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Highlights of the First International “Immunotherapy in Pediatric Oncology: Progress and Challenges” Meeting

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Abstract

The first annual conference on immunotherapy in pediatric oncology was held in Bethesda, MD, USA, from September 9–10, 2008 to discuss the state-of-the-art of immunotherapeutic strategies currently being explored in pediatric oncology. Major topics included targeting cell surface receptors, understanding and improving T cell-based therapies, augmenting innate immune strategies and enhancing graft-versus-leukemia for pediatric malignancies. As can be seen in the summaries of the individual presentations, significant progress has been made in developing preclinical models of pediatric tumors while a variety of novel immunobiologic therapies are approaching, or already in, the clinic. While there is much excitement about the potential utility of these agents, a great deal of challenges lie ahead in improving the efficacy of each of these modalities as well as getting them to patients in a timely fashion. The resulting discussions will hopefully lead to new collaborations and insight for further translational and clinical studies.

Introduction

Much progress has been made in understanding the tumor:immune interface. We now know that most tumors possess antigens that can be recognized by both the T cell and B cell arms of the immune system, and that natural killer (NK)-mediated anti-tumor effects can confer improved survival from malignant disease. At the same time, the earliest stages of tumor growth involve a dynamic interplay between the growing tumor and host immune responses that ultimately endow the tumor with immune-evasive properties. Patients with progressive tumor growth often maintain the capacity to recognize their tumor, and ongoing immune responses can contribute to the control of minimal residual neoplastic disease, however such responses are insufficient in most clinical settings.

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Despite progress in the larger field of cancer immunobiology, we still know very little about the specific interactions between pediatric tumors and the immune system. Identification of optimal immune targets and translation of effective immune-based therapies for pediatric cancer await studies that specifically focus on the immunobiology of the host:tumor interface in pediatric cancer. In September 2008, the National Institutes of Health (NIH) Office of Rare Diseases, the National Cancer Institute (NCI)-Center for Cancer Research's Center of Excellence in Immunology and the NCI-Pediatric Oncology Branch sponsored a two-day symposium aimed at bringing together researchers engaged in developing immune-based therapies for pediatric cancer. This summary highlights the translational and clinical work presented at the symposium followed by the individual abstracts submitted to this meeting.

Targeting Cell Surface Receptors on Pediatric Tumors

Monoclonal antibodies

Monoclonal antibodies (moAbs) represent the most effective form of immunotherapy for cancer to date. The administration of moAbs targeting CD20 and ErbB2 has substantially improved outcomes in adult lymphoma and breast carcinoma. However, pediatric lymphomas and leukemia rarely express CD20, and the ErbB2 gene is not amplified in pediatric cancers, rendering surface expression insufficient to induce anti-tumor effects with currently available moAbs. The GD2 antigen expressed on neuroblastoma however can be efficiently targeted with moAbs. 3F8 and hu14.18 are two anti-GD2 moAbs currently in clinical trials. The humanized 14.18 moAb has been conjugated to interleukin-2 (IL-2) (hu14.18-IL2) in an attempt to increase NK activation resulting in enhanced antibody-dependent cell-mediated cytotoxicity (ADCC)(1).

Dr. Paul Sondel (Univ. of Wisconsin) presented results of a recent phase II trial through the Children's Oncology Group (COG) on hu14.18-IL-2, which seems to work in preclinical models through antibody-dependent cellular cytotoxicity (ADCC) by natural killer (NK) cells. While no responses were seen in patients with bulky disease, there were complete responses (CRs) observed in patients with either MIBG-positive or bone marrow-positive minimal residual disease, as predicted by preclinical models. In a preclinical murine model, major histocompatibility complex (MHC) expression was higher in tumors that escaped from NK mediated immunotherapy. In contrast, tumors escaping from T cell mediated immunotherapy (FLT3 ligand) show downregulation of MHC expression. Recent studies in mice indicate that combination of NK and T cell mediated approaches (hu14.18-IL2 + FLT3 ligand) results in greater antitumor activity(2). Future studies will seek to confirm activity of hu14.18-IL2 in neuroblastoma patients with low tumor burden and explore the activity of this agent with other cytotoxic or immunomodulatory agents.

Dr. Nai-Kong Cheung (Memorial Sloan Kettering) presented results from the murine moAb 3F8, which also targets GD2, in the context of post-induction chemotherapy for neuroblastoma over the course of 2 decades. 3F8 appears to stay on tumors longer than other anti-GD2 moAbs, and induces 20–25% responses in the bone marrow as a monotherapy. 3F8 was given with subcutaneous GM-CSF to promote ADCC, which correlates with the FCGR2A (R/R and R/H) genotype polymorphism, in patients with refractory neuroblastoma. In an ongoing phase II trial, this combination induced CRs in the bone marrow in ~80% of patients(3), and ~40% CRs of MIBG⁺ disease. With 3F8, there was a survival advantage among patients receiving subcutaneous GMCSF versus intravenous GMCSF. Radiolabelling 3F8 with ¹³¹I for systemic disease does not add to long term survival, although it seems to be effective as part of salvage regimen for patients which brain metastases(4).

Dr. Robert Seeger (Children's Hospital of Los Angeles) emphasized the importance of a diverse immunotherapy portfolio when attempting to target malignancies with immune-based therapies. This observation is especially pertinent since the method of escape from one mode of immunotherapy may be optimally targeted by another modality. For example, MHC-mediated downregulation in response to T cell based therapies can render a tumor more receptive to NK cell-mediated therapies. Hence, Dr. Seeger has focused on combination therapies whereby moAbs are combined with agents that augment ADCC. While NK cells alone do not cure mice with neuroblastoma, NK cells combined with the anti-GD2 moAb ch14.18 cured 70% of mice if started 7 days after tumor challenge, whereas waiting 21 days diminished the benefit. Their group was able to significantly improve immunotherapy of 21-day tumors if they added bolus and metronomic cyclophosphamide, zoledronic acid and bevacizumab to NK plus moAb, which slowed tumor growth and prolonged survival in their model. He also mentioned how his group, and others, is using microarrays of neuroblastoma to determine prognosis(5' 6).

Dr. Alan Wayne (National Cancer Institute) discussed targeting CD22, which is highly expressed on the majority of blasts from patients with pediatric acute lymphoblastic leukemia (ALL), using BL-22, a recombinant immunotoxin composed of an anti-CD22 single chain variable fragment linked to modified *Pseudomonas* exotoxin. This agent reproducibly induces killing of ALL blasts *in vitro*, has been well tolerated in Phase I and II trials in adults, and has proven efficacy against hairy cell leukemia in adults. Administration of BL22 to children in a Phase I trial was well tolerated. Common reversible side effects included decrease in the serum albumin, increase in hepatic transaminases and microscopic proteinuria, as seen in adults(7). About 13% of patients developed neutralizing antibodies. Clinical activity was noted at all dose levels. He noted that treating patients with MRD seemed to increase the exposure to immunotoxin, consistent with rapid binding by CD22 positive blasts. A second generation antibody called higher affinity (HA)-22 is currently being studied in a Phase I trial at the NCI and St. Jude Children's Research Hospital.

As we move forward with moAb technology, it appears there are some salient points that must be addressed. We have learned that combining moAb with cytokines may enhance the efficacy of these agents by promoting ADCC, but possibly at the price of additive toxicity, such as the vascular leak observed with IL-2. It appears that the amount of time the moAb spends in contact with its target is critical, and treating MRD may be a more fruitful approach than treating bulky disease because it increases the half-life of the moAb in the serum. Lastly, licensing and proprietary issues associated with all of these technologies does restrict the ability to use these agents in combination with each other, and pharmaceutical companies need to share these compounds more freely so we can maximize our understanding of their immunobiology. For example, perhaps giving hu14.18 and 3F8 together may maximize targeting all GD2⁺ cells and lead to greater efficacy in neuroblastoma patients than as single agents.

Chimeric antigen receptors

MHC downregulation may limit the therapeutic efficacy of tumor-directed T cells. Several groups are currently investigating approaches to use gene therapy to exploit the potent cytotoxic activity of T cells while avoiding the need for T cells to recognize cell-surface tumor-derived antigenic peptides in the context of MHC. This can be accomplished by genetically modifying T cells to express "chimeric antigen receptors" (CARs). These endow the T cell with a receptor that harnesses the antigen-recognition of an antibody to activate the T cells to expand, persist, and kill.

Dr. Laurence Cooper (M.D. Anderson Cancer Center) discussed his group's use of T cells that have been genetically engineered to express a CAR that activates via CD3 and CD28

endodomains to target B-cell malignancies(8- 9). The CAR is introduced using a *Sleeping Beauty* (SB) transposon/transposase system to integrate the transgene into the primary T cell. When the two SB DNA plasmids are electro-transferred together, there is a 71% improvement in CAR expression compared with when transposon-expressing CAR is introduced without transposase(10). Current work is focused upon improving signaling through the CAR and translating this technology into clinical trials.

Dr. Xianzheng Zhou (Univ. of Minnesota) also presented work on a CAR targeting CD19 developed through a *Sleeping Beauty* transposon system, which he pointed out as providing stable integration at a low cost, and was less immunogenic than using a virus. It also might be safer because the integration is random rather than into the promoter region. His group modified both peripheral blood and umbilical cord blood-derived T- cells to express a CD19 CAR/CD20 dual gene. In this instance, the anti-CD20 moAb rituximab can be used to enrich CARs administered to a patient. This CAR/CD20 modified T-cell shows specific cytotoxicity against CD19⁺ cell lines, and prolongs survival in animals challenged with these tumors(11).

Dr. Gianpietro Dotti (Baylor College of Medicine) presented the results of a Phase I clinical trial in which patients with neuroblastoma received adoptive transfer of T lymphocytes genetically manipulated to express a CAR targeting GD2 expressed by neuroblasts. Baylor's group incorporated that CAR on virus-specific cytotoxic T lymphocytes (CTLs) such as Epstein-Barr-Virus-specific-CTLs (EBV-CTLs) in order to enhance the *in vivo* persistence of these cells, as they can receive a complete activation by the engagement of their native $\alpha\beta$ -T cell receptor with antigen-presenting cells expressing latent antigens of EBV(12). Patients with neuroblastoma received both autologous EBV-CTLs and activated T lymphocytes (ATCs) genetically manipulated to express a CAR targeting GD2. Three different cell dose levels were used, and they found that the CAR-EBV-CTLs persisted longer and at higher levels in the peripheral blood than the CAR-ATCs. The treatment did produce tumor responses, including one CR.

Dr. Michael Jensen (City of Hope) has focused efforts on developing CARs targeting IL13R α 2, which is expressed on medulloblastoma and most gliomas(13- 14). He emphasized the importance of developing approaches to enhance the survival of these cells *in vivo* following adoptive transfer, and presented data suggesting that genetic transfer into starting populations of central memory cells may result in superior long term survival than when non-selected T cells undergo gene transfer(15). Central memory T cells can be targeted by using EBV-specific T cells for gene transfer or by immunomagnetic enrichment for central memory populations prior to gene transfer.

Since the field of CAR technology is a bit newer than moAbs, most of the current work has been in preclinical development and testing, and we have less Phase I and II trial data to analyze. Although there is a great deal of promise for the agents presented, certainly other CARs have already been tested in children with neuroblastoma, demonstrating the potential for bringing these agents to the clinic(16). In addition, CARs that target other receptors are also in development that may offer new breakthroughs in lymphomas or medulloblastoma(17- 18). As we have mastered integrating receptors for a variety of targets, like CD19 or GD2, using the *Sleeping Beauty* or viral-based systems, identifying strategies that help these cells persist will be critical to the success of these therapies.

T Cell Based Therapies for Pediatric Malignancies

Several groups are involved in attempts to induce T cell immunity toward pediatric tumors. Dr. Crystal Mackall (National Cancer Institute) presented data from a recently completed Phase II trial of consolidative immunotherapy that incorporated autologous adoptive cell

transfer and dendritic cell (DC) vaccination for patients with high risk and recurrent pediatric sarcomas. The data demonstrated favorable overall survivals using autologous T cell transfer following cytoreductive chemotherapy, but immune responses to peptides targeted with DC vaccines were modest(19). As a result, future studies are utilizing tumor lysate-based vaccination, and newer approaches to DC maturation have been developed. A new Phase II trial for Ewing sarcoma, rhabdomyosarcoma or neuroblastoma combines regulatory T cell (Treg) depletion, which in preclinical models has improved the effectiveness of adoptive immunotherapy, with rhIL-7 administration to induce peripheral expansion of T cells and augment immune responses utilizing tumor lysate-based vaccination. She also presented data demonstrating that rhIL-7 is capable of safe, transient T cell expansion in humans associated with increased T cell repertoire diversity(20).

Dr. Stephan Grupp (Univ. of Pennsylvania) presented a similar approach for consolidative immunotherapy that exploits lymphopenia to expand T cells collected at diagnosis from neuroblastoma patients after tandem autologous stem cell transplants (SCTs). In a phase II trial, CD3/CD28 costimulated/expanded T cells were given to patients at day+12 after SCT, and patients showed robust recovery of CD4⁺ and CD8⁺ T cell counts. In a follow-up randomized pilot study, T cells were given at day+2 or day+90 to assess T cell recovery and responses to pneumococcal vaccination given 12 and 60 days post-SCT. The cohort that received T cells on day+2 had protective pneumococcal responses, but some patients developed a self-limited engraftment syndrome clinically similar to graft-versus-host-disease (GVHD). Survivin is a potentially universal cancer antigen, and is an anti-apoptotic protein expressed in high levels in neuroblastomas. The group has observed survivin-specific T cells in neuroblastoma patients(21), and future studies will aim to optimize a survivin-based, tumor-directed vaccine into this platform.

Dr. Helen Heslop (Baylor College of Medicine) discussed how Type 3 latency EBV⁺ lymphomas are very immunogenic such that adoptively transferred donor-derived T cell lines specific for EBV, given after allogeneic SCT as a means for targeting EBV-associated lymphoproliferative syndrome, have generated sustained CRs in 11/13 patients. Further, complete protection was observed when EBV-specific T cells were prophylactically administered to 101/101 high-risk patients. These T cells can persist for up to 8 years in patients. However treating immunocompetent patients is a greater challenge since their tumors have mechanisms of evasion in place. Her group manufactured polyclonal EBV-specific cytotoxic T cells and administered them to patients with advanced nasopharyngeal carcinoma in two phase I trials, and saw an overall response rate of 50% in patients with active disease. More recently, they used EBV-specific T cells that target the subdominant antigens LMP1 and LMP2 after chemotherapy or autograft in 24 patients with Type 2 latency EBV⁺ lymphoma, with 12/13 of the high risk patients remaining in remission up to 4.5 years after therapy and 9 of 11 patients with active disease having clinical responses(22).

Dr. Richard O'Reilly (Memorial Sloan Kettering) presented substantial data demonstrating that WT1 is highly expressed in a variety of adult and childhood malignancies including gliomas, acute and chronic leukemia, Wilm's tumor, desmoplastic small round cell tumor, and rhabdomyosarcoma. Moreover, several groups have demonstrated that WT1 is immunogenic and that immune responses toward WT1 can lyse tumor cells. Hence, Dr. O'Reilly has constructed a pool of pentadeca- (15-mer) peptides that span the Wilm's tumor antigen (WT-1) that can sensitize donor-derived T cells for treatment of WT-1⁺ leukemias(23). His group has utilized epitope mapping to select and define 26 relevant epitopes that elicit a robust immune response. Using a xenograft tumor model with a WT-1-expressing leukemia or solid tumor, they have shown that adoptive transfer of WT1-EBV transgenic T cells selectively causes regression of established tumors as well as prevents growth of new tumors when leukemias are infused at the time of the lymphocyte infusion. A

combined phase I/II trial is underway to look at infusing these transgenic T cells after a T cell-depleted allogeneic SCT for patients with WT-1 expressing leukemias or myelodysplastic syndrome.

Dr. Bryon Johnson (Medical College of Wisconsin) presented a murine model of neuroblastoma that is under study to optimize approaches for consolidative immunotherapy. Using a syngeneic SCT platform, his group implants a luciferase-expressing tumor (AGN2a) on day-8, supplements the graft with added T cells on day+0 of transplant, then gives a vaccine consisting of irradiated AGN2a cells genetically modified to express the immune stimulatory molecules CD54, CD80, CD86 and CD137L on days+2, 7 and 14 post-SCT. They have found that vaccinating during the first 2 weeks post-SCT induces anti-tumor immunity capable of eliminating established tumors. He also has used T cells sensitized to tumor antigens, and demonstrated that these T cells improve anti-tumor efficacy. The tumor responses mediated by T cells sensitized to tumor antigens are CD8⁺ dependent and are improved after CD4⁺ depletion in part due to the loss of Tregs, but long-term CD8⁺ memory responses were also lost(24). Selective elimination of Tregs similarly enhances tumor immunity but does not compromise CD8⁺ memory, suggesting that this may be a viable approach for generating potent long-term anti-tumor responses against neuroblastoma(25).

Dr. John Ohlfest (Univ. of Minnesota) presented data on targeting brain tumor stem cells (BTSCs) that form the therapy-resistant portion of gliomas. His group has found that BTSCs exhibit a heterogeneous expression of MHC class I or NK cell ligands and thus are difficult targets for immunotherapy(26). He emphasized the substantial heterogeneity present within clinical tumors, and the potential for targeting of a smaller, and perhaps more homogenous, population of BTSCs as a means for immunologic targeting of the subset responsible for tumor growth. He presented work aimed at developing T cell-based therapies for brain tumors. By combining administration of CpG nucleotides with a tumor lysate vaccine and intratumoral gamma interferon gene transfer in a murine model of glioblastoma multiforme, his group has demonstrated that gliomas can be made immunogenic, resulting in improved trafficking of effector cells, enhanced tumor cell lysis and overall survival(27). He is now moving these observations into a more clinically relevant canine model.

Dr. Alex Huang (Case Western Reserve) presented studies using intravital 2-photon microscopy to identify critical chemokines that regulate T cell trafficking to lymph nodes during the early stages of T cell priming and memory generation. Using genetically engineered tumors, Dr. Huang also demonstrated the enhancing effect of inflammatory chemokines in primary tumor rejection and in the generation of systemic anti-tumor immune responses that could ultimately be targeted in the clinical setting(28, 29).

Collectively, T cell-based therapies seem to be effective at expanding both CD4⁺ and CD8⁺ subsets, as well as promoting responses to infectious vaccines, but generation of Tregs appear to inhibit anti-tumor responses. Future studies will need to consider Treg depletion or inhibition of Treg function to be more effective. As observed with mAb trials, T cell-based therapies may function better in the setting of MRD than in patients with gross residual disease. We are also finally developing strategies to move these therapies past the blood-brain barrier to target brain tumors in a specific fashion.

Augmenting Innate Immunity for Pediatric Cancers

The innate immune system spans phagocytes, monocytes, macrophages, dendritic cells and NK cells. Each of these cells exhibit effector function in their own right, but in addition, the responses of these innate immune cells often serve to initiate the adaptive immune system, which can result in long term immunologic memory. Several groups are involved in

activating the innate immune system to induce direct anti-tumor effects and/or to help initiate or augment adaptive anti-tumor immunity.

Toll-like receptors

One approach to activate innate immunity is through the use of CpG oligodeoxynucleotides (ODNs), which mimic viral and bacterial DNA and therefore stimulate toll-like receptor (TLR)9 on innate immune cells and some tumor cells. Dr. Kirk Schultz (British Columbia Children's Hospital) presented studies demonstrating that CpGs can increase the immunogenicity of ALL cells, resulting in enhanced T cell-mediated reactivity(30). When CpGs were administered *in vivo* to mice bearing ALL xenografts, anti-tumor effects were observed, related both to tumor-directed increases in immunogenicity as well as anti-tumor effects mediated by the innate immune system *in vivo*. In addition, depletion of NK cells by anti-asialo-GM1 treatment significantly reduced the *in vivo* antileukemic activity of CpG ODN(31). Clinical trials to evaluate the efficacy of CpG ODNs for leukemia are being developed.

Dr. Bruce Blazar (Univ. of Minnesota) presented promising data from murine models showing enhanced rejection of AML in mice treated with CpGs as well. His group has shown that administering TLR9 agonists to naïve mice two days prior to AML challenge allowed mice challenged with AML to tolerate at least 100 times a lethal tumor dose, but giving CpG ODNs later after syngeneic SCT allowed recipients to tolerate 10 times a lethal dose(32). After a major-mismatched allogeneic SCT, giving TLR9 agonists on day 0 accelerated GVHD(33), but when it was used with a DLI 15 days after a T cell-depleted SCT, TLR9 agonists enhanced survival to AML challenge(32). Separate class I and class II antigen major mismatch SCT models demonstrated that TLR9 agonists led to host anti-donor rejection and low chimerism post-allogeneic SCT(33). Lastly, a phase I trial of TLR7 agonist (852A) for refractory solid tumors was presented, and an increase in alpha interferon levels was observed along with enhanced NK cell function(34). Preclinical allogeneic SCT models of TLR7/8 agonists given in the peri-SCT period also show accelerated GVHD and graft rejection, suggesting we should be cautious if TLR 7/8 or TLR9 agonists are to be used in humans early post-SCT.

Dr. Gregor Reid (The Children's Hospital of Philadelphia) presented preliminary work showing that CpG ODNs enhanced survival in mice bearing syngeneic murine ALL. The death of leukemia cells *in vivo* was independent of the ability of ALL cells to respond directly to CpG ODNs, and correlated with the production of pro-inflammatory cytokines by the host(31). CpG ODN stimulated ALL cells also showed increased co-stimulatory molecule expression and skewed T cells toward a T helper 1 cytokine profile(30, 35).

Exploitation of the tumor microenvironment for therapeutic benefit

Dr. Eugenie Kleinerman (M.D. Anderson) summarized her work in developing new approaches to treat lung metastases in patients with osteosarcoma. She discussed the studies demonstrating improved survival in pet dogs with osteosarcoma that were treated with MTP-PE following surgery compared to those treated with surgery alone. She also gave the most recent update from the Phase III COG trial in newly diagnosed osteosarcoma patients. This trial involved almost 700 patients and demonstrated a statistically superior long-term survival rate in those patients treated with MTP-PE together with either 3-drug or 4-drug chemotherapy compared to the group that received 3-drug or 4-drug chemotherapy without MTP-PE(36). In addition, Dr. Kleinerman presented her investigations using a human osteosarcoma lung metastasis nude mouse model which demonstrated that the expression of Fas on osteosarcoma cells correlates inversely with metastatic potential, and that up-regulating Fas expression resulted in the regression of established pulmonary metastases(37,

38). The *aerosol* administration of IL-12 gene therapy(39), gemcitabine(40) or liposomal 9-nitro camptothecin (L-9NC)(41), increased Fas expression in the pulmonary metastases with subsequent tumor regression. This effect was mediated by Fas ligand (FasL) in the lung(40). Aerosol gemcitabine showed no activity in FasL-deficient mice, showing for the first time that the tumor microenvironment plays a critical role in therapeutic efficacy. These agents may have potential in the treatment of patients with relapsed disease. Aerosolized L-9NC is being combined with oral temozolomide in a clinical trial at M.D. Anderson.

Leonid Metelitsa and his colleagues (Children's Hospital of Los Angeles) have investigated the immune microenvironment of neuroblastomas by analyzing gene expression in primary tumors and studying the infiltration of innate cells into these tumors(42). He discussed why neuroblastoma patients with better survival have increased numbers of NKT cells in their tumors. Because neuroblastoma cells are CD1d⁻, he hypothesized that NKT cells may target the CD1d⁺ cells of the tumor microenvironment. Dr. Metelitsa presented data that tumor-associated macrophages (TAMs) express CD1d and can be specifically recognized and killed by NKT cells. His group found that TAMs are the main source of IL-6 in primary tumors and metastatic bone marrows. IL-6 directly stimulates growth of neuroblastoma cells *in vitro* and *in vivo*, and expression of IL-6, IL-6R, and other TAM-related genes associates with poor survival. He concluded that killing of tumor-promoting TAMs may represent a novel mechanism of NKT cell anti-tumor activity.

NK cell-based therapies

NK cells represent another arm of the innate immune system that has been extensively studied in cancer immunobiology. They are attractive candidates for therapeutics since they do not require MHC expression by cancer cells and in fact, NK-mediated killing is increased when MHC expression is diminished. Recent studies have shed more light on the activating and inhibitory factors that control NK mediated killing of cancer cells.

Dr. Dario Campana (St. Jude Children's Research Hospital) also presented data evaluating the potential of *ex vivo*-expanded NK cells for treating leukemias and solid tumors of childhood. He used a genetically modified cell line (the leukemia cell line K562 transduced with CD137L and membrane-bound IL-15) to activate and expand human NK cells. A median expansion of more than 20-fold was observed after 7 days of culture, with no preferential expansion of any NK cell subset(43). The NK cells generated in this culture system express gene expression profiles that are different than those expressed by primary or IL-2-activated NK cells. Expanded NK cells showed a high cytotoxicity to myeloid leukemias and even some pediatric solid tumors, including Ewing sarcoma, rhabdomyosarcoma and neuroblastoma. Their cytotoxicity against ALL cells was relatively weak. To overcome this limitation, the group transduced these NK cells with a CAR against CD19 (anti-CD19-BB-z), which resulted in enhanced cytokine production of gamma interferon and GM-CSF, and markedly enhanced killing of ALL cells(43).

Dr. A.C. Lankester (Leiden Univ., the Netherlands) presented a series of studies investigating NK receptor ligands on Ewing sarcoma cell lines. These studies demonstrated that Ewing sarcomas express ligands for activating NK receptors, and that these receptors are critical for NK-mediated lysis of Ewing sarcoma. Notably, even chemoresistant Ewing sarcoma cell lines are susceptible to NK-mediated lysis. The efficacy of lysis was enhanced with IL-15(44).

We are steadily improving our ability to develop clinical-grade cell based-therapies for refractory tumors. Stimulation of TLRs appears to yield durable anti-leukemic responses, but caution must be used in the setting of allogeneic SCT. Regarding cell-based therapies, artificial APCs are going to play a major role in the future in expanding these cells further *ex*

vivo so that adequate doses can be delivered to patients in a timely and effective fashion. Additionally, studies involving the tumor microenvironment have demonstrated that effector cell trafficking to tumors is also a critical determinant of success, and must be dealt with simultaneously.

Augmenting Graft-versus-Leukemia for Pediatric Hematological Malignancies

Allogeneic hematopoietic SCT arguably represents the most effective form of immunotherapy for hematological neoplasms to date given that a large part of the curative effect of this procedure results from immunologically-mediated graft-versus-leukemia (GVL). Dr. Franco Locatelli (University of Pavia, Italy) presented the current state-of-the-art of allogeneic SCT for pediatric leukemia. Although it has been previously reported that the potency of GVL against ALL is less than for myeloid leukemias, Dr. Locatelli presented data demonstrating that the occurrence of chronic GVHD is associated with a lower risk of leukemia recurrence in children with ALL(45). Also, grade I/II/III acute GVHD improves survival for patients who undergo allogeneic SCT for ALL, preventing the incidence of cumulative of leukemia relapse. Furthermore, while in adult patients, allogeneic NK cells seem to produce better disease-free survival for AML than for ALL, he presented data that in pediatric leukemia, ALL may be the better target. Biological support to this observation is given by a recent paper documenting that in pediatric recipients of a haploidentical SCT, donor-derived alloreactive NK cells are generated and persist for many years(46). Finally, provocative data were presented demonstrating that in the setting of haploidentical SCT, maternal SCT donors lead to better survival as a result of both lower relapse rate and transplant-related mortality than paternal donors, even to female recipients(47). This clinical observation raises the issue of transplacental trafficking of host antigens sensitizing the mother; the few T cells transferred with the graft (i.e. a total of roughly 0.5 to 1 million cells), could undergo unopposed proliferation after transplantation by virtue of the absence of pharmacological GVHD prophylaxis.

Dr. R. Maarten Egeler (Leiden Univ., The Netherlands) presented data from the Dutch Oncology Children's Group on how MRD levels prior to SCT affect outcome for ALL patients(48). His group is investigating if early tapering of cyclosporine, followed by incremental donor lymphocyte infusions (DLIs), could be safely performed in patients with no or low grade GVHD. MRD detection assays on bone marrow were performed pre-SCT and patients were stratified into "MRD low" and "MRD high" cohorts. If patients were MRD low, there was no intervention, and their cyclosporine was tapered by day 100. If patients were MRD high, cyclosporine was tapered at week 4 or 5, and then 3–4 DLIs were given starting at weeks 9–10. The DLIs have not caused GVHD in any patients thus far. The results confirmed that pre-transplant MRD did predict a higher rate of relapse following transplant, showed that the rapid taper and DLI could be performed safely and raised the prospect that such therapy may delay recurrence of ALL following transplant.

Dr. Rupert Handgretinger (Univ. of Tuebingen, Germany) presented data using immune-based therapies following haploidentical SCT for patients with hematologic malignancies. Whereas most investigators have used CD34 selection to prevent T cell mediated-GVHD in this setting, Dr. Handgretinger illustrated the role for selective T cell depletion, which leaves substantial numbers of NK cells and NK precursors within the grafts of adult reduced-intensity SCT patients(49). Such grafts show greater NK expansion post-transplant, which may be associated with improved antitumor effects. They have learned that using G-CSF to mobilize CD34⁺ cells from donors may be deleterious on NK cells, and suggested exploring GM-CSF instead. He also presented the first clinical data on the *ex vivo* activation of NK cells with IL-15 and their subsequent infusion post-transplant. He also presented a large-

scale method of depleting $\alpha\beta$ -T cells to enrich peripheral blood stem cells for NK cells and gamma-delta T cells(50). Using this platform, he presented data infusing the moAb hu14.18 with NK cells or gamma-delta T cells for treating refractory neuroblastoma in a neuroblastoma mouse model.

Dr. Dagmar Dilloo (Heinrich Heine Univ., Germany) presented approaches to enhance GVL in ALL. She has demonstrated that modulation of the ALL blast can also be used to augment GVL, and improved immunogenicity is seen after CD40 activation, via CD70 and CD80/86 upregulation, resulting in substantial T cell priming(51-52). As blockade of CD70 prevents effector T cell expansion and reduces cytotoxicity, strategies facilitating upregulation of CD70 on antigen-presenting cells are critical for augmenting the quality of an anti-leukemic response against ALL. In a pilot clinical study for patients with relapsed ALL post-allogeneic SCT, her group employed DCs pulsed with ALL cell lysate as a vaccine in combination with DLI, and induced responses in all patients, with some surviving long-term. Future studies will aim to use CD40-activated ALL or lysate-pulsed professional DCs for T cell priming, with manipulation of the CD70:CD27 axis serving as a means for augmenting T cell reactivity not only toward ALL, but also to a variety of different tumor antigens.

Dr. A. John Barrett (National Heart, Lung and Blood Institute) presented clinical work aimed at augmenting GVL by selective depletion of alloreactive T cells. Initial studies used CD25 immunomagnetic bead-based depletion following mixed lymphocyte cultures to deplete alloreactive T cells. More recent studies have exploited the differential capacity for activated versus resting T cells to extrude a photosensitizer, which can also profoundly and selectively deplete alloreactive T cells when exposed to light. Early clinical results with this approach show minimal GVHD following alloreactive T cell depletion(53). Future studies will build upon this platform, and seek to augment tumor-directed immune reactivity by combining alloreactive T cell depletion with tumor antigen based vaccines.

Dr. Terry Fry (Children's National Medical Center) presented preclinical studies aimed at enhancing GVL using tumor vaccine-based approaches(54). His data demonstrate a critical role for both thymic-dependent immune reconstitution following transplant in order for effective immunotherapy to be undertaken because of the risk of immunosuppressive GVHD when T cell doses sufficient to induce meaningful antitumor responses are delivered via DLI. He also demonstrated potent immunosuppressive effects of GVHD on tumor-directed vaccines, thus emphasizing the importance of preventing GVHD if optimal effects of immune-based therapies are to be realized following allogeneic SCT.

The post-SCT environment appears to be a ripe platform for immunotherapy for already high-risk or refractory malignancies, and efforts appear to be headed toward making APCs better able to present tumor antigens, or making tumors better APCs themselves. In the allogeneic SCT setting, GVHD remains a significant obstacle, but a T cell-depleted platform may allow both NK cell-mismatch or delayed T cell add-back therapies to be administered. Therapies that can abrogate GVHD without impacting anti-tumor effects also remain essential.

Summary

While preclinical and clinical testing of immune-based therapies have been pursued for a variety of malignancies, to our knowledge, this meeting reflected the first time clinical and translational immuno-oncologists met to exclusively discuss progress and opportunities pertaining to the use of immune-based therapies specifically for the treatment of childhood malignancies. A growing body of preclinical and clinical data supports the anticipated clinical benefit of certain immune-based treatments for these childhood diseases as clinical testing proceeds. Participants agreed that worldwide collaboration in the testing of these

concepts will augment this progress, and has been aided by this meeting. As such, discussions are underway to convene a similar meeting, likely in ~2 years, and probably in Europe, to continue the development of a worldwide “community” of clinical-translational pediatric immuno-oncologists.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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