers, such as RNA and protein targets, can provide valuable insights into unique infiltrating immune cell populations and their activation states.
Here, we propose a novel approach that combines RNAscope™ and sequential immunofluorescence (seqIF™) protocols for simultaneously detecting RNA and protein targets. The integrated same-slide multiomics protocol is automated on the COMET™ platform, an advanced tissue staining and imaging platform with precise temperature control and full workflow automation, ensuring optimal efficiency and reproducibility.

We developed an integrated protocol for the automated detection of a 12-plex RNA panel, followed by consecutive cycles of seqIF™, with two protein markers detected per cycle. We included antibodies to detect infiltration of T cells, B cells, macrophages, and other immune cells in combination with RNA probes for key biomarkers such as chemokines and cytokines. Combining RNA and protein codetection, we gained extensive insights into the TME molecular landscape, uncovering co-expression patterns and relationships between RNA and proteins within individual cells.
Our results demonstrate the successful implementation of the combined RNAscope and seqIF™ protocols on COMET™. Preserving spatial context and intercellular relationships, this approach offers a more holistic understanding of the TME molecular landscape and the complex cellular interactions exhibited by different cell populations. Multiomics analysis will open new perspectives for personalized medicine and the discovery of novel therapeutic targets.

Biologic markers in Immuno-Oncology

Assessing immune cell functions in prostate tumor microenvironment

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Regulatory T (Treg) suppresses the immune response and maintains tolerance. Tregs` immunosuppressive mechanism(s) role in the context of programmed death 1 (PD-1) is not entirely understood. This study aims to explore the role of PD-1 on Treg cells and their impact on CD8+ T cell function in prostatic tumor microenvironment using transgenic adenocarcinoma of the mouse prostate (TRAMP) cells (TRAMP C1, C2, and C3) as a model system. We performed tumor induction studies to test this aim. Briefly, the C57BL/6 mice were administered with serial log concentrations of TRAMP (C1-3) cells. Interestingly, the TRAMP-C1 and TRAMP-C2 are tumorigenic, while the TRAMP-C3 cells do not form a tumor. Mice were sacrificed by cervical dislocation; tumor, lung, spleen, and draining lymph nodes were harvested when the tumor size reached approximately 20mm. Single-cell suspensions were prepared from different organs, cells were then stained with specific antibodies, and flow was analyzed for the expression of other immune markers. Our preliminary findings demonstrated that PD-1 expression on Foxp3+ Treg cells displayed greater suppressive capacity against CD8+ T cell function in tumor, lung, spleen, and draining lymph nodes when compared to the control. More importantly, Foxp3+ Treg(high) PD-1(high) interaction with PDL-1 induced immunosuppression by blocking CD8+ T cells function in the prostatic tumor microenvironment. Our data suggest that the expression of Foxp3 and PD-1 may enhance tumor progression; thus, targeting the PD-1 on Treg cells may be a possible therapy to treat prostate cancer.

Biologic markers in Immuno-Oncology

MUC2 Expression Modulates Immune Infiltration in Colorectal Cancer: Insights from In Vitro Investigations

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Background: A deeper understanding Colorectal cancer (CRC) tumor microenvironment and its interaction with cancer cells is crucial to devising effective therapeutic strategies. MUC2, a major component of the protective mucus layer in the gastrointestinal tract, has been implicated in CRC progression and immune response regulation.

Objectives: The goal of our study was to assess in-vitro in a 3D model the role of Muc2 in the immune invasion of colorectal solid tumors.

Method: In this study, we sought to elucidate the relationship between MUC2 expression and immune infiltration within CRC, using in-vitro models involving two well-established cell lines, HT-29 and LS-174T. By employing CRISPR-mediated MUC2 knockout, we investigated the influence of MUC2 on tumor invasion and its interplay with T cells and NK cells enriched peripheral blood mononuclear cells (PBMCs) in 3D spheroid cultures.

Results: Despite being more abundant in LS-174T cell lines compared to HT-29, the knockout of MUC2 resulted in increased immune invasion in HT-29 cell line but not in LS-174T. Our investigation revealed that the removal of MUC2 protein was compensated in LS-174T by increased expression of other gel forming mucin protein (Muc6, MUC5B) commonly expressed in colorectal epithelium, while this was not observed in HT-29 cell line.

Conclusion: We propose that the role of MUC2 documented in CRC progression can partially be explained by impairing immune invasion due to physical barrier established by the gel forming proteins such as MUC2 in mucinous CRC. On the other hand, the absence of MUC2 expression can be compensated by alternative gel forming mucin protein, thus not increasing tumor invasion but potentially favoring the metastatic process.

Biologic markers in Immuno-Oncology

Activation of PDL1 by Hippo pathway component, YAP Plays Significant Role in Drug Resistance and Cancer Immune Evasion in Breast Cancer

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Background: Chemotherapy resistance has been linked to the deregulation of signaling pathways, including the Hippo pathway. We hypothesize that Overexpression of YAP/TAZ in breast cancer has been suggested to foster drug resistance, possibly through the activation of the PD1/PDL1 pathway. PD-L1 expression has been observed to correlate with MDR1 and BCRP1 expression in breast cancer patients. Activation of PDL1 through the Hippo pathway may be significant in understanding the mechanism of cancer immune evasion.

Objectives: (1) Evaluate the putative role of the Hippo pathway components YAP/TAZ in regulating PD-L1 expression and thus regulate the drug resistance in breast cancer. (2) Delineate the molecular mechanism underlying the PD-L1-mediated drug resistance in breast cancer.

Methods: Development of a doxorubicin-resistant variant of the MDA-MB-231 cell line, down-regulating PD-L1 by siRNA, and inhibiting the YAP by vertiporfin and expression of MDR1, MRP1, and BCRP1.

Results: Down-regulating PD-L1 decreased the expression levels of MDR1, MRP1, and BCRP1 in the drug-resistant cells. Inhibiting the YAP/TAZ complex significantly affected PD-L1 expression and vice versa. Also, the expression of phosphorylated EGFR, AKT, and ERK was decreased with increased expression of PTEN upon PD-L1 down-regulation.

Conclusion: The YAP-TAZ-dependent activation of the EGFR-PI3K/AKT signaling pathway may participate in the regulation of PD-L1 expression and, thereby, the expression of drug-resistance proteins in chemotherapy-resistant breast cancer cells. The present work is the first substantiation that YAP/TAZ and PD-L1 positive feedback regulate EGFR and its downstream signaling and regulate the expression of multidrug resistance-associated proteins in breast cancer.

Biologic markers in Immuno-Oncology

Soluble Immune Targets Correlate With Response To Anti-PD-1 Treatment In Patients With Metastatic Melanoma

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Background

Although the advent of immunotherapy with immune checkpoints inhibitors (ICIs) has brought substantial benefits in terms of survival for metastatic melanoma (MM) patients, most of them develop resistance. Hence, the search of biomarkers, which could help in the selection of patients that could respond to therapy, represents an urgent clinical need.

Objectives

The aim of our study was to determine the amount of soluble blood factors (sFc), implicated in the modulation of the immune response, to find a possible correlation with clinical outcome of MM patients treated with ICI

Methods

Plasma samples were collected from 110 MM patients before starting ICI treatment. We evaluated the soluble forms of PD1, PD-L1, LAG-3, CTLA-4, CD73, CD74 and CD4 by ELISA kit. ROC analysis was used to identify the best cut-off values of sFs for stratifying MM patients according to RECIST clinical response to ICI.

Results

Basal level of sCTLA4 and sCD74 were significantly higher in the plasma of non-responders, while no significant difference was found in the plasma level of CD4, CD73, PD1 and PDL-1 between responders and non-responders.

The stratification by ROC analysis showed that baseline value of both sCTLA-4 and sPD1 higher than ROC correlated with either poor PFS and OS (sCTLA-4: both p0.0001; sPD1: p=0.0493 and 0.0192), while higher values of soluble PD-L1 and CD74 at baseline correlated with poor PFS (p=0.0394, p=0.0055, respectively).

Conclusion

Our results demonstrated that sFs could be dosed with a minimally invasive procedure and used for predicting the response to ICI in MM patients.

Biologic markers in Immuno-Oncology

Data Collection to Build a Time Dependent Predictive Model for Immune Suppression in Pancreatic Cancer with Radiation Therapy

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Background:

Patients with pancreatic cancer develop severe treatment-related lymphopenia, which correlates with worsened overall survival and disease free survival. However, there is no computational model to predict radiation (RT) plan dependent immune suppression. Optimizing treatment regimens with alterations in coverage volumes and/or doses and fractionation might benefit these patients with poor outcomes.

Objectives:

We evaluated lymphocyte reduction from radiation therapy in pancreatic cancer from the dose volume data, correlating the static organ dose volumes to immune suppression. We also evaluated the time dependent immune suppression function post radiation therapy.

Methods:

We analyzed 92 patients who received RT to the pancreas. The following structures were contoured using the RTOG contouring atlas: superior mesenteric artery, celiac artery, portal vein, inferior vena cava, aorta, liver, stomach, bowel, kidney, spleen, duodenum, and lymph nodes. For each patient for the above organs, integral dose, dose volumes: V2-V50, PTV volume, and dose fractionation were recorded. Statistical analysis was performed using IBM SPSS Statistics.

Results:

Lymphocyte drop is maximum at day 35 following initiation of RT, which is about a 79% reduction from Pre-Tx LYA value. Even at day 185, the reduction is 65%. However, if the total dose is less than or equal to 45 Gy, lymphocyte drop is less than 0.75×10^9 cells per L. Lymphocyte drop increases with the number of fractions. The highest Spearman rank correlations were observed for stomach, bowel, and kidney, suggesting the importance of these organs for dose sparing in pancreas radiation.

Conclusions:

Lymphocyte changes correlated most strongly with dose to stomach, bowel, and kidney and number of fractions of treatment.

Biologic markers in Immuno-Oncology

Pre-existing T-cell immunity and TCR Vβ Repertoire as Predictive Biomarkers for Immunotherapy in NSCLC Patients

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Background: T-cell mediated anti-tumoral responses may have a significant clinical relevance as immune biomarkers for immunotherapy response.

Objectives: We investigated the value of pre-existing tumor antigen specific T-cells (PreI) and TCR repertoire as predictive biomarkers before and during immunotherapy in NSCLC patients. Additionally, we analyzed the major differences in immune-cell phenotypes.

Methods: Blood was collected from 57 and tissue from 16 patients with stage IIIB/IV NSCLC before and during treatment with ICI-containing regimens. PreI was calculated as the percentages of CD3+IFNg+ cells after in-vitro co-cultures of PBMCs with peptides against 4 different TAA. TCR repertoire was assessed using the Ion Oncomine™ TCR Beta-SR NGS Assay for the identification and quantification of T-cell clonal expansion. Immunophenotyping of peripheral blood T-cells was performed using multicolor flow cytometry.

Results: PreI+ T-cells were detected in 42.3% of patients (PreI+ patients) with no significant changes during ICI treatment. Survival analysis revealed better OS in PreI+ than PreI– patients (log-rank=0.014). Comparison between blood and tissue revealed higher TCR diversity in blood of PreI+ patients (p= 0.035). Reduction in the number of clones during ICI treatment indicated significantly better PFS (log-rank=0.025) for PreI+ but not PreI– patients. Additionally, PreI+ patients had significantly higher numbers of possible exhausted CD3+CD8+PD-1+ cells compared to PreI- (p= 0.0053).

Conclusions: Pre-existing tumor antigen specific immunity before initiation of ICI in NSCLC patients could serve as a good predictive factor of response and could be related with TCR repertoire.

Cell therapy

Enhanced Antitumor Activity and Long-term Persistence of Novel Bi-specific CAR-T cells to Overcome Antigen Escape

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BACKGROUND:

Conventional second-generation CAR-T cells targeted at CD19 have demonstrated promising clinical outcomes using 41BB or CD28 co-stimulatory domains. However, the clinical data indicates that a significant number of patients undergoing this treatment experience relapse due to antigen escape. To tackle this issue, we developed bi-specific CAR constructs that incorporate diverse co-stimulatory domains.

OBJECTIVES:

1. Develop innovative CAR constructs incorporating various co-stimulatory domains.

2. Validate CAR expression and anti-tumor activity through in-vitro experiments.

3. Assess in-vivo efficacy and persistence of these CARs using NSG mice.

METHODS:

40 CAR constructs were designed, encompassing mono- & dual- domains with diverse co-stimulatory elements. CAR expression was evaluated using antibodies against ScFvs. T cells isolated from volunteers were transduced with lentiviral vectors expressing CAR transgenes. In-vitro anti-tumor activity was measured through bioluminescence. Tumor xenograft models were established using Raji cells in NOD-SCID IL2Rγnull (NSG) mice.

RESULTS:

The study demonstrated potent tumor-fighting efficacy of CD20-19 ICOS/41BB and CD22/19 ICOS/41BB CART cells in NSG mice. Over a 3–6-week monitoring period, mice treated with these CART cells exhibited significantly enhanced survival compared to 2nd gen CARs, emphasizing the promising nature of these CAR-T cell therapies for tumor treatment.

CONCLUSION:

This study addresses the limitations of conventional CAR T cells by designing bi-specific CAR constructs. These novel CARs effectively combat antigen loss by targeting multiple cancer antigens. Incorporating 41BB/ICOS co-stimulatory molecules significantly improves CART cell persistence, validating their potential for cancer immunotherapy and clinical application. The findings highlight a substantial stride towards more effective cancer treatment strategies.

Cell therapy

Bacterioruberin, a Rare Carotenoid, Induces Apoptosis and Suppresses Proliferation in Myeloid Leukemia Cells

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Background: Carotenoids are a promising source for complementary or alternative anti-cancer therapy because of their well-documented antioxidant and anticancer properties through their ability to modulate key cellular processes, including immunomodulatory activities, and possess minimal side effects. Our research focuses on bacterioruberin, an uncommon carotenoid derived from extremophilic haloarchaea. It has received limited study despite its significantly higher antioxidant capacity compared to conventional carotenoids. This characteristic raises the possibility that bacterioruberin may possess a superior antitumor potential. In this study, we evaluated its efficacy for the treatment of myeloid leukemia.

Objectives: Investigate bacterioruberin`s therapeutic potential as a natural agent in myeloid leukemia.

Methods: Bacterioruberin`s impact was assessed in vitro using K562 and THP1 myeloid leukemia cell lines. Increasing doses of bacterioruberin were administered (physiological concentrations), and cell toxicity (MTT), proliferation (CFSE), apoptosis (YO-PRO-1/PI), and cell cycle phases (PI) were monitored in time by spectral flow cytometry. Apoptosis was also examined morphologically through fluorescence microscopy (AO/EB).

Results: Bacterioruberin exhibits cytotoxicity in both cell lines (IC50K562: 42.9324h, 38.1848h, and 19.6872h µg/ml; IC50THP1: 43.5124h, 46.8048h, and 31.9572h µg/ml). Furthermore, bacterioruberin reduces cell proliferation in a time- and dose-dependent manner in both cell lines, causing alterations in the cell cycle. Importantly, cells undergo apoptosis in a time- and dose-dependent manner, which increases from the dose of 9.375 µg/ml.

Conclusion: Ex vivo bacterioruberin assays results shows a potential use for myeloid leukemia therapy with minimal side effects due to its natural origin, but further analysis is required to understand the underlying mechanisms.

Cell therapy

Potency of Anti-CD19 CAR-T Cells: Validation of the Killing Assay

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Background

For ATMPs the development of quantitative and functional potency assays is required. Assay validation in accordance with international guidelines is increasingly desirable to obtain reliable and consistent data.

Objectives

Our purpose was to validate the killing assay for the evaluation of CAR-T potency.

Methods

Killing test validation involved the use of CD3+ lymphocytes and anti-CD19 CAR-T cells as effector cells and REH (CD19+) or MOLM-13 (CD19-) cell line as target cells. After 24-hour co-culture of target and effector cells (1:1 ratio), samples are labelled with 7-AAD, anti-CD3 and anti-CD19 antibodies and the frequency of CD19+ dead cells was evaluated by flow cytometry. Potency, expressed as % of dead cells, is calculated subtracting the value of the background well (CD3+ and REH) from the co-culture well (CAR-T and REH).

Results

To verify the assay specificity, the co-colture between CAR-T and REH or MOLM-13 was performed. Percentage variation of potency values between the two conditions was 90% to demonstrate the high CAR-T specificity for the CD19+ target. Linearity and accuracy were evaluated performing scalar dilutions of CAR-T and REH co-colture. Established acceptance criteria were complied for both parameters (r2 ≥ 0.97 for linearity and average relative error ≤ 10% for accuracy). Moreover, the method is considered robust when performed between 20 and 24 hours of co-culture (CV ≤ 10%). Repeatability and intermediate precision, in terms of inter-assay and inter-day were also investigated. Results were always lower than the acceptance criteria (CV=3.13, 2.78 and 14.82, respectively).

Conclusion

Our cell killing assay is validated.

Cell therapy

Pre-planned interim results of a Phase 1B clinical trial of pembrolizumab (P) and dendritic cell vaccine (DC) in advanced pleural and peritoneal mesothelioma (M) patients

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Background:

Mesothelioma is a rare cancer with limited therapeutic options. Our data showed that DCvax induces the expression of PD-L1 on tumor cells so the addition of DCvax to antiPD1 antibody may sensitize patients to the effects of PD-1 blockade.

Methods:

MESOVAX is a Ph1b study evaluating safety of pembrolizumab 200 mg Q3W and an autologous DCvax in pretreated mesothelioma patients (pts) for a maximum of 6 cycles. Primary endpoint was safety; secondary were PD-L1 expression variations defined by immunohistochemistry (IHC) and efficacy (objective response rate [ORR], duration of response [DOR], progression-free survival [PFS], overall survival [OS]).

Results

As of 30 Aug 2023, 6 pts (median follow-up 20.1 mo) were treated and evaluable for safety. Median age was 64 yrs, all pts were male, 17%/83% were ECOG PS 0/1. All pts had epithelioid Mesothelioma. Any grade treatment-related adverse events (TRAEs) occurred in 5 pts (83%); most common were local reaction to injection site (67%), asthenia (33%) and fever (33%). None of the patients experienced grade 3–4 TRAEs. Concerning the treatment, 4 pts (67%) received 6 cycles of P+DC; 3 received maintenance P. In a preliminary analysis of tumor response, 2 had SD, 1 had PR and 3 had PD. PD-L1 expression increased from 0 to 70% in 1 responding patient.

Conclusions:

Preliminary results of MESOVAX trial of pembrolizumab combined with DC showed a manageable toxicity and demonstrated encouraging preliminary efficacy. The trial is actively recruiting.

Cell therapy

The Effect of Sorafenib and Adipose Tissue-Derived Stem Cells Conditioned Medium on Cell Cycle and Apoptosis of Hepatocellular Carcinoma Cells

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Background: Stem cells, such as adipose tissue-derived stem cells (ADSC) are investigated to address the problem of limited therapeutic options in advanced hepatocellular carcinoma (HCC). The current standard of care involves the usage of sorafenib, which targets the Ras/Raf/MEK/ERK pathway.
Objectives: To investigate the synergistic effect of ADSC with sorafenib on the cell cycle and apoptosis of HepG2 cell line.

Methods: The dose of sorafenib ($7.5\mu M$) was chosen based on the results of RT-qPCR (Ras/Raf/MEK/ERK gene expression). HepG2 cells were then cultured 1) alone - H, 2) with ADSC conditioned medium (CM) – H+CM, 3) with CM ADSC and sorafenib – H+CM+S or 4) with sorafenib – H+S for 48 hours.
To evaluate the effect of co-culture, we assessed the viability (MTT assay), apoptosis (flow cytometry FACS, annexin V stain), cell cycle (FACS, propidium iodide stain), the expression of CD133 cancer stem cell marker (FACS), RAS/RAF/MEK/ERK signaling genes (RT-qPCR), AFP and cyclin D proteins (immunofluorescence) in HepG2 cells.

Results: Sorafenib reduced the viability in H+S and H+CM+S by 38.62% and 35.23%, respectively, reduced the percentage of S-phase cells in both groups and increased the percentage of M-phase cells in H+S ($p < 0.05$). We did not observe changes in apoptotic activity or in CD133, AFP and cyclin D expression.

Conclusion: Although sorafenib reduced the viability of HCC cells and changed the cell cycle phases populations, CM ADSC did not show any synergistic effect with sorafenib.

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Cell therapy

Tracking Individual Natural Killer Cells by Machine Learning in a Killing Assay Sheds Light on Heterogeneity

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Background:
The FDA`s approval of NK cell-based cancer immunotherapy has highlighted the need for detailed individual cell analysis within large populations, which traditional technologies couldn`t provide.

Objectives:
To develop a platform that can investigate cell populations at the single-cell level in conditions mimicking in vivo environments, and permits isolation of living cells for subsequent studies.

Methods:
ARRALYZE, a pioneering digital cell biology platform, was developed to meet these objectives. Using miniaturized glass well arrays, it facilitates functional single-cell screening and continuous cell monitoring through brightfield and fluorescence microscopy. Our study employed K562 target cells and primary NK cells, co-cultured with a death-inducing dye to demonstrate the platform`s capabilities.

Results:
The platform detects killing events, marked by color changes in target cells. Tracking of the individual cells is also enabled, allowing to plot their motility in relation to their killing activity. The system provides valuable insights into immune cell dynamics and their efficiency in eliminating targets, using machine learning.

Conclusion:
ARRALYZE offers a revolutionary approach for professionals in immunotherapy, enabling in-depth analysis of individual cells in near in vivo conditions.

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Cell therapy

Dual Expression Of Vγ9Vδ2 and αβ MR1T TCRs Promotes Anti-Tumor Cooperativity

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Background

T lymphocytes expressing Vγ9Vδ2 TCR or αβ TCR restricted to MHC I-related molecule MR1 (MR1T) are stimulated by non-polymorphic molecules. These cell populations require the presence of different stimulatory/antigen-presenting molecules on target cells and show distinct specificity to ubiquitous self-antigens known to accumulate in cancer cells. Both cell populations have cytotoxic functions and boost anti-tumor inflammatory responses. TCR Vγ9Vδ2 cells are activated by butyrophilins 3A1 and 2A1 (BTN3A1 and BTN2A1) in the presence of phosphorylated intermediates of the mevalonate pathway (e.g., isopentenyl pyrophosphate, IPP), whereas MR1T cells are responsive to metabolites presented by MR1.

Objectives

To investigate whether co-expression of Vγ9Vδ2 and αβ MR1T TCRs improves T cell activation, tumor recognition, and tumor killing.

Methods

TCR Vγ9Vδ2 cells expanded from PBMCs of healthy donors were transduced with selected MR1T TCRs. TCRs co-operativity was assessed in vitro by measuring cytokine release from T cells in the presence of different antigen doses and by killing assays.

Results

Dual TCR cells recognized phospho-antigens similarly to non-transduced TCR Vγ9Vδ2 and gained reactivity towards MR1T-antigens. Remarkably, we observed a synergistic effect when dual TCR cells were challenged with antigens at sub-optimal doses, translating into broader recognition of tumor cell lines and their killing.

Conclusion

Our experimental model provides initial evidence of cooperativity between Vγ9Vδ2 and MR1T αβ TCRs in both the sensitivity to tumor antigens and the killing of tumor cell lines. This approach stands as a promising door to be opened in the immuno-oncology world.

Cell therapy

Next Generation CD44v6-Specific CAR-NK Cells Effective Against Solid Tumors

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There is a medical need to develop new and effective therapies against solid tumors. Chimeric antigen receptor (CAR) natural killer (NK) cells are a promising alternative to CAR-T cell therapy for cancer. A search for a suitable target in solid tumors, such as triple negative breast cancer (TNBC), identified CD44v6, an adhesion molecule that is implicated in tumorigenesis and metastases. We have developed a next-generation CAR targeting CD44v6 that incorporates IL-15 superagonist and checkpoint inhibitor molecules. We could show that CD44v6 CAR-NK cells demonstrated effective cytotoxicity against TNBC in 3D spheroid models. The IL-15 superagonist was specifically released upon recognition of CD44v6 on TNBC and contributed to the cytotoxic attack. PD1 ligands are upregulated in TNBC and contribute to the immunosuppressive tumor microenvironment (TME). Competitive inhibition of PD1 neutralized inhibition by PD1 ligands expressed on TNBC. In total, CD44v6 CAR-NK cells are resistant to TME immunosuppression and offer a new therapeutic option for the treatment of BC, including TNBC.

Cell therapy

Efficacy of taxane-Polyphenol fractions of Hazelnut Waste on Viability of tumor (Jurkat) Cells

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Background: Special attention has been paid to natural medicines among the new effective cancer treatment methods in recent years. paclitaxel can be isolated from the shells and leaves of the hazelnut (Corylus avellana L). Taxane-polyphenol synergism is known to enhance the efficacy and selectivity of chemotherapeutic drugs. Therefore, screening of taxane-polyphenol combinations is needed to select a highly effective complex, enhancing anticancer efficacy, and reducing taxane side effects through synergistic interaction with natural phenolic compounds.

Objectives: research aimed to study the effects of different polyphenol fractions obtained from hazelnut residue extracts on tumor (Jurkat) cells viability.

Methods: Compounds from the nut residue (hard shell) were extracted using solvents, shromoform, ethyl acetate, methanol, and ethanol. Chromatographic separation was performed on a high pressure liquid chromatography Agilent-1260 Infinity. The studies were performed on human leucemia transformed mature T-cells (Jurkat cells) and normal epithelial MDCK cells incubated with extracts (with and without liposomes); the viability of the cells was determined after 24h incubation by MTT assay.

Results: ethanol extract of hazelnut shells revealed the highest cytotoxity (more than xhloroform extract) against Jurkat cells and MCK cells.

Conclusion: Ethanol extract of hazelnut shells, characterized by a small paclitaxell content, revealed high cytotoxity against Jurkat cells and protected MDCK cells (more effective than chloroform extract). this may be related to the high content of polypheno, epicatechin gallate in ethanol extract of hazelnut shells, characterized with pro-oxidant activity, thereby exerting cytotoxity on cancer cells, which are more sensitive to oxidtiv stress than normal cells.

Future directions in Immuno-Oncology

The role of ADAM8 in PD-L1 shedding in glioblastoma: a future therapeutic target?

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Background: Glioblastomas (GB) are malignant brain tumours with a dismal prognosis. Metalloproteases (MPs) have been described for early GB diagnosis as essential for macrophage reprogramming whereas the PD-1/PD-L1 axis reflects immunosuppressive mechanisms in GB. Our groups have demonstrated that preclinical GL261 GB responding to Temozolomide (TMZ) treatment increased its PD-L1 expression, found strongly correlated with the expression of A disintegrin and metalloprotease 8 (ADAM8). Proteolytic processing of PD-L1 was described to be associated with MP expression, such as ADAM10/17, but not ADAM8.

Objectives: Our purpose was to investigate whether ADAM8 could be also involved in PD-L1 proteolytic processing in tumor and immune cells.

Methods: GL261 cells with a knockout for the ADAM8 gene were generated using the CRISPR/Cas9 methodology, further confirmed by western blot. Cells were incubated with medium containing mTNFα and mIFNγ (for PD-L1 expression increase) during 24h-48h. Conditioned medium was used for PD-L1 ELISA measurement. In addition, primary macrophages from wt and ADAM8 KO mice were also investigated.

Results: The ADAM8 knockout was confirmed via qPCR. The mRNA level of ADAM8 in GL261 wt cells increased after mIFNγ stimulation. Moreover, PD-L1 was strongly released after mIFNγ stimulation in GL261 wt cells, while the release in knockout cells was strongly decreased in a time-dependent manner. This release was also affected in macrophages (especially M1 subtype), although to a lesser extent.

Conclusions: The abrogation of ADAM8 prevented/decreased the release of PD-L1, suggesting that a new potential therapeutic target could emerge from such findings, of great interest for translational GB research.

Future directions in Immuno-Oncology

Effects Of Radiofrequency Radiation And Magnetic Nanoparticles On Proliferation And Apoptosis Of Human Chronic Lymphocytic Leukemia Cell Line

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Background: Chronic lymphocytic leukemia (CLL) is most common type of leukemia in Western countries and its pathogenesis associated with the defect in apoptosis. Since Radiofrequency radiation (RFR) has mechanism of altering the electrical properties of cell membrane, apoptosis may occur due to RFR exposure in certain frequencies and intensities which may change with respect to the cell lines.

Objectives: The aim of this study was to investigate whether RFR can enhance apoptotic activity of human MEC-1 CLL cell line alone or in the presence of magnetic nanoparticles Fe_3O_4.

Methods: In vitro exposure to 900 MHz, 120 µW/cm2 RFR and Fe_3O_4 nanoparticles with the concentration of 20 µg/mL ve 100 µg/mL during 3 hours was investigated in cultured MEC-1 cells. Cell proliferation via XTT method and ANNEXIN V FITC/PI via flow cytometry were used for detecting apoptotic cells due to RFR and/or magnetic nanoparticles application.

Results: The proliferation increased, whereas the rate of apoptosis decreased of MEC-1 cells due to RFR and nanoparticle treatments ($p < 0.05$).

Conclusion: Although our previous studies showed decrease in cell proliferation in different cancer cell lines, but the rate of proliferation is tended to increase in CLL cell lines. The reason of this result may be because of the role of iron in carcinogenesis described as a double-edged sword. Similarly, RFR has same type of effects, called "window effect" as dose-response implications of the transient nature of electromagnetic-field-induced bioeffects.

Future directions in Immuno-Oncology

Exploring the Interfacial Dynamics of Interactions between Deglycosylated Vitamin D binding protein (dgVDBP) and Interferon-gamma (IFN-γ) receptor.

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Vitamin D binding protein (VDBP), also known as GC-globulin or group-specific component, is a multifunctional protein primarily known for its role in transporting vitamin D metabolites in the bloodstream. While its primary function is related to vitamin D, VDBP can also interact with other molecules, including interferon-gamma (IFN-γ). Interferon-gamma (IFN-γ) is a cytokine produced by immune cells, particularly macrophages, T cells and natural killer cells, in response to infections and immune challenges. It plays a critical role in immune responses by promoting inflammation and activating immune cells to help fight off infections and regulate various immune functions as well an important effector molecule of anti-tumor immunity, capable of suppressing tumor growth through various mechanisms We studied the interaction between VDBP and IFN-γ, and here are some key points about this interaction: Binding of IFN-γ to VDBP: VDBP can bind to IFN-γ in the bloodstream. This interaction involves the physical binding of IFN-γ to specific sites on VDBP. Modulation of IFN-γ function: The binding of IFN-γ to VDBP can influence the activity of IFN-γ. While the exact mechanisms are not fully understood, some studies suggest that VDBP may act as a carrier protein for IFN-γ, potentially helping to regulate its availability and stability in the bloodstream. Immunomodulatory effects: Some research has suggested that VDBP-IFN-γ interactions may have immunomodulatory effects. For example, VDBP-IFN-γ complexes may influence the immune response by affecting the bioavailability and function of IFN-γ. Genetic variations: Genetic variations in the VDBP gene (GC gene) can lead to different forms of VDBP, which may have varying affinities for IFN-γ and other molecules. These genetic variations can influence an individual`s immune response. Current study for the first time as best of our knowledge reports the interfacial mechanism of protein-protein interactions between VDBP and IFN-γ using protein-protein docking and molecular dynamics simulations methods. Long run molecular dynamics simulations revealed the activated VDBP (VitD-dg-VDBP) to have strong interactions with IFN-γ protein specially in its active binding domain. Inter residual contact analysis (ICS) revealed that VitD-dg-VDBP have more residual contact to IFN-γ as compared with its glycosylated form (VitD-VDBP). That may give a clue about basic immune modulatory effect of (VitD-dg-VDBP) through blocking the immune-suppressive response of the IFN-γ.

Future directions in Immuno-Oncology

Molecular insights into effect of De-Glycosylation of VitD-dgVDBP (Immuno-D®) on its Structural Dynamics and immune modulatory mechanism

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Increasing interest in studying the role of vitamin D in cancer has been provided by the scientific literature during the last years. Vitamin D deficiency has been largely associated with various types of solid and non-solid human cancers such as colorectal, prostate and breast cancer. Conversely, several molecular studies are in agreement about the role of vitamin D in inhibiting tumor cell proliferation, growth and invasiveness, cell cycle arrest and inflammatory signaling, through which vitamin D may also regulate cancer microenvironment through the activation of different molecular pathways. Vitamin D3 complexed to deglycosylated vitamin D binding protein is a water-soluble vitamin D dimeric compound (VitD-dgVDBP). The curent study reports for the first time, as best of our knowledge, the structural and dynamics and immuno modulatory mechanism of the protein dimer, invesitigating the role of deglycsylation on the dimeric binding with Vitamin-D3. Immuno-D® was identified in the samples through SDS-PAGE followed by UV/VIS analysis to cofirm protein and Vitamin D 3 binding. Molecular Structure of VitD-dgVDBP was modelled to estimate the biding effecinecy with Vit D 3 in glycosylated and deglycosylated forms of protein and deglycosylated form showed the relatively stronger binding with VitD 3. Dynamical assesment of the modelled VitD-dgVDBP showed the deglycosylation having significant impact on confirmational dynamics, cross correlation movement , surface area and gyration, compactness of the protein. Furtheromore, molecular dynamics simulation suggest the deglycosylation to change whole strucutral dynamics of protein inducing the complete rotation due increase hydrogen bonding in glycosylation domain. Therefore, study suggest that deglycosylation of Vitamin-D binding protein in Immuno-D®(VitD-dgVDBP) enhaces the binding with VitD3 and thus increasing the availibility of Vit-D 3 in blood cells becomes major cause by its immuno-modulatory effects. Furthermore Deglycosylation changes the whole confirmational dynamics of protein and thus actiavting the the whole complex to interact with macrophages and T –cell receptors to enhance activation and reduces their immuno suppresive effect.

Future directions in Immuno-Oncology

Fc-competent multispecific PDL-1/TIGIT/LAG-3 antibodies potentiate superior anti-tumor T cell response

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The landscape of current cancer immunotherapy is dominated by antibodies targeting PD-1/PD-L1 and CTLA-4 that have transformed cancer therapy, yet their efficacy is limited by primary and acquired resistance. The blockade of additional immune checkpoints, especially TIGIT and LAG-3, has been extensively explored, but so far only a LAG-3 antibody has been approved for combination with nivolumab to treat unresectable or metastatic melanoma. Here we report the development of a PDL1 × TIGIT bi-specific antibody (bsAb) GB265, a PDL1 × LAG3 bsAb GB266, and a PDL1 × TIGIT × LAG3 tri-specific antibody (tsAb) GB266T, all with intact Fc function. In in vitro cell-based assays, these antibodies promote greater T cell expansion and tumor cell killing than benchmark antibodies and antibody combinations in an Fc-dependent manner, likely by facilitating T cell interactions (bridging) with cancer cells and monocytes, in addition to blocking immune checkpoints. In animal models, GB265 and GB266T antibodies outperformed benchmarks in tumor suppression. This study demonstrates the potential of a new generation of multispecific checkpoint inhibitors to overcome resistance to current monospecific checkpoint antibodies or their combinations for the treatment of human cancers.

Future directions in Immuno-Oncology

Tumor Adaptation To Immune Factors: New Ideas For Cancer Immunotherapy

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Background. The tumour is fully functional in the zone of action of immune mediators. Moreover, the tumour needs immune system mediators to survive. "Adaptation" refers to a tumour`s ability to withstand the effect of harmful elements. This gives birth to a new form of anti-tumour therapy: blocking tumour adaptability pathways. Objectives. i. tumour adaptation mechanisms as a result of pro-tumour immunoediting, ii. how understanding tumour adaptive mechanisms has led to ideas for developing cancer immunotherapies, and iii. prospects for using the adaptation theory to substantiate new approaches to tumour growth inhibition. Methods. Analysis of the modern concept of adaptation from literary sources and data from our own research over the past 25 years. Results. By considering the cancer problem through the lens of adaptability, a unique strategy for enhancing the efficacy of immunotherapy was proposed. The new approach is to utilise antisense treatment to erase the structural trace of adaptation in tumour cells, or to disadapt tumour cells by "turning off" the immune system before initiating immunotherapy. Conclusion. Destruction of tumor cell adaptation mechanisms may form the basis of a new paradigm for the treatment of oncological diseases.

Future directions in Immuno-Oncology

A Dual Immunogen of Kisspeptin and GnRH for Hormone-dependent Cancers

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GnRH immunogens are employed in sterilisation of animals but also for hormone-dependent cancers. However, they are not 100% efficacious. GnRH secretion is dependent on upstream stimulation by kisspeptin. We therefore hypothesised that a dual immunogen combining GnRH and kisspeptin may be more efficacious through targeting two levels of the axis. We studied over 300 days the efficacy of immunisation with a dual immunogen comprising GnRH linked to kisspeptin via a hepatitisB T helper peptide sequence (GKT) in rats. At all stages of development all immunised animals produced high titre antibodies to GnRH, kisspeptin and GKT. In adult, prepubertal and pubertal males testosterone and testes length were markedly reduced by 60 days and remained at low levels until day 150. Thereafter, testosterone recovered to pre immunisation levels and testes length increased to 40% of controls. 80% of males were infertile in three matings over 250 days. In prepubertal and pubertal female rats a single immunisation at day 0 reduced estradiol to low levels by day 60 which remained low until termination of the experiment on day 300. In matings of these females with fertile males on days 90, 120 and 250, 74% of prepubertal females were infertile and impressively, 100% (10/10) of pubertal females were infertile after a single immunisation on day 0. We have previously shown a GnRH vaccine is efficacious in humans with prostate cancer. Our current findings suggest that the dual immunogen will require a single vaccination and be more efficacious.

Future directions in Immuno-Oncology

The Role Of Neutrophils In The Therapeutic Phase Of Medical Rehabilitation Of Non-Small Cell Lung Cancer (pilot project)

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The clinical study is to find out the effect of a course of immunomodulatory drugs and detoxification scheme on finger bioelectroluminescence and neutrophil function and their correlation with changes in quality of life and life expectancy in patients with malignant diseases against the background of restorative treatment and rehabilitation.

Questions:

1) does the quality of life of patients with lung cancer change with the use of a course of immunomodulatory drugs and detoxification scheme?

2) does phagocytosis function, liposomal activity, mitochondrial function of neutrophils change against the background of the course?

Participants (n=36) will take Calcitreol capsules, Magnesium B-6 capsules, products containing quercetin flavonoids, Naderin (sodium deoxeribonucleate) daily for 21 days. before the course, after the course and after one year they will answer the QLQ-LC13, WHOQOL BREF, L.H. Garkavi adaptation self-assessment questionnaire and give blood for laboratory analysis of neutrophil function assessment.

Results:

Against the background of treatment with a Pearson coefficient of 0.978 (p0.001) according to the QLQ-LC13 test, the quality of life improved, which was maintained in the majority during the year and by the end of the trial was higher than before the start r=0.902 (p0.001). Also WHOQOL BREF scale r=0.951. spontaneous neutrophil membrane damage was significantly reduced r=0.902 (p0.001. Proportion of leukocytes with active mitochondrial conglomerate increased. And detected in 70% of cases the disturbance in Phagocytic activity of neutrophils (NST-test) and Examination of macrophage activity (latex test) after the course recovered, but in a year were below the norm.

Conclusions: Use of the selected treatment regimen improves quality of life in patients with lung cancer during the course and remains significantly higher after one year of follow-up. The quality of immunity is also improved. This qualitative analysis of neutrophil function can be used to personalize the prescribed regenerative therapy.

Future directions in Immuno-Oncology

Probing Novel Tumor-associated Antigen Presentation Inhibitors for Immunotherapy of Cancer

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Although the success of immune-checkpoint blockade has revolutionized cancer treatment, a number of tumors do not respond or develop resistance, especially in immunologically cold tumors. A potential mode of resistance is immune evasion of T-cell immunity involving myeloid-related dysregulation. To map such mechanisms of resistance we developed functional screening strategies to identify key regulators for antigen presentation (AP) as well as innate sensing. We built a comprehensive regulatory network for MHC-I modulation and defined a membrane-associated AP inhibitory axis involving SUSD6, TMEM127 and WWP2, as broadly applicable therapeutic targets for both leukemia and solid cancers. Our findings will facilitate our understanding in the tumor immune evasion mechanisms and foster the development of future antigen presentation inhibitor-guided immunotherapies for cancer and beyond.

https://pubmed.ncbi.nlm.nih.gov/37557169/

Future directions in Immuno-Oncology

MitoTam: A Promising Strategy for Immunogenic Cancer Treatment Revealed in RCC and Beyond

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Our MitoTam-01 study suggests a novel strategy for the treatment of RCC and other immunogenic tumors by targeting mitochondria in combination with ICIs. As a novel investigational drug, MitoTam has an excellent safety profile. Our results have direct clinical implications for the design of rational combination regimens in a prospective phase 2 trial in RCC and PDAC.

Our research in preclinical RCC models suggested at least an additive effect of MitoTam (2 mg/kg) with PD-L1 (400 µg/mouse) on tumour growth and CD8+ T cells and significantly improved survival compared to monotherapy. Neither CD8+ T cell infiltration nor their activity were affected by MitoTam. In addition, MitoTam did not affect PD-L1 levels in tumours from mice with syngeneic carcinomas. or in human patients, as later shown in the clinical trial. Our preclinical findings were the basis for the initial phase 1/1b monotherapy trial, which indeed showed an 83% (5/6) clinical benefit rate in RCC patients. All six patients were men with a median age of 69 years who had received at least three lines of palliative systemic therapy and had progressive disease prior to study entry. The histological subtype was consistent with clear cell RCC in the five responders and clear cell carcinoma with sarcomatoid features in the non-responder.

We believe our results would support a meaningful phase 2 trial of combination therapy in RCC or PDAC.

Selecting patients for treatment with immunotherapy

Molecular Ultrasound Imaging of Endothelial PD-L1 Expression as an Imaging Biomarker of Response to Immune Checkpoint Inhibitors in a Murine Colon Carcinoma Model

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Background: Tumor endothelial cells (ECs) can act to reduce tumor immunogenicity through overexpression of immunosuppressive molecules that block immune cell infiltration. Imaging EC anti-immune markers could provide additional real-time and bedside support to clinicians when selecting patients for immunotherapy.

Objective: Our objective is to introduce molecular ultrasound imaging (MUI) as a bedside real-time non-invasive tool to profile EC immunogenicity. Here, we developed MUI of EC expressing PD-L1 and evaluated MUI's potential to select responders to ICI therapies.

Methods: We first confirmed the in vitro adhesion of our MUI microbubble targeted contrast agent to EC PD-L1 through modulation of murine MS-1 EC PD-L1 using interferon-γ (IFN-γ) or Rapamycin. We then used target-ready lipid shelled microbubbles to image the expression of PD-L1 on ECs in CT26 tumors implanted in the lower flank of immunocompetent Balb/c mice. All mice were imaged at baseline, and longitudinally following either anti-PD-L1 or pro-PD-L1 IFN-γ therapy. Imaging was carried out using isotype (MUIiso) and PD-L1-targeted (MUIpdl1) microbubbles using the Visualsonics Vevo2100 ultrasound system. The differential targeted enhancement (dTE) MUI quantified parameter in arbitrary units (a.u.) was used to measure PD-L1 expression.

Discussion/Conclusion: In vitro experiments confirmed microbubble binding to PD-L1 following modulation using IFN-γ; 5-fold increased expression of PD-L1 was confirmed with FACS analyses. An elevated dTE signal in animals imaged with MUIpdl1 (~7.4 a.u,) vs. MUIiso (~2.7 a.u.) was observed. There was also a significant difference in dTE between antibody-blocked and IFN-γ induced groups. Finally, we observed evidence that MUIpdl1 can predict response to ICI therapy. In summary, MUI of EC PD-L1 and other immune markers could provide additional insight to clinicians to help select patients for immunotherapy.

Selecting patients for treatment with immunotherapy

Glycosphingolipids-Based Stratification of Paediatric Brain Tumours Prioritizes Group 4 Medulloblastoma And PFA Ependymoma For Anti-GD2 Based immunotherapy

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Background

Brain tumors are the most common type of solid tumor in children. Genomic and transcriptome-guided therapies have not improved the survival for patients with less prognostically favorable tumor subtypes, such as diffuse midline glioma (DMG), Medulloblastoma (MB) and Posteriora Fossa A Ependymoma (PFA-E). Emerging therapeutic approaches are considering gangliosides (GG) as a potential target for antitumor therapy. GG are amphiphilic glycosphingolipids (GSL) which carry N-acetylneuraminic acid residues, and play an important role in the maintenance of the nervous system. Their composition changes significantly during brain development and in cancer. The deregulation of GSL synthesis can lead to the accumulation of the GG GD2, a target for CAR-T cells and monoclonal antibodies therapies. GD2 is highly expressed in Neurobalstoma (NB), which are particularly sensitive to anti-GD2 therapies.

Objectives

To identify brain tumors subgroups with high expression of GD2

Methods

GD2 expression was analyzed by HPLC-ESI-MS2 in 12 MB, 10 Ependymoma and 2 DMG and compared to the expression in 15 NB.

Results

PFA-E had the highest GD2 expression, comparable to a NB. MB Group 4 and DMG had a similar level of GD2 expression but lower than PFA-E. Group 3 MB and SHH had a rather low or no expression of GD2. WNT MB did not express GD2.

Conclusion

GD2 expression is heterogeneous within the same tumor entity and quantification is required to identify subgroups with the highest expression. These findings will support basket clinical study programs with anti-GD2 based therapies for pediatric patients.

The current drugs available in immunotherapy (and combinations)

The role of nivolumab in palliative care for Head and Neck tumors: Real-world evidence from a Comprehensive Oncology Center

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Introduction: Head and Neck Cancer (HNC) is a significant and prevalent condition, with prognosis hinging on initial staging and treatment response. Immunotherapy has emerged as a pivotal approach in the palliative management of these tumors.

Objectives: To assess the clinical outcomes of palliative treatment with nivolumab in a cohort treated at a Comprehensive Oncology Center.

Methods: This retrospective observational cohort study included adult patients with recurrent/metastatic head and neck cancer who underwent treatment with nivolumab.

Results: The study encompassed 96 patients (88 men, 8 women) with a median age of 57 years. Predominant tumor sites were the oral cavity and hypopharynx (29.2% each), and 83.3% of patients were diagnosed at stage IV. Among the total population, 64.6% underwent radical treatment, most frequently receiving chemoradiotherapy (35.5%) or surgery followed by chemoradiotherapy (25.8%). Recurrence of disease was observed in over 50% of patients treated with a radical intent. Nivolumab was administered as first-line palliative treatment in 29 patients (30.2%) and as second-line treatment in 51%. Twenty-five patients experienced nivolumab-related toxicity, with 16% graded as severe (grade 3). Treatment discontinuation occurred in 82 patients, predominantly due to disease progression, but 6 cases involved suspension due to toxicity. The median overall survival was 7 months, with a 12-month survival rate of 28.8%.

Conclusions:
Nivolumab demonstrates benefit in a palliative context for patients with HNC, aiming to impact their survival with a good safety profile.

The current drugs available in immunotherapy (and combinations)

Classic Hodgkin Lymphoma- mixed cellularity- case report

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Abstract:

• Objectives: Follow-up of the clinical evolution and response to treatment of a 27-year-old patient with Hodgkin's Lymphoma- histological subtype mixed cellularity- chemorefractory.

• Material and method: Retrospective study in which was analysed the response of a refractory patient to different lines of chemotherapy.

• Outcomes: The results showed the evolution of the patient's disease with an initial complete response, with a relapse 18 months after the first line of chemotherapy, refractory to multiple subsequent treatments, but with a favorable response to the new type of treatment.

• Conclusions: Diagnosis and evaluation of treatment response in one patient with Hodgkin's Lymphoma refractory to multiple chemotherapy lines, with good evolution after FEAM-ASCT.

The current drugs available in immunotherapy (and combinations)

IFNg-induced changes in mitochondrial metabolism as a novel strategy for pancreatic cancer therapy

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Backgroung: Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease with no effective therapy. PDAC tumors show repression of anti-cancer immunity in their microenvironment. Immune checkpoint inhibitors can stimulate anti-cancer immune defenses via activation of T-lymphocytes producing IFNg, which was shown to induce cell death in malignant cells. Despite the well-established mechanism of IFNg-induced cell death, its effect on mitochondria and cellular metabolism remains elusive.

Objectives: Our goal was to study the effect of IFNg on regulation of mitochondrial biogenesis and its contribution to cell death.

Methods: C57BL6 mice grafted with KPC-1 tumor cells were treated twice per week by intraperitoneal administration with an anti-PD-1 (12ng/g), MitoTam (4mg/kg) or their combination.

Results: We show that IFNg or IFNg-producing immunotherapy represented by anti-PD-1 immune checkpoint inhibitor blocks aerobic glycolysis, prevents upregulation of a glucose transporter and lactate production. These changes in metabolism make pancreatic tumors more susceptible to OXPHOS inhibition by MitoTam, a new potential anti-cancer agent (Phase 1/1b clinical trial) that selectively targets highly polarized mitochondria of malignant cell, where induces electrochemical imbalance resulting in cell death. This combined therapy shows stabilization or rejection of tumors and significantly prolonged survival in all tested animals compared to immunotherapy or MitoTam alone.

Conclusion: Our study suggests a novel promising strategy for treatment of PDAC using immune checkpoint inhibitors and simultaneous targeting of mitochondrial metabolism.

The current drugs available in immunotherapy (and combinations)

Validation of Radiosensitization Therapy Using Gut Microbiota: Enhancing Radiotherapy Efficacy with Strains Regulating ROS and Apoptosis Markers in a Mouse Model, Including Radiation-Resistant Skin Cancer

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Title: Validation of Radiosensitization Therapy Using Gut Microbiota: Enhancing Radiotherapy Efficacy with Strains Regulating ROS and Apoptosis Markers in a Mouse Model, Including Radiation-Resistant Skin Cancer

Abstract: In this comprehensive investigation, we delve into the promising realm of utilizing gut microbiota to potentiate the effectiveness of radiotherapy. Our research focuses on the validation of radiosensitization therapy, employing specific bacterial strains adept at modulating reactive oxygen species (ROS) and apoptosis markers. Through a meticulous series of experiments in a mouse model, we have substantiated the remarkable enhancement in the therapeutic potential of radiotherapy. Furthermore, our findings extend to a particularly challenging scenario, as we have successfully demonstrated the augmentation of radiotherapy efficacy in a mouse model of radiation-resistant skin cancer. This study offers compelling evidence for the potential integration of gut microbiota-based interventions to optimize radiotherapy outcomes, especially in cases with inherent resistance.

Toxicity management of Immunotherapy drugs

Evaluation of immune-related endocrine toxicity of checkpoint inhibitors in clinical practice

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Relevance: The invention of new class of immune checkpoint inhibitors (ICTs) has significantly changed cancer treatment around the world. However, about 10% of patients receive immune-related side effects during ICIs therapy.

The purpose of this study is estimation of adverse effects onthyroid gland in patients with various malignant tumorsreceiving ICTs in the first and following lines of therapy.

Methods: Anamnestic, laboratory and instrumental research methods were used. Laboratory analysis included measurement of thyroid hormones in the blood. Analysis included 55 patients aged 24 to 79 years (mean age 55.9) receiving Pembrolizumab and Nivolumab in the period from May 2019 to May 2021.

The results of the study showed that the frequency of immune-related thyroiditis was 29%. The onset of thyroid dysfunction was diagnosed during the first 12-16 weeks of therapy and started with hyperthyroidism in the background of thyroid destruction, then progressed to persistent hypothyroidism within 1-3 months. The main difference was the time until the end of the AE: a transient and rapid course was noted in nivolumab (the average time was 38 versus 48 weeks). All patients continued ICIs after iAE registration.

Conclusion: it was shown that the safety of ICIs using in real practice doesn't differ from global practice. The frequency and spectrum of adverse events didn't depend on tumor localization. Early diagnosis of thyroid gland involvement using laboratory analysis is necessary for optimal and effective treatment. Knowledge of the timing of adverse effects development helps to timely diagnose, correct complications and continue effective therapy.

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Toxicity management of Immunotherapy drugs

Outcomes Of Immune-related Adverse Events In Patients Requiring Hospitalization In A Single UK Cancer Centre

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Background

Immune checkpoint inhibitors (ICIs) revolutionised cancer treatment, but they cause broad spectrum adverse events with different grades of severity and outcomes.

Objectives

Our aim was to report the incidence of patients required hospitalization for toxicity management and improve outcomes.

Methods

We collected retrospective data at the Cancer Centre of St George's University Hospitals NHS Foundation Trust to identify and characterise patients who were hospitalized due to immune-related adverse events (irAEs) between January 2021 and December 2022.

Results

29% (41/139) patients with an irAE required hospital admission for toxicity management. Of those, 29% had grade (G)2, 41% G3, 30% G4 toxicities. 49% had monotherapy, 34% dual immunotherapy and 17% ICIs in combination with chemotherapy/TKIs. 39% suffered from coliits followed by pneumonitis (22%) and hepatitis/cholangitis (17%). Less common were skin toxicity (7%), hypophysitis (5%), myocarditis (5%), encephalitis (2%), uveitis (2%). 95% received steroids. Of those, 13% required immune modulatory agents. Mean duration of hospital stay was 14 days and of immunosuppressive treatment 71 days . On follow up, immunotherapy discontinued in 65% patients, 17% had complete resolution of their irAE and continued immunotherapy. 12% deaths were reported -7% pneumonitis or colitis and 5% from their primary cancer.

Conclusion

irAEs can be severe and complex, requiring hospitalization for intense treatment with impact on resources and staff. Outcomes can vary but a significant number of patients had to discontinue their treatment and some of them died. Local management pathways and multidisciplinary meetings are recommended to enable early identification and intervention to achieve better response and complete recovery.

Evaluating response in Immunotherapy

Immunotherapy Related Pneumonitis: 5 Cases

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Immunotherapy has emerged as an important component of the treatment of patients with advanced cancer in recent years. Other than the well-known side effects such as fever, fatigue and diarrhea during treatment, pneumonitis due to immunotherapy is a lesser-known condition that is challenging to diagnose and carries significant morbidity and mortality risks.

In this presentation, we aimed to present five different cases of immunotherapy-induced pneumonitis. These cases involve two patients with non-small cell lung cancer receiving pembrolizumab, a mesothelioma patient receiving nivolumab, a patient with renal cell carcinoma receiving nivolumab and a patient with lung adenocarcinoma patient receiving nivolumab, all of whom applied to our clinic.

The hallmark symptoms in these cases were the presence of a persistent cough and shortness of breath. Hypoxic respiratory failure was also present. Notably, laboratory results exhibited elevated C-reactive protein (CRP) levels, while procalcitonin levels remained within normal ranges. Thorax computed tomography scans revealed bilateral ground-glass appearance and pleural effusion.

All patients were treated with corticosteroids and clinical and radiological improvement was achieved. Although pneumonia caused by immunotherapy is rare, it causes serious mortality and morbidity, its potential for severe morbidity and mortality underscores the need for vigilance regarding respiratory

symptoms during the follow-up of these patients. Early diagnosis and prompt initiation of treatment are
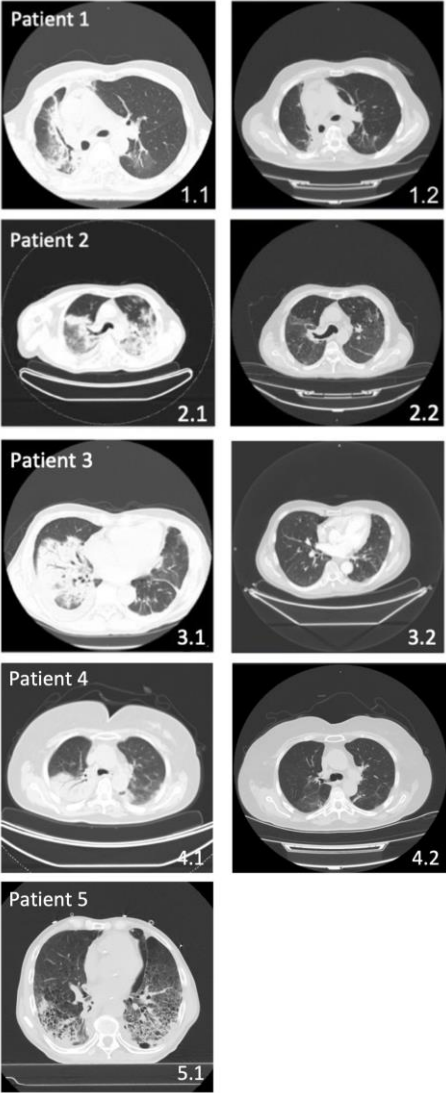
paramount to prevent the progression to respiratory failure.