A Scientific Overview

This page summarizes recent advancements and future directions as described at the 2007 2nd JMML International Symposium. In order to give you the true flavor of the symposium, acronyms and abbreviations used are footnoted rather than explained in the text.

Session 1: Therapeutic strategies and clinical results—Where have we been?

Session 1, held over dinner on the evening of December 6th, focused on what we have learned from clinical trials and practices in the US, Europe and Japan. The chair for this session was Brian Weiss of the University of Cincinnati.

Todd Cooper reported that the NAJP* trial, AAML0122, closed to accrual with 89 patients, short of its goal of 100. The data from this trial is still in the process of analysis, however the toxicity, tolerability and responsiveness of the FTI* Zarnestra® were determined in the phase II arm. Myelosuppression was the primary toxicity, with the dosage for future trials set at 300 mg/m². Response rates to the FTI were greater in the normalization of bone marrow (65%) compared to response in the liver and spleen normalization (5%). Event-free survival post-BMT* was ~40% with overall survival at ~55% (though statistical analysis is not complete).

From Japan, Seiji Kojima discussed the completed MDS99 study of 22 patients, which showed a 50% survival rate. MDS 03, using a new regimen is underway and currently has 16 patients enrolled. Kojima also described attempts to correlate prognosis with specific genetic mutations however the sample size was too small to correct for age at diagnosis.

Franco Locatelli related the findings of EWOG-MDS* in a study sample of 100 patients. The rate of survival for this study was 64%, with EFS* of 52%, a significant increase from the 1997 study (EFS of 31%). Both relapse and mortality rates increase with the age of diagnosis of the child; children over the age of 4 years at diagnosis have a significantly worse prognosis. Relapse prevention is a current focus of research at EWOG, with an emphasis on rapid withdrawal of immuno-suppression. A 2nd HSCT* after relapse has shown to be effective (3-yr survival of 50%) whereas DLI* has not.

Session 2: Scientific update–animal models and preclinical therapeutics

Coffee, breakfast, more coffee and Session 2 started out bright and early the morning of December 7th, with a look at the latest laboratory research dealing with molecular and mouse models of JMML.

The session chair, Rebecca Chan, reported her work with activating PTPN11 mutants. Three different mutations found in JMML were introduced into cultured mouse bone marrow cells and found to increase the numbers of macrophage and monocyte cells and to increase sensitivity to GM-CSF stimulation of colony formation.
by these cells. Using this cell culture system, Chan looked at the effects of the PTPN11 mutants on transcription factors, the proteins that regulate the timing and quantity of gene expression at the step when DNA is transcribed to RNA. The relative expression of the downstream transcription factor, GATA-2, was reduced while c-Jun was increased and PU.1 was unchanged. These altered transcription factor levels may lead to aberrant monocytic differentiation similar to that seen in JMML. Increases in cell number and colony formation could be counteracted by co-introduction of additional GATA-2, which is important for blood cell differentiation and whose expression is reduced in the leukemic cells. This normalization was dependent on the presence of the carboxy terminal zinc-finger domain of GATA-2.

Gordon Chan (from Ben Neel’s lab) described his introduction into mice of an inducible mutant version of the gene coding for Shp2 (PTPN11) that simulates JMML in the mouse. The same amino acid was mutated from aspartic acid to either tyrosine (as in some forms of JMML) or glycine (as in some forms of Noonan’s/JMML), with the tyrosine mutation resulting in greater severity and earlier fatality. In a bone-marrow engraftment assay, when both normal and JMML-like bone marrow cells are “transplanted” into mice, the JMML bone marrow cells, when present at a 20-fold excess, were not able to out-compete normal bone marrow cells. Some recipients of JMML-like spleen cells show engraftment of blood stem cells expressing the mutant allele, but surprisingly, these mice do not develop JMML-like diseases. Failure to produce disease by engraftment may be related to the rarity of the disease-initiating cells or their inability to engraft in a transplant setting.

Ben Braun discussed another mouse model of JMML, in which the mouse KRAS* gene is replaced by a JMML-mutated human KRAS, complete with the human gene regulatory regions. Similar to human JMML, mutant myeloid cells in these mice compete very well with normal cells and tend to dominate over them, however most mice develop acute leukemia leading to death. PTPN11 mutations were also analyzed in Braun’s lab: deletion of PTPN11 caused death, while activating mutations (as in JMML) caused myelo-proliferative disorders. The phosphatase activity of the Shp2* protein (coded for by the PTPN11 gene) appears to be required for leukemic growth but not for normal blood cell development and this may have therapeutic implications.

Jennifer Lauchle (from Kevin Shannon’s lab) described the use of mice in which the NF1* gene can be turned off in blood cell progenitors to study the relationship of NF1 to JMML and AML*. These mice develop a JMML-like MPD* and can be induced to frank AML by introduction of secondary retroviral-insertion mutants. Evaluation of therapeutic agents in this mouse line show that MEK* inhibitors, which have effects further downstream in the Ras pathway, have no effect on NF1-deficient MPD (JMML-like disorders), however they do temporarily inhibit disease progression in NF1-deficient AML mice. Drug resistance does develop to the MEK inhibitors and this appears to be related to the gene location of the retroviral insertion mutants. This system is promising for identifying genes that modulate response to targeted therapeutic agents.

The final talk in this session was by Malcolm Smith of the NCI*, who detailed the Pediatric Preclinical Testing Program. The PPTP* is designed to test potential therapeutic agents on childhood tumor or leukemia models in xenograft mice.
Xenografts are mice that basically have no immune system and are thus able to tolerate injection and engraftment of human tumor or bone marrow from a particular disease. Treatments for specific diseases can then be tested on the mice and gene expression in the cancer cells can be tested using gene array technology. This system helps to determine which drugs are worthy candidates for clinical trials in children and is a better indicator of clinical potency and tolerability than testing done in cell lines. Several targeted agents are being tested in the ALL* and CML* models and look quite promising. There is great interest in including a JMML xenograft mouse for the testing of new agents for future clinical trials.

Session 3: Scientific update – what can we learn from the patients?

Session 3 began after a quick coffee break. I’d just like to note here that we did NOT run out of coffee this year as we are well aware that science is actually fueled by coffee as much as it is by grant money. In this session the talks dealt with the molecular dissection of individual patient samples to give a better understanding of the disease and to help devise better assays for diagnosis and relapse assessment. Christian Kratz, the session chair introduced this topic.

Christian Flotho discussed the correlation in JMML between genotype, the exact genetic mutation in the DNA, and phenotype, how the disease looks or behaves. JMML patients currently fall into 4 genotypic groups: mutations in the RAS (20-25%), PTPN11 (35%) and NF1 (11%) genes or no gene mutation identified (30%). In 214 patients analyzed the gene that is mutated in an individual tends to correlate with survival and relapse, with mutations in PTPN11 and NF1 mutations giving a poorer prognosis than RAS or unknown mutations, however the age at diagnosis is a better predictor of both survival and relapse. In addition to the gene that is mutated, the exact location and amino acid change within the resulting protein can also affect the severity of the disease. Many JMML patients also have only a single copy of Chromosome 7, but at this point there is no correlation between clinical presentation and monosomy 7. Also, several other chromosomal defects in JMML patients that are too small to be seen on a chromosome spread have been identified using gene array technology.

Sophie Archambeault from Mignon Loh’s laboratory reported on the use of the new TaqMAMA* assay for MRD.* This assay makes use of the PCR* amplification of the specific genetic mutation from patient DNA to detect the presence of disease and is faster and more accurate than % donor chimerism. Using this assay, relapse can be detected by rising levels of mutant DNA much earlier than from clinical symptoms of relapse. These results were recently published in Blood and the assay, after validation, may also prove useful as a diagnostic tool. An interesting observation is that rare patients appear to be in clinical remission despite having low donor chimerism and no detectable DNA mutations as assessed by TaqMAMA. This raises the possibility that the JMML disease-initiating cell has been destroyed but that normal donor-specific stem cells have re-populated the blood and bone marrow. Finally, Archambeault discussed GM-CSF hypersensitivity colony assays that have been set up using patient samples to test responsiveness to potential therapeutic drugs.
Another fast new assay under development as a diagnostic tool is the Phospho-Flow assay described by Nikesh Kotecha. His group led by Garry Nolan and in collaboration with Mignon Loh, has developed a diagnostic panel that uses flow cytometry technology to detect GM-CSF* sensitive cells in the blood of JMML patients. This test looks for a small population of cells that have an abnormal signaling response within the first 15 minutes after GM-CSF exposure. The test can be completed within 1-2 days and thus significantly shortens the time it takes for patients and doctors to get the information needed to make clinical decisions. This test might also be a useful tool to track relapse and to tailor therapy based on specific signaling anomalies particular to each patient. It will need further validation but is very promising.

**Session 4: Future clinical trials for JMML**

After a lunch, a photo break, and of course, more coffee, Session 4 got underway with talks and discussion of future possibilities for JMML clinical trials. This session was chaired and introduced by Dr. Mignon Loh, also a member of The JMML Foundation Board of Directors.

Debbie Sakaguchi proposes the drug Sorafenib, which targets Raf1* a downstream effector of Ras, for an upfront, phase II window on a new North American clinical trial. In xenograft models of other forms of cancer in which the Ras pathway is critical, Sorafenib has been shown to reduce tumor burden. Primary objectives of the proposed study would be: (1) to estimate the response rate and define the acute toxicity of Sorafenib in newly diagnosed patients and (2) to estimate the 2-year EFS* following HSCT*, randomization to rapamycin, and rapid taper of conventional immunosuppression. Correlative biology studies would be included to validate assays including TaqMAMA, phosphoflow cytometry, and colony assays.

Shalini Shenoy discussed past and future improvements in HSCT* as determined by the HSCT-team transplant trial. Lessons learned are that improved HSCT (not necessarily in JMML patients) prognosis is associated with T-cell replete transplants, early withdrawal of immunosuppression, the presence of GVHD* and DLI*. Targets for improvement are reduction in TRM*, reduction of side effects, early recognition of relapse due to improved MRD* assays.

Charlotte Neimeyer discussed the upcoming EWOG* trial whose goal is to improve EFS*. There is, in Europe, a documented and significant difference in prognosis for JMML patients depending on the amount of experience their transplant center has with treating JMML. These center-dependent differences are larger than differences based on treatment regimen. In an attempt to right decrease this inequity in treatment quality, the study will standardize BMT procedures, donor selection and treatment regimens. The procedural focus of this study will be to tailor post-transplant immunosuppressive therapy to the individual’s risk of relapse.

Discussion at the close of each session was animated and interactive. Collaborations were proposed and suggestions made, all in the spirit of the best outcome for the children. A representative of the Children’s Oncology Group (COG) expressed interest in the possibility of a new JMML trial and encouraged international cooperation and a focus on preclinical testing of Sorafenib and other potential targeted therapeutic agents.
There is great enthusiasm at the prospect of including a JMML xenograft in NCI’s PPTP program, which would aid in the successful design and funding of a clinical trial protocol in the US. One suggestion for a potential project for The JMML Foundation was publication of transplant center guidelines to assist parents in choosing the highest quality of care for their JMML children. All in all, the 2nd JMML International Symposium was a great success due most significantly to the genuine interest and active participation of all our esteemed international participants (and not insignificantly to the abundant flow of coffee).

* NAJP (North American JMML Project)
* FTI (Farnesyl Transferase Inhibitor)
* BMT (Bone Marrow Transplant)
* EWOG-MDS (European Working Group on MDS in childhood)
* EFS (Event-Free Survival)
* HSCT (hematopoietic stem cell transplant)
* DLI (donor lymphocyte infusion)
* KRAS (historical name of gene from which the Kirsten RA At Sarcoma originated, entire Ras family of proteins is named after this gene)
* Shp2 (SH2 domain protein tyrosine phosphatase)
* NFI (Neurofibromin 1)
* AML (Acute myelogenous leukemia)
* MPD (myelo-proliferative disorder)
* MEK (MAP ERK Kinase) We won’t get into what MAP and ERK stand
* NCI (National Cancer Institute)
* PPTP (Pediatric Preclinical Testing Program)
* ALL (Acute Lymphoblastic Leukemia)
* CLL (Chronic Myelogenous Leukemia)
* TaqMAMA (Taq polymerase Mismatch Amplification Mutation Assay)
* MRD (Minimal Residual Disease)
* PCR (Polymerase Chain Reaction)
* Raf1 (Ras Activated Factor 1)
* TRM (Transplant Related Mortality)

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