IMMUNOTHERAPY

NOVEL TECHNOLOGIES AND BIOMARKERS FOR PERSONALIZED CANCER IMMUNOTHERAPY: MANAGING EFFICACY AND TOXICITY.

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Cancer immunotherapies are rapidly changing the treatment paradigms and therapeutic landscape for cancer patients. Despite the current successes, still a remarkable rate of patients do not respond to immunotherapy and, when they do, often experience clinical relevant toxicities. Thus, there is a growing need to identify predictive and prognostic biomarkers that enhance our understanding of the mechanisms underlying efficacy and toxicity before clinical signs will emerge. In particular, checkpoint inhibitors has been associated to unique toxicities, termed as immune-related adverse events (irAEs). Moreover, even the administration of antitumor monoclonal antibodies or Chimeric Antigen Receptors (CAR) T cells parallel important early promising results with the risk of potent side effects. Such toxicities are often associated to Cytokine release syndrome (CRS), a systemic inflammatory response leading to widespread reversible organ dysfunction. Clinical experience has shown that these immune events, when early recognized, are mostly reversible otherwise they can evoke severe or even life-threatening situations. Thus, researchers in the Immunotherapy WG will attempt to discover novel biological markers able to early predict toxic events in patients treated with immunotherapy approaches. The investigations, while carried within different immunotherapy protocols in place at each Institution, would generate data of transversal interest and provide investigational platforms accessible to the other components of the WG. In particular, an algorithm will be created considering that in the majority of the cases, irAEs arise from general immunologic enhancement, and temporary immunosuppression often used to modulate toxicity. The enrolled patients will be monitored by “omic” platform before and during immunotherapy for T cell activation status, myeloid cell expansion, plasma level of cytokines, microbiome, circulating miRNA, DNA, exosomes and metabolites. Several guidelines will be developed for the toxicity management as well as algorithms to help medical doctors and patients to better manage the novel side effects coming from innovative immunotherapy protocols.

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Abstract

IMMUNOTHERAPY

DEVELOPMENT OF A VIRTUAL BIOBANK FOR PATIENTS DEEPLY CHARACTERIZED IN THE IMMUNOTHERAPY WG-ACC.

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The activities of the Immunotherapy WG is expected to be of high impact in the clinical practice of patients affected by neoplasia and treated with several different immunotherapy approaches. Indeed, the major aim of the WG is to provide important information about the efficacy and adverse effects of immunotherapy interventions by controlling the variables that could affect the results of the treatment. Thus, data collected in a transversal manner between the WGs represent a tremendous opportunity to collect and storage information for further studies. Here the need to also create a biorepository that could accepts, processes, stores and distributes biospecimens of the enrolled patients and associated their deep characterization. The data associated with stored biospecimens will be increased in complexity from basics, such as date of collection and the diagnosis, to extensive information sets encompassing many aspects of patient phenotype, and extending into genetic, proteomic, and “immune-omics” information. Since the complexity of the proposed biobank that necessarily will involve several different IRCCS in ACC, we propose the model of a Virtual biobank to assist investigators locate biospecimens for testing and data mining from multiple biobanks in dispersed locations. For such virtual biobanks, researchers will have access using specialized software or web-portals designed to connect biobanks and investigators throughout the system in worldwide-fashion. The optimum collection, processing, storage, tracking and shipment of biospecimens are key to the outcome of a Virtual biobank, as well as compliance with the regulatory requirements, that include consent documentation of a complex structure, considering the serious ethical and legal issues associated with the area of genetics and bio-banking. In summary, we propose to develop a virtual biobank as an electronic database of biological specimens regardless of where the actual specimens are stored, and other related information produced in several different WGs of ACC, including the Immunotherapy-WG.

ACC Annual Meeting
3-5 October 2017 - ROME

Abstract

IMMUNOTHERAPY

NEW GENETIC STRATEGIES FOR REDUCING CHIMERIC ANTIGEN RECEPTOR TREATMENT-RELATED TOXICITY: INHIBITION OF IL-6 PATHWAY.

Iolanda Boffa, Domenico Orlando, Matilde Sinibaldi, Simona Caruso, Marika Guercio, Vinicia A. Polito, Rosaria Cristantielli, Claudia M. Arnone, Tamascia Belardinilli, Stefano De Cecca, Gerrit A. Weber, Francesca Del Bufalo, Ignazio Caruana, Biagio De Angelis, Franco Locatelli and Concetta Quintarelli.

IRCCS Ospedale Pediatrico Bambino Gesù, Dept. of Onco-haematology, Unit of Cell and Gene Therapy for peadiatric tumors, Rome Italy.

BACKGROUND:

T-cell-engaging immunotherapies are new emerging approaches to treat patients with cancer. Chimeric antigen receptor (CAR) T cells targeting CD19 have been shown a durable remissions rates in the relapsed, refractory ALL setting, despite a notable toxicity. Cytokine release syndrome (CRS), a systemic inflammatory response is the most significant treatment-related toxicity associated to CAR T cell treatment, characterized by fever and malaise, that can lead to widespread reversible organ dysfunction. IL-6 is a prominent cytokine in CRS, related to T-cell activation and detailed studies of the resultant immune activation produced by these novel therapies have led to more targeted approaches, to provide superior toxicity control without compromising efficacy. IL-6 can signal via membrane-bound or soluble forms of the IL-6R (sIL-6R) and in both cases IL-6/IL-6R complex formation triggers the recruitment of ubiquitous signal-transducing β-receptor gp130. It has become clear that sIL-6R signaling accounts for the IL6 pro-inflammatory properties and inhibition of this pathway is sufficient and sometimes even superior compared to the total blockade of IL-6. Soluble forms of gp130 act as natural inhibitors of trans-signaling sIL-6R-mediated, and its inhibition has been shown to be favorable in numerous inflammatory diseases.

PROPOSAL:

The purpose of the project is to implement the coding sequence of a specific CAR with a sequence for the soluble form of the gp130 protein, to test the CAR's ability to keep under control the development of a CRS, counteracting IL-6 produced by it same.

RESEARCH METHODOLOGY:

We will develop short-term and long-term cytotoxic assays to test whether genetic modification compromises CAR's killing activity. Then we will analyze the antitumor activity in mouse models, and eventually CAR toxicity. Finally we will proceed with CAR infusion in a CRS mouse model.

ACC Annual Meeting
3-5 October 2017 - ROME

Abstract

IMMUNOTHERAPY

LONG-PERSISTENT NK CELLS TO IMPROVE ADOPTIVE CELL THERAPY IN PATIENTS WITH CANCERS.

Simona Caruso, Iolanda Boffa, Domenico Orlando, Matilde Sinibaldi, Marika Guercio, Vinicia A. Polito, Rosaria Cristantielli, Claudia M. Arnone, Tamascia Belardinilli, Stefano De Cecca, Gerrit A. Weber, Francesca Del Bufalo, Ignazio Caruana, Biagio De Angelis, Franco Locatelli and Concetta Quintarelli.

IRCCS Ospedale Pediatrico Bambino Gesù, Dept. of Onco-haematology, Unit of Cell and Gene Therapy for peadiatric tumors, Rome Italy.

BACKGROUND:
Cell-based therapies are becoming more and more important for the treatment of disease progression in cancer and Natural killer (NK) cells are currently getting into the focus of interest as suitable and powerful effector cells for cellular therapy of cancer. NK cells spontaneously kill cells deemed to be dangerous to the host (cancer, foreign or virus-infected cells). It are usually defined as CD3−CD56+ cells in humans, they represent 5–15% of circulating lymphocytes and can be categorized into subpopulations with different maturation statuses and functional specificities. Today, NK cells are of great clinical interest because they are not responsible for the GvHD. However, ex vivo expansion of NK cells could lead to proliferation-induced senescence. NANOG is a master regulator of embryonic stem cell pluripotency involved in the cell cycle regulation. For example, NANOG induces HDAC1-driven epigenetic silencing of cell-cycle inhibitors CDKN2D and CDKN1B induced stem-like features.

PROPOSAL:
To contrast NK proliferation-induced senescence, we propose a system to genetically modify NK cells with a pluripotent gene (i.e. NANOG) in order to proliferation combined with a suicide gene to improve the safety of the approach.

RESEARCH METHODOLOGY:
Our experiments will be conducted in vitro and in vivo to test the ability of NANOG-NK cells to exert anti-tumor activity, proliferate upon stimulation and to be eliminate after activation of the suicide gene. The suicide gene activity will prevent the development of toxic effects associated with uncontrolled proliferation.

ACC Annual Meeting
3-5 October 2017 - ROME

Abstract

IMMUNOTHERAPY

VECTOR-GUIDED INSERTION TO IMPROVE ADOPTIVE T CELL THERAPY BASED ON THE GENETICALLY MODIFICATION OF T CELLS WITH TUMOR-PEPTIDE SPECIFIC TCR.

Domenico Orlando, Marika Guercio, Matilde Sinibaldi, Iolanda Boffa, Vinicia A. Polito, Rosaria Cristantielli, Claudia M. Arnone, Tamascia Belardinilli, Simona Caruso, Stefano De Cecca, Gerrit A. Weber, Francesca Del Bufalo, Ignazio Caruana, Biagio De Angelis, Franco Locatelli and Concetta Quintarelli.

IRCCS Ospedale Pediatrico Bambino Gesù, Dept. of Onco-haematology, Unit of Cell and Gene Therapy for peadiatric tumors, Rome Italy.

BACKGROUND:
T cells genetically modified to express antigen specific T-cell receptors (TCR) are a novel HLA-restricted immunotherapy tool. In particular, Cancer Testis Antigens (CTAs) are relevant cytoplasmic tumor proteins, with an overall restricted expression in tumor tissues of different origin. Among all the well-known CTAs, we focused our interest on the Preferential Antigen Expressed in Melanoma (PRAME), since its expression has been described in many solid and hematologic cancers. Results using PRAME-specific-TCR T cells genetically modified by retroviral transduction of exogenous PRAME-TCR reveal excellent cytotoxic activity and tumor growth control in vitro and in vivo. However, the transduction efficiency obtained is decreased because of the mispairing with the endogenous TCR a/b chains, decreasing the treatment efficacy and T cells persistence and activity.

PROPOSAL:
To overcome this issue and obtain a pure PRAME-specific-TCR T cells population, we hypothesize to guide the insertion of the retroviral vector in the TCR alfa locus. This approach will solve the problem of the TCR mispairing but also of the possible genotoxicity of the approach related to the insertion of the construct in multiple DNA sites, leading to an increased possibility of gene dysregulation.

RESEARCH METHODOLOGY:
We will subclone a retroviral variant in which LTRs will be modified to guide the insertion of the virus in the coding sequence of the costant region of the TCR alfa. We will test the specificity of the integration by vector integration analysis and the improving of the approach in terms of TCR pairing. After a starting proof-of-concept, this approach may be applied to other adoptive gene therapy approaches, including several different anti-tumor specific TCRs or Chimeric Antigen Receptors (CAR).

ACC Annual Meeting
3-5 October 2017 - ROME

Abstract

IMMUNOTHERAPY

BUILDING UP IPS/IS: A NOVEL WORKFLOW TO PREDICT RESPONSE TO IMMUNO-CHECKPOINT INHIBITORS THROUGH THE INTEGRATION OF MULTIPLE BIOMARKERS.

Matteo Pallocca, Fabio Palombo, Michele Milella, Frauke Goeman, Francesca De Nicola, Paolo Visca, Maurizio Fanciulli, Paola Nisticò, Gennaro Ciliberto.

Regina Elena National Cancer Institute.

BACKGROUND:
There are no available biomarkers able to accurately predict the response to Immuno-Checkpoint Inhibitors. Recently, the ImmunoPhenoScore (IPS), a transcriptional-based score was proposed as a possible in silico predictor of ICI response. Moreover, the Immunoscore™ (IS), a high-tech ImmunoHistoChemistry assay based on qualitative and quantitative measure of tissue samples, was defined as a prognostic factor in colorectal cancer. Here we propose an integrated Transcriptional/IHC approach in order to predict treatment response and eligibility for solid tumor patients.

METHODS:
Several public RNA-seq datasets were fetched and patients profiled based on ImmunoPhenoScores. Scores distributions were compared with current ICI eligibility guidelines such as PD-L1 expression.

RESULTS:
High IPS Ratio computed on public datasets showed an impressive similarity to overall responder ratio found in clinical trials. We found no significant correlation between IPS and PD-L1 expression, suggesting the IPS as a valuable marker in order to predict response in PD-L1-negative patients.

CONCLUSIONS:
Several possible limitations and aspects of the IPS model are herein discussed. In order to pair the IPS measurement with a structural characterization of the immune infiltrates, we plan to develop an integrated model based on both the Immunoscore™ and the ImmunoPhenoScore. The integrated IPS/IS model could serve as a valuable prediction method for ICI response in several solid tumors.

ACC Annual Meeting
3-5 October 2017 - ROME

Abstract

IMMUNOTHERAPY

PD1+ CELLS ACCUMULATE IN HIGHLY METABOLIC TUMORS IN PANCREATIC ADENOCARCINOMA.

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The pathways that regulate immune cell function and metabolism in cancer are tightly linked and metabolic dysfunction can severely impact on the efficacy of the anti-tumor immune response. Here we investigated the association of tumor metabolic activity and immune infiltration in Pancreatic Ductal Adenocarcinoma (PDAC), a microenvironment characterized by profound metabolic deregulations and a strong immunosuppression. Pancreatic juice from 30 patients with pancreatic pathologies surgically operated at Humanitas Clinical and Research Center was analyzed by Nuclear Magnetic Resonance to investigate the metabolomics. Immunohistochemical evaluation of metabolic and immune infiltrate markers was performed in a cohort of 40 PDAC patients. Tumor glycolysis was targeted in a murine PDAC cell line by knocking down the gene encoding for Phosphofructokinase (PFKKD). Glucose metabolism and tumor infiltrating leukocytes (TILs) were analyzed by multicolor flow cytometry comparing PFKKD tumors and control tumors. Metabolomic analysis of pancreatic juice indicates a metabolic signature that discriminates among ductal pancreatic tumors and other pancreatic pathologies. In human PDAC sections, a higher density of PD1-TILs correlates with tumors expressing higher levels of GLUT-1, suggesting that PD1-TILs accumulate in highly glycolytic tumors. In a preclinical model, PDAC tumors obtained from cell lines with different metabolic consumptions were differently infiltrated by T cells, with PD1+ CD8-TILs accumulating in highly metabolic tumors. Both in human and preclinical models of PDAC, tumor cell glycolysis impacts on the type of immune cell infiltration, underlining a key interplay between glucose metabolism and the antitumor immune response.

ACC Annual Meeting
3-5 October 2017 - ROME

Abstract

IMMUNOTHERAPY

IL-1R8: A NOVEL CHECKPOINT REGULATING ANTI-TUMOR AND ANTI-VIRAL ACTIVITY OF NK CELLS.

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IL-1R8 is an Interleukin-1 receptor family member that acts as a negative regulator of IL-1 family receptor and TLR signaling. Both murine and human NK cells express high levels of IL-1R8 but its functional role in this cell type has not been described so far.
Expression analysis showed that IL-1R8 was acquired during differentiation in human and murine NK cells. IL-1R8 deficiency in the mouse was associated with higher frequency of mature NK cells, higher levels of activating NK cell receptors and increased Interferon-γ (IFN-γ), GranzymeB and Fas ligand expression and degranulation. IL-18, which is a key regulator of NK cell activities and can be targeted by IL-1R8, was responsible for this phenotype. Indeed, IL-1R8 regulated IL-18-MyD88 axis during NK cell differentiation and IL-18-dependent activation of mTOR and JNK pathways increased in IL-1R8-deficient NK cells.
To assess the role of IL-1R8 in NK cells in pathology, we used models of MCA-induced lung metastasis, colon cancer-derived liver metastasis and DEN-induced hepatocellular carcinoma. The number and dimension of liver and lung metastasis and the liver disease severity were significantly reduced in Il1r8-/- mice. The depletion of NK cells in these models totally abrogated the protection observed in Il1r8-/- mice. Finally, we investigated the role of IL-1R8 in NK cell antiviral activity, in a model of MCMV infection. Il1r8-/- mice controlled the virus more efficiently in the liver and the protection was associated with enhanced NK cell degranulation and IFN-γ production. The adoptive transfer of Il1r8-/- NK cells conferred protection in both metastasis and viral infection models.
IL-1R8 plays a non-redundant role in the regulation of NK cell development and effector functions by tuning IL-18-dependent activities. IL-1R8 therefore emerges as a crucial regulator of NK cell antitumoral and antiviral potential.