CEP1430 and CEP1507 Novel Drug Candidates for Targeting Pancreatic Cancer Stem Cells and for Circulating Tumor Cells as Potential new Combinational therapy for Advanced Pancreatic Cancer Patients

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Introduction
Pancreatic cancer is the fourth leading cause of cancer mortality in the US, despite significant improvements in diagnostic imaging and surgical resections. The 5-year survival rate remains less than 6%. The low survival can be attributed to positive resection margins, poor tumor differentiation, a large tumor size, lymph node involvement, high levels of preoperative carbohydrate antigen 19-9 (CA19-9), and persistently elevated levels of postoperative CA 19-9. Other contributing factors to low survival include microscopic or gross metastatic disease at the time of diagnosis. The treatment options for surgically accessible patients are suboptimal and include chemotherapy and radiation. There are many advantages and disadvantages associated with these therapeutic approaches: chemotherapy is highly toxic, radiation is costly, and surgery can be delayed. Thus, there is a critical need for effective and less toxic treatments.

Methods:

In vitro study: Pancreatic tumor cells (parental) and CSCs were isolated from 10 terminal donor patients that had undergone chemotherapy and radiation treatments. The ages ranged from 35-65 years old, and included both genders. The tissues were consented and obtained under IRB and HIPPA guidelines. The tissues were transported from the surgical suites to Celprogen in Human Pancreatic CSC complete growth media [M36115-42S] within 24 hours following surgical resection. Upon receipt, the tissue was sectioned into three equal pieces and per section one section was maintained as the heterogeneous tumor population and cultured as parental cell culture. The other section was processed further and isolated with CSC biomarkers, in Celprogen Media [E36115-42S] and ECM [E36115-42-T25]. The third section was utilized for generating PDX models in SCID mice. For the CSC generation, once the cell cultures were established within 7-14 days the cells were characterized by Flow, IHC, Western Blot and Real Time PCR for Pancreatic Stem cell markers. Both the parental and the Pancreatic CSCs were evaluated for tumorigenicity by injecting 1000 cells subcutaneously in SCID mice. For in vitro studies, following characterization the cells were seeded at 10,000 cells per well in a 96 well format, pre-coated with Celprogen ECM [E36115-42S] and cultured in complete growth media [M36115-42S]. The drugs were tested on the cultures at various concentrations for 72 hours at 5% carbon dioxide, 5% oxygen, humidified 37°C incubator. Real time cell proliferation and viability were determined with Incucyte Zoom (Essen Bioscience). TGF-α curves were generated for the test compounds CEP1430 and CEP1507. In addition to the two test compounds we also tested Gemcitabine, Taxol, Fluorouracil, Oxaliplatin and found that they were not effective against Pancreatic Cancer Stem cell (CSC) but were effective on tumor cells (differentiated CSCs). We found that CEP1430 was effective against Pancreatic CSC targeting selected pathways whereas, CEP1507 was very effective against Pancreatic CTCs.

In vivo study: One thousand viable human pancreatic CSCs and parental cells were subcutaneously injected on the hind limb of SCID mice. After 10 days post injection when visible tumors were observed the mice were separated into control or experimental group of 10 mice/group. Mice received IP injections three times per week for a duration of two weeks of the test drugs or diluent control. Each week the tumor growth measurements were performed with calipers and tabulated. At the end of the two weeks the mice were sacrificed and the tumor tissues were fixed, H&E and IHC stained, cultured, Real-time PCR performed for specific genes from total RNA, and cells evaluated by staining and flow cytometry with various Stem cell. Table 2 indicates the study design.

Results:

Figure 1 Model for Human Pancreatic CSC screening. Human Pancreatic Cancer stem cells were inoculated subcutaneously (1000 cells/mouse). 6-10 days post injection blood samples were obtained from animals 200-300 mm³ sized tumors for PK/PD and ex-vivo Biochemical/IHC analysis. PDx model was generated from patient’s tumor.

Figure 2. Generation of donor specific cells from solid tumor: Utilizing Celprogen Pancreatic CSC 3D cell culture model for Human Pancreatic CSC treated with drug CEP 1430 and ECM. Also used in generation of PDX models in SCID mice.

Figure 3. A. SCID mice injected with 50 CD44*CD24*ESA+ cells and CEP1430+CD24*ESA+ cells. Tumor growth measurements were performed with calipers and tabulated. At 10 days post injection the tumor volume was reduced by 80% without signs of toxicity when tested in SCID mice PDX model administered twice daily via IP injections for 30 days. CEP1430 reduced the tumor volume by 80-90 % in the treated group when compared with the control group. CEP1507 inhibited circulating tumor cells from proliferating at the metastatic site in comparison to control groups. The combination treatment group had localized tumor reduction of 85%.

Table 1. Cancer Stem Cell general characterization Markers , and PDx model. 2. Positive Cells Markers for Human Pancreatic Parental Cancer cells , CTC and Cancer Stem Cells.

Table 2. In vivo study design in SCID mice. * 5 females and 5 Males.