2. **THE SCIENTIFIC ABSTRACT**

This multi-center, open label Phase 2 clinical protocol is designed to continue evaluation of Transgenic Lymphocyte Immunization (TLI) in subjects with Stage III melanoma under Cosmo Bioscience's IND (BB-IND-10891). As in the Phase 1 clinical trial, the proposed method of immunotherapy consists of the intravenous administration of autologous lymphocytes transfected with plasmid DNA which codes for selected peptide sequences from the reverse transcriptase of human telomerase (hTERT), an enzyme expressed in the vast majority (>85%) of cancer cells in humans.

The approach is termed TLI, and is centered around the immunogenic properties of adult B lymphocytes, which can be rendered transgenic by a method of spontaneous transgenesis (i.e., a process that does not require the use of facilitating molecules or electrical/magnetic fields). B lymphocytes represent 10-15% of cells in the peripheral blood, and much is known about their lineage characteristics as well as their capacity to serve as antigen presenting cells. B lymphocytes will be rendered transgenic with non-viral plasmid DNA coding for an immunoglobulin heavy (H) chain gene under the control of a B-cell specific promoter region. The Phase 2 Plasmid CB-10-01 codes for a chimeric immunoglobulin (Ig) heavy (H) chain gene under the control of an Ig promoter.

Per our agreement with the FDA, we modified the original \( \gamma_1 Y_{572}^{\text{NV}}_{2,540} \) plasmid (15.5Kb) which was used in production of the Phase 1 clinical trial material. Specifically, the SV40 promoter, the ampicillin-resistance gene, and part of the human genomic region were removed as requested. The Phase 2 plasmid (9727 kb), identified as CB-10-01, was constructed with elements from the pSV2-neo plasmid and the original plasmid. The resulting plasmid CB-I 0-01 contains the same functioning control and expression genes as the original Phase 1 plasmid.

The backbone of the plasmid is pSV2neo, a DNA of bacterial origin possessing the neomycin resistance gene, and the PBR322 origin of replication. The H chain gene is composed of a rearranged variable (V) region (Vh62) from a murine B cell hybridoma secreting an IgG1,k monoclonal antibody and a human IgG1 constant region from the pN\( \gamma_1 \) vector (Hybritech Corporation, San Diego, CA). The promoter for plasmid CB-10-01 is a constitutive Ig promoter derived from the murine B cell hybridoma H chain gene. The gene for neomycin resistance also confers kanamycin resistance for selective growth in prokaryotic cells.

This application is submitted with respect to an IgH chain gene coding for two hTERT acid peptides expressed in non-contiguous loops of the V region which are able to induce cytotoxic T lymphocytes in individuals of the HLA-2.1 genotype. In the native gene, the two human TRT regions flank an immuno-dominant CD4 T cell determinant.

Immunotherapy will be performed by injection of B lymphocytes harboring the gene described above, to elicit anti-tumor cytotoxic T cell responses targeted to tumor cells expressing hTERT. The steps through which cancer patients will be vaccinated by transgenic lymphocyte immunization are briefly outlined. 1) Subjects will undergo leukapheresis to obtain a sufficient number of lymphocytes for 3 infusions (a priming infusion and 2 booster infusions and transported to the cGMP facility where transgenic lymphocytes will be prepared. 2) Peripheral blood mononuclear cells (PBMC) will be incubated with plasmid DNA CB-10-01 for 60-90 minutes at 37° C. During this time a proportion of B lymphocytes internalize DNA and become transgenic. 3) Cells are
washed to remove non-internalized DNA, and incubated at 37°C overnight in serum free medium. 4) The next day the number of transgenic B lymphocytes is determined by FACS analysis, and cells are transported back to the clinical site for infusion into the same patient from which they had originated. Notably, cells to be infused will require no cell population enrichment or isolation. Transgenic lymphocytes for the primary infusion will remain unfrozen while cells for two booster infusions (administered approximately 28 days apart) will be frozen separately and stored in sterile freezing vials under cGMP standards in a dedicated liquid N2 tank.

Planned study enrollment is 40 subjects; 20 subjects will be enrolled and followed for 2 years from primary surgery, after which time an interim analysis will be performed. If the primary clinical endpoint is met, then the remaining 20 subjects will be enrolled.

The persistence of transgenic lymphocytes *in vivo* will be assessed in PBMC collected at regular intervals over the follow-up period. The nested-PCR method developed for this purpose is able to amplify the specific gene from a cell suspension containing less than 10 transgenic lymphocytes per 5x10^6 lymphocytes. Based on studies in mice we have determined that transgenic lymphocytes are eliminated from the blood and lymphoid organs after 3 weeks from injection. This is consistent with in vitro data indicating that, in transformed B cell lines, a transgene of the same type is lost after several days unless cells are kept under stringent selection conditions.