# Generation of IL-21 INDUKINE™ Molecules for the Treatment of Cancer

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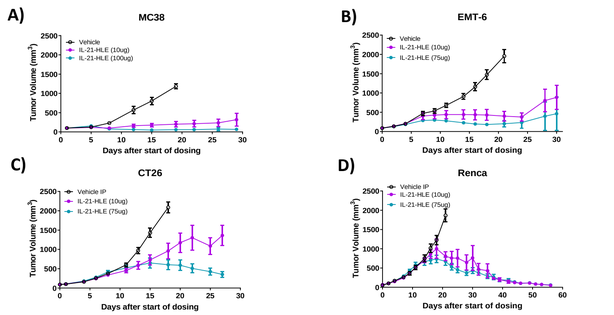
Abstract #1829

## Introduction

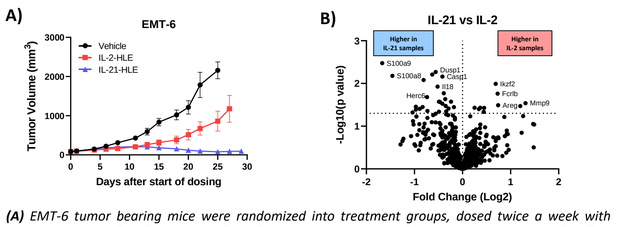
- Cytokines are potent immunomodulators with great promise for cancer treatment. However, the use of systemic cytokine therapy has been limited due to toxicity and poor pharmacokinetics (PK).
- Interleukin 21 (IL-21) is a pluripotent pro-inflammatory member of the common γ-chain family of cytokines.
- IL-21 activates anti-tumor T cell responses, induces B cell activation, and promotes the generation and maintenance of germinal centers and tertiary lymphoid structures.
- IL-21 acts on a broader range of cells than IL-2 and does not induce vascular leak syndrome.
- Clinical use of IL-21 has been hampered by its poor pharmacokinetic properties and adverse events at dose levels associated with efficacy.
- WTX-712 is an IL-21 INDUKINE polypeptide containing native human IL-21, an inactivation domain and a half-life extension domain tethered together by protease sensitive linkers.
- While WTX-712 is inactive in the periphery, dysregulation of the protease milieu in the tumor microenvironment (TME) results in cleavage of the linkers and the release of IL-21 intratumorally.
- WTX-712 contains native human IL-21 as the payload. Since human IL-21 has reduced potency on murine cells, we utilized mice expressing the human IL-21R for pre-clinical studies.

## Figure 1: Treatment with IL-21 Generates Robust Anti-Tumor Efficacy in Several Syngeneic Tumor Models



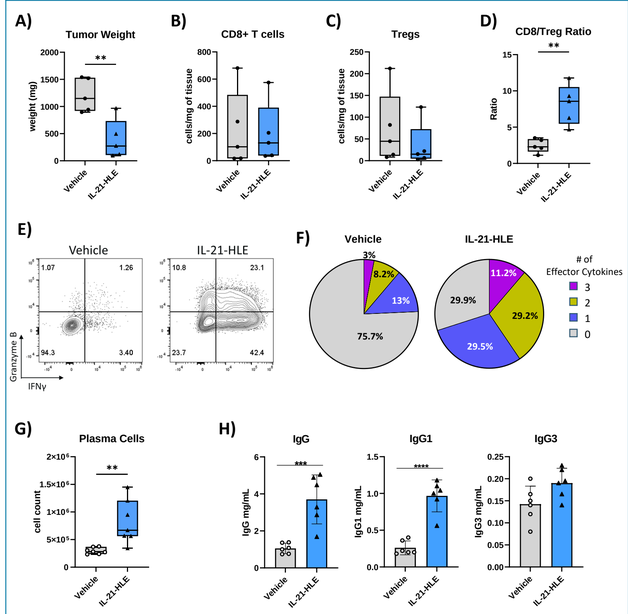

*Tumor growth curves of various syngeneic models **(A)** MC38, **(B)** EMT-6, **(C)** CT26, or **(D)** Renca tumors. Mice (n=8/group) were dosed twice a week with half-life extended (HLE) cytokines for 2 weeks, with doses reported in the figure legends. Tumor volumes were measured twice a week.*

## Figure 2: Differential Efficacy of IL-2 and IL-21 in PD-1 Resistant Syngeneic Tumor Model



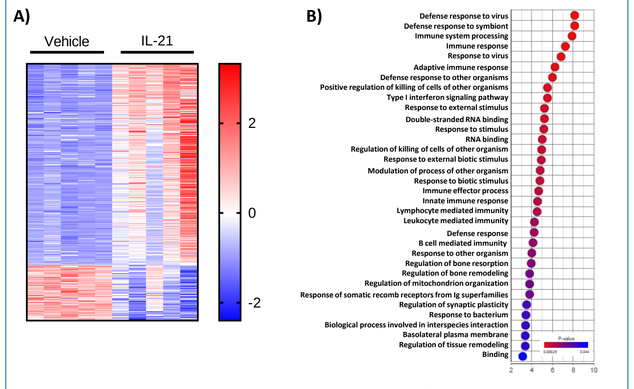

***(A)** EMT-6 tumor bearing mice were randomized into treatment groups, dosed twice a week with vehicle (black), IL-2-HLE (red) or IL-21-HLE (blue) for two weeks. Mice were dosed with IL-2-HLE at maximum tolerated dose. Tumor volumes were measured twice a week. **(B)** Nanostring analysis of tumors from EMT-6 tumors after dosing with IL-2-HLE or IL-21-HLE. Differentially expressed genes between IL-2-HLE and IL-21-HLE samples graphed as volcano plot comparing fold change and p value.*

## Figure 3: IL-21 Treatment Activates CD8+ T cells and Drives B cell Class Switching



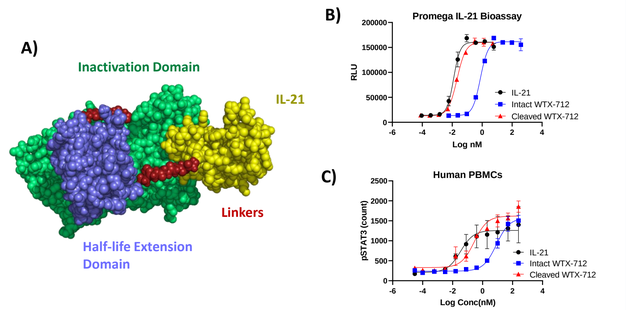

*EMT-6 tumor bearing mice were randomized into treatment groups, dosed twice a week with vehicle (grey) or IL-21-HLE (blue) for two weeks, and tumors were harvested for TIL analysis on day 12. **(A)** Weights of EMT-6 tumors analyzed on day 12. The cell counts of **(B)** CD8+ T cells, **(C)** CD4+ FoxP3+ Tregs, or the resulting **(D)** ratio of the two cell types is shown. **(E)** Representative flow plots of cytokine production from CD8+ T cells from TILs samples following restimulation. **(F)** The frequency of polyfunctional CD8+ T cells in the tumor based on co-expression of IFNγ, TNF, and Granzyme B is shown. **(G)** Total counts of plasma cells (based on expression of B220 and CD138) in the spleen of EMT-6 tumor bearing mice. **(H)** Quantification of antibody subclasses in the serum of EMT-6 tumor bearing mice. p values represent the results of a students T test at individual timepoints: ** = p<0.01; *** = p<0.001; **** = p<0.0001.*

## Figure 4: IL-21 Treatment Drives Transcriptional Changes in TILs of EMT-6 Tumors



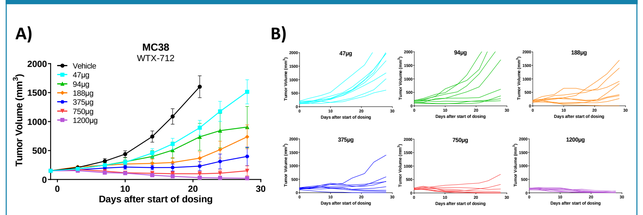

*EMT-6 tumor bearing mice were randomized into treatment groups (day 1), dosed twice a week with IL-21-HLE for two weeks, and tumors were harvested for Nanostring analysis of bulk RNA on day 12. **(A)** Heatmap of the differentially expressed genes between vehicle and IL-21-HLE treated tumors and **(B)** pathway analysis of the differentially expressed genes. Graph depicts pathways identified which were significantly enriched with a p value <0.05 and enrichment greater than 3.*

## Figure 5: WTX-712 is a Selectively Inducible IL-21 Pro-Drug



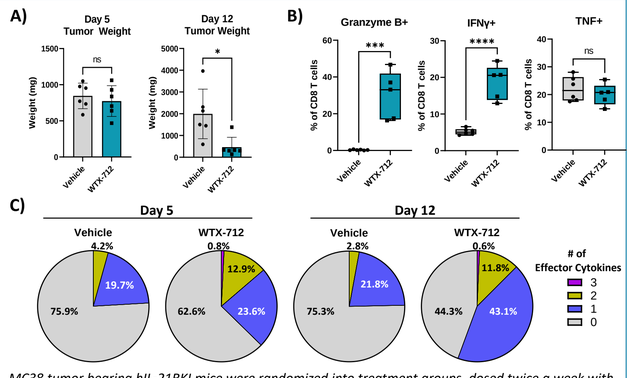

***(A)** Key features of the INDUKINE molecule WTX-712, including half-life extension domains (blue), native IL-21 (yellow), inactivation domains which block IL-21 receptor binding (green), and protease cleavable linkers (red). **(B)** In vitro activity of WTX-712 in the IL-21 Promega reporter assay or **(C)** measurement of pSTAT3 in human PBMCs after incubation comparing intact WTX-712 (blue squares) and in vitro cleaved/activated WTX-712 (red triangles) to recombinant IL-21 (black circles).*

## Figure 6: Treatment with WTX-712 Results in Robust Anti-Tumor Activity



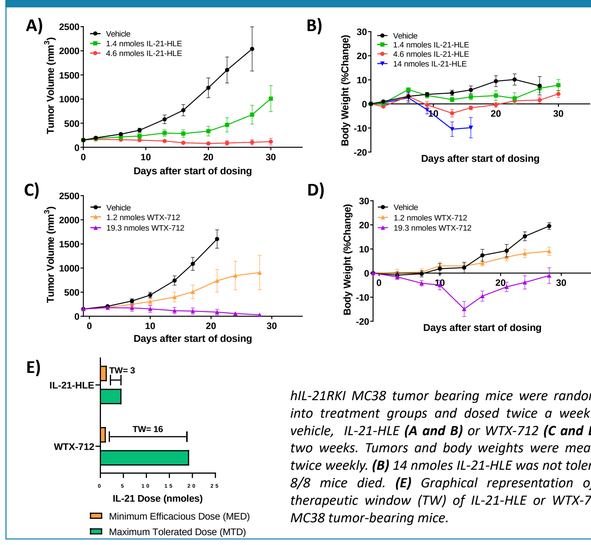

*hIL-21RKI mice bearing MC38 tumors were randomized into treatment groups, dosed twice a week with the specified agents for two weeks, and tumors were measured twice weekly. **(A)** Summary tumor burden for n=8 animals per group and **(B)** individual spider plots of tumor burden.*

## Figure 7: WTX-712 Treatment Reduces Tumor Burden and Activates CD8+ T cells




*MC38 tumor bearing hIL-21RKI mice were randomized into treatment groups, dosed twice a week with WTX-712 for two weeks, and tumors were harvested for TIL analysis at different time points. **(A)** Tumor weight at examined timepoints of vehicle (grey) and WTX-712 (teal) treated mice. **(B)** Frequencies of cytokine production from CD8+ T cells found in the tumor on day 12. **(C)** The frequency of polyfunctional CD8+ T cells in the tumor based on co-expression of Granzyme B, IFNγ, and TNF. p values represent the results of a students T test at individual timepoints: * = p<0.05, ***= p<0.001, ****= p<0.0001*

## Figure 8: WTX-712 Improves the Therapeutic Window of IL-21 to Minimize Systemic Toxicities




*hIL-21RKI MC38 tumor bearing mice were randomized into treatment groups and dosed twice a week with vehicle, IL-21-HLE **(A and B)** or WTX-712 **(C and D)** for two weeks. Tumors and body weights were measured twice weekly. **(B)** 14 nmoles IL-21-HLE was not tolerated, 8/8 mice died. **(E)** Graphical representation of the therapeutic window (TW) of IL-21-HLE or WTX-712 in MC38 tumor-bearing mice.*

## Conclusions

- Treatment with half-life extended IL-21 demonstrates potent anti-tumor activity in several syngeneic tumor models.
- The anti-tumor efficacy driven by IL-21 differs mechanistically from that of another potent common γc cytokine, IL-2, which is also in development for cancer treatment.
- IL-21 promotes the activation and polyfunctionality of CD8+ T cells and drives a favorable CD8:Treg ratio.
- IL-21 treatment leads to transcriptional changes in the TME, with the upregulation of several pro-inflammatory and immuno-stimulatory pathways with the TME.
- WTX-712, an INDUKINE polypeptide containing human IL-21, demonstrates *in vitro* and *in vivo* inducibility and activity.
- Systemic administration of WTX-712 drives anti-tumor immune responses resulting in reduced tumor burden and activation of CD8+ T cells in the syngeneic MC38 tumor model.
- WTX-712 has an improved therapeutic window compared to equimolar amounts of half-life extended IL-21.

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