Abstract for Oral Presentation

Safety Assessment of Sarah Nanotechnology in Swine Models

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Introduction:
The Sarah Nanotechnology (New Phase Ltd., Israel) is a medical device that comprises Sarah Nanoparticles (SaNPs) and an Electromagnetic Induction System (EIS). Sarah Nanotechnology strategy is aimed primarily for the treatment of stage IV small cell lung cancer (SCLC).
SaNPs are multicore NPs containing encapsulated iron oxide, administered intravenously (IV) to the patient and become localized via the Enhanced Permeability and Retention (EPR) effect into the tumor vicinity. Following delivery and accumulation in the malignant tissue, the patient undergoes a partial-body electromagnetic field (EMF) application with the EIS at 290 kHz ± 20%. The SaNPs absorb the electromagnetic energy and convert it to thermal energy, reaching a pre-determined temperature (50±3°C), thereby heating the malignant cells and causing hyperthermic cell death at sub-ablative temperatures.
For its therapeutic effect, the SaNPs need to accumulate in the tumor. However, accumulation in healthy organs is an important risk consideration and therefore, potential adverse effects and biodistribution were assessed in swine. Previous studies conducted by New Phase Ltd. have shown that SaNPs are stable, safe, and biocompatible *in vivo* in small animal studies (mice, Guinea pigs, and rabbits).
Pigs are considered to be one of the major animal species used in preclinical toxicology sharing similar anatomic and physiologic characteristics with human, particularly those related to thermo-physiology, such as comparable thermal mass, surface area, water loss through skin, metabolic energy per unit surface area, cardiac output, thermo-regulatory mechanisms, and electromagnetic and thermal properties, making them clinically relevant.

Aim:
Toxicity and biodistribution studies were performed in order to evaluate the effects of SaNPs with or without EMF application in healthy swine models.

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.
The following studies were conducted:

1. Sub-acute systemic toxicity and biodistribution of increasing SaNP doses of 60%, 80%, and 100% (without EMF) were examined in Sinclair minipigs for a follow up period of 30 days.
2. Sub-acute systemic toxicity and biodistribution were examined in Göttingen minipigs treated with 60% and 100% single doses of SaNPs followed by EMF application with the EIS (30 min. of continuous radiation at 30 mT) (e.g. full treatment) and a follow up period of 30 days.
3. Chronic systemic toxicity and biodistribution of repeated SaNP doses of 100% (without EMF) were evaluated in a Sinclair minipig that received 3 IV infusions at an interval of one month between each dosing session. The animal was followed up for 93 days to assess any potential safety issues.

Blood and tissue samples were collected for biodistribution analysis using particle electron paramagnetic resonance (pEPR) which is based on a low-field and low-frequency electron paramagnetic resonance enabling the quantitation of superparamagnetic iron oxide NPs. The main

advantage of the pEPR method relies on its ability to distinguish between endogenous and exogenous iron sources, showing greater sensitivity for exogenous magnetic NPs.

Results:

Doses of 60%, 80% and 100% of SaNP alone, and 60% and 100% doses of SaNP with EMF were well tolerated with no adverse reactions in any of the pigs up till 30 days.

All clinical parameters (blood pressure, heart rate, O_2, CO_2, and body temperature) were stable throughout the administration of SaNP doses.

There were no significant changes in the clinical pathology parameters (Hematology & Chemistry) and most changes observed were within normal ranges.

Histopathology analysis demonstrated no treatment-related toxicity in any of the organs examined (liver, lungs, kidney, spleen, brain, heart, lymph nodes) in all animals.

The presence of iron oxide containing NPs was identified by Prussian blue (PB) staining. Minimal multifocal PB stained macrophages were noted in the lungs but were not associated with any inflammation and therefore considered as not adverse. No such PB positive granules were noted in any of the other organs examined.

Analysis of iron content by pEPR showed that SaNPs accumulated primarily in the lungs, liver, and spleen. Excretion of SaNP from pigs that received 60%, 80%, and 100% doses (without EMF) was 83%, 45%, and 39%, respectively, after 30 days. For the pigs that received 60% and 100% doses (with EMF), the excretion of the SaNP was 75% and 59%, respectively, after 30 days. In the animal that received 3 repeated doses, the total SaNP percentage that accumulated in vital organs was 52% which corresponds to 48% SaNP that was cleared from the animal's body after 93 days.

Conclusions:

The main findings of the studies show that single doses as well as 3 repeated doses of SaNP without EMF application were well tolerated in swine models *in vivo*.

In the animals that received a full treatment, a dose level of 60% and 100% SaNP followed by EMF application was well tolerated. There were no SaNP-related or EMF-related clinical observations noted during the dosing, exposure, or observation periods.

The absence of inflammation, necrosis and adverse reactions suggest that the SaNPs remain intact in the body and do not degrade or cause toxicity or thermal damage, even after 3 repeated doses of SaNPs.

These safety assessments open new avenues for generating SaNPs and EMF application as a potential novel therapeutic modality for cancer patients.