Development of NAS911 as a medical countermeasure for radiation induced tissue injury

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Introduction

The risk of military or civilian exposure to ionizing radiation, from a purposefully initiated nuclear event, via accidental industrial release or by therapeutic application in a medical environment persists, as does the need for suitable medical countermeasures (MCM).

New Amsterdam Sciences is advancing pre-clinical development of NAS911 as an innovative MCM for the treatment of acute radiation syndrome (ARS) and other indications, including Delayed Effects of Acute Radiation Exposure (DEARE) or radiation-induced lung injury (RILI), which is often manifested as fibrotic interstitial lung disease (ILD). The most common ILD is Idiopathic Pulmonary Fibrosis (IPF), and preliminary studies using radiation as a model for IPF have garnered our compound Orphan Drug designation by FDA.

NAS911 consists of the synthetic peptide Sar^9, $met(O_2)^{11}$-Substance P, an analogue of the endogenous neuropeptide Substance P (SP) and a neurokinin-1 receptor (NK1R)-specific agonist displaying novel receptor activation characteristics. NAS911 has been shown to have a higher binding affinity for NK1R than SP, which implies a potential for less off target risk and side effects. NAS911 has also been shown to be resistant to proteolysis and dissociation from the NK1R, which promotes re-sensitization of the pro-inflammatory effects of SP.

Persistent association of NAS911 prevents NK1R recycling to the cell membrane and continued intracellular kinase pathway activation [2], possibly resulting in the suppression of neurogenic inflammation.

Our working hypothesis is that as Substance P is now recognized to play a role as an alarm molecule and effector of wound healing and tissue remodeling, coordinating complexities in cytokine and growth factor release and activity via activation of the NK1R and the stimulation of immune and other cells, the ability of NAS911 to affect ARS and DEARE could suggest a broader therapeutic role deserving exploration.

Materials and Methods

A Cesium-137 γ-ray source operating at dose rate of approximately 2.0 Gy/min was used for initial radiation exposures. Female, C57Bl/6J mice between 6–8 weeks of age (Jackson Laboratory, Bar Harbor, ME) were acclimated for one week prior to experimentation.

Study 1: Percent Survival: oral gavage administration Animals were exposed to an LD100/30 radiation therapy (RT) dose of 7.75 Gy total body irradiation (TBI) and 10 mg/kg NAS911 by oral gavage. Controls were no RT and saline administration. Mice (n=10) were dosed 14 days, starting on Day 1 post RT and another 10 mice were dosed every other day for 14 days, starting on Day 2. A radiation control group (n=10) received saline for 14 days, starting on Day 1. All mice were given acidified H_20 and antibiotic chow starting 14 days before RT stopping at 30 days post RT. Surviving animals were calculated daily.

Study 2: 16 week Club Cell Secretory Protein Gene Abundance Mice were exposed to 7.75 Gy TBI or had no RT. Groups were administered either 25 uL PBS, 2 mg/kg NAS911, or 20 mg/kg NAS911 via oral gavage at 24 hours post irradiation and daily for 2 weeks. The surviving irradiated mice (all of the NAS911 treated mice survived), received an additional 10 Gy lung radiation 8 weeks later, at 10 weeks, in a dual hit model. All animals were sacrificed at 16 weeks. RNA was isolated from one half of the left lung and 1 mg was reverse transcribed to cDNA for quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) for club cell secretory protein (CCSP) and Glyceraldehyde 3-phosphate dehydrogenase (G3PDH) with the following primer sequences. CCSP: CCACAAGAGACCAGGATA (upper) and GTGAGATGCTCGCAGTTT (lower). G3PDH: CCCAATGTGTCCGTCGTG (upper) and CCTGCTTCAC CACCTTCTTG (lower). CCSP gene expression was normalized to G3PDH expression.

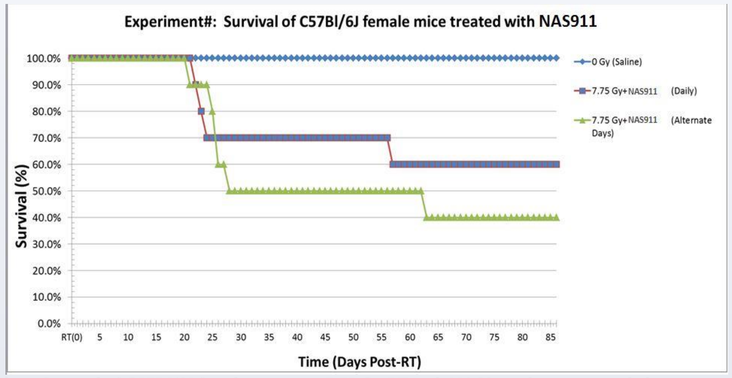
Study 3: T Lymphocyte and Neutrophil Cell Counts

Mice were exposed to a 5 Gy TBI followed by an 11 Gy lung radiation, designed to cause lung injury only. Dosing was performed at 3 different schedules. Acute dose of 20 mg/kg and started 3 days post-irradiation, continuing for 10 days. Animals were sacrificed at 2 weeks. A combination of both acute and chronic dosing was done and counts were done starting at 20 weeks post-RT. Chronic dose of 20 mg/kg started at 12 weeks post-irradiation, continuing for 10 days. Animals were sacrificed at 20 and 26 weeks. Sections of lungs from 2, 12, 20, and 26 week mice were obtained, T lymphocyte and neutrophil markers, CD3 and Gr-1 respectively, were visualized with IHC to enumerate their respective levels, and averaged from 10 images.

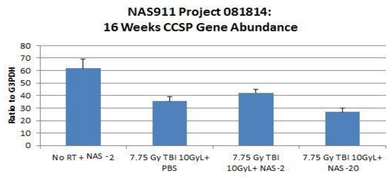
Study 4: Post-irradiation Fibrosis Markers in the Lungs

Mice were irradiated with a 5 Gy TBI dose plus an additional lung dose of 10 Gy. Animals were split into treated and non-treated groups, and beginning 24 hours post-RT, the treated groups were given 2 mg/kg of NAS911 orally, continuing for 10 days. Animals were sacrificed at 26 weeks and lungs were stained with H&E. A total of 10 lung images were used to quantify activated macrophages, which appear larger than resident macrophages. These are used as markers of radiation induced lung fibrosis.

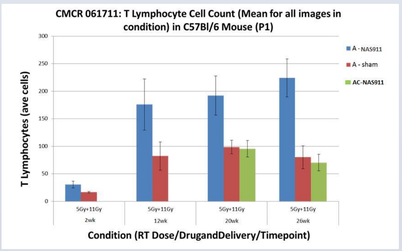
Results

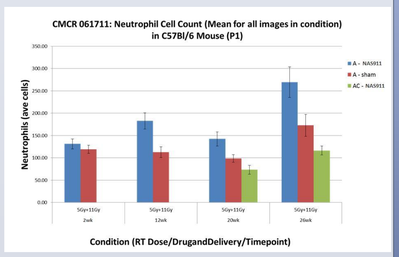


Study 1: Survival of C57Bl/6J female mice post 7.75 Gy RT with NAS911 administration daily or on alternate days. On Day 85, 40% of every other day treated and 60% of daily NAS911-treated animals were alive despite acidified water and antibiotics termination at day 30.

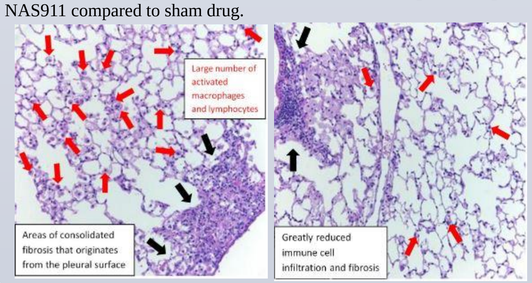


Study 2 (left): NAS911 dose post-radiation for weeks 1-2 protect all mice from ARS and partially restore progenitor club cells when measured at week 16, suggesting some long term preservation of airway progenitors. Gene abundance was normalized using the G3PDH housekeeping gene.





Study 3 (above): Both T lymphocyte (immune cell precursors) and PMN (immune response cells) recruitment, which are considered markers of radiation damage, were reduced by acute+chronic (AC) treatment with NAS911 as seen at 20 and 26 weeks. Average number of T Lymphocytes and Neutrophils at 2 weeks, 12 weeks, 20 weeks, and 26 weeks after 5 Gy and 11 Gy radiation therapy (RT) and acute NAS911, sham drug, or acute+chronic administration. Higher average T Lymphocyte counts in mice given NAS911 compared to sham drug.



Study 4 (left): H&E stained lung 26 weeks post-irradiation. The irradiated + non treated animals (left) show developing areas of heavy fibrosis, as well as activated macrophages and lymphocytes (arrows). NAS911 treatment resulted in less immune cell infiltration and fewer areas of fibrosis.

Discussion

A daily treatment regimen of NAS911 was able to arrest the lethality of ionizing radiation, with 60% survival to day 85 post-radiation, and with 40% survival at alternative day dosing. Thus, while both treatment regimens showed a radioprotective effect, the daily treatment was more effective. Note that animals are sickened by the radiation on the same timeline as untreated exposed animals (LD100 at 30 days) but fewer animals succumb at the two dosing paradigms tested. Additional testing will determine the optimal treatment regimen.

Club cell (non-ciliated bronchial epithelial cells that are progenitors involved in bronchoepithelial wound repair) preservation is associated with mitigation of the effects of radiation exposure [5] and CCSP expression is reduced by lung irradiation. Acute NAS911 treatment following total body irradiation results in approximately 20% increase in normalized (to the housekeeping gene G3PDH, see ref. 1) CCSP gene abundance compared to no drug treatment. Since this assessment is made weeks after both drug treatment and a second lung-only exposure, it suggests the ability of acute NAS911 treatment to have an impact prophylactically. The restoration of CCSP expression is equivalent (data not shown) to the effect of acute treatment with granulocyte colony stimulating factor (G-CSF), the only approved drug for hematopoietic ARS. The higher NAS911 dose (20mg/kg) was not as effective, but the exact reason remains unknown. While additional testing will identify the optimal dosage and timing for NAS911 treatment, the impact of the drug on airway precursor cell integrity is equivalent to that of the approved G-CSF, but in the double-hit model, suggesting this NAS911-induced preservation is pivotal to the survival benefit afforded by such post-exposure therapy, and that it may enable pre-exposure dosing to prevent delayed pulmonary sequelae, partially through preservation of bronchoepithelial precursors.

As a potential mechanism underlying protection from acute radiation, protection of hematopoietic precursors would be a prime candidate, as post-radiation neutropenia increases risk of infection and death. As experimental animals are routinely fed irradiated chow and given acidified water to minimize morbidity, survival data in the absence of such support beyond 30 days is noteworthy. Pulmonary ramifications of normalization are evident as acute NAS911 treatment elevates IHC-determined T-lymphocyte and neutrophil levels. NK1R-linked hematopoietic stem cell activation could also drive immune cell interactions with club cells and injured bronchiolar epithelium, impacting tissue repair processes in organs targeted in RILI or DEARE such as lung. Elevation of T-lymphocytes and neutrophils seen on acute treatment (when observed 2-26 weeks following radiation) could limit future damage. Indeed, imaging shows less fibrotic tissue and macrophage infiltration in the mice lung treated with NAS911 compared to the mice lung without any treatment. Macrophage infiltration drives repair of the damage tissue and recruitment of other immune cells [4], which could result in an excess of fibrous connective tissue associated with the healing and remodeling process. Thus, the diminution in macrophages shown in images could be associated with less connective tissue generation and consequently less fibrosis.

Collectively, NAS911 was shown to recruit and increase circulating blood cells which mitigates neutropenia of acute hematopoietic-ARS as well as aids in the repair and regeneration of damaged tissue and normalization of tissue healing, effectively halting fibrotic tissue formation. In conjunction with preliminary work showing diminished morbidity and mortality following influenza infection (not presented here), NAS911 might bring unique capabilities to post-radiation environments, both acutely and as prophylaxis, in civilian and war fighter settings.

References

[1] Barber, R. D., Harmer, D. W., et al. (2005). GAPDH as a housekeeping gene: analysis of GAPDH mRNA expression in a panel of 72 human tissues. Physiol Genomics 21(3):389-395.

[2] Cattaruzza, F., Cottrell, G., et al. (2009). Endothelin-converting enzyme 1 promotes re-sensitization of neurokinin 1 receptor-dependent neurogenic inflammation. British Journal of Pharmacology, 156(5), 730-739.

[3] Cottrell, G. S., Padilla, B. E., et al. (2009). Endosomal Endothelin-converting Enzyme-1. J Biol Chem, 284(33), 22411-22425.

[4] Ginhoux, F., Schultze, J. L., et al. (2016). New insights into the multidimensional concept of macrophage ontogeny, activation and function. Nature immunology, 17(1), 34-40.

[5] Manning, C. M., Johnston, C. J., et al. (2013). Exacerbation of Lung Radiation Injury by Viral Infection: The Role of Clara Cells and Clara Cell Secretory Protein. Radiat Res, 179(6), 617-629.

[6] Sagan, S., Chassaing, G., et al. (1996). Tachykinin peptides affect differently the second messenger pathways after binding to CHO-expressed human NK-1 receptors. J. Pharmacology and Experimental Therapeutics 276,1039-1048

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