Immunotherapy of Colorectal Cancer by the T-cell Targeted DART® Protein MGD007: Cellular Mechanisms of Action

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Abstract

Introduction: MGD007 (glycoprotein A33 x CD3), a DART protein designed to redirect T cells to target gpA33-expressing colon cancer, is presently undergoing clinical evaluation (NCT02238805). The gpA33 target was selected based on its universal expression profile across primary and metastatic colorectal cancer (CRC), including expression on putative cancer stem cell (CSC) populations. MGD007 activity in CRC cell cytolysis and its prolonged PK in nonhuman primates have previously been reported (Cancer Res 2014;74(19 Suppl): Abstract nr 669.1). Here we further characterize MGD007 cellular mechanisms associated with redirected T-cell killing, cytokine responses and modulation with steroids.

Methods: Redirected killing assays were performed using luciferase labelled gpA33$^+$ Colo205 or RECA0201-GF colorectal cancer stem-like cells (CSLC) with freshly isolated PBMC or fractionated T-cell populations; Treg cells (CD4$^+$, CD127lo, CD25$^+$) were expanded for 14 days in presence of IL-2 and rapamycin and confirmed to be suppressive; steroids (budesonide and dexamethasone) were evaluated at pharmacologically relevant concentrations; multi-parameter FACS and ELISA were performed to determine cell surface marker expression and cytokine levels respectively.

Results: MGD007 displays potent redirected T-cell killing of gpA33$^+$ CRC cells, including complete lysis of CRC stem cell-like models. Importantly, MGD007 mediated cytolysis can be supported by various T-cell populations, including Treg cells. Following prolonged in vitro exposure to MGD007 and gpA33$^+$ tumor cells, expanded T cells acquire a memory phenotype and retain potent CTL activity when challenged with fresh gpA33$^+$ target cells; however, much decreased cytokine release was observed compared to that observed following initial T-cell exposure. The addition of dexamethasone or budesonide to freshly isolated effector cells and gpA33$^+$ target cells also reduces cytokine release levels to baseline in the presence of MGD007, with minimal impact observed on MGD007-mediated killing.

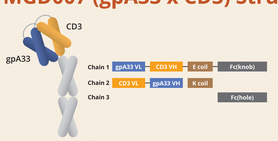
Discussion: MGD007 supports targeted lysis of CRC, including CSC subpopulations, and can leverage suppressive Tregs in addition to conventional T cells for cytolytic activity. Biological activity modulation is also feasible through induction of cytolytic Tmem cells with diminished cytokine release potential via sequential exposure to MGD007 or the simultaneous exposure to low-dose steroids. These data support further clinical development of MGD007 for the treatment of CRC patients.

Key Study Questions

- Can MGD007 target lysis of cancer stem cell (CSC) populations?
- Can MGD007 leverage all CD3 T-cell subpopulations — including suppressive T cells?
- What are the effects of prolonged MGD007 exposure on T-cell responses?
- What are the effects of steroids on MGD007 mediated biological activity?
- Does PD-1 blockade enhance MGD007 mediated anti-tumor activity?

Background

MGD007 (gpA33 x CD3) Structural Design



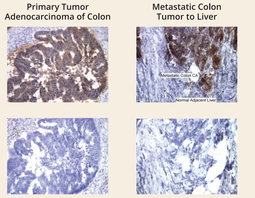
Anti-gpA33: humanized mAb selected from colon cancer stem-like cell immunization

Anti-CD3: humanized XR32 mAb

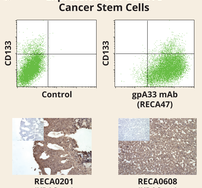
Human Fc: IgG1 with mutations to reduce undesired FcγR binding (ala,ala) and enhance heterodimerization (knob/hole); retains FcRN binding to enhance half-life

gpA33 is Broadly Expressed in Colorectal Cancer

A. Expression Across CRC



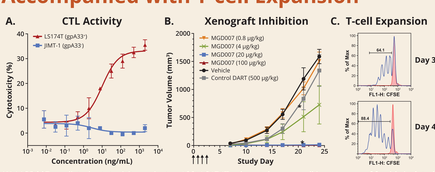
B. Expression on Putative Cancer Stem Cells



Frozen Tissue Type	Cases Evaluated	Score				gpA33 Positive Rate
		1+	2+	2-3+	3+	
Primary colon cancer	35	0	0	18	17	35/35
Metastatic colon cancer	17	0	0	3	14	17/17
Primary + metastatic colon cancer	52	0	0	21	31	52/52

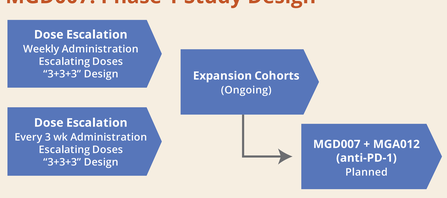
(A) Immunohistochemical (IHC) analyses performed across 52 CRC patients with anti-gpA33 mAb revealed 100% positivity at 2-3+ level. **(B)** FACS analyses of freshly isolated CRC biopsy epithelial cells reveals gpA33 expression across all cells including CD133$^-$ subset (top row); IHC analyses across panel of CRC-derived cancer stem-like cells (middle and bottom row).

MGD007 Mediates T-cell lysis of gpA33$^+$ CRC Accompanied with T-cell Expansion



(A) MGD007-mediated cytotoxicity against gpA33$^+$ LS174T colorectal cancer cell in presence of freshly isolated human PBMC (E:T = 30:1; 24h). **(B)** NOD/SCID mice (n = 8/group) implanted SC with activated human T cells co-mixed with LS174T tumor cells (E:T = 1:1) followed by indicated treatment with vehicle control, control DART, or 0.8-500 µg/kg MGD007 administered IV (arrow). Data represented as the mean ± SEM. *p < 0.001. **(C)** Proliferation of T-cells monitored by CFSE dilution in presence of MGD007 (blue) or control DART (red) incubated with gpA33+ LS174T (E:T = 10:1).

MGD007: Phase 1 Study Design



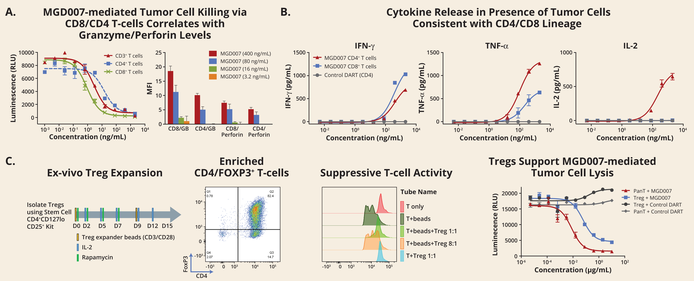
Objectives: Establish safety, determine MTD, characterize PK, evaluate alternate schedules, and describe early evidence of antitumors activity

Patient Population: Patients with relapsed/refractory metastatic colorectal carcinoma

Evaluations: RECIST and irRECIST

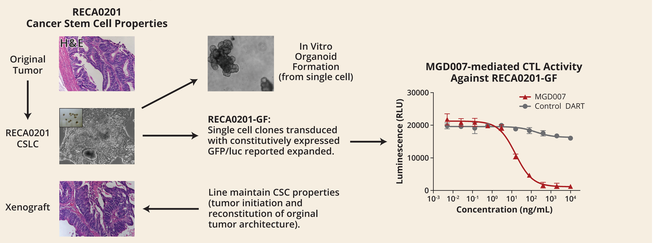
Results

MGD007 Can Recruit CD8, CD4, and Suppressive T Cells for Redirected T-cell Killing

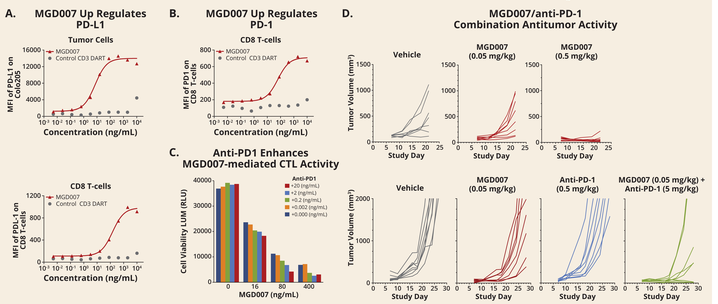


(A) MGD007-mediated cytotoxicity against gpA33$^+$ Colo205-Luc colorectal cancer cell line in presence of human T cells (CD3, CD8, or CD4 as indicated; E:T = 10:1) (left panel). Dose-dependent upregulation of granzyme B (GB) and perforin levels (right panel) following incubation of MGD007 with Colo205 and purified T cells (E:T = 10:1). **(B)** IFN-γ, TNF-α, and IL-2 observed in presence of Colo205 and T cells (E:T = 10:1) and MGD007. **(C)** Ex-vivo expanded Tregs expanded in vitro for 15 days (left panel) comprising > 80% CD4 FOXP3$^+$ and displaying functional suppression of conventional T-cells (CFSE dilution assay) in response to anti-CD3/CD28 activation, support MGD007 mediate cytotoxicity against gpA33$^+$ Colo205 (right panel).

MGD007 Mediates Lysis of Colorectal Cancer Stem-like Cells

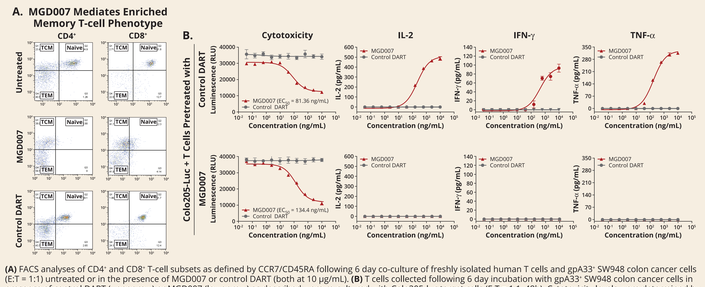


Anti-PD-1 Enhances MGD007-mediated Antitumor Activity



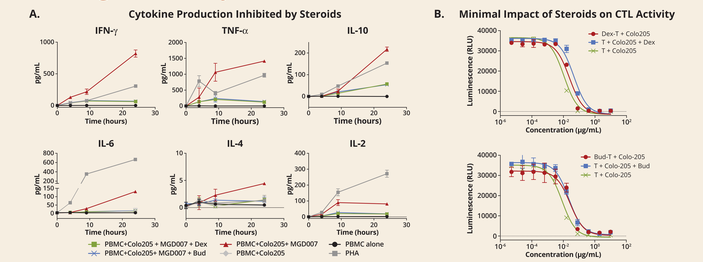
Cell surface expression of **(A)** PD-L1 on Colo205 (top), and CD8 T-cells (lower) and **(B)** PD-1 on CD8 T-cells in presence of MGD007 (E:T = 5:1; 24 hrs). **(C)** Redirected T-cell killing of Colo205 by MGD007 enhanced by addition of anti-PD1 mAb in presence of T cells (E:T = 3:1; 48 hrs). **(D)** MC38/hgpA33 colonic adenocarcinoma tumor growth in hCD3kI-Tg mice administered MGD007 alone (upper panels) or MGD007 ± anti-mouse PD-1 (lower panel). MGD007 dosed every 3–4 days; anti-PD-1 dosed on Days 0, 3 and 6.

T Cells Retain CTL Capacity but Reduced Cytokine Release Following Prolonged MGD007 Incubation



(A) FACS analyses of CD4$^+$ and CD8$^+$ T-cell subsets as defined by CCR7/CD45RA following 6 day co-culture of freshly isolated human T cells and gpA33$^+$ SW948 colon cancer cells (E:T = 1:1) untreated or in the presence of MGD007 or control DART (both at 10 µg/mL). **(B)** T cells collected following 6 day incubation with gpA33$^+$ SW948 colon cancer cells in presence of control DART (upper row) or MGD007 (lower row) as described were re-cultured with Colo205-luc target cells (E:T = 1:1, 48h). Cytotoxicity levels were determined by decrease in luciferase fluorescence (left column) while supernatants were evaluated for IL-2, IFN-γ, and TNF-α levels by ELISA.

Steroid Treatment Limits MGD007-induced Cytokine Release While Preserving CTL Activity



(A) Cytokine concentrations (mean ± SEM) measured in the culture supernatants of normal human PBMCs following 0, 4, 8, and 24 hours incubation in the presence of gpA33-expressing Colo205 target cells and MGD007 in medium with or without 1.38 µM Dexamethasone (Dex) or 10 nM Budesonide (Bud). **(B)** MGD007-mediated cytolysis of luciferase-expressing Colo205 tumor cells with purified human T cells (E:T = 10:1, 24 hours) with or without 1.38 µM Dexamethasone (top) or 10 nM Budesonide (bottom).

Conclusions

- Further evaluation of MGD007 redirected T-cell killing activity reveals ability to leverage various T-cell populations including suppressive T cells
  - Greater potency with CD8 vs CD4 T-cell subsets correlates with granzyme/perforin levels
  - Engagement of CD8 or CD4 associated with characteristic T-cell lineage cytokine release
- MGD007 mediates lysis of CRC derived cancer stem cell model cell lines
- T-cells expanded in presence of MGD007 and gpA33$^+$ tumor cells *in vitro* exhibit increased levels of tumor cell killing compared to cytokine release
  - Expanded T cells display increased memory phenotype
  - Potent redirected T-cell killing maintained upon re-exposure to MGD007 in presence of fresh gpA33$^+$ target cells, with limited IL-2, IFN-γ and TNF-α secretion
- Exposure of T cells to steroids in presence of MGD007 and gpA33$^+$ target cells limits cytokine release with minimal impact on cell killing
  - Comparable profiles observed with both dexamethasone and budesonide (gut-restricted steroid)
- MGD007-mediated antitumor activity enhanced in presence of anti-PD1 mAb
  - PD-1 and PD-L1 upregulated by MGD007 upon co-engagement of gpA33 expressing tumor cells with CD3 T cells
  - Co-incubation of anti-PD1 mAb augments MGD007-mediated in vitro CTL activity
  - Co-admistration of anti-PD-1 with MGD007 enhances antitumor activity in mouse syngeneic tumor model

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