## 234

### Synergistic Cytotoxicity of Gemcitabine, Clofarabine and Edelfosine (± DNA alkylating agent) in Lymphoma Cell Lines

*Ben C. Valdez [1], Axel Zander [2], Guiyun Song [1], David Murray [3], Yago Nieto [4], Yang Li [1], Richard E. Champlin [1], Borje S. Andersson [1]. [1] Stem Cell Transplantation & Cellular Therapy, UT MD Anderson Cancer Center, Houston, TX; [2] Abteilung Hama/Onkologie, Universitatsklinikum Hamburg-Eppendorf, Hamburg, Germany; [3] Experimental Oncology, Cross Cancer Institute, Edmonton, AB, Canada; [4] Stem Cell Transplantation & Cellular Therapy, UT M.D. Anderson Cancer Center, Houston, TX*

Lymphomas are malignancies of the B or T lymphocytes. Due to the heterogeneity of this disease, finding an effective form of treatment is challenging. One treatment option is hematological stem cell transplantation (HSCT), whose success partly depends on the efficacy of the pretransplant regimen. Nucleoside analogs such as gemcitabine (Gem) and clofarabine (Clo) are being used as part of pretransplant conditioning therapy for lymphoma patients. To improve the cytotoxicity of these drugs, we studied their synergism with edelfosine (Ed), an alkyl phospholipid with different mechanisms of antitumor activity, using cell line models. Exposure of the J45.01 and SUP-T1 (T-cell) and the OCI-LY10 (B-cell) lymphoma cell lines to $IC_{10}$ – $IC_{20}$ levels of the drugs resulted in strong synergistic cytotoxicity. While the individual drugs inhibited J45.01 cell proliferation by 3-19%, their combination inhibited proliferation by ~85%. 8-13% of J45.01 cells exposed to individual drugs underwent apoptosis, which increased to 71% for the triple-drug combination. Cell death correlated with increased phosphorylation of histone 2AX and KAP1, decreased mitochondrial transmembrane potential, increased production of reactive oxygen species, and release of pro-apoptotic factors. Apoptosis was indicated by cleavage of PARP-1, caspases 3 and 8, MCL1 and ANP32B. Exposure of caspase 8-negative I9.2 cells to [Gem+Clo+Ed] resulted in 40% inhibition of proliferation (as compared with 85% inhibition in caspase 8-positive J45.01 cells), suggesting the relevance of caspases in the antitumor activity of the three-drug combination. These observations are consistent with a decreased level of phosphorylation of the prosurvival protein AKT in cells exposed to [Gem+Clo+Ed]. The three-drug combination also activated the SAPK/JNK stress signaling pathway, which resulted in the phosphorylation and heterodimerization of the transcription factors ATF2 and c-Jun, two proteins involved in the DNA-damage response and apoptosis.

It is possible that [Gem+Clo+Ed] is stem cell sparing and should be investigated as salvage therapy in its own right; however, to further improve antitumor efficacy, we investigated the possible further synergism of the three drugs when combined with a DNA alkylating agent. Exposure of J45.01 to lower concentrations of the drugs (15 nM Gem, 25 nM Clo, 1.2 μg/ml Ed) in the presence of 6 μg/ml busulfan (Bu) or 0.18 μg/ml melphalan (Mel) resulted in stronger synergistic cytotoxicity. While the lower concentrations of [Gem+Clo+Ed] caused a moderate DNA damage response and apoptosis, addition of either Bu or Mel greatly enhanced the drug-mediated effects. Overall, these results can be used as a mechanistic impetus for evaluating [Gem+Clo+Ed]±DNA alkylator as salvage therapy for refractory NHL either in conventional dosing or as part of preparative regimens for patients undergoing HSCT.

## 235

### Outcome of Tandem Autologous/Allogeneic Hematopoietic Cell Transplantation in High-Risk Non Hodgkin's Lymphoma Patients: Stanford University Experience

*Kitsada Wudhikarn [1], Philip Lavori [2], Sally Arai [1], Laura Johnston [1], Ginna G. Laport [1], Robert Lowsky [1], David B. Miklos [1], Judith A. Shizuru [1], Richard T. Hoppe [3], Jonathan Benjamin [1], Everett Meyer [1], Robert Negrin [1], Wen-Kai Weng [1]. [1] Division of Blood and Marrow Transplantation, Stanford University Medical Center, Stanford, CA; [2] Health Research and Policy, Biostatistics, Stanford University School of Medicine, Stanford, CA; [3] Radiation Oncology, Stanford University Medical Center, Stanford, CA*

**Introduction:** Survival outcomes of chemotherapy resistant/refractory, relapsed and other high-risk Non Hodgkin's Lymphoma (NHL) patients are poor despite available salvage treatment strategies including immunotherapy, high dose chemotherapy and autologous hematopoietic cell transplant (HCT). Optimal subsequent therapies currently are not well defined. We explored the outcomes including efficacy and toxicities of the novel strategy with tandem autologous HCT followed by non-myeloablative (NMA) allogeneic HCT using total lymphoid irradiation and anti-thymocyte globulin regimen in high-risk NHL patients.

**Method:** Between November 2007 and December 2012, histologically proven NHL patients with high-risk features were prospectively enrolled to the institutional based tandem autologous HCT followed by NMA allogeneic HCT protocol (tandem HCT). Pre-transplant characteristics and transplant related parameters were recorded. We analyzed and reported post-transplant outcomes including event free survival (EFS), overall survival (OS), toxicities and adverse events.

**Results:** A total of 34 high-risk NHL patients were enrolled to the study between 2007 and 2012. Median age at autologous HCT was 59 years (range, 30-69 years). Diagnosis included 17 transformed diffuse large B cell lymphoma (DLBCL), 7 high-risk T cell NHL, 6 relapsed/refractory DLBCL, 2 double-hit NHL, and 2 refractory follicular lymphoma. Twelve patients were able to complete the pre-planned tandem HCT. Median duration between autologous and allogeneic HCT was 84.5 days (range, 66-211 days). Of 22 patients who did not undergo the pre-planned tandem HCT, three had allogeneic HCT under other available institutional based protocols. At the time of data analysis, with the median duration of follow up of 10 months, median EFS and OS for the entire group were 6.4 months and 13.3 months respectively. In 12 patients who underwent tandem HCT, median EFS and OS were 37.7 months and not reached respectively. The major causes of death were disease relapse (14 patients, 41.2%) and transplant related complication (5 patients, 14.7%). Of 12 tandem HCT patients, two patients (16.7%) died from disease relapse.

**Conclusion:** Survival outcomes of high-risk NHL are poor. Tandem autologous/allogeneic HCT might be a feasible and effective approach in these very high-risk patients. The most common cause of treatment failure remained disease relapse in these high-risk patients. Proper identification and selection of high risk NHL who will benefit from tandem autologous/allogeneic transplantation are challenging and warrant further studies.