3531. Discovery of GRC 65327: A Novel, Selective and Potent CBL-B E3 Ligase Inhibitor for the Treatment of Advanced Solid Cancers

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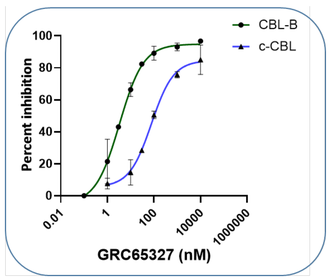
Background

Casitas B-lineage lymphoma-b (Cbl-b), a key member of the Cbl family of RING finger E3 ligases, regulates both innate and adaptive immunity by mediating the ubiquitination and degradation of signaling transducers[1]. Cbl-b is a master regulator downstream of tumor microenvironment (TME) receptors, including NK cells and CD28/CTLA4 (T cells), promoting an immunosuppressive TME that limits the anti-tumor functions of T and NK cells. This E3 ubiquitin ligase regulates both innate and adaptive immune cells, ultimately promoting an immunosuppressive TME in the absence of CD28 co-stimulation. The lack of CD28 co-stimulation has been linked to T-cell exhaustion[3-4]. Inhibition of Cbl-b activates T-cells even without CD28 co-stimulation, offering a potential therapeutic strategy to overcome immune suppression in the TME.

GRC 65327 is a potential best-in-class, highly active, and selective Cbl-b inhibitor. By inhibiting Cbl-b, GRC 65327 is expected to boost T-cell infiltration into tumors, improve antigen presentation by dendritic cells, and enhance the functionality of cytotoxic T lymphocytes. This could result in benefits across various tumor types, improving the efficacy of immune checkpoint inhibitors. Inhibition of Cbl-b function by GRC 65327 translates to activation of immune functions, resulting in efficacy in mouse models of colon cancer, CT26 and MC38 hPD-L1, and modulating immune biomarkers such as Notch1 and CD25, enhancing their expression on CD4+ and CD8+ T-cells. Additionally, a robust pharmacological response seen in the lymph nodes of monkeys at a low exposure is expected to translate to efficacy in patients. The toxicological profile of GRC 65327 has been characterized to support the First-in-Human (FIH) study.

Figure 1. Selective inhibition in a cell-free activity assay



Isoform	IC_{50}
Cbl-b	3.9 nM
C-Cbl	92.2 nM

Human Cbl-b and c-Cbl proteins were used in an activity assay in the presence of different concentrations of GRC 65327. The inhibition of activity was measured by Homogeneous Time-Resolved Fluorescence (HTRF).

Results

Figure 2. *In vitro* profile of GRC 65327

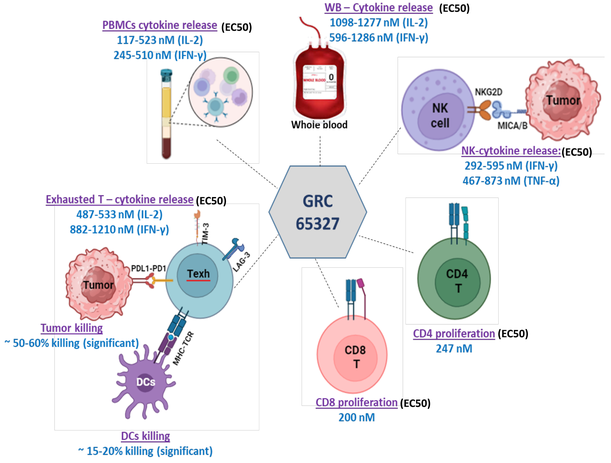
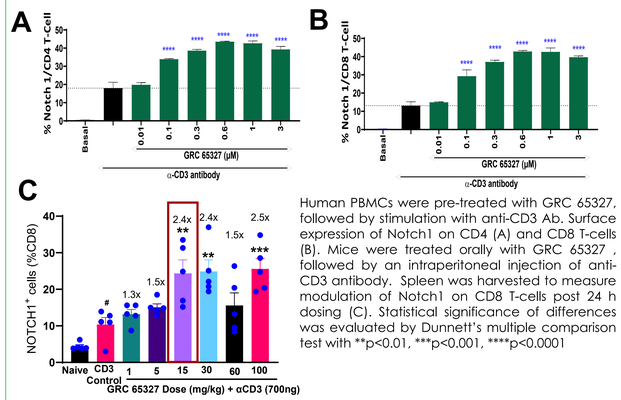
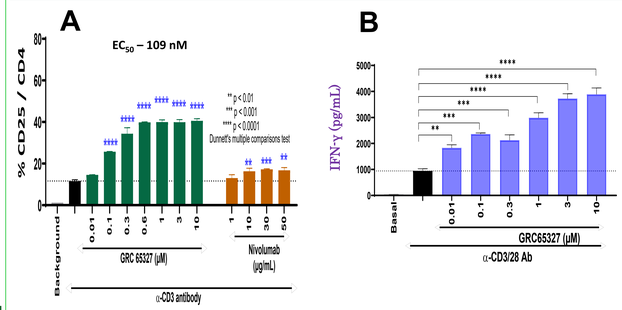


Figure 3. Significant upregulation of Notch1 by T cells upon treatment with GRC 65327



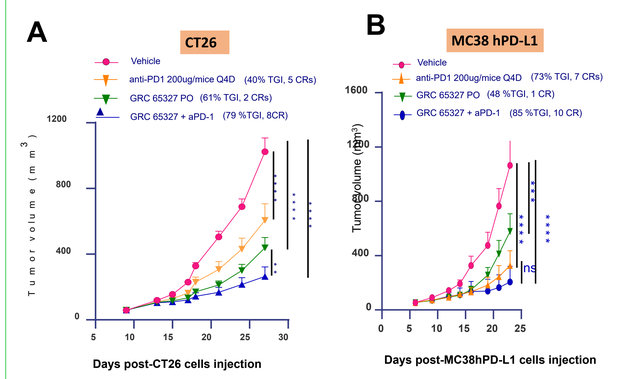
Human PBMCs were pre-treated with GRC 65327, followed by stimulation with anti-CD3 Ab. Surface expression of Notch1 on CD4 (A) and CD8 T-cells (B). Mice were treated orally with GRC 65327 , followed by an intraperitoneal injection of anti-CD3 antibody. Spleen was harvested to measure modulation of Notch1 on CD8 T-cells post 24 h dosing (C). Statistical significance of differences was evaluated by Dunnett's multiple comparison test with **p<0.01, ***p<0.001, ****p<0.0001

Figure 4. GRC 65327 enhanced upregulation of CD25 and IFN gamma in stimulated human PBMC (N=2 donors)



Human PBMCs were pre-treated with GRC 65327, followed by stimulation with anti-CD3 Ab. Surface expression of CD25 on CD4 T-cells (A) and IFN gamma release (B). Statistical significance of differences was evaluated by Dunnett's multiple comparison test with **p<0.01, ***p<0.001, ****p<0.0001

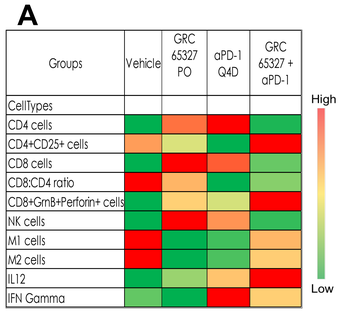
Figure 5. GRC 65327 as a single agent and in combination with anti-PD1 enhanced anti-tumor immune responses in CT26 and MC38hPD-L1 tumor models



Groups mean tumor volume ± SEM (A & B) in a xenograft mice bearing CT26 and MC38 hPD-L1. Statistical significance of difference in tumor volume was evaluated using 2-Way ANOVA followed by Bonferroni test comparison in between treatments with **p<0.01, ***p<0.001, ****p<0.0001

Results

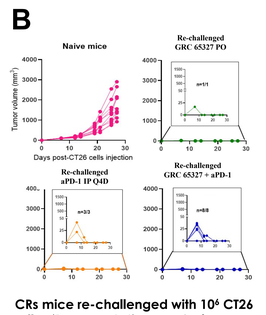
Figure 6. Immunophenotyping in CT26 tumours of treatment groups and CRs mice re-challenged with 10^6 CT26 cells



Groups	Vehicle	GRC 65327 PO	αPD-1 Q4D	GRC 65327 + αPD-1
CellTypes				
CD4 cells				
CD4+CD25+ cells				
CD8 cells				
CD8:CD4 ratio				
CD8+GrnB+Perforin+ cells				
NK cells				
M1 cells				
M2 cells				
IL12				
IFN Gamma				

High / Low

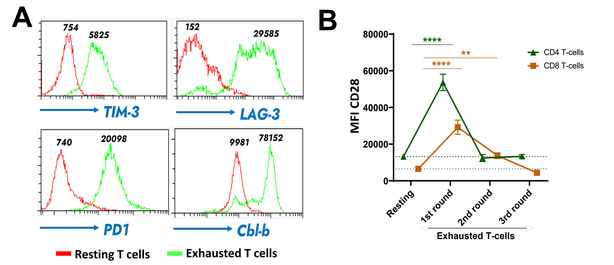
Immunophenotyping in tumours of treatment groups



CRs mice re-challenged with 10^6 CT26 cells (Representative graphs from one out of three studies)

Heat map (A) showing Immunophenotyping and cytokine signature in CT26 tumours. CRs mice re-challenged with 10x concentration of CT26 cells, both outer and inner graphs (B). Tumor growth kinetics post-re-challenged in CRs mice (inner graph, B).

Figure 7. GRC 65327 reverses phenotypic and functional exhaustion in the absence of anti-CD28 co-stimulation

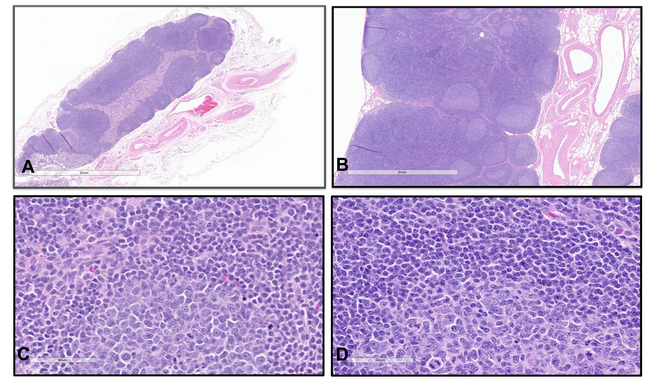


NX-1607: Nurix Cbl-b inhibitor clinical candidate; Nivo: anti-PD1 Ab (Nivolumab); TGN: anti-CD28 super agonist (Theralizumab)

Exhausted T-cells were generated *in vitro* and characterized for surface exhaustion markers (A) and expression of CD28 (B). Cells were also evaluated for the intracellular expression of CBL-b (A). Functional exhaustion of T cells assessed by production of IL-2 (C) and IFN-γ (D) in comparison to resting T-cells was evaluated in a cytokine release assay by ELISA. Exhausted T-cells were treated with GRC 65327 in the absence of co-stimulation to evaluate the proliferation (E) and cytokine release (F). Statistical significance of differences was evaluated by Dunnett's multiple comparison test with * p< 0.05; ** p< 0.01; *** p< 0.001; **** p< 0.0001

- IC50 for hERG = 16 μM, indicating minimal potential safety concerns.
- *In vitro* CEREP panel safety receptor profiling revealed minimal off-target activity.
- In a 1-month oral Good Laboratory Practice (GLP) study in mice, no adverse effects were observed at the maximum tested dose of 300 mg/kg/day.
- The target organ of toxicity observed in the dose-range finding study in dogs and monkeys is the liver.
- In a 1-month GLP toxicology study in monkeys, a dose-dependent diffuse increased cellularity was observed in the lymph nodes at a Day 30 $AUC_{0\text{-}24}$ of ~1500 ng.h/mL and no adverse findings were observed at the highest individual animal Day 30 $AUC_{0\text{-}24}$ of ~13400 ng.h/mL. Increased cellularity in the lymph node was observed in 9 out of 12 animals at mid and high doses, associated with gross enlargement of various lymph nodes at higher exposures. Further, an increased number of circulating immune cells was seen at the highest dose tested compared to controls and baseline levels (data not shown).
- No myeloproliferative effects or receptor tyrosine kinase (RTK)-mediated toxicities attributed to c-Cbl were observed at the highest tested dose in studies conducted in monkeys and mice.

Figure 8. Microscopic changes in a mesenteric lymph node in monkeys



Mesenteric lymph node: A & C: Control animal, a normal distribution of lymphoid cells (H&E, 2x & 40x); B & D: GRC 65327 administered animal on Day 30 at an $AUC_{0\text{-}24}$ of ~1500 ng.h/mL with a diffuse increased cellularity (H&E, 2x & 40x)

Conclusions

GRC 65327 is a highly selective, as demonstrated in a cell-free assay, and potent Cbl-b inhibitor with strong immunomodulatory effects. The compound enhances T cell activation and reverses T-cell exhaustion, resulting in a significant enhancement of the anti-tumor response in *in vivo* preclinical models, which leads to durable complete responses. GRC 65327 reversed functional exhaustion even in the absence of CD28 co-stimulation. When tested in nonhuman primates, GRC 65327 increased cellularity in the mesenteric lymph node in 9/12 animals at mid and high doses, along with an increase in immune cells in the peripheral blood at the high dose, highlighting its impact on immune activation. Based on these findings, a first-in-human clinical trial of GRC 65327 is scheduled to commence in patients with advanced solid cancers.

References

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Acknowledgements

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