therapy directed against CD19, such as blinatumomab and Inotuzumab. Clinical response was evaluated 1 to 2 months after CAR-T cell administration. One ALL patient died of sepsis before evaluation and one NHL patient is still awaiting his evaluation. Of 36 evaluated ALL patients, 24 (67%) achieved measurable residual disease (MRD) negative CR, 6 (17%) MRD positive CR and 5 patients (14%) progressed. One ALL patient with an initial response was treated a second time with CAR-T, but did not respond. Of 52 evaluated NHL patients, 32 (62%) achieved an objective response, including 16 complete remissions and 16 partial responses. Twenty (38%) patients had disease progression.[1,2] Notably, we recently show that CD19 CAR T-cells were able to induce remission in a patient with CD19+ AML with t (8; 21)(q22;q22.1) that relapsed 6 months post allogeneic transplant and failed re-induction. On day 28 post CAR-T CD19 infusion BM aspiration disclosed normal hematopoiesis with no excess blasts, full donor chimerism and lack of t (8; 21) by FISH confirming clinical and molecular remission.[3] We also assessed kinetic of cell phenotype on PBMCs of the CAR-T treated patients using multiparametric flow cytometry. The manufactured CAR-T products ($n = 9$) were also subjected to immunophenotypic analysis in order to elucidate the mechanisms of CAR-T cell trafficking and activity. We observed increased immunosuppressive phenotype as well as induction of T cell senescence/exhaustion in non-responding compare to responding patients.[4]

REFERENCES

1. Itzhaki O, Jacoby E, Nissani A, Levi M, Nagler A, Kubi A, et al. Head-to-head comparison of in-house produced CD19 CAR-T cell in ALL and NHL patients. J Immunother Cancer. 2020;8(1):e000148, http://dx.doi.org/10.1136/jitc-2019-000148.
2. Danylesko I, Chowers G, Shouval R, Besser MJ, Jacoby E, Shimoni A, et al. Treatment with anti CD19 chimeric antigen receptor T cells after antibody-based immunotherapy in adults with acute lymphoblastic leukemia. Curr Res Transl Med. 2020;68(January (1)):17–22, http://dx.doi.org/10.1016/j.retram.2019.12.001. Epub 2019 Dec 25.
3. Danylesko I, Jacoby E, Yerushalmi R, Shem-Tov N, Besser MJ, Vernitsky H, et al. Remission of acute myeloid leukemia with t(8;21) following CD19 CAR T-cells. Immunotherapy. 2020;34(7):1939–42, http://dx.doi.org/10.1038/s41375-020-0719-y.
4. Beider K, Besser MJ, Schachter J, Grushchenko-Polaq AH, Voevoda-Dimenshtein V, Wolf I, et al. Upregulation of senescent/exhausted phenotype of CAR-T cells and induction of both Treg and myeloid suppressive cells correlate with reduced response to CAR T cell therapy in relapsed/refractory B cell malignancies. ASH. 2019.

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SP 14

Treatment of sickle cell crises

Salam Alkindi

Sickle cell disease (SCD) is an inherited disorder prevalent in Sub-Saharan Africa, Middle East and parts of India. Its characterized by repetitive episodes of vaso-occlusive (VOC) process leading to recurrent painful episodes, hemolytic anemia and predisposition to infection. Sickle cell crises varies and this what brings patients to hospital including VOC leading to recurrent painful episodes, or organ specific complications such as acute chest syndrome, stroke, splenic sequestration, and many skeletal complications. Although the prognosis of patients with SCD has improved, however still these events contributes to decrease quality of life and increased risk of death. Also unfortunately, progress on the management of these acute complications is slow, and tended to be supportive including vaccination, use of antibiotics prophylaxis and blood transfusions. Better understanding of pathophysiology of the disease has allowed more accelerated progress on preventing these complications and development of more focused pharmacological therapies. Hemoglobin polymerization is a primary triggering event in the pathophysiology of the disease, leading to the sickling process, this usually ignite an inflammatory process/tissue ischemia and increased adhesions. This understanding of the pathophysiology has allowed scientist to develop drugs that interfere with these processes such as Voxeletor & Hydroxyurea (interfere with polymerization-both approved by FDA), L-glutamine and Omega 3 (interfere with inflammatory process and oxidative stress) and crizanlizumab and Tinzaparin (works by inhibiting adhesion molecules). This will allow patients and physicians the freedom for a number of therapeutic interventions including development of combinations protocols. SCD is very complex and require a drug with multi-faceted action such as Hydroxyurea and this is of the limiting factors in the new recently approved drugs, limiting the patients who can benefit from each of them. Further progress is also seen in the area of bone marrow transplant (including alternative donor pool) and gene therapy/gene editing.

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SP 15

Secondary acute leukemia evolving from myeloproliferative neoplasm (MPN)

Tariq Mughal

The natural history of myeloproliferative neoplasms (MPNs), both Philadelphia-chromosome positive – [chronic myeloid leukemia(CML)] and negative – [essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF)] has been well documented but the mechanism underlying the apparently inexorable progression from an initial, rather indolent or chronic phase (CP) to advance phase, a term including accelerated phase (AP) and blast crisis (BC) remains obscure. Most patients with MPNs present in the indolent phase, during which myeloid progenitor numbers are greatly increased in the bone marrow and blood. This phase may continue for as little as one year or as long as 20 years or more, but eventually it transforms into acute leukaemia (BC), in which an increasing proportion of blast cells are found in the marrow and peripheral blood. The risk associated with the development of advanced-phase disease differs depending on the MPN subtype and is influenced by a number of factors such as duration of disease, clinical factors, the presence of

unique molecular genetic features, and in some cases, the therapeutic interventions. ET probably carries the lowest rate of transformation to acute myeloid leukaemia (AML), whereas MF may carry a relatively high risk; lymphoid transformation has been reported in rare cases. The risk of transformation in CML to BC in the *ABL1*-tyrosine kinase inhibitors (TKI) era appears to be quite low, <2% per annum. Transformed disease in general tends to be difficult to be managed and is associated with a poor prognosis. The best treatment strategy, therefore, remains the prevention of transformation. Allogeneic stem cell transplantation is currently the only treatment that has been observed to confer long-term benefit to a small minority of patients who qualify for it. In this presentation, I will address the evolving genetic landscape, translational research efforts and investigational therapies for transformed MPNs.

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SP 16

The new European leukemianet recommendations for treating CML

Rüdiger Hehlmann

Twenty-two years after the first patients with chronic myeloid leukemia (CML) were treated with the tyrosine kinase inhibitor (TKI) imatinib, outcome exceeds all expectations: the vast majority of CML patients have achieved normal life expectancy and some patients in sustained deep molecular remissions (DMR) may even be operationally cured in durable treatment-free remissions (TFR). However, some expectations remain unmet. Most patients are not yet cured and require life-long maintenance therapy. Also, progression to blast crisis still occurs in 5–7% of patients and remains a challenge. CML has not become the expected model disease for treating other leukemias or cancers, but the principle of elucidating the pathogenesis as a successful approach for cancer treatment has been impressively demonstrated in CML.

New insights have emerged from maturing long-term academic and commercial clinical trials regarding optimum management of CML. Velocity of response has unexpectedly proved less important than hitherto thought, does not predict survival, and is of unclear relevance for TFR. Serious and cumulative toxicity has been observed with TKI that had been expected to replace imatinib. Generic imatinib has become cost-effective first-line treatment in chronic phase despite chronic low-grade side-effects in many patients. Earlier recognition of CML end-phase by genetic assessment might improve prospects for blast crisis. Treatment discontinuation and TFR has become an important new treatment goal of CML. Duration of DMR (MR4, MR4.5) may be the best predictor of success. To reflect this new situation, the European LeukemiaNet has recently revised and updated its recommendations for treating CML. The presentation will focus on recent developments and on current evidence for treating CML in 2020.

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SP 17

Ex vivo activation of pleural T cells in pleural malignancies

Vera S. Donnenberg, James D. Luketich, Albert D. Donnenberg

Introduction: MPE are uniformly fatal. It is estimated that the incidence of MPE in the United States is more than 150,000 cases per year, making this a common terminal pathway for a variety of cancers and a dire problem without a solution. Currently available cellular therapeutics are costly and often lack polyclonality, polyfunctionality, and the ability to persist as central memory. The treatment of this deadly complication is potentially at a turning point if the rich immune infiltrates that characterize the majority of effusions can be redirected to an efficacious anti-tumor response. Despite this promise, pleural immune infiltrates have not been used to generate effector cells for adoptive cellular therapy.

Objectives: We have exploited the heterogeneous cellular composition of MPE by piloting the generation of therapeutic T-cell products, using conventional methods used for expanding tumor-infiltrating lymphocytes (TIL). The advantages of plural T cells are: (1) Fewer cycles of expansion owing to several orders of magnitude greater starting number of T cells; (2) Greater initial clonal and functional heterogeneity; (3) Likelihood of preserving polyclonality, polyfunctionality and central memory.

Results: MPE have abundant tumor infiltrating CD3+ T-cells, CD19+ B-cells, CD14+ macrophages, and EpCAM-/Cytokeratin+ mesothelial cells. Regulatory T-cells, which may be abundant in TIL, are low or absent in MPE. Our laboratory's average recovery of viable nucleated cells per MPE is $7.8 \pm 4.0 \times 10^8$ cells, with viability exceeding 95%. The cellular composition (tumor, lymphocytes, macrophages, neutrophils, mesothelial cells) varies from patient to patient, but T-cell recovery averages $2.0 \pm 1.6 \times 10^8$ (mean, SD). In pilot experiments we cultured whole breast cancer MPE in the presence of anti-CD3/anti-CD28 Dynal beads, IL-2 and IL-7 for 96 h. CD3+ T cells were FACS-sorted and added to autologous tumor monolayer cultures and expanded for an additional passage (2 weeks). Expanded passage 2 T cells were compared to freshly isolated T cells (2nd MPE drainage) for ability to kill autologous tumor and non-tumor targets (live cell imaging). Expanded T cells were potently cytotoxic, whereas freshly isolated MPE had no activity against autologous tumor. Expanded T cells did not kill the autologous non-tumor target (adherent cells isolated from peripheral blood). Additionally, we tested freshly isolated breast cancer MPE T cells for the ability to secrete cytokines associated with expansion and effector generation (IL-2, IFNγ and TNFα). We also measured the immunosuppressive cytokine IL-10. Freshly isolated plastic nonadherent cells from a breast cancer MPE were incubated with TPA+ ionomycin for 1 h, followed by brefeldin for 2 h. CD4+ T cells (85%) and CD8+ T cells (9%) were gated on cells co-expressing intracellular IL-2 and IFNγ. Polyfunctional T cells, defined as IL-2+/IFNγ+/TNFα+/IL-10-, comprised 0.38%, and 0.82% of CD4+ and CD8+ T cells. Unstimulated control cultures constitutively secreted IL-10 and IFNγ but not IL-2 or TNFα.