We are grateful for the generous support of the When Everyone Survives Foundation to advance our research developing anti-sense oligonucleotides (AONs) to promote skipping within the Mpl gene. The Mpl gene is an important receptor for thrombopoietin (TPO), a factor that can stimulate leukemia growth. The Mpl protein is coded by an RNA composed of a 12 exons, each separated with an intervening intron sequence. Normally, the exons are spliced together sequentially, however, if exons 9 and 10 are skipped, a variant of Mpl, Mpl-TR, is formed which actually impairs Mpl function. Our original data used AONs (short DNA sequences) directed to the boundaries of exons 9 and 10, which interfered with splicing to cause Mpl-TR to form instead of full length Mpl. By having more Mpl-TR, we hypothesized leukemia cells would lose the ability to respond to the essential growth factor, TPO.

To improve the efficiency of our AONs in cells and to make them better adapted to be used as drugs, we worked with our collaborator, Dr. Jonathan Watts, a medicinal chemist to generate AONs with various chemical modifications to test in our cell system. We found that making the AONs in an alternative chemistry called MOE, made them bind better and resist breakdown. As a result. MOE AONs were more effective at causing Mpl-TR production.

In other work ongoing in our laboratory, we had learned the gene Ott1 could control Mpl-TR splicing by binding to a position within exon 10 at a sequence known as an “exonic splicing enhancer” (ESE). We generated MOE AONs to block the exon 10 ESE and found an even greater production of Mpl-TR than our original AONs. When we tested it on bone marrow, Mpl-TR levels were much higher and the cells did not respond to TPO. We are now using the exon 10 ESE AON as our lead compound and configuring the sequence to test on human leukemia cells.

Finally, we have been using fluorescent-tagged AONs injected into mice to show the AONs will target the bone marrow and spleen, where leukemias grow. Using special analytic equipment, we found the AONs efficiently enter the bone marrow and spleen. Many different types of blood cells take up the AON and we show the AON travels to the nucleus of the cell, where splicing takes place. We have been testing multiple doses and timing to identify the most effective combination. Our next step is to test the optimized AON in mice that have been transplanted with human leukemia cells to measure the inhibition of the leukemia, as an early pre-clinical step to developing the AON as a drug. This work was selected for an oral presentation at the American Society of Hematology’s Annual Meeting in Atlanta, GA.