Poster # 3740

Tusamitamab ravtansine induces immunogenic cell death and synergizes with anti-PD-1 or anti-PD-L1 antibody combination in solid tumor

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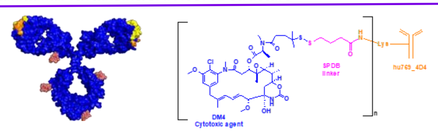
INTRODUCTION

- Carcinoembryonic antigen cell adhesion molecule 5, CEACAM5, is highly expressed on the cell surface of several epithelial tumors including colorectal cancer (CRC), pancreatic ductal adenocarcinoma (PDAC), gastric cancer (GC) and non-small cell lung cancer (NSCLC)[1].
- Tusamitamab ravtansine (also called SAR408701) is an antibody-drug conjugate (ADC) targeted against CEACAM5 and delivering DM4, a maytansinoid cytotoxic. SAR408701 has shown antitumor efficacy in CEACAM5-expressing tumoral cell lines and patient-derived xenograft models[2,3]. It is currently being evaluated in patients with advanced CEACAM5-positive non-squamous (NSQ) NSCLC, in a phase II study in combination with pembrolizumab, and a phase III trial as single agent in comparison with docetaxel.
- In this study we investigated the ability of tusamitamab ravtansine to induce immunogenic cell death (ICD) and if it could be beneficially combined with immune checkpoint inhibitors

tusamitamab ravtansine/SAR408701 structure

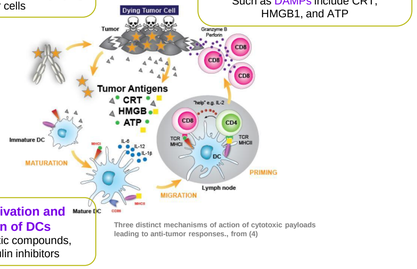


BACKGROUND

Immunogenic cell death

① Direct induction of tumor cell death by the cytotoxic payload, leading to release of **tumor antigens** by dying cancer cells

② Immunogenic cell death induced by certain classes of cytotoxic compounds resulting in the release of "danger signals" by the tumor cell. Such as DAMPs include CRT, HMGB1, and ATP



Three distinct mechanisms of action of cytotoxic payloads leading to anti-tumor responses., from (4)

③ Direct activation and maturation of DCs by some cytotoxic compounds, including tubulin inhibitors

- Preclinical and clinical investigations have shown that several cytotoxic agents currently utilized as payloads for antibody–drug conjugates (ADCs) have the ability to induce immunogenic cell death (ICD) of tumor cells and directly activate dendritic cells (DCs).Cytotoxic agents with these properties are therefore attractive candidates for combination with IO therapeutic agents.

Challenges for *in vivo* ICD determination and combination studies between ADC and immune checkpoint inhibitors

A fully immunocompetent environment is required for the investigations of ICD

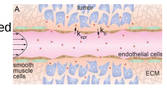
- Use of syngeneic tumors in immunocompetent mice

CEACAM5 ADC targets human CEACAM5 protein which is not expressed in rodents

HuCEACAM5 engineered murine tumors do not grow in immunocompetent mice

☞ Need to work with irrelevant ADC conditions

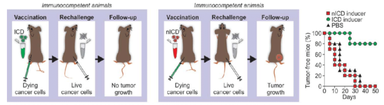
ADC will be administered at a high dose to exploit the enhanced permeability and retention effect observed in solid tumors subcutaneously implanted in mice. This leads to an improved delivery of macromolecular drugs to tumor site. Dose can be modulated to obtain different levels of antitumoral activity (inactivity, activity and/or high activity)



From (5)

RESULTS

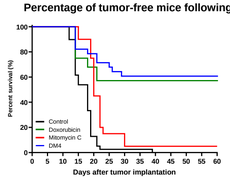
Operational determination of immunogenic cell death (ICD) by vaccination/rechallenge assay



Consensus guidelines for the detection of immunogenic cell death, from (6)

- The degree of immunogenicity of cell death induced by the cytotoxic under evaluation was reflected by the proportion of mice that do not develop subcutaneous tumors

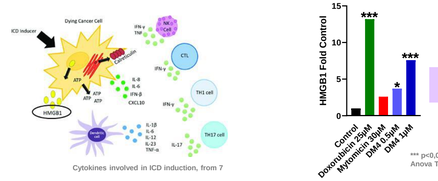
Percentage of tumor-free mice following re-challenge with live CT26 cells



60% of mice vaccinated by DM4-treated CT26 cells do not develop sc tumors

- **Tusamitamab ravtansine payload, DM4 induces robust immunogenic cell death.** CT26 cells cultured in presence of indicated drug were injected into the left flank of immunocompetent C57BL/6 mice; this was followed by injection of live tumor cells into the right flank a week later. Tumor incidence post-rechallenge was monitored for 60 days. Results are expressed in percentage of tumor-free mice following re-challenge with live CT26 cells. DM4 shows the same ability to induce ICD as doxorubicin (used as positive control).

Evaluation of HMGB1 release by DM4-treated CT26 cells



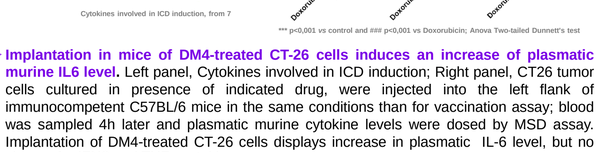
Cytokines involved in ICD induction, from 7

7.5-fold induction of release of HMGB1

*** p<0,0001 and * p<0,05 *vs* control
Anova Two-tailed Dunnett's test

- **Tusamitamab ravtansine payload, DM4 induces the release of danger signals by CT26 cells.** Left panel, Cytokines involved in ICD induction; Right panel, CT26 cells were incubated 24h with indicated drugs and HMGB1 was dosed from supernatant by ELISA. DM4 at 1 µM induces the release of HMGB1 by CT26.

Changes in plasmatic cytokines after DM4-treated CT26 cells implantation in mice

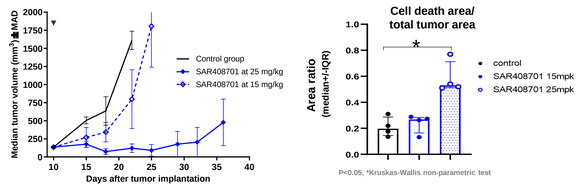


Cytokines involved in ICD induction, from 7

*** p<0,001 *vs* control and ### p<0,001 vs Doxorubicin; Anova Two-tailed Dunnett's test

- **Implantation in mice of DM4-treated CT-26 cells induces an increase of plasmatic murine IL6 level.** Left panel, Cytokines involved in ICD induction; Right panel, CT26 tumor cells cultured in presence of indicated drug, were injected into the left flank of immunocompetent C57BL/6 mice in the same conditions than for vaccination assay; blood was sampled 4h later and plasmatic murine cytokine levels were dosed by MSD assay. Implantation of DM4-treated CT-26 cells displays increase in plasmatic IL-6 level, but no change for IFNβ and TNFα cytokines.

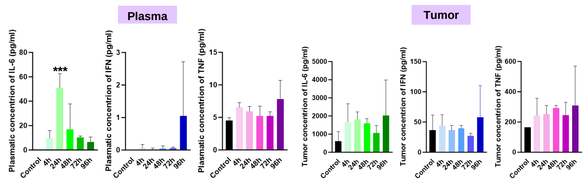
Efficacy evaluation of tusamitamab ravtansine in non-specific conditions in MC38 tumor



P<0.05, *Kruskas-Wallis non-parametric test

- **Tusamitamab ravtansine is able to induce a dose-dependent activity in MC38 tumor in non-specific conditions.** MC38 cells were injected into the flank of immunocompetent C57BL/6 mice. After tumor establishment (10 days), SAR408701 (tusamitamab ravtansine) was administered at 15 and 25 mg/kg following a single IV administration. SAR408701 leads to improved delivery of macromolecular drugs to the tumor site by exploiting the enhanced permeability and retention effect observed for solid tumors subcutaneously implanted in mice. Left panel, tumor volume evolution over time: Right panel, bars representing relative cell death area in the tumor (determined using Hematoxylin/Eosin/Saffron staining from tumors sampled 96h post ADC treatment).

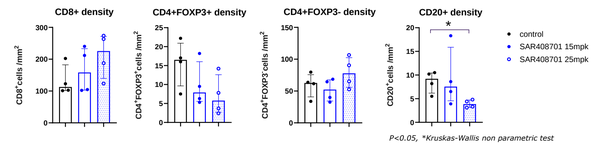
Murine cytokine levels after tusamitamab ravtansine treatment in mice bearing MC38 tumor



*** p<0,01 *vs* control; Anova Two-tailed Dunnett's test

- **Tusamitamab ravtansine treatment induces changes in murine cytokine levels in mice bearing MC38 tumor.** MC38 cells were injected into the flank of immunocompetent C57BL/6 mice. After tumor establishment (10 days) mice were treated IV by SAR408701 at 25mg/kg. Blood and tumors were sampled 4h, 24h, 48h, 72h, 96h after. Plasmatic and intra-tumor murine cytokine levels were dosed by MSD assay. Graphs represent plasmatic (left) and intra-tumoral (right) levels of murine cytokines. Increases in plasmatic IL6 was observed 24h post treatment by SAR408701 while IFNβ and TNFα increased are observed 96h post treatment. Intratumorally, slight increases in all cytokines are observed at each sampling time.

Tumor infiltrated lymphocytes in MC38 tumor after tusamitamab ravtansine treatment



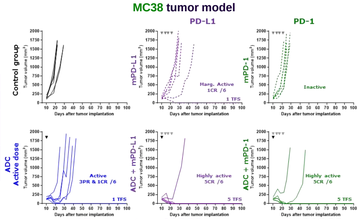
P<0,05, *Kruskas-Wallis non parametric test

- **Tusamitamab ravtansine induces a dose-dependent increase in cytotoxic T-cells and a dose-dependent decrease of Tregs and B cells in MC38 tumor.** . MC38 cells were injected into the flank of immunocompetent C57BL/6 mice. After tumor establishment, mice were treated IV by SAR408701 at 15 (inactive dose) and 25 mg/kg (active dose) . Tumors were sampled 96h post treatment. The intra-tumoral lymphocyte population was detected by CD20+, CD4+, CD8+, FoxP3+ by immunostaining 4-plex (Ventana Disco ULTRA) and the density of the different cell populations was measured. Graphs represent tumoral density of CD8+ T-Cells, CD4+FOXP3+, CD4+FOXP3- and CD20+ lymphocytes cells. SAR408701 treatment induced an increase in tumor infiltrating cytotoxic T-cells with a decrease in Treg cells and B cells.

In vivo evaluation of tusamitamab ravtansine in combination with IO mono-therapies in MC38 and CT26 tumor models

Summary of combination between ADC-DM4 and with IO mono-therapies

	MC38 syngeneic model (IO sensitive model; highly sensitive to ADC-DM4)			CT26 syngeneic model (IO poorly sensitive model; Moderately sensitive to ADC-DM4)		
ICI mAb		**PD-L1**	**PD-1**		**PD-L1**	**PD-1**
ICI activity	na	Marg. Active 1CR /6	Inactive	na	Inactive	Inactive
SAR408701, Dose active	Active 3PR & 1CR /6	Highly active 5CR /6 **Synergy**	Highly active 5CR /6 **Synergy**	Active No PR - CR	Active No PR – CR Combined effect	Active 1CR&1PR/8 Combined effect
SAR408701, Dose inactive	Inactive	Very active 1PR /6 Combined effect	Active 1CR&1PR /6 Combined effect	Inactive	Active No PR – CR Combined effect	Active 1CR /8 Combined effect

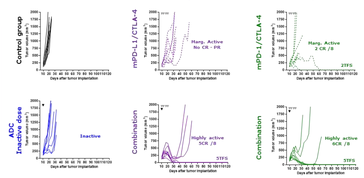


- **Tusamitamab ravtansine synergizes with IO mono-therapies in IO sensitive MC38 tumor model.** MC38 or CT26 cells were injected into the flank of immunocompetent C57BL/6 mice. After tumor establishment, SAR408701 was treated at 15 and 25 mg/kg following a single IV administration (black arrows), and PD-L1 or PD-1 mAbs were treated at 10 mg/kg following 4 biweekly IP administration (grey arrows). Murine environment with no huCEACAM5 expression. Left panel, table summarizing all tested combinations, Right panel, An example of SAR408701 activity as single agent or in combination. Combinations resulted in significantly better antitumor activity than single agent arms.

In vivo evaluation of tusamitamab ravtansine in combination with IO dual-therapies in MC38 and CT26 tumor models

Summary of combination between ADC-DM4 and with IO dual-therapies

	MC38 syngeneic model (IO sensitive model; highly sensitive to ADC-DM4)		CT26 syngeneic model (IO poorly sensitive model; Moderately sensitive to ADC-DM4)		
ICI mAb		**PD-L1/CTLA-4**	**PD-1/CTLA-4**	**PD-L1/CTLA-4**	**PD-1/CTLA-4**
ICI activity	na	Very active 3CR /6	Active 1PR & 1CR /6	na	Marg. Active No CR - PR	Marg. Active 2 CR /8
SAR408701, Dose active	Very active 1PR&1CR /6	Highly active 1PR & 3CR /6 **Synergy**	Highly active 1PR & 3CR /6 **Synergy**	Active No PR - CR	Highly active 2CR & 1PR /8 **Synergy**	Highly active 7CR & 1PR /8 **Synergy**
SAR408701, Dose inactive	Inactive	Highly active 2PR & 3CR /6 **Synergy**	Highly active 4CR /6 **synergy**	Inactive	Highly active 5CR /8 **Synergy**	Highly active 8CR /8 **Synergy**



- **Tusamitamab ravtansine synergizes with IO dual-therapies in IO sensitive MC38 and CT26 tumor model** MC38 or CT26 cells were injected into the flank of immunocompetent C57BL/6 mice. After tumor establishment, SAR408701 was treated at 15 and 25 mg/kg following a single IV administration (black arrows), and PD-L1 or PD-1 mAbs were treated at 5 (MC38) or 10 mg/kg (CT26) of PD-L1 + CTLA-4 or PD-1 + CTLA-4 mAbs following 4 biweekly IP administration (grey arrows). Left panel, table summarizing all tested combinations, Right panel, An example of SAR408701 activity as single agent or in combination. Combinations resulted in significantly better antitumor activity than single agent arms.

CONCLUSIONS

- **Tusamitamab ravtansine payload, DM4 induced robust immunogenic cell death**
- **DM4 induced the release of danger signals by CT26 cells**
- **Implantation of DM4-treated CT26 cells in mice induced an increase of plasmatic murine IL6 level**
- **Tusamitamab ravtansine induced a dose-dependent anti-tumoral activity in murine MC38 tumor in non-specific conditions**
- **Tusamitamab ravtansine induced changes in murine cytokine levels in blood and tumor in mice bearing MC38 tumor cells**
- **Tusamitamab ravtansine induced a dose-dependent increase of cytotoxic T-cell and a dose-dependent decrease of Tregs and B cells in MC38 tumor**
- **Combination of tusamitamab ravtansine with anti-PD-1 or anti-PD-L1 antibody led to complete responses and, even, tumor-free survivors at 120 days post tumor implantation while single agents werre inactive or moderately active**
- **These preclinical data show that tusamitamab ravtansine can induce robust ICD and could be combined with immune checkpoint inhibitors in clinic**

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DISCLOSURES:

Céline Nicolazzi, Anne-Marie Lefebvre, Nicolas Moindrot, Christelle Larois, Marion Classe, Sukhvinder Sidhu are Sanofi R&D Employees

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