The anti-BCMA Antibody-Drug Conjugate Belantamab Mafodotin (GSK2857916) Drives Immunogenic Cell Death and Immune-Mediated Anti-Tumor Responses, and in Combination with an OX40 Agonist Potentiates in vivo Activity

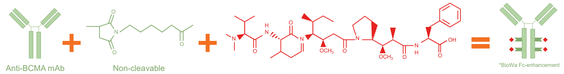
Abstract Code: PF558 - Myeloma and other monoclonal gammopathies – Biology & Translational Research

Rocio Montes de Oca[1], Sabyasachi Bhattacharya[1], Nick Vitali[1], Krupa Patel[1], Heather Kaczynski[1], Hong Z. Shi[1], Christina Blackwell[1], Laura Seestaller-Wehr[1], Eric Dobrzynski[1], Leslie Obert[1], Oliver Kepp[2], David Cooper[1], Lyuben Tsvetkov[1], Heather Jackson[1], Mark Creighton-Gutteridge[3], Chris Hopson[1], Jingsong Yang[1], Guido Kroemer[2], Axel Hoos[1], Patrick Mayes[1], Chris Shelton[1], Alireza Alavi[1]

[1]GlaxoSmithKline, Collegeville, United States, [2]Institut Gustave Roussy, Paris, France, [3]GlaxoSmithKline, Stevenage, Hertfordshire, United Kingdom.

Background

- Multiple myeloma (MM) is the second most common hematological malignancy and remains an incurable disease. Novel targeted therapies are urgently needed.
- B-cell Maturation Antigen (BCMA) is a cell surface receptor highly expressed in multiple myeloma and other B cell malignancies with a key role in plasma cell survival.
- **Belantamab mafodotin or GSK2857916** is a humanized, afucosylated, IgG1 anti-BCMA monoclonal antibody (aka., belantamab or GSK2857914) conjugated to monomethyl auristatin-F (MMAF). Belantamab mafodotin can kill tumor cells either by apoptosis after cytotoxic drug is released inside the cell or by antibody-dependent cellular cytotoxicity (ADCC).[1]



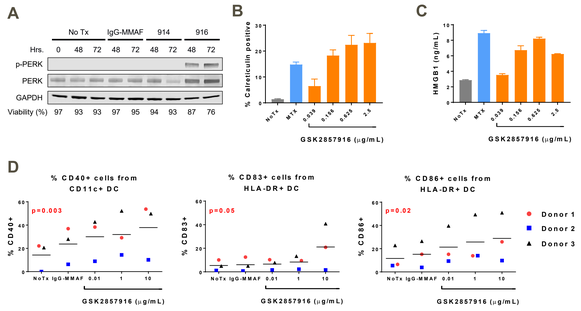
- In a Phase 1 clinical trial for relapsed and refractory multiple myeloma (RRMM), belantamab mafodotin achieved a 60% overall response rate, with a median progression-free survival of 12 months, and a median duration of response of 14.3 months.[2] These results highlight its potential as a promising drug candidate for the treatment of RRMM.

Aims

- MMAF is a member of the dolastatin family of microtubule inhibitors, potent anti-tumor agents that can lead to immunogenic cell death (ICD), a type of cell death that stimulates host immune responses.[3] We evaluated and characterized ICD as a novel mechanism of action for GSK2857916.
- We leveraged ICD and explored GSK2857916 immune-modulatory activities as single agent and in combination with immune-modulators, in particular OX40, in an immune-competent mouse model.

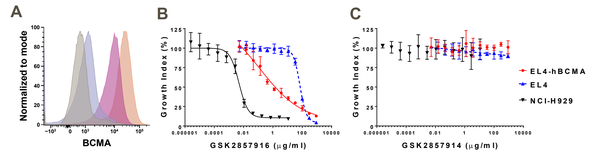
Results

Figure 1. GSK2857916 Induces Hallmarks of Immunogenic Cell Death and Dendritic Cell Activation in MM Cells



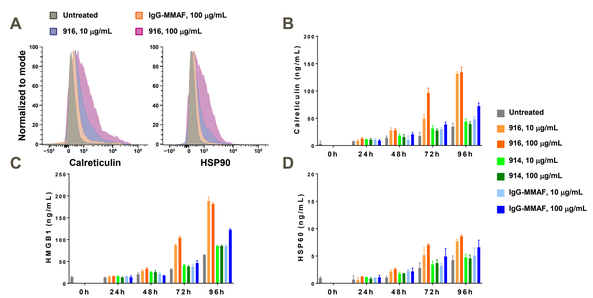
Treatment of NCI-H929 MM cells with GSK2857916 (916) induces endoplasmic reticulum (ER) stress **(A)** and ICD **(B-D)**. **(A)** Induction of phosphorylation of the ER stress kinase PERK (pPERK). **(B)** Increased surface exposure of calreticulin by flow cytometry and **(C)** secretion of HMGB1 by ELISA. Minimal or no effects were observed using control ADC (IgG-MMAF) or unconjugated antibody GSK2857914 (914). Mitoxantrone (MTX) as an ICD positive control. **(D)** Immature dendritic cells (iDC) from three healthy donors were co-cultured for 48 hours with NCI-H929 cells pre-treated with increasing concentrations of GSK2857916 or controls. Cell surface expression of CD40, CD83 and CD86, activation and maturation DC markers, significantly increased with GSK2857916 treatment.

Figure 2. Syngeneic EL4-hBCMA T cell Lymphoma Model Development and Characterization



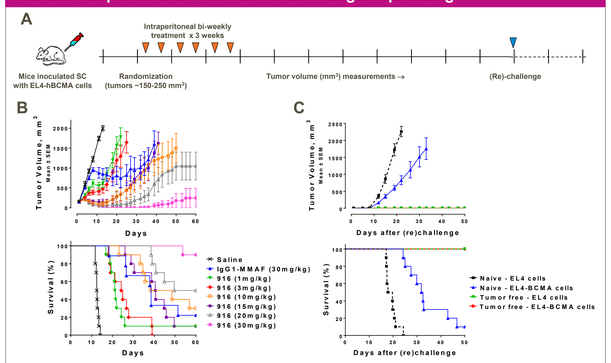
GSK2857916 does not cross-react with murine BCMA. The EL4 T cell lymphoma line was engineered to express human BCMA (EL4-hBCMA). **(A)** Expression of BCMA on the cell surface was evaluated by flow cytometry in NCI-H929 (□), EL4 parental (□) and EL4-hBCMA (□) cells with an anti-BCMA antibody or isotype (□; EL4-hBCMA). **(B)** Expression of hBCMA in the EL4 cell line (red) sensitizes it to GSK2857916 but not to the GSK2857914 unconjugated mAb, as determined by a 3 day cell viability assay.

Figure 3. GSK2857916 Induces Immunogenic Cell Death in EL4-hBCMA Cells



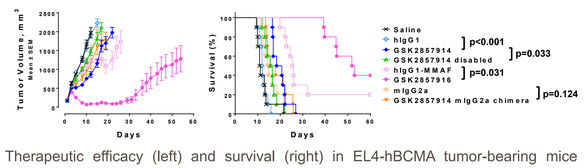
Treatment of EL4-hBCMA cells with GSK2857916 leads to ICD as measured by cell surface exposure of calreticulin and HSP90 **(A)** and increased secretion of calreticulin **(B)**, HMGB1 **(C),** and HSP60 **(D).** Minimal or no effects were observed using IgG-MMAF or unconjugated antibody (914) controls.

Figure 4. Anti-tumor Activity of GSK2857916 is Mediated by the Host Adaptive Immune Response and Promotes Tumor Antigen Spreading



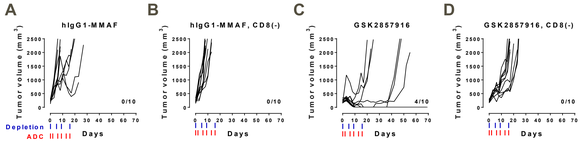
(A) Study design of dosing and (re)challenge, C57BL/6 mice bearing EL4-hBCMA tumors were treated with GSK2857916 or hIgG-MMAF. **(B)** Therapeutic efficacy (top) and survival (bottom) of animals treated as indicated (n=10 mice/group). **(C)** Animals with complete and sustained tumor regressions from (**B**; n=9/group) and age match naïve controls (n=10/group) were (re)challenged with EL4-hBCMA or EL4 parental cells on day 77. Therapeutic efficacy (top) and survival (bottom) were monitored for 50 days after cell inoculation.

Figure 5. Anti-tumor Activity of GSK2857916 is MMAF-dependent



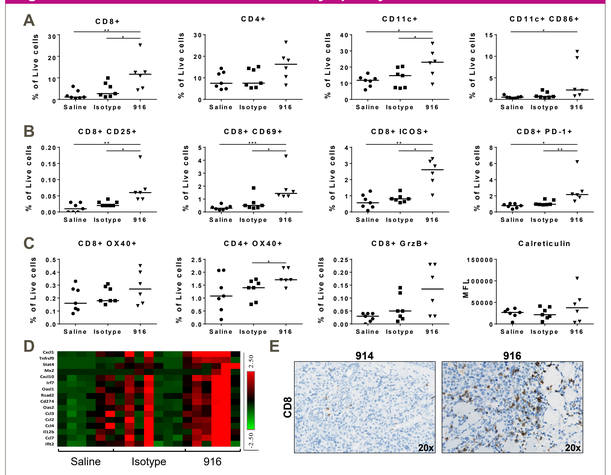
Therapeutic efficacy (left) and survival (right) in EL4-hBCMA tumor-bearing mice dosed with 30 mg/kg (as in **Figure 4**) with several versions of the GSK2857914 antibody or the GSK2857916 antibody drug conjugate, as indicated. Kaplan-Meier survival with significant p-values by a standard log-rank test (p<0.05; n=10 mice/group).

Figure 6. Depletion of CD8+ T Cells Abrogates GSK2857916 Anti-Tumor Activity



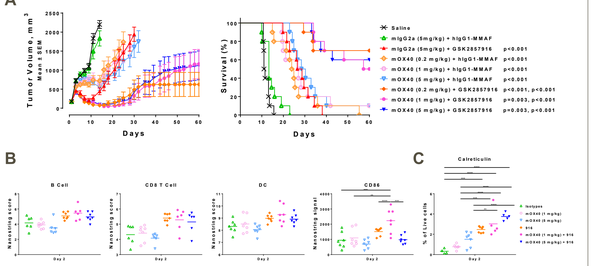
EL4-hBCMA tumors were established (as in **Figure 4**) and animals were treated on days 1, 3, 6, 9, 13, and 16 with GSK2857916 or IgG-MMAF (ADC, red ticks). T cell depleting doses of anti-CD8 (300 µg/mouse) were administered on days 1, 5, 9, and 16 (Depletion, blue ticks). Depletion of CD8+ T cells abrogated the anti-tumor activity of GSK2857916 **(C vs. D).** Depletion of CD8+ T cells was verified on day 15 by flow cytometry (not shown). The numbers in the bottom right corner indicate the number of tumor free mice.

Figure 7. GSK2857916 Promotes Tumor Lymphocyte Infiltration and Activation



PD changes following treatment with GSK2857916 in the EL4-hBCMA model. Mice were treated with 2 doses of GSK2857916 (15 mg/kg) and tumors were harvested 24 hours after the 2nd dose. Immunophenotyping revealed significant increases in tumor infiltration of CD8+ and CD4+ T cells and dendritic cells **(A),** activation markers CD86 **(A),** CD25, CD69 and ICOS **(B)**, the co-stimulatory molecule OX40 **(C)**, and cytotoxic Granzyme B (**C**). GSK2857916 also induced a modest but reproducible increase in calreticulin at the surface of tumor cell cells *in vivo* **(C)** and the type I interferon signature **(D)**, both ICD markers. Immunohistochemistry for CD8 reactive cells **(E)**.

Figure 8. Therapeutic Synergy by Combining GSK2857916 and an Anti-OX40 Agonist Antibody



Therapeutic efficacy (left) and survival (right) in EL4-hBCMA tumor-bearing mice dosed with 10 mg/kg of GSK2857916 concomitantly with mouse anti-OX40 IgG2a at 0.2, 1 and 5 mg/kg (as in **Figure 4**), controls as indicated. Kaplan-Meier survival with significant p-values by a standard log-rank test (p<0.05; n=10 mice/group), listed as compared to 1st or 2nd agent **(A)**. Mice were treated with 2 doses of GSK2857916 (10 mg/kg) and tumors were harvested 48 hours after the 2nd dose. **(B)** Gene expression signatures by NanoString. **(C)** Significant increase in calreticulin at the surface of tumor cell cells *in vivo* after treatment with GSK2857916 as single agent and in combination.

Conclusions

- This is the first study evaluating the mechanism of action of the clinical-stage belantamab mafodotin (GSK2857916) in a newly-developed immune-competent EL4-hBCMA mouse model.
- GSK2857916 potently delays tumor growth and promotes durable complete regressions *in vivo*.
- GSK2857916 anti-tumor activity is associated with increased tumor T lymphocyte and DC infiltration and activation, and is abrogated upon depletion of CD8+ T cells.
- Induction of ICD and engagement of the host immune system potentiate the anti-tumor activity of GSK2857916 in an immune-competent setting and constitute key mechanisms of GSK2857916 activity.
- In line with an engagement of the host immune system, combinations with immuno-modulatory agonists such as anti-OX40 synergize with GSK2857916.
- Results from this preclinical work provided rationale to support clinical evaluation of belantamab mafodotin in combination with an anti-OX40 agonist in a planed trial (DREAMM-5).

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References

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Acknowledgements

- R.MdeO, SB, NV, KP, HK, HZS, CB, LSW, ED, LO, OK, DC, LT, HJ, MC-G, CH, JY, PM, CS and AA are employees of GlaxoSmithKline and own stocks/shares. AH employee of and holds stocks/shares in GSK and a non-executive Director and shareholder of Imugene. GK and OK received grants/research support from: Bayer Healthcare, Biomérieux, GlaxoSmithKline, Eleor, Lytix Pharma, PharmaMar, Sotio, Vasculox/Tioma. GK and OK are founders and shareholders of Samsara Therapeutics. GK is founder and shareholder of everImmune. GK and OK received funds from GSK for this research.
- Drug linker technology is licensed from Seattle Genetics; monoclonal antibody is produced using POTELLIGENT® Technology licensed from BioWa. All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed by the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed.

Presented at the 24th Congress of the European Hematology Association (EHA), Amsterdam, The Netherlands, June 13-16, 2019

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