The data presented here provide evidence that TransCon IL-2 was well tolerated and showed expected slow-release kinetics. In mouse models, TransCon IL-2 α potency while losing IL-2R α⁺ immunosuppressive receptor with low IL-2R βγ binding yet relatively high IL-2R βγ binding in Biacore assays:

1) desired receptor selectivity
2) optimized exposure

Designed using TransCon technology to generate long-acting sustained release biased IL-2 prodrug plasma PK TransCon IL-2 prodrug levels by ELISA (A) or for Complete Blood Counts (B) using a hemocytometer. A) Analysis of TransCon IL-2 prodrug levels measured in IL-2 equivalents) via i.v. route. Peripheral blood was acquired pre- and post-dosing and was analyzed for plasma TransCon IL-2 β/γ prodrug levels and NK cell levels in mice.

We developed TransCon (transient conjugation) IL-2 (β/γ), a novel long-acting prodrug of a receptor-based IL-2 (β/γ) to optimally address each of these drawbacks. First, to block IL-2R binding yet retain IL-2R βγ activity, we created 52b by permanently attaching a small PEG moiety to an engineered cysteine placed at the IL-2R binding site. Second, to improve PK properties, we stabilized the receptor-based 6.2 (β/γ) to a TransCon carrier via a TransCon linker, shielding biodistribution and creating a prodrug. Under physiological conditions, TransCon 6.2 (β/γ) was designed for sustained release of the bioactive 6.2 (β/γ) from the PEG carrier, aiming for a much lower Cmax, and longer effective half-life of released 6.2 (β/γ) compared to aldesleukin.

In binding and cell-based functional assays, free 6.2 (β/γ) demonstrated desirable IL-2 receptor selectivity, maintaining IL-2R α potency while losing IL-2R α⁺ potency. In vitro, TransCon 6.2 (β/γ) showed expected slow-release kinetics. In mouse models, TransCon 6.2 (β/γ) demonstrated selective proliferation and activation. In cynomolgus monkeys (non-human primates (NHP)), a single dose of TransCon 6.2 (β/γ) was well tolerated and induced a more robust expansion of CD8 T cell subsets and NK cells relative to CD8 T cell subsets or exosorption as compared to daily aldesleukin treatment. TransCon 6.2 (β/γ) demonstrated a long-produg half-life in mice (22-260) and monkeys (23-126), supporting every three week clinical dosing. Consistent with these observations, TransCon 6.2 (β/γ) induced lower levels of systemic inflammatory cytokines and endothelial activation markers when compared to aldesleukin.

The data presented here provide evidence that TransCon 6.2 (β/γ) offers improved pharmacokinetic properties and optimal activation of cytotoxic lymphocytes with improved tolerability.