ENGINEERING AND PRE-CLINICAL EVALUATION OF A HUMAN ENZYME IMMUNE CHECKPOINT INHIBITOR FOR CANCER THERAPY

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The work presented here builds on the findings that the amino acid kynurenine (L-Kyn) synthesized via the enzymes indoleamine/tryptophan dioxygenase (IDO/TDO) mediates cancer immune suppression in a paracrine fashion and evaluates the hypothesis that systemic elimination of secreted Kyn in the extracellular space using an engineered human kynureninase enzyme (KYNase) to degrade it into inactive metabolites will have pronounced anti-tumor activity and overcome the limitations associated with IDO/TDO inhibitors.

The significance of L-Kyn production in cancer is well recognized and has led to the development of inhibitors of IDO and TDO; with at least one IDO inhibitor currently undergoing Phase II/III clinical evaluation. However IDO or TDO inhibition is problematic for cancer therapy because: (1) there are two isoforms of IDO and together with TDO, the inhibition of all possible pathways for Kynurenine generation at present requires the generation of multiple small molecule inhibitors; (2) resistance to inhibition can arise, and (3) as a single agent IDO/TDO inhibitors show very little efficacy.

Wild-type human KYNase has a strong preference for the degradation of 3'OH Kynurenine (OH-Kyn) with a $k_{cat}/K_M = 10^5\ M^{-1}s^{-1}$, about 1,000 fold higher than that displayed for L-Kyn degradation ($10^2\ M^{-1}s^{-1}$). In contrast some bacterial enzymes such as the KYNase from *P.fluorescens* displays a nearly equal and opposite substrate preference for Kyn over OH-Kyn. Although the *P.fluorescens* KYNase has ideal kinetics desired in an enzyme therapeutic targeting tumor L-Kyn, its high immunogenicity render it unsuitable as a clinical candidate, necessitating the engineering of a human KYNase with the requisite pharmacological properties.

Therefore we undertook a directed evolution campaign coupled with high throughput competitive genetic selections and screening strategies to engineer the human KYNase enzyme, creating variants with > 500 fold increases in catalytic activity towards L-Kyn. Engineered human KYNase enzymes were then PEGylated for long circulatory persistence and administered to mice bearing murine cancer allografts and evaluated for efficacy and PK/PD.

We found that administration of PEGylated engineered human KYNase enzymes resulted in lowered systemic L-Kyn levels accompanied by significant tumor growth retardation, extended survival and even complete regressions in a manner similar to that observed with immune checkpoint inhibitors such as anti-PD. Flow cytometric analysis showed a significant increase in the proportion of $TCRB^+$ T cells in the tumor-infiltrating lymphocytes (TILs) that is consistent with significant incorporation of BrdU in $CD4^+$ and $CD8^+$ T cells in the tumor-draining lymph nodes (dLNs) and TILs compared to control mice treated with deactivated enzyme. Additionally, marked elevation of $CD8^+$ and $CD4^+$ T cells expressing granzyme (Gzm)B and interferon (IFNG) was observed in the active-enzyme-treated mice, further highlighting the importance of L-Kyn in tumor evasion of immune surveillance.

As a monotherapy, small molecule inhibitors of IDO1 display at most marginal anti-cancer activity in animal models as well as in clinical trials likely due to the redundancy of Kyn biosynthetic pathways; necessitating combinations of IDO1/TDO inhibitors. The therapeutic enzyme approach using engineered human KYNase represents an effective "first in class" drug for restoring T-cell immunity for cancer eradication, without the limitations of IDO1/TDO inhibition. This work further demonstrates the utility of "enzymes as drugs" in targeting aberrantly regulated metabolites in disease states.