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

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Anna Galkin[1], Francesca Mercuri[2], Nicholas West[3], Ping Zhang[3], Weiguang Zeng[4], Grant McLachlan[1], Michael Bettess[5], Ian Holmes[5], David Jackson[4], Chris Smith[5], Christophe Demaison[2], Tobias Bald[6,7], Phil Kearney[1].

[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

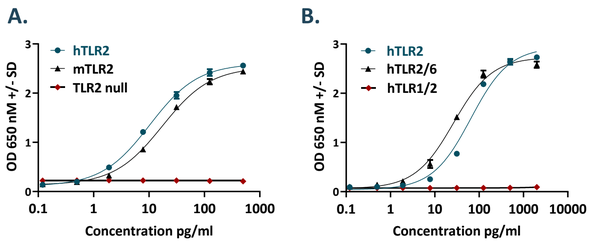
- Treatment approaches that engage both the innate and adaptive immune response have the potential to transform anti-cancer therapy, especially in settings of checkpoint inhibitor insensitivity or acquired resistance.
- Toll-like receptors (TLRs) mediate the initial cellular response to external pathogens 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

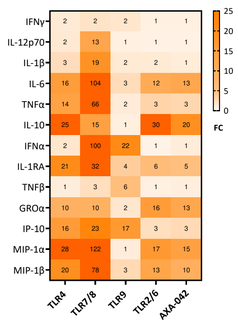


TLR	EC_{50} (pg/ml)
hTLR2	10.4
mTLR2	17.4
TLR2 Null	Not active

TLR	EC_{50} (pg/ml)
TLR2	26
TLR2/6	65
TLR1/2	Not active

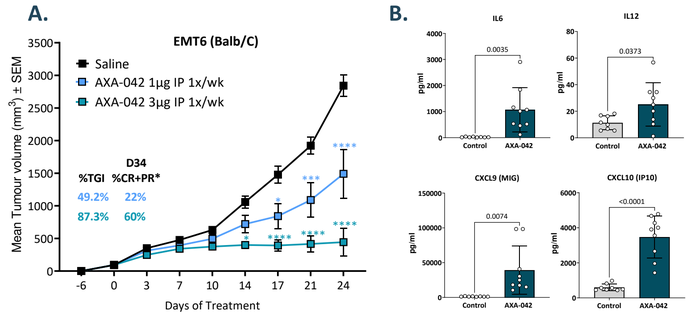
RESULTS

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



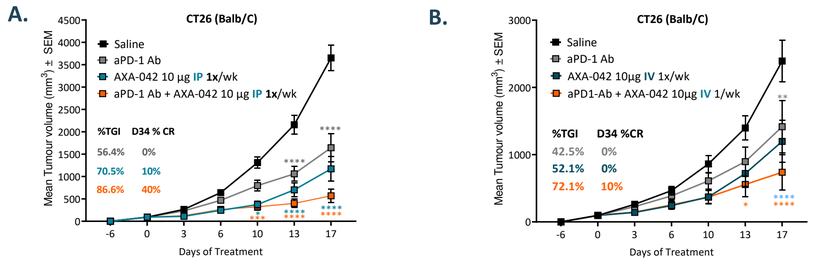
Human PBMCs were incubated for 6hrs with TLR agonists at 5x reported EC_{50}: TLR4 CRX-527 (0.25 ng/mL); TLR7/8 R848 (0.3µg/mL); TLR9 ODN2006 (5 µg/mL); TLR2/6 Pam2CSK4 (0.15 ng/mL), TLR2/6 AXA-042 (0.25 ng/mL). Supernatants were analyzed using the multiplex Luminex platform. Data is shown as median fold change (FC) relative to PBS (4 donors).

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



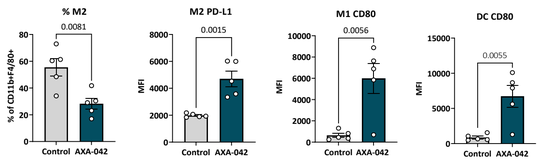
A. AXA-042 was delivered intraperitoneally (IP) 1x a week (QW) (day 0, 7, 14) for 3 weeks at 3 or 1 µg/mouse. Data is shown as Mean Tumor Volume (mm3) ± SEM (n=9-10 per group). %TGI = % tumor growth inhibition. CR = complete response. PR = ongoing regression. Statistical significance relative to control was determined using Two-Way Anova with Sidak's Multiple Comparisons Test, * p<0.05, *** p<0.001, **** 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



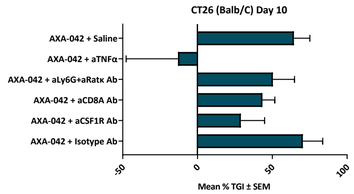
AXA-042 10 µg/mouse IP (**A**) or IV (**B**) in combination with anti-PD-1 antibody (Ab) (10 mg/kg IP 2x/wk). Data is shown as Mean Tumor Volume (mm3) ± SEM (n=10 per group). %TGI = % tumor growth inhibition. CR = complete response Statistical significance relative to control was determined using Two-Way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



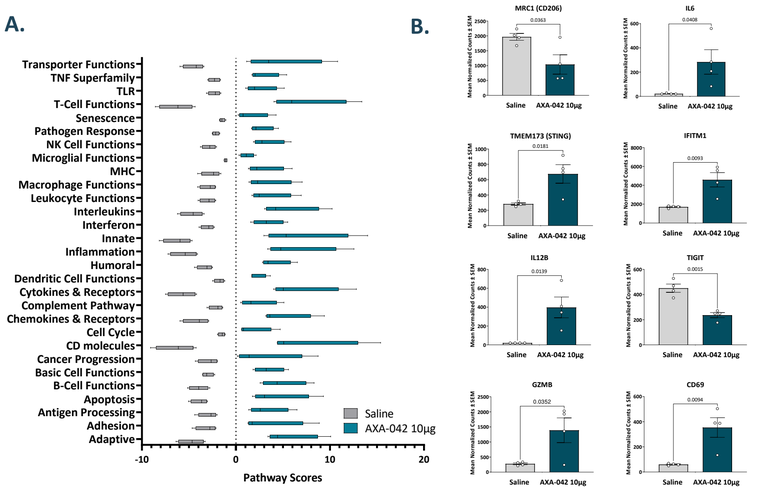
CT26 tumor tissues were collected 24 hrs after the 3rd dose of AXA-042 (10 µg/mouse, day 7) for FACS analysis. M2: CD45+CD11b+F4/80+ CD206+ MHCII low; M1: CD45+CD11b+F4/80+ CD206- MHCII +; DC: CD45+CD11c+ MHCII+. Data is presented as either mean % of events relative to the indicated gate or mean fluorescence 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).

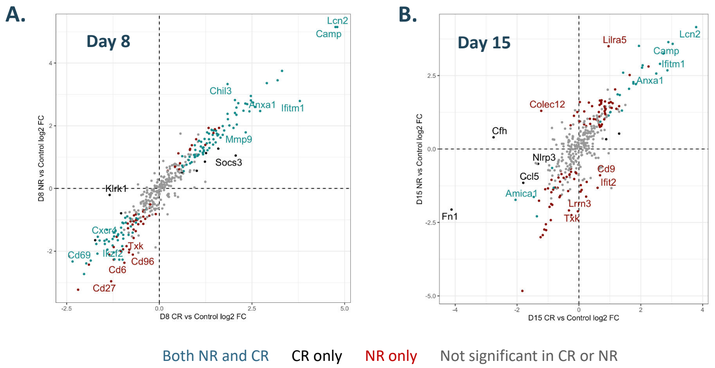
Figure 7. AXA-042 Engages the Innate and Adaptive Immune Response Pathways



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.**

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