COMMENTARIES

An April Fool's Day Commentary

What a difference a scale makes!

It is not widely known that fava beans produce large amounts of L-diorthophenylalamine, L-Dopa, the percussor of dopamine, the neurotransmitter deficient in Parkinson's disease. To use fava beans to treat PD, however, requires ingestion of about one cup three times daily. While this did cure constipation in the few patients who completed the 1-month protocol, three dropped out and three suffered witnessed cases of spontaneous combustion. Improvement in motor symptoms was statistically significant ($p<.05$) but minor in degree. Smoking sun-dried fava beans also reportedly improved symptoms but the report was confounded by the subjects' use of inhaled gingko and oral mink milk.

We made the obvious leap, when we realized that oral intake of fava beans would be difficult, to using brain implants. Based on a two-decade experience of sticking all sorts of things into human brains, from human and pig brain cells, human adrenal glands, electrodes, catheters, drug wafers, we reasoned that an all natural, organically grown, free-range fava bean would be more likely to be well tolerated than any artificial material. Use of fava bean is supported by the entire group of Mediterranean farming associations and is opposed by only a handful of biotech companies who failed in their attempts to patent the fava bean. They requested a hold on NIH funding of fava bean research until a patentable bean can be "invented." This is under review by the Bush administration.

The particular subspecies chosen, "favora Parkinsonian," named by a descendant of the legendary physician, was, ironically, found to contain the highest percentage of L-Dopa among all species of the plant. It is found growing naturally in one of the uninhabited Greek islands. PD patients unable to tolerate L-Dopa or dopamine agonists by mouth who were incapacitated by their worsening motor symptoms were asked to volunteer for this project. The protocol had been approved by the Culinary Institute Review Board of Johnson & Wales University and co-sponsored by the Agriculture Department of the Interior Department of United States and the Institute of Alternative Medicine at the National Institutes of Health. Informed consent was obtained prior to study entry.

The patients received 15 fragments per side of $1mm^3$ of the heart of the bean 3 days after a sprout was identified. 5 fragments were implanted in the caudate and 10 to the putamen (4 anterior & 6 posterior). The procedure was well tolerated, with no major adverse events identified. All subjects received fava bean implants. There were no sham procedures. Subjects were evaluated at baseline, one week, four weeks and then every four weeks post-implant using standard measures of Parkinson motor function. Subjects were compared on the Unified Parkinson's Disease Rating Scale, motor section, at each evaluation to their baseline score. Subjects were advised to avoid prolonged sunlight to prevent the implants from excessive growth. Surprisingly, to the author, and the sponsors, no benefit was noted over the 25 weeks of the trial. To avoid overlooking a potentially helpful and much needed intervention, a re-analysis was undertaken by the Independent Safety and Efficacy Monitoring Committee, underwritten by grants from Enron, and by the Oversight Committee, sponsored by the Arthur Anderson company. Using a scale frequently employed in recent neurosurgical trials, The Universal Open Label Unbiased Assessment Tool. {See Figure} This simple global impression scale represents a realistic evaluation of the subject as if he was a real life patient in a private practice. On this scale patients are given 5 points for being alive, 5 points if better, 10 points if much better, 15 points if even better than that, 25 points if better than they've ever been but not actually mentally impaired.

Using this scale a very different outcome was documented. On this scale subjects improved by significant numbers ($p<10^{-6}$) and the treatment was an obvious success.

This brief description of a clinical research project illustrates the importance of choosing the best assessment instruments for a particular study. In many areas, PD research is a good example; a particular test becomes embedded as a "gold standard." In assessing side effects of antipsychotic drugs, for example, the Simpson Angus Scale has been the gold standard for measuring parkinsonism, although no study by PD experts has ever or would ever validate this scale which is quite obviously extremely poorly designed. Yet for 30 years it has been popular.

Open label studies are particularly easy to "under power," that is, use too few subjects to attain statistical significance, so sensitive scales need to be utilized.

We thank our Oversight Committee for forcing us to reassess our data. We would welcome readers' suggestion of other analyses but unfortunately when the NIH asked to review the data we discovered, much to our chagrin, that the records had been shredded.

– Joseph H. Friedman, MD

Open Label Assessment Tool

Better = 1; Much Better = 2; Even Better than that = 3

Item

- Tremor
- Rigidity
- Dexterity
- Balance
- Memory
- Gait
- Speech
- Thinking

•If score < 10 double

•If score is between 15 & 20 add (20-score)x2

•If score is > 25 convert to 30

The Arrival and Departure of a Baffling Disease

The world cowers as it now confronts a succession of unfamiliar infectious diseases, new forms of environmental poisoning and novel ways of inflicting harm upon the human body. Many have wondered in recent years what unspeakable sins must have been committed by people to justify the terror wrought by wave after wave of new contagions. In earlier years, people contend, there were no such calamities as HIV/AIDS, legionnaire's disease, Lyme disease, Ebola fever, toxic-shock syndrome, Hanta fever and yet other microbial terrors. These newly arrived burdens, they conclude, must represent the microbiological prelude to a global Armageddon.

Yes, there are some 35 million humans currently afflicted with the HIV pathogen; and other infectious ailments such as Ebola fever, while perhaps not totally new, now constitute a palpable threat to the health of humans living beyond the African rain forests. Yet in terms of infectious disease, men and women have never been healthier. Smallpox has been made extinct and will stay so unless man, in his infinite cleverness, uses it as a deliberate terrorist weapon; polio and measles, by virtue of effective vaccines, are close to global eradication; still other diseases of childhood, including diphtheria, pertussis and rubella, are a thing of the past, at least in those populations wealthy enough to immunize their children. And still other diseases, once major scourges, through no known medical intervention have disappeared from the face of the earth.

In the category of spontaneously retreating diseases, consider a devastating neurological disease which, from 1917 to 1925, afflicted about 250,000 people in Europe, Australia, Japan and the United States. The first cases, at least in the recent medical literature, were described in an article submitted by an Austrian neurologist named Constantine von Economo to the April, 1917, issue of Vienna's weekly medical newsletter.

Von Economo portrayed an ailment which began innocently enough with moderate malaise, some fever, and a headache often accompanied by pains in the muscles. But within a day or so these symptoms were complicated by confusion, paralysis of some of the eyeball muscles and a dramatic somnolence [sleepiness]. This latter finding prompted von Economo to call the disease *encephalitis lethargica*.

Close to half of his patients recovered with no further difficulties. But many, either immediately following the acute phase or after a symptom-free interval of months or more, went on to develop a progressively worsening rigidity of their limb muscles, rhythmic tremors of their hands, an expressionless face [despite the coexistence of a high measure of anxiety], restlessness, increased salivation and an inversion of sleep pattern [sleeping during the day while remaining awake at night].

The mask-like face, the restless, jerky movements, the tremors, the rigidity of the limbs exhibited by those patients in the chronic phase of *encephalitis lethargica* closely resembled another neurological disease described by Parkinson in 1817.

Indeed, many neurologists called this new disorder post-encephalitic parkinsonism. But there were notable differences. The disease described by Parkinson appeared, most commonly, in the elderly with no preceding episode of encephalitis. The biphasic disorder described by von Eonomo, on the other hand, arose at any age, including childhood.

The cases of this seemingly new disease, described in 1917, may not have been the first to be encountered by the medical profession. In 1675, Sydenham described something he called *febris comatosa* [sleeping fever]; in 1712, in the German city of Tubingen, there was a cluster of cases with brain involvement called *Schlafkrankheit* [sleeping sickness]; and in Italy in the 1890s there was an ill-defined disease, called *nona*, distinguished by excessive sleepiness and other nervous system symptoms. But the numbers of people involved in each of these instances was small. And because the clinical descriptions were so meager, it is difficult to determine whether these prior cases were of the same nature as the 1917 cases. But one thing is certain: they never reached the magnitude or gravity which characterized the 1917-1925 outbreak of *encephalitis lethargica*.

Before the epidemic had run its course, an estimated 40,000 cases had been identified in the United States, many dying during the acute phase; a moderate number recovering completely and about half going on to the protracted parkinsonian phase. Large numbers of these patients populated the nursing homes, particularly on the East coast. A motion picture, *The Awakening*, has described their plight.

The epidemic in the United States coincided with the great influenza pandemic of 1918-19; and while some at first thought that one caused the other, this view has not been accepted by pathologists [who demonstrated very different microscopic changes in each of the two diseases], by epidemoiologists [who showed that each disease had its own trajectory] or by neurologists [who distinguished the two diseases solely by clinical examination].

In England there were an estimated 10,000 cases and in western Europe an additional 120,000 cases. The pathologic findings were consistent with an infection of viral origin although no virus had ever been convincingly recovered; admittedly, the technics for viral isolation in those days were primitive.

Two curious epidemiologic features bear noting. First, most cases arose in the late winter months, thus suggesting that insects were not involved in its dissemination. Second, this epidemic was virtually confined to Europe, the United States, Canada, Australia and Japan.

Encephalitis lethargica remains a two-fold mystery. First, what caused it? And second, what factors, either ecological or man-made, accounted for its essential disappearance?

Answers to these questions are of more than academic interest. Medicine still has no inkling of the causative agent of this disease; therefore it has no way of preventing the disease or identifying its pathogen. And since the profession doesn't know what factor or factors caused it to wane in the 1920s, it has no intervention to employ as a preventive weapon should it reappear.

When virologists somberly reflect on diseases which may arise unbidden in the foreseeable future, *encephalitis lethargica* is always included in their list of possible candidates.

– Stanley M. Aronson, MD, MPH

The Evolution of Transplantation in Rhode Island

Reginald Y. Gohh, MD, and Angelito F. Yango, MD

Organ transplantation has established itself as effective therapy for end-stage renal, hepatic, cardiac and pulmonary disease, with more than 20,000 grafts performed annually in North America.[1] Similarly, successes of hematopoietic cell transplantation have resulted in a large number of patients becoming long-term survivors of diseases that previously were fatal. Before 1994, such programs did not exist in Rhode Island. Area residents who required transplantation travelled to Massachusetts, Connecticut or farther to obtain treatment. Such inconveniences not only added to the total cost of the transplant procedure, but also heightened the already considerable stress and anxiety of patients, who had to undergo the procedure away from home, removed from family and friends.

This issue of *Medicine & Health/Rhode Island* focuses on the transplant services that have become available in Rhode Island within the last decade, with particular emphasis on kidney transplantation. Dr. Gerald Elfenbein, the director of the **Roger Williams Medical Center's** (**RWMC**) Stem Cell Transplant Program, will also provide information regarding the innovative therapies now available at his center.

To evaluate Rhode Islanders' need for a kidney transplant program, an analysis was undertaken to estimate the number of local residents who might benefit. Although the actual number of kidney transplants performed on RI residents in transplant centers within New England increased from 12 in 1983 to 28 in 1991, this value was not an accurate representation of need, since it did not include the number of Rhode Islanders who would have undergone transplantation if there had existed a transplant center within the state. National utilization rates for renal transplantation expressed in relationship to the total number of U.S. residents maintained on dialysis yielded a more accurate measure of need. (Figure 1) Using this formula, it was estimated that 43 patients should have received a renal allograft in 1991, yielding an unmet need of 15 transplants in that year. This striking difference persisted even after adjusting for patient age (since an older patient population may not be as suitable for transplantation). Thus, although Rhode Island had one of the largest dialysis populations per capita in the country, Rhode Islanders received fewer kidney transplant procedures annually than U.S. citizens on average. In short, a substantial number of Rhode Islanders did not have adequate access to transplantation and would have benefited from the creation of a local site.

Although the lack of a local program was clearly a factor in limiting access to transplantation, such procedures are impossible without the availability of healthy donor grafts. The supply of donor grafts is the major factor affecting access to transplantation. However, these limitations can be greatest for residents of regions lacking transplantation programs, in large part due to the policies that govern regional allocation of donor grafts to specific institutions. These policies give regional priority for the use of grafts to those centers that also retrieve them. Although hospitals in Rhode Island could harvest cadaver kidneys, they could not take advantage of their regional priority for utilizing them because no area hospitals performed transplant procedures. This meant that organs harvested in Rhode Island were not, as a matter of priority, assigned to local residents, even when local residents may have been in need.

Given the demonstration of local need, the Kidney Transplant Program at Rhode Island Hospital was established in 1996. Since performing its first cadaver kidney transplant in March 1997, the program has grown, with more than 250 kidney transplants performed to date (Table 1). More importantly, the creation of a local program has resulted in a steady increase in the number of Rhode Island residents receiving kidney transplants (Table 2). Over the last three years, the average annual transplant rate locally now exceeds the national rate (6.2 vs. 5.1 transplants/100 dialysis patients), representing a diametrical change from previous results. This is a clear indicator that accessibility to transplant services has truly improved in Rhode Island.

Figure 1. Estimate Need of Kidney Transplantation in Rhode Island[2,3]

Step 1: U.S Rate of Transplantation per Dialysis Population (1991): (# Kidney transplants)/ U.S. Dialysis Prevalent Population = 10,026/ 142,488 =

7 Kidney Transplants per 100 Dialysis Patients

Step 2: Applying U.S. Rate of Transplantation to RI Dialysis Population (1991) 7/100 x 612 =

43 RI Residents Needed Kidney Transplants in 1991

Table 1. Rhode Island Transplant Rate4

Year	US Transplant Rate (per 100 dialysis pt.)	Expected Number of RI Transplant Recipients	Actual Number of RI Transplant Recipients (Tx Rate)
1993	6.3	43	30 (4.3)
1994	5.8	41	42 (5.5)
1995	5.6	42	37 (4.8)
1996	5.5	43	53 (6.6)
1997	5.2	41	59 (7.0)
1998	5.2	46	56 (5.7)
1999	4.9	44	48 (5.8)

Despite these encouraging results, a more somber observation is the stagnant rate of organ donation both nationally and locally. Simply put, the number of kidney transplants performed each year cannot keep pace with the number of candidates on the waiting list. Despite a 25% increase in the number of kidneys recovered nationally from cadaver donors between 1990 to 1999, the kidney waiting list has more than doubled in the same period.[1] When the transplant program was established at Rhode Island Hospital, it was hoped that organ donation would be spurred by increased public awareness and acceptance of the issue and, ultimately, more willingness to donate organs. Furthermore, clinicians would be more motivated to improve community education regarding organ donation. Unfortunately, efforts to increase the cadaver donor pool have been generally unsuccessful. Data from the New England Organ Bank, the local organ procurement organization, show that the potential organ donor pool has remained unchanged within the last decade, resulting in longer waiting times for those individuals seeking a cadaver kidney. The increasing demand for organs has increased the pressure to identify new sources of donor organs. These include the use of "marginal donors" (as determined by age, cause of death, or hemodynamic instability) and in particular, increased reliance on living related and living unrelated donors. As experience with the latter has accumulated, the results have been surprisingly superior to those after cadaver transplantation.[4] Thus for the first time, relative volume of living donor transplantation now exceeds 50% of total transplant activity at our center. Dr. Paul Morrissey and Bette Hopkins-Garcia discuss the efficacy and difficult ethics behind the use of living donors, both locally and worldwide. Nevertheless, the shortage of cadaver organs continues to pose the most severe limitation to the number of patients who could potentially benefit from transplantation.

....the number of kidney transplants performed each year simply cannot keep pace with the number of candidates placed on the waiting list.

The unprecedented success of patients undergoing organ replacement therapy is strongly correlated to the development and introduction of new immunosuppressive agents to the clinical armamentarium. Depending on the organ transplanted and the donor source, graft survival in the 85-95% range at 1 year has become commonplace,[1] with acute rejection rates reported at less than 10%. Because many of these recipients are living longer and ultimately returning to the care of their referring physicians, we have provided a review of the newer immunosuppressive drugs. Particular emphasis is placed on issues relevant to the primary care physician, such as drug interactions and side effect profiles. As the incidence of acute allograft rejection has decreased dramatically in the past several years, increasing attention has focused on the management of long-term complications in transplant recipients. Among these, infectious complications remain a major cause of morbidity and mortality for transplant recipients. Dr. Staci Fischer briefly reviews the epidemiology and clinical behavior of infection in the post-transplant recipient.

The participation of Dr. Fischer, an infectious disease specialist, in this forum highlights a generally unappreciated aspect of the transplant process: any transplant procedure is highly complex, requiring the active involvement of a wide range of health care specialists in a multidisciplinary approach. Furthermore, transplant recipients often have complex medical and social histories and complications arising from their primary illnesses. It is imperative, therefore, that the patient's primary physician be available nearby to take an active role in clinical decisions that arise during the patient's hospitalization for the transplant. Thus, the creation of local transplant centers has not only directly provided vitally needed services to a number of Rhode Island residents, but has also provided indirect benefits for the entire state. By enhancing the development of a local system of health services catered to these individuals, the "culture of transplantation" has become firmly entrenched in Rhode Island.

Table 2. Kidney Transplants at Rhode Island Hospital

Year	Cadaver Donor Transplants	Living Donor Transplants
1997	26	9
1998	27	25
1999	29	24
2000	31	32
2001*	29	33

*As of October 8, 2001

References

1. Excerpts from the 2000 Annual Report of the U.S. Scientific Registry of Transplant Recipients and the Organ Procurement and Transplant Network.
2. Excerpts from 1991 End Stage Renal Disease Network of New England Annual Report.
3. Excerpts from the 1991 Annual Report of the U.S. Scientific Registry of Transplant Recipients and the Organ Procurement and Transplant Network.
4. UNOS Data Reports, 1993-1999.
5. Terasaki PI, Cecka JM, Gjertson DW, Takemoto S. High survival rates of kidney transplants from spousal and living unrelated donors. *NEJM* 1995;333: 333-6.

Reginald Y. Gohh, MD, is Medical Director, Division of Organ Transplantation, Rhode Island Hospital, and Assistant Professor of Medicine, Brown Medical School.

Angelito F. Yango, MD, is Associate Medical Director, Division of Organ Transplantation, Rhode Island Hospital, and Assistant Professor of Medicine, Brown Medical School.

Correspondence:
Reginald Y. Gohh, MD
Rhode Island Hospital
Divisions of Organ Transplantation
and Renal Diseases
593 Eddy Street, APC-921
Providence, RI 02903
phone: (401) 444-8345
fax: (401) 444-3283
e-mail: Rgohh@Lifespan.org

Blood and Marrow Stem Cell Transplantation at the Roger Williams Medical Center, 1999-2001

Gerald J. Elfenbein, MD, FACP

Exactly three years to the day of the submission date of this manuscript, I became Director of the **Roger Williams Medical Center's (RWMC's)** Cancer Center and Director of the RWMC's Blood and Marrow Transplant Program. These three years have been invigorated with the acquisition of talented colleagues by recruitment and the performance and communication of groundbreaking clinical research. I will describe some of that work. For the next three years, our program has two major thrusts: an exciting and novel form of immunotherapy in the setting of minimal residual disease and our version of the non-myeloablative allogeneic stem cell transplant that has recently become popular. Finally, I will close with a basic research concept, tying everything together in a nice, even if futuristic, package.

Five Pilot Studies:

Following are five of the studies that we completed by what may be a novel method.

1. Conventional chemotherapy has little to offer in terms of long-term disease-free survival for patients with high-risk lymphomas. High-dose chemotherapy and autologous (self-donated) stem cell transplantation offer these patients an opportunity for long-term disease free survival. At RWMC[1], we explored the therapeutic value of a well-tolerated, high-dose chemotherapy regimen, used for many years in patients with breast cancer, the so-called **CTC** regimen, consisting of 6 g/m2 of **cyclophosphamide**, 500 mg/mz of **thio-TEPA** and 900 mg/m2 of **carboplatin**, in 17 patients. Patient characteristics included: age - range 21 to 63 (median 50) years; gender - 11 males and 6 females; disease - 7 with **Hodgkin's disease** (**HD**; 6 nodular sclerosis and 1 lymphocyte depleted) and 10 with **non-Hodgkin's lymphoma** (**NHL**; 4 large cell, 3 follicular mixed, 1 mantle cell, 1 immunoblastic, and 1 follicular small cell); disease status - 9 (3 HD and 6 NHL) in first relapse, 3 (1 HD and 2 NHL) in second relapse, 2 (HD) in third relapse, 1 (NHL) in first complete remission, and 2 (NHL) in first partial remission. All patients were induced with 4 cycles of quite aggressive chemotherapy, which consisted of courses of continuous infusion of **cyclophosphamide and etoposide** (**CE**; developed by Elfenbein's team in Gainesville, FL[2], alternating with courses of **dexamethasone, cytosine arabinoside, and cisplatin** (**DHAP**). **Peripheral blood stem cell** (**PBSC**) collection (to be reinfused after CTC) was performed by leukapheresis (in collaboration with the Rhode Island Blood Center)- during recovery from the last cycle of DHAP. With a maximum follow up of 46 months and a minimum follow up of 11 months (median of 24 months) for survivors, the Kaplan-Meier estimate for overall survival at 25 months is 52% and for disease-free survival at 25 months is 47%. Of the subset of 4 "better" but still high-risk patient (3 with HD in first relapse and 1 with NHL in first complete remission), 2 are alive and free of disease and 2 have died (1 of sepsis and 1 from recurrent disease). For the 7 patients who progressed, the median time was 3 months (range 1 to 7 months). CTC offers cyclophosphamide at a very high dose and two new alkylating drugs, thio-TEPA and carboplatin, for lymphomas. CTC appears to be quite active in a broad range of lymphomas, is deliverable in the outpatient setting, and should be of use in consolidating first complete remissions in high-risk NHL and second complete remission of high-risk HD. Finally, CTC is sufficiently well tolerated (there were two only toxic deaths [12%] in this group of high-risk patients) and has a quick enough recovery of granulocyte count (median time to reach an **absolute granulocyte count** (**AGC**) of 500/uL was day 12) and platelet count (median time to recovery of **platelet** (**PLT**) count of 20,000/uL was day 15) after PBSC infusion to permit relatively early introduction of a post-transplant treatment strategy to reduce the probability of relapse after high dose chemotherapy followed by autologous stem cell transplantation for high-risk lymphomas. Comment: This Phase II study establishes the well-tolerated CTC regimen as a safe and effective regimen for lymphomas making it a

Pt No	Pt Age	Gender	NHL Grade (gr.) or HD Status	Marrow Biopsy+ for NHL	Prior Fludarabine or Nitrogen Mustard	CD34+ PBSC after Chemo-growth Factor x 10^6/kg	CD34+ PBSC after G-CSF Alone x 10^6/kg	Expanded Marrow Cells from 80 ml x 10^7/kg	Day That AGC >500 per uL	Day That PLT >20K per uL
1	54	F	Intermediate gr.	Yes	Yes	0.28*	1.0	3.0	14	>120
2	69	M	Intermediate gr.	No	No	0.04*	2.3	2.6	10	16
3	69	F	Low gr.	Yes	Yes	0.42*	0.5	6.6	14	>120
4	64	M	Low gr.	Yes	No	N.D.	0.81*	1.0	13	20
5	34	M	Primary Refractory HD	No	Yes	0.62*	N.D.	1.0	10	16

{Table note: * indicates first mobilization attempt and identifies poor mobilizer for study entry.}

Pt.	Char.	CD34+Cells/Infusion	Day AGC>500/uL	Day PLT>20,000/uL
1	54 M	1.4 x 10^6/kg	+12, +12, +11	+19, +18, +17
2	57 M	0.7 – 1.3 x 10^6/kg	+12, +13, +10	+17, NR[1], +18
3	44 F	3.0 x 10^6/kg	NR[2], NR[3], +9[4]	NR[1], NR[1], 23[4]
4	66 M	1.3[5] or 7.6[6] x 10^6/kg	9[7], 9[7], 9[7]	16[7], 11[7], 11[7]

{Table notes: 1. NR = not reached before next cycle of L-PAM initiated; 2. AGC = 410/uL on day −21; 3. AGC = 212/uL on day −21; 4. Days after unselected, back-up PBSC were given does not include the 13 days since the selected BPSC were given; and 5. CD34+ cell concentration of selected cells; 6. CD34+ cell concentration of the unselected, back-up PBSC.}

platform onto which post-transplant consolidation therapy can be built (see below).

2. Many experts have preferred peripheral blood stem cells as a source of stem cells for autologous transplantation (see below). Failure to collect sufficient numbers of CD34 (the surface marker identifying hematopoietic stem cells) positive (+) PBSC after mobilization with chemotherapy and growth factors (chemogrowth factor mobilization) has, however, been seen in patients who are heavily pretreated or are older aged and/or have had prior fludarabine therapy. Failed attempts at remobilization often disqualify these patients as candidates for autologous transplantation. At RWMC and in collaboration with the **Hackensack University Medical Center (HUMC)** and **Progenitor Cell Therapy (PCT)** in New Jersey,[3] we studied four heavily pretreated patients with NHL and one patient with primary refractory HD for whom we could not collect at least 2 x 10^6 /kg CD34+ PBSC (the standard minimum collection required to ensure engraftment after high dose chemotherapy) after the first course of mobilization. Four were mobilized first with cyclophosphamide, prednisone and G-CSF (our best chemogrowth factor mobilization regimen for hematologic malignancies). One was mobilized first with high doses of the growth factor G-CSF alone (10ug/kg/day for 3+ days). Second mobilization attempts were made in three patients with G-CSF. Only in one patient did we collect more than 2 x 10^6/kg CD34+ PBSC in a single mobilization attempt let alone cumulatively. Subsequently, all 5 patients were primed with G-CSF at 10ug/kg/day for 2 days. On the day of a full bone marrow harvest as a back-up collection, the first 80ml of marrow aspirated (2 to 2.5 ml/site) were cultured in the AastromReplicell™ by PCT for 12 days. Expanded marrow cells were infused 48-96 hours after the completion of high dose chemotherapy with the CTC regimen (see above) for the 4 patients at RWMC and after BVAC (a different high dose chemotherapy regimen) for the patient at HUMC. The day of infusion of expanded marrow is called day 0 of transplant. On day +l after transplant, all cryopreserved, stored PBSC were thawed and reinfused. Expanded marrow cells were well tolerated. Patient characteristics, CD34+ PBSC numbers, and expanded marrow cell numbers plus AGC and PLT count recovery times after autologous transplant following high dose chemotherapy are shown in Table 1.

With the expectation of delayed engraftment as a result of poor first PBSC collection, these patients would not have been considered autologous transplant candidates. With the addition of expanded marrow cells to PBSC, autologous transplantation was made possible for these five patients whose first collections were inadequate. AGC recovery was prompt (median day to AGC >500/uL was day 13). PLT recovery was prompt (median day to PLT count >20,000/uL was day 20), as well, except in the two patients who had received prior fludaribine therapy. We concluded that ex vivo marrow expansion is effective in providing a suboptimal PBSC collection with enough of the right cells to produce rapid granulocyte and platelet recovery. At this point in time, we don't know exactly what the "right" cells are - stem cells or accessory cells. All 4 NHL patients responded to high dose chemotherapy and have tolerated rituximab (antiCD20 antibody) to reduce the probability of relapse. The HD patient refused interferon after completing his post-transplant irradiation therapy. Comment: This small study demonstrated clearly that bone marrow may be a valuable source of expandable stem (or other) cells (see below). The biggest problem is how long will it take Aastrom to bring the Replicell™ to market?

3. **L-phenylalanine mustard (L-PAM)** is one of the most active agents for **multiple myeloma (MM)** available. The maximum tolerable dose of L-PAM is 240 mg/m^2 in high-dose chemotherapy and less is used in combinations with busulfan or total body irradiation. The usual "transplant" dose of L-PAM is 200 mg/m^2 followed by an autologous PBSC infusion. This usually produces a lengthy hospitalization with significant enteritis and mucositis. At RWMC,[4] we performed a feasibility study to determine if 3 cycles of L-PAM (followed by autologous PBSC infusion each time on the day after L-PAM) could be delivered at 100 mg/m^2 at 3-week intervals for a total of 300 mg/m^2 and on an outpatient basis. Four patients with chemoresponsive, stage III MM had PBSC mobilized with cyclophosphamide, prednisone and G-CSF. After CD34+ cell selection, PBSC were frozen in 3 aliquots. A back-up, unselected, PBSC collection was also cryopreserved. G-CSF was started day +1 and trovafloxacin and fluconazole were started day +5 after PBSC infusion. Of the 12 cycles, 1 cycle was delayed 1 week because of a central venous access line infection and 1 cycle was delayed because of a death in the patient's family. There were 7 admissions: 5 for neutropenic fever (42%) and 1 each for mild enteritis and disseminated Herpes zoster. The data from the study are shown in Table 2.

For patient #3, delayed AGC re-

Site	Trial Type	Pts	PBSC	Pts	BMSC	P-Value
Seattle	Randomized	81	16 (11-29)	91	21 (13-36)	< 0.001
RWMC	Sequential	15	12 (9-16)	7	11 (8-14)	0.0278

coveries were seen despite acceptable numbers of CD34+ cells infused; for course #3, back-up PBSC were given when AGC recovery appeared to be late again and prompt engraftment ensued. When patient #4 experienced late AGC recovery during cycle #1 with selected cells, we immediately infused back-up PBSC for that and both subsequent cycles; prompt engraftment was observed from then on. All 4 patients showed reduction in their M-proteins. After recovery from the third transplant, patients #l, #3, and #4 received alpha-interferon and patient #2 received thalidomide because of prior exposure to interferon. These 4 patients demonstrate that 300 mg/m^2 L-PAM can be delivered within a space of 9 weeks with acceptable non-marrow toxicities and with rapid recovery of AGC and PLT counts after each cycle especially after the third cycle which is the cycle that is the prelude to post-transplant therapy. Comment: This feasibility study delivered 50% more L-PAM in the same period of time as a standard autologous transplant and recovery from transplant (63 days or 2 months) with much less toxicity. Moreover the patients were in good enough hematologic shape and general health to start post-transplant immune based therapy.

4. In 1998, we initiated a strategy to improve clinical disease-free survival for patients with hematologic malignancies. The strategy has five steps:

a. Increased intensity of disease specific, induction therapy (maximal cytoreduction; CE/DHAC [carboplatin switched for cisplatin to minimize nephrotoxicity developed by Elfenbein's team in Tampa] for NHL and HD and DCIE [dexamethasone, cyclophosphamide, idarubicin, and etoposide developed by Elfenbein's team in Tampa[5]] for MM).

b. Mobilization of blood stem cells with chemotherapy and growth factor for fastest recovery of granulocytes and platelets (cyclophosphamide, prednisone and G-CSF) instead of just G-CSF alone and to provide an antitumor effect as well.

c. Positive selection of CD34+ cells, i.e., stem cells (which, also, purges the PBSC collection of tumor cells, i.e., negative selection of tumor cells).

d. High dose chemotherapy with known effective regimens (disease specific but with lowest known toxicity; CTC for NHL and HD and L-PAM x 3 for MM).

e. Consolidation with disease-specific immunotherapy (treating minimal residual disease [see below]). At RWMC,[6] we evaluated the first 18 consecutive patients who were transplant eligible and are in or have already completed the 5th step. Among the patients were 10 with NHL, 1 with HD, and 7 with MM. Only 5 (28%) would be considered to be good prognosis patients (first complete remission of NHL or first partial remission of MM). Only 7 (39%) had sufficient CD34+ cells collected to allow positive selection. The 11 lymphoma patients (63%) received CTC (see above) and the 7 MM (37%) received 3 cycles of L-PAM (see above). AGC recovery to > 500/uL (see below) was prompt in 16 pts (89%). Lymphoma patients received involved field irradiation for residual masses or history of bulky disease. One patient (5.5%) refused immunoconsolidation therapy after completing irradiation for HD (see above). The 12 patients (67%) whose disease was CD20 positive (a surface marker for B cells, 2 with MM and 10 with NHL) received all four planned weekly doses of antiCD20 monoclonal antibody (rituximab). Five

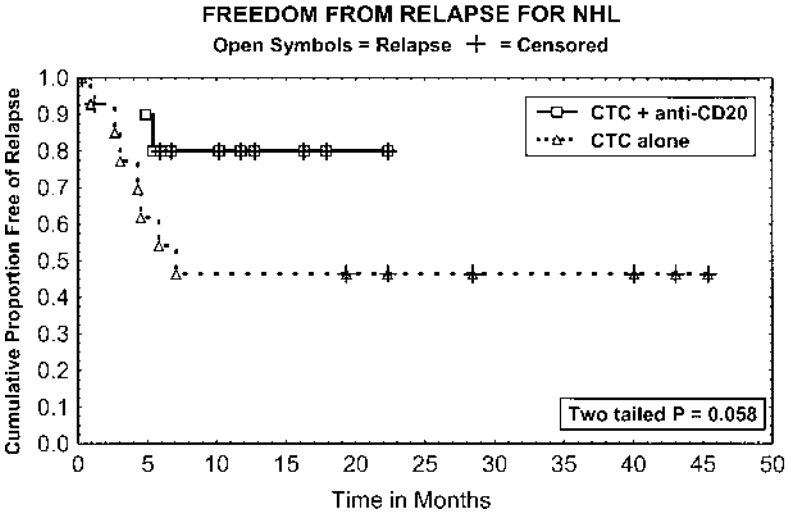

Figure 1.

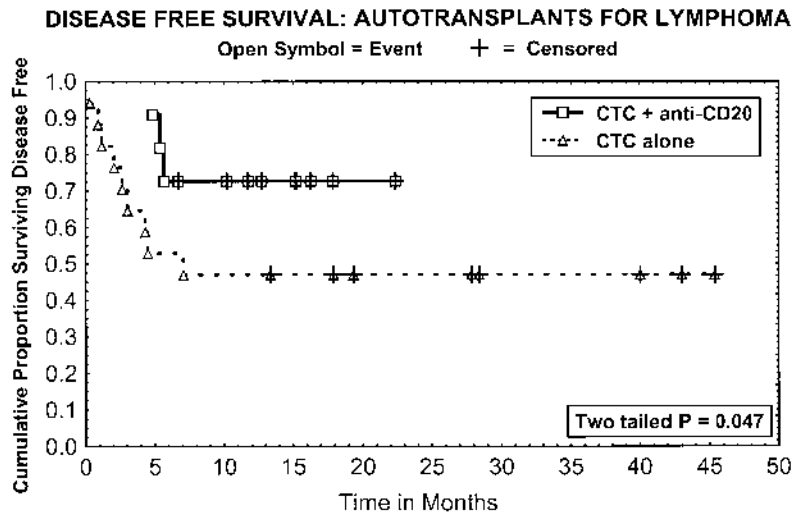

Figure 2.

of the 7 MM patients (71 %) were started on alpha-interferon but only 2 (40%) tolerated chronic administration of interferon. With a median follow-up of 15 months, there have only been 4 patients (22%) who developed disease progression. There have been 6 deaths (33%), 4 from progressive disease (22%) and 2 from late, trimethaprim-sulfamethoxazole resistant *Pneumocystis carinii pneumonia* (11%). We found it feasible to incorporate immunoconsolidation into the treatment after autologous transplant but it is too early to comment upon disease-free survival.[6] Comment: Now you can see where we were going all along with our strategy to improve disease-free and overall survival by better cytoreduction pre-transplant, less toxic transplant regimens, and immune based therapy post-transplant. We have compared the NHL patients in this study (#4) to the NHL patients in study #1. They (#4) are about as similar as you can get to historical controls (#1). They (#1) were treated nearly identically except for the post-transplant immunoconsolidation therapy (#4). With all the reservations intrinsic to comparing one small phase II study (#4) to another small phase II study (#1) performed at an earlier point in time, we did see a significant difference in freedom from relapse and disease-free survival for the patients who received the post-transplant anti-CD20 immunoconsolidation therapy (Figures 1 and 2).

5. There have been many debates whether blood and marrow derived stem cells engraft at significantly different rates after transplantation. Bensinger for the Seattle group (*NEJM* 2001;344:175-81) stated that their **randomized clinical trial (RCT)** "confirm(ed) that engraftment occurs more rapidly with peripheral-blood cells than with bone marrow" in allogeneic transplants. In 1995, Elfenbein's Tampa team published preliminary data[7] and, in 1999, they published the final report[8] "of the first prospective, stratified, randomized trial (also an

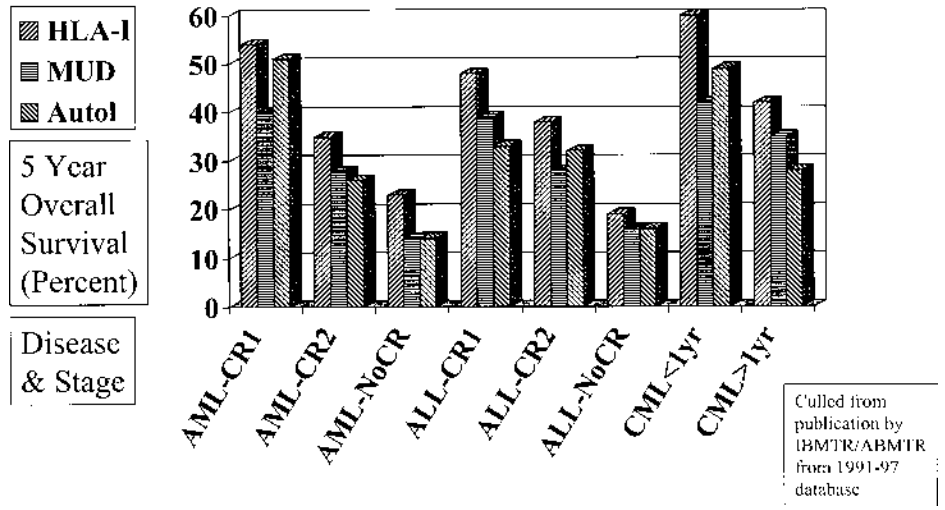

Figure 3.

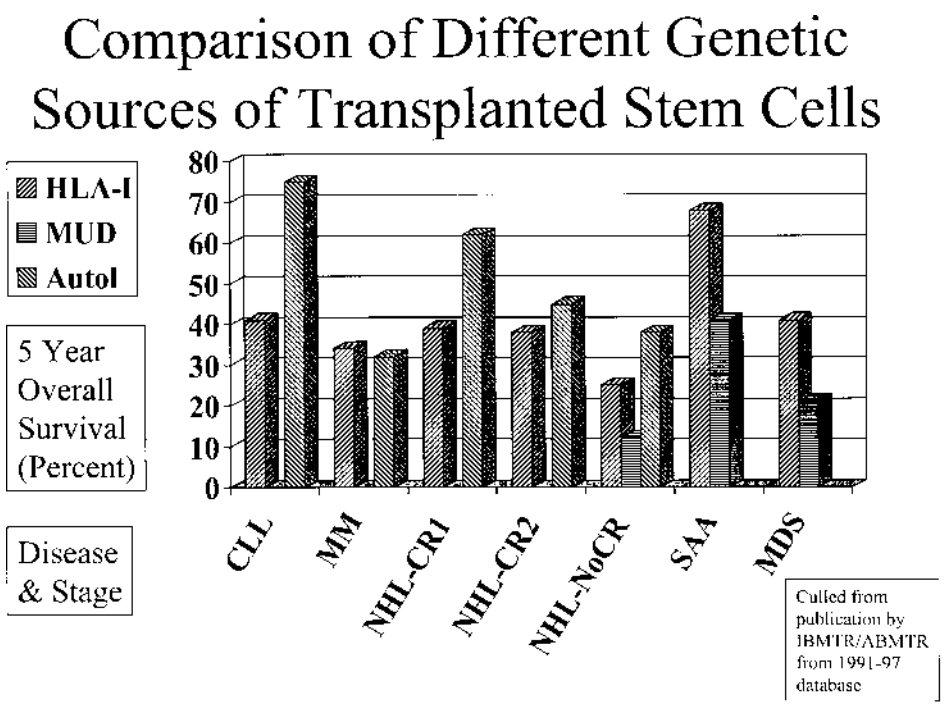

Figure 4.

RCT) comparing G-CSF primed bone marrow cells with G-CSF mobilized peripheral blood cells for pace of hematopoietic engraftment" demonstrating identical granulocyte and platelet recovery times for the two types of stem cells after autologous transplants. How could these two RCTs be in such stark contrast? The difference between the two RCTs is not whether the transplants were autologous or allogeneic, nor whether post-grafting therapy involved methotrexate or not. The difference is how the donor was treated prior to collection of the stem cells. In Bensinger, to collect PBSC the donor was pretreated with G-CSF at 16 ug/kg/day for 5 days whereas to collect stem cells from the bone marrow (BMSC) NO pretreatment was given. In Bensinger, cyclosporine and methotrexate were given as acute **graft versus host disease (GVHD)** prophylaxis. Myeloid engraftment was defined as the first of three consecutive days that the AGC was more than 500/uL. At RWMC,[9] we evaluated the results of our recent experience of 7 consecutive patients who had been transplanted with BMSC from healthy donors, all of whom had been pretreated with G-CSF at a dose of 10 ug/kg/day for 3 days. Only cyclosporine was utilized as GVHD prophylaxis. Prior to the return to BMSC, albeit G-CSF primed, we had performed a consecutive series of 15 G-CSF mobilized PBSC allotransplants that was rather novel in its time (starting June 1994). Table 3 summarizes the comparative study facts and presents engraftment results as the median day (range) for the AGC to exceed 500/uL.

We believe that large RCTs will confirm that G-CSF pretreated marrow will engraft just as rapidly as G-CSF mobilized peripheral blood and may, potentially, produce less GVHD. RCTs, no matter how large, may only be relied upon for interpretation within the bounds of their experimental design. Apparently, Bensinger et al. never anticipated that the confounding variable G-CSF was responsible for the effect they observed (difference in engraftment times) and not the anatomical site from which the stem cells were collected. Finally, these data demonstrate that post-grafting methotrexate delays the pace of granulocyte recovery considerably. Comment: The debate will rage on about bone marrow stem cells and blood-derived stem cells but I am convinced that the two are equivalent from the point of view of early engraftment. Bone marrow certainly offers advantages from the point of view of long-term engraftment while blood derived stem cells offer advantages from the point of view of antitumor activity. Both scenarios are dependent on the cells that contaminate the stem cell collections.

Two New Thrusts

I have been a "card carrying member" of two "brotherhoods", immunology and experimental hematology, since 1967 when I came under the tutorial wing of George Santos at Johns Hopkins. Therefore, I recruited Larry Lum from Wisconsin to develop a program in cellular immunotherapy but with an intriguing twist. Immunotherapy certainly has not been as successful as originally hoped. Why? What interested me so much in Larry's work is how low the effector-to-target ratios were in his *in vitro* cytotoxicity assays and that effectors could kill a second target cell in sequence. Potentially, this would help with the problem *in vivo* where the tumor vastly outnumbers the immune cell population. He does this by expanding and activating patient T cells *ex vivo* (call them activated T cells or ATC). But there is the problem of trafficking. How does he get the ATC to the tumor sites and, if they get there, why would they stay there? The answer to the first question is the cells arrive at the tumor sites in the blood at random unless they are directly injected into the tumor or are chemoattracted. Not easy to do, control, or ensure. The second question is more easily answered: "arm" the ATC with a bispecific antibody that, on the one hand, binds to the T cell and, on the other hand, binds to the tumor cell. Larry makes these "bi" antibodies in his own lab. His project is not only IRB-approved but also FDA-approved and looks at arming ATC with an antibody to HER2/neu found on some breast cancer cells and most prostate cancer cells. Patients with minimal residual disease are being treated now. There is also a project for metastatic breast cancer. In the not-too-distant future, we hope to have an antibody to EpCAM, which binds to a diverse number of carcinomas. Armed ATC may be considered the autologous ultimate in immunoconsolidation therapy.

Back to experimental hematology, I recruited Pete Quesenberry from Massachusetts, a world-class experimental hematologist whose success with low-dose total body irradiation and cellular immune therapy with HLA identical sibling donor cells in patients with hematologic malignancies made me take notice as this was the least toxic of all the "mini"-transplant regimens in the world. His goal was mixed chimerism (a state of co-existence of donor and host cells so that there would be no graft versus host disease and, because there was mixed chimerism, obviously there wasn't any rejection). But, even with mixed chimerism, there was an antitumor effect, the **graft versus tumor effect (GVT)**, else the diseases would not have gotten better. All this without major side effects. Simply marvelous pilot data. We activated this protocol at RWMC. Further, I have been struggling for many years with the problem of finding donors for patients when they don't have HLA-identical sibling donors, which happens more than 65% of the time. I have spent many years pursuing autologous transplants and study #4 is the ultimate expression of this research direction but it is limited by the number of antibodies that are currently available like rituximab for NHL. None yet for MM, for instance. HLA **matched, unrelated donor (MUD)** stem cells may now be obtained (about 65% of the time) from adults and from cord blood but the former transplants are very difficult to perform (read very toxic) and the latter are still rare in adults (because of low numbers of stored cord bloods and expansion problems). See Figures 3 and