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Mitazalimab, a potent CD40 agonist in combination with chemotherapy redirects and activates tumor infiltrating myeloid cells

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Mitazalimab – a CD40 agonist with best-in-class profile

- Mitazalimab is an FcγR crosslinking dependent CD40 agonistic antibody (IgG1) with a tumor-directed immune activation
- Mitazalimab binds with high affinity to a unique binding epitope on the CD40 receptor which allows for high efficacy and potency
- Mitazalimab has potential for superior clinical activity, since mitazalimab is the only FcγR-dependent CD40 agonist with tumor-directed activity that can be dosed >1 mg/kg

Mitazalimab in clinical development

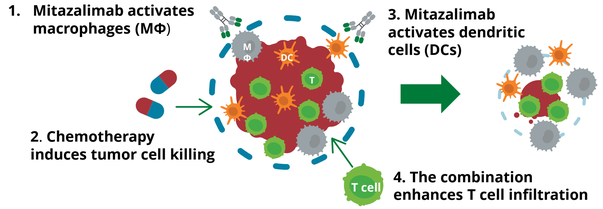
Phase I

- Mitazalimab has shown clinical activity and a manageable safety profile in Phase 1 studies
- NCT02829099: Mitazalimab monotherapy, 95 patients
  - Safety: Safe and tolerable up toresponse 1200 µg/kg i.v. and most drug-related adverse events grade 1-2
  - Responses: 1 patient with partial and 10 patients with stable disease (> 6 months)

Phase 1b/2

- OPTIMIZE-1 (NCT04888312) is an ongoing phase 1b/2, open-label, multicenter study designed to evaluate safety, tolerability, and efficacy of mitazalimab in combination with chemotherapy (mFOLFIRINOX) in adults diagnosed with previously untreated metastatic pancreatic cancer

Mode of action - mitazalimab combined with chemotherapy



- Mitazalimab binds to CD40, the key activation receptor on antigen presenting cells (APCs), i.e. dendritic cells (DCs), B cells and macrophages
- Mitazalimab activates tumor associated macrophages (TAMs) which have the ability to reshape the tumor infiltrating myeloid microenvironment leading to stromal degradation and enhanced chemo and immune cell penetration
- Mitazalimab activates DCs allowing priming of tumor specific T cells which results in more tumor specific T cells infiltrating and killing the tumor and improved anti-tumor efficacy
- Mitazalimab in combination with chemotherapy, that kills tumor cells, improves the release of tumor antigens that are picked up by DCs enhancing priming and infiltation of tumor specific T cells

Mitazalimab activates TAMs from human tumor samples

- Mitazalimab activates TAMs purified from human prostate and ovarian tumor samples, into a more pro-inflammatory and less immuno-suppressive phenotype

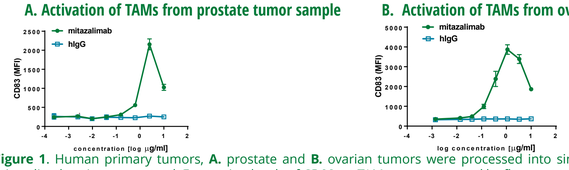


Figure 1. Human primary tumors, **A.** prostate and **B.** ovarian tumors were processed into single-cell suspensions and incubated with mitazalimab or isotype control. Expression levels of CD83 on TAMs were assessed by flow cytometry.

Mitazalimab alters the composition of myeloid cells in the tumor

- Mitazalimab activates tumoral and splenic DCs in human CD40 transgenic (hCD40tg) mice in vivo, which results in improved activation of both splenic and intratumoral T cells
- Mitazalimab alters the composition of tumor myeloid cells such that monocytes and macrophages are reduced in favor of granulocytic cells

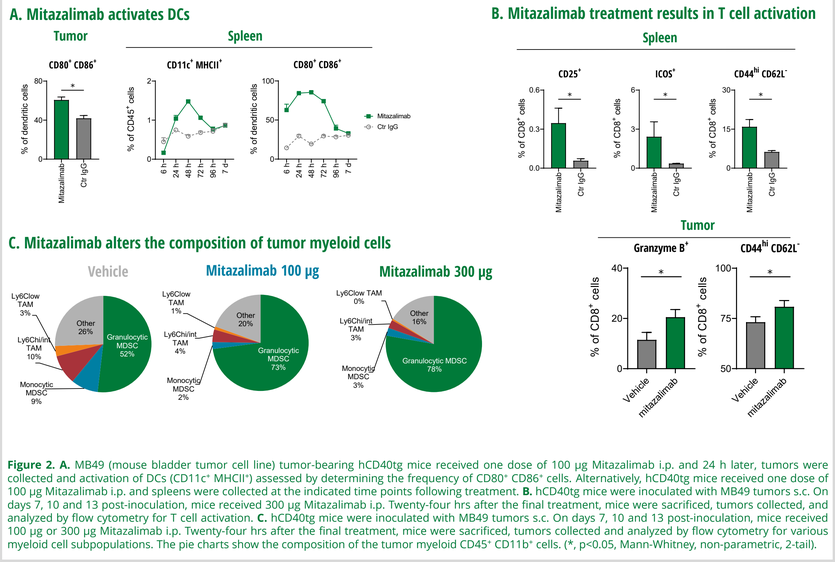


Figure 2. A. MB49 (mouse bladder tumor cell line) tumor-bearing hCD40tg mice received one dose of 100 µg Mitazalimab i.p. and 24 h later, tumors were collected and activation of DCs ($CD11c^+$ $MHCII^+$) assessed by determining the frequency of $CD80^+$ $CD86^+$ cells. Alternatively, hCD40tg mice received one dose of 100 µg Mitazalimab i.p. and spleens were collected at the indicated time points following treatment. **B.** hCD40tg mice were inoculated with MB49 tumors s.c. On days 7, 10 and 13 post-inoculation, mice received 300 µg Mitazalimab i.p. Twenty-four hrs after the final treatment, mice were sacrificed, tumors collected, and analyzed by flow cytometry for T cell activation. **C.** hCD40tg mice were inoculated with MB49 tumors s.c. On days 7, 10 and 13 post-inoculation, mice received 100 µg or 300 µg Mitazalimab i.p. Twenty-four hrs after the final treatment, mice were sacrificed, tumors collected and analyzed by flow cytometry for various myeloid cell subpopulations. The pie charts show the composition of the tumor myeloid $CD45^+$ $CD11b^+$ cells. (*, p<0.05, Mann-Whitney, non-parametric, 2-tail).

Mitazalimab plus chemotherapy improves immune activation

- The frequency of CD11b-expressing cells in the tumors was not affected after treatment. However, Mitazalimab + FOLFIRINOX reduced the proportion of CD206 expressing $CD11b^+F4/80^+$ TAMs, indicating a shift from immunosuppressive M2 to pro-inflammatory M1 macrophages.
- A direct effect on APCs was detected as a reduced total number of B cells in the periphery, while the remaining B cells displayed an enhanced activation profile ($CD86^+$)
- The proportion of activated $CD8^+$ T cells in the tumor was enhanced after the combined treatment of mitazalimab plus FOLFIRINOX

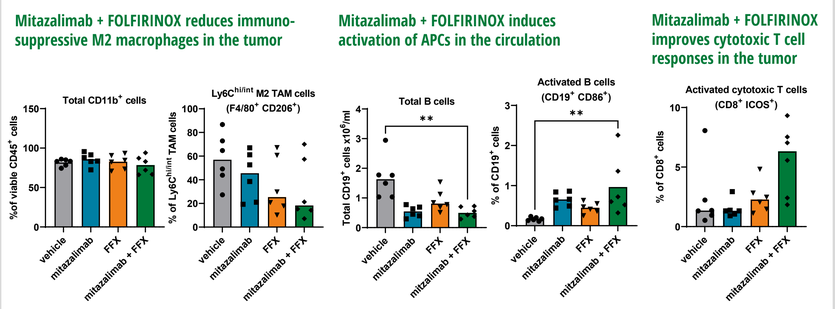


Figure 3. MB49 tumor-bearing hCD40tg mice received treatment with FOLFIRINOX (FFX; oxaliplatin, irinotecan, folinic acid and 5-fluorouracil) i.p. on days 14-15 and/or 100 µg mitazalimab i.p. on day 17 after tumor inoculation. 24 h later, tumors and blood were collected and analyzed by flow cytometry for various myeloid cell subpopulations, B cells and T cell populations and activation status. (**, p<0.01, Mann-Whitney, non-parametric, 2-tail).

Mitazalimab synergizes with anticancer treatments

- Mitazalimab administered as monotherapy reduces tumor growth and induces survival in preclinical tumor models
- Mitazalimab combined with chemotherapy, checkpoint inhibitor (anti-PD-1) or vaccine has a synergistic effect on survival in preclinical tumor models
- These preclinical data demonstrate opportunities for combination of mitazalimab with other treatment modalities in immuno-oncology

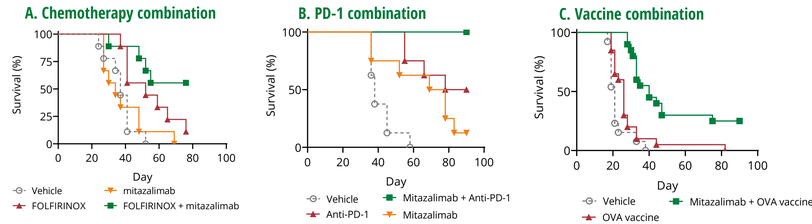


Figure 4. A. MB49 tumor-bearing hCD40tg mice received treatment with FOLFIRINOX (oxaliplatin, irinotecan, folinic acid and 5-fluorouracil) on days 7-8, 14-15 and 21-22, and/or 100 µg mitazalimab on days 10, 17 and 24. **B.** MB49 tumor-bearing hCD40tg mice received 100 µg mitazalimab every 2-3 days from day 7 until day 20, or 250 µg anti-PD-1 surrogate antibody on days 7, 10 and 13. **C.** hCD40tg mice received 100 µg mitazalimab and 200 µg ovalbumin protein (OVA) simultaneously on day 0 and 7. Controls received OVA only. Seven days following the second therapy dose, the mice were inoculated with E.G7-OVA tumors.

Mitazalimab is effective in a chemotherapy resistant tumor model

- FOLFIRINOX inhibits tumor growth in a chemotherapy sensitive mouse tumor model, but not in a chemotherapy resistant tumor model which was developed by sensitizing the tumor cell line MB49 with continuous low dose treatment of FOLFIRINOX
- The combined treatment of mitazalimab and FOLFIRINOX induces a strong anti-tumor response in chemotherapy resistant tumors

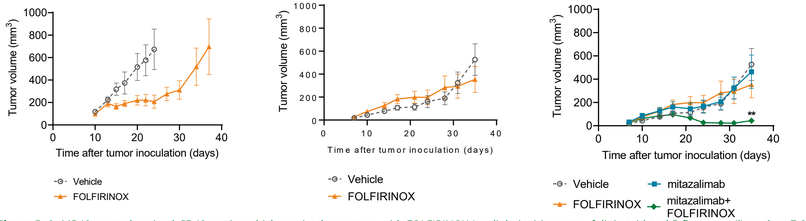


Figure 5. A. MB49 tumor-bearing hCD40tg mice which received treatment with FOLFIRINOX (oxaliplatin, irinotecan, folinic acid and 5-fluorouracil) on days 7-8, 14-15 and 21-22 had reduced tumor growth compared with vehicle treatment. **B.** Chemotherapy resistant MB49 cells, developed by continuous low dose treatment of FOLFIRINOX in vitro, were inoculated in tumor-bearing hCD40tg mice which received treatment with **B.** FOLFIRINOX on days 7-8, 14-15 and 21-22 alone or, **C.** in combinaton with 100 µg mitazalimab on days 10, 17 and 24 (**, p<0.01 mitazalimab + FOLFIRINOX vs vehicle, Mann-Whitney, non-parametric, 2-tail).

Summary and conclusions

- Preclinical data demonstrate that mitazalimab activates DCs and alters the composition of tumor infiltrating myeloid cells, resulting in enhanced activation of T cells and improved anti-tumor responses
- Mitazalimab has the potential to reshape the myeloid cell compartment in the tumor microenvironment. This was demonstrated as activation of TAMs purified from human tumor samples and as reduction in the proportion of intra-tumoral immuno-suppressive macrophages in preclinical tumor models
- Mitazalimab synergizes with other anti-cancer treatments, such as chemotherapy (FOLFIRINOX), vaccine and checkpoint inhibitor (anti-PD-1). The combined treatment of mitazalimab and FOLFIRINOX induces a potent anti-tumor response in chemotherapy resistant tumors demonstrating the opportunity for mitazalimab to overcome chemotherapy resistance in e.g. pancreatic cancer patients
- OPTIMIZE-1, a phase 1b/2 study with mitazalimab in combination with mFOLFIRINOX in pancreatic cancer is currently ongoing