Abstract for Oral Presentation

Sarah Nanotechnology: A Novel Approach for Metastatic Cancer Treatment

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Introduction:
The Sarah Nanotechnology, developed by NewPhase Ltd., Israel, is a medical device that comprises Sarah Nanoparticles (SaNPs) and a radiofrequency (RF) machine. Sarah Nanotechnology is aimed primarily for the treatment of small cell lung cancer (stage IV), while being studied in parallel for potential treatment applications in other cancer types.
The 4T1 triple negative mammary carcinoma is a transplantable tumor cell line that can be grown *in vivo* as a primary tumor in BALB/c mice. A major advantage of the 4T1 tumor is that 4T1 spontaneously metastasizes in a pattern that is analogous to human mammary cancer. When injected intravenously (i.v.), 4T1 cells are capable of metastasis to several organs affected in breast cancer and form metastases in the lungs.
SaNPs consist of a phase change material iron oxide nanoparticle (PCM NP) core surrounded by an encapsulating polymer crosslinker. The SaNPs are administered i.v. to the patient and localize on cancer cells. Following delivery and attachment of the SaNPs to malignant cells, the patient undergoes partial body RF non-ionizing irradiation (300 kHz) with the system's RF machine. The SaNPs convert the applied RF electromagnetic field (EMF) to thermal energy whereas the PCM NP absorbs energy without heating above the melting enthalpy point, controls, and stabilizes the temperature of the SaNP to 50^0C thereby, causing the attached malignant cells hyperthermic cell death in the primary tumor and tumor metastases. The main innovation of the SaNP is in the inner ability to control its temperature. The PCM core of the SaNP allows temperature control without inducing thermal ablation. The Sarah Nanotechnology offers two major advantages:
1. Systemic treatment - The SaNPs are administered i.v. and localize on cancer cells through the use of passive targeting based on the Enhanced Permeability and Retention (EPR) effect that enables the preferential retention of SaNPs in the tumor.
2. Total body irradiation - Because of the SaNP's unique properties, any tumor larger than 20 microns will be sensitive to the treatment, including vascular tumors. At this tumor size, the EPR effect is expected to be significant.
For its therapeutic effect, the SaNP needs to accumulate in the tumor. However, the accumulation of SaNP in healthy organs is an important risk consideration and therefore, potential toxic effects and the biodistribution of the SaNPs were assessed in mice in the following experiments. In addition, the efficacy of treatment as well as the effect of Sarah Nanotechnology on the survival of mice bearing 4T1 metastatic tumors were demonstrated.

Aim:
Safety, biodistribution, efficacy, and survival studies were performed in order to evaluate the effects of SaNPs in both healthy and 4T1 mCherry breast cancer bearing BALB/c mice.

Methods:
All protocols were reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) and followed officially approved procedures for the care and use of laboratory animals.

1. **Safety study**
   The potential toxic effects of SaNPs were assessed following an i.v. bolus injection to BALB/c healthy mice. Mice (n=60) were subjected to observation and terminated at 3

different timepoints, 3, 14, and 30-days after treatment. Measurements post-sacrifice included blood analyses (hematology, chemistry), necropsy, gross pathology, and histopathology of vital organs conducted by a Board-certified study Pathologist.

2. **Short distribution study**
   The distribution of SaNPs in vital organs, blood, and in tumor tissue over time, was assessed at 3 different timepoints (2, 4, and 8 hours) following a single i.v. injection of SaNPs, in the murine 4T1 mCherry breast cancer metastatic model in BALB/c female mice (n=12). The iron oxide content in the tissues and blood was determined by SQUID (Superconducting Quantum Interference Device) analysis that measures electromagnetic properties and can therefore detect the presence of the magnetic iron oxide nanoparticles of the SaNP in organic samples.
3. **Long distribution study**
   The long distribution and clearance of SaNP in vital organs and blood was evaluated over time. Healthy BALB/c female and male mice (n=30) were treated with Sarah Nanotechnology (SaNP injection, followed by 30 min. EMF application at 8 hours post injection). The animals were sacrificed at 14, 30 and 60/90 days post-treatment and the samples were analyzed by SQUID.
4. **Efficacy study**
   The efficacy of Sarah Nanotechnology was evaluated following 3 treatment cycles, in the murine 4T1 mCherry breast cancer metastatic model in BALB/c female mice (n=10). The treatment cycles (SaNP injection, followed by 30 min. EMF application at 8 hours post injection) were applied within 2-days intervals between each cycle. The primary endpoint of the study was the number of metastases in the lungs. At the end of the study, the mice were sacrificed and the lungs were subjected to macro analysis by visual count of the metastases, fluorescence imaging of the lungs using the Cri Maestro™ multispectral imaging system, in order to determine the fluorescence intensity of the lung metastases, and histopathology analysis.
5. **Survival study**
   In this study, the survival of BALB/c mice (n=10) bearing mCherry breast cancer metastatic tumors was assessed. The mice were treated with 5 cycles of Sarah Nanotechnology (SaNP injection, followed by 30 min. EMF application at 8 hours post injection) and followed-up until the last mouse died. The primary endpoint of the study was the survival of the mice; treated vs. untreated control.

Results:

The safety study indicated that no mortality occurred in none of the animals throughout the 3, 14, and 30-days observation periods. No abnormal clinical signs in response to treatment were observed throughout the study in any of the animals and they exhibited normal body weight gain. The histopathology evaluation did not find any treatment related changes in the organs of the mice, except of sporadic cases of pigment laden macrophages seen in the liver and lungs of treated animals, at all timepoints. As these changes were of minimal degree and were thought to reflect the accumulation of the pigment itself in these organs, were not associated with necrosis, significant inflammation, or any other pathological finding/s, these changes were not considered as adverse.

The results of the short distribution study showed that the amount of SaNP that reaches the lungs and lung metastases was the highest at 8 hours post injection. Based on this study, the time of EMF application post SaNP injection was set to 8 hours.

The long distribution study is still in progress. However, the same experiment with similar designs demonstrated 20-30% residual SaNP in the liver at 30-days post-treatment and therefore we expect to see similar results with the current design. At 60/90 days post-treatment most of the SaNPs are cleared from the body.

The main findings of the efficacy study demonstrated a 49.6% reduction in the number of lung metastases, based on the macro visual count; 22.5±10.7 metastases in the control group vs. 13.6±4.6

in the treatment group. Histopathology analysis demonstrated that there was a 70% reduction in the relative size of metastases in the lung sections of the treated compared to the control mice; 3.3795±1.219 mm^2 in the control vs. 1.0116±0.602 mm^2 in the treatment group. The fluorescence imaging analysis (Fig.1) showed a 91.3% reduction in the fluorescence intensity of metastases in the lungs thus, further supporting a reduction in the size of metastases due to treatment (i.e. efficacy).

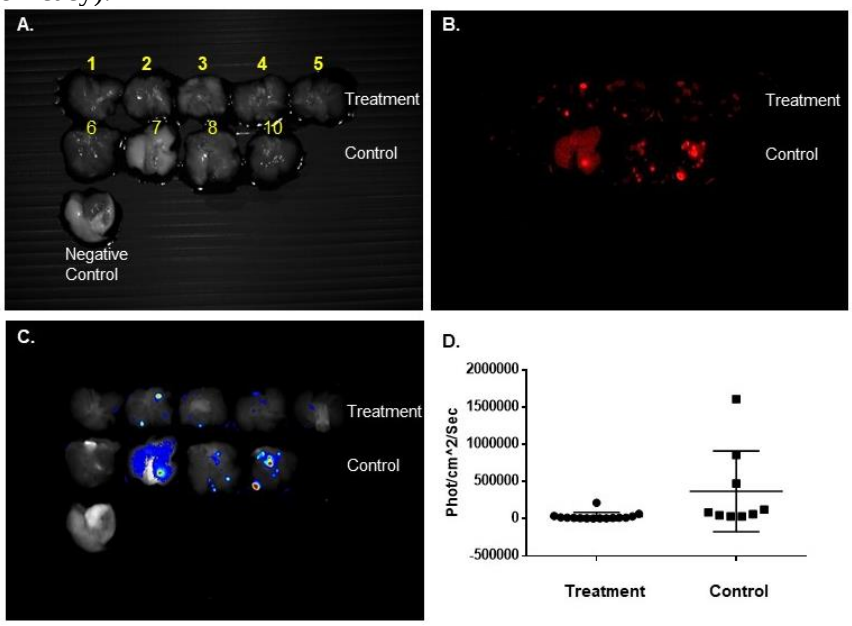

Figure 1: Maestro fluorescent *ex-vivo* imaging of lungs
Lungs (A); mCherry fluorescent imaging (red spots) (B); Heat map of viable metastases (blue spots); Quantitation of fluorescence intensity (D).

The results of the survival study showed that the number of mice surviving at the end of the experiment was significantly greater by more than 40% in the treated compared to the control mice group. Results are shown in Fig.2

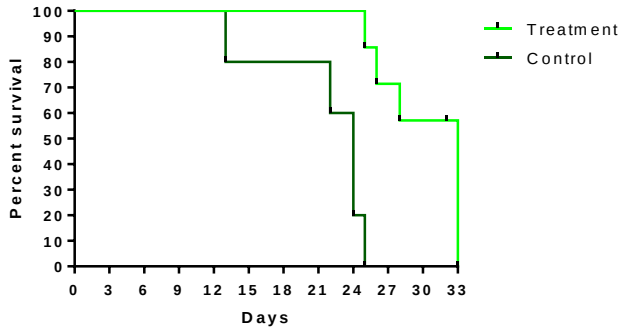

Figure 2: Sarah Nanotechnology treatment in metastatic breast cancer significantly prolongs survival
Kaplan-Meier analysis demonstrates a significant improvement in survival after treatment compared to the control group (p-value <0.005***).

Conclusions:

1. Systemic administration of SaNP followed by EMF application to mice, that were terminated at 3 different timepoints after treatment (3, 14, and 30 days), was not associated with any evident adverse reactions.
2. Based on the short distribution study, the optimal time for EMF application after i.v. injection of SaNP was 8 hours.
3. The percentage of residual SaNP at 30-days post treatment is expected to be ~20-30%.
4. The results of the efficacy study, following 3 treatment cycles of Sarah Nanotechnology treatment, showed that the primary endpoint of the study was reached as a significant reduction in the number of metastases was demonstrated as well as a significant reduction in their size.
5. The survival of treatment mice was significantly improved compared to untreated control mice, following 5 treatment cycles of Sarah Nanotechnology treatment.