mycophenolate mofetil, etanercept, extracorporeal photopheresis, anti-thymocyte globulin, alpha-1 antitrypsin, mesenchymal stromal cells, everolimus, or sirolimus can be used.

Clinical manifestations of cGVHD may be restricted to a single organ or widespread. The primary manifestations are skin involvement resembling lichen planus or cutaneous scleroderma, dry oral mucosa, ulcerations and sclerosis of the gastrointestinal tract, elevated serum bilirubin, and bronchiolitis obliterans. First-line treatment of cGVHD consists of steroids. For patients with mild cGVHD, localized/topical treatment can be preferred rather than systemic therapy. For initial treatment of moderate or severe cGVHD, systemic treatment with prednisone or methylprednisone at an initial dose of 1 mg/kg body weight/day should be used. The addition of azathioprine, mycophenolate mofetil, cyclosporine, thalidomide, or hydroxychloroquine to prednisone did not improve the response rate or other end-points in randomized trials. If symptoms progress during the first 4 weeks of first-line therapy or there is no improvement in symptoms within 8–12 weeks, second-line therapy should be initiated. For steroid refractory cGVHD patients ruxolitinib can be added to prednisone. Non-pharmacologic therapies such as extracorporeal phopheresis (ECP) has the advantage of being non-immunesuppressive. An immunosuppressive drug can be added to prednisone such as a calcineurin inhibitor or mycophenolate mofetil, but none shown to be effective. İbrutinib which is an inhibitor of Bruton's tyrosine kinase (BTK) has activity against cGVHD.

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Sp08

EVOLUTION OF THE PLEURAL SECRETOME ASSOCIATED WITH PLEURAL METASTASIS

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Malignant pleural effusions (MPE) are characterized by a distinct and complex secretome that varies little between malignancies. To understand the origin and functional significance, we measured 40 cytokines and chemokines in 356 MPE (mainly breast cancer, lung cancer and esophageal cancer), and compared them to benign effusions (n=18) and normal, non-effusate pleural fluid (n=27).

Pleural effusions were collected during therapeutic drainage. Normal (non-effusate) pleural fluid was aspirated during minimally invasive cardiac surgery. Samples were clarified by centrifugation and stored at -80°C until assay. Samples were analyzed with the Luminex platform, using the MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel - Premixed 38 Plex (Cat. No. HCYTMAG-60K-PX38), plus IL-6Rα (Cat. No. HANG2MAG-12K-01), and TGFβ (Cat. No. TGFBMAG-64K-01).

The baseline secretome in normal pleural fluid is dominated by IL-6Rα, CCL2, CXCL10, FGF2, TGFβ1 and CCL22. Effector cytokines (IFNα, IFNγ, CCL3, TNFα and TNFβ) and most stimulatory cytokines (GM-CSF, TGFα, G-CSF, IL-2, IL-5, IL-7, IL-9, IL-12p40, IL-12p70, IL-3) were absent in NPF.

Benign effusions, whether due to cardiac insufficiency or chronic inflammation (asbestosis without malignancy) resulted in a profound secretomic change, with statistically significant increases in IL-6, TGFβ1, GRO, IL-10 and IL-8, and decreases in FGF2 and IL-15.

All cytokines and chemokines present at elevated levels in benign effusions were also elevated in malignant effusions, with statistically significant increases in G-CSF, CX3CL1, GM-CSF, IFNγ, IL-1TNFα, IL1Rα, CCL4, VEGF, TNFβ, EGF, IFNα, IL-4 and IL-12p40, compared to benign pleural effusions.

Benign effusions can result from an imbalance between hydrostatic and oncotic forces or from inflammation. In both conditions our data indicate a dramatic and consistent change in the pleural environment dominated by IL-6, a highly pleotropic cytokine. When bound to sIL6-Rα, IL-6 induces pro-inflammatory trans-signaling that is markedly stronger than classic signaling and a potent driver of the epithelial to mesenchymal transition (EMT). Additionally, CXCL10, IL-8 and TGFβ1 are known to promote EMT, critical for the maintenance of the normal mesothelium, but dangerous when cancer cells reach the pleural environment, because EMT is associated with cell motility, invasion and therapy resistance.

It is unknown whether prior perturbation of the pleural environment is prerequisite to pleural metastasis, or alternatively, whether chance seeding of the pleura with metastatic tumor leads to secretomic changes similar to those seen benign effusions. In either case, the pleural environment is conditioned to promote tumor growth and inhibit anti-tumor immunity. The presence of cytokines such as VEGF and FGF2 in MPE further condition the pleural environment for tumor growth. The contained nature of the pleural space suggests that local interventions with protein therapeutics to block or augment key cytokines may alter this environment and render pleural metastases susceptible to chemo- or immunotherapy.

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Sp09

INTRAPLEURAL THERAPY TO DRIVE SYSTEMIC ANTI-TUMOR IMMUNITY

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Cancer metastatic to the pleura is uniformly fatal with a median survival of six months and quality of life that is diminished by dyspnea and discomfort. There is currently no curative treatment once metastatic disease has occurred. Current standard of care treatment for malignant pleural effusions (MPE) is exclusively palliative, consisting of drainage, followed by systemic therapy (chemotherapy, endocrine, or immunotherapy). Our institutional experience with systemic immune checkpoint blockers indicates a marginal

improvement in overall survival in a small subset of patients. Clearly, the incidence of MPE and lack of effective treatments has created an urgent unmet need to develop an effective treatment.

Our studies of the pleural secretome in non-small cell lung cancer and mesothelioma, as well as extensive secretomic data in MPE from other cancers, indicate that the IL-6/IL-6Rα axis is prominent in pleural effusions and drives the epithelial to mesenchymal transition (EMT). We have identified additional cytokines that are absent in normal pleural fluid but prominent in malignant effusions. We have also found that MPE T cells, removed from their environment, are capable of expansion in culture, polyfunctional cytokine response, and are cytolytic to autologous tumor. Because the pleural space is lined with mesothelial cells joined by tight junctions, we hypothesize that it acts as a cytokine-rich bioreactor which promotes EMT in cancer cells metastatic to the pleura, and redirects the abundant immune infiltrate to promote, rather than inhibit, tumor growth. We hypothesize that as a master cytokine, IL-6 and its soluble receptor drive this process. Therefore, local blockade of sIL-6Rα will alter the pleural cytokine milieu, inhibiting aggressive tumor behavior and promoting anti-tumor immune response. Once unleashed in the pleural space, tumor-specific T cells could be expected to migrate to the periphery through the draining lymphatics and respond to extra-pleural metastases. Further, based on our current data, we are confident that the 100 million MPE T cells that are routinely recovered during routine therapeutic MPE drainage can be expanded in culture for an adoptive cellular therapy product that is faster, better and cheaper than conventional solid-tumor derived culture-expanded tumor infiltrating lymphocytes (TIL).

What remains to be determined is whether blockade of dominant cytokines in MPE together with anti-PD-1/PD-L1 therapy can condition the pleural environment sufficiently to support and expand the existing anti-tumor responses.

Combining the knowledge derived from these studies we propose to devise a personalized combined treatment strategy that conditions the pleural environment without incurring systemic toxicities and facilitates local and systemic anti-tumor immune response.

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Sp10

CAR T-CELL

Francesco Saglio

Chimeric Antigen Receptor (CAR)-T cell therapy is emerging as one of the most powerful and promising therapeutictool for the treatment of malignat diseases. CAR-T cells are T-lymphocytes modified *in vitro* to harbor an artificial molecular construct (CAR) made by an extracellular domain consisting of a single-chain variable fragment (scFv) recognizing a specific tumor antigen joined to a transmembrane domain which is linked to the signaling unit CD3ζ and co-stimulatory units CD28 or 4-1BB of the T-cell receptor, making them capable to recognize and to kill tumor's cell in a HLA-independent manner. CAR T-cell therapy consists in the selection of patient's normal T-cells via leukapheresis, activation, transduction to express CARs using lentiviral or retroviral vectors, expansion of transduced cells and infusion of the final product back to the patient. After the CAR T-cells are infused back into the patient, the engineered cells proliferate, recognize and kill tumor cells bearing the specific antigen the CAR is directed against.

In recent years US Food and Drug Administration (FDA) and European Medicine Agency (EMA) approved CD19 CAR T-cells in patients affected by relapsed and refractory ALL under the age of 25 years, adult patients affected by Non-Hodgkin Lymphomas and more recently adult patients affected by Multiple Myeloma and this technology is moving from an experimental approach available for very selected patients treated in a small number of Centers to a standard-of-care therapy available almost worldwide.

The diffusion of commercially available CAR-T cells has increased the number of patients treated by this cell therapy products and has also permitted to confirm their safety and efficacy profile in the "real life".

The diffusion of this technology requires a re-definition of the role of all the other therapy options currently available including other forms of immuno-therapy as monoclonal antibodies, bi-specific monoclonal antibodies and, upon all, allogeneic hematopoietic stem cell transplantation (alloHSCT).

Until now data are limited, and the above-mentioned question is far from being answered but there are some observations derived from pivotal clinical trials that probably will help us in building future trials aimed to define this topic.

Another open question is represented by the persistence of these cells in the patients that is related to the definition of the need for patients responding to CAR-T cells to proceed to other therapies, especially to alloHSCT, to consolidate disease remission. Moreover CAR-T cells are characterized by some peculiar side effects as the Cytokines Release Syndrome or CNS toxicity that if are not properly detected and treated may lead to very severe consequences with a significant mortality rate.

Finally, some technological, practical and economical considerations need to be defined in order to extend the use of this technology worldwide, in respect to the other currently available therapies.

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Sp11

TARGETED THERAPY IN AML TREATMENT

Giovanni Martinelli

Prof. Martinelli will speak about new drugs in the treatment of acute leukemias, starting from mechanisms of actions of the compounds and explaining strategies for clinical research.

Venetoclax is a bcl-2 inhibitor that is entering in the therapy of AML. The use of venetoclax will be explored with particular attention to combination with purine and pyrimidine analogs and metabolism.