TECHNICAL ABSTRACT (Blancafort, P and Strahl, B.)

Background: Breast cancer stem cells (CSCs) represent a small a population of cells within a tumor that retain self-renewal capability and are able to generate tumors when injected in immunodeficient mice. In addition to their capability to originate tumors, tumor stem cells are resistant to chemotherapeutic agents, and are able to migrate and differentiate in all cell types of the mammary gland. These characteristics make cancer stem cells primordial therapeutic targets. In breast cancer patients, the "basal breast cancer subtype is found enriched in cancer stem cells. This subtype of breast cancer is associated with the poorest prognosis. Thus, there is a need in breast cancer therapeutics to develop novel approaches to target genes specifically dysregulated in these cells that are responsible for self-renewal and tumor initiation.

Objective/Hypothesis: The objective of this work is to create novel "chromatin engines", named Designed Epigenetic Remodeling Factors (DERFs). These factors will be designed to recognize 18-base pairs (bp) specific sequences in self-renewal gene promoters (found up-regulated in CSCs). These proteins will then incorporate specific silencing marks, which will stably trigger gene silencing. Thus, the IDEA is to direct the "chromatin editing" of the breast cancer stem genome, by altering the collection of the epigenetic marks at specific histone tails ("histone code" or "histone grammar") in CSCs. As a proof of principle, we will focus on the gene promoter SOX2, which plays a critical role controlling self-renewal of CSCs. Our specific hypothesis is that the delivery of sequence-specific ZF domains tethered to specific silencing enzymes will result on forced epigenetic silencing of SOX2, inhibition of CSC self-renewal and tumorigenicity.

Specific Aims: **1.** To design and construct Designed Epigenetic Remodeling Factors (DERFs) able to trigger epigenetic silencing of the endogenous SOX2 promoter in basal breast cancer cell lines. **2.** To analyze the phenotype of ATF-transduced cells (self-renewal assays, drug resistance assays). **3.** To investigate if DERFs are able to modulate tumor formation in immunodeficent mice.

Study design: We will engineer Designed Epigenetic Remodeling Factors (DERFs). The DERF is generated by linkage of two components, a highly specific DNA-Binding Domain (DBD) made of 6ZF domains, and a Chromatin Remodeling (CR) domain. The CR can switch-off a promoter by either eliminating positive epigenetic marks (which promote transcription, H3K4-demethylases) or by incorporating repressive marks into the nucleosomes (H3K9-, H3K36- and H3K27-methylatransferases. DERFs will be expressed in breast cancer stem cell lines using inducible retroviral vectors, and the phenotypic properties oftransduced cells will be analyzed in the cells and in a mouse model.

Innovation: We will develop a novel technology to direct the editing of the chromatin at specific gene promoters. Our strategy will allow to change the "histone code "of breast cancer stem cells, and to generate an epigenetic switch "off" in self-renewal gene promoters. This epigenetic switch will generate persistent chromatin silencing marks leading to inhibition of self-renewal in cancer stem cells. With the emergence of this novel chromatin-editing-grammar we hope to provide investigators with tools to "edit" the histone code of a variety of gene promoters, thereby modifying the epigenetic status of any desired promoter in a cancer cell.

Impact: Our technology will be able to inhibit proliferation of cancer stem cells, thereby inhibiting tumor formation. This will be especially important for the treatment of triple negative or basal breast cancer, which is very refractory to therapy. Another major achievement will be the idea to sensitize the CSCs to chemotherapy; meaning less dose of therapeutic agent will be required to destroy the tumor. This can potentially help patients preventing the relapse of the tumor/metastasis upon chemotherapeutic treatments, but also will help release the suffering and side effects of these drugs.