Background
Checkpoint inhibitors, including those of the PD-1/PD-L1 pathway, are immunotherapies used for the standard of care for cancer patients. New treatments or immunotherapies are compared to those that are currently approved for use. Continual focus is placed on cancers ranked amongst the most common worldwide, including breast, lung and colon cancer. In this study we assessed the ability of NCG mice to support engraftment of adult mobilized hCD34+ stem cells as a model for determining tumor growth modulation using anti-hPD-1 monotherapy driven cellular responses over time.

Methods
We evaluated the anti-tumor effects of an anti-human PD-1 checkpoint inhibitor in three human tumors (A549 lung, Colo-205 colon and MDA-MB-436 breast cancer) in NCGs humanized with adult healthy donor-mobilized hCD34+ stem cells. Humanized mice were implanted subcutaneously with cancer cells, and tumor bearing mice (TBM) were randomized into treatment groups when the average tumor size reached comparable volumes for each tumor type. Vehicle control mice were treated with isotype control IgG antibody, whereas TBM were dosed with anti-hPD-1 antibody. Clinical observations, body weights and tumor growth kinetics were recorded throughout the study. At time of euthanasia whole blood, spleen and tumor tissues were collected and processed for immune profiling by multi-color flow cytometry.

Results
In the mobilized hCD34+ humanized mice anti-hPD-1 monotherapy modulated tumor growth in lung, colon and breast cancer tumors compared to the IgG isotype control treated. We observed that growth kinetics varied between tumor types. At study termination common human lymphocytes were distributed in peripheral blood, spleen and tumors of surviving mice. After PMA/Ionomycin stimulation in vitro polyfunctional ex vivo T-cell responses were detected within tumor-infiltrating lymphocytes. NCG mice injected with adult healthy donor-mobilized hCD34+ stem cells sustained engraftment out to 34 weeks post-injection. The anticipated human immune cell types were detected in bone marrow, spleen and lung at ~18-19 weeks.

Conclusions
Adult healthy donor-mobilized hCD34+ humanized mice can be successfully grafted with human tumors and be used as a model for studying pharmacological interventions targeting immune cell responses. Levels of humanization were sustained throughout the course of the study. The ability to monitor tumor growth kinetics and observe a response to an anti-hPD-1 checkpoint inhibitor across multiple tumor types indicates that this model is useful when assessing immunotherapies. Future considerations of this model would expand upon monotherapies to evaluate additional treatment methods such as bispecific antibodies or cell-directed therapies.

Ethics Approval
Animal studies were executed in compliance with local Charles River IACUC guidelines, IACUC number IO33.