

Section 2: Scientific Abstract: K562/GM-CSF vaccine ation in combination with imatinib mesylate (Gleevec™) as booster for chronic myeloid leukemia (CML) patients previously vaccinated on protocol J0345/HUMAN GENE TRANSFER PROTOCOL # 0309-602

CML is a malignant clonal disorder of hematopoietic stem cells that affects myeloid cells, erythroid cells and platelets, leading to elevated cell counts in the blood and myeloid hyperplasia in the marrow. The genetic hallmark CML is the reciprocal translocation of chromosomes 9 and 22 forming the Philadelphia (Ph) chromosome, which results in rearrangements of regions of the BCR (chromosome 22) and ABL (chromosome 9) genes to form the hybrid BCR-ABL fusion gene. This translocation results in disrupted regulation of the tyrosine kinase domain of the ABL gene, resulting in its constitutive activation in the fusion protein. Such changes confer a proliferative and survival advantage of hematopoietic cells harboring the rearrangement, and their ultimate malignant transformation.

CML accounts for about 20 percent of newly diagnosed cases of leukemia in adults, and is most commonly identified in its chronic phase, which for years had been managed with hydroxyurea or busulfan. While these treatments are frequently successful in controlling the peripheral blood cell counts, in the absence of additional therapies, the chronic phase (typically lasting three to six years) invariably progresses to an accelerated phase followed by blast crisis, which is largely resistant to definitive therapy.

Treatment Options-

Chronic phase CML was among the first diseases shown to be curable by allogeneic bone marrow transplantation (BMT) in a significant percentage (70-80%) of younger patients having an HLA identical sibling. Notably, several aspects of the transplant experience in CML have firmly established CML as an immunologically responsive disease. First, an inverse correlation has been observed between the development of graft versus host disease (GVHD) and relapse from CML. Second, the relapse rate of CML patients receiving sygeneic transplants is significantly higher than in patients who receive allogeneic, HLA matched sibling donor grafts. Third, efforts to reduce the incidence and toxicity of graft versus host disease by T cell depletion of the graft led to a significant increase in leukemic relapse. Finally, the dramatic remissions induced by the infusion of donor lymphocytes in CML patients who relapse following allo-BMT, firmly established the potency of T cells in fully eradicating the malignant clone.

Whereas allogeneic BMT has been the treatment of choice for eligible CML patients for the past two decades or more, a significant fraction of these patients succumb to the toxicities of the procedure (GVHD, immunosuppression, and preparative regimen toxicity), and over two-thirds of patients either don't have a matched sibling donor or are considered too old to safely tolerate allogeneic BMT. Recent experience with non-myeloablative preparative regimens and alternate donor sources have attempted to deal with these limitations, although at present, these experimental therapies still carry a significant risk of morbidity and mortality.

In chronic phase CML, daily interferon- α can induce complete cytogenetic responses in 5 to 20% of patients and has been shown to prolong survival in such responders. Interferon- α affects proliferation and differentiation of the leukemic clone and augments immune responses against CML-associated antigens. Notably, HLA-A0201+ patients achieving a complete hematologic

remission (CHR) in response to interferon- α have been found to have a significant increase in the frequency of CD8+ T cells specific for an HLA-A0201 restricted epitope of proteinase 3. This enzyme is a normal constituent of the myeloid granules, but has been found to be over-expressed roughly ten-fold by CML cells. The correlation between clinical and immunological responses in CML patients treated with interferon further attests to the potential for immune mediated therapies in this disease. Unfortunately the majority of CML patients on interferon- α fail to achieve complete cytogenetic remissions, and many of those who do continue to harbor persistent molecular evidence of BCR-ABL transcripts. Furthermore, side effects of interferon can be intolerable and limit long-term treatment in a substantial number of patients.

In addition to interferon, imatinib mesylate (Gleevec) is now widely considered to be front-line therapy for CML. Imatinib is a potent inhibitor of the protein tyrosine kinase of the BCR-ABL fusion protein. The initial phase I clinical trials of imatinib revealed dramatic responses in CML patients who failed interferon α with 53 of 54 patients normalizing their white blood cell (WBC) count and platelet counts, usually within four weeks of initiating therapy. In this study, 54% of patients had cytogenetic responses (31% major and 13% complete), and these were demonstrable significantly earlier than with interferon. Several subsequent multi-institutional phase II trials have reported that greater than 90% of patients with interferon resistant chronic phase disease normalized their blood counts with imatinib, and nearly half had a major cytogenetic response, with complete cytogenetic responses obtained in over 40%. In the IRIS study, at a median 19 month follow-up, 95% of patients treated initially with imatinib had a complete hematologic response, and 85% had a major cytogenetic response, most of which were complete.

Despite these promising results with imatinib, resistance to this agent can occur. Some patients treated in chronic phase who initially respond to imatinib subsequently relapse with highly aggressive resistant disease. Recent reports have suggested that the primitive Ph chromosome positive CML stem cells are insensitive to imatinib in vitro, such that imatinib alone may be incapable of truly eradicating the disease. Furthermore, several studies examining molecular detection of BCR-ABL by RT-PCR have reported that most cytogenetic responses to imatinib, including complete responses, remain BCR/ABL positive. Indeed, an interim analysis of the IRIS study reported that only 2 of 28 patients receiving imatinib as first-line therapy reached PCR-undetectable transcript levels, and the rate of response slowed as a function of duration of therapy. Finally, of 21 patients who achieved a complete cytogenetic remission on imatinib with very low or RT-PCR-undetectable transcript levels, two-thirds had subsequent increases in tumor burden, with 19% having cytogenetic relapse, 27% having a sustained > 1 log increase in BCR/ABL transcripts, and 33% converting from undetectable to detectable disease status.

Accordingly, the central focus of this proposal is to extend the previous, J0345 CML pilot study, which tested the integration of imatinib and tumor-cell based vaccinations with imiquimod cream (Aldara™), a topical toll-like receptor agonist used as a vaccine adjuvant, to generate and/or augment a polyvalent endogenous T cell response to CML associated antigens. Results from the pilot study suggest clinical activity. The investigational agent (K562/GM-CSF) is an allogeneic tumor cell line derived from a patient with CML in blast crisis which has been transfected in vitro with a plasmid DNA encoding the human cytokine, GM-CSF. K562 cells have been shown to express several of the antigens found in CML patient samples. GM-CSF transduced tumor cell based vaccines have been extensively studied in rodent models and early phase clinical trials as a means

to augment T cell and antibody responses to a range of antigens expressed by the immunizing cell line.

Objectives:

The primary goals of this study are to determine whether 4 K562/GM-CSF, booster vaccinations (+/- imiquimod cream) can produce molecular remissions in subjects considered non-responders to imatinib mesylate + a series of K562/GM-CSF primary vaccines OR return subjects who have recurrent disease after an initial complete molecular remission following imatinib mesylate + and a series of K562/GM-CSF vaccines, to molecular negativity. In addition, the safety and tolerability of K562/GM-CSF booster vaccinations will be further assessed in subjects with CML who previously underwent primary vaccination, by monitoring laboratory parameters and adverse events.

The secondary goals are to characterize the impact of K562/GM-CSF booster vaccinations on the change in frequency of CML antigen-specific T-cell responses, determine the impact of said change on tumor burden, and determine the change in frequency of T-cells specific for individual CML associated antigens (BCR-ABL fusion protein, proteinase-3, WT-1, and survivin). The relationship will be assessed between an individual's initial vaccination response (molecular responder with loss of response vs non-responder) as well as the length of time between primary and booster vaccinations (within a year from last vaccine vs greater than a year) on the effect of the booster vaccinations.

Patient Population:

Up to 12 patients, who have been diagnosed with Ph chromosome positive (Ph+) CML, are in first or second chronic phase on imatinib (see Appendix for definitions) and completed the entire treatment plan of J0345, including the scheduled study follow-up of 36 weeks. They must manifest measurable evidence of CML, but with no history of dose limiting toxicities or toxicities defined as severe adverse events in the pilot study. Up to 6 of these patients will come from the group of "incomplete responders to J0345/Human Gene Transfer Protocol # 309-602", while the remaining 6 individuals will derive from the group of "responders to J0345/Human Gene Transfer Protocol # 309-602 who relapsed" (see Protocol for definitions).

Study Design:

Single institution open-label trial.

Treatment Plan:

Disease burden will be measured in patients with the above characteristics, within 4 weeks of the first planned vaccination boost. All subjects must remain in at least a minor cytogenetic response on imatinib and following their vaccines. In addition they must exhibit measurable levels of CML by detection of the BCR-ABL hybrid fusion gene, using FISH, cytogenetics, or PCR. All eligible patients will then be boosted with a series of 4 booster vaccinations, with each vaccine dose of 1×10^8 irradiated K562/GM-CSF Vaccine Cells, administered every 3 weeks. All subjects who received 5% imiquimod (Aldara™) with their original vaccinations will receive it again with the boost. Imatinib will be continued throughout the study, unless clinical status dictates otherwise. Yearly long-term follow up will then be performed per FDA guidelines.

Dose:

Each vaccine cycle will consist of 1×10^8 irradiated K562/GM-CSF cells divided into 10 intradermal injection sites of 1×10^7 cells each.

Endpoints:

The primary endpoints of the trial are: 1) the measurement of the change in disease burden over time as assessed by BCR/ABL FISH and qRT-PCR, comparing the pre and post-vaccination periods, and 2) the safety of K562/GM-CSF vaccination in CML patients taking imatinib, as assessed by standard clinical and laboratory parameters. The secondary endpoint of the trial are the measurements of change in frequency of T cells specific for four CML associated antigens shown to be expressed by K562 cells (BCR/ABL p210, proteinase-3, WT-1, and survivin). These measurements will be used to test the hypothesis that vaccination with K562/GM-CSF leads to the induction or amplification of T cell responses to one or more of these candidate antigens, and that such responses correlate with a reduction in tumor burden or conversion to complete molecular remission.

Product:

K562/GM-CSF

The K562 cell line was engineered by plasmid transfection to secrete human GM-CSF. These cells are grown in suspension, irradiated at 10,000 rads to arrest cell growth, formulated in a dimethyl-sulfoxide (DMSO)-containing cryoprotectant and frozen in liquid nitrogen. K562/GM-CSF cells are thawed just prior to administration and injected intradermally without further manipulation.