Pre-Clinical Development of a First in Class Half-Life Extended T Cell Engager Targeting ROR-1

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INTRODUCTION

- NVG-111 is a bispecific T cell engager (TCE) targeting ROR1xCD3 demonstrating efficacy with manageable toxicity in the clinic[1,2].
- It possesses a dual mechanism of action, targeting the Fzd domain of ROR1 to antagonize Wnt signaling whilst potently redirecting T cell cytotoxicity due to its membrane proximal epitope[3-5] (figure 1).
- Originally designed with a short $t_{½}$ for cIV infusion to maintain of high drug plasma concentrations but enable rapid cessation of therapy in the event of toxicity, the clinical responses observed support the development of a half-life extended (HLE) therapy providing more convenient dosing.
- NVG-222 is a 2nd generation ROR1-targeting TCE building on the active elements of NVG-111 at its core.

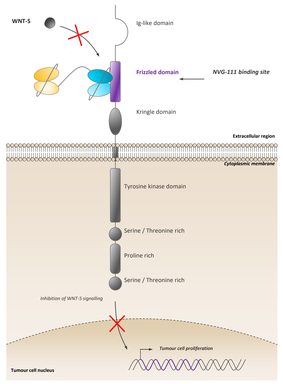

Figure 1. Structure of ROR1 and NVG-111 engagement

AIMS

1. Develop a 2nd generation ROR1 targeting TCE enabling a once weekly dosing regimen
2. Maintain the activity and CMC attributes of NVG-111

METHODS

- Several formats were designed and evaluated with different HLE technologies (figure 2), and NVG-111 cloned in-format with these, expressed using Expi293 cells and purified by our proprietary process.
- Biophysical properties were evaluated by determining the post-purification expression titer, aggregation profile, and serum stability at 37°C. Activity was measured using the T Cell Activation Bioassay (NFAT/IL2) (Promega) to determine the lead format for NVG-222.
- The cytotoxic potency of which was evaluated in co-culture assays using target cells and healthy donor T cells with responses measured by flow cytometry.

Figure 2. HLE technologies evaluated

RESULTS

1. Geometry of Binding for an HLE TCE

- Previously reformatting NVG-111 as a bispecific scFv-Fc resulted in a loss of cytotoxic potency in co-culture assays (figure 3a), which is likely due to an alteration in binding geometry. HLE formats that maintain this by using the tandem scFv format of NVG-111 at their core were therefore evaluated (figure 3b).

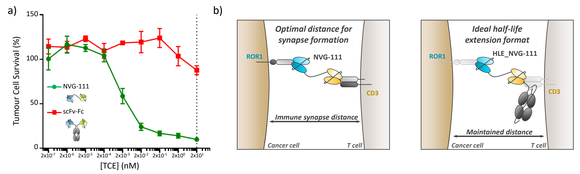

Figure 3. Activity of NVG-111 reformatted as bispecific scFv-Fc

2. Expression and Aggregation Characteristics of HLE Formats

- Fc fused NVG-111 displayed the highest levels of transient expression at ~150mg/L, with HSA-fused and parental NVG-111 expression levels 3-fold lower at ~50mg/L. The HSA-binding antibody fusion repeatedly demonstrated a complete loss of expression (figure 4).
- Analytical size exclusion chromatography (aSEC) also showed that the purified Fc-fused and parental NVG-111 were largely monomeric, with the HSA-fusion showing 42% aggregation (table 1).

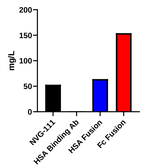

Figure 4. Expression levels of HLE constructs post purification

Construct	aSEC % Monomer
NVG-111	95
HSA-Binding Ab	N/A
HSA-Fusion	58
Fc-Fusion	98

Table 1. Aggregation levels of purified HLE formats

3. T Cell Activation and Stability

- T cell activation profile was identical for Fc-fused NVG-111 and parental NVG-111, with the HSA-fusion displaying ~100-fold loss of activity (figure 5).
- Following 7-day incubation in human serum at 37°C, target cell binding for the Fc-fusion was superior to compared with parental NVG-111, whereas the HSA-fusion was inferior (figure 6).

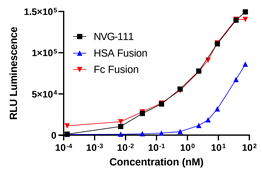

Figure 5. T cell activation induce by the HLE technologies

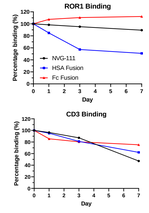

Figure 6. 7-day serum stability of HLE technologies as determined by residual target binding

4. Initial PK Profile for Fc-fused TCE

- The activity, biophysical and stability profile of the Fc-fused HLE construct enabled the selection of this architecture as the lead HLE format.
- The aim of this initial PK study was to gain definition of the terminal elimination profile in the β phase (figure 7), and potential half life of this format prior to more extensive studies.
- PK analysis determined that the $t_{1/2}$ of the Fc-fusion is 3.5 days in mice.
- Extended half-life of Fc-fusion suggests compatibility with weekly dosing in patients.

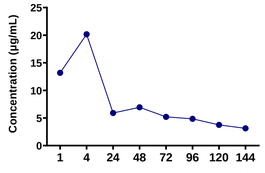

Figure 7. Initial PK profile of Fc-fused NVG-111

5. Silencing of Fc for NVG-222

- To further develop Fc-fused NVG-111 into the half-life extended variant NVG-222 it was imperative to silence the Fc effector functions to prevent any unwanted interactions with immune cells that could induce toxicity.

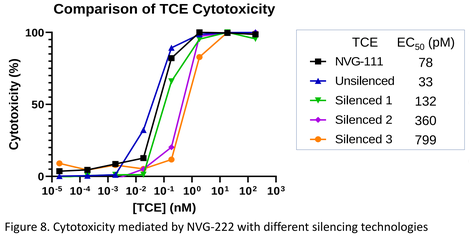

Figure 8. Cytotoxicity mediated by NVG-222 with different silencing technologies

- Evaluation of NVG-222 mediated cytotoxicity with different silencing technologies demonstrated broadly similar activity for silencing technology 1 compared with unsilenced NVG-222 and NVG-111 (figure 8). Silencing technologies 2 and 3 displayed some loss of potency.
- Analysis by aSEC demonstrated that all 3 technologies could be manufactured without aggregation (figure 9).
- Taking all data together, silencing technology 1 was selected as the lead for NVG-222 and is progressing into *in vitro* pharmacology studies, xenograft PD studies and PK studies in Tg32 mice to enable allometric scaling and dose prediction to aid a MABEL design for a Phase I trial.

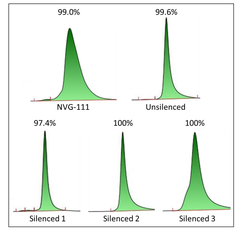

Figure 9. aSEC analysis of NVG-222 with different silencing technologies

CONCLUSIONS

- Heterodimeric bispecific scFv-Fc format loses cytotoxic activity, likely to due to altered geometry of binding
- Numerous formats that maintain NVG-111 binding geometry have been evaluated for a 2nd generation TCE targeting ROR1xCD3 named NVG-222
- Fusing NVG-111 to Fc provides the most favourable biophysical profile whilst maintaining the activity of the core molecule
- Applying silencing to the Fc does not impact on NVG-222 manufacturing characteristics or activity
- The initial PK profile suggests NVG-222 can be dosed weekly, which will be confirmed in ongoing pharmacology, PD and PK studies
- NVG-222 is progressing into IND enabling studies prior to clinical development

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