



Istituto Giannina Gaslini  
Ospedale Pediatrico IRCSS

# **New Frontiers in Molecular and Cellular Therapy**

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## **ABSTRACT BOOK**

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## Normal and Neoplastic Stem Cells

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The abstract is: "Following embryonic development, most of our tissues and organs are continuously regenerated from tissue/organ specific stem cells. The principal property that distinguishes such stem cells from their daughter cells is self-renewal; when stem cells divide they give rise to stem cells (by self-renewal) and progenitors (by differentiation). In most tissues only the primitive stem cells self-renew. Stem cell isolation and transplantation is the basis for regenerative medicine. Self-renewal is dangerous, and therefore strictly regulated. Poorly regulated self-renewal can lead to the genesis of cancer stem cells, the only self-renewing cells in the cancerous tumor. The Weissman lab has followed the progression from hematopoietic stem cells to myelogenous leukemias. They have found that the developing cancer clones progress at the stage of hematopoietic stem cells, until they become fully malignant. At this point, the 'leukemia' stem cell moves to a stage of a downstream oligolineage or multilineage progenitor that has evaded programmed cell death and programmed cell removal, while acquiring or keeping self-renewal. While there are many ways to defeat programmed cell death and senescence, there appears to be one dominant method to avoid programmed cell removal—the expression of the cell surface 'don't eat me' protein CD47, the ligand for macrophage SIRP-alpha. All cancers tested express CD47 to overcome expression of 'eat me' signals such as calreticulin and asialoglycoproteins. Antibodies that block the CD47–SIRP-alpha interaction enable phagocytosis and killing of the tumor cells in vitro and in vivo."

## **Human CMV drives rapid NK cell maturation after HSCT**

Alessandro Moretta

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It is well established that Natural killer (NK) cells play a crucial role in early immunity after hematopoietic stem cell transplantation because they are the first lymphocyte subset recovering after the allograft. Interestingly, in different patients undergoing umbilical CB transplantation discrete patterns of NK-cell development can be identified. In this context, we showed that, in a group of patients, a relevant fraction of NK cells already expressed a mature phenotype characterized by the KIR+NKG2A- signature 3-6 months after transplantation. In other patients, most NK cells maintained an immature phenotype even after 2 months. A possible role for cytomegalovirus in the promotion of NK-cell development was suggested by the observation that a more rapid NK-cell maturation together with expansion of NKG2C+ NK cells was confined to patients experiencing cytomegalovirus reactivation. In a fraction of these patients, an aberrant and hyporesponsive CD56-,CD16+,p75/AIRM1- NK-cell subset (mostly KIR+,NKG2A-) reminiscent of that described in patients with viremic HIV was detected. Our data support the concept that cytomegalovirus infection may drive NK-cell development after HSCT.

## **Transferring natural and adaptive immunity in haploidentical hematopoietic transplants**

Andrea Velardi

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In the 1990s, HLA haplotype-mismatched (“haploidentical”) transplants for leukemia patients became feasible through use of high-intensity conditioning regimens and transplantation of a mega-dose of extensively T cell-depleted peripheral blood hematopoietic progenitor cells (Aversa et al., NEJM 1998; JCO 2005). Because of T cell depletion, major issues were delayed immune reconstitution and high transplant-related/infectious mortality rate especially in adults (whose thymic output is limited) and potential lack of (T cell-mediated) GvL effect. On the other hand, the post-transplant immune recovery in the absence of any immune suppression created an opportunity for discovering innovative forms of immunotherapy. It favored natural killer (NK) cell development and revealed donor versus recipient NK cell alloreactions which eradicated acute myeloid leukemia, favored engraftment, protected from GVHD and improved survival (Ruggeri et al. Science 2002; Blood 2007). It also allowed effective donor T cell immunotherapies (devoid of GVHD potential) that protected against infections (Perruccio et al., Blood 2005). More recently, this setting provided evidence that infusion of naturally occurring freshly isolated donor regulatory T cells efficiently protected against otherwise lethal doses of conventional T cell add-backs given to improve immune reconstitution (Di Ianni et al., Blood 2011). Such Treg/Tcon approach is currently being assessed for its ability to improve immune reconstitution and mediate GvL effects (Di Ianni et al, EBMT 2013). As T regs are reported to down-regulate NK cell function, T regs might antagonize the graft versus leukemia (GvL) effects of donor versus recipient NK cell alloreactivity. Strikingly, we find T reg-based conditioning regimens preserve NK cell-mediated GvL effects and, additionally, provide an independent effector mechanism to control leukemia relapse.

## **Strategies to enhance T cell reconstitution**

Marcel R.M. van den Brink

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New York, NY-USA*

Recent studies have demonstrated that improved T cell reconstitution after allo-BMT can not only decrease TRM due to decreased post-transplant infections, but also result in decreased incidence of malignant relapse.

We have developed several strategies to enhance post-transplant T cell reconstitution, which are currently being tested in phase I and II clinical trials in allo-BMT recipients. A Phase I study with post-transplant Interleukin-7 administration demonstrated minimal toxicity and increased T cell recovery.



## **Genetic modifications of T cells for adoptive immunotherapy in cancer patients: from bench to the bedside**

Gianpietro Dotti

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The transfer of chimeric antigen receptor (CAR) genes that combine the antigen binding property of monoclonal antibodies with the cytolytic capacity of T-lymphocytes, represents one means of preparing T cells with a known antitumor specificity. There has been interest in generating T cells expressing CARs targeting antigens expressed in a broad array of hematological malignancies and solid tumors. Antitumor activity of redirected T cells both *in vitro* and *in vivo* are independent of MHC restriction and can be increased by co-expression of different costimulatory molecules within the CAR or by expressing CARs on T cells with a well defined antigen specificity, such as Epstein-Barr specific cytotoxic T cells (CTLs) or CMV-specific CTLs.

Dr Dotti will summarize the steps that led to clinical translation of this approach and focusing on CAR targeting the CD19 antigen as a model will review the procedures of gene transfer, generation of T cells for clinical use, T-cell subsets used in clinical trials, preconditioning of the host and finally safety issues directly associated with this technology.

## Targeting of Acute Myeloid Leukaemia (AML) by Cytokine-Induced Killer (CIK) Cells Redirected with a Novel CARs

Ettore Biagi

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Despite the progress in the treatment of acute myeloid leukaemia (AML), a significant number of patients are still refractory or relapse after conventional chemotherapy. Therefore it is necessary to develop novel alternative approaches. Immunotherapy with T cells genetically modified to express chimeric antigen receptors (CARs) represents a valid option. CARs are artificial T-cell receptors constituted by a specific antigen-binding domain, and a signaling region, that, upon antigen recognition, leads to T-cell activation and lysis of the target cells. AML is a potential optimal target because of over-expression of a number of surface antigens like CD33, CD123. Since CD33 is also expressed on normal haematopoietic stem/progenitors cells (HSPCs), resulting in a potential severe impairment of normal myelopoiesis, CD123 has recently emerged as a new attractive molecule based on its differential expression pattern, being widely overexpressed by AML population and less expressed on HSPCs.

CIK cells were transduced with an SFG-retroviral vector encoding a third-generation anti-CD123.CAR. The efficacy and safety profiles of transduced cells were characterized *in vitro* by short and long-term cytotoxicity assays. Their potential reactivity against HSPCs was evaluated by colony-forming unit assay after 4-hours co-incubation.

Anti-CD123.CAR<sup>+</sup>CIK cells strongly killed CD123<sup>+</sup> THP-1 cell line (60%±5.4%, Effector:Target –E:T- ratio of 5:1, n=3), as well as primary AML blasts (59%±5.4%, E:T ratio of 3:1, n=4) in 4-hours cytotoxicity assays. In long-term cytotoxicity assay we observed a leukaemic cell recovery for THP-1 of 3.5%±1.5% (n=5) and for primary AML cells of 2.4%±1.4% (n=3), when co-cultured with anti-CD123.CAR<sup>+</sup>CIK cells, compared to an average survival of up to 80%, when co-cultured with unmanipulated (NT) CIK cells. Interestingly, secondary colonies experiments after co-culture of cord

blood-derived HSPCs with anti-CD123.CAR<sup>+</sup>CIK cells demonstrated that anti-CD123.CAR better preserved a normal haematopoietic reconstitution in contrast to a previously generated anti-CD33.CAR (total number of colonies of 146.8±6.6, 66.4±5.1, 117.6±4.6, after co-culture with NT CIK cells, anti-CD33.CAR<sup>+</sup>CIK cells, anti-CD123.CAR<sup>+</sup>CIK cells respectively, n=4). Furthermore, a limited killing of normal CD123<sup>+</sup>monocytes and CD123-low expressing endothelial cells was measured. Taken together, our results (Tettamanti S, *British Journal of Hematology*, March 2013) indicate that anti-CD123.CAR strongly enhances anti-leukaemic CIK functions towards AML, while sparing HSPCs and normal CD123-expressing tissues, paving the way for the development of novel immunotherapy approaches for the treatment of resistant forms of AML.

*In vivo* experiments were further produced. Once injected into low-level AML engrafted NSG mice (median of hCD45+CD33+ 0.6% before treatment), genetically modified T cells had a potent antitumour effect. Indeed, the bone marrow of control untreated animals or mice treated with un-manipulated CIK cells was infiltrated by leukaemic cells (86% and 81% leukaemic engraftment), while in 7/8 anti-CD33-CD28-OX40- and in 8/10 anti-CD123-CD28-OX40-treated mice AML cells were not detectable. Similar results were obtained when T cells were injected in mice with an established high AML burden (median of hCD45+CD33+ 70% before treatment). One week after the last CIK injection the level of AML engraftment was 96%, 87%, 0.35% and 0.34% for untreated mice, mice treated with un-manipulated CIK cells and with anti-CD33-CD28-OX40- and anti-CD123-CD28-OX40- transduced CIK-cells, respectively. We performed secondary transplantation on residual AML cells and mice were treated again with transduced CIK cells. Residual AML cells were still sensitive to CARs approach, leading once again to an almost complete eradication of the disease (median level of hCD45+CD33+ engraftment was 98%, 0.02% and 0.04% respectively for untreated mice, anti-CD33-CD28-OX40- and anti-CD123-CD28-OX40-transduced CIK-cells).

Furthermore, a fundamental issue was to determine the safety profile of such approach against normal haematopoietic precursors. In

untreated mice injected with primary cord blood-derived CD34+ cells the level of engraftment of hCD45 compartment was 42%, whilst in mice treated with un-manipulated, anti-CD33-CD28-OX40- or anti-CD123-CD28-OX40- transduced CIK-cells the levels of human compartment was 40%, 11.7% and 26.3% respectively. Moreover, when we considered specifically the CD34+CD38- compartment, enriched in HSC, the level of engraftment was 1.92%, 1.02%, 0.55% and 0.83%.

These experiments should offer relevant information concerning the efficacy and safety of the proposed strategy particularly in the context of minimal residual disease in high-risk transplanted AML patients or for patients not eligible to receive high dose chemotherapy.

Ultimately, stable gene transfer of chimeric antigen receptors (CAR) is a powerful tool to redirect antigen specificity of effector T cells for adoptive immunotherapy of tumors. The development of efficient and safe nonviral vectors would rescue the safety and manufacture concerns that limit so far their clinical application. The *ex-vivo* use of the latest generation Sleeping Beauty (SB) Transposon-mediated gene transfer offers a valid alternative to viral vectors for human gene therapy. Here we used nucleofection of SB system and an optimized clinical-grade stimulation protocol to generate and propagate cytokine-induced killer (CIK) cells genetically modified to express CD123-specific 3rd generation CARs to redirect antigen specificity towards AML CD123+ blasts. With this system, the average transfection at 24hours was 68.9% ( $\pm 1.0$ , n=6) and mean survival percentage was 34.8% ( $\pm 5.6$ , n=5). Electroporation with the optimized stimulation protocol did not affect the phenotype of CIK cells, preserved their naïve and stem cell memory populations, and resulted in a fold increase of  $15.7 \pm 3.3$  at the end of the CIK culture at day 21. Transposed CIK cells displayed stable expression of Tr\_scFvCD123.3rdCAR ( $39.4\% \pm 8.6$ ), efficient lysis of AML target cells, and CAR-specific cytokine secretion. Finally, long-term expansion (fold increase  $423.8 \pm 301.4$ , n=2 at day 40) upon stimulation with AML cells in vitro promoted efficient selection of CAR+ CIK cells with potent cytotoxic activity towards AML target ( $92.9 \pm 1$ , n=2), suggesting long-term persistence of functional CIK cells in vivo. Efficient transfection of CARs together with a feasible

manufacturing practice (GMP) strategy to select and propagate CAR-expressing CIK cells will be instrumental in defining novel therapeutic approaches to target childhood acute myeloid leukemia.

## **Chimeric Antigen Receptor (CAR)-redirected T cells for lymphomas: old and new targets**

Barbara Savoldo

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Studies of adoptive transfer of T cells with a CD19-specific CAR to treat B-cell-malignancies from multiple centers show remarkable clinical efficacy. Dr. Savoldo will report on phase-I clinical studies with novel CARs in lymphoma patients.

The aim of the first study is to target B-cell malignancies more selectively, as the long-term persistence of T cells targeting CD19, a pan-B cell marker, results in the depletion of normal B lymphocytes and consequent severe hypo-gammaglobulinemia. Taking advantage of the clonal restriction of mature B-cell malignancies, which express either a  $\kappa$ - or a  $\lambda$ -light immunoglobulin (Ig) chains, a CAR specific for  $\kappa$ -light chain (CAR.  $\kappa$ ) was generated to selectively target  $\kappa^+$  lymphoma/leukemia cells, while sparing the normal B cells expressing the non-targeted  $\lambda$ -light chain, and thus minimizing the impairment of humoral immunity. After validation in preclinical experiments, a phase-I clinical trial was opened at Baylor College of Medicine in which patients (pts) with refractory/relapsed  $\kappa^+$  leukemia/lymphoma are infused with autologous T cells expressing a CAR.  $\kappa$  that includes the CD28 costimulatory domain. The protocol also allows for the inclusion of patients with multiple myeloma (MM) with the aim of targeting putative MM initiating cells. Eight pts were treated so far on this study and infusions were well tolerated without side effects. Persistence of infused T cells was assessed in blood by CAR.  $\kappa$ -specific Q-PCR assay, with molecular signals, peaking 1-2 wk post infusion, although at low levels and remained detectable for at least 6 wks. Six pts are currently evaluable for clinical response. Three had no response. One pt (with MM) had stable disease at the 6 wk evaluation, one (with transformed follicular lymphoma) achieved partial response 6 wk after the 1<sup>st</sup> infusion and complete remission following 2 more infusions, and one (with lymphoplasmacytic lymphoma) had 40% reduction of paraproteinemia at 6 wk.

The other study uses a CAR that target the CD30 molecule, which is expressed by Hodgkin's Lymphoma (HL) and some non Hodgkin's lymphoma (NHL), both at diagnosis and relapse. Also this CAR contains CD28 as a costimulatory endodomain. For this group of pts two trials have been opened. In one trial, the CAR is grafted on EBV-specific cytotoxic T lymphocytes to determine whether native costimulation can support their activation and expansion *in vivo*, while in the other one the CAR is grafted on activated polyclonal T cells. Pts with refractory/relapsed CD30+ HL or NHL are eligible for these phase-I dose escalation studies. Lines have been so far infused in 2 and 3 patients, respectively, with no significant adverse events, except for 1 pt experiencing flu-like symptoms. Molecular signals for CAR.CD30 increased post infusion, peaking at the 2 weeks follow-up, but declined by wk 6. One pt has stable disease and one a mixed response at the 6 wks follow-up. Clinical updates will be presented at the meeting.

## **Strategies to enhance T cell mediated GVT**

Marcel R.M. van den Brink

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New York, NY-USA*

Recent clinical studies using gene transfer of an anti-CD19 chimeric antibody receptor into T cells, so called CAR-T cells, have resulted in promising results in patients with refractory CLL and ALL. These data demonstrate the potential of genetically engineered T cells as immunotherapy of cancer. The importance of T cells as prime mediators of graft-versus-tumor (GVT) activity has been known for decades and many strategies have focused on optimization of GVT without aggravating graft-versus-host disease (GVHD). We have explored in preclinical models two approaches to enhance GVT.

We evaluated the relative effects of donor CD19-targeting CAR-T cells on the elimination of CD19+B cells and endogenous TCR-mediated GVHD in mouse models of allo-HSCT. We generated a panel of retroviral vectors encoding mouse CD19-specific CARs: (1) CD19-delta, a tail-less CAR lacking the CD3 signaling, as a negative control and (2) CD19-CAR, which can signal through CD28 and CD3 .

We found that CD19-CAR-T cells displayed (a) significant GVT activity against CD19+ malignancies, (b) caused persistent B cell aplasia, and (c) caused less GVHD due to decreased proliferation and intestinal homing of alloactivated CAR-T cells.

A second approach involves TNF-Related Apoptosis Inducing Ligand (TRAIL), which can induce apoptosis through death receptor (DR) 4 and 5 molecules (only DR5 in mice) in a variety of tumors without much toxicity to non-malignant cells. TRAIL is therefore an attractive candidate for genetic engineering of donor T cells to enhance their GVT potential.

Mature T cells derived from donor B6 splenocytes were transduced with a lentiviral TRAIL expression vector. Upon adoptive transfer of these cells with a B6 bone marrow allograft into lethally irradiated recipients, we found enhanced GVT activity against TRAIL\_sensitive tumors and, interestingly, less GVHD through fratricide of



alloreactive donor T cells and impairment of host APCs in recipients of allo-HSCT.

In conclusion, adoptive transfer of genetically engineered donor T cells, which overexpress a CD19-CAR or TRAIL, to allo-BMT recipients can enhance GVT activity while suppressing GVHD.

## **Haploidentical NK cells as post induction therapy for elderly patients with AML**

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### **ABSTRACT**

*Background:* The most common cause of treatment failure in elderly acute myeloid leukaemia (AML) patients is early relapse. Therefore, novel consolidation strategies, including stem cell transplantation, are actively investigated. Recent data suggest that KIR mismatched NK cells may be transferred and expanded, *in vivo*, and may significantly impact on tumor cell killing in elderly AML patients (Curti et al Blood 2011). *Aims:* Aim of this work is to establish the efficacy of adoptive immunotherapy with haploidentical KIR-L mismatched NK cells as consolidation therapy in elderly high-risk AML patients, who achieved CR. *Methods:* Fourteen patients with high-risk AML (2 in molecular relapse and 12 in morphological complete remission (CR); with a median age of 62 years (range 53-73) received highly purified CD56<sup>+</sup>CD3<sup>-</sup> NK cells from haploidentical KIR-ligand mismatched donors after fludarabine/cyclophosphamide-containing-immunosuppressive chemotherapy, followed by subcutaneous administration of interleukin-2 (10x10<sup>6</sup> IU/day, 3 times weekly for 2 weeks -6 doses total) after NK cell infusion. *Results:* The median number of infused NK cells was 2.74 x 10<sup>6</sup>/Kg. No NK cell-related toxicity, including graft-versus-host disease (GVHD), was observed. Hematological recovery was comparable to standard chemotherapy: median time to neutrophil count recovery (ANC > 0.5 x 10<sup>9</sup>/l) was 18 days (range 12-45), median time to platelet recovery (PLTs > 20 x 10<sup>9</sup>/l) was 20 days (range 13-45). Both patients in molecular relapse achieved molecular CR, which lasted 9 months for both patients. Among 12 patients in morphological CR, 7 patients are disease-free after 28, 25, 63, 59, 49, 10 and 9 months (median 28 months; range 9-

63), whereas 4 patients relapsed after 3, 5, 24 and 3 months, Three relapsed patients ultimately died due to disease progression, one is receiving re-induction chemotherapy. One patient died during the neutropenic phase due to overwhelming bacteria pneumonia. Seven AML patients, who had achieved CR after the same induction/consolidation therapy and with similar prognostic features as the study patients were excluded from NK therapy because they did not have KIR-ligand mismatched donors. Interestingly, analysis of their clinical outcome showed that 6/7 patients relapsed at a median of 4 months and died of disease progression, whereas one patient is alive and disease-free after 24 months after autologous stem cell transplantation. After infusion, donor NK cells were found in the peripheral blood (PB) of all evaluable patients (peak value on day 10). They were also detected in the bone marrow (BM) in some cases (peak value on day 5). An association between serum IL-15 concentration and donor chimerism after NK cell infusion was observed. Particularly, the rise in IL-15 serum level was followed by the increase of donor chimerism, thus supporting the conclusion that homeostatic IL-15 drives *in vivo* expansion and survival of adoptively transferred NK cells. Donor-versus-recipient alloreactive NK cells were demonstrated *in vivo* by the detection of donor-derived NK clones that killed recipient targets, including leukemic blasts. *Conclusions:* Adoptively transferred NK cells do show anti-leukemic activity thus inducing long-term disease-free survival in a significant proportion of in elderly patients with high risk AML.

## **Disease Modeling with iPS Cells**

George Q. Daley

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A major goal of stem cell research is the creation of personalized, patient-specific stem cells for use in disease modeling and as a foundation for gene repair in the context of autologous cell therapy. The ability to restore pluripotency to somatic cells through the ectopic co-expression of reprogramming factors has created powerful new opportunities for modelling human diseases. Some skepticism remains, however, whether subtle differences might impact research applications and therapeutic potential. We have derived induced pluripotent stem cells (iPSC) from patients with a variety of genetic bone marrow failure disorders, including Dyskeratosis Congenita, Fanconi's anemia, Shwachman-Diamond Syndrome, and Diamond Blackfan Anemia. Hematopoietic differentiation of these lines in vitro recapitulates certain aspects of these diseases and enables novel insights into disease mechanisms, thereby confirming their utility in disease modeling for studies of pathogenesis. Aspects of these studies highlighting advantages and limitations of iPS-based disease modeling will be presented.

## **Strategies for organ and tissue regeneration**

Izpisua Belmonte Juan Carlos

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Finding a cure for cardiovascular disease remains a major unmet medical need. Recent investigations have started to unveil the mechanisms of mammalian heart regeneration. The study of the regenerative mechanisms in lower vertebrate and mammalian animal models has provided clues for the experimental activation of proregenerative responses in the heart. In parallel, the use of endogenous adult stem cell populations alongside the recent application of reprogramming technologies has created major expectations for the development of therapies targeting heart disease. Together, these new approaches are bringing us closer to more successful strategies for the treatment of heart disease.

## **Standard therapies versus novel therapies in Lymphoma**

Gallamini Andrea

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The treatment outcome in Hodgkin's Lymphoma (HL) as well as in several non Hodgkin lymphoma (NHL) subtype has improved over time. Although no major advances have been achieved in patient treatment survival rate in HL increased in all age decades (even beyond 60 years) as a result of an improved overall standard of care. 18F-fluoro -2-deoxy-D-glucose (FDG) PET (FDG-PET) and subsequently PET combined with Computed tomography (PET/CT) has emerged as the most important advance in lymphoma management. Current prognostic models such as the International Prognostic Index (IPI) for NHL and the International Prognostic Score (IPS) for HL appeared unable to support a risk-adapted therapeutic strategy owing to a scarce predictive power in HL and, to a lesser extent, also in NHL. For these reasons a search for new prognostic tools has been addressed in the last few years to solve the dilemma whether an early identification of patients with a poor prognosis, potential candidates to an aggressive front-line therapy is possible and feasible, while sparing toxicity for the majority of them, curable with a traditional standard-intensity treatment. FDG-PET proved to play an ideal role in this search thanks to its ability to detect a residual FDG uptake due to a persisting metabolic activity and viability of surviving neoplastic cells.

New horizons opened in lymphoma treatment by the proteomic studies on different oncoproteins involved in cell-to-cell interaction, cell activation, mitotic activity, and lineage-specific differentiation and DNA studies on the expression level of different genes (Gene Expression Profile: GEP). This became first evident of B-cell signature in Diffuse Large B-Cell Lymphoma (DLBCL) when two different phenotypes have been discovered with different prognosis for the lymphoma cells with the so-called germinal-center phenotype (GCC) or Activated B-Cell (ABC) phenotype. Later on a number of different cell pathway activation has been studied such as the NfKB

nuclear proteins in Hodgkin lymphoma, the m-TOR pathway in different NHL subtype and the MAPK (Mitogen Activated Protein Kinases). The latter has been recently studied in Hairy Cell Leukemia (HCL) and an inhibitor BRAF, of a trigger protein to the MAPK cascade has shown a surprisingly high efficacy in the disease control of this rare B-lymphoproliferative disorder. Finally the discover of powerful activation markers such as the “cell activation” CD-30 antigen in neoplastic cell of different lymphoma entities, such as HL or Anaplastic Large Cell Lymphoma prompted the development of “targeted therapies” with use of Monoclonal Antibodies (MoAbs) conjugated with toxin such as Mono-Metil-Auristatin (MMA) to target the neoplastic cells. For CD 30 antigen an humanized antibody has been raised Brentuximab, conjugated with a toxin to for a stable complex called Brentuximab-Vedotin (BV). This drug proved very efficacious in phase II studies in HL and ALCL patient pre-treated with several chemotherapy lines and are now proposed now as first-line treatment in combination with standard chemotherapy for HL and ALCL.

The efficacy of these treatments, if confirmed, should be balanced with its cost: the estimated average cost of 12 BV administrations along with six courses of A(B)VD is 70.000€. For this reason the search of prognostic markers seems now even more important than in the past to warrant the use of these very active, but also very expensive drugs only in patients wit a very poor prognosis.

## **Brentuximab Vedotin: a revolution for treating patients with Hodgkin Lymphoma (HL) and Anaplastic Large Cell Lymphoma (ALCL)?**

Carmelo Carlo-Stella

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*Department of Medical Biotechnology and Translational Medicine  
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Brentuximab vedotin (SGN-35) is an antibody-drug conjugate (ADC) directed against the CD30 antigen expressed on Hodgkin lymphoma and anaplastic large cell lymphoma. SGN-35 consists of the cAC10 chimerized IgG1 monoclonal antibody SGN30, modified by the addition of a valine-citrulline dipeptide linker to permit attachment of the potent inhibitor of microtubule polymerization monomethylauristatin E (MMAE). In phase II trials, SGN-35 produced response rates of 75% in patients with Hodgkin lymphoma and 87% in patients with anaplastic large cell lymphoma. Responses to SGN-35 might be related not only to the cytotoxic effect due to release of MMAE within the malignant cell but also to other effects, including SGN-35-triggered signaling through CD30 ligation as well as effects on tumor microenvironment. The elimination of cells that provide growth factor support for Hodgkin/Reed–Sternberg cells could in fact further enhance the cytotoxic activity of SGN-35. The clinical activity of SGN-35 alone or in combination is actively being explored in patients with a variety of CD30-positive tumors and disease phases. The biology of SGN-35 and the clinical effects of SGN-35 administration will be reviewed.



## **Targeting the microenvironment (EphA3 –endothelium etc)**

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Angiogenesis is a process which, in the tumor microenvironment, occurs with an uncontrolled and unlimited kinetic being essential for tumor growth, invasion and metastasis. The growth of solid tumors is certainly angiogenesis-dependent, whereas the role of angiogenesis in the growth and survival of hematological malignancies has only recently rendered evident. During the last decade, a fundamental role of tumour microenvironment has been demonstrated in a number of malignancies with particular attention to Multiple Myeloma (MM). Multiple myeloma (MM) is a plasma cell cancer that resides in the bone marrow (BM). Numerous studies have demonstrated the involvement of the BM microenvironment supporting tumor growth, angiogenesis, bone disease and drug resistance. Reciprocal interactions between the different components of the BM microenvironment and the MM cells have been demonstrated to be necessary to regulate migration, differentiation, proliferation and survival of the malignant plasma cells. Basing on the crucial role of angiogenesis in supporting the malignant clone, different anti angiogenetic drugs have been developed and are currently approved for the treatment of MM patients. The Eph receptors (Ephs) are a large family of TK activated by ephrins binding. EphA receptors are expressed abundantly during all phases of embryogenesis in spatially and temporally highly-regulated patterns and they are implicated in different developmental processes. Originally identified as neuronal molecules regulating axon guidance and synaptic plasticity, they have been later recognized as modulators of several biological functions, including vascular development, and migration. Increasing evidences implicate Eph family proteins in some human cancers, especially in the more aggressive stages of tumor progression. Many reports suggest that Eph receptors are frequently overexpressed in a variety of solid tumors, including melanoma, prostate, breast, lung, gastric, colorectal carcinomas and it seems likely due to a deregulated re-emergence of Eph embryonic functions in the adult tissues. In

addition, EphA3 was demonstrated to represent a prognostic marker in colorectal carcinoma. Moreover, its overexpression has been demonstrated in some leukemic cell lines but not in normal hematopoietic cells. Finally, the over-expression of Eph is believed to be sufficient to confer tumorigenic potential although probably further mechanisms can occur to abnormally activate the receptor. Basing on the role of EphA3 in human cancer and in haematological malignancies, a first-in-class engineered IgG1 antibody targeting the EphA3 receptor tyrosine kinase was developed and it is now under Phase I clinical trial (Clinicaltrials.gov identifier: NCT01211691) in USA and Australia for the treatment of EphA3 overexpressing haematological malignancies refractory to conventional treatment. We have studied the role of EphA3 in MPNs, including CML and in acute leukemias (AL) (both AML and ALL) demonstrating that EphA3 is highly expressed in CD34+ compartment from MPN and in blast cells from acute leukemias but not in the normal counterpart. Functional experiments demonstrated that EphA3 is mainly involved in regulating cell contact and cell adhesion. In addition we showed that the monoclonal antibody KB004 is able to induce a selective apoptosis of CD34+ cells from leukemic patients but not those from healthy donors. Our data in the specific setting of MM showed that EphA3 mRNA is highly overexpressed in BM Endothelial cells (EC) from MM but not in HUVEC cells or in EC cell obtained from healthy subjects. In addition Immunofluorescence analysis carried out in EC from MM and healthy controls showed a significant expression of the protein in EC from MM but not in the normal counterpart. Our data strongly support the role of EphA3 in the MM endothelial cells and, more generally in the niche and, importantly its effective targeting seems to provide an antiangiogenetic activity in MM. It appears clear that, if this study will demonstrate, in a significant series of MM patients, that EphA3 is aberrantly expressed in MM niche and/or in the haematopoietic cells and that this receptor plays a pathogenetic role in this disease, we will provide the rational for a clinical trial with a new antibody. The availability of a monoclonal antibody design to selectively target EphA3 open new opportunities for innovative target therapies in different fields including MM.

## Molecular Basis for a Targeted Therapy in Hairy Cell Leukemia

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Hairy Cell Leukemia (HCL) has distinctive clinico-pathological features among mature chronic B-cell leukemias, including unique immunophenotype (e.g., co-expression of CD11c, CD103, CD25 and Annexin-1) and genome-wide expression profile, as well as exquisite sensitivity to chemotherapy with the purine analogs cladribine and pentostatin.

However, the genetic basis underlying HCL has been unknown until we recently discovered, by whole-exome sequencing, that an activating mutation (V600E) of the BRAF kinase is the disease-defining genetic lesion of HCL (Tiacci et al., *New Engl J Med* 2011;364:2305), similar to BCR-ABL1 in chronic myeloid leukemia. In fact, the BRAF-V600E mutation is present in virtually 100% of almost 500 HCL patients from all over the world we and others have analyzed. Conversely, this mutation is absent in virtually all 300 patients analyzed with HCL-like disorders (such as splenic marginal zone lymphoma and HCL-variant), that have a similar clinico-pathological picture but require a different therapeutic approach. To help in this differential diagnosis, we have also developed a simple PCR test to detect the BRAF-V600E mutation with high sensitivity and non-invasively in whole blood samples (Tiacci et al., *Blood* 2012;119:192).

The BRAF-V600E mutation leads to constitutive activation of the oncogenic MEK-ERK pathway in solid tumors harboring this mutation. In HCL, we observed that, *in vitro*, pharmacological inhibition of BRAF-V600E in primary hairy cells causes MEK and ERK dephosphorylation, followed by loss of the hairy morphology and eventually apoptosis. By immunohistochemistry of HCL patients' samples, we have also confirmed *in vivo* ubiquitous phospho-ERK expression in the anatomic sites (bone marrow and spleen) involved by the disease (Tiacci et al., *Haematologica*, Jan 24 2013 Epub ahead of print).

Our findings establish a strong biological rationale for inhibiting the BRAF-MEK-ERK cascade as a new, targeted therapeutic strategy in HCL. Indeed, small-molecule oral inhibitors exist that specifically block the mutated BRAF kinase (Vemurafenib and Dabrafenib) or the MEK kinase (Trametinib), and that have already shown remarkable (although typically not durable) clinical activity in melanoma patients harboring the BRAF-V600E mutation. We have, therefore, launched an academic, Italian multi-center, phase-2, open label clinical trial to explore Vemurafenib efficacy and safety in HCL patients early relapsing after or refractory to purine analogs.

In conclusion, the BRAF-V600E mutation and the consequent MEK/ERK phosphorylation represent an important new target in HCL, whose detection is becoming increasingly relevant from a diagnostic standpoint and it may become so also from a therapeutic perspective.

## **Jak2 and Mtor Inhibitors in Mpn**

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The chronic myeloproliferative neoplasms (MPN), which include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), are characterized by a V617F point mutation in the exon 14 of Janus Kinase 2 (*JAK2*). The *JAK2V617F* constitutively active protein provides a mechanistic explanation of the most prominent feature of MPNs: a constitutive activation of the JAK signal transducer and activator of transcription (STAT) signaling pathway that is largely responsible for the cytokine hypersensitivity and cytokine independent growth of the mutant cells, as exemplified by the erythropoietin-independent erythroid colonies (EEC) typically found in most PV patients. Transplant, transgenic and conditional knock-in murine models have shown that expression of mutated *JAK2V617F* is sufficient to recapitulate a MPN phenotype, indicating that constitutive activation of the JAK2/STAT pathway deserves a central role in the pathogenesis of MPNs. Therefore JAK2 inhibition has become an attractive target for MPNs treatment with the first-in-class ATP-competitive JAK2 inhibitor Ruxolitinib having been approved recently by FDA and European agency (EMA). Beyond the JAK/STAT network, the involvement of other signalling pathways has also been described in MPNs including the PI3K/Akt/mTOR pathway. PI3K pathway plays a critical role in cell physiology by fine regulating a signalling cascade originated by signals from membrane receptors. Therefore, PI3K pathway represents a potential target for treatment of some cancers, potentially including MPN. The mammalian target of rapamycin (mTOR) was originally described in yeast as the target of the macrolide rapamycin with a potent immunosuppressant activity. Several pathway inhibitors were developed from the founding members rapamycin including the allosteric everolimus, temsirolimus and AZD8055 mTORC1 inhibitors, perifosine and LY294002, respectively Akt and PI3K inhibitors or the dual mTORC1/mTORC2 inhibitors PP242 and AZD2014 up to the latest class of catalytic inhibitors BKM120,

BYL719 (PI3K-inhibitors) and BEZ235 (PI3K/mTORC1/mTORC2-inhibitor). Taken together, these observations lead to explore the effects of PI3K pathway inhibition in MPN models both *in-vitro* and *in-vivo* alone and in combination with JAK2 inhibitors in order to block at the same time two hyperactivated signalling networks in MPNs. Everolimus has been employed in a phase 1/2 clinical trial<sup>23</sup>; 39 MF patients were enrolled, 30 in phase 2, both primary (n=23) and secondary MF (n=16). The aim of the phase I was to establish the MTD, that was not reached at 10 mg per day that was thus employed for phase II. We observed significant reduction of the spleen in 13 patients (2 complete remission and 11 partial remission, according to EUMNET criteria) and a rapid and marked reduction of constitutional symptoms and pruritus in more than 80% of the subjects. According to IWG-MRT criteria, responses were categorized in 23% of the patients, with one partial remission and 20% clinical improvement responses in anemia were conversely observed in 25% of anemic subjects. Biomarker analysis showed that responder patients had significantly lower levels of CCDN1 mRNA in granulocytes (a target of mTOR) and also lower proportion of phosphorylated p70S6K. Treatment was well tolerated; low-grade mucositis and clinically irrelevant increase of lipids were the commonest side effect reported. Overall, these data provided a proof of evidence of the potential clinical efficacy of PI3K pathway inhibition in MPN treatment with mechanisms at least in part different from those described for ruxolitinib, since they were not apparently mediated by normalization of the inflammatory cytokine milieu.

In this presentation we will update information on the current research on the use of JAK2 and PI3K inhibitors, alone and in combination, in different cellular models of MPN.

## **New drugs in childhood ALL**

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### **Abstract**

Through the application of reliable prognostic factors and risk-oriented treatment protocols, childhood acute lymphoblastic leukemia (ALL) can be cured in almost 85% of cases. However, relapse, the most common cause of treatment failure in pediatric ALL, occurs in approximately 15-20% of patients. Although the relapsing clone may be undetectable after post-induction therapy, it can be initially evidenced as minimal residual disease (MRD) and subsequently as overt disease recurrence. Over the last decade, several biological and clinical differences have been identified between leukemic cells at diagnosis and relapse, including acquisition of new chromosomal abnormalities and gene mutations, and reduced responsiveness to chemotherapeutics. Allogeneic hematopoietic stem cell transplantation (HSCT) is widely used for patients with relapsed ALL. While many studies have documented that HSCT is superior to chemotherapy in early bone marrow (BM) relapse or in relapsed T-ALL, the benefit of HSCT for patients with late BM relapse has not been firmly established. Standard salvage regimens for relapsed ALL are mostly based on different combinations of the same agents used in frontline therapy. Innovative approaches are urgently needed to improve the outcome of children with relapsed ALL, especially when poor prognostic factors are present. The last decade has witnessed the development of novel pharmacological agents, including nucleoside analogues such as clofarabine and nelarabine, anti-CD22 monoclonal antibodies and bispecific, anti-CD3/CD19 antibodies, new formulations of existing chemotherapeutic agents and targeted molecules such as tyrosine kinase inhibitors and FLT3 inhibitors. This article summarizes the results of the most recent clinical trials with novel agents in childhood relapsed ALL and discusses how these molecules should be integrated into combination regimens with the aim at further improving clinical outcome.

## **Bcl2 Inhibitors in Hematological Malignancies**

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The Bcl2 family members play a central role in regulating programmed cell death (apoptosis) and arbitrating the cellular fate through an accurate balance between pro-apoptotic (Bax, Bak, BH3-only proteins) and pro-survival (Bcl-2 and its closest homologues, Bcl-xL, Bcl-w, Mcl-1) factors. Deregulation of Bcl2 family proteins contributes to programmed cell death evasion, that is a hallmark of human cancers and it is often related to (chemo)therapy resistance.

High Bcl2 levels have been detected in most human lymphoid malignancies, not limited to follicular lymphoma (where the role of Bcl2 overexpression is driven by the t[14;18] translocation), but also B-cell chronic lymphocytic leukemia (CLL) and multiple myeloma. For all these reasons, the opportunity to induce apoptosis by targeting Bcl2 proteins is considered a potentially promising therapeutic approach in hematological malignancies. Bcl2 family inhibition strategies currently explored in Phase I, II and III clinical trials are essentially two: 1) the use of antisense-based strategies to knockdown Bcl2 or Bclxl expression (e.g. oblimersen) or 2) the use of synthetic BH3 mimetics i.e small molecules binding to anti-apoptotic inhibitors thereby allowing the pro-apoptotic activity of BH3-only molecules (e.g. obatoclax, AT-101, ABT-737 and its derivative ABT-263). Several of these drugs demonstrated relevant clinical activity as single-agent or in combination therapy, with the most significant drawbacks in clinical use being represented by challenging pharmacokinetic profile (e.g. iv administration, high-levels of plasma proteins binding) and off-target side effects (e.g. gastrointestinal toxicity, thrombocytopenia).

Further clinical development of the current compounds is eagerly awaited and hopefully, in the next future, Bcl2 inhibitors (alone or in combination with immuno- and/or chemo-therapeutic agents) will represent target-specific drugs expanding our therapeutic armamentarium in the fight against hematologic malignancies.