Summary FDA IDE #G190165
Clinical Trials.gov Identifier: NCT04267575
Canady Helios Cold Plasma Scalpel Treatment
at the Surgical Margin & Macroscopic Tumor Site

Study Design, Participants

Canady Helios Cold Plasma (CHCP) Trial; Clinical Trials.gov identifier: NCT04267575 Canady Helios Cold Plasma Scalpel Treatment at the Surgical Margin & Macroscopic Tumor Site was a Phase I, multiple centered, open labelled, prospective controlled trial that enrolled eligible subjects undergoing surgery + intraoperative CHCP treatment for Stage IV metastatic or recurrent solid tumors. A number of prior treatments were permitted i.e. chemotherapy, radiation, immunotherapy or surgery. Full details of the trial methods, inclusion and exclusion criteria are described in Clinical Trials.gov. Patients were recruited from two major academic institutions: Rush University Medical Chicago, Illinois and Sheba Medical Center, Tel HaShomer Israel. All patients received intra-operative CHCP treatment after the macroscopic tumor was removed. The primary end point was safety, and the secondary end point was to demonstrate ablation and slowing down tumor growth in cancer patients.

Results

From March 2020 to April 2021, 20 patients were consecutively treated with CHCP intra-operatively (Zone 0 tumor margin site) after macroscopic tumor resection (See patient characteristics **Tables 1 – 5**).

Age ranges from 26 to 85 years (Mean 59). Age categories: < 18 years (0%), between 18 to 65 years 14 (70%), >65 years 6 (30%). Sex: 50% female (n=10) and 50% males (n=10). Ethnicity: White 18 (90%), Black or African American 1 (5%) and other 1 (5%). Karnofsky - index > 60% and ECOG < 2 was observed in all patients. Region of enrollment: United States 15 and Israel 5 patients respectively **(Table 1)**. Twenty patients were treated with CHCP power ranging from 20v – 40v and time 3 – 7 min at the tumor bed (Zone 0) with a median CHCP power 25v and time 5min. Single or multiple normal tissue samples from 9 out 20 (45%) patients were also treated with CHCP ranging from 20v – 40v and time 1 – 7 min (median 25v 5min) intra-operatively which resulted in no intra- or post-operative complications **(Table 3)**. All patients had prior surgery, chemotherapy, radiation or HIPEC neoadjuvant or adjuvant treatment. One patient received intraoperative radiation (IORT), HIPEC, and CHCP treatment **(Table 4)**. Types of cancers treated: metastatic recurrent colon, ovarian, anal, mesothelioma, myxofibrosarcoma, breast, non-small cell lung, chordoma, melanoma, squamous cell, adenoid cystic, and metastatic renal cell, cholangiocarcinoma, non-small cell lung, pleomorphic sarcoma, pleomorphic spindle cell sarcoma, angiosarcoma, desmoplastic small round cell sarcoma **(Table 5)**.

Intra-operative Physiological Data

Measurements of Oxygen saturation, End-Tidal CO_2, pulse, and body temperature were recorded before, during, and after CHCP treatment. During CHCP treatment, patient body temperature ranged from 35 – 36 °C with a mean of 35.9, O_2 saturation ranged from 97.3 - 100% with a mean of 97.3%, End-Tidal CO_2 ranged from 30 – 40 mmHg with a mean of 33.9 mmHg, and pulse ranged from 44.3 – 104.8 beats per minute with a mean of 69.3 beats per minute. No statistically significant changes were found between measurements taken during CHCP treatment and at any other time during surgery for the four types of vitals measurements. A Forward Looking InfraRed (FLIR) thermal camera was used to record the intra-operative CHCP temperature of the cold plasma beam (See intraoperative physiological data **Table 6**).

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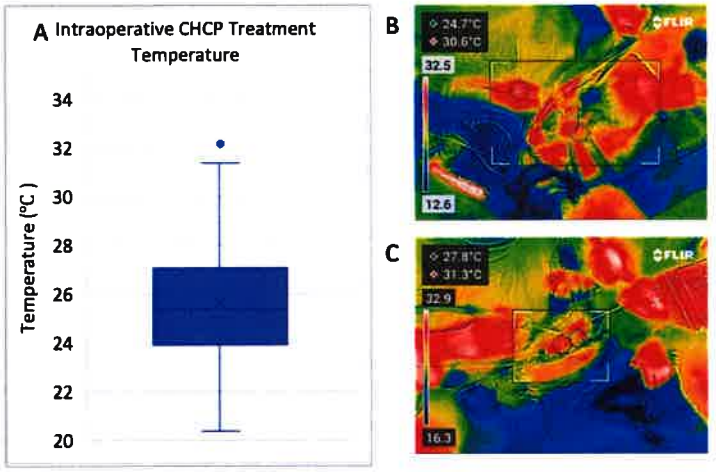

Table 6/Figure 21. Intraoperative CHCP temperature. During CHCP treatment a thermal camera (FLIR) was used to record the temperature of the cold plasma beam. (A) The mean of 25.6°C, median of 25.4°C, interquartile range of 23.9°C-27.1°C, minimum of 20.4°C, and maximum of 32.2°C measured by the FLIR camera during CHCP treatment. Representative thermal images (B) and (C) show the FLIR temperature measurement for the CHCP treatment area and surrounding tissue.

Histopathology Findings (see Table 2 and Table 8)

A surgical negative margin status (R0- status) was completed in 11 patients (55%). Nineteen out of 21 samples (90%) of normal tissues treated ex vivo with CHCP did not show any thermal damage or histological changes. Two samples demonstrated thermal damage, but a Bovie electrocautery device was used to dissect the tissue prior to CHCP treatment **(Table 2)**. Light micrographs displaying the morphology of the subject samples stained with H & E **(Table 8)** demonstrated CHCP – Induced Tumor cell death: 15/15 samples (100%), histological damages on normal tissue: 0/15 samples (0%), tumor detected in Zone 0: 5/9 samples (55%), tumor detected in Zone 1: 0/6 samples (0%) and tumor detected in normal tissue: 1/13 (7.7%).

Primary Tissue Culture (see Table 7)

Tumor samples of patients obtained at the clinical trial site Rush University Medical Center Chicago, IL were processed and cultured within 24 hours after resection at JCRI-ABTS, Takoma Park, MD USA. Primary cultures were established successfully from 10 tumor samples out of 15. Phase contrast images were taken with a Zeiss Axiocam ERc 5s (Zeiss, German) within 5 days of culture (Scale bars = 200 μm). Quantification of cell number or percentage of cell growth is plotted in the last column as Mean ± SEM (n = 3). Data analyzed by Student's t – test with differences considered statistically significant for *$p < 0.05$, ** $p< 0.01$.

Quantification plots demonstrated the following:

1. In 3 out of 10 cases (R0002, R0017, and R0018), no cancer cells survived after CHCP treatment.
2. In 5 out of 10 cases, fewer tumor cells survived from the CHCP – treated tissues compared to untreated tumor tissues. The morphological changes to the cells after CHCP treatment were also observed: fewer polygonal or rounded histocyte-like shaped (R0003, R0009, R0014) or vacuolated physaliferous (R0011) cancer cells. The majority of cells survived from R0014 were fibroblasts.
3. In 2 out of 10 cases, CHCP treatment did not significantly reduce the number of cancer cells or change the morphology (R0010 and R0013) because subjects R0010 and R0013 received sub-optimal CHCP treatment (6 min instead of 7 to 8 min) due to a malfunction of the connection between the cold plasma scalpel and CHCP generator. The tumor samples R0010 and R0013 were dissociated and cultured in JCRI-ABTS laboratory and treated with CHP with a lower tumor cell

density to demonstrate the potency of CHCP treatment on the melanoma and squamous cell carcinoma samples. CHCP demonstrate the capability of inducing cancer cell death at 20v 8min (Table 7 Figure 2) and 20v 4min (Table 7 Figure 3) for melanoma and squamous cell carcinoma, respectively.

Post-operative Course

There were no adverse events (CTCAE event version 4.03 to 5.0) within 30 days after Canady Helios Cold Plasma Scalpel treatment. Length of stay (LOS) total days ranged from 1 – 28 days with a median 5.5 days. Two patients had surgical complications (Clavien Dindo Grade III and IIIb) respectively. One patient underwent emergent angiogram and embolization by interventional radiology for intra-operative bleeding. A second patient return to the OR for a repair of floor of the mouth defect with buccal fat flap. (See patient characteristics **Table 1**).

Survival analysis

Overall survival was defined as the time of intraoperative CHCP treatment to death. The overall survival was calculated, and Kaplan-Meier survival curves were generated with RStudio, Version 1.4.1106. As of June 30, 2021, 17 out of 20 patients were still alive. Three patients died of their disease at 3, 4, and 10 months. The interim 16-month survival rate is 75.6% (95% confidence interval [CI], 53.3 – 100%).

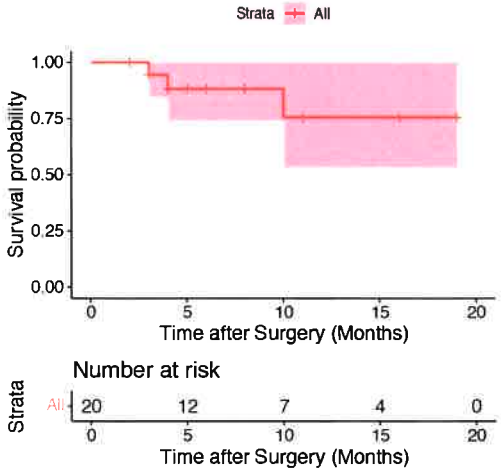

Figure 1. Kaplan-Meier overall survival curve for CHCP-treated patients (n=20).

Conclusion

We conclude that CHCP is a safe and effective new modality that effectively ablates and slows down tumor growth *in vitro*, *ex vivo* and in cancer patients.

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