

AXA-042 - A Novel Systemic TLR2/6 Agonist for Anti-Tumor Therapy.

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Anna Galkin¹, Francesca Mercuri², Nicholas West³, Ping Zhang³, Weiguang Zeng⁴, Grant McLachlan¹, Michael Bettess⁵, Ian Holmes⁵, David Jackson⁴, Chris Smith⁵, Christophe Demaison², Tobias Bald^{6,7}, Phil Kearney¹.

¹ Axelia Oncology, Melbourne, Victoria, Australia; ² ENA Respiratory, Melbourne, Victoria, Australia; ³ Systems Biology and Data Science, Mucosal Immunology Research Group, Menzies Health Institute Queensland Griffith University, Gold Coast campus, QLD, Australia; ⁴ Department of Microbiology and Immunology, the University of Melbourne, the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia; ⁵ Brandon Capital Partners, Melbourne, Victoria, Australia; ⁶ QIMR Berghofer Medical Research Institute, Australia; ⁷ Institute for Experimental Oncology, University Medical Center Bonn, Bonn, Germany

INTRODUCTION

- Treatment approaches that engage both the innate and adaptive immune response have the potential to transform anti-cancer therapy, especially in settings of checkpoint inhibitor insensitivity or acquired resistance.
- Toll-like receptors (TLRs) mediate the initial cellular response to external pathogens or endogenous alarmins, activating downstream pro-inflammatory cascades and leading to the activation and recruitment of key innate subsets.
- TLR2 is a cell surface receptor, expressed predominantly on macrophages, dendritic cells (DC), neutrophils and subsets of NK and T cells. TLR2 signaling plays an important role in NK and T cell cytolytic cell functions, as well as DC and macrophage activation.
- AXA-042 is a novel synthetic TLR2/6 agonist designed for systemic delivery to re-engage the innate immune response to help overcome tumor immune escape.

MATERIALS AND METHODS

- In vitro* potency and selectivity of AXA-042 was assessed across a panel of human and mouse HEK-blue TLR reporter assays.
- AXA-042 activity was profiled relative to other TLR agonists in human PBMC cytokine release assays.
- AXA-042 *in vivo* efficacy was evaluated in the EMT6 and CT26 syngeneic tumor models.
- The Nanostring nCounter™ Mouse PanCancer Immune Profiling Panel was used to identify AXA-042 tumor-localized and peripheral response signatures.

RESULTS

Figure 1. AXA-042 is a potent and selective TLR2/6 Agonist

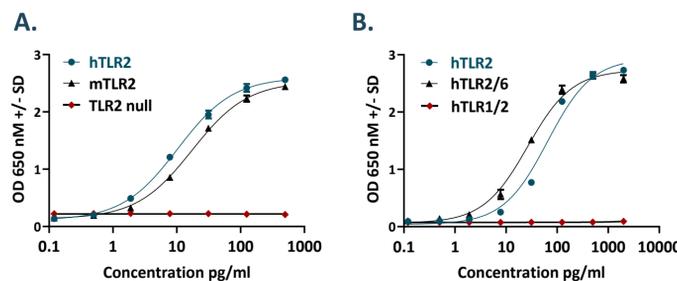
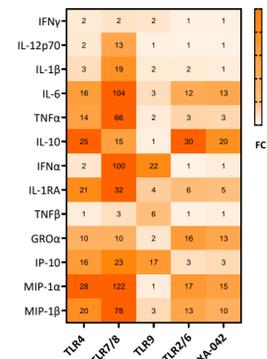


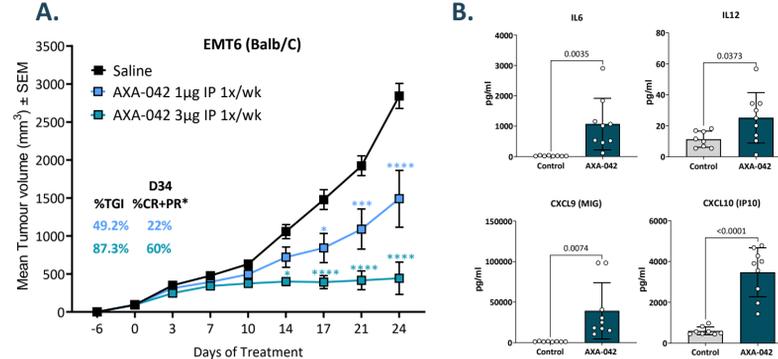
Figure 2. AXA-042 Displays a Distinct PBMC Cytokine Release Profile



Human PBMCs were incubated for 6hrs with TLR agonists at 5x reported EC₅₀: TLR4 CRX-527 (0.25 ng/mL); TLR7/8 R848 (0.3µg/mL); TLR9 ODN2006 (5 µg/mL); TLR2/6 Pam2CSK4 (0.15 ng/mL); TLR2/6 AXA-042 (0.25 ng/mL). Supernatants were analyzed using the multiplex Luminex platform. Data is shown as median fold change (FC) relative to PBS (4 donors).

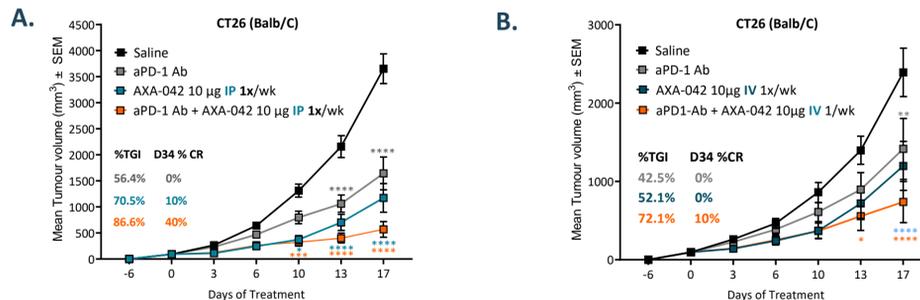
RESULTS

Figure 3. Systemic AXA-042 Efficacy in the EMT6 Tumor Model is Associated with Cytokine Release



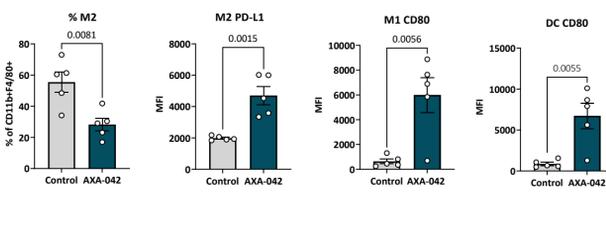
A. AXA-042 was delivered intraperitoneally (IP) 1x a week (QW) (day 0, 7, 14) for 3 weeks at 3 or 1 µg/mouse. Data is shown as Mean Tumor Volume (mm³) ± SEM (n=9-10 per group). %TGI = % tumor growth inhibition. CR = complete response. PR = ongoing regression. Statistical significance relative to control was determined using Two-Way Anova with Sidak's Multiple Comparisons Test. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. B. Plasma cytokines were assessed on day 14, 6 hrs post AXA-042 3 µg treatment. Statistical significance relative to control was determined using Student's T-Test

Figure 4. AXA-042 Enhances anti-PD-1 Ab Response in the CT26 Tumor Model



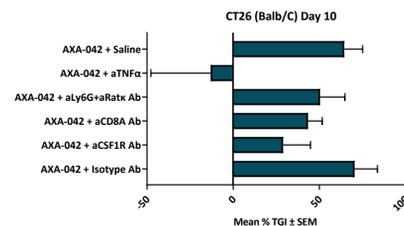
AXA-042 10 µg/mouse IP (A) or IV (B) in combination with anti-PD-1 antibody (Ab) (10 mg/kg IP 2x/wk). Data is shown as Mean Tumor Volume (mm³) ± SEM (n=10 per group). %TGI = % tumor growth inhibition. CR = complete response. Statistical significance relative to control was determined using Two-Way Anova with Sidak's Multiple Comparisons Test. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Figure 5. AXA-042 Induces DC and TAM Activation



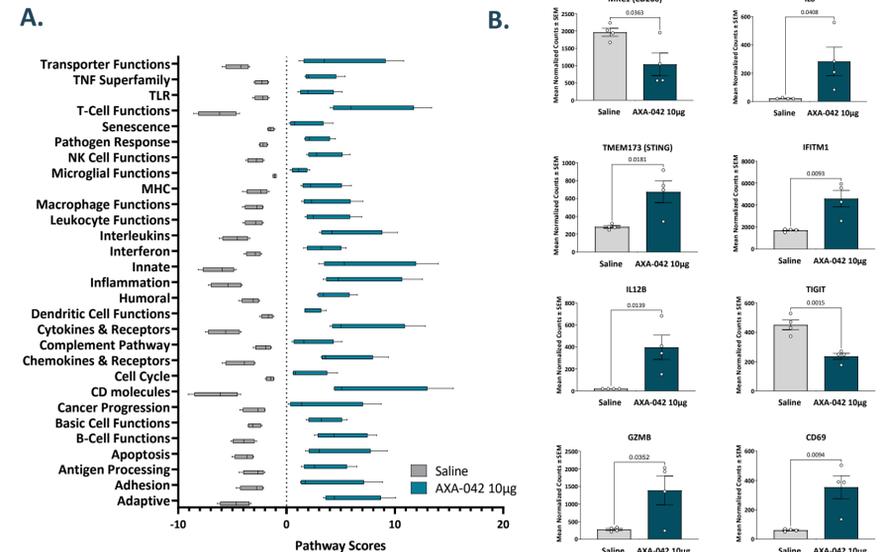
CT26 tumor tissues were collected 24 hrs after the 3rd dose of AXA-042 (10 µg/mouse, day 7) for FACS analysis. M2: CD45+CD11b+F4/80+ CD206+ MHCII low; M1: CD45+CD11b+F4/80+ CD206- MHCII +; DC: CD45+CD11c+ MHCII+. Data is presented as either mean % of events relative to the indicated gate or mean fluorescence intensity ± SEM (n=4-5 per group). Statistical significance relative to control was determined using Student's T-Test.

Figure 6. AXA-042 CT26 Efficacy is Macrophage and TNFα Dependent



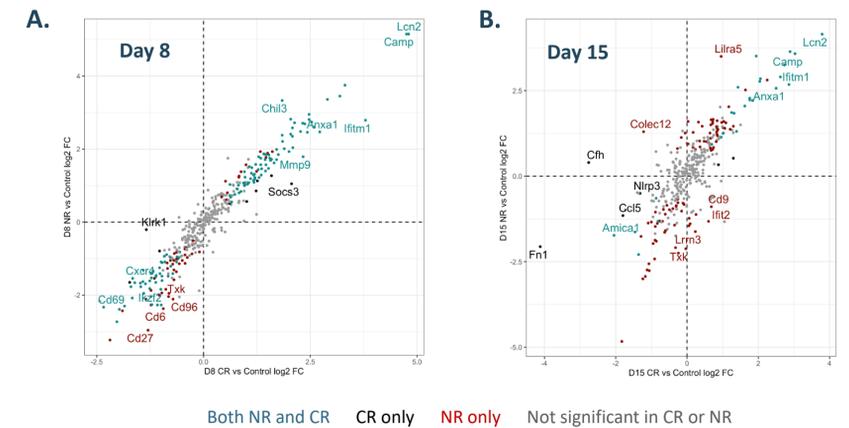
AXA-042 (10µg IP 1x/wk) efficacy was performed in presence of CSF1R (400µg 3x/wk), Ly6G (25µg QD +50 µg anti-Rat κ Ab EOD), CD8A (250 µg every 3 days) or TNFα (200 µg every 3 days) depletion Abs. Mean %TGI ± SEM is shown. % TGI for individual animals was calculated according to the following formula: ((Mean C_{D10}-C_{D0}) - (T_{D10} - T_{D0})) / ((Mean C_{D10}-C_{D0})¹⁰⁰), where T are AXA-042 treated tumors, and C are respective controls (saline + depletion Ab).

Figure 7. AXA-042 Engages the Innate and Adaptive Immune Response Pathways



CT26 tumors were collected 6 hrs after the 3rd dose of AXA-042 (10 µg/mouse, day 7) and immune gene expression analysis was performed using the Nanostring nCounter Mouse PanCancer Immune Profiling panel. A. Pathway scores were calculated using the Nanostring Pathway Module. B. Data is shown as mean normalized counts ± SEM (n=4/group). Statistical significance relative to control was determined using Student's T-Test.

Figure 8. AXA-042 Target Engagement Signature Identified in Whole Blood Non-Responders Display a Distinct Whole Blood DEG Signature



AXA-042 10 µg/mouse was delivered IP 1x/wk to CT26 tumor bearing mice for three weeks. Whole blood was collected on days 8 and 15 (24hrs post 2nd and 3rd dose) and immune gene expression analysis was performed using the Nanostring nCounter Mouse PanCancer Immune Profiling panel. A comparison of the differentially expressed genes (DEGs) between the complete responders vs control and the non-responders vs control on day 8 (A) or day 15 (B) is shown. The color coding indicates whether the DEG was significant (corrected p value < 0.05) in either or both cohorts relative to the control.

CONCLUSIONS

- Systemic delivery of AXA-042 was well tolerated *in vivo*, demonstrated innate response engagement and anti-tumor efficacy as monotherapy and in combination with anti-PD-1 Ab immunotherapy.
- AXA-042 has completed GLP toxicology studies and initiation of Phase 1/1b trial is planned for early 2022.

