Istituto Giannina Gaslini
Ospedale Pediatrico IRCSS

STEM CELLS, CANCER, IMMUNOLOGY AND AGING

Genoa (Italy), February 12-14, 2015

Organized by
ISTITUTO GIANNINA GASLINI

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MENARINI

ABSTRACT BOOK

Acquario di Genova
Area Porto Antico – Ponte Spinola

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CONTENTS

The achievements and developments of CAR-T cells in Leukemia

David Barrett
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Background
Relapsed acute lymphoblastic leukemia (ALL) poses a therapeutic challenge despite aggressive therapies. Chimeric antigen receptor (CAR)-modified T cells targeting CD19 may overcome many limitations of conventional therapies and induce remissions in patients with refractory disease.

Methods
Autologous T cells transduced with a CD19-directed CAR (CTL019) lentiviral vector were infused into patients with relapsed/refractory ALL at doses of 0.7-20.6x10^6 CTL019 cells/kg. Patients were monitored for response, toxicity, expansion and persistence of circulating CTL019 T cells.

Results
Twenty-five children and 5 adults with relapsed/refractory ALL received CTL019. Complete remissions (CR) were achieved in 27/30 patients (90%), including 2 blinatumomab-refractory patients and 15 with prior stem cell transplant (SCT). CTL019 cells proliferated *in vivo* and were detectable in blood, bone marrow, and cerebrospinal fluid of responding patients. Sustained remissions were achieved with 6-month event-free survival of 67% (95% confidence interval [CI], 51-88%) and overall survival of 78% (95% CI, 65-95%). Probability of 6-month CTL019 persistence was 68% (95% CI, 50-92%) and relapse-free B cell aplasia was 73% (95% CI, 57-97%).
All patients experienced cytokine release syndrome (CRS). Severe CRS, seen in 27% of patients, was associated with higher disease burden and effectively treated with the anti-IL-6 receptor antibody tocilizumab.

Relapses included both CD19 expressing and CD19 escape variant leukemias.

Conclusions

CAR-modified T cell therapy against CD19 is highly effective for relapsed/refractory ALL. CTL019 achieved a high remission rate even in patients for whom previous SCT had failed, and durable remissions up to 24 months were observed. Our findings support continued development of this therapy and have led to multicenter trials.

T cell therapy for ALL and Neuroblastoma

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Although great strides have been made in the improvement of outcome for newly diagnosed pediatric and adult ALL, the prognosis for relapsed leukemia has lagged behind significantly. Hence, development of therapies for patients failing HSCT is a desirable objective and the adoptive transfer of tumor-specific T cells is promising. Chimeric antigen receptors (CARs) are synthetic receptors that target surface molecules in a native conformation. The "generations" of CARs typically refer to the intracellular signaling domains. In particular, in pre-clinical models, T cells carrying second or third generation CARs possess greater effector function and improved in vivo persistence than first generation vectors. Recently published United States trials using T cells genetically modified to express a CAR against the CD19 antigen have demonstrated impressive responses when adoptively transferred to patients with B-cell malignancies. In particular, upwards of 20 early phase CAR.CD19 T-cell clinical trials are currently either recruiting or due to open world-wide, with the majority of this clinical activity occurring in major clinical centers in the United States. In the absence of one single optimized CAR.CD19 configuration identified in preclinical work, comparisons between individual trials are complicated not only by the testing of different configurations of applied CARs, but also by the variety of patient preconditioning regimes, CAR T-cell dose, scFv clone, tumor type, gene-transfer method, and overall manufacturing protocol. Moreover, clinical trials based on CAR.CD19 T cells for different malignancies have revealed some potential drawbacks, including limited in vivo persistence and expansion of genetically modified T cells. To maximize benefits from CAR-based therapies, we designed a third generation CAR vector carrying two co-stimulatory domains (i.e. CD28/4-1BB, CD28/OX40), to explore *in vitro* but also *in vivo* the best co-stimulatory signal for CAR-modified T cell persistence over time.

Safety issue is covered by including iC9 in the gene construct. To extend our proof-of-concept also to solid tumor treatment, we extended the CAR immunotherapy approach also to the field of neuroblastoma disease, targeting the ganglioside antigen G(D2). In particular, the genetically-modified T cells expressing specific T cell receptors or CARs are just now entering the clinical arena in the contest of solid tumors, showing great potential for anti-tumor responses.
However, continued investigations are necessary to improve the cell product quality so as to decrease adverse effects making adoptive T cell therapy a tool of choice for solid malignancies.

Harnessing T cell response

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The infusion of *ex vivo* expanded tumor-specific T cells to melanoma patients has clearly documented the efficacy of adoptive T cell therapy. However, the wide exploitation of this strategy is limited by the low frequency of high-avidity T cells naturally reactive against tumor cells. Indeed, tumor antigens are often overexpressed, unmodified self-antigens, subject to tolerance mechanisms. The thymus appears to be overprotective towards potentially self-reactive T cells, and this observation opens the window for engineering high-avidity tumor-reactive T cell receptors (TCR). TCR gene transfer has yielded promising clinical results in cancer patients. However, the genetic transfer of a novel TCR into polyclonal T cells holds some limitations. In particular, the tumor-specific and α and β TCR chains are expressed in lymphocytes that already bear an endogenous TCR on cell surface. Gene-modified cells thus express at least two different TCRs that compete for binding to the CD3 complex, and this bottleneck results in mutual TCR dilution and reduced avidity. Furthermore, since TCRs are heterodimers, the α and β chains of the endogenous and transgenic TCR can mispair to produce a new hybrid TCR, with unpredictable and potentially harmful specificity. To permanently remove the expression of the endogenous TCR and the risk of TCR chain mispairing, we developed a TCR gene editing approach, based on the combination of somatic knockout of the endogenous TCR genes (by transient exposure to α and/or β chain specific Zinc Finger Nucleases - ZFNs) and introduction of tumor-specific TCR genes by lentiviral vectors (Provasi, Genovese et al. Nat. Med. 2012). Challenges and opportunities of TCR gene transfer and TCR gene editing will be discussed.

New Strategies for Stem Cell Transplantation

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In 2010 the World Marrow Donor Association (WMDA) reported 46919 new searches for unrelated donors (UD): in the same yar 2010, the WMDA reported 9248 UD transplants from peripheral blood (PB), 4036 from cord blood (CB) and 3574 from bone marrow (BM) for a total number of transplants of 16858 .
This represents 37% of the 46919 activations, leaving over 60% of patients not transplanted. Family stem cell donors who share one HLA haplotype with the recipient, are an alternative option , and are referred to as haploidentical donors (HAPLO) .
Unmanipulated HAPLO transplants are being increasingly used, as shown by the a recent EBMT survey: particularly in Italy HAPLO grafts have increased in the last years, to over 35 /10^6 inhabitants, compared to 15/10^6 in Germany and less than 10/10^6 inhabitants in France.
There are two ways of performing such transplants: one is with graft manipulation, involving one or another form of T cell depletion (TCD), and the other is with unmanipulated BM or PB , also referred to as T cell replete. Both methods have been improved with time: TCD transplants have recently seen advances with selective removal or addition of T cell subpopulations. Similarly, unmanipulated HAPLO grafts have been improved by effective measures of preventing acute and chronic graft versus host disease (GvHD).
The outcome of HAPLO transplants, has recently been compared with UD and CB grafts, suggesting that HAPLO donors are an important alternative option for patients who lack an HLA identical donor.

Role of activating HLA class I-specific receptors and HCMV in NK cell maturation after HSCT

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Human Natural Killer (NK) cells express ITIM-bearing inhibitory receptors specific for HLA class I molecules such as the killer Ig–like receptors (KIRs), able to distinguish among different HLA-A, -B, and -C allotypes, and CD94/NKG2A heterodimer, specific for HLA-E. Activating counterparts exist that are characterized by a short cytoplasmic tail and, via the interaction with the ITAM-containing DAP-12 adaptor signaling molecule, enhance rather then inhibit NK cell function. These include KIR2DS1, KIR2DS2, and KIR3DS1 and the CD94/NKG2C heterodimer. NK cells are the first lymphocyte population recovering after hematopoietic stem cell transplantation (HSCT) and their role in early immunity, before T-cell immunity is fully recovered, is considered crucial. Human Cytomegalovirus (HCMV) infection, which can persist lifelong after primary infection, may become cause of life threatening complications in immunosuppressed patients. Interestingly, we have demonstrated that HCMV reactivation in transplanted recipients provides stimulatory signals to NK cells undergoing maturation and could be beneficial rather than detrimental. In particular, HCMV infection/reactivation in adult patients undergoing umbilical cord blood transplantation (UCBT) for high-risk leukemias can promote rapid development of highly differentiated NK cells expressing a memory-like surface phenotype ($NKG2A^{-}KIR^{+}NKG2C^{+}CD57^{+}CD16^{+}Siglec\text{-}7^{-}$).

More recently, we have analyzed the phenotype and function of maturing NK cells in HCMV-infected patients that were given UCBT from a donor carrying a homozygous deletion of the NKG2C gene. Our data indicate that, even in the absence of NKG2C, HCMV infection can drive rapid NK maturation, characterized by the expansion of $CD56^{dim}NKG2A^{-}KIR^{+}$ cells. Interestingly, this expanded mature NK cell subset express surface-activating KIR that trigger NK cell cytotoxicity, degranulation, and IFN-γ release. Thus, an emerging concept is that HCMV infection is capable of shaping the NK cell receptor repertoire, favoring the preferential expansion of memory" or “long-lived” NK cells that express activating receptors, persist over time and might contribute to the protection against leukemia relapse and to the control of virus infection/reactivation.

Innate Lymphoid Cells

Elisa Montaldo[1], Chiara Romagnani[2], Maria Cristina Mingari[3,4], Lorenzo Moretta[1]
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Innate lymphoid cells (ILCs) represent an emerging family of developmentally related cells. The most recent view on ILCs recognizes a subset of cytotoxic-ILC and three distinct subsets of helper-ILCs on the basis of their cytokine pattern and transcription factor required for their differentiation. Eomes$^+$ Natural Killer (NK) cells, which are involved in host protection against virus infected and neoplastic cells, represent cytotoxic-ILCs. Concerning helper-ILCs, Tbet$^+$ ILC1s, together with NK cells, mainly produce IFNγ. ILC2s require GATA3 and release IL-5, IL-13, and IL-4, thus contributing to type-2 responses. Finally, ILC3s are an heterogeneous subset of RORγt$^+$ cells that secrete IL-17 and IL-22. ILC3s drive the development of lymphoid tissue during fetal life and provide defence against extracellular pathogens. Collectively, ILCs play an important role in innate defences against different pathogens, in lymphoid organogenesis, in wound healing and in tissue remodelling. In the context of hematopoietic stem cell transplantation (HSCT), NK cells play a key role in anti-leukemic activity, and provide protections against re-activated or primary viral infections. Other ILCs may participate in host defense against infection or in the reconstitution of lymphoid tissues, damaged by the conditioning regimen. While the differentiation of human NK cells is now well characterized both *in vitro* and *in vivo*, limited information is available on the development of human helper-ILCs and of their relationships. In view of the possible role of helper-ILCs in immune reconstitution after HSCT, we analyzed CD34$^+$ HPC from umbilical cord blood (UCB), peripheral blood (PB), bone marrow (BM), and tonsil and showed that they differentiate towards both NK cells and ILC3s.

In tonsil and intestinal *lamina propria* we identified RORγt^{+}CD34^{+} HPCs. These cells share a distinct transcriptional signature with mature ILC3s and selectively differentiated toward ILC3 but not NK. Thus, RORγt^{+}CD34^{+} HPCs represent ILC3 committed progenitors while tonsil and intestinal *lamina propria* are their site of development.

Special session 'How to build a research based on iPS':

"Exploring the dedifferentiation of transplanted cord blood cells"

Valentina Gaidano
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The discovery that specific transcriptional factors could reverse the fate of finally differentiated cells into stem cells has led to the production of patient-specific induced pluripotent stem cells (iPS).
The main applications of this extraordinary tool have been the disease modeling, possibly coupled with drug screening, and the correction of genetically altered cells, theoretically followed by the reinfusion in the patient.
In both applications, we can identify two critical processes: the reprogramming phase to iPS and the differentiation phase from iPS to newly derived somatic cells.
The former can rely on very robust protocols, although concerns regarding safety –tumorigenicity in particular– and costs have prompt some groups to reconsider the direct conversion of a certain cell type into another type, bypassing the stem cell status, but often contemplating a partial dedifferentiation step.
The differentiation phase can still be very tricky: low efficiency, retention of a certain memory (of the starting cell or of the iPS phase) and partially functional engineered cells are common issues.
Preliminary data from our group suggests that transplanted CD34+ cord blood cells could reactivate some genes typically associated with stem cells, such as *Sox2* and *Nanog*.
Everyday clinical practice shows that these cells do not degenerate in teratomas, but efficiently differentiate into hematopoietic cells.
The study of this phenomenon could (*i*) detect a paraphysiological way that cord blood cells use, probably to proliferate exponentially, without running the risk of tumor, (*ii*) shed light on the early phase of differentiation, with the transition from a stem cell to a somatic cell

status, (*iii*) give further evidence of cell plasticity and (*iv*) partially explain why hematopoietic reconstitution is slower after cord blood transplantation compared to adult hematopoietic stem cell transplantation.

“Inflamma-aging” of the stem cell niche as an age-dependent driver of cancer

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Normal stem cells are harbored in well characterized tissue regions, “the niches” in which the stromal compartment steers their homeostasis. The cancer stem cell niche is far from being characterized, as it appears a more “functional” than “anatomic” entity.
Current literature supports the notion that aging is characterized by a progressive shift towards a systemic pro-inflammatory status called inflamm-aging. This inflammatory derangement recalls the notion of meta/para-inflammation occurring in cancer patients. A wealth of data support the tenet that inflammation is a cancer promoting condition and that inflammatory mediators share in the cancer stem cell niche. It can be therefore proposed that common mechanisms acts during aging and tumor promotion.
In particular, circulating factors such as exosomes and viral genomes are expected to fuel the “inflammaging of cancer stem cell niche”. Examples of such a scenario will be presented in the context of breast cancer and discussed as potential models that can unravel unexplored pathogenic pathways and therapeutic targets.

Hematopoietic Stem Cell Aging and Rejuvenation

Maria Carolina Florian
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The functional decline in hematopoietic function seen during aging involves a progressive reduction in the immune response and an increased incidence of myeloid malignancy, and has been linked to aging of hematopoietic stem cells (HSCs). The molecular mechanisms underlying HSC aging remain unclear. We demonstrate that elevated activity of the small RhoGTPase Cdc42 in aged HSCs is causally linked to HSC aging and correlates with a loss of polarity in aged HSCs. Pharmacological inhibition of Cdc42 activity functionally rejuvenates aged HSCs, increases the percentage of polarized cells in an aged HSC population, and restores the level and spatial distribution of histone H4 lysine 16 acetylation to a similar status as seen in young HSCs.
In addition we report an unexpected shift from canonical to non-canonical Wnt signalling due to elevated expression of Wnt5a in aged HSCs, which causes stem-cell aging. Wnt5a treatment of young HSCs induces aging-associated stem-cell apolarity, reduction of regenerative capacity and an aging-like myeloid–lymphoid differentiation skewing via activating the small RhoGTPase Cdc42. Conversely, Wnt5a haploinsufficiency attenuates HSC aging, whereas stem-cell-intrinsic reduction of Wnt5a expression results in functionally rejuvenated aged HSCs.
Collectively, our data suggest (1) a mechanistic role for Cdc42 activity in HSC biology and epigenetic regulation, (2) identify Cdc42 activity as a pharmacological target for ameliorating stem cell aging, and (3) demonstrate a critical role for stem-cell-intrinsic non-canonical Wnt5a-Cdc42 signalling in HSC aging.

In Vitro and in Vivo Strategies in Regenerative Medicine

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In modern societies, aging remains the leading risk factor for neurodegenerative disorders, cardiovascular diseases and cancer. Aging can be defined as the progressive decline in the ability of a cell or an organism to resist stress, damage and diseases. Hutchinson-Gilford progeria syndrome (HGPS) is a rare premature aging syndrome caused by mutations in the *LMNA* gene resulting in the accumulation of a truncated form of lamin A known as progerin that leads to abnormal nuclear morphology. Over the last decades, accumulating evidence has demonstrated that mitochondrial dysfunction and oxidative stress are among the phenotypes associated with the aging process. During life, a progressive decline in mitochondrial function leading to oxidative damage to macromolecules constitutes the central dogma of the "Free Radical" theory of aging. However, despite being classified as a premature aging syndrome, the role of mitochondrial dysfunction in progeria has not been investigated. We have generated induced pluripotent stem cells (iPSCs) from fibroblast obtained from progeria patients. Differentiation to vascular smooth muscle cells and mesenchymal stem cells (MSCs) recapitulates the premature aging phenotypes. Interestingly, progeria MSCs present a distinct transcriptome signature with significant alterations in central carbon metabolism including glycolysis and TCA cycle in addition to reduce mitochondrial respiration and increase oxidative stress. GC/MS and isotope tracing experiment reveals significant deficiency in glutamine metabolism in progeria MSCs. We propose that metabolic dysfunction could be one of the major drivers of premature aging in HGPS patients and hypothesize that genetic and pharmacological manipulations aiming to restore metabolic function will alleviate the devastating effects of this and other premature aging syndromes as well as normal aging.

The discovery of induced pluripotent stem cells (iPSCs) has reignited the enthusiasm for cell-based therapy. The ability of iPSCs to undergo unlimited division while maintaining genomic integrity provides a way to overcome the senescence barrier of aged somatic cells. The capacity of iPSCs to differentiate into cells of the three germ layers has been extensively documented in the field. Taken together, it is not hard to appreciate why human iPSC (hiPSC)-based autologous transplantation is heralded as the future of regenerative medicine. Pluripotent stem cells (PSCs) are especially amenable for genome editing because they can undergo extensive tissue culture manipulations, such as drug selection and clonal expansion, while still maintaining their pluripotency and genome stability. Human pluripotent stem cells (hPSCs) offer unprecedented opportunities to study cellular differentiation and model human diseases. In addition to expand our knowledge of aging, I will present and discuss the conceptual and practical issues to consider when attempting to model, diagnose and treat genetic diseases, both in vivo and in vitro, using pluripotent stem cells.

Heterogeneity of Leukemic Cells: Implication for treatment

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The recent availability of genome sequence and adequate analytical platforms, such as gene expression profiling, single nucleotide polymorphism arrays, and, more recently, next generation sequencing, has expanded the full repertoire of genetic lesions in childhood ALL. Some recent new genomic recurrent alterations occurring in ALL will be discussed including *CRLF2* rearrangements, *IKZF1* alteration, *JAK1/2* mutations, *BCR-ABL1*-like signature, and early T-cell precursor (ETP). Although many of these genomic studies have increased our knowledge of the pathogenesis of the disease significantly, there is no consensus on how new specific genotypes will affect the clinical management of children with ALL. It is likely that genomics will be translated into a better risk stratification that will drive tailored therapy in the near future. However, 2 major points need still to be addressed: (1) the impact of genomics in predicting response to therapy and thus to refine risk stratification and (2) how the identification of new targets will be translated into effective targeted therapy. Several factors should be considered in determining clinical significance and prognostic significance of a novel genetic discovery: treatment context, independent verification in multiple prospective clinical trials, independent prognostic value in multivariate analysis with MRD, and importance of the novel genetic aberration either as potential targets or as modifiers of specific therapies. Finally we need to consider that the definition of the full genetic repertoire of ALL and the progressive availability of new targeted therapy will make the subgroups of patients smaller and smaller. With that in mind, we should always consider the possibility of conducting International collaborative traditional trials to maximize recruitment, even if this would cause a significant increase in the organizational and regulatory burden.

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Menarini Foundation Symposia: 256