(DNMT1).

**Conclusions:** In this study, VR#2 could potentially be used protect the epidermis from UVB irradiation. In addition, results of this study suggest that UVB protection by vegetable-based resource is likely to be related to its expression of cell survival and anti-senescence-related genes such as PCNA, VEGF, TERT, HDAC1, and DNMT1 and reduction of pro-inflammatory cytokines against UVB-induced KSC damage.

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## P-211

### KCHO-1(Mecasin), anovel herbal anti-inflammatory compound, attenuates oxidative stress in ananimal model of amyotrophic lateral sclerosis.


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**Introduction:** Amyotrophic lateralsclerosis (ALS) is a neurodegenerative disease characterized by selective deathof motor neurons in the central nervous system. The main cause of the diseaseis still elusive but several mutations have been associated with the diseaseprocess. In particular, mutant SOD1 protein causes the neuro-inflammation byactivating glia cells and contributes to the motor neuron degeneration

**Materials and Methods:** The novel chemical compound KCHO-1 is a naturalethanol extract obtained from traditionally used herbal combinations whichinclude Curcuma longa, Salviamiltiorrhiza, Gastrodia elata, Chaenomeles sinensis, Polygala tenuifolia,Paeonia japonica, Glycyrrhiza uralensis, Atractylodes japonicam andprocessed Aconitum carmichaeli

**Results:** In this study, we aimed to determine whetherKCHO-1 can reduce the neuroinflammation and prevent the degradation of motorneurons in an ALS model. To address this issue, we orally administrated KCHO-1 tohSOD1 $^{G93A}$Tg mice. KCHO-1 administration delayed disease onset and improved motoractivity in symptomatic hSOD1 $^{G93A}$Tg mice. Importantly, we found that KCHO-1 could reduce M1 type microgliaexpression and mitigate neuroinflammation by reducing the gp91$^{phox}$ andiNOS expression in the spinal cord.

**Conclusions:** These data suggest that KCHO-1 has an anti-inflammatoryeffect on the ALS and could be an effective therapeutic agent.

## P-212

### Generation of canine induced pluripotent stem cells using a genomic integration-free method


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**Introduction:** Canine induced Pluripotent Stem Cells (iPSCs) can provide great potential for regenerative veterinary medicine and may assist in the development of new therapeutics and pre-clinical studies for both dogs and human. To date, there have been several reports on the generation of canine iPSCs using retroviral or lentiviral transduction of Yamanka's factors. However, there is no report of canine iPSCs generated by genomic integration-free methods. According to previous studies, a polycistronic and synthetic self-replicating RNA system was developed for generating human iPSCs by the RNA replicon of Venezuelan Equine Encephalitis (VEE) virus. The VEE replicon is a positive-sense and single-stranded RNA which is similar with cellular mRNA containing a 5' cap andpoly (A) tail. It is no potential for genomic DNA integration problems because it does not use a DNA intermediate.

**Materials and Methods:** Here, we generated canine iPSCs by a single transfection of the VEE-reprogramming factor (VEE-RF) RNA. To generate integration-free canine iPSCs, the VEE-OKS-iG RNA that expresses four reprogramming ORFs (hOct4, hKlf4, hSox2 and hGlis1) was transfected. Also, B18R mRNA was co-transfected to reduce immune response by VEE replicon.

**Results:** Putative canine iPSC colonies first appeared between day 15-25 and were identified by immunohistochemistry of live cells using TRA-1-60 antibody. The canine iPSC colonies also showed clear alkaline phosphatase (AP) activity.

**Conclusions:** Although further study will be needed, the non-integrating and self-replicating VEE RNA replicon system has the potential to make a great contribution to generating clinically applicable canine iPSCs.

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## P-213

### Effects of human neural stem cells transduced with cytosine deaminase and interferon-β on the growth of K562 human chronic myeloid leukemia cells in a xenograft model

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**Introduction:** Gene-directed enzyme/prodrug therapies have been found to be more advantageous compared to conventional cancer treatment method. One of these, a cytosine deaminase (CD)/5-fluorocytosine (5-FC) system, is known to induce apoptosis of cancer cells by converting 5-FC, a prodrug, to its metabolically active form, 5-fluorouracil. In this study, human neural stem cells (hNSCs) derived enzyme/prodrug therapy was used to treat leukemia. The parental hNSCs, HB1.F3, were engineered to express E. coli CD and/or human interferon-β.

**Materials and Methods:** To manufacture animal models xenografted with leukemia, K-562 cells ($1\times10^6$) were mixed with Matrigel at 1:1 volume ratio of Matrigel to PBS in 100 μl and injected subcutaneously (*s.c.*) into the back of athymic nude mice. This animal study was performed for 24 daysafter hNSCs injections. When tumor volume reached 500 $mm^3$, CM-Dilpre-labeled hNSCs ($4 \times 10^6$ cells per mouse) were injected subcutaneously adjacent to the tumor mass. Another group, CM-dil pre-labeled hNSCs ($4 \times 10^6$cells per mouse) were injected intravenously. hNSCs were injected on the first day of each week. Two days after the injection of hNSCs, all mice received i.p. injections of 5-FC (500 mg/kg/day in 100 μl PBS) everyday for 24 days. At 24 h after the last 5-FC treatment, the mice were euthanized and tumor masses were harvested for molecular analysis.

**Results:** In a xenografted mouse model administered with hNSCs intravenously or subcutaneously, hNSC significantly inhibits the growth of tumor mass and extends survival date in the presence of a prodrug. In addition, HB1.F3.CD.IFN-β treatment group showed more anti-tumor effects compared with HB1.F3.CD treatment group, indicating that IFN- β may have a synergistic effects for directly killing leukemia tumors.

**Conclusions:** The present results represent that engineered hNSCs and prodrug treatment inhibited the proliferation of leukemia. These results suggest that gene therapy employing genetically engineeredstem cells expressing CD and IFN-β may be effective for treating leukemia.

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## P-214

### Hypoxia Mitigates Mitochondrial Damage and Oxidative Stress Induced by Cisplatin in Human Malignant Mesothelioma Cells

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**Introduction:** Hypoxia induces drug resistance in human cancers. Human malignant mesothelioma (HMM), an aggressive tumor associated with exposure to asbestos fibers, is resistant to various chemotherapeutic regimens. This study was performed to determine if mitochondria are involved in the hypoxia-induced drug resistance in HMM.

**Materials and Methods:** Two HMM cell lines, MS1 and H513, were cultured in complete RPMI 1640 medium. Hypoxic condition (0.1% O2) was achieved by an air chamber system (Billups-Rothenberg Inc., USA). Cells were treated with 10 μM cisplatin. Cytotoxicity was assessed by MTT assay. Apoptosis was determined by Annexin V/propidium iodide analysis. Mitochondrial membrane potential (MMP) and permeability were measured by JC-1 ratio and calcein fluorescence. Mitochondrial stress was evaluated using Mitotracker Red fluorescence. Mitochondrial DNA (mtDNA) damage was analyzed by amplification efficiency of long mtDNA fragment relative to mtDNA copy number. Oxidative stress was determined using various fluorochromes specific for reactive oxygen species (ROS). Ultrastructure of mitochondria was scrutinized by transmission electron